Stem Cell Transplantation Reveals That Absence of Macrophage CD36 Is Protective Against Atherosclerosis

Maria Febbraio, Ella Guy, Roy L. Silverstein

Objective—CD36 is expressed on multiple cell types and has numerous functions, a subset of which can impact on atherogenesis. In previous work, we demonstrated that CD36 absence was protective against lesion formation. The current objective was to determine whether absence of macrophage CD36 alone was protective.

Methods and Results—Lethal irradiation and stem cell transfer were used to create chimeric mice that did or did not express macrophage CD36 in the context of the Apo E-null model of atherosclerosis. After engraftment, mice were fed a Western diet for 12 weeks. White cell counts, plasma levels of lipoproteins, triacylglycerol, and nonesterified fatty acids were determined, and glucose tolerance tests were preformed. Lesion area was assessed quantitatively after oil red O staining. Mice lacking CD36 in macrophages alone were profoundly protected against atherosclerosis (88.1% reduction of lesion area throughout the aortic tree). Re-introduction of macrophage CD36 resulted in a 2.11-fold increase in lesion area. There were no differences in engraftment, macrophage recruitment, glucose tolerance, weight, and total, low-density lipoprotein, and high-density lipoprotein cholesterol among the groups. Lesions contained similar percent macrophage antigen-positive area.

Conclusion—Protection in this model is primarily caused by loss of CD36 macrophage function. (Arterioscler Thromb Vasc Biol. 2004;24:2333-2338.)

Key Words: CD36 ■ atherosclerosis ■ Apo E-null mice ■ scavenger receptor ■ macrophages

Cellular cholesterol homeostasis is tightly regulated such that cells have an adequate supply under varying dietary conditions. For most cell types, excessive accumulation of cholesterol does not occur and would have toxic implications. Macrophages, by virtue of their scavenger function, internalize cholesterol-laden modified lipoproteins. Uncontrolled cholesterol accumulation in macrophages may result as an adaptive mechanism in response to excessive lipid load and lead to foam cell formation. These appear grossly as the initial lesion in atherosclerosis, the fatty streak. Oxidative modification of low-density lipoprotein (LDL) has been hypothesized to be a key event in the conversion of LDL to a pro-atherogenic form recognized by macrophage scavenger receptors. Thus, internalization of oxidized LDL (OxLDL) by macrophages is an early and potentially pivotal event which, if inhibited through intervention at the level of the scavenger receptor, may delay the atherosclerotic process.

Previous in vitro work has supported a significant role for the class B scavenger receptor, CD36, in atherogenesis. We and others demonstrated that CD36 bound OxLDL in a saturable manner; binding, internalization, and degradation of OxLDL were increased substantially in CD36 transfected cells. Regulation of CD36 expression was also highly suggestive of an important role in atherosclerosis. Unlike the LDL receptor, which is downregulated by cellular cholesterol/cholesterol ester, expression of CD36 was enhanced by cellular cholesterol/cholesterol ester content and downregulated by its depletion. We also showed that IL-4 and macrophage-colony stimulating factor cytokines present in atherosclerotic lesions, induced human monocyte expression of CD36 mRNA and protein, and that greater than half of the binding of OxLDL by human monocyte-derived macrophages was inhibited by specific anti-CD36 antibodies. Other studies have demonstrated that lipid-laden macrophages in human atherosclerotic lesions exhibited strong immunoreactivity to CD36, whereas in these same lesions there were low or moderate levels of immunoreactivity to the class A scavenger receptors. Genetic polymorphisms have been identified in Asian and African populations, which can lead to deficient or absent expression of CD36. Monocyte-derived macrophages isolated from homozygous mutant patients bound 40% less OxLDL and accumulated 40% less cholesterol ester than cells derived from normal controls. More recently, using macrophages derived from mice lacking both CD36 and the class A scavenger receptors, we demonstrated that the predominant receptor involved in recognition of pro-atherogenic LDL (either mildly oxidized by copper sulfate or oxidized by the myeloperoxide/hydrogen peroxide system)
peroxide/nitrite system) was CD36; the combined effect of absence of both scavenger receptors was not significantly greater than absence of CD36 alone.5

CD36 is expressed on multiple cell types in addition to monocytes/macrophages: adipocytes, skeletal muscle, platelets, megakaryocytes, microvascular endothelium, retinal pigment epithelium, and microglia.20–22 CD36 recognizes a broad array of ligands including OxLDL, anionic phospholipids, apoptotic cells, thrombospondin, collagen, effete photoreceptors, fatty acids, fibril β-amyloid deposits, and Plasmodium falciparum-infected erythrocytes.20–22 Unlike the α-receptors, fatty acids, fibrinogen, vitronectin, and fibrin, all may also impact lesion development.26

a pro-apoptotic, anti-angiogenic response to thrombospondin-1, which in turn has the potential to impact on atherosclerosis. CD36 is involved in fatty acid transport, and this has implications for fatty acid, glucose, and lipoprotein metabolism, which is especially true of CD36: fat and muscle are considered to be key tissues affecting insulin resistance, and both express CD36.

tissues affecting insulin resistance, and both express CD36. CD36 is involved in fatty acid transport, and this has implications for fatty acid, glucose, and lipoprotein metabolism, which in turn has the potential to impact on atherosclerosis. CD36 is also expressed on microvascular endothelium, where it mediates a pro-apoptotic, anti-angiogenic response to thrombospondin-1, and this may also impact lesion development.26

In a previous study, we observed that CD36-null mice crossed to the apo E-null (Apo E0) strain had a dramatic decrease in aortic lesion area.27 Interestingly, these results were confirmed indirectly by a study of the mechanism by which drugs used to treat HIV accelerated atherosclerosis.28

One such drug, ritonavir, upregulates CD36 and promotes lesion progression in the Apo E0 mouse. This effect was abrogated in Apo E0/CD360 mice, and the authors noted a 75% to 80% difference in lesion size in the 2 strains when treated with ritonavir. Their results indicate that upregulation of CD36 by a drug mimics upregulation of CD36 in the subendothelial space by pro-inflammatory factors, which also convert LDL into a ligand for CD36 and initiate a feed-forward loop in macrophages.14,15 The degree of protection afforded by absence of CD36 was nearly identical to our results.27

The current study was designed to differentiate the role of CD36 as a macrophage scavenger receptor from other functions it has in endothelium, muscle, adipocytes, and in regulating fatty acid transport and angiogenesis. Using stem cell transfer, the genotype of hematopoietic cells, including monocytes/macrophages, was exchanged in the background of presence or absence of CD36 in the Apo E0 model. Our results suggest rather conclusively that in the Apo E0 model, absence of macrophage CD36 is overwhelmingly protective, regardless of CD36 expression elsewhere, and indicates that specifically targeting macrophage CD36 can have important therapeutic implications.

Methods

Animals

CD36/Apo E double-null (CD360/Apo E0) and Apo E0 mice were derived from a cross between CD360 (created in our laboratory) and Apo E0 mice (Taconic, Germantown, NY). The strains are background-matched. The lines were established from littermates after the cross of heterozygotes. The genetic background is 96.9% C57Bl/6 and 3.1% 129Sv. Procedures were Institutional Animal Care and Use Committee–approved and performed in an Association for Assessment and Accreditation of Laboratory Animal Care International–accredited facility.

Stem Cell Transfer

One week before transplantation until 2 weeks after transplantation, recipient mice received 100 mg/L neomycin and 10 mg/L polymyxin B sulfate in acidified water. At 6 weeks of age, Apo E0 and CD360/Apo E0 mice were lethally irradiated (9 gray from a cesium gamma source; Gamma Cell 40). Four hours later, 1×107 bone marrow cells (from background- and gender-matched mice 6 to 12 weeks of age) were injected into the tail vein. Recipient mice received normal chow for 4 weeks. For the next 12 weeks, mice were fed a high-fat diet: 21% (wt/wt) adjusted calories from fat and 1.5% (wt/wt) cholesterol (no cholate; Harlan Teklad, TD 88137).

Assessment of Chimerism

Resident macrophages were collected by phosphate-buffered saline lavage. DNA was purified after proteinase K lysing by phenol–chloroform extraction/ethanol precipitation. For polymerase chain reaction of CD36, primers for the wild-type allele were: 5’ CD36: CAGGCTCATACATTCTAGTATGACG; 3’ CD36: GGTACAATCACAAGTGGTTTCTACGTGG (expected product ~600 bp). For the null allele, the same 5’ primer was used and the 3’ primer was CCGGTTCCTCGTGTTCATG (expected product ~800 bp). With all 3 primers present, polymerase chain reaction conditions were: 94°C, 1 minute; 65°C, 1 minute; and 70°C, 2 minutes, for 30 cycles.

Polymerase chain reaction at 12 weeks after irradiation showed varying degrees of chimerism, as has been reported by others (Figure I, available online at http://atvb.ahajournals.org). The majority of the animals screened had predominantly donor-derived marrow.

Assessment of Engraftment

Male mice receiving heterogenic marrow were assessed 14 weeks after irradiation (n=6/group) by flow cytometry to determine total white blood cell count. Equal volumes of blood were collected from the tail vein into heparinized tubes. After red cells were lysed, 10 000 Flow-Check Fluorospheres (Beckman Coulter) were added for volume quantification. Gates were set to include all white cells.

Morphometry

Hearts were perfused with phosphate-buffered saline and buffered formalin. The entire aorta from the heart, extending 5 to 10 mm after bifurcation of the iliacs and including the subclavian, right, and left carotid arteries, was dissected free of fat and opened longitudinally. After staining with oil red O, aortic sections were scanned and percent lesion area determined using Adobe Photoshop. Lesion area is expressed as percent±SE.

Assessment of Lesion Morphology

Hearts from male mice representing each group were infiltrated with 30% sucrose after fixation and embedded in OCT. Ten-micron sections were cut at the level of the aortic sinuses. Slides were either stained with oil red O or processed for immunohistochemistry with a macrophage-specific antibody (F4/80; Abcam), and counterstained with aqueous hematoxylin. Digital images of lesions at 200× magnification (Magnafire software) were assessed using Adobe Photoshop for percent oil red O or F4/80 positivity.

Glucose Tolerance Testing

Mice fasted overnight received an intraperitoneal injection of 1.5 mg glucose per gram of body weight. Tail vein blood was collected at intervals up to 120 minutes and glucose measured using a One Touch Basic glucometer (Johnson & Johnson). Area under the curve was
calculated using Graph Pad Prism 4 software, with individual baseline values set to control for the fasting hypoglycemia of mice lacking CD36.

Plasma Analyses
One week before euthanization, mice were fasted and blood collected for analysis. Total, LDL, and high-density lipoprotein (HDL) cholesterol and triacylglycerol were determined using kits from Sigma Diagnostics. (Note: the EZ-HDL cholesterol kit does not involve precipitation of HDL, which proved unreliable for determination of HDL levels in Apo E0 mice.) Nonesterified fatty acid concentration was determined using a kit from Wako.

Macrophage Recruitment Assay
Sterile thioglycollate broth (4%; Sigma) was injected into the peritoneal cavity of male mice (2 mL, n=4) receiving heterogeic marrow 4 days before euthanization, 16 weeks after irradiation. Total elicited macrophages were collected by phosphate-buffered saline lavage and counted using a hemocytometer.

Statistical Analyses
Significance was determined by Student unpaired t test before irradiation and ANOVA, followed by Bonferroni multiple comparison test after irradiation. P<0.05 was considered significant.

Results
Apo E0 mice receiving Apo E0 bone marrow had 3.2-fold greater lesion area compared with CD360/Apo E0 mice receiving CD360/Apo E0 marrow (Figure 1A, first bar versus third bar: 16.51±3.045% (n=12) versus 5.17±1.248%; n=18; P=0.01). This represents a 68.7% reduction in lesion in CD360/Apo E0 mice and compares well with our previous study in nonirradiated Apo E0 and CD360/Apo E0 mice, in which we found 76% reduction in lesion area in the CD360/Apo E0 group overall. In terms of gender, there was 3.87-fold greater lesion area in Apo E0 males versus CD360/Apo E0 males receiving syngeneic marrow (Figure 1B, first bar versus third bar: 9.83±1.736% versus 2.539±0.999%; P<0.05) and 2.55-fold greater lesion area in Apo E0 females versus CD360/Apo E0 females receiving syngeneic macrophage (Figure 1C, first bar versus third bar: 19.85±4.053% versus 7.79±1.974%; P<0.01). The most exciting finding from these studies, however, was that absence of CD36 in macrophages alone was profoundly protective: overall, Apo E0 mice receiving CD360/Apo E0 marrow had an 88.1% decrease in lesion area when compared with Apo E0 mice receiving syngeneic marrow (Figure 1A, second bar versus first bar: 1.96±0.384 (n=29) versus 16.51±3.045%; n=12; P<0.001). The parallel study supported these results: there was a 2.11-fold increase in lesion development when CD36 expression was restored in macrophages alone (CD360/Apo
E° mice receiving Apo E° marrow versus CD36°/Apo E° mice receiving syngeneic marrow) (Figure 1A, fourth bar versus third bar: 10.91 ± 1.362, n = 25 versus 5.165 ± 1.248, n = 18; P = 0.05). If the data were separated according to gender, we observed a 77.6% reduction in male Apo E° mice receiving CD36°/Apo E° marrow when compared with male Apo E° mice receiving Apo E° marrow (Figure 1B, second bar versus first bar: 2.216 ± 0.567% versus 9.83 ± 1.736%; P < 0.01) and a 91.9% reduction in female Apo E° mice receiving CD36°/Apo E° marrow when compared with female Apo E° mice receiving Apo E° marrow (Figure 1C, second bar versus first bar: 1.598 ± 0.473% versus 19.85 ± 4.053%; P < 0.001). In male CD36°/Apo E° mice receiving Apo E° marrow, there was a 3.69-fold increase in lesion area when compared with male CD36°/Apo E° mice receiving CD36°/Apo E° marrow (Figure 1B, fourth bar versus third bar: 9.362 ± 1.633% versus 2.539 ± 0.999%; P = 0.01); in female CD36°/Apo E° mice receiving Apo E° marrow, there was a 1.62-fold increase in lesion area when compared with female CD36°/Apo E° mice receiving CD36°/Apo E° marrow (Figure 1C, fourth bar versus third bar: 12.59 ± 2.187% versus 7.791 ± 1.974%; P > 0.05). Representative aortae are shown in Figure 1D and 1E.

Extensive analyses were performed to rule out an effect of irradiation and reconstitution with bone marrow-derived stem cells on parameters such as weight gain, plasma glucose, and lipid metabolism. Before irradiation, baseline values of weight, cholesterol, triacylglycerol, glucose, and nonesterified fatty acids were determined (Table I, available online at http://atvb.ahajournals.org). CD36°/Apo E° male mice were significantly heavier and had significantly higher triacylglycerol and nonesterified fatty acid levels compared with Apo E° male mice. Both male and female CD36°/Apo E° mice had significantly higher total cholesterol levels and fasting hypoglycemia as compared with Apo E° mice. The increased weight in the CD36°/Apo E° males may reflect many parameters, including litter size, which was not controlled for in this study. The increased cholesterol, triacylglycerol and fatty acids, and hypoglycemia may be attributed to the phenotype of absence of CD36 in its capacity as a fatty acid transporter, and were noted previously in the CD36-null. As demonstrated by fast protein liquid chromatography analysis in a previously published study, excess triacylglycerol in CD36°/Apo E° mice was contained within the very-low-density lipoprotein fraction, whereas excess cholesterol was contained within the very-low-density lipoprotein and LDL fractions. These studies indicated that CD36°/Apo E° mice had fatty acid and lipoprotein profiles that may be considered more proatherogenic as compared with Apo E° mice.

Before irradiation, both male and female Apo E° and CD36°/Apo E° mice cleared a bolus of glucose with similar kinetics (Figure II A and IIB, available online at http://atvb.ahajournals.org), and there were no significant differences in area under the curve. After irradiation and 8 weeks after the start of the high-fat diet, glucose tolerance testing was repeated in the different groups of mice, and there still were no significant differences in area under the curve (Figure IIC and IID).

At euthanization, there were no significant differences in weight, total cholesterol, HDL cholesterol, LDL cholesterol, and nonesterified fatty acids among the groups (Table II, available online at http://atvb.ahajournals.org). There were significant differences in triacylglycerol in female mice. There were significantly higher levels of triacylglycerol in Apo E° females receiving Apo E° marrow when compared with Apo E° females receiving CD36°/Apo E° marrow or CD36°/Apo E° females receiving CD36°/Apo E° marrow. CD36°/Apo E° mice of both sexes retained their fasting hypoglycemia after irradiation, regardless of genotype of bone marrow received.

To rule out possible differences in stem cell engraftment, we compared total white blood counts in mice (n = 6/group) 14 weeks after irradiation. Apo E° mice receiving CD36°/Apo E° bone marrow had 5981 ± 689 white blood cells per micro-liter of blood compared with 6170 ± 578 white blood cells per microliter in CD36°/Apo E° mice receiving Apo E° marrow. These values do not differ statistically and rule out this as a possible explanation for the decrease in lesions.

We also ruled out differences in macrophage recruitment as a potential explanation of our results in 2 ways. First, we used thioglycollate elicitation of peritoneal macrophages 16 weeks after irradiation as an assay of macrophage recruitment to a pro-inflammatory stimulus (n = 4). In Apo E° mice receiving CD36°/Apo E° bone marrow, we collected a total of 3.05 ± 0.21 × 10^7 macrophages. This was not different statistically from the 3.19 ± 0.15 × 10^7 macrophages collected from CD36°/Apo E° mice receiving Apo E° marrow.

To rule out the possibility that after irradiation there were differences in the ability of macrophages to migrate into lesions, hearts from a random sample of male mice from each group were sectioned at the level of the aortic sinus, stained with oil red O or immunohistochemically with a macrophage-specific antibody, and assessed microscopically. There were no obvious differences in lesion morphology among the groups; in all cases, a substantial proportion (mean percent area: 47 to 60) of the lesion consisted of oil red O-positive lipid. Apo E° mice receiving Apo E° marrow had the greatest percent oil red O lesion area, and this was statistically significant when compared with Apo E° mice receiving CD36°/Apo E° marrow and CD36°/Apo E° receiving CD36°/Apo E° marrow (Figure 2A). CD36°/Apo E° receiving Apo E° also had significantly greater oil red O-positive lesion area when compared with these 2 groups. However, there were no significant differences in F4/80 positivity among the groups (mean percent area: 15.31 to 17.13) (Figure 2B). Thus, our data strongly suggest that differences in lesion development were not related to a defect in macrophage stem cell engraftment after irradiation in any of the groups, and instead appear to reflect differences in lipid accumulation within individual macrophages.

Discussion

In previous work, we demonstrated that absence of CD36 had a profound effect on atherosclerotic lesion formation in the Apo E° model. Because CD36 expression is broad and has functions other than that of a scavenger receptor, there was the potential that these other roles were involved in the observed protective effect. In the present study, we controlled for the other functions of CD36 by transplanting hematopo-
sels examined are not likely to express endothelial CD36. We rule this out for several reasons. First, CD36 is expressed on microvascular endothelium and, thus, the ves-
er of atherosclerosis. For example, perhaps use of CD36 in macrophages and endothelial cells is also possible.

It is also possible that our results reflect differences in stem cell engraftment (and thus macrophage number) or macrophage recruitment because of a mismatch of the genotype of donor and recipient. We compared total white cell number between Apo E0 mice receiving CD360/Apo E0 marrow with that of CD360/Apo E0 receiving Apo E0 marrow and observed no statistical difference. We also found no difference in macrophage numbers in the peritoneal cavity after thioglycollate elicitation in these chimeras, indicating that there is no defect in macrophage ability to detect, react, and traffic to the site of an inflammatory stimulus caused by genotype mismatch. Finally, quantitative assessment of oil red O-positive lipid in lesion and area positive for a macrophage-specific antigen suggest similarity in overall lesion morphology but a difference in lipid accumulation.

In the studies contained in this report, we saw very few differences between the groups of mice after irradiation, with the exception of fasting hypoglycemia in CD360 hosts. Nonesterified fatty acids and total, HDL, and LDL cholesterol showed no statistically significant differences. Apo E0 female mice receiving Apo E0 bone marrow had significantly higher triacylglycerol levels when compared with CD360/Apo E0 females receiving Apo E0 or CD360/Apo E0 marrow. However, Apo E0 females receiving CD360/Apo E0 marrow had similar triacylglycerol levels to CD360/Apo E0 females receiving Apo E0 marrow, yet the lesion data were strictly dependent on the genotype of the engrafted cells. This strongly argues against alterations in plasma triacylglycerol levels playing a significant role. After irradiation and on the Western diet, all groups of mice showed some degree of insulin resistance. However, the absence of significant differences between the groups again strongly argues against an effect on pathogenesis in any one group.

The mechanism of protection afforded by absence of CD36 may be more than simple lack of macrophage uptake of OxLDL. OxLDL promotes a pro-inflammatory phenotype in macrophages and endothelial cells. Absence of CD36 may indirectly delay lesion progression by interfering with the expression of pro-inflammatory products, thereby delaying or inhibiting recruitment of other cells and secretion of cytokines and pro-oxidative factors involved in atherogenesis. We and others have shown that OxLDL and 2 oxidized linoleic acid metabolites, 9-HODE and 13-HODE, can stimulate CD36 gene expression in a mechanism involving activation of peroxisome proliferator-activated receptor (PPAR)-γ, a member of the nuclear hormone superfamily, which acts as a transcriptional regulator of genes encoding proteins involved in lipid and fatty acid metabolism/regulation, as well as inflammation.13–15,29–32 Absence of CD36 negates this feedforward loop and may not only alter the lipid metabolism state of the macrophage but also alter the inflammatory state.
of these cells, and this may account for the profound decrease in lesion formation observed.

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INSIGs.
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Figure I. PCR Analysis to Determine Degree of Chimerism.

Varying concentrations of genomic DNA extracted from resident peritoneal macrophages from Apo E\(^\circ\) and CD36\(^*/\)Apo E\(^\circ\) mice were mixed and subjected to PCR using primer sets specific to the CD36 wild type and null allele. These results were compared to those from irradiated mice reconstituted with bone marrow as indicated. Most of the mice examined showed predominance (>90%) of the donor marrow 12 weeks after transplant.

A. Lane 1. Molecular weight markers. Lane 2. No sample. Lane 3. 100% Apo E\(^\circ\). Lane 4. 90% Apo E\(^\circ\), 10% CD36\(^*/\)Apo E\(^\circ\). Lane 5. 75% Apo E\(^\circ\), 25% CD36\(^*/\)Apo E\(^\circ\). Lane 6. 50% Apo E\(^\circ\), 50% CD36\(^*/\)Apo E\(^\circ\). Lane 7. 25% Apo E\(^\circ\), 75% CD36\(^*/\)Apo E\(^\circ\). Lane 8. 10% Apo E\(^\circ\), 90% CD36\(^*/\)Apo E\(^\circ\). Lane 9. 100% CD36\(^*/\)Apo E\(^\circ\). Lane 10. CD36 wild type control (equivalent to Apo E\(^\circ\)). Lane 11. CD36 null control (equivalent to CD36\(^*/\)Apo E\(^\circ\)). Lane 12. CD36\(^*/\)Apo E\(^\circ\) receiving Apo E\(^\circ\) bone marrow. Lane 13. CD36\(^*/\)Apo E\(^\circ\) receiving Apo E\(^\circ\) bone marrow.

B. Lane 1. Molecular weight markers. Lane 2. No sample. Lane 3. CD36\(^*/\)Apo E\(^\circ\) receiving Apo E\(^\circ\) bone marrow. Lane 4. CD36\(^*/\)Apo E\(^\circ\) receiving Apo E\(^\circ\) bone marrow. Lane 5. CD36\(^*/\)Apo E\(^\circ\) receiving Apo E\(^\circ\) bone marrow. Lane 6. CD36\(^*/\)Apo E\(^\circ\) receiving Apo E\(^\circ\) bone marrow. Lane 7. CD36\(^*/\)Apo E\(^\circ\) receiving Apo E\(^\circ\) bone marrow. Lane 8. Apo E\(^\circ\) receiving CD36\(^*/\)Apo E\(^\circ\) bone marrow. Lane 9. Apo E\(^\circ\) receiving CD36\(^*/\)Apo E\(^\circ\) bone marrow. Lane 10. Apo E\(^\circ\) receiving Apo E\(^\circ\) bone marrow. Lane 11. Apo E\(^\circ\) receiving Apo E\(^\circ\) bone marrow.
Mice were fasted overnight and then given a bolus of glucose by intraperitoneal injection (1.5 mg glucose/g of body weight). Blood glucose was measured at the times indicated post administration. Area under the curve is shown in the inset. Glucose tolerance testing prior to irradiation/western diet in A. male mice and B. female mice. There were no significant differences in glucose clearance and area under the curve (ANOVA). Glucose tolerance testing 12 weeks post irradiation (8 weeks on western diet) in C. male mice and D. female mice. Although there is a flattening of the curve indicative of insulin resistance in all groups, there were no significant differences in area under the curve amongst the groups (ANOVA). Note that in general CD36 null mice (CD36°/Apo E°) display hypoglycemia.
Apo E<sup>0</sup> Male

Apo E<sup>0</sup> Male

Glucose (mg/dL)

Time (min)

Apo E<sup>0</sup>/CD36<sup>0</sup> Male

Apo E<sup>0</sup>/CD36<sup>0</sup> Male

Area

apo E<sup>0</sup>

apo E<sup>0</sup>/CD36<sup>0</sup>
C

Glucose (mg/dL) vs Time (min)

- M Apo E^0 (Apo E^0 BM)
- M Apo E^0 (Apo E^0/CD36^0 BM)
- M Apo E^0/CD36^0 (Apo E^0 BM)
- M Apo E^0/CD36^0 (Apo E^0/CD36^0 BM)

Recipient
- Apo E^0
- Apo E^0/CD36^0

Area

DONOR
- Apo E^0
- Apo E^0/CD36^0
- Apo E^0/CD36^0
Table I: Pre-Irradiation Values

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<td><strong>Weight</strong></td>
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<td><strong>Triacylglycerol</strong></td>
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<td><strong>Cholesterol</strong></td>
<td>469.7±39.8mg/dL</td>
<td>636±38.96mg/dL</td>
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<td><strong>LDL Cholesterol</strong></td>
<td>259.3±18.6mg/dL</td>
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<td><strong>HDL Cholesterol</strong></td>
<td>47.9±5.8mg/dL</td>
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<td><strong>Free Fatty Acids</strong></td>
<td>2.27±0.14mM/L</td>
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<td><strong>Glucose</strong></td>
<td>71.14±3.7mg/dL</td>
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<td>62.75±1.3mg/dL</td>
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Statistical Analysis Applied: Student’s t Test. Same symbol indicates the groups differ significantly.

^p=0.0001;  B-J p<0.05
### Table II: Post Irradiation Values

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<th>mApoE&lt;sup&gt;a&lt;/sup&gt; BM: ApoE&lt;sup&gt;a&lt;/sup&gt;</th>
<th>mApoE&lt;sup&gt;a&lt;/sup&gt; BM: CD36&lt;sup&gt;a&lt;/sup&gt;/ApoE&lt;sup&gt;a&lt;/sup&gt;</th>
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<th>fApoE&lt;sup&gt;a&lt;/sup&gt; BM: ApoE&lt;sup&gt;a&lt;/sup&gt;</th>
<th>fApoE&lt;sup&gt;a&lt;/sup&gt; BM: CD36&lt;sup&gt;a&lt;/sup&gt;/ApoE&lt;sup&gt;a&lt;/sup&gt;</th>
<th>fCD36&lt;sup&gt;a&lt;/sup&gt;/ApoE&lt;sup&gt;a&lt;/sup&gt; BM: CD36&lt;sup&gt;a&lt;/sup&gt;/ApoE&lt;sup&gt;a&lt;/sup&gt;</th>
<th>fCD36&lt;sup&gt;a&lt;/sup&gt;/ApoE&lt;sup&gt;a&lt;/sup&gt; BM: ApoE&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight</strong></td>
<td>29.36 ± 0.5g</td>
<td>28.14 ± 0.8g</td>
<td>26.49 ± 0.5g</td>
<td>26.76 ± 0.7g</td>
<td>22.68 ± 0.7g</td>
<td>21.62 ± 0.6g</td>
<td>20.46 ± 0.8g</td>
</tr>
<tr>
<td><strong>Triacylglycerol</strong></td>
<td>183.8 ± 73.3mg/dL</td>
<td>138.0 ± 20.1mg/dL</td>
<td>108.1 ± 9.1mg/dL</td>
<td>89.82 ± 12.6mg/dL</td>
<td>131.3 ± 11.7mg/dL&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.36 ± 7.1mg/dL&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.17 ± 6.4mg/dL&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Cholesterol</strong></td>
<td>617 ± 50.8mg/dL</td>
<td>614.5 ± 37.8mg/dL</td>
<td>664.7 ± 34.5mg/dL</td>
<td>720.4 ± 66.9mg/dL</td>
<td>653.8 ± 53.6mg/dL</td>
<td>627.4 ± 38.9mg/dL</td>
<td>758.7 ± 76.7mg/dL</td>
</tr>
<tr>
<td><strong>LDL Cholesterol</strong></td>
<td>354.1 ± 11.4mg/dL</td>
<td>384.4 ± 19.1mg/dL</td>
<td>235.4 ± 10.9mg/dL</td>
<td>405.6 ± 23.1mg/dL</td>
<td>328.2 ± 15.5mg/dL</td>
<td>443.5 ± 29.1mg/dL</td>
<td>291.0 ± 7.1mg/dL</td>
</tr>
<tr>
<td><strong>HDL Cholesterol</strong></td>
<td>71.59 ± 7.5mg/dL</td>
<td>75.26 ± 8mg/dL</td>
<td>57.73 ± 4.9mg/dL</td>
<td>69.28 ± 4.9mg/dL</td>
<td>69.63 ± 4.5mg/dL</td>
<td>63.95 ± 5.2mg/dL</td>
<td>58.22 ± 4.7mg/dL</td>
</tr>
<tr>
<td><strong>Free Fatty Acids</strong></td>
<td>1.45 ± 0.3mM/L</td>
<td>1.28 ± 0.2mM/L</td>
<td>1.24 ± 0.1mM/L</td>
<td>1.05 ± 0.1mM/L</td>
<td>0.93 ± 0.1mM/L</td>
<td>1.19 ± 0.1mM/L</td>
<td>1.17 ± 0.1mM/L</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td>105.8 ± 7.6mg/dL&lt;sup&gt;c&lt;/sup&gt;&lt;sup&gt;d&lt;/sup&gt;</td>
<td>71 ± 11.9mg/dL&lt;sup&gt;c&lt;/sup&gt;</td>
<td>55.8 ± 3.5mg/dL&lt;sup&gt;d&lt;/sup&gt;</td>
<td>58.75 ± 5.8mg/dL&lt;sup&gt;d&lt;/sup&gt;</td>
<td>100.5 ± 0.5mg/dL&lt;sup&gt;f&lt;/sup&gt;&lt;sup&gt;g&lt;/sup&gt;</td>
<td>100 ± 1mg/dL&lt;sup&gt;h&lt;/sup&gt;&lt;sup&gt;i&lt;/sup&gt;</td>
<td>75 ± 0mg/dL&lt;sup&gt;f&lt;/sup&gt;&lt;sup&gt;h&lt;/sup&gt;&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Statistical Analysis Applied: ANOVA, followed by Bonferroni's Multiple Comparison Test. Same symbol indicates the groups differ significantly.

A,B,D,F,H p=0.01; C,J p<0.05, E,G,J p<0.001