Altered mechanisms of endothelium-dependent dilation in skeletal muscle arterioles with genetic hypercholesterolemia

Phoebe A. Stapleton,1,3 Adam G. Goodwill,2,3 Milinda E. James,2,3 and Jefferson C. Frisbee2,3

1Division of Exercise Physiology, 2Department of Physiology and Pharmacology, and 3Center for Interdisciplinary Research in Cardiovascular Sciences, West Virginia University School of Medicine, Morgantown, West Virginia

Submitted 11 June 2007; accepted in final form 9 July 2007

Stapleton PA, Goodwill AG, James ME, Frisbee JC. Altered mechanisms of endothelium-dependent dilation in skeletal muscle arterioles with genetic hypercholesterolemia. Am J Physiol Regul Integr Comp Physiol 293: R1110–R1119, 2007. First published July 11, 2007; doi:10.1152/ajpregu.00410.2007.—With most cardiovascular disease risk factors, endothelium-dependent dilation of skeletal muscle resistance arterioles is compromised, although with hypercholesterolemia, impairments to reactivity are not consistently observed. Using apolipoprotein E (ApoE) and low-density lipoprotein receptor (LDLR) gene deletion male mouse models of hypercholesterolemia at 20 wk of age, we tested the hypothesis that arteriolar dilation would be maintained due to an increased stimulus-induced production of dilator metabolites via cyclooxygenase and cytochrome P-450 epoxygenase pathways. Arterioles from both strains demonstrated mild reductions in dilation to hypoxia and acetylcholine versus responses in C57/Bl/6j (C57) controls. However, although inhibition of nitric oxide synthase (NOS) attenuated dilation in arterioles from C57 controls, this effect was absent in ApoE or LDLR strains. In contrast, cyclooxygenase-dependent portions of dilator reactivity were maintained across the three strains. Notably, although combined NOS and cyclooxygenase inhibition abolished arteriolar responses to hypoxia and acetylcholine in C57 controls, significant reactivity remained in ApoE and LDLR strains. Whereas inhibition of cytochrome P-450 ω-hydroxylase and epoxygenases had no effect on this residual reactivity in ApoE and LDLR strains, inhibition of 12/15-epoxygenase with nordihydroguaiaretic acid abolished the residual reactivity. With both hypoxic and methacholine challenges, arteries from ApoE and LDLR strains demonstrated an increased production of both 12(S)- and 15(S)-hydroxyeicosatetraenoic acid, end products of arachidonic acid metabolism via 12/15-epoxygenase, a response that was not present in C57 controls. These results suggest that with development of hypercholesterolemia, mechanisms contributing to dilator reactivity in skeletal muscle arterioles are modified such that net reactivity to endothelium-dependent stimuli is largely intact.

skeletal muscle microcirculation; mouse models of cardiovascular disease

IT HAS BEEN WELL ESTABLISHED that development of the hypercholesterolemic condition is a profound risk factor for the evolution of coronary and peripheral arterial disease (1). From an epidemiological perspective, recent studies from the Centers for Disease Control have indicated that under conditions of dyslipidemia, a 10% reduction in total cholesterol levels can result in an estimated 30% reduction in the incidence of coronary artery disease (1). However, although hypercholesterolemia is a clear and profound risk factor for the initiation and progression of peripheral arterial disease, most notably through an increased likelihood for the risk of developing atherosclerotic depositions (16, 44), an understanding of the impact of hypercholesterolemia on the patterns of vascular reactivity has thus far demonstrated considerably less consensus.

In previous studies of human subjects afflicted with genetic dyslipidemia, particularly familial hypercholesterolemia [a genetic disorder resulting in exceptionally high low-density lipoprotein (LDL) level, in the face of an otherwise relatively normal lipid profile], arterial dilator reactivity during recovery from brief occlusion (i.e., flow-mediated dilation or reactive hyperemia) was found to be significantly attenuated compared with responses in normocholesterolemic subjects (2, 6, 8, 20, 29, 39). Furthermore, given the strong dependence of the flow-mediated response on endothelial nitric oxide (NO) bioavailability (22), many of these previous results have implicated oxidant radical scavenging of NO, thus reducing its bioavailability, as an underlying mechanism contributing to impaired reactivity (6, 8, 29, 42). However, results from other studies suggest that whereas dilator responses to NO-dependent stimuli are impaired with profound hypercholesterolemia, vasodilation in response to metabolic stimuli are largely intact (7), and this may suggest that compensatory mechanisms could be emerging to ameliorate the effects of any loss in reactivity owing to an impaired NO bioavailability. Notably, Panigagua et al. (34) demonstrated that shear stress-induced dilation of adipose tissue microvessels from hypercholesterolemic subjects was preserved despite a diminished activity of endothelial NO synthase (NOS). The maintenance of vascular reactivity under conditions of hypercholesterolemia has also been suggested by work from Laughlin’s group, since these investigators have demonstrated that a high-fat, high-cholesterol diet had only mild to moderate effects on coronary arteriolar (17) or femoral artery dilation (47) mediated through the vascular endothelium.

Previous studies have suggested that dilator responses of thoracic aortic rings (4, 5) and coronary arteries (25) from hyperlipidemic apolipoprotein E gene deletion mice remain largely intact while ingesting a normal diet. However, dilator responses in these mice were profoundly impaired following chronic ingestion of high-fat/high-cholesterol diets. Given the recent study by Wolfe and de Wit (46), wherein the endothelium-dependent dilator and conducted responses following challenge with acetylcholine were intact in the apolipoprotein E and low-density lipoprotein receptor gene deletion mouse

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
models of hypercholesterolemia, and the previous work of Pfister et al. (36), which suggests that the pathways of arachidonate acid-induced arterial dilation can be radically altered in hypercholesterolemic rabbits, the purpose of the present study was to determine the effects of profound hypercholesterolemia of genetic origin on mechanisms of endothelium-dependent dilator responses of skeletal muscle resistance arterioles. Using both the apolipoprotein E and low-density lipoprotein receptor gene deletion mouse models of hypercholesterolemia, we tested the hypothesis that endothelium-dependent dilator reactivity of skeletal muscle arterioles in these animals would be maintained, despite profound hypercholesterolemia, and that this would be manifested through an increased stimulus-induced production of dilator metabolites via cyclooxygenase and cytochrome P-450 epoxygenase pathways.

MATERIALS AND METHODS

Animals. The present study used three strains of mice, C57/Bl6J (C57) mice as the control strain, and the apolipoprotein E gene deletion (B6.129P2-Apoem1Unc/J; ApoE) and low-density lipoprotein receptor gene deletion (B6.129S7-Ldlrmd1Her/J; LDLR) mice on the C57/Bl6J background. All mice were purchased from Jackson Laboratories (Bar Harbor, ME) at 6 wk of age. The ApoE gene deletion mouse model of hypercholesterolemia manifests a type III hyperlipidemia in which both plasma cholesterol and triglyceride levels are elevated, although the elevations in LDL are not as severe as in the LDLR gene deletion mouse (30, 37). In contrast, the LDLR gene deletion mouse is a model of human familial hypercholesterolemia, manifesting a profound increase in serum LDL levels while ingesting a normal diet (19).

Male mice of each strain were fed standard chow and drinking water ad libitum and were housed in an American Association for Accreditation of Laboratory Animal Care-accredited animal care facility at the West Virginia University Health Sciences Center, and all protocols received prior Institutional Animal Care and Use Committee approval. At 20 wk of age, after an overnight fast, mice were anesthetized with injections of pentobarbital sodium (50 mg/kg ip) and received tracheal intubation to facilitate maintenance of a patent airway. In all mice, a carotid artery was cannulated for determination of arterial pressure. Blood aliquots were drawn from the jugular vein cannula for determination of glucose and insulin (Linco) and lipid profile levels (Waco). The 20-wk age was used to allow us to investigate alterations to microvascular structure/function in the presence of chronic dyslipidemia. Furthermore, at this age, the degree of the dysfunction was not so severe that it would not be amenable to amelioration via interventional strategies. Thus the use of this age range allowed us to examine both mechanisms underlying dysfunction as well as the efficacy of interventional strategies for improving microvascular outcomes.

Preparation of isolated skeletal muscle resistance arterioles. In anesthetized mice, the intramuscular continuation of the right gracilis artery was surgically removed and cannulated, as described previously for rats (14). These first-order arterioles were extended to their approximate in situ length and were equilibrated at 80% of the animal’s mean arterial pressure to approximate the in vivo intraluminal pressure experienced by the animal (26). Following equilibration, arteriolar reactivity was evaluated in response to 1) hypoxia, a reduction in superfuse and perfuse PO2 from ~135 mmHg (21% O2) to ~40 mmHg (0% O2); 2) acetylcholine (10–10–10–6 M; Sigma); 3) sodium nitroprusside (10–10–10–6 M; Sigma); and 4) prostacyclin (10–10–10–6 M; Biomol). Following assessments of arteriolar reactivity, the perfuse and superfuse were replaced with Ca2+-free physiological salt solution (PSS), and vessels were treated with 10–7 M phenylephrine until all reactivity and tone were abolished. Subsequently, arteriolar intraluminal pressure was altered, in 20-mmHg increments, between 0 and 140 mmHg, and the inner and outer diameter of arterioles was determined at each pressure. These data were used to calculate arteriolar wall mechanics, which were used as indicators of structural alterations to individual microvessels (13).

Removal of the vessel endothelium was accomplished by passing several air bubbles through the perfusate line into the isolated arteriole, the efficacy of which was determined from a loss of all dilator reactivity in response to application of 10–6 M acetylcholine (14). To assess the contribution of NO production or the generation of metabolites via cyclooxygenase as mediators of arteriolar dilator reactivity, isolated vessels were treated with the NOS inhibitor Nω-nitro-l-arginine methyl ester (l-NAME; 10–4 M; Sigma) or the cyclooxygenase antagonist indomethacin (10–6 M; Sigma), respectively. In addition, to determine the contribution of metabolites of arachidonic acid mediated via cytochrome P-450 enzymes, vessels were treated with the suicide substrate inhibitor 17-octadecynoic acid (17-ODYA; 10–5 M; Sigma). Previous studies have demonstrated that 17-ODYA profoundly attenuates both the ω-hydroxylation [producing 20-hydroxyeicosatetraenoic acid (20-HETE)] and epoxygenation [producing epoxyeicosatrienoic acids (EETs)] reactions of arachidonic acid through cytochrome P-450 (45), thus preventing changes to vascular levels of 20-HETE or EETs as contributing mediators to endothelium-dependent dilation (10, 14, 15, 33). To assess the contribution of lipxygenase metabolites to the patterns of arteriolar dilation, vessels were treated with nordihydroguaiaretic acid (NDGA; 3 × 10–5 M; Biomol), a selective inhibitor of 12- and 15-lipoxigenases (11, 38, 48).

Measurement of vascular nitric oxide bioavailability. From each animal, the aorta was removed and vascular NO production was assessed using amperometric sensors (World Precision Instruments). Briefly, aortas were isolated, sectioned longitudinally, pinned in a Silastic-coated dish, and superfused with warmed (37°C) PSS equilibrated with 95% O2-5% CO2. The NO sensor (ISO-NOPF 100) was placed in close apposition to the endothelial surface, and a baseline level of current was obtained. Subsequently, aortas were exposed to either acute reductions in PO2 from ~135 to ~40 mmHg (as described above) or increasing concentrations of methacholine (from 10–10 to 10–5 M), and changes in current were determined. To verify that the recorded data represented endothelium-dependent NO release, responses were reevaluated following acute treatment of aortas with l-NAME (10–4 M).

Determination of vascular metabolites of arachidonic acid. Vascular production of 6-keto-prostaglandin F1α (6-keto-PGF1α), the breakdown product of PG12 (31), 12(S)-HETE, the stable product from the reduction of 12(S)-hydroperoxyeicosatetraenoic acid [12(S)-HpETE], the 12-lipoxigenase metabolite of arachidonic acid (3, 40, 49), and 15(S)-HETE, the major hydroxy derivative of arachidonic acid when metabolized by 15-lipoxigenase (3, 27, 43, 48, 49), in response to hypoxia or methacholine within the three mouse groups was assessed using pooled conduit arteries (e.g., femoral, saphenous, iliac, carotid arteries) from each mouse. Vessels were incubated in microcentrifuge tubes in 1 ml of PSS for 30 min under control conditions (21% O2), after which time either the equilibration gas was switched to 0% O2 or methacholine (10–6 M) was added to the tube for an additional 30 min. After the second 30-min period, the PSS was removed from the tube, frozen in liquid N2, and stored at −80°C. Metabolite release by the vessels was determined using commercially available enzyme immunoassay kits for 6-keto-PGF1α (Cayman), 12(S)-HETE (Assay Designs), and 15(S)-HETE (Assay Designs).

Data and statistical analyses. Active tone of individual arterioles at the equilibration pressure was calculated as ΔD/Dmax × 100, where ΔD is the diameter increase from rest in response to Ca2+-free PSS and Dmax is the maximum diameter measured at the equilibration pressure in Ca2+-free PSS. Dilator responses of isolated arterioles following challenge with dilator agonists were fit with the three-
HYPERCHOLESTEROLEMIA AND VASCULAR REACTIVITY

Parameter logistic equation $y = \min + [(\max - \min)(1 + 10^{\log ED_{50} - y})$ where $\max$ and $\min$ represent the lower and upper bounds, respectively, of the change in arteriolar diameter with increasing agonist concentration, $x$ is the logarithm of the agonist concentration, and $\log ED_{50}$ represents the logarithm of the agonist concentration ($x$) at which the response ($y$) is half way between the lower and upper bounds.

The passive arteriolar incremental distensibility (%change in arteriolar diameter per mmHg) was calculated as $[(\Delta D/(ID \times \Delta P_{IL})) \times 100$, where $\Delta D$ represents the change in internal arteriolar diameter for each incremental change in intraluminal pressure ($\Delta P_{IL}$).

Data describing the vascular production of NO in response to methacholine challenge were fit with the linear regression equation $y = \alpha_0 + \beta_1(x)$, where $y$ represents the nitric oxide production, $\alpha_0$ represents the intercept term, and $\beta_1$ represents the rate of change in nitric oxide production for a change in methacholine concentration (slope).

Data are means ± SE. Statistically significant differences in measured and calculated parameters in the present study were determined using analysis of variance (ANOVA). In all cases, the Student-Newman-Keuls post hoc test was used when appropriate, and $P < 0.05$ was taken to reflect statistical significance.

RESULTS

Table 1 presents baseline characteristics of the three mouse groups used in the present study. Although all mice were of comparable mass at 20 wk of age, LDLR mice demonstrated a statistically significant elevation in mean arterial pressure and insulin resistance compared with values in either C57 or ApoE mice. Although both ApoE and LDLR mice manifested a profound hypercholesterolemia, most severe in LDLR animals, these animals also exhibited hypertriglyceridemia as well, which was most substantial in ApoE mice. In addition, isolated arterioles from ApoE and LDLR mice, while exhibiting diameters comparable those from C57 mice under active conditions, demonstrated a progressive reduction in inner diameter under passive (calcium-free) conditions.

Figure 1 presents dilator reactivity for isolated arterioles from C57, ApoE, and LDLR mice in response to challenge with hypoxia (A) and increasing concentrations of acetylcholine (B), sodium nitroprusside (C), and prostacyclin (D). In
response to hypoxia or acetylcholine, where arteriolar dilation is strongly endothelium dependent, responses in vessels from ApoE and LDLR mice were only modestly attenuated compared with responses determined in C57 control animals. In Fig. 1, C (sodium nitroprusside) and D (prostacyclin), where dilator responses to these stimuli are endothelium independent, arteriolar reactivity was also predominantly intact, demonstrating only mild attenuation.

Data describing the passive mechanical characteristics of the skeletal muscle resistance arteriolar wall in the mouse groups for the present study are summarized in Fig. 2. Under Ca\(^{2+}\)-free conditions, the increase in vessel diameter with elevated intraluminal pressure was consistently reduced in isolated arterioles of LDLR compared with C57 mice, with the majority of this impact being observed at higher levels of pressure (Fig. 2A). Although a directionally consistent effect was also determined in a comparison of ApoE with C57 mice, this effect was not as pronounced. However, calculated incremental distensibility, although demonstrating a consistent trend toward a

Fig. 2. Intraluminal pressure-induced expansion (A) and arteriolar wall incremental distensibility (B) of isolated skeletal muscle microvessels from C57, ApoE, and LDLR mice under passive (Ca\(^{2+}\)-free) conditions. Data are means ± SE (n = 8 animals for each group). *P < 0.05 vs. C57.

Fig. 3. Dilator responses of isolated skeletal muscle resistance arterioles of C57, ApoE, and LDLR mice in response to hypoxia (A) and increasing concentrations of acetylcholine (B–D) for arterioles under control conditions and following pharmacological inhibition of nitric oxide synthase (NOS) with L-nitro-arginine methyl ester (L-NAME), cyclooxygenase with indomethacin (INDO), or combined inhibition of both enzymatic pathways (see text for details). Data are means ± SE (n = 5–10 animals for each group). *P < 0.05 vs. control conditions. †P < 0.05 vs. no response.
reduced deformability of the vessel wall in the hypercholesterolemic animals, was not significantly different among the three mouse groups (Fig. 2B).

Figure 3 presents vascular reactivity to hypoxia (A) and to increasing concentrations of acetylcholine (B–D) in isolated arterioles of C57, ApoE, and LDLR mice following inhibition of NOS, cyclooxygenase, and both pathways. In response to hypoxia, C57 mice demonstrated an arteriolar reactivity that was dependent on the release of both NO and PGI2 from the vascular endothelium. Alternatively, in both ApoE and LDLR mice, arteriolar reactivity to hypoxia demonstrated no statistically significant contribution from NOS, although a significant contribution for metabolites of arachidonic acid via cyclooxygenase remained intact (Fig. 3A). Interestingly, combined inhibition of NOS and cyclooxygenase in both hypercholesterolemic mouse strains did not abolish arteriolar dilation to hypoxia, since a significant reactivity to reduced PO2 remained intact. Vascular responses to challenge with acetylcholine demonstrated a pattern comparable to that for hypoxia. In arterioles from C57 mice (Fig. 3B), dilation to acetylcholine was an integrated response mediated through contribution of NO and PGI2. In ApoE (Fig. 3C) and LDLR mice (Fig. 3D), the NO-dependent portion of arteriolar dilation in response to acetylcholine was attenuated and the cyclooxygenase-dependent portion remained intact. Furthermore, a significant acetylcholine-induced arteriolar dilation remained in ApoE and LDLR mice, despite combined treatment with both l-NAME and indomethacin.

Figure 4 presents data describing vascular NO and PGI2 production from C57, ApoE, and LDLR mice in response to hypoxic and methacholine challenge. Hypoxia-induced NO production was pronounced in aortas from C57 mice and demonstrated a progressive attenuation in ApoE and LDLR mice such that this response was entirely abolished in the latter strain (Fig. 4A). A similar pattern was present in response to methacholine challenge, since the agonist-induced generation of NO in C57 mice was abrogated in both ApoE and LDLR mice (Fig. 4B). In contrast, production of prostacyclin (from 6-keto-PGF1α levels) from pooled arteries was comparable among C57, ApoE, and LDLR mice in response to either reduced PO2 (Fig. 4C) or increasing concentrations of methacholine (Fig. 4D).

The role of metabolites of arachidonic acid from cytochrome P-450 enzymes in contributing to hypoxia- or acetylcholine-induced vasodilation in C57, ApoE, and LDLR mice are summarized in Fig. 5. Hypoxic dilation of isolated arterioles from these mice was unaffected by application of 17-ODYA (Fig. 5A), and combined application of 17-ODYA with both l-NAME and indomethacin resulted in a reduction in dilator reactivity that was extremely similar to that determined for NOS and cyclooxygenase inhibition alone (as shown in Fig. 3A). This pattern was repeated with acetylcholine challenge in arterioles of C57 (Fig. 5B), ApoE (Fig. 5C), and LDLR mice (Fig. 5D), since treatment with 17-ODYA alone had minimal impact on acetylcholine-induced dilation, and combined treat-
ment with 17-ODYA, l-NAME, and indomethacin had an effect that was nearly identical to combined administration of l-NAME and indomethacin in the absence of 17-ODYA.

The effects of 12/15-lipoxygenase inhibition with nordihydroguaiaretic acid (NDGA) on hypoxia- and acetylcholine-induced arteriolar dilation in the mouse groups of the present study are presented in Fig. 6. With acute reductions in PO2 (Fig. 6A), treatment with NDGA alone had no substantial impact on responses in C57 mice but significantly reduced hypoxic dilation in arterioles of ApoE and LDLR mice. Combined treatment of arterioles with l-NAME, indomethacin, and NDGA abolished the responses of vessels from all groups in response to hypoxia. Comparable results were also determined in response to acetylcholine challenge, since NDGA treatment had minimal impact on arteriolar dilation in response to acetylcholine in C57 mice (Fig. 6A) but significantly reduced these responses in ApoE (Fig. 6C) and LDLR mice (Fig. 6D). Combined treatment with l-NAME, indomethacin, and NDGA abolished all arteriolar acetylcholine-induced reactivity in the hypercholesterolemic mice.

Figure 7 presents data describing the vascular production of 12(\(S\))-HETE and 15(\(S\))-HETE from the mouse groups in the present study in response to hypoxia and challenge with methacholine. The production of 12(\(S\))-HETE from pooled arteries following exposure to hypoxia (Fig. 7A) or methacholine (Fig. 7B) was minimal in C57 mice but was significantly increased in both ApoE and LDLR mice. Similarly, arterial production of 15(\(S\))-HETE was also significantly increased over that in C57 mice in ApoE and LDLR mice following challenge with hypoxia (Fig. 7C) or methacholine (Fig. 7D). In all cases, incubation of vessels with NDGA abolished stimulus-induced vascular release of 12(\(S\))- or 15(\(S\))-HETE.

**DISCUSSION**

Although the development of many cardiovascular disease risk factors is associated with profound alterations to vascular reactivity (9, 12, 18) and most commonly with impaired endothelium-dependent dilation (32), the development of hypercholesterolemia has somewhat less predictable effects on vasodilator responses. Impairments to numerous indexes of endothelium-dependent dilation have been found in hypercholesterolemic humans (2, 6, 8, 20, 29, 39), but this is not universally observed (7, 34). In addition, although some animal models of hypercholesterolemia have exhibited blunted patterns of endothelium-dependent dilation (41, 42), studies have also suggested that these impairments can be mild (17, 24, 47), if they are present at all (46). Given this lack of clarity within the existing literature, the present study was designed to evaluate the impact of genetic hypercholesterolemia in mice on endothelium-dependent and -independent dilation of skeletal muscle resistance arterioles.

Fig. 5. Dilator responses of isolated skeletal muscle resistance arterioles of C57, ApoE, and LDLR mice in response to hypoxia (A) and increasing concentrations of acetylcholine (B–D) for arterioles under control conditions and following pharmacological inhibition of cytochrome P-450 enzymes with 17-octadecynoic acid (17-ODYA), either alone or in combination with l-NAME and INDO. Data are means ± SE (n = 6–7 animals for each group). *\(P < 0.05\) vs. control conditions in the indicated strain. †\(P < 0.05\) vs. no response.
The primary observation of this study was that dilator reactivity of skeletal muscle resistance arterioles of ApoE and LDLR mice was not strikingly different from that determined in C57 control animals. As is evident from Fig. 1, dilator reactivity to the endothelium-dependent stimuli of hypoxia and acetylcholine, although somewhat blunted, was largely intact in arterioles from both ApoE and LDLR mice. Furthermore, responses to the endothelium-independent stimuli of PGI2 and the NO donor sodium nitroprusside were also generally intact and manifested only mild reductions in the magnitude of dilation, if they were present at all. When combined with observations of arteriolar wall mechanics presented in Fig. 2, wherein the present results suggest that passive expansion of arteriolar diameter with elevated intraluminal pressure was blunted in ApoE and LDLR mice, resulting in a mild (but not statistically significant) reduction in incremental distensibility, the present results support three initial conclusions: 1) endothelial function, with regard to net dilator reactivity, is largely intact in skeletal muscle resistance arterioles of ApoE and LDLR mice; 2) vascular smooth muscle reactivity to exogenously supplied prostacyclin and NO appears to be near normal in these microvessels; and 3) impairments to dilator reactivity at this stage may partially reflect developing alterations to the mechanics of the arteriolar wall rather than simply compromised endothelial or vascular smooth muscle function.

The data presented in Figs. 3 and 4 suggest that although the net dilator reactivity of individual arterioles in response to hypoxia and increasing acetylcholine concentration remains largely intact in ApoE and LDLR compared with C57 mice, the predominant signaling molecules that contribute to this reactivity may be substantially altered. Specifically, these results suggest that the portion of hypoxia- or acetylcholine-induced dilation that is mediated by endothelium-dependent generation of NO in C57 mice is lost with the development of hypercholesterolemia. This interpretation is supported by observations that the impact of l-NAME on dilator reactivity of isolated arterioles in response to hypoxia or challenge with acetylcholine was nearly abolished in ApoE and LDLR mice and that the stimulus-induced generation of NO from either hypoxia or methacholine was profoundly attenuated in arteries of hypercholesterolemic mice. The loss of vascular NO bioavailability with profound hypercholesterolemia has been reported previously (21, 23), and these results support those of previous studies. In contrast, the contribution of endothelium-derived prostacyclin to both hypoxia- and acetylcholine-induced dilation was not substantially impacted by development of the hypercholesterolemic condition in ApoE and LDLR mice. This was evident in both the consistent impact of indomethacin on dilator responses across the three strains of mice and the comparable level of 6-keto-PGF1α production in arteries of C57, ApoE, and LDLR mice in response to challenge with either hypoxia or methacholine. Notably, the results of these experiments suggest that the arteriolar dilator response to...
reduced oxygen tension or increased acetylcholine challenge in C57 mice was overwhelmingly the result of the production and release of endothelium-derived NO and prostacyclin. However, in both ApoE and LDLR models of hypercholesterolemia, significant dilator reactivity to both hypoxia and acetylcholine remained, despite pharmacological inhibition of both NOS and cyclooxygenase with l-NAME and indomethacin, respectively. These observations implicate the emergence of an additional dilator signaling pathway that may compensate for the loss of vascular NO bioavailability during hypercholesterolemia.

Previous studies have suggested that metabolites of arachidonic acid produced via either the \( \omega \)-hydroxylation (producing 20-HETE) or epoxygenation (producing EETs) reactions of cytochrome P-450 enzymes can contribute to arteriolar dilation in response to both hypoxia (14) and acetylcholine (28). However, results from the present study suggest that this is not the case in skeletal muscle resistance arterioles from C57 control mice or with the development of hypercholesterolemia in either ApoE or LDLR mice. Treatment of arterioles with 17-ODYA, either alone or in combination with l-NAME and indomethacin, had no impact on dilator responses to hypoxia or increased concentration of acetylcholine in any of the three mouse strains.

It has previously been demonstrated that the development of diet-induced hypercholesterolemia in rabbits may result in an increased role for lipoxygenase metabolites in contributing to endothelium-dependent dilator reactivity (35). To address the possibility that products of arachidonic acid metabolized through lipoxygenases may contribute to the residual dilation to hypoxia and acetylcholine in ApoE and LDLR following treatment with l-NAME and indomethacin, arterioles were treated with NDGA, either alone or in combination with l-NAME and indomethacin (Fig. 6). Although NDGA had no significant impact on dilator reactivity in C57 mice, it blunted dilator responses to hypoxia and acetylcholine in arterioles from ApoE and LDLR mice when given alone and abolished responses in arterioles from these strains when given following pretreatment with l-NAME and indomethacin. These results provide compelling evidence that the generation of metabolites of arachidonic acid via lipoxygenase may provide a compensatory mechanism to maintain arteriolar dilator reactivity in ApoE and LDLR mice. In support of this concept, data presented in Fig. 7 provide evidence that vascular production of 12(\( \omega \))-HETE and 15(\( \omega \))-HETE, from 12- and 15-lipoxygenase, respectively, although minimal in C57 mice in response to either hypoxia or methacholine challenge, was profoundly elevated in arteries of both ApoE and LDLR mice following exposure to both of these stimuli.

Recent studies have provided some compelling insight into the patterns of arteriolar reactivity in mice with diet-induced hypercholesterolemia. In wild-type mice, 2 wk of high-cholesterol diet has previously been demonstrated to result in an impaired dilator reactivity of cremasteric arterioles (in situ) to \( 10^{-5} \) M acetylcholine (41). However, an additional study from Kim et al. (24) suggested that the impairments to arteriolar dilation with diet-induced hypercholesterolemia in wild-type mice may be a function of the arteriolar proximity to a paired venule, since the degree of dilator impairment was inversely proportional to the diffusion distance from the venule. Interestingly, both of these studies have provided evidence that the dysfunction may be most tightly predicted by profound eleva-
tions in oxidant stress (24, 41) and elevations in P-selectin-mediated cellular adhesion (24). The results from these previous studies may provide compelling avenues for future study.

Throughout these studies, the production of vasodilator metabolites in response to challenge with either hypoxia or methacholine was determined from larger conduit arteries or aortic segments. These data were then employed to provide insight into the mechanical responses of skeletal muscle resistance arterioles following exposure to either reductions in oxygen tension or challenge with acetylcholine. As a result, these results should be interpreted with some caution, since mechanisms underlying vascular reactivity in response to specific stimuli are not necessarily consistent across all vessels and can demonstrate considerable heterogeneity. Ongoing studies are needed to determine whether the present results acquired using conduit arteries are maintained in the peripheral microcirculation. Together, the results of the present study suggest that with the development of genetic hypercholesterolemia in ApoE and LDLR mice, the dilator reactivity of skeletal muscle resistance arterioles is largely intact, although the signaling mechanisms responsible for these responses are altered. Although the contribution of prostacyclin to endothelium-dependent dilation appears to be maintained, vascular levels of NO bioavailability are dramatically reduced. However, with the development of hypercholesterolemia, the production of dilator metabolites via 12- and 15-lipoxygenase emerges as a compensatory mechanism in ApoE and LDLR mice and help to maintain net dilator reactivity in these vessels despite the loss of components dependent on NO bioavailability. Focused efforts into the signaling mechanisms responsible for the reduction in vascular NO bioavailability and the progressive compensation for this loss by lipoxygenases may represent exciting avenues for future investigation. In addition, the consequences of both the chronic reductions in NO bioavailability and the increased generation of arachidonic acid metabolites via lipoxygenases for other microvascular outcomes (e.g., microvessel network structure, wall mechanics, antithrombotic processes, spatiotemporal regulation of perfusion) also require considerable future study.

GRANTS

This study was supported by American Heart Association Awards SDG 0330194N and EIA 0720194N and National Institute of Diabetes and Digestive and Kidney Diseases Grant R01 DK-64668. We also gratefully acknowledge support provided through the “Translational Research Initiative: Cardiovascular Health in Appalachia—from Mechanisms to Policy” at the West Virginia University Health Sciences Center in the performance of this study.

REFERENCES


