Impairment of Endothelium-Dependent Arterial Relaxation
By High-Fat Feeding in ApoE-Deficient Mice
Toward Normalization By Human ApoA-I Expression

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Background—Atherogenic lipoproteins can impair the endothelium-dependent arterial relaxation, and circumstantial evidence suggests a beneficial role of plasma high density lipoproteins and apolipoprotein (apo) A-I in counteracting the endothelium dysfunction. In the present study, vascular reactivity was determined in control, apoE-deficient mice (apoE-KO mice), and apoE-deficient mice expressing human apoA-I (apoE-KO/HuAITg mice).

Methods and Results—In the first part of the study, control and apoE-KO mice were fed a low-fat or a high-fat diet for 23 weeks, and the vasoactive responses of isolated thoracic aortic segments to norepinephrine, sodium nitroprusside, and acetylcholine (ACh) were determined. Whereas norepinephrine, sodium nitroprusside, and ACh evoked similar vascular responses in control and apoE-KO mice fed the low-fat diet, high-fat feeding in apoE-KO mice produced a significant 3-fold increase in the mean concentration required to produce a half-maximal relaxing effect (EC50) of ACh as compared with control mice. This reflects a weaker sensitivity to ACh of the aortic segments from the apoE-deficient animals. In the second part of the study, the mean EC50 for ACh after high-fat feeding was found to be 4.4-fold lower in apoE-KO/HuAITg mice than in apoE-KO mice, indicating that the reduced sensitivity to ACh of the thoracic aorta from the apoE-KO mice fed the high-fat diet is improved by the expression of human apoA-I.

Conclusions—The present study demonstrates that the endothelium-dependent arterial relaxation is impaired in apoE-KO mice fed the high-fat diet. The endothelium dysfunction tends to be normalized by human apoA-I expression. (Circulation. 1999;100:1230-1235.)

Key Words: apolipoproteins ■ mice ■ atherosclerosis ■ vascular reactivity

In addition to their ability to initiate morphological disorders at the vessel wall, atherogenic lipoproteins have also been shown to influence vascular reactivity, ie, the ability of blood vessels to develop either a vasoconstrictor or a vasodilator response to biological or pharmacological compounds. Studies in humans1–3 and animal models4–8 have demonstrated that hypercholesterolemia is associated with alterations of vasodilation and vasoconstriction. More precisely, the nitric oxide-mediated, endothelium-dependent arterial relaxation evoked by acetylcholine (ACh) has been shown to be impaired in the earliest stage of atherosclerosis in humans or hyperlipidemic rabbits.5,9 In more advanced stages, the endothelium-independent relaxation, as assessed by the direct action of nitrovasodilators on arterial smooth muscle cells, can also be affected.10–11,12 In addition, hyperlipidemia and atherosclerosis can be associated with abnormal vasoconstric-

reported in hyperlipidemic rabbits.11,12 On aortic rings mounted in organ chambers, oxidized LDL can mimic the arterial dysfunctions observed in dyslipidemic subjects, and 2 oxidized LDL derivatives, lysolecithins18,19 and cholesterol oxides,20 constitute potent inhibitors of the endothelium-dependent arterial relaxation through the inhibition of the nitric oxide release.21

Several evidences support a beneficial role of HDL and apoA-I in counteracting the dysregulation of the vascular tone. Indeed, a direct relationship between plasma HDL cholesterol levels and the vasodilation of coronary artery in response to ACh was reported among patients with or without evidence for coronary atherosclerosis.22 Complementary ex vivo studies on arterial rings further supported the role of HDL and apoA-I in reversing the oxidized LDL-induced impairment of endothelium-dependent arterial relaxation.23,24 Whereas apoE-deficient mice (apoE-KO mice) recently arose as one of the most relevant animal models for studying

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human atherosclerosis, the role of lipoproteins in regulating the vascular tone in apoE-KO mice has not been clearly established. Recent studies by Bonthu et al reported significant impairment of the endothelium-dependent and -independent relaxations of thoracic aortas in apoE/LDL receptor double knock-out mice. Considering the growing interest for mouse as a model of human atherosclerosis, as well as the importance of the role of the arterial tone regulation in vascular biology, the present studies were designed to investigate the effects of alterations in lipoprotein metabolism on vascular reactivity in the mouse. To this end, the contractile response of aortas to norepinephrine (NE) as well as their relaxing response to ACh and sodium nitroprusside (SNP) were studied in apoE-KO mice fed either low-fat or high-fat diets. In order to bring more insights into the role of HDL and apoA-I in influencing the arterial tone in vivo, complementary studies were conducted by comparing vasomotor responses in apoE-KO and apoE-KO mice expressing human apoA-I.

Methods

Animals

Three distinct mouse lines were used in the present studies: C57BL/6 control female mice, homozygous apoE-KO mice28 and homozygous apoE-KO female mice expressing human apoA-I (apoE-KO/HuA1Tg mice).29

Study Protocol

The first part of the study was designed to search for putative alterations in vascular reactivity in apoE-KO versus control mice. To this end, 6-week-old female mice were placed for 23 weeks either on a low-fat diet (LF diet; Regular mouse chow, Piétrement) or on a high-fat diet containing 15.8% by weight fat, 1.25% by weight cholesterol, and 0.5% by weight sodium cholate (HF diet, TD 88051, Teklad).

In the second part of the study, the effect of human apoA-I expression on vascular reactivity was studied by comparing apoE-KO mice and apoE-KO/HuA1Tg mice. To this end, 14-week-old female mice of either genotype were fed the high-fat diet for 23 weeks.

Preparation of Blood Vessels

Blood vessel treatments were conducted as previously described. Mice were anesthetized with intraperitoneal sodium pentobarbital injection, and thoracic aorta was rapidly removed and transferred into a Krebs’ solution (composition in mmol/L: NaCl 119, KCl 4.7, KH2PO4 1.18, MgSO4 1.17, CaCl2 2.5, EDTA 0.027, glucose 11, and NaHCO3 25) bubbled with 95% O2 and 5% CO2. Loose connective tissue was carefully removed under a binocular magnifier. Two 2-mm arterial segments of thoracic aorta were cut off between the end of the aortic arch and the branch point of the celiac artery with special care to preserve endothelium integrity. Aortic rings were suspended horizontally between 2 wire hooks in 20-mL jacketed organ baths containing oxygenated, 37°C Krebs’ solution. One hook was fixed to the support and the other was connected to a UFI force transducer (Pioden Ltd). Changes in isometric tension were continuously monitored on a Mac Laboratory 8 system (AD Instruments Ltd). The resting tension of the aortic rings was set to 1 g, a value that was shown to be optimal in preliminary studies. After a 30-minute equilibration period, the contractile response of vascular smooth muscle was checked by incubation with 30 mmol/L of KCl. After washout and equilibration, the aortic rings were contracted by cumulative additions of NE in the 0.001- to 3-μmol/L concentration range. After a washout and a 1-hour recovery period, aortic segments were precontracted with NE at a concentration that gave approximately 75% of the maximal contraction as determined from the preceding NE concentration-response curve. After the precontraction to NE reached a plateau value, the aortic rings were relaxed by cumulative additions of ACh in the 0.003- to 10-μmol/L concentration range. Finally, aortic rings were washed and reequilibrated for 1 hour in Krebs’ buffer. They were precontracted again with NE at the same concentration giving 75% of the maximal contraction, and they were relaxed by the cumulative addition of SNP in the 0.001- to 10-μmol/L concentration range.

Analysis of Contractile and Relaxing Responses of Arteries

The contraction of aortic rings to NE was expressed in mg, and the relaxations to ACh and SNP were expressed as percentage of the contraction to NE. Maximal relaxation (Emax) and half-maximal relaxing effect (EC50) values for NE, ACh, and SNP were determined from experimental data. EC50 values correspond to the concentration required to produce a half-maximal vasoactive effect, and they reflect the sensitivity of arterial segments to the various compounds. EC50 values were calculated after fitting each curve according to a sigmoidal equation of the form: Y = P1 + P2/(1 + e-P3(logX-P4)), in which X is the agonist concentration, P1 is the lower plateau response, P2 is the range between the lower and the maximal plateaus of the concentration-effect curve, P3 is a negative curvature index indicating the slope independently of the range, and P4 is logEC50. Total cholesterol levels in plasma samples were determined on a COBAS-Fara centrifugal analyzer (Roche) by the enzymatic method from Boehringer. Data are expressed as mean±SEM. The significance of differences between data means was determined by using the Mann-Whitney U test.

Results

Vascular Responses in ApoE-KO Mice Versus Control Mice Fed the Low-Fat Diet

The first part of the study was designed to test the effect of apoE-deficiency on vascular reactivity in the mouse. To this end, 6-week-old female mice that were deficient (apoE-KO group, n=5) or not (control group, n=5) in apoE were placed on a low-fat diet for 23 weeks. In accordance with previous studies, apoE-KO mice were hypercholesterolemic when fed the regular mouse chow (total cholesterol: 350±34 mg/dL in apoE-KO mice versus 67±6 mg/dL in control mice; P<0.01).

At the end of the dietary period, the vasoactive responses of the thoracic aorta to NE (a vasoconstrictor), ACh (an endothelium-dependent vasodilator), and SNP (an endothelialum-independent vasodilator) were determined as described in Methods. Aortic segments presented very similar responses to NE, SNP, or ACh whether or not mice were deficient in apoE, and neither the Emax values nor the EC50 values obtained with distinct pharmacological agents differed between the 2 groups (Figure 1). In particular, the concentration response curves to ACh were very similar in control and apoE-KO mice (Figure 2), indicating that the endothelium-dependent relaxation of aortic segments is not significantly affected by apoE deficiency per se.

Vascular Responses in ApoE-KO Mice Versus Control Mice Fed the High-Fat Diet

As expected from previous studies, the hypercholesterolemic state associated with apoE deficiency was considerably worsened when 6-week-old apoE-KO female mice were given a high-fat diet (see Methods). The mean total plasma cholesterol levels were 3080±250 mg/dL in apoE-KO mice.
(n=9) versus 427±55 mg/dL in control mice (n=5) after 23 weeks of high-fat dietary intake (P<0.01).

Again, as observed under the low-fat diet, NE, ACh, and SNP evoked very similar maximal responses whether or not the mice were deficient in apoE (Figure 3). In addition, EC50 values calculated from the concentration response curves to NE and SNP did not differ between the control and apoE-KO groups (Figure 3). In contrast, the concentration-response curve to ACh was shifted to the right in apoE-KO mice fed the high-fat diet as compared with control mice fed the same experimental diet, and the percent relaxation evoked by the 30, 100, and 300 nmol/L ACh concentrations ([ACh] log M: −7.5, −7.0, and −6.5, respectively) was significantly lower in apoE-KO mice than in control mice (P<0.01 in all cases, Figure 4). The mean EC50 value determined from the concentration response curve to ACh was approximately 3-fold higher in the apoE-KO mice than in control animals (Figure 3), reflecting a significantly weaker sensitivity to ACh of the aortic segments from the apoE-KO mice when fed the high-fat diet.

**Effect of Human ApoA-I Expression on Vascular Reactivity in ApoE-Deficient Mice Fed the High-Fat Diet**

In an additional series of experiments, we tested the effect of human apoA-I gene expression on the arterial response to NE, ACh, and SNP in apoE-KO mice. To this end, 14-week-old apoE-KO female mice (n=9) and apoE-KO female mice expressing human apoA-I (apoE-KO/HuAITg mice, n=12) were fed the high-fat diet for 23 weeks, and the reactivity of thoracic aortic rings was assessed as described above. In agreement with previous studies,29 hypercholesterolemia in apoE-KO/HuAITg mice was similar to that observed in apoE-KO mice (3330±260 mg/dL and 3350±210 mg/dL, respectively; P=NS). In addition, the apoE-KO/HuAITg

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female mice were previously shown to exhibit 2- to 3-fold higher HDL cholesterol levels and greater plasma apoA-I concentrations than apoE-KO female mice.29

As shown in Figure 5, the maximal responses to NE, ACh, and SNP were similar whether or not the apoE-KO mice expressed human apoA-I, and EC50 values calculated from the concentration-response curves to NE and SNP did not differ between the apoE-KO and apoE-KO/HuAITg groups (Figure 5). In contrast, human apoA-I expression induced a shift to the left of the concentration response curve to ACh (Figure 6). The percent relaxation evoked by all the ACh concentrations was constantly greater in apoE-KO/HuAITg mice than in apoE-KO mice, and the difference reached the statistical significance for the 30 nmol/L ACh concentration ([ACh] log M: -7.5; * P < 0.05). Overall, a significant 4.4-fold decrease in the mean EC50 value was observed in apoE-KO/HuAITg mice (Figure 5). These observations indicate that the sensitivity to ACh of the thoracic aorta from the apoE-KO mice fed the high-fat diet is significantly improved by the expression of human apoA-I.

Discussion
The present study demonstrated that the severe hypercholesterolemia induced by fat feeding in apoE-KO mice produces a selective alteration of the endothelium-dependent relaxation of the thoracic aorta, whereas the contractile and the endothelium-independent arterial responses remained unchanged. Additional investigations in apoE-KO/HuAITg mice brought direct evidence in favor of the role of human HDL apoA-I in preventing the endothelium dysfunction in vivo.

Genetically engineered mice with introduced transgenes or knocked-out endogenous genes were recently created in several laboratories in order to bring more insights into apolipoproteins, enzymes, or receptors that are involved in lipoprotein metabolism.25,26,31 In particular, apoE-KO mice were developed by gene targeting.28,32 Hypercholesterolemia observed in apoE-KO heterozygotes, and to a greater extent in apoE-KO homozygotes, is associated with the development of atherosclerotic lesions that resemble human lesions in their morphology and distribution.28,30,33,34 Although the linear parts of the descending aorta do not constitute privileged sites for the development of atherosclerosis in apoE-deficient mice, minimal fatty streaks and foam cell lesions can be detected at 10 weeks of age in apoE-deficient mice fed a chow diet.34 Early lesions were also observed in the present study through the histological analyses of a few thoracic arterial segments from apoE-KO mice that were fed the low-fat diet for 23 weeks (results not shown). In these animals, we observed no alterations of the vasoactive response of linear thoracic arterial segments. In particular, no endothelium dysfunction was observed, confirming recent observations of Bonthu et al.27 who reported a normal relaxation of thoracic aortic segments from chow-fed, 19-week-old apoE-KO mice that displayed only minimal atherosclerotic lesions.

In addition to the advanced fibroproliferative lesions which predictably occurred in the linear aortic segments from apoE-KO mice fed a high-fat diet,28,30,34 the present study demonstrated, for the first time, that the exaggeration of hypercholesterolemia in apoE-KO mice by high-fat feeding produces a selective alteration of the endothelium-dependent relaxation of the thoracic aorta. Because only the ACh-induced and not the SNP-induced relaxation varied according to the mouse genotype under the high-fat diet, endothelium dysfunction, and not smooth muscle dysfunction accounted for the decrease in the relaxing response in apoE-KO mice fed the high-fat diet. Therefore, these animals arise as a model to target disturbances in the endothelium-dependent control of vasomotor tone in vivo. They may constitute a convenient alternative to the use of double knock-out mice with concomitant LDL receptor and apoE deficiency that is also known to combine severe hypercholesterolemia and endothelium dysfunction.27 As suggested for the acceleration of atherosclerosis development in apoE-KO mice,28,30,32,35 the disruption of the endothelium-dependent arterial relaxation in apoE-KO mice fed a high-fat diet may relate to both increased plasma
levels of atherogenic apoB-containing lipoproteins and decreased efficiency of the reverse cholesterol transport pathway mediated by HDL.

The present study brings the first in vivo evidence for the positive role of human HDL apoA-I in preventing the endothelium dysfunction. More precisely, the endothelium-dependent arterial relaxing response to ACh tended to be normalized in apoE-KO/HuAIItg mice fed the high-fat diet, indicating that the ability of isolated HDL-apoA-I to reverse the oxidized LDL-induced impairment of arterial relaxation in vitro may be of pathophysiological relevance. Although human apoA-I was previously reported to significantly reduce the severity of atherosclerosis lesions, but not a complete restoration of advanced fibroproliferative lesions.29,36 As previously reported,29 human apoA-I expression in apoE-KO female mice is accompanied by a 50% increase in the concentration of total plasma apoA-I (primarily human in apoE-KO/HuAIItg animals). The human apoA-I concentration in apoE-KO/HuAIItg mouse plasma (approximately 70 mg/dL) clearly remains below the levels that are normally found in human plasma (apoA-I concentration range: 110 to 220 mg/dL). Therefore, it appears that the beneficial effect of the human apoA-I on the endothelium-dependent arterial relaxation may have been underestimated by its low expression in the apoE-KO/HuAIItg mice studied. Similarly, in the same apoE-KO/HuAIItg mouse line, only significant decreases in the number and severity of atherosclerosis lesions, but not a complete restoration of the vasculature integrity, were reported by Paszty and coworkers.29 Because severe hypercholesterolemia is not corrected in apoE-KO/HuAIItg mice, HDL-apoA-I is likely to exert its own direct protective effect against the LDL-mediated endothelium dysfunction, independently of the plasma atherogenic lipoprotein levels. The improvement of endothelium-dependent relaxation by HDL might be a specific property of HDL-bound apoA-I, because only phospholipid/apoA-I complexes and neither apoA-I nor phospholipids alone were shown to mimic the beneficial effect of plasma HDL on vascular reactivity in vitro.24

In conclusion, the endothelium-dependent arterial relaxation is significantly impaired in apoE-KO mice fed a high-fat diet but not in apoE-KO mice fed a low-fat diet. The expression of human apoA-I in apoE-KO mice can significantly improve the vascular reactivity that is now recognized as a fundamental aspect of vascular biology. These observations provide an explanation for the previously reported association of high plasma HDL levels with normal endothelium-dependent arterial relaxation in humans.22,39

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