The role of macrophage leptin receptor in aortic root lesion formation

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Surmi BK, Atkinson RD, Gruen ML, Coenen KR, Hasty AH. The role of macrophage leptin receptor in aortic root lesion formation. Am J Physiol Endocrinol Metab 294: E488–E495, 2008. First published January 8, 2008; doi:10.1152/ajpendo.00374.2007.—Plasma leptin is often elevated in obese individuals, and previous studies have suggested leptin as a factor that links obesity and atherosclerosis. Because macrophages play a key role in atherogenesis and are responsive to leptin, we hypothesized that leptin increases aortic root lesion formation, in part, through macrophage leptin receptor (LepR). Three different bone marrow transplantation studies were conducted in which bone marrow, with or without LepR, was transplanted into lethally irradiated 1) LDL receptor-deficient (LDLR−/−) mice with moderate hyperleptinemia due to Western diet (WD) feeding, 2) LDLR−/− mice with WD feeding plus pharmacologically induced hyperleptinemia (daily injection of 125 μg leptin), or 3) obese, hyperleptinemic, LepR-deficient LDLR−/− (LepRΔ/db/db-LDLR−/−) mice. Minor differences in plasma parameters such as cholesterol, triglycerides, and insulin were observed in some groups; however, a consistent trend for the role of LepR on these parameters was not detected. In each of the studies, macrophage LepR expression did not have an effect on aortic root atherosclerotic lesion formation. These results suggest that nonhematopoietic cells may have a more significant role than macrophages in leptin-mediated effects on aortic root lesion formation.

cardiovascular disease; db/db mouse; hyperlipidemia

IN THE CURRENT WORLDWIDE OBESITY EPIDEMIC, more than 1 billion adults are overweight, and at least 300 million of these individuals are obese. In the United States, cardiovascular disease (CVD) is the number one cause of death, and more than 80% of CVD deaths are due to atherosclerosis-related diseases. Due to the compounding effect of obesity on atherosclerosis (10, 26, 31, 36, 39), understanding mechanisms by which obesity increases risk of atherosclerosis is critical to providing knowledgeable treatment and prevention of this disease.

Leptin is a 16-kDa protein secreted by adipocytes and represents one potential molecular link between obesity and atherosclerosis. Circulating plasma leptin levels correlate with the percentage of body fat and/or body mass index in normal-weight and obese rodents and humans (9, 24). Although leptin is a satiety factor that normally reduces appetite and promotes energy expenditure, many obese humans display hyperleptinemia and are considered leptin resistant (1). Leptin has multiple biological effects, both centrally and peripherally, and there is evidence (35, 38) that hyperleptinemia is an independent risk factor for myocardial infarction and atherosclerosis.

There are six leptin receptor (LepR) isoforms. Unlike the other isoforms, the long form of the receptor, isoform B, has a 302-amino acid cytoplasmic tail and participates in intracellular signaling. The long form of the LepR is highly expressed in hypothalamic neurons, and LepR-deficient (LepRΔ/db/db) mice develop extreme obesity and hyperleptinemia. Peripheral cells such as macrophages also express this isoform (25, 28), and leptin has been shown to have several potentially atherogenic effects in macrophages. Treatment of macrophages with recombinant leptin affects their cytokine production (20, 23, 32, 33), lipid metabolism (11, 28), phagocytic function (11, 23), proliferation (11, 23, 32, 33), and oxidative stress (19). Because macrophages are key in the development and progression of atherosclerosis (22), it is possible that hyperleptinemia could promote atherogenesis via some of these macrophage-specific effects.

Recently, Bodary et al. (3) investigated the possible role of hyperleptinemia in atherosclerosis and thrombosis. In their study, atherosusceptible apolipoprotein E-deficient (apoE−/−) mice were injected daily with recombinant leptin for 4 wk. Mice receiving the leptin injections had a significant increase in lesion surface area in their brachiocephalic and carotid arteries as well as their thoracic aorta. Although these experiments indicated that leptin may be a molecular link between obesity and CVD, the cell type mediating leptin’s effect was not identified. In the experiments described in our current study, we have tested the hypothesis that hyperleptinemia promotes atherosclerosis at the aortic root via macrophage LepRs.

MATERIALS AND METHODS

Mice. LDL receptor-deficient (LDLR−/−) and LepRΔ/db/db mice were originally purchased from Jackson Laboratories (Bar Harbor, ME) and were on the C57BL/6 background. The LDLR−/− and LepRΔ/db/db mice were crossed to generate LDLR−/−/LepRΔ/db/db mice. LepR genotype was determined according to the protocol of Kowalski et al. (18). Primers were as follows: db-F1 5′-AGT CTC 3′, db-R4-WT 5′-ACT GAC TTT TGC 3′, db-Rb-db 5′-CAC TTC AGT GTA AAC CAT GTT TTT C3, and db-Rb-db 5′-GAG ACT CTT TGA AGT CTC 3′, db-Rb-db 5′-GAG ACT CTT TGA AGT CTC 3′, and db-Rb-db 5′-GAG ACT CTT TGA AGT CTC 3′.

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**Plasma analyses.** Blood glucose was measured from whole blood using a OneTouch glucometer (LifeScan, Milpitas, CA). Total cholesterol (TC) and triglyceride (TG) levels were quantified using enzymatic colorimetric kits (Raichem, San Diego, CA) according to the manufacturer’s instructions and adapted to a microtiter plate. Plasma nonesterified fatty acid (NEFA) levels were quantified with a NEFA C kit (Wako, Richmond, VA). Plasma leptin and insulin were quantified in Vanderbilt’s Hormone Assay Core laboratory using radioisotope assays.

**BMT.** Donor mice were killed by cervical dislocation under anesthesia, and bone marrow was collected from the femurs and tibias. For each donor genotype, bone marrow was pooled, and ~2–5 x 10^6 bone marrow cells were injected into lethally irradiated recipient mice via the retroorbital venous plexus. Recipient mice were given antibiotic water (5 mg of neomycin and 25,000 U polymyxin B sulfate/l) for 1 wk prior to and 2 wk following BMT. After transplantation, whole blood was collected from select recipient mice in BMT study 1 and DNA prepared using the Qiagen DNeasy tissue kit. DNA was extracted from spleen tissue from a representative recipient mouse in each bone marrow transplant group in BMT studies 2 and 3 using the Qiagen DNeasy blood and tissue kit. PCR for LepR genotype was performed as described above to confirm complete reconstitution with donor marrow. Three separate BMT experiments were conducted as described below and diagrammed in Fig. 1.

**BMT study 1.** Male and female LepR^+/+;LDLR^−/− mice were lethally irradiated at 6–9 wk of age and transplanted with marrow from LepR^+/+;LDLR^−/− or LepR^db/db;LDLR^−/− donors. Eight weeks post-BMT, mice were fed a high-fat Western diet (WD; 0.15% cholesterol, 42% of calories from milkfat; Harlan Teklad, Madison, WI). Mice were killed at 21 wk post-BMT.

**BMT study 2.** Female LepR^+/+;LDLR^−/− mice were lethally irradiated at 9–16 wk of age and transplanted with marrow from LepR^+/+;LDLR^−/− or LepR^db/db;LDLR^−/− donors. Starting at 4 wk post-BMT, mice were fed WD through the end of the study (8 wk total). After 4 wk of WD feeding, mice began to receive daily injections of leptin or PBS control for 4 wk and were then killed.

**BMT study 3.** Male and female obese, hyperleptinemic LepR^db/db;LDLR^−/− mice were lethally irradiated at 9–13 wk of age and transplanted with marrow from LepR^db/db;LDLR^−/− or LepR^+/+;LDLR^−/− donors. Mice remained on chow diet throughout the study and were killed at 16 wk post-BMT.

**Leptin injections.** Mice in BMT study 2 received leptin or vehicle injections daily for 4 wk. Murine recombinant leptin (National Hormone & Peptide Program, Torrance, CA) was dissolved in PBS, and 125 μg was injected intraperitoneally in a volume of 200 μl, as previously done by Bodary et al. (3). Leptin solution was made fresh each week and frozen at −20°C until use.

For the leptin half-life study, 9-wk-old LepR^+/+;LDLR^−/− female mice were injected with leptin (125 μg). Plasma samples were obtained at baseline (2 h before injection) and 30 min, 1 h, 3 h, 12 h, and 22 h post-leptin injection.

**Atherosclerosis quantification.** Hearts were preserved in optimal cutting temperature and immediately frozen on dry ice. Fifteen 10-μm sections were collected over a 300-μm range beginning at the aortic root according to the method of Paigen et al. (29). Aortic root sections were then stained for neutral lipids with oil red O (ORO) and counterstained with hematoxylin according to standard protocol. Lesions were captured with a Q-Imaging MicroPublisher camera and quantified using Kinetic Histomixtrix 6 imaging and analysis software (Kinetic Imaging, Durham, NC).

**Measurement of tissue mass.** During BMT study 2, mice were assessed for adipose and muscle mass by NMR using a Bruker Minispec (Woodlands, TX) in the Mouse Metabolic Phenotyping Center at Vanderbilt University. After the mice were killed, the liver and abdominal fat pads were weighed.

**Statistical methods.** Unpaired Student’s t-test was used to assess statistical significance. A P value of <0.05 was considered to be statistically significant. Results are shown as means ± SE.

**RESULTS.** To test our hypothesis that hyperleptinemia promotes atherosclerosis via macrophage LepR, three different BMT experiments were conducted. All donor and recipient mice were LDLR deficient, with or without LepR. Figure 1 shows a diagram of the three BMT studies. After transplantation, reconstitution with the correct donor marrow was confirmed by performing PCR for the LepR on DNA from whole blood or spleen tissue from recipient mice. As shown in Fig. 2, mice were completely reconstituted and expressed the LepR genotype of the donor marrow.

**BMT study 1:** mice with an absence of macrophage LepR and mild hyperleptinemia. The first BMT was designed to test the hypothesis that the absence of macrophage LepR would be atheroprotective in a setting of mild hyperleptinemia. At 6–9 wk of age, LepR^+/+;LDLR^−/− mice were lethally irradiated and transplanted with bone marrow from LepR^+/+ or LepR^db/db donors. This BMT design resulted in mice with an absence of...
functional LepR in their hematopoietic cells, including macrophages. Comparisons were made for male and female recipients separately (Table 1). At 5 mo post-BMT, body weight, glucose, NEFA, insulin, and leptin levels were not different between the recipients of LepR+/- LDLR/- or LepR/db/db, LDLR/- marrow. TC and TG levels in male mice receiving LepR/db/db LDLR/- marrow were increased compared with controls (P < 0.05); however, no differences in plasma lipids were noted in female mice. Measurement of aortic root lesion area by ORO staining revealed an increase in lesion area in female compared with male mice (Fig. 3); however, within each sex the absence of LepR on hematopoietic cells did not affect lesion area.

BMT study 2: mice with an absence of macrophage LepR and pharmacologically induced extreme hyperleptinemia. Because the presence or absence of LepR on hematopoietic cells did not impact atherosclerotic lesion area in LepR+/-; LDLR/- recipient mice when they were exposed to only mild elevations in leptin, a similar experiment was conducted using daily injections of leptin to induce extreme hyperleptinemia. At 9–16 wk of age, female LepR+/-; LDLR/- mice were lethally irradiated and transplanted with bone marrow cells either with or without LepR expression. After 4 wk on WD, mice began receiving leptin (125 μg) or vehicle (PBS) injections for an additional 4 wk. The resulting groups have been labeled according to the genotype of their bone marrow and their injection treatment type: 1) LepR+/-; LDLR/- vehicle, 2) LepR/db/db; LDLR/- vehicle, 3) LepR+/-; LDLR/- leptin, and 4) LepR/db/db; LDLR/- leptin.

Body weight, abdominal adipose tissue, and liver mass, as well as total body adipose tissue and muscle mass, were measured (Fig. 4). At baseline, no differences in body weight were observed among the four groups (Fig. 4A). All groups had increased body weight after 4 wk on WD. After initiation of leptin injection, both groups receiving leptin had a significant decrease in body weight (P < 0.05 at 2 and 4 wk postinjection; Fig. 4A and Table 1). Both leptin-treated groups had significantly less abdominal adipose tissue compared with vehicle-treated groups after 4 wk of leptin injections (LepR+/-; LDLR/- leptin vs. LepR+/-; LDLR/- vehicle, P < 0.005; LepR/db/db; LDLR/- vehicle, P = 0.0001; Fig. 4B). Liver mass was also decreased in both leptin-treated groups, although this reached significance only for the LepR/db/db; LDLR/- leptin group (P < 0.005; Fig. 4B). Total body adipose tissue mass (as measured by NMR) was decreased by 45% in the LepR+/-; LDLR/- leptin group and by 49% in the LepR/db/db; LDLR/- leptin group compared with their respective controls (P < 0.0001; Fig. 4C). Muscle mass decreased nonsignificantly, by 3%, in mice receiving leptin.

Table 1. Plasma parameters and body weight for LepR+/-; LDLR/- recipients from BMT study 1 at 5 mo post-BMT

<table>
<thead>
<tr>
<th>Donor Marrow</th>
<th>Females</th>
<th>Males</th>
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<tbody>
<tr>
<td>LepR+/-; LDLR/-</td>
<td>LepR/db/db; LDLR/-</td>
<td>LepR+/-; LDLR/-</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td><strong>Body weight, g</strong></td>
<td>21.4 ± 1.1</td>
<td>20.8 ± 1.0</td>
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| **Cholesterol, mg/dl** | 873 ± 59 | 765 ± 44 | 904 ± 37 | 1,093 ± 72 |*
| **Triglycerides, mg/dl** | 357 ± 38 | 276 ± 50 | 323 ± 65 | 568 ± 53 |*
| **NEFA, mEq/l** | 0.71 ± 0.08 | 0.87 ± 0.07 | 0.88 ± 0.10 | 0.40 ± 0.12 |*
| **Glucose, mg/dl** | 146 ± 11 | 147 ± 12 | 118 ± 5 | 130 ± 8 |*
| **Insulin, ng/ml** | 3.43 ± 1.58 | 1.13 ± 0.20 | 3.40 ± 1.53 | 0.73 ± 0.11 |*
| **Leptin, ng/ml** | 16.9 ± 2.9 | 12.8 ± 1.8 | 18.9 ± 3.9 | 12.4 ± 1.6 |*

Results are shown as means ± SE. LepR, leptin receptor; LDLR, LDL receptor; BMT, bone marrow transplantation; NEFA, nonesterified fatty acids. LepR+/-; LDLR/- recipients were transplanted with LepR+/-; LDLR/- or LepR/db/db; LDLR/- bone marrow. Mice were placed on Western diet (WD) 8 wk post-BMT and killed 5 mo post-BMT. Plasma parameters were assayed as described in MATERIALS AND METHODS. Statistics were performed using Student's t-test to compare parameters of LepR+/- and LepR/db/db recipients within each sex. *P < 0.05 vs. LepR+/-; LDLR/- group.
The presence or absence of macrophage LepRs did not impact any plasma parameters when mice within the vehicle- or leptin-treated groups were compared (Table 2). When leptin- vs. vehicle-treated recipients of the same marrow were compared, some differences in plasma parameters were noted (Table 2). Leptin-treated LepR<sup>−/−</sup>LDLR<sup>−/−</sup> recipients demonstrated a reduction in TC and TG levels compared with vehicle-treated controls (P < 0.05). Leptin-treated LepR<sup>+/+</sup>; LDLR<sup>−/−</sup> recipients displayed a reduction in TC and an increase in NEFA levels compared with vehicle-treated controls (P < 0.01). Plasma insulin levels were significantly reduced in the leptin-treated LepR<sup>+/+</sup>;LDLR<sup>−/−</sup> group (P < 0.05) and trended toward a decrease in the leptin-treated LepR<sup>ob/db</sup>;LDLR<sup>−/−</sup> group compared with their respective vehicle controls. Contrary to our initial expectations, a statistically significant decrease in plasma leptin levels was observed in both leptin-treated groups at time of death (P < 0.05). No differences in aortic root lesion area were detected among the groups (Fig. 5).

**Half-life of injected leptin.** Because end point circulating leptin levels were significantly lower in mice injected with leptin, we sought to investigate the half-life of injected recombinant leptin. Female LepR<sup>+/+</sup>;LDLR<sup>−/−</sup> mice were injected with 125 μg of leptin and plasma leptin concentrations measured for ≤22 h postinjection (Fig. 6). Plasma leptin levels were dramatically elevated at 30, 60, and 180 min postinjection (P < 0.0001). By 12 h post-leptin injection, plasma leptin levels had returned to baseline in all of the mice. These half-life data were used to calculate an average daily leptin concentration in the mice from BMT study 2 of ~350 ng/ml (Table 2).

**BMT study 3: mice with the presence of macrophage LepR and severe hyperleptinemia.** As a third test of our hypothesis, obese hyperleptinemic LepR<sup>ob/db</sup>;LDLR<sup>−/−</sup> mice were transplanted with bone marrow from LepR<sup>ob/db</sup> or LepR<sup>+/+</sup> donors. The recipients had naturally occurring hyperleptinemia (>100 ng/ml) due to their deficiency of LepR and were also hyperlipidemic, similar to leptin-deficient LDLR<sup>−/−</sup> mice (13, 15). Thus, they did not require the use of a high-fat diet to induce atherogenic lesion formation. This BMT was designed to test the atherogenicity of LepR when it is expressed only on macrophages and other hematopoietic cells in the presence of obesity accompanied by high plasma leptin levels. Mice were 9–13 wk old at the time of transplantation and were killed 16 wk post-BMT.

There were no differences in body weight, plasma lipids, or leptin levels between the two groups at death; however, plasma insulin levels were significantly lower in the LepR<sup>+/+</sup>; LDLR<sup>−/−</sup> recipients (P < 0.05; Table 3).

No sexual dimorphism was detected among the groups when aortic root lesions were quantified by ORO staining; therefore, data from male and female mice were combined. Differences in lesion size were not observed between the mice with a global absence of LepR (LepR<sup>ob/db</sup>;LDLR<sup>−/−</sup>) group and those expressing LepR only on their hematopoietic cells (LepR<sup>+/+</sup>; LDLR<sup>−/−</sup> group), as shown in Fig. 7.

**DISCUSSION**

We have addressed the role of macrophage LepR in aortic root atherosclerotic lesion formation through three distinct BMT studies. First, we tested the hypothesis that an absence of LepR on macrophages would be atheroprotective in lean LDLR<sup>−/−</sup> mice (BMT study 1). Second, we tested this same hypothesis under pharmacologically induced hyperleptinemia (BMT study 2). Finally, we tested the hypothesis that the presence of LepR only on hematopoietic cells such as macrophages would be proatherogenic in obese hyperleptinemic mice (BMT study 3). Despite the use of these three approaches...
LEPTIN, MACROPHAGES, AND ATHEROSCLEROSIS

Table 2. Plasma parameters and body weight for LepR<sup>+/−</sup>;LDLR<sup>−/−</sup> recipients from BMT study 2

| Donor Marrow | LepR<sup>+/−</sup>;LDLR<sup>−/−</sup> | Vehicle | LepR<sup>+/−</sup>;LDLR<sup>−/−</sup> | Leptin
<table>
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<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>10</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>20.8 ± 0.9</td>
<td>21.0 ± 0.3</td>
<td>18.3 ± 0.4*</td>
<td>18.6 ± 0.7§</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>820 ± 59</td>
<td>817 ± 46</td>
<td>691 ± 35</td>
<td>662 ± 34†</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>261 ± 14</td>
<td>287 ± 24</td>
<td>216 ± 8†</td>
<td>226 ± 16†</td>
</tr>
<tr>
<td>NEFA, mEq/l</td>
<td>0.96 ± 0.08</td>
<td>1.11 ± 0.19</td>
<td>1.36 ± 0.10‡</td>
<td>1.16 ± 0.11</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>108.10 ± 7</td>
<td>127.5 ± 7</td>
<td>105.2 ± 6</td>
<td>107.10 ± 10</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>1.05 ± 0.21</td>
<td>0.89 ± 0.13</td>
<td>0.67 ± 0.06*</td>
<td>0.63 ± 0.04</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>10.6 ± 2.3</td>
<td>9.3 ± 1.4</td>
<td>4.8 ± 0.6*</td>
<td>4.7 ± 0.9†</td>
</tr>
<tr>
<td>Estimated average daily leptin, ng/ml</td>
<td>10.6 ± 2.3</td>
<td>9.3 ± 1.4</td>
<td>4.8 ± 0.6*</td>
<td>4.7 ± 0.9†</td>
</tr>
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</table>

Results are shown as means ± SE. Female LepR<sup>+/−</sup>;LDLR<sup>−/−</sup> recipients were transplanted with LepR<sup>+/−</sup>;LDLR<sup>−/−</sup> or LepR<sup>deldel</sup>;LDLR<sup>−/−</sup> bone marrow. Mice were placed on WD 4 wk post-BMT. Eight weeks post-BMT, mice were last injected daily with leptin (125 μg) or vehicle (PBS) for 4 wk. Mice were last injected with leptin or vehicle 21–25 h before being killed. Student’s t-tests were used to compare recipients of different bone marrow within treatment groups and recipients of the same bone marrow in different treatment groups. The average daily leptin levels were estimated using the area under the curve from Fig. 6 to calculate the average elevation above basal leptin levels in leptin-injected mice. *P < 0.05 vs. LepR<sup>+/−</sup>;LDLR<sup>−/−</sup> vehicle group; †P < 0.05 vs. LepR<sup>deldel</sup>;LDLR<sup>−/−</sup> vehicle group; ‡P < 0.01 vs. LepR<sup>+/−</sup>;LDLR<sup>−/−</sup> vehicle group.

However, our data, derived from three separate bone marrow protocols, do not support a role for macrophages in hyperleptinemia-associated aortic root lesion formation.

There is evidence (35, 38) that hyperleptinemia is associated with increased risk of CVD in humans. Recent mechanistic studies related to the role of leptin in vascular health (6, 27, 30, 34, 35a) have focused on the four major cell types involved in atherogenesis: endothelial cells, smooth muscle cells, T lymphocytes, and macrophages. Full-length LepR has been shown (6, 27, 30, 34, 35a) to be expressed on and capable of signal transduction in all four of these cell types. Potential means by which leptin could cause macrophages to become proatherogenic include increased inflammatory cytokine secretion (20, 23, 33), proliferation (11, 23, 32, 33), and oxidative stress (2, 19) as well as impaired lipid metabolism (6, 28). Thus, we speculated that macrophages could be the cell type responsible for increased lesion formation in a setting of hyperleptinemia. However, our data, derived from three separate bone marrow

![Fig. 5. Aortic root lesion area in female LepR<sup>+/−</sup>;LDLR<sup>−/−</sup> mice from BMT study 2. Lethally irradiated female LepR<sup>+/−</sup>;LDLR<sup>−/−</sup> recipients were reconstituted with LepR<sup>+/−</sup>;LDLR<sup>−/−</sup> or LepR<sup>deldel</sup>;LDLR<sup>−/−</sup> bone marrow and fed WD for 8 wk, followed by daily leptin injections for 4 of the 8 wk. Aortic root lesions were stained with ORO and quantified as described in MATERIALS AND METHODS. Bone marrow genotype of the recipients and injection treatment are indicated as follows: LepR<sup>+/−</sup>;LDLR<sup>−/−</sup> vehicle (a), LepR<sup>deldel</sup>;LDLR<sup>−/−</sup> vehicle (b), LepR<sup>+/−</sup>;LDLR<sup>−/−</sup> leptin (c), and LepR<sup>deldel</sup>;LDLR<sup>−/−</sup> leptin (c); n = 8–10 mice/group.

![Fig. 6. Measurement of plasma leptin following intraperitoneal leptin injection. Lean female LepR<sup>+/−</sup>;LDLR<sup>−/−</sup> mice were injected with recombinant leptin (125 μg). Blood samples were collected at baseline (2 h before injection) and at 30 min, 1 h, 3 h, 12 h, and 22 h after injection, and plasma leptin values were quantified. #P < 0.0001 vs. baseline; n = 3–5 mice/time point.](http://ajpendo.physiology.org/Downloadedfrom)
changes in aortic root lesion area in LDLR$^{-/-}$ mice, then the leptin-treated LepR$^{+/+}$;LDLR$^{-/-}$ recipient mice in BMT study 2 would have been expected to have more atherosclerosis than the vehicle-treated LepR$^{+/+}$;LDLR$^{-/-}$ recipients. Third, it is possible that leptin has a greater effect in more advanced lesions. The lesions in apoE$^{-/-}$ mice fed Western diet for 8 wk are more advanced than those in LDLR$^{-/-}$ mice under the same conditions. Fourth, the location of the lesions quantified was different between the two studies. It is possible that leptin mediates more potent effects at sites other than the aortic root. Last, macrophages from LepR$^{db/db}$ mice are a known model of diabetic macrophages. They have been shown (14, 21, 40) to have altered insulin signaling, cell morphology, and cytokine secretion compared with normal macrophages. Therefore, an intriguing possibility is that the atheroprotective effect that we expected in mice without macrophage LepR isoform B was obscured due to the LepR$^{db/db}$;LDLR$^{-/-}$ macrophages being proinflammatory or having other defects (21).

In both the study by Bodary et al. (3) as well as a recent study by Chiba et al. (8), leptin was shown to mediate changes in the abdominal and thoracic aorta as well as smaller arteries such as the brachiocephalic. This difference from our current finding in the aortic root is important to note, because these other vessels are encompassed by adipose tissue. Leptin can be secreted from perivascular adipose tissue (16) and may modulate the activity of macrophages more directly in these vessels. Our original hypothesis was that leptin would increase macrophage-driven atherogenesis by mediating inflammatory or lipid-related processes. However, recent data from our laboratory (12) have shown that leptin is a potent monocyte/macrophage chemoattractant. Our current studies in the aortic root are unable to address the question of whether perivascular-derived leptin can influence macrophage recruitment to the artery wall.

Our current data leave open the possibility that non-bone marrow-derived cells such as endothelial cells and smooth muscle cells mediate the proatherogenic effects of leptin detected in other studies (1, 3). LepRs are expressed on coronary endothelial cells, and hyperleptinemia has been shown to induce endothelial dysfunction (17) and nitric oxide production (37). Other investigators (5) have shown that leptin induces oxidant stress in endothelial cells as well as their proliferation and expression of matrix metalloproteinases, a process likely involved in plaque rupture. Oda et al. (27) have shown that leptin stimulates the proliferation and migration of rat aortic

Table 3. Plasma parameters and body weight for LepR$^{db/db}$;LDLR$^{-/-}$ recipients from BMT study 3

<table>
<thead>
<tr>
<th>Donor Marrow</th>
<th>LepR$^{db/db}$;LDLR$^{-/-}$</th>
<th>LepR$^{+/+}$;LDLR$^{-/-}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>48 ± 1</td>
<td>45 ± 1</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>775 ± 29</td>
<td>718 ± 46</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>430 ± 41</td>
<td>348 ± 32</td>
</tr>
<tr>
<td>NEFA, mEq/l</td>
<td>0.86 ± 0.06</td>
<td>0.87 ± 0.08</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>17.8 ± 2.3</td>
<td>11.1 ± 1.5*</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>153 ± 10</td>
<td>127 ± 14</td>
</tr>
</tbody>
</table>

Results are shown as means ± SE. LepR$^{db/db}$;LDLR$^{-/-}$ recipients were transplanted with LepR$^{db/db}$;LDLR$^{-/-}$ or LepR$^{+/+}$;LDLR$^{-/-}$ bone marrow and killed 16 wk post-BMT. *P < 0.05 vs. LepR$^{db/db}$;LDLR$^{-/-}$ group.
smooth muscle cells, a process that is involved in both intermediate atherosclerotic lesion formation and in restenosis after balloon injury. Thus, it is possible that the atherogenic effects of leptin are via activation of these nonhematopoietic cells involved in lesion formation.

The leptin-treated mice in BMT study 2 responded physiologically to the exogenously induced hyperleptinemia by losing weight, including a ~50% reduction in adipose tissue mass, which concurs with previous studies (3, 7). At the time they were killed, despite 4 wk of daily leptin treatment, both leptin-treated groups had significantly lower plasma leptin levels than the vehicle-treated groups. Thus, induction of hyperleptinemia via daily intraperitoneal injection resulted in reduced end point plasma leptin levels, probably due to the significant loss of adipose tissue, the main endogenous source of leptin. To test the length of time that injected leptin stayed in the circulation, plasma leptin levels were measured for ≤22 h postinjection (Fig. 6). Although this leptin half-life study did not indicate the effect of chronic leptin injections, it suggested that the leptin-treated mice in BMT study 2 were exposed to hyperleptinemia for ≤12 h out of each day during the 4-wk treatment period. This short period of extreme hyperleptinemia was sufficient to induce loss of adipose tissue mass; however, the overall result was decreased circulating leptin levels at the time of death. Other methods of leptin treatment, such as twice daily injections, miniosmotic pumps, or adenovirus vectors, might induce hyperleptinemia more effectively in lean mice (4, 7) and therefore allow obesity and hyperleptinemia to be separately assessed.

In conclusion, although in vitro data (6, 11, 28, 33) have suggested a role for macrophages in the leptin-mediated development of atherosclerotic lesions, our results, from three different in vivo BMT studies, do not support a role for macrophages in aortic root lesion formation.

REFERENCES


