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Hypercholesterolemia potentiates aortic endothelial response to inhaled diesel exhaust

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Abstract

Background—Inhalation of diesel exhaust induces vascular effects including impaired endothelial function and increased atherosclerosis.

Objective—To examine the *in vivo* effects of subchronic diesel exhaust exposure on endothelial cell transcriptional responses in the presence of hypercholesterolemia.

Methods—ApoE (−/−) and ApoE (+/+) mice inhaled diesel exhaust diluted to particulate matter levels of 300 or 1000 $\mu\text{g}/\text{m}^3$ vs. filtered air. After 30 days, endothelial cells were harvested from dispersed aortic cells by fluorescent-activated cell sorting (FACS). Relative mRNA abundance was evaluated by microarray analysis to measure strain-specific transcriptional responses in mice exposed to dilute diesel exhaust vs. filtered air.

Results—Forty-nine transcripts were significantly dysregulated by >2.8-fold in the endothelium of ApoE (−/−) mice receiving diesel exhaust at 300 or 1000 $\mu\text{g}/\text{m}^3$. These included transcripts with roles in plasminogen activation, endothelial permeability, inflammation, genomic stability, and atherosclerosis; similar responses were not observed in ApoE (+/+) mice.

Conclusions—The potentiation of diesel exhaust-related endothelial gene regulation by hypercholesterolemia helps to explain air pollution-induced vascular effects in animals and humans. The observed regulated transcripts implicate pathways important in the acceleration of atherosclerosis by air pollution.

Keywords

Diesel engine exhaust; endothelium; gene expression; microarray; apolipoprotein E; hypercholesterolemia; air pollution

Introduction

Air pollution contributes to cardiovascular disease, including atherosclerosis (Künzli et al. 2005), acute myocardial infarction (Peters et al. 2004), and vascular endothelial dysfunction

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Declaration of interest

The authors report no declaration of interest.

(Mills et al. 2005). Traffic-related emissions, as a major contributor to ambient air contamination, have been identified as a likely driver of vascular effects (Hoffmann et al. 2007). Pathophysiology of the endothelium (including impaired vasoregulation and anticoagulation, as well as enhanced cellular adhesivity and permeability) is exacerbated by atherosclerosis, hypertension, and diabetes; emerging evidence now shows a contribution from exposure to air pollution as well (Brook 2008). The proximity of endothelial cells to circulating, inhaled toxicants underscores their potential susceptibility. However, demonstrating the effects of inhaled particulate matter (PM) on endothelial cells has been difficult, owing to the tiny volume occupied by the endothelium in any tissue.

The effects of air pollution on atherosclerosis have been explored in animal models of hypercholesterolemia. These include ApoE (–/–) and LDL (–/–) mice and Watanabe hyperlipidemic rabbits, which display accelerated atherosclerosis upon exposure to air pollutants, including diesel engine particulates (Yatera et al. 2008), concentrated ambient air nanoparticles (CAPS) (Araujo et al. 2008; Ying et al. 2009). Pollution-related expression of adhesion molecules in the rabbit model reveals a clear effect on the systemic vascular endothelium (Yatera et al. 2008) and the responses of ApoE (–/–) mice to CAPS include dysfunctional endothelial physiology (Ying et al. 2009). Importantly, exposure to CAPS results in augmented atherogenesis in a murine model of hypercholesterolemia (ApoE [–/–]) compared with wild type (C57BL/6) (Sun et al. 2005).

The responses of cultured endothelial cells to specific model particulates have been assessed in microarray studies (Yamawaki and Iwai 2006). However, cultured endothelium exposed to various components of air pollution represents an oversimplified model, given the complex set of responses that are likely to influence the vasculature *in vivo*. It has been shown that ultrafine PM from the lung is translocated to other organs, including the endothelium (Furuyama et al. 2009). However, the lung also acts to modify and amplify the response to PM by the secondary release of humoral mediators (Arimoto et al. 2007) and by the promotion of oxidative (Li et al. 2008) and nitrosative (Rundell et al. 2008) stress. Furthermore, PM exposures are coincident with exposure to pollutant gases, such as carbon monoxide, oxides of nitrogen, and volatile organic compounds, all of which have differing uptake and disposition. With the growing recognition of a systemic vascular effect of inhaled pollutants, ascertaining endothelial-specific transcriptional responses to complex “real-world” air pollutant mixtures will be useful. Our analysis of freshly isolated endothelial cells from ApoE (–/–) mice exposed to engine exhaust *in vivo* is a robust approach to discerning authentic vascular responses to air pollution.

Materials

Animals

BL/6 ApoE (–/–) and Tie2 GFP mice (strain name B6.129P2-Apoe^{tm1Unc}/J [stock #002052] and Tg[TIE2GFP]287Sato [stock #003658], from Jackson Labs, Bar Harbor, ME) were interbred and genetically selected to obtain Tie2 GFP/ApoE (–/–) mice on an approximately 1:1 mixed genetic background of C57Bl/6 and FVB/N. All mice received standard chow. A cholesterol assay (Cayman Chemical, Ann Arbor, MI; cat. #10007640) was used according to the manufacturer’s instructions to measure total cholesterol in the sera of male mice of the strain Tie2 GFP/ApoE (–/–) and both parental strains Tg(TIE2GFP)287Sato and B6.129P2-Apoe^{tm1Unc}. Tie2 GFP and Tie2 GFP/ApoE (–/–) mice were designated as ApoE (+/+) and ApoE (–/–) in this study for simplicity of nomenclature.

Diesel engine exhaust exposures

Four-month-old male mice underwent unrestrained (whole body) inhalation exposure in their cages to diesel exhaust generated from a Yanmar Diesel engine under controlled conditions of load. Exhaust particulate concentrations in a flow-through exposure chamber (model H2000; Hazleton Systems, Maywood, NJ) were monitored continuously with a nephelometer (model 8200; TSI Inc., Shoreview, MN) calibrated against determinations made gravimetrically with a Pallflex filter (Pall Inc., New York, NY) and were adjusted to 300 and 1000 $\mu\text{g PM}/\text{m}^3$ by dilution with HEPA-filtered air. NO_x levels in these atmospheres were 12 and 30 ppm, respectively, and CO levels were 3 and 100 ppm, respectively. Greater detail regarding volatile organic compounds, other pollutants, and uncertainty ranges have been previously published (McDonald et al. 2004). Exposures were performed 4 h/day, 7 days/week for 4 weeks in both ApoE (–/–) and ApoE (+/+) strains; littermates exposed to HEPA-filtered air were designated as controls. Separate groups containing four experimental and four control mice of each strain were utilized for each dose of PM. Mice were euthanized and aortic samples from each group were pooled within 24 h of the last exposure for subsequent processing and analysis.

All studies were approved by the Animal Care and Use Committees of the University of Texas Southwestern, Dallas, TX (the previous institution of JGM and RVS), and the Lovelace Respiratory Research Institute, Albuquerque, NM.

Tissue collection and extraction

Animals were euthanized by CO_2 asphyxiation. Aortae were excised by dissection and freed of adherent tissue from the iliac bifurcation to the aortic root. Luminal blood was rinsed and aortas were sliced into 1-mm segments. Segments pooled from four animals were suspended in 5 mL of Dulbecco's phosphate-buffered saline (PBS) with 2 mg/mL of dextrose. The suspension was combined with 5 mL of prewarmed PBS containing 10 mg/mL type II collagenase (Worthington Biochemical, Lakewood, NJ), and 60 units/mL deoxyribonuclease I, agitated continuously at 37°C on a shaking platform, and triturated 10 times every 10 min for a total digestion period of 40 min to generate a single cell suspension. The cell suspension was maintained at 0–4°C throughout the remainder of the isolation, which lasted a total of 2–3 h. The suspension was then combined with 10 mL of 10% fetal bovine serum (FBS) in Dulbecco's modified Eagle's medium (DMEM) and cells were collected by centrifugation and resuspended in 10 mL of PBS followed by filtration through a sterile 40- μm mesh filter to remove undigested tissue fragments. Following centrifugation, the cellular pellet was resuspended in 0.3 mL PBS containing 0.5 mM ethylenediaminetetraacetic acid (EDTA), 30 U/mL deoxyribonuclease I, 3% FBS, and 2 mg/mL dextrose and once again filtered through a 40- μm mesh filter.

The resulting aortic cell suspensions were sorted using a MoFlo from Dako Cytomation (Carpinteria, CA). Cells were excited by a 488 nm laser and GFP signals collected at 510 to 550 nm. A pressure of up to 30 psi was utilized, generating 5000 to 10,000 events per second. Positive cells were collected directly into Trizol (Invitrogen, Carlsbad, CA) and RNA was isolated (with the addition of glycogen) according to the manufacturer's protocol.

RNA amplification

RNA obtained from 10,000 endothelial cells obtained by FACS was amplified with the SPIA Aminoallyl™ system (Nugen, CA) according to the manufacturer's instructions, producing at least 4 μg of cDNA.

cDNA hybridizations

Two-color array hybridizations were performed. For each hybridization, 2 µg of amplified cDNA was labeled with Cy3 and Cy5 with CyDye™ Post-Labeling Reactive Dye Pack (GE Healthcare, Piscataway, NJ) according to the manufacturer's instructions. cDNA probes were combined, purified, and hybridized for 14 h at 42°C in a total volume of 35 µL of a hybridization solution containing 25% formamide, 3× SSC (0.45 M NaCl, 0.045 M Na citrate, pH 7.0), 0.2% sodium dodecyl sulfate (SDS), 1 µg of murine Cot1 DNA, 4 µg of polyA RNA, and 4 µg of tRNA. Microscopic slides pin-spotted with 32,000 long oligonucleotides of the Operon V3 set representing the entire mouse genome were produced by the UT Southwestern Microarray Core (Dallas, TX). Stringency washing at room temperature was sequentially performed for 10 min in each of the following solutions: 2× SSC containing 0.1% SDS, 0.1× SSC containing 0.1% SDS, and finally, 0.1× SSC. Performance of each experiment by microarray analysis was duplicated with the Cy3 and Cy5 labeling order reversed.

Data analysis

Slides were scanned with a Genepix 4000B dual channel scanner and the resultant images analyzed with Genepix and Acuity software (Molecular Devices, Sunnyvale, CA). Genepix 6.1 default settings were employed: median local background pixel intensity was subtracted, and features found “bad,” “absent,” “not found,” were flagged; features with a ratio of 635/532 <0.1 or >10 were excluded from normalization. Results were normalized by the ratio of medians method and filtered to exclude those with any of the following characteristics: a percentage of saturated pixels >3, a signal/noise ratio <3, a (regression ratio 635/532)² <0.6, or a Genepix flag. Data passing the filter and exceeding threshold fold changes in replicate, dye-reversed analyses were considered to be dysregulated. For statistical analysis, the one sample *T*-test, with Benjamini–Hochberg correction, was applied to the combined results obtained at 300 and 1000 µg PM/m³ (4 microarrays per strain).

Immunolocalization

Tissue sections (5 µm) obtained from formalin-fixed, paraffin-embedded aortae were deparaffinized and exposed to the following: 95°C sodium citrate at 100 mM for 20 min, 0.3% (v/v) H₂O₂ for 5 min, and PBS containing 1% (w/v) bovine serum albumin and 5% (v/v) donkey serum for 20 min. Immunohistological localization was performed with goat anti-Terf1 (Santa Cruz Inc., Santa Cruz, CA) at 4 µg/mL for 16 h followed by biotinylated donkey anti-goat (Jackson ImmunoResearch, West Grove, PA) at 1.0 µg/mL for 1 h. Slides were then incubated with ABC Elite™ and visualized with VIP™ substrate (both from Vector Labs, Burlingame, CA) according to the manufacturer's instructions.

Results

ApoE (–/–) mice were hypercholesterolemic vs. ApoE (+/+), with cholesterol elevation comparable with the ApoE-deficient parental strain BL/6 ApoE (–/–) (see Figure 1). Furthermore, Tie2 GFP/ApoE (–/–) mice developed aortic atheroma (results not shown), as reported for the BL/6 ApoE (–/–) strain (Breslow 1996).

Microarray results were submitted to the Gene Expression Omnibus database (US NCBI) as series GSE13160. A total of 176 transcripts were found to be down-regulated (<0.35-fold) or up-regulated (>2.8-fold) in the ApoE (–/–) strain in at least one experimental dose; only two transcripts appeared dys-regulated in the ApoE (+/+) strain. A subset of transcripts possessing known RefSeq identities are shown in Tables A and B of the appendix. Thirty days of exposure to diesel exhaust resulted in >50 transcripts appearing dysregulated in

ApoE (–/–) mice at both 300 and 1000 $\mu\text{g PM}/\text{m}^3$ doses, with no transcripts appearing commonly dysregulated in ApoE (+/+) mice.

In the combined microarray results of ApoE (–/–) mice exposed to 300 and 1000 $\mu\text{g PM}/\text{m}^3$, 49 of the highly dys-regulated transcripts appeared statistically significant (P -value <0.05); no transcripts appeared statistically significant in pollution-exposed ApoE (+/+) mice. These results were subjected to hierarchical cluster analysis, displayed as a heat map shown in Figure 2, which indicates a high level of concordance of responses to either concentration of diesel exhaust in ApoE (–/–) mice. Transcripts lacking RefSeq identity are not displayed.

A literature search revealed important pathophysiological roles for a subset of these transcripts within the vasculature. Supporting references are provided in Table C of the appendix. The highly dysregulated transcripts observed within the ApoE (–/–) strain were subjected to analysis with the DAVID bioinformatics tool according to default settings (Huang da et al. 2009) to detect enrichment of annotation terms: Fisher's exact t -test with Benjamini correction revealed no evidence of significant enrichment of annotation terms (all P -values >0.05).

As displayed in Figure 3, a subset of transcripts appearing highly dysregulated in ApoE (–/–) mice exposed to diesel exhaust at 300 and 1000 $\mu\text{g PM}/\text{m}^3$ possess important and overlapping roles in vascular function. Transcripts responding to diesel exhaust in ApoE (–/–) mice include molecules known to maintain normal vascular homeostasis or contribute to pathology in other models of vascular disease. *Pdgfra*, *Vcam1*, *Cav1*, and *Clu*/Clusterin appear down-regulated in response to diesel exhaust in our study. The progression of atherosclerosis in ApoE (–/–) mice can be suppressed pharmacologically with an inhibitor of PDGF signaling, Imatinib (Lassila et al. 2004) or by a peptide of Clusterin (Navab et al. 2005); atherogenesis in the ApoE (–/–) can be decreased by genetic deficiency of *Cav1* (Frank et al. 2004) or *Vcam1* (Dansky et al. 2001). Down-regulated transcripts, *Anxa2*/Annexin A2 and *Srgn*/serglycin, possess known roles in plasminogen activation and plasminogen intracellular trafficking, respectively (He et al. 2008; Kolset and Tveit 2008). Endothelial permeability has been shown to be increased by *Angptl4* (Cazes et al. 2006) as well as both components of the adrenomedullin receptor complex, *Ramp2* (Ichikawa-Shindo et al. 2008) and *Calcrl* (Temmesfeld-Wollbrück et al. 2007), all of which appear down-regulated by diesel exhaust. Transcripts of lipid metabolic enzymes dysregulated by diesel exhaust included up-regulated *Bcdo2* and down-regulated *Adipsin*; lipid-binding proteins *Clu* and *Fabp6* both appear down-regulated in our study. Transcripts up-regulated upon exposure to diesel exhaust included a glutamate receptor, *Gria3*, and a glutamate transporter, *Slc1a3*.

As shown in Figure 3, exposure of ApoE (–/–) mice to diesel exhaust resulted in dysregulation of inflammatory signaling transcripts within the aortic endothelium, including up-regulation of *Nfkbie* and down-regulation of *Vcam1* and *Sdcbp*. Our observed increase in transcription of *Nfkbie* potentially reflects a compensatory mechanism to limit ongoing NF- κ B signaling associated with responses to diesel engine particulate, similar to those observed in lung epithelium (Shukla et al. 2000).

As shown in Figure 4, immunolocalization revealed up-regulation of *Terf1* in the aortic endothelium of ApoE (–/–) mice exposed to diesel exhaust at 1000 $\mu\text{g PM}/\text{m}^3$, consistent with the 6-fold up-regulation indicated by our microarray analysis. *Terf1* is an inhibitor of telomerase activity; telomere shortening has been observed in circulating endothelial progenitors obtained from patients with coronary artery disease (Satoh et al. 2008).

Discussion

We report substantial transcriptional responses in the aortic endothelium of ApoE (–/–) mice exposed to diesel engine exhaust. Importantly, our analysis revealed a much lower degree of dysregulation in identically exposed wild-type mice, suggesting that: (1) healthy endothelial cells are able to maintain homeostasis over a prolonged exposure to diesel emissions and (2) hypercholesterolemia increases the sensitivity of the endothelial transcriptional response to air pollution. The high degree of concordance of the responses of ApoE (–/–) mice exposed to either 300 or 1000 $\mu\text{g PM}/\text{m}^3$ implies a threshold of activation below 300 $\mu\text{g PM}/\text{m}^3$, a concentration of diesel particulate observed in mining and other occupational settings (Pronk et al. 2009); due to their similarity, these data sets were combined for statistical evaluation.

Interestingly, studies of ApoE (–/–) mice exposed to gasoline or diesel emissions for up to 50 days in the presence of a high-fat diet revealed enhancement of matrix metalloproteinase (MMP), heme oxygenase-1, and endothelin-1 gene expression in the whole aorta, accompanied by evidence of enhanced remodeling and monocyte invasion of the vascular wall (Lund et al. 2007, 2009). The results of the present study of normally fed mice, wherein gene regulation in non-endothelial cell types has been minimized by FACS isolation, did not reveal MMPs or other previously reported transcripts. Furthermore, serum cholesterol levels of ApoE (–/–) mice receiving normal chow used in our study were >2-fold lower than those receiving a high fat (BioServ #S3282, results not shown), thus more closely approximating levels found in clinical hypercholesterolemia. These differences highlight the importance of understanding cell-specific responses that drive whole-organ outcomes.

In an earlier study, acute exposure of rats to diesel exhaust at 300 $\mu\text{g PM}/\text{m}^3$ for 5 h resulted in endothelial-dependent changes in coronary responsiveness to endothelin-1 (Cherng et al. 2009). One might expect prolonged exposure of wild-type mice to diesel exhaust at 300 $\mu\text{g PM}/\text{m}^3$ would lead to transcriptional changes as well; however, it is possible that biochemical effects in the absence of gene regulation, or homeostatic mechanisms counterbalanced these effects upon 30 days of exposure. In the present study, substantial transcriptional responses in the endothelium to the effects of diesel exhaust were apparent only in the ApoE (–/–) strain. This differential response is attributable to dyslipidemia, impaired macrophage lipid scavenging activity (Atkinson et al. 2008), or other metabolic differences characteristic of the ApoE (–/–) strain. Such susceptibility in the ApoE (–/–) strain further demonstrates diesel exhaust and other forms of air pollution as cardiovascular risk factors, which may become manifest only in the added presence of impaired lipid disposition.

Our data indicate that chronic exposure of ApoE (–/–) mice to diesel exhaust affects regulators of endothelial adhesion (Nfkb1 and Vcam1), permeability (Angptl4, Calcr1, Ramp2), invasion (Erbb2ip), and genomic stability (Terf1 and Lmna). Not all of these transcripts were up-regulated; the complex response may reflect homeostatic regulation of these processes or selective survival of endothelial cells that exhibit these changes. Our findings of responses to diesel exhaust among modulators of endothelial function are consistent with reports of accelerated atherogenesis in the aortae of ApoE (–/–) mice exposed to air pollution (Sun et al. 2005; Araujo et al. 2008).

A subset of dysregulated transcripts identified in our analysis possesses known vascular function and thus provides a molecular foundation for the epidemiological association of air pollution with accelerated atherogenesis. The present study combines realistic exposure and cell-specific genomic inquiry to obtain robust clues to *in vivo* mechanisms underlying the augmentation of atherosclerosis by diesel exhaust. Future work using similar methods will

enable a greater understanding of the biological outcomes following exposures to the complex mixtures that comprise combustion-source air pollution.

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Appendix

Table A

Transcripts down-regulated in aortic endothelium in ApoE (+/+) and ApoE (–/–) mice in response to exposure to diesel exhaust for 30 days.

Marker symbol	Name	Ref Seq	Average fold change ^a			
			ApoE (+/+)		ApoE (–/–)	
			300 µg/m ³	1000 µg/m ³	300 µg/m ³	1000 µg/m ³
Anxa2	Annexin A2	NM_007585	0.88	1.0	0.29	0.52
Cd93	Cd93 antigen, C1q receptor	NM_010740	1.2	0.77	0.34	0.29
Vcam1	Vascular cell adhesion protein 1	NM_011693	0.88	0.91	0.32	0.39
Fxyd5	FXYD domain-containing ion transport regulator 5	NM_008761	1.5	1.12	0.21	0.43
Pdgfra	Platelet-derived growth factor receptor, alpha polypeptide	NM_011058	1.4	1.9	0.41	0.27
Lmna	Lamin A	NM_019390	1.2	1.0	0.25	0.34
Irf6	Interferon regulatory factor 6	NM_016851	1.3	0.34	0.30	0.30
Ahr	Aryl hydrocarbon receptor	NM_013464	0.92	1.1	0.32	0.51
Cxcl2	Chemokine (C-X-C motif) ligand 2	NM_009140	1.2	3.9	0.80	0.22
Apb2	Amyloid beta (A4) precursor protein-binding, family B, member 2	NM_009686	1.2	1.12	0.31	0.43
Angptl4	Angiotensinogen-related protein 4	NM_020581	1.3	1.7	0.33	0.45
Vcl	Vinculin	NM_009502	1.1	1.2	0.36	0.32
Sdcbp	Syntenin 1	NM_016807	1.2	0.87	0.30	0.42
Gdap2	Ganglioside-induced differentiation-associated protein 2	NM_010269	1.0	1.1	0.39	0.34
Srgn	Serglycin	NM_011157	1.0	1.10	0.33	0.34
Tbc1d15	TBC1 domain family, member 15	NM_025706	0.81	0.91	0.49	0.33
Clc4	Chloride intracellular channel 4	NM_013885	1.1	1.1	0.23	0.48
Cav1	Caveolin 1, caveolae protein	NM_007616	0.98	0.95	0.25	0.37
Pls3	Plastin 3 T isoform	NM_145629	1.2	0.92	0.32	0.38

Marker symbol	Name	Ref Seq	Average fold change ^a			
			ApoE (+/+)		ApoE (-/-)	
			300 µg/m ³	1000 µg/m ³	300 µg/m ³	1000 µg/m ³
Serp1	Stress-associated endoplasmic reticulum protein 1	NM_030685	1.1	1.2	0.47	0.31
Spon1	Spondin 1 extracellular matrix protein	NM_145584	1.4	0.81	0.33	0.24
Adams8	A disintegrin and metalloproteinase with thrombospondin motifs 8	NM_013906	0.75	2.0	0.29	1.1
Clu	Clusterin	NM_013492	0.73	0.85	0.33	0.40
Ramp2	Receptor activity-modifying protein 2	NM_019444	0.91	0.84	0.33	0.38
Calcr1	Calcitonin gene-related peptide type 1 receptor	NM_018782	0.70	0.69	0.28	0.34
S100a4	S100 calcium-binding protein A4	NM_011311	0.57	0.85	0.27	0.44
Adn	Adipsin	NM_013459	0.86	1.8	0.31	0.26
Ptp4a3	Protein tyrosine phosphatase 4a3	NM_008975	1.2	1.1	0.30	0.37
Fstl	Follistatin-like 1	NM_008047	1.2	0.64	0.36	0.25
Cd74	Cd74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	NM_001042605	0.68	1.8	0.29	0.35
P4hb	Prolyl 4-hydroxylase, beta-polypeptide	NM_011032	0.84	0.89	0.34	0.37
Adfp	Adipose differentiation-related protein	NM_007408	0.63	2.0	0.34	0.43
Mrpl33	Mitochondrial ribosomal protein L33	NM_025796	0.95	0.98	0.52	0.32
Rbm7	RNA-binding motif protein 7	NM_144948	0.93	0.88	0.37	0.31

^aValues in bold were down-regulated 0.35.

Table B

Transcripts up-regulated in aortic endothelium in ApoE (+/+) and ApoE (-/-) mice in response to exposure to diesel exhaust for 30 days.

Marker symbol	Name	Ref Seq	Average fold change ^a			
			ApoE (+/+)		Apo E (-/-)	
			300 µg/m ³	1000 µg/m ³	300 µg/m ³	1000 µg/m ³
Terf1	Telomeric repeat-binding factor 1	NM_009352	1.4	1.1	3.8	6.1
Fabp6	Fatty acid-binding protein 6, ileal (gastrotropin)	NM_008375	0.87	0.53	2.61	3.8
Gria3	Glutamate receptor, ionotropic, alpha 3	NM_016886	0.97	0.94	4.3	5.0
Nfkbie	NF-κB inhibitor epsilon	NM_008690	1.3	0.78	3.2	4.6
Gk2	Glycerol kinase 2	NM_010294	1.1	0.95	3.2	4.4
Bcdo2	Beta-carotene oxygenase 2	NM_133217	0.98	0.55	2.4	4.0
Gcnt3	Glucosaminyl (N-acetyl) transferase 3, mucin type	NM_028087	1.2	1.4	2.4	4.3
Dmrt1	Double sex and mab-3-related transcription factor 1	NM_015826	1.1	1.3	2.9	3.1
Hydin	Hydrocephalus-inducing	NM_172916	1.1	0.88	3.8	5.5
Grb10	Growth factor receptor-bound protein 10	NM_010345	1.1	0.88	3.0	4.6

Marker symbol	Name	Ref Seq	Average fold change ^a			
			ApoE (+/+)		Apo E (-/-)	
			300 µg/m ³	1000 µg/m ³	300 µg/m ³	1000 µg/m ³
H2-M10.4	Histocompatibility 2, M region locus 10.4	NM_177634	1.2	1.1	2.8	4.3
Cpne5	Copine V	NM_153166	1.5	1.2	3.6	5.0
Rab40c	Rab40c, member RAS oncogene family	NM_139154	1.0	1.1	2.3	4.4
Inpp4a	Inositol polyphosphate-4-phosphatase, type I	NM_030266	1.1	0.71	3.3	6.5
Camsap111	Calmodulin-regulated spectrin-associated protein 1-like 1	NM_001081360	1.1	1.2	3.5	5.6
Ttn	Titin	NM_176926	1.3	0.88	3.2	3.6
ErbB2ip	ErbB2-interacting protein	NM_001005868	1.0	0.87	1.6	3.3
Vtcn1	V-set domain-containing T-cell activation inhibitor 1	NM_178594	1.1	0.98	2.5	3.5
Slc1a3	Solute carrier family 1 (glial high-affinity glutamate transporter), member 3	NM_148938	0.78	1.0	2.7	4.7

^aValues in bold were up-regulated 2.8.

Table C

Transcripts with known vascular functions, which appear dysregulated in aortic endothelium of ApoE (-/-) mice in response to 30 days of diesel exhaust.

Marker symbol ^a	Ref Seq	Role in vasculature
Anxa2 (↓)	NM_007585	Annexin A2 heterotetramer with S100A10/p11 is endothelial locus for tPA and plasmin activation (He et al. 2008)
Cd93 (↓)	NM_010740	Cd93 is prominently expressed on endothelium and contributes to removal of apoptotic cells (Fonseca et al. 2001; Norsworthy et al. 2004)
Vcam1 (↓)	NM_011693	Vcam1 domain 4-deficient (D4D) +/+ mice crossed with Apo E (-/-) mice exhibit decreased atherogenesis (Dansky et al. 2001)
Pdgfra (↓)	NM_011058	Imatinib/Gleevec (blockade of PDGFr) down-regulates atherogenesis induced by type I diabetes model in Apo E mice (Lassila et al. 2004)
Terf1 (↑)	NM_009352	Terf1 regulates telomere length. Endothelial progenitor cell telomere length is shorter in CAD patients and <i>in vitro</i> EPC treated with oxidative treatments (Okamoto et al. 2008; Satoh et al. 2008)
Ahr (↓)	NM_013464	Benzopyrene leads to up-regulation of ICAM-1, which is disrupted by Cav1 deficiency (Oesterling et al. 2008)
Apb2 (↓)	NM_009686	Amyloid precursor cleavage by gamma secretase is inhibited by Apo E (Irizarry et al. 2004)
Angptl4 (↓)	NM_020581	Angiopoietin-like 4 inhibits endothelial permeabilization (Cazes et al. 2006)
Sdcbp (↓)	NM_016807	TNF-inducible in cultured endothelium (Stier et al. 2000)
Srgn (↓)	NM_011157	Serpin binds plasminogen activator in endothelium (Kolset and Tveit 2008)
Cav1 (↓)	NM_007616	Knockout of Cav1 confers resistance to atherogenesis in the Apo E (-/-) mouse (Frank et al. 2004)
Spon1 (↓)	NM_145584	F-spondin (an ECM protein) affects amyloid precursor processing by interacting with Apo E receptor (Hoe et al. 2005)
Fabp6 (↑)	NM_008375	Fabp6 levels are regulated by sterol receptors FXR, LXR, and SREBP1c (Besnard et al. 2004)

Marker symbol ^a	Ref Seq	Role in vasculature
Clu (↓)	NM_013492	Potentially protects against the effects of modified LDL. Clu/ApoJ peptide mimetic suppresses atherosclerosis in Apo E mice (Navab et al. 2005)
Ramp2 (↓)	NM_019444	Ramp2 underexpression is associated with increased vascular permeability (Ichikawa-Shindo et al. 2008)
Gria3 (↑)	NM_016886	Vasodilation by glutamate is mediated by carbon monoxide produced by heme oxygenase (Parfenova et al. 2003)
Nfkbie (↑)	NM_008690	I kappa B epsilon is involved in c-Rel-associated ICAM-1 induction in endothelium (Spiecker et al. 2000)
Bcd02 (↑)	NM_133217	Asymmetric cleavage of beta-carotene
Calcr1 (↓)	NM_018782	Calcr1 knockout resembles adrenomedullin knockout; adrenomedullin protects against endothelial barrier breakdown; AM AMBP instillation protects against endothelial dysfunction in rat model of sepsis (Dackor et al. 2006; Temmesfeld-Wollbrück et al. 2007; Zhou et al. 2007)
S100a4 (↓)	NM_011311	S100a is required for metastasis (Grigorian et al. 2008)
Adn (↓)	NM_013459	Up-regulated in endothelium of model of Type I diabetes (Maresh and Shohet 2008)
Grb10 (↑)	NM_010345	Grb10 inhibits Vegf-r2 internalization (Murdaca et al. 2004)
Ptp4a3 (↓)	NM_008975	Expression in endothelium of tumors (Bardelli et al. 2003)
Fstl (↓)	NM_008047	Fstl/Tsc-36 promotes endothelial function in ischemic tissue (Ouchi et al. 2008)
H2-M10.4 (↑)	NM_177634	MHC Class II, M region locus 10.4
Cpne5 (↑)	NM_153166	Ca ²⁺ -dependent phospholipid-binding protein (Tomsig and Creutz 2002)
Cd74 (↓)	NM_001042605	MHC Class II invariant chain
Inpp4a (↑)	NM_030266	PI3 kinase signal regulation (Ivetac et al. 2005)
Adfp (↓)	NM_007408	Adfp antisense oligo decreases hepatic insulin resistance (Varela et al. 2008)
Mrp133 (↓)	NM_025796	Mitochondrial ribosomal protein with nuclear code
ErbB2ip (↑)	NM_001005868	Endothelium from aged mice susceptible to tumor cell invasion in <i>in vitro</i> assay and this process requires ErbB EGF signaling cross talk (Price et al. 2004)
Vtcn1 (↑)	NM_178594	Negative modulator of T-cell response (Suh et al. 2006)
Lmna (↓)	NM_001002011	Mutant lamin A (progerin) accumulation in dermal vascular cells of Hutchinson–Gilford progeria (McClintock et al. 2006)

^aArrows indicate direction of dysregulation in the Apo E (–/–) strain; n.c. indicates no change upon exposure to exhaust at 300 µg/m³.

Figure 1.

Total serum cholesterol levels in ApoE (+/+), ApoE (-/-), and BL/6 ApoE (-/-) mice ($n = 3$). * indicates a P -value < 0.05 .

Figure 2.

Microarray results of endothelial responses to diesel engine exhaust *in vivo*. Heat map of aortic endothelial transcripts dysregulated by >2.8- or <0.35-fold in ApoE (-/-) and ApoE (+/+) mice exposed for 30 days to fresh exhaust from a Yanmar diesel engine diluted with air to particulate concentrations of 300 or 1000 $\mu\text{g PM}/\text{m}^3$. Transcripts shown were significantly dysregulated in the combined analyses of the ApoE (-/-) strain ($n = 4$, $P < 0.05$). Each rectangle represents the average results of two microarray analyses of pooled endothelium from four mice exposed to exhaust at 300 or 1000 $\mu\text{g PM}/\text{m}^3$ vs. four control mice exposed to HEPA-filtered air. Red indicates up-regulation, green indicates down-regulation, and black indicates no change.

Figure 3.

Selected transcripts dysregulated by >2.8- or <0.35-fold in ApoE (-/-) mice exposed for 30 days to fresh exhaust from a Yanmar diesel engine diluted with HEPA-filtered air to engine exhaust at 300 or 1000 $\mu\text{g PM/m}^3$. Transcripts shown have recognized roles in vascular pathophysiology. Fold changes are presented on a log₂ scale with each bar representing the average results of two microarray determinations of pooled endothelium from four mice exposed to exhaust vs. four control mice exposed to air.

Figure 4.

Representative immunolocalization of Terf1, indicated by elevated staining (purple) in the aortic endothelium of ApoE (-/-) mice following exposure to dilute diesel exhaust at 1000 $\mu\text{g PM}/\text{m}^3$ for 30 days vs. purified air. Arrows indicate endothelia; L indicates luminal space. Tissue sections lacking primary antibody displayed minimal background staining.