Glycative Stress Research

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Original article

Effect of continuous walking exercise program on the glycative stress marker in the elderly.

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Abstract

***Objective*:** The author at al. have been practicing a health promoting activity centering on a pedometer-based physical activity program in the Yurin Study in Kyoto since 2008. The purpose of this study is to evaluate the effect of pedometer-based physical activity for three years on the physical information of the elderly, with a focus on their glycative stress index in particular.

***Methods*:** The subjects were 12 males and 8 females (73.7 ± 4.6, 66 ~ 85 years old) who had received all three Anti-Aging Medical Checkups at 0M (0 months), 12M (12 months) and 24M (24 months) out of the elderly who participated in the pedometer-based physical activity program. The subjects were provided with a pedometer (HJ-720IT) for 12M ~ 24M and the number-of-step information recorded in the pedometer was confirmed through an interview once a month, during which the subjects were encouraged to continue the exercise. At Anti-Aging Medical Checkups, in addition to usual biochemical examinations, serum IGF-I and DHEA-s concentrations, muscle mass by bioelectrical impedance method (Physion MD), heel bone strength by supersonic method (A-1000), higher brain function by Wisconsin card sorting test, arteriosclerosis degree by acceleration plethysmogram (SDP-100) and the fluorescence strength (AF value) of AGEs (advanced glycation end products) in skin as glycative stress index were measured and the results were evaluated as functional ages by Age Management Check TM (Ginga Kobo). This research was conducted with the approval of the Ethical Committee of Doshisha University.

***Results*:** Although the number of subjects decreased year by year, the average numbers of steps a day (males: 10,648 steps and females: 7,272 steps) during the 13M ~ 24M period remained higher than the average numbers of steps a day of the elderly in their ‘70s (males: 4,800 steps and females 3,800 steps) by the guidelines given by the Ministry of Health, Labour and Welfare. Functional ages were compared with chronological ages. Muscle ages (0M: 56.5 ± 2.6 years, 12M: 57.1 ± 2.6 years and 24M: 57.6 ± 2.4 years) and vascular ages (0M: 62.0 ± 6.9 years, 12M 66.1 ± 9.5 years and 24M: 70.1 ± 9.0 years) were significantly young at all measuring times, and bone ages (0M: 65.5 ± 13.4 years) and hormone ages (24M: 69.5. ± 8.8 years) were significantly young in some measuring times. As for glycative stress index, AF values (0M: 2.47 ± 0.37, 12M: 2.24 ± 0.28, and 24M: 1.98 ± 0.37) and HbA1c (0M: 64 ± 1.2%, 12M: 6.0 ± 0.7% and 24M: 5.9 ± 0.6%) decreased as time proceeded ($p < 0.05$).

***Conclusion*:** This research showed that continued walking exercise maintains skeletal muscle mass and favorably affects glycometabolism, and as a result, it reduces glycative stress.

***KEY WORDS*:** functional age, Anti-Aging Medical Checkup, pedometer-based physical activity program, skeletal muscle and advanced glycation end products (AGEs)

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Introduction

The average life span of Japanese people is among the highest in the world. In 2014, the average was 80.50 years for males and 86.83 for females, which broke the past record in 2014[1]. Meanwhile, the population ageing is progressing rapidly in association with the decline in birthrate. The population aging rate was 23.1% in 2010 and Japan entered the era of the super-aging society so to speak.

The aging is not the problem of Japan only. It is expected that the world population will be aging significantly which has never been experienced before, so "aging and health" has become a global problem[2]. Therefore, Japan has been approaching this problem ahead of other nations. What is important is not only the extension of average life expectancy but also measures to extend the healthy life expectancy, where they live in good health independently, are required.

Most people have some physical weaknesses in association with aging, which are gradually worsening, leading to the loss of health. It is known that the elderly who exhibit healthy longevity, living independently beyond 100 years are not late in progression of aging, in particular, but they evenly age in the whole body[3]. In Anti-Aging Medicine, the functional ages of muscle, bone, hormone, nerve and blood vessels are evaluated as physical functions, and immune stress, oxidant stress, mental and physical stresses, lifestyle habit and glycative stress are evaluated as aging factors. It is considered that it is possible to extend healthy life expectancy by an individual's most aged function and biggest aging factor being found out and intensively remedied.

Glycative stress, one of the risk factors of aging, is now getting more attention. It is a comprehensive concept of biological stress caused by reducing sugar and aldehyde loading and the reactions that follow. In the case of glycation reaction, reducing sugars such as glucose and fructose derived from meals react with protein non-enzymatically and irreversibly, and as a result, advanced glycation end products (AGEs) are generated. It is known that AGEs are risk factors of atherosclerosis and bone fractures, and are also involved in the worsening of degenerative diseases including Alzheimer's, dementia, and the progression in diabetic complications[4]. The result of research on centenarians shows that the centenarians who suffered from diabetes account for approximately 6% and their prevalence of diabetes in their 70's and 80's were approximately 20%, which is a very low rate[5]. This finding suggests that it is important to solve the problem of glycative stress in order to achieve healthy longevity as a centenarian.

The elderly tend to be inactive in daily life, which often leads to the lowering of physical functions. It has been validated that those whose amounts of exercise and activity are larger are lower in the risk of the incidence of non-communicable diseases (NCDs) including ischemic heart disease, diabetes and osteoporosis, and as a result, the number of people in a bedridden state and death rate decrease[6-9]. It is also clarified that the physical activity and exercise are not only involved in reducing the incidence of NCDs, but also related to the prevention of the lowering of the functions necessary for engaging in social life, such as the prevention of the lowering of cognitive function and locomotive function of the elderly[10]. For the purpose of increasing physical activity, walking exercise is encouraged because many can easily do it in daily life[11]. The National Health Promotion Movement in the 21st century (Healthy Japan 21 – the second) presented by the Ministry of Health, Labour and Welfare is aiming at a long-term "increase of the number of steps" and "increase of the proportion of people with exercise habits" as the indexes for the measures of physical activity and exercise on a long time basis.

Our laboratory has implemented health promotion activity centering on a pedometer-based physical activity program as Yurin Study in the Kyoto Yurin Area since 2008[12-16]. The purpose of this research is to comprehensively evaluate physical functions and verify the effect of the continued practice of walking exercise on physical information of the elderly, with a focus on glycative stress in particular.

Method

Subjects

The subjects in this research were the participants in the exercise of previous research[12] (started Dec. 2008 and the late participants included), where they were motivated to participate in an exercise program using a pedometer and a handout. The participants were middle-aged and elderly persons living independently in the Yurin area, Shimogyou-ku, Kyoto, Japan and had no contraindication to exercise. The number of subjects at the start of this program were 37 (17 males and 20 females, aged 66 ∼ 85), the average age was 72.6 ± 6.0 (average ± standard deviation) and the average period of participation in the program was 3.9 years. The subjects associated with the result were those who had participated in the program more than three years and received all three Anti-Aging Medical Checkups.

The subjects could decide to participate, or not, of their own will, and even if they discontinued the participation that they had decided for certain reasons, they would not face any disadvantage due to its discontinuation.

Research design

The research period and research design are shown in *Fig. 1*. The 37 subjects received the first Anti-Aging Medical Checkup in April 2012 (0M), the second medical checkup in April 2013 (12M) and the third medical checkup in 2014 (24M). The number of steps was measured every month from April 2011 (-12M) to April 2014.

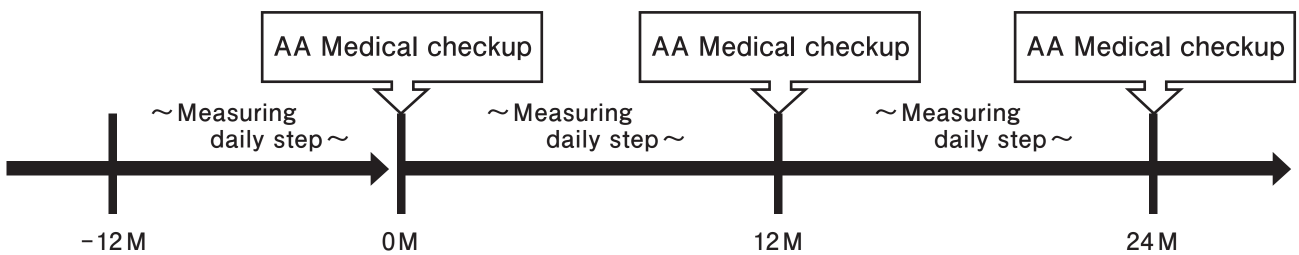

Fig. 1. **Study design.**
AA, Anti-Aging.

Intervening method

The 37 subjects were provided with a two-axis accelerometer-type pedometer (Walking Style HJ-720OT; Omron Health Care, Inc., Kyoto, Japan) and instructed to continuously wear the pedometer at the waist except sleeping time, bathing time and other times of being exposed to water, and walk every day during the research period with their own living style without any special instructions about the timing of exercise.

It was confirmed that the accuracy of this pedometer does not change either by lean person, overweight person or obese person in the past research [17].

The pedometer could record the number-of-steps data for 42 days. The data were recorded on a PC more than once a month (from –12M to 24M) using a special software (BI-Link Professional Edition 2.0: Omron Health Care, Inc.) for the purpose of the collection of data.

In order to exclude the days when the subjects forgot to wear their pedometer, only the data of the days when they wore it longer than 12 hours a day were used.

Distribution of printed matter

For the motivation of exercise, a handout was prepared once a month and given to the subjects after the interview. The contents of the handout were the individual's and whole participant groups' number-of-step record tables [18].

In the individual's number-of-step record table, the accumulated exercise volume from the start of current year based upon the collected number-of-step data was recorded. The individual average number of steps a day of the current month was calculated and recorded in the individual number-of-step record table together with target number of steps of the next month. The target number of steps of the next month was set based on the fluctuations of number of steps by season, individual physical condition and ailment taken into consideration so that the subjects could increase the number of steps effortlessly. At the time of interview, they were encouraged to continue the exercise by focusing on the following three points: 1) it was made possible for the subjects to be able to confirm their own position on the concrete course based on the their cumulative distance from the start of the current year in order to enhance the willingness to exercise, 2) the names and numbers of steps of the top ten subjects by gender and the average number of steps per day of the current month of the whole participant group were recorded in the whole participant groups' number-of-step record table. Furthermore, the comprehensive evaluations by gender for current month were recorded. 3) The information concerning exercise suited to each subject's conscious level was provided, which was written in a simple style, technical terms being avoided wherever possible.

Anti-Aging Medical Checkup

For the purposes of motivational support and the verification of achievement, Anti-Aging Medical Checkups were conducted at the frequency of once every 12 months (0M. 12M and 24M), which included body measurement, the evaluations of five functional ages including muscle, bone, hormone, nerve and blood vessel, and glycative stress test.

For the calculation of functional ages at Anti-Aging Medical Checkups, The Age Management Check^{R} system (Ginga Kobo, Nagoya, Aichi, Japan) was used, and relative functional ages were calculated from these databases and presented to each subject [18,19]. Functional ages have the merit that they easily became familiar to the subjects and easily connected to their behavior modification by being presented in the form of "equivalent to how old." As the results of Anti-Aging Medical Checkups were presented to them, the subjects could understand the weak point of their own aging and their behavior modification caused by the consciousness aiming at rejuvenation of their deteriorated functions were expected.

The evaluation of subjective symptom

Subjective symptom was classified into "physical symptom" and "mental symptom" and each ofof them were divided into five stages and evaluated using Anti-Aging QOL common questionnaire (AAQol) [20].

Body measurement

As for body measurement, body height, body weight and body composition were measured. For body composition, a bioelectrical impedance muscle measuring instrument (Physion MD; Nippon Shooter Ltd., Tokyo, Japan) was used.

Biochemical examination of blood

As for biochemical examination of blood, the blood concentrations of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, fasting blood glucose level, Hemoglobin Alc (HbAlc) (National Glycohemoglobin Standardization Program (NGSP)), insulin, insulin-like growth factor-1 (IGF-1), dehydorepiandrosterone-sulfate (DHEA-s) and coltisol were measured.

Measurement of muscle Age

The muscle mass to be used for the calculation of muscle age was evaluated with weight bearing index (WBI) and basal metabolism (kcal/day) as indexes using a bioelectric impedance method (high-precision muscle mass meter, Physion MD) in accordance with the method used in past research [18, 21-24].

Measurement of bone age

The bone density to be used for the calculation of bone age was calculated with the stiffness value of heel bone and % young adult means (%YAM) as indexes using a supersonic method (A-1000; GE Yokokawa Systems Ltd., Hino City, Tokyo).

Measurement of hormone age

IGF-I and DHEA-2, the blood makers of aging used for the calculation of hormone age were measured by biochemical examination of blood [25].

Measurement of neural age

The higher brain function to be used for the calculation of neural age was measured by Wisconsin card sorting test (WCST) [26-29].

Vascular age

The degree of arteriosclerosis to be used for the calculation of vascular age was measured using an acceleration plethysmogram (Dynapulse SDP-100, Fukuda Denshi Co., Ltd., Bunkyo-ku, Tokyo)[30, 31] and the estimated vascular age obtained was expressed as vascular age[32].

Glycative stress index

As reported already[33, 34], for glycative stress, the AF value (Auto Fluorescence value) of integrated data of AGEs was measured using an AGE Reader ™ (DiagnOptics, Groningen, Netherlands). The estimated AF value (y) was calculated from the regression equation, $y = 0.0175x + 1.1121$, based on the age-related changes and distribution of skin-auto-fluorescence of Japanese, using real age (x)[35].

AGE Reader™ is used to measure the accumulation of AGEs in skin by measuring auto-fluorescence of AGEs excited by being irradiated with ultraviolet light, non-invasively and easily. It is confirmed from the study of skin verification for diabetic patients and dialysis patients that skin AF is correlated to the accumulation amounts of typical AGEs including pentosidine and N^{ε}-(carboxymethyl) lysine (CML)[33]. The measurement was made at the 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[36]. It is proven that the higher the AF 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[37].

Statistical analysis

SPSS Statistics 23 (IBM Japan Ltd., Tokyo, Japan) was used for a comparative analysis of each measured value. A paired t-test was used for the comparison of each functional age and chronological age, Tukey's one-way analysis of variance was used for the change ofof functional age by each 12M and a Pearson's correlation analysis was used for the relationship between the number of steps and functional age. The percentage of risk less than 5% by two-sided test indicated that there was significant difference.

Ethical norm

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).

Result

Counting of the number of steps

The number of subjects participating in all medical checkups for three years was 20 (12 males and 8 females), their average period of participation in this program was 4.2 years and their average age at the starting time of this research was 73.7 ± 4.6 years.

The average numbers of steps a day for each of the 12 months in the period of this research (from the minus 12th month to the 24th month) were shown in ***Fig. 2***.

The average steps a day of all subjects during the research period of 36 months were 9,977.7 ± 6,642.4 steps. The average steps in each 12 months show that it was 10,937.3 ± 6,179.1 steps in 0M (from the minus 12th month to 0M), 9,697.9 ± 7,019.8 steps in 12M (from the first month to 12th month) and 9,297.8 ± 6,978.3 steps in 24M (from the 13th month to 24th month).

The average number of steps a day through research period of three years by gender was 11,219.6 ± 8,380.2 for male and 8,114.7 ± 1,670.2 for female. The average number of steps a day of each year of males was 11,977.7 ± 7,700.1 steps in 0M, 11.032.9 ± 8.834.2 in 12M and 10,648.4 ± 8,804.8 steps in 24M. That of females was 9,376.7 ± 2,413.5 in 0M, 7,695.5 ± 1,864.8 in 12M and 7,272.0 ± 1,592.7 in 24M. These values were kept higher than the average numbers of steps of septuagenarians recommended by the Exercise Guideline Small Committee, the Ministry of Health, Labour and Welfare (4,800 steps for male and 3,800 steps for female)[11].

The changes of individual's daily number of steps were shown in ***Fig. 3***. From the changes of individual's number of steps from 0M to 24M, four subjects increased their number of steps and 16 subjects decreased, and the maximum of change value was 3,450 steps per day and the minimum of change value was − 7,358 steps.

Evaluation of subjective symptom by common questionnaire

There was no significant difference in "physical symptom", "mental symptom" and all items of lifestyle habits from 0M to 24M. The questionnaire items with average scores higher than 2.9 out of 33 items of physical symptoms were the following four items: "tired eye (3.1 ± 0.9)," "hair loss (3.5 ± 0.9)," "gray hair (2.9 ± 0.7)" and "aching joint (3.0 ± 1.2)." The item with an average score higher than 2.9, out of 21 items of the mental symptoms, was only "lapse of memory."

Body measurements

The results of body measurements and body composition tests by a bioelectrical impedance analysis are shown in ***Table 1***. As the results of the comparison of measurement values at 0M and 24M, there was no significant difference in all items of body measurements or body composition tests.

Biochemical examination of blood

The results of measurements of blood concentration of each item by biochemical examination of blood are shown in ***Table 2***. As the results of the comparison of the measured values in 0M and 24M, HbAlc significantly improved (0M: 6.4 ± 1.2%, 24M: 5.9 ± 0.6%, p = 0.048). The value of cortisol was significantly lowered from 12.5 ± 3.0 µg/dl in 0M to 11.1 ± 3.4 µg/dl in 24M (p = 0.014).

Anti-Aging Medical Checkup

The changes of functional ages calculated are shown in ***Table 3*** and ***Fig. 4***.

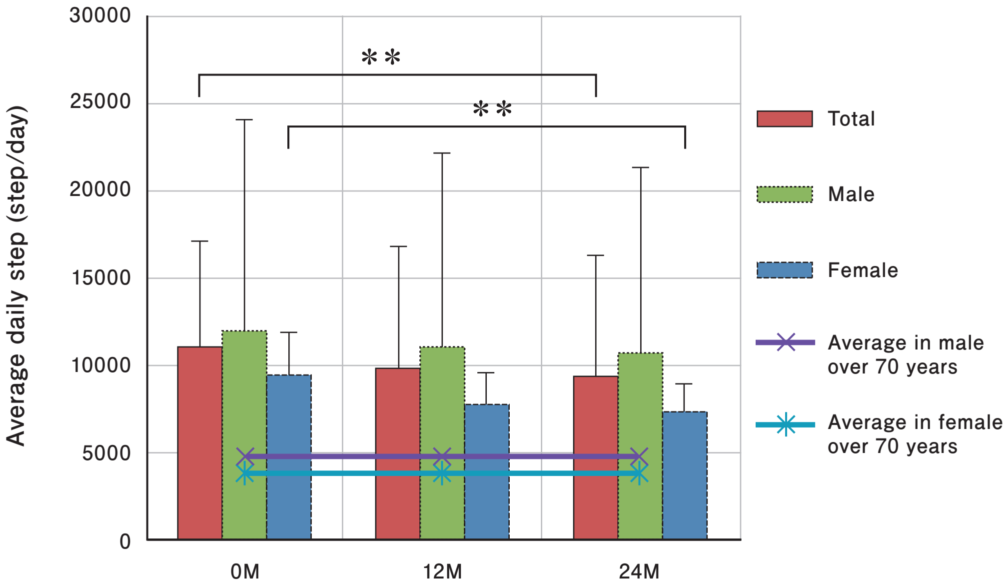

Fig. 2. **Changes of average daily step.**

Results are expressed as mean ± standard deviation, ** $p < 0.01$, Tukey test vs 0M, total n = 20 (male n = 12, female n = 8). 0M, average value from –12M to 0M; 12M, average value from 1M to 12M; 24M, average value from 13M to 24M.

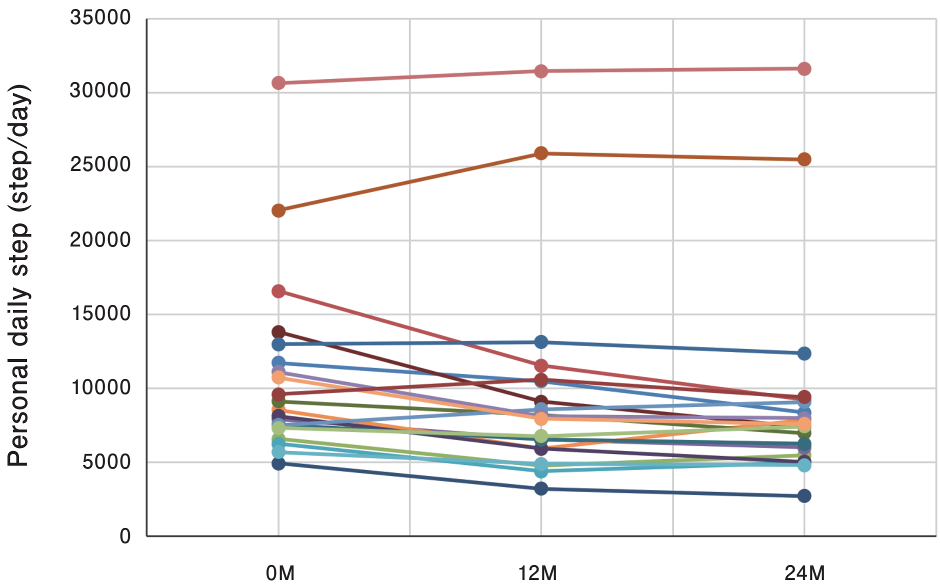

Fig. 3. **Changes of personal daily step.**

Table 1. Anthropometry.

		0M	12M	24M	p value
Height	cm	160.1 ± 7.5	159.0 ± 7.2	157.8 ± 7.4	0.598
Weight	kg	58.2 ± 9.1	57.0 ± 8.5	56.8 ± 8.9	0.996
BMI	kg/m²	22.6 ± 2.9	31.5 ± 2.4	22.7 ± 2.5	1.000
Systolic blood pressure	mmHg	142.1 ± 22.3	137.1 ± 29.3	143.5 ± 21.7	0.984
Diastolic blood pressure	mmHg	78.6 ± 11.9	74.9 ± 14.4	76.4 ± 12.7	0.849
(Body composition)					
Body fat	kg	14.7 ± 4.4	15.9 ± 3.6	16.3 ± 3.9	0.443
Percentage body fat	%	25.1 ± 5.7	27.8 ± 4.2	28.2 ± 4.4	0.121
Lean body mass	kg	43.0 ± 7.6	41.1 ± 6.3	41.3 ± 6.5	0.707
Percentage Lean body mass	%	74.9 ± 5.7	72.2 ± 4.2	71.8 ± 4.4	0.121
Water content	kg	31.8 ± 5.2	30.1 ± 4.6	30.2 ± 4.8	0.545
Percentage water	%	54.8 ± 4.2	52.9 ± 3.1	52.6 ± 3.2	0.121
Muscle mass	kg	23.3 ± 6.6	22.1 ± 4.9	21.5 ± 6.3	0.628
Percentage muscle	%	39.6 ± 8.6	37.7 ± 6.1	36.9 ± 7.3	0.482
Bone mass	kg	10.3 ± 2.9	9.7 ± 2.5	9.5 ± 2.7	0.607
Percentage bone	%	17.5 ± 3.5	16.9 ± 2.7	16.3 ± 2.8	0.406
Basal metabolic rate	kcal	1246.1 ± 175.3	1196.8 ± 159.3	1186 ± 161.7	0.489

Results are expressed as mean ± standard deviation, p value by Tukey test vs 0M, n = 20. BMI, body mass inde

Table 2. Blood chemistry.

		0M	12M	24M	p value
Total cholesterol	mg/dL	220.8 ± 33.5	212.0 ± 27.4	219.6 ± 37.1	0.826
HDL cholesterol	mg/dL	70.9 ± 18.9	68.5 ± 16.8	66.5 ± 14.8	0.247
LDL cholesterol	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**
Insulin	μU/mL	4.4 ± 2.0	3.5 ± 1.4	5.0 ± 4.5	0.168
IGF-I	ng/mL	104.2 ± 23.8	100.1 ± 26.0	102.1 ± 25.2	0.694
DHEA-s	μg/mL	77.3 ± 48.0	83.1 ± 62.5	79.3 ± 47.3	0.261
Cortisol	μg/dL	**12.5 ± 3.0**	**13.2 ± 3.5**	**11.1 ± 3.4 ***	**0.014**

Results are expressed as mean ± standard deviation. * $p < 0.05$, Tukey test vs 0W, n = 20. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FPG, fasting plasma glucose; IGF-I, insulin-like growth factor-I; DHEA-s, dehydroepiandrosterone-sulfate.

Table 3. Changes of functional ages

		0M	12M	24M	p value
Chronological age	years	73.7 ± 4.6	74.7 ± 4.6	75.7 ± 4.6	
Muscle age	years	56.5 ± 2.6	57.1 ± 2.6	57.6 ± 2.4	0.391
Bone age	years	64.5 ± 13.4	71.2 ± 14.1	72.0 ± 13.2	0.290
Hormone age	years	71.0 ± 10.2	70.7 ± 9.9	69.5 ± 8.8	0.876
Neural age	years	69.7 ± 13.4	72.2 ± 15.4	73.6 ± 13.3	0.669
Vascular age	years	62.0 ± 6.9	66.1 ± 9.5	70.1 ± 9.0 *	**0.010**

Results are expressed as mean ± standard deviation, * $p < 0.05$, Tukey test vs 0W, n = 20.

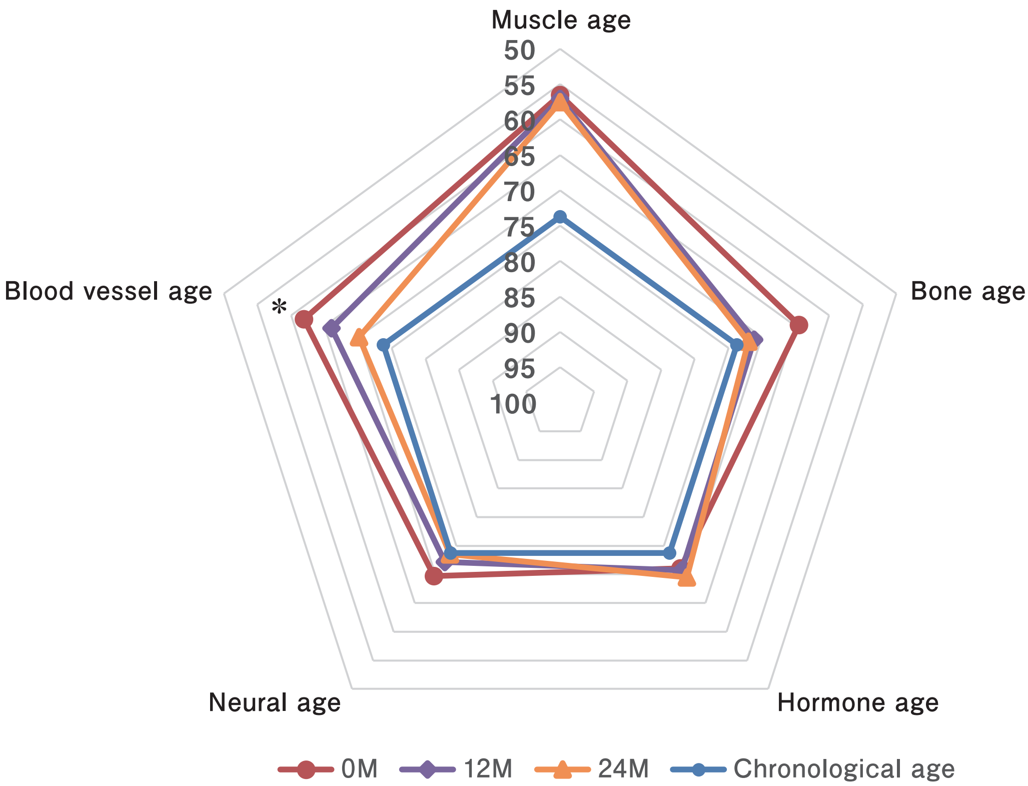

Fig. 4. **Changes of the functional age.**
* p < 0.05, Tukey test vs 24M, n = 20.

・Muscle age

The results of measurements of muscle mass by site, by using the bioelectrical impedance method, are shown in *Table 4*. The comparison of muscle ages and chronological ages shows that muscle age was younger than chronological age at any measurement time (0M: 56.5 ± 2.6 years, p < 0.001, 12M: 57.1 ± 2.6 years, p < 0.001 and 24M: 57.6 ± 2.4 years, p < 0.001). The comparison of measured values in 0M and 24M showed no significant difference in each site.

・Bone age

The results of measurements of bone mass by ultrasound method are shown in *Table 5*. The comparison of bone ages and chronological ages, bone ages were significantly younger in 0M measuring time (0M: 65.5 ± 13.4 years, p = 0.016). As the result of the comparison of the measured values at 0M and 24M, bone stiffness value (0M, 76.7 ± 23.0, 24M: 74.5 ± 14.4, p = 0.010) and YAM% (0M: 81.3 ± 11.3, 24M: 75.1 ± 13.1, p = 0.008) significantly deteriorated.

・Hormone age

The results of the measurements of the concentrations of IGF-I and DHEA-s in blood are shown in *Table 2*. The comparison of hormone ages and chronological ages showed that hormone ages were significant younger in 24M (24M: 69.5 ± 8.8 years, p = 0.002). As the result of the comparison of the measured values in 0M and 24M, no significant variation was observed in IGF-I, DHEA-s or hormone age.

・Neural age

The results of the measurements of higher brain functions by WCST are shown in *Table 6*. The comparison of neural ages and chronological ages showed that there was no significant difference at any measuring time point. As the result of a comparison of the measured value at 0M and 24M, there was no significant variation in each parameter, reaction time or neural age.

・Vascular age

The comparison of vascular ages were measured using an acceleration plethysmogram and chronological ages showed that the vascular age was significantly higher at any measuring time (0M: 62.0 ± 6.9 years, p < 0.001, 12M: 66.1 ± 9.5 years, p = 0.001 and 24M: 70.1 ± 9.0 years, p = 0.017). As the result of the comparison of the measuring values in 0M and 24M, vascular age significantly advanced (p = 0.010).

Table 4. Muscle mass.

		0M	12M	24M	p value
Right Side					
Upper arm	kg	0.60 ± 0.17	0.57 ± 0.16	0.57 ± 0.17	0.796
Forearm	kg	0.49 ± 0.12	0.47 ± 0.10	0.47 ± 0.11	0.830
Upper limb	kg	1.09 ± 0.29	1.04 ± 0.26	1.05 ± 0.27	0.801
Thigh	kg	3.48 ± 0.96	3.25 ± 0.76	3.27 ± 0.79	0.659
Leg	kg	1.49 ± 0.41	9.17 ± 35.03	1.39 ± 0.49	1.000
Lower limb	kg	4.97 ± 1.30	4.59 ± 1.06	4.68 ± 1.18	0.668
Left Side					
Upper arm	kg	0.60 ± 0.17	0.55 ± 0.15	0.52 ± 0.15	0.184
Forearm	kg	0.51 ± 0.12	0.45 ± 0.10	0.48 ± 0.15	0.684
Upper limb	kg	1.11 ± 0.28	4.02 ± 13.41	1.00 ± 0.28	0.999
Thigh	kg	3.60 ± 1.00	3.25 ± 0.80	3.35 ± 1.23	0.663
Leg	kg	1.45 ± 0.47	1.39 ± 0.41	1.37 ± 0.40	0.734
Lower limb	kg	5.05 ± 1.41	4.62 ± 1.13	4.71 ± 1.56	0.657
Total					
Upper limb	kg	2.14 ± 0.53	1.99 ± 0.44	2.05 ± 0.54	0.786
Lower limb	kg	9.83 ± 2.71	9.23 ± 2.15	9.40 ± 2.68	0.819
Right-Left balance					
Upper arm	%	100.68 ± 10.53	95.25 ± 19.78	92.06 ± 12.84	0.129
Forearm	%	105.11 ± 13.65	96.31 ± 9.04	101.06 ± 12.55	0.457
Upper limb	%	102.57 ± 9.67	97.49 ± 10.01	95.79 ± 8.77	0.051
Thigh	%	103.63 ± 8.37	99.89 ± 7.78	100.94 ± 14.46	0.639
Leg	%	97.18 ± 11.74	104.28 ± 11.56	98.23 ± 11.27	0.942
WBI					
Right		0.87 ± 0.19	0.83 ± 0.14	0.82 ± 0.14	0.508
Left		0.90 ± 0.21	0.82 ± 0.13	0.84 ± 0.24	0.570

Results are expressed as mean ± standard deviation, p value by Tukey test vs 0M, n = 20. Upper limb = forearma + upper arm; lower limb = thigh + leg; WBI, weight bearing index.

Table 5. Bone stiffness.

		0M	12M	24M	p value
Stiffness		**76.7 ± 23.0**	**74.9 ± 12.3**	**74.5 ± 14.4 ***	**0.010**
%YAM	%	**81.3 ± 11.3**	**75.6 ± 12.0**	**75.1 ± 13.1 ****	**0.008**

Results are expressed as mean ± standard deviation, * $p < 0.05$, ** $p < 0.01$, Tukey test vs 0W, n = 20. Stiffness, bone stiffness is measured by ultrasonographic ankle bone examination; YAM, young adult mean.

Table 6. Wisconsin card sorting test.

		0M	12M	24M	p value
CA		4.7 ± 1.5	4.4 ± 1.6	4.1 ± 1.7	0.302
NUCA		5.5 ± 6.5	6.1 ± 9.8	7.9 ± 10.7	0.301
TE		12.7 ± 5.2	14.6 ± 6.0	13.3 ± 3.7	0.981
PEN		2.5 ± 3.8	3.1 ± 3.4	1.1 ± 1.7	0.531
PEM		1.4 ± 2.7	1.6 ± 2.1	1.7 ± 1.6	0.748
UE		0.2 ± 0.4	0.2 ± 0.5	0.1 ± 0.4	0.766
Response time	second	161.9 ± 101.4	200.4 ± 73.3	159.4 ± 53.6	0.996

esults are expressed as mean ± standard deviation, p value by Tukey test vs 0M, n = 20. CA, categories achieved; NUCA, numbers of response cards used until the first category achieved; TE, total errors; PEN, perseverative errors of Nelson; PEM, perseverative errors of Milner; UE, unique errors.

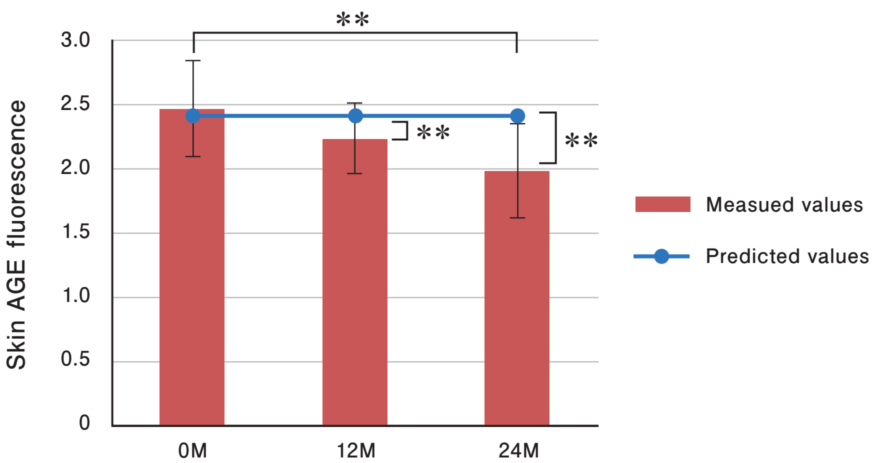

Fig. 5. **Changes of skin AGE fluorescence.**
** p<0.01, Tukey test vs 0M or paired t test vs predicted values, n = 20. Predicted values (y) of skin AGE fluorescence in each age (x) are calculated by the equation as follows; y = 0.0175x + 1.1121.

・Glycative stress

The measuring results of the fluorescence strengths of AGEs in the skin of the subjects' forearm (AF value) by AGE Reader TM are shown in ***Fig. 5***. Fluorescence strength (AF value) of AGEs in skin decreased over time (0M: 2.47 ± 0.37, 12M: 2.24 ± 2.28 and 24M: 1.98 ± 0.37) and AF value significantly decreased in 24M compared to that in 0M ($p < 0.001$). The actual measured value of the fluorescence strength of AGEs in the skin (AF value) was significantly low at 12M ($p = 0.008$) and 24M ($p < 0.001$).

Effect of walking exercise

In order to evaluate the effect of walking exercise amounts, a correlation analysis between the absolute number of steps a day for 12 months immediately before each measurement and functional age was conducted. The absolute number of steps had a negative correlations with bone age (0M: $r = -0.502$, $p = 0.024$, 24M: $r = -0.444$, $p = 0.050$) and vascular age (0M: $r = -0.491$, $p = 0.028$, 12M: $r = -0.417$, $p = 0.047$). In other words, the correlation of "the larger the absolute number of steps was, the younger the functional age is" was observed.

In order to evaluate the effect of change of the number of steps that occurred, due to the continuation of exercise, a correlation analysis between the change of number of steps a day from −12M to 24M, and the change of functional age from 0M to 24M, was conducted. The change of the number of steps had a negative correlation with the changes of bone age. In other words, the more greatly the number of steps decreased, the more greatly bone age advanced (0M ~ 24M: $r = -0.594$, $p = 0.006$). Furthermore, the change of the number of steps had a positive correlation with the change of vascular age. In other words, there was a correlation that the more the number of steps increased, the more the vascular age advanced (0M ~ 24M: $r = -0.557$, $p = 0.011$).

Discussion

The exercise program

According to recent reports, the number of people who are conscious of the importance of physical activity and exercise and the elderly who have an active attitude towards going out have been increasing. On the other hand, the comparison of data of 1997 and 2009 showed that the average number of steps a day of Japanese people 15 years old or older, both male and female, decreased by about 1,000 steps and the proportion of the people with exercise habits defined as those who continue the exercise for 30 minutes twice a week more than a year had not changed [1]. In other words, the current state is that although people have become more conscious of the importance of physical activity and exercise and highly motivated to practice, they have the problem that they cannot actually do it. The current situation of the elderly is that the average number of steps a day of the elderly 65 years old or older is 5,628 for male and 4,585 for female, and the proportion of the elderly with exercise habits is said to be 41.9% [38]. However, in the case of the subjects of this research, although their number of steps a day for each 12 month period decreased, compared with elderly people in general, their average number of steps for 36 months was about twice for male and about 1.8 times for female, and it showed that they maintained a very high level. Furthermore, 80% of the subjects had exercise habits, which were also at high level. Because there was no variation in cortisol, a stress index was observed, it may be said that the exercise intervention of this time was a reasonable body burden.

It is also considered that the participation in the exercise program of this research caused the effect of making friends, because they practiced the same activity not only the exercise together with the people of the same generation. From the viewpoint of the establishment of exercise habits, it is said to be important to do exercise effortlessly. Being conscious of the existence of friends doing exercise together with and knowing their own level of physical strength [39], so that the walking exercise program for this research is considered to be an effective method from these viewpoints. In order to achieve healthy longevity when the aging society is advancing, the propagation of simple and familiar training methods like this is considered to be meaningful.

Anti-Aging Medical Checkups

Evaluation of subjective symptom by body measurement and common questionnaire

There was no significant change in body measurement, body composition tests by bioelectrical impedance method and the evaluation of subjective symptoms by AAQol common questionnaire during 24 months of intervention period.

Although no significant difference in the comparison of average values, the number of subjects who were diagnosed with obesity (BMI ≧ 25) was six in 0M but it decreased to four in 24M and the number of subjects diagnosed with low body weight (BMI ≦ 18.5) was one in 0M but it was zero in 24M. Although this research set no diet restriction, in particular, it is considered that they could healthfully reduce BMI through walking exercise, without lapsing into low body weight.

Biochemical examination of blood

It is said that aerobic exercises like walking, accelerates heart rate and facilitates the flow of blood, and as a result, they can improve the metabolism of glycolipid and the levels of HbAlc and cholesterol in blood and it is important to continue the exercises, in particular [40]. It is also reported that the significant improvements of the values of HDL-C and adiponectin were recognized in the discussion of the effectiveness of pedometer-based physical activity type walking instruction as the same as this research [18].

In this research, no significant change in total cholesterol, LDL-C or HDL-C was observed. Even though no significant improvement was observed, the average values of these items of the subjects fell in standard values. Therefore, it was considered that because of their participation in this program, over four years in average, they could maintain constant values of these items.

Muscle age

Their muscle ages were significantly young compared to chronological ages. It is said that more than 60% of WBI is required for the independent activities of daily living (ADL) and the lowering of WBI leads directly to the need of long-term nursing care and a bed-ridden state. Because age-related factors are less involved in WBI in middle age, it can be interpreted within the context of body weight factor only that muscles cannot simply deal with the increase of body weight [41]. However, the more people age, the more they are affected by various aging factors other than body-weight factors.

The decrease of muscle mass caused by aging is pointed out. Generally speaking, about 1% of muscle mass decreases a year with only daily living activity over 40 years of age and it decreases 1% in two days in the case of the elderly in bed-ridden state [21]. There was no person who was bed-ridden or requiring nursing care among the subjects of this research. The proportion of those having daily exercise habits was large among the subjects and their number of steps a day was much larger than that of elderly people in general. It was suggested that the continuation of walking exercise inhibits the decrease of muscle mass and keeps muscles young.

Bone age

Bone age had a negative correlation with the absolute number of steps and age-related bone change had a negative correlation with number-of-step change. In other words, there was a correlation that the larger the absolute number of steps is, the younger the functional age is. As the number of steps decreased as a whole, bone age also resulted in aging.

There are many reports that exercise therapy has an effect on the prevention of osteoporosis. In the report concerning the effect of exercise increasing bone mass, it was concluded that although there is no large effect, low bone mass can be inhibited by exercise enforcement [42]. Walking exercise is simple and less risky and its effect on the increase of lumbar spine bone density [43] and that of femoral neck bone density [44] are suggested.

Even though the subjects of this research were more active than elderly people in general, and their average number of steps a day also was larger, their bone ages advanced with the decrease of the number of steps in the period of the program.

Generally speaking, the intensity of exercise is said to greatly affect the improvement of bone density, and therefore, exercise with strong intensity is considered to be desirable. However, in the case of the elderly whose bone mass has been already lowered, it is impossible to do complicated and high intense exercise and it is difficult to make them manage their own exercise without the management and intervention of physical therapists and other specialists.

Therefore, in order to persuade them to improve their bone age, it is necessary to investigate the relationship between the intensity of exercise within the range where the elderly can practice on a daily basis and bone age. It is also important to conduct exercise guidance using indexes other than the number of steps, such as metabolic equivalents (METS), the index of intensity of exercise and the amount of exercise (Ex) that can express physical activity amount disregarding the difference in individual body weight.

Hormone age

In this research also, no significant changes in IGF-I and DHEA-s were observed and no significant change in hormone age also was observed. It might be possible to maintain the status quo with the number of steps of the subjects of this research but it could not improve hormone age. As this research set no diet restriction, and if it is aimed at achieving the improvement of hormone age more than the status quo, it is necessary to consider diet therapy, including supplement, drug, and hormone replacement therapies.

Neural age

Regarding neural age, it is reported that walking favorably affects nerve activity. According to a cross-sectional research on the 18,766 women aged 70 ～ 81 who were regularly practicing walking for long-term, their cognitive function was significantly superior and the lowing of their cognitive function also was mild[45]. According to a cross-sectional research on the 2,257 men aged 71 ～ 93, walking had a correlation with the decreased risk of developing cognitive dysfunction[46]. These findings suggest the possibility that active lifestyle habits such as walking prove useful for the maintenance of cognitive function and prevention of dementia in the elderly.

In this research, neural age was cited as the weak point for aging among the large number of subjects. Even so, it is not that neural age greatly ages compared to chronological age, but that because the other functions were kept in a very young condition compared to chronological age, neural age was cited as a weak point.

Anti-Aging Medicine attaches great importance in keeping the five functional ages of muscle, bone, hormone, nerve and vascular age in good balance and a hypothesis has been proposed that healthy longevity can possibly be extended by neural age judged as weak point being improved. In past research where the neural ages of the same group of this research (independently living elderly people) evaluated by WCST and Δ neural age (Δ neural age = neural age – chronological age) were compared with those of persons needing support and those requiring long-term care, the neural age of independently living elderly people was 71.1 ± 16.7 years, that of persons needing support was 81.2 ± 9.1 years and that of persons requiring long-term care was 88.5 ± 5.8 years; and Δ neural age of independently living elderly people was 0.3 ± 11.8 years, that of persons needing support was 3.2 ± 13.4 years and that of persons requiring long-term care was 4.8 ± 6.8 years[47]. The neural age of independently living elderly people is maintained younger compared to persons needing support and those requiring long-term care and it is described that in order to live an independent life, it is important to keep neural age young.

In this research, no significant improvement of neural age by walking exercise was observed. This exercise program is not only for exercise but useful for the communication with others and it is also useful for conscious behavior modification. Establishing an effective method for the rejuvenation of neural age by the methods including exercise and other approaches makes it possible to realize well-balance aging and extend healthy longevity to live an independent life.

Vascular age

Vascular ages were significantly younger in all measurements compared to chronological ages.

In the study of the relationship among absolute amount and change of step number and the value of Brachial-ankle pulse wave velocity (baPWV), one of the vascular functional indexes that increases with aging, there is a report that the increase of baPWV value by the increase of the number of steps in improved the vascular function[13]. However, in this research, vascular age has a significant negative correlation with the absolute amount of step number. In other words, the larger the absolute number of steps is, the younger the vascular function is, and on the other hand, regarding the variation of step number, the opposite result was shown that the more the number of steps increases, the more the vascular age advances. In this respect, focus on a subject whose step number greatly decreased (more than – 5,000 steps) from the minus 12th month to the 24th month and it was found that his average number of steps a day is more than 10,000 in the minus 12th month. Although the number of steps greatly decreased, this subject maintained a larger number of steps than the average value of the subjects. From the fact that the number of steps and vascular ages were distributed in this way and that absolute amount of step number and vascular age have negative correlation, the absolute amount of step number is more important than variation of step number for the improvement of vascular function.

Glycative stress

During the period of this research, AF value and HbA1c greatly decreased and the improvement of glycative stress was observed due to continued walking exercise for three years. Aerobic exercises including walking and jogging are said to be effective for the improvement of insulin resistance. In this research, no significant change in insulin resistance was observed but none of the subjects had an abnormality in insulin resistance. HbA1c is an index of long-term disorder of carbohydrate metabolism reflecting blood glucose control for the past 1 ～ 2 months and it was improved by the continued walking exercise for the long term. Although AGEs in skin are said to correlate with age and be accumulated in the body as a result of long-term lifestyle habits and life rhythm, it is significant that despite the fact that subjects were elderly, such a great rejuvenation was observed.

As the reason why the result like this was achieved, the muscle of the subjects was maintained at a very young condition (Δ muscle age: about – 17 years). Muscle is a very important organ that consumes sugar and generates energy, and during exercise, AMP kinase senses the elevations of adenosine monophosphate (AMP) and adenosine triphosphate (ATP) in association with muscle contraction of skeletal muscles can decrease blood glucose levels by increasing the uptake of glucose. And, as mentioned above, the average number of steps per day of the subjects was very large compared to elderly people in general. It was considered that, by consciously doing walking exercise in the condition where the muscle was maintained at a young state like the subjects of this research, synergetic effects can be obtained. It is reported that the accumulated amount of AGEs in tissue of non-diabetic smokers is larger than that of non-smokers [48]. The fact that there was no smokers among the subjects contributed to this result.

Recently, glycative stress is paid attention to as one of the risk factors of aging together with oxidant stress. Aerobic organisms have obtained the protective mechanism against oxidant stress over a long period of history in order to adapt to the environment where the concentration of oxygen levels were elevated 2.5 billion years ago. On the other hand, the history of glycative stress started from the discovery of Maillard reaction by Louis Camille Maillard in 1912 and the concept of glycative stress was born about 50 years ago. In recent years, in particular, glycative stress has been proven to be caused by excess nourishment and inadequate exercise by changes in lifestyle habits including diet, and our protective mechanism against glycative stress is not enough. It is known that AGEs are risk factors of atherosclerosis and bone fracture and they are involved in the worsening of degenerative diseases including Alzheimer-type dementia and the progression of complications of diabetes [4].

In the research on centenarians in Japan, it is reported that the incidence of diabetes is very low among ultra-centenarians over 110 years [5,49]. Because it is possible to reduce the risk of complications of diabetes and incidence of diabetes by reducing glycative stress, it is considered to be significant to alleviate glycative stress in order to achieve 100 years. Although the glycative stress index of the subjects of this research measured in 0M was in the same level with the average value of the people of the same generation, it was greatly improved by the continued walking exercise. The intervention into the subjects of this research will be continued and the validation of whether the subjects of this research who could alleviate their glycative stress can achieve 100 years or not will be continued in the future.

Conclusion

The continued exercise is effective for the alleviation of glycative stress by maintaining muscle mass and consuming glucose. Although it works differently for different functions, it was suggested that it was also effective in the improvement of the functions of bone and blood vessels.

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Conflict of interest

The authors claim no conflict of interest in this study.

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