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;24:1-6.)

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 atherogenic 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 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/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 toll receptors, fatty acids, fibril lipids, apoptotic cells, thrombospondin, collagen, effete photoreceptors, fatty acids, fibril β-amyloid deposits, and Plasmodium falciparum-infected erythrocytes.20–22 Unlike the class A receptors, which recognize the oxidized apoprotein portion of the lipoprotein particle, CD36 binds to the lipid moity of OxLDL, and is competed by anionic phospholipids.7,23 Recently, the specific ligands for CD36 on OxLDL have been defined as a family of structurally related oxidized choline glycerophospholipids.24,25 It is important to note that scavenger receptors are expressed on tissues other than macrophages that may effect disease outcome. This is especially true of CD36: fat and muscle are considered to be key 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 feedback 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). 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 IACUC-approved and performed in an AAALAC-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 protease K lysing by phenol–chloroform extraction/ethanol precipitation. For polymerase chain reaction of CD36, primers for the wild-type allele were: 5’ CD36: CAGTCATACATTTGTTATGATG; 3’ CD36: GGTACAATACTGGTTGCTGCG (expected product ~600 bp). For the null allele, the same 5’ primer was used and the 3’ primer was CGGCTCTGCTGCTGCGTGAC (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 Fluospheres (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, aortae 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 sinus. 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/H11005 4) receiving heterogeneic 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 marrow (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 bone 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±3.045% (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 E0 mice receiving Apo E0 marrow versus CD360/Apo E0 mice receiving syngeneic marrow) (Figure 1A, fourth bar versus...
published study, excess triacylglycerol in CD36\(^{-}\)/Apo E\(^{0}\) mice by fast protein liquid chromatography analysis in a previously and were noted previously in the CD36-null. As demonstrated of absence of CD36 in its capacity as a fatty acid transporter, eters, including litter size, which was not controlled for in this erol and nonesterified fatty acid levels compared with Apo E\(^{0}\) significantly heavier and had significantly higher triacylglyc-

In previous work, we demonstrated that absence of CD36 had significant differences in triacylglycerol in female mice. There were significantly higher levels of triacylglycerol in Apo E\(^{0}\) females receiving Apo E\(^{0}\) marrow when compared with Apo E\(^{0}\) females receiving CD36\(^{-}\)/Apo E\(^{0}\) marrow or CD36\(^{-}\)/Apo E\(^{0}\) females receiving CD36\(^{-}\)/Apo E\(^{0}\) marrow. CD36\(^{-}\)/Apo E\(^{0}\) 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\(^{0}\) mice receiving CD36\(^{-}\)/Apo E\(^{0}\) 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\(^{0}\) mice receiving Apo E\(^{0}\) 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\(^{0}\) mice receiving CD36\(^{-}\)/Apo E\(^{0}\) bone marrow, we collected a total of 3.05 ± 0.21 × 10\(^{5}\) macrophages. This was not different statistically from the 3.19 ± 0.15 × 10\(^{5}\) macrophages collected from CD36\(^{-}\)/Apo E\(^{0}\) mice receiving Apo E\(^{0}\) 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\(^{0}\) mice receiving Apo E\(^{0}\) marrow had the greatest percent oil red O lesion area, and this was statistically significant when compared with Apo E\(^{0}\) mice receiving CD36\(^{-}\)/Apo E\(^{0}\) marrow and CD36\(^{-}\)/Apo E\(^{0}\) receiving CD36\(^{-}\)/Apo E\(^{0}\) marrow (Figure 2A). CD36\(^{-}\)/Apo E\(^{0}\) receiving Apo E\(^{0}\) 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\(^{0}\) model.27 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 hematopoietic stem cells, allowing us to isolate the scavenger receptor function of macrophage CD36 from most other functions.
expressed on microvascular endothelium and, thus, the ves-
vessel examined are not likely to express endothelial CD36.

We rule this out for several reasons. First, CD36 is
result of endothelial cell CD36 rather than macrophage
oxidation.

Our data provide strong support for the hypothesis that
inhibition of CD36 function in macrophages alone will have
large therapeutic impact.

This contention is supported in 2 ways. Absence of CD36
in macrophages transplanted into mice that expressed CD36
in all other nonhematopoietic tissues resulted in an 88.1%
decrease in aortic lesions. Alternatively, presence of CD36
only in macrophages resulted in a 52.7% increase in lesion
area. The lesion area in this group was nearly indistinguish-
able from atherosclerosis in Apo E0 mice transplanted with
syngeneic marrow. These results provide strong rationale for
therapeutic blockade of macrophage CD36 to slow
atherogenesis.

There is a nonstatistically significant trend in the data that
suggests that absence of CD36 in macrophages is more
protective than whole-body absence of CD36. This intriguing
result points to the multifaceted nature of this protein. It is
possible that CD36 expression and function as a fatty acid
transporter in adipocytes and muscle may provide a level of
protection against atherosclerosis. For example, perhaps use
of lipoproteins by these tissues decreases potential for

It is theoretically possible that the effect we observed is a
result of endothelial cell CD36 rather than macrophage
CD36. We rule this out for several reasons. First, CD36 is
expressed on microvascular endothelium and, thus, the ves-
sels examined are not likely to express endothelial CD36.
Second, about 0.1% of in vivo endothelium turns over each
day. Even if we were to assume that all new endothelium was stem
cell-derived (which is probably not the case), 4 months after
irradiation <15% of the endothelium in irradiated mice
would be donor-derived. The dramatic nature of our results,
believe, is more consistent with an effect on macrophages,
although an additional effect from CD36 absence on endo-
thelial 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 thiogly-
collate 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 mismat-
ch. 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 differ-
ces 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 cyto-
kines 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 via a mechanism involving activation
of 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/regula-
tion, as well as inflammation. Absence of CD36
negates this feed-forward 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.
Acknowledgments
This work was supported by grants from the National Heart, Lung, and Blood Institute of the NIH, R01 HL070083, (M.F.), and P01 HL 72942 (R.L.S.). We thank Jonathan Smith and his laboratory (CCF) for excellent technical expertise in the sectioning of the aortic sinus.

References
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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° and CD36°/Apo E° 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°. Lane 4. 90% Apo E°, 10% CD36°/Apo E°. Lane 5. 75% Apo E°, 25% CD36°/Apo E°. Lane 6. 50% Apo E°, 50% CD36°/Apo E°. Lane 7. 25% Apo E°, 75% CD36°/Apo E°. Lane 8. 10% Apo E°, 90% CD36°/Apo E°. Lane 9. 100% CD36°/Apo E°. Lane 10. CD36 wild type control (equivalent to Apo E°). Lane 11. CD36 null control (equivalent to CD36°/Apo E°). Lane 12. CD36°/Apo E° receiving Apo E° bone marrow. Lane 13. CD36°/Apo E° receiving Apo E° bone marrow.

Figure II. Glucose Tolerance Testing

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<sup>−/−</sup>/Apo E<sup>−/−</sup>) display hypoglycemia.
**Figure A**

- **Graph**: Plot showing glucose levels over time for two groups: Apo E<sup>0</sup> Male and Apo E<sup>0</sup>/CD36<sup>0</sup> Male.

- **X-axis**: Time (min)
- **Y-axis**: Glucose (mg/dL)

**Bar Graph**

- **Groups**: apo E<sup>0</sup> and apo E<sup>0</sup>/CD36<sup>0</sup>
- **Y-axis**: Area

The data suggests a significant difference in glucose levels and area between the two groups.
Graph D:

- F Apo E<sup>0</sup> (Apo E<sup>0</sup> BM)
- F Apo E<sup>0</sup> (Apo E<sup>0</sup>/CD36<sup>0</sup> BM)
- F Apo E<sup>0</sup>/CD36<sup>0</sup> (Apo E<sup>0</sup> BM)
- F Apo E<sup>0</sup>/CD36<sup>0</sup> (Apo E<sup>0</sup>/CD36<sup>0</sup> BM)

Glucose (mg/dL) vs. Time (min)

Recipient:
- Apo E<sup>0</sup>
- Apo E<sup>0</sup>/CD36<sup>0</sup>

Area:

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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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<td>108.1 ± 9.1mg/dL</td>
<td>89.82 ± 12.6mg/dL</td>
<td>131.3 ± 11.7mg/dL&lt;sup&gt;A,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></td>
<td></td>
<td></td>
<td>108.1 ± 34.5mg/dL</td>
<td>720.4 ± 66.9mg/dL</td>
<td>653.8 ± 53.6mg/dL</td>
<td>627.4 ± 38.9mg/dL&lt;sup&gt; &lt;/sup&gt;</td>
<td>758.7 ± 76.7mg/dL&lt;sup&gt; &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>700.4 ± 66.9mg/dL</td>
<td>758.7 ± 76.7mg/dL</td>
<td>758.7 ± 76.7mg/dL</td>
<td>701 ± 58.7mg/dL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>235.4 ± 10.9mg/dL</td>
<td>405.6 ± 23.1mg/dL</td>
<td>328.2 ± 29.1mg/dL</td>
<td>443.5 ± 71mg/dL</td>
<td>291.0 ± 7.1mg/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 ± 29.1mg/dL</td>
<td>443.5 ± 71mg/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&lt;sup&gt;L&lt;/sup&gt;</td>
<td>1.17 ± 0.1mM/L&lt;sup&gt;L&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td>105.8 ± 7.6mg/dL&lt;sup&gt;C,D,E&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;E&lt;/sup&gt;</td>
<td>100.5 ± 0.5mg/dL&lt;sup&gt;F,G&lt;/sup&gt;</td>
<td>100 ± 1mg/dL&lt;sup&gt;H,J&lt;/sup&gt;</td>
<td>75 ± 0mg/dL&lt;sup&gt;F,H,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.

<sup>A,B,D,F,H</sup> p=0.01,  <sup>C,J</sup> p<0.05,  <sup>E,G,I</sup> p<0.001