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Lycopene in serum, skin and adipose tissues after tomato-oleoresin supplementation in patients undergoing haemorrhoidectomy or peri-anal fistulotomy

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Lycopene, the main carotenoid found in tomatoes and tomato-based products, has been reported to be protective against several types of cancer. Assessment of changes in plasma concentration of carotenoids following ingestion of lycopene-rich food sources does not necessarily predict changes in lycopene concentration or distribution of its isomers in other body tissues. Our aim was to determine the relationship between concentrations of lycopene and other tomato carotenoids in human serum and body tissues after tomato-oleoresin supplementation. Tomato lycopene oleoresin (30 mg/d) or a placebo was administered for 1 to 7 weeks to seventy-five volunteers undergoing elective haemorrhoidectomy or peri-anal fistulotomy. Carotenoid concentration and isomer distribution in blood and in the surgically removed skin and adipose tissues was measured by HPLC. The serum concentration of lycopene increased after supplementation from 0·26 (SD 0·12) to 0·52 (SD 0·25) μmol/l (n 35; $P<0·0001$). In the placebo group (n 40), lycopene serum concentration did not change significantly. Serum lycopene concentration after treatment was 2·2-fold greater in the lycopene group than in the placebo group, a slightly higher ratio than that found in skin and adipose tissue (1·6- and 1·4-fold higher than the placebo, respectively). A significant correlation between serum and tissue concentrations was found for both β-carotene and lycopene in the placebo group, whereas in the lycopene-supplemented group the correlation between serum and tissues remained the same for β-carotene but for lycopene was weak. Lycopene supplementation did not significantly change the proportion of all-*trans v. cis* isomers in the serum and tissues, despite the fact that more than 90 % of the supplemented lycopene was in the all-*trans* form. These results show that tomato-oleoresin supplementation increases lycopene concentrations in serum and in adipose tissue and skin. The ability to increase lycopene levels in tissues is one of the prerequisites for using it as a food supplement with health benefits.

Lycopene: Carotenoids: Bioavailability: Tomato-oleoresin supplementation

Lycopene, the main carotenoid in tomatoes, is one of the major carotenoids in human serum (Parker, 1989; Stahl & Sies 1992; Khachik *et al.* 1997). Epidemiological studies have suggested that intake of foods rich in lycopene decreases the risk of several types of human malignancies, such as those of the prostate (Giovannucci *et al.* 1995), breast (Zhang *et al.* 1997), lung (Michaud *et al.* 2000) and digestive tract (Franceschi *et al.* 1994). Giovannucci (1999) reviewed data from clinical and epidemiological studies and found that most of these studies show an inverse association between tomato intake or blood lycopene level and cancer risk. Chronic ingestion of lycopene decreased spontaneous mammary tumour development in mice and enhanced the immune response by increasing the number of helper T lymphocytes (Nagasawa *et al.* 1995; Kobayashi *et al.* 1996). It has been found that lycopene is more potent than α- or β-carotene in inhibiting cell growth of various human cancer cell lines (Levy *et al.* 1995). Two preliminary intervention studies reported that supplementation with natural tomato preparations in men with prostate cancer reduced the levels of serum prostate-specific antigen and positively modulated other parameters of the disease (Chen *et al.* 2001; Kucuk *et al.* 2001).

The mechanism underlying the inhibitory effects of lycopene and other carotenoids involves interference in several intracellular pathways related to cancer cell proliferation. Such pathways include induction of gap-junctional communication between cells (Zhang *et al.* 1991, 1992), inhibition of the cell cycle progression (Karas *et al.* 2000; Nahum *et al.* 2001) and modulation of transcription by ligand-activated nuclear receptors or other transcription factors (Ben-Dor *et al.* 2001). To achieve

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such intracellular effects the carotenoids should gain access to cells and tissues. Several studies have assessed the bioavailability of lycopene; however, results have been inconclusive, suggesting poor (Brown *et al.* 1989; Micozzi *et al.* 1992; Stahl & Sies 1992), moderate (Gartner *et al.* 1997; Johnson *et al.* 1997), or even good (Bowen *et al.* 1993) bioavailability. This ambivalence is probably due to differences in the food matrix or fat intake. Most of these studies measured bioavailability as an increase in plasma lycopene level. However, the increase in plasma carotenoid concentrations is not a direct measure of the amount of carotenoids absorbed. Therefore, it is important to measure carotenoids in tissues alongside plasma to learn about their distribution. But since tissue biopsies are very invasive and rarely available, this important issue is very difficult to assess. One exception is the buccal mucosa cells, which were used in two recent studies for monitoring tissue concentrations of carotenoids since these cells can be readily collected in a non-invasive manner (Paetau *et al.* 1999; Richelle *et al.* 2002). The findings showed a significant correlation between plasma and buccal mucosa cells concentrations of lutein, β-cryptoxanthin, α-carotene and β-carotene, suggesting that plasma carotenoid concentrations are good biomarkers for tissue concentrations of these carotenoids. In one of these studies, however, the correlations for lycopene were weak and not significant (Paetau *et al.* 1999), indicating that, unlike for most of the other major dietary carotenoids, buccal mucosa cells do not reflect plasma lycopene concentrations. On the other hand, Chen *et al.* (2001) found a positive correlation between serum lycopene and prostate lycopene concentrations in a recent study on patients with prostate cancer who consumed tomato-sauce-based pasta dishes (30 mg lycopene/d) for 3 weeks preceding their scheduled radical prostatectomy.

One factor influencing the bioavailability of lycopene is its stereochemistry. Lycopene is a highly unsaturated molecule containing thirteen double bonds, eleven of which are conjugated. The lycopene in tomatoes and tomato products comprises more than 90 % of the all-*trans* geometric isomer (Stahl *et al.* 1992; Richelle *et al.* 2002). In contrast, more than 50 % of lycopene isomers in human tissues and blood are in several *cis* configurations.

The objective of the present study was to determine the bioavailability of tomato carotenoids in human tissues after tomato-oleoresin supplementation and to evaluate the relationship between serum and tissue concentrations. Another objective was to determine if the distribution of isomers in the body is affected by the supplementation of a higher proportion of the all-*trans* isomer. To address these questions, blood, skin and adipose tissue samples were acquired from patients who were given supplements of a natural source of tomato oleoresin before undergoing elective proctologic procedures.

Experimental methods

Subjects and treatment

The study had a double-blind, randomised, design with a tomato-oleoresin treatment group *v.* a placebo group. Volunteers (*n* 90), candidates for elective haemorrhoidectomy or peri-anal fistulotomy, were recruited from the local community a few days to a few weeks before surgery during the period November 1998–October 2000. The participation of patients in the study did not interfere with their treatment plan and did not influence the time between diagnosis and surgery, which was determined solely by clinical considerations. The study was approved by the local medical ethics committee and the Israeli Ministry of Health. Subjects were asked to sign an informed consent form before entering the study and were randomly divided between the lycopene and placebo groups. Information on BMI, health habits and medical condition was obtained from the subjects and clinical records. The demographic characteristics of the two groups are displayed in Table 1. There were no major differences between the groups that could affect the results.

The volunteers were given supplements of a natural source of lycopene or a placebo for a variable period of time before surgery (depending on the time between referral and surgery). Lycopene was administered as tomato oleoresin (LYC-O-MATO®; LycoRed Natural Products Industries Ltd, Beer-Sheva, Israel), which is manufactured from proprietary lycopene-rich tomato varieties and formulated into soft-gel capsules. Each capsule contains 15 mg lycopene, and about 1·5 mg phytoene, 1·4 mg phytofluene, 0·4 mg β-carotene and 5 mg tocopherols. The

Table 1. Demographic characteristics of the patients in the tomato-oleoresin and placebo groups

(Mean values and standard errors of the mean)

Study group...	Tomato-oleoresin		Placebo	
	Mean	SEM	Mean	SEM
No. of patients	35		40	
Period of treatment (d)	24	3	25	2
Range of period of treatment (d)	6–43		8–52	
Sex (*n*)				
Males	23		29	
Females	12		11	
Age (years)	49	2	46	2
Age range (years)	26–76		20–70	
Country of birth or ethnic group (*n*)				
Israel	11		11	
Bedouin	6		8	
North Africa	9		10	
Eastern Europe	6		11	
Other	3		0	
Smoking status (*n*)				
Smoker	13		9	
Non-smoker	21		28	
Ex-smoker	1		3	
Weight (kg)	70*	2	74†	2
Height (m)	1·66‡	0·02	1·71§	0·02
BMI (kg/m^2)‖	25·3‡	0·7	25·6§	0·8

* *n* 31.
† *n* 36.
‡ *n* 22.
§ *n* 26.
‖ Values for height and weight were missing for some patients and thus BMI was not calculated for all patients.

placebo contained refined soya oil instead of the oleoresin component. The subjects were asked to take one capsule twice daily (30 mg lycopene/d) with meals and not to change their regular diet.

Two blood samples (before and after treatment), and normal adipose and skin-tissue samples (after treatment) that would normally be discarded as part of the surgical procedure were acquired for analysis. The haemorrhoidectomy samples were 10–20 mm in diameter and included: normal skin, which is the external part of the haemorrhoid; the mucocutaneus junction, which includes the transitional zone between the rectum and the anal verge; the distal rectal mucosa, which covers the internal part of the haemorrhoid. Underneath these three parts lies normal adipose connective tissue that includes abnormal dilated veins. Fistulotomy entails 'unroofing' the normal tissue above the anorectal fistula. This tissue is composed of the same parts described for haemorrhoids; however, there are no dilated veins in the adipose connective tissue of the fistular tract. After completing the excision, the removed tissue samples were handled by regular scissor dissection to separate skin and adipose connective tissue. Sera and tissue samples were kept at −70°C. The medical and laboratory personnel were blind to the treatment status of the blood and tissue samples.

Carotenoid extraction and analysis

Tissue homogenisation, extraction and analysis were performed as described previously (Stahl *et al.* 1993) under dim light to prevent photo-oxidation. Tissue was weighed, minced and homogenised (polytron) on ice. To improve carotenoid extraction, the homogenate was enzyme-treated in a phosphate buffer containing butylated hydroxytoluene and internal standard (β-apo-8′carotenal) as described by Wingerath *et al.* (1998). The homogenate was incubated with collagenase type IV and lipase type VII for 2·5 h at 37°C and subsequently with pronase E for 2·5 h under the same conditions. All enzymes were from Sigma Chemicals (St Louis, MO, USA). Triton X-100 was added for an additional 2·5 h. Serum samples were not enzyme-digested before extraction. Enzyme-digested tissues and sera were extracted with 6 vol. n-hexane–dichloromethane (5:1, v/v) containing 0·01 % (w/v) butylated hydroxytoluene. The samples were then centrifuged at 2000 ***g*** for 10 min at 16°C, and 5 ml supernatant fraction was removed. The solvent was evaporated under a gentle stream of N_2. The residues were dissolved in 180 μl HPLC mobile phase plus 20 μl dichloromethane. Chromatography of carotenoids was carried out using a Hewlett-Packard 1100 HPLC with a diode-array detector. A reverse-phase Suplex pKb 100 column (Supelco, Bellefonte, PA, USA) was used, with isocratic elution and a mobile phase consisting of methanol–acetonitrile–2-propanol (54:44:2, by vol.). A representative chromatograph of carotenoids from serum and tissue (skin) is shown in Fig. 1. Three *cis* geometric isomers of lycopene were usually present. The main one, eluting immediately after the all-*trans* isomer, was 9-*cis* lycopene, followed by small amounts of 13- and 15-*cis* isomers. Carotenoid concentrations in extracts were calculated from calibration curves generated from the ratio of peak area of the carotenoids to that of internal standard (β-apo-8′carotenal). The same standards were used for the all-*trans* and *cis* isomers of lycopene and β-carotene. The intra- and interassay precision varied between tissues, and CV ranged from 5 to 15 %. The extraction recovery of the internal standard was >75 % and the analysis detection limit was 0·01 nmol/g wet tissue. Under these HPLC conditions, lutein co-eluted with zeaxanthin. Standards for these two carotenoids and for β-cryptoxanthin were available only during the last year of the study and therefore the data presented for these carotenoids are from only part of the collected samples.

Statistical analysis

All results are presented as mean values and standard deviations. The results for lycopene are given also as median levels. The comparison between the placebo and the lycopene-treated groups was performed with the *t* test and the comparison of carotenoid values before and after supplementation was performed with the paired *t* test. The Pearson product correlation coefficient was employed to check correlations between tissue and serum concentrations.

Results

The study was completed by ninety subjects. Of these, fifteen were excluded due to lack of compliance or other reasons (Table 2). From the seventy-five remaining subjects, thirty-five were in the lycopene group (receiving tomato oleoresin) and forty in the placebo group. The average length of treatment was 24 (SEM 3) and 25 (SEM 2) d for the lycopene and placebo groups, respectively, with a range of 6 to 52 d.

Serum lycopene concentration increased in all lycopene-group patients but one, while in the placebo group the concentrations changed erratically (Fig. 2). Table 3 displays both the mean and the median levels of lycopene. The similarity of the values of these two parameters suggests that the distribution of results is normal and supports our conclusions. Serum lycopene concentration after treatment was significantly higher (2·2-fold) in the lycopene group than in the placebo group (Table 3) whereas the concentration before treatment did not differ between the two groups. Tissue lycopene concentration was also significantly higher in the lycopene group compared with the placebo group, but the difference in concentration was smaller (1·6- and 1·4-fold for the skin and adipose parts of the tissue, respectively). A possible explanation for this difference is that a longer time is needed to achieve a steady-state level in tissues as compared with serum. However, there was no positive correlation between time of treatment and tissue concentration (not shown).

As shown in Table 4, a small but significant increase was noted also in serum β-carotene and α-tocopherol concentration in the tomato-oleoresin-supplemented group, two compounds that are found at low levels in the tomato oleoresin. In the placebo group no significant change was found in any of the carotenoids (not shown). A difference in β-carotene tissue concentration between the two groups was

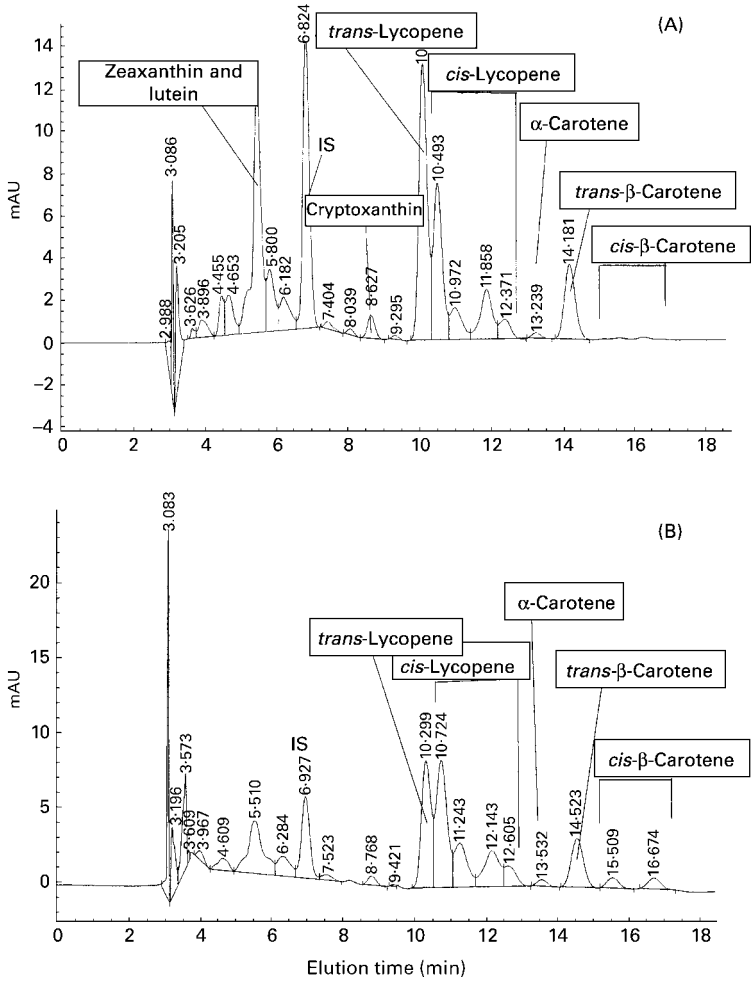

Fig. 1. Representative tracings of carotenoid chromatography from a serum sample (A) and a haemorrhoid skin sample (B). Samples are from a non-treated haemorrhoid patient. The samples were extracted and analysed as described on p. 761. IS, internal standard.

Table 2. Number of patients recruited to or excluded from the study

Description	Haemorrhoid	Fistula	Total
Completed the study	71	19	90
Excluded due to compliance problems*	7	2	9
Excluded due to subjective side effects†	5	–	5
Excluded due to clinical problems‡	1	–	1
Patients included in the study	58	17	75

* Lack of compliance was defined as more than a 35% difference between the number of capsules that had to be taken and the number that was actually consumed.

† A small number of patients (four in the lycopene group; one in the placebo group) reported sensitivity to the compound (mainly skin rash). Since it was not possible to ascertain if sensitivity resulted from the use of the compound, the participation of these patients was immediately discontinued.

‡ A gastrointestinal tumour together with a large part of the normal tissue had been removed in the past; this caused absorption problems that were manifested in very low blood and tissue carotenoid levels.

found only for skin (0·235 (SD 0·035) nmol/g for the lycopene group and 0·165 (SD 0·022) nmol/g for the placebo group; $P=0·047$). No significant difference between the lycopene and the placebo groups was found in the serum (Table 4) and tissue (not shown) concentrations of the other carotenoids. The significant increase in skin β-carotene concentration suggests that the accessibility to skin of this carotenoid, which is present at low levels in the oleoresin, is better than that of lycopene. In order to further analyse the accessibility of lycopene to tissues, the correlations between its serum and tissue concentration were calculated and compared with that found for β-carotene (Table 5). In the placebo group, under non-supplemented conditions, the *r* values for lycopene were similar to those of β-carotene. In contrast, the correlation after supplementation with tomato oleoresin was much weaker for lycopene than for β-carotene in both skin and adipose tissues.

The relative size of total body adipose tissue, which is indicated by BMI, may affect the concentrations and the distribution of carotenoids in serum and tissues because adipose tissue may act as a 'reservoir' for these lipophilic compounds. The latter assumption was tested by examining the relationship between BMI and lycopene concentration in the serum and tissues of patients supplemented with lycopene, but no correlation was found. An inverse association was suggested in the adipose tissue but the degree of association was very low (*r* 0·33).

The distribution of lycopene isomers between the all-*trans* and the sum of all the *cis* isomers is shown in

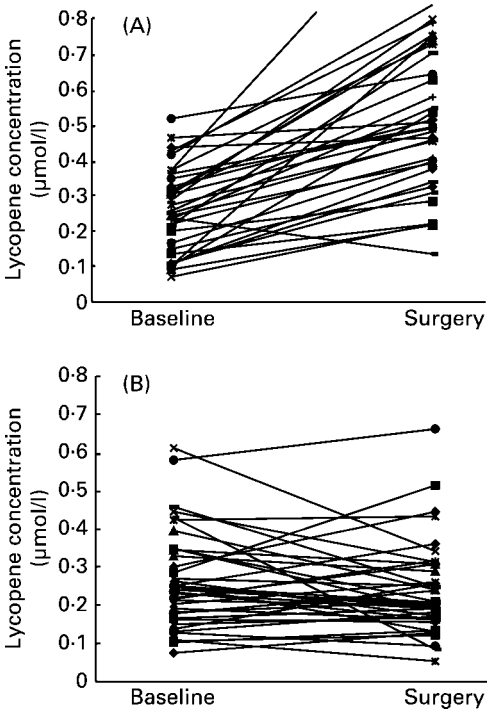

Fig. 2. Changes in serum lycopene levels of individual patients. (A), Tomato-oleoresin-treated patients; (B), placebo-treated patients. The first data point is the concentration at baseline and the second at time of surgery, after supplementation.

Table 6. Although, in the same way as tomatoes, the supplemented lycopene consists of more than 90 % all-*trans* isomer, lycopene supplementation did not significantly change the isomer distribution, which was 40–50 % all-*trans* isomer in the serum and 30–40 % in both tissues. In contrast to lycopene, almost all of the β-carotene, more than 98 % in serum and 85–90 % in both tissues, was present as the all-*trans* isomer.

Discussion

The main finding of the present study is that tomato-oleoresin supplementation increased lycopene concentrations in serum, skin and adipose tissue with the increase in tissue concentration being smaller than that in serum. Since this was a short-term study, these results may imply that a longer time is needed to achieve a new equilibrium between concentrations in serum and tissues. Regrettably, the time of intervention could not be extended as it was determined solely by clinical considerations. Indeed, after a longer intervention period (8 weeks), with a similar tomato extract containing 25 mg lycopene, it was found that the increases in plasma and buccal mucosa cells lycopene concentrations were significantly correlated (Richelle *et al.* 2002). In a similar though shorter study (Paetau *et al.* 1999) (4 weeks as in the present study), using even higher lycopene supplementation (70–75 mg), no such correlation between plasma and buccal mucosa cells was achieved for lycopene; however, good correlation was found for the other carotenoids. In agreement with this latter study, after supplementation with tomato oleoresin for a short time (average 25 d), the present study found good correlation between serum and tissue concentrations for β-carotene but not for lycopene. A good correlation between lycopene serum and tissue concentrations was found in the placebo group at the end of the intervention and in both groups before intervention, suggesting that equilibrium between serum and tissues can be achieved also for lycopene. Porrini & Riso (2000) and Porrini *et al.* (2002) found that the lycopene level in lymphocytes increased 2- to 3-fold after tomato purée supplementation for 2–3 weeks. This increase was similar to that in plasma, and was much higher than that reported in the present paper and in the other studies discussed earlier. However, because lymphocytes are in direct contact with plasma these findings cannot be compared directly with the results for other non-blood tissues.

An important question is whether the haemorrhoidectomy and fistulotomy samples are representative of other tissues. It should be emphasised that there was no clinical need for a dietary change for our patients and they were asked to continue their usual diet. The main problem in haemorrhoid tissue is the dilated veins, which do not exist in the fistulotomy samples. Since there were no differences in the average carotenoids levels in the two types of

Table 3. Lycopene concentration in serum and tissues of tomato-oleoresin and placebo groups* (Mean values and standard deviations and median values)

Study group...	Tomato-oleoresin			Placebo				
	Mean	SD	*n*	Mean	SD	*n*	T/P	*P* value
Serum baseline (μmol/l)								
Mean	0·257	0·119	35	0·266	0·128	40	0·97	0·374
Median	0·250			0·245				
Serum after supplementation (μmol/l)								
Mean	0·524	0·248	35	0·240	0·121	39	2·19	<0·0001
Median	0·489			0·208				
Skin (nmol/g)								
Mean	0·478	0·195	31	0·304	0·184	34	1·57	0·0003
Median	0·458			0·258				
Adipose tissue (nmol/g)								
Mean	0·336	0·229	30	0·233	0·164	34	1·44	0·02
Median	0·243			0·174				

T, tomato-oleoresin; P, placebo.
* For details of participants and procedures, see Tables 1 and 2 and p. 760.

Table 4. Carotenoid and α-tocopherol serum concentration (μmol/l) at baseline and after tomato-oleoresin supplementation*
(Mean values and standard deviations)

	Baseline		After supplementation			
	Mean	SD	Mean	SD	*n*	*P* value
Lycopene	0·257	0·119	0·524	0·248	35	<0·0001
α-Carotene	0·055	0·046	0·048	0·035	35	0·081
β-Carotene	0·164	0·127	0·188	0·122	35	0·047
α-Tocopherol	25·2	19·0	28·8	21·2	26	0·037
Zeaxanthin and lutein	0·293	0·112	0·304	0·121	9	0·413
Cryptoxanthin	0·361	0·481	0·338	0·455	9	0·327

* For details of participants and procedures, see Tables 1 and 2 and p. 760.

Table 5. Pearson correlation (*r*) between serum and tissue carotenoid concentrations after tomato-oleoresin or placebo treatment§

Tissue...	Skin				Adipose tissue			
Study group...	Tomato-oleoresin		Placebo		Tomato-oleoresin		Placebo	
	r	*n*	*r*	*n*	*r*	*n*	*r*	*n*
Lycopene	0·137*	31	0·393†	32	0·161*	30	0·418†	33
β-Carotene	0·507‡	31	0·507‡	33	0·368†	30	0·425†	33

* NS.
† $P<0·05$.
‡ $P<0·01$.
§ For details of participants and procedures, see Tables 1 and 2 and p. 760.

Table 6. Distribution of lycopene and β-carotene isomers in serum and tissues after tomato-oleoresin or placebo treatment*
(Mean values and standard deviations)

Study group...	Lycopene all-*trans* isomer (%)						β-Carotene all-*trans* isomer (%)					
	Tomato-oleoresin			Placebo			Tomato-oleoresin			Placebo		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Serum baseline	49·3	13·4	35	52·6	15·0	40	97·9	10·3	35	99·8	1·0	40
Serum after supplementation	46·2	14·2	35	50·9	15·6	39	99·5	1·5	35	98·5	7·1	38
Skin	39·2	14·3	31	41·7	16·8	34	88·0	5·6	31	86·0	14·1	34
Adipose tissue	39·2	16·8	30	39·8	14·3	32	87·7	7·0	30	85·7	12·3	32

* For details of participants and procedures, see Tables 1 and 2 and p. 760.

samples and there were no pathological conditions in the surgically removed tissues it is reasonable to assume that the studied samples represent normal skin and adipose tissues. However, it should be understood that the distribution of carotenoids into other body tissues is not necessarily the same as in the skin and adipose tissues used in the present study. Adipose tissue in particular is a depot for fat-soluble compounds and its carotenoids composition is an indicator of long-term dietary exposure (Kohlmeier *et al.* 1997). Thus, the conclusion reached with these tissues should be taken with caution when discussing other tissues.

The increase in serum lycopene concentration after tomato-oleoresin supplementation was about 2-fold, which is in good agreement with previous reports on bioavailability of lycopene (Agarwal & Rao 1998; Paetau *et al.* 1998; Pellegrini *et al.* 2000; Olmedilla *et al.* 2002). Supplementation with α-carotene, β-carotene or lutein (Olmedilla *et al.* 2002) resulted in increases in serum levels of 14-fold, 5-fold and 5-fold, respectively. In this latter study, which was a multicentre project involving 400 healthy male and female volunteers from five European regions, lycopene supplementation (from tomato paste) resulted in only a 2-fold increase in serum lycopene, similar to our results. Thus, plasma lycopene concentration does not increase to a great extent, as found for β-carotene, and usually does not exceed a concentration of 1–2 μm. It should be stressed that although the duration of treatment was variable (from 6–52 d) most patients still showed an increase in plasma lycopene level. This is in agreement with previous studies with lycopene doses higher than

20 mg/d, in which a significant increase in serum lycopene level, up to 90 % of equilibrium levels, even after a week of supplementation was reported (Paetau *et al.* 1999; Edwards *et al.* 2003). However, equilibrium of serum lycopene level was not reached in a very short time, especially with small doses. For example, with 5 mg/d, equilibrium was achieved only after 2 weeks of supplementation (Bohm & Bitsch 1999). Thus, the fact that five out of thirty-five subjects were supplemented with lycopene for only about 1 week may have some effect on the variability of the results in the serum and even more so in the tissues. Variability in the results could also be due to differences in the basal tissue levels of lycopene, which were not measured. However, based on the large variations found in the basal blood level of both the placebo- and the lycopene-treated groups (Fig. 2), it can be assumed that there is a large variability also in the tissue levels. This variability may be an effect of the different ethnic backgrounds of the patients, which influence their dietary habits, or seasonal variation that can change tomato-product intake. Variability in basal tissue levels can further explain the lack of correlation between time of treatment and tissue concentration. However, the similar distribution of basal blood levels in the placebo and the lycopene groups (Fig. 2) suggests that the variability in the results did not have a major effect on the ratio of tissue lycopene levels between the two treatment groups.

The isomeric composition of plasma and tissue lycopene is significantly different from that of the tomato oleoresin and was not affected by supplementation. These results are in good agreement with previous reports examining isomer distribution in plasma after tomato-product supplementation (Holloway *et al.* 2000; Olmedilla *et al.* 2002). However, in another study some increase in the all-*trans* lycopene isomer was detected after tomato-sauce supplementation (van Breemen *et al.* 2002). A lack of change in isomer distribution after supplementation suggests that the *cis* isomers are preferentially absorbed (Boileau *et al.* 1999), or that the all-*trans* isomer is changed to *cis* isomers in the acid gastric milieu, as suggested by Re *et al.* (2001).

In conclusion, the present study demonstrated that lycopene supplementation, as a natural tomato extract, increased the concentration of lycopene in the human tissues measured. Thus, serum carotenoid concentration provides a measure, at population level, of concentrations in the skin and adipose tissue and probably for other tissues not sampled in the present study.

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References

Agarwal S & Rao AV (1998) Tomato lycopene and low density lipoprotein oxidation: a human dietary intervention study. *Lipids* **33**, 981–984.

Ben-Dor A, Nahum A, Danilenko M, *et al.* (2001) Effects of acyclo-retinoic acid and lycopene on activation of the retinoic acid receptor and proliferation of mammary cancer cells. *Arch Biochem Biophys* **391**, 295–302.

Bohm V & Bitsch R (1999) Intestinal absorption of lycopene from different matrices and interactions to other carotenoids, the lipid status, and the antioxidant capacity of human plasma. *Eur J Nutr* **38**, 118–125.

Boileau AC, Merchen NR, Wasson K, Atkinson CA & Erdman JW Jr (1999) Cis-lycopene is more bioavailable than trans-lycopene in vitro and in vivo in lymph-cannulated ferrets. *J Nutr* **129**, 1176–1181.

Bowen PE, Garg V, Stacewicz-Sapuntzakis M, Yelton L & Schreiner RS (1993) Variability of serum carotenoids in response to controlled diets containing six servings of fruits and vegetables per day. *Ann N Y Acad Sci* **691**, 241–243.

Brown ED, Micozzi MS, Craft NE, *et al.* (1989) Plasma carotenoids in normal men after a single ingestion of vegetables or purified b-carotene. *Am J Clin Nutr* **49**, 1258–1265.

Chen L, Stacewicz-Sapuntzakis M, Duncan C, *et al.* (2001) Oxidative DNA damage in prostate cancer patients consuming tomato sauce-based entrees as a whole-food intervention. *J Natl Cancer Inst* **93**, 1872–1879.

Edwards AJ, Vinyard BT, Wiley ER, *et al.* (2003) Consumption of watermelon juice increases plasma concentrations of lycopene and beta-carotene in humans. *J Nutr* **133**, 1043–1050.

Franceschi S, Bidoli E, La Vecchia C, Talamini R, D'Avanzo B & Negri E (1994) Tomatoes and risk of digestive-tract cancers. *Int J Cancer* **59**, 181–184.

Gartner C, Stahl W & Sies H (1997) Lycopene is more bioavailable from tomato paste than from fresh tomatoes. *Am J Clin Nutr* **66**, 116–122.

Giovannucci E (1999) Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *J Natl Cancer Inst* **91**, 317–331.

Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA & Willett WC (1995) Intake of carotenoids and retinol in relation to risk of prostate cancer. *J Natl Cancer Inst* **87**, 1767–1776.

Holloway DE, Yang M, Paganga G, Rice-Evans CA & Bramley PM (2000) Isomerization of dietary lycopene during assimilation and transport in plasma. *Free Radic Res* **32**, 93–102.

Johnson EJ, Qin J, Krinsky NI & Russell RM (1997) Ingestion by men of a combined dose of beta-carotene and lycopene does not affect the absorption of beta-carotene but improves that of lycopene. *J Nutr* **127**, 1833–1837.

Karas M, Amir H, Fishman D, *et al.* (2000) Lycopene interferes with cell cycle progression and insulin-like growth factor I signaling in mammary cancer cells. *Nutr Cancer* **36**, 101–111.

Khachik F, Spangler CJ, Smith JC Jr, Canfield LM, Steck A & Pfander H (1997) Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. *Anal Chem* **69**, 1873–1881.

Kobayashi T, Itjima K, Mitamura T, Torilzuka K, Cyong J & Nagasawa H (1996) Effect of lycopene, a carotenoid, on

intrathymic T cell differentiation and peripheral CD4/CD8 ratio in a high mammary tumor strain of SHN retired mice. *Anticancer Drugs* **7**, 195–198.

Kohlmeier L, Kark JD, GomezGracia E, *et al.* (1997) Lycopene and myocardial infarction risk in the EURAMIC Study. *Am J Epidemiol* **146**, 618–626.

Kucuk O, Sarkar FH, Sakr W, *et al.* (2001) Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy. *Cancer Epidemiol Biomarkers Prev* **10**, 861–868.

Levy J, Bosin E, Feldman B, *et al.* (1995) Lycopene is a more potent inhibitor of human cancer cell proliferation than either α-carotene or β-carotene. *Nutr Cancer* **24**, 257–267.

Michaud DS, Feskanich D, Rimm EB, *et al.* (2000) Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *Am J Clin Nutr* **72**, 990–997.

Micozzi MS, Brown ED, Edwards BK, *et al.* (1992) Plasma carotenoid response to chronic intake of selected foods and beta-carotene supplements in men. *Am J Clin Nutr* **55**, 1120–1125.

Nagasawa H, Mitamura T, Sakamoto S & Yamamoto K (1995) Effect of lycopene on spontaneous mammary tumor development in SHN virgin mice. *Anticancer Res* **15**, 1173–1178.

Nahum A, Hirsch K, Danilenko M, *et al.* (2001) Lycopene inhibition of cell cycle progression in breast and endometrial cancer cells is associated with reduction in cyclin D levels and retention of p27(Kip1) in the cyclin E-cdk2 complexes. *Oncogene* **20**, 3428–3436.

Olmedilla B, Granado F, Southon S, *et al.* (2002) A European multicentre, placebo-controlled supplementation study with alpha-tocopherol, carotene-rich palm oil, lutein or lycopene: analysis of serum responses. *Clin Sci (Lond)* **102**, 447–456.

Paetau I, Khachik F, Brown ED, *et al.* (1998) Chronic ingestion of lycopene-rich tomato juice or lycopene supplements significantly increases plasma concentrations of lycopene and related tomato carotenoids in humans. *Am J Clin Nutr* **68**, 1187–1195.

Paetau I, Rao D, Wiley ER, Brown ED & Clevidence BA (1999) Carotenoids in human buccal mucosa cells after 4 wk of supplementation with tomato juice or lycopene supplements. *Am J Clin Nutr* **70**, 490–494.

Parker RS (1989) Carotenoids in human blood and tissues. *J Nutr* **119**, 101–104.

Pellegrini N, Riso P & Porrini M (2000) Tomato consumption does not affect the total antioxidant capacity of plasma. *Nutrition* **16**, 268–271.

Porrini M & Riso P (2000) Lymphocyte lycopene concentration and DNA protection from oxidative damage is increased in women after a short period of tomato consumption. *J Nutr* **130**, 189–192.

Porrini M, Riso P & Oriani G (2002) Spinach and tomato consumption increases lymphocyte DNA resistance to oxidative stress but this is not related to cell carotenoid concentrations. *Eur J Nutr* **41**, 95–100.

Re R, Fraser PD, Long M, Bramley PM & Rice-Evans C (2001) Isomerization of lycopene in the gastric milieu. *Biochem Biophys Res Commun* **281**, 576–581.

Richelle M, Bortlik K, Liardet S, *et al.* (2002) A food-based formulation provides lycopene with the same bioavailability to humans as that from tomato paste. *J Nutr* **132**, 404–408.

Stahl W, Schwarz W, Sundquist AR & Sies H (1992) cis-trans isomers of lycopene and beta-carotene in human serum and tissues. *Arch Biochem Biophys* **294**, 173–177.

Stahl W & Sies H (1992) Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. *J Nutr* **122**, 2161–2166.

Stahl W, Sundquist AR, Hanusch M, Schwarz W & Sies H (1993) Separation of beta-carotene and lycopene geometrical isomers in biological samples. *Clin Chem* **39**, 810–814.

van Breemen RB, Xu X, Viana MA, *et al.* (2002) Liquid chromatography-mass spectrometry of cis- and all-trans-lycopene in human serum and prostate tissue after dietary supplementation with tomato sauce. *J Agric Food Chem* **50**, 2214–2219.

Wingerath T, Sies H & Stahl W (1998) Xanthophyll esters in human skin. *Arch Biochem Biophys* **355**, 271–274.

Zhang L-X, Cooney RV & Bertram J (1991) Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10T1/2 cells: relationship to their cancer chemopreventive action. *Carcinogenesis* **12**, 2109–2114.

Zhang LX, Cooney RV & Bertram JS (1992) Carotenoids up-regulate connexin-43 gene expression independent of their provitamin-A or antioxidant properties. *Cancer Res* **52**, 5707–5712.

Zhang SM, Tang GW, Russell RM, Mayzel KA, Stampfer MJ, Willett WC & Hunter DJ (1997) Measurement of retinoids and carotenoids in breast adipose tissue and a comparison of concentrations in breast cancer cases and control subjects. *Am J Clin Nutr* **66**, 626–632.