# Absorption of lycopene from single or daily portions of raw and processed tomato

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To study the relationship between lycopene intake and plasma concentration, ten healthy female subjects were given one or more portions of tomato purée or fresh raw tomato containing 16·5 mg total lycopene (all-*trans* + *cis* forms). In Expt 1 subjects (*n* 9) were randomly assigned the single portions of the two tomato products and blood samples were collected to follow the change in plasma carotenoid concentrations within the first 12 h and on each of the following 5 d (104 h). In Expt 2 subjects (*n* 10) were divided into two groups of five each receiving daily dietary portions of tomato purée or fresh raw tomato containing 16·5 mg total lycopene for 7 d. Fasting blood samples were collected daily. In Expt 1 the plasma total lycopene (all-*trans* + *cis* forms) concentration, after the single portions of tomato purée and raw tomato, varied significantly over time, with a first peak reached after 6 h, a further increase after 12 h and a slow decrease until 104 h. In Expt 2, when the tomato products were given daily, there was a day-by-day increase in the plasma total lycopene concentration, and through the following week of a diet without tomato there was a gradual decrease. However, values did not return to basal concentrations. Plasma total lycopene concentration was higher after the tomato purée intake than after the raw tomato in both the first ($F$ (1,8) 7·597; $P < 0·025$) and the second experiments ($F$ (1,8) 12·193; $P < 0·01$) demonstrating a significant effect of food matrix on absorption.

**Absorption: Lycopene: Tomato**

Several studies (Bendich & Olson, 1989; Di Mascio *et al.* 1989; Miller *et al.* 1996) have reported that lycopene, a carotenoid having no pro-vitamin A activity, may have an important role in the antioxidant defence system of man. It has been demonstrated that the antioxidant activity of lycopene *in vitro* is double than that of $\beta$-carotene (Di Mascio *et al.* 1989). Moreover, other studies report a high inverse correlation between the intake of this carotenoid in the diet and the development of some types of cancer, demonstrating its activity *in vivo* also (Gerster, 1993; Franceschi *et al.* 1994; Parfitt *et al.* 1994). Studies on the distribution of lycopene in the body indicate that it is present at relatively high concentrations in plasma and in selected organs (testes, adrenal glands, liver and kidney) where it may have specialized functions (Stahl & Sies, 1996).

The main sources of lycopene in the diet are tomato and tomato products. Very little information is available, however, regarding lycopene availability from food sources and the relationship between lycopene intake and its plasma concentration. There are highly variable data in the literature, mainly regarding the absorption of $\beta$-carotene in man, which range between 2 and 52 % (Goodman *et al.* 1966; Blomstrand & Werner, 1967), and regarding plasma response after the intake of single or multiple doses of the carotenoid from supplements or foods (Willett *et al.* 1983; Dimitrov *et al.* 1988; Micozzi *et al.* 1992).

Recently the uptake of lycopene from a food source (tomato juice) was investigated by Stahl & Sies (1992) who found a greater absorption of the carotenoid from a heat-processed tomato juice, underlining the importance of processing of foods on carotenoid availability. They also found a better absorption of the *cis*-isomers of lycopene compared with the all-*trans* form. They did not investigate, however, the time course of the absorption within the same day of supplementation.

In the present paper results are reported for plasma lycopene levels in response to two food sources when consumed in single or daily portions.

## Materials and methods

### Subjects

Ten female subjects were selected on the basis of their eating habits and plasma carotenoid profile. Eating habits were assessed using a food-frequency questionnaire and a

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food preference list (Porrini *et al.* 1995). Subjects who did not eat fruit and vegetables and those who followed a specific diet or regimen (vegetarians, vegans, macrobiotics, etc.) were excluded. Fasting plasma lycopene concentrations were analysed by means of Box and Whisker Plot (Frigge *et al.* 1989) analysis to omit outliers. All subjects selected participated in both studies. Informal written consent was obtained from each participant and the protocol was approved by the Local Ethical Committee.

### Foods

To study the relationship between lycopene intake and plasma concentration a tomato purée (Sainsbury's double concentrate tomato purée) and a fresh raw tomato (Ramato of Sicily) were used.

Each subject received about 60 g (one small measure, corresponding to a mean weight of 57 g) of tomato purée or 300 g raw tomato, both containing 16·5 mg total lycopene (all-*trans* + *cis* forms as determined by HPLC) together with 10 g olive oil on a slice of bread (40 g). This provided a mean of 0·56 (SD 0·07) μmol total lycopene/kg body weight from both the sources.

### Experimental procedure

All the subjects were asked to consume a controlled diet low in carotenoids and free of lycopene for 7 d (t = −7) before starting the tomato intake (t = 0) and for the subsequent period of experimentation.

Subjects were given clear written instructions about allowed and forbidden foods plus a basal daily menu. For example, the lunch menu consisted of a first course and a second course freely chosen from those reported in the list of allowed foods, a fixed portion of a specific vegetable (lettuce, potato, aubergine, cauliflower, fennel) and a specific fruit (apple, banana, pear, pineapple, strawberry). The dinner consisted of a first and a second course freely chosen from those reported in the list of allowed foods; no vegetables or fruit were allowed. An expert dietitian was also available to answer any questions about the diet and to check the compliance with the diet.

Menus were designed to have a carotenoid content lower than 0·6 mg/d. The content of carotenoids in plasma was analysed at the beginning and the end of the first 7 d of the diet without tomato. Two experiments were scheduled: in

order to use a single batch of fresh raw tomato, limiting product variability, its consumption was concentrated in the shortest period of time (17 d) as reported in Fig. 1. A 10 d 'wash out' period (low-carotenoid diet) was observed between each session of tomato intake.

### Expt 1: effect of a single portion of tomato purée or raw tomato

In this experiment all the subjects had single portions of tomato purée and raw tomato on two experimental occasions. In order to use a single batch of fresh raw tomato all the subjects consumed the fresh tomato at the same time, while the tomato purée was randomized (Fig. 1).

Subjects were instructed to fast from 22.00 hours the night before the day of the experiment and to come to the laboratory early in the morning to consume the tomato with the slice of bread and the olive oil.

To follow the increase in plasma concentrations of carotenoids, blood samples were collected just before eating and every 2 h for 12 h after eating. Then blood samples were collected at 24, 32, 56, 80 and 104 h (5 d) to verify plasma clearance.

In order to improve the compliance with the diet, on the first day of experimentation all the meals were eaten in the laboratory.

### Expt 2: effect of daily portions of tomato purée and raw tomato

In this experiment subjects were randomly divided into two groups, five receiving daily portions of tomato purée and the other five receiving raw tomato for seven consecutive days (Fig. 1). The two sources were consumed each day at home with the main meal, together with the same quantity of olive oil as in Expt 1. Compliance with the diet was checked daily by an expert dietitian. Blood samples were collected from fasting subjects daily during the first 5 d then on day 7 (end of the supplementation) and on days 9 and 11 to follow plasma clearance.

### Collection of blood samples

Blood samples (about 500 μl) were taken by pricking the finger tip with disposable Glucolet lancets and collected in lithium-heparin microtubes. Blood was then centrifuged and
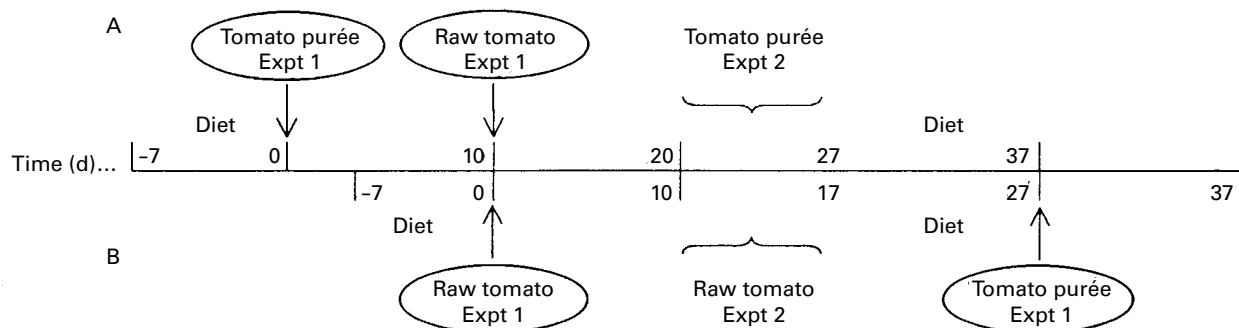


**Fig. 1.** Experimental procedures for group A (five subjects) and group B (five subjects).

plasma obtained was stored at −80° until analysis. All the analyses were performed within 3 months.

### Extraction and HPLC analysis of foods and plasma

The extraction and the HPLC analysis of carotenoids from vegetable foods and plasma were performed according to a method previously described, partially modified in the extraction phase (Riso & Porrini, 1997). Briefly, tomato samples were extracted exhaustively with tetrahydrofuran, minimizing isomerization and degradation by performing the operation in the dark and using butylated hydroxytoluene as antioxidant. The extract was then recovered in petroleum ether and portions of the organic phase evaporated under $N_2$ in the dark and redissolved in the HPLC mobile phase.

Plasma extraction was performed on 100 μl using 100 μl ethanol (with echinen one as external standard) and 200 μl hexane. After vortex-mixing for about 1 min and centrifuging for 5 min at 1000 $g$, 150 μl of the supernatant fraction was evaporated under $N_2$ in the dark and redissolved in 100 μl of the mobile phase.

The HPLC analysis was performed by using a 5 μm Vydac 201 TP 54 $C_{18}$ column (250 × 4·6 mm, i.d.), fitted with a $C_{18}$ guard column and biocompatible frits. The eluent consisted of methanol-tetrahydrofuran (95:5, v/v) at a flow rate of 1 ml/min. The detector was set at 445 nm using a u.v.-visible detector (Varian 2010; Varian, Mildford, CA, USA). An example of a plasma chromatogram is presented in Fig. 2.

Carotenoid concentrations were calculated by means of a mix of standards containing lutein, zeaxanthin, $\beta$-cryptoxanthin (Hoffman–La Roche, Basel, Switzerland), $\alpha$-carotene and $\beta$-carotene (Sigma Chemical Co., St. Louis, MO, USA). Stock solutions of these carotenoids were prepared monthly and kept under $N_2$ at −20°. Lycopene (Sigma) solution was divided into portions in brown glass vials and dried under $N_2$ before storing at −20° (Hart & Scott, 1995). To avoid degradation, the lycopene working solution was prepared daily at the same concentration, in the range of linearity evaluated in our working conditions (Riso & Porrini, 1997), and injected separately. Concentrations of all standards were calculated from absorption values (E1 % 1 cm) using a spectrophotometer (Varian DMS 90; Varian, Mulgrave, Australia). Carotenoid purity was checked by HPLC analysis. Carotenoid concentrations in samples were corrected by the recovery of the external standard. The recovery was between 80 and 100 %.

### Data analysis

Only complete sets of data in each experiment were analysed. As one subject missed the blood sampling in the days after raw tomato intake, only nine out of the ten subjects were considered in the first experiment.

Plasma total lycopene concentrations before and after the 7 d of diet low in carotenoids (t = −7 and t = 0) were analysed by means of Student's $t$ test. The relationships between the decrease in plasma lycopene concentration during this period of diet and its basal concentrations with the subsequent increase after tomato consumption (expressed as area under the curve, AUC) were analysed by means of Spearman's rank correlation test.

The AUC were calculated using a trapezoidal approximation. Baseline was not subtracted. A two-way ANOVA for repeated measures design was used to verify the effect of the type of tomato and time (from the consumption of the single portion, h) on lycopene and the other carotenoid concentrations (Expt 1). A two-way ANOVA with type of tomato as independent factor and time (of supplementation, d) as dependent factor was used in Expt 2. The least significant difference test was performed to evaluate specific significance. The AUC for tomato purée and raw tomato were compared by means of Student's $t$ test.

## Results

The carotenoid contents of the raw tomato and the tomato purée are reported in Table 1. Volunteer characteristics are reported in Table 2. The mean plasma total lycopene concentration after the 7 d of the controlled diet low in carotenoids and free of lycopene, 0·30 (SD 0·11) μmol/l, was significantly lower than values at recruitment ($t$ 5·75, $P = 0·0004$). The change of plasma lycopene concentration during this period of diet was 0·28 μmol/l (95 % CI 0·21, 0·35).

The Spearman rank correlation test showed that neither the basal concentrations nor the decrease of lycopene concentrations during the 7 d of diet were related to the subsequent increase of plasma lycopene (expressed as AUC) ($r_s$ 0·52, $P = 0·15$ and $r_s$ 0·43, $P = 0·24$).

### Expt 1

In Fig. 3 the mean plasma response curves of total lycopene, all-*trans* and *cis* lycopene after the ingestion of a single portion of tomato purée and raw tomato are reported. The mean initial plasma lycopene concentrations are reported in Table 3. Values were not significantly different.

After the consumption of tomato there was a rapid increase in plasma lycopene concentration with a first

**Table 1.** Carotenoid content (mg/kg) of raw tomato and tomato purée

| Tomato product | All-*trans* lycopene | *Cis* lycopene | $\beta$-Carotene |
|---|---|---|---|
| Raw tomato | 42 | 13 | 3 |
| Tomato purée | 280 | 10 | 10 |

**Table 2.** Characteristics of volunteers at recruitment (t = −7)

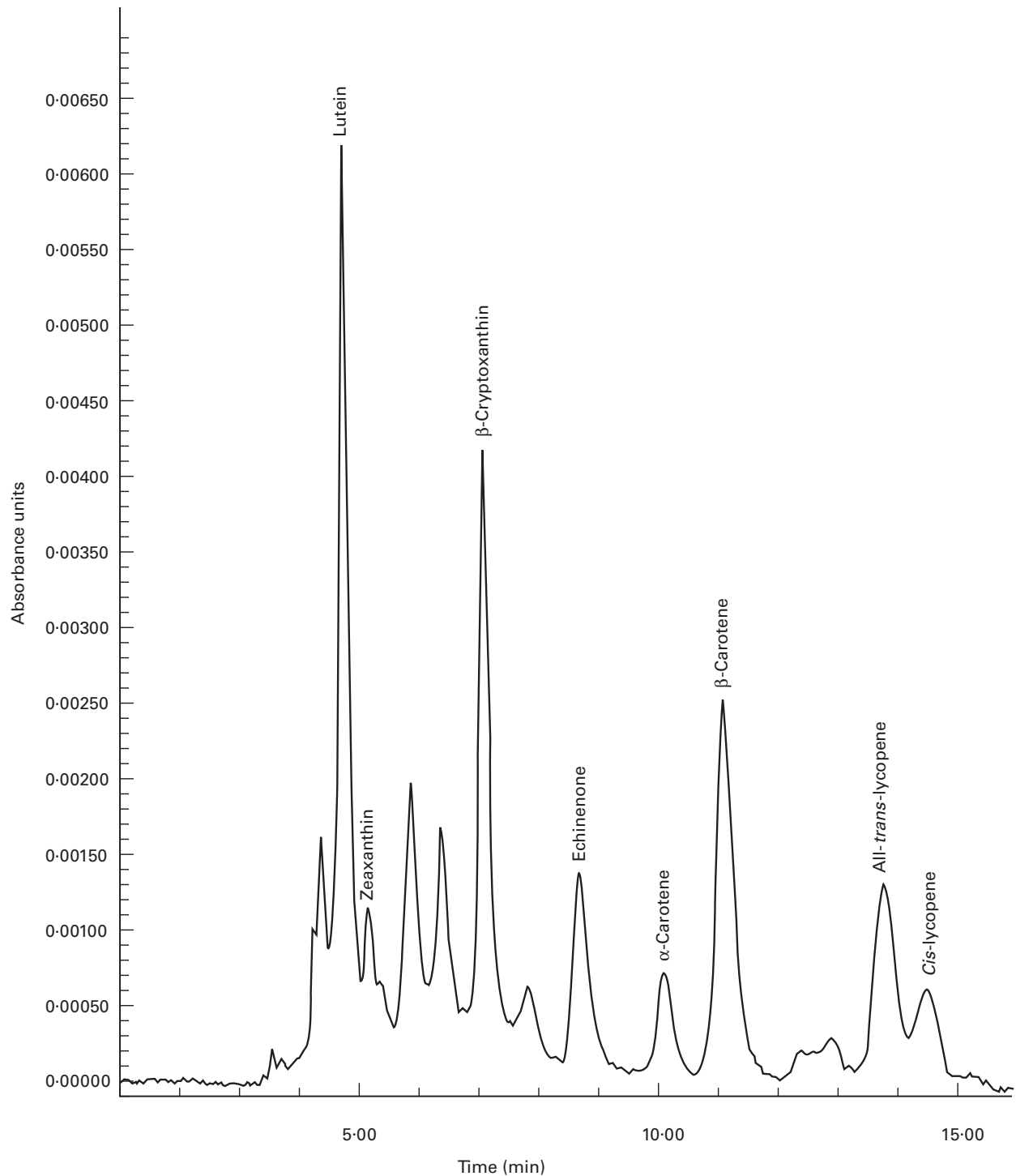| | Mean | SD |
|---|---|---|
| Age (years) | 27·5 | 3·8 |
| Weight (kg) | 54·1 | 6·6 |
| Fasting plasma concentrations | | |
| Total lycopene (μmol/l) | 0·59 | 0·06 |
| All-*trans* lycopene (μmol/l) | 0·37 | 0·08 |
| *Cis* lycopene (μmol/l) | 0·22 | 0·05 |
| Lutein (μmol/l) | 0·47 | 0·03 |
| Zeaxanthin (μmol/l) | 0·05 | 0·02 |
| $\beta$-Cryptoxanthin (μmol/l) | 0·24 | 0·05 |
| $\alpha$-Carotene (μmol/l) | 0·09 | 0·03 |
| $\beta$-Carotene (μmol/l) | 0·58 | 0·17 |

**Fig. 2.** Chromatogram of carotenoids in a plasma sample.

peak at 6 h (individual range 4–8 h) for raw tomato and at 6–8 h for tomato purée, and a further peak at 12 h. There was a significant decline in plasma lycopene concentration at 24, 32 and 56 h, with respect to 12 h, and lycopene concentrations were always higher after tomato purée consumption than raw tomato ($P < 0.03$). After 104 h the concentrations returned to values not significantly different from the basal ones.

ANOVA showed an effect of type of tomato ($F(1,8)$ 7·597; $P = 0.025$) and time on lycopene concentrations ($F(11,88)$ 10·047; $P < 0.0001$). Although the curves for the two types of tomato were similar over time, significantly higher values for total lycopene were registered in the range 2–80 h (always $P < 0.05$) for the tomato purée with respect to the raw tomato. The interaction between type of tomato and time was not significant.
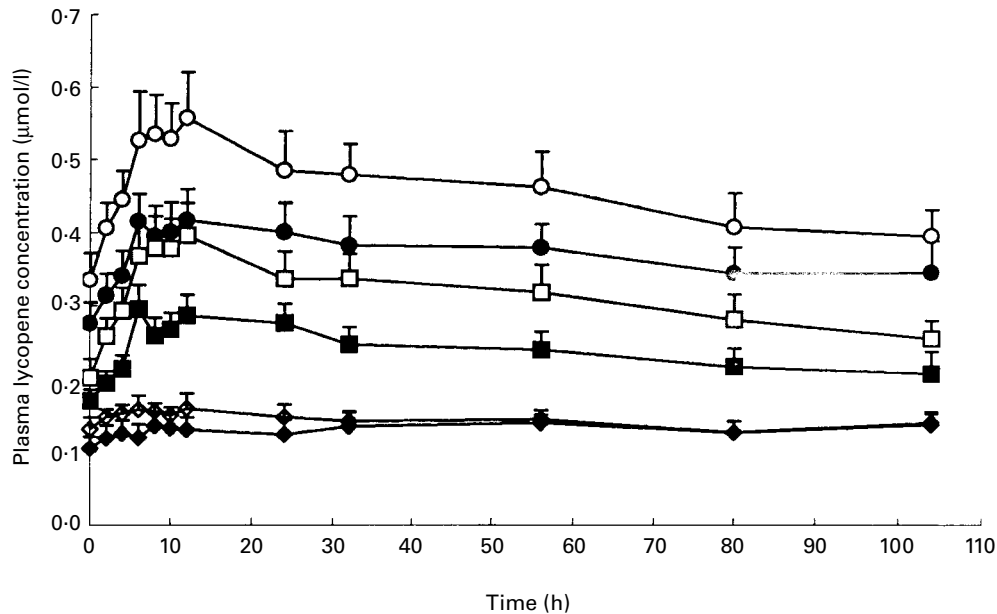
**Fig. 3.** Expt 1. Plasma lycopene concentrations over time in subjects consuming a single portion of raw tomato or tomato purée. Values are means for nine subjects, with standard errors represented by vertical bars. (O), Total lycopene, tomato purée; (●), total lycopene, raw tomato; (□), all-*trans* lycopene, tomato purée; (■), all-*trans* lycopene, raw tomato; (◇), *cis* lycopene, tomato purée; (◆), *cis* lycopene, raw tomato. For details of subjects and procedures, see Table 2 and pp. 353–355.

**Table 3.** Carotenoid concentrations in the serum of subjects who subsequently received supplements containing raw tomato or tomato purée in two different experiments*

(Mean values and standard deviations for nine subjects (Expt 1) and five subjects (Expt 2))

|  | Expt 1 | | | | Expt 2 | | | |
|---|---|---|---|---|---|---|---|---|
|  | Raw tomato | | Tomato purée | | Raw tomato | | Tomato purée | |
|  | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Total lycopene (μmol/l) | 0·28 | 0·09 | 0·33 | 0·11 | 0·24 | 0·09 | 0·34 | 0·11 |
| All-*trans* lycopene (μmol/l) | 0·17 | 0·05 | 0·20 | 0·08 | 0·15 | 0·04 | 0·22 | 0·09 |
| *Cis* lycopene (μmol/l) | 0·10 | 0·05 | 0·13 | 0·05 | 0·09 | 0·06 | 0·12 | 0·03 |
| Lutein (μmol/l) | 0·31 | 0·11 | 0·43 | 0·14 | 0·34 | 0·11 | 0·36 | 0·03 |
| Zeaxanthin (μmol/l) | 0·03 | 0·02 | 0·05 | 0·03 | 0·05 | 0·03 | 0·03 | 0·02 |
| β-Cryptoxanthin (μmol/l) | 0·16 | 0·09 | 0·24 | 0·12 | 0·14 | 0·08 | 0·24 | 0·08 |
| α-Carotene (μmol/l) | 0·06 | 0·03 | 0·08 | 0·04 | 0·07 | 0·04 | 0·05 | 0·04 |
| β-Carotene (μmol/l) | 0·38 | 0·17 | 0·50 | 0·16 | 0·36 | 0·14 | 0·42 | 0·22 |

* For details of subjects and procedures, see pp. 353–355.

**Table 4.** Expt 1. Individual and mean areas under the curve (μmol.h/l) for total lycopene and its all-*trans* and *cis* isomers, calculated from 0 to 104 h after consumption of raw tomato or tomato purée

| Subject | Raw tomato | | | Tomato purée | | |
|---|---|---|---|---|---|---|
|  | Total | All-*trans* | *Cis* | Total | All-*trans* | *Cis* |
| 1 | 49·6 | 32·6 | 17·0 | 61·1 | 44·3 | 16·7 |
| 2 | 54·3 | 30·5 | 23·9 | 57·7 | 38·7 | 19·0 |
| 3 | 38·1 | 24·2 | 13·9 | 42·2 | 28·4 | 13·7 |
| 4 | 34·3 | 21·7 | 12·6 | 45·6 | 30·2 | 15·4 |
| 5 | 26·2 | 16·9 | 9·2 | 36·2 | 24·1 | 12·1 |
| 6 | 38·0 | 24·1 | 14·0 | 62·1 | 43·9 | 18·2 |
| 7 | 22·5 | 13·8 | 8·7 | 29·6 | 18·7 | 11·0 |
| 8 | 40·0 | 25·9 | 14·1 | 59·5 | 41·9 | 17·6 |
| 9 | 44·1 | 33·7 | 10·3 | 31·6 | 22·5 | 9·1 |
| Mean | 38·6* | 24·8* | 13·7 | 47·3 | 32·5 | 14·8 |
| SD | 10·2 | 6·8 | 4·6 | 13·1 | 9·9 | 3·5 |

Mean values were significantly different from the corresponding values for tomato purée, *$P < 0.04$.

**Table 5.** Expt 2. Individual and mean areas under the curve (μmol.d/l) for total lycopene and its all-*trans* and *cis* isomers, calculated from 0 to 11 d; subjects consumed raw tomato or tomato purée daily for the first 7 d

| | Raw tomato | | | | Tomato purée | | |
|---|---|---|---|---|---|---|---|
| Subject | Total | All-*trans* | *Cis* | Subject | Total | All-*trans* | *Cis* |
| 1 | 3·7 | 2·6 | 1·1 | 6 | 7·8 | 5·6 | 2·2 |
| 2 | 7·8 | 6·0 | 1·8 | 7 | 9·0 | 6·3 | 2·6 |
| 3 | 5·6 | 4·6 | 1·0 | 8 | 7·2 | 5·3 | 1·8 |
| 4 | 4·6 | 3·5 | 1·1 | 9 | 7·1 | 5·9 | 1·2 |
| 5 | 3·7 | 2·9 | 0·8 | 10 | 7·6 | 5·8 | 1·8 |
| Mean | 5·1* | 3·9* | 1·2* | Mean | 7·7 | 5·8 | 1·9 |
| SD | 1·7 | 1·4 | 0·4 | SD | 0·8 | 0·4 | 0·5 |

Mean values were significantly different from the corresponding values for tomato purée, *$P < 0.03$.

In Table 4 the individual and mean AUC values are reported. Mean AUC values were always higher after the tomato purée than after the raw tomato ($t$ 2·506; $P < 0.04$). As regards individual values, apart from one subject the AUC after tomato purée were higher than after raw tomato. The major contributor to the total lycopene concentrations was always the all-*trans* form as shown in Fig. 3. ANOVA again showed an effect of the type of tomato ($F (1,8)$ 7·855; $P = 0.023$) and the time ($F (11,88)$ 14·820; $P < 0.0001$) on the levels of all-*trans* lycopene, while the concentration of the *cis* forms was independent of these factors.

The ANOVA did not show any effect of the type of tomato on plasma responses of the other carotenoids. Significant modifications over time were seen for lutein, zeaxanthin, β-cryptoxanthin and α-carotene ($P < 0.05$), but not for β-carotene (results not shown).

### Expt 2

In Fig. 4 the plasma response curves of total lycopene, all-*trans* and *cis* lycopene registered after daily portions of tomato purée and raw tomato are reported. The mean initial lycopene concentrations are presented in Table 3. Data were not significantly different.

The consumption of both the tomato products for 7 d produced a significant daily increase of the plasma lycopene concentration until the last day of supplementation and a gradual decrease on days 9 and 11 as demonstrated by ANOVA ($F (7,56)$ 22·548; $P < 0.0001$). The significant effect of type of tomato ($F (1,8)$ 12·193; $P = 0.008$) confirms that again higher values of lycopene concentration were reached after the consumption of the tomato purée. The post hoc comparison by least significant difference test showed significant differences at each time (t = 1, 2, 3, 4 and 7 d
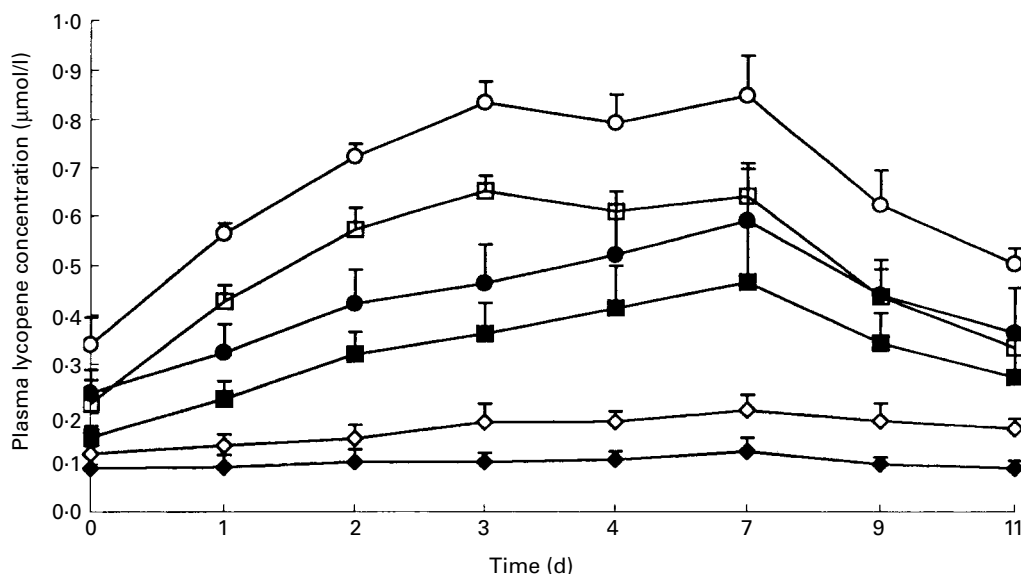


**Fig. 4.** Expt 2. Plasma lycopene concentrations over time in subjects consuming daily portions of raw tomato or tomato purée from days 1 to 7. Values are means for five subjects, with standard errors represented by vertical bars. (O), Total lycopene, tomato purée; (●), total lycopene, raw tomato; (□), all-*trans* lycopene, tomato purée; (■), all-*trans* lycopene, raw tomato; (◇), *cis* lycopene, tomato purée; (◆), *cis* lycopene, raw tomato. For details of subjects and procedures, see Table 2 and pp. 353–355.

$P < 0.0001$, t = 9 and 11 d $P < 0.04$). A 5 d period of diet without tomato was not sufficient to reduce plasma lycopene concentrations to basal values.

The slight bending of the curve on day 5 after the tomato purée intake was due to two subjects who showed decreases in the lycopene concentration after only 4 d of supplementation; this probably explains the significant effect of the interaction (type of tomato × time) found ($F(7,56)$ 2·191; $P = 0.049$).

In Table 5 the individual and mean AUC values are reported. A significantly higher AUC was registered after the tomato purée intake ($t$ 3·164; $P < 0.013$).

As regards the concentrations of the two isomers (Fig. 4), the all-*trans* form was again predominant and was the main contributor to the lycopene concentration (effect of type of tomato ($F(1,8)$ 11·595; $P = 0.009$), effect of time ($F(7,56)$ 19·212; $P < 0.0001$). The *cis* lycopene concentrations were statistically different after the two tomato products ($F(1,8)$ 6·089; $P = 0.039$); higher mean values were registered after the tomato purée particularly from days 4 to 7 of supplementation ($F(7,56)$ 5·294; $P = 0.0001$). The ANOVA did not show any effect of the type of tomato on plasma responses of the other carotenoids, while there was an effect of time for $\alpha$-carotene and $\beta$-carotene ($P < 0.05$) (results not shown).

## Discussion

Apart from the physiological variables, many dietary factors may significantly affect the digestion and absorption of carotenoids. The food matrix as well as the processing of foods seem to be important determinants for the release of carotenoids. While many studies report data on $\beta$-carotene absorption (van Vliet, 1996; Parker, 1997), little information is available on lycopene. To study lycopene absorption we used two tomato products: a raw tomato generally eaten fresh and a tomato purée that had undergone a process of homogenization and heating (industrial production). The results obtained clearly show that one portion of both the raw and processed tomatoes, corresponding to about 0·56 µmol lycopene/kg body weight, was able to increase significantly the plasma lycopene concentration. This amount of lycopene, however, although higher than that generally eaten with a free diet, was not high enough to reach the concentrations obtained with daily portions of tomato purée.

The absorption of lycopene from the processed tomato was greater than from raw tomato. In both the experiments the AUC values we found were always higher after the tomato purée than after the raw tomato. This fact could be attributed to a lower availability of the carotenoid from raw product where it is probably trapped in the matrix, while in the tomato purée heat could have induced the rupture of the cell walls accompanied by the release of lycopene.

Stahl & Sies (1992) found that heating 700 ml tomato juice for 1 h before consumption led to an increase in lycopene serum concentration with respect to the same tomato juice not heated.

Analysis of the plasma lycopene concentration over time after the single portion of tomato showed that a first peak was rapidly reached at about 6 h, and another peak after 12 h. From our results it is not possible to state whether after this time there was a further increase in plasma lycopene concentration as we did not follow subjects between 12 and 24 h (night time). Results reported in the literature vary, owing to differences in the methodological approach. Stahl & Sies (1992) found that the peak serum concentration (after a dose of three different amounts of lycopene: 0·35, 1·25, 2·5 µmol/kg body weight) was always reached between 24 and 48 h and that the carotenoid disappeared with a half-life of 2–3 d; however they collected blood samples once daily and did not follow the increase of the lycopene concentration in the first 12 h as we did. From our results, approximately 3 d were sufficient to have plasma concentrations returned close to the baseline.

In a study where monkeys were supplemented with labelled lycopene, the maximum plasma concentration was reached within 8–48 h, while in rats the peak was seen between 4 and 8 h (Matthews-Roth *et al.* 1990), similar to our observations. More recently Bierer *et al.* (1994) studied the plasma response to 20 mg lycopene in the preruminant calf; they found a peak after 16 h, while for lutein the peak was after 12 h and for $\alpha$- and $\beta$-carotene after 24 h.

Kostic *et al.* (1995) studied the absorption of lutein and $\beta$-carotene in human subjects, giving about the same amounts of carotenoids per kg body weight as in the current study. They found a peak for lutein absorption at 16 h (range 12–32 h), while for $\beta$-carotene there was a first peak at 6 h (range 4–12 h), a decrease at about 8 h and a new increase at 32 h (range 16–32 h); after that there was a continuous decline to reach values near baseline by 128 h. From our results lycopene seems to be more similar to $\beta$-carotene than to lutein; this may be due to their similar hydrophobic structures. So, as already suggested, the absorption kinetics may differ among the carotenoids depending on their polarity. Kostic *et al.* (1995) suggested that the peak of $\beta$-carotene at 6 h may derive from the chylomicron-borne $\beta$-carotene, whereas the subsequent peak at 32 h may include the release of newly absorbed $\beta$-carotene.

The absorption of carotenoids is thought to be quite a slow process that involves a passive diffusion of the compounds through the intestinal membrane determined by a density gradient that depends on the concentration of the carotenoid in the lumen and inside the enterocyte. The rate of incorporation into chylomicrons is certainly an important factor and explains the role of the presence of fat in the lumen in increasing absorption. Other researchers (Dimitrov *et al.* 1988) have already reported the importance of this factor, so we decided to add a fixed quantity of oil to the tomato in order to standardize the influence of this variable within the subject's diet.

Among the factors able to influence absorption efficiency of carotenoids from food sources, their mutual interaction could play a significant role. To minimize this factor subjects had to consume a diet with a controlled carotenoid content for 7 d before the experimentation and throughout the experimental period, and the compliance with the diet was checked. From our results it seems that lycopene intake did not modify the original plasma concentrations of the other carotenoids.

In the second experiment subjects who consumed daily

portions of tomato purée seemed to reach a steady state for lycopene concentrations between days 4 and 7 after the start of intake; this did not happen after the consumption of raw tomato, probably as a consequence of the lower availability of lycopene from this source. At the end of the period of supplementation (day 7 of experiment) the plasma lycopene concentrations began to reduce significantly, although they had not reached the basal values after 5 d (day 11).

From these results it would seem that the increases and decreases in plasma lycopene concentrations are easily obtained by adding natural sources to, or removing them from the diet. This is in contrast to the hypothesis that plasma concentrations of carotenoids are the result of long-term diet rather than short-term intake. Brown *et al.* (1989) suggested that the steady-state plasma concentration of carotenoids is changed only slightly by a single large intake of a green leafy or yellow vegetable, but they studied mainly $\beta$-carotene. From our results it seems that a reasonable intake of tomato is able to increase significantly plasma lycopene concentrations.

Brown *et al.* (1989) also reported the profile of plasma carotenoids for a subject fed on a low-carotenoid diet for 14 d: they did not find a decline for $\beta$-carotene, $\alpha$-carotene or cryptoxanthin, but noted a progressive decline for lycopene. While the authors explained this fact by indicating that their subjects had a high basal consumption of tomato-containing foods, such as pizza, it could also be hypothesized that plasma lycopene is more responsive than $\beta$-carotene to the amount ingested. In fact after 7 d of the low-carotenoid diet we found a significant decrease in mean plasma lycopene concentration (about 50 %) indicating once again a clear responsiveness to the diet.

Micozzi *et al.* (1992) also registered a sharp decline of lycopene concentration in their placebo group following a control-diet period and a subsequent rise after change to a self-selected diet. A similar result was found by Rock *et al.* (1992) who investigated the effect of a low-carotenoid diet on plasma response and indicated that there was a profound and rather immediate influence on carotenoid concentrations with respect to that observed with the fat-soluble vitamins A and E. They followed plasma concentrations for 13 weeks and found a marked decrease in the first days of diet followed by a lower decrease in the subsequent period and suggested that two pools of carotenoids exist in the body: one which responds rapidly to changes in the carotenoid intake, and another which is more resistant to depletion and may represent tissue stores.

Carotenoids seem to concentrate in those tissues with a large number of LDL receptors and a high rate of lipoprotein uptake (i.e. liver, adrenal gland, testes). Furthermore it has been recently reported (Zhu *et al.* 1997) that fat-free mass may play a more active role than adipose tissue in the disposition of newly absorbed carotenoids during the non-steady-state condition (supplementation period) and this may support the hypothesis of the two pools of carotenoids. It has been suggested that differences in fat-free mass between subjects could be a potential cause of individual differences in plasma response to a carotenoid load and may contribute towards the inter-individual variability that is generally found in these kinds of studies.

All these results should be carefully considered in the epidemiological studies where plasma concentrations of carotenoids are correlated to risk factors for specific diseases. In this case, just one blood sample may not be representative of the habitual dietary pattern of the subjects studied. This could also be the problem of the low correlation found between plasma concentrations (from one blood sample) and dietary intake evaluated by more days of food registration or by diet history methods (Ascherio *et al.* 1992; Scott *et al.* 1996).

As regards the two different geometrical isomers of lycopene, it would seem that the all-*trans* form is absorbed to a greater extent than the *cis* form from both the tomato products. Furthermore, the different all-*trans:cis* ratios of the two products, 3·2 in raw tomato and 28 in tomato purée, were not reflected in plasma, where 12 h after the single portion of raw tomato and tomato purée the ratios were 2·3 (SD 0·4) and 2·5 (SD 0·4) respectively, and after 7 d of daily portions were 4·2 (SD 2·5) and 3·2 (SD 0·7) respectively. From these data it may be suggested that *cis* lycopene is absorbed more efficiently than the all-*trans* form.

Stahl & Sies (1992), analysing the increase in plasma concentrations following the consumption of tomato, hypothesized a higher availability of the *cis* isomers (9-13-*cis* lycopene) compared with the all-*trans* form. In our study the higher plasma *cis* lycopene concentrations reached after the daily consumption of the tomato purée *v.* raw tomato support the hypothesis of a better availability of both the *trans* and *cis* forms from the processed product.

A recent study (Stahl *et al.* 1995) provided evidence for a preferential accumulation of all-*trans* $\beta$-carotene in chylomicrons and VLDL compared with the 9-*cis* isomer and suggested an efficient isomer-selective mechanism for intestinal uptake or a very rapid elimination into tissues (25 % in the liver, 10 % in adrenals). This may be hypothesized for lycopene too, as results in the literature show that the all-*trans* form and the *cis* isomers of lycopene are present in different concentrations in the plasma and tissues (Stahl *et al.* 1992).

## Conclusion

In conclusion, this study showed that there is a fast response of plasma lycopene concentration when the diet is modified (both when dietary intake increases or decreases). Consequently the regular consumption of lycopene-containing foods is necessary to maintain plasma concentrations.

Industrial processing of tomato (e.g. homogenization and heating) improves lycopene availability, and this fact may be particularly important when considering the food habits of Northern populations who do not have a high consumption of fresh tomato. Recently it has been suggested that the lower oxidative status (evaluated by means of biochemical variables) of an Italian population (Naples) compared with an English population (Bristol) could be attributed to the higher tomato intake (Parfitt *et al.* 1994).

The introduction into the diet of a tomato purée, which is easily available and acceptable (and can be added to habitual foods without changing the diet completely) may represent a relatively easy way to increase the plasma lycopene concentration so decreasing the risk of diseases associated with oxidative stress.

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