

Short communication

Reduction of exercise-induced asthma oxidative stress by lycopene, a natural antioxidant

Background: Lycopene has previously been shown to have high antioxidative activity. In view of the controversy regarding the beneficial effect of antioxidants on asthma, the acute effects of lycopene (LYC-O-MATO™) on airway hyperreactivity were assessed in patients with exercise-induced asthma (EIA).

Methods: Twenty patients with EIA participated in our study to verify the antioxidative effects. The test was based on the following sequence: measurement of baseline pulmonary function, 7-min exercise session on a motorized treadmill, 8-min rest and again measurement of pulmonary function, 1-week, oral, randomly administered, double-blind supplementation of placebo or 30 mg/day of lycopene (LYC-O-MATO), measurement of pulmonary function at rest, 7-min exercise session, and 8-min rest and again measurement of pulmonary function. A 4-week washout interval was allowed between each protocol.

Results: All patients given placebo showed significant postexercise reduction of more than 15% in their forced expiratory volume in 1 s (FEV₁). After receiving a daily dose of 30 mg of lycopene for 1 week, 11 (55%) patients were significantly protected against EIA. Serum analyses of the patients by high-pressure liquid chromatography detected in the lycopene-supplemented patients an elevated level of lycopene compared to the placebo group, with no change in retinol, tocopherols, or in the other carotenoids.

Conclusions: Our results indicate that a daily dose of lycopene exerts a protective effect against EIA in some patients, most probably through an *in vivo* antioxidative effect.

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The view that exercise training in children with asthma has a beneficial effect on aerobic conditioning and psychosocial behavior warrants consideration from a general health perspective (1). Recent evidence shows that strenuous exercise increases the production of reactive oxygen species (ROS) associated with depletion of the antioxidant defense in vital tissues of the body (2). Strenuous exercise may promote free radical production, leading to lipid peroxidation and tissue damage. On the other hand, exercise training seems to reduce the extent of oxidative damage (3), and dietary supplementation with antioxidant vitamins has been shown to be beneficial in combating oxidative stress (2–6).

There is evidence that dietary supplementation with antioxidants, such as vitamin C and β -carotene, protects against exercise-induced asthma (EIA) (7, 8). Studies on the use of vitamin C in antigen-inhalation challenges, histamine inhalation, and methacholine-inhalation tests have yielded contradictory results. Proper assessment of the vitamin C effect on airways requires measurement of alterations in airway tone and airway obstruction, such as occurs after exercise-induced bronchospasm, or in

NO₂-induced airway hyperresponsiveness. In asthmatic patients, pretreatment with ascorbic acid has been shown to prevent the significant alterations in pulmonary functions induced by exercise (7). A natural isomer mixture of β -carotene from the alga *Dunaliella bardawil* was recently shown to prevent EIA (8).

The present study was undertaken in order to evaluate the prevention of EIA by lycopene supplementation. Recent studies have indicated that lycopene and other carotenoids play an important role in human health and resistance to degenerative conditions (9). LYC-O-MATO™ is a new enriched lycopene product of tomato containing other bioactive ingredients such as tocopherols, carotenoids, phytoene, and phytofluene. The effect of LYC-O-MATO on hyperreactive airways was examined at rest and following the physical provocation of exercise.

Material and methods

A group of 20 patients with proven EIA participated in

the study. There were 13 males and seven females ranging in age from 10 to 43 years (mean age 23). All patients underwent an initial measurement of pulmonary function by Spirograph, Jaeger Compacttransfer, Germany, in which all of them showed a reduction of at least 15% in their forced expiratory volume in 1 s (FEV₁) after a 7-min run on a motorized Quinton treadmill followed by an 8-min rest. The workload was the same for each patient exercising up to a submaximal effort of 80% of the theoretical maximal heart rate, as indicated by an Omeda 3700e pulse oxymeter. All subsequent exercise testing was performed under the same ambient conditions for all patients and with the same workload that produced EIA at the baseline evaluation. Pulmonary functions were measured at the same time of the day with similar ambient conditions to avoid any variability. Exercise testing as described above was performed before and after a 7-day supplementation period of 30 mg lycopene or placebo, administered randomly in a double-blind fashion. Each patient underwent this protocol twice, receiving either lycopene or placebo, with a 4-week interval between each protocol to allow washout. Twelve hours before evaluation of the pulmonary functions, all patients discontinued their regular medications, which consisted of antihistamines, short-action bronchodilators, and inhaled steroids. The dose of inhaled steroids was constant for several weeks before each challenge. Challenge was not done within several weeks of a respiratory tract infection. Patients avoided allergen exposure for several weeks before each challenge.

The lycopene preparation used was LYC-O-MATO, an oleoresin product manufactured by LycoRed Natural Products Industries Ltd, Beer Sheva 84102, Israel. LYC-O-MATO contains 6% lycopene, 1.6% tocopherols, 1% phytoene and phytofluene, 0.25% β -carotene, and other minor phytochemicals extracted from tomato. LYC-O-MATO was introduced in the form of soft capsules of 15 mg (6%) lycopene.

Lycopene, carotenoids, retinol, and tocopherol serum concentrations were measured on blood samples collected after supplementation of placebo or lycopene. Blood samples were taken after overnight fasting from 7 to 9 am. Blood was separated for routine blood tests and for vitamin analysis. Serum was obtained by centrifugation at 3000 *g* at 5°C and stored at -70°C under nitrogen until analysis, usually within 7 days. Ethanol (2.5 ml) was added to 1 ml serum, and after vigorous mixing the lipophilic fraction was extracted with 5 ml *n*-hexane through phase separation and centrifugation at 2000 *g* at 5°C for 5 min. The upper phase was removed and the water-ethanol phase extracted a second time with 3 ml *n*-hexane. The two hexane extracts were combined, evaporated to 1 ml for spectrophotometric quantitative analysis, and then to dryness by a stream of nitrogen. The dried residue was dissolved in 100 μ l methylene chloride before being injected into the HPLC

system. This was a Waters HPLC system (Millipore, Marlborough, MA, USA). The system included pumps 501 and 510 and a Waters 996 photodiode array detector attached to Waters Millennium 2010, Chromatography Manager, Version 2.10, run on an IBM-compatible computer connected to an HP DeskJet 1200 plotter (Hewlett Packard, Avondale, PA, USA). The column was a Vydac 201 TP54 stainless steel column of 25 cm x 4.6 mm (internal diameter) packed with C-18 reversed-phase material with particle size of 5 μ m and a pore size of 30 nm (The Separation Group, Hysperia, CA, USA). The column was maintained at 30 $\pm$ 0.2°C in an HPLC Column 7955 Heater/Chillier (Jones Chromatography, Glamorgan, UK). The column was protected by a 5-cm C-18 ODS guard column (Shimadzu, Kyoto, Japan) and with a small preguard column, a Guard-Pak, inserted with a C-18 μ Bondapak cartridge (Waters Chromatography, Milford, PA, USA). Elution was performed at 30 $\pm$ 0.2°C with an isocratic solvent, HPLC grade methanol:acetonitrile (9:1 by vol), at a constant flow of 1.0 ml/min, which is a well-documented system for distinguishing the different carotenoids and their isomers (12, 28, 30). The mobile phase was flushed with nitrogen to avoid air gassing in the solvents. Samples were injected with a 7725i syringe-loading sample injector fitted with a 5- μ l loop (Rheodyne, Inc., Cotati, CA, USA). Peak responses were measured and assessed at their maximum wavelengths with the photodiode array and detected by the Millennium 3-D "Max" absorption, as described previously. Peak responses of carotenoids were measured at 450 nm. Excel (Microsoft, USA) was used for quantification of the HPLC data. The standards, lutein, β -apo-8'-carotenal, β -cryptoxanthin, all-*trans*- β -carotene, α -carotene, α -tocopherol, and lycopene, were purchased from Sigma (St Louis, MO, USA). All standards were kept at -70°C under nitrogen, and dried by a stream of nitrogen before being analyzed and injected into the HPLC system in methylene chloride. The concentration of the standards was determined by spectral measurement and calculated with the appropriate extinction coefficients in ethanol. β -apo-8'-carotenal was used as internal standard in all runs. An amount of 2.5 μ g of standard were added to the serum with THF:MeOH, and then the internal standard was extracted as described above for injection into the HPLC at 125 ng/5 μ l. We did not note any loss in the quantity of the internal standard along the extraction process; nevertheless, we ensured linearity by using three concentrations of the standard.

Statistical analysis

Comparisons of means $\pm$ SD between the groups supplemented with either or lycopene or placebo were made by the paired *t*-test. *P* < 0.05 was considered to be significant.

Results

All 20 patients taking placebo revealed a significant postexercise reduction of more than 14% in their FEV₁. Eleven patients out of the 20 (55%) that were supplemented with a total dose of 210 mg of the tomato lycopene in 7 days showed a postexercise reduction of less than 14% in FEV₁ (Table 1). Of the 11 patients that benefited from the lycopene, six were males and four females. As a group, the placebo-supplemented patients had a mean reduction after exercise of $-26.5\% \Delta\text{FEV}_{1\text{}}$, while the lycopene-supplemented patients had a mean reduction after exercise of $-14.7\% \Delta\text{FEV}_{1\text{}}$. The patients that responded more favorably to lycopene reported a subjective feeling of well-being as opposed to the less responsive patients, who did not report any change. Table 2 summarizes quantitatively the content of retinol, tocopherols, and carotenoids in the patient sera after the supplementation of placebo or lycopene. Lycopene, but not the other components, significantly increased in the lycopene-supplemented patients.

Discussion

Several studies have shown a beneficial association between fruit and vegetable intake and lung function (10–12). Intake of both fruits and vegetables above the median level was positively associated with pulmonary function in three European countries (13). Intake of the three antioxidants, vitamin C, vitamin E, and β -carotene

above median level tended to be positively associated with pulmonary function (14–19). In previous clinical evaluations (7–9), we have shown that both vitamin C and natural β -carotene may be beneficial to the exercising asthmatic in the prevention of EIA. Epidemiologic studies show associations among oxidant exposure, respiratory infections, and asthma. There is evidence that oxidants produced endogenously by overactive inflammatory cells can contribute to ongoing asthma (20–22). It has been observed that mononuclear phagocytes, alveolar macrophages, and blood monocytes release higher quantities of reactive oxygen species in asthmatic patients than in healthy subjects (23, 24).

Antigen bronchial challenge produces airspace inflammation that may develop, in part, as a consequence of enhanced reactive oxygen species metabolism of airspace cells. Reactive oxygen species may cause bronchial constriction and mucus secretion, affect airway vasculature, and increase airway responsiveness (25, 26). The role of reactive oxygen species in airway disease has been largely neglected. If reactive oxygen species participate in the inflammatory response in airway disease, the radical scavengers or antioxidants could play a useful role in therapy.

Patients with asthma are known to generate increased amounts of reactive oxygen species from peripheral blood cells and from cells recovered from bronchoalveolar lavage. These reactive oxygen species produce many of the pathophysiologic changes associated with asthma and may contribute to its pathogenicity (24–26).

Table 1. FEV₁ values before and after exercise in EIA patients protected by lycopene

Patient age (years)	Placebo-treated					Lycopene-treated				
	Before exercise		After exercise		Change %	Before exercise		After exercise		Change %
	Liter	% of predic.	Liter	% of predic.		Liter	% of predic.	Liter	% of predic.	
29	3.24	86	2.00	53	–38	2.60	69	2.08	55	–20
10	1.56	105	1.24	84	–21	1.68	113	1.64	111	–2
33	3.84	117	1.92	58	–50	3.4	103	2.36	72	–31
26	3.72	89	3.16	75	–15	3.96	94	3.96	94	0
17	4.88	138	3.76	105	–23	4.88	138	4.48	126	–8
17	3.48	86	2.52	62	–28	4.00	99	3.12	77	–22
26	2.72	75	1.88	52	–31	2.76	76	2.64	73	–4
25	3.37	82	2.23	54	–34	3.16	77	2.84	69	–10
12	2.32	91	1.88	74	–19	2.2	86	2.00	78	–9
22	2.36	76	1.96	63	–17	2.88	93	2.56	83	–11
11	1.52	80	0.76	40	–50	1.64	87	1.24	66	–24
39	3.09	76	2.50	62	–14	4.02	104	3.84	99	–4
25	2.28	73	1.84	59	–19	1.92	62	1.40	45	–27
15	3.80	113	2.84	84	–25	3.64	108	2.84	84	–22
25	4.76	102	4.08	88	–14	4.84	104	3.76	81	–22
25	3.93	93	1.96	46	–50	4.08	97	2.64	63	–35
43	2.88	90	2.28	72	–21	2.64	83	2.64	83	0
25	3.76	97	3.24	83	–14	3.68	95	3.40	88	–8
16	2.96	86	2.24	65	–24	2.88	84	2.68	78	–7
22	3.20	73	2.48	56	–23	2.92	66	2.12	48	–27
23 ± 9	3.18 ± 0.9	84.8 ± 30.8	2.34 ± 0.79	67 ± 16	–26.5 ± 12.1	3.19 ± 0.95	92 ± 18	2.71 ± 0.86	79 ± 20	–14.7 ^a ± 11.0

Mean ± SD.

^a Significant improvement.

Table 2. Serum measurements of values for lycopene, carotenoids, retinol, and tocopherols in EIA patients supplemented with placebo or lycopene (LYC-O-MATO)

	Serum variable (mg/l)						
	Retinol	Tocopherols	Carotenoids	Lycopene	Lutein	β-Cryptoxanthin	α- and β-Carotenes
Placebo (n=20)	0.23 ± 0.08	23 ± 12	0.31 ± 0.26	0.04 ± 0.02	0.14 ± 0.05	0.06 ± 0.04	0.12 ± 0.06
Lycopene (n=20)	0.28 ± 0.05	25 ± 11	0.36 ± 0.20	0.08 ± 0.02 ^a	0.18 ± 0.08	0.08 ± 0.05	0.10 ± 0.04

Mean $\pm$ SD.^a Significantly different from placebo ($P < 0.05$).

In patients with steroid-dependent bronchial asthma, the free radical presence is more intensive than in non-steroid-dependent patients (27), and reactive oxygen species may induce an autonomic imbalance between the muscarinic receptor-mediated contraction and the β -adrenergic-mediated relaxation of the pulmonary smooth muscle. Such autonomic imbalance might be involved in the genesis of bronchial hyperreactivity during lung inflammation (28). The reactive oxygen species induce bronchoconstriction, elevate mucus secretion, and cause microvascular leakage (29, 30). If reactive oxygen species participate in the inflammatory response occurring in airways disease, antioxidants should prove beneficial in therapy (30). Indeed, ingestion of 1 g of vitamin C or 30 mg of β -carotene diminished the markers of lipid peroxidation at rest and after exercise, but did not prevent the exercise-induced increase in oxidative stress (31). The amount of oxidative damage depends on the exercise intensity and could be reduced through dietary supplementation with antioxidants, such as vitamins C and E or β -carotene (32), all of which appear to be very good quenchers of activated forms of singlet oxygen and free radicals (33, 34). Conventional treatment has not yet been able to correct either enhanced lipid peroxidation or weak antioxidant defense (35).

Therapeutic action aimed at increasing antioxidant defense mechanisms is still a clinical challenge. Nevertheless, bronchial asthma patients who received antioxidants in addition to conventional therapy were found to exhibit a more pronounced lowering of chemiluminescence in the blood, as well as of the plasma malondialdehyde content, than did patients who received conventional therapy alone. With regard to the use of vitamin C to alleviate asthma and to enhance athletic activity, the available evidence is contradictory or inconclusive. Thus, for example, Malo et al. (36) found no significant changes in FEV₁ and forced vital capacity after ascorbic acid administration as compared to placebo administration. It was concluded that ascorbic acid has no acute bronchodilator effect and does not alter bronchial responsiveness in subjects with asthma. Recent studies support the thesis that vitamin C has no bronchodilatory effect, as ingestion of ascorbic acid had no detectable effect on the degree of bronchodilation of the resting asthmatic (36–38). However, a deficiency

of vitamin C was recorded in the majority of bronchial asthma patients during exacerbation of their disease. Furthermore, pretreatment with ascorbic acid obviated the significant alteration in airway geometry which is induced in asthmatic patients by exercise (39, 40), and also significantly reduced the bronchospasm normally observed 5 min after exercise. The latter finding concurs with our own results. It has been suggested that ascorbic acid exerts its effect by altering arachidonic acid metabolism (7).

Lycopene was administered to the subjects in soft capsules of LYC-O-MATO. It is evident that lycopene content increases in the serum of the lycopene-supplemented patients. If lipophilic lycopene functions as an efficient quencher of singlet oxygen and other free radicals (41), it may prevent the consequent *in vivo* formation of reactive oxygen species. Nutritional supplementation that contains lycopene will provide strong protection against damage by free radicals through the effective anti-oxidation of lycopene. Lycopene seems to suppress the attack of cellular and reactive oxygen species, slow the formation of more radicals, and therefore prevent the destruction of the lipophilic parts of the cell or the membrane. Asthmatic subjects represent a classical case study where the cellular level of free radicals is basically high and increases upon strenuous exercise. A combination of different antioxidants, hydrophilic and lipophilic, such as vitamin C, a mixture of stereoisomers of β -carotene, and lycopene, may under certain conditions provide a better antioxidative effect than the use of one type of quencher.

Experimental nutritional and medical studies with natural carotenoids originating from different plant sources specifically, or synergistically with polar antioxidants, have been limited, and such research is in its infancy. The present study shows that even within the scope of lycopene itself, more attention should be paid to the origin of the supplement and to the mode of its intake. We used LYC-O-MATO lycopene made from the whole tomato, which contains, aside from a high concentration of lycopene, other different minor constituents common to the fruit. The possibility of synergistic effects and the possible beneficial potency of the plant nutrients are still unknown and warrant further research.

The findings of the present study clearly support the assumption that in most patients, dietary supplementation with lycopene protects against EIA. Physical activities are beneficial for asthmatic patients, and ingestion of vitamin C or β -carotene may enable them to enjoy full participation in such

activities. In summary, it is suggested that natural plant antioxidants such as lycopene, vitamin C, carotenoids, and stereoisomers of carotenoids should be integrated in the prevention of exercise asthma. A significant number of asthmatic patients will benefit from these antioxidants.

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