Macrophage Fatty-acid Synthase Deficiency Decreases Diet-induced Atherosclerosis*§

Received for publication, January 2, 2010, and in revised form, May 11, 2010 Published, JBC Papers in Press, May 17, 2010, DOI 10.1074/jbc.M110.100321

Jochen G. Schneider†1,2, Zhen Yang†1, Manu V. Chakravarthy†, Irfan J. Lodhi†, Xiaochao Wei†, John Turk‡, and Clay F. Semenkovich†§3

From the †Department of Medicine, Division of Endocrinology, Metabolism, and Lipid Research, and ‡Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri 63110

Fatty acid metabolism is perturbed in atherosclerotic lesions, but whether it affects lesion formation is unknown. To determine whether fatty acid synthesis affects atherosclerosis, we inactivated fatty-acid synthase (FAS) in macrophages of apoE-deficient mice. Serum lipids, body weight, and glucose metabolism were the same in FAS knock-out in macrophages (FASKOM) and control mice, but blood pressure was lower in FASKOM animals. Atherosclerotic extent was decreased 20–40% in different aortic regions of FASKOM as compared with control mice on Western diets. Foam cell formation was diminished in FASKOM as compared with wild type macrophages due to increased apoAI-specific cholesterol efflux and decreased uptake of oxidized low density lipoprotein. Expression of the anti-atherogenic nuclear receptor liver X receptor α (LXRα; Nr1h3) and its downstream targets, including Abca1, were increased in FASKOM macrophages, whereas expression of the potentially pro-atherogenic type B scavenger receptor CD36 was decreased. Peroxisome proliferator-activated receptor α (PPARα) target gene expression was decreased in FASKOM macrophages. PPARα agonist treatment of FASKOM and wild type macrophages normalized PPARα target gene expression as well as Nr1h3 (LXRα). Atherosclerotic lesions were more extensive when apoE null mice were transplanted with LXRα-deficient/FAS-deficient bone marrow as compared with LXRα-replete/FAS-deficient marrow, consistent with anti-atherogenic effects of LXRα in the context of FAS deficiency. These results show that macrophage FAS deficiency decreases atherosclerosis through induction of LXRα and suggest that FAS, which is induced by LXRα, may generate regulatory lipids that cause feedback inhibition of LXRα in macrophages.

Atherosclerotic cardiovascular disease remains the leading cause of death in Western countries, and its prevalence is likely to increase due to the current obesity epidemic (1). Major risk factors such as elevated serum cholesterol, hypertension, smoking, diabetes, and others were identified decades ago, yet these known mediators may account for only about half of cases of atherosclerotic vascular disease (2). Roughly 40% of coronary deaths occur in nonsmokers with cholesterol levels below the mean of the general population (3), and many patients whose initial presentation of atherosclerosis is myocardial infarction or sudden death are not previously identified as being at risk by conventional risk measures (4). Treating elevated cholesterol with statins decreases the risk of vascular events in clinical trials, but the residual risk is substantial even with aggressive cholesterol lowering (5). These observations suggest that unidentified or underappreciated mechanisms are involved in the initiation and progression of atherosclerotic lesions.

Cholesterol, one of the major components of atherosclerotic plaques, is not the only class of lipids in the diseased vasculature. In addition to cholesterol (predominantly in the form of cholesteryl esters), the intima of human fatty streaks also accumulates phospholipids and triglycerides (6). Cholesteryl esters, phospholipids, and triglycerides all contain fatty acids. Oleate, palmitate, and linoleate are the most abundant fatty acids in fatty streaks (7) and atheromatous plaques (8) from humans. Fatty acid distribution varies within different regions of plaques as well as between intact and ruptured lesions. For example, concentrations of saturated fatty acids such as palmitate are increased at the edge of disrupted plaques (9). The source of fatty acids in human plaques is unknown, although dogma holds that these fatty acids are mostly derived from diet. This assumption may be true for polyunsaturated fatty acids, because their plaque concentrations are correlated with their serum and adipose content in humans, but such a relationship does not appear to hold for saturated fatty acids (10), suggesting that fatty acids like palmitate may be synthesized in lesions. In fact, a series of studies dating from nearly half a century ago demonstrated the capacity of the vasculature for de novo lipogenesis (11–13). In rabbit and pigeon models, atherosclerosis accelerates vascular fatty acid synthesis, and the plaque itself appears to be the predominant site of synthesis (14, 15).

Fatty acid synthesis is an energy-consuming process that requires the multifunctional enzyme fatty-acid synthase (FAS).4 After priming with acetyl-CoA, FAS utilizes malonyl-
CoA as substrate and NADPH as cofactor to generate palmitate and other saturated fatty acids (16). FAS is expressed in essentially all human tissues (17); no loss of function mutations have been described in humans, and its germ line absence is embryonically lethal in mice (18), indicating that FAS is critical for normal development. Tissue-specific knock-out of FAS is feasible and has provided unexpected insight into the signaling role of the enzyme. Inactivation of FAS in liver or brain impairs expression of genes regulated by peroxisome proliferator-activated receptor α (PPARα) that is restored by PPARα agonist treatment (19, 20). These results suggest that FAS contributes to the generation of regulatory lipid molecules that affect gene expression, and a discrete FAS-dependent phosphatidylcholine species was recently identified as an endogenous activator of PPARα (21). Given the key roles played by macrophages in the formation of fatty streaks as well as the subsequent progression of atherosclerotic lesions (22), and the demonstration of fatty acid synthesis in plaques (14, 15), we tested the hypothesis that inactivation of FAS in macrophages affects diet-induced atherosclerosis in apoE null mice.

**EXPERIMENTAL PROCEDURES**

**Animals**—The Washington University Animal Studies Committee approved these experiments. Mice with loxP-flanked Fasn alleles (19) and lysozyme M-Cre mice (23) were mated with apolipoprotein E knock-out and were crossbred to yield FAS knock-out in macrophage (FASKOM) animals that were at least N5 in the C57BL/6 background with conditional deletion of FAS in the myelomonocytic lineage. Animals were genotyped using FAS- and Cre-specific primer sets (19), weaned to chow providing 6% calories as fat, and subsequently fed a Western-type diet containing 0.15% cholesterol with 42% calories as (TD 88137, Harlan) for 8 weeks for atherosclerosis experiments.

**FAS Enzyme Activity and Analytical Procedures**—FAS enzyme activity (19) was determined by first adding 10 μl of freshly harvested macrophage lysate to 80 μl of assay buffer (2 mM EDTA (pH 8.0), 2 mM dithiothreitol, 0.4 mg/ml NADPH) and monitoring NADPH oxidation at 340 nm. Then substrate-dependent activity was determined by subtracting the baseline NADPH oxidation rate from the rate following addition of 10 μl of 0.85 mg/ml of malonyl-CoA (Sigma). Serum chemistry assays, insulin measurements, and glucose tolerance as well as insulin tolerance tests were performed as described previously (24, 25). Enzyme-linked immunosorbent assays for adiponectin and tumor necrosis factor-α were performed with commercial reagents (Alpco Diagnostics, BD Biosciences).

**Macrophage Analyses**—Macrophages were elicited by injecting mice intraperitoneally with a 4% solution of thioglycollate media (Sigma), culturing isolated cells in DMEM plus 10% fetal bovine serum, and harvesting cells for RNA or protein as described previously (25, 26). Adherent cells used for experiments consisted of ~90% macrophages. There was no difference in the yield of macrophages from WT and FASKOM mice. For RT-PCR assays, total RNA (1 μg) was treated with DNase, reverse-transcribed, and subjected to PCR using primer and probe sets as described previously (19, 25). All assays were performed in triplicate and included a negative control with RNA not subjected to reverse transcription, and results were normalized to signal for the invariant message for the mouse ribosomal protein L32. Western blotting was performed as described previously (25) using a mouse monoclonal antibody against ABCA1 (Abcam).

**Blood Pressure Determinations and Atherosclerosis Quantification**—Systolic and diastolic blood pressures in conscious mice were measured, as described previously (27), after the animals were habituated to the tail-cuff apparatus for several days. Multiple measurements at several sessions generated an average value for each animal that was used to calculate the mean results for each genotype. For atherosclerosis assays, all comparisons were performed with littermates using the en face technique (25). Results are reported as percentage involvement of the intimal surface for three regions of the aorta as captured by an image-processing program.

**Lipid Content**—Lipids from peritoneal macrophages and freshly isolated aortic arches were extracted with chloroform/methanol (2:1 v/v), dried under nitrogen, and reconstituted for enzymatic assays using commercial reagents (19). Cholesteryl ester content was determined as the difference between total cholesterol and free cholesterol. Phospholipids in macrophages were assayed using commercial reagents. Phospholipids in aortic arches were assayed as total lipid phosphorus using an assay involving perchloric acid treatment of lipid extracts (28). Fatty acid composition of lipid extracts of macrophages and triacylglycerol composition of lipid extracts of aortic arches were analyzed by electrospray ionization-mass spectrometry on a Finnigan TSQ-7000 triple stage quadrupole mass spectrometer as described previously (21).

**Cholesterol Efflux**—The efflux assay, performed essentially as described by Traves et al. (29), utilized murine peritoneal macrophages plated on day 1 in 6-well plates in DMEM containing 10% fetal bovine serum. On day 2, the cells were washed and incubated for 24 h in DMEM containing 2% bovine serum albumin (fatty acid-free) supplemented with [3H]cholesterol (1 μCi/ml). On day 3, the cells were washed and incubated overnight in DMEM containing 2% bovine serum albumin (fatty acid-free). On day 4, the cells were washed and incubated for 5 h in DMEM containing 0.2% bovine serum albumin (fatty acid-free) in the absence or presence of apolipoprotein AI (10 μg/ml). After incubation, the medium was collected; cells were lysed in 1 ml of 0.2 M NaOH containing 0.1% SDS at room temperature for 30 min, and radioactivity was determined by liquid scintillation counting. The apolipoprotein AI-dependent efflux of radioactive cholesterol from the cells into the medium was determined as the percentage of medium-derived radioactivity relative to the total radioactivity for the medium and cells.

**Cholesterol Uptake**—Murine peritoneal macrophages were plated in 6-well plates in DMEM containing 10% fetal bovine serum. Cells were extensively washed and then incubated with 3,3′-dioctadecyldimercaptopropane-oxidized low density lipoprotein (Intralcel) at a concentration of 1 mg/ml in DMEM at 37 or 4 °C in a 5% CO₂ incubator for 2 h. Cells were subse-
Macrophage FAS and Atherosclerosis

A

Targeted allele

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| P1 | | | | | | | | |
| P2 | | | | | | | | |
| Post-Cre allele

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| P1 | | | | | | | | |
| P2 | | | | | | | | |

317bp

B

FASKOM

WT

1 2 3 4 5 6 7 8 9

1 2 3 4 5 6 7 8 9

C

Relative FAS mRNA expression

WT

FASKOM

D

FAS activity

WT

FASKOM

Liver

30 40 50

0 100

FIGURE 1. Targeting FAS in macrophages. A, top shows the targeted allele (FAS locus with loxP-flanked exons 4 – 8 (with the loxP sites indicated as triangles)) and the post-Cre allele. P1 and P2 represent primers used to detect a 317-bp fragment indicating Cre excision at the FAS locus. The bottom shows a simplified breeding scheme (omitting intermediate progeny) to produce FASKOM mice. B, PCR analyses of DNA from FASKOM and control mice using P1 and P2. DNA sources for both FASKOM and WT were as follows: lane 1, liver; lane 2, spleen; lane 3, lung; lane 4, kidney; lane 5, heart; lane 6, aorta; lane 7, adipose tissue; lane 8, peritoneal macrophages; and lane 9, negative control. C, FAS mRNA expression in peritoneal macrophages by quantitative RT-PCR. Data are expressed relative to L32 mRNA. *, p < 0.05; D, FAS enzyme activity in peritoneal macrophages. *, p < 0.001. The inset shows FAS enzyme activity in liver. Each bar in C and D represents the mean ± S.E. of eight mice for each genotype.

TABLE 1

Body weight, serum chemistries, and insulin

Data are presented as mean ± S.E. for ≥10 animals per genotype. p values represent comparisons within the same genotype as follows: body weight at base line versus 4 (a, p < 0.001) or 8 weeks (b, p < 0.01) of high fat feeding; cholesterol at base line versus 4 or 8 weeks of high fat feeding (c, p < 0.0001); free fatty acid (FFA) at base line versus 4 (a, p < 0.001) or 8 weeks (b, p < 0.01) of high fat feeding; glucose at base line versus 4 (c, p < 0.0001) or 8 weeks (b, p < 0.01, and c, p < 0.001) of high fat feeding; and insulin at base line versus 4 (a, p < 0.05) or 8 weeks of high fat feeding (a, p < 0.01).

| Genotype          | WT | FASKOM |
|-------------------|----|--------|
|                   | Base line | 4 weeks | 8 weeks | Base line | 4 weeks | 8 weeks |
| Body weight (g)   | 23.2 ± 3.4ab | 27.1 ± 3.8 | 29.8 ± 4.8 | 23.4 ± 2.7bc | 27.0 ± 4.2 | 31.1 ± 5.6 |
| Cholesterol (mg/dl)| 316 ± 116 | 1772 ± 153 | 1705 ± 137 | 387 ± 103bc | 1810 ± 104bc | 1722 ± 232bc |
| Triglycerides (mg/dl)| 188 ± 58 | 223 ± 60 | 244 ± 130 | 290 ± 85 | 243 ± 42 | 143 ± 141 |
| FFA (mg/dl)       | 1.32 ± 0.37abcd | 2.26 ± 0.62 | 2.11 ± 0.93 | 1.44 ± 0.41abcd | 2.46 ± 0.51 | 2.19 ± 1.51 |
| Glucose (mg/dl)   | 206 ± 39abc | 325 ± 57 | 341 ± 102 | 197 ± 45cd | 352 ± 57 | 381 ± 129 |
| Insulin (pg/ml)   | 765 ± 104ab | 1389 ± 401 | 2400 ± 837 | 834 ± 117abc | 1286 ± 188 | 2001 ± 329 |

B

Bone Marrow Transplantation—LXRa−/− mice in the C57BL/6 background, kindly provided by David Mangelsdorf (University of Texas Southwestern), were crossbred with apoE−/− and FASKOM animals to generate FASKOM-LXRa+/−-apoE−/− and FASKOM-LXRa−/−-apoE−/− mice. Bone marrow was isolated from the femurs and tibias of these animals at 12 weeks of age by flushing bones with cold phosphate-buffered saline. Marrow was washed, triturated using a 24-gauge needle, collected by centrifugation at 1250 rpm for 4 min, and diluted with phosphate-buffered saline. After lysis of erythrocytes using 0.05% sodium azide, cells were counted to obtain a defined concentration of unfractonated bone marrow. Recipient FASKOM-apoE−/− mice were lethally irradiated...
with 10 gray from a cesium-137 γ-cell irradiator and infused with ~5 × 10⁶ donor marrow cells within 6 h of irradiation. The fidelity of the process was confirmed by verifying the lethality of the radiation dose in animals not reconstituted with marrow, and by histological staining of recipient femurs. Four weeks after transplantation, recipients were placed on a Western type diet; serum chemistries were monitored, and atherosclerosis lesion extent by the en face technique was determined after 8 weeks.

**Statistical Analyses**—Statistical significance of differences was calculated using the Student’s unpaired t test for parametric data involving two groups, analysis of variance for parametric data involving more than two groups with the Dunnett or Bonferroni post tests, the Mann-Whitney test for nonparametric data involving two groups, and the Kruskal-Wallis test for nonparametric data for more than two groups with Dunn’s post test.

**RESULTS**

**Generation of FASKOM Mice**—To determine whether de novo lipogenesis in macrophages impacts atherosclerosis, we generated FASKOM mice by crossing FAS floxed animals previously used to inactivate FAS in other models (19, 20, 32) with lysozyme M-Cre mice expressing the Cre recombinase in myelomonocytic lineages. Fig. 1A shows a diagram of the targeted allele (with loxP sites indicated by triangles) and the post-Cre allele following removal of exons 4–8. A simplified scheme that omits intermediate breeding steps is also shown for FASKOM mouse generation. These animals were produced in the setting of apoE deficiency; appropriate genotypes during the breeding strategy were observed at predicted Mendelian frequencies, and a tissue distribution survey for rearrangement of the FAS gene is shown in Fig. 1B. A 317-bp product (produced using primers P1 and P2 as denoted in Fig. 1A) indicating deletion of exons 4–8 was detected in macrophage-rich tissues of FASKOM mice, including the spleen (Fig. 1B, lane 2), lung (lane 3), aorta (lane 6), and adipose tissue (lane 7) as well as in peritoneal macrophages (lane 8). As expected, no such band was detected in wild type tissues. FAS mRNA expression (Fig. 1C) and enzyme activity (Fig. 1D) were substantially decreased in peritoneal macrophages isolated from FASKOM as compared with WT mice. As expected, liver FAS enzyme activity was unaffected (Fig. 1D, inset). These results are consistent with the selective inactivation of FAS in macrophages in FASKOM mice.

**Metabolic Phenotype and Atherosclerotic Susceptibility of FASKOM Mice**—Our breeding strategy yielded three potential control groups of apoE null mice with different genotypes as follows: those with Cre but no floxed FAS alleles, those with no floxed FAS alleles and no Cre, and those with two floxed alleles and no Cre. There were no phenotypic differences between these groups (data not shown), so subsequent comparisons were made between FASKOM mice (apoE⁻/⁻/Faslloxflox/Cre⁺) and WT mice...
FIGURE 3. Cholesterol metabolism in peritoneal macrophages. A, Oil Red O staining of peritoneal macrophages. Magnification ×40 with bar indicating 50 μm. B, lipid content of peritoneal macrophages. *, p < 0.05. Similar results were seen in three independent experiments. C, representative electrospray ionization-mass spectrometry analysis of fatty acids extracted from WT and FASKOM peritoneal macrophages. The vertical axis represents relative abundance and the horizontal axis m/z values. The peak with m/z 311.3 is an internal standard. D, apoAI-specific cholesterol efflux using macrophages from n = 3 mice of each genotype. Cells were loaded with acetylated LDL in the presence of [3H]cholesterol and then treated with apoAI for 48 h. *, p < 0.05. E, Western blot of proteins extracted from WT and FASKOM macrophages and detected with an anti-ABCA1 antibody (top panel) and an anti-actin antibody (bottom panel). F, cholesterol uptake at 4 and 16 h after exposure to fluorescently labeled oxidized LDL using cells from n = 3 mice of each genotype. *, p < 0.05.
(apoE<sup>−/−</sup>-<i>Fas</i><sup>fl<sup>ox/</sup>fl<sup>ox</sup>-<i>Cre</i><sup>−</sup>) to simplify breeding strategies for the production of littermates. There were also no metabolic differences between the sexes within genotypes, so experiments included equal numbers of males and females.

Body weight, lipids, fasting glucose, and insulin measurements for FASKOM and WT mice were similar at base line and showed the same changes over the span of 8 weeks of eating a Western diet (Table 1). At base line on a chow diet, systolic and diastolic blood pressures were 9 and 10 mm Hg lower, respectively, in FASKOM versus control mice (Fig. 2A, upper panel). After 8 weeks of high fat feeding, systolic and diastolic blood pressures increased in each genotype but remained 7 mm Hg lower in FASKOM as compared with control littermates (Fig. 2A, lower panel). Lipoproteins assayed by fast protein liquid chromatography (Fig. 2B) with chow feeding and after 8 weeks of high fat feeding were similar in each genotype, an expected observation given the lack of differences in total lipids (Table 1). Although atherogenic lipoproteins were similar in both genotypes, FASKOM mice had 20% less atherosclerosis at the aortic arch, 23% less at the thoracic aorta, and 40% less at the abdominal aorta (Fig. 2C) as compared with WT mice. Thus, FAS deficiency in macrophages of apoE<sup>−/−</sup>-<i>Fas</i><sup>fl<sup>ox/</sup>fl<sup>ox</sup>-<i>Cre</i><sup>−</sup>) mice protected FASKOM mice from accelerated atherosclerosis as compared with littermate controls despite similar cholesterol levels. The reduced blood pressure observed in FASKOM mice did not appear to make a considerable contribution to the atherosclerosis phenotype because there were no significant correlations between systolic or diastolic blood pressure and atherosclerotic lesion extent (supplemental Table 1).

Given the growing recognition of the potential importance of macrophages in insulin resistance (which is associated with atherosclerosis), we further evaluated glucose metabolism in fat-fed FASKOM mice. There were no significant differ-
Macrophage FAS and Atherosclerosis

![Cell type distribution in atherosclerotic lesions](image)

**FIGURE 5. Cell type distribution in atherosclerotic lesions.** WT and FASKOM mice were fed a high fat diet for 8 weeks, and then the aortic root was sectioned using a cryostat. Slides were subjected to Oil Red O staining for lesion quantification by computerized image processing, and to fluorescent immunocytochemical quantification using an anti-macrophage antibody (MOMA) and an anti-smooth muscle antibody (SMA). A, representative immunocytochemistry images from WT (top panels) and FASKOM (bottom panels) sections. B, atherosclerotic lesion area for four WT and five FASKOM mice. A.U., arbitrary units. *, p < 0.05. C, macrophage content normalized to lesion area in the same lesions quantified in B.

To determine the effects of macrophage FAS deficiency on lipid content of atherosclerotic lesions, we repeated the feeding studies with separate cohorts of age-matched mice. After 8 weeks of Western diet feeding, animals were sacrificed, and aortic arches visually selected for differences in lesion extent were cleaned and immediately subjected to a lipid extraction protocol (as opposed to being fixed and mounted as for Fig. 2C). Decreased lesions in FASKOM aortas were associated with decreased content of total cholesterol, free cholesterol, and cholesteryl esters (Fig. 4A). Cholesteryl ester content exceeded that of free cholesterol in atherosclerotic aortas, unlike peritoneal macrophages (Fig. 3B), for both genotypes. There were no differences between WT and FASKOM vessels for total lipid phosphorus (Fig. 4B), a measure of phospholipids) or for total triglycerides (Fig. 4C). Fig. 4D shows an analysis of aortic arch triglycerides by mass spectrometry. For example, peaks with m/z 837 (representing 16:0/16:1/18:1 triglycerol and isomers, i.e. 50:2 triglycerols), m/z 863 (52:3 triglycerol isomers), and others had the same relative abundance in WT and FASKOM aortas.

**Cell Type Distribution in Atherosclerotic Lesions**—Aortic roots of these age-matched mice after 8 weeks of Western diet feeding were analyzed for cell types. As expected, the early atherosclerotic lesions in this model were composed of macrophages, with smooth muscle cells detected only in the aortic media (representative images are shown in Fig. 5A). Despite the

To determine the effects of macrophage FAS deficiency on lipid content of atherosclerotic lesions, we repeated the feeding studies with separate cohorts of age-matched mice. After 8 weeks of Western diet feeding, animals were sacrificed, and aortic arches visually selected for differences in lesion extent were cleaned and immediately subjected to a lipid extraction protocol (as opposed to being fixed and mounted as for Fig. 2C). Decreased lesions in FASKOM aortas were associated with decreased content of total cholesterol, free cholesterol, and cholesteryl esters (Fig. 4A). Cholesteryl ester content exceeded that of free cholesterol in atherosclerotic aortas, unlike peritoneal macrophages (Fig. 3B), for both genotypes. There were no differences between WT and FASKOM vessels for total lipid phosphorus (Fig. 4B), a measure of phospholipids) or for total triglycerides (Fig. 4C). Fig. 4D shows an analysis of aortic arch triglycerides by mass spectrometry. For example, peaks with m/z 837 (representing 16:0/16:1/18:1 triglycerol and isomers, i.e. 50:2 triglycerols), m/z 863 (52:3 triglycerol isomers), and others had the same relative abundance in WT and FASKOM aortas.

**Cell Type Distribution in Atherosclerotic Lesions**—Aortic roots of these age-matched mice after 8 weeks of Western diet feeding were analyzed for cell types. As expected, the early atherosclerotic lesions in this model were composed of macrophages, with smooth muscle cells detected only in the aortic media (representative images are shown in Fig. 5A). Despite the
presence of less extensive atherosclerosis at the aortic root in FASKOM mice (determined by Oil Red O staining and image processing, Fig. 5B), macrophage content normalized to lesion area was the same in WT and FASKOM mice, suggesting that differences in lesions are due to reduced lipid accumulation in macrophages as opposed to reduced numbers of macrophages.

Gene Expression in FASKOM Macrophages—To identify potential mechanisms responsible for increased cholesterol efflux and decreased cholesterol uptake, candidate gene expression was assayed in elicited macrophages. Message levels were increased for LXRα (Nr1h3) (Fig. 6A), an antiatherogenic nuclear receptor that interacts with oxysterols, and in FASKOM as compared with WT macrophages. There was no effect on LXRβ (Nr1h2) mRNA in FASKOM macrophages (supplemental Fig. 2). The expression of the LXRα target gene Abca1 (Fig. 6B) was increased, consistent with the detection of increased ABCA1 protein by Western blotting in FASKOM macrophages (Fig. 3D). The induction of FASKOM ABCA1 expression provides a potential explanation for the increased cholesterol efflux demonstrated with these cells (Fig. 3D) because the ABCA1 protein mediates sterol efflux to lipid-poor apoAI (35). Expression of another LXRα target gene, SREBP1 (Sreb1) (Fig. 6C), was also increased in FASKOM cells.

There was no effect of macrophage FAS inactivation on message levels for Ppara (Fig. 6D), but expression of the PPARα target genes Cpt1a and Acox1 were both decreased, a pattern also present in liver and brain with inactivation of FAS (19, 20). FAS inactivation had no effect on message levels for Pparg or its target gene aP2 (Fabp4) (Fig. 6, G and H). However, Cd36 gene expression was decreased in FASKOM macrophages (Fig. 6I). This difference in message was reflected at the protein level because cell surface CD36 protein was decreased as determined by cell sorting (supplemental Fig. 3). This finding is consistent with the decreased uptake of oxidized LDL seen in these cells (Fig. 3F).

These data point to interactions between PPARα and LXRα in macrophages, and indirect evidence suggests that adiponectin may link these signaling pathways in macrophages (36), but the inactivation of FAS in macrophages had no effect on adiponectin levels (supplemental Fig. 4A). PPARs and LXRs affect the inflammatory status of macrophages, but serum levels of tumor necrosis factor-α after treatment with lipopolysaccharide was the same in FASKOM and littermate control mice (supplemental Fig. 4B). Release of the proatherogenic cytokine MCP-1 was also not different between FASKOM and control mice (data not shown). Collectively, these results suggest that FAS inactivation in macrophages does not have a major anti-inflammatory effect.

Effects of PPARα Activation in FASKOM Macrophages—In liver and brain, the loss of PPARα-dependent gene expression caused by FAS inactivation is restored by treatment with the PPARα agonist Wy14,643 (19, 20). When elicited macrophages

FIGURE 6. Gene expression in peritoneal macrophages. Message expression was assayed by quantitative RT-PCR using cells from WT (open symbols) and FASKOM (solid symbols) mice for LXRα (Nr1h3) (A), Abca1 (B), SREBP1 (Sreb1) (C), Ppara (D), Cpt1a (E), Acox1 (F), Pparg (G), aP2 (Fabp4) (H), and Cd36 (I). Data are expressed relative to L32 (Rpl32) mRNA as mean ± S.E. using RNA from cells derived from three to five mice per condition. *, p < 0.05. Positive results were replicated in one or more independent experiments.
Macrophage FAS and Atherosclerosis

from FASKOM and WT mice were incubated for 24 h with 100 

μM Wy14,643 in carrier (Wy in Fig. 7A), decreased expression of the PPARα-dependent gene Acox1, seen when cells were incubated with the carrier alone (Control in Fig. 7A), was restored. In the same cells, Wy14,643 treatment had little effect on LXRα (Nr1h3) expression in FASKOM macrophages (Fig. 7B, compare the solid bars for the Control and Wy conditions) but instead increased expression in WT cells (Fig. 7B, compare the open bars for the Control and Wy conditions). Because Wy14,643 activates PPARα, a major inducer of fatty acid oxidation, one interpretation of these results is that Wy14,643 treatment induces PPARα-dependent genes like Acox1 in FASKOM cells, and disinhibits LXRα (Nr1h3) in WT cells perhaps by accelerating metabolism of a putative lipid species that normally suppresses LXRα (Nr1h3) gene expression.

Atherosclerosis in Mice Transplanted with LXRα-deficient Marrow—We studied LXRα expression in FASKOM cells because this nuclear receptor participates in the regulation of cholesterol efflux, and our data (Fig. 3D) implicate efflux as mediating at least a part of decreased atherosclerosis induced by macrophage FAS deficiency. Although considerable data support the notion that LXRα is anti-atherogenic, it is possible that such effects are context-specific. For example, the lack of FAS in macrophages might lead to alterations in membrane lipids or signaling in such a way that the increased expression of LXRα would be ineffectual at antagonizing the atherosclerotic process.

To answer the question of whether LXRα is anti-atherogenic specifically in the setting of macrophage FAS deficiency, we transplanted two groups of littermate LXRα+/+ FASKOM apoE−/− mice with bone marrow from either LXRα+/+ FASKOM apoE−/− mice or LXRα−/− FASKOM apoE−/− mice. Recipient mice were allowed a period of 4 weeks for engraftment and appeared healthy. After 8 weeks of Western diet feeding, animals were sacrificed; bone marrow histology was examined, and en face atherosclerosis was quantified. Mice transplanted with either LXRα+/+ FASKOM apoE−/− or LXRα−/− FASKOM apoE−/− marrow manifested no differences in serum chemistries at baseline and achieved the same diet-induced elevations in serum cholesterol over the 8-week period of eating a Western diet (Table 2). Representative photomicrographs of bone marrow documenting successful transplantation are shown in supplemental Fig. 5. Atherosclerosis was significantly more extensive at the aortic arch in mice transplanted with LXRα-deficient (Fig. 8, open diamonds) as compared with LXRα-replete (Fig. 8, solid squares) bone marrow. A similar trend was present at the thoracic aorta. These results indicate that bone marrow LXRα decreases diet-induced atherosclerosis in the context of macrophage FAS deficiency.

**DISCUSSION**

Atherosclerotic plaques are metabolically active (37) and have the capacity to synthesize fatty acids (14, 15), but it is unknown if disrupting fatty acid biosynthesis in cells relevant to plaque biology can affect atherogenesis. Here, we show that inactivation of FAS, which catalyzes the first committed step in fatty acid biosynthesis, in macrophages decreases diet-induced atherosclerosis in apoE null mice (Fig. 2). FASKOM macrophages less avidly become foam cells through a combination of increased cholesterol efflux and decreased cholesterol uptake (Fig. 3). The former is probably due to an induction of LXRα and its target Abca1, and the latter results at least in part from

**TABLE 2**

Serum chemistries in transplanted animals

| Donor | LXRα+/+ FASKOM apoE−/− | LXRα−/− FASKOM apoE−/− |
|-------|------------------------|------------------------|
|       | Base line | 4 weeks | 8 weeks | Base line | 4 weeks | 8 weeks |
| Cholesterol (mg/dl) | 405 ± 71 | 1596 ± 54a | 1469 ± 43a | 353 ± 44 | 1460 ± 129a | 1382 ± 48a |
| Triglycerides (mg/dl) | 214 ± 23 | 191 ± 21 | 128 ± 10a | 171 ± 31 | 202 ± 39 | 174 ± 20 |
| FFA (mg/dl) | 1.68 ± 0.03 | 1.82 ± 0.13 | 2.15 ± 0.15a | 1.20 ± 0.13 | 1.99 ± 0.23a | 2.79 ± 0.15a |
| Glucose (mg/dl) | 189 ± 12 | 315 ± 21a | 185 ± 11 | 170 ± 7 | 306 ± 29a | 175 ± 23 |

a p < 0.05 compared with base line.
Macrophage FAS and Atherosclerosis

In WT macrophages (Fig. 9, left side), LXRα is known to induce Fasn gene expression (39). This likely involves both direct effects at the Fasn promoter (40) as well as indirect effects by inducing SREBP1c, which induces Fasn and other lipogenic genes. In turn, FAS contributes to the generation of regulatory lipids with the capacity to inhibit LXRα and activate PPARα. Feedback inhibition of LXRα by FAS-generated regulatory lipids would be beneficial because unabated stimulation of fatty acid synthesis by LXRα, an effect that occurs even in the setting of sterol loading when SREBP-1c is suppressed (40), would be expected to overload macrophages with lipid and exhaust energy stores (because synthesis of palmitate by FAS requires ATP).

Although the findings that PPARα target genes have decreased expression that is restored with a synthetic PPARα agonist are consistent with the involvement of FAS in the generation of an endogenous PPARα ligand in macrophages, additional data will be required to establish this link. This relationship appears to exist in liver and brain (19, 20), and a phosphatidylcholine species was recently identified as an FAS-dependent lipid that activates PPARα in liver (21), raising the possibility that a related molecule may also exist in macrophages. Identifying this species will require the characterization of lipids bound to the nuclear receptor purified from FAS-replete and FAS-deficient cells and demonstration of bioactivity of the identified lipid(s).

The effects of PPARs on scavenger receptors are complex and species-specific. However, treatment of apoE null mice with the PPARα agonist Wy14,643 increases Cds36 expression in a PPARα-dependent manner in the vasculature in early stages of atherosclerosis (41), and the same agonist promotes the appearance of Cds36 on the plasma membrane (42), consistent with the current data. The role of Cds36 in mouse atherosclerosis is under debate. The protein has been implicated in foam cell development and the extent of lesion formation by some (43) but not by others (44). More recent studies have implicated Cds36 (in concert with another scavenger receptor) in the development of lesion complexity (45), and other potential mechanisms for the involvement of this protein in atherosclerosis have been identified (46). If Cds36 is even minimally proatherogenic, this effect could be amplified by a relative decrease in ABCA1 associated with less than maximal expression of LXRα due to feedback inhibition induced by FAS-generated regulatory lipids.

In FASKOM macrophages (right side of Fig. 9), the absence of FAS would be expected to deplete the regulatory lipid pool, impairing activation of PPARα-dependent genes. However, the major effect of depleting this pool might be due to decreasing levels of putative lipid(s) that cause feedback inhibition of LXRα. Thus, depletion of this pool through FAS deficiency inhibits LXRα leading to its increased expression. LXRα activation with synthetic agonists in mice decreases atherosclerosis (47); transplanting mice with LXR-deficient marrow increases atherosclerosis (48), and macrophage overexpression of LXRα in transgenic mice decreases atherosclerosis (49). These effects are mediated in part through ABCA1. Recent data show that physiological effects of ABCA1 are most important at the liver (50), but transgenic overexpression of ABCA1 in macrophages decreased Cds36 expression (Fig. 6 and supplemental Fig. 3). PPARα target genes are decreased in FASKOM macrophages; their expression is restored by PPARα agonist treatment, and the same treatment, which promotes fatty acid oxidation, disinhibits LXRα (Nr1h3) expression in WT macrophages (Fig. 7). Bone marrow LXRα deficiency promotes atherosclerosis in the setting of macrophage FAS deficiency (Fig. 8). One unifying interpretation of these results suggests that FAS generates regulatory lipids with access to the nucleus of macrophages with some of these lipids leading to LXRα inhibition and others activating PPARα expression. A parsimonious model incorporating the current data is shown in Fig. 9.

Blood pressure was reduced in FASKOM mice (Fig. 2). This effect did not appear to contribute to lesions in a major way as there was no significant relationship between blood pressure and atherosclerosis (supplemental Table 1). Our unexpected blood pressure results suggest that FAS in either macrophages or neutrophils (sites of Cre expression in this model) could affect systolic and diastolic blood pressure. In rats, the secretion of vascular endothelial growth factor-C by macrophages has been implicated in salt-dependent hypertension (38).
Macrophage FAS and Atherosclerosis

decreases atherosclerosis (51). LXRx activation in macrophages induces ABCA1 and suppresses CD36 (52). LXRx inhibition of PPARx signaling (53) may also amplify these effects in FASKOM macrophages.

This model provides a mechanistic framework for our finding that macrophage FAS deficiency decreases vascular disease. FAS has been crystallized (54), and its domains are considered appropriate targets for the development of inhibitors. The enzyme has drawn renewed interest because FAS is expressed in many malignancies, raising the possibility that its inhibition could treat cancers (55). Should suitable FAS inhibitors become available, targeting them to macrophages might represent a novel approach for treating atherosclerosis.

While this paper was in revision, another group reported that fatty acid synthesis is required for the acquisition of phagocytic capacity in human monocytes and that inhibition of fatty acid synthesis can inhibit lipoprotein uptake during monocyte differentiation (56). Our findings in mice are consistent with these observations in human cells and provide proof of principle that inhibition of macrophage FAS antagonizes foam cell development and vascular disease.

In summary, tissue-specific inactivation of FAS in macrophages decreases diet-induced atherosclerosis in mice through mechanisms that appear to involve the induction of LXRx. The results suggest that FAS generates lipids involved in feedback inhibition of LXRx. Identification of FAS-associated regulatory lipids in macrophages and their subsequent manipulation could provide new insight into the pathophysiology of premature vascular disease.

REFERENCES

1. Stewart, S. T., Cutler, D. M., and Rosen, A. B. (2009) *N. Engl. J. Med.* 361, 2252–2260
2. Wilson, P. W., D’Agostino, R. B., Levy, D., Belanger, A. M., Silbershatz, H., and Kannel, W. B. (1998) *Circulation* 97, 1837–1847
3. Pekkanen, J., Tervahauta, M., Nissinen, A., and Karvonen, M. J. (1993) *Cardiology* 82, 181–190
4. Kannel, W. B., and Schatzkin, A. (1985) *J. Am. Coll. Cardiol.* 1, 58–65
5. LaRosa, J. C., Grundy, S. M., Waters, D. D., Shear, C., Barter, P., Fruchart, J. C., Gotto, A. M., Greten, H., Kastelein, J. J., Shepherd, J., and Wenger, N. K. (2005) *N. Engl. J. Med.* 352, 1425–1435
6. Insull, W., Jr., and Bartsch, G. E. (1966) *J. Clin. Invest.* 45, 513–523
7. Lang, P. D., and Insull, W., Jr. (1970) *J. Clin. Invest.* 49, 1479–1488
8. Stachowska, E., Dolegowska, B., Chlubek, D., Wesołowska, T., Ciechowlowicz, H., and Turowski, R. (2004) *Eur. J. Nutr.* 43, 313–318
9. Fenton, C. V., Crook, D., Davies, M. J., and Oliver, M. F. (1997) *Arterioscler. Thromb. Vasc. Biol.* 17, 1337–1345
10. Felton, C. V., Crook, D., Davies, M. J., and Oliver, M. F. (1994) *Lancet* 343, 1195–1196
11. Lollard, H. B., Clarkson, T. B., and Artom, C. (1960) *Arch. Biochem. Biophys.* 88, 105–109
12. Howard, C. F., Jr. (1968) *J. Lipid Res.* 9, 254–261
13. Whereat, A. F. (1971) *Adv. Lipid Res.* 9, 119–159
14. Whereat, A. F. (1964) *J. Atheroscler. Res.* 4, 272–282
15. Lollard, H. B., Jr., Mouri, D. M., Hoffman, C. W., and Clarkson, T. B. (1965) *J. Lipid Res.* 6, 112–118
16. Semenkovich, C. F. (1997) *Prog. Lipid Res.* 36, 43–53
17. Semenkovich, C. F., Coleman, T., and Fiedorek, F. T., Jr. (1995) *J. Lipid Res.* 36, 1507–1521
18. Chirala, S. S., Chang, H., Matzuk, M., Abu-Elheiga, L., Mao, J., Mahon, K., Finegold, M., and Waki, S. J. (2003) *Proc. Natl. Acad. Sci. U.S.A.* 100, 6358–6363
136–145
47. Joseph, S. B., McKilligin, E., Pei, L., Watson, M. A., Collins, A. R., Laffitte, B. A., Chen, M., Noh, G., Goodman, J., Hagger, G. N., Tran, J., Tippin, T. K., Wang, X., Lusis, A. J., Hsueh, W. A., Law, R. E., Collins, J. L., Willson, T. M., and Tontonoz, P. (2002) Proc. Natl. Acad. Sci. U.S.A. 99, 7604–7609
48. Tangirala, R. K., Bischoff, E. D., Joseph, S. B., Wagner, B. L., Walczak, R., Laffitte, B. A., Daige, C. L., Thomas, D., Heyman, R. A., Mangelsdorf, D. J., Wang, X., Lusis, A. J., Tontonoz, P., and Schulman, I. G. (2002) Proc. Natl. Acad. Sci. U.S.A. 99, 11896–11901
49. Teupser, D., Kretzschmar, D., Tennert, C., Burkhardt, R., Wilfert, W., Fengler, D., Naumann, R., Sippel, A. E., and Thiery, J. (2008) Arterioscler. Thromb. Vasc. Biol. 28, 2009–2015
50. Brunham, L. R., Singaraja, R. R., Duong, M., Timmins, J. M., Fievet, C., Bissada, N., Kang, M. H., Samra, A., Fruchart, J. C., McManus, B., Staels, B., Parks, J. S., and Hayden, M. R. (2009) Arterioscler. Thromb. Vasc. Biol. 29, 548–554
51. Van Eck, M., Singaraja, R. R., Ye, D., Hildebrand, R. B., James, E. R., Hayden, M. R., and Van Berkel, T. J. (2006) Arterioscler. Thromb. Vasc. Biol. 26, 929–934
52. Chawla, A., Boisvert, W. A., Lee, C. H., Laffitte, B. A., Barak, Y., Joseph, S. B., Liao, D., Nagy, L., Edwards, P. A., Curtiss, L. K., Evans, R. M., and Tontonoz, P. (2001) Mol. Cell 7, 161–171
53. Ide, T., Shimano, H., Yoshikawa, T., Yahagi, N., Amemiya-Kudo, M., Matsuzaka, T., Nakakuki, M., Yatoh, S., Iizuka, Y., Tomita, S., Ohashi, K., Takahashi, A., Sone, H., Gotoda, T., Osuga, J., Ishibashi, S., and Yamada, N. (2003) Mol. Endocrinol. 17, 1255–1267
54. Maier, T., Leibundgut, M., and Ban, N. (2008) Science 321, 1315–1322
55. Kridel, S. J., Lowther, W. T., and Pemble, C. W., 4th (2007) Expert Opin. Investig. Drugs 16, 1817–1829
56. Ecker, J., Liebisch, G., Englmaier, M., Grandl, M., Robenek, H., and Schmitz, G. (2010) Proc. Natl. Acad. Sci. U.S.A. 107, 7817–7822