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Perivascular Visceral Adipose Tissue Induces Atherosclerosis in Apolipoprotein E Deficient:

Mice Öhman: Perivascular Fat and Atherosclerosis

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Abstract

Objective—picardial adipose tissue is associated with coronary artery disease, however the causal relationship between perivascular adipose tissue and local atherogenesis is unclear.

Methods and Results—Apolipoprotein E deficient (*ApoE*$^{-/-}$) mice underwent transplantation of visceral or subcutaneous adipose tissue immediately adjacent to the right common carotid artery. Carotid arteries with fat transplants were analyzed for atherosclerosis by surface oil-red-O staining and cross-sectional analysis. Vascular function of the carotid arteries was assessed using pressure myography. Visceral fat transplants were also performed to *ApoE*$^{-/-}$ mice with neutralization of P-selectin glycoprotein ligand-1 (Psgl-1). Atherosclerosis surface area and lesion thickness were greater in mice receiving the perivascular visceral fat compared to the subcutaneous fat. Mice with visceral fat transplants also displayed more complicated atherosclerotic lesions with evidence of atherothrombosis. Serum Mcp-1 was higher in mice receiving visceral fat transplants compared to subcutaneous transplants. Visceral fat transplantation also caused impaired endothelial-dependent relaxation of the carotid artery. Psgl-1 deficiency or neutralization of Psgl-1 with an anti-Psgl-1 antibody was protective against perivascular visceral adipose tissue-induced atherosclerosis and was associated with reduced Mcp-1 levels.

Conclusions—Perivascular visceral fat leads to endothelial dysfunction and accelerated atherosclerosis. This proatherogenic effect of perivascular adipose tissue is blocked by neutralization of Psgl-1.

Keywords

atherosclerosis; adipocyte; cytokine; visceral obesity

INTRODUCTION

Excessive visceral adipose tissue is independently associated with cardiovascular events [1]. In addition to triggering a systemic inflammatory state [2], visceral adipose tissue may also

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DISCLOSURES
None.

exert effects on local, adjacent tissues. For example, increases in the volume of the adipose tissue that overlays the coronary arteries, the epicardial fat, is associated with increased presence of noncalcified coronary plaques [3, 4] and total coronary occlusions [5]. This association is independent of total visceral fat mass, hypertension, dyslipidemia, and diabetes, suggesting there may be a direct local effect of the epicardial fat on coronary atherosclerosis. However, the causal role of perivascular fat on the progression of atherosclerosis in arteries directly underlying the adipose tissue is unknown.

We have previously shown that transplantation of adipose tissue can be used as a tool to study effects of inflammatory fat (i.e. fat with increased macrophage content compared to lean endogenous fat) on clinically relevant endpoints without other confounding metabolic effects of obesity [6]. To determine whether perivascular, inflammatory adipose tissue is sufficient to cause endothelial dysfunction and atherosclerosis, we performed transplantation of visceral and subcutaneous fat to the common carotid artery of atherosclerosis-prone, apolipoprotein E deficient ($ApoE^{-/-}$) mice. We also examined the effect of P-selectin glycoprotein ligand-1 (Psgl-1) deficiency on the effect of perivascular transplanted fat on atherosclerosis since deficiency of Psgl-1 has been shown to be protective against both visceral adipose tissue inflammation and atherosclerosis [7, 8].

MATERIALS AND METHODS

Mice

10 week old male $ApoE^{-/-}$ and combined $ApoE^{-/-}$,$Psgl\text{-}1^{-/-}$ mice were used, all on the C57BL/6J background strain. Original breeding pairs were purchased from Jackson Laboratory (Bar Harbor, ME). $ApoE^{-/-}$,$Psgl\text{-}1^{-/-}$ mice were generated by first crossing $ApoE^{-/-}$ mice to $Psgl\text{-}1^{-/-}$ mice and then intercrossing $ApoE^{+/-}$,$Psgl\text{-}1^{+/-}$ breeders to produce $ApoE^{-/-}$,$Psgl\text{-}1^{-/-}$ and $ApoE^{-/-}$,$Psgl\text{-}1^{+/+}$ littermates. Mice were housed in specific pathogen-free facilities and were fed a normal chow diet (Laboratory Rodent Diet 5001, 5% fat, LabDiet, New Brunswick, NJ) prior to and 4 wks following the surgery (to facilitate healing), after which they were switched to Western diet (Rodent Western Diet #D12079B, Research Diet, New Brunswick, NJ) for 4 wks. Mice were sacrificed 8 wks post-operatively.

All procedures conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by the University of Michigan Committee on Use and Care of Animals.

Adipose Transplantation to Carotid Artery

All recipient mice were anesthetized with intraperitoneal (i.p.) injection of sodium pentobarbital (67 mg/kg). Sixty mg of visceral (epididymal) adipose tissue was removed from $ApoE^{-/-}$ donors, and implanted adjacent to the right common carotid artery of the recipient $ApoE^{-/-}$ and $ApoE^{-/-}$,$Psgl\text{-}1^{-/-}$ mice. Control $ApoE^{-/-}$ mice received 60 mg of subcutaneous inguinal fat. Sham-operated $ApoE^{-/-}$ mice underwent the same surgery without a fat transplant. Skin was sutured with 6-0 nylon filament.

Measurements of Mcp-1, insulin, glucose and cholesterol

Blood samples from mice were collected by retro-orbital bleeding using capillary tubes (Kimble Chase, Vineland, NJ). Commercially available murine ELISA kits were used to measure monocyte chemoattractant protein-1 (Mcp-1) (R&D Systems, Minneapolis, MN) and fasting insulin (Crystal Chem Inc., Downers Grove, IL). Glucose was measured after overnight fasting with a glucometer using test strips (Ascensia ® Contour, Bayer HealthCare LLC, Mishawaka, IN) and total cholesterol was measured with a colorimetric assay (Wako Chemicals USA, Inc., Richmond, VA).

Atherosclerosis Quantification

Eight weeks after the operation, mice were euthanized with sodium pentobarbital (67 mg/kg) and blood was collected via cardiac puncture. Animals were perfused with PBS at physiologic pressure, and then fixed using formalin with a 25-gauge needle inserted into the left ventricle, at a rate of 1 ml/min. The carcass was fixed in formalin and the arterial tree was then dissected and placed in 70% ethanol. After removing the connective tissue from the arterial trees, the right common carotid arteries were left intact, stained with oil-red-O and pinned on wax. Atherosclerotic lesion area was assessed as % coverage of the right carotid tube. To quantitate lesion thickness, right carotid arteries were analyzed by cross sections. Sections of the paraffin embedded right common carotid arteries were cut at 5 μm intervals at the site of the adipose transplant. 3 different areas were sampled along the carotid artery spaced 100 μm apart. All images were analyzed with Image-Pro Plus software (Media Cybernetics).

Immunohistochemistry

Immunohistochemistry was performed to characterize lesion composition. Carotid artery cross sections were stained with hematoxylin & eosin (H&E), monoclonal antibodies to Mac-3 (BD Biosciences, San Jose, CA), and α-smooth muscle actin (Millipore (Chemicon), Billerica, MA) as previously described [9] to quantify macrophages and smooth muscle cells, respectively. A goat anti-mouse fibrin(ogen) polyclonal antibody (Nordic Immunological Laboratories, The Netherlands), and Masson Trichrome staining (Sigma-Aldrich, St Louis, MO) were used to quantify fibrin(ogen) and connective tissue elements in the plaque, respectively. Transplanted adipose tissue was removed from the dissected artery at the time of sacrifice, fixed in formalin and embedded in paraffin. Adipose tissue cross sections were cut at 5 μm intervals and stained with the Mac-3 monoclonal antibody (BD Biosciences, San Jose, CA) for macrophages and rabbit polyclonal antibody to CD31 (PECAM-1) (Abcam, Cambridge, MA) for endothelium. Three fields (40x magnification) were studied per slide.

Pressure myography

Carotid artery fat transplantation was performed to 10 week old *ApoE*$^{-/-}$ mice with visceral and subcutaneous adipose tissue. Mice were fed with standard chow for one week post-operatively followed by Western diet for 1.5 weeks. Mice were sacrificed earlier at 2.5 weeks following transplantation in attempts to avoid development of severe lesions in the transplanted carotid artery, which might confound the myograph procedure. Following anesthesia with sodium pentobarbital (67 mg/kg, i.p.) and exsanguination via right ventricle phelobotomy, a segment of the right common carotid artery distal to the fat transplant site was removed and placed into a silastic-elastomer lined petridish filled with Physiological Salt Solution (PSS) (equilibrated with 5% CO_2- 95% O_2) containing (in mmol/L): NaCl 120, KCl 4.7, $MgSO_4$ 1.18, $CaCl_2$ 2.5, KH_2PO_4 1.18, $NaHCO_3$ 25, glucose 5.5, EDTA 0.026, pH 7.4. The surrounding connective tissue was gently cleared and a vessel segment 3 mm in length was mounted onto glass cannulas of the pressure myograph (Living Systems, VT). One cannula was adjusted with axial direction of vessel until walls of vessel were parallel without any stretch. Vessels were then equilibrated in PSS at 37°C (60 minutes, 80 mmHg intraluminal pressure). The real-time dimension of the vessel wall was detected and analyzed with a video dimension analyzer (Living Systems, VT). Vascular activity was tested under no-flow condition. After equilibration, vascular contraction was assessed by measuring the constrictive response of lumen diameter to cumulatively applied phenylephrine (PE) (Sigma-Aldrich, St. Louis, MO) (10^{-9} to 10^{-3} mol/L). After washing and equilibration for one hour, endothelium-dependent relaxation was assessed by measuring the dilatory response to acetylcholine (Ach) (Sigma-Aldrich, St. Louis, MO) (10^{-9} to 10^{-5} mol/L) in PE precontracted vessels (10^{-5} mol/L).

Antibody injections to animals

Starting two weeks post-operation, a rat anti-mouse Psgl-1 antibody (4RA10) or control IgG k isotype (BD Pharmingen, Franklin Lakes, NJ) was injected via tail vein (50μg in 200μl 1xPBS) into transplanted mice once a week for 3 weeks. Mice were fed standard chow for 2 weeks post-operatively, followed by 6 weeks of Western diet. Mice were sacrificed 8 weeks post-operatively.

Statistical Analysis

The statistical significance of differences between groups was determined by 1-way ANOVA followed by Tukey's Studentized Range (HSD) post-hoc test for multiple comparisons, or Student's *t* test when comparisons were made between 2 groups. Values are expressed as mean ± SEM. $P<0.05$ are considered significant.

RESULTS

Perivascular inflammatory adipose tissue increases local atherosclerosis

To first determine if an adipose tissue transplant overlying the right common carotid artery would lead to formation of a local atherosclerotic plaque, *ApoE*$^{-/-}$ mice underwent visceral fat transplant or sham operation to the mid right common carotid artery, a site that typically does not develop spontaneous atherosclerosis. Eight weeks after the transplantation, mice receiving the visceral fat transplant (n=5) had large lipid-rich atherosclerotic lesions of the right common carotid artery while sham operated (n=4) mice did not display any lesions (Fig. 1A, 1B). To next determine whether the type of fat affected the local lesion formation, another experiment was performed transplanting *ApoE*$^{-/-}$ mice with subcutaneous fat (n=5). Mice with subcutaneous adipose transplants had significantly smaller lipid-rich lesion area than mice with visceral fat transplants, while there was no significant difference compared to sham operated animals (Fig. 1C). To determine whether the effect of the different types of fat on atherosclerosis was related to macrophage content of the transplanted fat, Mac3 immunostaining was performed on transplanted fat and endogenous fat depots. Transplanted visceral adipose tissue revealed marked increase in macrophages compared to the endogenous visceral (epididymal) depot (38.1±2.2 vs. 7.5±2.0%, $p<3.7\times10^{-6}$) (Fig. 2), while there was no difference between transplanted visceral and transplanted subcutaneous fat (38.1±2.2 vs. 33.6±2.0%, p=NS). Transplanted fat was also stained with antibody to CD31 (PECAM-1) to determine vascularization of the tissue. There was no difference in the number of vessels between visceral and subcutaneous fat transplants (1.5±0.5 vs 1.6±0.1 vessels per 40x field, p=NS).

In order to further characterize the atherosclerotic lesions triggered by the fat transplant, new groups of *ApoE*$^{-/-}$ mice were transplanted with visceral (n=9) and subcutaneous (n=9) adipose tissue to the right carotid artery for cross-sectional analysis of lesion thickness and composition. Mice receiving visceral fat had greater lesion area compared to mice receiving subcutaneous fat transplants (152446±48691 vs. 5349±3617 μm^2, $p<0.008$) (Fig. 3) and higher intima/media ratio (0.6±0.2 vs. 0.02±0.01, $p<0.02$). There was no difference in the medial area between *ApoE*$^{-/-}$ mice with visceral fat and *ApoE*$^{-/-}$ mice with subcutaneous fat transplants (268902±28899 vs. 227659±26969 μm^2, p=NS).

There were no differences in body weight (29.9±0.6 vs. 30.3±0.5 g, p=NS), fasted insulin (1.5±0.8 vs. 1.0±0.3 ng/ml, p=NS), fasted glucose (115.8±15.1 vs. 127.4±13.5 mg/dl, p=NS) and total cholesterol levels (222.1±13.1 vs. 205.8±29.1 mg/dl, p=NS) between *ApoE*$^{-/-}$ mice with visceral versus subcutaneous fat transplants.

Perivascular visceral adipose tissue increases serum Mcp-1 and triggers more complicated lesion formation

Since Mcp-1 is a marker of adverse vascular effects related to visceral fat inflammation, circulating Mcp-1 was measured from recipient mice 8 weeks post-operation. *ApoE*$^{-/-}$ mice with visceral fat transplants had significantly higher serum Mcp-1 compared to *ApoE*$^{-/-}$ mice with subcutaneous fat transplants (66.0±5.6 vs. 46.7±2.0 pg/ml, p<0.006). Comparison of serum Mcp-1 levels of the fat transplanted *ApoE*$^{-/-}$ mice to age-matched control *ApoE*$^{-/-}$ mice without fat transplantation (n=7) revealed no difference between the control mice and mice with subcutaneous fat transplants (48.9±5.1 vs. 46.7±2.0 pg/ml, p=NS, respectively). Mice with visceral fat transplants had significantly higher Mcp-1 compared to non-transplanted control mice (66.0±5.6 vs. 48.9±5.1, p<0.05, respectively). Cross sectional analysis of carotid artery atherosclerotic lesions revealed more area occupied by macrophages (41.7±14.6 vs. 4.6±0.9 μm^2, p<0.05) and more extensive fibrin deposition (24610±7474 vs. 2903±2698 μm^2, p<0.05) in mice with visceral fat transplants compared to subcutaneous transplants, respectively. Visceral fat transplanted mice showed large subocclusive plaques with areas of intense fibrin staining suggestive of intraplaque hemorrhage (Fig. 4), a finding not observed in mice with subcutaneous transplants. There were no differences between *ApoE*$^{-/-}$ mice with visceral fat compared to *ApoE*$^{-/-}$ mice with subcutaneous fat in the lesion content of connective tissue (18.4±10.3 vs. 9.0±7.7 μm^2, p=NS) or smooth muscle cells (11.4±5.3 vs. 5.4±3.1 μm^2, p=NS). Cross sections from sham-operated *ApoE*$^{-/-}$ mice did not display any lesions in the same area of the right common carotid artery.

Perivascular visceral adipose tissue impairs endothelial function

To study the effect of perivascular adipose tissue on vascular function of carotid arteries from *ApoE*$^{-/-}$ mice transplanted with visceral or subcutaneous adipose tissue, pressure myography was performed with segments of carotid arteries immediately distal to the fat transplant. These studies were performed at an earlier time point (18 days), prior to development of severe lesions. Vasoconstrictor responses to PE were similar between mice receiving visceral fat (n=4) and those receiving subcutaneous fat (n=5) (Fig. 5A). However, endothelial relaxation responses to Ach were significantly reduced in mice receiving visceral fat compared to those receiving subcutaneous fat (p<0.05) (Fig. 5B).

Deficiency of Psgl-1 is protective against perivascular inflammatory fat-induced local atherosclerosis

Since Psgl-1 deficiency has been shown to be protective against fat inflammation triggered by obesity [8], we tested the role of Psgl-1 deficiency on the development of atherosclerosis in this model. Visceral adipose tissue was therefore transplanted to the right carotid artery of *ApoE*$^{-/-}$,*Psgl-1*$^{-/-}$ mice (n=5). Eight weeks post-operatively, there was no significant difference in total cholesterol (222.1±13.1 vs. 169.0±30.4 mg/dl, p=NS) or fasted insulin (1.2±0.5 vs. 1.5±0.7 ng/ml, p=NS) between *ApoE*$^{-/-}$,*Psgl-1*$^{-/-}$ mice and *ApoE*$^{-/-}$,*Psgl-1*$^{+/+}$ mice with visceral fat. The macrophage content of transplanted visceral adipose tissue in *ApoE*$^{-/-}$,*Psgl-1*$^{-/-}$ mice was significantly lower compared to visceral transplants harvested from *ApoE*$^{-/-}$,*Psgl-1*$^{+/+}$ mice (23.5±1.3 vs. 38.1±2.2 %, p<0.0006). *ApoE*$^{-/-}$,*Psgl-1*$^{-/-}$ mice had no detectable lipid-rich atherosclerotic plaques in the right carotid artery while *ApoE*$^{-/-}$,*Psgl-1*$^{+/+}$ mice with visceral fat transplants all had plaques (0.0±0.0% vs 24.1±7.2% of total area, p=0.01). Circulating levels of Mcp-1 were lower in *ApoE*$^{-/-}$,*Psgl-1*$^{-/-}$ mice compared to *ApoE*$^{-/-}$,*Psgl-1*$^{+/+}$ mice (26.8±4.2 vs. 66.0±5.6 pg/ml, p=0.003).

To test a potential therapeutic strategy, the effect of an anti-Psgl-1 antibody on the proatherogenic effect of transplanted fat was determined. Carotid fat transplantation was

performed with visceral adipose tissue to *ApoE*$^{-/-}$ mice, and mice were then injected once a week with either anti-Psgl-1 antibody (n=8) or control IgG k isotype (n=10) for 3 consecutive weeks post-operatively. Eight weeks after the transplantation, *ApoE*$^{-/-}$ mice injected with anti-Psgl-1 antibody had significantly lower serum Mcp-1 compared to controls (54.1±3.3 vs. 81.1±7.4 pg/ml, p=0.007). Cross sections of right common carotid artery at the location of the fat transplant were analyzed for lesion thickness. Mice injected with anti-Psgl-1 antibody had significantly smaller lesion size (23314±12764 vs. 63124±14386 μm^2, p=0.03) and intima/media ratio (0.2±0.1 vs. 0.9±0.3, p=0.03) compared to controls.

DISCUSSION

Obesity is a risk factor for cardiovascular events and mortality from all causes [10]. The mechanism(s) by which obesity increases vascular risk is unclear. Visceral adiposity in particular appears to be most strongly associated with features of the metabolic syndrome and vascular risk [1, 11]. In addition to systemic proinflammatory effects, visceral adipose tissue may exert local effects on adjacent blood vessels [12, 13]. Consistent with this hypothesis, clinical studies have demonstrated that epicardial adipose tissue volume is correlated with total visceral adipose tissue mass and metabolic syndrome[14, 15], and is also independently associated with the presence of noncalcified coronary plaques[3, 4] and total coronary occlusions[5]. Inflammatory epicardial fat, in particular, independently correlates with the presence of coronary atherosclerosis [3, 16]. Both human and animal studies have shown that obesity[17, 18], high-fat feeding[19] and hypercholesterolemia without obesity[20] can increase inflammation in perivascular adipose tissue, which may contribute to the effects of these manipulations on atherosclerosis. Inflammatory perivascular adipose tissue may in turn secrete proatherogenic cytokines[12], and cause local endothelial dysfunction [17, 21], thus contributing to progression of systemic and local vascular disease.

Epidemiologic and preclinical data support a strong association between perivascular adipose tissue with local atherosclerosis, however, a direct causal relationship remains to be established. One way to address this is by the perivascular addition of adipose tissue using fat transplantation techniques. Since the process of fat transplantation is associated with chronic inflammation in the transplanted depot [6], this also provides a model of inflammatory fat which may mimic the perivascular inflammation that occurs in the obese state [17]. Although the mechanism(s) by which inflammation occurs in the setting of fat transplantation compared to endogenous obesity are likely to be different, the downstream adverse effects of the inflammatory fat may be similar. We have previously shown that transplantation of 400 mg of visceral adipose tissue to the dorsal aspect of the mouse leads to a viable graft capable of secreting adipokines such as leptin for at least 1 year [6]. Although the transplant becomes revascularized, as shown in this study, it is associated with chronic inflammation with features similar to those observed in visceral depots in the setting of obesity [6]. This visceral fat transplantation protocol to *ApoE*$^{-/-}$ mice is sufficient to accelerate atherosclerosis systemically, throughout the vascular tree [6].

In the current study, we transplanted a much smaller amount of fat (only 60 mg) to a site immediately adjacent to the common carotid artery to determine if inflammatory fat in a perivascular location could affect vascular function and trigger formation of a local atherosclerotic lesion. The mid carotid artery site was chosen since this site typically remains free of atherosclerosis in *ApoE*$^{-/-}$ mice at the ages we were studying.

The transplanted perivascular visceral adipose tissue led to local impaired vascular relaxation in our model. The important role of perivascular adipose tissue in regulation of vascular tone has been previously reported [22, 23] and is likely the precursor to subsequent

atherosclerotic lesion formation. In obesity, endothelial dysfunction is likely secondary to the inflammation and hypoxia-induced changes in periadventitial fat [24]. Activated macrophages in perivascular fat may be particularly important towards the loss of anticontractile activity [25]. Transplanted adipose tissue is similar to obese adipose tissue in regards to inflammation and hypoxic damage to tissue and our results support the recent human studies which have shown abnormalities in endothelium-dependent vasodilatation involving small arteries from obese patients [17]. As we expected, mice subjected to a sham operation did not show evidence of atherosclerosis in the mid common carotid arteries. However, severe complex lesions occurred in mice in which a piece of visceral fat was placed adjacent to the mid common carotid. These lesions showed frequent evidence of necrotic, fibrin-positive cores representing either plaque rupture with thrombosis or plaque hemorrhage. Perivascular fat may also be triggering local thrombus formation which could serve as a nidus for plaque growth. However, in a group of mice sacrificed 1 week after the transplant, there was no evidence of thrombus or lesion suggesting acute thrombus formation related to the procedure was not responsible for lesion formation. Because there were some plaques without thrombus, we suspect that plaque formation preceded the thrombosis or hemorrhage. This is a particularly interesting finding since human studies have shown an association between epicardial adipose tissue volume and unstable coronary plaques[4]. Although we cannot completely rule out hemodynamic effects from the fat transplant on lesion formation in the adjacent carotid artery, there was no obvious mechanical extrinsic obstruction apparent at the time of fat transplantation, or later at the time of sacrifice. In addition, transplantation of subcutaneous fat of identical mass did not produce the same effect as visceral fat, despite a similar inflammatory infiltrate. We cannot rule out a greater effect of visceral adipose tissue on characteristics of the local carotid adventitia as accurate quantitation of the adventitial was not possible in this study. The difference between visceral and subcutaneous fat on local atherosclerosis suggests that an interaction between visceral adipocytes and inflammatory cells is playing an important role toward the proatherogenic effect of perivascular fat. This effect is not due to inflammatory fat effects on insulin resistance since no differences in glucose or insulin levels were noted between the groups of transplanted mice. Interestingly, Mcp-1, which may be a biomarker of accelerated vascular disease induced by inflammatory fat [9], was elevated in the presence of visceral compared to subcutaneous perivascular fat transplantation.

Therapeutic targeting of the macrophage infiltration into visceral fat may reduce the subsequent adverse vascular effects of perivascular inflammatory adipose tissue. Psgl-1 deficiency has been shown to reduce obesity-induced visceral fat macrophage infiltration by reducing the adhesive characteristics of the endothelium induced by obesity [8]. To determine if Psgl-1 deficiency in the recipient $ApoE^{-/-}$ mice would be sufficient to attenuate the proatherogenic effect of visceral fat, $ApoE^{-/-}$,$Psgl\text{-}1^{-/-}$ mice were generated. $ApoE^{-/-}$,$Psgl\text{-}1^{-/-}$ were completely protected from the effects of the visceral fat transplant with no evidence of atherosclerosis at the site of fat transplant. Antibody blockade of Psgl-1 with weekly injections of anti-Psgl-1 antibody for only 3 weeks following the fat transplant was also effective in reducing the proatherogenic effect of the transplanted fat and was associated with reduced levels of Mcp-1. It has been previously shown that a single injection of this anti-Psgl-1 antibody is capable of reducing neointima formation 28 days following injury to the carotid artery in $ApoE^{-/-}$ mice [26], thus interference with Psgl-1 may have long term effects, especially in the setting of acute or subacute injury.

In conclusion, transplanted perivascular visceral adipose tissue induces endothelial dysfunction and triggers formation of local complex atherosclerotic plaques. Inhibition of the leukocyte ligand, Psgl-1, may provide a therapeutic approach to attenuate the effects of inflammatory visceral adipose tissue on atherosclerosis.

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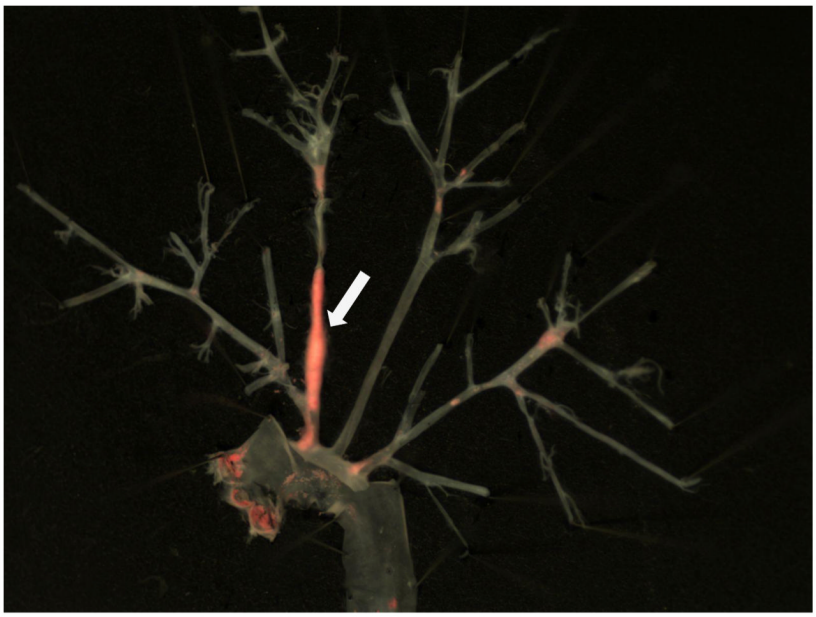

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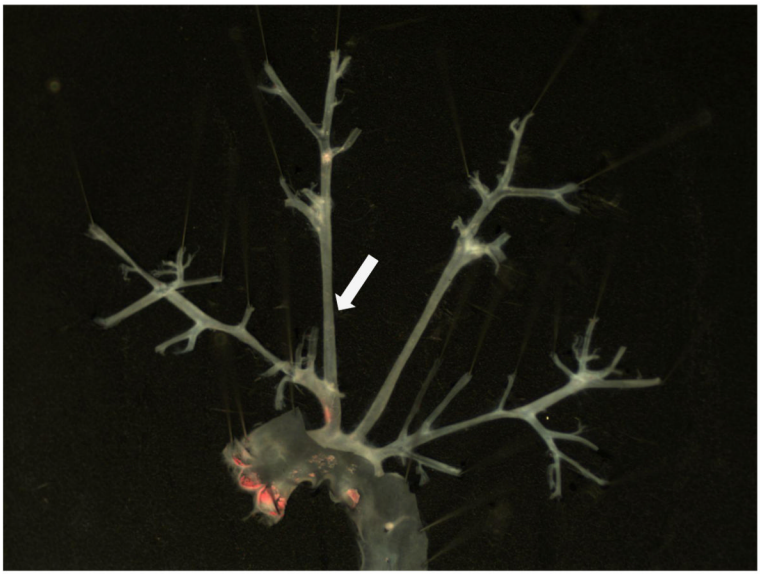


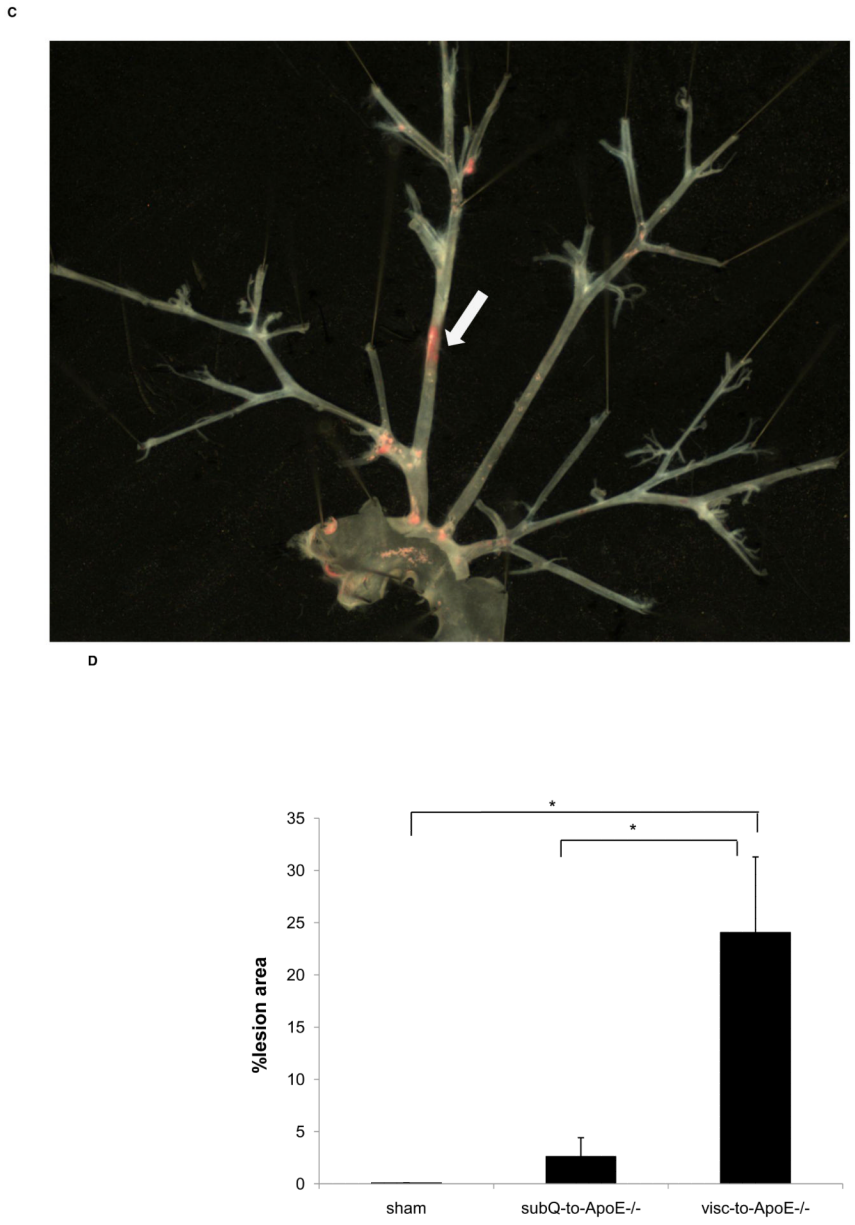


Figure 1. Local atherosclerosis increased in mice with perivascular visceral fat transplantation

Oil-red-o stained aortic arch and major branches of *ApoE*$^{-/-}$ mouse transplanted with **A)** visceral (visc-to-ApoE−/−) adipose tissue, **B)** sham operated *ApoE*$^{-/-}$ mouse and **C)** *ApoE*$^{-/-}$ mouse transplanted with subcutaneous (subQ-to-ApoE−/−) adipose tissue. **D)** Lesion surface area in operated *ApoE*$^{-/-}$ mice. *p<0.05. Arrows point to the site of fat transplantation on the right common carotid arteries.

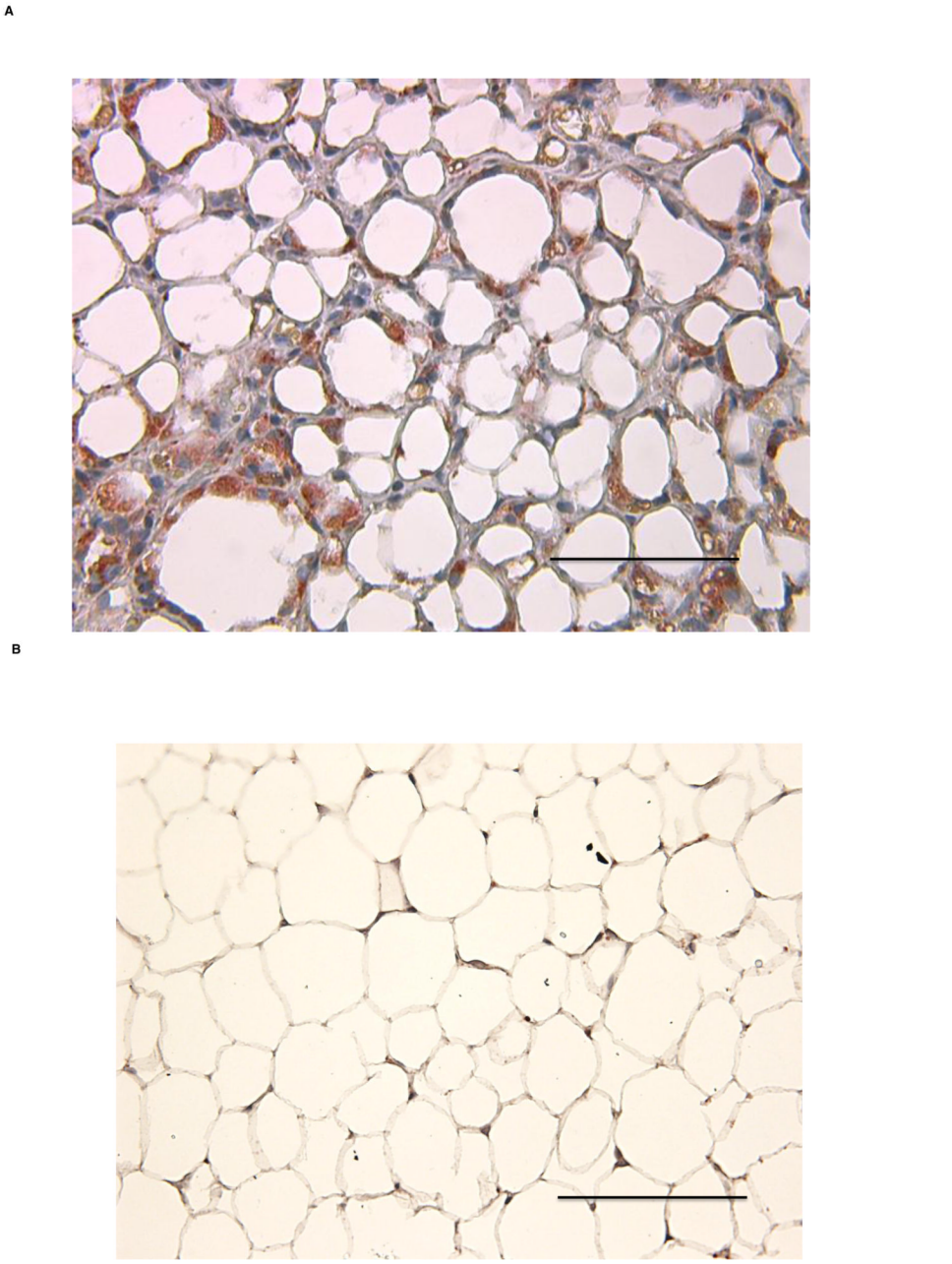
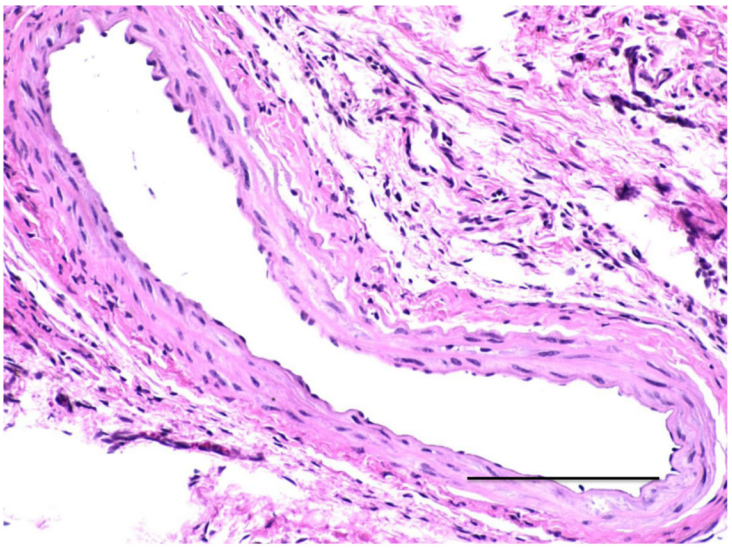


Figure 2. Inflammatory infiltrate in transplanted adipose tissue

A) Transplanted visceral adipose tissue and **B)** endogenous visceral adipose tissue stained with Mac3 antibody. Magnification 40x, scale bar =100 μm.

A



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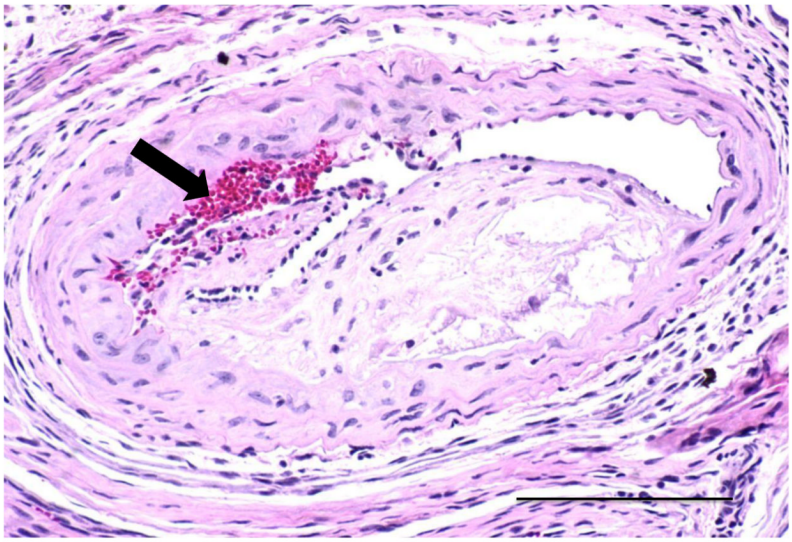

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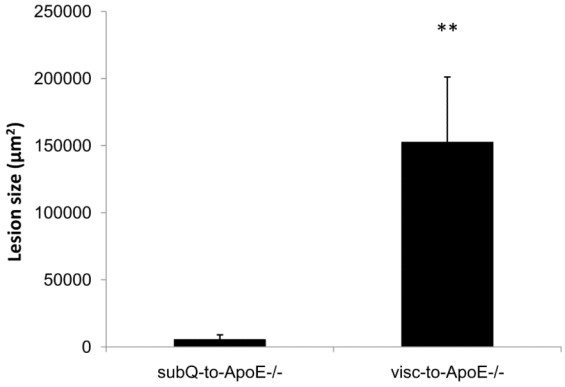
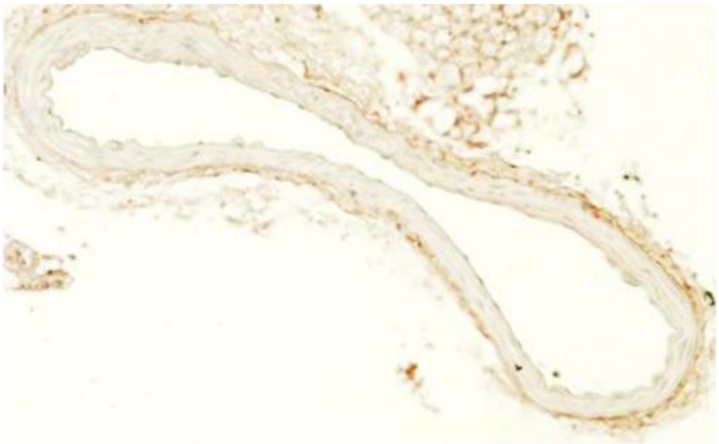


Figure 3. Perivascular visceral adipose tissue increases lesion thickness

H&E stained cross sections of the right common carotid artery from *ApoE*$^{-/-}$ mice with **A)** subcutaneous and **B)** visceral adipose tissue transplant. Arrow pointing to a potential intraplaque hemorrhage. Scale bar = 100 μm, magnification 40x. **C)** Lesion size in operated *ApoE*$^{-/-}$ mice. **p<0.01.

A



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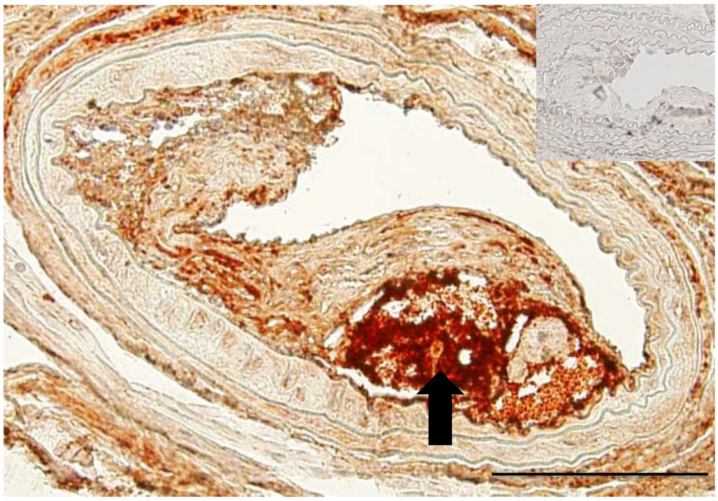

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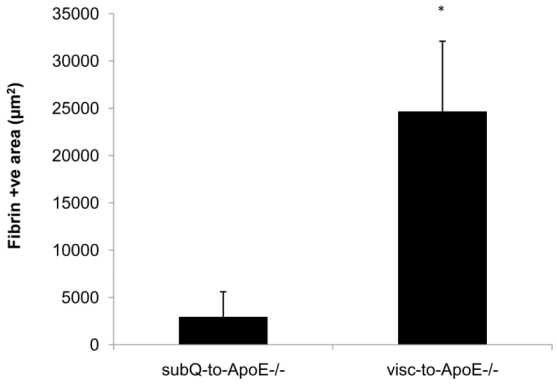

Figure 4. *ApoE*$^{-/-}$ mice transplanted with visceral fat display complicated lesions

A) Cross section of carotid artery stained with fibrin(ogen) antibody from *ApoE*$^{-/-}$ mice transplanted with **A)** subcutaneous and **B)** visceral fat. Inset shows negative control without primary antibody. Arrow points to necrotic core of the lesion. Scale bar = 100 μm, magnification 40x. **C)** Fibrin-positive area in lesions in operated mice. *p<0.05.

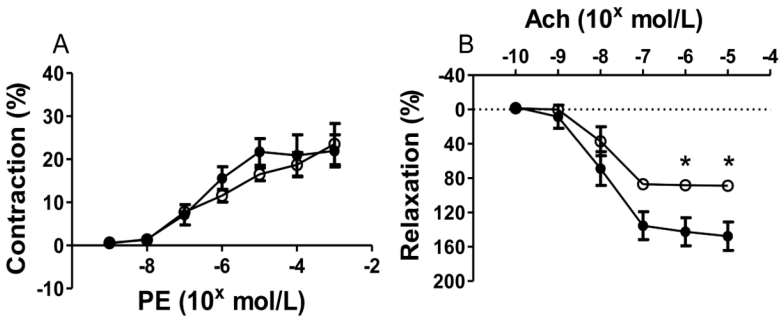

Figure 5. Perivascular visceral fat transplant leads to endothelial dysfunction

A) Vasoconstriction responses to phenylephrine (PE). **B)** Relaxation responses to acetylcholine (Ach). *ApoE*$^{-/-}$ mice with visceral (○) and subcutaneous (●) fat transplants. *p<0.05.