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Improvement of vascular endothelial function by intake of lycopene-rich tomato juice in healthy adults: a randomized, placebo-controlled, double-blind, parallel-group comparative study

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Maintaining normal vascular endothelial function is important for preventing arteriosclerosis. Flow-mediated dilatation (FMD) is widely used to evaluate vascular endothelial function. Lycopene, a carotenoid abundant in tomatoes, has been reported to improve FMD, but its effect after 4 weeks of intake remains unclear. We conducted a randomized, placebo-controlled, double-blind, parallel-group trial to assess the effects of lycopene. Seventy-five healthy adults with borderline FMD (4–7%) were randomly assigned to placebo juice (0.7 mg lycopene), tomato juice (TJ, 15.0 mg), or high-lycopene tomato juice (HLTJ, 26.7 mg) groups. FMD, serum lycopene, oxidative stress markers (malondialdehyde, 8-hydroxy-2′-deoxyguanosine, 8-iso-prostaglandin F2α), and nitrogen oxide concentrations were measured every 4 weeks over a 12-week intervention. The participants analyzed included 23 (placebo), 25 (TJ), and 24 (HLTJ). Data were analyzed using a linear mixed effects model adjusting for baseline covariates and a *post hoc* Dunnett's test with Bonferroni correction for intergroup comparisons. At week 12, FMD was significantly higher in the TJ (6.1 ± 0.5%, $p < 0.001$) and HLTJ groups (7.0 ± 0.7%, $p < 0.001$) than in the placebo group (5.4 ± 0.6%). In the HLTJ group, FMD at weeks 4 (6.2 ± 1.0%, $p < 0.001$) and 8 (6.7 ± 0.7%, $p < 0.001$) was significantly higher than in the placebo group (5.4 ± 0.9% and 5.3 ± 0.6%, respectively). Serum lycopene concentrations were significantly increased in TJ and HLTJ groups than in the placebo group. No significant differences were observed in oxidative stress or nitrogen oxide levels. These findings suggest that lycopene intake improves vascular endothelial function, though the mechanism remains unclear. Trial registration: UMIN000051176.

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Introduction

The prevalence of cardiovascular disease (CVD) is increasing globally, and trends over the past decade have indicated a worsening of this burden. CVD is the largest contributor to the global disease burden, and atherosclerotic diseases are the primary mediators of CVD burden and trends.[1] Atherosclerotic disease is the major cause of death in Japan, and the Japan Atherosclerosis Society published the Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases in 2022 to prevent atherosclerotic CVD, which presents non-invasive diagnostic methods for arteriosclerosis prevention.[2] Vascular endothelial function is one of them and is evaluated by measuring changes in forearm blood flow and the diameter of the brachial artery in response to endothelium-dependent increases in blood flow caused by drugs such as acetylcholine and reactive hyperemia after forearm ejection. One of the most commonly used techniques is flow-mediated dilatation (FMD), which is used to evaluate the degree of brachial artery dilation caused by reactive hyperemia after 5 min of restricted forearm blood flow. Given that FMD declines in the early stages of atherosclerosis,[3,4] it is useful for the initial assessment of atherosclerotic CVDs. Normal FMD is ≥7%; 4–7% is borderline; and <4% is abnormal.[5]

Lycopene is a carotenoid primarily found in tomatoes and tomato products, with strong singlet oxygen scavenging activity.[6] Consumption of tomato-based products has been shown to be inversely associated with the risk of CVD in a cohort study.[7] Another cohort study reported that lycopene intake was inversely associated with the incidence of CVD.[8] Lycopene exhibits anti-inflammatory properties[9] and inhibits the migration of endothelial cells by inhibiting the expression of vascular endothelial growth factor and increasing NO production.[10] A human interventional study reported that

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consuming 70 g of tomato paste containing 33 mg of lycopene for 15 days significantly increased the FMD.[11] Additionally, the consumption of tomato juice (intake amount was not specified) for 2 months significantly increased FMD.[12] In addition, a meta-analysis that integrated three interventional studies evaluating the effect of continuous intake (for ≥1 week) of tomato-containing foods on FMD showed that tomato intake significantly increased FMD.[13] In contrast, the ingestion of 70 g of tomato puree containing 46.2 mg of lycopene for 1 week resulted in no significant increase in FMD.[14] Additionally, the ingestion of 80 g of tomato paste for 1 week (the amount of lycopene was not specified) resulted in no significant increase in postprandial FMD.[15] To date, no placebo-controlled studies have investigated the effects of pure lycopene on FMD.

The effects of lycopene intake on FMD were significantly affected by the amount and duration of lycopene intake. The guidance issued by the European Food Safety Authority (EFSA), "Guidance for the scientific requirements for health claims related to antioxidants, oxidative damage, and cardiovascular health", states that an increase in fasting FMD caused by the continuous consumption of food ingredients for more than 4 weeks has a beneficial physiological effect on vascular endothelial function.[16] Considering these reports, it would be meaningful to clarify the effect of lycopene on vascular endothelial function by evaluating the effect of lycopene intake for ≥4 weeks on FMD. In addition, there have been no placebo-controlled studies evaluating the effect. Therefore, we conducted a randomized, placebo-controlled, double-blind, parallel-group comparison study in healthy adults with borderline FMD values to evaluate the change in FMD based on the long-term (≥4 weeks) intake of lower doses of lycopene compared to those in previous reports. In addition, the effects of lycopene on oxidative stress markers and nitrogen oxides (NO_x), which may be related to the mechanism of lycopene function, were evaluated.

Methods

Study overview

Ethical review and protocol registration. This study was approved by the Kobuna Orthopedic Clinic Ethics Review Committee (approval number: MK-2305-02; approval date: May 25, 2023), which is a third party and not involved in the study. This study was conducted in accordance with the World Medical Association Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research involving human subjects. The study protocol was registered with the University Hospital Medical Information Network Clinical Trial Registry (UMIN-CTR; ID number: UMIN000051176) on May 26, 2023, and was conducted from May 27, 2023, to October 29, 2023.

Study design. This was a randomized, placebo-controlled, double-blind, parallel-group comparison study. The study design is illustrated in Fig. 1. We entrusted KSO Corporation (Tokyo, Japan), a Contract Research Organization (CRO), for conducting the study. Study participants were recruited *via* a website operated by KSO Corporation. The study was conducted at the Maebashi North Hospital (Maebashi, Japan). Written informed consent was obtained from all participants before the screening examination (SCR). Data on FMD, hematological, blood biochemical, and urinary parameters; blood pressure; anthropometric parameters; medical questionnaires; and salt intake status were collected during the SCR. Participants were selected based on their SCR results, and a pre-intervention examination (pre-exam) was conducted. Data on FMD; oxidative stress marker, nitric oxide, and serum lycopene concentrations; virus tests; and medical questionnaires

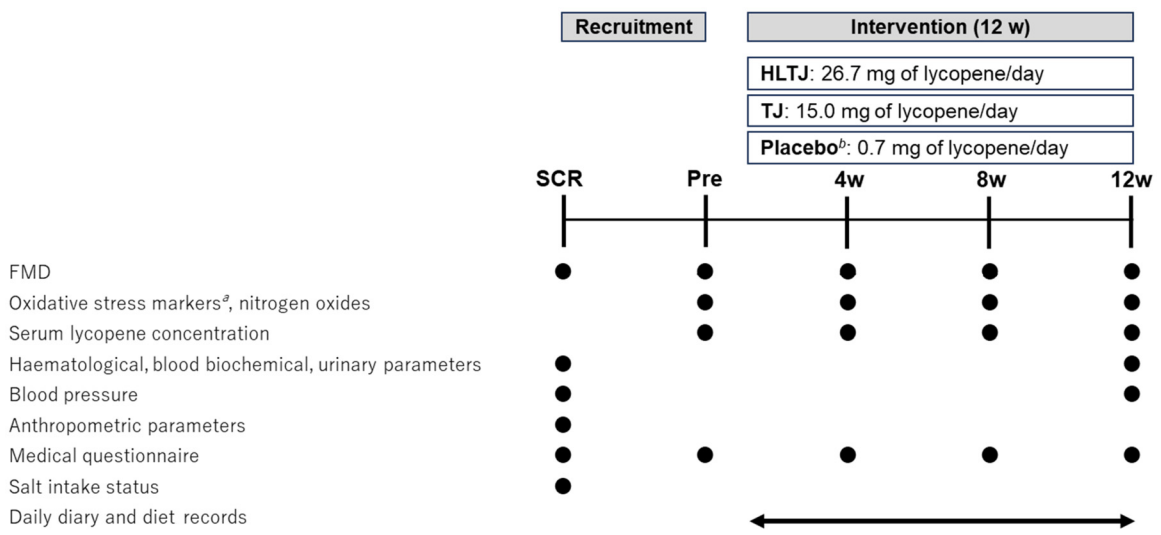

Fig. 1 Study design. FMD, flow-mediated dilatation; SCR, screening examination; Pre, pre-intervention examination; HLTJ, high-lycopene tomato juice; TJ, tomato juice. [a] Serum malondialdehyde (MDA), urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG), and 8-iso-prostaglandin F2α (8-isoprostane). [b] The placebo juice was prepared by removing lycopene from tomato juice using centrifugation and membrane filtration.

were collected during the pre-examination. After the eligible participants were selected, they were randomly allocated to the placebo group, tomato juice (TJ) group, and high-lycopene tomato juice (HLTJ) groups at an allocation ratio of 1 : 1 : 1. Each participant consumed placebo juice containing 0.7 mg lycopene, TJ containing 15.0 mg lycopene, or HLTJ containing 26.7 mg lycopene for 12 weeks. Participants visited the hospital at 4, 8, and 12 weeks after starting the intervention, and data on FMD, oxidative stress markers, NO_x, serum lycopene concentrations, virus tests, and medical questionnaires were collected. Hematological, blood biochemical and urinary parameters and blood pressure were measured at 12 weeks. The participants maintained their daily diaries and dietary records during the intervention period.

Participants

Inclusion and exclusion criteria. The inclusion criteria were as follows: 1, healthy adults aged ≥40 and <65 years (except for premenopausal women); 2, subjects with ≥4% FMD; 3, subjects who received a sufficient explanation of the purpose and content of this study, were able to fully understand and consent to it, and had voluntarily applied for participation with written informed consent.

The exclusion criteria were as follows: 1, subjects with a chronic disease and were undergoing treatment; 2, subjects with a history or current medical history of serious liver, kidney, gastrointestinal, heart, respiratory, endocrine, thyroid, adrenal, or other metabolic disease; 3, subjects diagnosed with arrhythmia during the SCR; 4, subjects who were judged as unsuitable for this study based on the SCR; 5, subjects with a history of gastrointestinal disease or gastrointestinal surgery that affected digestion and absorption; 6, subjects with allergies to drugs or foods (particularly test foods); 7, subjects who did not stop taking any supplement or a healthy food that may affect vascular endothelial function or blood pressure during the study period; 8, subjects who consumed tomato (≥40 g) or tomato products (*e.g.*, ≥100 mL tomato juice or meals with a lot of tomato ketchup or tomato sauce) for ≥3 days a week; 9, subjects from whom >200 mL of blood was collected within 1 month or those from whom 400 mL of blood was collected within 3 months prior to the date of providing informed consent; 10, subjects with a history of drug dependence or alcohol dependence; 11, subjects who consumed ≥20 g of pure alcohol per day for ≥3 days a week; 12, subjects who smoked ≥21 cigarettes per day on an average (including e-cigarettes); 13, subjects who were shift workers or late night workers; 14, subjects who were pregnant and subjects who intended to conceive during the study period, and breastfeeding patients; 15, subjects who were participating in research involving other foods or medicines, subjects who used other study cosmetics or medicines, subjects who had participated in other studies within 1 month prior to the date of informed consent, and subjects who were willing to participate in other studies; and, 16, subjects who were considered unsuitable for this study by the investigators or researchers.

Selection of the participants. The SCR included 400 healthy adults aged ≥40 and <65 years (except for premenopausal women), of which 150 who met the following three criteria were selected: 1, subjects who were considered healthy by the investigators; 2, subjects who did not meet the exclusion criteria; 3, subjects whose FMD was within the top 150 among those whose FMD was ≥3% and ≤7%. The pre-intervention examination was conducted on 150 individuals selected through the SCR. Among them, 75 individuals with an FMD between ≥4% and ≤7% were selected as participants, in ascending order of FMD.

Sample size calculation. A study that analyzed 19 individuals in the control and interventional groups reported that vascular endothelial function improved upon the consumption of tomato paste containing 33.3 mg of lycopene for 15 days.[11] Although the lycopene intake amount in this study was 15.0 mg or 26.7 mg, lower than the amount in the previous study, the interventional period in this study (12 weeks) was longer than that in the previous study. Therefore, we set the target sample size to 20, which is similar to that of a previous study, and estimated a maximum dropout rate of 20%, with 25 participants in each group.

Randomization and allocation. A randomization manager who was not directly involved in this study randomly assigned participants to three groups by stratified block randomization based on sex, age, FMD, salt intake status, and number of cigarettes smoked per day. Subsequently, an allocation manager who was not directly involved in this study allocated participants to three groups. The allocation table was sealed by the allocation manager and kept sealed until the study was completed. The allocation information was not disclosed to anyone other than the manager.

Activities requiring compliance during the study period. Compliance with restrictions during the study period was confirmed using daily diaries and dietary records. Activities requiring compliance during the study period were as follows: 1, experimental foods were to be consumed by the study participants and not shared with others; 2, experimental foods were to be consumed as directed by the principal investigator; 3, alcohol consumption was prohibited from 2 days before each examination until the end of each examination; 4, eating, drinking (except for water), and smoking was prohibited from 9:00 pm on the day before each examination until the end of the examination; 5, strenuous exercise such as running, swimming, or triathlon for >90 min was prohibited from the day before each examination until the end of the examination; 6, the intake of any supplements and healthy foods that may affect vascular endothelial function and blood pressure was prohibited; 7, the intake of raw tomatoes and tomato products was restricted to twice a week at the following amounts per day: 1/4 raw tomato or 2 cherry tomatoes or 100 mL of tomato juice or a meal using tomato ketchup (Napolitan or ketchup rice, *etc.*) or a meal using tomato sauce (pasta with tomato sauce, *etc.*), or a slice of watermelon; 8, a regular lifestyle was to be maintained, and an irregular lifestyle was to be avoided (reckless drinking/overeating, extreme loss of weight, changing

eating habits, discontinuing previous exercise, starting new exercise, changing drinking and smoking habits, *etc.*); 9, consuming excessive amounts of alcoholic beverages was prohibited (maximum daily amount: ≤20 g equivalent per day of pure alcohol); 10, the remaining experimental foods were to be kept and brought to the hospital on the day of the visit; 11, the daily diary and diet records were to be filled out every day during the intervention period and brought on the day of the visit; 12, participation in other studies involving consuming other foods or medicines or applying cosmetics or medicines was prohibited; 13, medicines were only to be used with permission from the principal investigator or sub-investigator, except in cases of emergency. The reason for use, name of the drug used, amount used, and period of use were recorded in a daily diary; and, 14, divulging any information about this study or experimental foods to anyone, including posting on social networking services, was prohibited.

Dietary intervention

Experimental foods. HLTJ (26.7 mg of lycopene), TJ (15.0 mg of lycopene), and placebo juice (0.7 mg of lycopene) were manufactured at Kagome Co., Ltd (Nagoya, Japan). HLTJ and TJ were prepared using tomato raw materials with differing lycopene concentrations. The placebo juice was produced by removing lycopene from tomato juice through centrifugation and membrane permeation. Each experimental food had a capacity of 190 g and was packed in a plain white can to ensure blinding. The nutritional content of each experimental food is presented in Table 1.

Distribution and intake of the experimental foods. Thirty cans of the experimental food were sent to each participant before starting the intervention. The experimental food was consumed once per day during the intervention period. To ensure blinding, the participants were asked to drink the experimental food directly from the can without pouring it into another container. The remaining cans of experimental foods were counted and retrieved at the hospital, and 30 cans of the experimental food were given to each participant every 4 weeks.

Data collection

Flow-mediated dilatation. This was measured in accordance with the Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2022.[2] The measurements were performed after a participant rested for approximately 30 min in a quiet room with a constant temperature (22–26 °C). The ultrasound diagnostic device ARIETTA E70 (Hitachi, Ltd, Tokyo, Japan) was fixed to an arm/instrument holder MIST-100H (Saraya Co., Ltd, Osaka, Japan), and the resting artery diameter of the upper arm was measured using the image analysis software MISTPILOT (Saraya Co., Ltd). The forearm was compressed with a cuff for 5 min to induce vascularization, and the maximum dilated artery diameter was measured using image analysis software in the same manner as described above after the dilation. Flow-mediated dilatation (%) was calculated using the following formula:

$$\begin{aligned}\text{FMD}\,(\%) = &(\text{the maximum dilated artery diameter} \\ &- \text{the resting artery diameter}) \\ &\times 100/\text{resting artery diameter}\end{aligned}$$

The arm to be measured for each participant, *i.e.*, left or right, was determined at the time of the SCR, and measurements were made on the same arm during the study period.

Blood and urinary oxidative stress markers and urinary nitrogen oxide. Measurements such as serum concentration of malondialdehyde (MDA) and urinary concentrations of 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-iso-prostaglandin F2α (8-isoprostane) were outsourced to Nikken SEIL Co., Ltd (Fukuroi, Japan), which has extensive experience in measuring oxidative stress markers. MDA was measured using the thiobarbituric acid reactive substances method. The enzyme-linked immunosorbent assay was used to measure 8-OHdG and 8-isoprostane. Urinary NO was measured using a colorimetric method at LSI Medience Co., Ltd (Tokyo, Japan), a leading clinical laboratory in Japan.

Serum lycopene concentration. The serum lycopene concentration was quantified by Kagome Co., Ltd. Lycopene was extracted and measured in accordance with previously described methods.[17,18] We used a high-performance liquid chromatograph with a photodiode array detector, Prominence Nexera X2 LC-30AD/Nexera X2 SPD-30A (Shimadzu Corporation, Kyoto, Japan), for the measurements.

Medical questionnaire. The principal investigator or co-investigator conducted medical interviews to select the study participants and collect information on adverse events. Data on the subjects' health conditions, past medical history, usage of medicines and healthy foods, presence of food allergies, and lifestyle habits, including eating, alcohol consumption, and smoking habits, were collected during the SCR. Data on health conditions and adverse events were collected during other examinations.

Anthropometric parameters. The height, weight, systolic and diastolic blood pressure, and pulse rate of the participants were measured at the hospital. The body mass index (BMI) was calculated using height and weight.

Hematological, blood biochemical, and urinary parameters. The measured hematological parameters included white blood cell count, red blood cell count, platelet count, hemoglobin

Table 1 Nutritional content of the experimental foods

	Placebo	TJ	HLTJ
Energy, kcal	45.6	36.1	30.4
Protein, g	2.3	1.5	1.5
Fat, g	N. D.	N. D.	N. D.
Carbohydrate, g	9.3	7.8	6.8
Sugars, g	9.1	6.8	5.7
Dietary fiber, g	0.2	1.0	1.1
Lycopene, mg	0.7	15.0	26.7

Values represent the content per 190 g serving. TJ, tomato juice; HLTJ, high-lycopene tomato juice.

level, hematocrit level, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. The blood biochemical parameters measured included total protein, albumin, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltranspeptidase, lactate dehydrogenase, total bilirubin, alkaline phosphatase, creatinine, urea nitrogen, uric acid, sodium, chloride, potassium, calcium, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein-cholesterol, triglyceride, glucose, and hemoglobin A1c levels. The urinary parameters measured included sodium, potassium, protein, sugar, urobilinogen, and bilirubin levels; pH; specific gravity; urinary ketone bodies; and occult blood reactions. Virus tests included serum hepatitis B surface antigen, hepatitis C virus antibody III, human immunodeficiency virus antigen/antibody, and qualitative syphilis (rapid plasma reagin test and *Treponema pallidum* antibody test) tests. All the measurements were outsourced to LSI Medience Co., Ltd.

Daily diary and diet records. The participants filled out the following items in a daily diary during the intervention: (1) intake of experimental food; 2, changes in physical conditions; 3, changes in living conditions; 4, menstruation; 5, smoking (including number of cigarettes); 6, exercise and its details, and (7) use of drugs. All meals consumed (including alcohol) were recorded in the dietary records during the intervention period; however, the nutritional intake was not calculated.

Salt intake status. The salt intake status of the participants was investigated using a salt check sheet,[19,20] and the results were used for participant allocation.

Endpoints

Primary endpoint. The primary endpoint was FMD at 12 weeks.

Secondary endpoints. The secondary endpoints were FMD at 4 and 8 weeks; urinary NO, 8-OHdG, and 8-isoprostane levels; and serum MDA and lycopene levels at 4, 8, and 12 weeks.

Safety analysis

Safety analysis was based on the incidence of side effects and adverse events during the intervention period and the results of hematological, blood biochemical, and urinary examinations.

Statistical analysis

Data were expressed as mean value ± standard deviation, and EZR (Ver. 1.61)[21] was used for statistical analysis. We used a linear mixed-effects model (LMM) to examine the effects of time and intervention on the outcome variable, while adjusting for baseline measurements. The model included the fixed effects of time (Pre, 4, 8, and 12 weeks), intervention group (Placebo, TJ, HLTJ), and baseline outcome values. A random intercept was included to account for individual variability across subjects. If a significant effect was detected by LMM, estimated marginal means were calculated and a *post hoc* Dunnett's test for intergroup comparisons between the placebo and TJ groups was performed. Bonferroni correction was applied to adjust the *p*-value for each comparison because of 3 comparisons by Dunnett's test. Specifically, the original *p*-value was tripled and adjusted *p*-values are reported for each test. All tests were performed at a 5% significance level.

Results

Flow diagram and participant characteristics

Fig. 2 shows a flow diagram of the study participants. A total of 400 potential participants were screened for eligibility, and 65 were excluded because they did not meet the inclusion criteria. Furthermore, the top 150 patients were selected from among those with FMD ranging from 3% to 7%, and a pre-intervention examination was conducted. Five individuals who declined to participate in the study were excluded, and 75 participants with FMD ≥4% were selected in the ascending order of FMD. They were randomly allocated to the placebo (n = 25), TJ (n = 25), and HLTJ (n = 25) groups. Two participants (one in the placebo group and one in the HLTJ group) were excluded for personal reasons (injuries unrelated to the study), and 73 participants completed the study. One participant did not comply with the restrictions (intake of healthy foods that can affect vascular endothelial function), and 72 participants (placebo group, n = 23; TJ group, n = 25; HLTJ group, n = 24) were included in the analysis. The rate of adherence to the experimental food was 100% in all groups. The participant characteristics during the SCR examination were not significantly different between the groups (Table 2).

Primary and secondary endpoints

The mean value in FMD is shown in Fig. 3. The LMM revealed significant interaction between time and intervention (p for interaction <0.001). The *post hoc* Dunnett's test revealed that FMD at 12 weeks in the TJ (6.1 ± 0.5%, $p < 0.001$) and HLTJ groups (7.0 ± 0.7%, $p < 0.001$) was significantly higher than that in the placebo group (5.4 ± 0.6%). In the HLTJ group, FMD at 4 weeks (6.2 ± 1.0%, $p < 0.001$) and 8 weeks (6.7 ± 0.7%, $p < 0.001$) was significantly higher than that in the placebo group (5.4 ± 0.9%, 5.3 ± 0.6%, respectively). Detailed results of FMD are shown in Table S1. The results of secondary endpoints are shown in Table 3. No significant differences between the groups were observed in blood and urinary oxidative stress markers or urinary nitric oxide levels. A significant interaction between time and intervention was observed for serum lycopene concentrations in the LMM (p for interaction <0.001). The *post hoc* Dunnett's test revealed that serum lycopene concentrations were significantly higher in the TJ and HLTJ groups than those in the placebo group ($p < 0.001$ at 4, 8, and 12 weeks).

Safety analysis

The hematological, biochemical, and urinary parameters are shown in the SI (Table S2). Two markers (white blood cell count and lactate dehydrogenase) were significantly different in the placebo and TJ groups; however, no significant differ-

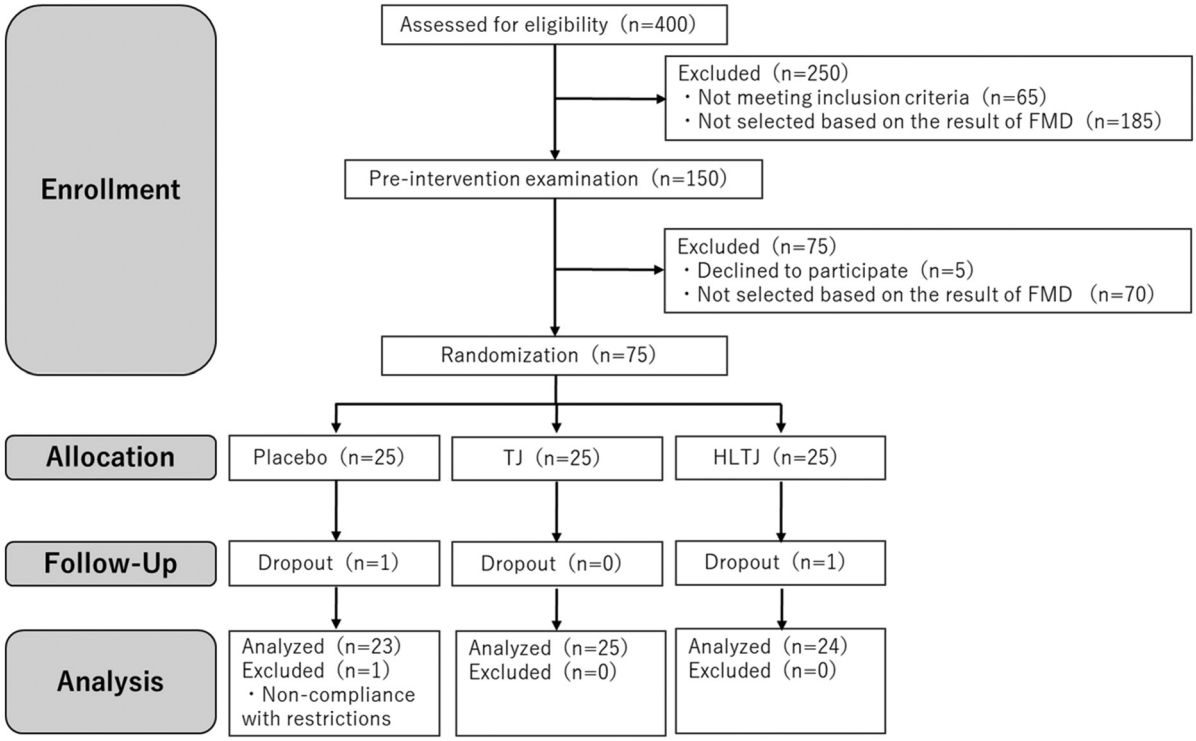

Fig. 2 Flow diagram of the study participants. TJ, tomato juice; HLTJ, high-lycopene tomato juice; FMD, flow-mediated dilatation.

Table 2 Participant characteristics

	Placebo	TJ		HLTJ	
n	23	25		24	
M/F	9/14	9/16		8/16	
Age, years	54.1 ± 6.6	54.6 ± 5.3	$p = 0.921$	54.7 ± 5.2	$p = 0.915$
Height, cm	161.8 ± 7.9	164.7 ± 8.9	$p = 0.363$	160.6 ± 7.8	$p = 0.827$
Weight, kg	59.0 ± 10.5	65.4 ± 13.8	$p = 0.135$	60.9 ± 12.4	$p = 0.811$
BMI, kg m^{-2}	22.4 ± 2.7	23.9 ± 3.6	$p = 0.176$	23.4 ± 3.2	$p = 0.436$
SBP, mmHg	123.7 ± 10.0	123.9 ± 10.7	$p = 0.996$	124.1 ± 10.2	$p = 0.986$
DBP, mmHg	77.2 ± 8.4	76.7 ± 9.0	$p = 0.970$	75.1 ± 10.0	$p = 0.662$
FMD, %	4.8 ± 0.7	5.0 ± 1.0	$p = 0.699$	4.9 ± 0.9	$p = 0.880$

Data are measured at the timing of SCR and expressed as numbers or mean ± standard deviation. The *p*-value is a comparison with placebo (Dunnett's test). TJ, tomato juice; HLTJ, high-lycopene tomato juice; M, male; F, female; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FMD, flow-mediated dilatation.

ences were observed between the placebo and HLTJ groups. There were five adverse events (elevation of blood TG, occult blood reaction, fracture of the right elbow, left knee meniscus posterior horn injury, and fatigue) during the intervention period; however, none of the symptoms were related to the intake of the experimental foods (Table S3). All serum samples returned negative results for virus testing.

Discussion

This randomized, placebo-controlled, double-blind, parallel-group comparison study examined the effects of long-term lycopene intake on FMD in healthy adults with borderline FMD values. The results showed that FMD significantly increased after consuming tomato juice containing 15.0 mg of lycopene for 12 weeks or consuming tomato juice containing 26.7 mg of lycopene for ≥4 weeks. These results showed that long-term lycopene intake improved the FMD even at lower doses than those previously reported.[11]

The effect of continuous lycopene intake (for more than 1 week) on FMD is influenced by the amount and duration of lycopene intake.[11,12,14] Xaplanteris *et al.* reported that consuming 70 g of tomato paste (33 mg of lycopene) for 15 days significantly increased FMD compared to that in the control group.[11] Furthermore, Samaras *et al.* reported a significant

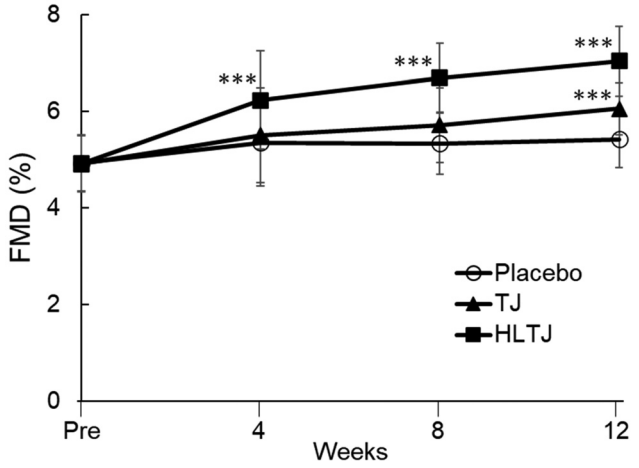

Fig. 3 Mean values of FMD at each time point. Pre, pre-intervention examination; TJ, tomato juice; HLTJ, high-lycopene tomato juice; FMD, flow-mediated dilation. Data are presented as mean ± standard deviation. ***$p < 0.001$ *vs.* placebo (adjusted p-value by Dunnett's test and Bonferroni correction).

increase in FMD after drinking tomato juice (the amount of lycopene was not specified) for 2 months compared to that before the intervention.[12] In contrast, Stangl *et al.* reported that consuming 70 g of tomato puree containing 46.2 mg of lycopene for 1 week did not significantly increase FMD.[14] In our study, consuming 26.7 mg of lycopene for >4 weeks or 15.0 mg of lycopene for 12 weeks significantly increased the FMD. These results suggest that the effect of lycopene on improving vascular endothelial function is more likely to be exerted by consuming lycopene for >4 weeks. Although lycopene is gradually depleted from the blood over time,[17,22,23] blood lycopene concentrations have been reported to increase significantly with continuous ingestion of tomato juice for 3 to 6 weeks.[24–26] In this study, blood lycopene concentrations increased significantly after 4 weeks of tomato juice ingestion. Continuous intake of tomato juice for more than 4 weeks likely causes accumulation of lycopene in the body, which may explain the significant increase in FMD observed after 4 weeks of intake. The guidelines issued by the EFSA state that "an increase in fasting FMD caused by the continuous consumption of food ingredients for >4 weeks has a beneficial physiological effect on vascular endothelial function".[16] Increasing FMD by lycopene intake for >4 weeks in our study strongly supports the beneficial effect of lycopene on vascular endothelial function.

The FMD significantly increased in the placebo group after 8 and 12 weeks compared to the values before intake ($p < 0.001$, preliminary intragroup comparison by Dunnett's test). The Framingham Heart Study, a long-term ongoing cardiovascular cohort study, showed that FMD changes seasonally, increasing from winter to summer but not from summer to autumn.[27] Given that the intervention period of this study was from summer to autumn, the increase in FMD in the placebo group was not considered to result from seasonal changes. Although the placebo food contained a very small amount (0.7 mg) of lycopene, the effect of lycopene was considered negligible because there was no significant increase in serum lycopene concentration in the placebo group. Water-soluble tomato components remained in the placebo food even though lycopene was almost completely removed by membrane permeation. Water-soluble tomato extract has anti-platelet aggregation effects[28] and anti-inflammatory effects in human umbilical vein endothelial cells.[29] The potential active ingredients in the water-soluble tomato components include adenosine, rutin, and chlorogenic acid.[28,29] Based on these reports, the placebo food might have contained these water-soluble ingredients, which could have influenced the FMD. Previous reports[11,12,14,15] evaluating the effects of tomato products on FMD compared the effects of tomato products on the test group with those not undergoing intervention; no food

Table 3 Mean values of secondary endpoints measured at each time point

Outcomes	Group	Pre	4 weeks	8 weeks	12 weeks	P for interaction
Urinary NO_x (μmol mg^{-1} creatinine)	Placebo	1.1 ± 0.6	1.3 ± 0.9	1.1 ± 0.5	1.2 ± 1.0	0.918
	TJ	1.2 ± 0.8	1.3 ± 0.6	1.5 ± 1.3	1.6 ± 2.2	
	HLTJ	1.2 ± 0.7	1.2 ± 0.7	1.3 ± 0.9	1.5 ± 0.9	
Urinary 8-OHdG (ng mg^{-1} creatinine)	Placebo	12.5 ± 17.6	9.7 ± 2.7	9.6 ± 3.8	10.2 ± 2.9	0.706
	TJ	10.3 ± 4.5	9.8 ± 3.5	9.8 ± 3.4	10.5 ± 3.2	
	HLTJ	9.3 ± 3.1	10.1 ± 3.5	9.1 ± 2.5	10.0 ± 2.9	
Urinary 8-isoprostane (ng mg^{-1} creatinine)	Placebo	4.5 ± 1.7	3.9 ± 1.3	3.8 ± 1.4	3.5 ± 1.0	0.998
	TJ	4.3 ± 1.3	3.8 ± 1.5	3.8 ± 1.2	3.6 ± 1.0	
	HLTJ	4.1 ± 0.9	3.7 ± 1.4	3.6 ± 1.1	3.3 ± 1.3	
Serum MDA (nM)	Placebo	608.7 ± 159.0	657.0 ± 190.9	655.7 ± 181.4	598.7 ± 182.8	0.483
	TJ	616.0 ± 197.0	730.4 ± 232.6	702.8 ± 230.1	694.8 ± 227.0	
	HLTJ	617.5 ± 208.7	665.0 ± 155.6	630.8 ± 122.1	601.3 ± 152.5	
Serum lycopene (nM)	Placebo	447.0 ± 237.1	422.2 ± 203.5	376.1 ± 153.5	419.2 ± 148.2	<0.001
	TJ	495.7 ± 273.5	759.6 ± 295.4***	781.2 ± 305.8***	842.0 ± 371.4***	
	HLTJ	508.9 ± 235.7	852.8 ± 348.5***	842.1 ± 288.7***	943.7 ± 293.8***	

Data are expressed as mean ± standard deviation. ***$p < 0.001$ *vs.* placebo (adjusted p-value by Dunnett's test and Bonferroni correction). Pre, pre-intervention examination; TJ, tomato juice; HLTJ, high-lycopene tomato juice; FMD, flow-mediated dilation; NO_x, nitrogen oxides; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; MDA, malondialdehyde.

was set as an appropriate placebo. Our study evaluated the effect of lycopene on FMD more accurately than previous reports using a placebo food containing the water-soluble components of tomatoes.

The placebo juice in this study contained 0.8 g and 0.9 g less dietary fiber than TJ and HLTJ, respectively, because it was produced by removing lycopene from tomato juice through centrifugation and membrane permeation. A human interventional study reported that a meal containing 20.3 g of dietary fiber does not significantly increase postprandial FMD,[30] suggesting that the amount of dietary fiber in TJ and HLTJ likely has little effect on FMD. In addition, centrifugation and membrane permeation may have removed other carotenoids (β-carotene, phytoene, and phytofluene). Although these components were not quantified in this study, previous reports indicated that 190 g of tomato juice contained approximately 0.3 mg of β-carotene, 3.8 mg of phytoene, and 1.7 mg of phytofluene.[25,26,31] A human interventional study showed that ingestion of palm oil containing 21 mg of carotenes for 8 weeks did not increase FMD.[32] Moreover, no human intervention studies have evaluated the effects of phytoene and phytofluene on vascular endothelial function. Although the contributions of β-carotene, phytoene, and phytofluene to the increase in FMD by tomato juice intake in this study cannot be completely ruled out, the effects are considered to be minimal.

NO is a substance secreted from the vascular endothelium that has beneficial effects on the vascular endothelium[33] and plays an important role in improving FMD.[3] An *in vitro* study showed that lycopene suppressed endothelial cell migration by suppressing vascular endothelial growth factor expression and increasing NO production.[10] Furthermore, an animal study using a hyperhomocysteinemia rat model showed that administration of 20 mg kg^{-1} pure lycopene dissolved in corn oil for 12 weeks increased serum NO levels, ameliorated endothelial dysfunction, and prevented early arteriosclerosis induced by hyperhomocysteinemia.[34] A human interventional study evaluating the effects of tomato paste on postprandial FMD showed a significant increase in urinary NO levels by tomato paste intake, but no significant change in FMD was observed.[15] Xaplanteris *et al.* proposed that antioxidants such as lycopene in tomato paste may change the redox status of vascular endothelium and increase the production of vasodilators such as NO; however, NO was not measured in the study.[11] Our study found no significant difference in the urinary NO level between the groups, even though the mean NO concentration at 12 weeks was higher in the TJ and HLTJ groups than in the placebo group. Measuring the serum NO concentration may be necessary to examine the relationship between lycopene-induced FMD improvement and NO production because a previous animal study showed an increase in serum NO concentration and amelioration of endothelial dysfunction.[34]

The increased production of reactive oxygen species is thought to reduce NO bioavailability and worsen vascular endothelial function.[35] A human intervention study showed that the intake of tomato paste containing lycopene increased the FMD and improved the oxidative status measured by the total amount of plasma lipid peroxides.[11] Another human intervention study showed that daily intake of 15 mg of lycopene supplement increased the activity of superoxide dismutase, an antioxidant enzyme, and improved reactive hyperemia peripheral arterial tonometry, an indicator of vascular endothelial function. Additionally, a correlation was observed between the changes in these two measures.[36] Urinary 8-isoprostane and serum MDA levels, which are indicators of lipid oxidation, and the urinary 8-OHdG level, an indicator of DNA oxidation, were measured as indicators of oxidation status in our study, but no significant changes were observed by lycopene intake. Therefore, the antioxidant activity of lycopene might have contributed little to the increase in FMD observed in this study. In addition to its direct effects on oxidative stress, lycopene exerts antioxidant activity by modulating transcription factors such as NF-kB and Nrf2.[37] The relationship between lycopene's effects on these transcription factors and its impact on vascular endothelial function remains to be investigated and is a topic for future study.

Oxidized LDL is thought to cause impairment of vascular endothelial function by decreasing the activity of NO synthase and increasing vascular endothelial adhesion factors.[38] The plasma oxidized LDL concentration is positively correlated with the plasma LDL-C concentration.[39] Additionally, blood LDL-C concentration is negatively correlated with FMD.[40] A human interventional study showed that a 3-week intake of a high-tomato diet (400 mL TJ and 30 mg tomato ketchup daily) significantly reduced LDL-C levels.[41] Another human intervention study found that intake of 50 g semi-dried tomato (providing 22.0–27.8 mg lycopene per day) for 12 weeks significantly reduced LDL-C levels.[42] In addition, a meta-analysis of six human interventional studies showed that tomato supplementation was associated with significant reductions in LDL-C.[13] Reduction in blood LDL-C concentration might be involved in the effect of lycopene on improving vascular endothelial function based on these reports. The serum LDL-C concentration was measured as a blood biochemical marker for safety analysis in our study. However, the relationship between changes in FMD and blood LDL-C concentration was unclear.

A safety analysis was conducted based on hematology, blood biochemistry, and urinary examination results and adverse event data collected from the daily diaries; however, no information concerning the safety of lycopene was obtained. Lycopene is thought to be an extremely safe component because synthetic lycopene and lycopene extract from tomatoes have received GRAS certification,[43,44] and there have been few reports of serious adverse events caused by lycopene intake.

This study had some limitations. First, markers of oxidative stress and NO were measured to elucidate the mechanism of action of lycopene; however, the mechanism was not clear in our study alone. It would be necessary to measure other markers not assessed in our study, such as blood NO_x, LDL-C, and oxidized LDL concentrations, to elucidate the underlying mechanism. Second, the effects of components other than lycopene could not be completely excluded, although our

study evaluated the effect of lycopene more accurately than previous reports by using a placebo food containing water-soluble components of tomatoes. This is a common limitation of studies evaluating the physiological functions of food ingredients; however, an interventional study using lycopene supplements containing high concentrations of lycopene is important.

Conclusions

This randomized, placebo-controlled, double-blind, parallel-group comparison study showed that lycopene intake from tomato juice increased FMD, a representative indicator of vascular endothelial function. These findings support the beneficial effects of lycopene on vascular endothelial function. In the future, it will be important to evaluate markers such as blood NO_x, LDL-C, and oxidized LDL concentrations to elucidate the underlying mechanisms.

Author contributions

K. Y.: conceptualization, data curation, formal analysis, methodology, validation, and writing – original draft; Y. N.: conceptualization, formal analysis, methodology, investigation, validation, and writing – review and editing; S. T.: conceptualization, methodology, project administration, and writing – review and editing; S. S.: supervision and writing – review and editing.

Conflicts of interest

KAGOME Co., Ltd supported this work and provided support in the form of salaries for authors K. Y., Y. N., S. T., and S.S.

Data availability

The data supporting this article have been included as part of the SI. Supplementary information is available. Detailed results of FMD are shown in Table S1. The results of hematological, biochemical, and urinary parameters are shown in Table S2. Detailed information of the adverse events during the intervention period is shown in Table S3. See DOI: **https://doi.org/10.1039/d5fo01397f**.

Other datasets are not readily available because the de-identified data described in the article, code book, and analytic codes will be made available upon request for the approval of an application for data use and execution of a Data Use Agreement and/or Material Transfer Agreement with KAGOME Co., Ltd. Requests to access the datasets were made by the corresponding author.

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