β cell and Hyperglycemia in Type 2 Diabetes: Glucotoxicity and Lipotoxicity

KyuChang Won

Department of Internal Medicine
Yeungnam University College of Medicine
Daegu, Korea
Glucotoxicity, Lipotoxicity, Glucolipotoxicity

- Adverse or toxic influence on pancreatic β cell function caused by excessive glucose and/or lipids

- Glucotoxicity (1985, Unger RH et al, Diabetologia)
- Lipotoxicity (1995, Unger RH, Diabetes)
- Glucolipotoxicity (2002, Prentki M et al, Diabetes)
  - lipotoxicity is dependent on glucose levels
Schema of five stages of progression of diabetes

(Weir GC et al Diabetes 53:S17, 2004)
Contribution of glucotoxicity and glucolipotoxicity to the development of type 2 diabetes
Glucotoxicity

Chronic exposure to high glucose in β-cell lines:

- Insulin mRNA
- Insulin promoter activity
- Insulin gene transcription factors
  
  (STF-1/PDX-1/IPF-1/IDX-1, MafA, RIPE3b1 activator)

- Glucose-stimulated insulin secretion
- Insulin content

Chronic exposure to low glucose

Glucotoxicity and Oxidative Stress

• In vitro evidence
  - β cell lines
    1) HIT-T15
    2) INS-1 cell
    3) βTC-6 cell
  - Isolated islets

• In vivo evidence
  - Zucker diabetic fatty rat (ZDF rat)
  - db/db mice
Biochemical pathways through which elevated glucose can form excessive levels of reactive oxygen species (ROS)
The glucotoxic effect on insulin gene expression via loss of PDX-1 and MafA

(RP Robertson  J Biol Chem. 2004 Jul 16)
Time-dependent disappearance of MafA binding relative to the disappearance of PDX-1 binding to islet DNA during continuous culturing and weekly passaging of HIT-T 15 cells.

(Harmon JS et al, Diabetes 1998)
Effect of H$_2$O$_2$ on insulin gene expression
The effects of high glucose on the intracellular peroxide level and GSIS in the INS-1 cells and rat islets

Chronic exposure to high glucose concentration induces glucotoxicity

(Park KG et al. Diabetes 56:431-37, 2007)
Clinical correlations between glucotoxicity and oxidative stress of the β cell
Evidence in human diabetes mellitus

- Defective plasma antioxidant defenses and enhanced susceptibility to lipid peroxidation in uncomplicated IDDM.  
  (Santini SA et al Diabetes 46:1853-1858, 1997)

- New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus.  

- Increased oxidative damage to all DNA bases in patients with type II diabetes mellitus.  
  (Rehman A et al FEBS Lett 488:120-122, 1999)

- Effect of oxidative stress on glutathione pathway in red blood cells from patients with insulin-dependent diabetes mellitus.  
  (Dincer Y et al Metabolism 51:1360-1362, 2002)

- The effect of repaglinide on insulin secretion and oxidation stress in type 2 diabetic patients.  

- Parameters of oxidative stress in children with Type 1 diabetes mellitus and their relatives.  
Expression of $\gamma$ GCS m RNA and GSH level in INS-1 cell

(Won KC et al. J Kor Diabetes Assoc 31:302-309, 2007)
GSH level at pancreatic tissues in OLETF and LEUTO rats

(Data are mean ± SE from 3 separate experiments.)
Expression of γ GCS mRNA and GSH level in leukocytes, mesothelial cells from patients with T2DM

* p<0.05 vs Diabetics
GSH, Ox-LDL, and MDA in serum from patients with type 2 DM
\( \gamma \)-GCS Expression: Summary & Conclusion

- Decreased GSH level, \( \gamma \)-GCS expression, GSIS and increased peroxide level in INS-1 cells exposed to high glucose.

- Decreased GSH level, \( \gamma \)-GCS expression and increased Ox-LDL, MDA level at leukocytes and mesothelial cells from patients with T2DM esp, poorly controlled patients.

- Insufficient antioxidant defense by the GSH pathway may be one of the factors responsible for development of complications in patients with type 2 diabetes.
Lipotoxicity

• Acquired cause of impaired β-cell function

• Short term exposure of islets to FFA
  - stimulates insulin secretion
  - LCFA → Fatty acyl CoA → DAG → PKC → Exocytosis

• Long term exposure of islets to FFA
  - inhibit insulin secretion
  - through Randle cycle

(McGarry JD Diabetes 51: 7-18, 2002)
Effects of glucose on lipid partitioning in the β cell
Prolonged exposure to increased FFA concentration induces beta-cell death

(McGarry JD Diabetes 51: 7-18, 2002)
Antioxidant strategies to protect the β cells from hyperglycemia
Oxidative stress

Adaptive response

Activation of antioxidant defenses

TF activation (e.g. AP-1, NF-κB, Nrf2)

Immediate-early gene induction (e.g. c-fos)

Induction of antioxidant genes:
- Glutathione peroxidase
- Catalase
- SOD
- γ-Glutamylcysteine synthase
- Glutathione reductase
- Thioredoxin
- Thioredoxin reductase
- Quinone reductase
- Metallothionein
- Heme oxygenase
- Ferritin...

Repression of ROS-producing systems

TF inhibition (e.g. NFI)

Specific decrease in RNA stability

CYP1A1 repression

Mitochondrial activity shutdown +

Transferrin receptor decrease

NADPH oxidase inhibition

EPO gene repression

Activation of several other genes (e.g. cytokines)

Model for the mechanism by which metformin mediates effects on lipid and glucose metabolism.
**pAMPK levels in the hypothalamus by metformin treatment**

• Metformin restores insulin secretion altered by chronic exposure to free fatty acid or high glucose
  

• Activation of the AMP-activated protein kinase by the anti-diabetic drug metformin in vivo
  

• Metformin, but not leptin, regulated AMP-activated protein kinase in pancreatic islets: impact on GSIS
  
Lipotoxicity in Human Pancreatic Islets and the Protective Effect of Metformin

<table>
<thead>
<tr>
<th></th>
<th>Insulin release (pmol · islet(^{-1} \cdot 45 \text{ min}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.3 G</td>
</tr>
<tr>
<td>Control</td>
<td>22.1 ± 3.1</td>
</tr>
<tr>
<td>FFA</td>
<td>18.9 ± 6.3</td>
</tr>
<tr>
<td>Metformin</td>
<td>20.7 ± 2.9</td>
</tr>
<tr>
<td>FFA + metformin</td>
<td>22.0 ± 6.3</td>
</tr>
</tbody>
</table>

(Diabetes 51 (Suppl. 1):S134–S137, 2002)
Islet perifusion experiments showing the dynamics of insulin secretion

(Diabetes 51 (Suppl. 1):S134–S137, 2002)
Metformin in β cells

• To evaluate
  Whether
  1) glucose, metformin regulates AMPK in INS-1 cell
  2) changes in AMPK modulate insulin secretion
  3) metformin prevents glucose toxicity
Intracellular Peroxide Level, AMPK activity, GSIS after 3 days subculture: INS-1 cell

(Unpublished data)
Intracellular Peroxide Level after 3 days subculture (1 day preexposure of metformin)

* p<0.05 compared with 5.6 mM G
** p<0.05 compared with 30 mM G

Data are mean ± SE from 3 separate experiments
INS-1 Cell

GSIS after 3 days subculture (1 day preexposure of 25 µg/ml metformin)

Data are mean ± SE from 3 separate experiments
Metformin: Summary & Conclusion

- High glucose increases oxidative stress and decreases GSIS in INS-1 cell
- Glucose decreases AMPK phosphorylation in INS-1 cell
- Metformin stimulates AMPK and regulates oxidative stress and insulin secretion in INS-1 cell

At 30 mM glucose: metformin increases GSIS

- Metformin may protect glucose toxicity & lipotoxicity
- Activation of AMPK by metformin might contribute to the beneficial effects of metformin in treating diabetes
GLP-1 Preserved Morphology of Human Islet Cells *In Vitro*

Islets treated with GLP-1 in culture were able to maintain their integrity for a longer period of time.

Adapted from Farilla L et al *Endocrinology* 2003;144:5149–5158.
Des-fluoro-sitagliptin (DFS):
11-Week Treatment in HFD/STZ Diabetic Mice

<table>
<thead>
<tr>
<th>Non-diabetic</th>
<th>Diabetic + vehicle</th>
<th>Diabetic + DFS 0.1%</th>
<th>Diabetic + DFS 0.4%</th>
<th>Diabetic + DFS 1.1%</th>
</tr>
</thead>
</table>

• **Antioxidant enzymes:**
  Superoxide dismutase, Catalase, Glutathione Peroxidase

• **Heat Shock Proteins:**
  HSP 70
  Heme Oxygenase-1
• Complementary action of antioxidant enzymes in the protection of bioengineered insulin-producing RINm5F cells against the toxicity of reactive oxygen species.  (Tiedge M et al Diabetes 47:1578-1585, 1998)

• Protection against the co-operative toxicity of nitric oxide and oxygen free radicals by overexpression of antioxidant enzymes in bioengineered insulin-producing RINm5F cells.
  (Tiedge M et al Diabetologia 42:849-855, 1999)

• Stable expression of manganese superoxide dismutase in insulinoma cells prevents IL-1beta-induced cytotoxicity and reduces nitric oxide production.  (Hohmeier HE et al J Clin Invest 101:1811-1820, 1998)

• Contribution of adenoviral-mediated superoxide dismutase gene transfer to the reduction in nitric oxide-induced cytotoxicity on human islets and INS-1 insulin-secreting cells.
Fig. 6. Comparison of the interleukin-1β (IL-1β) and aminoguanidine (AG) exposure for 24 h on the expression of heat shock protein 70 (hsp 70) in cultured rat islet. A: Western blot analysis with the quantification of levels of expression of hsp 70 shown in B. Density of hsp 70 immunoreactivity was quantitated by computer program (Bio ID version 6). Data are mean ± S.D. from 4 separate experiments. * p<0.05 vs control islets cultured in medium containing 11 mM glucose. (Won KC et al. J Kor Diabetes Assoc 25:273-285, 2001)
**Heme degradation pathway**

1. Heme
2. \( \text{Fe}^{3+}, \text{CO}^* \) → Biliverdin
3. Biliverdin → Biliverdin Reductase → Bilirubin*

- Carbon monoxide protects pancreatic beta-cells from apoptosis and improves islet function/survival after transplantation

*(Gunther L et al. Diabetes 51: 994-999, 2002)*
Protective effect of Heme Oxygenase-1 in INS-1 cell

- Intracellular Peroxide Level
  - Fluorescence Intensity
  - 5.6 mM G: 2
  - 30 mM G: 4

- HO-1 expression
  - 5.6 mM G: 2
  - 30 mM G: 4

- GSIS
  - Insulin Secretion (mU/ml)
  - 5.6 mM G: 600
  - 30 mM G: 1600

- HO-1 activity
  - nmol bilirubin/mg protein/hr
  - 5.6 mM G: 2
  - 30 mM G: 4

* p<0.05 vs 5.6 mM G

**INS-1 Cell**

- 5.6 mM G
- 30 mM G
- 0.1 mM 1 mM 10 mM Hemin

**Rat Islets**

- 11.1 mM G
- 50 mM R
- 0.1 mM 1 mM 10 mM Hemin

*GSIS after 3 days subculture (1 day preexposure of Hemin)*

* *p<0.05*
After HO-1 adenovirus infection

GSIS after 3 days subculture (2hrs exposure of HO-1 adenovirus): INS-1 cell
• Heme Oxygenase-1 seems to mediate protective responses of pancreatic islets against oxidative stress due to hyperglycemia
Lipotoxicity and Type 2 Diabetes
Relative risk for T2DM by BMI in women aged 30-55 years

FFAs in the pathogenesis of type 2 diabetes

(Wilding JPH Diabet Med 24:934-945, 2007)
## Randomized, controlled Type 2 DM prevention studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention</th>
<th>Relative risk for Type 2 diabetes</th>
<th>Percentage decrease in FFA with intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes Prevention Program</td>
<td>Metformin</td>
<td>31%</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>Lifestyle modification</td>
<td>58%</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>Troglitazone</td>
<td>45%</td>
<td>31%</td>
</tr>
<tr>
<td>Finnish Diabetes Prevention Program</td>
<td>Lifestyle modification</td>
<td>58%</td>
<td>30%</td>
</tr>
<tr>
<td>TRIPOD</td>
<td>Troglitazone</td>
<td>55%</td>
<td>31%</td>
</tr>
<tr>
<td>STOP-NIDDM</td>
<td>Acarbose</td>
<td>25%</td>
<td>No significant effect</td>
</tr>
<tr>
<td>South African cohort study</td>
<td></td>
<td>0.11</td>
<td>12–26%</td>
</tr>
<tr>
<td>Xendos</td>
<td>Orlistat</td>
<td>33.7%</td>
<td>25%</td>
</tr>
<tr>
<td>DREAM*</td>
<td>Rosiglitazone</td>
<td>60%</td>
<td>12%</td>
</tr>
<tr>
<td>SOS</td>
<td>Bariatric surgery</td>
<td>97%</td>
<td>40–50%</td>
</tr>
</tbody>
</table>

*Primary end point of diabetes or death.
DREAM, Diabetes REduction Assessment with ramipril and rosiglitazone Medication; FFA, free fatty acid; SOSS, Swedish Obese Subjects study; STOP-NIDDM, SStudy tO Prevent-Non-Insulin Dependent Diabetes Mellitus; TRIPOD, TRoglitazone In the Prevention Of Diabetes.
Glucotoxicity vs. Lipotoxicity

Glucolipotoxicity
Most individuals with increased circulating lipid levels have normal \( \beta \)-cell function

Lipotoxicity only occurs in the context of chronic hyperglycemia

(Kelpe BI et al. Diabetes 51: 662-668, 2002)

Long term exposure of islets to FFA : inhibit insulin secretion

(McGarry JD Diabetes 51: 7-18, 2002)
Consequences of treating ZDF rats with the lipid-lowering drug bezafibrate or the glucose-lowering agent phlorizin agent for 6 weeks beginning at 6 weeks of age

(Harmon JS et al. Diabetes 50:2481-2486, 2001)
Fatty acid translocase cluster determinant 36 (FAT/CD36) and glucolipotoxicity in INS-1 cells
Absorbed Cholesterol and Triglycerides are Packaged into Chylomicrons and Secreted into the Lymph

Intestinal Lumen

Enterocyte (Jejunum)

Lymph

Free Cholesterol

Cholesterol

Cholesterol Ester

NPC1L1

ACAT

MTP

ABC5G5/8

2 Fatty Acid

DGAT

Monoglyceride

2 Fatty Acid

Triglyceride

Lipase

2 Fatty Acid + Monoglyceride

DGAT

ACAT: Acyl-Co-A:Cholesterol AcylTransferase; DGAT: DiacylGlycerol AcylTransferase; MTP: Microsomal Triglyceride Transfer Protein

High glucose levels increase cholesterol absorption

- The study proposes that glucose-mediated intestinal cholesterol may contribute to increasing circulating cholesterol and, consequently, the risk of developing CHD, a feature of T2DM.

High glucose levels increase cholesterol absorption

- Effect of glucose concentrations on the protein expression of transporters mediating cholesterol influx. Caco 2/15 cells were cultured for 24 h in DMEM containing 5 or 25 mM glucose.
- Western blot was used to analyze the protein expression of NPC1L1 (A), CD36 (B)

The higher glucose levels, the higher protein expression of NPC1L1 and CD36, which result in higher cholesterol uptake.
• To determine whether prolonged exposure of pancreatic islets to a glucolipotoxic condition disrupts CD36 or increases CD36

• To evaluate a role of CD36 in pancreatic islets against glucolipotoxicity
FAT/CD36

0.5mM Palmitate

Glucose

5.6mM

11.1mM

30mM

% of control

*: p<0.05

* p<0.05
Insulin mRNA

- 0.5mM Palmitate
- Glucose: 5.6mM, 11.1mM, 30mM

* p<0.05
• At only lipotoxic condition (5.6 mM glucose and 0.5 mM palmitate), PDX-1 and insulin mRNA in INS-1 cells were not decreased compared to 5.6 mM glucose media.

• The insulin mRNA levels, PDX-1 and GSIS were decreased in INS-1 cells exposed to glucolipotoxic condition (30 mM glucose and 0.5 mM palmitate) compared to 5.6 mM glucose media.

• At INS-1 cells exposed to glucolipotoxic condition (30 mM glucose and 0.5 mM palmitate), CD 36 was significant increased compared to normal glucose media.
• These results suggest that the influx of fatty acid to islet at high glucose condition can be increased by increasing of CD36

• At islets exposed to high glucose, high fatty acid may be exacerbating β cell dysfunction via increasing of CD 36
Summary
• There are ample evidences at the cellular level

1) Glucotoxicity & oxidative stress: Glucose form ROS
2) Pancreatic islets contain an usually low complement of antioxidant proteins and activity but normal γ-GCS expression
3) Antioxidant drugs improve pancreatic islet survival in the face of oxidative stress
4) Gene transfer techniques can be used to overexpress antioxidant enzymes or HO-1 which provide enhanced protection of pancreatic beta cells against oxidative stress
5) At islets exposed to high glucose, high fatty acid may be exacerbating β cell dysfunction via increasing of CD 36
6) Because hyperglycemia is a prerequisite for lipotoxicity to occure, the term glucolipotoxicity, rather than lipotoxicity, is more appropriate
• In human studies of type 2 diabetes

1) Markers of oxidative stress are increased in type 2 diabetics

2) Reducing plasma FFA levels may decrease the relative risk for type 2 diabetes

3) Glucolipotoxicity are secondary phenomena that are proposed to play a role in all forms of type 2 diabetes

4) What new strategies might be utilized to protect the β cell against glucolipotoxicity in type 2 diabetes?
   : NAC? GLP-1? DPP IV inhibitors? etc….
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