

Critical Role of Macrophage 12/15-Lipoxygenase for Atherosclerosis in Apolipoprotein E-Deficient Mice

Yuqing Huo, Lei Zhao, Matthew Craig Hyman, Pavel Shashkin, Brian L. Harry, Tracy Burcin, S. Bradley Forlow, Matthew A. Stark, David F. Smith, Sean Clarke, Suseela Srinivasan, Catherine C. Hedrick, Domenico Praticò, Joseph L. Witztum, Jerry L. Nadler, Colin D. Funk and Klaus Ley

Circulation. 2004;110:2024-2031; originally published online September 27, 2004;
doi: 10.1161/01.CIR.0000143628.37680.F6

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://circ.ahajournals.org/content/110/14/2024>

An erratum has been published regarding this article. Please see the attached page for:
<http://circ.ahajournals.org/content/110/19/3156.4.full.pdf>

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

Reprints: Information about reprints can be found online at:
<http://www.lww.com/reprints>

Subscriptions: Information about subscribing to *Circulation* is online at:
<http://circ.ahajournals.org/subscriptions/>

Critical Role of Macrophage 12/15-Lipoxygenase for Atherosclerosis in Apolipoprotein E-Deficient Mice

Yuqing Huo, MD, PhD*; Lei Zhao, MD*; Matthew Craig Hyman, BS; Pavel Shashkin, PhD; Brian L. Harry, BS; Tracy Burcin, MS; S. Bradley Forlow, PhD; Matthew A. Stark, BS; David F. Smith, MS; Sean Clarke, BS; Suseela Srinivasan, PhD; Catherine C. Hedrick, PhD; Domenico Praticò, MD; Joseph L. Witztum, MD; Jerry L. Nadler, MD; Colin D. Funk, PhD; Klaus Ley, MD

Background—Mice lacking leukocyte type 12/15-lipoxygenase (12/15-LO) show reduced atherosclerosis in several models. 12/15-LO is expressed in a variety of cells, including vascular cells, adipocytes, macrophages, and cardiomyocytes. The purpose of this study was to determine which cellular source of 12/15-LO is important for atherosclerosis.

Methods and Results—Bone marrow from 12/15-LO^{-/-}/apoE^{-/-} mice was transplanted into apoE^{-/-} mice and vice versa. Deficiency of 12/15-LO in bone marrow cells protected apoE^{-/-} mice fed a Western diet from atherosclerosis to the same extent as complete absence of 12/15-LO, although plasma 8,12-*iso*-iPF_{2α}-IV, a measure of lipid peroxidation, remained elevated. 12/15-LO^{-/-}/apoE^{-/-} mice regained the severity of atherosclerotic lesion typical of apoE^{-/-} mice after replacement of their bone marrow cells with bone marrow from apoE^{-/-} mice. Peritoneal macrophages obtained from wild-type but not 12/15-LO^{-/-} mice caused endothelial activation in the presence of native LDL. Absence of 12/15-LO decreased the ability of macrophages to form foam cells when exposed to LDL.

Conclusions—We conclude that macrophage 12/15-LO plays a dominant role in the development of atherosclerosis by promoting endothelial inflammation and foam cell formation. (*Circulation*. 2004;110:2024-2031.)

Key Words: atherosclerosis ■ cell adhesion molecules ■ endothelium ■ lipids

Atherosclerosis is a chronic inflammatory disease of the arterial vessel wall that progresses from fatty streak to fibrofatty matrix and fibrous plaque. Monocyte recruitment to the vessel wall and transformation to lipid-enriched foam cells initiate and sustain atherosclerosis.¹

12/15-Lipoxygenase (12/15-LO) is a nonheme iron-containing dioxygenase that forms 12-hydroperoxy-eicosatetraenoic acid (12-HPETE) and 15-HPETE and oxidizes esterified fatty acids in lipoproteins (cholesteryl esters) and phospholipids.^{2,3} On the basis of its product from arachidonic acid, it is classified as 15-lipoxygenase (15-LO) in humans and rabbits^{4,5} and as “leukocyte-type” 12-lipoxygenase (12-LO) in pig, rat, and mouse.⁶ 12/15-LO can also produce 13-hydroperoxy-octadecadienoic acid (13-HPODE) from linoleic acid.^{2,3} Mouse leukocyte 12/15-LO is highly related to 15-LO in humans in that they are ≈74% identical in primary structure, and both are dual-specificity lipoxygenases.⁷ Mouse 12/15-LO probably represents the orthologue of 15-LO in humans.^{3,7}

Pharmacological inhibition of 15-LO in hypercholesterolemic rabbits resulted in attenuation of atherosclerosis.^{8,9} In the apoE^{-/-}, LDLR^{-/-}, and apobec-1^{-/-}/LDL-R^{-/-} mouse models of atherosclerosis, disruption of the 12/15-LO gene significantly retarded the initiation and progression of atherosclerosis.^{10–12} A variety of vascular cells are able to express 12/15-LO, including endothelial cells,^{13,14} smooth muscle cells,^{13,14} and monocytes/macrophages.^{13,15} Overexpression of 12/15-LO in mouse endothelial cells¹⁶ and rabbit monocytes/macrophages¹⁷ resulted in completely opposite effects: The former was proatherogenic and the latter antiatherogenic, which indicates a possibility that different cellular expression of 12/15-LO may have different effects on atherosclerosis or that species differences may exist.

In the present study, we used bone marrow transfer to determine the role of 12/15-LO in macrophages and vascular cells (including endothelial cells and smooth muscle cells) in the development of atherosclerosis. We

Received May 23, 2004; revision received July 10, 2004; accepted July 21, 2004.

From the University of Virginia (Y.H., M.C.H., P.S., B.L.H., T.B., S.B.F., M.A.S., D.F.S., S.C., S.S., C.C.H., J.L.N., K.L.), Charlottesville, Va; University of Pennsylvania (L.Z., D.P., C.D.F.), Philadelphia, Pa; and University of California (J.L.W.), San Diego, Calif.

*Drs Huo and Zhao contributed equally to this work.

Correspondence to Yuqing Huo, MD, PhD, Cardiovascular Division and Vascular Biology Center, University of Minnesota, MMC 508, 420 Delaware St SE, Minneapolis, MN 55455. E-mail Yuqing@umn.edu

© 2004 American Heart Association, Inc.

Circulation is available at <http://www.circulationaha.org>

DOI: 10.1161/01.CIR.0000143628.37680.F6

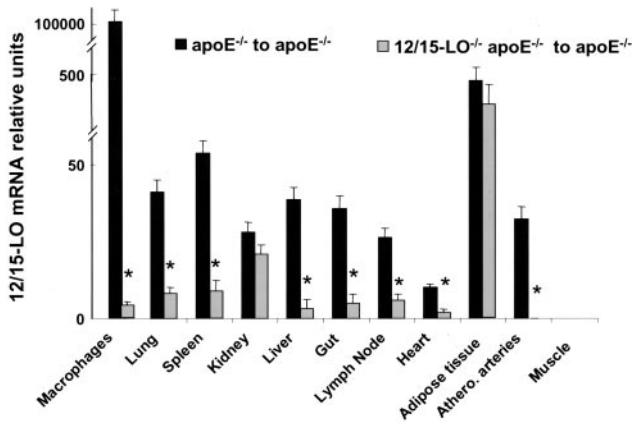


Figure 1. 12/15-LO mRNA expression. Comparison of 12/15-LO mRNA expression in various tissues from apoE^{-/-} mice transplanted with bone marrow of apoE^{-/-} mice with those from apoE^{-/-} mice transplanted with bone marrow of 12/15-LO^{-/-}/apoE^{-/-} mice. n=4. *P<0.01. Athero indicates atherosclerotic.

also examined the effects of 12/15-LO on endothelial activation and monocyte adhesion using in vitro endothelium-monocyte interaction systems. Finally, we compared in vitro foam cell formation in bone marrow-derived and peritoneal macrophages isolated from 12/15-LO^{-/-} or wild-type mice.

Methods

Mice: Bone Marrow Transplantation

The generation and genotype analysis of 12/15-LO^{-/-}/apoE^{-/-} mice have been described previously.¹⁰ Mouse bone marrow transplantation (BMT) was performed as described previously.¹⁸ Four weeks after recovery from BMT, mice were fed a Western diet for 12 weeks.

Determination of 12/15-LO mRNA Expression With Real-Time Reverse Transcription-Polymerase Chain Reaction

Total RNA was isolated with an RNeasy Mini Kit (Qiagen Inc). The primers used to analyze mRNA for mouse 12/15-LO were CTCT-CAAGGCCTGTTTCAGGA (sense) and GTCCATTGTCGCCA-GAACCT (antisense). Reverse transcription-polymerase chain reaction (RT-PCR) was performed on the iCycler (Bio-Rad Laboratories) with SYBR Green I (Molecular Probes).

Preparation of Mouse Aortas and Quantification of Atherosclerosis

The aortas of mice were collected and stained with oil red O.¹⁹ Images were scanned into a Macintosh computer, and the percent surface areas occupied by lesions were determined with Image-ProPlus (Media Cybernetics).

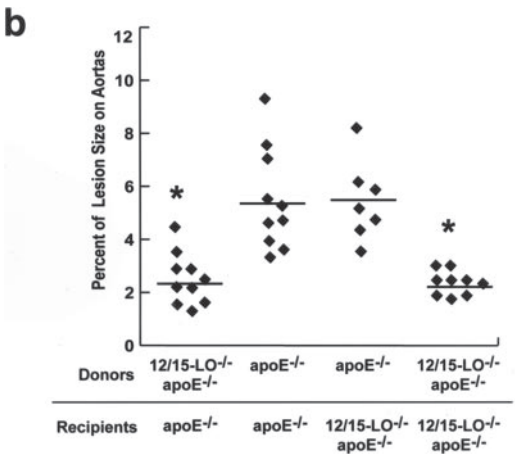
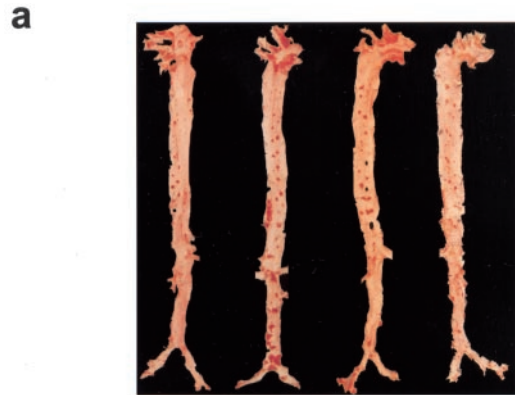


Figure 2. 12/15-LO in bone marrow cells plays a crucial role in formation of atherosclerotic lesions in apoE^{-/-} mice. En face analysis of aortas of bone marrow chimeric mice fed Western diet (12 weeks). Lesion size of apoE^{-/-} mice receiving bone marrow of 12/15-LO^{-/-}/apoE^{-/-} mice was reduced 53% compared with that of apoE^{-/-} mice receiving bone marrow of 12/15-LO^{+/+}/apoE^{-/-} mice and was equal to that of 12/15-LO^{-/-}/apoE^{-/-} mice receiving bone marrow from 12/15-LO^{-/-}/apoE^{-/-} mice. a, Representative oil red O-stained aortas from chimeric apoE^{-/-} mice. b, Quantitative data on aortic lesion size. Each data point represents value from a single mouse. *P<0.01.

Measurement of Plasma Lipids, Isoprostanes, and Autoantibody Titers Against Oxidized LDL Epitopes

Plasma triglyceride and total cholesterol levels were determined via an automated enzymatic technique (Boehringer Mannheim GmbH). Plasma 8,12-iso-iPFα-VI levels were measured by gas chromatography/mass spectrometry.¹¹ The titers of IgG and IgM autoantibodies against malondialdehyde LDL (MDA-LDL) and oxidized LDL (OxLDL) were analyzed as described previously.²⁰

Experimental Design for BMT Study

Donors	Recipients	Chimeric Mice
12/15-LO ^{-/-} /apoE ^{-/-} mice	apoE ^{-/-} mice	12/15-LO in non-bone marrow-derived cells and long-lived tissue macrophages
apoE ^{-/-} mice	12/15-LO ^{-/-} /apoE ^{-/-} mice	12/15-LO in bone marrow-derived cells
apoE ^{-/-} mice	apoE ^{-/-} mice	12/15-LO in all expressing cells
12/15-LO ^{-/-} /apoE ^{-/-} mice	12/15-LO ^{-/-} /apoE ^{-/-} mice	No 12/15-LO in any cells

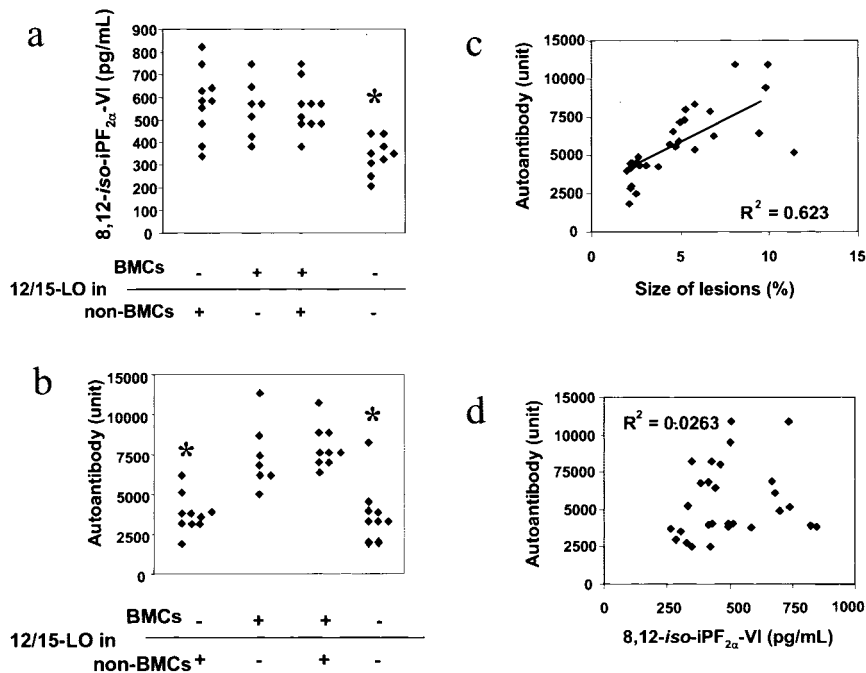


Figure 3. Measurement of lipid peroxidation and autoantibodies to OxLDL in BMT mice. **a**, Lipid peroxidation, reflected by plasma isoprostanes, was decreased in chimeric 12/15-LO^{-/-}/apoE^{-/-} mice with 12/15-LO^{-/-}/apoE^{-/-} bone marrow (BMCs) but not in any other group. **P*<0.05. **b**, Levels of autoantibody (IgM Ab against copper-modified LDL) are significantly lower in 12/15-LO^{-/-}/apoE^{-/-} mice and apoE^{-/-} mice receiving bone marrow of 12/15-LO^{-/-} apoE^{-/-} mice than in those receiving apoE^{-/-} bone marrow. **P*<0.01. **c**, Levels of autoantibodies correlate with size of atherosclerotic lesions in chimeric mice and their controls. **d**, Levels of autoantibodies (IgM Ab against copper-modified LDL) do not correlate significantly with levels of isoprostanes.

Isolation and Culture of Murine Aortic Endothelial Cells and Macrophages

Endothelial cells from mouse thoracic aortas were isolated and cultured as described previously.²¹ Endothelial cells from passage 3 to 5 were used in this study. Macrophages used in this study were either harvested by peritoneal lavage or differentiated from bone marrow cell by cytokines that included interleukin-4 (IL-4), granulocyte-macrophage colony-stimulating factor (GM-CSF), and macrophage colony-stimulating factor (M-CSF).

Monocyte Adhesion Assay

Confluent murine endothelial monolayers were washed and cultured in serum-free DMEM supplemented with Nutridoma-HU (Roche Diagnostics GmbH). LDL (Biomedical Technologies, Inc) was added at a final concentration of 200 μg/mL. After 20 hours, cell media were removed, and the endothelial monolayer was washed 3 times for the monocyte adhesion assay. The confluent murine endothelial monolayer was cocultured with macrophages for 24 hours in serum-free DMEM supplemented with Nutridoma-HU in the presence of LDL at a concentration of 200 μg/mL. Then, washed endothelial monolayers were incubated with 10⁶ carboxyfluorescein diacetate succinimidyl ester-labeled WEHI78/24 cells (a murine monocytic cell line) suspended in 1 mL of binding buffer for 20 minutes. After a vigorous wash, adherent cells were determined by fluorescence intensity.

In Vitro Foam Cell Assay

Thioglycollate-elicited or bone marrow-derived macrophages were used. For some experiments, macrophages were stimulated with IL-4. LDL, OxLDL, or acetylated LDL (Biomedical Technologies, Inc) was added at different concentrations for different periods. Then, macrophages were washed with PBS, fixed with 4% paraformaldehyde, and stained with oil red O. Images were scanned into a Macintosh computer, and the percentage of oil red O-positively stained cells or surface area occupied by oil red O-stained droplets in each cell was determined with Image-Pro Plus.

Statistical Analysis

Statistical analysis was performed with InStat software (GraphPad Software). Data are represented as mean±SE. Data were compared with either 1-way ANOVA followed by Bonferroni correction post

hoc test or Student *t* test to evaluate 2-tailed levels of significance. The null hypothesis was rejected at *P*<0.05.

Results

Expression of 12/15-LO mRNA In Vivo

To investigate potential tissue-specific roles of 12/15-LO in atherosclerosis, we first surveyed 12/15-LO mRNA expression in atherosclerotic mice using real-time RT-PCR. Of all tissues surveyed, the highest mRNA expression level was found in peritoneal macrophages. 12/15-LO mRNA in elicited peritoneal macrophages was ≈1000 times higher than that in other tissues, including lung, heart, atherosclerotic vessel, liver, gut, spleen, lymph node, kidney, and adipose tissue. 12/15-LO was undetectable in brain and muscle (Figure 1). 12/15-LO was decreased by 90% in most tissues 12 weeks after 12/15-LO^{-/-} bone marrow replacement (Figure 1), which suggests that 12/15-LO exists mainly in macrophages in these tissues. Alternatively, 12/15-LO expression of native cells in these tissues may be regulated by 12/15-LO-expressing macrophages, although no autocrine and paracrine loops of 12/15-LO expression have been reported. The amount of 12/15-LO mRNA in kidney and adipose tissues did not change significantly as a result of transplantation with 12/15-LO^{-/-} bone marrow, which suggests 12/15-LO expression in tissue-resident cells. Under the conditions employed, 12/15-LO mRNA was not detectable in atherosclerotic arteries of apoE^{-/-} mice that received 12/15-LO-deficient bone marrow, which indicates that 12/15-LO in atherosclerotic lesions exists mainly in macrophages/foam cells (Figure 1).

Role of Bone Marrow-Derived 12/15-LO in Atherosclerosis

Bone marrow-derived macrophages contribute to foam cell formation in atherosclerotic lesions.²² To determine

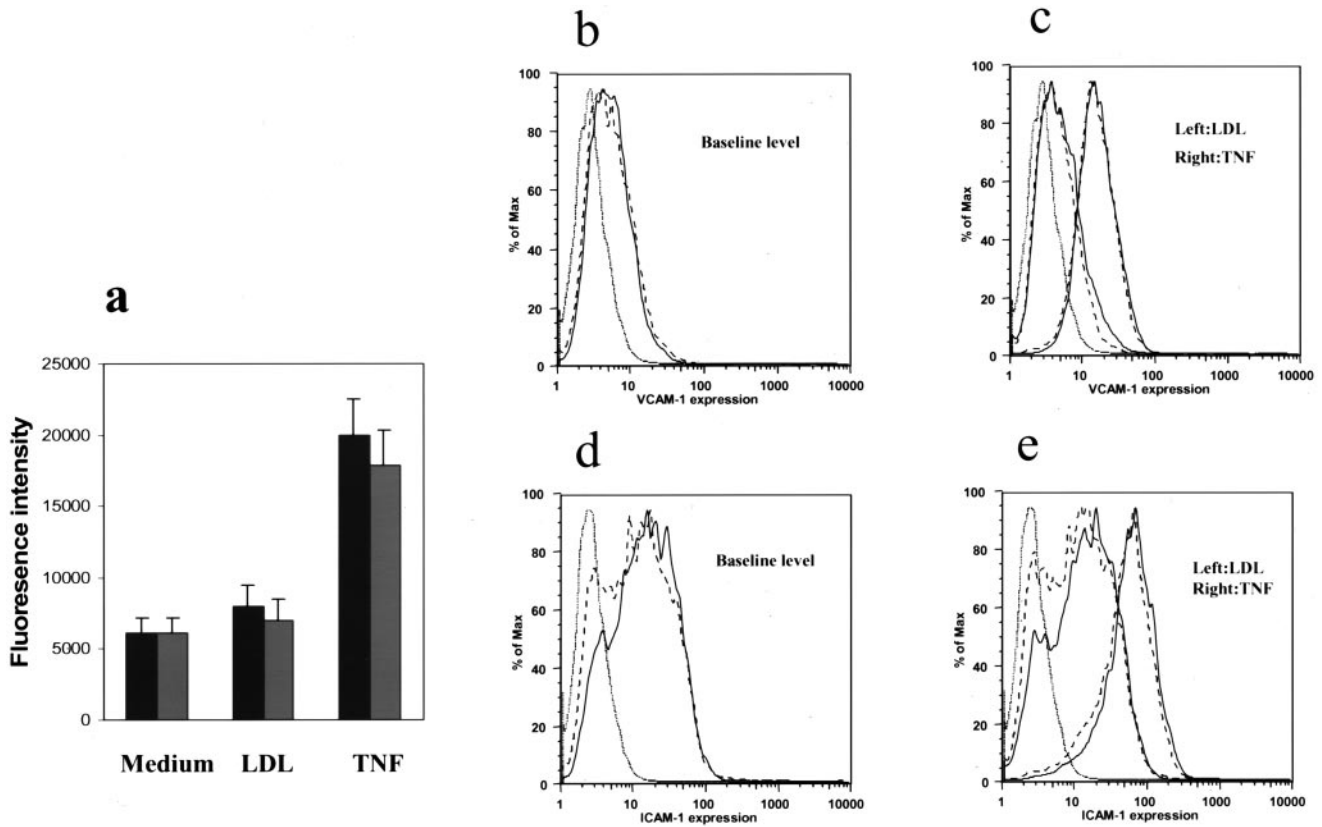


Figure 4. Endothelial activation by autocrine 12/15-LO products. a, Adhesion of fluorescently labeled WEHI24/78 cells (arbitrary units) on murine aortic endothelial monolayer was not dependent on presence (black) or absence (gray) of endothelial 12/15-LO either under resting condition or treated with LDL or tumor necrosis factor (TNF). n=6. Expression of adhesion molecules including VCAM-1 and ICAM-1 did not show significant difference either under resting conditions (b, d) or with exposure to LDL (200 μ g/mL for 20 hours) or TNF (10 ng/mL for 5 hours; c, e). Figures b through e represent 1 of 3 experiments; solid line indicates wild type; broken line, 12/15-LO^{-/-}. d, Serum-free cell medium conditioned by 12/15-LO^{+/+} macrophages slightly increased endothelial VCAM-1 expression. e, In presence of LDL, 12/15-LO^{+/+} macrophages cocultured with endothelial cells significantly increased expression of VCAM-1 on endothelial cells. n=4. Max indicates maximum.

the influence of macrophage 12/15-LO in the formation of atherosclerotic lesions, we generated 4 groups of chimeric mice (Table) by transplanting bone marrow from 12/15-LO^{-/-}/apoE^{-/-} mice into apoE^{-/-} mice and vice versa, as well as 2 control groups. BMT did not affect blood monocyte counts or tissue macrophages as reflected by the number of macrophages in the peritoneal cavity of thioglycollate-challenged mice (data not shown). Aortic lesion sizes in the 4 groups are shown in Figure 2b. ApoE^{-/-} mice reconstituted with bone marrow of apoE^{-/-} mice had lesions that covered 4.5% to 8.7% of the aortic surface. The lesion size in apoE^{-/-} mice receiving the bone marrow of 12/15-LO^{-/-}/apoE^{-/-} mice, similar to that in 12/15-LO^{-/-}/apoE^{-/-} mice receiving the bone marrow of 12/15-LO^{-/-}/apoE^{-/-}, was reduced by 50% (2.0% to 4.2%) compared with that in their controls (Figure 2b). This suggests that 12/15-LO from bone marrow-derived cells is critical for lesion development in this model. Consistent with this interpretation, reconstitution of 12/15-LO^{-/-}/apoE^{-/-} mice with bone marrow of apoE^{-/-} mice fully restored their lesion sizes to the levels of apoE^{-/-} mice.

Plasma Lipids, Lipid Peroxidation, and Immune Response in Reconstituted apoE^{-/-} Mice

Mean cholesterol and triglyceride levels in bone marrow-transferred mice in different groups were not different (data not shown). Because the apoE^{-/-} mice reconstituted with bone marrow from 12/15-LO^{-/-}/apoE^{-/-} mice had small atherosclerotic lesions, indistinguishable from the lesion size found in mice completely lacking 12/15-LO, we tested whether plasma 8,12-iso-iPF_{2 α} -VI levels in these mice would also be suppressed. Interestingly, isoprostane levels in these mice were the same as in apoE^{-/-} or 12/15-LO^{-/-}/apoE^{-/-} mice reconstituted with the bone marrow of apoE^{-/-} mice (Figure 3a).

In atherosclerosis, an immune response to oxidized lipids is prominent and results in the formation of autoantibodies of the IgM and IgG isotypes to modified LDL.²³ In all groups of BMT mice, autoantibody levels, especially levels of IgG autoantibodies, were much lower than those of age-matched controls, presumably because the immune system had not completely recovered from the lethal irradiation and reconstitution at the time serum was harvested. Interestingly, autoantibody (IgM Ab against

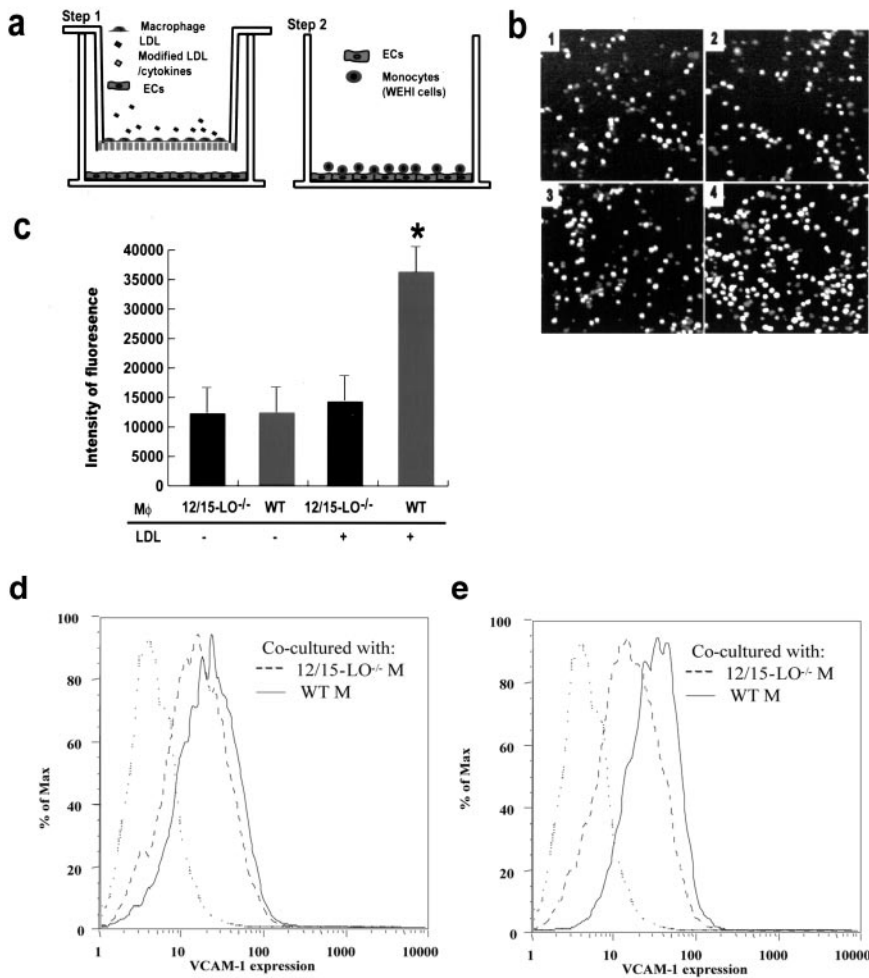


Figure 5. Endothelial activation by macrophage 12/15-LO activity. **a**, Coculture system used to evaluate effect of macrophage 12/15-LO on endothelial activation. Step 1: peritoneal macrophages were incubated in upper well of transwell system (pore size 0.4 μ m) with endothelial cells (ECs) in lower well with or without addition of LDL. Step 2: After a period of 20 to 24 hours, fluorescently labeled WEHI24/78 cells were loaded on washed endothelial monolayer to determine adhesiveness of endothelium. **b**, In absence of LDL, wild-type (1) or 12/15-LO-deficient (2) macrophages caused similar low level of adhesion of WEHI24/78 cells to endothelial cells. LDL-containing media conditioned by 12/15-LO^{+/+} (4) but not 12/15-LO^{-/-} (3) macrophages promoted cell adhesion. **c**, Quantification of WEHI24/78 adhesion to endothelial cells (arbitrary units). * $P < 0.01$. M ϕ indicates macrophages; WT, wild type. **d**, Serum-free cell medium conditioned by 12/15-LO^{+/+} macrophages slightly increased endothelial VCAM-1 expression. **e**, In the presence of LDL, 12/15-LO^{+/+} macrophages co-cultured with endothelial cells significantly increased the expression of VCAM-1 on endothelial cells. $n = 4$.

copper-modified LDL) levels were higher in mice reconstituted with the bone marrow of apoE^{-/-} mice than in those receiving the bone marrow of 12/15-LO^{-/-}/apoE^{-/-} mice, irrespective of the genotype of the recipient (Figure 3b). Consistent with protection of mice receiving 12/15-LO^{-/-}/apoE^{-/-} bone marrow, we found a highly significant positive correlation of autoantibody (IgM Ab against copper-modified LDL) levels with lesion size (Figure 3c). Autoantibody levels did not correlate with levels of plasma isoprostanes (Figure 3d).

Endothelial Activation by Autocrine 12/15-LO Products

Aortic endothelium of apoE^{-/-} mice fed a Western diet is activated and expresses various adhesion molecules.²⁴ Because endothelial cells are known to express 12/15-LO,¹³ we tested whether endothelial activation is caused by endothelial cell 12/15-LO by an autocrine mechanism. Figure 4 shows that there was no difference in the expression of either vascular cell adhesion molecule-1 (VCAM-1) or intercellular adhesion molecule-1 (ICAM-1) between cultured wild-type and 12/15-LO-deficient aortic endothelial cells, either incubated with LDL, stimulated with tumor necrosis factor- α , or under resting conditions.

Similarly, adhesion of WEHI78/24 cells, a murine monocytic cell line, also was not dependent on endothelial 12/15-LO (Figure 4a).

Endothelial Activation by Macrophage 12/15-LO Activity

To test whether macrophage 12/15-LO has an influence in endothelial activation and endothelium-monocyte interactions, we developed a coculture system (Figure 5a) in which peritoneal macrophages from 12/15-LO^{-/-} or wild-type mice were incubated in the upper well of a transwell system with and without native (nonmodified) LDL, and endothelial cells in the lower well were used as indicators of endothelial activation, measured by adhesion of WEHI78/24 cells. In the absence of LDL, neither wild-type nor 12/15-LO-deficient macrophages induced much endothelial activation (Figures 5b and 5c). By contrast, when LDL was added to the culture media, wild-type but not 12/15-LO-deficient macrophages had a large and significant activating effect on endothelial cells, as demonstrated by a 3-fold elevation of WEHI78/24 cell adhesion (Figures 5b and 5c) and significant upregulation of VCAM-1 on endothelial cells (Figures 5d and 5e).

Foam Cell Formation by 12/15-LO–Deficient Macrophages

Foam cell formation is a hallmark of atherosclerosis.²⁵ Therefore, we tested whether 12/15-LO might influence foam cell formation in peritoneal macrophages loaded with acetylated LDL. After 24 and 48 hours in culture, oil red O uptake as a marker of lipid accumulation was indistinguishable in macrophages from wild-type and 12/15-LO^{-/-} mice (Figure 6a). Similar results were obtained with OxLDL (data not shown).

When 12/15-LO–deficient peritoneal macrophages were cultured in the presence of native LDL, their lipid uptake was much reduced compared with wild-type controls. The intensity of oil red O staining was decreased in peritoneal macrophages from 12/15-LO^{-/-} mice (Figures 6b and 6c). The difference was further enhanced in macrophages stimulated with IL-4 (Figures 6b and 6c). This may be due to an increase of 12/15-LO protein and activity induced by IL-4 *in vitro*.²⁶ Similar results were obtained on macrophages differentiated from bone marrow cells in the presence of GM-CSF, IL-4, and M-CSF (data not shown).

Discussion

Our study demonstrates that 12/15-LO in macrophages is critical for the formation of atherosclerotic lesions. Mice reconstituted with bone marrow from apoE^{-/-}/12/15-LO^{-/-} mice show >50% reduced lesion sizes compared with mice receiving apoE^{-/-} bone marrow. Double-knockout mice regained the severity of atherosclerotic lesion typical of apoE^{-/-} mice after receiving bone marrow from apoE^{-/-} mice. Thus, macrophage 12/15-LO mediates endothelial activation and foam cell formation, 2 key steps in the process of atherosclerosis.

The BMT study suggests that macrophage 12/15-LO is proatherogenic in mice. This is inconsistent with previous studies by Shen et al,¹⁷ who found that 15-LO in monocytes/macrophages is antiatherogenic in a rabbit atherosclerotic model. The conflicting conclusions from these studies may be due to differences in species (rabbit versus mouse), lipoxygenase gene products (ratio of 12HETE:15HETE), and genetic manipulation (knockout versus transgenic overexpression). The expression level of the enzyme in transgenic monocyte-derived rabbit macrophages is >20-fold higher than in macrophages of normal rabbits and comparable to that of highly activated human monocytes by cytokines.²⁷ 12/15-LO and its products at high concentrations may play a role different from that at their physiological concentration. Recent studies have shown that several 12/15-LO products are ligands of peroxisome proliferator–activated receptor- γ (PPAR γ).²⁸ PPAR γ ligands have potent antiinflammatory effects at a high concentration.²⁹ Overexpression of 15-LO in monocytes/macrophages may result in substantial accumulation of 12/15-LO products and consequently trigger the PPAR γ pathway in these cells.

Circulating precursor cells can be recruited to the vessel wall and differentiate to various vascular cells under

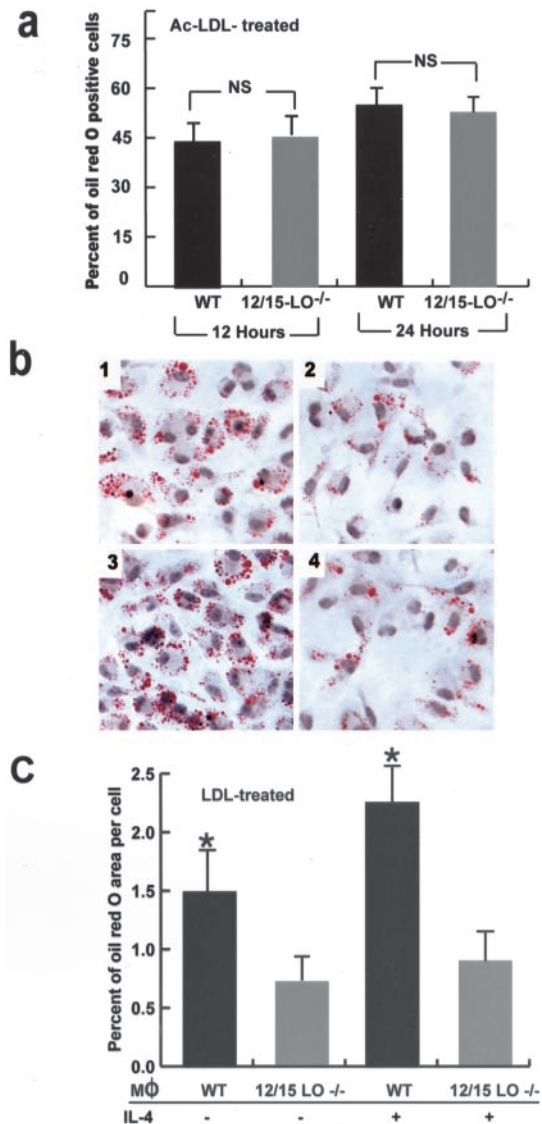


Figure 6. Effect of 12/15-LO on foam cell formation *in vitro*. a, No difference in percentage of oil red O–positive cells was found when peritoneal wild-type (WT) and 12/15-LO–deficient macrophages were exposed to acetylated LDL (Ac-LDL). n=5. b, After incubation with native LDL, intensity of oil red O staining was much lower in 12/15-LO–deficient macrophages (2) than in wild-type macrophages (1). This difference in intensity of oil red O staining was further enhanced when wild-type (3) and 12/15-LO deficient (4) macrophages were treated with IL-4 before incubation with LDL. c, Quantitative data on foam cell formation. Amount of lipid per macrophage was quantified by measuring area of oil red O staining. n=4. *P<0.01. M ϕ indicates macrophages.

different pathological conditions, especially in the setting of vascular injury.³⁰ In spontaneous atherosclerotic vessels, only a very limited number of endothelial cells and smooth muscle cells are replaced by differentiated donor-derived stem cells within 8 to 12 weeks after BMT.³⁰ In the same time frame, 12/15-LO^{-/-}/apoE^{-/-} mice receiving bone marrow from apoE^{-/-} mice developed the same extent of atherosclerotic lesions as control mice. There-

fore, the contribution of circulating precursor cells to the observed effects, if there is any, is likely to be small.

Human monocytes and macrophages express a 15-LO that can also produce 12-HETE and probably represents the human orthologue of mouse 12/15-LO.³ The presence of specific 15-lipoxygenase products,³¹ 15-LO protein and 15-LO mRNA,³² in human atherosclerotic arteries has been clearly demonstrated in samples obtained from patients aged 15 to 37 years. In a recent report, Spanbroek et al³³ did not detect 15-LO in very advanced atherosclerotic arterial samples. The present study was performed at the time when mice start to develop atherosclerosis, showing participation of macrophage 12/15-LO in the early phase of atherogenesis in mice, which is consistent with the findings in humans.

In the absence of LDL, macrophage 12/15-LO causes little increase in endothelial activation. However, in the presence of LDL, macrophage 12/15-LO significantly increases expression of endothelial adhesion molecules and endothelial-monocyte interactions. A suppression of lipid uptake and foam cell formation in macrophages lacking 12/15-LO was observed after incubation with native LDL but not modified LDL.¹² These results suggest that the proatherogenic role of macrophage 12/15-LO may be related to its oxidative action on LDL.^{10–12} However, in this BMT study, levels of isoprostane do not correlate with the sizes of atherosclerotic lesions. Overall levels of isoprostane may not reflect the local accumulation of oxidative products generated by 12/15-LO in the vessel wall, which may be important for the formation of atherosclerotic lesions. Alternatively, other 12/15-LO-dependent mechanisms may be involved. A recent study has demonstrated a decrease in production of IL-12 in 12/15-LO-deficient macrophages,³⁴ which suggests a reduced immune response. This correlates with lower titers of autoantibodies to OxLDL in BMT mice in the present study. Therefore, in addition to its oxidative action, macrophage-derived 12/15-LO may also be involved in regulating the immune response during initiation and progression of atherosclerosis.

Acknowledgments

This work was supported by NIH HL-58108 to Dr Ley, HL53558 to Dr Funk, P01 HL55798 to Dr Nadler, HL071141 to Dr Hedrick, and American Heart Association grants 030211N to Dr Pratico, 0120404U to Dr Huo, and 0225369U to Dr Zhao. We thank Michele Kirkpatrick and Jennifer Tripp for mouse husbandry.

References

- Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med*. 1999; 340:115–126.
- Yamamoto S. Mammalian lipoxygenases: molecular structures and functions. *Biochim Biophys Acta*. 1992;1128:117–131.
- Funk CD. The molecular biology of mammalian lipoxygenases and the quest for eicosanoid functions using lipoxygenase-deficient mice. *Biochim Biophys Acta*. 1996;1304:65–84.
- Kuhn H, Belkner J, Suzuki H, et al. Oxidative modification of human lipoproteins by lipoxygenases of different positional specificities. *J Lipid Res*. 1994;35:1749–1759.
- Belkner J, Stender H, Kuhn H. The rabbit 15-lipoxygenase preferentially oxygenates LDL cholesterol esters, and this reaction does not require vitamin E. *J Biol Chem*. 1998;273:23225–23232.
- Takahashi Y, Glasgow WC, Suzuki H, et al. Investigation of the oxygenation of phospholipids by the porcine leukocyte and human platelet arachidonate 12-lipoxygenases. *Eur J Biochem*. 1993;218: 165–171.
- Yoshimoto T, Takahashi Y. Arachidonate 12-lipoxygenases. *Prostaglandins Other Lipid Mediat*. 2002;68–69:245–62.
- Sendobry SM, Cornicelli JA, Welch K, et al. Attenuation of diet-induced atherosclerosis in rabbits with a highly selective 15-lipoxygenase inhibitor lacking significant antioxidant properties. *Br J Pharmacol*. 1997;120:1199–1206.
- Bocan T, Rosebury WS, Mueller SB, et al. A specific 15-lipoxygenase inhibitor limits the progression and monocyte-macrophage enrichment of hypercholesterolemia-induced atherosclerosis in the rabbit. *Atherosclerosis*. 1998;136:203–216.
- Cyrus T, Witztum JL, Rader DJ, et al. Disruption of the 12/15-lipoxygenase gene diminishes atherosclerosis in apo E-deficient mice. *J Clin Invest*. 1999;103:1597–1604.
- Cyrus T, Pratico D, Zhao L, et al. Absence of 12/15-lipoxygenase expression decreases lipid peroxidation and atherogenesis in apolipoprotein e-deficient mice. *Circulation*. 2001;103:2277–2282.
- George J, Afek A, Shaish A, et al. 12/15-Lipoxygenase gene disruption attenuates atherogenesis in LDL receptor-deficient mice. *Circulation*. 2001;104:1646–1650.
- Kim JA, Gu JL, Natarajan R, et al. A leukocyte type of 12-lipoxygenase is expressed in human vascular and mononuclear cells: evidence for upregulation by angiotensin II. *Arterioscler Thromb Vasc Biol*. 1995;15:942–948.
- Patricia MK, Natarajan R, Dooley AN, et al. Adenoviral delivery of a leukocyte-type 12 lipoxygenase ribozyme inhibits effects of glucose and platelet-derived growth factor in vascular endothelial and smooth muscle cells. *Circ Res*. 2001;88:659–665.
- Asada Y, Hara S, Tsuneyoshi A, et al. Fibrin-rich and platelet-rich thrombus formation on neointima: recombinant tissue factor pathway inhibitor prevents fibrin formation and neointimal development following repeated balloon injury of rabbit aorta. *Thromb Haemost*. 1998;80:506–511.
- Harats D, Shaish A, George J, et al. Overexpression of 15-lipoxygenase in vascular endothelium accelerates early atherosclerosis in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol*. 2000;20: 2100–2105.
- Shen J, Herderick E, Cornhill JF, et al. Macrophage-mediated 15-lipoxygenase expression protects against atherosclerosis development. *J Clin Invest*. 1996;98:2201–2208.
- Jung U, Ley K. Mice lacking two or all three selectins demonstrate overlapping and distinct functions of each selectin. *J Immunol*. 1999; 162:6755–6762.
- Nunnari JJ, Zand T, Joris I, et al. Quantitation of oil red O staining of the aorta in hypercholesterolemic rats. *Exp Mol Pathol*. 1989;51:1–8.
- Horkko S, Miller E, Branch DW, et al. The epitopes for some antiphospholipid antibodies are adducts of oxidized phospholipid and beta2 glycoprotein 1 (and other proteins). *Proc Natl Acad Sci U S A*. 1997;94:10356–10361.
- Hatley ME, Srinivasan S, Reilly KB, et al. Increased production of 12/15 lipoxygenase eicosanoids accelerates monocyte/endothelial interactions in diabetic db/db mice. *J Biol Chem*. 2003;278: 25369–25375.
- Fazio S, Babaev VR, Murray AB, et al. Increased atherosclerosis in mice reconstituted with apolipoprotein E null macrophages. *Proc Natl Acad Sci U S A*. 1997;94:4647–4652.
- Palinski W, Horkko S, Miller E, et al. Cloning of monoclonal autoantibodies to epitopes of oxidized lipoproteins from apolipoprotein E-deficient mice: demonstration of epitopes of oxidized low density lipoprotein in human plasma. *J Clin Invest*. 1996;98:800–814.
- Nakashima Y, Raines EW, Plump AS, et al. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the apoE-deficient mouse. *Arterioscler Thromb Vasc Biol*. 1998;18: 842–851.
- Glass CK, Witztum JL. Atherosclerosis: the road ahead. *Cell*. 2001; 104:503–516.
- Cornicelli JA, Welch K, Auerbach B, et al. Mouse peritoneal macrophages contain abundant omega-6 lipoxygenase activity that is inde-

- pendent of interleukin-4. *Arterioscler Thromb Vasc Biol.* 1996;16:1488–1494.
27. Shen J, Kuhn H, Petho-Schramm A, et al. Transgenic rabbits with the integrated human 15-lipoxygenase gene driven by a lysozyme promoter: macrophage-specific expression and variable positional specificity of the transgenic enzyme. *FASEB J.* 1995;9:1623–1631.
 28. Huang JT, Welch JS, Ricote M, et al. Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase. *Nature.* 1999;400:378–382.
 29. Daynes RA, Jones DC. Emerging roles of PPARs in inflammation and immunity. *Nat Rev Immunol.* 2002;2:748–759.
 30. Sata M, Saiura A, Kunisato A, et al. Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. *Nat Med.* 2002;8:403–409.
 31. Kuhn H, Heydeck D, Hugou I, et al. In vivo action of 15-lipoxygenase in early stages of human atherogenesis. *J Clin Invest.* 1997;99:888–893.
 32. Yla-Herttuala SF, Rosenfeld ME, Parthasarathy SF, et al. Gene expression in macrophage-rich human atherosclerotic lesions: 15-lipoxygenase and acetyl low density lipoprotein receptor messenger RNA colocalize with oxidation specific lipid-protein adducts. *J Clin Invest.* 1991;87:1146–1152.
 33. Spanbroek R, Grabner R, Lotzer K, et al. Expanding expression of the 5-lipoxygenase pathway within the arterial wall during human atherogenesis. *Proc Natl Acad Sci U S A.* 2003;100:1238–1243.
 34. Zhao L, Cuff CA, Moss E, et al. Selective interleukin-12 synthesis defect in 12/15-lipoxygenase-deficient macrophages associated with reduced atherosclerosis in a mouse model of familial hypercholesterolemia. *J Biol Chem.* 2002;277:35350–35356.

Corrections

In the article “Omega-3 Fatty Acids in Cardiac Biopsies From Heart Transplantation Patients: Correlation With Erythrocytes and Response to Supplementation,” by Harris et al, which appeared in the September 21, 2004, issue of the journal (*Circulation*. 2004;110:1645–1649), the authors have identified 2 errors. On page 1646, under the heading “Plasma Lipids and Lipoproteins,” the abbreviation in parentheses on the third line should be HDL-C, not LDL-C. Also, in the final line in column 1 of page 1648, the word “not” was omitted. The sentence should read, “We found that RBC EPA+DHA was not altered in postprandial blood from 10 healthy volunteers. . .” The authors regret these errors.

DOI: 10.1161/01.CIR.0000148060.90440.08

Regarding the Correspondence page “Dietary Intervention Combined With Exercise Improves Vascular Dysfunction but Also Obstructive Sleep Apnea in Obese Children,” which appeared in the September 21, 2004 issue of the journal (*Circulation*. 2004;110:e314), it has been brought to our attention that this title is misleading. The 2 letters on this page pertained to an article published in the April 27, 2004, issue of the journal by Woo et al (*Circulation*. 2004;109:1981–1986) titled “Effects of Diet and Exercise on Obesity-Related Vascular Dysfunction in Children.” The second letter (available at <http://circ.ahajournals.org/cgi/content/full/110/12/e314>), written by Tsung O. Cheng, should have been titled “Childhood Obesity Among the Chinese.” We regret this error.

DOI: 10.1161/01.CIR.0000148059.96278.F9

The article “Antibodies From Preeclamptic Patients Stimulate Increased Intracellular Ca²⁺ Mobilization Through Angiotensin Receptor Activation” by Thway et al, which appeared in the September 21, 2004, issue of the journal (*Circulation*. 2004;110:1612–1619), was inadvertently published without the authors’ corrections. The corrected version is available online at <http://circ.ahajournals.org/cgi/content/full/110/12/1612>. We regret these errors.

DOI: 10.1161/01.CIR.0000149087.81831.11

In the article “Critical Role of Macrophage 12/15-Lipoxygenase for Atherosclerosis in Apolipoprotein E-Deficient Mice” by Huo et al that appeared in the October 5, 2004, issue of the journal (*Circulation*. 2004;110:2024–2031), an error appeared in the legend of Figure 4. The following sentences should have been deleted: d, Serum-free cell medium conditioned by 12/15-LO^{+/+} macrophages slightly increased endothelial VCAM-1 expression. e, In presence of LDL, 12/15-LO^{+/+} macrophages cocultured with endothelial cells significantly increased expression of VCAM-1 on endothelial cells. n=4.

DOI: 10.1161/01.CIR.0000149088.58961.0C
