Lycopene and the Lung

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The human lung, due to the oxidative and ozone stress to which it is exposed, is particularly vulnerable to oxidative damage. Concentrations of dietary antioxidants in the lung epithelial lining and lining fluids may provide protection against oxidative damage. A randomized clinical trial was conducted to study the effects of supplemental, carotenoid-rich vegetable juice (V-8) on lung function macrophage levels of carotenoids and in moderating ozone-induced lung damage. Healthy young adults (n = 23) were exposed to 0.4 ppm ozone in a chamber for 2 hr after either 2 weeks of antioxidant supplementation (including one can of V-8 juice daily) or placebo. Mean lung concentrations of lycopene increased by 12%, and lung epithelial cell DNA damage as measured by the Comet Assay decreased 20% in supplemented subjects. No change in peripheral blood lymphocyte DNA damage was observed as evidenced by no change in mean comet area or length in supplemented or placebo subjects. We were not able to separate the effects of lycopene from other carotenoids or antioxidants administered in this study; however, lycopene is the predominant carotenoid in V-8 (it represents 88% of total carotenoids). A review of the epidemiologic literature providing evidence for the effect of lycopene (diet or serum) or tomatoes on the risk of lung cancer reveals 27 observational epidemiologic studies (18 case-control and nine cohort studies) reporting relative risk (RR) estimates. RR estimates for cohort studies ranged from 0.63 to 1.24 (mean RR = 0.93, SD = 0.16). Odds ratios (OR) for case-control studies ranged from 0.27 to 0.93 (mean OR = 0.61, SD = 0.16). Both plasma levels (RR = 1.01, OR = 0.37) and estimated intakes of lycopene from dietary sources (mean RR = 0.93, RR range = 0.80–1.05; mean OR = 0.67, OR range = 0.27–0.93) were examined. Seventeen studies, three of which were cohorts, reported their results at the level of tomato consumption rather than, or in addition to, lycopene consumption (mean RR = 0.89, RR range = 0.63–1.24; mean OR = 0.61, OR range = 0.37–0.80). The published epidemiologic literature shows an interaction between study design and the relationship between lycopene and/or tomatoes and risk of lung cancer. Overall, cohort studies did not show an association, whereas case-control studies showed a decreased risk with greater consumption of lycopene and tomatoes. Altough lycopene can be found in the human lung, and there is evidence, albeit weak, for a protective association with lung cancer, its biologic role remains to be elucidated. Exp Biol Med 227:894–899, 2002

Key words: lycopene; antioxidants; lung function

The lung is a large organ that is extremely susceptible to oxidative stresses. The volume of oxygen that passes through the lung is so great that it is likely to be extremely vulnerable unless there are multiple levels of antioxidant protection. The lung operates under a higher oxygen pressure environment than other internal organs. This can result in the conversion of high concentrations of antioxidants into pro-oxidants. In addition, the presence of other oxidants such as ozone can enhance risk of oxidative damage (1). Ozone is prone to react with and change the structures of organic compounds. Levels of ambient ozone in many areas of the U.S. exceed the National Ambient Air Quality Standards (2). Among other things, ozone has been shown to functionally affect the lung capacity (3, 4).

To protect against these vulnerabilities, a number of antioxidant defenses are present in the lung epithelial lining fluids, as well as within the lung cells. Ascorbic acid and tocopherols have been studied in this context (5). Tocopherols are believed to be, by sheer mass, the first line of defense against membrane oxidation. Ascorbic acid levels are known to be positively related to pulmonary function (6). Both lycopene and β-carotene have been measured in lung tissue, from autopsy study and from bronchial lavage, lending credence to the possibility that carotenoids are a lung defense mechanism despite the fact that their concentrations are much lower than that of α-tocopherol (7–10). The roles of the carotenoids are not well defined, although it can reasonably be hypothesized that if carotenoids serve as antioxidants, their effect in this environment should be evident. Human studies are needed to prove the bioavailability of lycopene and other carotenoids in the lung, the responsiveness of these substances to insult, and their potential for prevention of functional damage.

Lycopene and Other Lung Functions

Aside from the risk of lung cancer, which is a rare event and is strongly associated with smoke exposure, there is potential for carotenoids to protect lung integrity and lung function. Greater levels of lycopene and other carotenoids in the lung may provide an additional level of protection

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1535-3702/02/22710-0894$15.00

against oxidative and ozone induced damage. Several biochemical mechanisms might explain the protective effects of carotenoids in the human lung. Concentrations of carotenoids in addition to the other antioxidant vitamins in the lung epithelial lining and lining fluids can provide protection against oxidative damage.

The relationships between dietary antioxidants and clinical outcomes of lung dysfunction, such as wheezing, bronchitis, emphysema, asthma, and chronic, nonspecific lung disease have been examined in observational studies (reviewed in Refs. 11 and 12). These have confirmed the associations with vitamin C often (13–15) and vitamin E less often (15).

Other epidemiologic studies have examined the relationship between dietary antioxidants and markers of lung function (6, 16–18). In the NHANES 1 study, a random sample of the U.S. population in the 1970s, spirometric measures in 2500 adults were positively associated with vitamin C intakes, in particular with FEV1 as the outcome (6). In The Netherlands, both vitamin C and β-carotene intakes were associated with higher FEV1 and forced vital capacity (FVC) in a large (n = 6555) adult population aged 20–59 (16). In the UK, reported intakes of both vitamins C and E were associated with greater FEV1 and FVC (17). In a study of men from three European countries, FEV was positively associated with vitamin E intake in Finland and with β-carotene intake in The Netherlands (18). Another population of 1600 adults free from respiratory disease also demonstrated an association between lower vitamin E (or lutein/zeaxanthian) and lower forced vital capacity (19).

Although controlled clinical trials have confirmed the vitamin C and vitamin E effects on lung function (20–23), the examination of carotenoids, particularly those other than β-carotene, has been largely overlooked. To study the uptake of lycopene in the lung and the effects of exercise and ozone exposure on plasma levels and lung levels, we conducted a randomized clinical trial of placebo versus vegetable juice in combination with a supplement of vitamins E and C. This involved bronchial lavage after exposure to air or ozone and exercise in a temperature- and moisture-controlled chamber.

Antioxidants and Lung: The Antioz Study

In collaboration with the Environmental Protection Agency (EPA) to test whether antioxidants can modify lung damage, we have studied the effects of supplementation of carotenoid-rich vegetable juice (V-8) on lung macrophage levels of carotenoids. We investigated whether dietary carotenoids are found in the lung, if they respond to oxidative stress, and if they impact lung function. In this randomized clinical trial, we found that the lung accumulated lycopene, and that the supplementation reduced DNA damage as measured by the Comet Assay.

The ANTIOZ Study: Ozone Exposure and Antioxidant Supplementation. Ozone is a potent oxidant and has been shown to decrease lung function and increase inflammation in humans, which points to a causative role for ozone in lung diseases such as asthma and lung cancer. A randomized placebo-controlled clinical trial was conducted at the EPA to examine whether antioxidant supplementation could protect the human lung from the adverse effects of ozone exposure.

Twenty-three healthy, nonsmoking subjects (21 men and two women) between the ages of 18 and 35 years were enrolled in the study. Volunteers were excluded if their medical history revealed any of the following: cardiovascular disease, pulmonary disease and/or allergy including hay fever or dust allergies, asthma, chronic bronchitis, chronic exposure to dusts, irritants or allergens, hypertension, allergy to lidocaine or other local anesthetics, or any food allergies. Most subjects were not regular vitamin supplement users, but those that took supplements were instructed to stop supplement use at least 90 days prior to entry into the study.

Subjects were placed on a fruit- and vegetable-restricted diet for 1 week. All subjects were then exposed to ambient air while exercising intermittently for 2 hr in a Plexiglas and steel chamber as described previously (24). Blood samples were drawn and lung function was assessed before and after exposure. After the exposure, a bronchoscopy and bronchoalveolar lavage (BAL) was performed. Subjects were then randomized into either placebo (n = 12) or supplement groups (n = 11), and they maintained the low fruit and vegetable diet for 2 weeks. Supplemented subjects received one can of vegetable juice, 250 mg of vitamin C, and 50 IU of α-tocopherol daily, and placebo subjects received one can of orange soda and a placebo pill consisting of a corn oil capsule daily. After 2 weeks of intervention, subjects were exposed to 0.4 ppm ozone in the chamber with intermittent exercise for 2 hr. Again, blood samples were drawn, lung function was assessed before and after the exposure, and another bronchoscopy and BAL were performed on all subjects post-exposure. The study timeline is presented in Figure 1.

The main findings of the study regarding the effects on lung function and inflammation were recently published

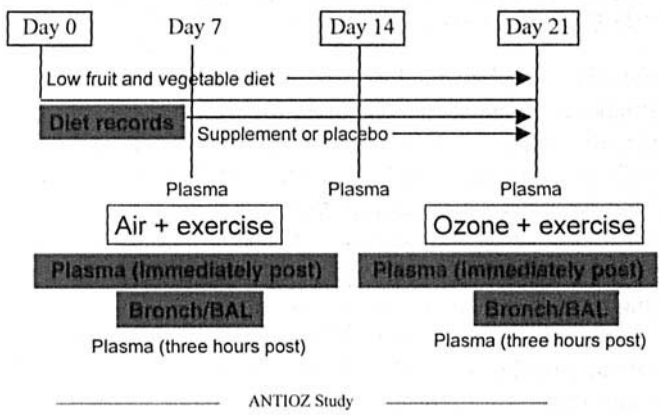

Figure 1. ANTIOZ study timeline and sampling

Table I. Epidemiological Case-Control Studies of Lycopene Intake from Diet or Plasma and Tomato Consumption and Risk of Lung Cancer

Reference	Country	Cases	Dietary assessment	Consumption	OR (95% CI)
Agudo, 1997	Spain	103	33-item FFQ: 1 year prior to diagnosis	Tomatoes (High intake)	0.45 (0.22–0.91)[b]
Axelson, 1996	Sweden	308 M	80-item FFQ: 3 years prior to interview	Tomatoes (daily)	0.67 (0.43–1.05)[e]
Bond, 1987	Texas, USA	308 Deaths	FFQ prior to diagnosis	Tomatoes (4–6 servings/week)	0.44 (0.12–1.53)[g]
				(1 servings/day)	0.42 (0.14–1.33)[g]
Brennan, 2000	Multi-European	506	FFQ	Tomatoes daily	0.5 (0.4–0.6)
Candelora, 1992	Florida, USA	124	60-item Block FFQ: over past 5 years	Lycopene (Highest quartile)	0.6 (0.3–1.2)[f]
De Stafani, 1999	Uruguay	541	64-item FFQ: 1 year prior to symptoms	Tomatoes (>79 servings/year)	0.76 (0.55–1.07)[g]
Forman, 1992	Yunnan, China	183 Tin miners	27-item FFQ	Tomatoes	0.42 (NA)[h]
Garcia-Closas, 1998	Spain	103	33-item FFQ: 1 year prior to diagnosis	Lycopene (>1972.3 mcg/day)	0.56 (0.26–1.24)[g]
Goodman, 1992	Hawaii, USA	463 M	130-item diet	Tomatoes	
		212 F	history	>50 grams/day M	0.77 (NA)[g]
				>42 grams/day F	0.50 (NA)[g]
Harris, 1991	United Kingdom	96	Interview: contains food items	Tomatoes (>29.1 grams/day)	0.69 (NA)[d]
Le Marchand,	Hawaii, USA	597 M	130-item diet	Lycopene	
1989		268 F	history	Highest Quartile M	0.43 (NA)[g]
				Highest Quartile F	0.27 (NA)[g]
				Tomatoes	
				Highest Quartile M	0.63 (NA)[g]
				Highest Quartile F	0.42 (NA)[g]
Le Marchand,	Hawaii, USA	230 M	130-item diet	Lycopene	
1993		102 F	history	>4200 mcg/day M	0.67 (NA)[g]
				>3600 mcg/day F	0.77 (NA)[g]
Li, 1997	United States	93	Unknown	Lycopene (plasma)	0.37 (NA)[h]
Mayne, 1994	New York, USA	413	26-item FFQ: since age 25	Tomatoes	
				Highest Quartile M	0.80 (NA)[h]
				Highest Quartile F	0.76 (NA)[h]
Muscat, 1996	New York, USA	94	FFQ	Tomatoes	0.50 (0.2–1.4)[g]
Sankaranarayanan, 1993	India	281 M	45-item FFQ	Tomatoes (3–6 servings/week)	0.56 (0.30–1.03)[e]
Swanson, 1992	Yunan, China	428	31-item FFQ: during adult life	Tomatoes (>0.8 servings/month)	0.70 (NA)[e]
Ziegler, 1996	New Jersey, USA	1084	44-item FFQ	Lycopene >9055 mcg/day	0.93 (0.58–1.49)[h]

Note. OR, odds ratio; CI, confidence interval; M, male; F, female; FFQ, food frequency questionnaire.
[a] Unadjusted.
[b] Adjusted for smoking.
[c] Adjusted for age.
[d] Adjusted for age and smoking.
[e] Adjusted for age, smoking, and SES.
[f] Adjusted for age, smoking, and energy.
[g] Multivariate adjustment includes age, smoking, and others.
[h] Unable to determine adjustment.

(24). The supplemented subjects experienced 30% and 24% smaller decrements in FEV_1 and FVC, respectively, than the placebo subjects. Markers of inflammation in the lung, however, were not different between the supplemented and placebo subjects. This suggests that antioxidants may have a role in protecting the lung from the adverse effects of ozone exposure on lung function, but not in mediating the inflammatory response.

We also measured DNA damage using the Comet Assay in peripheral blood leukocytes before and after each exposure and in lung epithelial cells after each exposure. We found no significant change in DNA damage in the blood leukocytes in response to either ozone or antioxidant supplementation (25). However, we did observe a significant 20% increase in single-strand breaks in lung epithelial cells of placebo subjects from the baseline bronchoscopy after air exposure to the second bronchoscopy after ozone exposure, and no change in single-strand breaks in lung epithelial cells of supplemented subjects (26). This suggests that antioxidants may protect the lung from DNA damage associated with increased oxidative stress caused by ozone exposure.

We were not able to separate the effects of lycopene from those of other carotenoids or antioxidants administered

Table II. Epidemiological Cohort Studies of Lycopene Intake from Diet or Plasma and Tomato Consumption and Risk of Lung Cancer

Reference	Country	Cases	Dietary assessment	Consumption	OR (95% CI)
Comstock, 1997	Maryland, USA	258	Blood serum before diagnosis	Lycopene (serum) Highest 5th	1.01 (NA)[a]
Fraser, 1991	Seventh Day Adventist, California, USA	61	51-item FFQ at baseline	Tomatoes ≥7 servings/week	1.24 (0.51–2.99)[g]
Knekt, 1991	FMCHES, Finland	117	Interview: habitual diet during past year	Lycopene	Case mean = 684 mcg/day non-case mean = 718 mcg/day
Knekt, 1999	FMCHES, Finland	138	Interview: habitual diet during past year	Lycopene >41 mcg/day	1.00 (0.67–1.50)[d]
Kvale, 1983	Norway	168	Dietary questionnaire	Tomatoes (>14 servings/month)	0.63 (NA)[h]
Michaud, 2000	Health Professional & Nurses, USA	275 M 519 F	FFQ at baseline	Lycopene >18,195 mcg/day	0.86 (0.59–1.25)
Speizer, 1999	Nurses Health Study, USA	593 F	FFQ at baseline	Lycopene Highest 5th Tomatoes (1+/day)	0.8 (0.6–1.1)[g] 0.8 (NA)[g]
Steinmetz, 1993	Iowa Women's Health, USA	179	Willett FFQ at baseline: during past year	Tomatoes (≥3 servings/week)	1.00 (0.61–1.64)[g]
Voorrips, 2000	Netherlands Cohort	939 M	FFQ at baseline	Lycopene >2035 mcg/day	1.05 (0.75–1.46)

Note. RR, relative risk; CI, confidence interval; M, male; F, female; FFQ, food frequency questionnaire.
[a] Unadjusted.
[b] Adjusted for smoking.
[c] Adjusted for age.
[d] Adjusted for age and smoking.
[e] Adjusted for age, smoking, and SES.
[f] Adjusted for age, smoking, and energy.
[g] Multivariate adjustment includes age, smoking, and others.
[h] Unable to determine adjustment.

in this study. However, lycopene was the predominant carotenoid in the vegetable juice supplement. The amount of lycopene in the vegetable juice supplement, as measured by high-performance liquid chromatography (HPLC), was 23 mg/day as compared with 2 mg/day β-carotene, 0.7 mg/day α-carotene, 0.4 mg/day lutein, and 0.1 mg/day zeaxanthin. We measured carotenoids using HPLC in the lung macrophages obtained from the BAL procedure and found that lycopene was also the predominant carotenoid following supplementation, representing 55% of total carotenoids found in these cells (Steck S, Arab L, Craft N, Samet J, unpublished data). After the supplementation period, lung macrophage concentrations of lycopene increased on average 0.56 ng/100,000 cells, representing a 12% increase from baseline (27). It is not clear how well lung macrophage carotenoid concentrations reflect lung epithelial cell concentrations. Only one study has examined this question and observed a correlation of $r = 0.33$ ($P = 0.19$) between BAL cell and lung tissue total carotenoid concentration in 12 smokers (10).

Lycopene and Lung Cancer: Observation Epidemiology. Many case-control and cohort studies have examined lycopene-rich diets and lung cancer (28–54). Tables I and II identify 27 observational studies (18 case-control and nine cohort) that have reported RR estimates for the intake of tomato products, lycopene from dietary sources, or lycopene in serum and risk of lung cancer. The majority of RR estimates for the highest categories of intake or serum concentrations suggest a protective effect. In fact, every case-control OR for the highest category of consumption compared with the lowest is below the null value. The ORs reported in case-control studies ranged from 0.27 to 0.93. Whereas three of nine cohort studies reported RR estimates greater than 1.0, none of these were statistically significant. The RR estimates for cohort studies ranged from 0.63 to 1.24. Therefore, no consistent relationship among cohort studies is evident.

The range of intake across studies showed considerable heterogeneity. As noted in Tables I and II, high intakes for tomato consumption ranged from greater than 0.8 servings

per month to greater than or equal to daily consumption. The range for the highest categories of lycopene from diet was greater than 0.01 mg per day to more than 18.2 mg per day. Closer inspection of the highest categories across all case-control studies revealed that daily consumption of tomatoes was associated with the lowest ORs (28–32) as compared with the highest categories in other studies that set the cut-point for tomato consumption to include lower frequency and quantities of use. This trend was not observed in cohort studies (33) where daily consumption of tomatoes was not associated with risk, except for results from the Nurses Health Study (34) where a modest reduced risk (RR = 0.80) with at least daily consumption was reported. No dose-response relationship between increasing tomato consumption and decreasing risk of lung cancer was observed.

Examining the influence of study design, cohort studies assess exposures prior to the onset of disease, whereas case-control studies evaluate exposures subsequent to disease. Case-control studies can misclassify individuals as a result of recall bias, whereas cohort studies with a single measurement do not account for changes in diet over time. In the case-control studies represented in this review, most dietary assessment tools focused on the year or years (28–29) prior to diagnosis or interview. Recall bias is likely when disease influences an individual's recent diet, and that person is more likely to recall recent diet.

Other sources of bias may include the measurement tool, and in most studies, a single food frequency questionnaire is the method to assess exposure status. The food frequency questionnaire in these studies ranged from 27 to 130 items. Misclassification could be introduced if all sources of lycopene are not represented or, if coded, sources were not adjusted for bioavailability differences between raw and cooked foods. For lycopene, the major sources are tomatoes and tomato products (tomato juice and tomato sauces). Nonetheless, it is uncertain why RR estimates from case-control studies and cohort studies were consistently divergent.

Discussion

The plausibility of a relationship between lycopene and oxidative protection in the lung rests upon the known chemistry of lycopene, *in vitro* studies on its antioxidant potential, and the documented presence of lycopene in lung epithelium and lung macrophages. The epidemiologic literature suggests that diets rich in tomato products are associated with lower lung cancer rates. However, this can also be said for β-carotene, yet interventions failed to show a protective effect. The epidemiology also suggests an interaction between study design and the relationship between lycopene and/ or tomatoes and risk of lung cancer. Cohort studies do not show an association, whereas case-control studies consistently show a protective effect for the highest categories of consumption. This may be due to the decreased attention to quantitative dietary assessment in cohort studies, measurement error in case-control studies, or the differences in bioavailability assessment between studies. Also, cohort studies in general have fewer cases in the exposed subgroups than do case-control studies. All told, the strength of association in epidemiologic studies between lycopene intake and lung cancer risk is inconsistent.

Regarding the hypothesis that supplementation with food-based carotenoids can influence the lung environment, we have been able to demonstrate the uptake of lycopene into the lung after supplementation and ozone exposure. This finding opens the door to research on ways by which we can prevent environmental damage to the lung through dietary selection.

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