Leptin deficiency suppresses progression of atherosclerosis in apoE-deficient mice

Atherosclerosis, 2007
Chiba T, Shinozaki S, Nakazawa T, et al.

Present by
Sudaporn Pummuang

Apolipoprotein Apolipoprotein E (apoE)

- a glycoprotein that is synthesized in the liver, brain, and other tissues in both humans and mice
- a ligand for receptors that clear remnants of chylomicrons and VLDLs

Apolipoprotein Apolipoprotein E (apoE)

- Nascent chylomicrons
- Chylomicrons
- Chylomicron remnants
- HDL

Leptin & leptin receptor deficiency

- obesity in humans & rodents

Lack of apoE

- expected to cause accumulation of cholesterol-rich remnants in plasma

ApoE-/- mice is an animal model of spontaneous atherosclerosis

High circulating leptin

- coronary heart disease
- inflammation

Deficiency of leptin or its receptor prevents thrombus formation in injured arteries and exogenous leptin can reverse the effect in those studies.
Leptin

Is suggested to be proatherogenic hormone but none of them showed that leptin accelerates atherosclerosis directly.

This study aims to clarify the effect of leptin on atherosclerosis.

Materials & Methods

Animals

apoE^+ (atherogenic mice)

ob/ob, apoE^- (leptin-deficiency apoE^- mice)

apoE^- (C57BL/6J) mice + heterozygous ob/+ (C57BL/6J) mice

(The Jackson Laboratory)

Materials & Methods

Animals were housed under specific pathogen-free conditions in static micro isolate cages.

• Only male mice were used.

• The protocols were approved by the Animal Welfare Committee of Tokyo Medical and Dental University.

Material & Methods

Serum levels of lipids and adipocytokines

normal diet ad libitum

atherogenic diet (1.25% cholesterol, 12.5% fat, 0.5% sodium cholate)

or semipurified diet of similar components (free of sodium cholate) for 4 or 16 weeks

Enzymatic assay

• total cholesterol
• triglyceride

ELISA kits

• leptin
• TNF-α
• adiponectin
• serum amyloid A
**Material & Methods**

Quantitative analysis of atherosclerotic area

- 4% paraformaldehyde
- oil red O

Immunohistochemical analysis

- Immunoperoxidase protocol (ABC Elite kit and diaminobenzidine)
- Smooth muscle cells (anti-α-actin antibody)
- Macrophage cells (anti-murine pan macrophages antibody)

Leptin administration

- apoE−/− mice (fed an atherogenic diet for 16 weeks from 8th wk of age)
- Recombinant mouse leptin (5 µg/day)
- Saline

Statistical analysis

- Two-way ANOVA, paired t-test
- Unpaired t-test
- P values < 0.05

**Results**

Characteristics of ob/ob; apoE−/− mice

ob/ob; apoE−/− mice were heavier than apoE−/− mice
**Results**

- *ob/ob; apoE<sup>−/−</sup> mice had greater amount of visceral fat
  
  ![Graph showing visceral adipose tissue (g) over weeks](image)

- Both groups of mice had increased TC level (***P < 0.001***)
  
  ![Graph showing total cholesterol (mg/dL) over weeks](image)

- TG increased then declined after 4<sup>th</sup> week
  
  ![Graph showing triglyceride (mg/dL) over weeks](image)

- Leptin-deficiency suppressed progression of atherosclerosis
  
  ![Graph showing atherosclerotic area (%) over weeks](image)

- Atherosclerotic area was less in leptin-deficient mice
  
  ![Image of atherosclerotic area in leptin-deficient mice](image)
Results

Atherosclerotic area was less in leptin-deficient mice

apoE-/-

ob/ob; apoE-/-

Serum atherogenic cytokines were comparable in two groups of mice

P < 0.001

Results

Leptin-deficiency suppressed progression of fatty streaks to fibrous plaques

HE stain

Elastica van Gieson stain

Leptin-deficiency suppressed progression of atherosclerosis

Anti-SMC α-actin stain

Anti-macrophage stain

Leptin-deficiency suppressed progression of atherosclerosis

Sodium cholate did not affect atherosclerosis of leptin-deficient mice

4 weeks 16 weeks

<table>
<thead>
<tr>
<th></th>
<th>apoE-/-</th>
<th>ob/ob; apoE-/-</th>
<th>apoE-/-</th>
<th>ob/ob; apoE-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>25.1 ± 0.9</td>
<td>45.8 ± 2.0***</td>
<td>27.8 ± 0.8</td>
<td>59.0 ± 2.6***</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>1217.1 ± 79.8</td>
<td>3215.8 ± 343.4***</td>
<td>1697.3 ± 158.7</td>
<td>1581.1 ± 201.0</td>
</tr>
<tr>
<td>Atherosclerosis area (%)</td>
<td>1.62 ± 0.27</td>
<td>5.88 ± 0.42***</td>
<td>27.97 ± 4.42</td>
<td>15.2 ± 1.36***</td>
</tr>
</tbody>
</table>

Exogenous leptin increased atherosclerosis in apoE-/- mice

P < 0.05

Results

N = 9 N = 10
Results

Exogenous leptin decreased B.W of apoE<sup>−</sup> mice

Exogenous leptin decreased serum adiponectin in apoE<sup>−</sup> mice

Exogenous leptin did not change inflammatory biomarkers in apoE<sup>−</sup> mice

Discussion

apoE<sup>−</sup> mice and ob/ob; apoE<sup>−</sup> mice had comparable serum levels of
- adiponectin
- cholesterol
- TNF-α
Discussion

*ob/ob; apoE<sup>−/−</sup> mice* vs *apoE<sup>−/−</sup> mice*

- had higher serum level of triglyceride
- greater area of fatty streaks (an earlier stage of lesions)
- fewer plaques (at 16th week)

Leptin deficiency

- suppressed the progression from fatty streaks to fibrous plaques
- accelerates the development of atherosclerosis

Wu KK et al., 2005

- apoE<sup>−/−</sup>; db/db mice fed a normal chow diet until 20 weeks of age
- hypercholesterolemia and greater atherosclerosis than apoE<sup>−/−</sup> mice

Hasty AH et al., 2001 & Mertens A et al., 2003

- ob/ob; LDL-R<sup>−/−</sup> mice developed more fatty streaks than LDL-R<sup>−/−</sup> mice when fed a normal chow diet until 26 weeks of age
- contradict to this study ob/ob; apoE<sup>−/−</sup> mice lesser fatty streaks than apoE<sup>−/−</sup> mice

Potential mechanism of how leptin accelerates the progression of atherosclerosis

Leptin might induce platelet aggregation

- platelet + leptin

Platelet aggregation

- Increased the intimal thickening of injured arteries (Schaffer K et al., 2004)
Discussion

PDGF
bFGF
TGFβ etc.

stimulate migration
& proliferation of SMC

platelet aggregation

progression of fatty streaks to fibrous plaques

Discussion

Leptin might exaggerate inflammation in the artery

Th1 lymphocytes
- IFN-γ
- IL-18

Leptin

Th2 lymphocytes
- IL-4
- IL-10

increases mRNA & protein levels of iNOS, TNF-α, COX2 etc. in macrophages and endothelial cell

(Loffreda S et al., 1998 and Yamagishi SI et al., 2001)

Discussion

Leptin might directly/indirectly affect on adiponectin level

In this study

exogenous leptin decreased serum adiponectin levels in apoE−/− mice

may account for the increase in atherosclerotic area

Discussion

Leptin might directly accelerate foam cell formation

ob/ob mice peritoneal macrophages

decreased expression of scavenger receptors & cholesterol accumulation

(Kjerrulf et al., 2006)

Discussion

Leptin might directly/indirectly affect on adipogenesis level

in this study

exogenous leptin decreased serum adiponectin levels in apoE−/− mice

may account for the increase in atherosclerotic area

Discussion

Administration of recombinant leptin into apoE−/− mice promoted atherosclerosis and thrombosis

Summary

Proatherogenic effect of leptin that is independent of food intake was confirmed by using leptin-deficient mice

Explanation for the positive correlation between plasma leptin level and CHD

Present study Bodary et al, 2005

**Apolipoprotein classifications**

<table>
<thead>
<tr>
<th>Apolipoprotein</th>
<th>Lipoprotein Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoA-I (CI)</td>
<td>HDL</td>
</tr>
<tr>
<td>ApoA-II (B)</td>
<td>HDL</td>
</tr>
<tr>
<td>ApoA-III (B)</td>
<td>HDL</td>
</tr>
<tr>
<td>ApoB</td>
<td>LDL</td>
</tr>
<tr>
<td>ApoC-I</td>
<td>LDL</td>
</tr>
<tr>
<td>ApoC-II</td>
<td>LDL</td>
</tr>
<tr>
<td>ApoC-III (C)</td>
<td>LDL</td>
</tr>
<tr>
<td>ApoE</td>
<td>LDL</td>
</tr>
<tr>
<td>ApoE-2 (E2)</td>
<td>LDL</td>
</tr>
<tr>
<td>ApoE-3 (E3)</td>
<td>LDL</td>
</tr>
<tr>
<td>ApoH</td>
<td>LDL</td>
</tr>
</tbody>
</table>

**Foam cell formation**

**ABC method**