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Effects of lycopene supplementation on oxidative stress and markers of endothelial function in healthy men☆

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ABSTRACT

Objective: The objective was to determine the effects of lycopene supplementation on endothelial function assessed by reactive hyperemia peripheral arterial tonometry (RH-PAT) and oxidative stress.
Methods: Healthy men ($n=126$) were randomized to receive placebo ($n=38$), 6 mg ($n=41$), or 15 mg ($n=37$) lycopene daily for 8-week.
Results: Serum lycopene increased in a dose-dependent manner after 8-week supplementation ($P<0.001$). The 15 mg/day group had greater increase in plasma SOD activity ($P=0.014$) and reduction in lymphocyte DNA comet tail length ($P=0.042$) than the placebo group. Intragroup comparison revealed a 23% increase in RH-PAT index from baseline (1.45 ± 0.09 vs. 1.79 ± 0.12; $P=0.032$) in the 15 mg/day group after 8-week. hs-CRP, systolic blood pressure, sICAM-1 and sVCAM-1 significantly decreased, and β-carotene and LDL-particle size significantly increased only in the 15 mg/day group. Interestingly, the beneficial effect of lycopene supplementation on endothelial function (i.e., RH-PAT and sVCAM-1) were remarkable in subjects with relatively impaired endothelial cell function at initial level. Changes in RH-PAT index correlated with SOD activity ($r=0.234$, $P=0.017$) especially in the 15 mg lycopene/day group ($r=0.485$, $P=0.003$), lymphocyte DNA comet tail moment ($r=-0.318$, $P=0.001$), and hs-CRP ($r=-0.238$, $P=0.011$). In addition, changes in lycopene correlated with hs-CRP ($r=-0.230$, $P=0.016$) and SOD activity ($r=0.205$, $P=0.037$).
Conclusion: An increase in serum lycopene after supplementation can reduce oxidative stress which may play a role in endothelial function.

1. Introduction

Lycopene is the most powerful antioxidant among the major carotenoids and has received attention for its potential role in preventing cardiovascular disease in humans [1]. Low serum and adipose tissue lycopene concentrations are associated with excess incidence of acute coronary events and stroke [2]. Further, plasma lycopene is inversely associated with early atherosclerosis, as measured by intima-media thickness [3], and an independent inverse relationship between circulating lycopene and arterial stiffness was reported in healthy subjects [4,5]. Recently, lycopene was reported to attenuate atherogenesis in rabbits fed on a high-fat diet and this effect was comparable to fluvastatin [6].

Oxidative stress can inactivate nitric oxide, impairing endothelium-dependent vasodilation [7]. However, the studies of the relationship between lycopene and early atherosclerosis are inconsistent [8]. Dietary intervention studies involving either lycopene-containing foods or lycopene supplementation have shown potential short-term improvement in LDL oxidation [9], oxidative damage of lymphocyte DNA [10], and blood pressure [11]. However, studies that measure effects on a robust marker of cardiovascular health, such as endothelial function using precise amounts of lycopene supplements, are sparse.

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Endothelial function is considered one of the best indicators of vascular health, and its dysfunction is viewed as the common pathway between coronary risk factors and the development of atherosclerosis [12]. Measurement of flow-mediated dilatation of the brachial artery has been the standard method of assessing endothelial function, but in recent years, reactive hyperemia peripheral arterial tonometry (RH-PAT) [6,13], an equally reliable, less operator-dependent, non-invasive test for endothelial function has been developed for use in clinical research.

Our hypothesis was that an increase in serum lycopene levels following supplementation with capsules containing natural lycopene rich tomato extract would reduce oxidative stress and improve endothelial function in healthy men with frequently smoke cigarettes or consume alcohol and low intake of fruits and vegetables. A randomized double-blind, placebo-controlled study was designed to assess the changes in serum lycopene levels, oxidative stress and endothelial function in response to an 8-week treatment with lycopene supplementation.

2. Materials and methods

2.1. Study subjects

This was a randomized double-blind, placebo-controlled intervention trial conducted by the Laboratory of Clinical Nutrigenetics/Nutrigenomics (project#: 2010-0015017 and M10642120002-06N4212-00210) at Yonsei University. The study design was approved by the Institutional Review Board of Yonsei University. One hundred twenty-six healthy men aged 22–57 years, who frequently smoked cigarettes or consumed alcohol, were enrolled in this study. Subjects were excluded if they consumed more than 3 servings/day of vegetables and fruit, or more than one serving/week of lycopene-rich foods; took antioxidant, vitamin, or mineral supplements; had any history of chronic disease; or were taking lipid-lowering or antihypertensive medications.

2.2. Lycopene sources and study design

The subjects were randomly assigned to receive placebo, 6-mg, or 15-mg lycopene capsule per day for 8-week. The lycopene source was Lyc-O-Mato® 6% (natural tomato lycopene complex in viscous gel type, LycoRed Natural Products Industries Ltd., Israel) consisting of 6.3% lycopene, <0.2% β-carotene, >1.5% tocopherols, >1.0% phytoene and phytofluene [10,11]. Study substances were suspended in soybean oil and given in acid-soluble gelatin capsules. A placebo containing soybean oil (100%) was supplied in identical-looking capsules. All the subjects were instructed to consume one capsule per day right after any meal of their choice and to store the study substance in a refrigerator. All participants were encouraged to maintain their usual lifestyle and dietary habits. Compliance was assessed at the end of the study using pill counting, food records, and measurement of plasma lycopene levels.

2.3. Anthropometric parameters, blood pressure, and blood collection

Body weight and height were measured in the morning in unclothed subjects without shoes to calculate Body Mass Index (kg/m^2). Blood pressure (BP) was measured in the left arm of seated patients with an automatic BP monitor (TM-2654, A&D, Tokyo, Japan) after a 20-min rest. Study subjects were interviewed for smoking and drinking behavior during their visit. After a 12-h fasting period, venous blood specimens were collected in EDTA-treated and plain tubes, centrifuged to produce plasma or serum, and stored at −70 °C until analysis.

2.4. Serum lipid profile, fasting glucose, and hs-CRP

Fasting total-cholesterol and triglycerides were measured using commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd., Tokyo, Japan). After precipitation of serum chylomicrons with dextran sulfate magnesium, the concentrations of LDL- and HDL-cholesterol in the supernatants were measured enzymatically. Fasting glucose levels were measured by a glucose oxidase method using a Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA, USA). Serum hs-CRP (C-reactive protein) concentrations were measured with an Express Plus™ auto-analyzer (Chiron Diagnostics Co., Walpole, MA, USA) using a commercially available, high-sensitivity CRP-Latex(II) X2 kit (Seiken Laboratories Ltd., Tokyo, Japan).

2.5. Serum lycopene and β-carotene

Ultraperformance liquid chromatography (UPLC; Waters, Milford, MA) was used to determine lycopene and β-carotene in serum. Data were collected and processed using Empower chromatographic software (Waters, Milford, MA, USA). Concentrations of lycopene and β-carotene in serum were reported as uncorrected or corrected concentrations, normalized to the sum of the serum total cholesterol and triglycerides.

2.6. Antioxidative enzyme activities

Plasma superoxide dismutase (SOD) activity was measured based on the generation of superoxide radicals produced by xanthine and hypoxanthine using a kit (Cayman Chemical, Ann Arbor, USA). Absorbance was read at 450 nm with a Wallac Victor^2 multilabel counter (Perkin Elmer Life Sciences, Turka, Finland).

2.7. Finger pulse wave amplitude

Reactive hyperemia peripheral arterial tonometry (RH-PAT) index was used for endothelial function. PAT is a noninvasive technology that captures beat-to-beat plethysmographic recording of the finger arterial pulse wave amplitude with pneumatic probes. The measurements were performed using a modification of the methods of Bonetti et al. [13]. RH-PAT index values from one finger probe placed on the index finger of the hand undergoing occluded testing were normalized to a second PAT probe placed on the non-occluded index finger. The measurement equipment used for this purpose (Endo-PAT 2000, Itamar Medical Ltd., Caesarea, Israel) comprises a finger probe to assess digital volume changes accompanying pulse waves.

2.8. Alkaline comet assay for DNA damage

DNA damage was analyzed as described by Devaraj et al. [10] and Green et al. [14]. For the comet assay, whole blood was mixed with phosphate buffered saline and poured gently over a peripheral blood lymphocyte separation solution (Histopaque-1077). After centrifugation at 1450 rpm for 4 min, peripheral blood lymphocytes were transferred to another tube, mixed with 0.7% low-melting agarose, and added to slides precoated with 0.5% agarose. The slides were immersed in freshly prepared cold lysing solution and then placed into an electrophoresis tank. Following electrophoresis, the slides were washed in neutralizing buffer and then treated with ethanol. Measurements were made using image analysis software (Komet 5.0, Kinetic Imaging, UK) and fluorescence microscopy (Leica, Germany) equipment with filters. Images from 50 cells were analyzed on each slide and the parameters recorded were tail DNA (%), tail length (μm) and tail moment (% of DNA in the tail × tail

Table 1
Baseline clinical characteristics and nutrient intake.

	Placebo ($n=38$)	Low dose ($n=41$)	High dose ($n=37$)	P
Age (year)	33.5 ± 1.13	34.8 ± 1.28	34.7 ± 1.23	0.696
BMI (kg/m^2)	24.9 ± 0.50	25.3 ± 0.60	23.9 ± 0.49	0.149
Cigarette smoker, *n* (%)	15(30.0)	21(42.0)	14(28.0)	0.422
Cigarette (cigarettes/day)	13.4 ± 1.86	13.2 ± 1.15	13.4 ± 1.55	0.996
Alcohol drinker, *n* (%)	33(30.3)	39(35.8)	37(33.9)	0.053
Alcohol intake (g/day)	19.0 ± 3.79	18.9 ± 3.28	19.5 ± 3.52	0.991
Fasting glucose (mg/dL)[a]	87.7 ± 1.07	87.2 ± 1.48	88.1 ± 1.71	0.889
Total-cholesterol (mg/dL)	183.9 ± 5.22	187.5 ± 5.43	191.5 ± 5.22	0.607
LDL-cholesterol (mg/dL)	112.0 ± 4.90	117.4 ± 4.41	120.4 ± 4.92	0.455
HDL-cholesterol (mg/dL)	41.5 ± 1.78	41.8 ± 1.78	46.3 ± 1.65	0.099
Triglyceride (mg/dL)[a]	142.9 ± 12.0	141.4 ± 10.4	132.4 ± 11.1	0.817
Diastolic BP (mmHg)	83.5 ± 1.62	81.0 ± 1.36	84.0 ± 1.50	0.311
Estimates of daily nutrient intakes				
Total energy intake (kcal)	2522.6 ± 72.7	2510.6 ± 57.9	2520.3 ± 61.9	0.990
Carbohydrate (% of energy)	59.2 ± 0.83	60.4 ± 0.90	59.3 ± 1.38	0.662
Protein (% of energy)	16.7 ± 0.29	16.4 ± 0.39	16.5 ± 0.69	0.857
Fat (% of energy)	24.1 ± 0.70	23.7 ± 0.84	24.3 ± 1.06	0.907
Total energy expenditure (kcal)	2511.7 ± 48.0	2497.1 ± 44.7	2514.4 ± 42.0	0.959

Mean ± S.E.
[a] Tested by logarithmic transformation. Tested by ANOVA.

length). All steps were performed under dimmed light and the electrophoresis tank was covered with black paper to avoid additional light-induced DNA damage.

2.9. Plasma sVCAM-1, sICAM-1 and LDL particle size

Plasma sVCAM (Soluble Vascular Cell Adhesion Molecule)-1 and sICAM (Soluble Inter-Cellular Adhesion Molecule)-1 were measured using Bio-Plex™ Reagent Kit with the Bio-Plex™ (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's instructions. Particle size distribution of LDL (d1.019–1.063 g/mL) isolated by sequential flotation ultracentrifugation was examined by a pore-gradient lipoprotein system (CBS Scientific, CA, USA) on commercially available non-denaturing polyacrylamide slab gels containing a linear gradient of 2–16% acrylamide (Alamo Gels Inc., San Antonio, TX, USA). Gels were scanned with a GS-800 Calibrated Imaging Densitometer (Bio-Rad, Graz, Austria).

2.10. Assessment of dietary intake and physical activity level

Dietary intake was assessed with a 24-h recall method and semi-quantitative food frequency questionnaire. Dietary energy values and nutrient content were calculated using the Computer Aided Nutritional analysis program (CAN-pro 2.0. Korean Nutrition Society, Seoul, Korea). Total energy expenditure was calculated from activity patterns including basal metabolic rate (BMR), physical activity over a 24-h period [15], and specific dynamic action of food. The BMR of each participant was calculated using the Harris–Benedict equation.

2.11. Statistical analysis

Statistical analyses were performed using SPSS version 12.0 for Windows (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA). For intra-group tests, we conducted paired *t*-tests. For inter-group comparison, one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) method was used to compare initial value or absolute (net) differences. Pearson's correlation coefficients were used to examine relationships between variables. We determined whether each variable was normally distributed before statistical testing, and logarithmic transformation was performed on skewed variables. A two-tailed value of $P<0.05$ was considered statistically significant.

3. Results

Among enrolled subjects ($n=126$), 10 dropped out for personal reasons and 116 subjects completed the study. Among the dropouts, 4 were in the placebo group, 1 was in the 6-mg lycopene supplement/day group, and 5 were in the 15-mg lycopene supplement/day group. Thus, compliance was high (92.1%) and no serious adverse reactions due to lycopene supplementation were noted. Participants were supplied with 30 capsules of placebo pills, low-dose or high-dose lycopene pills at 0-week and at 4-week respectively. They were also asked to return unconsumed capsules at the next visit (4-week and 8-week respectively). All the participants are reinforced to regularly consume the pills by a dietitian through every 2 week's phone call check during the intervention period. Compliance was verified by counting the remained capsules. If the capsules are consumed more than 80%, compliance was considered good. Among the 10 dropped-out, 4 in placebo group, 1 in low-dose group and 1 in high-dose group had personal problems, and 4 in high-dose group showed no good-compliance on capsule consumption.

3.1. Baseline clinical characteristics and nutrient intake

Table 1 shows the baseline clinical characteristics and nutrient intake of the study subjects. There were no significant differences among the groups. Lycopene supplementation had no significant effect on BMI, lipid profiles, diastolic blood pressure, and estimates of daily nutrient intake (data not shown). In addition, lycopene supplementation at any dose, did not affect any of the safety parameters in subjects, including liver and kidney function tests, and complete blood count (data not shown).

3.2. Serum lycopene and β-carotene concentrations

Lycopene supplementation resulted in a dose-dependent increase in serum lycopene levels in each supplemented group compared to baseline ($P<0.001$) but not in placebo at 8-week (Fig. 1). The increase in serum lycopene concentration was significantly greater for the 15-mg/day group (0.13 ± 0.01 μg/mL) than the 6-mg/day group (0.06 ± 0.01 μg/mL). In addition, circulating lycopene levels after 8-week were higher in subjects supplemented with 15-mg lycopene/day compared to the 6-mg/day or placebo groups ($P<0.001$). Lipid standardized lycopene levels showed similar changes (data not shown). Serum β-carotene

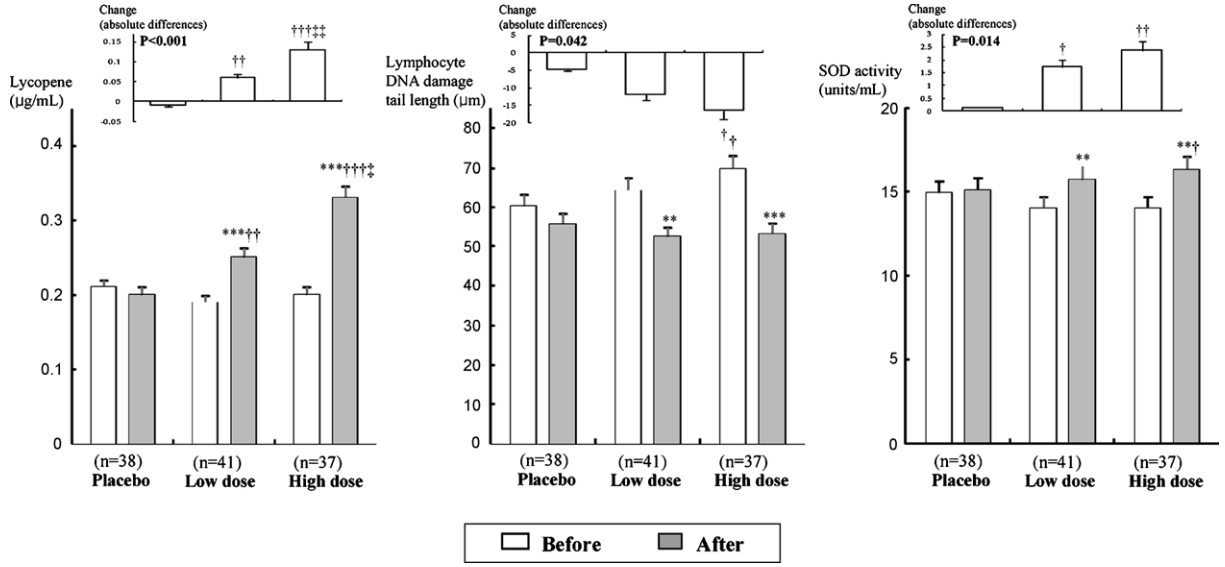

Fig. 1. Effects of 8-week lycopene supplementation on serum lycopene, lymphocyte DNA damage, and plasma SOD activity $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ compared to baseline within the group. $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$, and $^{\dagger\dagger\dagger}P<0.001$ compared to the placebo group. $^{\ddagger}P<0.01$, $^{\ddagger\ddagger}P<0.001$ compared to the low dose group. Each comparison was tested by ANOVA (LSD method). *P*-values are for comparing differences among the change values. Changes are absolute differences.

concentration increased from baseline after 8-week only in subjects supplemented with 15-mg lycopene/day ($P<0.010$; Table 2), and the changes (absolute differences) in this high dose group (0.08 ± 0.02 μg/mL) was significantly higher than that in low dose group (6-mg/day) or placebo group.

3.3. Oxidative stress biomarkers

The comet assay showed a significant decrease in comet tail lengths (Fig. 1), comet tail DNA and comet tail moment (Table 2), indicating decreased lymphocyte DNA damage at 8-week compared to baseline in each supplemented group but not placebo. The changes were significantly different for comet tail length ($P=0.042$; Fig. 1), with significantly greater reduction in comet tail length for the 15-mg/day group (-16.5 ± 3.43 μm) than for the placebo group (-4.61 ± 2.65 μm). Plasma SOD activity was significantly increased following 8-week of lycopene supplementation compared to baseline. The changes in plasma SOD activity were significantly different across groups ($P=0.014$; Fig. 1). Subjects supplemented with 15-mg lycopene/day had a mean SOD activity increase of 2.37 ± 0.63 units/mL compared to 1.73 ± 0.48 units/mL in the 6-mg/day group and a decrease of 0.15 ± 0.50 units/mL in the placebo group. Plasma LDL particle size ($P=0.039$) increased after 8-week compared to baseline only in subjects supplemented with the 15-mg lycopene/day (Table 2), but these changes were not significantly different across groups.

3.4. Endothelial function, systolic blood pressure, and hs-CRP

RH-PAT index increased after 8-week compared to baseline only in subjects supplemented with 15-mg lycopene/day (1.45 ± 0.09 vs. 1.79 ± 0.12; $P=0.032$; Table 2). Systolic blood pressure ($P=0.037$), plasma concentrations of sICAM-1 ($P=0.008$) and sVCAM-1 ($P=0.02$), and serum concentrations of hs-CRP ($P=0.046$) were significantly decreased following the 8-week 15-mg lycopene/day supplementation compared to baseline (Table 2).

Table 2
Effects of 8-week lycopene supplementation on systolic BP, β-carotene levels, and markers of oxidative stress, endothelial function, hs-CRP, and RH-PAT index.

	Placebo ($n=38$)		Low dose ($n=41$)		High dose ($n=37$)	
	Pretreatment	Post-treatment		Post-treatment	Pretreatment	Post-treatment
Systolic BP (mmHg)	125.0 ± 1.96	124.4 ± 1.81	123.5 ± 1.59	122.4 ± 1.69	126.0 ± 2.16	122.8 ± 1.78*
β-Carotene (μg/mL)[a]	0.38 ± 0.04	0.39 ± 0.03	0.37 ± 0.03†	0.36 ± 0.03	0.41 ± 0.03‡‡	0.49 ± 0.04**,‡
Lymphocyte DNA damage						
Tail DNA (%)	10.7 ± 0.33	9.87 ± 0.37	10.8 ± 0.55	9.39 ± 0.38*	11.2 ± 0.52	9.30 ± 0.36***
Tail moment	7.28 ± 0.34	6.30 ± 0.42	8.34 ± 0.81	5.73 ± 0.34**	9.28 ± 0.72†	5.91 ± 0.41***
LDL particle size (nm)	23.76 ± 0.10	23.79 ± 0.10	23.63 ± 0.10	23.74 ± 0.10	23.80 ± 0.10	23.93 ± 0.09*
sICAM-1 (pg/mL)	203.1 ± 7.90	189.7 ± 7.91	199.9 ± 7.85	183.0 ± 7.64*	203.7 ± 7.92	173.8 ± 8.40**
sVCAM-1 (pg/mL)	276.3 ± 9.76	267.4 ± 8.67	286.2 ± 10.5	254.7 ± 9.65**	276.4 ± 11.3	239.5 ± 10.3*,†
hs-CRP (mg/dL)[a]	1.14 ± 0.22	1.10 ± 0.27	1.39 ± 0.33	1.40 ± 0.37	1.25 ± 0.44	0.54 ± 0.10*
RH-PAT index	1.38 ± 0.07	1.48 ± 0.09	1.41 ± 0.07	1.52 ± 0.09	1.45 ± 0.09	1.79 ± 0.12*,†

Mean ± S.E.

[a] Tested by logarithmic transformation.

* $P<0.05$ compared with baseline values in each group tested by paired *t*-test.

** $P<0.01$ compared with baseline values in each group tested by paired *t*-test.

*** $P<0.001$ compared with baseline values in each group tested by paired *t*-test.

† $P<0.05$ compared with the value in the placebo group.

‡ $P<0.05$ compared with the value in the low dose group at the post-treatment tested by ANOVA (LSD method).

‡‡ $P<0.001$ compared with the value in the low dose group at the post-treatment tested by ANOVA (LSD method).

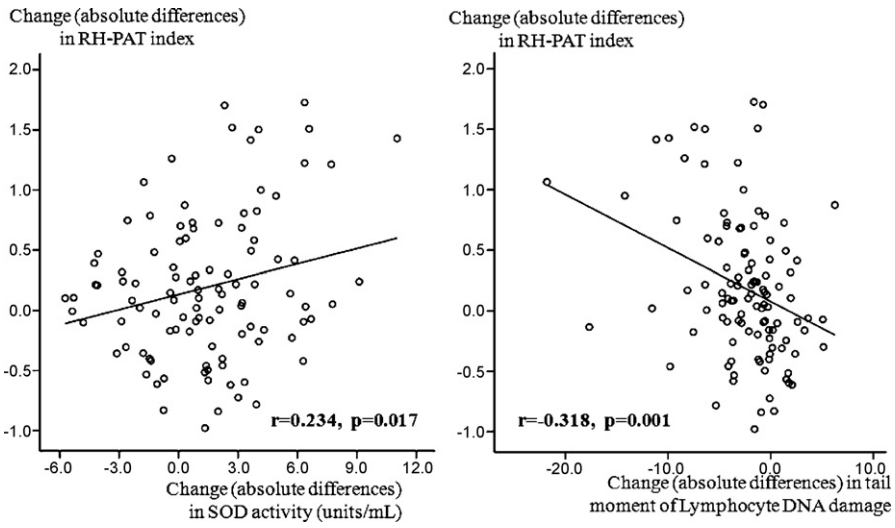

Fig. 2. Relationship between changes in antioxidative and oxidative stress markers, and RH-PAT index *r*: correlation coefficient, Pearson's correlation analysis.

No significant differences were found in the initial levels of RH-PAT index, systolic blood pressure, sICAM-1, sVCAM-1 and hs-CRP among the 3 groups.

3.5. Relationship between changes in lycopene levels, oxidative stress markers, and RH-PAT index

In all subjects, changes in lycopene levels correlated positively with changes in SOD activity ($r = 0.205$, $P = 0.037$) and changes in β-carotene levels ($r = 0.596$, $P < 0.001$), but negatively with changes in hs-CRP ($r = -0.230$, $P = 0.016$). Overall, changes in RH-PAT index correlated positively with changes in SOD activity ($r = 0.234$, $P = 0.017$; Fig. 2) and negatively with changes in percentage of lymphocyte DNA in the tail ($r = -0.223$, $P = 0.020$), comet tail length ($r = -0.269$, $P = 0.005$), tail moment ($r = -0.318$, $P = 0.001$; Fig. 2), and changes in hs-CRP levels ($r = -0.238$, $P = 0.011$). In particular, for subjects supplemented with 15-mg lycopene/day, changes in RH-PAT index showed strong positive correlation with changes in SOD activity ($r = 0.485$, $P = 0.003$). In all subjects, changes in hs-CRP also correlated negatively with changes in SOD activity ($r = -0.283$, $P = 0.003$) and positively with systolic blood pressure ($r = 0.194$, $P = 0.038$).

3.6. Effects of a 8-week lycopene supplementation on the markers of endothelial cell functions and RH-PAT index in subjects with impaired endothelial cell functions group

Study subjects were subdivided into two groups based on their initial levels of markers for endothelial cell function (sVCAM-1, sICAM-1 and RH-PAT): 'impaired endothelial cell functions' and 'normal function'. Subjects were categorized into 'impaired endothelial cell functions' if the initial level of at least one of these markers was upper tertile (sVCAM-1 or sICAM-1) or lower tertile (RH-PAT). The other cases were into 'normal function' group. Finally, 76 subjects were in 'impaired endothelial cell functions' group and the other 40 were in 'normal endothelial cell functions'. Fig. 3 shows the effects of a lycopene supplementation on sICAM-1, sVCAM-1, and RH-PAT index in subjects with impaired endothelial cell functions. There were no significant differences in initial levels among three groups (placebo, $n = 25$, low dose lycopene, $n = 28$, high-dose lycopene, $n = 23$). The absolute differences were significantly different for sVCAM-1, with remarkably greater reduction ($P = 0.048$) in the high-dose group (-71.0 ± 20.5 pg/mL) than in the placebo group (-29.2 ± 10.2 pg/mL). In addition, absolute differences in RH-PAT index was remarkably increased in high-dose group than the other groups. On the other hand, there is no significant inter-group differences in subjects with normal endothelial cell function (data not shown).

4. Discussion

In this randomized, double-blind, placebo-controlled study, lycopene supplementation had beneficial effects on biomarkers of oxidative stress, as evidenced by decreased oxidative DNA damage and increased plasma SOD activity. In addition, changes in RH-PAT index, a measure of peripheral microvascular endothelial function [13], correlated with the increase in SOD activity and negatively

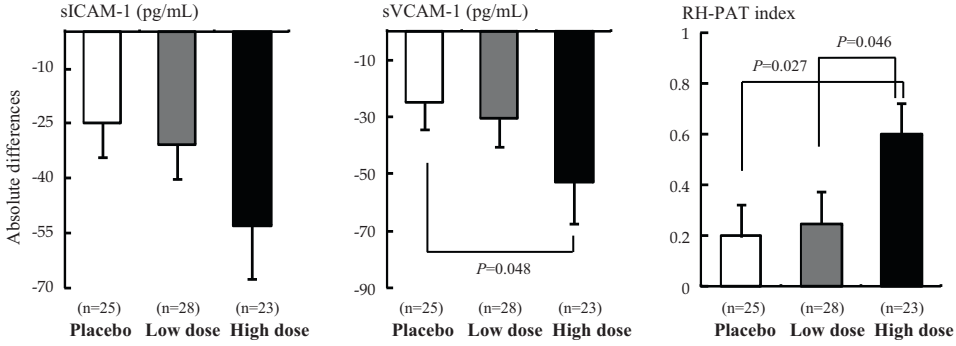

Fig. 3. Effects of a 8-week lycopene supplementation on the markers of endothelial cell functions and RH-PAT index in subjects with impaired endothelial cell functions group ($n = 76$) Each comparison was tested by one-way analysis of variance (ANOVA) with least significant difference (LSD) method. Impaired endothelial cell functions group: initial levels were lower tertile (sVCAM-1 or sICAM-1) or upper tertile (RH-PAT) group.

with changes in oxidative DNA damage and hs-CRP levels. This result suggests that the antioxidant effects of lycopene may play a role in the improvement in endothelial function, which is closely related to inflammatory markers. The reduction in oxidative DNA damage after lycopene supplementation is consistent with previous findings of up to 50% protection of lymphocyte DNA following supplementation with tomato puree, which provided about 7- or 16-mg lycopene per day [16]. Recently, Devaraj et al. [10] also found an exclusive role for lycopene as a cellular antioxidant in reducing lymphocyte DNA susceptibility to oxidative damage in healthy subjects consuming purified lycopene. In this study, lycopene supplementation significantly increased serum lycopene levels from baseline in each supplemented group compared to placebo. This result confirms compliance among the study subjects and agrees with previous suggestions of identical bioavailability of tomato-based lycopene [17].

SOD, an antioxidative enzyme that forms a major cellular defense against superoxide and formation of peroxynitrite, has been suggested to play a major role in preventing the pathophysiological effects of superoxide on the vasculature [18]. Plasma SOD reflects extracellular SOD activity and reduction of its activity is most likely a consequence of attenuated nitric oxide (NO) [19], since extracellular SOD expression is induced by endothelial NO [18]. In addition, controlling the amount of superoxide is reportedly critical for preserving NO bioactivity in the vessel wall [18]. Therefore, the favorable impact of lycopene supplementation on SOD activity and endothelial function seen in this study could be partly related to the preservation of NO bioactivity. Changes in lycopene, the most potent singlet oxygen quencher among the natural carotenoids [20], correlated positively with changes in SOD activity, which in turn, showed a strongly positive correlation with changes in RH-PAT index in subjects supplemented with 15-mg lycopene/day ($r = 0.485$, $P = 0.003$).

Endothelial functions, as measured by RH-PAT, sICAM-1 or sVCAM-1 were significantly improved by consuming lycopene, particularly 15-mg lycopene/day for 8-week. Furthermore, the effect was remarkably significant when subjects had relatively impaired endothelial cell function at initial level (Fig. 3). From these results, 'lycopene supplementation' particularly high-dose supplementation may play a beneficial role in endothelial function, which is more remarkable in subjects in sedentary life style with relatively impaired endothelial cell function. Similarly, systolic blood pressure and hs-CRP decreased only in subjects consuming 15-mg lycopene daily for 8-week. These results suggest that the beneficial effect of lycopene on endothelial function, blood pressure, and inflammatory markers might be related to the daily intake of lycopene. Engelhard et al. [11] also demonstrated that 4-weeks of daily oral supplementation with tomato extract (15-mg lycopene, 0.375 mg β-carotene) significantly decreased blood pressure and lipid peroxidation. The antioxidant activity of the carotenoids such as lycopene has been shown to quench singlet oxygen and decrease levels of reactive oxygen species, which inactivate nitric oxide, thus impairing endothelium-dependent vasodilation [1,20]. In fact, increased oxidative stress was found to be a contributing mechanism for endothelial dysfunction [21], and various studies demonstrated the ability of antioxidant carotenoids to improve vascular function and blood pressure [11].

A reduction in hs-CRP in the 15-mg lycopene/day group and the inverse correlation between changes in lycopene and changes in hs-CRP in this study, suggest that lycopene may play a role in inflammatory processes by interfering the action of cytokines. Further, changes in hs-CRP correlated negatively with RH-PAT index and positively with systolic blood pressure. This result is in agreement with previous reports of an inverse relationship between endothelial function and CRP in human subjects [20,22]. In fact, the presence of an independent inverse relationship between circulating lycopene and arterial stiffness was reported in healthy subjects [4,5]. Therefore, the relationships among lycopene, CRP, RH-PAT index, and blood pressure in this study could support the previous suggestion of a possible protective effect of carotenoids on atherosclerosis through an influence on inflammatory processes and endothelial function [22]. Jacob et al. [23] also found that consuming tomato juice (21-mg lycopene) daily for 2-weeks reduced the inflammatory marker CRP in healthy subjects. Of interest, an inverse relation between lycopene and CRP has been reported in men but not in women [22].

Subjects supplemented with 15-mg lycopene daily for 8-week also showed reduction in other cardiovascular risk factors, for example, an increase in LDL particle size. Since the lycopene capsule used in this study also contains β-carotene (>0.5 mg), the subjects in the 15-mg lycopene/day group had a 65% increase in lycopene concentration and a 20% increase in β-carotene, which is a known effective antioxidant that inactivates free radicals, inversely correlates with CRP, and slows the progression of atherosclerosis [23]. Therefore, a synergistic effect of lycopene and β-carotene in the 15-mg lycopene/day group likely increased the beneficial effects on these atherosclerosis risk factors. These beneficial effects occurred unaccompanied by changes in diet, enhanced physical activity, or changes in serum concentrations of total cholesterol, lipoproteins, triglycerides, or BMI because these variables were not significantly changed before and after supplementation.

Baseline serum lycopene levels in subjects of this study (0.2 μg/mL) were lower than those reported in typical Koreans (0.230 μg/mL) [4]. This is due to the exclusion of subjects who consumed >3 servings/day of vegetables and fruit or >1 serving/week of lycopene-rich foods. Thus, it is possible that the low baseline lycopene level in this study shows a better response to lycopene supplementation. In addition, because gender is an important factor in determining endothelial function, this study specifically focused on a representative group of middle-aged Korean men, so the results cannot be generalized to women or other ethnic, age, or geographical groups. Despite these limitations, in healthy men, 8-week of low (6-mg/day) or high (15-mg/day) lycopene intake increased serum lycopene and plasma SOD activity and decreased oxidative DNA damage in a dose-dependent manner. Changes in endothelial function as measured by RH-PAT correlated positively with SOD activity and negatively with changes in oxidative DNA damage and hs-CRP levels. Additionally, changes in lycopene negatively correlated with hs-CRP. These results add to the growing literature on potential protective effects of the antioxidant lycopene in atherosclerosis through an anti-inflammatory effect and preserving endothelial function.

Conflict of interest

There is no potential conflict of interest.

Acknowledgements

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