JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2006,** *54,* 2563−2566 **2563**

Effect of a Tomato-Based Drink on Markers of Inflammation, Immunomodulation, and Oxidative Stress

PATRIZIA RISO,*,† FRANCESCO VISIOLI,§ SIMONA GRANDE,§ SERENA GUARNIERI,† CLAUDIO GARDANA,† PAOLO SIMONETTI,† AND MARISA PORRINI†

Department of Food Science and Microbiology, Division of Human Nutrition, and Department of Pharmacological Sciences, University of Milan, Milan 20133, Italy

Regular consumption of tomato and its products is being consistently associated with lower risk of several types of cancer and, to a lesser extent, coronary heart disease. Among the many tomato components credited with healthful properties, carotenoids and particularly lycopene are being actively investigated. Given the recognized role of immune/inflammatory processes in atherogenesis, the effects of a tomato-based drink (Lyc-o-Mato), which was previously shown to afford DNA protection from oxidative stress, on the modulation of immune and inflammatory markers (by enzyme immunoessay), on basal lymphocyte DNA damage (by comet assay), and on F_2-isoprostane excretion (by LC-MS/MS), were investigated in 26 healthy young volunteers. In a placebo-controlled, double-blind, crossover study, Lyc-o-Mato (5.7 mg of lycopene, 3.7 mg of phytoene, 2.7 mg of phytofluene, 1 mg of β-carotene, and 1.8 mg of α-tocopherol) or a placebo drink (same taste and flavor, but devoid of active compounds) were given for 26 days, separated by a wash-out period. During the study subjects maintained their habitual, hence unrestricted, diet. TNF-α production by whole blood was 34.4% lower after 26 days of drink consumption, whereas the other parameters were not significantly modified by the treatment. In turn, modest effects of the regular intake of a tomato drink, providing small amounts of carotenoids, were found on the production of inflammatory mediators, such as TNF-α, in young healthy volunteers. Future intervention trials in subjects with low carotenoid status and/or compromised immune system will resolve the issue of whether carotenoids modulate immune parameters in humans.

KEYWORDS: Tomato; inflammation; immunomodulation; oxidative stress; carotenoids; lycopene; TNF-α

INTRODUCTION

Regular consumption of tomato and its products is being consistently associated with lower risk of several types of cancer and, to a lesser extent, coronary heart disease (*1*). Among the many tomato components, for example, vitamin C and polyphenols, credited with healthful properties, carotenoids and particularly lycopene are being actively investigated. Carotenoids are potent antioxidants that have also been proposed to affect the immune function, possibly because of their ability to modulate cellular redox environment and cell-to-cell interaction (*2*).

Evidence of a preventive effect of carotenoid (including lycopene) consumption on cardiovascular disease and atherosclerosis is, to date, circumstantial but rapidly accumulating. As an example, we have shown that the regular intake of tomato products for 3 weeks decreases lipid peroxidation markers associated with cardiovascular disease (*3*). The increasingly recognized role of immune/inflammatory processes in atherogenesis (*4*) suggests that the intake of potentially immunomodulating and anti-inflammatory compounds might inhibit the initial damage to the arterial wall, largely by lowering the production of cytokines (*5*−*7*). In agreement with this hypothesis, higher intakes of lycopene have been found to be inversely correlated with the severity of atherosclerosis, as evaluated by intima/media thickness (*8*). Moreover, it is known that nuclear factor κ B (NFκB)-mediated expression of cellular adhesion molecules and pro-inflammatory cytokines are implicated in arterial wall thickening (*9*). Interestingly, lycopene inhibits NFκB activation (*2*), as shown, for example, in dendritic cells (*10*). It is noteworthy that other antioxidants abundant in the Mediterranean diet, namely, resveratrol, oleuropein, and hydroxytyrosol, have been shown to inhibit endothelial NFκB-mediated activation (*11*). The same inflammation/NFκB-mediated pathways are implicated in mutagenesis and carcinogenesis (*12*); hence, the potential exists for lycopene and carotenoids to prevent degenerative disease by lessening the production of inflammatory mediators, for example, TNF-α.

Recently, we reported that daily intake of a tomato drink (Lyc-o-Mato), formulated with a lycopene, phytoene, phytofluene,

* Author to whom correspondence should be addressed (telephone +39.0250316073; e-mail patrizia.riso@unimi.it).
† Department of Food Science and Microbiology.
§ Department of Pharmacological Sciences.

10.1021/jf053033c CCC: $33.50
Published on Web 03/10/2006

and α-tocopherol oleoresin increases plasma and lymphocyte carotenoid concentrations and augments cellular antioxidant protection (*13*). Now, we have further investigated the effects of the same drink on the modulation of immune and inflammatory markers, on basal lymphocyte DNA damage, and on F_2-isoprostane excretion, an in vivo marker of lipid peroxidation.

MATERIALS AND METHODS

Materials. Lyc-o-Mato (Lycored Natural Products Industries Ltd., Beer-Sheva, Israel) is a clear reddish soft drink prepared by a microemulsification technique. A bottle (250 mL) of Lyc-o-Mato contains 5.7 mg of lycopene, 3.7 mg of phytoene, 2.7 mg of phytofluene, 1 mg of β-carotene, and 1.8 mg α-tocopherol, as evaluated by HPLC analysis, a fruit syrup, and flavors. The placebo drink contained the same fruit syrup and flavors, and a colorant, but was devoid of carotenoids, including lycopene, and α-tocopherol. Both beverages had identical appearance, taste, and flavor.

Study Design. This was a double-blind, crossover study, approved by the Local Ethic's Committee. Twenty-six healthy men and women were recruited from within the University of Milan. They had no history of cardiovascular, renal, hepatic, or gastrointestinal disease; pregnant women or subjects routinely taking supplements were not enrolled. The selected volunteers had homogeneous eating habits (in particular for fruit and vegetable consumption), as evaluated by a validated questionnaire (*14*, *15*). Moreover, they did not follow alternative diets (e.g., vegetarian, macrobiotic, or others).

All subjects gave written informed consent to the study and were instructed to maintain their own habitual diet, which was monitored by weekly interviews. In particular, the frequency of consumption of fruits and vegetables was checked to verify the compliance with what was reported in the recruitment phase. Throughout the study, plasma levels of carotenoids, different from that present in the drink, were analyzed as further markers of fruit and vegetable intake.

Male and female subjects were randomly divided into two groups of 13 individuals each: group 1 (age, 25.7 ± 2.1 years; BMI, 21.2 ± 2.2 kg/m^2) was assigned to the sequence placebo/wash-out/Lyc-o-Mato, whereas group 2 (age, 25.9 ± 3.4 years; BMI, 20.9 ± 1.9 kg/m^2) was allocated to the sequence Lyc-o-Mato/wash-out/placebo. Each period lasted for 26 days. Both Lyc-o-Mato and placebo bottles were given once a week to the subjects, who were instructed to store them in the refrigerator and drink one of them per day.

Experimental Procedures. Blood was drawn in the morning before and after each experimental period from the antecubital vein of fasting volunteers into evacuated tubes containing Li^+ heparin as the anticoagulant. Twenty-four hour urine was gathered on the day of blood collection. Aliquots were stored at −80 °C.

Cytokine Determination. Under sterile conditions, 1 mL of blood was diluted with 9 mL of RPMI medium containing penicillin and streptomycin (Sigma, St. Louis, MO). For each volunteer, three test tubes containing 1 mL of diluted blood were prepared. One tube was used as the control; the second tube was treated with 10 μL of lipopolysaccharide (LPS; Sigma) and was incubated at 37 °C for 24 h; the third tube was treated with 12 μL of phytohemagglutinin (PHA; Sigma) and was incubated at 37 °C for 72 h.

After the incubation periods, all samples were centrifuged at 3000*g* for 6 min. Supernatants were stored at −80 °C.

The quantitative determination of human cytokines was performed by enzyme immunoassay, following the instructions of the manufacturer (AL-ImmunoTools, Friesoythe, Germany). In particular, the samples treated with LPS were used to quantify tumor necrosis factor α (TNF-α) and were diluted 1:5 with dilution buffer (PBS with 1% BSA and 0.05% Tween-20). The samples stimulated with PHA were used for the determination of interferon γ (IFN-γ) and were diluted 1:8 with dilution buffer. For each determination, the respective untreated samples were used as controls.

Assessment of DNA Damage. Endogenous lymphocyte DNA damage (mainly single-strand breaks) was evaluated by means of the comet assay (*16*). Briefly, separation of cells was performed on 70 μL of whole blood by density gradient centrifugation using Histopaque 1077 (Sigma). Separated cells were fixed with agarose on fully frosted microscope slides (Richardson Supply Co., London, U.K.).

Slides were incubated in cold lysis buffer for 1 h at 4 °C in the dark and then in fresh alkaline electrophoresis buffer in a horizontal electrophoresis tank (Scotlab, Coatbridge, U.K.) for 40 min, followed by 20 min of electrophoresis (25 V, 300 mA). Slides were subsequently neutralized, stained with ethidium bromide (2 μg/mL, in neutralizing buffer), washed with PBS, drained, and covered with coverslips.

DNA damage was observed under an epifluorescence microscope (BX60; Olympus Italia, Milan, Italy) attached to a high-sensitivity CCD video camera (Variocam; PCO Computer Optics, Kelheim, Germany) and via a computer provided with an image analysis system. One hundred cells for each slide were electronically captured and analyzed for fluorescence intensity. Damaged DNA is recognized as a fluorescent core followed by a tail, which is due to the electrophoretic migration of fragments. DNA damage was calculated as percent DNA in the tail.

Quantitation of Urinary 8-Isoprostaglandin-$F_{2\alpha}$ (8-Iso-$PGF_{2\alpha}$). At each experimental point, aliquots from the 24-h urine were stored at −80 °C.

Urine samples (200 μL) were extracted with ethyl acetate (400 μL) and vortexed. The upper layer (300 μL) was brought to dryness under nitrogen. The residue was dissolved in methanol (100 μL), centrifuged at 1000*g*, and injected in a LC-MS/MS system (20 μL) for 8-iso-$PGF_{2\alpha}$ quantitation.

We used the chromatographic system Alliance model 2695 (Waters) coupled with a Quattro micro triple-quadrupole mass spectrometer (Micromass, Beverly, MA) equipped with an electrospray ionization (ESI) interface. The instrument was operated in the negative ion mode with the capillary voltage set to 3.5 kV, the sampling cone voltage to 30 V, and the source temperature to 120 °C. The argon gas pressure was set to 2.5×10^{-3} mbar. The collision energy was 27 eV. The analyzers were set in the multiple-reaction monitoring mode (MRM) using the transition 353 → 193 $(m/z)^-$. An analytical column 4 μm Synergy Max-RP C12 150 × 2.0 mm (Phenomenex, Torrance, CA) was used. The mobile phase consisted of 2 mmol/L CH_3COONH_4, pH 5.2 (solvent A), and acetonitrile (B). The flow rate was 0.2 mL/min, and the separation was carried out with a linear solvent gradient program starting at 20% B and ramping to 40% B in 20 min.

Calibration curves were obtained by dissolving an 8-isoprostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) standard (Cayman, Ann Arbor, MI) in methanol (1 mg/mL). The mother solution was portioned and stored at −80 °C. The calibration curve was prepared by diluting the mother solution with methanol, in the range of 0.2−10 ng/mL.

Statistical Analysis. Statistical analysis was performed using Statistica as the software (Statsoft Inc., Tulsa, OK). A repeated-measures analysis of variance (ANOVA) with the sequence of treatments (placebo drink followed by Lyc-o-Mato or vice versa) as the independent factor was initially used to investigate the effect of Lyc-o-Mato intake on the variables under study. In the absence of a carry-over effect, all data from the two groups of subjects were matched and analyzed together using ANOVA for repeated measure design with the treatment (Lyc-o-Mato or placebo) as the factor. Differences between means were further evaluated by the least significant difference test. Differences were considered to be significant at $P < 0.05$.

RESULTS

The production of IFN-γ by stimulated blood cells was significantly increased after 26 days of placebo intake [from 9.3 (6.2) to 21.3 (15.7) ng/mL]. Conversely, no significant modulation was noted after the period of Lyc-o-Mato consumption (**Figure 1**).

Concerning TNF-α, the production of which was not modified by the administration of placebo, consumption of Lyc-o-Mato resulted in significantly (34.4%) lower concentrations quantified in challenged whole blood, as shown in **Figure 2**.

Endogenous lymphocyte DNA damage was low and did not change significantly following the intake of either the placebo or the tomato drink (placebo, 2.6 ± 1.3−2.6 ± 1.1; Lyc-o-

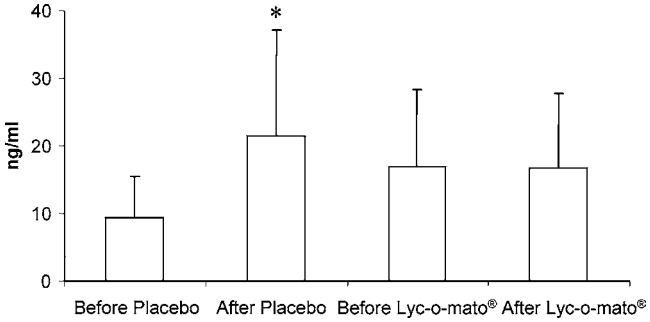

Figure 1. Interferon-γ concentrations in PHA-challenged whole blood of volunteers administered Lyc-o-Mato and placebo drinks in a crossover fashion. Data are means ± SD. *, $p < 0.05$.

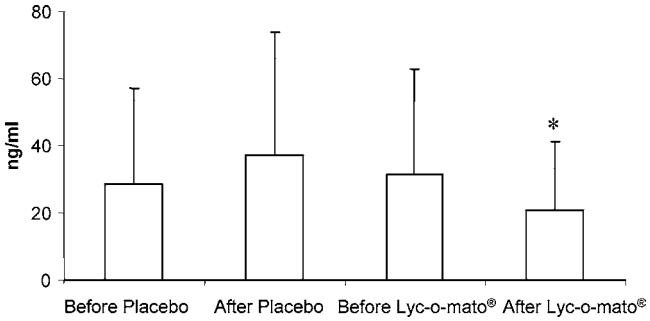

Figure 2. TNF-α concentrations in LPS-challenged whole blood of volunteers administered Lyc-o-Mato and placebo drinks in a crossover fashion. Data are means ± SD. *, $p < 0.05$.

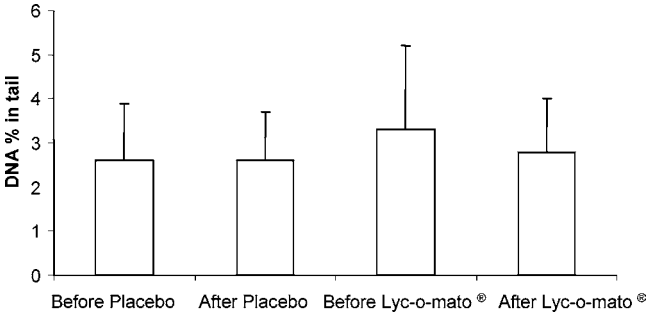

Figure 3. DNA damage of lymphocytes isolated from volunteers administered Lyc-o-Mato and placebo drinks in a crossover fashion. Data are means ± SD.

Mato, 3.3 ± 1.9−2.8 ± 1.2%; DNA in tail, mean ± SD), even if a trend toward a reduction was observed after Lyc-o-Mato intake (**Figure 3**).

Finally, 8-iso-$PGF_{2\alpha}$ excretion, a marker of lipid peroxidation, was unmodified by either soft drink [placebo, from 2.4 (0.2) to 2.1 (0.2); Lyc-o-Mato, from 2.3 (0.2) to 2.4 (0.2) ng/mL; mean ± SD; **Figure 4**].

DISCUSSION

The immunomodulatory activity of carotenoids (namely, β-carotene) has been demonstrated by several animal studies (*17*), whereas data from human intervention studies with supplements or carotenoid-rich foods are still controversial (*18*). The potential ability of carotenoids to influence the immune system may be at least in part explained by the fact that they are lipophilic compounds able to situate in or within the cell membrane, where surface molecules modulate the primary immune response and where they could moderate the activity of redox-sensitive transcription factors, also implicated in mutagenesis and carcinogenesis (*12*).

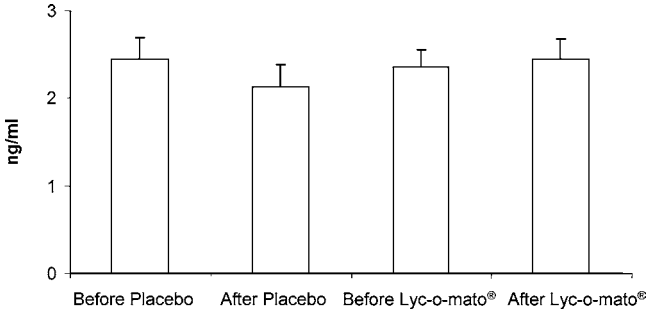

Figure 4. Urinary 8-iso-$PGF_{2\alpha}$ concentrations of volunteers administered Lyc-o-Mato and placebo drinks in a crossover fashion. Data are means ± SD.

We have previously reported that the intake of a tomato drink (Lyc-o-Mato) for 26 days is able to increase not only plasma but also lymphocyte carotenoid, namely, lycopene (+105%), phytoene (+159%), phytofluene (+84%), and β-carotene (+51%) concentrations. After the intervention, the ability of lymphocytes to resist DNA damage induced by an ex vivo oxidative stress was significantly increased (*13*). In the present study, we did not find any effect of Lyc-o-Mato on endogenous DNA damage evaluated on cells not exposed to oxidative stress. Conversely, Pool-Zobel et al. (*19*) reported decreased levels of endogenous DNA strand breaks in subjects who followed 2 weeks of tomato juice (330 mL/day) intervention. The different results might be interpreted as being due to the fact that the volunteers enrolled in our study were all young healthy subjects with very low (2−3%) basal DNA damage. Hence, basal variations are likely to be negligible and of little biological significance, whereas antioxidants, for example, carotenoids, are expected to protect against exogenous oxidative stimuli.

In this study, we did not witness an effect of Lyc-o-Mato on the immune function, as evaluated by analyzing IFN-γ secretion in stimulated blood cells. Actually, we recorded an increase in IFN-γ production after the administration of placebo. This is currently difficult to interpret, as the placebo drink did not contain bioactive components that might be able to exert biological effects.

As opposed to IFN-γ, a marked modulation of TNF-α production was observed. In fact, TNF-α production was significantly decreased (−34%) by the intake of Lyc-o-Mato (**Figure 2**). TNF-α is a cytokine produced by macrophages and T cells that has multiple functions in the immune response. For example, it is primarily involved in systemic inflammation and the acute phase response. Moreover, it is implicated in the development of apoptosis (*20*), and it has also been suggested that sustained elevation of TNF-α levels may be injurious during the effector phase of the immune response (*21*).

Our data disagree with those of Briviba et al. (*22*), who did not record any effect in TNF-α production after 2 weeks of supplementation with Lyco-o-mato oleoresin. Differences between the two studies include their duration (26 vs 14 days, respectively) and the formulation of Lyc-o-Mato, which in the Briviba et al. study was administered as a capsule rather than as a soft drink. Other published data differ from ours: as an example, 8 weeks of tomato juice consumption (330 mL/day providing 47.1 mg/day of lycopene) by well-nourished elderly subjects significantly increased TNF-α concentrations by activated peripheral blood mononuclear cells (PBMC) (*23*). It has been reported that the production of proinflammatory cytokines such as TNF-α is higher in elderly people than in young subjects (*24*). However, Watlz et al. also recorded a tendency to increased production of TNF-α in the control group (consuming the same

amount of mineral water). Finally, PBMC cultured in medium containing 5% FBS (free of carotenoids) or 5% autologous serum (high in lycopene) produced the same amount of TNF-α, underscoring inconsistent effects of tomato juice consumption on cytokine secretion by the elderly (*23*). Another important observation by the same authors was that subjects on a low-carotenoid diet (obtained by restricting fruit and vegetable intake) exhibited reduced T-lymphocyte function, which could be restored by reinstating tomato juice. However, the observed modulation was not explained by changes in plasma carotenoid concentrations, suggesting the involvement of other compounds.

In addition to the modulation of cytokine production, another important potential mechanism that explains the anti-inflammatory effect of bioactive compounds is the inhibition of arachidonic acid metabolism. In particular, F_2 isoprostanes are specific end-products of COX-independent free-radical-catalyzed oxidation of arachidonic acid and are a sensitive and reliable measure of in vivo lipid peroxidation (*25*).

The urinary excretion of F_2 isoprostanes was not modified by Lyc-o-Mato consumption, in contrast to our previous results obtained with, however, tomato products (raw, sauce, and puree) (*3*). This outcome is fairly plausible, as Lyc-o-Mato was administered to healthy volunteers who were not assigned a restricted diet, which, conversely, was adopted in the previous study (*3*). Another lipophilic antioxidant, namely, vitamin E, has been shown not to influence F_2-isoprostane excretion when administered to healthy volunteers (*26*). Also, we now measured F_2 isoprostanes by mass spectrometry instead of immunoassay, thus excluding potential interference in the assay by other molecules.

In conclusion, we report modest effects of the regular intake of a tomato drink providing small amounts of carotenoids on the production of inflammatory mediators such as TNF-α in young healthy volunteers. These data add to the controversy of whether lycopene, and the other carotenoids, affect immune response. In addition, we did not find in these healthy subjects any significant effect of the tomato drink on in vivo lipid peroxidation or endogenous DNA damage. Further intervention trials will follow, in subjects with low carotenoid status and/or compromised immune system.

ACKNOWLEDGMENT

LycoRed Natural Products Industries Ltd., Beer-Sheva, Israel, supplied the Lyc-o-Mato drink.

LITERATURE CITED

(1) Stahl, W.; Sies, H. Bioactivity and protective effects of natural carotenoids. *Biochim. Biophys. Acta* **2005**, *1740*, 101−107.
(2) Hughes, D. A. Effects of carotenoids on human immune function. *Proc. Nutr. Soc.* **1999**, *58*, 713−718.
(3) Visioli, F.; Riso, P.; Grande, S.; Galli, C.; Porrini, M. Protective activity of tomato products on in vivo markers of lipid oxidation. *Eur. J. Nutr.* **2003**, *42*, 201−206.
(4) Frei, B. On the role of vitamin C and other antioxidants in atherogenesis and vascular dysfunction. *Proc. Soc. Exp. Biol. Med.* **1999**, *222*, 196−204.
(5) Getz, G. S. Thematic review series: the immune system and atherogenesis. Immune function in atherogenesis. *J. Lipid Res.* **2005**, *46*, 1−10.
(6) Laberge, M. A.; Moore, K. J.; Freeman, M. W. Atherosclerosis and innate immune signaling. *Ann. Med.* **2005**, *37*, 130−140.
(7) Daugherty, A.; Webb, N. R.; Rateri, D. L.; King, V. L. Thematic review series: the immune system and atherogenesis. Cytokine regulation of macrophage functions in atherogenesis. *J. Lipid Res.* **2005**, *46*, 1812−1822.
(8) Gianetti, J.; Pedrinelli, R.; Petrucci, R.; Lazzerini, G.; De Caterina, M.; Bellomo, G.; De Caterina, R. Inverse association between carotid intima-media thickness and the antioxidant lycopene in atherosclerosis. *Am. Heart J.* **2002**, *143*, 467−474.
(9) Stocker, R.; Keaney, J. F., Jr. Role of oxidative modifications in atherosclerosis. *Physiol. Rev.* **2004**, *84*, 1381−1478.
(10) Kim, G. Y.; Kim, J. H.; Ahn, S. C.; Lee, H. J.; Moon, D. O.; Lee, C. M.; Park, Y. M. Lycopene suppresses the lipopolysaccharide-induced phenotypic and functional maturation of murine dendritic cells through inhibition of mitogen-activated protein kinases and nuclear factor-κB. *Immunology* **2004**, *113*, 203−211.
(11) Carluccio, M. A.; Siculella, L.; Ancora, M. A.; Massaro, M.; Scoditti, E.; Storelli, C.; Visioli, F.; Distante, A.; De Caterina, R. Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of Mediterranean diet phytochemicals. *Arterioscler. Thromb. Vasc. Biol.* **2003**, *23*, 622−629.
(12) Garg, A.; Aggarwal, B. B. Nuclear transcription factor-κB as a target for cancer drug development. *Leukemia* **2002**, *16*, 1053−1068.
(13) Porrini, M.; Riso, P.; Brusamolino, A.; Berti, C.; Guarnieri, S.; Visioli, F. Daily intake of a formulated tomato drink affects carotenoid plasma and lymphocyte concentrations and improves cellular antioxidant protection. *Br. J. Nutr.* **2005**, *93*, 93−99.
(14) Porrini, M.; Gentile, M. G.; Fidanza, F. Biochemical validation of a self-administered semi-quantitative food-frequency questionnaire. *Br. J. Nutr.* **1995**, *74*, 323−333.
(15) Fidanza, F.; Gentile, M. G.; Porrini, M. A self-administered semiquantitative food-frequency questionnaire with optical reading and its concurrent validation. *Eur. J. Epidemiol.* **1995**, *11*, 163−170.
(16) Riso, P.; Visioli, F.; Gardana, C.; Grande, S.; Brusamolino, A.; Galvano, F.; Galvano, G.; Porrini, M. Effects of blood orange juice intake on antioxidant bioavailability and on different markers related to oxidative stress. *J. Agric. Food Chem.* **2005**, *53*, 941−947.
(17) Bendich, A. β-carotene and the immune response. *Proc. Nutr. Soc.* **1991**, *50*, 263−274.
(18) Chew, B. P.; Park, J. S. Carotenoid action on the immune response. *J. Nutr.* **2004**, *134*, 257S−261S.
(19) Pool-Zobel, B. L.; Bub, A.; Muller, H.; Wollowski, I.; Rechkemmer, G. Consumption of vegetables reduces genetic damage in humans: first results of a human intervention trial with carotenoid-rich foods. *Carcinogenesis* **1997**, *18*, 1847−1850.
(20) Zhivotovsky, B.; Orrenius, S. Caspase-2 function in response to DNA damage. *Biochem. Biophys. Res. Commun.* **2005**, *331*, 859−867.
(21) Rutigliano, J. A.; Graham, B. S. Prolonged production of TNF-α exacerbates illness during respiratory syncytial virus infection. *J. Immunol.* **2004**, *173*, 3408−3417.
(22) Briviba, K.; Schnabele, K.; Rechkemmer, G.; Bub, A. Supplementation of a diet low in carotenoids with tomato or carrot juice does not affect lipid peroxidation in plasma and feces of healthy men. *J. Nutr.* **2004**, *134*, 1081−1083.
(23) Watzl, B.; Bub, A.; Blockhaus, M.; Herbert, B. M.; Luhrmann, P. M.; Neuhauser-Berthold, M.; Rechkemmer, G. Prolonged tomato juice consumption has no effect on cell-mediated immunity of well-nourished elderly men and women. *J. Nutr.* **2000**, *130*, 1719−1723.
(24) Rink, L.; Cakman, I.; Kirchner, H. Altered cytokine production in the elderly. *Mech. Ageing Dev.* **1998**, *102*, 199−209.
(25) Rokach, J.; Kim, S.; Bellone, S.; Lawson, J. A.; Pratico, D.; Powell, W. S.; FitzGerald, G. A. Total synthesis of isoprostanes: discovery and quantitation in biological systems. *Chem. Phys. Lipids* **2004**, *128*, 35−56.
(26) Meagher, E. A.; Barry, O. P.; Lawson, J. A.; Rokach, J.; FitzGerald, G. A. Effects of vitamin E on lipid peroxidation in healthy persons. *JAMA−J. Am. Med. Assoc.* **2001**, *285*, 1178−1182.

Received for review December 5, 2005. Revised manuscript received February 14, 2006. Accepted February 15, 2006.

JF053033C