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Original Article

The Effects of Long-term Intake of Kale Juice on the Aging of Physical Functions - Cross Sectional Study -

Chie Tarumizu [1)], Sayuri Matsuoka [1)], Kei Yui [1)], Motohiko Sugai [2)], Umenoi Hamada [3)], Wakako Takabe [4)], Yoshikazu Yonei [4)]

1) FANCL Research Institute, FANCL Corporation, Totsuka-ku, Yokohama, Japan
2) Sakura Clinic, Mitaka, Tokyo
3) A-KIT. Co., Ltd, Kyotanabe, Kyoto, Japan
4) Anti-Aging Medical Research Center and Glycative Stress Research Center, Graduate School of Life and Medical Sciences, Doshisha University, Kyotanabe, Kyoto, Japan

Abstract

***Objective*:** To verify the effects of kale juice on physical functional age by a cross sectional study.
***Methods*:** An anti-aging medical checkup was performed on 110 females aged 50-69 years who had been consuming one or more cups of kale juice (FANCL Corporation) daily for at least 1 year (hereinafter referred to as the "kale group"). The subjects were examined once and the results were compared with a general, countrywide data group already accumulated and age-adjusted (n = 1,063). The study was only commenced once approved by the appropriate ethics committee.
***Results*:** Concerning physical functional age, the results of the anti-aging medical checkup indicated a nervous system age of 53.5 ± 12.8 years (chronological age; -4.8 ± 12.0 years) and a muscle age of 48.7 ± 3.9 years (chronological age; -9.7 ± 4.7 years) in the kale group; both were significantly lower than the corresponding values in the control group. Blood biochemistry indicated significantly higher HDL cholesterol and significantly lower triglycerides, fasting blood glucose, HbA1c, insulin, and homocysteine in the kale group than in the control group. Urine analysis showed a significantly lower isoprostane production rate in the kale compared to control group. Finally, an anti-aging questionnaire measuring quality of life (QOL) showed lower scores in the kale group for "palpitations," "shortness of breath," "thirst," "anorexia," "early satiety," "coughing and sputum," "diarrhea," "constipation," "memory lapse" and "inability to solve problems", compared to control data.
***Conclusion*:** These results suggested that long-term kale consumption could be effective for the prevention of physical and mental aging.

***KEY WORDS*:** kale (*Brassica oleracea* var. acephala), aging, physical functional age, risk factor, quality of life (QOL)

Introduction

The recent and rapid aging in Japan is inducing increased medical expenses and emerging problems associated with specialized medical care. Anti-aging medicine falls under preventive medicine and integrated medical care surpassing specific clinical specialties [1)], and aims to "enhance health, improve quality of life, and achieve long life in good health." Regressive changes associated with aging emerge as various symptoms that can be expressed and understood as the functional age of organs, and which can be used to evaluate the therapeutic effect of integrative medicine. For instance, the Japanese Respiratory Society has already presented the notion of lung age [2)].

The Anti-Aging Medical Research Center, Doshisha University is undertaking research into the development of an aging evaluation system based on anti-aging clinical data collected thus far [3, 4)]. Their system is defined as a "method to evaluate aging with an anti-aging medical checkup and to present the results as functional age," that is designed to motivate users to enhance their health, and, for physicians and researchers, is suitable for evaluating health promotion programs. Data on approximately 10,000 persons across the country have already been accumulated. The reversal and prevention of functional age can be an indicator of health enhancement and a motivator for the need to take health food.

*Address Correspondence to: Chie Tarumizu
FANCL Research Institute, FANCL Corporation
Address: 12-13 Kamishinano, Totsuka-ku, Yokohama, Kanagawa, 244-0806, Japan
Tel.: +81-45-820-3443 Fax: +81-45-820-3526 E-mail: chie_0904@fancl.co.jp
Co-authors: Matsuoka S, matsuoka@fancl.co.jp ; Yui K, ke-yui@fancl.co.jp ;
Sugai M, motohiko@pg7.so-net.ne.jp ; Hamada U, hamada@yonei-labo.com ;
Takabe W, wtakabe@mail.doshisha.ac.jp ; Yonei Y, yyonei@mail.doshisha.ac.jp

Kale is a plant of the Brassicaceae family (the original species of cabbage), which contains remarkably more vitamins, antioxidative components and minerals such as calcium than other vegetables, and there have been many reports about its health benefits. We have reported various aspects of the functionality of kale such as its potential for the prevention of bone loss[5], palliation of knee joint pain[6], palliation of cedar pollen allergy symptoms[7] and atopic dermatitis[8,9], as well as improvement in alcohol metabolism [10]. Other possible effects of kale include cancer prevention, benefits for intestinal health and immunity, reduction of blood pressure and cholesterol, protection of the gastric mucosa, and alleviating or reducing stress; however, there have been no reports that investigated the effects on aging.

The present study therefore compared the effects on aging of physical functions in persons who have consumed kale juice for a long time compared to the general Japanese population based on data already accumulated.

Methods

Subjects

The study group comprised 110 females aged 50 to 69 years who had been consuming one or more cups of kale as juice produced by FANCL Corporation (Honshibori-aojiru; Premium-Reito or Premium (frozen or powder), both equated to approximately 120 g of raw leaves), daily and continuously for at least 1 year (hereafter referred to as kale group). Subjects were excluded under the following criteria: 1) serious kidney, liver, heart, brain, blood or immune disease; 2) contraindicated for the use of the test apparatus; 3) blood not able to be collected from the cubital fossa vein; and 4) judged as unsuitable by the principal investigator.

The significance of the study, test method, and participants' rights were properly explained to the candidate participants, and their participation thereafter was determined by signing a consent form.

Study design

The study was designed as a cross-sectional study, testing each person in the kale group 1 time, and was performed at Tokyo Synergy Clinic (Chuo-ku, Tokyo) from May 2013 to May 2014.

Subject food

Table 1 shows a summary of the kale product (frozen or powder, both equivalent to approximately 120 g of raw leaves) corresponding to the inclusion criteria of subjects.

Test method

Subjects were prohibited from performing more exercise than daily activities necessitated and from drinking alcohol beverages on the day before testing, and were instructed to

Table 1. Kale Component (/a pack)

	Honshibori-aojiru Premium-Reito (frozen100 g)	Honshibori-aojiru Premium (powder10 g)
Energy	11 ~ 30 kcal	25 ~ 44 kcal
Dietary fiber	0.10 ~ 0.60 g	0.81 ~ 2.0 g
Sodium	–	18 ~ 44 mg
Sodium chloride equivalent	0.06 ~ 0.15 g	–
Potassium	138 ~ 349 mg	161 ~ 445 mg
Calcium	73 ~ 226 mg	91 ~ 215 mg
Magnesium	19 ~ 33 mg	17 ~ 48 mg
Iron	0.14 ~ 0.78 mg	0.19 ~ 0.36 mg
β-Carotene	935 ~ 2,378 μg	555 ~ 2,029 μg
Vitamin C	200 mg	76 ~ 229 mg
Vitamin E	0.32 ~ 2.1 mg	0.46 ~ 1.6 mg
Vitamin K	92 ~ 209 μg	98 ~ 222 μg
Folic acid	38 ~ 127 μg	39 ~ 93 μg
Total chlorophyll	16 ~ 35 mg	14 ~ 44 mg
SOD activity	49,000 ~ 110,000 unit	27,000 ~ 78,000 unit
Lutein	1.3 ~ 3.1 mg	0.97 ~ 2.7 mg
DFA III (Difructose anhydride III)	500 mg	500 mg

SOD, superoxide dismutase; DFA, difructose anhydride.

finish dinner before 21:00 and to sleep for an appropriate time. Urine was collected and analyzed in the morning on the test day. Patients were prohibited from eating, drinking except for water, or smoking from rising until after completion of the tests. In addition, oral drugs were only administered under the direction of the attending physician. Testing was performed during the morning sequentially after checking the health condition on the day. Test parameters included anthropometry, blood pressure after resting, anti-aging medical checkup (functional age evaluation: muscle age, bone age, hormonal age, nervous system age and vascular age), blood biochemistry, urine analysis, and subjective symptoms, in reference to previous reports [11-13].

(1) Anti-aging medical checkup (functional age evaluation)

Relative functional age of muscles, bones, hormones, nerves, and blood vessels was calculated for each subject using the system database of the population by the Age Management Check^{R} system (Ginga Kobo, Naka-ku, Nagoya, Japan). To determine muscle age, weight bearing index, basal metabolic rate, body mass index (BMI), and body fat percentage were measured using a bioelectrical impedance body composition meter (Physion MD; Physion, Shimogyo-ku, Kyoto). For bone age, the stiffness was measured by ultrasonic bone densitometry (A-1000; GE Yokogawa Medical Systems Ltd., Hino, Tokyo). For hormonal age, blood somatomedin-C (insulin-like growth factor-I; IGF-I) and dehydroepiandrosterone-sulfate (DHEA-s) were analyzed. For nervous system age, the Wisconsin card-sorting test (WCST) [14] was performed to test higher brain function. Vascular age was measured with a blood pressure/pulse wave meter (Cardio Ankle Vascular Index (CAVI); Fukuda Denshi Co., Ltd., Bunkyo-ku, Tokyo) and accelerated plethysmography (Dyna Pulse SDP-100; Fukuda Denshi Co., Ltd., Bunkyo-ku, Tokyo) as indicators of arteriosclerosis.

(2) Blood biochemistry

Hematology measurements included leukocyte count, erythrocyte count, hemoglobin (Hb), hematocrit, and platelet count. Biochemistry measurements included total bilirubin, aspartate aminotransferase (AST), alanine transaminase (ALT), γ-glutamyl transpeptidase (γ-GTP), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), and creatinine. Lipid metabolism was investigated by measuring total cholesterol (TC), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), and triglycerides (TG), while sugar metabolism was investigated by measuring fasting plasma glucose (FPG). HbA1c [NGSP] and insulin (immune reactive insulin; IRI), and protein metabolism was investigated by measuring the total protein (TP). The measured electrolytes were sodium (Na), potassium (K), chloride (Cl), calcium (Ca) and iron (Fe). Endocrine function was assessed by measuring cortisol, DHEA-s, IGF-I, progesterone, and estradiol (E_2), while glycative stress index was measured based on pentosidine. Finally, levels of highly sensitive C-reactive protein (hsCRP) and total homocysteine were also measured. These analyses were contracted out to LSI Medience Corporation (Minato-ku, Tokyo). The LDL cholesterol/HDL cholesterol (L/H) ratio as the arteriosclerosis index and insulin resistance index (HOMA-R) were also calculated.

(3) Urine analysis

The first morning urine was used to measure the oxidative stress indices of 8-OHdG (8-hydroxydeoxyguanosine) and isoprostane (1,5-isoprostane F2t: 8-epi-PGF2α/ 8-isoPGF2α) production rates as well as creatinine levels. Analysis was contracted out to LSI Medience Corporation (Minato-ku, Tokyo).

(4) Subjective symptoms

Subjective symptoms were evaluated using the Anti-Aging QOL Common Questionnaire (AAQol) [15] that consisted of 56 questions on complaints concerning "physical symptoms" and "mental symptoms" at 5 grades, as follows: "1. Not at all", "2. Almost not", "3. Slightly", "4. Moderately", "5. Severe." For lifestyle-related behavior, an interview was conducted regarding smoking, alcohol consumption, exercise, sleeping hours, water intake, and visual display terminal (VDT) working hours.

(5) Diet survey

The current status of nutrient and food consumption was investigated as consumption frequency using a brief self-administered diet history questionnaire (BDHQ) [16, 17].

(6) Skin AGEs measurement

The skin deposition of advanced glycation endproducts (AGEs) was evaluated non-invasively by the AGE reader™ (DiagnOptics, Groningen, The Netherlands) [18].

(7) General data

For the control group, females aged 50 to 69 years and matched to the subject group were selected from the first visit data of anti-aging examinees (6,016 cases as of November 2012) owned by the Anti-Aging Medical Research Center, Doshisha University, and 1,063 individuals who had undergone bone densitometry and WCST required for the calculation of functional age were extracted from them. There were no differences in chronological age distribution between the control group and the kale group.

Ethics

The study was performed in compliance with the ethics principles that have their origin in the Declaration of Helsinki, the Law for the Protection of Computer - Processed Personal Data Held by Administrative Organs, and the "Ethical Guidelines for Epidemiological Research" of the Ministry of Health, Labour and Welfare and the Ministry of Education, Culture, Sports, Science and Technology. The present study was also performed with the approval of the clinical research ethics board of FANCL Corporation (Approval No. F12-031), the ethics board of Tokyo Synergy Clinic, and the ethics board of Doshisha University (Approval No. #1318).

Statistical analysis

Results are shown as mean ± standard deviation (SD). For the functional age of the control group, there are differences in the number n, because the data were obtained with different models of analysis and thus only data using the same model as used for the kale group were included in the present study for some parameters. Comparison between groups was performed using an independent t test for parametric data and the Mann-Whitney U-test for nonparametric data. The statistical analysis software used was SPSS 21.0 for windows (SPSS Japan, Shibuya-ku, Tokyo), with a two-tailed significance level of 5%.

Results

(1) Subjects

All collected data of 110 persons in total (mean age 58.4 ± 5.6 years) were used for analysis of the kale group. The control group was set as 1,063 females aged 50 to 69 years of matching age, sex, and ethnicity (Japanese) as the general data, and it was confirmed that there were no differences in chronological age distribution from the kale group.

Table 2 shows lifestyle-related behaviors, and ***Table 3*** shows the status of kale intake in the kale group as subject background. Among the lifestyle-related behaviors, alcohol consumption and frequency of alcohol drinking were significantly higher in the kale group compared to controls. The kale intake status in the kale group confirmed that almost all had consumed 1 cup of frozen juice daily for 3 years. The combined use of supplements containing functional components that overlap with those of kale was reflected in high percentages of vitamin C (15%) and calcium (15%) in the kale group, but most (~73%) did not use any supplements. The daily food consumption status of the kale group was generally the standard for Japanese people. As for the glycative stress index, blood pentosidine in the kale group was 0.0453 ± 0.0147 μg/mL, which was slightly higher than the standard range according to the kit manufacturer (0.00915 to 0.0431 μg/mL [ELISA]). AGEs accumulation (AF) in the kale group was 2.2 ± 0.3, which was similar to the mean value (2.09 ± 0.36 at age 50 to 60 years, and 2.46 ± 0.57 at age 60 to 70 years) supplied by the manufacturer of the AGEs Reader.

(2) Functional age

Table 4 represents the comparison of functional age; muscle age in kale group was younger than that in control group ($p < 0.001$), bone age in kale group older than in control group ($p = 0.024$), hormonal age in kale group older in control group ($p = 0.005$), nervous system age in kale group younger than in control group ($p < 0.001$), and vascular age in kale group was not significantly different from that in control group ($p = 0.244$).

(3) Functional age calculation factors

Table 5 represents the comparison of functional age factors. Among muscle age factors, the basal metabolic rate was significantly higher while BMI and body fat percentage were significantly lower in the kale group than in controls. As for the bone age factor, stiffness was significantly lower in the kale group. Among vascular age factors, estimated vascular age by accelerated plethysmography was higher

Table 2. Lifestyle-related behaviors.

		Control group (CON)			Kale intake group (KL)			p-value
		n	mean	SD	n	mean	SD	(CON vs KL)
Smoking	Cigatettes/day	1,063	1.25 ±	5.09	110	0.65 ±	3.22	0.154
Alcohol consumption	mL/day	1,063	0.45 ±	0.76	102	0.72 ±	0.90	0.000 **
Frequency of alcohol drinking	times/week	1,050	1.49 ±	2.29	108	2.46 ±	2.57	0.000 **
Exercise	days/week	1,063	1.76 ±	2.08	109	1.68 ±	2.18	0.319
Sleeping hours	hours/day	1,063	6.21 ±	1.13	108	6.21 ±	0.93	0.590
Water consumption	L/day	1,063	1.21 ±	0.51	100	1.24 ±	0.48	0.456
VDT working hours	hours/day	1,023	3.61 ±	2.50	107	4.30 ±	3.31	0.184

**$p<0.01$, Mann-Whitney U test. SD, standard deviation; VDT, visual display terminals.

Table 3. Kale intake background (KL).

			n	%
Ingestion history	Over 3 years		107	97
	More than 1 year less than 3 years		3	3
Kale type	Honshiboriaojiru	Premium-Reito (frozen 100 g)	100	91
	Honshiboriaojiru	Premium (powder 10 g)	10	9
Consumption and frequency of Kale juice	1 cup/day		107	97
	1 ~ 2 cups/day		1	1
	over 2 cups/day		2	2
Supplement combination (The main components that overlap with kale juice)	Vitamin C		16	15
	Vitamin E		3	3
	Calcium		16	15
	No combination		80	73

in the kale group than in controls, while no difference was observed in pressure/pulse wave measurement (CAVI) between the groups. As for nervous system age factors, categories achieved (CA) was significantly higher while numbers of response cards used until the first category achieved (NUCA) and Time were significantly lower in the kale group than in controls by the higher brain function test. Among hormonal age factors, IGF-I was significantly lower in the kale group than in controls, while no difference was observed in DHEA-s between the groups.

Table 4. Functional age by Anti-aging check system.

		Control group (CON)			Kale intake group (KL)			p-value
		n	mean	SD	n	mean	SD	(CON vs KL)
Age	years	1,063	59.4	± 5.5	110	58.4	± 5.6	
Muscle age	years	1,063	53.7	± 11.4	110	48.7	± 3.9	<0.001 **
Bone age	years	1,063	56.7	± 14.3	110	60.4	± 16.2	0.024 *
Hormonal age	years	1,063	61.5	± 9.9	110	64.3	± 9.9	0.005 **
Nervous system age	years	1,063	60.9	± 14.4	110	53.5	± 12.8	<0.001 **
Vascular age	years	1,063	65.2	± 13.0	110	64.1	± 8.9	0.244

**p<0.01, *p<0.05, independent t-test. SD, standard deviation; Functional age is estimated by Age Management Check (Ginga Kobo).

Table 5. Anti-aging medical checkup.

		Control group (CON)			Kale intake group (KL)			p-value
		n	mean	SD	n	mean	SD	(CON vs KL)
Anthropometry								
Height	cm	1,063	156.1	± 5.3	108	157.9	± 5.5	0.001 **
Weight	kg	1,063	54.0	± 8.2	108	53.0	± 6.7	0.152
Systolic blood pressure	mmHg	1,063	122.6	± 18.6	110	120.4	± 15.7	0.230
Diastolic blood pressure	mmHg	1,063	74.9	± 11.0	110	76.4	± 10.6	0.181
Muscle age								
R-WBI		79	0.7	± 0.1	108	0.7	± 0.1	0.085
L-WBI		79	0.7	± 0.1	108	0.7	± 0.1	0.189
BMR	kcal/day	142	1,098.4	± 166.4	108	1,144.6	± 106.2	0.008 **
BMI	kg/m^2	1,063	22.1	± 3.1	108	21.3	± 2.6	0.001 **
Body fat percentage	%	1,046	28.5	± 6.5	108	25.1	± 4.8	<0.001 **
Bone age								
Stiffness value		985	80.1	± 15.2	110	76.1	± 16.7	0.009 **
Hormonal age								
IGF-I	ng/mL	1,063	128.9	± 40.8	110	111.1	± 27.5	<0.001 **
DHEA-s	μg/dL	1,063	835.7	± 446.0	110	862.8	± 425.4	0.542
Nervous system age								
CA		1,063	4.8	± 1.4	110	5.5	± 0.7	<0.001 **
NUCA		1,063	4.5	± 8.2	110	1.3	± 1.5	<0.001 **
Response time	second	1,063	154.2	± 120.0	110	109.8	± 39.3	<0.001 **
Vascular age								
SDPTG-estimated vascular age	year	24	60.7	± 7.1	109	64.5	± 9.3	0.032 *
R-CAVI		69	8.0	± 0.9	110	7.9	± 0.9	0.304
L-CAVI		69	7.9	± 0.9	110	7.8	± 0.9	0.394

**p<0.01, *p<0.05, independent t-test. WBI, weight bearing index measured by Physion MD; BMR, basal metabolic rate; BMI, body mass index; IGF-I, insulin-like growth factor-I; DHEA-s, dehydroepiandrosterone-sufate; CA, categories achieved; NUCA, numbers of response cards used until the first category achieved. CA, NUCA and response time are measured by Wisconsin card sorting test. SDPTG-estimated vascular age is measured by Dyna Pulse SDP-100; CAVI, cardio ankle vascular index.

(4) Blood biochemistry and urine analysis

Table 6 data compares the blood chemistry and urine analysis. Regarding lipid metabolism, HDL cholesterol was significantly higher, while triglycerides and L/H ratio were significantly lower in the kale group than in the control group, and of the sugar metabolism factors, FPG, HbA1c, insulin, and HOMA-R were significantly lower in the kale group. Hormonal status showed significantly higher estradiol levels in the kale group compared to controls, while progesterone and cortisol were significantly lower in the kale group. Homocysteine as an index of arteriosclerosis was significantly lower in the kale group. In addition, although the leukocyte count, hemoglobin, hematocrit, protein, and LDH were significantly lower, and the bilirubin, Cl and creatinine were significantly higher in the kale group than in controls, all data were within the range of standard values. Urine analysis showed that the isoprostane production rate (ng/kg/hr) was significantly lower in the kale group compared to the control group.

(5) Subjective symptoms

Table 7 shows the comparison of subjective symptoms. Among physical symptoms, the eight items of palpitations, shortness of breath, thirst, anorexia, early satiety, coughing and sputum, diarrhea, and constipation showed a significantly lower score (milder symptoms) in the kale group than the control group; conversely, the three items of skin problems, gray hair, and easily breaking into a sweat showed a significantly higher score in the kale group. As for mental symptoms, the two items of memory lapse, and inability to solve problems showed a significantly lower score in the kale group than the control group.

Adverse events

There was one case of edema of the right lower leg due to blood pressure/pulse wave measurement (self-reported), but the symptom was mild and did not require any specific treatment.

Discussion

The kale group showed better results than the control group for many parameters analyzed in this cross-sectional study, with the main differences showing in memory, intelligence and gastrointestinal symptoms such as constipation among the subjective physical and mental symptoms. The risk factor analysis showed good results for markers of lipid metabolism and sugar metabolism in addition to body composition, and this could explain the lower muscle age and nervous system age in the kale group compared to control data among functional age parameters measured.

Nervous system age was lower in the kale group in this study than in controls. This is notable because for the control group, results from multiple centers using different analyzers were mixed to determine muscle age, bone age, hormonal age, and vascular age, but nervous system age was tested by the same test (PC version: Wisconsin card sorting test) in all persons, and thus the results are more valid in terms of statistical significance. In addition, blood cortisol (stress), homocysteine (Alzheimer risk factor [19]), urine isoplastane (oxidative stress), and mental symptom scores (memory lapse, and inability to solve problems) were lower in the kale group than the control group, supporting the results for nervous system parameters.

Plant species of the Brassicaceae family have organic sulfur compounds (such as sulforaphane) in common, and there have been reports on the neuroprotective effect of extracts of broccoli sprouts, which also belong to the Brassicaceae family in the Alzheimer cell model (SH-SY5Y cells) [20], and on the protective effect of Brussels sprouts and the phytochemicals they contain on amyloid β (Aβ) peptide-induced neurotoxicity in ICR mice [21]. Since kale is also considered to contain antioxidative polyphenols such as sulforaphane, and the SOD activity of kale is also high (in-house analysis: 49,000 to 110,000 units), it is likely to also have an anti-oxidative effect on cranial nerves.

Muscle age was lower in the kale group mainly because of the body composition (being thin), and it can be assumed based on the blood biochemistry results that it is possible that the long-term effects on sugar metabolism and lipid metabolism indirectly contributed to weight loss. The general benefits of Brassicaceae plants including kale on health have been widely reported in relation to cancer, inflammation, cardiovascular disease, and metabolic diseases including diabetes [22, 23]. Reports have also implicated kale consumption in the prevention of blood sugar elevation (in-house data), a decrease in LDL-C, an increase in HDL-C, and increased glutathione peroxidase activity in males with hyperlipidemia [24], and it can be said that these data support the present results. In addition, findings that kale supernatant promoted the *in vitro* growth of *Bifidobacterium longum* [25], effects on intestinal flora and beneficial effects on bowel movements due to the contained dietary fiber are also expected, and it is possible that kale could help to control obesity. In fact, users of the target products gave the highest rating for improvement in constipation.

The overall comparison herein also indicated that bone age was significantly higher in the kale group than the control group, namely by +2.0 years compared with the chronological age. Bone age reflects the bone mineral density, and the physical constitution (being thin) of the kale group is considered to be a contributing factor in these results. However, it is unlikely that the bone mineral density decreased due to kale consumption, because factors that maintain bone substance including sugar metabolism and homocysteine levels were good in the kale group, and kale contains abundant vitamin K and Ca. Thus, it is possible that the bone mineral density results were more reflective of the subject age and the likelihood of bone loss inhibition in postmenopausal females [5].

Moreover, while the glycative stress index markers were slightly higher in the kale group in the present study, the glycation suppression effect of kale has been shown in an *in vitro* study (in-house data), and the suppression of glycated protein has been reported for leaf mustard of the same Brassicaceae family [26], and thus it is unlikely that the components of kale promote glycation. Since the consumption of alcoholic beverages, confectionery, and fruit (fructose) was higher in the kale group than the results of a national nutrition survey, it is possible that glycation progressed even though sugar metabolism-related markers were low. Since

Table 6. Blood biochemistry and urine analysis.

		Control group (CON)			Kale intake group (KL)			p-value
Blood biochemistry		n	mean	SD	n	mean	SD	(CON vs KL)
Leukocyte count	/μL	23	5,731.3 ±	1,123.7	110	4,378.2 ±	1,208.2	<0.001 **
Erythrocyte count	× 10^4/μL	25	432.1 ±	25.5	110	421.9 ±	31.0	0.128
Hemoglobin	g/dL	22	13.2 ±	0.7	110	12.8 ±	0.9	0.040 *
Hematocrit	%	21	41.0 ±	2.5	110	39.6 ±	2.5	0.019 *
Platelet count	× 10^4/μL	22	23.3 ±	5.8	110	22.5 ±	4.9	0.517
Total bilirubin	mg/dL	19	0.6 ±	0.2	110	0.8 ±	0.2	0.001 **
AST	U/L	26	22.0 ±	5.7	110	21.0 ±	4.6	0.332
ALT	U/L	26	19.5 ±	7.8	110	17.0 ±	6.1	0.081
γ-GTP	U/L	27	28.3 ±	21.1	110	22.2 ±	13.7	0.069
LDH	U/L	19	209.4 ±	62.4	110	177.2 ±	28.3	0.039 *
BUN	mg/dL	20	13.6 ±	3.8	110	13.9 ±	3.6	0.740
Creatinine	mg/dL	22	0.6 ±	0.1	110	0.6 ±	0.1	0.032 *
Total cholesterol (TC)	mg/dL	174	222.5 ±	36.8	110	216.7 ±	33.1	0.180
HDL-C	mg/dL	1,043	64.8 ±	15.5	110	72.6 ±	15.3	<0.001 **
LDL-C	mg/dL	1,058	124.9 ±	30.9	110	126.0 ±	29.6	0.721
Triglyceride (TG)	mg/dL	1,056	93.4 ±	48.4	110	77.9 ±	36.0	<0.001 **
FPG	mg/dL	1,053	97.6 ±	16.8	110	84.6 ±	10.3	<0.001 **
HbA1c	%	1,041	5.3 ±	0.6	110	5.2 ±	0.3	<0.001 **
Insulin (IRI)	μU/mL	193	5.8 ±	4.2	110	3.7 ±	4.4	<0.001 **
Total protein (TP)	g/dL	20	7.2 ±	0.5	110	6.8 ±	0.4	<0.001 **
Na	mEq/L	29	142.2 ±	1.9	110	141.6 ±	1.8	0.126
K	mEq/L	28	4.0 ±	0.3	110	4.1 ±	0.3	0.368
Cl	mEq/L	13	104.5 ±	1.8	110	105.9 ±	1.5	0.002 **
Ca	mg/dL	13	9.4 ±	0.3	110	9.6 ±	0.4	0.126
Fe	μg/dL	64	93.2 ±	30.8	110	99.7 ±	24.4	0.130
Cortisol	μg/dL	1,061	9.3 ±	3.8	110	7.7 ±	2.8	<0.001 **
Progesterone	ng/mL	71	0.4 ±	1.1	110	0.1 ±	0.0	<0.001 **
Estradiol (E2)	pg/mL	130	16.9 ±	30.4	110	25.2 ±	51.0	0.001 **
Homocysteine	nmol/mL	133	9.5 ±	2.7	110	6.4 ±	1.6	<0.001 **
hsCRP	mg/dL	998	6.5 ±	39.0	110	0.0 ±	0.1	<0.001 **
LH ratio		1,043	2.1 ±	0.8	110	1.8 ±	0.7	0.001 **
HOMA-R		191	1.3 ±	1.0	110	0.8 ±	1.5	0.003 **
Urine analysis								
8-OHdG production rate	ng/kg/hr	67	6.9 ±	3.5	110	6.7 ±	3.3	0.721
Isoprostane production rate	ng/kg/hr	64	2.0 ±	1.4	110	1.2 ±	0.9	<0.001 **

**p<0.01, *p<0.05, independent t-test or Mann-Whitney U test. FPG, fasting plasma glucose; IRI, immuno reactive insulin; hsCRP, high-sensitive C-reactive protein; LH ratio, LDL-C : HDL-C ; HOMA-R, homeostasis model assessment insulin resistance; 8-OHdG, 8-hydroxydeoxyguanosine.

Table 7. Physical symptom and Mental symptom score in AAQol

	Control group (CON)			Kale intake group (KL)			p-value
Physical symptoms	n	mean	SD	n	mean	SD	(CON vs KL)
Tired eyes	1,063	3.19	± 0.98	110	3.33	± 1.01	0.162
Blurry eyes	1,063	2.63	± 1.04	110	2.72	± 1.07	0.516
Eye pain	1,063	2.01	± 0.97	108	1.92	± 0.99	0.257
Stiff shoulders	1,063	3.64	± 1.13	109	3.50	± 1.24	0.318
Muscular pains/stiffness	1,063	3.07	± 1.14	110	2.89	± 1.21	0.097
Palpitations	1,063	2.09	± 0.93	110	1.76	± 0.87	<0.001 **
Shortness of breath	1,063	2.07	± 0.93	110	1.70	± 0.81	<0.001 **
Tendency to gain weight	1,063	3.00	± 1.27	110	2.94	± 1.37	0.626
Weight loss, thin	1,063	1.56	± 0.79	109	1.56	± 0.85	0.692
Lethargy	1,063	2.48	± 0.96	110	2.50	± 1.06	0.966
Lack of sense of wellness	1,063	2.35	± 0.97	109	2.31	± 0.95	0.705
Thirst	1,063	2.14	± 0.98	110	1.92	± 0.98	0.011 *
Skin problems	1,063	2.28	± 0.90	110	2.51	± 1.00	0.025 *
Anorexia	1,063	1.72	± 0.72	110	1.56	± 0.74	0.010 *
Early satiety	1,063	2.06	± 0.92	110	1.84	± 0.80	0.021 *
Epigastralgia	1,063	2.00	± 0.89	110	1.90	± 0.86	0.264
Liability to catch colds	1,063	2.08	± 0.89	110	2.05	± 0.91	0.675
Coughing and sputum	1,063	2.13	± 0.94	110	1.87	± 0.92	0.003 **
Diarrhea	1,063	1.90	± 0.82	109	1.66	± 0.81	0.001 **
Constipation	1,063	2.44	± 1.15	110	2.15	± 1.21	0.004 **
Hair loss	1,063	2.47	± 0.93	109	2.50	± 0.91	0.608
Gray hair	1,063	3.68	± 0.95	110	3.95	± 0.81	0.009 **
Headache	1,063	2.35	± 1.01	110	2.33	± 1.08	0.737
Dizziness	1,063	1.99	± 0.88	110	1.85	± 0.90	0.078
Tinnitus	1,063	1.93	± 1.01	109	1.85	± 1.01	0.383
Lumbago	1,063	2.89	± 1.16	110	2.75	± 1.06	0.200
Arthralgia	1,063	2.50	± 1.13	110	2.55	± 1.12	0.464
Edematous	1,063	2.32	± 1.04	110	2.42	± 1.05	0.222
Easily breaking into a sweat	1,063	2.56	± 1.19	110	2.81	± 1.22	0.029 *
Frequent urination	1,063	2.31	± 1.02	110	2.25	± 1.10	0.436
Hot flashes	1,063	2.08	± 0.98	110	1.95	± 0.93	0.201
Cold skin	1,063	2.82	± 1.21	110	2.59	± 1.12	0.113
	Control group (CON)			Kale intake group (KL)			p-value
Mental symptoms	n	mean	SD	n	mean	SD	(CON vs KL)
Irritability	1,063	2.51	± 0.85	110	2.41	± 0.86	0.212
Easily angered	1,063	2.37	± 0.81	110	2.25	± 0.81	0.092
Loss of motivation	1,063	2.29	± 0.88	110	2.22	± 0.86	0.521
Unhappy	1,063	1.91	± 0.85	110	2.02	± 0.81	0.124
Nothing to look forward to in my life	1,063	1.91	± 0.87	110	2.00	± 0.90	0.295
Daily life is not enjoyable	1,063	1.90	± 0.83	110	2.01	± 0.86	0.210
No confidence	1,063	2.09	± 0.85	110	2.17	± 0.89	0.262
Reluctance to talk with others	1,063	1.85	± 0.81	110	1.90	± 0.82	0.569
Depressed	1,063	1.90	± 0.81	110	1.94	± 0.88	0.802
Feeling useless	1,063	1.88	± 0.78	110	1.89	± 0.82	0.937
Shallow sleep	1,063	2.52	± 1.12	109	2.33	± 1.11	0.063
Difficulty falling asleep	1,063	2.36	± 1.11	110	2.18	± 1.09	0.087
Pessimism	1,063	2.29	± 0.92	110	2.45	± 1.01	0.118
Memory lapse	1,063	3.12	± 0.84	110	2.92	± 0.86	0.007 **
Inability to concentrate	1,063	2.46	± 0.85	109	2.32	± 0.78	0.129
Inability to solve problems	1,063	2.17	± 0.77	110	1.95	± 0.71	0.008 **
Inability to readily make judgments	1,063	2.15	± 0.77	109	2.03	± 0.75	0.123
Inability to sleep due to worries	1,063	2.21	± 0.87	110	2.12	± 0.90	0.232
Felling tense	1,063	2.46	± 0.86	110	2.45	± 0.81	0.668
Felling anxious for no particular reason	1,063	1.98	± 0.85	110	1.90	± 0.85	0.329
Vague feeling of fear	1,063	1.76	± 0.78	110	1.65	± 0.71	0.168

**$p<0.01$, *$p<0.05$, Mann-Whitney U test. SD, standard deviation; AAQol, Anti-Aging QOL Common Questionnaire.

glycation is also a cause of osteoporosis [27], it may also be a contributor to the higher bone age in the kale group.

Interestingly, the control group used for comparison in the present study consisted of anti-aging examinees, and may be a population that is more health-conscious and continues more physical activity than the average person. This assumption is also supported by a comparison between executives who had an anti-aging medical checkup and ordinary males in the same age group in a major iron and steel company, showing 7.1% higher bone mineral density and 5.3 years younger bone age in the former group [28]. Moreover, mixed testing was performed using X-rays (dual-energy X-ray absorptiometry; DEXA) and ultrasonic method using different models of analyzers, thus the test method is a problem to be addressed in future studies.

Hormonal age was significantly higher in the kale group than the control group and was +5.9 years older than the chronological age. This could be related to the IGF-I level being significantly lower in the kale group. IGF-I is a hormone that repairs damaged cell tissues by cell proliferation and growth promotion via growth hormone, it is known to decrease with increasing age and is used as an index of aging, and the reported factors of higher IGF-I include cancer, obesity, metabolic syndrome-related factors [29], exercise [30], and milk (casein) consumption [31]. Thus, the hormonal index is complex and has at least two aspects of relevance to this study. Since milk consumption was high and insulin was low in the kale group, it is assumed that BMI is a factor of the low IGF-I. It is also possible that a large amount of soybean products (estrogen-like product rich) [32], which inhibit the effect of IGF-I, were consumed in the kale group based on the consumption of beans, and soybean product consumption may also have a greater or lesser effect. However, because the IGF-I value in the kale group is within the normal range, it is assumed that there is hardly any effect of kale consumption.

The above-mentioned comparison between executives and ordinary males of the same age group also showed 15% higher IGF-I in the former group [28]. It is therefore possible that IGF-I was higher in the anti-aging examinees (former group) than the general health checkup examinees (latter group) due to differences in lifestyle-related behavior, and the same can be said of the overall control group.

Functional age is one of the developing evaluation method, and discussions are therefore ongoing regarding the obtained laboratory test values as valid risk factors and quality of life (QOL) indicators regardless of the results. Notably, the kale group tested herein showed good laboratory test values and QOL in general.

The limitations of the present study are that the survey region of both groups could not be unified and thus the difference in background information of subjects such as lifestyle-related behaviors and consumption status of meals and health food cannot be estimated or analyzed, and that a sufficient number of cases for analysis considering the kale consumption status could not be found in the kale group. Despite these limitations, the result of nervous system age can particularly be appreciated among the present findings. There has been generally been limited efficacy of Brassicaceae plants including kale on the neuronal function, and the evaluation in humans in particular is very difficult. We will therefore verify the effects of kale in this field in the future.

Conclusion

The present study compared aging of physical functions between persons who consumed kale for at least 1 year and a control group representing the general population. Functional ages, particularly muscle and nervous system age, were significantly lower in the kale group than in controls, and the kale group was functionally better based on laboratory test values and a comprehensive anti-aging QOL questionnaire. These data suggest that kale consumption could have a beneficial and preventative effect on mental and physical aging.

Statement of Conflict of Interest

The present study was partly supported by FANCL Corporation. FANCL Corporation was not involved in the statistical analysis of the results.

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