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DIFFERENTIAL IMPACT OF FAMILIAL HYPERCHOLESTEROLEMIA AND COMBINED HYPERLIPIDEMIA ON VASCULAR WALL AND NETWORK REMODELING IN MICE

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Abstract

Genetic familial hypercholesterolemia (FH) and combined hyperlipidemia (FCH) are characterized by elevated plasma LDL (FH) and LDL/triglycerides (FCH), with mouse models represented by LDL receptor (LDLR) and apolipoprotein E (ApoE) gene deletion mice, respectively. Given the impact of FH and FCH on health outcomes, we determined the impact of FH/FCH on vascular structure in LDLR and ApoE mice. LDLR, ApoE and control mice were utilized at 12–13 and 22–23 weeks when gracilis arteries were studied for wall mechanics and gastrocnemius muscles were harvested for microvessel density measurements. Conduit arteries and plasma samples were harvested for biochemical analyses. Arteries from ApoE and LDLR exhibited blunted expansion versus control, reduced distensibility and left-shifted stress versus strain relation (LDLR>ApoE). Microvessel density was reduced in ApoE and LDLR (ApoE>LDLR). Secondary analyses suggested that wall remodeling in LDLR was associated with cholesterol and MCP-1, while rarefaction in ApoE was associated with TNF- α , triglycerides and vascular production of TxA₂. Remodeling in ApoE and LDLR appears distinct; as that in LDLR is preferential for vascular walls, while that for ApoE is stronger for rarefaction. Remodeling in LDLR may be associated with cellular adhesion, while that in ApoE may be associated with pro-apoptosis and constrictor prostanoid generation.

Keywords

vascular wall mechanics; microvascular density; vascular wall distensibility; rodent models of dyslipidemia

INTRODUCTION

Numerous previous studies have determined that severe dyslipidemia, including both hypercholesterolemia and hypertriglyceridemia represent significant risk factors for the development of peripheral artery diseases and negative health outcomes (10,13,15,23,31). Overwhelmingly, these address alterations to patterns of atherosclerotic plaque, lesion and fatty streak depositions within the vascular lumen, the propensity for the creation of emboli, the contribution to occlusive disease states, and downstream tissue/organ outcomes (3,15,20,39). While unquestionably of considerable clinical significance, these overt pathological states do not provide a complete understanding of the alterations to vascular structure and function that accompany dyslipidemia.

We have recently determined that in two mouse models of genetic dyslipidemia on the C57/Bl6/J background, the low density lipoprotein receptor gene deletion (B6.129S7-*Ldlr*^{tm1Her}/J; LDLR) and the apolipoprotein E gene deletion (B6.129P2-*ApoE*^{tm1Unc}/J; ApoE) the patterns of endothelium-dependent arteriolar reactivity are significantly altered from their control strains (C57; refs: 18,40). In terms of clinical relevance, the LDLR mouse represents a model of familial hypercholesterolemia (FH; refs: 27,37), characterized by striking elevations in plasma LDL levels, while the ApoE mouse represents familial combined hyperlipidemia (FCH; refs: 35,36,45), characterized by elevated plasma LDL (although not to levels in FH) and triglycerides. Specifically, both the LDLR and ApoE mouse models exhibit a striking loss of vascular nitric oxide bioavailability, as demonstrated by vascular responses to nitric oxide-dependent stimuli (11,40,41,44). However, this is more complicated than a simple loss of NO bioavailability. Previous studies in our laboratory (40) and by others (1) have suggested that this loss of NO bioavailability may be partially compensated for through alterations to arachidonic acid metabolism via lipoxygenases. Further, other reports exist suggesting that there may also be an additional shift in arachidonic acid metabolism with hypercholesterolemia, increasing the production of thromboxane A₂ (TxA₂) versus prostacyclin (PGI₂) which also has the potential to negatively impact vascular function (18,25). Finally, taken in combination with recent work from Wolfle and de Wit, suggesting that conducted responses in microvessels of LDLR mice were largely intact (43), and from Pfister *et al.* indicating an alteration in arachidonic acid metabolism in vascular tissue from hypercholesterolemic rabbits (34), and it becomes clear that the net vascular outcomes from dyslipidemia can be very complicated.

From the perspective of vascular outcomes and the determination of tissue perfusion, one area that requires further attention is that of the vascular remodeling that accompanies genetic dyslipidemia, and this should incorporate two different elements of remodeling; vascular wall mechanics and microvessel network structure. While studies exist which describe wall stiffening with genetic dyslipidemia (2,7,16), these are limited by the analyses utilized and do not provide for a more thorough understanding. Additionally, the study of microvessel density under conditions of dyslipidemia has been extremely limited, although what evidence is available suggests that this may be reduced with hypercholesterolemia (9,24).

The purpose of the present study was to determine differences in skeletal muscle microvascular remodeling (i.e., resistance artery wall mechanics and microvessel density) under conditions of FH and FCH utilizing the LDLR and ApoE mouse models of these conditions. The current study was designed to test the hypothesis that the progression of FH and FCH, while causing a remodeling of the skeletal muscle vasculature in both conditions, will be distinct in their vascular outcomes owing to differences in lipid and inflammatory profiles and impacts on endothelial function. This study will not only provide information regarding relevant changes to two key indices of vascular structure, it will also evaluate the

temporal development of these alterations, involving mice at 12–13 and 22–23 weeks of age. Finally, these results are compared with an extensive analysis of biomarkers between the three strains to determine if differences in outcomes may be associated with a specific clustering of contributing elements. It is believed that this process may provide a framework for targeted interventional procedures to ameliorate the poor vascular outcomes associated with genetic dyslipidemia.

MATERIALS AND METHODS

Animals

This study utilized three strains of mice; C57/B16/J as the controls, and the LDLR and ApoE mice as models of FH and FCH, respectively. All mice were purchased from Jackson Laboratories (Bar Harbor, ME). Male mice of each strain were fed standard chow and drinking water *ad libitum* and were housed in the animal care facility at the West Virginia University Health Sciences Center and all protocols received prior IACUC approval. At 12–13 or 22–23 weeks of age, mice were anesthetized with injections of sodium pentobarbital (50 mg·kg⁻¹ i.p.), and received tracheal intubation to facilitate maintenance of a patent airway. In all mice, a carotid artery was cannulated for determination of arterial pressure. After surgical removal of gracilis muscle resistance arteries (see below), deeply anesthetized mice received a bilateral pneumothoracotomy followed by cardiac puncture, wherein venous blood aliquots were drawn into tubes containing either heparin (for lipid determination) or K₂-EDTA (for all other analyses). Samples were immediately frozen in liquid N₂ and processed as batches for lipid profiles (Wako) and inflammatory markers (Millipore) using commercially available kits.

Preparation of Isolated Skeletal Muscle Resistance Arterioles

In anesthetized mice, the right gracilis artery was removed, cannulated, and extended to its approximate *in situ* length (14). Following equilibration, the perfusate and superfusate were replaced with Ca²⁺-free physiological salt solution and vessels were treated with 10⁻⁷ M phenylephrine until all tone was abolished. Subsequently, intraluminal pressure was altered, in 20 mmHg increments, between 0 mmHg and 160 mmHg and the inner and outer diameter of arteries was determined at each pressure. To ensure that a negative intraluminal pressure was not exerted on the vessel, 5 mmHg was used as the “0 mmHg” intraluminal pressure point; all other values of intraluminal pressure were multiples of 20 mmHg up to 160 mmHg. Specific pressures were randomized to prevent the occurrence of ordering effects. After 10 minutes at each intraluminal pressure, the inner and outer diameter of the passive arteriole was determined. These data were used to calculate arteriolar wall mechanics which were used as indicators of structural alterations to individual microvessels (19). Vessel wall thickness was calculated as:

$$WT = \frac{(OD - ID)}{2}$$

where WT represents wall thickness (μm) and OD and ID represent arteriolar outer and inner diameter, respectively (μm). Arteriolar cross-sectional wall area (CSWA; μm²), assuming the arteriole is cross-sectionally round, is calculated as:

$$CWSA = \left[\pi(OD/2)^2 \right] - \left[\pi(ID/2)^2 \right]$$

Incremental arteriolar distensibility ($DIST_{INC}$; % change in arteriolar diameter/mmHg) was calculated as:

$$DIST_{INC} = (\Delta ID / (ID_i \times \Delta P_{IL})) \times 100$$

where ΔID represents the change in internal arteriolar diameter for each incremental change in intraluminal pressure (ΔP_{IL}), and ID_i represents the initial internal diameter prior to the pressure change.

Circumferential wall stress is a measure of the average amount of distending force (in this case pressure; expressed in N/m^2) exerted on a deformable object. For the calculation of circumferential stress, intraluminal pressure was converted from mmHg to N/m^2 , where 1 mmHg = $1.334 \times 10^2 N/m^2$. Circumferential stress (σ) was then calculated as:

$$\sigma = (P_{IL} \times ID) / (2WT)$$

Circumferential strain is a measurement of the degree of deformation exhibited by an object (in this case the vessel wall) as a result of the imposed stress. Circumferential strain (ϵ) was calculated as:

$$\epsilon = (ID - ID_5) / ID_5$$

where ID_5 represents the internal arteriolar diameter at the lowest intraluminal pressure (i.e., 5 mmHg).

The tangential elastic modulus (E_T) describes an object's tendency to be deformed elastically in response to an applied stress. A vessel's E_T is defined as the slope of its circumferential stress versus strain relation. To determine tangential elastic modulus (E_T), the stress versus strain curves from each vessel were fit (OLS, $r^2 > 0.85$) with the following exponential equation:

$$\sigma = \sigma_5 e^{\beta \epsilon}$$

where σ_5 represents circumferential stress at ID_5 and β is the slope coefficient, which will be a function of the independent variable, ϵ . E_T was then estimated at different values of circumferential stress from the derivative of the exponential curve:

$$E_T = d\sigma / d\epsilon = \beta \sigma_5 e^{\beta \epsilon}$$

Determination of Microvessel Density

While under anesthesia, the right gastrocnemius muscle from each mouse was removed, rinsed in physiological salt solution and lightly fixed in 0.25% formalin. Muscles were embedded in paraffin and cut into 5 μm cross sections, which were stained with *Griffonia simplicifolia* I lectin (Sigma), and mounted on microscope slides, as described previously (14). Using epifluorescence microscopy, localization of labeled microvessels was performed with a Nikon E600 upright microscope with a 20x objective lens (Plan Fluo phase NA 0.5). Excitation was provided by a 75 watt Xenon Arc lamp through a Lambda 10-2 optical filter changer (Sutter Instrument Company, Novato, CA) controlling a 595nm excitation filter and a 615 nm emission filter. All acquired images from individual sections were analyzed for

number of microvessels and number of skeletal muscle fibers using commercially available software.

Measurement of Vascular Nitric Oxide Bioavailability

From each mouse, the aorta was removed and vascular nitric oxide production was assessed using amperometric sensors (World Precision Instruments). Briefly, aortae were sectioned longitudinally, pinned in a silastic coated dish and superfused with warmed (37°C) physiological salt solution equilibrated with 95% O₂/5% CO₂. The nitric oxide sensor (ISO-NOPF 100) was placed in close apposition to the endothelial surface and a baseline level of current was obtained. Subsequently, aortae were exposed to acute challenge with methacholine (10⁻⁶ M) and changes in current were determined. To verify that recorded data represented nitric oxide release, responses were re-evaluated following acute treatment of aortae with L-NAME (10⁻⁴ M).

Determination of Vascular Metabolites of Arachidonic Acid

Vascular production of 6-keto-prostaglandin F_{1α} (6-keto-PGF_{1α}; the stable breakdown product of PGI₂; ref. 32), and 11-dehydro-thromboxane B₂ (11-dehydro-TxB₂; the stable breakdown product of TxA₂; ref. 8) in response to challenge with arachidonic acid within the three mouse strains was assessed using pooled conduit arteries (femoral, saphenous, iliac, carotid arteries) from each mouse. Vessels were incubated in microcentrifuge tubes in 1 ml of physiological salt solution for 30 minutes under control conditions (21% O₂), after which time arachidonic acid (10⁻⁵ M) was added to the tube for an additional 30 minutes. After the second 30 minute period, the solution was transferred to a new tube, frozen in liquid N₂ and stored at -80°C. Metabolite release by the vessels was determined using commercially available kits for 6-keto-PGF_{1α} and 11-dehydro-TxB₂ (Cayman).

Statistical Analyses

All data are presented as mean±SEM. Differences in passive mechanical characteristics and microvessel density between mouse strains were assessed using analysis of variance (ANOVA) or regression analyses, with Student-Newman-Keuls-test post-hoc, as appropriate. Data describing tangential elastic modulus versus stress relations were fit with semi-logarithmic regression equations, each of which provided a significant F-statistic for the regression and an adjusted r²>0.90. Differences between regression coefficients were evaluated using ANOVA with post-hoc tests, as appropriate. In all cases, p<0.05 was taken to reflect statistical significance.

RESULTS

At the time of use, body mass was not different between the three strains of mice at either the 12–13 week or 22–23 week ages. At 12–13 weeks, C57 averaged 22±2 g, while ApoE and LDLR averaged 21±2 and 22±2 g, respectively. At 22–23 weeks, C57 averaged 31±2 g, as compared to 30±3 g for ApoE and 31±2 g for LDLR. While no differences were observed for mean arterial pressure across the three strains at 12–13 weeks (84±6 mmHg for C57; 86±5 mmHg for ApoE and 90±5 mmHg for LDLR), LDLR mice exhibited a significant increase in blood pressure by 22–23 weeks, reaching 112±6 mmHg, as compared to 84±5 mmHg in C57 and 92±5 mmHg in ApoE.

Figure 1 presents the severity of hypercholesterolemia (Panel A) and hypertriglyceridemia (Panel B) across the three mouse strains in the present study. While both ApoE and LDLR were significantly hypercholesterolemic as compared to C57 at both 12–13 and 22–23 weeks of age, total cholesterol was also significantly elevated in LDLR as compared to ApoE at both ages (Panel A). In contrast, plasma triglyceride levels were only elevated in

ApoE mice, and this effect was present at both age ranges (Panel B). Between age groups, within a strain, there were no differences in plasma cholesterol or triglycerides.

Figure 2 summarizes data describing basic deformational alterations to skeletal muscle arteries of C57, ApoE and LDLR mice at 12–13 and 22–23 weeks of age in response to increasing intraluminal pressure. The increase in arterial inner (Panel A) and outer diameter (Panel B) in response to increasing intraluminal pressure was blunted in ApoE and LDLR as compared to responses in C57. This blunting was greater with regard to inner diameter in LDLR mice, and as such there was a tendency for wall thickness to be elevated in LDLR with increasing age (Panel C). Total cross-sectional wall area demonstrated some decline with age within a strain, but with the exception of comparisons to the younger C57 mice, this did not exhibit a consistent pattern (Panel D). The tendency for an increased wall thickness with increasing hypercholesterolemia was also evident in terms of wall:lumen ratio (Panel E), as the older group of LDLR exhibited a significantly greater ratio over the range of intraluminal pressure. Finally, arterial wall incremental distensibility was reduced in LDLR with age as compared to either ApoE or C57 mice throughout the early range of intraluminal pressures (Panel F). However, beyond these points, all of the curves became superimposable.

Figure 3 presents data describing the changes in arterial circumferential wall stress versus strain (Panel A) and the tangential elastic modulus versus stress (Panel B) relations across the three strains. With increasing age and dyslipidemia, there was a progressive left-shifting of the stress versus strain relation that was most pronounced in LDLR as compared to ApoE (where no statistically significant shift in the curve was determined). This was also apparent in the E_T versus stress relation, where a modest trend toward an increasing slope in arteries from ApoE versus C57 was present, but that a striking shift in this relation in arteries from LDLR was evident compared to either other group.

The changes in microvessel density with age in control and dyslipidemic mice are summarized in Figure 4. While microvessel density was stable in skeletal muscle of C57 mice between 12–13 and 22–23 weeks of age, a consistent and increasing rarefaction in muscle of ApoE mice was exhibited, such that it was reduced at 12–13 weeks and a further rarefaction continued to 22–23 weeks of age. In contrast, there was no evidence for a significant rarefaction of the skeletal muscle microcirculation of LDLR at 12–13 weeks of age. However, the data presented in Figure 4 suggest that microvascular rarefaction was delayed in LDLR as compared to ApoE, as a significant reduction in microvessel density was evident by 22–23 weeks of age.

Data describing the plasma levels of specific biomarkers or contributors to vascular dysfunction are summarized in Figure 5. Plasma nitrotyrosine, an indicator of chronic vascular oxidant stress, was significantly elevated in both ApoE and LDLR as compared to C57, and this was evident at both age ranges (Panel A). However, no differences were determined between ApoE and LDLR in terms of nitrotyrosine. Plasma concentrations of tumor necrosis factors- α (TNF- α) were elevated in both ApoE and LDLR as compared to C57 at both age ranges, although the magnitude of this increase was greater in ApoE mice (Panel B). In both ApoE and LDLR, plasma interleukin-1 β (IL-1 β) was elevated as compared to levels determined in C57 at both 12–13 and 22–23 weeks of age, although no differences were determined between the two dyslipidemic strains (Panel C). Panel D presents plasma levels of monocyte chemoattractant protein-1 (MCP-1) between the three strains. While ApoE mice consistently demonstrated a significant increase in MCP-1 versus C57, this effect was much more pronounced in LDLR, where MCP-1 levels were also increased over that in ApoE.

Using conduit arteries, indices of vascular endothelial function are summarized in Figure 6. Following challenge with arachidonic acid, pooled arteries from all strains demonstrated a comparable, maintained ability to produce PGI_2 , as estimated from its breakdown product, 6-keto- $\text{PGF}_{1\alpha}$ (Panel A). In contrast, arachidonic acid-induced production of TxA_2 , estimated from its breakdown product 11-dehydro- TxB_2 , was significantly increased at both 12–13 and 22–23 weeks of age in arteries from ApoE as compared to C57 mice (Panel B). A similar pattern was also evident for thromboxane production in arteries from LDLR mice, although this lagged behind that in ApoE. Finally, vascular bioavailability of nitric oxide, estimated from its production following challenge with methacholine was dramatically attenuated in arteries from both ApoE and LDLR versus control at both 12–13 and 22–23 weeks (Panel C). Treatment of arteries with L-NAME abolished methacholine-induced nitric oxide production in all cases (data not shown).

DISCUSSION

While previous studies involving dyslipidemic humans and animal have demonstrated that elevated plasma cholesterol and triglycerides represent a significant risk factor for the development of atherosclerotic vascular disease (10,13,15,23,31), less thoroughly evaluated are alterations to vascular mechanics and almost completely unknown are the distal impact of these conditions on muscle vascularity. The results of the present study revealed several key observations. First, while both FH and FCH in mice resulted in an extensive remodeling of the skeletal muscle microcirculation, the specifics of the remodeling varied between strains. In FH, this remodeling was predominantly at the level of the resistance arterial wall through in increased wall stiffness, while in FCH, the remodeling was localized more at the level of the microvascular networks through an increased rarefaction. Further, the results from the present study identify potential contributors for future interrogation under each condition. Specifically, in FH, predictors such as cholesterol severity and the expression of inflammatory markers for cell attraction/adhesion are implicated for the developing stiffness of the resistance arterial wall. Alternatively, in FCH, the severity of hypertriglyceridemia, the presence of pro-apoptotic markers of inflammation, and alterations to endothelial metabolism of arachidonic acid were identified as correlates of the microvascular network remodeling.

The initial observation from the present study was that, while both familial hypercholesterolemia and familial combined hyperlipidemia can impact vascular wall mechanics, the alterations to wall mechanics with FH (in LDLR mice) develop more rapidly and are more severe than those determined under conditions of FCH (in ApoE mice). While the blunted distention of the arterial wall with increased pressure was in keeping with the observations of a decreased deformability of the vessel wall with chronic dyslipidemia in both strains, this was considerably pronounced in LDLR. When combined with the trend toward an increased wall thickness determined in arteries from LDLR mice, this resulted in significant elevations in wall:lumen ratio, an observation that has also been demonstrated in human subjects with chronic hypercholesterolemia (12,21) and in the ApoE/LDLR double knockout mouse (6). Taken together, these factors also resulted in the reduction in arterial wall incremental distensibility that was determined for both dyslipidemic strains, although more pronounced in LDLR than in ApoE.

These data describing the mechanical characteristics of the resistance arterial wall become more informative when placed in the context of circumferential stress versus strain relations (Figure 3A) and the relationship between tangential elastic modulus (E_T) versus circumferential stress (Figure 3B). The left-shifting of the stress versus strain relation, as indicated by the increased magnitude of the β coefficient from the exponential equation fit to the data, demonstrates that the stiffness of the resistance artery is increased with FH in

LDLR mice. While this process also develops in ApoE, it is considerably less robust and slower to develop. The shape of the E_T versus stress curves reveals an interesting insight into the behavior of the vascular wall. Had these data been best approximated by a linear model, this would have suggested that the increased stiffness of the arterial wall in LDLR as compared to C57 developed via contributions throughout the range of the intraluminal pressure range. However, these data were better approximated by a non-linear equation (with a superior adjusted r-squared), with the majority of the differences between responses in LDLR and C57 being accounted for over the range of relatively low stresses. From the perspective of functional restraints on perfusion, this suggests that the remodeling that develops in the arterial wall of LDLR may be primarily relevant in terms of restricting diameter at lower intraluminal pressures. Future investigation into the physical alterations to the architecture of the vessel wall, and how this contributes to the shift in these mechanical responses, especially in LDLR mice appears to be well justified.

The other major observation from the present study was that the remodeling of skeletal muscle microvascular networks (microvessel rarefaction) was most pronounced under conditions of familial combined hyperlipidemia. Notably, while the reduction in microvessel density with familial hypercholesterolemia in LDLR mice was present by 22–23 weeks of age, the rarefaction that developed in skeletal muscle of ApoE mice was much more robust, and was clearly identifiable by 12–13 weeks with increasing severity in the older age range. This observation, when taken in context with the impacts of the two models of genetic dyslipidemia on vascular wall mechanics, has a potentially significant implication. Specifically, familial hypercholesterolemia and familial combined hyperlipidemia, while ultimately leading to directionally consistent outcomes in terms of vascular remodeling, preferentially impact different sites along the vascular tree. Familial hypercholesterolemia is associated with an outcome that more rapidly and severely impacts vascular wall distensibility with effects on microvessel density that are delayed and more muted. Conversely, the vascular remodeling associated with familial combined hyperlipidemia is more targeted to microvessel rarefaction, with vascular wall remodeling being less pronounced.

At the outset, the obvious issue to be addressed is how familial hypercholesterolemia and familial combined hyperlipidemia differ with respect to systemic markers of cardiovascular disease risk and vascular function. While both ApoE and LDLR exhibit a significant hypercholesterolemia (Figure 1), this is considerably greater in LDLR than in ApoE. Further, ApoE mice also demonstrate a significant hypertriglyceridemia that is not present in LDLR. The data presented in Figure 5 may provide additional insight in how systemic markers of inflammation may help to discriminate between the impact of FH and FCH. Plasma levels of TNF- α were much higher in ApoE mice at both ages as compared to LDLR, while levels of MCP-1 exhibited a reversed relationship. However, neither the systemic marker of chronic oxidant stress (nitrotyrosine), nor the inflammatory marker IL-1 β , while elevated in ApoE and LDLR versus C57, were able to provide for discrimination between the two dyslipidemic strains.

Previous studies have suggested that alterations to endothelial function can play a critical role in both vascular wall (17,38) and vascular network (26) remodeling. As such, we utilized three indices of endothelial function in arteries as potential discriminating factors between FH and FCH in terms of vascular remodeling; methacholine-induced nitric oxide bioavailability, and arachidonic acid-induced PGI $_2$ or TxA $_2$ production (Figure 6). Vascular nitric oxide bioavailability represented a poor discriminator between ApoE and LDLR mice, as methacholine-induced NO production was rapidly and severely attenuated in both strains. Similarly, the generation of PGI $_2$ (through the measurement of 6-keto-PGF $_{1\alpha}$) was also a poor discriminator between the dyslipidemic mice, as this parameter was largely intact in

both strains at both age ranges. The vascular production of TxA₂, as estimated from the arachidonic acid-induced generation of 11-dehydro-TxB₂ was a stronger discriminator, showing a rapid and significant elevation in ApoE mice as compared to C57, which was maintained to 22–23 weeks. In contrast, 11-dehydro-TxB₂ production in arteries of LDLR was less robust and lagged behind that in arteries in ApoE.

Taken together, these results may begin to provide for a framework distinguishing vascular structural outcomes between familial hypercholesterolemia in LDLR mice and familial combined hyperlipidemia in ApoE mice. In LDLR mice, the development of FH is associated with a more rapid and robust remodeling of the arterial wall, leading to an increased stiffness at lower intraluminal pressures which continues to impact vessel dimension at higher levels of distending pressure. In contrast, microvascular rarefaction develops more slowly under these systemic conditions, reaching significance by 22–23 weeks. Based on the results of the present study, this vascular structural outcome may be well predicted by severity of the hypercholesterolemia and increased MCP-1 expression. This potential linkage has been tentatively identified previously, as in mouse (28,42) and rabbit (30) models of hypercholesterolemia and in humans afflicted with FH (5,29), a particularly strong correlation between MCP-1, cholesterol and atherogenesis has been identified. One of the most compelling possibilities for the role of MCP-1 in contributing to vascular wall remodeling in dyslipidemia was identified in a recent study by Jagavelu *et al.* (22). In this study, the authors investigated the importance of MAP kinase-activated protein kinase-2 (MK2) in contributing to endothelial dysfunction and atherogenesis. As a key regulator of inflammatory processes, the systemic level of activation of MK2 was correlated with the level of endothelial dysfunction and lipid/macrophage in the vessel wall in LDLR mice, an observation that was independent from the level of pro-atherogenic lipoproteins. With direct relevance to the present study, systemic gene deletion of MK2 in LDLR mice or siRNA silencing of MK2 in endothelial cells was associated with a decreased aortic expression of VCAM-1 and MCP-1, key mediators of macrophage recruitment into the vessel wall. However, to our knowledge, this previous work has not been extended significantly at present and does not include vascular wall mechanics.

In the ApoE mouse, the development of FCH is associated with a microvascular rarefaction that develops quickly, while arterial wall remodeling is delayed. This outcome appears to be well predicted by a severe hypertriglyceridemia in the face of significant hypercholesterolemia, and is well tracked by an increase in the plasma level of TNF- α and the vascular production of TxA₂. While existing studies have linked plasma levels of TNF- α (33) and altered arachidonic acid metabolism (4) with microvascular rarefaction in other models of CVD risk factors, this possibility represents a novel concept in mouse models of genetic dyslipidemia and will require further verification and, assuming validation, investment of resources to determine underlying mechanistic contributors and ameliorative interventions.

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Figure 1. Plasma total cholesterol (Panel A) and triglycerides (Panel B) in C57, ApoE and LDLR mice at 12–13 weeks or 22–23 weeks of age. Data are presented as mean±SEM, n=8 animals for each group. * p<0.05 vs. C57 at that age; † p<0.05 vs. ApoE at that age.

Figure 2.

Passive mechanical characteristics of the skeletal muscle resistance arterial wall in C57, ApoE and LDLR mice at 12–13 weeks or 22–23 weeks of age. Data (mean±SEM) are presented for arterial inner diameter (Panel A), arterial outer diameter (Panel B), arterial wall thickness (Panel C), arterial cross sectional wall area (Panel D), wall:lumen ratio (Panel E) and incremental distensibility (Panel D) over a range of increasing intraluminal pressure, n=8 animals for each group. * p<0.05 vs. that strain at 12–13 weeks; † p<0.05 vs. C57 at that age; ‡ p<0.05 vs. ApoE at that age.

Figure 3.

Circumferential wall stress versus strain (Panel A) and tangential elastic modulus versus wall stress (Panel B) relationships in passive skeletal muscle resistance arteries from C57, ApoE and LDLR mice at 12–13 weeks or 22–23 weeks of age. Data are presented as mean $\pm$ SEM, n=8 animals for each group. * p<0.05 vs. that strain at 12–13 weeks; † p<0.05 vs. C57 at that age; ‡ p<0.05 vs. ApoE at that age.

Figure 4. Gastrocnemius muscle microvessel density in C57, ApoE and LDLR mice at 12–13 weeks or 22–23 weeks of age. Data are presented as mean±SEM, n=8 animals for each group. * p<0.05 vs. C57 at that age; † p<0.05 vs. ApoE at that age.

Figure 5. Plasma nitrotyrosine (Panel A), TNF- α (Panel B), IL-1 β (Panel C) and MCP-1 (Panel D) in C57, ApoE and LDLR mice at 12–13 weeks or 22–23 weeks of age. Data are presented as mean $\pm$ SEM, n=8 animals for each group. * p<0.05 vs. C57 at that age; † p<0.05 vs. ApoE at that age.

Figure 6.

Data describing the production of nitric oxide (from aortae, Panel A), 6-keto-PGF_{1α} (from pooled arteries, Panel B) or 11-dehydro-TxB₂ (from pooled arteries, Panel C) of C57, ApoE and LDLR. Data, presented as mean±SEM, are shown in response to application of 10⁻⁴ M methacholine (Panel A) or 10⁻⁶ M arachidonic acid (Panels B and C). n=6 animals for each group, with each n representing either an aorta or pooled arteries from an individual mouse; please see text for details. * p<0.05 vs. C57 at that age; † p<0.05 vs. ApoE at that age.

Table 1

Initial values for the dimensions of isolated skeletal muscle resistance arteries from C57, ApoE and LDLR mice at the lowest intraluminal pressure (5 mmHg).

	C57		ApoE		LDLR	
	12-13W	22-23W	12-13W	22-23W	12-13W	22-23W
Inner Diameter (μm)	45.0 $\pm$ 4.5	54.5 $\pm$ 5.1	52.7 $\pm$ 4.9	58.8 $\pm$ 4.5	56.0 $\pm$ 4.6	63.8 $\pm$ 4.9
Outer Diameter (μm)	93.3 $\pm$ 5.0	104.1 $\pm$ 4.9	100.7 $\pm$ 5.5	108.7 $\pm$ 4.9	105.1 $\pm$ 4.3	101.5 $\pm$ 5.3
Wall Thickness (μm)	48.3 $\pm$ 4.0	49.5 $\pm$ 3.9	48.0 $\pm$ 4.1	49.8 $\pm$ 3.9	49.0 $\pm$ 4.0	38.0 $\pm$ 4.0 ^{†‡}
CSWA (μm^2)	5250 $\pm$ 306	6173 $\pm$ 321 [*]	5785 $\pm$ 319	6559 $\pm$ 266 [*]	6200 $\pm$ 228 [†]	4914 $\pm$ 285 ^{†‡}
Wall:Lumen	1.08 $\pm$ 0.10	0.96 $\pm$ 0.09	0.96 $\pm$ 0.11	0.88 $\pm$ 0.09	0.89 $\pm$ 0.09	0.60 $\pm$ 0.10 ^{†‡}

* p<0.05 vs. 12-13W in that strain;

[†] p<0.05 vs. C57 at that age;

[‡] p<0.05 vs. ApoE at that age.