Lycopene Uptake and Tissue Disposition in Male and Female Rats[1]

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Abstract. **Epidemiologic and clinical studies suggest that tomato consumption may reduce the risk of cancer. Lycopene, a hydrocarbon carotenoid, is the major carotenoid in tomatoes and, as a potent singlet oxygen quencher, has been considered by some to be the biologically active agent responsible for the reduction of cancer risk associated with tomato consumption. However, little is known concerning lycopene absorption or biological activity in rodent models of cancer. Therefore, the present study was designed to provide information regarding the uptake and tissue disposition of lycopene and related carotenoid after feeding a diet containing a carotenoid mixture extracted from tomatoes (Betatene). Betatene was added to the diet at 2.3, 0.9, 0.45, 0.23, 0.09 and 0 (m*M*/kg diet) and fed to male and female Fischer-344 rats for a period of 10 weeks. Using reverse phase HPLC methods, it was found that approximately 55% of administered lycopene was excreted in the feces. In both males and females, lycopene concentrations were highest in the liver (120–42 μg/g wet wt.); physiologically significant levels were detected in prostate (97–47 ng/g), lung (227–134 ng/g), mammary gland (309–174 ng/g) and serum (285–160 ng/ml). Tissue concentrations were related to dose with the exception of serum, and differences between males and females were minimal. Other carotenoids present in Betatene (i.e., phytoene, phytofluene, z-carotene and β-carotene) were also absorbed and stored in the liver. These results indicate that lycopene, when incorporated into the semipurified AIN-76A diet, is absorbed in both male and female rats in a dose-related manner and can be detected at nanogram levels in a variety of target organs.**

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Epidemiological (1, 2) and clinical (3) studies suggest that tomato consumption may exert anticancer effects in humans particularly with regard to gastrointestinal and prostate cancer. Lycopene, which is the major carotenoid present in tomatoes, is a hydrocarbon carotenoid that does not serve as a vitamin A precursor and is present at high levels in human milk and serum, often at higher levels than β-carotene (4).

There are a number of plausible mechanisms by which lycopene could exert anticancer effects. Lycopene is a powerful singlet oxygen quencher (5); it protects against membrane damage caused by the nitrogen dioxide radical (6); and it has been shown *in vitro* to block the cell cycle selectively (7). In addition, lycopene has been shown to enhance gap junction communication in cultured neoplastic cells and to inhibit *in vitro* neoplastic transformation of normal human and mouse cells (8). Recently Khachik *et al.* have isolated and characterized several oxidative metabolites of lycopene suggesting that the metabolic transformation of lycopene involves oxidation-reduction reactions (9).

The earliest reports on the biological activities of lycopene were in 1959 by Ernster and colleagues who showed that intraperitoneally injected lycopene increased the survival of irradiated mice and facilitated resistance to bacterial infections (see Ref. 10 for review). Recently, it was reported that crystalline lycopene added to the diet at very low levels (0.5 PPM) significantly suppressed spontaneous mammary tumor development in mice (11). The inhibition was accompanied by suppression of mammary gland thymidylate syn-

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thetase, serum prolactin levels, and enhancement of immune functions (12). Lycopene has also been shown to inhibit colonic aberrant crypt formation in rats (13). [For a review of the anticarcinogenic effects of lycopene and other carotenoids, see Gerster (14).] Preliminary blood level studies indicated that lycopene was inversely associated with cancers of the cervix (15, 16) and pancreas (17).

A number of gaps remain in our understanding of the anticancer effect of lycopene. Inconsistencies in the epidemiological evidence have been reported. Steinmetz *et al.* (18) for example, reported no association between lung cancer risk and consumption of three food groups defined as "high carotenoid" or tomatoes; and Järvinen *et al.* (19) found no significant relationship between intake of β-carotene, lycopene, or lutein and the occurrence of breast cancer in a prospective cohort study conducted in Finland. Moreover, little is known concerning the uptake and tissue distribution of dietary lycopene in rodent models commonly used for cancer bioassays. Therefore, the purpose of the present study was to assess (1) the stability of lycopene in the diet; (2) the maximal tolerated dose (MTD); and (3) tissue uptake and blood levels of lycopene and related carotenoids in female and male rats. The rationale for using a tomato extract rather than pure lycopene was based on the fact the epidemiological evidence suggested that tomatoes, not lycopene *per se* provide protection (20). Hence, interplay among the entire carotenoid mixture of the tomato, rather than lycopene alone, may act to decrease cancer risk.

To this end, we have fed rats varying levels of a concentrated carotenoid mixture derived from tomatoes (Betatene) and measured body weight changes and carotenoid levels in blood, feces and major target organs. Because lycopene is a lipid-soluble molecule with antioxidant properties, we also tested the possibility of sparing effects with other lipid soluble (Vitamin E, Vitamin A) and water soluble (glutathione) components of the cellular antioxidant system. These studies are considered to be a prelude to bioassays designed to test the chemopreventive effects of lycopene and other related carotenoids in rodent models of breast, lung, colon, and prostate cancer.

Methods

The tomato oleoresin, "Betatene," used in this study was provided *gratis* by Dr. J. Clark (Henkel Corp., La Grange, IL). Betatene consists of a carotenoid concentrate suspended in medium chain triglyceride (MCT). By HPLC it was found that "Betatene" contained 5.7% carotenoids; approximately 3.7% as lycopene and 2% as a mixture of other carotenoids including β-carotene, zeta-carotene, phytoene, phytofluene, and an oxidative metabolite of lycopene, 2,6-cyclolycopene-1,5-diol (Table I).

Fischer (F-344) male and female 50-day-old rats were allocated to six experimental groups, 10 animals per treatment group and 20 animals as controls (Table II). In terms of lycopene the concentrations ranged from a high of 1240 PPM to a low of 50 PPM. The tomato oleoresin was incorporated into the AIN-76A semipurified diet, (5% corn oil) which contains no carotenoids. MCT was added to the diets as needed in order to maintain equal amounts of lipid in all the treatment groups. Diets were made up in 15-kg batches and stored at 4°C in the dark for no more than 2 weeks. Diets were fed in J-type feeders, and food cups were emptied and cleaned every 2 days. Animals were fed the lycopene-containing diets for 10 weeks; before sacrifice, animals were placed in metabolism cages, and feces were collected. Blood was obtained by heart puncture under anesthesia. Following sacrifice, liver, lung, mammary, colon, and prostate tissue were collected and frozen at −70°C. Animal weights were recorded on a weekly basis to assess food aversion or weight loss consequent to lycopene consumption.

Table I. Qualitative and Quantitative Carotenoid Profile of Betatene

Carotenoid	μg/g	%	% Total carotenoid
1. Lycopene	37,504	3.7	66
2. Beta-carotene	12,443	1.2	22
3. Phytofluene	3,349	0.3	6
4. Phytoene	2,794	0.28	5
5. Zeta-carotene	440	0.04	0.7
6. 2,6-cyclolycopene-1,5-diol	471	0.04	0.7
Total carotenoids	57,001	5.7	100

Table II. Study Design[a]

Group	No. animals		Lycopene quantity		
	Males	Females	(m*M*/kg diet)	(m/kg diet)	(% diet)
1	10	10	2.3	1240	0.124
2	10	10	0.9	496	0.050
3	10	10	0.45	248	0.024
4	10	10	0.23	124	0.012
5	10	10	0.09	50	0.005
6	20	20	0	0	0
Total *N*	70	70			

[a] Based on Lycopene MW = 536. The Betatene suspension contained 5.7% carotenoids, 3.7% of which was pure lycopene.

For the qualitative and quantitative assessment of carotenoids and Vitamins A and E, diets and tissues were prepared according to methods developed by one of the authors (FK) (4). Extraction of carotenoids from the diet was by mechanical agitation with a mixture of acetonitrile (85%), hexane (2.5%), methanol (10%), and methylene chloride (2.5%) followed by centrifugation and removal of the organic fraction. The efficiency of extraction of carotenoids into the organic layer, using echinenone, an oxygenated derivative of β-carotene not present in tomatoes as internal standard, varied from 73%–100%.

Extraction from lung and colon involved homogenization with the extraction mixture in a Polytron homogenizer in the presence of internal standard, followed by centrifugation to remove cellular debris. In prostate and mammary gland, samples were first saponified to remove fat and then

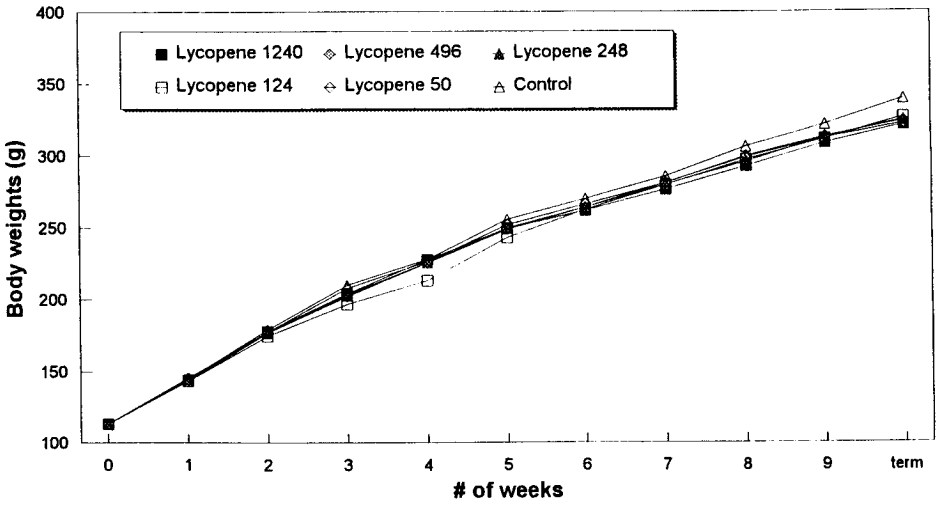

Figure 1. Effect of lycopene on mean absolute weights in male rats plotted as a function of time in weeks. Overall and pairwise comparisons were NS for all groups.

subjected to the above extraction procedures. Saponification was with 50% NaOH plus 25% ascorbate overnight at 30°C under N_2 gas. Extraction from liver slices was with tetrahydrofuran (THF) using sonication. After five extractions, the THF extracts were evaporated to dryness under N_2 gas and the residue treated with methanolic KOH (10%) under N_2 gas for 1 hr at room temperature to remove lipid. The carotenoids were then partitioned between NaCl (10%) and dichloromethane (v/v). The organic layer was decanted, dried under N_2 gas, and the residue brought up in injection solvent prior to analysis.

Extraction from 1 ml serum involved addition to sample of 1 ml ethanol and internal standard (echinenone) followed by centrifugation to remove precipitated protein. Two milliliters of hexane were then added to the ethanol fraction, and the hexane layer was decanted twice and the combined extract evaporated to dryness under N_2 gas. The residue, containing carotenoids, was then brought up in the injection solvent prior to HPLC analysis.

Lycopene and secondary carotenoids were analyzed by reverse-phase HPLC as previously described (4). Analyses were conducted on a Waters model 510 HPLC (Milford, MA) equipped with a Rainin Microsorb 5 μm *cis* column (Emeryville, CA) and a Shimadzu SPD-10A UV-Visible detector (Kyoto, Japan). Retention times using this system were: lycopene, 32 min (470 nm); zeta carotene, 3 min (400 nm); phytofluene, 41–42 min, (350 nm); phytoene, 43–44 min (290 nm).

Changes in Vitamins E and A were assessed by the HPLC method of Milne and Botnen (21). For the determination of glutathione, liver slices were homogenized (10% w/v) in 5% w/v metaphosphoric acid using an all-glass Ten-Broeck homogenizer. Acid extracts were obtained by centrifugation and stored at −70°C until analysis by HPLC as described by Kleinman and Richie (22).

Results

Consumption of the lycopene-containing diet had no adverse effects on weight gain, behavior (Figs. 1 and 2), or coat appearance with the exception of a brownish discoloration of the tail in a few rats.

The efficiency of extraction from diet varied from 73%–100% depending on the concentrations of lycopene added (Table III). Lycopene was stable in the diet for 48 hr at room temperature and for 2–3 weeks at 4°C in the dark (Table IV). However, after 3 weeks of storage at 4°C there

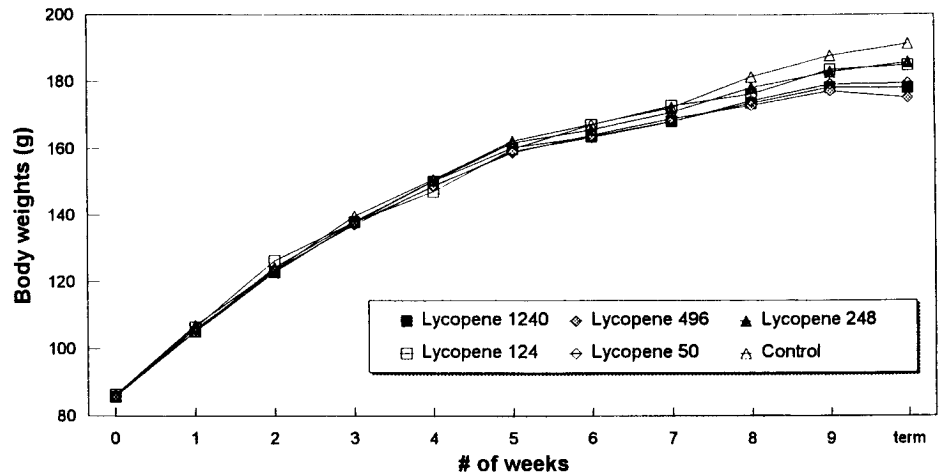

Figure 2. Effect of lycopene on mean absolute weights in female rats plotted as a function of time in weeks. Overall and pairwise comparisons were NS for all groups.

Table III. Efficiency of Extraction of Lycopene from the AIN-76A Diet[a]

Group no.	Conc lycopene added to diet	Lycopene measured	% Efficiency
1	1240	975	80
2	496	362	73
3	248	192	77
4	124	112	90
5	50	57	100
6	—	—	—

[a] One-half gram of diet was extracted with 100 ml (Groups 1 and 2) and 50 ml (Groups 3–5) extraction solvent; 20 µl of extract injected onto HCLC column.

Table IV. Stability of Lycopene in Diet Stored at 4°C[a,b,c]

Group no.	Number of days stored (4°C)	Concentration (µg/g)	%
1	1	748	100
2	8	728	98
3	14	672	90
4	21	534	72

[a] AIN-76A diet.
[b] Internal standard.
[c] Taking concentration of diet, 748 µg/g, as 100%.

Table V. Stability of Lycopene Incorporated in the AIN-76A Diet at Ambient Temperature[a]

Sample no.	Days in food cup	Concentration (µg/g)	%
1	0	744[a]	100
2	2	702	94
3	3	616	83
4	6	547	74

[a] Based on 744 mg lycopene/g diet as 100%.

was a marked decrease in lycopene levels (Table IV). At ambient conditions, 25% of lycopene was lost after 6 days (Table V). The full complement of carotenoids was present when extracted from the diet following formulation (Table VI).

Approximately 55% of ingested lycopene was recovered in the feces. The major part of absorbed lycopene was stored in the liver (Table VII). The liver contained µg/g wet weight levels of lycopene and all five other carotenoids present in Betatene. There was a distinct dose-related uptake in the liver (Table VII) and much higher levels of phytofluene and phytoene than would be expected based on their relative percent in the diet. This suggests selective uptake of these compounds by the liver. Also there was increased relative amounts of the lycopene 2,6-cyclolycopene-1,5-diol, suggesting that either the selective uptake or oxidative metabolism of lycopene occurred in the liver. Vitamin A and E levels in the liver were closely associated with lycopene levels, a finding that argues for a sparing effect by lycopene on Vitamin A or E uptake (Table VIII), and against a negative compensatory effect.

Lycopene was present in lung in both male and female tissue (Table IX), mammary gland, and prostate (Table X) in the 100–300 ng/g range. These levels closely reflected dietary concentrations. Serum levels of lycopene ranged from 80–370 ng/ml in both males and females but were not reflective of dietary intake (Table XI). This suggests that serum lycopene concentrations are controlled by compensatory homeostatic mechanisms, perhaps involving storage and release from the liver based on increased or decreased dietary intake.

Liver (Table XII) and kidney (not shown) glutathione levels were significantly increased at the higher levels of lycopene intake. This increase in glutathione may also be indicative of a sparing effect of lycopene and/or secondary carotenoids on tissue glutathione levels. No changes were seen in circulating glutathione levels under any of the dietary conditions in either male or female rats.

Summary and Conclusions

Lycopene is stable in the diet for a period of 48 hr at room temperature and at 4°C for 3 weeks and is therefore suitable for animal feeding studies. The lycopene-containing diets were avidly consumed, and lycopene was absorbed from the diet at all doses, with no toxic side effects, in both male and female F-344 rats. Lycopene was concentrated in the liver at levels two orders of magnitude higher

Table VI. Carotenoid Concentration of Rat Diets Supplemented with the Suspension of Tomato Oleoresin[a]

Diets	m*M*/kg	µg/gm					
		Lycopene	β-Carotene	α-Carotene	Phytofluene	Phytoene	2,6-Cyclo-lycopene-1,5-diol
1	2.3	1240	284	1	58	70	2
2	0.9	496	138	9	28	23	1.4
3	0.45	248	69	4	14	0.3	0.8
4	0.23	124	37	2	8	5	0.5
5	0.09	50	8	0	2	1	0.1
6	0	0	0	0	0	0	0

[a] One half gram of diet was saturated with 100 ml of 50 ml of extraction solvent and injected into HPLC column. Detection of various carotenoids was by photo diode array detector (FK).

Table VII. Carotenoid Concentration of Livers of Rats Fed Diets Supplemented with Tomato Oleoresin[a,b,c]

Rats	Diet mM/kg	μg/g					
		Lycopene	β-Carotene	α-Carotene	Phytofluene	Phytoene	2,6-Cyclo-lycopene-1,5-diol
Female							
1	2.3	120	11	17	106	66	9
2	0.9	64	6	8	48	35	5
3	0.45	66	8	7	50	40	7
4	0.23	49	7	2	46	46	4
5	0.09	42	4	4	33	38	6
6	0	0	0	0	0	0	0

[a] 3 Rat livers from each group pooled.
[b] Carotenoids were degraded during processing in male group.
[c] Linear regression analysis was used to test dose was used to test for dose-response relationship: $P < 0.05$ for all but 2,6-cyclolycopene-1,5-diol.

Table VIII. Concentration of Lycopene, Retinol, and α-Tocopherol in Rat Liver[a,b]

Group no. Female	Lycopene in diet (mM/kg)	Lycopene (μg/g)	Retinol (μg/g)	α-Tocopherol (μg/g)
1	2.3	120	1,302	47
2	0.9	64	768	38
3	0.45	66	858	39
4	0.23	49	888	46
5	0.09	22	555	32
6	0	0	120	13

[a] Rat livers from each group pooled.
[b] Carotenoids, Vitamin A, and Vitamin E were degraded during processing in male rats.

Table IX. Concentration of Lycopene in Rat Lung[a,b]

Group no.	mM kg diet	Ave. conc. in lung (ng/g)	Range
Male			
1	2.3	190	193, 187
2	0.9	214	170, 257
3	0.45	375	325, 424
4	0.23	201	239, 162
5	0.09	151	167, 135
6	0	0	0
Female			
1	2.3	227	184, 270
2	0.9	246	280, 211
3	0.45	193	243, 142
4	0.23	211	208, 214
5	0.09	134	144, 124
6	0	0	0

[a] $n = 2$.
[b] Linear regression analysis for dose-response trend: $P < 0.755$ (males); $P < 0.208$ (females).

than in other organs. Relatively more of the secondary carotenoids were present in the liver than in the diets, suggesting selective uptake of these carotenoids. The high Vitamin A levels in the livers of Betatene fed rats may be the result of selective uptake of dietary Vitamin A, but it is

Table X. Lycopene Concentration of Rat Prostate and Mammary Gland (ng/g)[a,b]

Group no.	mM/kg diet	Mean (SD)	Median	Range
Prostate				
1	2.3	97 (17)	99	79–112
2	0.9	95 (48)	83	54–147
3	0.45	50 (37)	35	23–93
4	0.23	52 (26)	52	26–77
5	0.09	47 (16)	47	32–63
6	0	0	0	0
Mammary				
1	2.3	309 (131)	235	232–460
2	0.9	200 (030)	197	172–231
3	0.45	215 (062)	220	139–282
4	0.23	229 (054)	217	181–288
5	0.09	174 (057)	143	139–239
6	0	0	0	0

[a] $n = 6$.
[b] Linear regression analysis for dose-response trend: $P < 0.002$ prostate; and $P < 0.09$ breast.

Table XI. Lycopene Concentration of Rat Serum (ng/ml)[a,b]

Group no.	mM/kg diet	Mean (SD)	Median	Range
Female				
1	2.3	187 (43)	205	123–232
2	0.9	169 (42)	180	109–211
3	0.45	243 (83)	210	174–366
4	0.23	313 (47)	308	262–369
5	0.09	145 (53)	152	81–207
6	0	0	0	0
Male				
1	2.3	168 (36)	160	134–230
2	0.9	227 (68)	225	148–326
3	0.45	228 (66)	285	174–372
4	0.23	231 (64)	215	153–328
5	0.09	171 (49)	177	100–238
6	0	0	0	0

[a] $n = 6$.
[b] Linear regression analysis for dose-response trend: $P < 0.283$ (females) and $P < 0.172$ (males).

Table XII. Lycopene Study: Liver Glutathione[a]

Group no.	Lycopene (ppm)	Glutathione (μmol/g)	
		Female	Male
1	1240	7.4 (0.70)	7.2 (1.95)
2	496	5.9 (0.50)	6.9 (1.10)
3	248	4.9 (1.10)	4.6 (1.37)
4	124	6.5 (1.10)[b]	5.4 (0.36)
5	50	6.0 (0.30)	6.8 (1.53)
6	0	5.5 (0.37)	6.1 (0.52)

[a] Values are mean ± SD, $n = 5$.
[b] Significantly different from untreated control, $P < 0.05$.

more likely due to the efficient conversion of the β-carotene in the Betatene to Vitamin A by rat intestinal 15′,15-dioxygenase followed by storage in the liver. The increases observed in hepatic Vitamin E and glutathione levels in rats fed high levels of lycopene may be indicative of an overall decrease in oxidative stress in these animals.

Uptake from the diet into distant organs such as lung, mammary, prostate gland, and colon (data not shown) at ng/g levels indicate that both male and female F-344 rats provide good models for cancer chemoprevention studies.

The consumption of lycopene in this study relative to human consumption can be estimated based on an estimated human lycopene consumption of 2000–6000 μg/day (1). On a daily basis, rats consumed 280–7000 μg lycopene/day in the present study. On a weight basis, assuming the average weight of a rat to be 200 g and that of a human as 70,000 g, rats in this experiment consumed approximately 350 times the average human lycopene consumption. On a surface area basis, assuming the average surface area of a rat to be 324 cm^2 and human as 18,000 cm^2, rats consumed approximately 55 times more lycopene than average human consumption. Future studies using levels of lycopene more closely approaching human intake may prove of value prior to conducting cancer chemoprevention bioassays in rodent models.

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