Glycative Stress Research

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Original article

Effect of red wine on AGEs in blood

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Abstract

***Purpose*:** The purpose of this study was to select wines with strong anti-glycation effects based on grape variety, region, and vinification method through basic experiments, and to clarify whether consumption of these wines has an effect on the inhibition of AGEs accumulation in human blood.

***Methods*:** Study subjects were 60 men and women between the ages of 20 and 65 who were able to consume alcohol. The study design was a prospective crossover open-labeled randomized controlled trial. In the basic study, various wines were added to a human serum albumin (HSA)-glucose glycation model and the effect of glycation inhibition was evaluated by measuring fluorescent AGEs production. Study participants consumed 125 mL per day of either wine or mineral water, which has strong anti-glycation effects in basic research and is available for human comparative studies, 6 days per week for 4 continuous weeks. The AGEs accumulation level in the body was measured by an AGEs sensor and the stress level by a stress measuring instrument (VM500) before and after ingestion for a total of 4 times. In addition, an email questionnaire regarding lifestyle influences on AGEs accumulation was administered weekly.

***Results*:** The target wine for intake was a red wine (La Forge Estate Pinot Noir containing 13.5 mL/100 mL of alcohol. The intake was set at 125 mL with the IC_{50} calculated based on the anti-glycation effect of aminoguanidine. There were 48 research collaborators. Nine persons whose response rate to the questionnaire results was less than 50 % and six persons who were absent from at least one measurement session were excluded. A decrease in AGEs levels was observed in the wine intake group, and an increase in AGEs levels was observed in the mineral water group, Although the difference was not significant ($p = 0.16$). A comparison of the Week 2-5 group (early June to mid-July) and s Week 8-11 group (late July to early September) groups showed a strong decreasing trend in AGEs levels before and after wine consumption ($p = 0.06$). Women also showed a trend toward lower AGEs after wine consumption ($p = 0.07$). Overlapping analysis with the time of administration showed a significant decrease in AGEs in women in the Week 8-11 group who consumed wine ($p = 0.02$).

***Conclusion*:** Wine consumption was suggested to inhibit the accumulation of AGEs. In particular, the effect of AGEs due to ultraviolet light was less pronounced in women who used sunscreen on a daily basis, suggesting that the effect of AGEs accumulation inhibition was achieved in the latter half of the group, when the amount of ultraviolet light reaches its peak.

***KEY WORDS*:** advanced glycation end products (AGEs), red wine

Background

In recent years, "glycation" is thought to be one of the factors that accelerate aging [1]. Glycation is a Maillard reaction in the body, in which excess carbohydrates ingested from food and other sources combine with proteins in the body to eventually produce aging-causing substances called glycation end products (AGEs) [2]. These AGEs are difficult for the body to break down and are thought to cause various diseases by affecting skin coloration, wrinkle formation due to hardening of collagen proteins, and hardening of blood vessels [3, 4]. Foods such as tea [5], vegetables [6], fruits [7] and yogurt [8] have been reported to inhibit this glycation reaction.

Anti-glycation substances, which inhibit the glycation reaction that occurs in the body, are contained in foods that we consume in our daily lives, and their efficient intake

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can be expected to have a preventive effect on aging and disease. For example, wines differ in grape variety, region, and brewing method, and the characteristics of each brand of wine have been expressed by its unique taste, aroma, and color. Furthermore, wine is known to contain antioxidants and anti-glycation substances such as polyphenols. We investigated the anti-glycation effects of wine according to grape variety, region of origin, and brewing method, and identified brands with strong anti-glycation effects by comparing them in terms of their ability to inhibit the production of AGEs. In the present study, we wondered whether wine consumption might be effective in inhibiting AGEs accumulation in the body, which might lead to the prevention of aging and disease.

The objective of this study was to identify wines with strong anti-glycation effects through basic experiments and to determine whether consumption of that brand of wine has an effect on the inhibition of AGEs accumulation in human blood.

Methods

Basic Research

Commonly available wines were selected (***Table 1***) and the anti-glycation effect of the wines was determined. The anti-glycation effects of the following wines were evaluated by adding them to a human serum albumin (HSA)-glucose glycation model [9] and measuring fluorescent AGEs production. Aminoguanidine (AG) [10, 11], an inhibitor of AGEs production that has been reported to prevent or inhibit the progression of nephropathy, retinopathy, and neuropathy, was used as a positive standard.

Clinical Research

Study design

Prospective crossover open-labeled randomized controlled trial.

Study subjects

60 men and women between the ages of 20 and 65 who were able to consume alcohol were included in the study. Those with alcohol intolerance or an underlying disease adversely affected by alcohol consumption were excluded. Study participants who requested a written report were informed of the results.

Allocation methods

Alcohol-capable men and women between the ages of 20 and 65 years participated in the study. Study participants were assigned to two groups using random numbers at the time consent forms were obtained.

Protocols

Wine with a strong inhibitory effect of AGEs production, selected from the results of basic research, was used as the test product, and mineral water (Acqua Panna, Tuscany, Italy) was used as the control product. Participants consumed either red wine or mineral water, 125 mL per day, 6 days per week for 4 weeks, followed by a 2-week period of no consumption to allow the active ingredient to be excreted from the body. After that, the red wine and mineral water were switched, and the participants consumed 125 mL each day, 6 days a week, for 4 weeks. The AGEs accumulation level in the body was measured by an AGEs sensor four times before and after ingestion. Fatigue stress was also measured at the same time; AGEs were measured using an AGEs sensor (Air Water Biodesign Inc., Osaka, Japan) was used to measure AGEs, and VM500 (Fatigue Science Institute Co., Ltd., Yokohama, Japan) was used to measure stress. A summary of the intake

Table 1. Profile of wine used in this research

ID	Color	Winery	Product name	Vintage	Alcohol % (v/v)
①	White	Domaines Paul Mas	Les Tannes en Occitanie Chardonnay 375	2019	13.3
②	Red	Domaines Paul Mas	Les Tannes en Occitanie Cabernet Sauvignon 375	2019	13.5
③	White	Domaines Paul Mas	Les Tannes en Occitanie Sauvignon Blanc	2019	12.1
④	Red	Domaines Paul Mas	Les Tannes en Occitanie Merlot	2019	13.6
⑤	Red	Domaines Paul Mas	Les Tannes en Occitanie Pinot Noir	2019	13.1
⑥	White	Domaines Paul Mas	La Forge Estate Unwooded Chardonnay	2019	13.5
⑦	White	Domaines Paul Mas	La Forge Estate Chardonnay	2019	13.0
⑧	White	Domaines Paul Mas	La Forge Estate Viognier	2019	13.8
⑨	White	Domaines Paul Mas	La Forge Estate Sauvignon Blanc	2018	12.4
⑩	Red	Domaines Paul Mas	La Forge Estate Cabernet Sauvignon	2019	13.8
⑪	Red	Domaines Paul Mas	La Forge Estate Merlot	2019	13.8
⑫	Red	Domaines Paul Mas	La Forge Estate Syrah	2019	13.8
⑬	Red	Domaines Paul Mas	La Forge Estate Pinot Noir	2019	13.5
⑭	Red	Shalauri Wine Cellars	Saperavi	2016	15.0
⑮	Red	Cantina Goretti	Montefalco Sagrantino	2015	14.2

Table 2. Schedule of clinical studies

	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	9 weeks	10 weeks	11 weeks
Drinking period		Drinking (4weeks) wine or mineral water				No drinking (2weeks) ←→		Drinking (4weeks) wine or mineral water			
Questionnaire by email	Every Day ←→										
Measurement session	(1) Measure				(2) Measure		(3) Measure			(4) Measure	

periods and measurements is shown in ***Table 2***.

Questionnaires were administered via email during the study period. The questionnaire asked about the quality of sleep, the presence or absence of mid-onset awakenings, sleep duration, frequency of breakfast, what to start eating at meals, presence or absence of defecation, the nature of stools, whether or not alcoholic beverages other than the study wine and sun protection were consumed. Constipation was also defined as having no stool for more than 3 days.

The group was divided into the Week 2-5 group (early June to mid-July) and the Week 8-11 group (late July to mid-September) by the time of wine consumption. Excluding those who had nail polish on their fingers and those who answered less than 50 % of the questionnaire, the analysis was conducted from four perspectives: duration of consumption, gender, stool condition, and stress level.

Statistical processin

Statistics were obtained using a correspondence nonparametric test (Wilcoxon signed rank sum test).

Ethical Considerations

This study was conducted after approval (2020-18) by the Ethics Committee of Doshisha Women's University.

Results

Basic Research

In basic research, we measured the inhibitory effect of wine on AGEs production. All red wines measured in this study had a stronger inhibitory effect on AGEs production than the standard substance aminoguanidine (AG), even at 5-fold dilution (***Fig. 1***).

In the undiluted solution, the inhibitory effect on AGEs production was stronger in the following order:
10→13→14→15→12→11→2→4→5→3→9→8→6→7→1;
in the 5-fold dilution,
11→15→13→14→10→4→12→5→2→8→3→9→7→1→6;
in the 25-fold dilution,
15→2→13→14→4→10→12→11→7→3→5→1→8→9→6
(red wine is indicated by underbar).

Depending on the results of basic research the wine to be consumed was a red wine (La Forge Estate Pinot Noir containing 13.5 mL/100 mL of alcohol. The intake of red wine was calculated from the IC_{50} of red wine based on the AGEs production inhibitory effect of AG, and was set at 125 mL. The grape variety of La Forge Estate Pinot Noir was Pinot Noir and the producer was Domaines Paul Mas, a wine produced by a grape grower in Pécenas near Carcassonne, Languedoc-Roussillon, France since 1892.

Clinical Research

Of the 48 research collaborators, 9 participants with a response rate of less than 50 % to the questionnaire results and 6 participants who were absent from at least one measurement session were eliminated. The status of the study participants is shown in the flowchart (***Fig. 2***).

Difference by intake period

Comparison of pre- and post-wine intake showed a decrease in AGEs values in the wine intake group and an increase in AGEs in the mineral water group, but the difference was not significant ($p = 0.16$). In a comparison by treatment period, the first half group (early June to mid-July) and the second half group (late July to early September) showed a strong decreasing trend in AGEs values before and after wine intake in the first half group compared to those before and after wine intake in the second half group ($p = 0.06$, ***Fig. 3***). In contrast, there was no difference in AGEs in the second half group ($p = 0.39$, ***Fig. 4***). AGEs increased gradually in the 20s, 30s, and 60s, and decreased in the 40s and 50s (***Fig. 5***).

Differences by gender

Since AGEs generally increase with smoking and sun exposure, we compared changes in AGEs between men and women, assuming that women who use sunscreen on a regular basis are more likely to have lower AGEs. The results showed that for men, there was no difference in AGEs levels between the wine intake and mineral water groups during all intake periods. On the other hand, women showed a trend toward lower AGEs after wine intake ($p = 0.07$). Furthermore, when analyzed overlaid with the time of administration, AGEs were significantly lower in women in the group that consumed wine from summer to fall ($p = 0.02$, ***Fig. 6***).

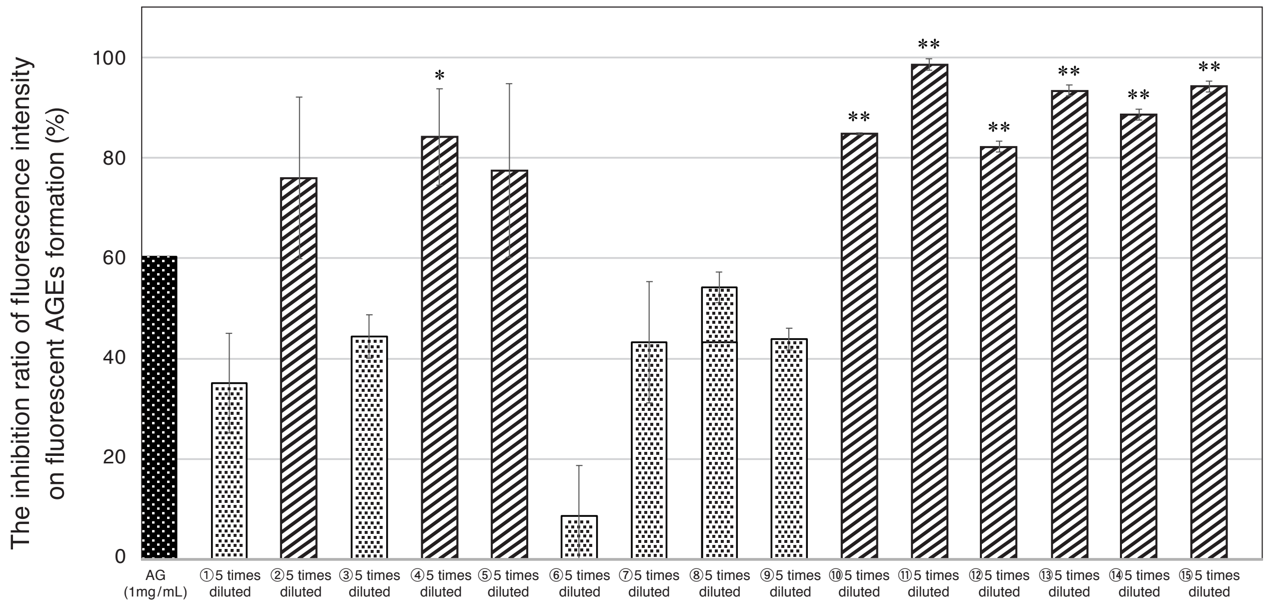

Fig. 1. **Differences in inhibitory effect on fluorescent AGEs formation between wine varieties.**

Fifteen variety of wines were used to determine the inhibitory effect of fluorescent AGEs formation. All data were shown as the mean ± SD, n = 3. $^*p < 0.05$, $^{**}p < 0.01$ vs. AG. AG, aminoguanidine; AGEs, advanced glycation end product; SD, standard deviation.

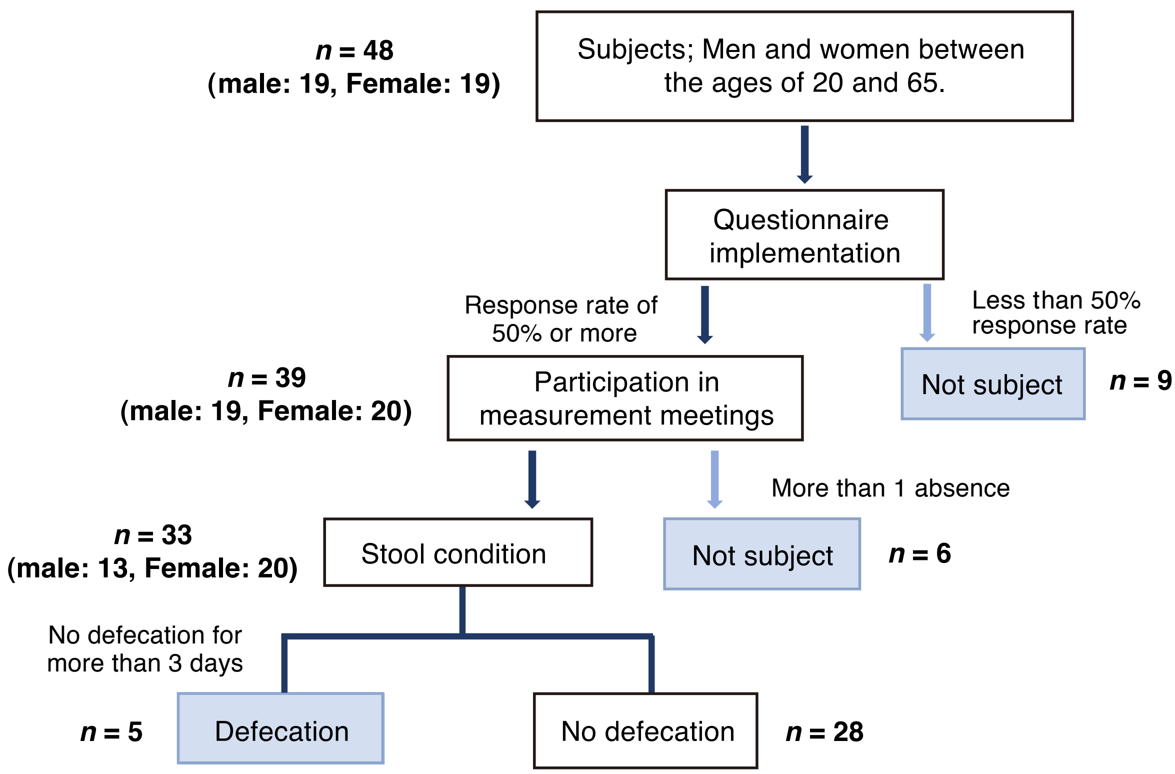

Fig. 2. **Flowchart of clinical research participants.**

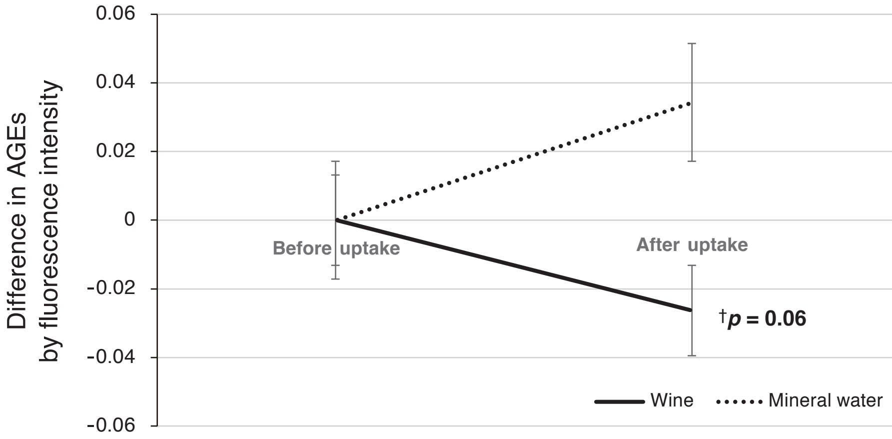

Fig. 3. **Difference in AGEs levels before and after intake (early June to mid-July).**

All data were shown as the mean ± SD, Wine: n = 16, Mineral water: n = 17. †$p < 0.1$ vs. mineral water. AGEs, advanced glycation end product; SD, standard deviation.

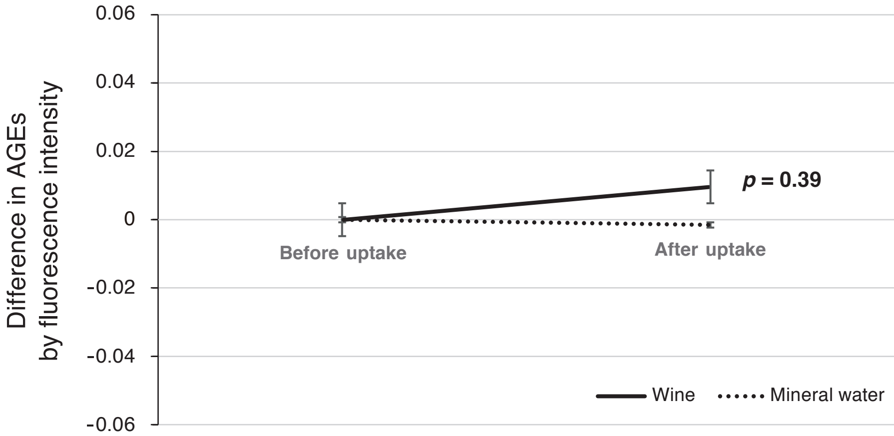

Fig. 4. **Difference in AGEs levels before and after intake (late July to mid-September).**

All data were shown as the mean ± SD, Wine: n = 17, Mineral water: n = 16. AGEs, advanced glycation end product; SD, standard deviation.

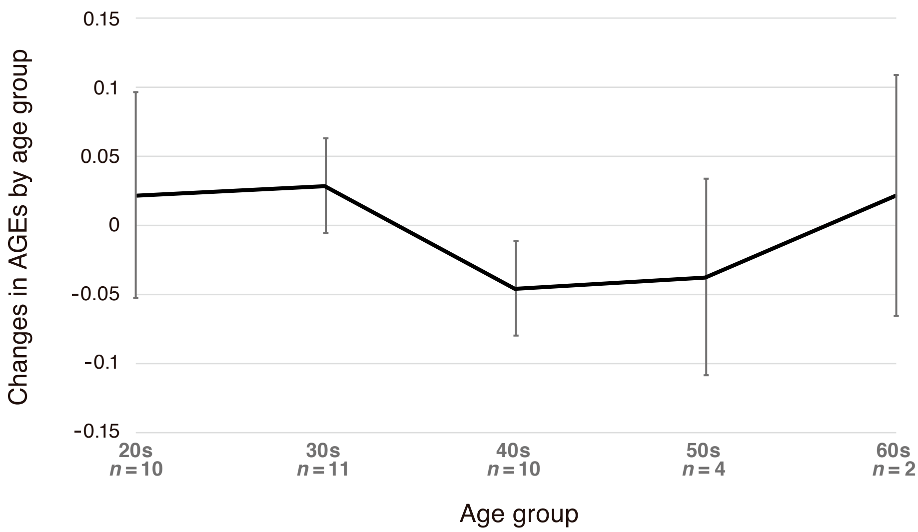

Fig. 5. **Changes in AGEs by age group as a result of wine consumption.**

Data includes subjects who responded to less than 50 % of the questionnaires. All data were shown as the mean ± SD. AGEs, advanced glycation end products; SD, standard deviation.

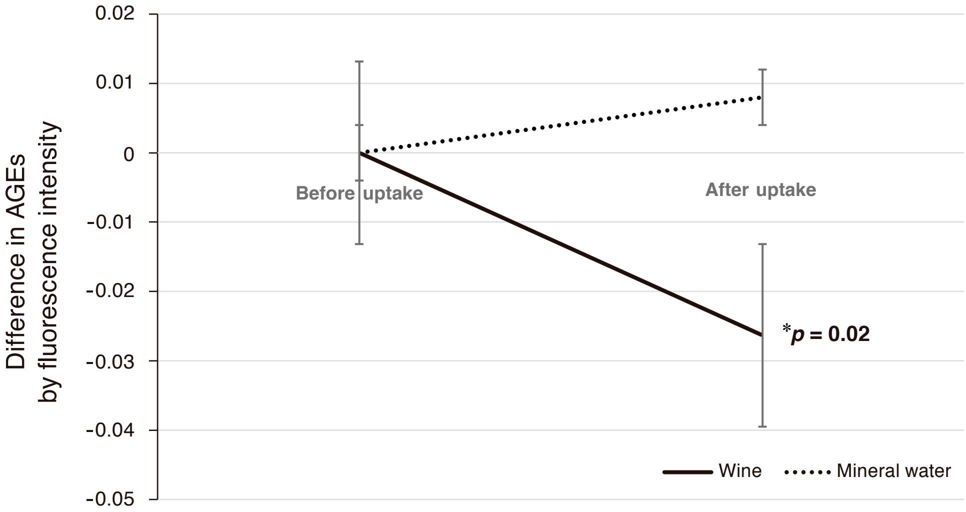

Fig. 6. **Difference in AGEs levels before and after intake for women (late July to mid-September).**

All data were shown as the mean ± SD, Wine: n = 10, Mineral water: n = 10. *p < 0.05 vs. mineral water. AGEs, advanced glycation end product; SD, standard deviation.

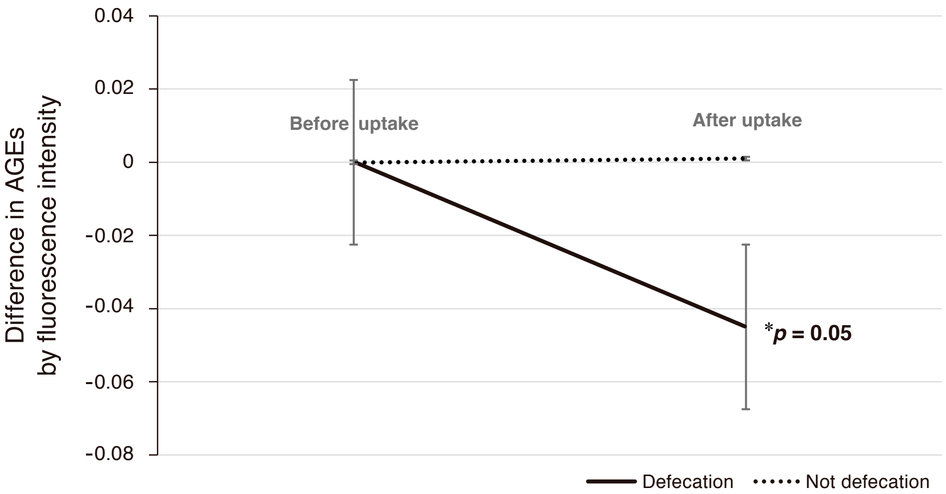

Fig. 7. **Changes in AGEs lowering between constipation and normal stool groups with wine consumption.**

All data were shown as the mean ± SD, Defecation: n = 28, Not defecation: n = 5. *p < 0.05 vs. not defecation. AGEs, advanced glycation end product; SD, standard deviation.

The effects of constipation

Since AGEs are absorbed from the gastrointestinal tract, we compared changes in AGEs values between the constipation group and the normal stool group for the purpose of investigating the possible effect of AGEs absorption due to constipation. AGEs levels were significantly higher in the constipation group than in the normal stool group. Comparison of the reduction in AGEs with wine consumption showed a greater reduction in AGEs in the normal stool group than in the constipation group. (p = 0.05, ***Fig. 7***).

Stress

Stress was evaluated by dividing the group into two groups: one with autonomic disturbance and predominantly activated sympathetic nervous system (stress group) and a normal group, as determined by VM500. In the stress group (n = 10), there was no significant difference in AGEs values after wine consumption (p = 0.64), while in the normal group, AGEs values tended to decrease before and after wine consumption (p = 0.06). The stress counties with disturbed autonomic nervous system tended to weaken the inhibitory effect of wine on AGEs accumulation.

Discussion

Depending on the period of consumption, there was a tendency for AGEs to decrease in the group that consumed wine from early June to mid-July, but there was no predominant difference, whereas there was no change in the group that consumed wine from late July to mid-September. However, when compared by gender, the female group that consumed wine from late July to early September showed a predominant decrease in AGEs. This is thought to be due to the fact that from early June to mid-July was the rainy season with many cloudy days, which reduced the effects of ultraviolet rays, and both men and women showed a tendency to lower AGEs levels due to wine consumption. In addition, from late July to mid-August, the amount of ultraviolet rays reached its peak, and the increase in AGEs due to ultraviolet rays was thought to have had an effect. In a comparison between men and women, men who did not care about sunburn and did not have the habit of using a parasol were less likely to benefit from wine consumption than women, but among women, the predominant decrease in AGEs in the wine consumption group suggested that the use of sunscreen on a daily basis affected the effect of wine on suppressing the increase in AGEs. This suggests that the daily use of sunscreen may have influenced the effect of wine on AGEs inhibition.

In comparison between the constipation and non-constipation groups, the constipation group did not show any decrease in AGEs due to wine consumption. In general, AGEs are thought to be absorbed from the intestinal tract by about 10 %, and the increased absorption of AGEs from the intestinal tract due to constipation was thought to affect the inhibition effect of wine on AGEs increase. The results also suggested that constipation increases the absorption of AGEs.

It is generally believed that sympathetic nerves are activated predominantly in high-stress situations and blood glucose levels rise [12]. Since an increase in blood glucose levels leads to an increase in AGEs, we compared the relationship between stress and the inhibition of AGEs increase by wine. The results showed that AGEs in the normal group tended to decrease with wine consumption, whereas AGEs in the stress group remained unchanged. This indicates that stress inhibits the wine-induced AGEs lowering effect. In other words, the results suggest that stress affects aging.

Acknowledgements

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Conflict of interest statement

This research was partly supported by Mottox Inc. (Osaka, Japan). The wine used in this study was also provided by Mottox, Inc.

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