UNCOVERING CIRCULATING FACTORS LINKING PREDIABETES TO DIABETES

Ying Liu    PhD
Mar.07/2017
DIABETES MELLITUS: A MULTI-ORGAN DISEASE

Initiation Stage

Liver

Brain

Pancreas

Disrupted Metabolic Homeostasis

Adipose Tissue

Skeletal Muscle

Gut
DIABETES MELLITUS: A MULTI-ORGAN DISEASE

Hyperglycemia
**DIABETES MELLITUS**

- **Type 1 Diabetes**: beta cell destruction
- **Type 2 Diabetes**: beta cell failure in response to peripheral metabolic demands
- **Gestational Diabetes**: Insufficient beta cell function in pregnancy

Red: Insulin (β cells)
Green: Glucagon (α cells)
Blue: Nucleus
DIABETES MELLITUS: A PROGRESSIVE DISEASE

Compensatory Stage

Normal Prediabetes Diabetes Severe

Disease Stage

Insulin Resistance

Beta Cell Dysfunction

Insulin Secretion

β cell mass
WHAT CAN WE DO???

- Early Detection
  - Prevent disease progression

- Therapeutic Targets
  - Prevent loss of or restore β cell function

- β cell Replacement
  - Therapy to restore β cell function

<table>
<thead>
<tr>
<th>Normal</th>
<th>Prediabetes</th>
<th>Diabetes</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Insulin Secretion
- β cell mass
WHAT CAN WE DO???

1. DISCOVER
   Discover novel metabolites which cause β cell failure

2. PREDICT & DETECT
   Establish novel and sensitive biomarkers to predict and detect Diabetes
**DISCOVER NOVEL METABOLITE**

**Cohort 1**

GDM: Toronto/Canada  
BMI, Age, Race Matched  
3rd trimester

*One Step Diagnosis*

NGT: 24  
75g OGTT:  
- Fasting > 5.1 mmol/L  
- 1hr > 10.0 mmol/L  
- 2hr > 8.5 mmol/L

**Cohort 2**

T2D: Shanghai/China  
BMI, Age, Sex Matched  
Prospective Cohort  

*Pre-diabetes Diagnosis*

Normal: 50  
- Fasting: 5.6-6.9 mmol/L or  
- 75g OGTT 2hr: 7.8-11 mmol/L

**Diabetes Diagnosis**

- Fasting ≥ 7.0 mmol/L or  
- 75g OGTT 2hr ≥ 11 mmol/L

**Newly Diagnosed Diabetes, No Drug Intervention**

GDM, Prediabetes and Diabetes conditions were determined based on ADA criteria (American Diabetes, 2014)
DISCOVER NOVEL METABOLITE

Metabolomics: Advancing Technology for Biological Discovery

Blood Sample

Amino Acids

Carbohydrate

Lipids

Nucleotides
**DISCOVER NOVEL METABOLITE**

**Cohort 1: Toronto – GDM & Cohort 2: Shanghai – T2D**

342 metabolites

**Up-regulation**

- Lipids
- Amino Acids

**Down-regulation**

- Xenobiotics
- Amino Acids
- Vitamins
- Carbohydrates
- Peptides
- Nucleotides
- Lipids

Prentice et. al., Cell Metabolism, 2014
**Cohort 1: Toronto - GDM**

- pyroglutamine
- N6-acetyllysine
- 3-hydroxyisobutyrate
- tiglyl carnitine
- 2-hydroxybutyrate (AHB)
- arginine
- creatine
- 2-aminobutyrate
- 1,5-anhydroglucitol (1,5-AG)
- eicosapentaenoate (EPA; 20:5n3)
- docosapentaenoate (n3 DPA; 22:5n3)
- nonadecanoate (19:0)
- 10-nonadecenoate (19:1n9)
- eicosenoate (20:1n9 or 11)
- dihomo-linoleate (20:2n6)
- arachidonate (20:4n6)
- docosadienoate (22:2n6)
- arachidonate (20:4n6)
- adrenate (22:4n6)
- 2-hydroxystearate
- 2-hydroxypalmitate
- 3-carboxy-4-methyl-5-propyl-2...
- hexanoylcarnitine
- octanoylcarnitine
- decanoylcarnitine
- cis-4-decenoyl carnitine
- laurilcarnitine
- 3-hydroxybutyrate (BHBA)
- 1-palmitoylglycerophosphoinositol
- pregnenolone sulfate

**Cohort 2: Shanghai – T2D**

Prentice et. al., Cell Metabolism, 2014
## WHAT IS CMPF? **FURAN FATTY ACID METABOLITE**
3-CARBOXY-4-METHYL-5-PROPYL-2-FURANPROPANOIC ACID

### Occurrence of Furan FA in Different Organs and Blood of Lipid Fractions in Animals

<table>
<thead>
<tr>
<th>Organism</th>
<th>Organ/blood</th>
<th>PL</th>
<th>CLE</th>
<th>TG</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>Liver</td>
<td>—</td>
<td>++++</td>
<td>++</td>
<td>26,28–34</td>
</tr>
<tr>
<td>Fish</td>
<td>Testes</td>
<td>++</td>
<td>++</td>
<td>++++</td>
<td>23,26,28–34</td>
</tr>
<tr>
<td>Fish</td>
<td>Ovaries</td>
<td>—</td>
<td>++++</td>
<td>—</td>
<td>35</td>
</tr>
<tr>
<td>Fish</td>
<td>Sperm</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>35</td>
</tr>
<tr>
<td>Fish</td>
<td>Egg</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>35</td>
</tr>
<tr>
<td>Fish</td>
<td>Roe</td>
<td>++</td>
<td>+</td>
<td>—</td>
<td>31</td>
</tr>
<tr>
<td>Fish</td>
<td>Muscle</td>
<td>+</td>
<td>++++</td>
<td>—</td>
<td>26,33,34</td>
</tr>
<tr>
<td>Fish</td>
<td>Blood</td>
<td>—</td>
<td>++++</td>
<td>—</td>
<td>31</td>
</tr>
<tr>
<td>Crayfish</td>
<td>Hepatopancreas</td>
<td>+</td>
<td>++++</td>
<td>+</td>
<td>39,40</td>
</tr>
<tr>
<td>Crayfish</td>
<td>Muscle</td>
<td>++++</td>
<td>+</td>
<td>—</td>
<td>41</td>
</tr>
<tr>
<td>Sponges</td>
<td></td>
<td>—</td>
<td>++</td>
<td>—</td>
<td>42</td>
</tr>
<tr>
<td>Beef</td>
<td>Liver</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>53</td>
</tr>
<tr>
<td>Beef</td>
<td>Blood</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>53</td>
</tr>
<tr>
<td>Humans</td>
<td>Blood</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>54–56</td>
</tr>
<tr>
<td>Plants</td>
<td>Grasses, dandelion, olive</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>48–50,52</td>
</tr>
<tr>
<td>Plants</td>
<td>Cell culture</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>50</td>
</tr>
<tr>
<td>Algae</td>
<td></td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>46,47</td>
</tr>
</tbody>
</table>

*PL, phospholipid, CLE, cholesterol ester. The symbols +, ++, ++++, and ++++ indicate relative abundances of furan FA (F-acids) in the different species. PL containing F-acids readily undergo oxidative decomposition during chromatographic separation on silica gel columns. Therefore, the absence of F-acids in the PL fraction may be due to complete decomposition (31).*
CMPF IN UREMIC PATIENTS

Elsevier

Accumulation of furancarboxylic acids in uremic serum as inhibitors of drug binding

Toshimitsu Niwa a, Naohito Takeda b, Kenji Maeda a, Masao Shibata and Akira Tatematsu b

Fig. 4. Correlation between serum level of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid and duration on hemodialysis.

Niwa et al., 1988; Allegra et al., 1994; De Marchi et al., 1987; DeFronzo, 1978; Nakamura et al., 1985
**DISCOVER NOVEL METABOLITE**

**Cohort 1: Toronto - GDM**

![Graph showing CMPF levels in Pregnant and Postpartum conditions](image)

Prentice et. al., Cell Metabolism, 2014
DISCOVER NOVEL METABOLITE

Cohort 2: Shanghai – T2D

Prospective Cohort

Liu et. al., Cell Reports, 2016
DISCOVER NOVEL METABOLITE

Cohort 2: Shanghai – T2D

Relation between the change in CMPF levels and the risk of future diabetes development, with adjustment for Age, Sex, BMI and estimated glomerular filtration rate

<table>
<thead>
<tr>
<th>Change in CMPF levels during 4-5 years follow up period</th>
<th>Development of diabetes compared to the maintenance of a non-diabetic state</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Likelihood Ratio</td>
</tr>
<tr>
<td>1st quartile</td>
<td>Base line</td>
</tr>
<tr>
<td>2nd quartile</td>
<td>1.74</td>
</tr>
<tr>
<td>3rd quartile</td>
<td>5.41</td>
</tr>
<tr>
<td>4th quartile</td>
<td>7.35</td>
</tr>
<tr>
<td>Continuous</td>
<td>9.57</td>
</tr>
</tbody>
</table>

A large elevation in circulating CMPF concentrations is associated with increased risk of diabetes development

Liu et. al., Cell Reports, 2016
What role does CMPF play during the progression of diabetes?
CMPF and Beta Cell Function

Diet Predisposed Model (DIO)
- Sucrose matched chow
- Vehicle
- 60% high fat diet
- CMPF (6mg/kg/day)
- 2wk i.p. injection
- 6wk

Genetic Predisposed Model (Ob/Ob)
- Chow
- DIO
- Vehicle
- CMPF (6mg/kg/day)
- 2wk i.p. injection
- Ob/Ob
- Ob/Ob (w/CMPF)
CMPF AND BETA CELL FUNCTION

Glucose Tolerance Test

Insulin Secretion *in-vivo*

Liu et. al., Cell Reports, 2016
Elevated CMPF Impairs Glucose-Stimulated Insulin Secretion and may Potentiate the Development of Diabetes in Rodent Models

Liu et. al., Cell Reports, 2016
INSULIN SECRETION IN BETA CELL

Glucose Sensing (Glycolysis) x
Metabolism x
ATP production x
Insulin Biosynthesis x
Exocytosis x

CMPF
CMPF: GLUCOSE SENSING (GLYCOLYSIS)

Glucose Uptake

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CMPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>20</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>40</td>
<td>60</td>
<td>80</td>
</tr>
</tbody>
</table>

* statistically significant difference

Glycolysis

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>DIO</th>
<th>DIO (w/CMPF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>20</td>
<td>40</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>40</td>
<td>60</td>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

20mM Glucose

ECAR (AUC, pmol/min)

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>DIO</th>
<th>DIO (w/CMPF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>20</td>
<td>40</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>40</td>
<td>60</td>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

Pyruvate Dehydrogenase (PDH)

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>DIO</th>
<th>DIO (w/CMPF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>20</td>
<td>40</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>40</td>
<td>60</td>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

PDH Activity (HG/LG, ΔOD450/min)

CMPF Impairs Glycolysis

Liu et. al., Cell Reports, 2016
CMPF: MITOCHONDRIAL METABOLISM

Mitochondrial Membrane Potential (MMP)

Liu et al., Cell Reports, 2016
CMPF: MITOCHONDRIAL METABOLISM

Glucose Oxidation

Mitochondrial Biogenesis

CMPF Impairs Glucose Metabolism without affect Mitochondrial Biogenesis

Liu et. al., Cell Reports, 2016
CMPF: MITOCHONDRIAL METABOLISM

Mitochondrial Membrane Potential (MMP)

CMPF Impairs Glucose Metabolism by Metabolic Remodeling to Enhance Fatty Acid Metabolism

Fatty Acid Oxidation

Liu et al., Cell Reports, 2016
CMPF: METABOLIC REMODELING

**ROS**

<table>
<thead>
<tr>
<th>Chow</th>
<th>DIO</th>
<th>DIO (w/CMPF)</th>
<th>Ob/Ob</th>
<th>Ob/Ob (w/CMPF)</th>
</tr>
</thead>
</table>

**Apoptosis**

![Graph showing apoptosis](image)

**Advanced Glycation End-products (AGEs)**

![Graph showing AGEs](image)

**CMPF Increases Advanced Glycation End-Products and Oxidative Stress**

Liu et. al., Cell Reports, 2016
CMPF: INSULIN SECRETION IN BETA CELL
CMPF: INSULIN BIOSYNTHESIS & EXOCYTOSIS

**Insulin Content**

![Bar chart showing insulin content with Ob/Ob and Ob/Ob (w/CMPF) groups, along with Chow, DIO, and DIO (w/CMPF) conditions.](chart)

*Graph showing gene expression (relative to Actb, %) for Pdx1, MafA, Ins1, CpE, Pc1, and Pc2 with and without CMPF treatment.*

Liu et. al., Cell Reports, 2016
CMPF: INSULIN BIOSYNTHESIS & EXOCYTOSIS

Transmitted Electron Microscopy (TEM)

Chow  DIO  DIO (w/CMPF)  Ob/Ob  Ob/Ob (w/CMPF)

Glucose  G6P  Pyruvate  Glycolysis  ATP

Immuno-Gold Insulin Staining

Liu et. al., Cell Reports, 2016
The constitutive secretory pathway present in all cell types does not require stimulation for exocytosis.

**Constitutive Secretory Pathway**
- Secretonary granules bud from the TGN as immature secretory granules (ISGs)
- Clathrin-coated ISCs contain missioned non-granule proteins (eg. Furin, lysosomal enzymes, etc.)
- ISGs remove missioned proteins and clathrin coats by budding off constitutive-like vesicles
- After further acidification and condensation, ISGs become mature
- The mature granules (MSGs) are secreted via triggering by a secretagogue

**Regulated Secretory Pathway**
- Secretory granules bud from the TGN as immature secretory granules (ISGs)
- Clathrin-coated ISCs contain missioned non-granule proteins (eg. Furin, lysosomal enzymes, etc.)
- ISGs remove missioned proteins and clathrin coats by budding off constitutive-like vesicles
- Constitutive-like vesicles
- Lysosome

Liu et al., Cell Reports, 2016
**CMPF: INSULIN BIOSYNTHESIS & EXOCYTOSIS**

Transmitted Electronic Microscopy (TEM)

### Results

**Chow**
- Dense Core
- Non-dense Core
- Immature

**DIO**
- Dense Core
- Non-dense Core
- Immature

**DIO (w/CMPF)**
- Dense Core
- Non-dense Core
- Immature

**Ob/Ob**
- Dense Core
- Non-dense Core
- Immature

**Ob/Ob (w/CMPF)**
- Dense Core
- Non-dense Core
- Immature

---

Liu et. al., Cell Reports, 2016
CMPF: INSULIN BIOSYNTHESIS & EXOCYTOSIS

**Cellular**

**Exocytosis: Secretion**

 CMPF Increases Proinsulin and Impairs Insulin Granule Maturation

Liu et. al., Cell Reports, 2016
Human Study
1. CMPF significantly elevated in Prediabetes and Diabetes population.
2. Rapid elevation may be a high risk for future diabetes development

Animal Study
1. CMPF impairs glucose metabolism by introducing preferential fatty acid oxidation
2. CMPF induces beta cell dysfunction shown as impaired insulin secretion

Rapid Elevation of CMPF May Act as a Tipping Point Towards β Cell Dysfunction During Diabetes Development
ACKNOWLEDGEMENTS

University of Toronto
Toronto General Hospital
Dr. Michael Wheeler
Dr. Kacey Prentice
Amina Allalou
Andrea Eversley
Dr. Feihan Dai

Kaiser Permanente
Oakland, USA
Dr. Erica Gunderson

Hospital for Sick Children
Toronto, Canada
Analytical Facility for Bioactive Molecules

6th People’s Hospital,
Shanghai, China
Dr. Weiping Jia
Dr. Wei Li
Dr. Cheng Hu
**WHAT CAN WE DO??**

1. **DISCOVER**  ✔ CMPF
   Discover novel metabolites which cause β cell failure

2. **PREDICT & DETECT**
   Establish novel and sensitive biomarkers to predict and detect Diabetes
Establish Biomarkers to Predict and Detect Diabetes

Metabolomic Signature: it is more than just hyperglycemia

What can we do with this data???
ESTABLISH SIGNATURE PANELS TO PREDICT AND DETECT DIABETES

Fasting Blood Glucose/OGTT

HbA1c

Clinical Practice Guidelines

NGT  IGT

Prediction of Future DM Within 5 Years

Population Risk

10%  40-50%

Allalou et. al., Diabetes, 2016
Establishe Signature Panels to Predict and Detect Diabetes

- Identify metabolites associated with GDM/T2D
- Develop an SRM method for quantification of each metabolite
- Validate/quantify each metabolite in test plasma
- Assay Prospective Cohort

181 metabolites chosen for testing:
- Carbs
- Amino Acids
- Fatty acids
- Other

- Identify Ion Spectra for each metabolite and validate in blood

Allalou et al., Diabetes, 2016
ESTABLISH SIGNATURE PANELS TO PREDICT AND DETECT DIABETES

SWIFT Prospective GDM Cohort

V0: Pregnancy

V1 (Baseline) PP: 6-9 weeks

nonT2D 1010

T2D 21

非T2D 1035

T2D 21

非T2D 912

T2D 58

非T2D 801

T2D 47

T2D 19

V2 PP: 1 year

V3 PP: 2 years

V4 2 years+

By 2.5 years postpartum, 124 women (10.3%) have developed T2D

Allalou et. al., Diabetes, 2016
Establish Signature Panels to Predict and Detect Diabetes

Over 20 metabolites associated with future T2D status

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Class</th>
<th>Fold Change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hexose</td>
<td>1.097</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>2</td>
<td>SMC20:2</td>
<td>0.819</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>Tyr</td>
<td>1.121</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>Val</td>
<td>1.094</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5</td>
<td>SMC18:1</td>
<td>0.891</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>6</td>
<td>Leu</td>
<td>1.098</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>7</td>
<td>2-AAA</td>
<td>1.20</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>8</td>
<td>Ile</td>
<td>1.095</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>9</td>
<td>SMC24:1</td>
<td>0.913</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>10</td>
<td>Trp</td>
<td>1.057</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>11</td>
<td>Thr</td>
<td>1.097</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>12</td>
<td>PCaeC42:5</td>
<td>0.916</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>13</td>
<td>SMC18:0</td>
<td>0.919</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>14</td>
<td>Gly</td>
<td>0.897</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>15</td>
<td>C16:1n9</td>
<td>0.890</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>16</td>
<td>SM(OH)C16:1</td>
<td>0.914</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>17</td>
<td>SM(OH)C22:2</td>
<td>0.977</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>18</td>
<td>PCaeC40:5</td>
<td>0.906</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>19</td>
<td>PCaeC44:5</td>
<td>0.922</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>20</td>
<td>AC3</td>
<td>1.104</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>21</td>
<td>AC10</td>
<td>0.907</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Allalou et. al., Diabetes, 2016
EStABLISH SIGNATURE PANELS TO PREDICT AND DETECT DIABETES

Machine Learning: Decision Tree Algorithm

- **PCaeC40:5**
  - ≤3.4: T2D
  - >3.4: Hexose
    - ≤4.9: BCAA
      - ≤427: nonT2D
      - >427: T2D
    - >4.9: SM(OH)C14:1
      - ≤4.4: nonT2D
      - >4.4: T2D

Allalou et. al., Diabetes, 2016
ESTABLISH SIGNATURE PANELS TO PREDICT AND DETECT DIABETES

SWIFT Prospective GDM Cohort

Machine Learning Algorithm Accurately Predicts Future T2D

Allalou et. al., Diabetes, 2016
WHAT WE HAVE ACHIEVED SO FAR???

1. DISCOVER ✔ CMPF
   Discover novel metabolites which cause β cell failure

2. PREDICT & DETECT ✔ Predictive Signature Panel
   Establish novel and sensitive biomarkers to predict and detect Diabetes
ACKNOWLEDGEMENTS

University of Toronto
Toronto General Hospital
Dr. Michael Wheeler
Dr. Kacey Prentice
Amina Allalou
Andrea Eversley
Dr. Feihan Dai

Kaiser Permanente
Oakland, USA
Dr. Erica Gunderson

Hospital for Sick Children
Toronto, Canada
Analytical Facility for
Bioactive Molecules

6th People’s Hospital,
Shanghai, China
Dr. Weiping Jia
Dr. Wei Li
Dr. Cheng Hu
We need more prospective cohort to test our signature metabolite panel for future diabetes prediction!!!

INTERESTED???