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Evaluation of the Effect of Dietary Lycopene, the Main Carotenoid in Tomato (*Lycopersicon esculentum*), on the *In Vivo* Renal Reducing Ability by a Radiofrequency Electron Paramagnetic Resonance Method

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ABSTRACT: Although it has been reported that dietary lycopene, the main carotenoid in tomato, improved drug-induced nephropathy, there are no reports on the effect of orally administered lycopene on the *in vivo* renal reducing (i.e., antioxidant) ability. The radiofrequency electron paramagnetic resonance (EPR) method is a unique technique by which the *in vivo* reducing ability of an experimental animal can be studied. In this study, the *in vivo* changes in the renal reducing ability of rats orally administered lycopene were investigated using a 700 MHz EPR spectrometer equipped with a surface-coil-type resonator. Rats were fed either a control diet or a diet containing lycopene. After 2 weeks, *in vivo* EPR measurements were conducted. The renal reducing ability of lycopene-treated rats was significantly greater than that of the control. This is the first verification of *in vivo* antioxidant enhancement via dietary lycopene administration.

KEYWORDS: Tomato, carotenoids, lycopene, antioxidant, renal reducing ability, electron paramagnetic resonance

INTRODUCTION

The kidney is the organ that excretes and reabsorbs various substances in the blood. If relatively greater amounts of oxidative substances pass through the kidney, the increased oxidative stress, such as reactive oxygen species (ROS) production, will cause nephropathy. Thus, prevention of renal oxidative stress by antioxidants is effective in suppressing nephrotoxicity.[1,2]

Lycopene is naturally contained in tomato and tomato-based products and has attracted considerable attention as an antioxidant.[3] Lycopene is most likely involved in the scavenging of two ROS, singlet molecular oxygen[4] and peroxynitrite.[5]

On the basis of these antioxidant activities, lycopene may contribute to the prevention of oxidative damage in various organs.[6]

It was reported that dietary lycopene improved drug-induced nephropathy.[7−9] In these reports, the antioxidant ability of orally ingested lycopene was indirectly suggested on the basis of the suppression of oxidative markers, such as lipid peroxidation. To our knowledge, however, there have been no reports where the *in vivo* antioxidant ability of orally administered lycopene was directly proven.

The radiofrequency electron paramagnetic resonance (RF-EPR) method is a unique technique by which the *in vivo* reducing (i.e., antioxidant) ability of an experimental animal can be studied.[10−14] In this method, electron spins in free radicals can be directly detected, in which a RF less than 1 GHz is used as an EPR frequency because of the frequency characteristics of the dielectric loss of water in an experimental animal and wavelength allowed with the sample size (diameter). A surface-coil-type resonator (SCR) is a device used in the RF-EPR method.[15] An SCR comprises a single-turn coil and a transmission line, equivalent to an LC resonance circuit, which provide the inductance and the capacitance, respectively. It can be positioned in several possible sites of an experimental animal, and EPR can be observed at the organ surface near the single-turn coil without the influences of other organs.[10,14] The *in vivo* renal half-life of the nitroxide radical administered to experimental animals can be obtained by EPR measurements of the kidney using an SCR. Because endogenous reducing agents in a biological system reduce nitroxide radicals to EPR-silent compounds, the decay rate (i.e., half-life) of this radical reflects the reducing ability at the EPR measurement area. The *in vivo* reducing ability is subject to a balance between the amounts of oxidants and reductants in target areas.[11−13]

Whereas the *in vivo* renal reducing ability in drug-induced nephropathy has been estimated by EPR measurements using an SCR in previous reports,[11−13] there have been no reports on the effect of orally ingested food constituents on the *in vivo* renal reducing ability. In this study, the *in vivo* changes of the renal reducing ability by orally ingested lycopene to rats were investigated by *in vivo* EPR measurements using an SCR.

In the kidney, antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), play

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important roles in protecting the kidney from oxidative stress. In previous reports, it was suggested that lycopene affected these antioxidant enzymes and improved drug-induced nephropathy.[7−9] In this study, the activities of these antioxidant enzymes in the kidney were investigated because the changes in these activities by the dietary lycopnene might influence the *in vivo* renal reducing ability.

It was reported that lycopene showed stronger antioxidant activity in coexistence with other antioxidants, such as α-tocopherol,[16,17] and that α-tocopherol was protected and recycled by other antioxidants, such as ascorbic acid.[18] To examine the protection of α-tocopherol by lycopene, the α-tocopherol concentration was also measured.

■ MATERIALS AND METHODS

***In Vitro* EPR Study.** To examine the direct chemical reaction between lycopene and nitroxide radical, *in vitro* EPR measurements were conducted as follows. Lycopene (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was dissolved in hexane at a final concentration of 200 μM in a brown measuring flask. The lycopene concentration was set at the maximal level to dissolve in hexane, where concentrations comparable to 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPOL) in the rat kidney could be achieved.[10,19] The lycopene solution was deoxygenated by bubbling with 100% argon gas for 60 min. The nitroxide radical, TEMPOL (Wako), was dissolved at a concentration of 200 μM in ethanol and deoxygenated as above. Deoxygenated lycopene solution and deoxygenated TEMPOL solution were mixed in equal amounts in dark vials that had been filled with argon gas in advance. After the mixing of lycopene and TEMPOL solutions for 2 and 30 min, a capillary tube containing the mixture was located at the center of the cavity resonator of a conventional X-band EPR spectrometer (TE-200, JEOL, Akishima, Japan) and EPR measurements were made. The EPR conditions were as follows: irradiation power, 8 mW at 9.44 GHz; center magnetic field, 336.5 mT; magnetic field sweep speed, 5 mT/s at a width of 10 mT; time constant, 1 ms; magnetic field modulation width, 0.1 mT at 100 kHz; and accumulation number, 10.

Preparation of the Lycopene-Containing Diet. Lycopene for the animal diet was extracted and purified from tomato oleoresin, Lyc-O-Mato 6% (lycopene content, 6%; Lycored, Yavne, Israel). Tomato oleoresin was dissolved in dichloromethane and methanol and was saponified with 60% potassium hydroxide for 60 min at 50 °C. After suction filtration, the residues were dissolved in dichloromethane and methanol and were recrystallized at 4 °C overnight. After a second suction filtration, the residues were dried in a vacuum overnight to obtain purified lycopene. Lycopene purity was determined to be 92% by measuring the absorption in benzene solution at 487 nm using an ultraviolet−visible spectrometer (UV-1800, Shimadzu, Kyoto, Japan). The composition of the other 8% was unknown. The high-performance liquid chromatography (HPLC) results indicate that the impurities do not contain carotenoids. The proportion of *cis* isomers in the whole lycopene was determined to be ca. 3% by HPLC. In the determination of lycopene purity, these *cis* isomers were regarded as purified lycopene.

Purified lycopene and CR-LPF powder diet (Oriental Yeast Co., Ltd., Itabashi, Japan) were mixed and solidified at a concentration of 250 mg/100 g to obtain lycopene-containing solid diets. The composition of the CR-LPF powder diet was as follows: moisture, 7.6%; crude protein, 16.8%; crude fat, 4.2%; crude ash, 6.1%; crude fiber, 4.5%; nitrogen-free extract, 60.9%.

The aim of this study is the *in vivo* verification of the reducing ability of lycopene; therefore, the lycopene dosage was set at a maximum level. No changes in renal lycopene concentrations between rats that received diets containing lycopene at 50 mg/100 g and 500 mg/100 g of diet were reported,[20] indicating that saturation of lycopene in the kidney occurs between these dietary concentrations. Thus, a lycopene concentration of 250 mg/100 g of solid diet, which is a clearly saturating level, was set as the dosage in this study.

Animals. A total of 16 male 5-week-old Wistar rats were obtained from Japan SLC, Inc. (Hamamatsu, Japan). During the maintenance period (1 week), a basal CR-LPF solid diet (Oriental Yeast) and water were given *ad libitum*. Fresh diets were provided to the rat every 2 days. Changes in the lycopene concentration in the diets 2 days after preparation were less than 10%. All rats were individually housed in plastic cages at a room temperature of 23 ± 1 °C and humidity of 50 ± 5% with a 12 h light/dark cycle (lights on from 7:00 to 19:00). After the maintenance period, rats were divided into two groups with similar average body weights: the control (n = 8) and lycopene (n = 8) groups. The former and latter animals were fed CR-LPF solid diet and lycopene-containing diet, respectively. The diets and water were given *ad libitum* for 2 weeks.

The experiment protocol was approved by the Committee on the Care and Use of Laboratory Animals of the International University of Health and Welfare (10103) and the Kagome Animal Use Committee (2009.003).

RF-EPR Spectrometer. A 700 MHz RF-EPR spectrometer constructed at Yamagata Promotional Organization for Industrial Technology (Yamagata, Japan) has already been described in detail.[14,19,21] It consisted of an EPR resonator, a main electromagnet, a pair of magnetic field sweep coils, a pair of magnetic field modulation coils, power supplies, a personal computer, 700 MHz RF circuits for homodyne detection, and intermediate frequency circuits for lock-in detection at a magnetic field modulation frequency of 100 kHz.

An SCR was used as an EPR resonator. The SCR inductor was fabricated from a circular single-turn one-loop coil, constructed from copper wire, 0.5 mm in diameter. The coil diameter was 10 mm. Two semi-rigid coaxial cables with a characteristic impedance of 50 Ω (diameter of 3.5 mm) were used for the SCR transmission lines. On the basis of the inductance of the inductor and a resonant frequency of 700 MHz, their lengths were calculated to be 270 mm.[15]

***In Vivo* EPR Study.** The rats in each group were anesthetized with intraperitoneal administration of 50 mg/kg of body weight of sodium pentobarbital. Under anesthesia, the right kidney of the rats was exposed by a dorsal incision and each rat was restrained in a static magnetic field. A single-turn coil of the SCR was placed on the kidney. The TEMPOL solution (0.2 M), dissolved in a physiological saline solution, was injected via the tail vein at a dose of 1.5 mL/kg of body weight. The EPR measurements were repeated every 5 s for 40 s after the injection of TEMPOL. The EPR conditions were as follows: irradiation power, 50 mW at ca. 720 MHz; center magnetic field, 25.6 mT; magnetic field sweep speed, 10 mT/s at the width of 10 mT; time constant, 1 ms; magnetic field modulation width, 0.2 mT at 100 kHz; and accumulation number, 4.

After the *in vivo* EPR study, the kidneys were removed, immediately frozen, and kept at −80 °C until required.

Estimation of the Renal Reducing Ability. When a single-exponential decay of the EPR signal intensity was observed in a target area, the half-life can be used as a parameter to estimate the decay rate of TEMPOL in that area. Therefore, the equation expressing the TEMPOL concentration in a target organ (X_n) as a function of time (t) approximates

$$X_n = X_{n0} \exp(-k_{0n} t) \quad (1)$$

where X_{n0} is the initial value of the concentration and k_{0n} is a first-order rate constant (=log 2/half-life). However, the organs are actually connected to each other via the blood circulatory system. Thus, the temporal change in X_n can be expressed by

$$dX_n/dt = k_{1n}X_v - k_{2n}X_n - k_{3n}X_n \quad (2)$$

where k_{1n}, k_{2n}, and k_{3n} are the rate constant of the inflow, outflow, and reduction of TEMPOL, respectively, in the target organ and X_v is the TEMPOL concentration in the blood. Using data from a previous EPR kinetics study of multiple organs in rats[11,14]

$$X_n = C_{1n} \exp(-k_{3n}t) + C_{2n} \exp(-k_v t) \quad (3)$$

can be obtained from eq 2, where $C_{2n} = k_{1n}X_{v0}/(k_{3n} - k_v)$, $C_{1n} = X_{n0} - C_{2n}$, k_v is the rate constant of the TEMPOL concentration change in the blood, and X_{v0} is the initial value of X_v. C_{1n} and C_{2n} reflect the degree of influence of the target and other areas to the EPR signal decay observed in the target area, respectively. The conditions under which the curve of eq 3 could fit the curve of eq 1 were simulated when the values obtained from the prior EPR measurements were substituted for k_{0n}, k_v, X_{n0}, and X_{v0} in these equations.[11,14] The results of simulation showed $k_{0n} \sim k_{3n}$ and $C_{1n} \gg C_{2n}$, indicating that X_n is not influenced by k_v, and k_{0n} reflects k_{3n}, indicating that the rate constant (i.e., half-life) of TEMPOL measured in the kidney (i.e., the target organ) reflects the reduction of TEMPOL in this area and is not influenced by the reduction in the other areas.

Renal Lycopene Concentration. Extraction of lycopene from the kidney was conducted on the basis of a previous report.[22] A part of the kidney (ca. 0.5 g) was homogenized in a mixture of 0.01% 2,6-di-*t*-butyl-4-methylphenol (Wako) ethanol solution and 5 ppm of diethylenetriaminepentaacetic acid (Wako) physiological saline solution in brown vials. The homogenate was saponified with 60% potassium hydroxide for 30 min at 50 °C. After the addition of β-carotene standard solution as an internal standard, the homogenate was vigorously stirred and extracted with hexane/dichloromethane (4:1, v/v) for 10 min twice. The supernatant fraction was dried by nitrogen gas and reconstituted in hexane/acetone/ethanol/toluene (10:7:6:7, v/v/v/v) solvent. The resultant was filtered with a 0.20 μm filter, and HPLC analyses were performed using a photodiode array detector (SPD-M10, Shimadzu) at a detecting wavelength of 472 nm and a C30 carotenoid column (250 × 4.6 mm, 5 μm; YMC, Wilmington, NC).

Mobile phase A (A) consisted of a 75:15:10 mixture of methanol/*tert*-butyl-methyl-ether/water. Mobile phase B (B) consisted of an 8:90:2 mixture of methanol/*tert*-butyl-methy-ether/water. Pumps were programmed to perform the following gradient at a flow rate of 1 mL/min: start at 100% A, a 25 min linear gradient to 100% B, 3 min at 100% B, a 2 min gradient back to 100% A, and 10 min at 100% A. Under these conditions, the concentration of lycopene was measured and corrected using an internal standard (β-carotene).

Renal Antioxidant Enzyme Activities. The activities of SOD and GSH-Px in the kidney were investigated. A part of the kidney (ca. 0.1 g) was homogenized in a buffer containing 0.25 M sucrose, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, and 1 mM ethylenediaminetetraacetic acid (pH 7.4). The final concentration of the sample was 10% (w/v). The homogenates were centrifuged at 10000*g* and 4 °C for 60 min to determine SOD activities, which was measured by the SOD Assay Kit-WST (Dojindo Laboratories, Kumamoto, Japan). In this method,[23] the reduction of WST-1 (water-soluble tetrazolium salts) by superoxide was determined at 560 nm.

A part of the kidney (ca. 0.1 g) was homogenized in phosphate buffer, and the final concentration of the sample was 10% (w/v). The homogenates were centrifuged at 15000*g* and 4 °C for 30 min to determine GSH-Px activities, which were measured using the NWLSS Glutathione Peroxidase Assay (Northwest Life Science Specialties, LLC, Vancouver, British Columbia, Canada). In this method,[24] the disappearance of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) by glutathione reductase, which was coupled to oxidation of the reduced form of glutathione by GSH-Px, was determined at 340 nm.

Renal α-Tocopherol Concentration. The α-tocopherol concentration in kidney was determined by the method by Ueda and Igarashi,[25] in which 2,2,5,7,8-pentamethyl-6-chromanol (PMC) was used as an internal standard.

A part of the kidney (ca. 0.1 g) was cut into small pieces and saponified with 0.1 mL of 1% sodium chloride solution, 1.8 mL of 6% ethanolic pyrogallol, 0.2 mL of PMC (Wako) ethanol solution, and 0.2 mL of 60% potassium hydroxide at 70 °C for 60 min. After cooling on ice, a mixture of the resultant and 4.5 mL of 1% sodium chloride solution was extracted with *n*-hexane/ethyl acetate (9:1, v/v) and centrifuged at 1500*g* for 5 min. The supernatant fraction was dried by argon gas and reconstituted in hexane. The resultant was filtered using a 0.20 μm filter, and HPLC analyses were performed using a fluorescence detector (RF-10A, Shimadzu) at detecting wavelengths of 297 nm (excitation) and 327 nm (emission) and a 5NH$_2$ column (150 × 4.6 mm, 5 μm, Nomura Chemical Co., Ltd., Seto, Japan).

For the mobile phase, *n*-hexane/2-propanol (97:3, v/v) was used at a flow rate of 1 mL/min. Under these conditions, the concentration of α-tocopherol was measured and corrected using an internal standard (PMC).

Statistical Analysis. The data were expressed as the mean ± standard error. Differences between group means were analyzed using an unpaired *t* test. Results were considered statistically significant at $p < 0.01$.

Table 1. Body Weight, Increase in Body Weight, Right and Left Kidney Weights, and Renal Lycopene Concentration of Rats in the Control and Lycopene Groups[a]

	control (n = 8)	lycopene (n = 8)
final body weight (g)	211.30 ± 2.32	205.88 ± 2.25
increase in body weight[b] (g)	77.09 ± 1.07	75.85 ± 1.13
right kidney weight (g)	0.77 ± 0.01	0.80 ± 0.03
left kidney weight (g)	0.78 ± 0.01	0.77 ± 0.02
renal lycopene concentration (ng/g of tissue)	0.00 ± 0.00	55.64 ± 10.19[c]

[a] The data were expressed as the mean ± standard error. [b] The increase in body weight was calculated by subtracting the body weight measured at the onset of dietary protocols from the final body weight. [c] $p < 0.01$.

RESULTS

***In Vitro* EPR Study.** A three-line EPR spectrum (a^N = 1.7 mT), which originates from the hyperfine interaction of an electron spin ($I = 1/2$) with a nitrogen nucleus spin ($I = 1$) of a nitroxide radical, was observed in the mixture of lycopene and TEMPOL solutions. No changes in the spectra obtained 2 and 30 min after mixing were observed. This finding suggests a low possibility of the direct chemical reaction between lycopene and TEMPOL.

Growth Data and Renal Lycopene Concentration. The growth data and renal lycopene concentration of rats in each group are shown in Table 1. The body weights of rats in the *in vivo* EPR study were about 200 g, which was almost the same as in previous *in vivo* EPR studies.[12,13] There were no significant differences in body weight, weight gain, and kidney weight, indicating that the general status of rats was not altered by lycopene intake. The renal lycopene concentration of the rats administered lycopene was about 50 ng/g of tissue (Table 1). The ratio of all *trans* and *cis* isomers of lycopene in the kidney were determined to be ca. 60 and 40%, respectively, by HPLC.

It was previously reported that the renal lycopene concentration rose to 150−200 ng/g of tissue in F344 rats fed lycopene (50−500 mg of lycopene/100 g of diet) for 8 weeks.[20] In this study, the renal lycopene concentration was about one-third of that observed previously, because the lycopene feeding period in this study was relatively short (2 weeks).

Table 2. Renal SOD and GSH-Px Activities and α-Tocopherol Concentration[a]

	control ($n = 8$)	lycopene ($n = 8$)
SOD activity (units/mg of tissue)	57.74 ± 2.64	56.31 ± 1.61
GSH-Px activity (units/mg of protein)	0.54 ± 0.06	0.51 ± 0.06
α-tocopherol concentration (μg/g of tissue)	17.76 ± 0.61	18.04 ± 0.79

[a] The data were expressed as the mean ± standard error.

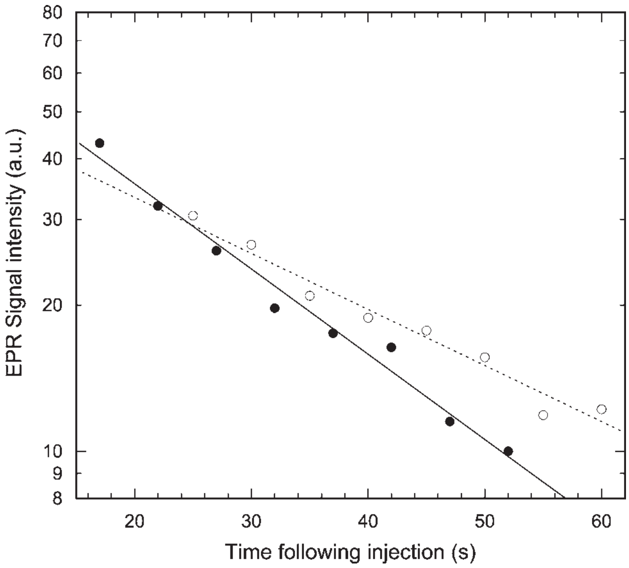

Figure 1. Typical semi-logarithmic plots of EPR signal intensity against time after the injection of TEMPOL in a representative rat from the control (○) or lycopene (●) group. Rats in the control or lycopene group were fed either a control diet or a diet containing lycopene (lycopene concentration, 250 mg/100 g of solid diet), respectively, for 2 weeks.

Renal SOD and GSH-Px Activity and α-Tocopherol Concentration. Table 2 shows the renal activities of SOD and GSH-Px and the renal concentrations of α-tocopherol in each group. There were no significant differences between the two groups, indicating that lycopene intake had no influence on SOD, GSH-Px, and α-tocopherol.

***In Vivo* EPR Study.** The three-line EPR spectrum, of which the hyperfine structure (a^N = 1.7 mT) was the same as that of the *in vitro* EPR study, was observed in the rat kidney. As noted in previous reports,[10−13] the peak-to-peak height of the lowest magnetic field component of the three-line EPR spectrum of TEMPOL defined the EPR signal intensity. In all groups, good linearity on a semi-logarithmic plot was observed (the absolute value of the correlation coefficient was >0.99), which means that signal intensity decays exponentially. Typical plots are shown in Figure 1. Because the temporal change of the EPR signal intensity shows a single exponential decay, its half-life can be used as an index of the decay rate. The half-life of EPR signal intensity was determined from each semi-logarithmic plot. The results are summarized in Figure 2. The EPR signal half-life in the kidney of the lycopene group was significantly shorter than in the control group ($p < 0.01$).

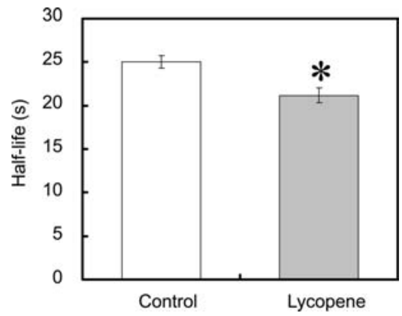

Figure 2. Renal half-life of TEMPOL in the control and lycopene groups. (*) $p < 0.01$. The shortening of the half-life means enhancement of the *in vivo* reducing ability of the kidney.

DISCUSSION

In this study, it was found that the renal half-life of the nitroxide radical (TEMPOL) in rats administered lycopene was significantly shortened. Nitroxide radicals ($R_2N{-}O\bullet$) are stable free radicals. An electron spin is located at the nitrosyl group (>N−O•) in a nitroxide radical (this is the nitroxide radical center). The stability of the nitrosyl group originates from the strong three-electron nitrogen−oxygen bond. Further, the tetramethyl group protects the nitrosyl group from attacks by other molecules.[26] However, nitroxide radicals are easily reduced to hydroxyl amines ($R_2N{-}O\bullet + e^- + H^+ = R_2N{-}OH$), which are not EPR-detectable, by reducing agents, such as ascorbic acid, because the reduction potential of nitroxide radicals is relatively low.[27,28] Therefore, the effect of reactions other than reduction is negligible in the metabolism of nitroxide radicals in biological systems.

The simulation showed that the half-life of TEMPOL measured in the target area (i.e., kidney) reflects the reduction of TEMPOL in the kidney and is not influenced by the reduction in the other organs. Therefore, the shortening of the renal half-life in the lycopene group observed in this study indicates enhancement of the *in vivo* reducing ability of the kidney as a result of the dietary antioxidant lycopene.

In this study, the TEMPOL half-life was decreased about 3 s in the lycopene group as compared to the control. Because this degree of shortening in the half-life was equivalent to that for dimethyl sulfoxide and dimethylthiourea, which are known as scavengers of hydroxyl radicals, thereby protecting the kidney,[13] we believe that lycopene has the same antioxidant ability as these drugs.

On the other hand, direct chemical reaction between lycopene and TEMPOL was not observed. Some have reported that lycopene directly reacts with other free radicals.[29−31] However, it is thought that direct chemical reaction between lycopene and TEMPOL is unfavorable because TEMPOL is a relatively stable radical.[26] In the *in vivo* relationship between lycopene and TEMPOL, the important elements are not only their direct chemical reactions but also the indirect effects to other reduction systems.

In this study, we measured renal SOD and GSH-Px activities and the α-tocopherol concentration to evaluate the effects of lycopene on *in vivo* reduction systems. No changes in these reduction systems as a result of lycopene intake were observed. The amount of α-tocopherol is much higher than lycopene in the kidney. However, lycopene is not present in the normal kidney. Thus, exogenous lycopene might indirectly influence other reduction systems.

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It is thought that the mechanisms of *in vivo* lycopene reduction relate to the field of reduction. For example, TEMPOL is reduced in the renal mitochondria.[32] An *in vivo* EPR study using TEMPOL revealed that adriamycin impaired renal mitochondria and lowered the renal reducing ability.[12] These findings indicate that the renal reducing ability depends upon the mitochondrial functions in the kidney. Because lycopene is a lipophilic antioxidant,[33] affinity of lycopene to the mitochondrial membrane will be high. In fact, it was reported that the mitochondrial function was altered by lycopene.[34−36] Therefore, lycopene might act in the kidney mitochondrial membrane and augment the renal reducing ability.

In conclusion, we directly investigated the *in vivo* renal reducing ability after oral ingestion of lycopene using an EPR spectrometer equipped with an SCR. This is the first verification of the *in vivo* enhancement of the antioxidant ability of lycopene, a typical antioxidant agent in food.

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■ ABBREVIATIONS USED

EPR, electron paramagnetic resonance; GSH-Px, glutathione peroxidase; HPLC, high-performance liquid chromatography; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; PMC, 2,2,5,7,8-pentamethyl-6-chromanol; RF, radiofrequency; ROS, reactive oxygen species; SCR, surface-coil-type resonator; SOD, superoxide dismutase; TEMPOL, 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl

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