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Original article

Anti-glycation effects of water-soluble vitamins

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Abstract

***Objective*:** Glycation in the body is initially caused by the formation of carbohydrate-derived aldehydes secondary to postprandial hyperglycemia, enhanced by fatty acid-derived aldehydes, which convert tissue proteins into AGEs (advanced glycation end products), eventually deteriorating protein function. In this study, we examined the anti-glycation and antioxidant effects of representative water-soluble vitamins with the aim of systematically organizing the functions of vitamins. ***Method*:** Seven types of water-soluble vitamins were used as samples. Anti-glycation effects were evaluated using a human serum albumin-glucose glycation reaction model to assess the inhibition of the formation of fluorescent AGEs, pentosidine, 3-deoxyglucosone (3DG), glyoxal (GO), and methylglyoxal (MGO) . Aldehyde trap and antioxidation effects were also evaluated. ***Results*:** Formation of fluorescent AGEs, pentosidine, GO, and MGO were inhibited by L(+)-ascorbic acid, nicotinic acid, pyridoxine, and D-biotin. Inhibition effects on 3DG formation were not observed in any of the vitamins tested. DPPH radical scavenging activity was observed only in L(+)-ascorbic acid. ***Conclusion*:** These findings suggest that the mechanism of action of the anti-glycation effects of water-soluble vitamins may differ depending on the type.

***KEY WORDS*:** water-soluble vitamins, anti-glycation, antioxidant, aldehyde trap

Introduction

Glycation in the body is primarily triggered and caused by the formation of carbohydrate-derived aldehydes due to postprandial hyperglycemia, subsequently forming lipid-derived aldehydes due to the oxidation (including the attack by other aldehydes) of fatty acids, which converts tissue proteins through various processes into advanced glycation end products (AGEs), eventually causing a decrease in protein function[1,2]. Protein denaturation due to glycation, as well as the accumulation of AGEs, are involved in the aging manifestation and aging-related diseases including diabetes and its complications, arteriosclerosis, osteoporosis, cancer, and Alzheimer's disease[3-5]. Glycative stress refers to an excessive load of aldehydes, and oxidation is said to promote glycative stress because new aldehydes are formed by the oxidation of fatty acids and glucose. Methods for suppressing glycative stress (anti-glycation) include suppressing postprandial hyperglycemia and subsequent aldehyde formation, suppressing glycation reactions, and decomposing and excreting AGEs[6].

Aminoguanidine has an AGE formation inhibition effect, and has been reported to prevent and inhibit the progression of nephropathy, retinopathy, and neuropathy, but it has side effects such as anemia, liver damage, and vitamin B6 deficiency, and has not been put to practical use[7,8]. Phytochemicals, such as polyphenols (*e.g.*, flavonoids, catechins, anthocyanins) and carotenoids contained in plant foods, have also been reported to have anti-glycation effects[9-13]. Previous studies have reported that food ingredients such as tea, herbal tea, vegetables, herbs, and fruits have anti-glycation effects, and these effects are believed to be due to substances such as polyphenols and carotenoids[14-16].

Vitamins are organic compounds that are essential for maintaining life and are difficult to synthesize in the body, and have various physiological functions such as metabolic regulation and antioxidant effects[7]. Previous studies have shown that pyridoxamine not only directly captures intermediates in glycation reactions, but also inhibits the formation of AGEs and ALEs (advanced lipoxidation end products) by reducing oxidative stress

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through its antioxidant effect, and that clinical trials have shown that it may delay the progression of renal function deterioration in patients with diabetic nephropathy [18-22]. Thiamine and its derivative, benfotiamine, have been confirmed to reduce the accumulation of harmful intermediate metabolites required for the formation of AGEs by adjusting the glucose metabolic pathway, *i.e.*, by distributing glucose to the pentose phosphate system, and have been reported to contribute to the prevention of diabetic complications such as diabetic retinopathy and nephropathy [23-26]. In addition, ascorbic acid has been reported to suppress the progression of nonenzymatic glycation reactions accompanied by oxidation by removing reactive oxygen species and free radicals, thereby preventing the accumulation of AGEs [11,27].

On the other hand, there are only a limited number of examples that systematically summarize the specific mechanism of vitamins' anti-glycation effects, their relationship with antioxidant effects, and the differences in effects depending on the type of vitamin. In this study, we examined the anti-glycation and antioxidant effects of water-soluble vitamins with the aim of systematically summarizing the anti-glycation effects of vitamins and elucidating the effects of vitamins on glycative stress.

Methods

Reagents

Human serum albumins (HSA, lyophilized powder, ≥ 96%, agarose gel electrophoresis) were used as a model protein for glycation reactions. HSA was purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). Other reagents were of special grade or HPLC grade. Reagents were purchased from Fujifilm Wako Pure Chemical Industries, Ltd. (Osaka, Japan) or Nacalai Tesque, Inc. (Kyoto, Japan).

Sample preparation

In this study, water-soluble vitamins were focused and used as follows: L(+)-ascorbic acid (VC), nicotinic acid (NA), pyridoxine hydrochloride (VB6), D-biotin (VB7), vitamin B1 hydrochloride (VB1), nicotinamide (NAM), sodium D-pantothenate (VB5), vitamin B2 (VB2), vitamin B12 (VB12), and folic acid, purchased from Nacalai Tesque Inc. Aminoguanidine (AG) was used as a positive control. Each reagent was dissolved in purified water to prepare a solution with a concentration of 0.2 mol/L, which was used as the sample. NA and VB7 were dissolved in 1% dimethyl sulfoxide (DMSO). VB2, VB12, and folic acid were excluded, since they were not dissolved in purified water or DMSO. Thus, a total of seven types of solutions were used as samples. The structures of the reagents used are shown in ***Fig.1***.

Verification of anti-glycation effect

A human serum albumin-glucose glycation reaction model was used to verify the anti-glycation effect [28]. A solution was prepared by mixing 0.2 mol/L phosphate buffer (pH 7.4), 40 mg/mL HSA solution, 2.0 mol/L glucose solution, and

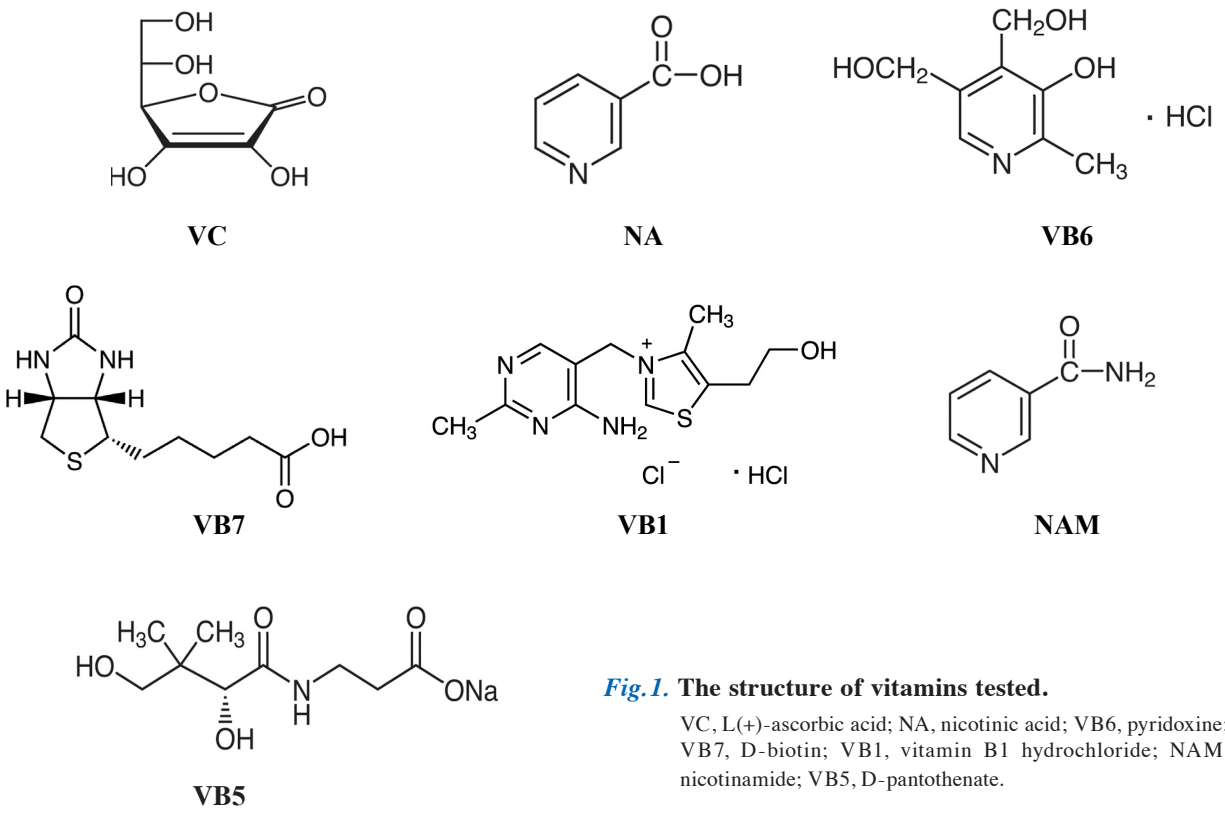

Fig.1 **The structure of vitamins tested.**

VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, D-pantothenate.

the sample, and reacted at 60 °C for 40 hours. Fluorescent AGEs were measured using a microplate reader to measure the AGE-derived fluorescence value (excitation wavelength 370 nm/detection wavelength 440 nm). Pentosidine and glycation intermediates, aldehyde-type dicarbonyl compounds: 3-deoxyglucosone (3DG), glyoxal (GO), methylglyoxal (MGO) were measured by HPLC [29-32]. As a control (Reference: Ref), an equal amount of purified water or DMSO was added instead of the sample and reacted under the same conditions. Aminoguanidine (AG), which is known as an AGE formation inhibitor, was used as a positive control for the inhibition test on fluorescent AGE formation. The inhibition rate (%) of formation of fluorescent AGEs, pentosidine, and dicarbonyl compounds was calculated based on **Equation 1**. In this study, the anti-glycation effect was evaluated as follows: if the inhibition rate was less than 10 %, there was no inhibition; if it was between 10 % and 20 % (excluding 20 %), there was an extremely weak inhibition; if it was between 20 % and 40 % (excluding 40 %), there was a weak inhibition; if it was between 40 % and 60 % (excluding 60 %), there was a moderate inhibition; if it was between 60 % and 80 % (excluding 80 %), there was a strong inhibition; and if it was 80 % or more than 80 %, there was an extremely strong inhibition.

(Equation 1)

Inhibition rate (%) = 100 – {(Sample Glucose (+) – Sample Glucose (–))/(Ref Glucose (+) – Ref Glucose (–))} × 100

Aldehyde trap effect

Aldehyde trap capacity was measured according to the method of Yagi M et al.[33]. The reaction was initiated by each test solution in sodium phosphate buffer (pH 7.4) and adding the same concentration of aldehyde, followed by incubation at 37 °C for 1 hour. The reaction was stopped by adding TFA-acetonitrile solution, then, free aldehyde remaining in the reaction solution was derivatized with DBD-H, and the fluorescence intensity (Ex 450 nm, Em 565 nm) was measured. The remaining aldehyde concentration was simultaneously quantified using a derivatized calibration curve. The aldehyde trap rate was calculated from the rate of decrease in aldehyde concentration in the sample-added solution compared to the reference solution (sample solvent only).

Verification of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity (nmol-Trolox equivalent/μmol) was measured by calculating the equivalent amount using Trolox as the standard substance [34,35].

Statistical analysis

Measurements were expressed as mean ± standard deviation (n = 3). Tukey's multiple comparison test was used to compare measurements. Statistical analysis results were considered significant at a risk level of less than 5 %.

Results

1. Inhibition effect on fluorescent AGE formation

Table 1 and ***Fig. 2*** show the fluorescent AGE formation inhibition rates of seven water-soluble vitamins.

VC had an inhibition rate of 80 % or more, and had an extremely strong inhibition effect. In addition, VC had a significantly higher rate than the other six vitamins ($p < 0.001$).

NA, VB6, VB7, and VB1 had inhibition rates between 60 % and 80 %, and had a strong inhibition effect. NAM and VB5 had inhibition rates of less than 10 %, and did not have a fluorescent AGE formation inhibition effect.

The inhibition effect of VC was similar to that of AG (1.0 mg/mL, ***Fig. 3***).

2. Inhibition effect on pentosidine formation

Table 2 and ***Fig. 4*** show the pentosidine formation inhibition effect of vitamins tested.

VC, NA, VB6, and VB7 had inhibition rates of 80 % or more, and had an extremely strong effect. The inhibition effects were stronger than that of AG at the concentrations measured (***Fig. 5***). NAM and VB5 had inhibition rates of less than 10 %, and showed no effect. For VB1, the value for Sample Glucose (+) was extremely high, resulting in a calculated inhibition rate of –1341.65, and it was determined that evaluation was not possible.

Table 1. Inhibition rate of fluorescent AGE formation.

Sample	Inhibition rate of fluorescent AGE formation [%]	Sample Glucose (+)	Sample Glucose (–)	Reference Glucose (+)	Reference Glucose (–)
VC	108.65 ± 0.49	431.66 ± 3.77	467.75 ± 2.77	475.21 ± 6.03	57.77 ± 0.97
NA	74.30 ± 0.07	284.16 ± 0.60	59.19 ± 0.14	945.49 ± 33.80	70.01 ± 0.32
VB6	73.49 ± 0.36	690.72 ± 2.23	580.05 ± 1.41	475.21 ± 6.03	57.77 ± 0.97
VB7	72.62 ± 0.55	301.50 ± 6.29	61.77 ± 1.47	945.49 ± 33.80	70.01 ± 0.32
VB1	68.44 ± 4.75	874.61 ± 8.19	742.88 ± 17.03	475.21 ± 6.03	57.77 ± 0.97
NAM	4.72 ± 0.72	455.03 ± 3.19	57.28 ± 0.29	475.21 ± 6.03	57.77 ± 0.97
VB5	-6.11 ± 3.75	502.19 ± 14.04	59.25 ± 1.68	475.21 ± 6.03	57.77 ± 0.97

Results are expressed as mean ± standard deviation, n = 3. AGE, advanced glycation endproducts; VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, D-pantothenate.

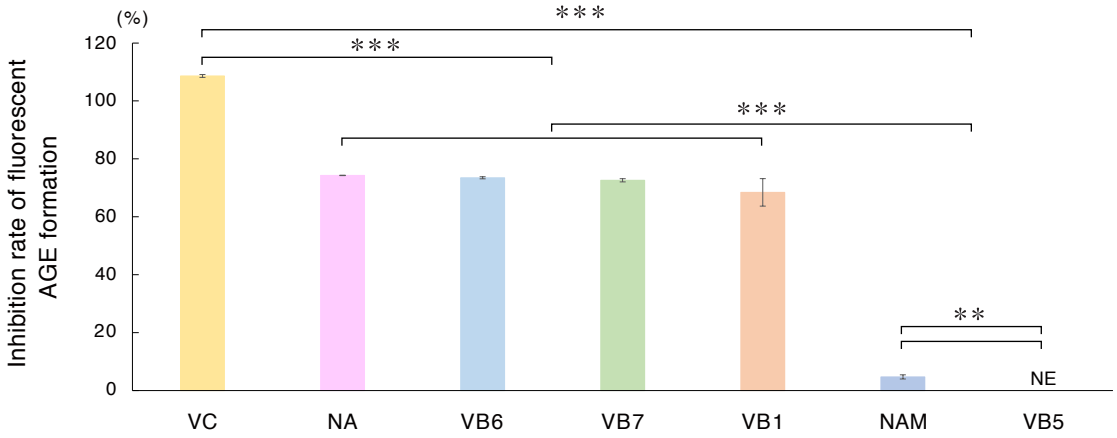

Fig. 2. **Inhibition rate of fluorescent AGE formation.**

Results are expressed as mean ± standard deviation, n = 3. ** $p < 0.01$, *** $p < 0.001$, Tukey's multiple comparison test. Final concentration of each sample: 0.04 mol/L. AGE, advanced glycation endproduct; NE, no effect; VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, D-pantothenate.

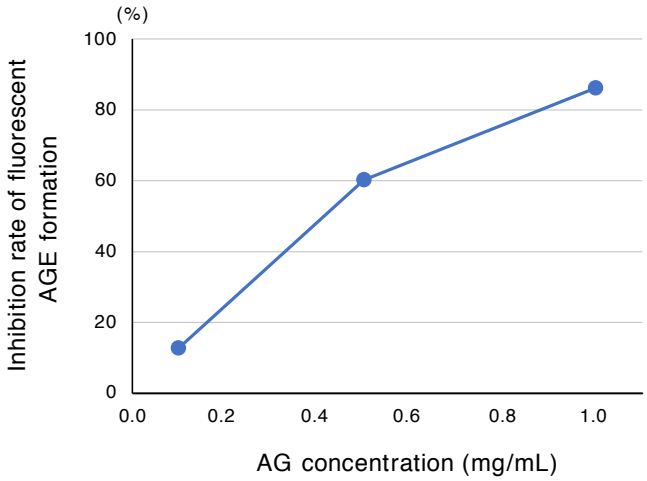

Fig. 3. **Inhibition rate of fluorescent AGE formation by AG.**

AGE, advanced glycation endproduct; AG, aminoguanidine.

Table 2. Inhibition rate of pentosidine formation.

Sample	Inhibition rate of pentosidine formation [%]	Sample Glucose (+) [ng/mL]	Sample Glucose (−) [ng/mL]	Reference Glucose (+) [ng/mL]	Reference Glucose (−) [ng/mL]
VC	84.30 ± 4.26	1.59 ± 0.21	0.83 ± 0.00	5.04 ± 0.00	0.18 ± 0.00
NA	90.65 ± 0.02	0.49 ± 0.00	0.16 ± 0.00	3.76 ± 0.03	0.21 ± 0.00
VB6	94.44 ± 0.07	0.45 ± 0.00	0.18 ± 0.00	5.04 ± 0.00	0.18 ± 0.00
VB7	92.84 ± 3.44	0.25 ± 0.12	0.00 ± 0.00	3.76 ± 0.03	0.21 ± 0.00
VB1	-1341.65 ± 94.22	70.22 ± 4.57	0.23 ± 0.01	5.04 ± 0.00	0.18 ± 0.00
NAM	1.65 ± 0.23	4.95 ± 0.01	0.17 ± 0.00	5.04 ± 0.00	0.18 ± 0.00
VB5	5.53 ± 0.07	4.77 ± 0.00	0.19 ± 0.00	5.04 ± 0.00	0.18 ± 0.00

Results are expressed as mean ± standard deviation, n = 3. VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, D-pantothenate.

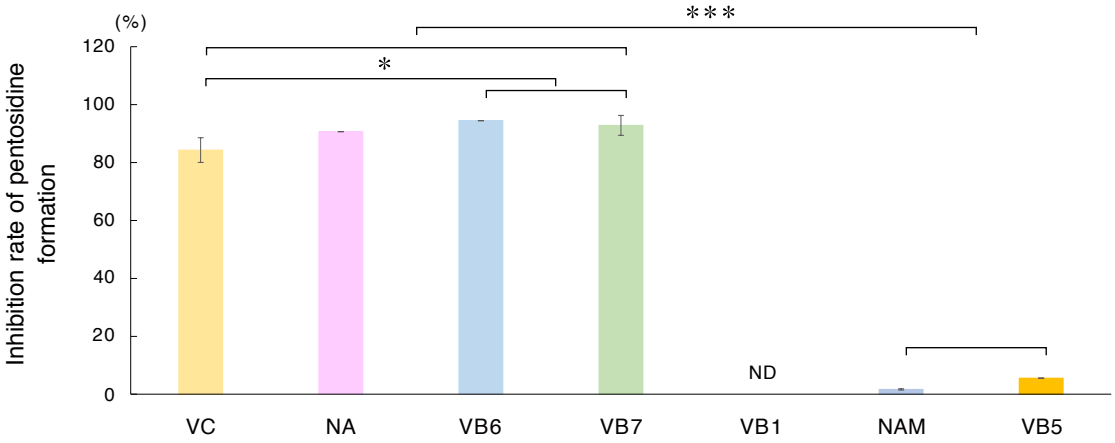

Fig. 4. **Inhibition rate of pentosidine formation.**

Results are expressed as mean ± standard deviation, n = 3. *p < 0.05, *** p < 0.001, Tukey's multiple comparison test. Final concentration of each sample: 0.04 mol/L. ND, not determined; VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, D-pantothenate.

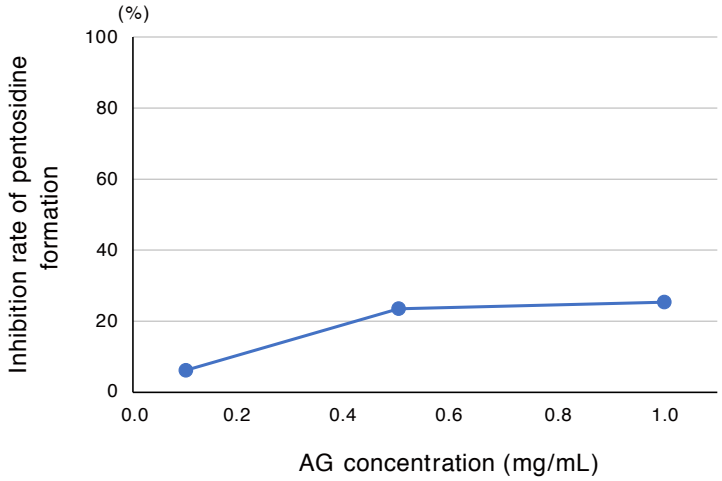

Fig. 5. **Inhibition rate of pentosidine formation by AG.**

AG, aminoguanidine.

3. Inhibition effect on dicarbonyl compound formation

3-Deoxyglucosone (3DG) formation

Table 3 and ***Fig. 6*** show the 3DG formation inhibition effect of vitamins tested.

VB6 had an inhibition rate between 10 % and 20 %, and was judged as an extremely weak inhibition effect. VB6, however, had a significantly higher inhibition rate than the other vitamins ($p < 0.001$).

VC, NA, VB7, VB1, NAM, and VB5 showed inhibition rates of less than 10 %, and judged no effect.

The inhibition effects of these substances were weaker than that of AG (0.5 mg/mL, ***Fig. 7***).

Glyoxal (GO) formation

Table 4 and ***Fig. 8*** show the GO formation inhibition effect of vitamins tested.

VC had an inhibition rate of 80 % or more, showing an extremely strong effect. Also, VC had a significantly higher inhibition rate than the other vitamins ($p < 0.001$).

VB6 showed an inhibition rate between 60 % and 80 %, and was judged as a strong inhibition effect. NA and VB7 showed inhibition rates between 40 % and 60 %, indicating that they had a moderate effect. NAM and VB5 showed inhibition rates of less than 10 %, indicating that they had no effect.

The inhibition effects of VC and VB6 were between those of AG (0.5 mg/mL) and AG (1.0 mg/mL) (***Fig. 9***).

VB1 showed the calculated rate value of 181.26 %. This is because the peak area of GO was higher in the Glucose (−) sample than in the Glucose (+) sample. Therefore, it is not possible to judge the effect.

Methylglyoxal (MGO) formation

Table 5 and *Fig. 10* show the MGO formation inhibition rates of seven water-soluble vitamins.

VC showed an inhibition rate of over 80%, indicating a very strong inhibition effect. VC had a significantly higher inhibition rate than the other vitamins ($p < 0.001$). The inhibition effect of VC was equal to or slightly stronger than that of AG (1.0 mg/mL, *Fig. 11*).

NA, VB6, and VB7 had inhibition rates between 40% and 60%, indicating a moderate effect. While, VB1, NAM, and VB5 had inhibition rates of less than 10%, judged as no effect.

4. Aldehyde trap effect

VB6 and NAM were found to have a 3DG trap capacity of over 10% (*Fig. 12*). VB6, VB1, and NAM were found to have a GO trap capacity of over 10% (*Fig. 13*). NA and VB7 were found to have an MGO trap capacity of over 5% (*Fig. 14*).

5. DPPH radical scavenging activity

The DPPH radical scavenging activity of seven water-soluble vitamins is shown in *Fig. 15*.

Of these, NA, VB7, and VB5 had low DPPH radical scavenging activity and showed no concentration dependency, judged as no effect in this study.

The radical scavenging activity of the remaining four vitamins was, in descending order, VC, VB1, NAM, and VB6. Furthermore, the radical scavenging activity of VC was significantly more than 1,000 times higher than that of VB6, VB1, and NAM ($p < 0.001$).

Table 3. Inhibition rate of 3DG formation.

Sample	Inhibition rate of 3DG formation [%]	Sample Glucose (+) [ng/mL]	Sample Glucose (−) [ng/mL]	Reference Glucose (+) [ng/mL]	Reference Glucose (−) [ng/mL]
VC	-78.12 ± 0.38	23.40 ± 0.16	4.34 ± 0.14	10.71 ± 0.15	0.01 ± 0.00
NA	-30.82 ± 1.10	17.47 ± 0.12	0.02 ± 0.03	13.31 ± 0.23	-0.03 ± 0.00
VB6	13.48 ± 2.23	9.31 ± 0.24	0.06 ± 0.00	10.71 ± 0.15	0.01 ± 0.00
VB7	-52.57 ± 2.02	20.35 ± 0.27	0.00 ± 0.00	13.31 ± 0.23	-0.03 ± 0.00
VB1	-20.78 ± 11.55	12.92 ± 1.24	0.00 ± 0.00	10.71 ± 0.15	0.01 ± 0.00
NAM	-5.31 ± 0.64	11.28 ± 0.07	0.01 ± 0.00	10.71 ± 0.15	0.01 ± 0.00
VB5	-15.46 ± 0.03	12.35 ± 0.00	0.00 ± 0.00	10.71 ± 0.15	0.01 ± 0.00

Results are expressed as mean ± standard deviation, n = 3. 3DG, 3-deoxyglucosone; VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, D-pantothenate.

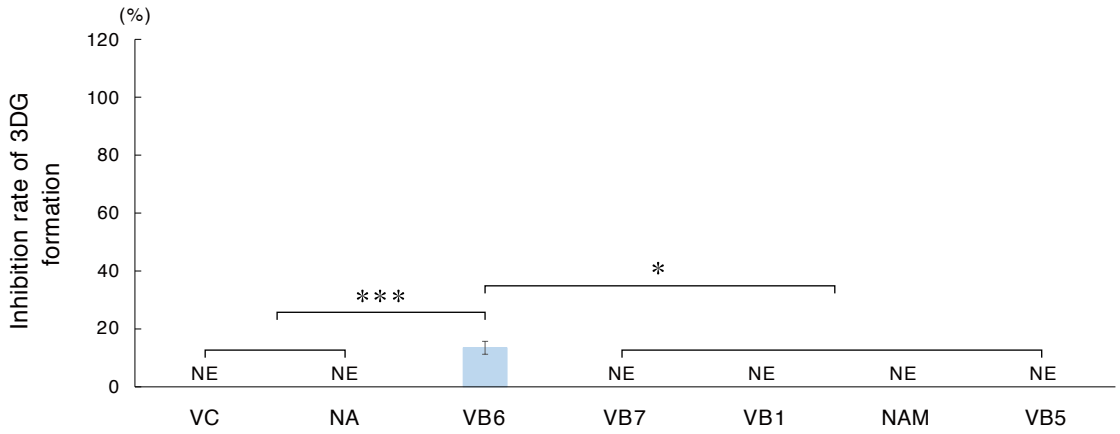

Fig. 6. **Inhibition rate of 3DG formation.**

Results are expressed as mean ± standard deviation, n = 3. * $p < 0.05$, *** $p < 0.001$, Tukey's multiple comparison test. Final concentration of each sample: 0.04 mol/L. 3DG, 3-deoxyglucosone; NE, no effect; VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, D-pantothenate.

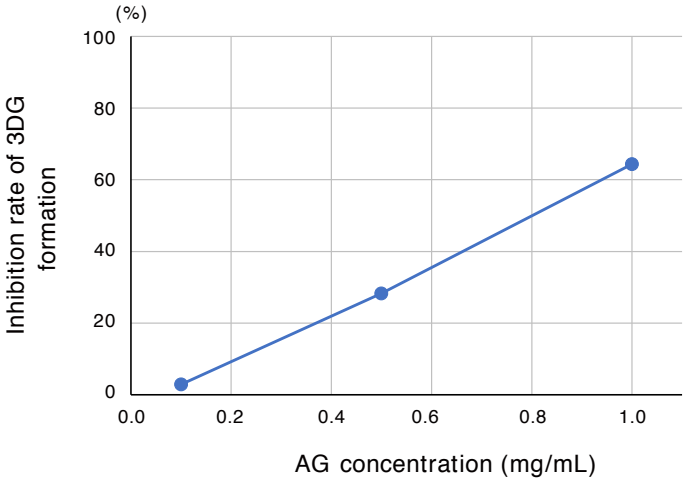

Fig. 7. **Inhibition rate of 3DG formation by AG.**
3DG, 3-deoxyglucosone; AG, aminoguanidine.

Table 4. Inhibition rate of GO formation.

Sample	Inhibition rate of GO formation [%]	Sample Glucose (+) [ng/mL]	Sample Glucose (−) [ng/mL]	Reference Glucose (+) [ng/mL]	Reference Glucose (−) [ng/mL]
VC	95.93 ± 0.02	-0.16 ± 0.00	-0.19 ± 0.00	0.64 ± 0.03	-0.21 ± 0.00
NA	43.57 ± 1.14	0.11 ± 0.00	-0.20 ± 0.00	0.34 ± 0.01	-0.21 ± 0.00
VB6	74.50 ± 0.03	0.01 ± 0.00	-0.21 ± 0.00	0.64 ± 0.03	-0.21 ± 0.00
VB7	48.77 ± 0.06	0.07 ± 0.00	-0.21 ± 0.00	0.34 ± 0.01	-0.21 ± 0.00
VB1	181.26 ± 43.32	30.95 ± 0.03	31.65 ± 0.39	0.64 ± 0.03	-0.21 ± 0.00
NAM	-19.78 ± 0.95	0.81 ± 0.01	-0.21 ± 0.00	0.64 ± 0.03	-0.21 ± 0.00
VB5	-17.04 ± 0.85	0.79 ± 0.01	-0.21 ± 0.00	0.64 ± 0.03	-0.21 ± 0.00

Results are expressed as mean ± standard deviation, n = 3. GO, glyoxal; VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, D-pantothenate.

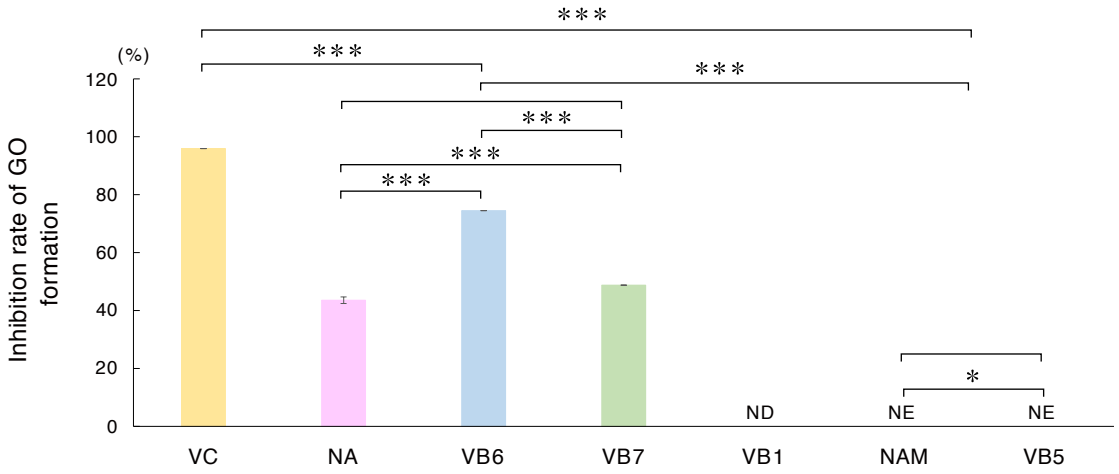

Fig. 8. **Inhibition rate of GO formation.**
Results are expressed as mean ± standard deviation, n = 3. * $p < 0.05$, *** $p < 0.001$, Tukey's multiple comparison test. Final concentration of each sample: 0.04 mol/L. GO, glyoxal; ND, not determined; NE, no effect; VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, D-pantothenate.

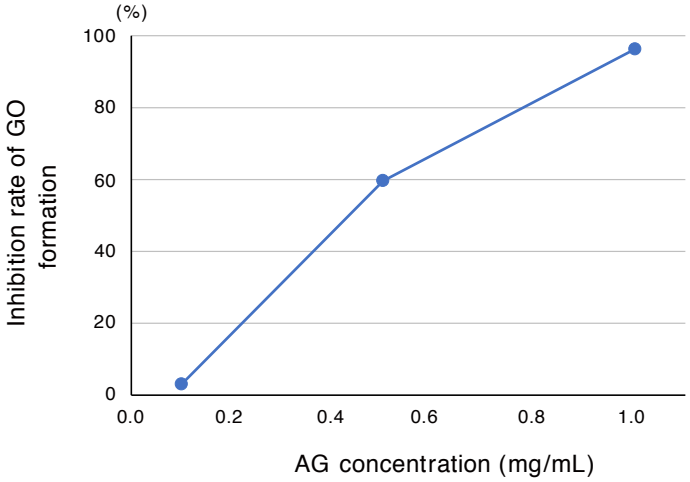

Fig. 9. **Inhibition rate of GO formation by AG.**
GO, glyoxal; AG, aminoguanidine.

Table 5. Inhibition rate of MGO formation.

Sample	Inhibition rate of MGO formation [%]	Sample Glucose (+) [ng/mL]	Sample Glucose (–) [ng/mL]	Reference Glucose (+) [ng/mL]	Reference Glucose (–) [ng/mL]
VC	85.44 ± 0.59	0.07 ± 0.00	0.04 ± 0.00	0.12 ± 0.00	-0.09 ± 0.00
NA	40.94 ± 4.82	0.10 ± 0.01	-0.08 ± 0.01	0.21 ± 0.00	-0.09 ± 0.00
VB6	54.22 ± 3.62	0.00 ± 0.01	-0.09 ± 0.00	0.12 ± 0.00	-0.09 ± 0.00
VB7	54.25 ± 0.14	0.05 ± 0.00	-0.09 ± 0.00	0.21 ± 0.00	-0.09 ± 0.00
VB1	1.20 ± 8.55	0.26 ± 0.02	0.05 ± 0.00	0.12 ± 0.00	-0.09 ± 0.00
NAM	-15.57 ± 0.29	0.15 ± 0.00	-0.09 ± 0.00	0.12 ± 0.00	-0.09 ± 0.00
VB5	-15.37 ± 0.33	0.15 ± 0.00	-0.09 ± 0.00	0.12 ± 0.00	-0.09 ± 0.00

Results are expressed as mean ± standard deviation, n = 3. MGO, methylglyoxal; VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, -D-pantothenate.

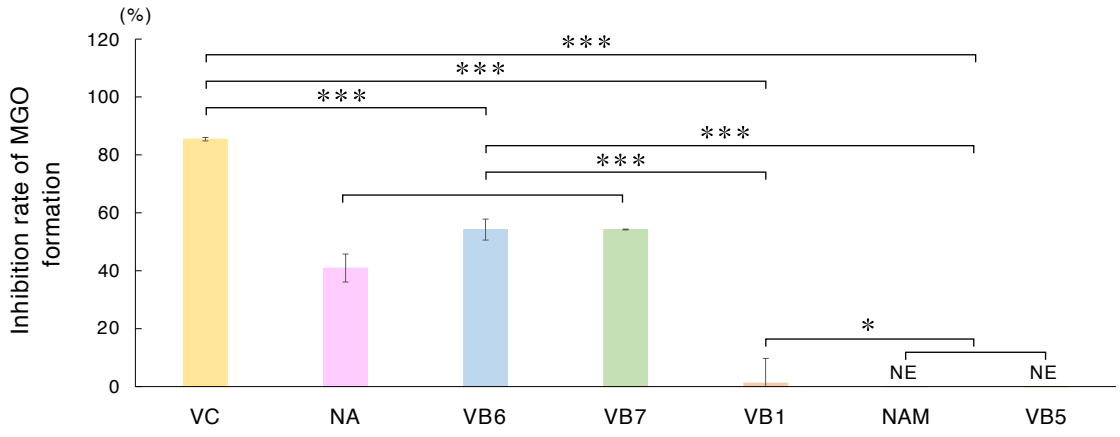

Fig.10. **Inhibition rate of MGO formation.**
Results are expressed as mean ± standard deviation, n = 3. * $p < 0.05$, *** $p < 0.001$, Tukey's multiple comparison test. Final concentration of each sample: 0.04 mol/L. MGO, methylglyoxal; NE. no effect; VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, D-pantothenate.

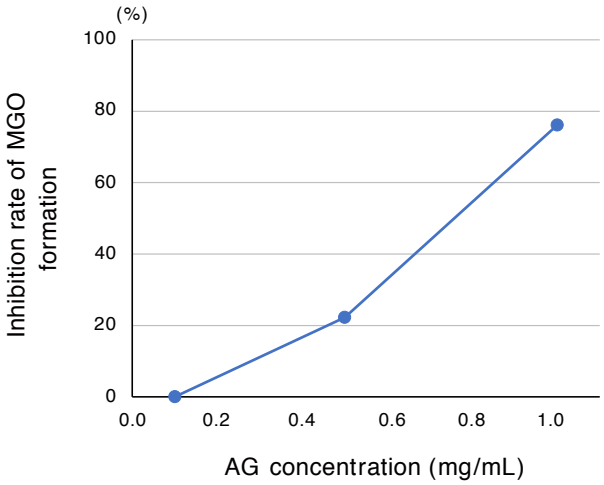

Fig. 11. **Inhibition rate of MGO formation by AG.**
MGO, methylglyoxal; AG, aminoguanidine.

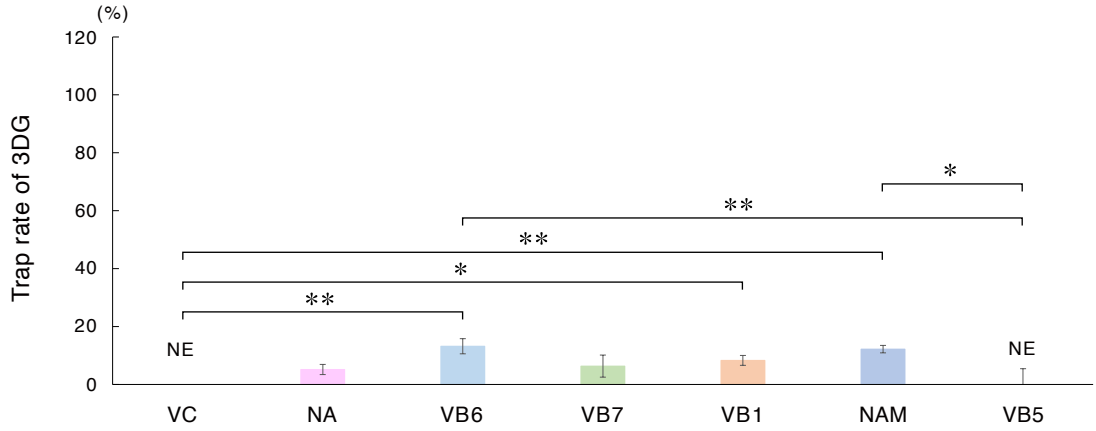

Fig. 12. **3DG trap effects.**
Results are expressed as mean ± standard deviation, n = 3. * $p < 0.05$, ** $p < 0.01$, Tukey's multiple comparison test. 3DG, 3-deoxyglucosone; NE, no effect; VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, D-pantothenate.

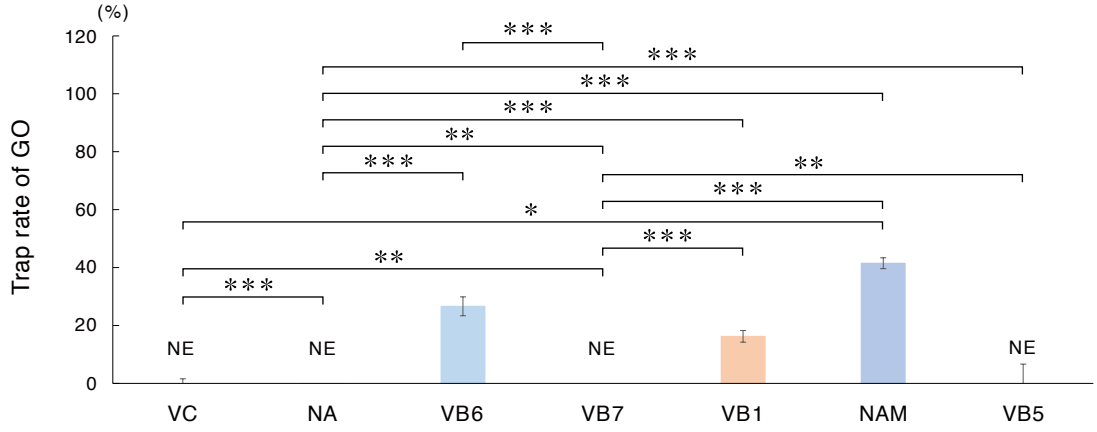

Fig. 13. **GO trap effects.**
Results are expressed as mean ± standard deviation, n = 3. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Tukey's multiple comparison test. GO, glyoxal; NE, no effect; VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, D-pantothenate.

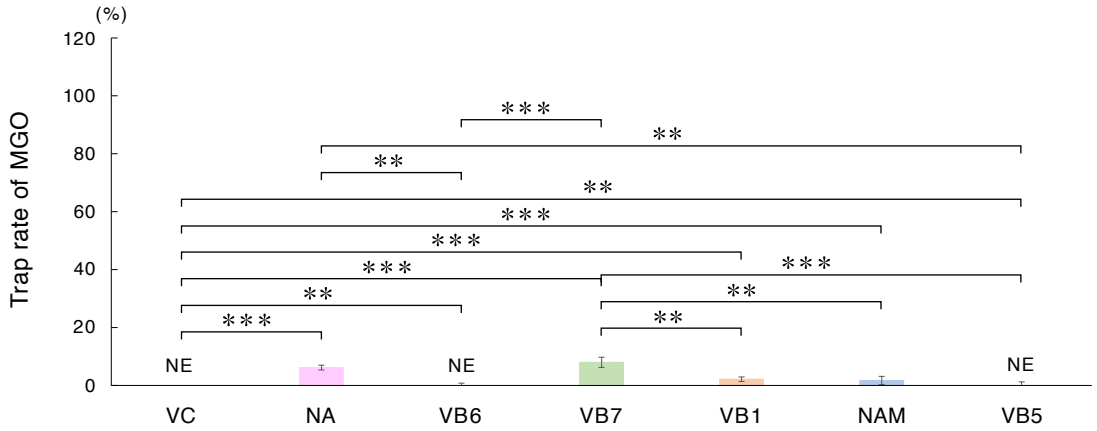

Fig. 14. **MGO trap effects.**

Results are expressed as mean ± standard deviation, n = 3. ** p < 0.01, *** p < 0.001, Tukey's multiple comparison test. MGO, methylglyoxal; NE, no effect; VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, D-pantothenate.

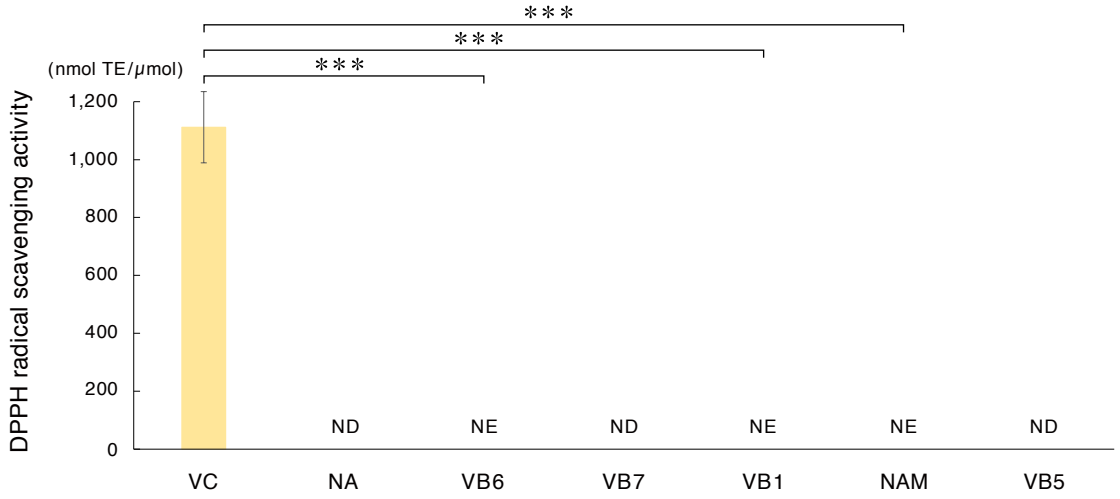

Fig. 15. **DPPH radical scavenging activity.**

Results are expressed as mean ± standard deviation, n = 3. *** p < 0.001, Tukey's multiple comparison test. DPPH, 1,1-diphenyl-2-picrylhydrazyl; NE, no effect; ND, not determined; VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, D-pantothenate.

Discussion

1. Grouping by anti-glycation and antioxidant effect

In this study, the anti-glycation and antioxidant effects of seven water-soluble vitamins (VC, NA, VB6, VB7, VB1, NAM, and VB5) were examined. As a result, the vitamins tested were classified into four groups based on their inhibition effects on the formation of fluorescent AGEs, pentosidine, 3DG, GO, and MGO, and the presence or absence of DPPH radical scavenging activity (***Table 6***).

The first group had inhibition effects on formation of fluorescent AGEs, pentosidine, GO, MGO and DPPH radical scavenging activity. The second group had inhibition effects on formation of fluorescent AGEs, pentosidine, GO, and MGO. The third group had only fluorescent AGE formation inhibition effects. The fourth group had none of these effects. In other words, the results of this study suggest that the presence or absence of anti-glycation and antioxidant effects may differ depending on the type of water-soluble vitamin, and that the mechanism of action of the anti-glycation effect may differ.

The mechanisms of glycation reaction inhibition include: 1. scavenging free radicals; 2. blocking carbonyl (-CHO) groups (blocking carbonyl or dicarbonyl groups contained in reducing sugars, Schiff bases, or Amadori products); 3. chelating metal ions (chelating transition metal ions involved in the formation of AGEs and suppressing their presence); 4. inhibiting the formation of late Amadori products (blocking the formation of Amadori products in the late stage of glycation reactions); 5. cleaving the cross-linked structure of AGEs (cutting off the cross-links of already formed AGEs and reducing the functional effects of AGEs); and 6. blocking RAGE (preventing binding to the AGE receptor (RAGE) and suppressing the occurrence of oxidative stress and inflammatory reactions in cells)[36]. In this literature, it has been reported that free radical scavenging removes hydroxyl radicals and superoxide radicals generated in the early stages of glycation and reduces the generation of reactive carbonyl and dicarbonyl groups[36]. This phenomenon may occur in *in vitro* experimental systems where the reaction temperature is high, however, it is unclear whether this phenomenon actually occurs in the body. We speculate that the antioxidant effect of VC may be of great significance in reducing the formation of lipid-derived aldehydes due to fatty acid oxidation.

The first four mechanisms are prominent as characteristics of anti-glycation ingredients contained in natural foods, while mechanisms 5 and 6 are often used mainly for pharmacological inhibition[36]. As mentioned above, the results of this study suggest that the mechanism of action of anti-glycation effects of water-soluble vitamins may differ depending on the type. Therefore, it is considered that the seven water-soluble vitamins targeted in this study have their own inhibition mechanisms, mainly focusing on mechanisms 1, 2, 3, and 4.

2. Estimation of the mechanism of action of anti-glycation and antioxidant effects.

L(+)-ascorbic acid (VC)

VC had extremely strong effects in inhibiting the formation of fluorescent AGEs, pentosidine, GO, and MGO. It also had a strong antioxidant effect.

Since glycation reactions are promoted by oxidation, substances with antioxidant properties, such as polyphenols, are known to have the effect of inhibiting glycation reactions by suppressing oxidation[36]. VC is known to have a strong antioxidant effect due to its many hydroxyl groups, and the results of this study also showed a strong antioxidant effect. VC is also known to have a carbonyl scavenging effect[27]. Therefore, it was suggested that VC may exhibit an anti-glycation effect by suppressing the formation of AGEs, mainly due to its strong antioxidant and carbonyl scavenging effects.

Pyridoxine (VB6)

VB6 showed a strong inhibition on fluorescent AGE formation, an extremely strong inhibition on pentosidine formation, a strong inhibition on the GO formation, and a moderate inhibition on the MGO formation. On the other hand, it did not have a strong antioxidant effect comparable to that of VC.

Pyridoxine, a type of vitamin B6, is said to have an anti-glycation effect due to the carbonyl trap of the nucleophilic functional group present on the molecule, and

Table 6. Anti-glycation and antioxidant activities of water-soluble vitamins tested.

Group	Sample	Fluorescent AGE inhibition	Pentosidine inhibition	3DG inhibition	GO inhibition	MGO inhibition	DPPH scavenging
1	VC	+	+	−	+	+	+
	VB6	+	+	−	+	+	−
2	NA	+	+	−	+	+	−
	VB7	+	+	−	+	+	−
3	VB1	+	−	−	−	−	−
4	NAM	−	−	−	−	−	−
	VB5	−	−	−	−	−	−

"+" indicates an inhibition rate of 40 % or more, meaning that it has an inhibition effect. " − " indicates an inhibition rate of 40 % or less, meaning that it has no inhibition effect. In this table, the water-soluble vitamins are arranged in descending order according to the number of inhibitory actions that achieve a suppression rate of 40 % or higher. AGEs, advanced glycation endproducts; 3DG, 3-deoxyglucosone; GO, glyoxal; MGO, methylglyoxal; DPPH, 1,1-diphenyl- 2-picrylhydrazyl; VC, L(+)-ascorbic acid; NA, nicotinic acid; VB6, pyridoxine; VB7, D-biotin; VB1, vitamin B1 hydrochloride; NAM, nicotinamide; VB5, D-pantothenate.

the metal chelating effect in which the hydroxyl group, amino group, and nitrogen of the pyridine ring present in the molecule form strong complexes with transition metal ions *i.e.*, copper and iron, thereby expectedly inhibiting oxidation reactions catalyzed by metal ions [22]. Since pyridoxine has a nucleophilic functional group, a hydroxyl group and a pyridine ring, in the molecule, it is speculated that it is highly likely to have a carbonyl trap effect and a metal chelating effect similar to pyridoxine. Therefore, pyridoxine does not show a direct antioxidant effect, but it is suggested that it may have an anti-glycation effect by suppressing the AGE formation through its carbonyl scavenging and metal chelating effects.

Nicotinic acid (NA) and D-biotin (VB7)

NA and VB7 had a strong fluorescent AGE formation inhibition effect, an extremely strong pentosidine formation inhibition effect, a moderate GO formation inhibition effect, and a moderate MGO formation inhibition effect. Whereas, they did not have a strong antioxidant effect comparable to VC.

Previous research has revealed that pyridoxamine and polyphenols, even substances that do not have direct antioxidant ability, have an anti-glycation effect due to their carbonyl scavenging and metal ion chelating effects [22, 37]. It is possible that a similar mechanism of action may be applied to NA and VB7.

The results of this study showed that NA and VB7 had low DPPH radical scavenging activity, making it difficult to measure their antioxidant effects, but they did have a moderate inhibition effect on the formation of GO and MGO. Therefore, it was suggested that NA and VB7 do not exhibit direct antioxidant effects, but may exhibit anti-glycation effects by suppressing the AGE formation through their carbonyl scavenging and metal chelating effects.

Vitamin B1 (VB1)

VB1 had a strong inhibition effect on the formation of fluorescent AGEs. Whereas, it did not have a strong antioxidant effect comparable to VC. In addition, the inhibition rate of pentosidine formation by VB1 was –1341.65%, and the inhibition rate of GO formation was 181.26%, and the values of the other formation inhibition rates also showed a large variation. Therefore, it was determined that it was difficult to assess the effectiveness.

In this study, each inhibition rate was calculated based on **Equation 1**. That is, if the ratio to the difference with the reference exceeds 1.0, such as when the value of Sample Glucose (+) increases due to the influence of the sample and the value of (Sample Glucose (+) – Sample Glucose (–)) becomes significantly higher, the calculated inhibition rate will be 0% or less. If the value of Sample Glucose (–) increases due to the influence of the sample and the value of (Sample Glucose (+) – Sample Glucose (–)) becomes negative, the calculated inhibition rate will be 100% or more. In other words, if the obtained inhibition rate is 0% or less or 100% or more, it cannot be said that the actual inhibition effect is accurately reflected, and the background effect from the sample must be considered. In this study, the peak area value of pentosidine in VB1 was about 14 times higher than that of the reference. In addition, the peak area value of GO in VB1 was higher in Sample Glucose (–) than in Sample Glucose (+). The results calculated according to **Equation 1** showed that the inhibition rate of VB1 for pentosidine formation was –1341.65%, and the inhibition rate for GO formation was 181.26%.

This result suggests that pentosidine may have been produced by heating with VB1, or another compound with similar wavelength characteristics may have been produced. This case requires further investigation [23-26].

Nicotinamide (NAM) and D-pantothenate (VB5)

NAM and VB5 did not have inhibition effects on the formation of fluorescent AGEs, pentosidine, 3DG, GO, and MGO, nor did they have a strong antioxidant effect comparable to VC. Therefore, it was suggested that NAM and VB5 may not have sufficient anti-glycation effects by themselves.

On the other hand, in living organisms, there are cases where a substance alone does not show anti-glycation effects, but an anti-glycation effect is shown by synergistic effects with other ingredients, so NAM and VB5 may also show anti-glycation effects by combining with other factors [11]. It is considered necessary to conduct experimental systems and *in vivo* verification taking other factors into account in the future to make a comprehensive judgment on the anti-glycation effects of NAM and VB5.

3. Aldehyde trap effects

We are currently conducting measurements and collecting information on the aldehyde-trap effects of other substances. The effectiveness of the test products in this study was shown to be a trap rate of 10% or more for 3DG with VB6 and NAM, and a trap rate of 20% or more for GO with VB6 and 40% or more with NAM. The significance of these values will be discussed after the results of other substances have been compiled.

Conclusion

The seven water-soluble vitamins used in this study (VC, NA, VB6, VB7, VB1, NAM, and VB5) were classified into four groups based on their inhibition effects on the formation of fluorescent AGEs, pentosidine, 3DG, GO, and MGO, and on the presence or absence of DPPH radical scavenging activity. This suggests that the mechanism of action of the anti-glycation effect of water-soluble vitamins may differ depending on the type.

Conflict of interest declaration

No conflicts of interest to note.

Acknowledgement

Part of the results of this study were presented at the 25th General Meeting of the Japanese Society of Anti-Aging Medicine (June 13-15, 2025, Osaka).

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