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Original article

Survey of fluorescence wavelength range reflecting human tissue aging

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Abstract

***Purpose*:** To determine the fluorescence wavelength range reflecting the degree of tissue aging in the human body, the skin fluorescent spectrum was measured at 375 (±5) nm excitation and 420-750 nm emission.
***Method*:** Skin fluorescence data and measurements reflecting aging as well as data on lifestyle were collected from 65 healthy people (mean chronological age: 40.9±17.2 years). The correlation of fluorescence intensity, chronological age, skin auto fluorescence (AF), tissue age (vascular age and stiffness), and number of risky lifestyle behaviors were assessed using Pearson's correlation coefficient (r) which was then age-adjusted (r_{age}).
***Results*:** Chronological age (420-627.7 nm; $r_{max} = 0.372$, $p = 0.002$ at 507.5 nm), skin AF (473.0-502.1 nm; $r_{max} = 0.275$, $p = 0.028$ at 485.8 nm), vascular age (447.3-532.6 nm; $r_{max} = 0.317$, $p = 0.031$ at 485.3 nm), and number of risky lifestyle behaviors (436.2-525.5 nm; $r_{max} = 0.325$, $p = 0.024$ at 484.0 nm) were all positively correlated with relative fluorescence. Further, only number of risky lifestyle behaviors (421.3-525.5 nm; $r_{age\ max} = 0.336$, $p = 0.024$ at 451.0 nm) remained positively correlated with relative fluorescence after age-adjusted analysis.
***Conclusion*:** Our present findings suggest that several ranges of fluorescence intensity may be positively correlated with vascular age. Further, the degree of human tissue aging might be reflected by spectrofluorimetry values.

***KEY WORDS*:** fluorescence spectra, glycation, advanced glycation endproducts (AGEs), tissue aging, arteriosclerosis

Introduction

Advanced glycation end products (AGEs) are considered an index of human aging given their documented relationships with age- and lifestyle-related diseases [1-3]. As several AGEs exhibit a characteristic fluorescence, detection of such fluorescence intensity may facilitate evaluation of the degree of AGE formation—and subsequently the degree of glycation of the body's protein [4-6]. The AGE Reader which is a commercially-produced fluorescence-measuring machine widely used to determine the degree of glycation in the body [2,7]. Given the large number of fluorescent substances in the human body, this reader has a broad mean intensity (420-600 nm) [8]. A more precise wavelength range might more accurately reflect aging and disease.

If a specific wavelength range for the fluorescence of AGEs derived from glycation can be identified, that range can then be used to accurately evaluate the degree of aging of the human body. We therefore developed a method of directly measuring AGE fluorescence (at 370 nm/420-750 nm) using light electronic diodes (LEDs) as a light source for practical application [9]. Then, several AGEs are accumulated tissue, as for skin, bone, vascular and internal organs [1-4]. So tissue aging is thought to be more important in determining risk of developing several diseases than chronological age, as increased tissue age is correlated with reduced functional mechanism in humans [10]. However, the correlation between which wavelength of AGE fluorescence and human tissue age is unclear.

Here, to determine the relationship between fluorescent intensity and human tissue ageing, we measured the fluorescent spectrum under conditions of excitation wavelength 375 (±5) nm and evaluated the fluorescence intensity in the range of 420-750 nm. In addition, we assessed the degree of aging and lifestyle behaviors based on measured values and survey of subjects.

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Methods

Subjects

A total of 65 Japanese men and women, aged 14 to 74 years (mean chronological age ± standard deviation: 40.9 ± 17.2 years) who had been admitted to the Anti-Aging Medical Research Center at Doshisha University were enrolled in the present study (***Table 1***).

Measurement of tissue fluorescence

Excitation light (375 ± 5 nm) through a fluorometer (C10988MA; Hamamatsu Photonics, Shizuoka, Japan) was produced by light-emitting diodes (LEDs; Nichia Corporation. Co., Ltd., Tokushima, Japan) [9,11,12]. Measurements were obtained using excitation light at an angle of 45° through a 6-mm diameter hole for set periods and measuring auto fluorescence (AF) intensity in the dark. The LED light source was fitted with visible light cut-off filters (IUV-365; Isuzu Glass Co., Osaka, Japan) to separate stray light from excitation light.

The underlying index of skin fluorescence was measured using an AGE Reader™ (DiagnOptics, Groningen, Netherlands) [13-15]. Excitation light (wavelength: 300-420 nm) was projected onto 1 cm^2 of skin inside the upper arm, approximately 10 cm above the elbow, and the intensity of emitted light (420-600 nm, representing AF) was then measured with a fluorometer. The skin AF (arbitrary units [AUs] × 100) was calculated from the mean value of the emitted light intensity divided by the excitation light intensity.

Survey of lifestyle-related behaviors

Subjects were questioned about their lifestyle using the Anti-Aging Common Questionnaire [16], which questioned them about "Smoking habit (number of cigarettes/day)," "Frequency of alcohol drinking (number of times/week)," and "Sleeping duration (number of hours/day)" [17].

Measurement of tissue age

The degree of atherosclerosis was evaluated by acceleration plethysmography (SDP-100; Fukuda Denshi Co. Ltd., Tokyo, Japan) [18,19], and the results were expressed as vascular age [20].

Bone age was determined from the stiffness of the calcaneus bone as measured via ultrasonography (A-1000; GE Yokogawa Medical Systems, Ltd., Tokyo, Japan). High correlation has been reported between stiffness and bone mineral density determined using dual-energy X-ray absorptiometry (DEXA), with the correlation coefficient in the calcaneus being found to be r = 0.6-0.8 [21,22]. Results were expressed as stiffness values and % young adult mean (%YAM).

Statistical analysis

The correlation between fluorescence intensity, tissue age, and skin AF was assessed using Pearson's correlation coefficient (r) and plotted against fluorescence wavelength. In addition, the age-adjusted correlation coefficient (r_{age}) was also calculated and plotted against fluorescence wavelength. Relative fluorescence intensity was calculated using the following expression:

$$\text{Relative fluorescence intensity} = \frac{\text{Intensity of each wavelength}}{\text{Intensity of 375mm}}$$

Ethical approval

The study followed the guidelines ('The Ethical Principles Concerning Epidemiologic Study') published by the Japanese Ministry of Health, Labour and Welfare, and the Doshisha University Ethics Committee for Clinical Studies approved the study protocol (approval number #0832). All participants provided informed, written consent, and data were not linked to subjects' personal information.

Results

Relationship between tissue aging and skin AGE fluorescence

Chronological age (420-627.7 nm; $r_{max} = 0.372$, $p = 0.002$ at 507.5 nm), skin AF (473.0-502.1 nm; $r_{max} = 0.275$, $p = 0.028$ at 485.8 nm), vascular age (447.3-532.6 nm; $r_{max} = 0.317$, $p = 0.031$ at 485.3 nm), and number of risky lifestyle behaviors (436.2-525.5 nm; $r_{max} = 0.325$, $p = 0.024$ at 484.0 nm) were all positively correlated with relative fluorescence (***Fig. 1***). On age-adjusted analysis, only number of risky lifestyle behaviors (421.3-525.5 nm; $r_{age\ max} = 0.336$, $p = 0.024$ at 451.0 nm) remained positively correlated with relative fluorescence.

AGE fluorescence

In the analysis of AGE fluorescence at 375/440 nm, chronological age ($r = 0.314$, $p = 0.011$) and number of risky lifestyle behaviors ($r = 0.298$, $p = 0.039$) were positively correlated (***Fig. 2***). On age-adjusted analysis, only number of risky lifestyle behaviors was positively correlated ($r_{age} = 0.327$, $p = 0.028$).

Table 1. Subjects' background

	Chronological age (years)	Skin AF	Vascular age (years)	Stiffness	Number of risky lifestyle behaviors
mean	40.9	1.39	50.2	91.3	0.88
SD	17.2	0.35	18.5	20.9	0.73
N	65	64	46	40	48

SD, standard deviation; AF, auto fluorescence

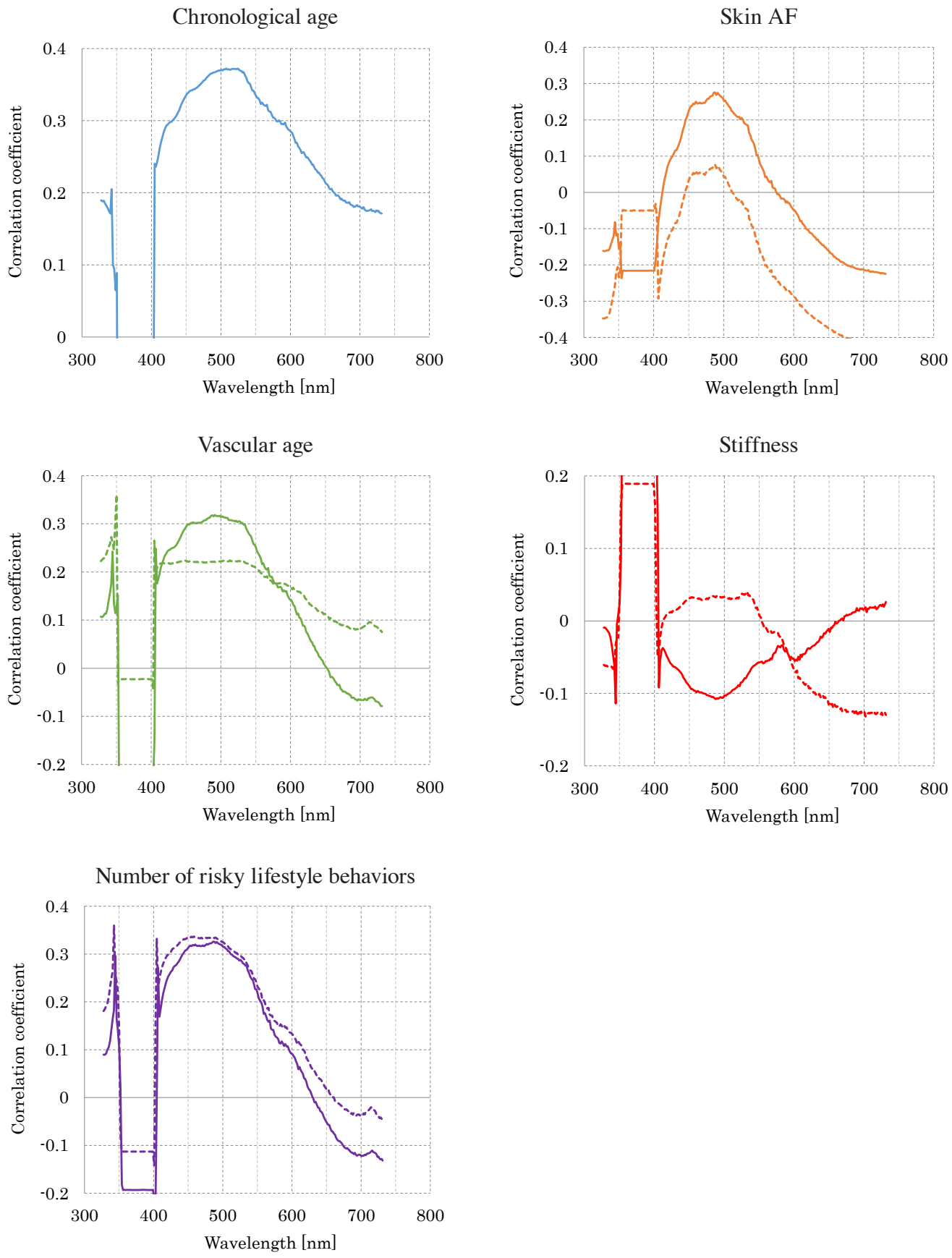
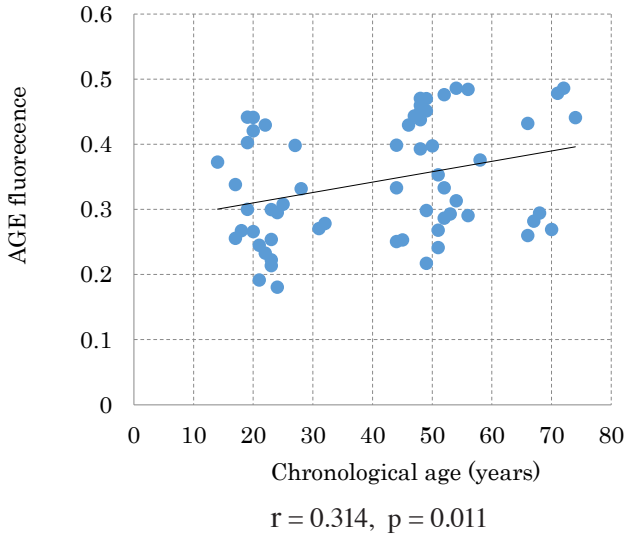


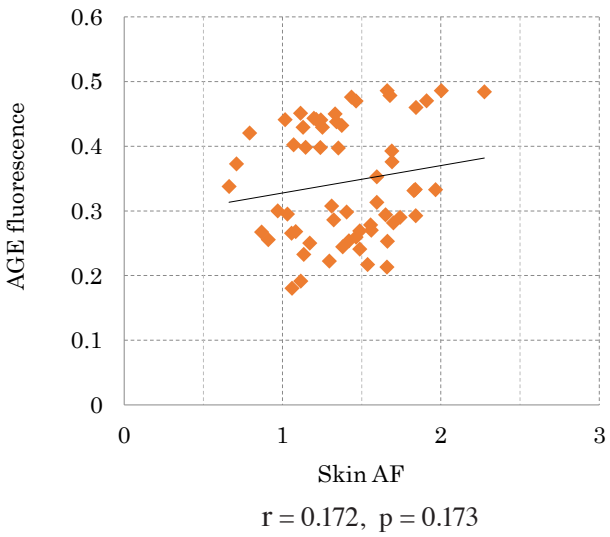
Fig 1. **Correlation coefficient spectrum for several aging indices.**

——— r: Correlation coefficient

---------- r_{age}: Correlation coefficient after age adjustment

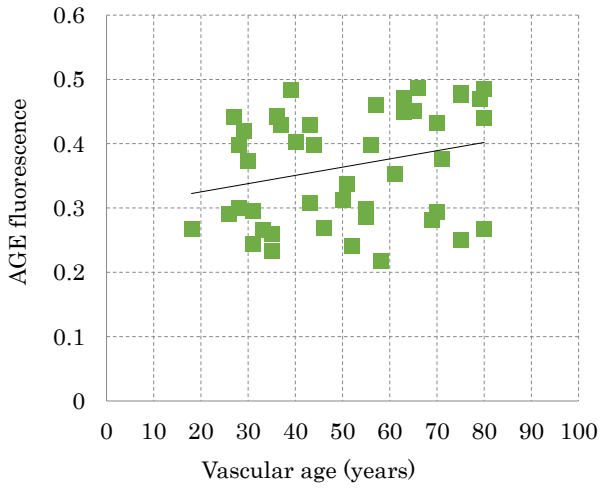


r = 0.314, p = 0.011



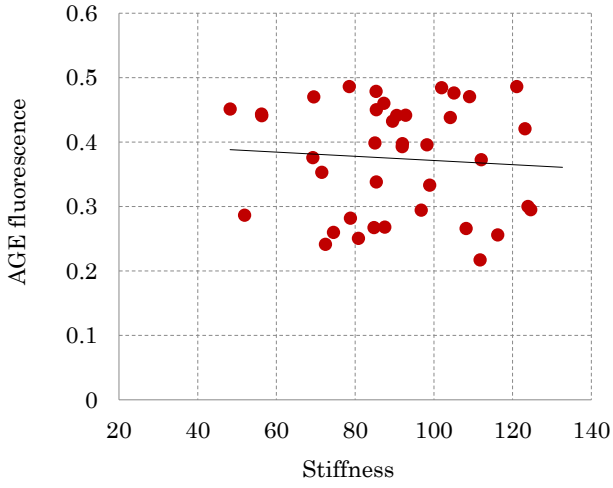
r = 0.172, p = 0.173

r_{age} = -0.037, p = 0.867



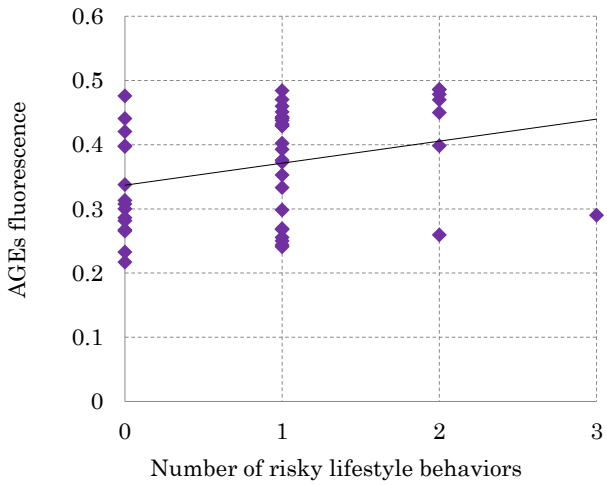
r = 0.272, p = 0.068

r_{age} = 0.221, p = 0.155



r = -0.079, p = 0.630

r_{age} = 0.022, p = 0.897



r = 0.298, p = 0.039

r_{age} = 0.327, p = 0.028

Fig 2. **Correlation with AGE fluorescence for several aging indices.**

AGE fluorescence refers to the relative fluorescence at 370/440 nm. Excitation light wavelength produced by LEDs was set at 375 (±5) nm.

Skin AF

In the analysis of skin AF, chronological age ($r = 0.611$, $p < 0.001$), vascular age ($r = 0.418$, $p = 0.005$), number of risky lifestyle behaviors ($r = 0.347$, $p = 0.018$) were positively correlated (***Fig. 3***). On age-adjusted analysis, only stiffness was positively correlated ($r_{age} = 0.373$, $p < 0.023$).

Discussion

Chronological age

Chronological age was positively correlated with relative fluorescence in the range of 420-627.7 nm, the broadest range of the variables examined (***Fig. 1***). This finding indicates that generation and accumulation of fluorescent substances substantially increase with chronological age. Fluorescence intensity in the range of 420-600 nm has been reported to be higher in diabetic subjects than in non-diabetic ones [23], and the fluorescent intensity of this region has been found to be higher in elderly people than in younger ones.

Skin AF (473.0-502.1 nm; $r_{max} = 0.275$, $p = 0.028$ at 485.8 nm) (***Fig. 1***), and number of risky lifestyle behaviors (436.2-525.5 nm; $r_{max} = 0.325$, $p = 0.024$ at 484.0 nm) (***Fig. 1***) were positively correlated with relative fluorescence. These indices were thought to be related with chronological age [10,17], especially, the number of risky lifestyle behaviors were correlated around the relative intensity of 440 nm were really interested. A lot of fluorescent AGEs have peak intensity around 440 nm [2,4], number of risky lifestyle behaviors might be shown the value of fluorescence AGEs. It is because the people who have higher number of risky lifestyle behaviors have a custom of smoking, drinking alcohol, and may be having a lot of sugar, they are the substance of producing AGEs when reacted with protein.

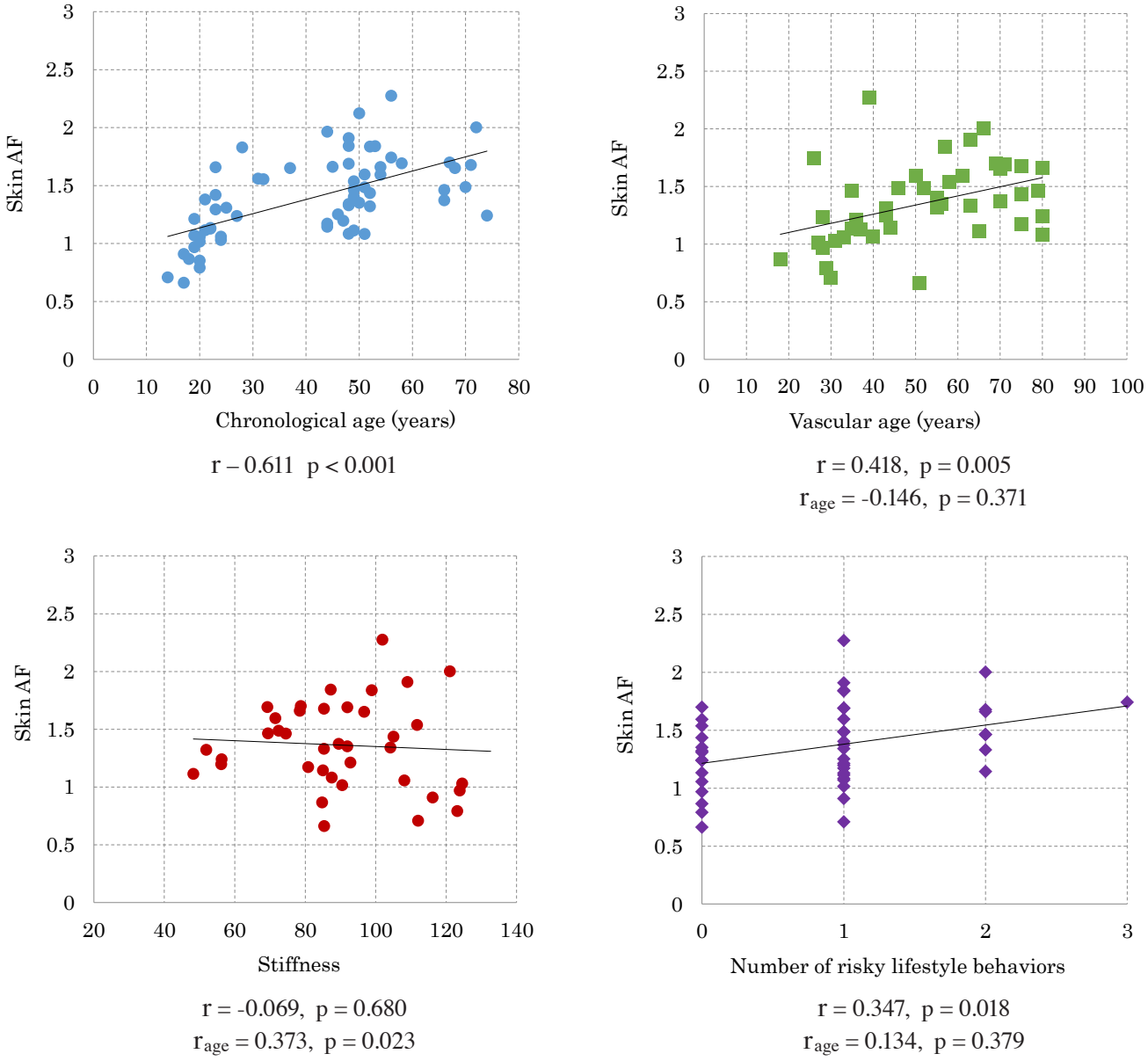


Fig 3. **Correlation with skin AF for several aging indices.**
Skin AF was measured using an AGE Reader.

Arteriosclerosis

AGEs (both fluorescent and non-fluorescent AGEs) andlipoperoxidation products excessively generated by metabolic derangement were thought to promote vascular age (which was one of the tissue age) by decreasing elasticity of blood vessel wall. Decreasing elasticity was related with arteriosclerosis which promote age-related diseases like hypertention. Non-elderly people have possibility to have age related diseases if they have too much AGEs. So the purpose of using spectrofluorimetry was checking the value of fluorescent AGEs. In this study, vascular age was positively correlated with relative fluorescence in the range of 447.3-532.6 nm (***Fig. 1***).

Bone density

Stiffness was not correlated with relative fluorescence, as the r value was negative (***Fig. 1***). The fluorescent AGE pentosidine is found in skin and bone, and its accumulation increases with chronological age [3,24,25]. Given that stiffness in the present study reflected the bone density of the ankle, future studies should consider evaluating density at other measurement points.

AGE fluorescence

In this study, AGE fluorescence (370/440 nm) was measured using a light source of 375 (±5) nm emission. On analysis, chronological age ($r = 0.314$, $p = 0.011$) and number of risky lifestyle behaviors ($r = 0.298$, $p = 0.039$) were positively correlated with relative fluorescence (***Fig. 2***). However, the highest correlation coefficient (r_{max}) was around 485 nm.

A previous report found that the peak value of the fluorescence spectrum of glycation products from a reaction using human serum albumin was 440 nm [7]. When doing an *in vitro* fluorescence analysis, keratin or collagen in the skin might influence the fluorescent spectrum [29]. Given that our present analysis involved evaluating degree of arteriosclerosis and lifestyle behaviors, a range around 450-490 nm might be more appropriate in future studies.

Conclusion

Our present findings suggest that several ranges of fluorescence intensity may be positively correlated with vascular age. Further, the degree of human tissue aging might be reflected by spectrofluorimetry values.

Acknowledgements

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Conflicts of interest statement

The authors have no conflicts of interest related to this study to declare.

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