The role of sphingolipids in pancreatic β-cell dysfunction
CONTENTS

• Sphingolipid metabolism

• Ceramide & β-cell
  – Apoptosis & Dysfunction
  – Ceramide effect on TXNIP in pancreatic β-cell
Sphingolipid Metabolism
Sphingolipid

- Structural components of cellular membranes
- Key intracellular signaling molecule
  - cell cycle arrest, proliferation, apoptosis, senescence, stress response
History of Sphingolipid

- “Sphinx-like”
- first described in 1884

J. L. W. Thudichum
### Structure of Sphingolipids

![Structure of Sphingolipids](image)

<table>
<thead>
<tr>
<th>Substituent (R)</th>
<th>Sphingolipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>Ceramide</td>
</tr>
<tr>
<td>Phosphocholine</td>
<td>Sphingomyelin</td>
</tr>
<tr>
<td>Sugar(s)</td>
<td>Glycosphingolipid</td>
</tr>
</tbody>
</table>
Sphingolipid biosynthesis

Ceramide: ‘metabolic hub’

J Clin Invest. 2011;121(11):4222-4230
I. De novo pathway

- Palmitoyl-CoA
- Serine
- SPT
- CO₂
- 3-keto-Sphinganine
- KSA-Reductase
- NADPH
- Sphinganine (Dihydro-Sphingosine)
- CerS
- Acyl-CoA
- Dihydro-Ceramide
- DES
- NADPH
- Ceramide
- Complex Sphingolipids (SM, GlycoSLm)
- Complex Sphingolipids
- Ceramide desaturase
- Ceramide synthase
- (ER)
- (Golgi)
- Sphingosine
- Ceramidase
- ATP
- Sphingosine-1P
- SO-Kinase
- SO₁P-lyase
- Hexadecenal
- Ethanolamine-P

Serine Palmitoyltransferase
Ceramide synthase
Ceramide desaturase

Lipids in Health and Disease 2010 9:84
II. Sphingomyelin (Hydrolytic) pathway

(Plasma membrane)

Ceramide

Sphingomyelin

Sphingosine

Sphingosine-1-phosphate
III. Salvage pathway

(Late endosome/lysosome)
Compartmentalization of pathways of sphingolipid metabolism
Transport and transbilayer movement of sphingolipids
Sphingolipid “rheostat”
Sphingolipid & Diabetes
Endogenous ceramide accumulation block insulin signaling

Role of ceramide in β-cell apoptosis
**Sphingolipids and Type 1 Diabetes**

☑ β-cell cytokine signaling

- IL-1β promoted decrease SM?
- IL-1β increase in ceramide mass?
- Inhibitors of ceramide synthesis or SMase activity alter IL-1β-stimulated responses?
- Activation of the SM/ceramide signaling pathway is involved in cytokine-induced β-cell death?
- IL-1β and TNFα augment S1P?

- Are alteration in sphingolipid metabolism playing major roles in cytokine-mediated β-cell destruction in the context of T1D?

*Diabetes. 1999;48(7):1372-80*
β-cell failure in the pathogenesis of T2DM
Lipid-induced β-cell failure: ceramide-mediated

**Diagram:**
- FFA
- PKC / ceramides
  - Oxidative stress / ER stress
  - Inflammation (e.g. IKKβ / JNK activation)
- Impaired β-cell insulin signaling?
- β-cell failure

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- ▼ -- Potential input from glucotoxicity (overlapping / additive mechanisms)
- ▼ -- Potential input from glucotoxicity (synergistic mechanisms)

*Am J Physiol Endocrinol Metab. 2011 300(2):E255-62*
Ceramide accumulation induce β-cell apoptosis in ZDF obesity

Inhibition of ceramide synthesis reduce lipotoxicity

Unger RH et al. PNAS 1998 95(5): 2498-502
SPT inhibitors prevents lipoapoptosis

Serine +Palmitoyl-CoA

\[\text{SPT} \quad \text{cycloserine myriocin} \]

\[\text{Dihydropshinganine} \]

\[\text{Dihydroceramide} \]

\[\text{Ceramide} \]

\[\text{Apoptosis} \]

Human islet: β-cell apoptosis is dependent on ceramide pathway

Survival of human islets exposed
(48 h, 2.0 mmol/l FFA mixture)

DCI: serine protease
Myriocin: CeS inhibitor
Specific Cer species and pathway are important

• Palmitate levels ↑ → ceramide generation ↑
  – involves both ‘de novo’ and ‘salvage pathways’

• Harmful effect of palmitate on β-cells
  – Cer(14:0), Cer(16:0), Cer(20:1), and Cer(24:0) species
  – generated by the salvage pathway
Contradictory result?

- Some studies with β-cell apoptosis have reported only modest increase in ceramide upon treatment with FFA

*POKO mouse: PPARγ(-/-) Lep(ob)/Lep(ob)

Palmitate increase GlcCer, not Cer in MIN6
Possible explanation

- Increase in ceramide at particular cellular location, without altering the total ceramide mass may be sufficient to induce apoptosis
  
  *Biochem J 2004, 382:527 – 533*

- Conversion of ceramide to another sphingolipid metabolite after apoptotic induction
  
  *Biochem J 2011, 435:267 – 276*

- Apoptotic potential of ceramide may vary with different isoforms
  
  *Biochem J 2011, 438:177 – 189*
Gluco-lipotoxicity induce ceramide deposition
Glucolipotoxicity induce apoptosis: Dual mechanism

• ↑ De novo synthesis
  – Dihydrosphingosine, (dihydro)ceramides

• Up-regulation of Ceramide synthase 4 (CeS4)
  – C18:0, C22:0 and C24:1 species ↑
Mechanisms of ceramide induced β-cell apoptosis
Ceramide: the lipid “second messenger”
Activation of extrinsic apoptotic pathway

Agent that induces ceramide accumulation
→ extrinsic pathway of apoptosis ↑
→ β-cell apoptosis ↑
Activation of extrinsic apoptotic pathway

• Cytokine alone (IL-1β, IFN-γ, TNK-α) does augment neither cellular ceramide content nor SMase activity

• Ceramide plays a minor role in cytokine action
  – but activates or disturbs distinct signaling pathways that synergistically augment apoptosis of insulin-secreting cells.
Alteration of mitochondrial membrane permeation
Free radical generation

- Ceramide-induced free radical (ROS/RNS) generation
  - Activation of NADPH oxidase
  - Initiation of mitochondrial dysfunction
  - Induction of iNOS gene expression
  - Down regulation of anti-apoptotic Bcl-2 proteins and mRNA expression

- Bidirectional interaction: ROS/RNS → Ceramide ↑
  - Inducing SMase
  - Inhibiting ceramidase

*J Cell Physiol. 2004 May;199(2):310-5.*
Induction of ER stress

(De novo synthesis)

Palmitate

SPT

Cycloserine

Myriocin

Ceramide?

ER stress

β-cell apoptosis

Chronic palmitate induces ER stress via increases in a subpool of ceramide, without altering total ceramide mass.
Ceramide: defective protein trafficking

- Ceramide (De novo > salvage pathway)
  - Disrupt ER–to-golgi protein trafficking
  - Activation of lipotoxic ER stress
- Overexpression of GCS protects MIN6 cells from lipoapoptosis, terminal ER stress and from the palmitate-induced delay in protein trafficking

![Graphs showing GluCer/Cer ratio, CHOP, Apoptosis, and VSVG localization](Image)
ER stress $\rightarrow$ ceramide accumulation $\uparrow$

: Activation of iPLA2$\beta$

Strong ER stress

$\uparrow$ Ca$^{2+}$ - independent phospholipase A2 (iPLA2$\beta$)

$\uparrow$ Neutral SMase

SM $\rightarrow$ Cer $\uparrow$

Intrinsic mitochondrial pathway of apoptosis

JBC 2008; 283(50):34819
Inhibition of Akt

• A major mechanism through which ceramide induces β -cell apoptosis
  – Ceramide inhibits Akt activation in cultured cells and rats
  – Inhibition of ceramide biosynthesis restored the Akt activity

• Chronic exposure to moderately elevated palmitate activates Akt??

  Biol Chem 2003; 278:30015-21
  Diabetes Obes Metab 2006; 8:228-33
Amyloid deposits induce ceramide accumulation
Adiponectin inhibit ceramide induced apoptosis

Trends in Cell Biology, 22(1), 2012, 50–60
Ceramide and insulin synthesis
Ceramide synthesis is a mechanism for palmitate inhibition of insulin gene expression

- Exogenous ceramide inhibits insulin mRNA levels, whereas blockade of \textit{de novo} ceramide synthesis prevents palmitate inhibition of insulin gene expression.
Mechanism of insulin gene transcription inhibition by ceramide

- Ceramide activates JNK which inhibits insulin gene transcription
  - c-jun-dependent
  - Oxidative stress-dependent pathways

- Ceramide directly activates PKCζ which phosphorylates and inactivates PDX-1


Glucolipotoxicity:
De novo ceramide synthesis activate ERK1/2
Ceramide accumulation enhance C/EBPβ expression

- CERT inhibition: Ceramide accumulation ↑
  - ↓ PDX-1 and Maf A binding
  - ↑ C/EBPβ binding to the promoter of proinsulin genes

- Down-regulation of C/EBPβ could block ceramide impairment of proinsulin gene expression

Cell Physiol Biochem 2010;26:717-728
Ceramide and insulin secretion defect

• Ceramide analogs have only marginal effects on glucose-stimulated insulin secretion
  
  *Diabetes* 1999; 48:1372-80

• Inhibitors of de novo ceramide synthesis do not overcome the secretory defects due to lipotoxicity

  *J Cell Biochem* 2010; 111:497-507
Down-regulation of SMS1 or SMS2 significantly reduces insulin secretion

- localized, or more subtle, alterations in sphingolipid metabolism might still impact on stimulus-secretion coupling

SphK activity and S1P levels are critical for glucose-stimulated insulin secretion

• High glucose induces SphK activity, leading to increases in S1P levels and stimulation of insulin secretion.
Ceramide stress

TXNIP

Oxidative stress ↑

β-cell dysfunction/apoptosis

β-cell
Ceramide reduced β-cell survival and insulin gene expression

A. INS-1 cell

- Apoptosis (%)

C2-Cer (uM) | 25 | 50 | -
D2-Cer (uM) | - | - | 50

B. Primary rat islet

- Apoptosis (%)

C2-Cer(50uM) | - | +

C. Insulin mRNA

- Relative Insulin mRNA

C2-Cer | - | +

D. PDX-1 mRNA

- Relative PDX1 mRNA

C2-Cer | - | +

(unpublished data)
Ceramide induce Mitochondrial dysfunction and ROS production

A.

C2-CER (uM)

- 25 50

B.

Cytosolic Cyto-C

β - actin

C.

D2-CER (uM)

C2-CER (uM)

- 50 - 25 50

Cleaved Caspase-3

β - actin

D.

Relative ROS level

(Fold of Control)

- 25 50

* P< 0.05 vs Cont.

E.

Time (hr) 0 3 6 12 24

C2-CER

P-JNK2

P-JNK1

P-P38MAPK

β-Actin

(unpublished data)
Ceramide induce TXNIP mRNA, protein expression and reduce Trx activity

* P< 0.05, ** P< 0.005 vs Cont.

(unpublished data)
Mitochondrial shuttling of TXNIP in response to ceramide

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>C2-CER (50uM)</th>
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<tr>
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<td><strong>TXNIP</strong></td>
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<td><img src="image2" alt="C2-CER 6hr TXNIP" /></td>
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<tr>
<td><strong>MITOTRACKER</strong></td>
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<td><img src="image5" alt="C2-CER 6hr MITOTRACKER" /></td>
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<tr>
<td><strong>MERGE</strong></td>
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<td><img src="image8" alt="C2-CER 6hr MERGE" /></td>
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</table>

(unpublished data)
Ceramide enhance NF-κB activation

A. Time (hr) 0 1 3 6 12 IL-1β
   C2-CER
   P-P65
   P65
   β-actin

B. Time (hr) 1 3
   C2-CER
   P-P65
   DAPI
   MERGE

C. NF-κB DNA binding activity
   Absorbance 450nm
   C2-CER - + IL1β
   (unpublished data)
Inhibition of CD36 reduce ceramide-induced NF-κB activation

A.

<table>
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<th>0</th>
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<th>3</th>
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<td>P-P65</td>
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<tr>
<td>β-Actin</td>
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</table>

B.

C2-CER

SSO

P-P65

C.

C2-CER

SSO

NF-κB DNA binding activity

Absorbance 450nm

(unpublished data)
CD36 inhibitor decrease ceramide-mediated TXNIP expression

A.

B.

C.

(unpublished data)
Inhibition of CD36 recovered ceramide reduced Insulin, PDX1 mRNA and apoptosis

A.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>60</th>
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<th>30</th>
<th>60</th>
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</thead>
<tbody>
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<td>P-AKT(308)</td>
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<tr>
<td>T-AKT</td>
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<tr>
<td>β-Actin</td>
<td></td>
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</tr>
</tbody>
</table>

B.

C2-CER

SSO

Relative Insulin mRNA

C.

C2-CER

SSO

Relative PDX1 mRNA

D.

C2-CER

SSO

Apoptosis (%)

E.

C2-CER

SSO

C-Caspase 3

β-Actin

(unpublished data)
(unpublished data)
Summary

• Ceramide is the “second lipid messenger” responsible for β-cell apoptosis

• Ceramide blunt insulin gene expression
  – Akt, C/EBP β

• CD36 : new target of ceramide effects on TXNIP?
Take Home message

• Sphingolipids have varied roles in many critical aspects of β-cell biology.
  – Apoptosis, insulin gene expression & secretion

• “mass” & “flux”

• need more therapeutic target
Thank you for your Attention!