# Red Wine Does Not Reduce Mature Atherosclerosis in Apolipoprotein E–Deficient Mice

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***Background***—Red wine polyphenols and ethanol reduce fatty streak formation (early atherosclerosis) in various animal models. These experimental results support the observation that alcoholic beverages protect against myocardial infarction in humans. However, fatty streaks may not reflect the pathology of mature and clinically relevant atherosclerosis. The present study examined the effects of red wine polyphenols and ethanol on mature atherosclerosis in apolipoprotein E–deficient mice.

***Methods and Results***—Eighty-four 7-week-old mice were randomized to receive water, red wine (diluted to 6% ethanol v/v), 6% ethanol v/v, or red wine powder in water. All mice were fed a normal chow diet. At 26 weeks of age, the mice were killed. HDL cholesterol was raised 12.0% (95% CI, 4.0% to 20.0%) and 9.2% (95% CI, 1.5% to 16.9%) by red wine and ethanol, respectively. At the end of study, all mice exhibited advanced atherosclerosis in the aortic bulb, whereas less mature atherosclerosis predominated in the brachiocephalic trunk. The amount of atherosclerosis in the aortic bulb and the brachiocephalic trunk were similar in all groups ($P=0.92$ and $P=0.14$, respectively). To evaluate whether ethanol or red wine polyphenols were protective by stabilizing atherosclerotic plaques rather than reducing their size, we measured the percentage of collagen-poor areas in left coronary sinus plaques as a morphological criterion of plaque stability. The percentage of collagen-poor areas did not differ between groups ($P=0.71$).

***Conclusions***—Neither ethanol nor red wine polyphenols reduced mature atherosclerosis or changed the content of collagen in plaques in apolipoprotein E–deficient mice. **(*Circulation*. 2001;103:1681-1687.)**

**Key Words:** atherosclerosis ■ alcohol ■ antioxidants



The inverse association between ethanol consumption and myocardial infarction is among the most consistent reported in epidemiology,[1] but causality remains controversial for rational and irrational reasons. Rationally, many studies, if not all, are likely to have been confounded by a number of differences between drinkers and abstainers. Irrationally, the nature of ethanol as an intoxicating and habituating substance adds to the controversy.

Coronary atherosclerotic plaque disruption with superimposed thrombosis is the main cause of myocardial infarction.[2] It follows that the reduced incidence of myocardial infarction seen with drinkers could be caused by an inhibition of atherosclerosis, a stabilization of plaques, a reduction of plaque intrinsic thrombogenicity, or a reduction of the systemic propensity to thrombosis. Inhibition of atherosclerosis is a comprehensible pathway because ethanol consumption, as its hallmark, increases HDL cholesterol.[3]

A number of epidemiological studies have suggested a special protective effect of red wine,[4] and this hypothesis has been supported by experimental research. Red wine polyphenols protect LDL from oxidation ex vivo[5] and were recently shown to inhibit smooth muscle cell proliferation in vitro.[6] Both properties could mediate an antiatherogenic effect.

Previous animal experiments have, with 1 exception,[7] examined the effects of ethanol and red wine polyphenols only on fatty streaks (early atherosclerosis) because of either limitations of the animal model[8–14] or a short study period.[15,16] However, the development of fatty streaks may not reflect the pathogenesis of clinically relevant atherosclerosis.

Apolipoprotein E–deficient ($apoE^{-/-}$) mice develop advanced, human-like atherosclerotic plaques in the aortic bulb, aortic arch, and the main aortic branches on a low-fat diet within 6 months of age.[17] These plaques appear vulnerable by human morphological criteria but rarely rupture (Erling Falk, MD, PhD, unpublished data, 1999). We tested the hypothesis that treatment with ethanol or red wine polyphenols would reduce mature atherosclerosis or increase plaque stability by morphological criteria in $apoE^{-/-}$ mice.

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**TABLE 1. Intake of Red Wine Polyphenols**

| | Red Wine | Red Wine Powder |
|---|---|---|
| Colorimetric assay, μg/mouse per day | | |
| Condensed tannins | 5940±760 | 6010±1570 |
| Anthocyanidins | 710±90 | 1950±510 |
| HPLC assay, μg/mouse per day | | |
| Catechin | 73±9 | ND |
| Epicatechin | 42±5 | 2.0±0.5 |
| Procyanidin dimers | 225±29 | 17±5 |
| Polymeric phenols | 1933±250 | 991±259 |
| Quercetin glycosides | 85±11 | ND |
| Quercetin | 31±4 | ND |
| Myricetin glycosides | 6.3±0.8 | ND |
| Myricetin | 4.4±0.6 | ND |
| Anthocyanin glycosides* | 333±43 | 186±49 |
| Polymeric anthocyanins | 89±11 | 68±18 |
| Gallic acid | 63±8 | 43±11 |
| Other phenolic acids† | 173±22 | 2.0±0.5 |
| trans-Resveratrol | 5.9±0.8 | ND |
| cis-Resveratrol | ND | ND |
| TAS assay, μmol/mouse per day | | |
| Trolox equivalents | 62±8 | 42±11 |

Values are mean±SD. Numbers are averages of consumption throughout the study period. HPLC indicates high-performance liquid chromatography; ND, not detectable.

*Delphinidin, cyanidin, peonidin, petunidin, and malvidin glycosides.

†Caftaric, caffeic, p-coumaric, and coutaric acids.

## Methods

Eighty-four male homozygous apoE$^{-/-}$ mice, backcrossed 9 generations into the C57BL/6 background, were obtained from Bomholtgaard Breeding and Research Center, Ry, Denmark. The study and all procedures were approved by the National Animal Ethics Committee.

At 7 weeks of age, mice were randomized into 4 groups and given water (n=24), red wine (n=20), ethanol (n=20), or suspended red wine powder (n=20) as their only source of fluid. All mice were fed normal mouse chow (Altromin 1314). Red wine (Chateau de Paillas, Cahors, 1996, matured in steel vats) was diluted to an ethanol concentration of 6% v/v. Ethanol was prepared in a 6% v/v solution. Red wine powder (Clarét), was kindly provided by Poul Olsen, Pharmex Medical Aps, Hadsund, Denmark. Clarét is a spray-dried extract of unspecified Austrian red wine matured in steel vats. Fluid consumption was recorded, and all fluids were changed 3 times weekly. Body weights were recorded weekly. Comparability between red wine and red wine powder groups was sought by clamping the intake of condensed tannins, as measured by the colorimetric method of Ribereau-Gayon and Stonestreet[18] with the correction for condensed tannins introduced by Glories.[19] This was done by adjusting the concentration of the red wine powder suspension to correct for differences in fluid intake between the red wine and red wine powder groups.

After completion of the study, a more detailed analysis of the polyphenol profiles of the red wine and red wine powder was done by ETS Laboratories, St Helena, California. Briefly, red wine and red wine powder (dissolved in methanol:water at 1:1 and sonicated for 5 minutes) were centrifuged at 10 000 relative centrifugal force for 3 minutes. Wine phenolic compounds were then separated and quantitated by high-performance liquid chromatography on the basis of comparison with known standards. Total antioxidant status (TAS) of the consumed fluid was evaluated using the kit from Randox Laboratories. Intakes of polyphenols and antioxidant equivalents, as measured by these methods, are listed in Table 1.

**TABLE 2. Distribution of Blood Ethanol Levels in Mice Fed Red Wine or Ethanol**

| Sampling Session | <0.005% | 0.005% to 0.025% | 0.025% to 0.040% |
|---|---|---|---|
| 9 weeks of age | 25 | 10 | 4 |
| 15 weeks of age | 22 | 10 | 6 |
| 26 weeks of age | 34 | 2 | 1 |

Values are no. of animals.

### Ethanol in Blood

Ethanol concentrations in blood were measured at 9, 15, and 26 weeks of age in all mice in the red wine and ethanol groups, as well as in control animals using headspace gas chromatography with an internal standard. At 9 and 15 weeks, blood was obtained from the retroorbital venous plexus. Results are expressed as percent wt/wt.

### End of Study

At 26 weeks of age, nonfasting mice were anaesthetized (20 mg/kg midazolam IP, 40 mg/kg fluanisone IP, and 1.5 mg/kg fentanyl citrate IP) and exsanguinated by withdrawing blood from the right ventricle into heparin-coated tubes. Thereafter, the mice were flushed with St Thomas cardioplegic solution containing heparin, perfusion-fixed at ≈100 mm Hg with 4% phosphate-buffered formaldehyde (pH 7.2) via the left ventricle, and then immersed in the fixative for 6 hours before storing in cold phosphate buffer.

### Pathoanatomical Examination

The heart, including the ascending aorta, was cut in half; this was followed by paraffin embedding. The half containing the aortic bulb was sectioned serially at 4-μm intervals. Once the aortic sinuses appeared, every other section was collected on glass slides. Five sections taken at 80-μm intervals, spanning 320 μm of the aortic bulb from the commissures of the aortic leaflets and upward, were stained with orcein and evaluated microscopically. The plaque area was measured blindly by the same person (J.F.B.) using computer-assisted image analysis (Olympus BX50 light microscope, Sony DXC-151P color video camera, Imagraph Precision frame grabber, and SigmaScan Pro from Jandel Scientific Software). The amount of atherosclerosis in the aortic bulb was expressed as mean plaque size of the 5 sections.

Sections (4 μm) of the brachiocephalic trunk were cut at 100-μm intervals from the aortic arch to the appearance of the subclavian–common carotid bifurcation. Plaque area was measured as outlined above. Numbers are given as mean plaque size throughout the brachiocephalic trunk.

The percentage of collagen-poor areas was assessed in the left coronary sinus plaque. A section from the level of maximal plaque size was stained with Sirius Red, and collagen was detected by its birefringency using polarized light microscopy. Images were captured in Adobe Photoshop 5.0. Collagen-poor areas were defined as areas with a gray-scale level <50 units above background (Histogram function).

### Plasma Analysis

Plasma total cholesterol, HDL cholesterol (HDL-C), and triglycerides were measured enzymatically on a Cobas Fara analyzer (Roche) using kits from Roche Diagnostics. TAS was determined with the kit from Randox Laboratories. Each sample was assayed twice with a coefficient of variation between duplicate samples of 7.7%. Lag times of the $Cu^{2+}$ oxidation of LDL+VLDL were measured in 8 mice per group as previously described.[20]

### Statistical Methods

One-way ANOVA was used for comparisons of means. If means were different by ANOVA, we used *t* tests to determine the origin of

**TABLE 3. Lipids**

| | Water (n=23) | Red Wine (n=18) | Ethanol (n=20) | Red Wine Powder (n=19) |
|---|---|---|---|---|
| Total cholesterol, mmol/L | 14.6±2.6 | 13.2±4.4 | 14.1±2.2 | 14.3±2.2 |
| HDL cholesterol,* mmol/L | 1.97±0.25 | 2.21±0.25‡ | 2.15±0.25† | 2.05±0.23 |
| Triglycerides,* mmol/L | 1.46±0.57 | 0.98±0.30‡ | 1.25±0.35 | 1.35±0.28 |

Values are mean±SD.
*$P<0.05$ by ANOVA; †$P<0.05$ vs water; ‡$P<0.01$ vs water.

the difference. Triglycerides and plaque areas in the aortic bulb and brachiocephalic trunk were log-transformed to normalize the distribution. We used a *t* test to calculate 95% confidence intervals (95% CI) of the effect (ratio of geometric means) of red wine, ethanol, and red wine powder on aortic bulb and brachiocephalic trunk atherosclerosis. SPSS 8.0 was used for the calculations.

## Results

Eighty mice survived until the end of study. One was killed for health monitoring. No pathogenic microorganisms or antibodies against murine viruses were detected. One mouse from each of the red wine, red wine powder, and water groups was killed for failure to thrive.

### Body Weight and Fluid Intake

Mice tolerated red wine, ethanol, and red wine powder well. Fluid intake was 5.42±1.18, 4.88±0.63, 5.08±0.40, and 5.61±1.31 mL/day in the water, red wine, ethanol, and red wine powder groups, respectively. Weight gain during the study was similar in all groups, with final body weights of 33.1±3.2, 32.4±1.3, 31.8±2.6, and 32.7±2.3 g in the water, red wine, ethanol, and red wine powder groups, respectively, ($P=0.41$ by ANOVA).

### Ethanol in Blood

Sampling for determination of blood ethanol levels were performed at 9 AM at 9 weeks of age and at 9 PM at 15 weeks of age (Table 2). At these sessions, 36% and 42%, respectively, of the animals given alcohol had detectable blood ethanol levels (>0.005%) that ranged up to 0.040%. At the end of study, blood ethanol levels were much lower, probably due to lack of fluid intake during the final transportation of the animals from the stable to the laboratory.

### Lipids

HDL-C rose 12.0% (95% CI, 4.0% to 20.0%) and 9.2% (95% CI, 1.5% to 16.9%) after administration of red wine and ethanol, respectively (Table 3). Total cholesterol was similar in all groups. Surprisingly, mice given red wine had low triglycerides, whereas neither ethanol nor red wine powder caused significant changes in triglycerides.

### Antioxidant Parameters

Blood obtained at death was analyzed for antioxidant parameters, TAS, and lag times of LDL+VLDL oxidation (Table 4). As far as we know, plasma TAS values for apoE$^{-/-}$ mice have not been reported previously. We found low plasma TAS compared with humans[21] and C57BL mice.[12] Paradoxically, plasma TAS of the water-fed mice was higher than those of the other groups. However, TAS correlated with triglyceride ($r=0.33$, $P=0.003$) and total cholesterol levels ($r=0.28$, $P=0.012$), and the higher TAS of the water group seemed due, in part, to the effect of higher lipid levels in this group. Ethanol and red wine increased the lag times of LDL+VLDL oxidation, but red wine powder had no effect. Red wine did not increase lag time compared with ethanol ($P=0.86$ by *t* test). Lag times correlated inversely with triglyceride levels ($r=0.53$, $P=0.002$).

### Atherosclerosis

At 26 weeks of age, all mice had mature atherosclerosis in the aortic bulb resembling advanced human lesions (Figure 1). Mean plaque area was similar in all groups ($P=0.92$ by ANOVA; Figure 2). The 95% CIs of the effect of red wine, ethanol, and red wine powder were −24% to 65%, −23% to 66%, and −29% to71%, respectively. To extend these observations, we quantitated atherosclerosis in the brachiocephalic trunk by a novel method. Atherosclerosis of the brachiocephalic trunk spanned from no lesion in the distal part over a fatty streak to a mature plaque near the aortic arch (Figure 3). Again, no differences in the amount of atherosclerosis were detected between groups ($P=0.14$ by ANOVA; Figure 4). The 95% CIs of the effect of red wine, ethanol, and red wine powder on brachiocephalic trunk atherosclerosis were 2% to 144%, 1% to 135%, and −29% to 96%, respectively. However, these values were not significant by ANOVA.

The amount of atherosclerosis in the brachiocephalic trunk and the aortic bulb did not correlate ($r=0.08$, $P=0.49$). None

**TABLE 4. Markers of Antioxidant Activity**

| | Water | Red Wine | Ethanol | Red Wine Powder |
|---|---|---|---|---|
| Lag time of LDL+VLDL | 71±29 | 109±21‡ | 107±27† | 69±26 |
| $Cu^{2+}$ oxidation,* min | (n=8) | (n=8) | (n=8) | (n=8) |
| Total antioxidant status,* | 0.83±0.10 | 0.76±0.10† | 0.73±0.09‡ | 0.74±0.11‡ |
| mmol/L TEAC | (n=23) | (n=18) | (n=20) | (n=19) |

Values are mean±SD. TEAC indicates Trolox-equivalent antioxidant capacity.
*$P<0.01$ by ANOVA; †$P<0.05$ vs water; ‡$P<0.01$ vs water.

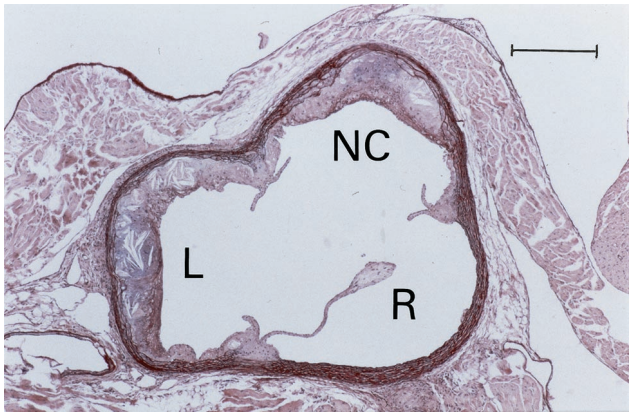


**Figure 1.** Cross-section of aortic bulb from mouse given red wine powder. Advanced atherosclerotic plaques containing lipid-rich (cholesterol crystals) and fibrous-rich components are present in left coronary and noncoronary aortic sinuses (L and NC, respectively). No plaque is seen in right coronary sinus (R). Orcein stained elastin black. Bar=300 $\mu$m.

of the potential risk factors (body weight, total cholesterol, HDL-C, triglycerides, TAS, and lag time) correlated with atherosclerosis in the aortic bulb or the brachiocephalic trunk (data not shown).

## Plaque Composition

Atherosclerotic plaques in the aortic bulb consisted of 2 main components: a collagen-poor atheromatous (cholesterol crystals) and a collagen-rich sclerotic component (Figure 5). The relative size of the collagen-poor component is a morphological criterion of plaque vulnerability in humans.[22] Regarding plaques in the 3 aortic sinuses of the bulb (see Figure 1), the largest plaque area was consistently found in the left coronary sinus. To evaluate whether ethanol or red wine polyphenols might influence the stability of atherosclerotic plaques, we quantitated collagen content in the large left coronary sinus plaque. The percentage of collagen-poor areas in left coronary sinus plaques did not differ between groups (63.8±11.0%, 61.2±12.4%, 61.2±13.8%, and 58.9±16.7% in the water, red wine, ethanol, and red wine powder groups, respectively; $P$=0.71 by ANOVA).

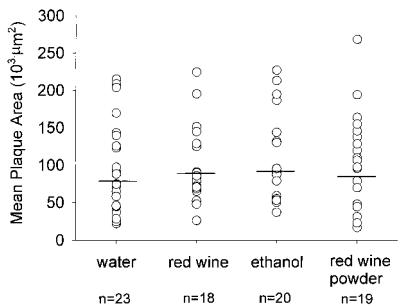


**Figure 2.** Amount of atherosclerosis in aortic bulb was similar in all groups ($P$=0.92 by ANOVA). Mean plaque area was 78 700 $\mu$m$^2$ (95% CI, 58 700 to 105 400 $\mu$m$^2$), 88 000 $\mu$m$^2$ (95% CI, 67 700 to 114 300 $\mu$m$^2$), 89 000 $\mu$m$^2$ (95% CI, 68 500 to 115 600 $\mu$m$^2$), and 86 400 $\mu$m$^2$ (95% CI, 60 500 to 123 500 $\mu$m$^2$) in water, red wine, ethanol, and red wine powder groups, respectively.

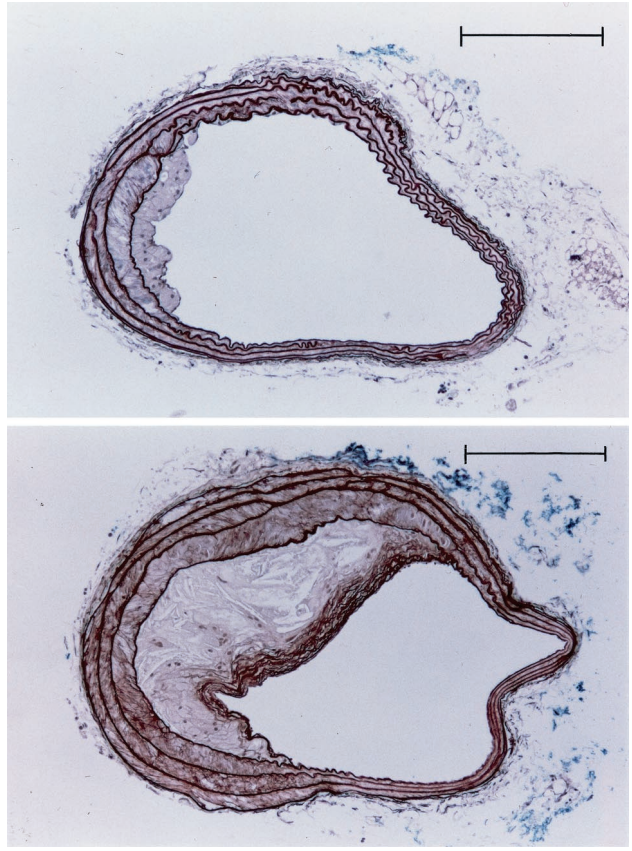

**Figure 3.** Cross-sections of brachiocephalic trunk illustrating a fatty streak (foam cells only) located distally in artery (top) and a mature plaque (cholesterol crystals and fibrous tissue) near aortic arch (bottom). Orcein stained elastin black. Bar=100 $\mu$m.

## Discussion

The present study does not suggest a protective effect of either ethanol or red wine polyphenols on the amount or composition of advanced atherosclerosis in apoE$^{-/-}$ mice. Consistent with the experience of other groups,[23–25] we found large variations in the amount of atherosclerosis in apoE$^{-/-}$ mice. Despite this, the 95% CIs of the effects of red wine, ethanol, and red wine powder left little chance for overlooking a substantial antiatherogenic effect. On the question of whether these substances could have an aggravating effect in apoE$^{-/-}$ mice, our data are not informative.

Obviously, this lack of effect could be a true lack of antiatherogenic effect of ethanol and red wine polyphenols, but at least 2 other possibilities must be discussed. First, insufficient dosing would preclude a response. Second, a deviation of the biology of atherosclerosis in apoE$^{-/-}$ mice from humans could obscure a true antiatherogenic effect.

## Ethanol

The full cardioprotective effect of ethanol in epidemiological studies is seen with intakes of ≥1 half-drink per day.[1] In the present study, mice drank ≈5 mL of 6% ethanol per day. This translates into 6 drinks (of 12 g of ethanol) per day in a 70-kg man when differences in metabolic capacities between mice and man are taken into account.[26] The data on blood ethanol levels support this level of alcohol consumption. Also, the

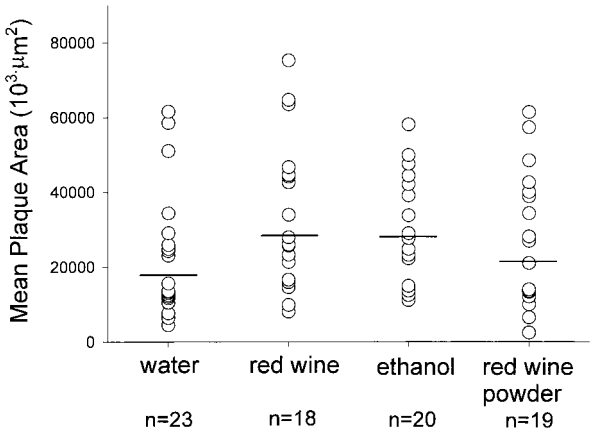


**Figure 4.** Amount of atherosclerosis in brachiocephalic trunk was similar in all groups ($P$=0.14 by ANOVA). Mean plaque area was 17 800 μm$^2$ (95% CI, 13 000 to 24 300 μm$^2$), 28 100 μm$^2$ (95% CI, 20 400 to 38 700 μm$^2$), 27 400 μm$^2$ (95% CI, 20 600 to 36 300 μm$^2$), and 21 000 μm$^2$ (95% CI, 13 400 to 32 800 μm$^2$) for water, red wine, ethanol, and red wine powder groups, respectively.

magnitude of the HDL-C increase in the red wine and ethanol groups corresponded to what is observed in human intervention studies of ≈3 to 4 drinks (of 12 g of ethanol) per day.[3] Thus, whether judged on intake, blood concentration, or HDL-C response, the present model constitutes an appropriate level of ethanol consumption.

In contrast to the HDL-C elevation caused by the expression of a human apolipoprotein A-I (apoA-I) transgene,[23] ethanol-induced HDL-C increase did not protect against atherosclerosis in apoE$^{-/-}$ mice. This may reflect a difference in quality between the 2 modes of HDL-C elevation and may apply to humans. Not all kinds of HDL-C elevation in humans seem to be protective.[27] However, it is not clear whether the protective effect demonstrated in the human apoA-I transgene studies arose from increased HDL-C as such or from the introduction of the human genotype. Particularly intriguing, inactivation of the mouse apoA-I gene did not aggravate atherosclerosis in apoE$^{-/-}$ mice in the single study reported.[28] More needs to be learned about the physiology of mouse apoA-I before this point can be elucidated.

## Red Wine Polyphenols

We did not measure plasma phenolic levels or urinary phenolic excretion in the present study, but absorption of catechin and quercetin has been demonstrated previously in the apoE$^{-/-}$ mouse model. Hayek et al[15] gave apoE$^{-/-}$ mice Cabernet Sauvignon red wine containing a total polyphenol content of 50 μg of catechin equivalents per day and detected catechin and quercetin in LDL at concentrations of 3.65 nmol/mg LDL protein and 3.00 nmol/mg LDL protein, respectively. This treatment lengthened lag time by 120 minutes and reduced fatty streak formation by 48% compared with 1.1% ethanol-fed animals. We fed mice in the red wine group a higher dose (containing 73 μg of monomeric catechin alone per day) but saw no effect on mature atherosclerosis. In the present study, red wine did not lengthen lag times compared with ethanol.

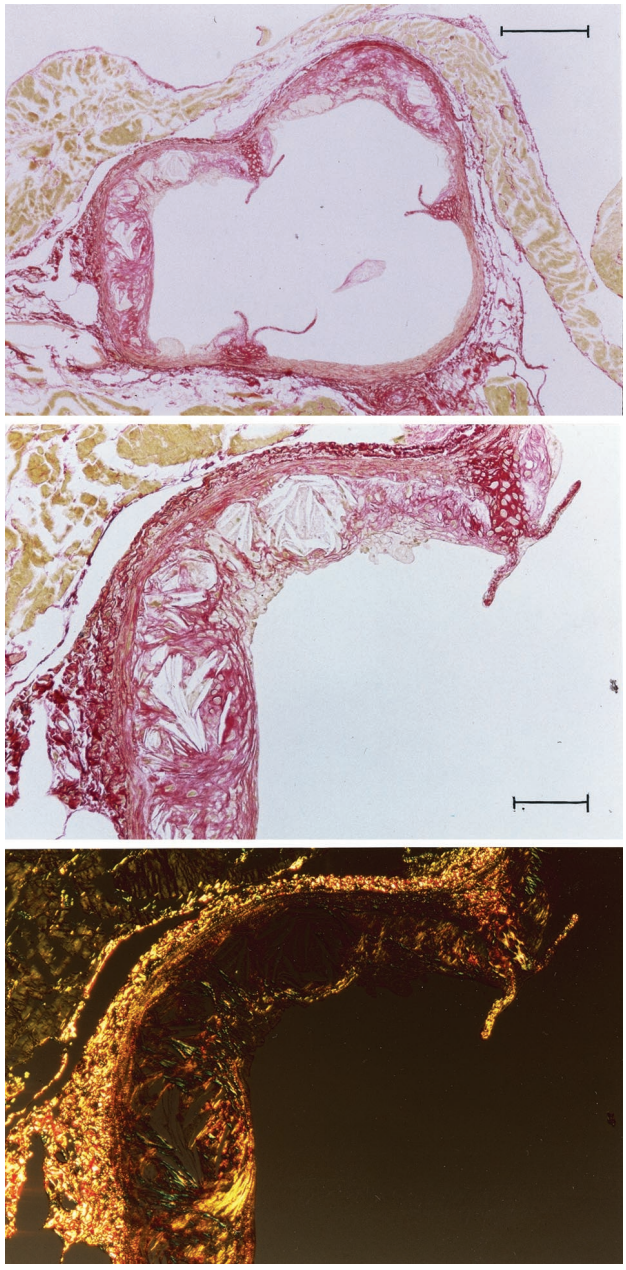

**Figure 5.** Top, Sirius Red–stained section corresponding to one shown in Figure 1. Plaque in left coronary sinus is shown at higher magnification (middle), and collagen is identified by its birefringency when viewed under polarized light (bottom). Bar=300 μm (top) and 100 μm (middle).

This discrepancy is not due to differences in polyphenol profiles between the used wines because Hayek et al[15] found that 50 μg of catechin per day alone reduced fatty streak formation to almost the same extent (39%) and significantly lengthened lag time by 40 minutes.

Although we clamped the intake of condensed tannins between red wine and red wine powder groups, the detailed high-performance liquid chromatography assay revealed that polyphenol intakes were not comparable between these groups. The virtual absence of monomeric polyphenols in the red wine powder makes it difficult to dismiss the possibility

that the lack of effect could be caused by a poor absorption of the contained oligomeric and polymeric polyphenols.

The fact that we did not demonstrate an ex vivo antioxidant effect of red wine polyphenols with the TAS assay or with the measurements of lag times may lead to questioning the appropriateness of the apoE$^{-/-}$ mouse as a model to study antioxidant effects on atherosclerosis. However, a number of observations points toward the apoE$^{-/-}$ mouse as a suitable model for exactly that. First, LDL oxidation occurs in the atherosclerotic plaques of these mice.[29] Second, although unmodified VLDL remnant particles might be atherogenic in humans through apoE-mediated macrophage internalization, the lack of apoE in apoE$^{-/-}$ mice abolishes this pathway, thus exaggerating the role of oxidation and other kinds of lipoprotein modification. Convincingly, a disruption of the gene for scavenger class A receptors that internalize oxidized LDL in macrophages attenuates advanced atherosclerosis in apoE$^{-/-}$ mice by 58%.[24] Third, the antioxidants vitamin E[30] and N,N′-diphenyl-1,4-phenylenediamine[31] inhibit advanced atherosclerosis in this model, although it remains uncertain if the effect of these drugs can be attributed to their mere antioxidant activity because another antioxidant, probucol, accelerates atherosclerosis in apoE$^{-/-}$ mice.[28]

### Fatty Streaks Versus Mature Atherosclerosis

Our finding that neither red wine nor ethanol protect against mature atherosclerosis in apoE$^{-/-}$ mice is in contrast with a number of experiments in animal models of fatty streak formation.[7–16] Although not necessarily in conflict with these studies, it challenges the implications that have been drawn from them. Red wine polyphenols and ethanol may inhibit fatty streak formation even in humans, but if this effect is lost with the progression of atherosclerosis, it is of limited interest clinically. In apoE$^{-/-}$ mice, numerous studies have identified treatments capable of inhibiting fatty streak formation,[32] but the clinical relevance of these are hampered by recent results that suggest qualitative differences in the pathogenesis of early versus late stages of atherosclerosis in apoE$^{-/-}$ mice. Antibodies against the macrophage-colony–stimulating factor receptor c-*fms*,[25] transplantation of apoE-transduced bone marrow,[33] and fibrinogen deficiency[34] all reduce fatty streaks in apoE$^{-/-}$ mice but do not reduce atherosclerosis at the late stage. Our study adds red wine polyphenols to this growing list. The discrepancies are not caused by a general insensitivity of the advanced atherosclerotic process to intervention, because the advanced stage can be attenuated by a number of substances, including high-dose vitamin E[30] and N,N′-diphenyl-1,4-phenylenediamine,[31] reversed by liver-directed apoE gene transfer,[35] and aggravated by probucol[28] or by feeding the mice a Western-type diet.[17] Recently, a genetic disruption of CD154 was shown to reduce advanced atherosclerosis in apoE$^{-/-}$ mice without influencing fatty streak formation.[36] The possibility of important pathogenetic differences between fatty streaks and mature atherosclerosis has become a topic of increasing awareness.[37]

## Conclusions

Neither ethanol nor red wine polyphenols reduced the speed of progression or changed the collagen content of mature atherosclerosis in apoE$^{-/-}$ mice, despite an ethanol-induced HDL-C increase comparable to that seen in humans. Our finding does not support the hypothesis of an anti-atherogenic effect of these substances.

### Study Limitations

Better understanding of the physiology of mouse apoA-I is necessary to fully understand the relevance of the mouse as a model to examine the effects of ethanol-induced HDL-C elevation. Also, the surprising antioxidant parameter findings and lowering of triglycerides with intake of red wine raise the question of the generalizability from this genetically defined animal model to human atherosclerosis.

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