Tomato Lycopene and Low Density Lipoprotein Oxidation: A Human Dietary Intervention Study

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ABSTRACT: Increase in low density lipoprotein (LDL) oxidation is hypothesized to be causally associated with increasing risk of atherosclerosis and coronary heart disease. In recent epidemiological studies, tissue and serum levels of lycopene, a carotenoid available from tomatoes, have been found to be inversely related to risk of coronary heart disease. A study was undertaken to investigate the effect of dietary supplementation of lycopene on LDL oxidation in 19 healthy human subjects. Dietary lycopene was provided using tomato juice, spaghetti sauce, and tomato oleoresin for a period of 1 wk each. Blood samples were collected at the end of each treatment. Serum lycopene was extracted and measured by high-performance liquid chromatography using an absorbance detector. Serum LDL was isolated by precipitation with buffered heparin, and thiobarbituric acid-reactive substances (TBARS) and conjugated dienes (CD) were measured to estimate LDL oxidation. Both methods, to measure LDL oxidation LDL-TBARS and LDL-CD, were in good agreement with each other. Dietary supplementation of lycopene significantly increased serum lycopene levels by at least twofold. Although there was no change in serum cholesterol levels (total, LDL, or high-density lipoprotein), serum lipid peroxidation and LDL oxidation were significantly decreased. These results may have relevance for decreasing the risk for coronary heart disease.

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Coronary heart disease is one of the leading causes of death in North America. Epidemiological, clinical, and biochemical studies indicate convincingly that increased serum low density lipoprotein (LDL) cholesterol concentration is associated with increased risk of atherosclerosis (1,2). Oxidative damage to LDL caused by reactive oxygen species may play an important role in the etiology of coronary heart disease (3–5). Antioxidants are believed to slow the progression of atherosclerosis because of their ability to inhibit LDL oxidation (6–9).

In recent epidemiological studies, lycopene levels in serum and adipose tissue were inversely associated with the risk of coronary heart disease (10–13). Lycopene is an antioxidant carotenoid without provitamin-A activity. Tomatoes and tomato products are the main sources of lycopene in the human diet. *In vitro* studies have shown it to be one of the most potent antioxidants (13–15) while epidemiological, animal, and tissue culture studies have indicated its anticarcinogenic potential (for review see Ref. 13). In humans lycopene levels are related to dietary habits and lifestyle (16). The aim of this study was to investigate the effect of dietary supplementation of lycopene on LDL oxidation in healthy human subjects.

MATERIALS AND METHODS

Subjects. Nineteen healthy subjects (10 male and 9 female), age group 25 to 40 yr (average age 29 yr), nonsmokers, not pregnant, and not taking any medication or vitamin supplements, were chosen for this study. Average weight and body mass index of the subjects were 67.6 ± 11.6 kg and 24.0 ± 2.8 kg/m^2, respectively. The subjects maintained their body weight and had no adverse symptoms during the entire study period.

Study design. A randomized, cross-over study design was used. All subjects completed all four treatments including a placebo (0 mg lycopene); 126 g spaghetti sauce (39.2 mg lycopene), provided by Hunt-Wesson Inc., Fullerton, CA; 540 mL tomato juice (50.4 mg lycopene), provided by H.J. Heinz Co. of Toronto, Ontario, Canada; and 1.243 g of 6% lycopene oleoresin from tomatoes (75.0 mg lycopene), provided by LycoRed Natural Product Industries Ltd., Beer-Sheva, Israel, in the form of capsules. Each treatment was for 1 wk with a 1-wk washout phase. Dietary lycopene in the form of tomato products or capsules was consumed once a day with a standardized breakfast consisting of pasta, margarine, grated Parmesan cheese, banana, and BECEL® margarine (Lipton, Toronto, Ontario, Canada) during the treatment period. Test products were consumed as a part of breakfast to standardize the delivery of lycopene. Subjects consumed their regular breakfast during the washout periods. They were advised to avoid consuming tomatoes and tomato products and any other sources of lycopene throughout the study period (treatment and washout phases) and were provided with a list of food products containing tomatoes as a guide. They also main-

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Abbreviations: BHT, butylated hydroxytoluene; CD, conjugated dienes; HDL, high-density lipoprotein; LDL, low density lipoprotein; TBARS, thiobarbituric acid-reactive substances.

tained their food records and a symptoms diary during the study period, which were used to check compliance. The study protocol was approved by the Human Ethics Committee of the University of Toronto. Blood samples were collected from overnight fasting subjects at the end of each treatment phase. Serum was separated from fresh blood and stored at −70°C for analysis. All the samples from one subject were analyzed at the same time to avoid the sample-handling variability.

Lycopene analysis. Serum lycopene was extracted using hexane/methylene chloride (5:1) containing 0.015% butylated hydroxytoluene (BHT) and analyzed by high-performance liquid chromatography using Vydac 201HS54 reverse-phase analytical column (The Separations Group, Hesperia, CA) and a mixture of acetonitrile/methanol/methylene chloride/water (7:7:2:0.16, by vol) as mobile phase (16,17). Lycopene was measured as single peak containing *all trans* and *cis* isomers using an absorbance detector set at 470 nm. An external standard of lycopene (Sigma Chemical Co., St. Louis, MO) was used as reference standard. The coefficient of variance was 4.0%.

Serum cholesterol analysis. Serum was analyzed for total cholesterol, triglycerides, and high-density lipoprotein (HDL)-cholesterol after magnesium chloride precipitation using a Technicon CH1000 (Technicon Inc, Tarrytown, NY) with chemical methods of the Lipid Research Clinic (LRC) Project (18). LDL cholesterol was derived using a modification of the Freidewald formula. The precision and accuracy for total cholesterol, triglyceride, and HDL cholesterol measurements were certified by the Centers for Disease Control—National Heart, Lung and Blood Institute (CDC—NHLBI) Lipid Standardization Program. Internal and external quality control procedures were followed (18).

LDL isolation. For LDL oxidation analysis, serum LDL were isolated by precipitation with buffered heparin (19). The LDL precipitate was separated by centrifugation at $1000 \times g$ and resuspended in saline for further analysis. LDL cholesterol contents were estimated enzymatically (20) using Cholesterol Assay Kit (Sigma Chemical Co.). The coefficient of variance was 1.7%.

Malondialdehyde analysis. Malondialdehyde, as a measure of lipid peroxidation, was estimated using the thiobarbituric acid (TBA) reaction. Results are expressed as TBA-reactive substances (TBARS). Freshly thawed serum or freshly prepared LDL samples were incubated with TBA and orthophosphoric acid, in the presence of BHT, for 45 min at 95°C, cooled to room temperature, and extracted with *n*-butanol. Absorbance of the butanol phase was measured at 535 nm (16,21,22). Results were calculated using the extinction coefficient 1.56×10^5 M^{-1}. Serum-TBARS are reported as μmol/L of serum and LDL-TBARS as mmol/mol LDL. The coefficients of variance for serum-TBARS and LDL-TBARS were 2.8 and 5.3%, respectively.

Conjugated diene (CD) analysis. CD analysis was also done to measure lipid peroxidation. Lipids from LDL were extracted by chloroform/methanol (2:1), dried under nitrogen, redissolved in cyclohexane, and analyzed spectrophotometrically at 234 nm using the molar extinction coefficient 2.95×10^4 M^{-1} (23). LDL-CD are reported as mmol/mol LDL. The coefficient of variance was 2.5%.

Statistical analysis. All statistical calculations were performed by using Excel 5.0 (Microsoft Corp., Redmond, WA). *P* values were assessed by analysis of variance, Dunnett's test, two samples paired Student's *t*-test, and Student-Newman-Keuls multiple range test (SAS Institute, Cary, NC). *P* values of <0.05 were considered statistically significant. Results are expressed as mean ± SEM.

RESULTS

Figure 1 shows the effect of dietary lycopene on serum lycopene. Serum lycopene levels increased significantly with daily intakes of 126 g of spaghetti sauce (39.2 mg lycopene), 540 mL of tomato juice (50.4 mg lycopene), or 1.24-g capsule of tomato oleoresin (75 mg lycopene) as a part of breakfast for a period of 1 wk each over the placebo when no lycopene was consumed. The average increase in serum lycopene levels for any treatment over placebo was at least twofold. However, there were no differences in the serum lycopene levels between the different treatments.

Dietary intake of tomato products had no significant effects on either cholesterol (total, LDL, or HDL) or triglycerides. All dietary lycopene treatments significantly lowered serum LDL oxidation over the placebo (Fig. 2). Both LDL-TBARS and LDL-CD decreased significantly with each tomato product. The average decrease over placebo was 25% for LDL-TBARS and 13% LDL-CD for the tomato products treatment. There were no significant differences between

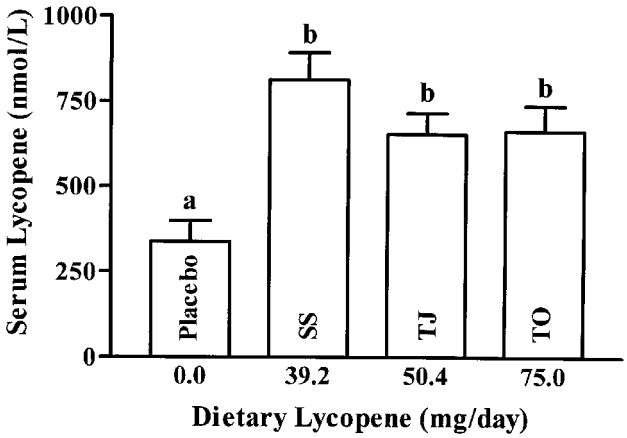

FIG. 1. Effect of dietary lycopene supplementation on serum lycopene concentration. Dietary lycopene was provided to healthy human subjects in the form of spaghetti sauce (SS), tomato juice (TJ), or tomato oleoresin capsules (TO) with a standardized breakfast for a period of 1 wk in a random order. Fasting blood samples were collected at the end of each treatment. Serum lycopene was extracted by hexane/methylene chloride and analyzed by high-performance liquid chromatography (16,17). Results are mean ± SEM. Bars with different letters are statistically significant ($P < 0.05$).

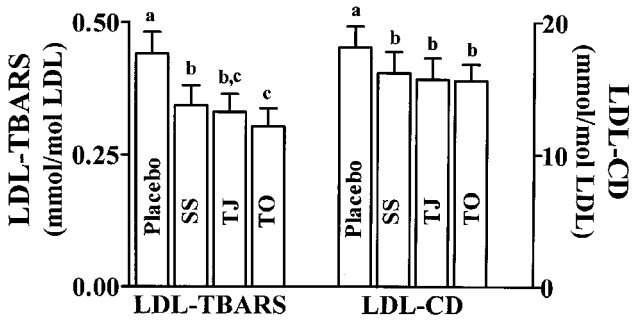

FIG. 2. Effect of dietary lycopene supplementation on serum low density lipoprotein (LDL) oxidation [LDL-thiobarbituric acid-reactive substances (TBARS) and LDL-conjugated dienes (CD)]. Dietary lycopene was provided to healthy human subjects in the form of SS, TJ, or TO with a standardized breakfast for a period of 1 wk in a random order. Fasting blood samples were collected at the end of each treatment. Serum LDL was separated by complexation with heparin. LDL oxidation was measured by spectrophotometric thiobarbituric acid-malondialdehyde and CD assay. Results are mean ± SEM. Bars with different letters are statistically significant ($P < 0.05$). For other abbreviations see Figure 1.

LDL-TBARS and LDL-CD between different sources of lycopene. Both methods of detecting LDL oxidation, LDL-TBARS and LDL-CD, yielded similar results and were highly correlated ($r = 0.89$, $P < 0.05$) with each other. Also LDL-TBARS were significantly correlated ($r = 0.90$, $P < 0.05$) with the serum-TBARS.

DISCUSSION

Our study indicates that human subjects absorb lycopene from traditional sources of processed tomato products including spaghetti sauce and tomato juice as well as from tomato oleoresin. Consuming the tomato products for 1 wk resulted in significantly higher levels of serum lycopene when compared to the placebo. Although no baseline (washout phase) measurements were made, the carryover effect is not important because plasma half-life of lycopene is about 2–3 d (24), and we allowed 1 wk washout during which no tomato products were consumed. Lycopene levels dropped significantly when the subjects consumed lycopene-free diet (16). Moreover, a randomized, cross-over study design provides for a balance in which the order of treatments was assigned to the subjects (25). The consumption of spaghetti sauce, containing much lower amounts of lycopene, resulted in an increase in serum lycopene almost identical to that with tomato oleoresin, which contained a higher lycopene amount, indicating a differential absorption rate for lycopene from different tomato products. These differences can probably be attributed to the differences in food processing. Lycopene from heat-processed tomatoes is more bioavailable than from fresh tomatoes (26).

Intake of tomato products protected the serum LDL cholesterol from oxidative damage and increased the serum lycopene levels. For oxidation analysis, the LDL were separated by precipitation following complexation with heparin at an isoelectric point. The technique was particularly chosen because of its simplicity and rapidity, which avoided artifactual oxidation during processing (27). Moreover, a highly significant correlation between precipitation method and ultracentrifugation or quantitative electrophoresis techniques for plasma LDL analysis had already been established (19,28).

TBARS and CD analyses were used to estimate serum LDL oxidation. Since TBARS assay as a measure of lipid oxidation lacks sensitivity and specificity, we modified the method in this study by using isolated LDL together with BHT during heating and butanol extraction of the pigment. These procedures contributed to increasing specificity and sensitivity of TBARS assay (22). Additional measurement of LDL oxidation was carried out by measuring total serum-TBARS and LDL-CD. The results obtained from these different analyses were in good agreement with each other. Highly significant correlations between LDL-TBARS and serum-TBARS and between LDL-TBARS and LDL-CD, as observed in this study, would further support the validity of using the TBARS test to measure oxidative stress. Our results indicate that both TBARS and CD are good indicators of LDL oxidation. Others reported similar results (29).

Several studies suggest that lipid peroxidation products are cytotoxic and genotoxic and play an important role in the etiology of several degenerative diseases (5–8,30–32). Oxidized LDL is involved in the formation of foam cells that eventually lead to the formation of arterial plaques (5,33), making oxidized LDL a risk factor for coronary heart disease. That the intake of lycopene lowered not only serum lipid peroxidation but also LDL oxidation may have an important physiological relevance in decreasing the risk for coronary heart disease.

In this study, the different tomato products were consumed in one or two servings per day for 1 wk. These consumption levels are easily achievable and are consistent with the current dietary guideline for healthful eating (34,35). Daily consumption of tomato products providing at least 40 mg lycopene was enough to alter LDL oxidation; however, intakes lower than this may also be effective. Future long-term studies should be undertaken in healthy human subjects as well as subjects at risk for coronary heart disease to obtain further information about the role of dietary lycopene in the prevention of the disease.

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REFERENCES

1. Brown, M.S., and Goldstein, J. (1983) Lipoprotein Metabolism in the Macrophage, *Annu. Rev. Biochem. 52*, 223–261.

2. Steinberg, D., and Witztum, J.L. (1990) Lipoproteins and Atherogenesis, *J. Am. Med. Assoc. 264*, 3047–3052.
3. Cathcart, M.K., Morel, D.W., and Chisolm, G.M. (1985) Monocytes and Neutrophils Oxidize Low Density Lipoproteins Making It Cytotoxic, *J. Leukocyte Biol. 38*, 341–350.
4. Hiramatsu, K., Rosen, H., Heineckle, J.W., Wolfbauer, G., and Chait, A. (1987) Superoxide Initiates Oxidation of Low Density Lipoprotein by Human Monocytes, *Arteriosclerosis 7*, 55–60.
5. Witztum, J.L. (1994) The Oxidation Hypothesis of Atherosclerosis, *Lancet 344*, 793–795.
6. Esterbauer, H. (1993) Cytotoxicity and Genotoxicity of Lipid-Peroxidation Products, *Am. J. Clin. Nutr. 57(suppl.)*, 779s–786s.
7. Halliwell, B., and Gutteridge, J.M. (1984) Lipid Peroxidation, Oxygen Radicals, Cell Damage, and Antioxidant Therapy, *Lancet 1*, 1396–1397.
8. Jialal, I., and Devaraj, S. (1996) Low-Density Lipoprotein Oxidation, Antioxidants, and Atherosclerosis: A Clinical Biochemistry Perspective, *Clin. Chem. 42*, 498–506.
9. Rimm, E.B., Stampfer, M.J., Ascherio, A., Giovannucci, E., Colditz, G.A., and Willett, W.C. (1993) Vitamin E Consumption and the Risk of Coronary Heart Disease, *New Engl. J. Med. 328*, 1450–1456.
10. Kohlmeir, L., Kark, J.D., Gomez-Gracia, E., Martin, B.C., Steck, S.E., Kardinaal, A.F.M., Ringstad, J., Thamm, M., Masaev, V., Riemersma, R., Martin-Moreno, J.M., Huttunen, J.K., and Kok, F.J. (1997) Lycopene and Myocardial Infarction Risk in the EURAMIC Study, *Am. J. Epidemiol. 146*, 618–626.
11. Gomez-Aracena, J., Sloots, L., Graciarodriguez, A., Vantveer, P., Gomez-Garcia, C., Garciaalcantara, A., Martin-Moreno, J.M., Kok, F.J., and Navajas, J.F.C. (1997) Antioxidants in Adipose Tissue and Myocardial Infarction in a Mediterranean Area: the EURAMIC Study in Malaga, *Nutr. Metab. Cardiovascul. Disease 7*, 376–382.
12. Kristenson, M., Zieden, B., Kucinskiene, Z., Elinder, L.S., Bergdahl, B., Elwing, B., Abaravicius, A., Razinkoviene, L., Calkauskas, H., and Olsson, A. (1997) Antioxidant State and Mortality from Coronary Heart Disease in Lithuanian and Swedish Men: Concomitant Cross Sectional Study of Men Aged 50, *Br. Med. J. 314*, 629–633.
13. Rao, A.V., and Agarwal, S. (1998) Role of Lycopene as Antioxidant Carotenoid in the Prevention of Chronic Diseases: A Review, *Nutr. Res.*, in press.
14. DiMascio, P., Kaiser, S., and Sies, H. (1989) Lycopene as the Most Effective Biological Carotenoid Singlet Oxygen Quencher, *Arch. Biochem. Biophys. 274*, 532–538.
15. Miller, N.J., Sampson, J., Candeias, L.P., Bramley, P.M., and Rice-Evans, C.A. (1996) Antioxidant Activities of Carotenes and Xanthophylls, *FEBS Lett. 384*, 240–246.
16. Rao, A.V., and Agarwal, S. (1998) Effect of Diet and Smoking on Serum Lycopene and Lipid Peroxidation, *Nutr. Res. 18*, 713–721.
17. Stahl, W., Schwarz, W., Sundquist, A.R., and Sies, H. (1992) *cis-trans* Isomers of Lycopene and β-carotene Human Serum and Tissues, *Arch. Biochem. Biophys. 294*, 173–177.
18. Association of Official Analytical Chemists (1980) *AOAC Official Methods of Analysis*, Association of Official Analytical Chemists, Washington, DC.
19. Wieland, H., and Seidel, D. (1983) A Simple Specific Method for Precipitation of Low Density Lipoproteins, *J. Lipid Res. 24*, 904–909.
20. Allain, C.A., Poon, L.S., Chan, C.S.G., Richmond, W., and Fu, P.C. (1974) Enzymatic Determination of Total Serum Cholesterol, *Clin. Chem. 20*, 470–474.
21. Jentzsch, A.M., Bachmann, H., Furst, P., and Biesalski, H.K. (1996) Improved Analysis of Malondialdehyde in Human Body Fluids, *Free Radical. Biol. Med. 20*, 251–256.
22. Draper, H.H., Squires, E.J., Mahmoodi, H., Wu, J., Agarwal, S., and Hadley, M. (1993) A Comparative Evaluation of Thiobarbituric Acid Methods for the Determination of Malondialdehyde in Biological Materials, *Free. Radical Biol. Med. 15*, 353–363.
23. Ahotupa, M., Ruutu, M., and Mantyla, E. (1996) Simple Methods of Quantifying Oxidation Products and Antioxidant Potential of Low Density Lipoproteins, *Clin. Biochem. 29*, 139–144.
24. Stahl, W., and Sies, H. (1996) Lycopene: A Biologically Important Carotenoid for Humans? *Arch. Biochem. Biophys. 336*, 1–9.
25. Ratkowsky, D.A., Evans, M.A., and Alldredge, J.R. (1993) *Cross-Over Experiments: Design, Analysis and Application*, Marcel Dekker, New York.
26. Gärtner, C., Stahl, W., and Sies, H. (1997) Lycopene Is More Bioavailable from Tomato Paste Than from Fresh Tomatoes, *Am. J. Clin. Nutr. 66*, 116–122.
27. Klienveld, H.A., Hak-Lemmers, H.L.M., Stalenhoef, A.H.F., and Demacker, P.N.M. (1992) Improved Measurement of Low-Density Lipoprotein Susceptibility to Copper-Induced Oxidation: Application of a Short Procedure for Isolating Low-Density Lipoprotein, *Clin. Chem. 38*, 2066–2072.
28. Väisänen, S., Gävert, J., Julkunen, A., Voutilainen, E., and Penttilä, I. (1992) Contents of Apolipoprotein A-I, A-II and B of the Human Serum Fractions for High-Density and Low-Density Lipoproteins Prepared by Common Precipitation Methods, *Scand. J. Clin. Invest. 52*, 853–862.
29. Esterbauer, H., Striegl, G., Puhl, H., Oberreither, S., Rotheneder, M., El Saadani, M., and Jurgens, G. (1989) The Role of Vitamin E and Carotenoids in Preventing Low Density Lipoprotein, *Ann. N.Y. Acad. Sci. 570*, 254–267.
30. Boyd, N.F., and McGuire, V. (1990) Evidence of Lipid Peroxidation in Premenopausal Women with Mammographic Dysplasia, *Cancer Lett. 50*, 31–37.
31. Mukai, F.H., and Goldstein, B.D. (1975) Mutagenicity of Malondialdehyde, a Decomposition Product of Peroxidized Polyunsaturated Fatty Acids, *Science 191*, 868–869.
32. Pincemail, J. (1995) Free Radicals and Antioxidants in Human Disease, in *Analysis of Free Radicals in Biological Systems* (Favier, A.E., Cadet, J., Kalyanaraman, B., Fontecave, M., and Pierre, J.-L., eds.), pp. 83–98, Birkhäuser Verlag, Basel.
33. Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., and Witztum, J.L. (1989) Beyond Cholesterol: Modifications of Low Density Lipoproteins That Increase Its Atherogenicity, *N. Engl. J. Med. 320*, 915–924.
34. Suber, A., Heimendinger, J., Kreb-Smith, S., Patterson, B., Kessler, R., and Pivonka, E. (1992) *5-a-Day for Better Health: A Baseline Study of American's Fruit and Vegetable Consumption*, National Cancer Institute, Washington, DC.
35. Health Canada (1992) *Canada's Food Guide to Healthy Eating*, Ministry of Supply and Services Canada, Cat. No. H39-253/1992E, Ottawa.

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