Appendix A: Detailed summary and evaluation of the nutritional information for Lyc-O-Mato®

Contents

1. Dietary lycopene intake and bioavailability

Lycopene occurs in the human diet predominantly in tomatoes and tomato products (see Table 1.1). They provide over 85% of the total lycopene consumed and occur in popular dishes such as chili con carne, pizza or spaghetti with tomato sauce and tomato-based meat sauce. Because of the frequency of consumption, tomato ketchup is also a major contributor of lycopene in many diets (reviewed by Gerster, 1997).

Table 1.1 Natural lycopene concentrations in food (Gerster, 1997; Tonucci *et al*., 1995*)

Food	Form	Lycopene concentration (µg/100g)
Tomatoes	Fresh, raw	3100 (range 879-0200) 9270± 1020*
Tomatoes	Fresh, cooked	3703
Tomatoes	Sauce, canned	6205 17980 ±1470*
Tomatoes	Paste, canned	6500 (range 5400-15000) 55450 ±4330*
Tomatoes	Juice, canned	8580 (range 5000-11600) 10770± 1070*
Tomatoes	Ketchup	9900 17230±2180*
Apricot	Dried	864
Apricot	Canned, drained	65
Apricot	Raw	5
Grapefruit	Pink, raw	3362
Guava	Juice	3340
Guava	Raw	5400 (range 5340-5500)
Rosehip	Puree	780
Watermelon	Canned	4100 (range 2300-7200)
Papaya	I Fresh -	12000-6300

The all-trans form is the most stable isomer of lycopene. Due to the high number of double bonds, carotenoids can undergo trans to cis isomerisation if exposed to heat or light (within the absorption range). In all lycopene-containing fruit and vegetables, all-trans lycopene is the predominant form, with variable amounts of cis-isomers.

The estimated intakes from food sources in the Netherlands is on average 1.05 ± 1.56 mg/day in men, and 1.33 ± 1.88 mg/day in women (Goldbohm *et al*, 1998).

Intake data from the US show higher intakes (Forman *et al*., 1993), a daily intake of lycopene of about 3.7 mg/day was found. Depending on the actual product use, daily intake can be as high as 15-30 mg from normal daily servings (or even higher in the case of red palm oil use which may contain up to 20 g lycopene per 100 g). In a more recent report from the US, an average daily intake of 5.08 mg lycopene was calculated (Schweizer et al., 1999).

In a British study (Scott *et al*., 1996) the daily consumption of lycopene-rich food was equivalent to a lycopene intake of about 1.1 mg/day. A lower intake was determined in the Finnish Mobile Clinic Health Examination Survey with a mean intake of 0.7 mg/day for men and 0.9 mg/day for women (Jarvinen, 1995). The Nordic Council of Ministers (Strube and Dragsted, 1999) reports an estimated lycopene intake for Sweden and Finland of 0.34 and 0.26 mg/day.

- *Lycopene absorption and postprandial metabolism*

Lycopene is fat-soluble and absorbed in parallel with fat digestion and absorption. Mastication and gastric action are required, as well as digestive enzyme activity, such as pancreatic lipase, to dissociate lycopene from the (food) matrix and to effect dissolution/dispersion in the lipid phase (fat globules). In the presence of bile acids, free fatty acids, monoglycerides and phospholipids, lycopene becomes incorporated in the mixed micelles. These mixed micelles mediate uptake of lycopene into the enterocyte after passive diffusion across the unstirred water layer. In the intestinal mucosa lycopene becomes incorporated in the chylomicron fraction, which is released into the lymphatic system and subsequently in the blood stream (reviewed by Clinton (1998) and by Johnson (1998)).

The efficiency of absorption therefore depends on the same factors that influence fat absorption, but especially disposition of lycopene from the (food) matrix, and the efficiency of incorporation into the mixed micelles. Absence of bile or any generalised malfunction of the lipid absorption system will interfere with absorption of lycopene (reviewed by Clinton (1998) and by Johnson (1998)).

In plasma, lycopene is transported exclusively by lipoproteins, approximately 75% by LDL and 25% by HDL and VLDL.

Lycopene is predominantly found in testes and adrenals, but significant amounts are also found in the liver, adipose tissue, prostate, kidney and ovary (reviewed by Clinton (1998)

and by Johnson (1998)). More than 50% of total lycopene in human tissue and serum exists of 9cis, 13cis and 15cis-isomers, while the 5-cis and 9-cis are the main cis-isomers in plasma (Stahl *et al*., 1992; Schierle *et al*., 1997; Nguyen and Schwartz, 1999).

In contrast to other carotenoids, plasma concentrations of lycopene are not found to be higher in women than in men, become lower with increasing age, and are not inversely related to alcohol intake (reviewed by Johnson, 1998).

- *Effect of processing and of isomerisation on lycopene bioavailability*

Food processing of tomatoes may result in degradation (oxidation) and isomerisation of lycopene. Thermal processing, especially heating, may cause losses and results in isomerisation of all-trans (all-E) to cis (Z) forms. Dehydration has a similar effect. The extent of degradation reported varies widely. In some studies, losses up to 60% were found after heating tomato pulp, while others reported only minimal losses during bench-top food preparation of fruits and vegetables (for review see Nguyen & Schwartz, 1999; Shi & Le Maguer, 2000). In general, processed (canned) tomato products have a higher cis-trans ratio than raw tomatoes. Isomerisation affects the chemical and physical characteristics of the product, such as a lower colour intensity.

Recent studies indicate that the extent of isomerisation is probably less relevant in terms of bioavailability, as under *in vivo* conditions significant isomerisation occurs during passage through the gastrointestinal tract as a result of the higher temperature (ca. 37ºC), and the presence of catalysts (such as the acid conditions in the stomach) and oxidants (such as Fe^3) (Holloway *et al*., 2000). Remarkably, food processing has been shown to improve bioavailability of lycopene. Stahl and Sies (1992) were the first to demonstrate that lycopene bioavailability from tomatoes can be greatly improved by heating in the presence of fat. Boiling tomato juice with 1% corn oil for 1 hour resulted in a two to threefold increase in serum lycopene concentrations as compared to unprocessed juice.

This is likely explained by a positive effect on the dissolution and solubilisation of lycopene into the lipid phase, related to the crystalline nature of lycopene as present in the plant chromoplast.

In Table 1.2 reported bioavailability studies, mainly based upon plasma responses, after a single dose, or after longer term intervention (1-4 week), with lycopene from different food sources or concentrates (supplements) are summarised. From the results it can be concluded that the plasma all-trans and cis lycopene can be increased after a single

dose/meal of processed tomatoes (juice/puree), but hardly, or not after, feeding raw tomatoes. The increase in plasma level is considered to reflect absorption, but does not allow any conclusions with respect to the absolute absorption rate.

In Table 1.2 the effect on other markers measured in blood (cells), such as effects on DNA damage and lipid oxidation are also presented. From the results summarised in this table it can be seen that lycopene decreased DNA damage and lipidperoxide levels after consumption of tomato products. From the studies reported by Paetau *et al.* (1998) and Bohm & Bitsch (1999), it can be concluded that lycopene, given as encapsulated oleoresin or as a beadlet preparation, has at least the same bioavailability as tomato sauce or juice. In the study from Bohm & Bitsch (1999) oleoresin capsules prepared from the 6% lycopene preparation in natural oils of tomatoes (5 mg lycopene from Lyc-O-Mato®) were compared with tomato juice and raw tomatoes.

Additional data on lycopene bioavailability from various formulations containing Lyc-O-Mato® were reported by Bohm (data provided by LycoRed). He compared plasma lycopene responses after a 4 week supplementation period (preceded by a 2 week period of lycopene depletion), with various hard and soft gel capsules containing 5 mg lycopene (1 capsule per day; n=8 volunteers per treatment group).

Results are summarised in Table 1.3. Significant increases in the plasma lycopene levels were obtained with capsules no.1 (LYCRGR2), 2 (LYCRGGS3), 3 (LYCDWG4) and 4 (LYCHLF1), but not with capsules no. 5 (LYCCWD2) and 6 (LYCALB1). The lycopene content in plasma was higher after supplementation with soft gel capsules (nr.1-3) than after supplementation with hard gel capsules (nr. 4-6).

In one study (by Johnson *et al.*, 1997) a synergistic effect of a combined dose of â-carotene and lycopene on lycopene absorption was observed which was explained by an enhanced solubilisation of lycopene in the presence of â-carotene.

Earlier studies from Brown *et al.*, (1989) and Micozzi *et al.* (1992) showed no response in serum lycopene levels after 12 mg lycopene, however, the studies summarised in Table 1.2 (except Hininger *et al.*, 1997) do show that supplementation with lycopene (either as capsule or tomato product) leads to an increase in plasma lycopene concentrations (up to 6.94 µg/ml).

Table 1.2 Lycopene bioavailability (as plasma lycopene response) from the diet of different food matrices; effect on biomarkers of oxidative stress.

Source	Lycopene content	Study design	Plasma values	Other markers	Reference
60 g tomato puree / tomato free diet	16.5 mg lycopene/d	10 (2 x 5) healthy women, crossover, 3 weeks	Plasma lycopene increase of 0.5 µmol/l in group of tomato diet and decrease of 0.2 µmol/l in group which consumed tomato-free diet.	Comet assay; DNA damage; 30-40% decrease, after H_20_2 treatment.	Riso *et al*, 1999
Soft gel capsules containing tomato oleoresin Tomatoes (80-230 g/d) Tomato juice (59 g/d)	5 mg lycopene	22 females (20-27 yr); depletion 2 weeks. Randomised study with three groups. 6 weeks treatment	Sign. 1 plasma lycopene (0.10 to 0.25 µmol/l) after two weeks of supplementation with lycopene soft gel capsules. Sign. 1 plasma lycopene (0.12 to 0.22µmol/l) after supplementation with tomato juice. Maintenance of these levels during the next four weeks of supplementation. No significant change of the plasma levels after intake of tomatoes.		Bohm & Bitsch, 1999
Week 1-2: tomato juice (330 ml/d) Week 3-4: carrot juice (330 ml/d) Week 5-6: spinach powder (10g/d)	Week 1-2: 40 mg/d lycopene and 1.5 mg/d â-carotene Week 3-4: 21.6 mg/d â-carotene. 15.7 mg/d á-carotene, and 0.5 mg/d lutein. Week 5-6: 11.3 mg/d lutein and 3.1 mg/d â-carotene	23 (non-smoking) men (27-40 yr). 2 weeks depletion.	Sign 1. from baseline after wk 4 (after tomato juice supplementation) of: - mean plasma all-trans-lycopene (0.16 µmol/l to 0.38 µmol/l) - mean cis-lycopene (0.14 to 0.34 µmol/l - lycopene epoxides (0.04 µmol/l to 0.14 µmol/l). After week 8 sign. 1 to appr. Baseline levels of all concentrations.		Muller *et al.*, 1999

Table 1.2 Lycopene bioavailability (as plasma lycopene response) from the diet of the different food matrices; effect on biomarkers of oxidative stress (continued)

Source	Lycopene content	Study design	Plasma values	Other markers	Reference
Placebo Spaghetti sauce (126 g/d) Tomato juice (540 ml/d) Lycopene oleoresin from tomatoes	0 mg lycopene 39.2 mg lycopene 50.4 mg lycopene 75.0 mg lycopene	19 healthy subjects (25-40yr; 10M & 9F): randomised cross-over design, each treatment was for 1 week with a 1 week wash-out phase.	1 average serum lycopene levels for any treatment over placebo was at least two fold. No differences serum lycopene levels between the different treatments. 1 serum lycopene by consumption of spaghetti sauce (39.2 mg lycopene) almost identical to oleoresin (75 mg lycopene)	Average decrease over placebo was 25% for LDL-TBARS and 13% LDL-CD for the tomato products treatment. No significant differences between LDL-TBARS and LDL-CD between different sources of lycopene.	Agarwal & Rao, 1998
Exp 1 (single portion) 300 g Raw tomato 60 g Tomato puree Exp 2 (daily portion 7 days) 300 g Raw tomato 60 g Tomato puree	Portion 16.5 mg total lycopene Raw tomato: 42 mg/kg all-trans lycopene 13 mg/kg cis-lycopene 3 mg/kg â-carotene Tomato puree: 280 mg/kg all-trans lycopene 10 mg/kg cis-lycopene 10 mg/kg â-carotene	10 healthy women (average age of 27.5 years). 7 days depletion. Exp 1: single portion of both raw tomato and tomato puree on different occasions. Exp 2: Two groups receiving daily portions of raw tomato or tomato puree during 7 days.	Exp 1: Plasma total lycopene concentration, after the single portions of tomato puree and raw tomato, varies significantly over time, with a first peak after 6h, a further increase after 12h and a slow decrease after 104h. Exp 2: Day-by-day increase in the plasma total lycopene concentration, and through the following week of a diet without tomato there was gradual decrease, however no return to baseline.		Porrini *et al.*, 1998

Table 1.2 Lycopene bioavailability (as plasma lycopene response) from the diet of the different food matrices; effect on biomarkers of oxidative stress (continued)

Source	Lycopene content	Study design	Plasma values	Other markers	Reference
476 ml tomato juice 4 oleoresin soft-gel capsules 15 lycopene beadlet capsules	74.9 mg/d 75.4 mg/d 70 mg/d	7 men and 9 women (healthy; 33-61 yrs); randomised, crossover design with 4 wk treatment periods (6 wk washout between each period)	Changes from baseline in plasma lycopene concentration over the 4 treatment periods were 0.17 µmol/l for tomato juice, 0.24 µmol/l for oleoresin, 0.28 µmol/l for lycopene beadlets and –0.16 µmol/l for placebo. All plasma concentrations were significantly different from placebo.		Paetau *et al.*, 1998
Carrot juice Tomato juice Dried spinach	22.3 mg â-carotene 40 mg lycopene 11.3 g lutein	23 (non-smoking) subjects, 2 wk depletion, 3x2 week supplementation		*Comet assay*: Reduction in endogenous strand breaks. Reduction in base oxidation only with carrots.	Pool-Zobel *et al.*, 1997
Single dose 400 g fresh tomatoes 40 g tomato paste	Fresh tomatoes: 22.2 mg total lycopene 21.1 mg *all-trans* 1.16 mg *cis* Tomato paste: 23.6 mg total lycopene 22.8 mg *all-trans* 0.78 mg *cis*	2 males and 3 females. 3 days depletion and 2 experimental days 2 weeks apart	No distinct changes in lycopene concentrations were observed (data not shown). After consumption of tomato paste, total and all-trans lycopene concentrations showed a slight time-dependant increase (non-significant). After consumption of fresh tomatoes, total and all-trans lycopene concentrations remained constant.	The chylomicron lycopene response was significantly higher after consumption of tomato paste than of fresh tomatoes (AUC total lycopene 109.3 and 28.4 nmol*h/l, respectively).	Gartner *et al.*, 1997

Table 1.2 Lycopene bioavailability (as plasma lycopene response) from the diet of the different food matrices; effect on biomarkers of oxidative stress (continued)

Source	Lycopene content	Study design	Plasma values	Other markers	Reference
Capsules: Lycopene â-Carotene Lycopene + â-Carotene	60 mg of *all-trans* lycopene 60 mg of *all-trans* â-carotene Combined: 60 mg of both	10 healthy men (mean age 38 yr). Single portions of each dose each, 2 weeks apart (wash-out)	Sign. 1 serum lycopene from baseline at 5h after the lycopene dose and returned to baseline thereafter. Ingestion of a combined dose resulted in a Sign. 1 in serum lycopene at 24h. The 24h area under the curve for lycopene was significantly greater after consumption of the combined dose than after lycopene alone (3074 and 12668 nmol/l*h, respectively).		Johnson *et al.*, 1997
Fruits and vegetables 150 g carrots/d 200 g tomatoes/d 300 g spinach/d	10 mg â-carotene/d 10 mg lycopene/d 10 mg lutein/d	22 smokers/non smokers (men and woman), 2 weeks	11 smokers baseline after 2 weeks: no effect on lycopene		Hininger *et al.*, 1997
Dehydrated fruit and vegetable extract (Juice Plus+®) twice daily with meals	No data provided	15 subjects, 28 days. Fasting plasma and serum samples at 0,7,14 and 28 days	After 28 days plasma lycopene sign. 1 (0.137 to 2.94 µg/ml)	Decrease in lipidperoxide levels.	Wise *et al.*, 1996
Controlled high fruit and vegetable diet (16.2 mg/d high carotenoid content) Addition of 205 g/d broccoli	2.3mg lutein, 0.2mg lutein isomer, 0.3mg zeaxanthin, 1.1 mg cryptoxanthin, 2.2mg á-carotene, 0.2mg 13-cis-â-carotene, 6mg all-trans-â-carotene, 0.6mg ζ-carotene+9-cis-â-carotene, 3.3mg lycopene	36 healthy men and women:crossover design of 2 15-d periods (with and without addition).	Significant 1 in cis-and trans-lycopene (high fruit and vegetable diet) and significantly increased further after addition of broccoli.		Yeum *et al.*, 1996

Table 1.2 Lycopene bioavailability (as plasma lycopene response) from the diet of the different food matrices; effect on biomarkers of oxidative stress (continued)

Source	Lycopene content	Study design	Plasma values	Other markers	Reference
Heated and unheated tomato juice	0.35 µmol lycopene/kg body wt	6 healthy subjects (22-66 yr, 1F and 5M); single lycopene dose of heated tomato juice (n=3), unheated tomato juice (n=2) and no tomato juice (n=2)	Total dose of 1.4 µmol/kg body wt led to increase of tomato lycopene by 320 nmol/l over the base level.		Stahl & Sies, 1992

Table 1.3 Lycopene bioavailability from various formulations containing Lyc-O-Mato® (reported by Bohm; data provided by LycoRed; 5 mg lycopene per treatment)

Treatment	T=-2wk	T=0 (start treatment)	T=1 wk	T=2 wk	T=3 wk	T=4 wk
Tomatoes (n=6)	0.22+0.08	0.13±0.07	0.1810.05	0.19±0.07	0.2010.08	0.14±0.08
Tomato juice	0.27±0.08	0.12±0.05	0.19±0.05	0.22±0.08	0.23±0.07	0.21±0.11
Regular oleoresin	0.17±0.09	0.10±0.05	0.18±0.06	0.25±0.08	0.24±0.08	0.25±0.08
LYCCWD2 (n=7) Lyc-O-Mato® dry formulations	0.27±0.19	0.14±0.16	0.20±0.14	0.20±0.12	0.24±0.16	0.25±0.18
LYCRGGS3 Lyc-O-Mato® ground & surfactant	0.27±0.15	0.13±0.10	0.27±0.08	0.34±0.10	0.36±0.12	0.32±0.08
LYCDWG4 Lyc-O-Mato® dewaxed	0.31±0.15	0.08±0.11	0.23±0.12	0.31±0.19	0.34±0.13	0.38±0.12
LYCRGR2 Lyc-O-Mato® ground	0.19±0.08	0.04±0.06	0.27±0.06	0.34+0.11	0.34±0.18	0.35±0.15

2. Potential health benefit properties of lycopene

Epidemiological studies have demonstrated that a diet rich in fruits and vegetables is associated with a lower risk for several types of cancer (Block *et al*., 1992). Similar observations have been reported with respect to atherosclerotic vascular disease (Mayne, 1996). These health beneficial effects might be attributed to carotenoids, but also to other bioactive compounds present in fruits and vegetables.

With respect to the carotenoids, most of the earlier studies were focussed on ß-carotene, and less on lycopene. However, more recently, lycopene intake has been associated with several health benefits, related to its antioxidant properties, its effect on cell-cell communication, or cell growth.

In addition to its effects on specific cancers and cardiovascular disease, other proposed beneficial health properties of lycopene are prevention of diseases of the central nervous system, such as Alzheimer's disease, Parkinson's disease and multiple sclerosis.

Excellent, comprehensive reviews on the functions and actions of lycopene, as well as on the reported beneficial health potential, are available from Stahl and Sies (1996), Gerster (1997), Clinton (1998), Nguyen and Schwartz (1999), Rao and Agarwal (1999), Giovannucci (1999) and from Arab and Steck (2000).

In this chapter the most relevant studies on lycopene efficacy and safety are summarised and discussed. This includes both studies reported in the literature and studies with Lyc-O-Mato®.

2.1 Reported data on the health benefits of (tomato) lycopene

2.1.1 Antioxidant action

The antioxidant properties of carotenoids, including lycopene, are hypothesised to explain most of the associated health beneficial effects. Lycopene has been reported to be the most efficient singlet oxygen quencher *in vitro*, and in biological systems (di Mascio *et al*., 1989). The antioxidant action of lycopene is likely restricted to lipophilic environments. Studies have shown that lycopene is more protective than ß-carotene in protecting lymphocytes against NO radicals (for review see Stahl and Sies (1996); Clinton (1998)). Although there is evidence from *in vitro* studies that lycopene could limit

oxidative damage, especially protection of cell membranes against lipid peroxidation by Reactive Oxygen Species (ROS) (Britton, 1995), the experimental evidence of an antioxidant effect *in vivo, i.e.* in humans is still limited.

Table 1.2 reported effects of lycopene supplementation on biomarkers of oxidative stress, such as effects on DNA damage and lipid oxidation are summarised (and discussed in the sections below). From the results summarised in this table it can be concluded that lycopene decreased DNA damage and lipidperoxides levels after consumption of tomato products.

In a randomised crossover study, serum lycopene levels increased significantly with daily intake of spaghetti sauce (126 g/day) containing 20.5 or 39.2 mg of lycopene, tomato juice (540 ml/day) containing 50.4 mg of lycopene, or tomato oleoresin (125 or 250 g/day) containing 75.0 or 150.0 mg of lycopene (Rao and Agarwal, 1998). All lycopene treatments (tomato juice, sauce and oleoresin) resulted in significantly lower serum TBARS than in the placebo group. However, there were no differences between different sources and levels of lycopene. Although not statistically significant, a tendency towards lowered protein and DNA oxidation (measured by respectively higher serum thiol concentrations in subjects consuming tomato products, and 8-oxodG analysis) was observed. There was also an indication that the lycopene levels increased in a dose-dependent manner in the case of spaghetti sauce and tomato oleoresin.

2.1.2 Lycopene and UV skin exposure

Exposure of the skin to UV light resulted in 31 to 46% reductions in skin lycopene concentrations (Ribaya-Mercado *et al*., 1995). Exposure of the skin to UV light results in skin injury. The short-term effects include sunburn and tanning; cumulative UV exposure results in photoaging and increased risk of skin cancer. At least a portion of the adverse events seem to be mediated by singlet oxygen, hydroxyl radicals and other free radicals that can seriously affect membranes by formation of lipid peroxides, by reaction with proteins, and by damaging DNA and RNA Because carotenoids are consumed in the process of quenching free radicals, Ribaya-Mercado *et al*. (1995) conclude that their data suggest that lycopene may be an important defence mechanism against adverse effects of UV irradiation of the skin.

Earlier studies in mice showed that i.p. injection of lycopene ameliorated toxicity from total body irradiation (Forssberg *et al.*, 1959). UV exposure of the skin is also associated with photosensitivity and increased free radical and singlet oxygen generation.

2.1.3 Lycopene and cardiovascular disease

A protective effect of lycopene was observed in the EURAMIC multicentre study, conducted in men with myocardial infarction and matched controls in 10 European countries (Kohlmeier *et al*., 1997). Adipose tissue needle aspiration biopsies were obtained from each participant and analysed for carotenoid profiles and tocopherols. After adjusting for age, body mass, socioeconomic status, smoking, hypertension and family history, the lycopene concentration remained independently protective, with an odds ratio (OR) of 0.52 for the 10^{th} versus the 90^{th} percentiles. This study together with three other epidemiological studies in which the relation between lycopene and cardiovascular disease was investigated are summarised in Table 2.1. The ORs ranged between 0.39 and 0.81.

Low levels of serum lycopene were reported in an elderly population in Austria at risk for microangiopathy related cerebrovascular disease (Schmidt *et al*, 1997).

Arab and Steck (2000) stratified data from the EURAMIC study by smoking status and examined the association between adipose lycopene concentrations and myocardial infarction. Higher lycopene concentrations tended to be most effective against myocardial infarctions in those men who had never smoked (OR: 0.3 for never smokers, 0.4 for ex-smokers and 0.6 for smokers). Because this result was contrary to what the authors expected (if lycopene is protective by being an antioxidant, then its effect should be strongest in smokers who are in a high oxidant state), it was concluded that lycopene is working by some other mechanism than as an antioxidant.

Table 2.1 Lycopene and cardiovascular disease: epidemiological studies (from: Arab and Steck, 2000)

References	Study design	N	Lycopene measurement	Outcome	Odds ratio (95% Cl)	P for trend	Factors controlled for in analysis
Street *et al*., 1994	Nested case-control study	123 cases, 246 controls	Serum sample	Myocardial infarction	0.75[1] (Cl not shown)	0.54	None.
Iribarren *et al*., 1997	Case-control study (ARIC study)	231 cases, 231 controls	Fasting serum sample	Intima media thickness	0.81 (0.60, 1.08)	Data not shown	Age, blood storage time, total cholesterol and triacylglycerols, education level, former smoking, current smoking, BMI, ethanol intake, hypertension, diabetes mellitus, and vitamin supplement use
Kohlmeier *et al*., 1997		662 cases, 717 controls	Adipose tissue needle aspiration biopsy	Myocardial infarction	0.52 (0.33, 0.82)	0.005	Age, centre, BMI, smoking, family history of disease, and history of high blood pressure
Gomez-Aracena *et al*., 1997		100 cases, 102 controls	Adipose tissue needle aspiration biopsy	Myocardial infarction	0.39[1] (0.13, 1.19)	0.04	Age, family history of coronary heart disease, and cigarette smoking

1: Odds ratios were inverted for comparability, using the lowest quintile as the referent value.

It has also been suggested that lycopene inhibits cholesterol synthesis and augments LDL receptors, as demonstrated with J-774 A.1 macrophage cell line (Fuhrman *et al*., 1997b).

Dietary supplementation of 60 mg lycopene per day to six males for three months was associated with a 14% reduction in plasma LDL cholesterol concentrations. The authors concluded that lycopene may be a modest hypocholesterolemic agent secondary to the inhibition of macrophage 3-hydroxy-3methyl-glutaryl-coenzyme-A (HMGCoA) reductase, the rate limiting enzyme in cholesterol biosynthesis.

2.1.4 Lycopene and risk of cancer

There is accumulating evidence that a diet high in fruits and vegetables results in lower risk of several types of cancer (Block et al, 1992). Carotenoids present in fruit and vegetables have been postulated to play a role in these protective effects. Experimental studies, including intervention trials, have mainly focused on â-carotene, but were, up to now, without positive effects.

Lycopene has been shown to inhibit growth of malignant brain and mammary cells, both in *in vitro* and *in vivo* animal models (Nishino, 1998; Sharoni *et al*, 1997; Nagasawa *et al*, 1995; Wang *et al*, 1989). In an *in vitro* test system on Aflatoxin B1-induced mutagenesis in S. *typhimudum* TA 100 and TA 98 lycopene was found to be inactive, while canthaxanthin and â-cryptoxanthin reduced the mutagenic potential of promutagens (He and Campbell, 1990).

Observational studies have shown that a high tomato consumption is associated with a lower risk for digestive tract cancer, *i.e*. oesophageal and gastric and colon cancer (Franceschi et al, 1994). However, this effect is not observed in all studies (for review see Clinton (1998); Giovannucci (1999)). At least two prospective studies have shown a significantly lower risk for prostate cancer with higher tomato consumption, especially from processed tomatoes (tomato sauce, pizza) (Mills *et al*, 1989; Giovannucci *et al*, 1995).

In addition to free radical scavenging, other mechanisms have been hypothesised, such as antiproliferative effect (through inhibition of insulin-like growth factor (IGF-1) (Levy *et al*., 1995)), or up-regulation of gapjunction communication by increasing expression of connexin 43 (Zhang *et al*., 1992).

Recently, a study was reported showing a marked decrease in endogenous levels of DNA strand breaks (using the Comet assay) in lymphocytes after supplementation with tomato products (ca. 16 mg lycopene/day; Riso *et al*., 1999).

These observational data provide evidence that there is a relationship between lycopene dietary intakes or plasma lycopene values. However, confounding factors and bias, such as a healthy lifestyle, including a high intake of tomatoes (rich in lycopene), but not necessarily related to the protective effect, cannot be excluded. Stronger and convincing evidence should come from intervention studies, but up to now, no studies with lycopene have been reported.

Table 2.2 shows a summary of the epidemiological data reported on the potential effect of lycopene on risk of cancer at various sites (from Gerster, 1997).

In a recent review of the epidemiological literature on tomato (products) and lycopene on cancer, Giovanucci (1999) concluded that the evidence for a beneficial effect of lycopene on cancer risk is indeed strongest for the prostate, lung and stomach, but does not, as yet, allow conclusions on a causative relationship.

Table 2.2 Epidemiological data on the potential effect of Lycopene on cancer risk (from: Gerster, 1997)

Cancer site	Type of study	N	Variables	Findings	Reference
All	Prospective cohort study in US elderly (Massachusetts)	1271	Mortality Frequency of intake of different types of vegetables	Tomato intake 1/week:RR of death from cancer at all sites = 0.5 (95% Cl 0.3-0.8) No effect of other carotenoids-rich fruits and vegetables	Colditz *et al.*, 1985
Oesophagus	Case-control	637 oesophageal patients and 488 other tumour patients	Factors identified as potentially causally related to cancer of the oesophagus	Significant 40% risk reduction ($p<0.001$) by at least weekly tomato consumption	Cook-Mozaffari *et al.*, 1979
Stomach	Case-control study in high-versus low-risk areas for gastric cancer	1016 cases and 1159 controls	Retrospective dietary intake pattern and other life-style factors	OR for upper tertile of tomato (lycopene) intake = 0.7 ($p<0.001$). Risk reduction also by other raw vegetables	Buiatti *et al.*, 1989
Stomach	Cross-sectional study in four regions of Japan with different stomach cancer rates	170-175 from each area (random selection)	Mortality Plasma values of micronutrients Urinary excretion of salt and nitrosamines	Mean lycopene plasma values = 0.08 µmol/l ± 0.07 in high-risk area of Yokote and 0.27µmol/l ± 0.21 in low-risk area of Ishikawa. No protection by other micronutrients.	Tsugane *et al.*, 1992

Table 2.2 Epidemiological data on the potential effect of Lycopene on cancer risk (from: Gerster, 1997) (continued)

Cancer site	Type of study	N	Variables	Findings	Reference
Mouth/pharynx (314) Oesophagus (85) Stomach (723) Colon (955) Rectum (629)	Series of case-control studies on digestive tract cancer	2706 cases and 2879 controls	Frequency of tomato intake (quartiles)	Consistent pattern of protection with intakes in the upper quartile for all sites (OR=0.5-0.6); for colon (OR=0.39), rectum (OR=0.42) and stomach (OR=0.43) ($p<0.01$).	Franceschi *et al.*, 1994
Prostate	Prospective cohort study of dietary fruit and vegetables intakes	812 cases among 47894 participants	Estimated intakes of individual carotenoids	Lycopene was the only carotenoids whose intake was inversely related to prostate cancer risk. Of the tomato-based products tomato sauce showed strongest inverse association (RR=0.62; 95% Cl=0.39-0.99)	Giovannucci *et al.*, 1995
Prostate	Case-control study of prediagnostic serum micronutrients values	103 cases and 103 matched controls	Serum micronutrient concentrations	Odds ratio for highest versus lowest serum Lycopene values = 0.50 (95% Cl=0.20-1.29); p value for trend = 0.26. No association with other carotenoids or tocopherol.	Hsing *et al.*, 1990

Table 2.2 Epidemiological data on the potential effect of Lycopene on cancer risk (from: Gerster, 1997) (continued)

Cancer site	Type of study	N	Variables	Findings	Reference
Pancreas	Case-control study of prediagnostic serum micronutrients values	22 cases and 44 matched controls	Serum micronutrient concentrations	Lower serum values of lycopene in cases than controls (OR=6.4; 95% Cl=1.75-24.2; p<0.025), Se also significantly different, but no other micronutrients.	Burney *et al.*, 1989
Cervix	Case-control study of serological markers in Latin American women (high prevalence)	387 cases and 670 controls	Serum values of retinal, different carotenoids and tocopherol at early stage of the disease	The only carotenoid inversely associated with risk was â-carotene.	Potischman *et al.*, 1991
Cervix	Case-control study of serological markers in Latin American women	696 cases and 1217 control	Serum values of retinal, different carotenoids and tocopherol in different stages of the disease	A small trend for decreasing serum concentrations with disease progression was observed for lycopene, lutein and retinal. The decreased values could reflect a disease effect.	Potischman *et al.*, 1994
Cervix	Case-control study of prediagnostic serum micronutrients values	49 cases and 98 matched controls	Serum values of retinal, different carotenoids, tocopherol and selenium	Odds ratio for lowest and middle versus highest tertile of serum lycopene=2.52 (95% Cl=0.96-6.62). á and â-carotene appeared to provide higher protection.	Batieha *et al.*, 1993

Table 2.2 Epidemiological data on the potential effect of Lycopene on cancer risk (from: Gerster, 1997) (continued)

Cancer site	Type of study	N	Variables	Findings	Reference
Cervix	Case-control study of dietary intake and serum values of carotenoids	102 cases and 102 matched controls	Intake data and serum values of carotenoids	OR for lowest versus highest quartile of lycopene intake=5.4 (95% Cl=1.3-23.3); OR for lowest versus highest lycopene serum values=3.8 (95% Cl=1.1-2.4). No clear relationship with á- and â-carotene or â-cryptoxanthin.	Van Eewyck *et al.*, 1991
Cervix	Cross sectional sample of women with a histopathological diagnosis of cervical intraepithelial neoplasia (CIN) or cervical cancer	125 cases of CIN, 15 carcinoma and 95 control	Plasma lycopene (and other micronutrients) and tissue specimens	Mean plasma lycopene levels were significantly lower in women with CIN and cervical cancer	Palan *et al.*, 1996
Bladder	Case-control study of prediagnostic serum micronutrient values	35 cases and 70 matched controls	Serum micronutrient concentrations	OR of lowest versus highest tertile of serum lycopene values = 2.2 (95% Cl=0.67-6.35). No association with â-carotene	Holzlsouer *et al.*, 1989

Table 2.2 Epidemiological data on the potential effect of Lycopene on cancer risk (from: Gerster, 1997) (continued)

Cancer site	Type of study	N	Variables	Findings	Reference
Breast	Case-control study in women with cancer or BBD (=controls) using food frequency questionnaire and serum sampling before final diagnosis	83 cases and 113 controls	Carotenoid and retinal intake values Serum carotenoids and retinal concentrations	No significant inverse relation of lycopene values to breast cancer risk (RR=0.6 for highest versus lowest category). Serum â-carotene was lower in cases	Potischman *et al.*, 1990
Breast	Case-control study of life-style and other risk factors in post-menopausal women with cancer, proliferative BBD and in controls	377 cases, 173 BBD, 403 controls	Serum carotenoids, retinal and tocopherol values Known breast cancer risk factors (parity, *etc*)	No associations between risk of BBD or cancer and serum lycopene or other carotenoids.	London *et al.*, 1992
Lung	Reanalysis of population-based case-control study using updated food composition tables	332 cases and 865 matched controls	Intake values of á- and â-carotene, lutein, lycopene and â-cryptoxanthin	High á- and â-carotene and lutein intakes were inversely related to cancer risk esp. in combination; no such relation was found for lycopene and â-cryptoxanthin.	Le Marchand *et al.*, 1993
Lung	Nested case-control study in postmenopausal women	138 cases and 2814 randomly selected controls	Intake of different vegetable and food groups and of single carotenoids	The adjusted OR for all vegetables and fruit was 5.05 (95% Cl=0.02); p=0.02. The adjusted OR for lycopene was only 1.21 (95% l=0.69-2.10); p=0.53. No relationship was observed with other carotenoids either.	Steinmetz *et al.*, 1993

2.1.5 Other conditions

Lycopene has also been discussed in relation to other conditions associated with increased oxidative stress, such as in inflammations and in eye disease.

Reduced lycopene levels were observed in HIV positive women and children (Coodley *et al*., 1993; Periquet *et al*., 1995).

In a prospective, observational study the antioxidant vitamin concentrations were determined in sixteen consecutive patients with septic shock (Goode *et al*., 1995). Plasma lycopene concentrations were undetectable (<15 pg/I) in eight (50%) patients, and below the reference range (<154 µg/l; obtained from a comparable group of healthy controls) in the remaining patients.

In a case-control study the serum carotenoid levels of cases with retinal pigment abnormalities with the presence of soft drusen or with late age-related macular degeneration (ARMD) or neovascular and exudative macular degeneration were compared with controls (Mares-Perlman *et al*., 1995). Low levels of lycopene were related to an increased likelihood of ARMD (adjusted odds ratio (95% confidence interval) of 2.2). The authors suggest that this finding may be related to the great ability of lycopene to quench singlet oxygen, a reactive oxygen species in the eye, or that it might reflect a systemic effect of this carotenoid on oxidant stress, which indirectly influences the macula. It should be noted that in other studies lutein was found to be the relevant carotenoid present in the macula. The authors mention that their findings need confirmation, because of the small number of analyses performed.

2.2 Studies with tomato oleoresin (Lyc-O-Mato®)

Studies performed with the intended product Lyc-O-Mato®, *i.e*. the tomato oleoresin prepared from the lycopene rich tomatoes, are summarised below.

Fuhrman et al. (1997a): Effect of lycopene and ß-carotene on macrophage-mediated oxidation of LDL

In this study macrophages or LDL were enriched with the carotenoid, followed by analysis of cellmediated oxidation of LDL. Enrichment of LDL with lycopene or â-carotene (8 times enriched) resulted in a 50% reduction in LDL oxidation. Enrichment of J-774 A.1

macrophages with lycopene (7 times enriched) or with â-carotene (2 times) did not significantly affect cell-mediated oxidation of LDL. However, both carotenoids reduced macrophage lipid peroxidation in the presence of ferrous ions (by 30%).

The antioxidative effects against LDL oxidation of lycopene and â-carotene were examined.

Seven LDLs, out of 12 samples, responded to their enrichment with 3 µM of lycopene (supplied by LycoRed) or with 3 µM of synthetic all trans â-carotene by reduced susceptibility to oxidation, whereas in five LDLs there was no effect of LDL enrichment with carotenoids on LDL oxidisability. In the “responder" LDLs, supplementation of the LDL with 3 µM tomato lycopene or with 3 µM â-carotene resulted in a significant inhibition of LDL oxidation when induced by $CuSO_4$ (by 65% and 38% respectively), by the free radicals generator, AMVN (by 68% and 52% respectively), or by J-774 A.1 macrophages (by 51% and 48% respectively).

The vitamin E content in LDL, whose oxidation was inhibited by lycopene or by â-carotene supplementation was, however, significantly higher than that found in the "non-responder" LDLs. Therefore the effect of the carotenoids in combination with vitamin E, on the susceptibility of LDL to copper ion-induced oxidation was studied. Because a synergistic interaction in the inhibition of LDL oxidation between vitamin E and the carotenoids was shown, the effect of tomato oleoresin (5% lycopene, 0.1% â-carotene, and 1% vitamin E, supplied by LycoRed) against LDL oxidation was studied. *In vitro* supplementation of human LDLs with tomato oleoresin resulted in a dose-dependent inhibition of copper ion-induced LDL oxidation as shown by the prolongation of the lag phase (from 90 minutes to 240 minutes by addition of 5 µg/ml tomato oleoresin), and by a reduction in the lipoprotein-associated TBARS, by up to 90%.

In the same study (Fuhrman *et al*., 1997a) four groups of apolipoprotein E deficient mice, 20 mice in each, at the age of 6 weeks, were supplemented with carotenoids for a period of six weeks. Dietary supplementation of 50 ìg of purified lycopene/mouse/week, 50 µg of â-carotene/mouse/week or 1 mg of tomato oleoresin extract/mouse/week resulted in a substantial enrichment of the LDL with carotenoids, by 7.5, 3.2 or 2.5 fold, respectively. $CuSO_4$-induced LDL oxidation was inhibited by 90% and 22% after tomato oleoresin and lycopene-supplementation, respectively. No significant effect was observed after â-carotene consumption.

Fuhrman et al. (1997b): Effect of lycopene on in vitro and in vivo cholesterol synthesis.

In a J-774 A.1 macrophage cell line, the effect of 'rotene" or lycopene (10 µM) on cellular cholesterol synthesis from [3H]-acetate was studied and compared to the inhibition of cellular cholesterol synthesis in cholesterol-enriched cells and in cells treated with fluvastatin (cholesterol synthesis inhibitor).

[3H]-Acetate incorporation into newly synthesised cholesterol was inhibited in a dose-dependent manner, reaching a maximum inhibition of 88% and 98% on using 60 µg of LDL cholesterol/ml, or 1 µg of fluvastatin/ml, respectively.

Similarly, supplementation of the cells with increasing concentrations of â-carotene or lycopene resulted in a dose-dependent inhibition of macrophage cholesterol biosynthesis from [3H]-acetate. However, lycopene, when added at a similar concentration as â-carotene, inhibited cellular cholesterol biosynthesis more effectively than â-carotene along all studied concentrations. Cholesterol synthesis was inhibited by 73% or by 63%, after incubation with 10 µM of lycopene and â-carotene, respectively. In agreement with these *in vitro* observations, dietary supplementation of tomato's lycopene (60 mg/day, supplied by LycoRed) to 6 males for a 3 months period resulted in a significant ($p<0.02$) 14% reduction in their plasma LDL cholesterol concentrations.

Fuhrman et al. (2000): Synergistic antioxidant effect of lycopene with other natural antioxidants on LDL oxidation

Fuhrman *et al*. incubated LDL (100 µg of protein/ml) with increasing concentrations of lycopene or of tomato oleoresin (lipid extract of tomatoes containing 6% lycopene, 0.1% â-carotene, 1% vitamin E, and some polyphenols), which was subsequently oxidised with 5 µmol/l CUS04.

Tomato oleoresin exhibited a fivefold greater antioxidant capacity against LDL oxidation (about 90%) in comparison to 50 µmol/l of pure lycopene (as measured by a maximal of 22% and 27% inhibition in TBARS and in lipid peroxides formation respectively).

Also possible co-operative interactions of lycopene with other natural antioxidants were investigated. Vitamin E (10 µmol/l) inhibited copper ion-induced LDL oxidation in a dose dependent manner, reaching 94% and 91% inhibition in TBARS and lipid peroxides formation, respectively. When 5 µmol/l lycopene was added to 1, 2.5 and 5 µmol/l of

vitamin E, the inhibition of LDL oxidation exceeded the expected additive contribution of the individual antioxidants by 10%, 25% and 45% for the TBARS formation and by 8%, 23% and 53% for the lipid peroxides formation, respectively.

A combination of lycopene with tocotrienol showed no synergistic effect.

A maximal inhibition of 94%, 93% and 99% was achieved by the addition of 6.75 µmol/l of glabridin, or 225 µmol/l of rosmarinic acid or camosic acid. The inhibition of LDL oxidation by glabridin (1 µmol/l), rosmarinic acid (25 µmol/l), or camosic acid (25 µmol/l), in combination with 5 µmol/l of lycopene, exceeded the calculated additive effects by 32%, 32% and 15%, respectively. The LDL oxidation by â-carotene together with á-tocopherol (5 µmol/l), glabridin (1 µmol/l), camosic acid (25 µmol/l), rosmarinic acid (25 µmol/l), or with garlic (250 µmol/l), exceeded the expected additive inhibition by 6%, 9%, 15% and 4%, respectively. Similarly to lycopene, the combination of â-carotene with tocotrienol (5 µmol/l) also showed no synergistic effect. Addition of increasing concentrations of garlic (100-500 µg/ml), together with a constant lycopene concentration (5 µmol/l), inhibited LDL oxidation in a synergistic manner.

After an oral dose with 30 mg of lycopene (as tomato oleoresin), administered with a fatty meal, the postprandial plasma lycopene concentration was elevated by 70% in four normolipidemic subjects (aged 30-45, non smokers; no medication or vitamin supplement use). LDL isolated from the postprandial plasma fraction (5 hours after meal consumption) exhibited a significant reduced susceptibility to oxidation by 21 %.

Aviram et al. (1999a): Comparative bioavailability of lycopene from different tomato oleoresin formulations

Four normolipidemic subjects aged 30-45, non smokers, under no medication or vitamin supplements, consumed a fatty meal of 1200 calories (18% protein, 45% fat, 37% carbohydrates) including 30 mg lycopene that was administered as tomato oleoresin prepared in different forms as following:

1. tomato oleoresin 12% (DW), which is depleted of waxes;
2. tomato oleoresin 2% (SG), which is a suspension of tomatoes crystals to which is added lecithin and sucrose esters;
3. tomato oleoresin emulsion in water (800 ppm, or 0.08% lycopene), containing soybean oil, ascorbic acid, citric acid, sodium benzoate and tween 80;

4. tomato oleoresin 6%;
5. tomato oleoresin beadlets 5%.

Blood samples were collected before meal consumption in the fasting state, and in the postprandial state, 2, 4, and 6 hours after meal consumption.

Lycopene was absorbed most efficiently when administered as oleoresin 2%, in 3 out of 4 subjects. Lycopene administered as an oleoresin beadlet was absorbed in all subjects, however to a different extent. In contrast, administration of oleoresin 6%, or oleoresin emulsion increased plasma lycopene levels in only 2 out of 4 subjects. Oleoresin 12% DW ingestion resulted in a moderate increase in plasma lycopene levels in 3 out of 4 subjects.

Consumption of a fatty meal had no effect on the susceptibility of LDL to oxidation in 3 out of 4 subjects, therefore ingestions of the fatty meals which contained lycopene supplements had no inhibitory effects on LDL oxidation. In one subject, a 50% increase in the susceptibility of LDL to oxidation was observed 4 hours after consumption of the fatty meal. However, consumption of a fatty meal which included tomato oleoresin 12%, 6%, 2% or oleoresin beadlets, but not oleoresin emulsion abolished the stimulation of LDL oxidation by the fatty meal with no carotene.

Aviram et al. (1999b): Antioxidative effect of lycopene in combination with tocotrienol

The following preparations were studied:

1. Crystalline lycopene (1 mM) in tetrahydrofuran (THF).
2. Lyc-O-Mato® DW (10%), tomato oleoresin which does not contain waxes (de-waxed) and contains 10% lycopene, was dissolved in THF to a concentration of 10 mg/ml.
3. A mixture of tocotrienols was dissolved in THF 1 mM.

Lycopene alone inhibited copper ion-induced LDL oxidation by 13%. Tomato oleoresin DW (containing 5 µM of lycopene) alone, inhibited copper ion-induced LDL oxidation by 27%. However, addition of increasing concentrations of tocotrienol together with 5 µM of tomato oleoresin DW-lycopene resulted in an additive inhibition in LDL oxidation measured as TBARS formation.

Aviram et al. (1998a): Antioxidative effect of lycopene in combination with natural vitamin E

The following preparations were studied:

1. Crystalline lycopene in tetrahydrofuran (THF) (1 mM).
2. Lyc-O-Mato® (6%), tomato oleoresin containing 6% lycopene (10 mg/ml).
3. Lyc-O-Mato® DW (10%), tomato oleoresin which does not contain waxes (de-waxed) (10 g/ml).
4. Natural vitamin E (a mixture of tocopherols containing at least 537 mg of D-alpha tocopherol, 1 mM.

It was found that a minimal concentration of 5 µM of tomato oleoresin-lycopene inhibited LDL oxidation induced by copper ions by 22%. The IC50 (concentration at which 50% inhibition occurs) of Lyc-O-Mato® (DW) in the copper ions-induced LDL oxidation is 8 µM for TBARS formation inhibition and 8.4 µM for inhibition of lipid peroxide formation.

Synergistic effects were observed when vitamin E (dissolved in ethanol) was added at concentrations of 0.5 µM, 1.0 µM, 5.0 µM and 10 µM, in combination with tomato oleoresin DW containing 5 µM of lycopene. The greatest effect was obtained with 5.0 µM of vitamin E (dissolved in ethanol), which resulted in a substantial inhibition in TBARS or lipid peroxides formation by 80% or 79%, respectively (separately: vitamin E 39% or 30%; lycopene 17% or 19%, respectively). When vitamin E was dissolved in THF, the greatest synergistic effect was obtained with 1.0 µM of vitamin E, which resulted in a substantial inhibition in TBARS or lipid peroxides formation by 45% or 25%, respectively (separately: vitamin E no effect; lycopene 12% or no effect, respectively).

Addition of 0.5 µM vitamin E (dissolved in ethanol) together with 6 µM of tomato oleoresin-lycopene resulted in a synergistic inhibitory effect of 74% (separately: vitamin E no effect; lycopene 38%). This effect was only shown when measuring formation of lipid peroxides but not TBARS formation. Addition of 1.0 µM of vitamin E alone (dissolved in THF) together with 6 µM of tomato oleoresinlycopene resulted in a synergistic inhibitory effect of 68% inhibition of TBARS formation and 49% inhibition of lipid peroxides formation (separately: vitamin E no effect; lycopene 42% and 32% respectively).

Aviram et al. (no date): Antioxidative effect of lycopene in combination with camosic acid, rosmarin extract or with glabridin

The following preparations were studied:

1. Crystalline lycopene was dissolved in tetrahydrofuran (THF) (1 mM).
2. Lyc-O-Mato® DW (10%).
3. Camosic acid.
4. Rosmarinic acid.
5. Glabridin.
6. Ginger extract.

A maximum inhibition of 98% and 88% in copper-ion-induced TBARS and lipid peroxides formation, respectively, was obtained by 100 µg/ml of camosic acid.

A combination of 25 µg/ml camosic acid together with Lyc-O-Mato® DW (5 µg lycopene/ml) resulted in a substantial inhibition in TBARS or lipid peroxides formation by 63% or 91%, respectively (separately: camosic acid 19% or 40%; Lyc-O-Mato® 11 % or 15%, respectively).

The lag time (the time during which no lipid peroxidation occurs due to LDL endogenous antioxidants) was 2.5 times greater after addition of camosic acid together with Lyc-O-Mato® (5 µg of lycopene/ml), or with lycopene (5 µg/ml) (50 minutes) than addition of camosic acid (25 µg/ml) alone (20 minutes).

No co-operative antioxidant effect of rosmarinic acid with Lyc-O-Mato® or with lycopene was observed on $CuSO_4$-induced LDL-oxidation.

Addition of glabridin together with lycopene (5 µg/ml) inhibited TBARS formation by 71% and there was no synergistic effect in inhibition of lipid peroxides formation by glabridin in combination with lycopene (separately 0.3 µg/ml glabridin 31% and 10% respectively). Addition of glabridin together with Lyc-O-Mato® (5 mg lycopene/ml) inhibited TBARS formation or lipid peroxides formation by 100% and by 45% respectively. Addition of glabridin together with Lyc-O-Mato® (5 µg lycopene/ml) retarded the onset of LDL lipid peroxidation by 80 minutes, 30 minutes more than the effect induced by glabridin alone.

In the system of AAPH-induced LDL oxidation, no co-operative effects of either camosic acid, rosmarinic acid, glabridin or ginger in combination with Lyc-O-Mato® or with

lycopene have been found. However, when LDL oxidation was measured as lipid peroxides formation, a synergistic inhibitory effect was observed between glabridin in combination with Lyc-O-Mato®, as well as with lycopene.

Aviram and Fuhrman (1999): Effect of a combination of lycopene and soybean extract on LDL oxidation

The following preparations were studied:

1. Lyc-O-Mato® (6%), tomato oleoresin containing 6% lycopene was dissolved in THF to a concentration of 10 mg/ml, equivalent to 1.2 mM of lycopene.
2. Soy extract containing 40% isoflavones (10 mg/ml stock solution) was filtered through a 0.45 filter, and the filtrate was used in the experiments.

No cooperative antioxidant effect of soy extract with lycopene was observed by addition of Lyc-O-Mato® (5 µM lycopene) to low concentrations of soy extract ranging between 1 to 25 µg/ml. When using 25 µM or 50 µM of soy extract LDL oxidation was inhibited.

Aviram and Fuhrman (1997): Antioxidative effect of garlic-lycopene against LDL oxidation

The addition of both garlic (6.25 allicin equivalents/ml) and lycopene (5 µg/ml) to LDL incubated with 5 µM Cu decreased TBARS or lipid peroxides formation by 64% and 72%, respectively. Garlic and lycopene added alone inhibited LDL oxidation by 25% and 18%.

When tomato oleoresin (3 µg lycopene/ml) or garlic (2.5 allicin equivalents/ml) were added individually no significant inhibition of LDL oxidation was observed. However, upon addition of both antioxidants LDL oxidation was inhibited by 54%. Addition of tomato oleoresin in combination with increased concentrations of garlic (6.25 µg of allicin equivalents/ml), a 95% reduction in LDL oxidation was obtained.

No co-operative effect of garlic and lycopene was observed on AAPH-induced LDL oxidation. The addition of garlic and tomato oleoresin decreased TBARS or lipid peroxides formation by 68% and 50%, respectively (separately: garlic 26%, tomato oleoresin 18%).

Aviram et al. (provided by LycoRed): Antioxidative effect of garlic in combination with Lyc-O-Mato® (1.5%), â-carotene, vitamin E and ginger.

The following preparations were studied:

1. Lyc-O-Mato® (1.5%).
2. Garlic powder (0.25 mg of allicin equivalents/ml).
3. Natural vitamin E (1 mM).
4. â-Carotene (10 mg/ml).
5. Ginger (10 mg/ml).

Addition of 2.5 µg allicin equivalents/ml of garlic together with Lyc-O-Mato® 1.5%, 1.5 µg lycopene/ml, resulted in a substantial inhibition in TBARS or lipid peroxides formation by 87% and 72% respectively. This inhibition is greater than the sum of the inhibition observed with each compound individually.

No co-operative antioxidative effects of garlic in combination with either Lyc-O-Mato® 1.5% (1.5 µg lycopene/ml), or with vitamin E (1 µM), or with â-carotene (5 µg/ml), or with ginger (5 µg/ml), were observed in the system of AAPH-induced LDL oxidation.

2.3 Conclusions

Experimental (*in vitro*) studies show that lycopene is an excellent singlet oxygen quencher and has antioxidant capacity. Lycopene has been shown to be effective in scavenging of NO radicals in lymphocytes, and increased consumption of tomatoes, a rich source of lycopene, had a positive effect on biomarkers of oxidative stress, *i.e.* DNA damage and lipid oxidation.

The protective, health beneficial effects of lycopene might be related to its antioxidant potential. In (other) conditions associated with an increased exposure of free radicals (reactive oxygen species), such as smoking, UV (skin) exposure and inflammation, low serum lycopene levels have been reported.

Besides its antioxidant properties, lycopene was demonstrated *(in vitro*) to have an effect on cell-cell communication, and cell growth/differentiation.

Experimental and observational (epidemiological) studies indicate that consumption of tomato products, containing lycopene, are associated with lower cancer risk, especially in

the case of prostate cancer. Lycopene, as tomato oleoresin, has also been demonstrated, both *in vitro* as *in vivo*, to inhibit LDL oxidation and have an inhibitory effect on cholesterol synthesis. These findings offer the potential for protection against cardiovascular disease.

In one case-control multicentre study a negative association between lycopene content in adipose tissue (as a marker for long term exposure) and incidence of myocardial infarction in men was observed. This study together with three other epidemiological studies showed odds ratios between 0.39 and 0.81.

The health beneficial effects of fruits and vegetables, especially from tomato products, might be attributed to lycopene, but a role for other bioactive compounds cannot be excluded. The findings from observational and experimental (animal and *in vitro*) studies need therefore to be extended and confirmed in larger scale, controlled intervention studies.

Studies performed with the intended product Lyc-O-Mato®, show that the lycopene contained in the tomato oleoresin, is absorbed and results in a significant increase in serum lycopene level, at least comparable with that obtained with equivalent amounts of processed tomatoes (tomato puree), using intake levels of at least 5 mg.

3. Nutritional safety of Lyc-O-Mato®

To our knowledge, no adverse effects of lycopene in humans have been reported (see Appendix B). With the exceptions of a 12 month study (as yet unpublished) using high levels of lycopene on prostate cancer patients, no other data are available on long term dosing studies, nor from studies performed in children, and pregnant/lactating women.

There is no evidence for interaction at the level of absorption or postprandial metabolism of high dose lycopene intake with other carotenoids. Although pro-oxidants effects cannot be excluded, these are not anticipated to occur at intake levels in the nutritional/dietary range (ca. 6 till maximum 45 mg/day). No safe upper limit of intake for humans has been established by nutritional authorities (see Appendix B).

- *Effect on the bioavailability of nutrients from the diet or any adverse physiological effects*

Carotenoid interactions have been reported (van den Berg, 1999). In the case of lycopene no interactions have been reported with respect to other carotenoids, or other components. There is one report of an *in vitro* study showing competitive inhibition of the â-carotene-15, 15'-dioxygenase activity, the enzyme responsible for cleavage of provitamin A carotenoids into retinal, in rabbit intestinal cells (Ershov *et al*, 1993). However, this was not confirmed in another study with the rat intestinal enzyme (van Vliet *et al*, 1996).