

# A Murine Model of Obesity With Accelerated Atherosclerosis

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The epidemic of obesity sweeping developed nations is accompanied by an increase in atherosclerotic cardiovascular diseases. Dyslipidemia, diabetes, hypertension, and obesity are risk factors for cardiovascular disease. However, delineating the mechanism of obesity-accelerated atherosclerosis has been hampered by a paucity of animal models. Similar to humans, apolipoprotein E-deficient (apoE<sup>-/-</sup>) mice spontaneously develop atherosclerosis over their lifetime. To determine whether apoE<sup>-/-</sup> mice would develop obesity with accelerated atherosclerosis, we fed mice diets containing 10 (low fat (LF)) or 60 (high fat (HF)) kcal % from fat for 17 weeks. Mice fed the HF diet had a marked increase in body weight and atherosclerotic lesion formation compared to mice fed the LF diet. There were no significant differences between groups in serum total cholesterol, triglycerides, or leptin concentrations. Plasma concentrations of the acute-phase reactant serum amyloid A (SAA) are elevated in both obesity and cardiovascular disease. Accordingly, plasma SAA concentrations were increased fourfold ( $P < 0.01$ ) in mice fed the HF diet. SAA was associated with both pro- and antiatherogenic lipoproteins in mice fed the HF diet compared to those fed the LF diet, in which SAA was primarily associated with the antiatherogenic lipoprotein high-density lipoprotein (HDL). Moreover, SAA was localized with apoB-containing lipoproteins and biglycan in the vascular wall. Taken together, these data suggest male apoE-deficient mice are a model of metabolic syndrome and that chronic low level inflammation associated with increased SAA concentrations may mediate atherosclerotic lesion formation.

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## INTRODUCTION

The prevalence of obesity is at an epidemic proportion in the United States and is accompanied by an increase in morbidity and mortality from cardiovascular diseases, including atherosclerosis. Obesity is often associated with a high prevalence of traditional risk factors for atherosclerosis including hyperlipidemia, hypertension, and insulin resistance, as well as with emerging risk factors such as chronic inflammation. However, the mechanisms by which obesity increases atherosclerosis are not fully understood, in part due to a paucity of animal models of obesity-accelerated atherosclerosis.

Although obesity is associated with increased prevalence of metabolic perturbations and increased cardiovascular risk, not all subjects with obesity have these abnormalities (often termed obese, metabolically normal), and some subjects with normal BMI have considerable metabolic abnormalities (termed normal weight, metabolically obese). Accumulating evidence suggests that the location of the excess adipose tissue is of importance to the development of metabolic dysfunction, with abdominal or visceral fat depots thought to convey the

greatest risk. Clinical investigations have played a key role in identifying these associations, but animal models are necessary to elucidate the mechanisms by which obesity accelerates atherosclerosis.

Recent studies have demonstrated that obesity is associated with chronic inflammation of the adipose tissue itself, which is thought to contribute to systemic inflammation, and possibly play a role in the development and progression of atherosclerosis. A number of proinflammatory molecules are secreted by adipocytes and other cells resident within adipose tissue. Moreover, transplantation of visceral fat from obese mice into lean apolipoprotein E (apoE<sup>-/-</sup>) mice increased inflammatory markers, including leptin and monocyte chemoattractant protein-1, and atherosclerotic lesion formation (1). Leptin, an adipokine having a profound effect on appetite regulation, is a marker of total body fat. Although some investigators have proposed that leptin may play a role in atherogenesis (2,3), this has not been supported by other studies (4). Adipose tissue macrophages have been shown to contribute to the pathogenesis of insulin resistance observed in obesity (5,6). However, the

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link between insulin resistance or hyperglycemia with atherosclerosis and cardiovascular disease is also unclear. Obesity is associated with modestly but chronically elevated plasma concentrations of acute-phase reactants serum amyloid A (SAA) and C-reactive protein (7,8), which have been shown to be predictive of cardiovascular disease events. SAA has been proposed to play a role in atherogenesis, whereas there is no evidence that C-reactive protein has a direct proatherogenic role at this time (9–11). Reductions in body weight decrease serum SAA concentrations (8,12,13) and the reduction in SAA concentrations in response to caloric restriction is independent of dietary composition (13) suggesting a direct link of SAA concentrations with adipose mass. Data from a small clinical study suggests that increased SAA production from adipose tissue of obese individuals significantly contributes to the systemic SAA pool (8). Therefore, adipose tissue SAA may play a critical role in the development and/or progression of atherosclerosis.

Animal models of obesity-accelerated atherosclerosis are necessary to elucidate the mechanisms involved in the pathogenesis of atherosclerosis. Rodent models are desirable due to their short lifespan, sequenced genome with availability of genetic manipulation, and relative low cost. However, models that reliably develop features of the metabolic syndrome and obesity-accelerated atherosclerosis are lacking. The goal of this study was to develop a murine model of diet-induced obesity with accelerated atherosclerosis. Similar to humans, apoE<sup>-/-</sup> mice develop atherosclerosis spontaneously over their lifetime. Despite previous studies that have reported that apoE<sup>-/-</sup> mice are resistant to diet-induced obesity and accelerated atherosclerosis (14,15), we demonstrate that diets high in saturated fat induce obesity, insulin resistance, modest systemic inflammation and accelerated atherosclerosis in apoE<sup>-/-</sup> mice.

## METHODS AND PROCEDURES

### Animals

Male apoE<sup>-/-</sup> mice backcrossed 10 times onto a C57BL/6 background were obtained from the Jackson Laboratories (Bar Harbor, ME). Mice were housed in specific pathogen-free rooms and fed a normal laboratory diet (Harlan Teklad diet 2918) prior to commencement of studies. All studies were approved by the University of Kentucky Institutional Animal Care and Use Committee. Eight-week-old male apoE<sup>-/-</sup> mice were fed diets containing 10 or 60% kcal from fat (D124508 and D12492, respectively; Research Diets, New Brunswick, NJ). These diets contain no added cholesterol, and thus their total cholesterol content is 0.002 and 0.003% by weight, respectively (compare to 0.15% cholesterol by weight in the standard atherogenic Western diet TD88137, Harlan Teklad). The mice received diets ad lib for 17 weeks and had free access to water. Systolic blood pressure was measured in conscious mice by tail cuff using a Visitech BP-2000 Platform (16). Systolic pressure was defined as the mean of 10 individual measurements for each individual mouse per recording session. Mice were acclimated for 5 days prior to data collection. Blood pressure was measured 5 days during the final week (week 17) of diet.

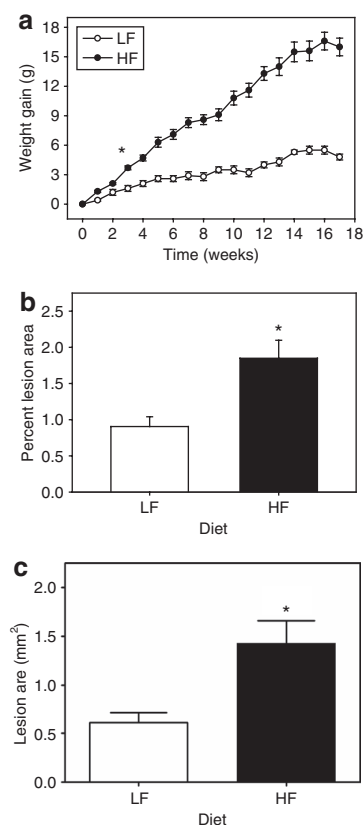
### Metabolic analyses

Intraperitoneal glucose tolerance test was performed in all mice after 16 weeks. Mice were fasted overnight (12 h) then injected intraperitoneal with glucose (20% solution) at a dose of 2 g glucose/kg body weight. Plasma glucose was measured by glucometer (Freestyle) prior to and at 30, 60, 90, and 120 min after glucose injection. After 17 weeks

of diet mice were killed, blood was collected and plasma leptin, SAA, and adiponectin concentrations were measured by specific enzyme-linked immunosorbent assays or Milliplex assay (Linco Research, Biosource, and Millipore, respectively) according to manufacturers' directions. SAA isoforms were characterized by isoelectric focusing, as described previously (17). Serum cholesterol and triglyceride concentrations were measured by enzymatic colorimetric assay (Wako Chemical Company) as described previously. Plasma aliquots from individual mice were separated by fast protein liquid chromatography as previously described (18) and cholesterol and triglyceride content of each fraction quantified. Equal aliquots of fractions from the peaks of each lipoprotein compartment (very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL)) were analyzed by western blot for SAA content (antibody from Lab Logix, Belmont, CA). Liver triglyceride content was extracted by Bligh Dyer Method and triglycerides measured by enzymatic colorimetric assay (Wako Chemical).

### Cytokine/chemokine analyses

Plasma monocyte chemoattractant protein-1, interleukin-6, and tumor necrosis factor- $\alpha$  concentrations were measured by Milliplex assay (Millipore, Billerica, MA). Total RNA was isolated from mouse liver using the Aurum Total RNA Mini Kit (BioRad, Hercules, CA). 1  $\mu$ g of RNA was reverse transcribed into cDNA using an iScript cDNA Synthesis Kit (BioRad). After 20-fold dilution, 5  $\mu$ l was used as a template for quantitative PCR. Primers used in this study are listed in **Supplementary Table S1** online. Amplification was done for 40 cycles using iQ SYBR Green Supermix (BioRad) on a My iQ Cycler (BioRad). Both internal control (18S rRNA) and negative control (minus reverse transcriptase) were included. Values of each RNA sample were the average of triplicate assays normalized toward 18S rRNA (internal control) levels.



**Figure 1** Feeding a high fat diet increases (a) weight gain and atherosclerotic lesion formation in the (b) descending aorta, and (c) aortic sinus in apoE<sup>-/-</sup> mice. Data represents mean  $\pm$  s.e.m. ( $n = 22$ – $26$  mice/group; \*denotes  $P \leq 0.001$ ;  $P = 0.02$ ;  $P = 0.016$ , respectively).

### Atherosclerosis quantification

Mouse aortas were removed and fixed in paraformaldehyde overnight. Aortas were cut, pinned, and photographed for *en face* measurements of atherosclerosis. Aortic root sections were collected beginning at the appearance of the valve leaflets, stained with Oil Red O, and lesions were quantified over 400  $\mu\text{m}$ . Images of aortas and aortic roots were captured by a digital camera (Nikon DXM1200, Nikon, Melville, NY), and lesion area was measured by Image-Pro software. Quantitative analysis of atherosclerosis was performed as described previously (19). Adjacent sections were immunostained using antibodies against apoB (BioDesign, Saco, ME), biglycan (R&D Systems, Minneapolis, MN) and SAA (Santa Cruz Biotechnology, Santa Cruz, CA) as previously described (11,20).

### Statistical analyses

Data were analyzed by Student's *t*-test or ANOVA with repeated measures, as appropriate. Significant interactions identified by ANOVA were analyzed using a Tukey post hoc test for all pairwise comparisons. Nonparametric data was analyzed by Mann-Whitney Rank sum test or significant interactions were analyzed using a Holm-Sidak multiple comparison, where appropriate. All data analyses were performed using SigmaStat 3.5 software (SPSS, Chicago, IL). All data are represented as means  $\pm$  s.e.m.  $P < 0.05$  values were considered to be statistically significant.

## RESULTS

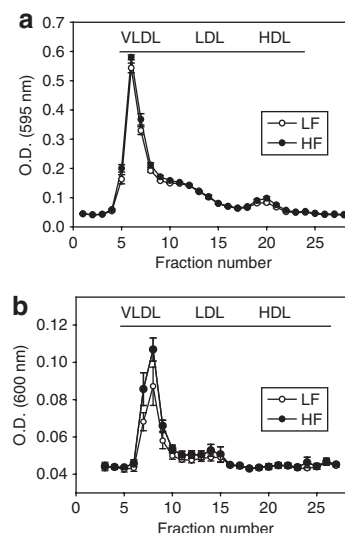
To determine the effect of obesity on atherosclerosis, 8–10-week-old male apoE<sup>-/-</sup> mice were placed on either a low fat (LF), or high fat (HF) diet, 10 or 60 kcal % fat respectively, for 17 weeks. Body weight was measured weekly throughout the 17 week time course of the study. Body weight significantly differed between groups following 3 weeks of feeding the HF diet compared to LF diet (Figure 1a). Moreover, body weight increased linearly in mice fed the HF diet, throughout the time course of the study. Although, mice fed the LF diet consumed more food on a daily basis than those fed a HF diet; caloric consumption was increased in HF-fed mice compared to those on the LF diet (Table 1). The distribution of adiposity was measured in liver, epididymal, and retroperitoneal adipose tissue. Mice fed the HF diet had a marked increase in percent

**Table 1** Body and tissue weights

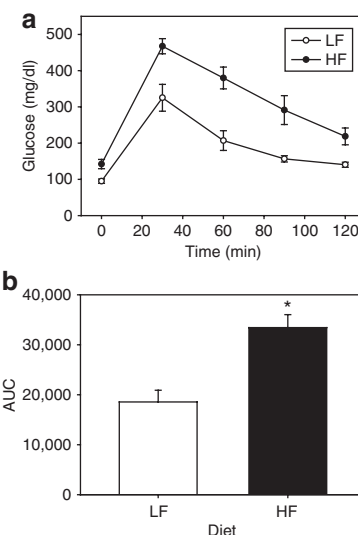
	LF diet	HF diet	<i>P</i> value
Body weight (beginning, g)	24.5 $\pm$ 0.5	23.8 $\pm$ 0.4	NS
Body weight (ending, g)	29.5 $\pm$ 0.7	40.1 $\pm$ 1.0	$P < 0.001$
Epididymal fat (percent body weight)	1.41 $\pm$ 0.16	4.30 $\pm$ 0.43	$P < 0.001$
Retroperitoneal fat (percent body weight)	0.39 $\pm$ 0.04	1.85 $\pm$ 0.18	$P < 0.001$
Liver (percent body weight)	4.28 $\pm$ 0.28	4.11 $\pm$ 0.29	NS
Food consumption (g/day)	3.57 $\pm$ 0.07	2.92 $\pm$ 0.03	$P < 0.001$
Caloric density consumption (kcal/g/day)	13.46 $\pm$ 0.32	15.32 $\pm$ 0.17	$P < 0.001$

NS, nonsignificant.

body weight of both epididymal and retroperitoneal adipose tissue depots; however, feeding a HF diet did not alter percent body weight of the liver (Table 1). To determine if the obese mice had increased atherosclerosis, atherosclerotic lesion area was quantified in the descending aorta and aortic sinus. Atherosclerosis was markedly increased in both sites in mice fed the HF diet (Figure 1b,  $P = 0.02$ ; Figure 1c,  $P = 0.016$ ; Supplementary Figure S1a). Moreover, increased weight gain correlated with increased atherosclerotic lesion formation (Supplementary Figure S1b;  $R^2 = 0.287$ ;  $P = 0.001$ ).



**Figure 2** Feeding a high fat diet does not alter (a) cholesterol lipoprotein distribution, but (b) increased the distribution of triglyceride into very low-density lipoprotein in apoE<sup>-/-</sup> mice. Data represent mean  $\pm$  s.e.m. ( $n = 10$  mice/group).



**Figure 3** Glucose tolerance is altered in apoE<sup>-/-</sup> mice fed a high fat diet. (a) Mice were injected with a 20% glucose solution (2g glucose/kg body weight) and plasma glucose levels were measured by glucometer every 30 min over a 120 min time period. (b) Area under the curve was measured in individual mice. Data represents mean  $\pm$  s.e.m. ( $n = 13$  mice/group). \* $P = 0.003$ .

Total serum cholesterol concentrations were modestly, but not significantly increased in mice fed the HF diet (LF:  $513 \pm 15$  vs. HF:  $601 \pm 23$  mg/dl;  $P =$  nonsignificant) and the cholesterol lipoprotein distribution was not different between the two groups (Figure 2a). Consumption of the HF diet for 17 weeks did not alter total plasma triglyceride concentrations compared to consumption of the LF diet (LF:  $96 \pm 31$  vs. HF:  $101 \pm 25$  mg/dl,  $P =$  nonsignificant); however, the distribution of triglyceride among lipoproteins was modestly increased in VLDL fraction from mice fed the HF diet (Figure 2b). Moreover, liver content of triglyceride was increased in mice fed the HF diet (Supplementary Figure S2a online). However, liver expression of a number of inflammatory genes was not changed in response to feeding a HF diet for 17 weeks (Supplementary Figure S2b online). Previous studies have demonstrated that neither Western nor diabotogenic diets altered serum glucose concentrations in apoE<sup>-/-</sup> mice (15). In marked contrast, fasting glucose concentrations were markedly increased in male apoE<sup>-/-</sup> mice fed the HF diet for 17 weeks (LF:  $103 \pm 11$  vs. HF:  $147 \pm 7$  mg/dl;  $P < 0.001$ ). To further characterize the hyperglycemia, we performed an intraperitoneal glucose tolerance test following 16 weeks of feeding the diets. Glucose levels peaked 30 min following intraperitoneal loading of glucose (Figure 3a). There was a marked increase in the area under the curve in mice fed the HF diet compared to LF diet demonstrating impaired glucose tolerance in mice fed the HF diet (Figure 3b,  $P = 0.003$ ). These data suggest that obesity due to consumption of a HF diet induces glucose intolerance in male apoE<sup>-/-</sup> mice.

Systolic blood pressure was measured during the final week of diets and did not differ between the two groups of mice (LF:  $118 \pm 3$  vs. HF:  $113 \pm 1$  mm Hg,  $P =$  nonsignificant). As expected, leptin concentrations were increased in obese HF-fed mice compared to lean mice (Table 2), although this

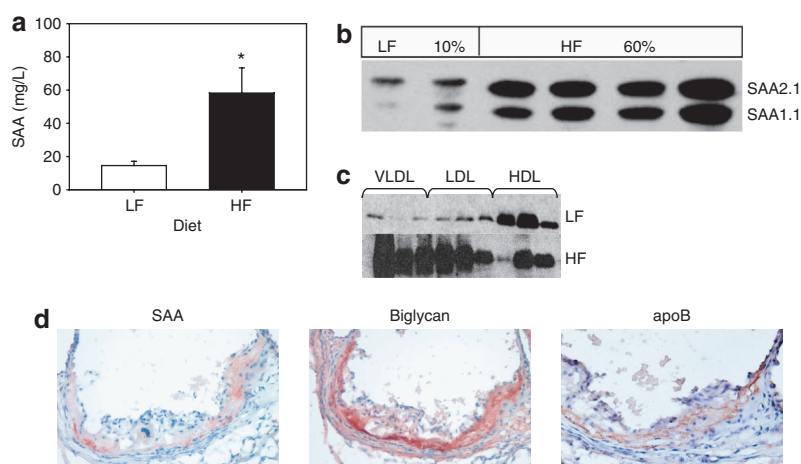
did not reach statistical significance ( $P = 0.068$ ). However, as expected increases in body weight are highly correlated ( $R^2 = 0.9132$ ;  $P < 0.001$ ) with increases in leptin concentrations (data not shown). Obesity is associated with increased levels of inflammatory markers. Cytokines and chemokines, known to be associated with obesity, including adiponectin, monocyte chemoattractant protein-1, interleukin-6 and tumor necrosis factor- $\alpha$  were measured after 17 weeks of diet, and no significant differences between groups were observed (Table 2). However, serum SAA concentrations were markedly elevated in mice fed the HF diet compared with LF diet (Figure 4a). Isoelectric focusing demonstrated that both acute-phase SAAs (SAA1.1 and SAA2.1) were increased in serum from mice fed the HF diet compared to the LF diet (Figure 4b). Although SAA is primarily carried on HDL, previous studies have suggested that HF diets may promote a redistribution of SAA to the proatherogenic lipoproteins VLDL and LDL (9,21,22). In agreement with these findings, western blot analyses demonstrated a marked increase in SAA content in VLDL and LDL fractions in mice fed the HF diet (Figure 4c).

Recently, we demonstrated that SAA stimulated the synthesis of vascular proteoglycans, increased their LDL binding affinity, and specifically up-regulated biglycan (11).

**Table 2 Serum adipokine concentrations**

	LF diet	HF diet	<i>P</i> value
Leptin (ng/ml)	$5.1 \pm 1.4$	$17.4 \pm 3.4$	$P = 0.068$
Adiponectin (mg/ml)	$1.0 \pm 0.5$	$1.1 \pm 0.5$	$P = 0.181$
MCP-1 (pg/ml)	$27.4 \pm 5.0$	$18.3 \pm 4.6$	$P = 0.179$
IL-6 (pg/ml)	$26.3 \pm 7.0$	$13.5 \pm 2.0$	$P = 0.249$
TNF- $\alpha$ (pg/ml)	$5.5 \pm 0.6$	$5.5 \pm 0.5$	$P = 0.949$

IL, Interleukin-6; MCP-1, monocyte chemoattractant protein-1; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .



**Figure 4** A high fat (HF) diet increases (a) plasma concentrations of serum amyloid A. Data represent the mean  $\pm$  s.e.m. ( $n = 22$ – $26$  mice/group).  $*P = 0.01$ . (b) Isoelectric focusing demonstrates that both SAA 1.1 and 2.1 expression are increased in plasma from mice fed a HF diet. Shown are representative blots with each lane representing one mouse from each group. (c) Immunoblot analysis demonstrates that SAA is highly associated with plasma very low-density lipoprotein and LDL in mice fed a HF diet. Shown are representative blots from one mouse from each group. Lanes were loaded with equal aliquots of (b) plasma and (c) fast protein liquid chromatography fractions. (d) Immunocytochemistry demonstrates SAA colocalizes with biglycan and apoB. Shown are representative adjacent sections from an atherosclerotic lesion from an apoE<sup>-/-</sup> mice fed a HF diet. (200 $\times$  magnification).

We proposed that this would be proatherogenic as increased vascular proteoglycan (biglycan) content would be expected to lead to increased lipoprotein retention (23–26). Accordingly, immunohistochemical analyses of atherosclerotic lesions demonstrated colocalization of SAA with biglycan and apoB within atherosclerotic lesions from mice fed a HF diet (Figure 4d).

## DISCUSSION

Taken together, these data demonstrate that consumption of a HF diet promotes obesity-accelerated atherosclerosis in apoE<sup>-/-</sup> mice, accompanied by development of a metabolic syndrome phenotype. Similar to humans, the obese mice developed obesity, impaired fasting glucose, impaired glucose tolerance, modest dyslipidemia, and increased levels of the inflammatory marker SAA. The single major feature of metabolic syndrome lacking in this model is that of hypertension. Plasma leptin concentrations were increased in mice that became obese as expected, albeit this increase did not reach statistical significance. Thus, apoE<sup>-/-</sup> mice fed HF diets are an animal model of obesity with accelerated atherosclerosis.

Furthermore, this murine model allows some understanding of mechanisms by which obesity may accelerate atherosclerosis development. The LF and HF-fed mice did not differ in many of the major known risk factors for atherosclerosis formation including total cholesterol levels, lipoprotein cholesterol distribution, or blood pressure. However, HF-fed mice did have impaired glucose tolerance, increased triglyceride-rich lipoprotein particles, a trend towards increased leptin levels, and markedly increased plasma SAA levels with altered SAA lipoprotein distribution compared to LF-fed mice. Any or all of these metabolic factors may have contributed to the atherosclerosis development. Although the impaired glucose tolerance observed in HF-fed mice may contribute towards the increased atherosclerosis observed, large clinical trials have struggled to clearly identify a link between glucose concentrations and cardiovascular diseases. Similarly, clinical studies are controversial as to whether elevated plasma triglycerides are a risk factor for cardiovascular disease. Although we did not find a difference in total plasma triglycerides between groups, we did observe increased triglyceride-rich lipoproteins and increased hepatic triglycerides in HF-fed mice, and thus we cannot exclude a contribution of these lipoproteins to the increased atherosclerosis observed. The role of leptin in atherosclerosis formation is also controversial. The leptin receptor is expressed on vascular cells under both normal and pathological conditions, as well as on inflammatory cells that have migrated into the vascular wall during the development of atherosclerosis (27). Previous studies have demonstrated that exogenous administration of leptin augments atherosclerosis (2). Paradoxically, deficiency of either leptin or its receptor also increased lesion formation (4,28). However, deficiency of leptin or its receptor resulted in marked increases in both plasma cholesterol and triglyceride concentrations that may have confounded interpretation of the direct effect of leptin on atherosclerotic lesion formation. Therefore, we cannot exclude the possibility that

increases in leptin may have contributed to the increase in atherosclerotic lesion formation observed in mice fed the HF diet. However, the most marked difference observed between HF and LF-fed mice was in the total SAA concentration and its lipoprotein distribution. This increase in SAA was due to increased amounts of both acute-phase isoforms, SAA1.1 and SAA2.1. During an acute-phase response plasma SAA levels can increase up to a 1,000-fold. Interestingly, the fourfold increase in plasma SAA observed in the obese mice is similar to the chronic modest elevations of SAA observed in obese patients (8,12,29).

Clinical studies have demonstrated an association between elevated levels of the acute-phase proteins SAA and C-reactive protein with increased risk of cardiovascular events (30–32). C-reactive protein is not an acute-phase protein in mice, but SAA protein and mRNA have been detected in both human and mouse atherosclerotic lesions (33). Moreover, SAA mRNA expression has been localized to smooth muscle cells, endothelial cells, and macrophages derived from atherosclerotic plaques (33). Previous studies demonstrated that plasma SAA concentrations correlate with atherosclerotic lesion size in hyperlipidemic mice (9,21), and this correlation was independent of plasma cholesterol concentrations. Although these studies do not prove a causal role for SAA in the development and progression of atherosclerosis, they are suggestive that SAA may play a fundamental role.

During an acute-phase response the liver is the primary source of SAA production. The acute-phase response in humans results in SAA association primarily with HDL. Interestingly, our data demonstrates that a large concentration of SAA associates with the proatherogenic lipoproteins VLDL and LDL in apoE<sup>-/-</sup> mice with diet-induced obesity. SAA association with VLDL and LDL is also markedly increased in LDL receptor-deficient mice fed diets enriched in saturated fat (9,22). We propose that both the elevated SAA and SAA lipoprotein redistribution observed in HF-fed mice may contribute to the increased atherosclerosis observed.

The response-to-retention hypothesis suggests that lipoprotein binding to proteoglycans in the subendothelial space is a key step in the development and/or progression of atherosclerosis (24,34). This concept is supported by data demonstrating that atherosclerotic lesion formation is attenuated in mice expressing proteoglycan-binding-defective lipoproteins (34). SAA itself is able to bind proteoglycans, which is likely relevant to its role in vascular pathology (9,21). The basic C-terminal residues of SAA are particularly important for its binding to proteoglycans. Additionally, we recently demonstrated that SAA increased proteoglycan synthesis by vascular smooth muscle cells, and in particular, upregulated biglycan (11). This is of particular interest as we and others have demonstrated that biglycan is the proteoglycan most closely associated with apoB, thus may have a major role in atherosclerosis development (11,20,35,36). In addition, SAA itself can bind to proteoglycans, and HDL containing SAA has greater proteoglycan binding than HDL without SAA (9,21). Previous work by Flood et al has demonstrated that proteoglycan

binding proteins can act cooperatively to increase lipoprotein-proteoglycan interactions (37). In the present study, we demonstrate the colocalization of SAA in the subendothelial space with biglycan and apoB. These findings could be due to either increased retention of apoB-containing lipoprotein particles that also contain SAA (as in **Figure 4c**), or increased retention of lipoprotein particles due to SAA-induced upregulation of biglycan, or a combination of the above.

Previous studies have suggested that apoE<sup>-/-</sup> mice are resistant to the development of obesity; however, those studies used a diabetogenic diet (14,15). Conversely, our data demonstrates that as early as 3 weeks following consumption of a diet enriched in lard, apoE<sup>-/-</sup> mice gain significant body weight, which increased linearly for the 17 week time course of these studies. The diabetogenic diet, which did not cause obesity, contains 35.5 g% fat and 36.3 g% carbohydrate, primarily sucrose. The HF diet we used, which did cause obesity, was fairly similar in composition, containing 34.9 g% fat and 26.3 g% carbohydrate, also primarily sucrose. The concentration and the primary source of fat (lard) is similar between our HF diet and the previous diabetogenic diets, suggesting that the increase in body weight observed in the present study is not solely attributable to the fat in the diet and may be accounted for by differences in other constituents in the diet (14,15). An important feature of the diets used in the present study is that they only differed in concentrations of constituents, rather than diet composition. In contrast, previous studies investigating the effect of diabetogenic diets on obesity and atherosclerotic lesion formation compared diets which had a marked difference in dietary constituents.

In summary, feeding apoE<sup>-/-</sup> mice a HF diet enriched in lard induces a metabolic phenotype which is characterized by obesity, modest dyslipidemia, impaired glucose tolerance, and accelerated atherosclerosis. Similar to humans, the increase in obesity and increased cardiovascular disease is associated with increases in plasma SAA concentrations. We speculate that the increase in SAA and the association of SAA with proatherogenic lipoproteins in obese mice contributes to the increase in atherosclerotic lesion formation observed.

#### SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/oby>

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#### DISCLOSURE

The authors declared no conflict of interest.

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