Evidence for Oxidative Activation of c-Myc–Dependent Nuclear Signaling in Human Coronary Smooth Muscle Cells and in Early Lesions of Watanabe Heritable Hyperlipidemic Rabbits

Protective Effects of Vitamin E

Filomena de Nigris, PhD; Tammam Youssef, MD; SilviaAnna Ciafre, PhD; Flavia Franconi, MD; Vittorio Anania, PhD; GianLuigi Condorelli, MD; Wulf Palinski, MD; Claudio Napoli, MD

Background—Oxidized LDL (oxLDL) promotes atherogenesis, and antioxidants reduce lesions in experimental models. OxLDL-mediated effects on c-Myc are poorly characterized, and those on c-Myc nuclear pathways are completely unknown. c-Myc stimulates smooth muscle cell (SMC) proliferation and could be involved in atherosclerosis. We investigated the early effects of oxLDL and \( \alpha \)-tocopherol on c-Myc, its binding partner Max, and the carboxy-terminal domain–binding factors activator protein-2 and elongation 2 factor in human coronary SMCs. We also investigated whether 9-week treatment of Watanabe heritable hyperlipidemic (WHHL) rabbits with diet-enriched \( \alpha \)-tocopherol reduces c-Myc expression and oxLDL in the left coronary artery.

Methods and Results—OxLDL enhanced c-Myc/Max expression and transcription by cotransfection assay and the nuclear activities of E2F and activator protein-2 by binding shift and supershift in coronary SMCs. \( \alpha \)-Tocopherol significantly reduced these molecular events. Furthermore, \( \alpha \)-tocopherol reduced early lesions, SMC density, and the immunohistochemical presence of c-Myc, which colocalized with oxLDL/foam cells in the coronaries of WHHL rabbits.

Conclusions—We provide the first evidence that oxLDL and \( \alpha \)-tocopherol may influence c-Myc activation and several c-Myc–dependent signaling pathways in human coronary SMCs. The observation that in vivo, an antioxidant reduces both c-Myc and oxLDL in early coronary lesions of rabbits is consistent with, but does not prove, the hypothesis that c-Myc–dependent factors activated by oxidative processes contribute to atherogenesis and coronary heart disease. (Circulation. 2000;102:2111-2117.)

Key Words: lipoproteins ■ coronary disease ■ antioxidants ■ atherosclerosis

Oxidized LDL (oxLDL) and oxidative byproducts contribute to early atherogenesis,\(^1\) as seen in fetuses\(^2\) and children.\(^4\) A corollary of this theory is that antioxidants have beneficial effects on atherosclerosis.\(^1\) Although some of the intervention trials have so far been mostly unsuccessful,\(^5\) antioxidants have been shown to reduce atherogenesis in most experimental models,\(^6\) and human studies in a randomized clinical trial have shown that \( \alpha \)-tocopherol reduces the incidence of coronary heart disease\(^7\) and can be beneficial in its secondary prevention.\(^6\)

Vitamin E inhibits atherogenesis not only by protecting LDL against oxidation but also by interference with oxidation-sensitive signaling pathways regulating cytokine expression and other factors.\(^5,7\) One such factor is c-Myc, an early response gene,\(^8,9\) which after heterodimerization with its physiological binding partner, Max,\(^10\) is a potent activator of transcription coding for phosphonuclear proteins. c-Myc is involved in cell growth and differentiation\(^8,9\) and in smooth muscle cell (SMC) proliferation.\(^11\) Enhanced expression of c-Myc-mRNA has been demonstrated in human SMCs cultured from aortic plaques,\(^12\) carotid atherosclerotic lesions,\(^13\) and vein graft SMC hyperplasia.\(^14\) Exposure of human SMCs to native LDL (nLDL) for 1 hour also increased c-Myc expression.\(^15\)

c-Myc recognizes DNA sequences through Max,\(^9,10\) and it has reciprocal regulatory effects at the carboxy-terminal domain; c-Myc/Max also controls other transcription factors\(^9\) by sequestering or interacting with them, including activator
protein (AP)-2\(^a\) and elongation 2 factor,\(^{16}\) which are involved in the basal machinery of cell. To date, oxLDL-mediated effects on c-Myc are poorly understood and are limited to mRNA detection in fibroblasts exposed to minimally modified LDL.\(^{17}\) Effects of oxLDL on c-Myc–dependent nuclear signaling pathways are completely unknown. The goal of the present study was to investigate the effects of cell exposure to oxLDL and \(\alpha\)-tocopherol on c-Myc, its binding partner Max, and AP-2 and E2F in human coronary SMCs. We also investigated whether treatment with \(\alpha\)-tocopherol in Watanabe heritable hyperlipidemic (WHHL) rabbits interferes with c-Myc expression in the left coronary artery.

**Methods**

**Lipoproteins**

Plasma was obtained from healthy nonsmoking males (n=4, 21 \pm 2 years), and LDL was rapidly isolated by ultracentrifugations in KBr, as described.\(^{18}\) LDL was immediately used to minimize spontaneous peroxidation.\(^{18}\) Proteins were determined by the Lowry assay.\(^{19}\) LDL (300 mg/mL) was incubated for 12 hours at 37°C with 1 mmol/L copper.\(^{16}\) Malondialdehyde was assayed by the thiobarbituric acid method.\(^{18}\) LDL was oxidized, dialyzed against phosphate buffer,\(^{18}\) and then added to the medium in the presence or absence of newly added \(\alpha\)-tocopherol.

**Cell Culture**

Primary human coronary SMCs were cultured in delipidated DMEM, as described.\(^{20}\) Our preliminary experiments and others\(^{19}\) had shown that nLDL induced c-Myc mRNA immediately after the start of the cell cycle (15 to 20 minutes). After time-course experiments, we exposed the cells to oxLDL with or without \(\alpha\)-tocopherol for only 2 hours. We did not investigate possible mitogenic effects induced by c-Myc and/or other mediators, which would become evident only after 8 hours (ie, S phase).

**Preparation of Nuclear Proteins**

The nuclear extracts were prepared as described\(^{20,21}\) and assayed by the method of Lowry et al.\(^{19}\)

**Western Blot Analysis**

Whole-cell extracts (50 mg)\(^{20,21}\) were transferred to Immobilon-P membranes (Millipore). Membranes were incubated for 1 hour with the following antibodies (1:1000 in 5% nonfat milk buffer): anti-human c-Myc (C-19), Max (C-17), E2F-1 (C-20), and AP-2 and E2F in human coronary SMCs. We also investigated whether treatment with \(\alpha\)-tocopherol in Watanabe heritable hyperlipidemic (WHHL) rabbits interferes with c-Myc expression in the left coronary artery.

**Immunoprecipitation**

Nuclear extracts (100 mg) were incubated overnight at 4°C with c-Myc (C-33) or Max (H-2) antibodies (Santa Cruz). Antibody–protein complexes were immunoprecipitated with protein G plus agarose for 1 hour at 4°C, and beads were washed 3 times in lysis buffer consisting of 0.5% NP-40 (Sigma), 50 mmol/L HEPES (pH 7.5), 250 mmol/L NaCl, 5 mmol/L EDTA, 50 mmol/L NaF, 0.5 mmol/L sodium orthovanadate, 0.5 mmol/L phenylmethylsulfonyl fluoride, 5 mg/mL aprotinin, and 5 mg/mL leupeptin and then boiled for 5 minutes. Anti-c-Myc immunoblots were probed with E2F-1 (C-17), AP-2α (C-18), and Max (C-20) antibodies, and anti-Max immunoblots were probed with c-Myc (C-19) antibody.

**Electrophoretic Mobility Shift Assay**

An electrophoretic mobility shift assay was performed as described.\(^{20}\) During supershift experiments, 10 mg of c-Myc (C-33) antibody was added. The oligonucleotide probes were as follows: consensus binding site for E2F-1 (2507, Santa Cruz), 5'-ATTTAAGTTCTCGCCCTTTCTCA-3'; binding site for AP-2α (2513, Santa Cruz), 5'-GATCGAAGTGAACCGGGCGGGCCGT-3'.

**Transient Transfection Assay**

Confluent SMCs were cotransfected with 10 mg of the plasmid pSVluciferase (Promega), into which the c-Myc/Max binding site sequence was cloned,\(^{22}\) and 2 mg of \(\beta\)-galactosidase reporter vector. Cells were transfected by 30 mg lipofectamine according to the procedure of Life Technologies. After transfection (24 hours), the medium was changed, and after an additional 24 hours, cells were assayed for luciferase and \(\beta\)-galactosidase activities (Promega).

**In Vivo Effects of \(\alpha\)-Tocopherol on c-Myc**

Male 8-month-old WHHL rabbits (Harlan-Nossan) were used to study c-Myc expression in vivo and \(\alpha\)-tocopherol effects in the left coronary artery.\(^{23}\) in accordance with the Guidelines of the American Physiological Society. The control group (n=9) was fed a regular diet containing 0.005% (wt/wt) \(\alpha\)-tocopherol; the antioxidant group (n=9) received the same diet supplemented with 0.5% (wt/wt) \(\alpha\)-tocopherol, a dose proven to be effective in rabbits.\(^7\) After 9 weeks, rabbits were euthanized by an overdose of ketamine. Under a stereo microscope, the left coronary artery was dissected.\(^{23}\) Lesions were determined as the area staining for oil red O in 15 to 20 sections per coronary artery by computer-assisted imaging.\(^2,4\) Oil red O usually identifies arterial lipid accumulations.\(^2,4\) Additional paraffin-embedded sections were immunostained with AB-2/c-Myc antibody (Calbiochem), RAM-11 (macrophages), MDA-2 (oxLDL), and NP-1539, an apoB antibody.\(^2,4\) Antibodies were used at 1:250 to 1000 dilutions and detected by avidin-biotin–peroxidase.\(^2,4\) Sections were analyzed with the investigator blinded to the sample identity. SMC density was determined by use of Alcian blue.\(^2\) Vitamin E concentrations in plasma, LDL, and tissue were determined by high-performance liquid chromatography.\(^2,4\)

**Statistics**

Results were analyzed by 1-way ANOVA followed by the Bonferroni correction or by the Scheffé multiple comparison test; a value of \(P<0.05\) was considered significant. Immunohistochemical data were analyzed for mean \pm SE and variance, kurtosis, and skew. Correlations were evaluated by linear regression analysis. Data were analyzed by the SPSS statistical package.

**Results**

Under our experimental conditions, nLDL contained 0.8 \pm 0.4 mmol malondialdehyde/mg protein, and oxLDL contained 12.9 \pm 2.5 mmol malondialdehyde/mg protein (\(P<0.001\)). Electrophoretic mobility on agarose was 0.5 \pm 0.2 and 1.9 \pm 0.3 cm for nLDL and oxLDL, respectively (\(P<0.05\)). We tested several doses of \(\alpha\)-tocopherol on c-Myc signaling pathways, but only results with 10 and 50 \(\mu\)mol/L are presented. At lower doses (ie, 300 \(\mu\)mol/L to 3 \(\mu\)mol/L), densitometry of blots and shifts showed no significant differences between oxLDL with and without freshly added \(\alpha\)-tocopherol (not shown). Concentrations of \(\alpha\)-tocopherol >50 \(\mu\)mol/L (ie, 100 to 300 \(\mu\)mol/L) produced results similar to those at 50 \(\mu\)mol/L (\(P=NS\)).

**c-Myc Expression in Human SMCs**

Western blots of total protein extracts from SMCs exposed to oxLDL for 2 hours showed that 10 and 50 \(\mu\)mol/L \(\alpha\)-tocopherol progressively prevented accumulation of the 64- to 67-kDa c-Myc protein (Figure 1A). c-Myc in cells exposed to oxLDL, compared with nLDL, was significantly increased.
Figure 1B shows a significant reduction of c-Myc expression at 4 to 8 hours and the lowering effects of vitamin E.

c-Myc/Max Complex

To investigate whether c-Myc was in its active form (ie, heterodimerized with Max and capable of binding DNA), we immunoprecipitated the nuclear extracts. Figure 2A shows an immunoprecipitation with anti–c-Myc followed by plotting with an equal dose of anti–c-Myc. Densitometry revealed a significant increase of c-Myc/Max in cells exposed to oxLDL compared with nLDL. The heterodimerization of c-Myc was inhibited dose-dependently by α-tocopherol. Figure 2B shows an immunoprecipitation with equal doses of anti-c-Myc followed by anti–Max. Again, densitometry showed an increase of Max in nuclear extracts from oxLDL-treated cells (P<0.0001 versus nLDL), which was inhibited by α-tocopherol. Taken together, they indicated that 68±6% of the increased amount of c-Myc induced by oxLDL was coupled to Max. α-Tocopherol (50 μmol/L) almost abolished the elevation of c-Myc/Max induced by oxLDL (ie, −72±3%) but did not appear to affect the ratio of binding of c-Myc to Max. Also, in this case, there was a dramatic reduction of Max at 4 to 8 hours (Figure 2C). Vitamin E significantly reduced Max expression at 1 and 2 hours (Figure 2C). Finally, to assess the effects on transcription, moderate basal luciferase activity was detected in SMCs treated with nLDL for 24 hours, as conventional time for luciferase measurements (Figure 2D). In contrast, an ∼7-fold increase of luciferase activity was found in cells exposed to oxLDL (P<0.0001); this was reduced progressively by α-tocopherol. Thus, increased nuclear expression of the c-Myc/Max complex induced by oxLDL was associated with its enhanced transcriptional activity.

E2F and AP-2 Factors

We also investigated whether c-Myc/Max complex was associated with carboxy-terminal domain–binding factors, such as AP-2 and E2F, which may be complexed at the c-Myc DNA-binding site. Figure 3 shows the immunoprecipitation with c-Myc, followed by anti-E2F or anti–AP-2 (panels A and B, respectively). Densitometry showed an increase in c-Myc/E2F or c-Myc/AP-2 complexes in response to oxLDL; α-tocopherol decreased both complexes dose-dependently.

An electrophoretic mobility shift assay was used to investigate whether the increase of AP-2 and E2F complexes in the nucleus also reflected their DNA-binding capacity. Figure 4A (left) shows that oxLDL significantly increased the binding of nuclear extracts to E2F. This was progressively reduced in cells treated with α-tocopherol. We then examined by supershift (Figure 4A, right) whether the E2F complex contained c-Myc. Incubation of nuclear extracts from cells exposed to oxLDL with the c-Myc antibody resulted in a change in electrophoretic mobility of the band recognized by the E2F probe. This band was supershifted, and it migrated slowly, indicating that E2F was complexed with c-Myc. In cells treated with α-tocopherol, we observed a progressive reduction of the supershifted band, indicating reduced formation of E2F/c-Myc complexes.

Figure 4B (left) shows increased binding to the AP-2 oligonucleotide in nuclear extracts from cells exposed to oxLDL. According to E2F data, this increase was reduced by α-tocopherol. Similarly, when the extracts were preincubated with the c-Myc antibody, a change in electrophoretic mobility of the AP-2 supershifted band was observed (Figure 4B, right). Thus, oxLDL causes an increase of nuclear AP-2 and E2F activities that is dose-dependently inhibited by α-tocopherol. More important, the E2F and AP-2 complex may cooperate in the nucleus with c-Myc, and α-tocopherol actions on c-Myc are also extended to E2F and AP-2. In all of the above experiments, treatment of cells with nLDL plus 50 μmol/L α-tocopherol (n=3) or 50 μmol/L α-tocopherol alone (n=4) did not result in significant changes (not shown). However, at greater doses (100 to 300 μmol/L), α-tocopherol resulted in a small additional decrease of c-Myc and c-Myc/Max complexes (P=NS).

In Vivo Studies

Plasma cholesterol in WHHL rabbits at the end of the 9-week intervention was similar in the vitamin E and control groups.
(721±38 and 743±42 mg/dL, respectively; P=NS). Plasma vitamin E was 65.8±6 μmol/L in the control group and 325.8±12 μmol/L in the treated group (P<0.0001). Vitamin E on LDL was 5.2±0.4 and 28.5±1.3 molecules per LDL particle in the control and treated groups, respectively (P<0.001). Tissue vitamin E in the intima and media of the coronary arteries of the control and vitamin E–treated groups was 1.5±0.2 and 5.8±0.4 ng/mg, respectively, in microscopic lesion-free coronary sections (P<0.001). These values increased to 15.6±0.9 and 48.5±1.3 ng/mg, respectively, in microscopically detectable early lesions (P<0.001). Susceptibility of LDL to oxidation was determined as a measure of the biological activity of α-tocopherol. As expected, LDL from the vitamin E group showed reduced oxidation (2.8±0.5 versus 10.2±2.1 nmol malondialdehyde/mg protein, P<0.001).

Computer-assisted imaging of sections of the entire left coronary artery showed that early lesions were significantly reduced by α-tocopherol (32 080±3180 versus 19 030±2260 μm², P<0.01). In absolute terms, these lesions are small.
compared with the aorta, where extensive atherosclerosis occurred in 10-month-old WHHL rabbits. Although the quantitative differences between groups may be of relative relevance, the Table reports atherosclerosis in the left coronary arteries. Atherogenesis was significantly decreased in the main trunk of the left coronary artery (LMT) and in the left anterior descending (LAD) and left circumflex (LCX) coronary arteries in the vitamin E–treated group compared with the control group. Cells immunostained for c-Myc were mainly distributed in the media, and the same cells were also immunostained for SMC actin. In coronaries without significant lesions, 6.2 ± 1.5% of all intimal and medial cells (identified by their nuclei) were stained for c-Myc/AB-2 antibody used at a low dilution (1:1000). In lesions, significantly more cells were stained for c-Myc (48.8 ± 5.8%, P < 0.0001), and c-Myc–positive cells were frequently grouped together.

c-Myc expression was significantly reduced in both normal and atherosclerotic coronary arteries by α-tocopherol. Figure 5 (top) shows staining for c-Myc in the left coronary arteries of control and vitamin E–treated WHHL rabbits. The media contained 694 ± 95 SMC nuclei/mm² in the control group and 503 ± 81 SMC nuclei/mm² in the vitamin E–treated group (P < 0.05). In analogy, the intimal/medial ratio decreased after α-tocopherol administration (0.178 ± 0.06 vs. 0.355 ± 0.196 in controls, P < 0.01). Figure 5 also reports percentages of positive sections for c-Myc (AB-2), oxLDL (MDA-2), nLDL (NP-1539), and macrophages (RAM-11) in left coronary arteries (LMT, LAD, and LCX) from control and vitamin E–treated WHHL rabbits.

### Atherosclerosis in Left Coronary Arteries of Control and Vitamin E–Treated WHHL Rabbits

<table>
<thead>
<tr>
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<th>LMT</th>
<th>LAD</th>
<th>LCX</th>
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<tbody>
<tr>
<td>Control (n=9)</td>
<td>11.5 ± 1.8 (9.1–14.0)</td>
<td>6.8 ± 1.0 (5.3–8.2)</td>
<td>28.5 ± 3.4 (23.4–34.6)</td>
</tr>
<tr>
<td>Vitamin E (n=9)</td>
<td>9.1 ± 0.6* (7.9–10.3)</td>
<td>4.1 ± 0.5† (2.9–6.1)</td>
<td>18.5 ± 2.2‡ (14.9–22.8)</td>
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Values are mean ± SEM (range), and differences were determined by Scheffé test.

Severity of atherosclerosis (5 sections/coronary segment) is expressed as a score: 0 points, no lesions; 1 point, stenosis <15%; 2 points, stenosis ≥15% to 30%; 3 points, stenosis ≥30% to 60%; and 4 points, stenosis ≥70%. Scores are shown to permit comparisons with coronary lesions previously reported. A score of 4 was found in only 2 control LCXs.

P<0.0321, †P=0.0232, and ‡P=0.0384 vs controls.
rophanes. However, α-tocopherol significantly reduced macrophages in the LAD and LCx but not in the LMT, suggesting that reduced immunostaining for c-Myc is related to the reduction in macrophages. A separate analysis of the colocalization of c-Myc with other components throughout the coronary artery indicated that c-Myc staining correlated well with oxLDL (r = 0.77, P < 0.001) and, to a lesser extent, with macrophages (r = 0.34, P < 0.05). Because of the selective decrease of c-Myc and oxLDL in Figure 5, their colocalization with macrophages and nLDL also decreased in vitamin E–treated rabbits (P = NS).

**Discussion**

We demonstrate that exposure to oxLDL enhances c-Myc/Max expression and transcription and the nuclear activities of E2F and AP-2 in human coronary SMCs. α-Tocopherol significantly reduces these events in cultured SMCs, and prolonged α-tocopherol treatment of WHHL rabbits reduces the expression of c-Myc in early atherosclerotic coronary lesions, as well as SMC density and lesion size. Neither the in vitro nor the in vivo data provide evidence for a causal role of c-Myc in atherogenesis. However, they show convincingly that oxLDL activates c-Myc signaling in coronary cells. Given that oxLDL is abundant in atherosclerotic lesions and that c-Myc seems to be involved in the proliferation of SMCs (and of other intimal cells as well), these data are consistent with the hypothesis that modulation of atherogenesis by oxLDL and α-tocopherol also involves c-Myc signaling.

Activation of c-Myc pathways was inhibited by α-tocopherol, the predominant LDL antioxidant. During LDL oxidation, antioxidants are rapidly depleted, whereas supplementation with α-tocopherol increases the resistance of LDL to oxidation.25 Although the micromolar concentrations used in the present study are severalfold greater than the natural levels, α-tocopherol is easily incorporated into vessels and plaques, reaching much higher concentrations in arteries than in plasma. Given that incubation of SMCs with α-tocopherol alone or in combination with nLDL had little effect on the c-Myc–dependent pathways, a direct cellular effect independent of oxLDL seems improbable. The fact that oxLDL was oxidized before addition of the antioxidant also suggests that α-tocopherol did not act exclusively by preventing LDL oxidation. It may limit the activation of c-Myc signaling induced by oxLDL, either by inhibiting further oxidative modification of oxLDL during cell incubation or by interfering with oxygen radical–mediated signaling upstream from c-Myc. This is supported by studies carried on the same experimental conditions,20 which demonstrated that α-tocopherol at the time of LDL exposure to oxidants did not modify the reduction of the oxLDL-modified induction of transcription factors.

In vivo vitamin E data are consistent with the assumption that vitamin E contributes to the reduction of atherosclerosis by downregulating the expression of c-Myc–dependent factors. However, the present study was not designed to provide evidence for causality. It must be kept in mind that α-tocopherol influences multiple other mechanisms potentially affecting atherogenesis (such as cytokines and leukocyte adhesion and their targeting to endothelium) and monocyte transmigration.7

A rapid increase of c-Myc/mRNA was induced in SMCs15 by nLDL prepared by conventional centrifugation (which is likely to induce spontaneous oxidation) and in fibroblasts by minimally modified LDL.17 In the present study, we provide the first evidence that this is not limited to c-Myc itself but that oxLDL also rapidly induces c-Myc/Max transcriptional activity and the formation of complexes with E2F and AP-2. Such complexing of transcription factors is increasingly recognized to be functionally relevant.27 α-Tocopherol reduced the expression of some c-Myc–dependent upstream events, indicating that its actions may also extend to nuclear activities and to other pathways, such as AP-1 and nuclear factor-κB.20 Micromolar concentrations of α-tocopherol may decrease c-Myc/mRNA26,29 and negatively regulate E2F transactivation activity29 in tumors. Therefore, it is conceivable that α-tocopherol also affected c-Myc–dependent pathways in arteries.

c-Myc activation and target genes8 may have long-term effects on SMC proliferation, which may contribute to the growth of atherosclerotic lesions. This may represent a single-clonemutation event (monoclonal hypothesis of atherosclerosis),30–33 SMCs cultured from human plaques retain transforming potential and display enhanced c-Myc expression.12,13 Transforming genes and c-Myc overexpression were also found in coronary SMCs when plaque DNA was transfected into fibroblasts and the transformed cells were injected into a nude mouse.34 SMCs from human plaques display chromosome instability,15 microsatellite genomic instability in the transforming growth factor-β receptor (that may disable apoptosis and allows monoclonal selection16), or a single pattern of X inactivation indicating monoclonality.37 Although other data do not support the monoclonal hypothesis (eg, no consistent trends were found in glucose-6-phosphate dehydrogenase isoenzyme distribution in the different layers of human plaques),31,33 the above findings provide ample evidence of proliferative events in plaques involving c-Myc signaling.

The present study and many others12–14,34 have found c-Myc expression in plaques, but its causal role in the pathophysiology of atherosclerosis remains unclear. Although in vivo experiments explore different aspects of the biological effects of vitamin E, we provide the first evidence that α-tocopherol reduces both c-Myc expression and the onset of early atherogenesis in the coronary arteries of WHHL rabbits. This is remarkable for 2 reasons. First, a reduction of atherosclerosis by vitamin E has been previously reported in New Zealand White rabbits.7 Chronic treatment with α-tocopherol also inhibits the SMC neosynthesis stimulated by oxidized lipids, as well as other signals, during restenosis in cholesterol-fed rabbits,8 whereas in WHHL rabbits, α-tocopherol treatment had little effect on advanced aortic lesions.39 α-Tocopherol–induced beneficial effects and a reduction of c-Myc pathways may also occur in early coronary lesions, in which intimal SMC proliferation still plays a subordinate role. Thus, α-tocopherol–mediated protection was associated with reduced c-Myc–dependent nuclear signaling, which may have an impact on events other than SMC proliferation, or the associated phenomena are independent in the causal relationship.

Little is known about the role of α-tocopherol and c-Myc signaling on human atherosclerosis. The interference of vitamin E with c-Myc–dependent and other pathways20 may
favor plaque stabilization. The Gruppo Italiano per lo Studio della Streptochinasi nell’Infarto Miocardico (GISSI)-Prevenzione and the Heart Outcomes Prevention Evaluation (HOPE) trials have shown apparent negative results, but the statistical analysis and the criteria for patient selection are still controversial. Interestingly, compared with the control the statistical analysis and the criteria for patient selection are still controversial.

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References

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