Monocyte Chemoattractant Protein-1 Deficiency Protects Against Visceral Fat–Induced Atherosclerosis

Miina K. Öhman, Andrew P. Wright, Kevin J. Wickenheiser, Wei Luo, Hana M. Russo, Daniel T. Eitzman

Objective—To determine the role of monocyte chemoattractant protein-1 (Mcp-1) on the progression of visceral fat–induced atherosclerosis.

Methods and Results—Visceral fat inflammation was induced by transplantation of perigonadal fat. To determine whether recipient Mcp-1 status affected atherosclerosis induced by inflammatory fat, apolipoprotein E–deficient (ApoE−/−) and ApoE−/− and Mcp-1–deficient (Mcp-1−/−) mice underwent visceral fat transplantation. Intravital microscopy was used to study leukocyte-endothelial interactions. To study the primary tissue source of circulating Mcp-1, both fat and bone marrow transplantation experiments were used. Transplantation of visceral fat increased atherosclerosis in ApoE−/− mice but had no effect on atherosclerosis in ApoE−/−Mcp-1−/− mice. Intravital microscopy revealed increased leukocyte attachment to the endothelium in ApoE−/− mice compared with ApoE−/−Mcp-1−/− mice after receiving visceral fat transplants. Transplantation of visceral fat increased plasma Mcp-1, although donor adipocytes were not the source of circulating Mcp-1 because no Mcp-1 was detected in plasma from ApoE−/−Mcp-1−/− mice transplanted with Wt fat, indicating that recipient Mcp-1–producing cells were affecting the atherogenic response to the fat transplantation. Consistently, transplantation of Mcp-1−/− fat to ApoE−/− mice did not lead to atheroprotection in recipient mice. Bone marrow transplantation between Wt and Mcp-1−/− mice indicated that the primary tissue source of circulating Mcp-1 was the endothelium.

Conclusion—Recipient Mcp-1 deficiency protects against atherosclerosis induced by transplanted visceral adipose tissue. (Arterioscler Thromb Vasc Biol. 2010;30:1151-1158.)

Key Words: cytokines ■ macrophages ■ obesity ■ adipocyte ■ adipose tissue ■ inflammation

Obesity is associated with an increased risk for cardiovascular disease.1,2 This risk is primarily due to central or visceral adiposity, and it is associated with systemic markers of inflammation.3–4 Features of adipose depots that may confer increased cardiovascular risk include leukocyte infiltration with evidence of increased adipose tissue macrophage activity.5–7 Chemokines such as monocyte chemotactant protein-1 (Mcp-1) have been shown to be elevated in adipose tissue of obese humans and animals.8,9 Mcp-1 expression has been shown to be higher in visceral adipose tissue depots compared with subcutaneous depots, and Mcp-1 may play a role in regulating inflammatory characteristics of adipose tissue.10–12 Visceral adipose tissue transplantation leads to heightened inflammation in the fat transplant and is sufficient to promote atherosclerosis in atherosclerotic-prone mice.13 However, the specific mediator(s) of increased atherosclerosis in this model of inflammatory fat are unknown.

In the present study, we determined the role of Mcp-1 in atherosclerosis induced by visceral adipose tissue transplantation.
barbital (67 mg/kg), visceral (perigonadal) adipose tissue was removed from donors, weighed, and implanted subcutaneously into 4 dorsal incisions for a total of 424±16 mg of fat per recipient. Nylon filament (6-0) was used for wound closure. An additional group of ApoE−/− mice received an equivalent amount of subcutaneous inguinal fat (440±35 mg) from Wt donor mice using the same protocol. The transplanted visceral fat represents an ~60% increase in total visceral fat mass, based on previous quantitation of fat depots, whereas the transplanted subcutaneous fat represents an ~50% increase in the total amount of subcutaneous fat in an adult, 25 g body-weight, chow-fed C57BL/6 mouse (subcutaneous estimate based on calculation of total fat mass minus visceral and brown fat mass).14

Bone Marrow Transplantation
Bone marrow transplantation (BMT) was performed as previously described.15 Wt mice were used as recipients for Wt and Mcp-1−/− donor mice, and Mcp-1−/− mice were used as recipients for Wt donor mice. Each recipient mouse was irradiated (2x650 rad [0.02×6.5 Gy]) and injected with 4×10⁶ bone marrow cells via the tail vein. To induce an acute inflammatory reaction, BMT recipients were injected intraperitoneally with lipopolysaccharide (LPS) (Sigma-Aldrich, St. Louis, Mo) (1 mg/kg), and blood samples were collected 6 hours after LPS injection. A subset of bone marrow–transplanted Wt and Mcp-1−/− mice, receiving Mcp-1−/− and Wt marrow, respectively, underwent fat transplantation 4 weeks after BMT, with all mice receiving Mcp-1−/− deficient visceral adipose tissue.

Measurements of Cytokines, Insulin, Glucose, Lipids, and Body Fat Percentage
Blood samples from mice were collected by retro-orbital bleeding using capillary tubes. Fat homogenates were prepared at euthanization from 100 mg of transplanted adipose tissue as described earlier.13 Commercially available murine ELISA kits (R&D Systems, Minneapolis, Minn.) were used to measure Mcp-1, osteopontin, and soluble vascular cell adhesion molecule-1 levels. Glucose was measured after overnight fast with a glucometer using test strips (Ascensia Contour, Bayer HealthCare LLC, Mishawaka, Ind) 8 weeks after the fat transplantation. At the same time point, fasted insulin levels were measured with an ELISA kit (Crystal Chem Inc., Downers Grove, Ill). Serum collected at euthanization after overnight fast was used to measure nonesterified fatty acids (Wako, Richmond, Va) and cholesterol levels (Wako) with colorimetric assays. Body fat percentage was measured using a Dual-Energy X-ray Absorptiometry scanner Lunar PIXImus2 (GE Healthcare Bio-Sciences Corp, Piscataway, NJ).

Atherosclerosis Quantitation
At the time of euthanization (24 weeks of age, 16 weeks after fat transplantation), all ApoE−/−, Mcp-1−/− and ApoE−/− mice (both fat-transplanted and nontransplanted control mice) were anesthetized with sodium pentobarbital (67 mg/kg), perfused with saline at physiological 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 meticulously dissected and placed in 70% ethanol. After staining with oil red O and pinning on wax, the surface area occupied by atherosclerosis was quantitated at the aortic arch and major branches, with aortic tree cross-sections were also markedly reduced in ApoE−/−, Mcp-1−/− mice receiving Wt fat compared with ApoE−/− mice receiving Wt fat (243±1140 versus

**Results**

Effect of Mcp-1 Deficiency on Visceral Fat–Induced Atherosclerosis
To determine whether Mcp-1 deficiency would attenuate the proatherogenic effect of inflammatory visceral fat, we quantified atherosclerosis by oil red O staining of the aortic trees in ApoE−/−, Mcp-1−/− (n=6) and ApoE−/− (n=8) mice that received Wt fat transplants. When comparing nontransplanted ApoE−/− mice (n=5) with their fat-transplanted littermates, there was a significant increase in atherosclerosis in transplanted ApoE−/− mice (Figure 1). However, the ApoE−/−, Mcp-1−/− mice were completely protected against the increase in atherosclerosis induced by inflammatory visceral fat compared with nontransplanted littermate ApoE−/−, Mcp-1−/− mice (n=10) (Figure 1). Macrophage-rich lesion area determined from aortic root cross sections was also markedly reduced in ApoE−/−, Mcp-1−/− mice receiving Wt fat compared with ApoE−/− mice receiving Wt fat (243±1140 versus
65584±13470 μm², \( P = 0.002 \) (Figure 2). Fasting glucose (226.5±13.9 versus 186.8±14.0 mg/dL, \( P = \text{not significant} \) [NS]), insulin (2.00±0.94 versus 1.96±0.55 ng/mL, \( P = \text{NS} \)) and adiposity (fat%; 12.52±0.82 versus 13.30±0.56%, \( P = \text{NS} \)) in fat-transplanted \( \text{ApoE}^{-/-} \) and \( \text{ApoE}^{-/-}\text{Mcp-1}^{-/-} \) mice receiving \( Wt \) fat were not different between the groups 8 weeks postoperatively.

We also quantified atherosclerotic lesion area in \( \text{ApoE}^{-/-} \) mice that received \( \text{Mcp-1}^{-/-} \) fat transplants (n=8). These mice displayed similar findings to those observed in \( \text{ApoE}^{-/-} \) mice receiving \( Wt \) fat, ie, their surface lesion area was significantly greater than that of nontransplanted control \( \text{ApoE}^{-/-} \) mice (7.5±1.1 versus 2.9±0.5, respectively, \( P < 0.05 \)), and they developed an amount of atherosclerosis similar to that of \( \text{ApoE}^{-/-} \) mice receiving \( Wt \) fat (7.5±1.1 versus 7.4±1.5%, respectively, \( P = \text{NS} \)). Thus, the lack of Mcp-1 in donor adipocytes does not explain the decrease in the atherosclerotic burden induced by fat transplantation. In nontransplanted control \( \text{ApoE}^{-/-} \) and \( \text{ApoE}^{-/-}\text{Mcp-1}^{-/-} \) mice, which were fed the same normal chow diet as transplanted mice, no differences in atherosclerotic lesion area were detected (2.9±0.5 versus 3.7±0.6%, respectively, \( P = \text{NS} \)).

Total cholesterol and free fatty acids were measured at euthanization from all groups of fat-transplanted and control mice. There was no difference in cholesterol between \( \text{ApoE}^{-/-} \) and \( \text{ApoE}^{-/-}\text{Mcp-1}^{-/-} \) mice receiving \( Wt \) fat (398.2±32.8 versus 508.6±58.8 mg/dL, respectively, \( P = \text{NS} \)), or between \( \text{ApoE}^{-/-} \) mice receiving \( Wt \) fat and \( \text{ApoE}^{-/-}\text{Mcp-1}^{-/-} \) mice receiving \( \text{Mcp-1}^{-/-} \) fat (398.2±32.8 versus 452.2±21.1 mg/dL, respectively, \( P = \text{NS} \)). There was no difference in free fatty acids between the \( \text{ApoE}^{-/-} \) and \( \text{ApoE}^{-/-}\text{Mcp-1}^{-/-} \) mice receiving \( Wt \) fat (1.52±0.12 versus 1.92±0.13 mmol/L, respectively, \( P = \text{NS} \)) or between

\( \text{ApoE}^{-/-} \) mice receiving \( Wt \) fat and \( \text{ApoE}^{-/-} \) mice receiving \( \text{Mcp-1}^{-/-} \) fat (1.52±0.12 versus 1.56±0.05 mmol/L, respectively, \( P = \text{NS} \)).

**Effect of Mcp-1 Deficiency on Fat Inflammation**

To characterize the role of Mcp-1 deficiency on inflammation in transplanted visceral fat pads, transplanted fat pads were removed from \( \text{ApoE}^{-/-} \) and \( \text{ApoE}^{-/-}\text{Mcp-1}^{-/-} \) mice at the time of euthanization and analyzed for macrophage content. \( Wt \) fat transplants removed from \( \text{ApoE}^{-/-}\text{Mcp-1}^{-/-} \) mice contained a reduced percentage of macrophages compared with \( Wt \) fat transplants removed from \( \text{ApoE}^{-/-} \) mice (Figure 3). The \( \text{Mcp-1}^{-/-} \) fat pads transplanted to \( \text{ApoE}^{-/-} \) mice were also analyzed for macrophage content, and no differences were found in macrophage content compared with \( Wt \) fat pads analyzed from \( \text{ApoE}^{-/-} \) mice (26.1±5.6 versus 38.2±4.9, respectively, \( P = \text{NS} \)). Thus, the adipocyte Mcp-1 status does not regulate macrophage infiltration into fat in this model.

**Effect of Fat Transplantation on Leukocyte-Endothelial Interactions**

To determine whether visceral fat transplantation was associated with increased systemic adhesive interactions between the endothelium and leukocytes, intravital microscopy was used to visualize interactions between leukocytes and endothelial cells in \( \text{ApoE}^{-/-}\text{Mcp-1}^{-/-} \) and \( \text{ApoE}^{-/-} \) mice following visceral fat transplantation. Fat-transplanted \( \text{ApoE}^{-/-}\text{Mcp-1}^{-/-} \) mice displayed significantly less leukocyte firm attachment compared with fat-transplanted \( \text{ApoE}^{-/-} \) mice (20.3±3.5 versus 44.1±5.3 firmly attached cells/mm vessel, \( P = 0.002 \)) (Figure 4).

We have previously shown that transplantation of subcutaneous adipose tissue does not promote atherosclerosis in \( \text{ApoE}^{-/-} \) mice and that mice with subcutaneous fat transplants have lower circulating levels of Mcp-1 compared with
mice transplanted with visceral fat.13 Interestingly, ApoE<sup>−/−</sup> mice that received subcutaneous fat transplants also exhibited reduced leukocyte firm attachment compared with ApoE<sup>−/−</sup> mice receiving visceral fat transplants (18.6±6.5 versus 44.1±5.3 adherent cells/mm vessel, *P<0.01) (Figure 4). No difference in soluble vascular cell adhesion molecule-1<sup>22</sup> levels were observed between ApoE<sup>−/−</sup> and ApoE<sup>−/−</sup>Mcp-1<sup>−/−</sup> mice receiving visceral fat (571.0±69.8 versus 639.7±38.9 ng/mL, respectively, *P=NS) or between ApoE<sup>−/−</sup> mice receiving visceral fat and ApoE<sup>−/−</sup> mice receiving subcutaneous fat (799.7±63.2 versus 803.0±26.0 ng/mL, respectively, *P=NS). Plasma osteopontin<sup>23–25</sup> levels were not different between ApoE<sup>−/−</sup> mice receiving visceral fat and ApoE<sup>−/−</sup> mice receiving subcutaneous fat (29.5±2.3 versus 30.5±5.6 ng/mL, respectively, *P=NS).

Source of Circulating Mcp-1

To determine whether the transplanted adipose tissue contributed to the plasma levels of Mcp-1 following the fat transplantation procedure, Mcp-1 levels were measured at 4 weeks following transplantation of wild-type visceral fat into ApoE<sup>−/−</sup>Mcp-1<sup>−/−</sup> and ApoE<sup>−/−</sup> mice. Levels of circulating Mcp-1 increased significantly in ApoE<sup>−/−</sup> mice receiving Wt fat compared with pretransplant levels (78.7±11.2 versus 31.4±2.9 pg/mL, respectively, *P<0.01). The plasma Mcp-1 in ApoE<sup>−/−</sup>Mcp-1<sup>−/−</sup> mice receiving Wt fat was barely detectable (0.1±0.1 pg/mL) 4 weeks after the operation indicating that the donor adipocytes are not the source of elevated Mcp-1 following adipose transplantation.

To determine the contribution of transplanted adipocytes toward local Mcp-1 concentrations, Mcp-1 was also measured from fat homogenates prepared from transplanted Wt adipose tissue collected from ApoE<sup>−/−</sup>Mcp-1<sup>−/−</sup> and ApoE<sup>−/−</sup> recipient mice. Mcp-1 levels in fat pads were significantly lower in ApoE<sup>−/−</sup>Mcp-1<sup>−/−</sup> mice compared with ApoE<sup>−/−</sup> mice (77.8±28.8 versus 626.8±187.5 pg/mL, *P=0.02) indicating that host cells (eg, endothelial and leukocytes) infiltrating the fat transplant contribute to the levels of Mcp-1 in adipose tissue.

To determine whether adipose tissue Mcp-1 varied with harvest site in this transplant model, Mcp-1 levels were measured in fat homogenates prepared from ApoE<sup>−/−</sup> mice (n=8) that received subcutaneous fat transplants. Interestingly, Mcp-1 levels were significantly lower in subcutaneous fat transplants compared with visceral fat transplants (246.8±79.7 versus 626.8±187.5 pg/mL, *P=0.04).

Because the above experiments revealed that the transplanted adipocyte cellular pool was not an important contributor to circulating systemic Mcp-1 levels, BMT was used to determine the potential of monocytes/macrophages as a source of circulating Mcp-1. Ten weeks after BMT, plasma Mcp-1 levels were measured in 3 different BMT groups, Wt mice with Wt marrow (n=9), Mcp-1<sup>−/−</sup> mice with Wt marrow (n=6), and Wt mice with Mcp-1<sup>−/−</sup> marrow (n=6). Wt mice with Wt marrow had higher levels of plasma Mcp-1 compared with Wt mice with Mcp-1<sup>−/−</sup> marrow (94.5±9.2 versus 66.8±3.2 pg/mL, respectively, *P<0.05). The lowest levels of circulating Mcp-1 were measured in Mcp-1<sup>−/−</sup> mice with Wt marrow (15.6±7.2 pg/mL), compared with the 2 other groups (*P<0.0002 for both comparisons), indicating a greater contributory role of the endothelium toward plasma Mcp-1 levels in the basal state. To investigate whether an acute inflammatory stimulus would change this pattern, BMT mice were given intraperitoneal LPS injection, and 6 hours...
later the circulating Mcp-1 levels were measured (Figure 5). Plasma Mcp-1 increased markedly in Wt mice with Wt marrow and in Wt mice with Mcp-1−/− marrow. However, compared with the other 2 groups, Mcp-1−/− mice with Wt marrow exhibited a relatively minor increase in circulating Mcp-1 levels (Figure 5). These data indicate that the main source of circulating Mcp-1 is not bone marrow–derived at baseline or after acute inflammatory stimulus with LPS.

We next evaluated the extent to which the host endothelial cells growing into the fat transplants contribute to the Mcp-1 levels in transplanted adipose tissue compared with the monocytes/macrophages infiltrating the fat pads. To determine this, we performed a combination of BMT followed by visceral fat transplantation. Two different BMT groups, Wt mice with Mcp-1−/− marrow (n=4) and Mcp-1−/− mice with Wt marrow (n=8) all received fat transplants from Mcp-1−/− donor mice 4 weeks after BMT. In this experiment, the Mcp-1 in transplanted fat pads could only come from either infiltrating vasculature or infiltrating leukocytes from the host. Six weeks after the fat transplantation, fat pads were removed, and Mcp-1 levels were measured from fat homogenates. Mcp-1−/− mice with Wt bone marrow had significantly higher levels of Mcp-1 in their fat pads compared with Wt mice with Mcp-1−/− bone marrow (108±26.5 versus 6.3±1.9 pg/mL, respectively, P<0.02), indicating that the bone marrow–derived cells were the major source of local Mcp-1 in the transplanted adipose tissue.

We also measured circulating Mcp-1 levels from the mice that received combined BMT and fat transplantation. Mcp-1−/− mice with Wt marrow and Mcp-1−/− fat displayed lower plasma Mcp-1 compared with the Wt mice with Mcp-1−/− marrow and Mcp-1−/− fat (23.6±3.5 versus 36.2±1.5 pg/mL, respectively, P<0.02). Thus, even though bone-marrow derived cells play a major role in regulating local tissue levels of Mcp-1, the endothelium contributes more to circulating levels of Mcp-1 after both acute inflammatory stimulus (ie, LPS) and after a more chronic, low-grade inflammatory challenge as occurs in the adipose tissue transplantation model.

**Discussion**

Obesity is an independent risk factor for cardiovascular disease.5,26 The mechanisms linking obesity and risk for cardiovascular disease are not completely understood. A recent large study demonstrated that central obesity, which reflects accumulation of visceral adipose tissue, is strongly associated with mortality.27 Excess visceral adiposity has also shown to be a risk factor for prevalent atherosclerosis and a trigger of markers of inflammation that are strongly associated with complications of atherosclerosis such as myocardial infarction and stroke.28,29 Thus, visceral adiposity may trigger a systemic inflammatory state which affects the development of atherosclerosis. The specific mediators of vascular risk associated with visceral obesity are unknown, although obese adipose tissue has been shown to express and secrete multiple factors that may promote atherosclerosis.10,11,30–32

To further dissect the specific role of adipose tissue on comorbidities of obesity such as vascular disease, models may be helpful in which confounding variables induced by severe obesity, such as diabetes and extreme hyperlipidemia, do not occur. Surgical implantation of adipose tissue to the dorsal surface of a mouse from a donor of the same background strain leads to a long term viable graft capable of secreting adipose-specific products, such as leptin, at physiological concentrations.13 Transplantation of visceral but not subcutaneous adipose tissue leads to acceleration of atherosclerosis in ApoE−/− mice.13 One of the circulating factors shown to be elevated in the visceral transplant model is Mcp-1.13 However, whether Mcp-1 plays a role in the proatherogenic effect of inflammatory visceral fat is unknown. There are likely multiple steps in the pathway linking visceral fat inflammation and atherosclerosis that could potentially serve as therapeutic targets. For example, adipose tissue inflammation may be initiated by overnutrition causing adipocyte stress, followed by release of factors leading to leukocyte infiltration into adipose tissue.6,7,33 A state of monocye/macrophage activation may then occur, leading to release of cytokines or activated macrophages, which in turn affect the macrovascular endothelium.33,34 The activated macrovascular endothelium may then lead to further monocyte/macrophage recruitment.35,36 Thus, there are several steps where Mcp-1 could affect the atherogenic response to inflammatory visceral adipose tissue. The goal of the current study was to determine whether Mcp-1 was a mediator of the increased atherosclerosis induced by inflammatory visceral fat.

Mcp-1 is a potential mediator of both adipose tissue inflammation and atherosclerosis,37,38 and atherosclerosis,39,40 based on previous investigations. Mcp-1 deficiency in mice prone to atherosclerosis via deletion of the low-density lipoprotein receptor, apoE or overexpression of apoB is associated with protection from atherosclerosis when challenged by a high-fat diet.39–42 In the setting of obesity, Mcp-1 and its receptor have been shown to contribute to macrophage infiltration into adipose...
tissue, insulin resistance, and hepatic steatosis in some studies, whereas other studies have not demonstrated effects of Mcp-1 on adipose tissue macrophage infiltration. Because Mcp-1 may play a role in both adipose tissue inflammation and atherosclerosis, we hypothesized that Mcp-1 may be one of the factors linking inflammatory adipose tissue and atherosclerosis. As several investigators have found increased expression of Mcp-1 in obese adipose tissue stores, we first tested whether the rise in plasma Mcp-1 observed in our fat transplant model was due to increased production from the inflammatory adipose transplant. To determine this, we performed visceral adipose transplantation from Wt mice to ApoE<sup>−/−</sup>Mcp-1<sup>−/−</sup> mice. To our surprise, Mcp-1 was undetectable in the plasma of ApoE<sup>−/−</sup>Mcp-1<sup>−/−</sup> mice, indicating that in this model, cells of the donor visceral fat transplant are not a major source of circulating Mcp-1. If the adipocyte was a relevant source of circulating Mcp-1, we should have detected it, because we have previously shown that plasma levels of leptin are chronically reconstituted in this model when wild-type adipose tissue is transplanted to leptin deficient mice. Conversely, transplantation of adipose tissue into ApoE<sup>−/−</sup> mice produced an increase in circulating Mcp-1 levels. This indicates that either recipient monocytes or the endothelium is producing Mcp-1 in response to a stimulus released from the transplanted adipose tissue. To further differentiate the recipient endothelial cells from the recipient bone marrow–derived pool as a source of circulating Mcp-1, bone marrow transplants were performed between Mcp-1<sup>−/−</sup> and Wt mice. Mcp-1 levels in Mcp-1<sup>−/−</sup> mice receiving Wt bone marrow transplants were lower 10 weeks after the transplant, whereas Mcp-1 levels in Wt mice receiving Mcp-1<sup>−/−</sup> marrow were similar to control Wt mice receiving Wt marrow. A relatively minor bone marrow contribution to circulating Mcp-1 levels was present after LPS administration. Thus, at baseline and in response to an acute inflammatory stimulus, the bone marrow–derived cells are not the major contributor to circulating levels of Mcp-1. However, the bone marrow–derived cells were a relevant source of Mcp-1 content within the transplanted visceral adipose tissue.

Because the circulating systemic Mcp-1 levels are endothelial derived in the basal state and after acute and chronic inflammatory challenges, we hypothesize that following macrophage infiltration into the transplanted fat is a systemic increase in adhesion characteristics of the endothelium that could promote atherosclerosis. Consistent with this hypothesis, more leukocyte firm attachment was observed in cremaster venules of ApoE<sup>−/−</sup> mice with visceral fat transplants compared with ApoE<sup>−/−</sup>Mcp-1<sup>−/−</sup> mice with visceral fat transplants. An effect of Mcp-1 on leukocyte firm attachment has previously been observed under flow conditions using in vitro models. Interestingly, the reduction in firm attachment in ApoE<sup>−/−</sup>Mcp-1<sup>−/−</sup> mice was similar to that observed in ApoE<sup>−/−</sup> mice receiving a subcutaneous fat transplant. The explanation for differences between visceral and subcutaneous fat on vascular disease and circulating Mcp-1 levels remains unclear. We have previously demonstrated that adipocyte-derived factors leptin and adiponectin are not different between ApoE<sup>−/−</sup> mice receiving visceral and subcutaneous fat transplants. Although the factor(s) responsible for triggering endothelial Mcp-1 and the mechanism(s) for differences between subcutaneous and visceral fat are unknown, the current data provide additional evidence that Mcp-1 may be one of the critical mediators of inflammatory visceral fat–induced atherosclerosis and may contribute to the different vascular effects of visceral and subcutaneous fat.

To provide proof that Mcp-1 plays a contributory role in the proatherogenic effect of visceral inflammatory adipose tissue, visceral fat transplants were performed into ApoE<sup>−/−</sup>Mcp-1<sup>−/−</sup> mice maintained on a normal chow diet. Although previous studies indicate that deficiency of Mcp-1 is protective against atherosclerosis in atherosclerotic-prone mice in the absence of fat transplantation, these previous studies were performed with a Western diet challenge that induces extreme hyperlipidemia and accelerates atherosclerosis. Interestingly, a Western diet triggers macrophage activation, and these macrophages then exhibit enhanced adhesive characteristics and contribute to atheroma. In the current study, no difference in atherosclerosis was noted between ApoE<sup>−/−</sup>Mcp-1<sup>−/−</sup> mice on a normal chow diet without adipose transplantation. Following visceral adipose tissue transplantation, the proatherogenic effect of the transplanted fat was observed in ApoE<sup>−/−</sup>Mcp-1<sup>−/−</sup> mice but was completely neutralized in ApoE<sup>−/−</sup>Mcp-1<sup>−/−</sup> mice. Thus, the protective effect of Mcp-1 deficiency on atherosclerosis appears to require an inflammatory trigger such as a high-fat diet or inflammatory visceral adipose tissue. In the setting of visceral adipose tissue inflammation, Mcp-1 may be a regulator of vasculopathic factors released by inflammatory visceral adipose or may mediate the endothelial response of the macrovasculature to atherogenic factors produced by the fat. Although we cannot rule out a differential effect of ApoE release from the donor fat on atherosclerosis, we believe this is unlikely, as the ApoE status was the same in all donor fat pads. Interestingly, the Mcp-1 status of the donor adipocytes does not appear to play an important role in regulating macrophage infiltration into fat or in atherosclerotic lesion formation, because ApoE<sup>−/−</sup> mice that received Mcp-1<sup>−/−</sup> visceral fat displayed equal amount of inflammation in adipose transplants and atherosclerotic burden as ApoE<sup>−/−</sup>Mcp-1<sup>−/−</sup> mice transplanted with Wt visceral fat.

In conclusion, recipient Mcp-1 deficiency leads to protection against the proatherogenic effects of inflammatory visceral adipose tissue. Mcp-1 may serve as a therapeutic target in subjects with excessive visceral adiposity at high risk for vascular events.

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Disclosures
None.

References


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