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Effects of β-carotene and α-tocopherol on the levels of tissue cholesterol and triglyceride in hypercholesterolemic rabbits

Kamalakshi C. Sulli, Jidong Sun, David W. Giraud, Rodney A. Moxley,* and Judy A. Driskell

*Department of Nutritional Science and Dietetics and *Department of Veterinary and Biomedical Sciences, University of Nebraska, Lincoln, NE USA*

Male New Zealand white rabbits were made hypercholesterolemic by feeding an atherogenic diet (0.5% cholesterol, 3% peanut oil, and 3% coconut oil) with and without β-carotene (25 mg/kg body weight given intravenously twice weekly) and/or α-tocopherol (0.5% of diet) for 8 weeks. Rabbits treated with combined β-carotene and α-tocopherol had significantly lower cholesterol contents in liver, heart, and plasma than control animals; heart and plasma cholesterol contents were also significantly lower in animals treated with β-carotene than in controls. Treatment with both antioxidants significantly increased triglyceride contents of liver and triceps, but not heart and plasma. Rabbits given both β-carotene and α-tocopherol had significantly lower values for tissue α-tocopherol than animals treated with α-tocopherol only, and significantly higher values for tissue β-carotene than animals treated with β-carotene only. Atherosclerotic lesion areas in the aortic arch, thoracic aorta, and abdominal aorta were positively correlated (r = 0.36–0.42) with plasma cholesterol concentrations and negatively correlated (r = −0.34––0.60) with plasma, liver, triceps, and heart α-tocopherol levels. Moderate levels of α-tocopherol supplementation with or without β-carotene may have a beneficial effect on tissue cholesterol contents and on the development of aortic atherosclerotic lesions in rabbits fed an atherogenic diet. (J. Nutr. Biochem. 9:344–350, 1998) © *Elsevier Science Inc. 1998*

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Introduction

The antioxidants vitamin E and β-carotene appear to be protective nutrients against the development of coronary heart disease.[1–4] Various factors, including foam cell accumulation[5,6] and endothelial damage,[7] may increase oxidation of low density lipoproteins (LDLs), the synthesis of autoantibodies, and the incidence of atherosclerosis.[8]

Carotenoids may have protective effects against many chronic degenerative diseases in which reactive oxygen species play a role.[9] Within tissues and LDLs, β-carotene functions as a chain-breaking antioxidant.[10] α-Tocopherol, biologically and chemically the most active form of vitamin E, also functions as a chain-breaking antioxidant in LDLs and tissues.[11,12] It has been observed in vitro that as long as there are antioxidants in LDLs, the rate of lipid peroxidation is low. Among these endogenous antioxidants, β-carotene is the last to be consumed when LDL is exposed to oxidizing conditions.[6] α-Tocopherol is by far the most abundant antioxidant in LDL. Almost all of the in vivo studies that employed supplementation with α-tocopherol found significantly increased resistance of LDL to oxidation by Cu^{2+}, macrophages, and endothelial cells.[6,13] β-Carotene has been reported to significantly decrease aortic atherosclerotic lesions in hypercholesterolemic rabbits,[14] as has α-tocopherol.[12,15] β-Carotene is the more effective quencher of singlet oxygen at low oxygen pressures, whereas vitamin E is more effective at high oxygen pressures.[16] Dietary antioxidants protect LDL against oxidation[17,18] and may help

Address correspondence and reprint requests to Dr. Judy A. Driskell, Department of Nutritional Science and Dietetics, University of Nebraska, Lincoln, NE 68583-0806, USA.
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prevent experimental atherosclerosis.[12,14,15] Epidemiologic evidence suggests that antioxidants also help to prevent the manifestations of clinical coronary artery disease.[1,19]

This study was designed to investigate the effects of β-carotene and α-tocopherol, or a combination of both, on the tissues of hypercholesterolemic rabbits. The objectives were to investigate whether intravenous injection of β-carotene or dietary supplementation of α-tocopherol, or a combination of both, would affect the cholesterol and triglyceride contents in liver, triceps muscle, and heart muscle of rabbits fed an atherogenic diet. The tissue contents of cholesterol, triglyceride, β-carotene, and α-tocopherol were correlated with the development of aortic atherosclerotic lesions in these animals. Treatment of these rabbits with both antioxidants did inhibit the development of atherosclerotic lesions.[20]

Materials and methods

Animals and experimental design

New Zealand white male rabbits (Gary Smith Rabbitry, Harlan, IA USA), each weighing approximately 2.5 kg, were housed individually in wire-bottomed stainless steel cages in a room kept at 16°C, 50% humidity, and alternate 12-hour periods of light and dark. These 28 rabbits were initially fed Purina Certified Rabbit Chow (No. 5322, Purina Mills, Inc., St. Louis, MO USA). The rabbits were observed daily and weighed weekly. The University of Nebraska's Institutional Animal Care and Use Committee approved all procedures performed on the animals.

The rabbits utilized in the current study were also used in a previously published investigation in which the animal treatment was described in greater detail.[20] All four groups of animals were fed Purina Certified Rabbit Chow supplemented with 0.5% (w/w) cholesterol, 3% (w/w) peanut oil, and 3% (w/w) coconut oil. The diets were stored at 4°C until used. The control (CN) group received the atherogenic diet with no additional antioxidants. The β-carotene and α-tocopherol contents of this atherogenic diet were 0.0028% and 0.0074% (w/w), respectively. Animals in the β-carotene (BC) group were given 25 mg/kg BW β-carotene (Atherotene, 40 mg/mL, CardioSpectrum, Inc., East Walpole, MA USA) intravenously twice weekly to avoid possible intestinal conversion of β-carotene to vitamin A.[21,22] This route also is the most efficient way to get β-carotene into the internal organs and peripheral tissues of the rabbits. Differences are known to exist by species as to how efficiently oral doses of carotenoids are absorbed. The α-tocopherol (AT) group received an additional 0.5% (w/w) dl-α-tocopherol added to their diets. One group (AT + BC) received both α-tocopherol and β-carotene treatments. After storage for 10 months, the diets were confirmed to contain the appropriate levels of α-tocopherol by analysis.

Tissue collection

Animals were sacrificed at the end of the 8-week experimental period following a 12-hour fast. The liver, left triceps muscle, and heart muscle were excised, thoroughly blotted, and quickly put on dry ice. These tissues were then stored at −70°C for later lipid and antioxidant analyses.

Determination of tissue cholesterol, triglyceride, β-carotene, and α-tocopherol concentrations

Tissue cholesterol concentrations were determined using the method of Abell et al.[23] Tissue triglyceride concentrations were determined using kit no. 405 from Sigma Chemical Co. (St. Louis, MO USA). Tissue concentrations of β-carotene and α-tocopherol were determined by high performance liquid chromatography using an adaptation[20] of the method developed by Nierenberg and Nann.[24]

Determination of extent of aortic atherosclerosis

Aortic atherosclerotic lesion areas of these animals were estimated by the methods previously described.[20]

Statistical analyses

All data were evaluated by general linear models with differences between groups being determined using least squares means. Pearson correlation coefficients were determined between aortic atherosclerotic lesion areas and values for tissue biochemical variables. A *P*-value of less than 0.05 was taken to indicate statistical significance. Results were expressed as least square means ($LS\bar{x}$) and least square standard errors (LSSE). All statistical analyses were performed using SAS version 6.08 (SAS Institute Inc., Cary, NC USA, 1990).

Results

Body weight, plasma concentrations of various lipids, and aortic atherosclerotic lesion area data of the rabbits in the current study were previously published.[20] Initial weights of the rabbits were 2.66 ± 0.04 kg (mean ± SEM) and those after 8 weeks were 3.45 ± 0.05 kg (mean ± SEM), with no significant differences between groups.

Organ weights of animals

Liver, triceps, and heart weights of the animals are given in *Table 1*. Liver weights of animals in the AT + BC group were significantly lower ($P < 0.05$) than those in the CN and AT groups. Animals in the BC group had lower liver weights also, but they were not significantly different from those of the CN and AT groups. No significant differences were observed in triceps and heart muscle weights among the different treatment groups.

Cholesterol and triglyceride levels

Cholesterol and triglyceride contents of organs were calculated per gram and per organ; data are presented in *Figure 1* and *Table 2* on the organ basis.

Liver, heart, and plasma but not triceps cholesterol levels of animals in the AT + BC group were significantly lower ($P < 0.05$) than those in the CN and AT groups (*Figure 1*). Heart and plasma cholesterol levels were also significantly lower ($P < 0.05$) in BC rabbits than in CN and AT groups. Triceps muscle cholesterol levels of AT + BC rabbits were significantly higher ($P < 0.05$) than those of other groups.

Table 1 Organ weights of rabbits by group

Organ	Group				LSSE
	Control	β-carotene	α-tocopherol	α-tocopherol + β-carotene	
Liver (g)	147.5—a	126.08—a,b	145.96—a	110.43—b	8.46
Triceps (g)	42.98—a	40.60—a	45.51—a	50.86—a	2.93
Heart (g)	6.79—a	6.77—a	7.24—a	7.12—a	0.22

Values are LS$\bar{x}$. Values among groups having different letters for each variable are significantly different at $P < 0.05$.
n/group = 7.
LSSE, least square standard errors.

Liver triglyceride levels of BC and AT + BC animals were significantly higher ($P < 0.05$) than those of CN and AT groups (*Table 2*). Triglyceride contents of triceps muscles were significantly higher ($P < 0.05$) in the AT + BC group than in other groups, with no significant differences among the CN, BC, and AT groups. Heart and plasma triglyceride levels of rabbits in the four treatment groups were not significantly different.

β-*Carotene and* α-*tocopherol levels*

β-Carotene and α-tocopherol contents of organs were calculated per gram and per organ; data are presented in *Table 2* on the organ basis. The BC group had significantly lower ($P < 0.05$) levels of β-carotene in their livers, triceps muscles, heart muscles, and plasma than animals in the AT + BC group, but significantly higher ($P < 0.05$) than the CN and AT groups. The AT + BC group had significantly lower ($P < 0.05$) levels of α-tocopherol in their livers, triceps muscles, heart muscles, and plasma than the AT group, but significantly higher ($P < 0.05$) levels than the CN and BC groups. The combined antioxidant treatment (AT + BC) resulted in significantly lower values for tissue α-tocopherol compared with the group given α-tocopherol only (AT) and significantly higher tissue β-carotene levels than the group given β-carotene only (BC).

Aortic atherosclerotic lesion areas

The percentage of intimal aortic surface areas covered by atherosclerotic lesions are given in *Figure 2*. The extent of

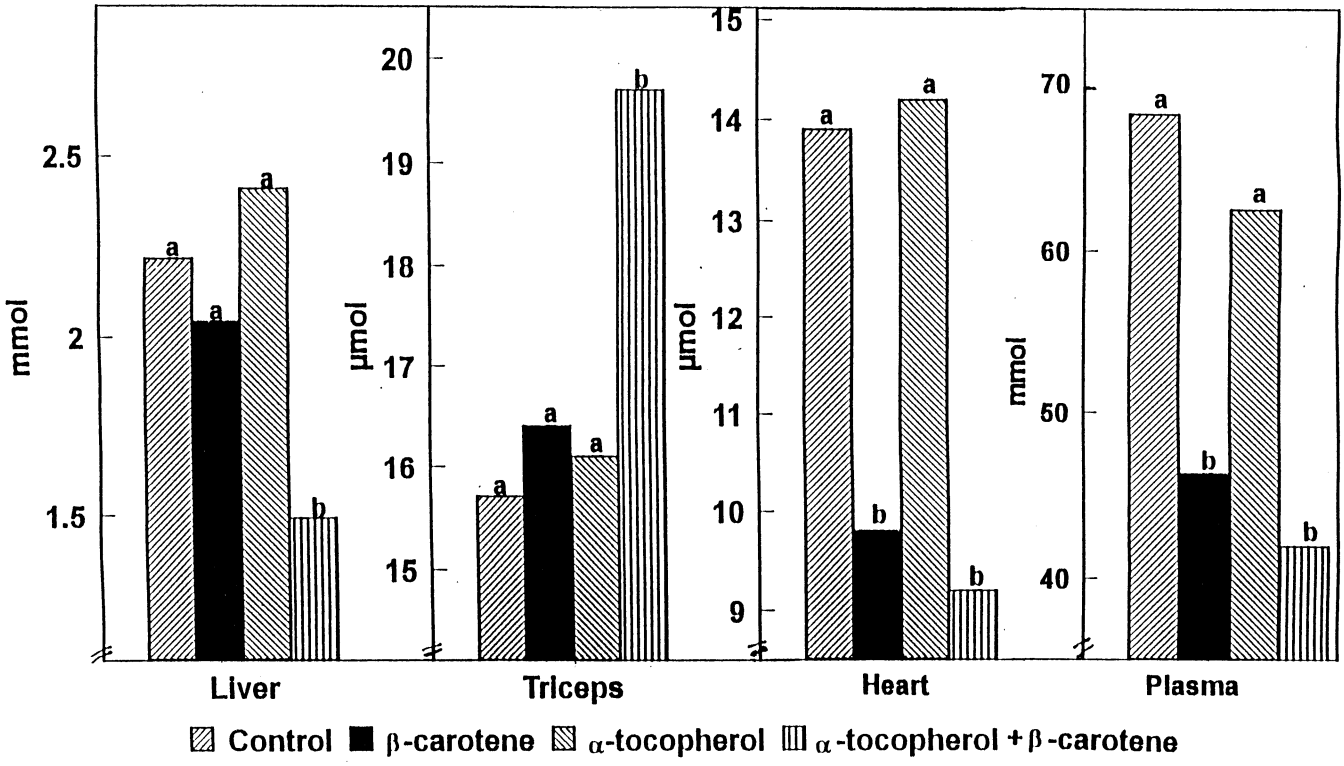

Figure 1 Cholesterol contents of liver, triceps, heart, and plasma of rabbits by group. Values represent least square means. Least square standard error values were 0.164 mmol for liver, 1.352 μmol for triceps, 1.144 μmol for heart, and 5.055 mmol for plasma. a,b, values among groups having different letters for each variable are significantly different at $P < 0.05$.

Table 2 Tissue triglyceride, α-tocopherol, and β-carotene concentrations of rabbits by group

Variable	Group				LSSE
	Control	β-carotene	α-tocopherol	α-tocopherol + β-carotene	
Triglyceride					
Liver (mmol/liver)	3.13—a	5.54—b	3.79—a	5.15—b	0.51
Triceps (mmol/tricep)	0.40—a	0.42—a	0.41—a	0.51—b	0.03
Heart (mmol/heart)	0.20—a	0.17—a	0.18—a	0.21—a	0.01
Plasma (mmol/L)	6.42—a	6.51—a	6.59—a	6.31—a	0.20
β-Carotene					
Liver (μmol/liver)	2.74—a	174.44—b	0.27—a	260.32—c	28.69
Triceps (nmol/tricep)	0.00—a	140.03—b	2.93—a	421.34—c	27.84
Heart (nmol/heart)	10.24—a	65.95—b	10.33—a	146.23—c	8.62
Plasma (μmol/L)	0.25—a	125.74—b	0.06—a	276.89—c	14.32
α-Tocopherol					
Liver (mmol/liver)	14.52—a	5.99—a	365.41—b	52.83—c	22.24
Triceps (μmol/tricep)	0.00—a	66.65—a	2356.97—b	1358.64—c	139.29
Heart (μmol/heart)	14.82—a	10.71—a	1295.94—b	389.87—c	104.58
Plasma (μmol/L)	43.02—a	23.73—a	1026.59—b	413.98—c	26.84
No. animals	7	7	7	7	

Values are LSx̄. Values among groups having different letters for each variable are significantly different at $P < 0.05$.
LSSE, Least square standard errors.

atherosclerotic lesions in the aortic arch was significantly less ($P < 0.05$) in the AT group than in the CN group, whereas those in the BC and AT + BC groups were statistically similar to both groups. The extent of atherosclerotic lesions in the thoracic aorta was significantly less ($P < 0.05$) in the BC, AT, and AT + BC groups than those in the CN group. The extent of lesions in the abdominal aorta was significantly less ($P < 0.05$) in the AT group than in the CN and BC groups, but not the AT + BC group, and significantly less ($P < 0.05$) in the AT + BC group than in the CN group but not the BC group, with no significant differences between the CN and BC groups.

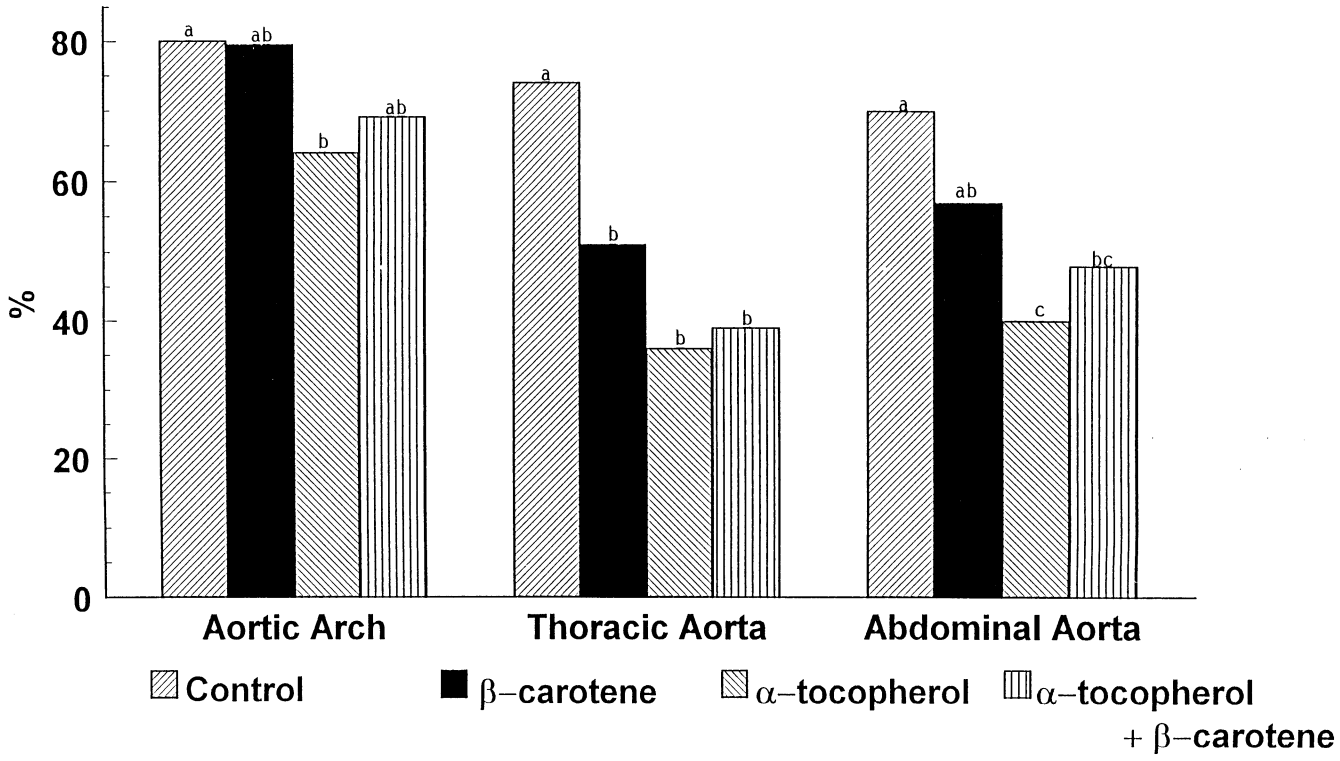

Figure 2 Percentages of intimal aortic surface areas covered by atherosclerotic lesions in rabbits by group. Values represent least square means. Least square standard error values were 5.363% for aortic arch, 7.726% for thoracic aorta, and 5.353% for abdominal aorta. a–c, values among groups having different letters for each variable are significantly different at $P < 0.05$.

Table 3 Significant correlations (*r*) between aortic atherosclerotic lesion areas and biochemical variables

Variables	Aortic arch	Thoracic aorta	Abdominal aorta
Lesion area			
Plasma cholesterol	0.36[1]	0.42[2]	0.39[4]
Heart β-carotene	NS	−0.40[2]	NS
Plasma α-tocopherol	−0.37[1]	−0.40[2]	−0.51[4]
Liver α-tocopherol	−0.43[2]	−0.38[2]	−0.50[2]
Triceps α-tocopherol	−0.39[2]	−0.47[2]	−0.60[4]
Heart α-tocopherol	−0.40[2]	−0.34[3]	−0.44[2]

Correlations were calculated per L plasma and per organ.
[1]$P = 0.06$.
[2]$P < 0.05$.
[3]$P = 0.08$.
[4]$P < 0.01$.
NS, not significant.

Correlations between aortic atherosclerotic lesion areas and tissue biochemical variables

Significant correlations between aortic lesion areas and tissue biochemical variables of all animals are given in *Table 3*. Atherosclerotic lesion areas were positively correlated with plasma cholesterol concentrations in the thoracic aorta ($r = 0.42$, $P < 0.05$), abdominal aorta ($r = 0.39$, $P < 0.01$), and aortic arch ($r = 0.36$, $P = 0.06$). Atherosclerotic lesion areas in the thoracic aorta were significantly correlated (inversely) with the β-carotene content of the heart ($r = -0.40$, $P < 0.05$). Atherosclerotic lesion areas were negatively correlated with plasma, liver, triceps, and heart α-tocopherol levels ($r = -0.34$–-0.60), with all but two ($P = 0.06$, $P = 0.08$) of these correlations being significant ($P < 0.05$). All other correlations between aortic atherosclerotic lesion areas and tissue biochemical variables were not significant.

Discussion

In the present study, liver, heart, and plasma cholesterol levels of AT + BC animals and heart and plasma cholesterol values of BC animals were significantly lower than those of CN and AT animals. Triceps muscle cholesterol and triglyceride levels of AT + BC rabbits were significantly higher than those of other groups. AT + BC animals had significantly lower values for tissue α-tocopherol than AT animals and significantly higher values for tissue β-carotene than BC animals. Compared with CN rabbits, AT animals had significantly less aortic atherosclerotic lesions in all three locations (aortic arch, thoracic aorta, and abdominal aorta), AT + BC animals had significantly less in two locations, and BC animals had significantly less in one location. Atherosclerotic lesion areas were positively correlated with plasma cholesterol concentrations, negatively correlated with plasma α-tocopherol concentration, and not significantly correlated with plasma β-carotene concentrations.

In reviewing previous studies, Wilson et al.[25] surmised that total lipid levels in livers, spleens, kidneys, and lungs of rabbits were elevated and became fatty when the animals were fed large amounts (0.5–3%) of cholesterol. Although initial levels of organ cholesterol and triglyceride levels were not determined in the present study, plasma levels of cholesterol and triglycerides in these rabbits were significantly increased as a result of consumption of the atherogenic diet.[20] Indeed, all the rabbits showed visual deposition of lipids throughout most body organs, particularly the liver.

Some researchers have noted a marginally protective influence of vitamin E on cholesterol-induced serum cholesterol and atherosclerosis in rabbits.[26,27] Though mean plasma cholesterol levels of rabbits fed the atherogenic diet with α-tocopherol supplementation were lower than those of controls, differences were not significant in the present study. This is in agreement with the findings of Brattsand.[28] The BC + AT group had significantly lower liver, heart, and plasma cholesterol than other groups and significantly higher liver and triceps triglyceride levels than CN and AT animals. Hence, the combined antioxidant treatment in the current study resulted in the AT + BC animals having different tissue distributions of cholesterol and triglyceride compared with those of the control (CN) group.

Schwenke and Carew[29] suggested that plasma is the source of most of the cholesterol esters present in atherosclerotic lesions. In lesion development LDL enters the arteries intact at increased rates. LDL has been associated with atherosclerotic lesions in several species.[30] In the present study, we observed lower mean aortic atherosclerotic lesion areas, significantly lower in the thoracic aorta, in rabbits supplemented with β-carotene than in controls. This may be due to β-carotene having an inhibitive effect on LDL oxidation[31] or it may be associated with its antihypercholesterolemic effect[20,32] or both. Cohn and Kuhn[33] demonstrated the importance of the LDL-receptor pathway for vitamin E clearance in normal rabbits. α-Tocopherol's putative function is to protect cells and tissues against the destructive effects of lipid peroxidation.[34] Vitamin E and β-carotene appear to have a synergistic effect against experimental atherosclerosis in rabbits.[28] β-Carotene undergoes autooxidation in vivo, which generates oxygen species that are capable of initiating further oxidation[35] and diminishing the antioxidant effect of β-carotene.[31] The presence of α-tocopherol helps to prevent this oxidation. In the current study, the vitamin E only treatment was more effective with regard to decreased atherosclerotic lesion areas than treatment with both antioxidants. Perhaps α-tocopherol is needed more to protect β-carotene from oxidation when both antioxidants are present than when α-tocopherol alone is present. Vitamin E functions in protecting other antioxidants from oxidation.

As expected, liver, triceps muscle, heart muscle, and plasma β-carotene levels were significantly higher in BC rabbits than in CN and AT animals. Similarly, the organ and plasma α-tocopherol levels were significantly higher in AT rabbits than in CN and BC animals. The combination of the two antioxidants significantly decreased α-tocopherol levels of these organs and plasma, compared with values of animals given only α-tocopherol. However, the β-carotene levels were significantly higher in organs and plasma of animals given both antioxidants than in those given only β-carotene, whereas levels of both groups were significantly higher than those of the CN and AT groups. α-To-

copherol and β-carotene, when given in larger quantities, may compete with each other during absorption or transport. Alam et al.[36] reported that weanling Wistar Kyoto rats treated with diets containing different types of dietary fat and excessive amounts of vitamin E and 10% of β-carotene, had lower levels of liver β-carotene concentration than rats given coconut oil. Hence, an excess of vitamin E seems to affect the tissue levels of β-carotene. Nair et al.[37] indicated that α-tocopherol supplementation not only restored α-tocopherol concentrations, but also reversed the elevation of β-carotene in humans. Both β-carotene and α-tocopherol are transported by LDLs and high density lipoproteins in plasma.[38,39]

Keaney et al.[40] indicated that consumption of high-cholesterol diets was associated with the elevation of α-tocopherol levels in plasma and tissues in humans. Massive doses of vitamin E may have a marginally protective effect against hypercholesterolemia.[41] Diplock and Lucy[42] suggested that tocopherol is a constituent part of the membranes. The cell may require α-tocopherol in order to synthesize membranes.

Atherosclerotic lesions were observed in aortas of the rabbits fed the atherogenic diet in this study (*Figure 2*). Rabbits in the BC group had significantly lower atherosclerotic lesion areas in the thoracic aorta than the CN group, whereas AT animals had significantly reduced atherosclerotic lesion areas at the aortic arch, thoracic aorta, and abdominal aorta than the CN group. Animals given both antioxidants had significantly lower atherosclerotic lesion areas in the thoracic aorta and abdominal aorta than the CN group. The greatest reduction in atherosclerotic lesion areas was observed in the group given α-tocopherol alone. Dietary vitamin E has been reported to inhibit the development of atherosclerotic lesions in Dutch-Belted rabbits.[41,43] Mongrel rabbits treated with diets containing cholesterol and coconut oil with vitamin E have been reported to have decreased atherosclerotic plaque development.[44]

Female New Zealand white rabbits fed a high cholesterol (0.5 g/kg/d) diet supplemented with vitamin E (40 mg/kg/d) for 4 months showed significantly inhibited development of aortic atherosclerotic lesions.[45] In the present study, this may be related to the significant inverse correlations (though *r* values were modest) observed between lesion areas and α-tocopherol contents of the four tissues. Rabbits given α-tocopherol alone had lower mean aortic atherosclerotic lesion areas and high mean plasma, liver, triceps muscle, and heart muscle contents of α-tocopherol than animals that received both α-tocopherol and β-carotene, β-carotene alone, or no added antioxidants (the control group). In rabbits that received both antioxidants, α-tocopherol probably functioned in protecting the added β-carotene from oxidation, resulting in their having lower tissue α-tocopherol levels than observed in animals given only the added α-tocopherol.

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