The effect of carotenoids on the expression of cell surface adhesion molecules and binding of monocytes to human aortic endothelial cells

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Abstract

Several large epidemiological studies have shown a correlation between elevated plasma carotenoid levels and decreased risk of cardiovascular disease (CVD). One proposed mechanism for the beneficial effect of carotenoids is through functional modulation of potentially atherogenic processes associated with the vascular endothelium. To test this, we incubated confluent human aortic endothelial cell (HAEC) cultures (passages 4–8) for 24 h with each of the five most prevalent carotenoids in human plasma, which are α-carotene, β-carotene, β-cryptoxanthin, lutein, and lycopene, at an approximate concentration of 1 μmol/l. Carotenoids were solubilized in 0.7% (v/v) tetrahydrofuran and incorporated into FBS before adding to cell culture medium. Due to disparate solubilities in aqueous medium, final concentrations of α-carotene, β-carotene, β-cryptoxanthin, lutein, and lycopene were 1.7, 1.1, 0.7, 0.9, and 0.3 μmol/l and monolayers accumulated 647, 158, 7, 113, and 9 pmol/mg protein, respectively. Monolayers were then stimulated with IL-1β (5 ng/ml) for 6 h with subsequent determination of cell surface expression of adhesion molecules as measured by an enzyme-linked immunosorbent assay (ELISA). To assess endothelial cell adhesion to monocytes, IL-1β-stimulated monolayers were incubated for 10 min with 51Cr-labeled U937 monocytic cells and adhesion determined by isotope counting. Pre-incubation of HAEC with β-carotene, lutein and lycopene significantly reduced VCAM-1 expression by 29, 28, and 13%, respectively. Pre-incubation with β-carotene and lutein significantly reduced E-selectin expression by 38 and 34%, respectively. Pre-treatment with β-carotene, lutein and lycopene significantly reduced the expression of ICAM-1 by 11, 14, and 18%, respectively. While other carotenoids were ineffective, lycopene attenuated both IL-1β-stimulated and spontaneous HAEC adhesion to U937 monocytic cells by 20 and 25%, respectively. Thus, among the carotenoids, lycopene appears to be most effective in reducing both HAEC adhesion to monocytes and expression of adhesion molecules on the cell surface. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Carotenoids; Lycopene; Adhesion molecule; Endothelial cell; U937

1. Introduction

Cardiovascular disease (CVD) remains a leading cause of morbidity and mortality in the United States and atherosclerosis is a key factor in the pathogenesis of myocardial and cerebral infarction, gangrene, and loss of function in the extremities [1]. Although numer-
Intercellular adhesion molecules are involved in the interactions of endothelial cells (EC) and immune cells. Intercellular adhesion molecules are important regulators of direct cell-cell interactions; inflammatory responses in atherogenesis are directed by regulation and expression of these molecules [12]. Adhesion molecule expression in low-density lipoproteins (LDL) receptor-knockout mice fed atherogenic diets showed a significantly reduced incidence of fatty streaks supporting a role for adhesion molecules in atherogenesis [13]. Constituents of each of the main families of adhesion molecules are involved in the interactions of endothelial cells (EC) and immune cells. Intercellular adhesion molecule (ICAM)-1 and -2 (CD54 and CD106) are members of the immunoglobulin superfamily expressed on EC. Of these, ICAM-1 and VCAM-1 increase in response to various inflammatory cytokines. E-Selectin and P-selectin (CD62E and CD62P) on EC also play an early role in adhesion between these two cell types. ICAM-1 and -2 bind to the LFA-1 counterligand; VCAM-1 binds to VLA-4; the selectins recognize certain carbohydrate structures on opposing cells [14,15].

Oxidative stress and expression of adhesion molecules on vascular EC are considered to be important features in the pathogenesis of atherosclerosis and other inflammatory diseases [16,17]. Studies suggest a molecular linkage between an antioxidant-sensitive transcriptional regulatory mechanism and expression of adhesion molecule genes that expands on the notion of oxidative stress as an important regulatory signal in the pathogenesis of atherosclerosis [18]. In the inflammatory response of the arterial wall, leukocyte recruitment to the endothelium is mediated by the interaction of adhesion molecule receptors expressed on the surface of EC and immune cells.

While the pathogenic oxidation of LDL is currently one postulated mechanism for the etiology of atherosclerosis, the role of dietary antioxidants in suppressing the deleterious oxidation of LDL has produced conflicting results, suggesting that other factors and possibly alternate mechanisms are involved. The specific contribution of carotenoids in the atherosclerosis process is inconclusive since carotenoids have been shown to protect LDL in some studies but not in others. In fact, the extent of in vitro LDL oxidation and protection by an antioxidant may not indicate in vivo atherosclerotic status as well as was recently emphasized [19]. However, in one study β-carotene supplementation in rabbits retarded aortic lesion formation [20]. Also, in a more recent study β-carotene supplementation in combination with vitamins E and C reduced aortic valve lesion formation in LDL receptor-knockout mice [21], which may indicate a potential effect by carotenoids in the final pathological outcome. Our contention is that carotenoids may act via alternate mechanisms within the endothelial cell to modulate adhesion molecule expression and thus reduce subsequent leukocyte binding. As a result, the focus of this research was to examine in vitro the effect of the five most prevalent plasma carotenoids on expression of key adhesion molecules involved in the atherosclerosis process, and determine the subsequent binding of U937 monocytic cells when carotenoids are incorporated into human aortic endothelial cells (HAEC). Our results indicate that among the test carotenoids, lycopene appears to be the most effective in reducing immune and endothelial cell interaction.

2. Materials and methods

2.1. Human aortic endothelial cell and U937 cell cultures

HAEC were purchased from Clonetics Laboratories (San Diego, CA) and cultured in M-199 medium (Gibco, Grand Island, NY). Culture medium contained 10% fetal bovine serum (FBS) (Sigma, St. Louis, MO), 5 μg/ml EC-derived growth factor, 100 U/ml heparin, 100 U/ml penicillin, 100 U/ml streptomycin, and 1.25 μg/ml amphotericin B (Sigma). HAEC were cultured on 1% gelatin-coated flasks in Corning 24-well and 96-well plates, and T-25 and T-75 flasks (Corning, NY). The medium was replaced every second day until the cells attained confluence. Fourth to eighth passage cells were employed, and all experiments conducted within 72 h post-confluence using triplicate and quadruplicate wells per experimental treatment. Under inverted microscopy (Zeiss, West Germany), confluent HAEC monolayers displayed a cobblestone phenotype typical of quiescent EC and were characterized by the presence of von
Willebrand factor antigen using immunofluorescent microscopy. Cells were grown to confluence in a humidified atmosphere of 95% air and 5% CO₂. Following treatment of HAEC with 0.05% trypsin for 1–2 min or until 80% of the cells were detached, cell viability was determined by trypan blue exclusion and expressed as the percent of cells that excluded the dye. The monocytic U937 cell line was originally derived from a human histiocytic lymphoma and was procured from American Tissue Type Collection (Rockville, MD). U937 cells were seeded at 5 × 10⁵ cells/ml, grown in suspension culture in RPMI-1640 medium containing 10% FBS, 100 U/ml penicillin, and 100 U/ml streptomycin, and routinely subcultured at a 1:5 ratio two times per week.

Confluent HAEC grown in 24-well plates were supplemented with carotenoids by incubating them in medium alone or containing ~1 μmol/l α-carotene, β-carotene, β-cryptoxanthin, lutein or lycopene for 24 h. Based on our pilot dose response studies and the absence of any apparent pro-oxidant effects, we selected 1 μmol/l as a biologically relevant test concentration. Following supplementation, supernatant was removed, HAEC were washed with phosphate buffered saline (PBS), and medium alone or containing 5 ng/ml human recombinant interleukin (IL)-1β (Pharmingen, San Diego, CA) was added to wells for 6 h to activate cells. Expression of adhesion molecules and adhesion of U937 monocytes were determined (see below).

2.2. Carotenoid supplementation

Stock solutions of carotenoids containing ~ 5 μmol/l were prepared in absolute ethanol and stored under a nitrogen blanket in amber tubes at -70°C. To supplement the culture media with carotenoids, the required amount of carotenoid was transferred from stock solution and dried under nitrogen. Carotenoids were redissolved in tetrahydrofuran (THF) to achieve a final concentration of 0.7% (v/v) in the culture medium. Carotenoids were then mixed with aliquots of FBS and incubated at 37°C for 15 min with gentle mixing by inversion every 5 min. The HAEC were incubated for 24 h in medium containing vehicle or the indicated carotenoid incorporated into the FBS.

2.3. Carotenoid determination

Cellular association of test carotenoids by HAEC and concentrations in media were measured by high performance liquid chromatography (HPLC). Briefly, 2 μg echinenone (gift from Hoffmann-La Roche, Nutley, NJ) was added to homogenates of HAEC cells or media as an internal standard. Aliquots (0.5 ml) of HAEC homogenates were saponified in the presence of 2% pyrogallol (Sigma) at 70°C for 30 min after addition of 30% KOH (0.1 ml). After extraction of cells or medium with hexane containing 0.02% butylated hydroxytoluene (BHT), the samples were dried under nitrogen and reconstituted in absolute ethanol (0.04 ml). Carotenoid peaks were separated according to the method of Yeum et al. [22] using a Pecosphere C18 reverse phase column (0.46 × 8.3 cm × 3 μm) protected by a Perkin-Elmer (Norwalk, CT) guard column (0.46 × 3.3 cm × 3 μm). The mobile phase was acetonitrile-tetrahydrofuran-water (50:20:30: v/v/v, solvent A, and 50:44:6, v/v/v, solvent B, with 1% ammonium acetate in water). The gradient procedure at a flow rate of 1 ml/min was as follows: 60% solvent A and 40% solvent B were used for 1 min followed by a 9 min linear gradient to 83% solvent B, a 4 min hold at 83% solvent B, then a 2 min linear gradient to 100% solvent B, a 2 min hold at solvent B, and finally a 2 min gradient back to 60% solvent A and 40% solvent B. The HPLC system was equipped with a Waters 490E programmable multiwavelength detector (Waters Corporation, Milford, MA) with the wavelength set at 450 nm; peaks were integrated using the Waters Millenium chromatography system.

2.4. Antibodies

Purified mouse anti-human monoclonal antibodies (PharMingen) were used to quantify the expression of VCAM-1. Mab 51-10C9 VCAM-1 (CD 106; mouse IgG1, k) reacts with the 110 kD glycoprotein of VCAM-1 (CD106), also known as INCAM-110, that is expressed at high levels on the surface of cytokine-stimulated endothelium. Mab 68-5H11 reacts with the 97–115 kD cell surface glycoprotein E-selectin (CD62E) also known as endothelial-leukocyte adhesion molecule-1 (ELAM-1) that is expressed on cytokine-stimulated endothelium and is thought to be involved in the early interaction of leukocytes with inflamed endothelium. Mab HA58 reacts with the 85–110 kD integral membrane glycoprotein ICAM-1 (CD54) that is expressed on EC and both resting and activated lymphocytes and monocytes.

2.5. Enzyme-linked immunosorbent assay (ELISA) for adhesion molecule expression

Endothelial cells were cultured in 96-well plates (3.2 × 10⁵ cells/200 μl/well) until confluent, as described above. After 24 h pre-incubation with carotenoids, cultures were washed (PBS with 0.5% sodium dodecyl sulfate, SDS), fixed for 30 min with 1% paraformaldehyde in PBS containing 2% FBS and incubated with blocking buffer (PBS containing 10% FBS) for an additional 60 min. After washing, monoclonal primary antibodies were added by incubating with blocking buffer containing either VCAM-1 (2.5 μg/ml), E-se-
lectin (2.5 μg/ml) or ICAM-1 (1 μg/ml) for 2 h at 25°C. After thorough washing, horseradish peroxidase-conjugated goat anti-mouse antibody (Bio-Rad, Hercules, CA) was diluted and added to the fixed monolayers. After 1 h, cultures were washed and peroxide substrate (Bio-Rad, Hercules, CA) added for 30 min with subsequent addition of stop solution (48% N,N-dimethylformamide containing 20% SDS). After color development, absorbances were determined at 405 nm to estimate relative adhesion molecule expression.

2.6. Assay for U937 monocytic adhesion to HAEC

Adhesion of U937 cells to HAEC cultures was determined as previously described [23,24]. Briefly, HAEC were plated into 24-well plates and grown to confluence before experimental use. At 100% confluence, M199 alone or containing the indicated concentration of carotenoid was added, and the monolayers were incubated for 24 h. After washing to remove unincorporated carotenoid, M199 alone or containing IL-1β (5 ng/ml) was added for 6 h at 37°C. U937 cells (10⁷) were labeled for 60 min at 37°C with 100 μCi ⁵¹Cr as sodium chromate (New England Nuclear, Boston, MA) in 1 ml RPMI-1640 containing 10% FBS. The labeled cells were washed by centrifugation, resuspended in RPMI-1640, and 10⁵ viable cells were added per well after washing the HAEC monolayers to remove cytokine. After 10 min at 37°C to allow binding, non-adherent ⁵¹Cr-U937 cells were removed by repeated washes with PBS, and the cells (HAEC and bound U937) lysed by addition of 0.5 ml harvest solution (PBS containing 0.1% SDS and 1 mM EDTA). After mechanical disruption, each solution was transferred to a 5 ml polypropylene tube for quantitation of total radiolabel associated with each well by γ-ray spectroscopy (Canberra, Sydney, Australia). The number of U937 cells bound to endothelial cells was determined from the specific activity (cpm/cell) of the initial cell suspensions and is expressed as the percentage of added cells that are bound to HAEC. Spontaneous release of ⁵¹Cr by ⁵¹Cr-U937 was less than 5% during the binding phase of the assay. Experiments were repeated 2–3 times with each performed in triplicate or quadruplicate.

2.7. Statistical analysis

Significant differences in the measured parameters between treatment groups were calculated using the unpaired, two-tailed Student’s t-test. Values are presented as means ± SEM and P values < 0.05 were considered significant.

3. Results

Incubation of HAEC for 24 h with complete cell culture medium containing approx. ~1 μmol/l carotenoids increased cellular levels as shown in Table 1. Disparate solubilities due in part to intrinsic chemical characteristics of the carotenoids precluded obtaining equivalent final concentrations of the five test carotenoids in culture medium. However, we were able to incorporate each carotenoid into HAEC to a measurable level although there was a 6-fold difference in medium concentrations (Table 1). Cellular association varied over a 74-fold range and from 0.6–15.6% of total added carotenoid suggesting in addition to varying solubility, there was concentration-dependent association that possibly was also carotenoid- and tissue-specific. In control medium and cell monolayers incubated in unsupplemented medium, carotenoids were not detectable. Pre-incubation with carotenoids did not appear to be cytotoxic to monolayers.

For assessment of the impact of carotenoid incubation on expression of key adhesion molecules, monolayers were incubated overnight with the test carotenoids, washed and further incubated with the pro-inflammatory cytokine IL-1β (5 ng/ml) for 6 h. Previous studies have shown that IL-1β-stimulated expression of VCAM-1, E-selectin and ICAM-1 is maximal at 6 h and IL-1β at 5 ng/ml is an optimal concentration for inducing expression of most adhesion molecules [25]. Additionally, the cobblestone morphology of quiescent

<table>
<thead>
<tr>
<th>Carotenoids concentration</th>
<th>Medium (μmol/l)</th>
<th>Cells (pmol/mg protein)</th>
<th>Associated carotenoid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>α-carotene</td>
<td>1.7 ± 0.3</td>
<td>646.8 ± 78.3</td>
<td>15.6</td>
</tr>
<tr>
<td>β-carotene</td>
<td>1.1 ± 0.1</td>
<td>153.7 ± 16.0</td>
<td>5.6</td>
</tr>
<tr>
<td>β-cryptoxanthin</td>
<td>0.7 ± 0.1</td>
<td>7.4 ± 1.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Lutein</td>
<td>0.9 ± 0.2</td>
<td>113.0 ± 8.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.3 ± 0.1</td>
<td>8.8 ± 2.1</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* n.d., not detected.
Fig. 1. The effect of 24-h incubation with \( \alpha \)-carotene (AC), \( \beta \)-carotene (BC), \( \beta \)-cryptoxanthin (CRY), lutein (LUT), and lycopene (LYC) on IL-1\( \beta \) stimulated VCAM-1 adhesion molecule expression. Human aortic endothelial cell (HAEC) cultures were incubated for 24-h in M199 medium alone (CTL) or containing the indicated carotenoid at \( \sim 1 \) \( \mu \)mol/l. After washing monolayers with PBS, cells were stimulated for 6 h with human recombinant IL-1\( \beta \) (5 ng/ml) and VCAM-1 expression was determined by ELISA as described in Section 2. Values are means \( \pm \) SEM of at least three experiments, each performed in triplicate. Asterisks above the bars indicate significant differences between the IL-1\( \beta \) stimulated CTL and each IL-1\( \beta \) stimulated carotenoid treatment group. Differences were significant (\( P < 0.05 \)) between unstimulated control (CTL without cross-bar) and IL-1\( \beta \) stimulated (CTL with cross-bar) although for clarity no asterisk is presented. There were no significant differences between unstimulated carotenoid treatment groups.

HAEC became spindle-shaped after IL-1\( \beta \) exposure, which is typical of activated endothelium. Basal VCAM-1 expression was low but increased 8.2-fold after incubation with IL-1\( \beta \) (Fig. 1). Pre-incubation with \( \beta \)-carotene, lutein, and lycopene significantly (\( P < 0.05 \)) reduced VCAM-1 expression by 29, 28 and 13\%, respectively. However, neither \( \alpha \)-carotene nor \( \beta \)-cryptoxanthin affected IL-1\( \beta \)-stimulated expression of this molecule. Stimulation of HAEC by IL-1\( \beta \) increased E-selectin expression by 14.7-fold above basal level (Fig. 2). Pre-incubation with \( \beta \)-carotene and lutein significantly reduced E-selectin expression by 38 and 34\%, respectively. However, neither lycopene nor \( \alpha \)-carotene or \( \beta \)-cryptoxanthin markedly affected IL-1\( \beta \)-stimulated expression of this adhesion molecule. HAEC have a high constitutive expression of ICAM-1 and IL-1\( \beta \) treatment further increased expression by 50\% (Fig. 3). Similar to VCAM-1, the expression of ICAM-1 was significantly reduced by 11, 14, and 18\% in monolayers pre-incubated with \( \beta \)-carotene, lutein and lycopene, respectively. Neither \( \alpha \)-carotene nor \( \beta \)-cryptoxanthin had any impact on ICAM-1 expression. Carotenoids did not affect constitutive expression of adhesion molecules on the surface of HAEC (Fig. 4).

Since upregulated expression of several key adhesion molecules including VCAM-1, E-selectin and ICAM-1 are integral in promoting adhesion of monocytes to the vascular endothelium, we next incubated HAEC with \( ^{51} \)Cr-U937 human monocytic cells to determine if...
carotenoids modulate HAEC adhesion to these cells. The basal level of $^{51}$Cr-U937 binding to unstimulated HAEC plateaued at 24% and was not markedly affected by pre-incubation with β-carotene, α-carotene, β-cryptoxanthin or lutein. However, pre-incubation of unstimulated cultures with lycopene significantly reduced $^{51}$Cr-U937 binding by 18%. Stimulation of HAEC with IL-1β significantly increased binding twofold to 47% and pre-incubation with lycopene significantly reduced $^{51}$Cr-U937 binding to HAEC by 20%. In contrast, pre-incubation of HAEC with β-cryptoxanthin, α-carotene, β-carotene or lutein did not affect IL-1β-stimulated binding to the HAEC monolayer. Binding of $^{51}$Cr-U937 to plastic was less than 6% and viability of $^{51}$Cr-U937 was > 95%.

4. Discussion

Although the effectiveness of supplemental β-carotene in reducing coronary heart disease has not been confirmed [26,27], consumption of carotenoid-containing fruits and vegetables has been inversely correlated with the incidence of numerous degenerative diseases [28,29]. One proposed mechanism of action of carotenoids is their antioxidant activity, which may prevent LDL oxidation, although studies have shown conflicting results [30,31]. However, one study showed that β-carotene supplementation reduced fatty lesion formation in rabbits [20]. More recently, β-carotene, in combination with vitamins E and C, was shown to reduce aortic valve lesion formation in an LDL receptor-knockout mouse model [21]. In our study, we investigated the effect not only of β-carotene and but also of other major carotenoids commonly present in human plasma on endothelial cell function and the resultant modulation of interactions between immune cells and EC, an event associated with development and progression of atherosclerosis.

To this end, we supplemented HAEC with the five most prevalent carotenoids in human plasma and found that lycopene, relative to the other test carotenoids, was the most effective in reducing adherence of HAEC to U937 cells and suppressing expression of two molecules involved in cellular adhesion. Relative to its molar concentration in EC, lycopene reduced VCAM-1 expression in IL-1β stimulated cells approx. 6-fold more than lutein and 8-fold more than β-carotene. It was also 16-fold better than lutein and 25-fold better than β-carotene in reducing ICAM-1 expression in HAEC. ICAM-1 mediates the attachment and spreading of monocytes, and VCAM-1 preferentially contributes to firm monocyte adhesion [32,33]. Lycopene was the only test carotenoid that reduced HAEC adherence to U937 cells in both activated and inactivated states. Interestingly, the lycopene concentration in HAEC was significantly lower than other carotenoids, yet it had the greatest impact on both adhesion of monocytes and expression of adhesion molecules.

The final concentration of carotenoids delivered to tissue culture medium after dissolution in THF [34] and incorporation into FBS ranged from 0.3–1.7 μmol/l. The α-carotene concentration was 10-fold higher than concentrations of β-carotene, lutein and β-cryptoxanthin, and was approximately twice the concentration normally found in plasma of healthy adults [35]. The level of lycopene in culture medium, however, was similar to normal plasma levels. Lycopene is the major carotenoid in human plasma and comprises ~40% of the total plasma pool with a concentration of 0.2–1.1 μmol/l [36,37]. Dietary lycopene occurs predominantly in tomatoes and tomato products. Intake is approx. 3 mg/d in the US [38].

The low concentration of lycopene in HAEC was due in part to insolubility of carotenoids in media and inherent physical characteristics of carotenoids as suggested by Britton [39]. Increasing carotenoid concentrations in culture medium required larger volumes of organic solvent vehicle, viz., THF, which caused cytotoxicity.

Endothelial cells recruit leukocytes by selectively expressing three types of adhesion molecules in response...
to inflammatory stimuli such as IL-1β [40]. Pro-inflammatory cytokines are commonly found in atherogenic lesions, and can induce chemotactic factors, other cytokines, and adhesion molecules which collectively contribute to the inflammatory process [41]. As a result, we focused on the impact of cell-associated carotenoids and the expression of key adhesion molecules often coupled with atherosclerotic events. We found that three of the five tested carotenoids, viz., β-carotene, lutein, and lycopene, significantly reduced both VCAM-1 and ICAM-1 expression in IL-1β-stimulated HAEC cultures. While β-carotene and lutein significantly suppressed E-selectin expression as well, lycopene had no impact on E-selectin.

Upregulation of adhesion molecule expression by cytokine-induced oxygen radical formation results in part from activation of NF-κB [42,43]. NF-κB is a redox-sensitive transcriptional factor that mediates gene expression in inflammatory, immune and acute phase responses [44]. Modulation of adhesion molecule expression by reactive oxygen radicals and antioxidants has been investigated and evidence supports the role of a redox mechanism in the initiation of atherosclerosis and suggests the potential for dietary intervention with such antioxidants [45,46]. Experimental data clearly demonstrate that cellular accumulation of antioxidants including N-acetylcyesteine (NAC), pyrroline dithiocarbamate (PDTC), and α-tocopherol modulate adhesion molecule expression and monocyte adhesion to EC models by blocking activation of NF-κB when stimulated by cytokines, phorbol esters, hydrogen peroxide and lipopolysaccharide [18,47,48]. A number of diverse compounds including aspirin, isoflavones, flavonoids, phytoalexins, and antibiotics have also been shown to reduce both endothelial adhesion molecule expression and subsequent leukocyte binding [49–51]. Several potentially non-antioxidant mechanisms have been proposed for these and other compounds including inhibition of protein tyrosine kinases [49], inhibition of proteasomes [52], and decreased degradation of IκB [53], as well as modulation of cyclic AMP levels [50] and protein kinase C activity [54]. Additionally, it has been reported theVCAM-1, ICAM-1, and E-selectin may share a common regulatory signal immediately after receptor activation by receptor inducers, but ICAM-1 and E-selectin gene expression may be modified by gene-specific signal transduction mechanisms [45].

Carotenoids have been reported to function in immunomodulation, cellular gap junction communication, enzyme induction, and as retinoid precursors [8,39]. In addition to forming potentially bioreactive retinoids, carotenoids may be metabolized to a number of compounds through central and/or eccentric cleavage to form apocarotenals, as well as forming oxidative metabolites [6,7].

In our studies, cellular association was readily detectable in HAEC over a range consistent with values reported in the literature for several mammalian cell types, including hepatoma cells [55], human hepatocytes [56], and buccal mucosal cells [57]. Carotenoids have been detected in lipid-rich atherosclerotic lesions, and in particular β-carotene and lycopene, although their concentrations have been reported to be low [58]. The elevated level of cellular α-carotene suggests concentration-dependent association which may be tissue-specific, and may depend on properties of α-carotene as well.

Expression of endothelial adhesion molecules may reflect endothelial activation, although leukocyte adhesion may not be substantially altered [59]. Our results indicate that β-carotene and lutein modulate adhesion molecule expression, but these two carotenoids did not reduce binding of monocytes to EC. In contrast, lycopene, or one of its metabolites, significantly reduced monocyte binding to both unstimulated and IL-1β-stimulated HAEC suggesting a structure-specific mechanism of action [7]. Numerous studies have demonstrated variable patterns of adhesion molecule expression and differing effects on monocyte binding when antioxidants were incorporated into cells. Faruqi et al. [60] and Vandermeeren et al. [47] found that incubation of EC with α-tocopherol or dimethylfumarate significantly reduced both adhesion molecule expression, viz., VCAM-1, E-selectin and ICAM-1, and U937 binding. However, Weber et al. [61] demonstrated that PDTC and NAC dose-dependently reduced cytokine-induced VCAM-1, but not ICAM-1, expression in HUVEC, although PDTC decreased adhesion of U937 cells to EC. Furthermore, Faruqi et al. [60,62] reported that the antioxidants α-tocopherol, probucol and NAC inhibited binding of U937 to EC which correlated with E-selectin expression, but other antioxidants including ascorbic acid, BHT, and desferral did not inhibit binding. Conversely, Marui et al. [18] showed that in IL-1β stimulated EC, VCAM-1 expression was reduced 90% by PDTC and NAC, but ICAM-1 and E-selectin were insensitive to antioxidant inhibition. Erl et al. [63] reported that IL-1β-stimulated expression of VCAM-1 and E-selectin in EC, but not ICAM-1, was inhibited by α-tocopherol succinate in a time- and dose-dependent manner. Vanhee et al. [40] demonstrated that stimulation of EC with cytokine increased expression of ICAM-1 but did not affect VCAM-1 or E-selectin, which resulted in increased binding of U937 to EC presumably through an ICAM-1 adhesion pathway. Thus, cytokine- and cell-specific induction of adhesion molecules and subsequent binding of U937 appear to be modulated in an antioxidant-specific manner.

Structurally, individual carotenoids may cause unique membrane modification that alters the expression of adhesion molecules and/or function. Britton [39]...
suggests that carotenoids are located in precise locations and orientations in cellular structures, and their physicochemical properties are strongly influenced by neighboring molecules. Lutein and lycopene possess exceptionally high antioxidant activity compared to other carotenoids [7] and in fact lycopene possesses the greatest physical quenching capacity [64]. Woodall et al. [65] have shown that lycopene with its opened rings and extended conjugated diene system reacts rapidly with oxidizing agents suggesting key function as an in vivo antioxidant.

Lycopene is one of the major carotenoids in the diet of North Americans and Europeans. Epidemiological studies have suggested that intake of lycopene, in addition to the prevention of cancers of the prostate or gastrointestinal tract [38,66,67], may be also associated with reduced risk of CVD. The recent European study (EURAMIC multicenter study) evaluated the association between adipose tissue concentrations of carotenoids and the risk of myocardial infarction [4]. A significant inverse association for lycopene alone and acute myocardial infarction was evident [68]. Most recently, a population-based case-control study in Costa Rica also reported that a lower adipose content of lycopene was associated with increased risk of myocardial infarction. Circulation 1998;98:1–374.

Our in vitro data are in concurrence with the findings of these studies in that among the carotenoids tested, only lycopene modulated adhesion molecule expression and reduced binding of U937 monocytes suggesting a pivotal role for lycopene in attenuating atherogenesis. As a result, further elucidation of the metabolic and functional contributions of lycopene and/or its metabolites in the vascular endothelium are necessary to assess potential protective roles.

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