Bilirubin Improves Obesity and Insulin Resistance in Diet-Induced Obese Mice

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1. Type 2 diabetes is a disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency.

2. 347 million individuals worldwide and 26 million in the United States—11% of the population—have diabetes, with another 79 million (35%) have pre-diabetes.

3. Obesity is a major cause of diabetes, Over 80% of type 2 diabetes is related to being overweight.

4. The US has the highest rate of obesity in the world (68% are overweight and 34% (that’s 72.5 million people) are obese.)
Heme Catabolism by Heme Oxygenase Yields Bioactive Products
HO-1 is a Stress-Response Gene

- H$_2$O$_2$
- Hypoxia
- Hyperoxia
- UVA
- Endotoxin
- Hypoxia
- Heavy Metals
- Cytokines
- Nitric Oxide (NO)
Protective Effects of HO-1

HO-1

Fe^{2+}  CO  BV  BR

• MAPK, sGC, STAT, NO, NF-κB
• MAPK, Antiox Power
• MAPK, sGC, p21, p53

Apoptosis  Anti-Inflammatory  Proliferation

EC, HC, β cell, T cell, fibroblast  Leukocytes, EC, Platelets  SMC, T cell, EC
Our Hypothesis

HO-1 induction/bilirubin administration reduces hyperglycemia and increase insulin sensitivity in mouse models of obesity.
Mouse Model I

BKS.Cg-Dock7m +/- Leprdb/J (db/db)mice:

10–14 days: Elevations of plasma insulin
3–4 wk: Obese.
4–8 wk: Elevations of blood sugar, polyphagic, polydipsic, and polyuric, severe depletion of β cells
10m: Death
Mouse Model II

Diet-Induced Obese mice:

C57BL/6 fed with 58 kcal% fat w/sucrose
Treatment Protocol

**HO-1:** Cobalt protophophyrin (CoPP) at 10 or 20 mg/kg, every other day for 14 days.

**Bilirubin:** 20μmol/kg, i.p. twice per day for 14 days.
HO-1 Induction Reduces Hyperglycemia and Increases Insulin Sensitivity in db/db Mice
Insulin Signal Transduction in Skeletal Muscle
### HO-1 Induction Leads to Activation of Insulin Signaling Pathway in Muscle

<table>
<thead>
<tr>
<th></th>
<th>CTR</th>
<th>10mg/kg</th>
<th>20mg/kg</th>
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</thead>
<tbody>
<tr>
<td><strong>Y612-IRS1</strong></td>
<td>![Image]</td>
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<tr>
<td><strong>308-AKT</strong></td>
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<td><strong>473-AKT</strong></td>
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<td><strong>HO-1</strong></td>
<td>![Image]</td>
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<td>![Image]</td>
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<tr>
<td><strong>Insulin</strong></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- = negative, + = positive
**HO-1 Promotes Membrane Transport of Glut4 in Muscle Cells**

**Figure 3.**

**A**

<table>
<thead>
<tr>
<th></th>
<th>CTR</th>
<th>CoPP 10mg/kg</th>
<th>CoPP 20mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**B**

![Bar chart showing relative Glut4 expression with error bars for different conditions.](chart.png)

HO-1 promotes membrane transport of Glut4 in muscle cells.
Mouse Model II

Diet-Induced obese mice:

C57BL/6 fed with 58 kcal% fat w/sucrose
Bilirubin Reduces Body Weight of DIO Mice

A

B

[Graph showing body weight changes over days for different diets and treatments.]

[Bar chart comparing diet consumption (g/mouse/day) for HFD+Vehicle and HFD+Billirubin.]
Bilirubin Improves Liver Steatosis
Bilirubin Reduces Blood Glucose Level and Increases Insulin Sensitivity in DIO Mice
Bilirubin Promotes Phosphorylation of Insulin Receptor in Muscle and Liver of DIO Mice

- CHOW
- HFD+Br
- HFD+Vehicle

**Insulin:**
- **p-IR**
- **Total IR**
- **p-AKT**
- **Total akt**

**CHOW**

**HFD+Br**

**HFD+Vehicle**

**Graphs:**
- **p-IR/IR**
- **p-AKT/AKT**
Summary

• HO–1 induction significantly improves obesity and insulin resistance in the db/db mice.

• Bilirubin mimics the protective effect of HO–1 induction.

• Bilirubin may be used as an insulin sensitizer to improve obesity and insulin resistance in obese and diabetes patients.
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