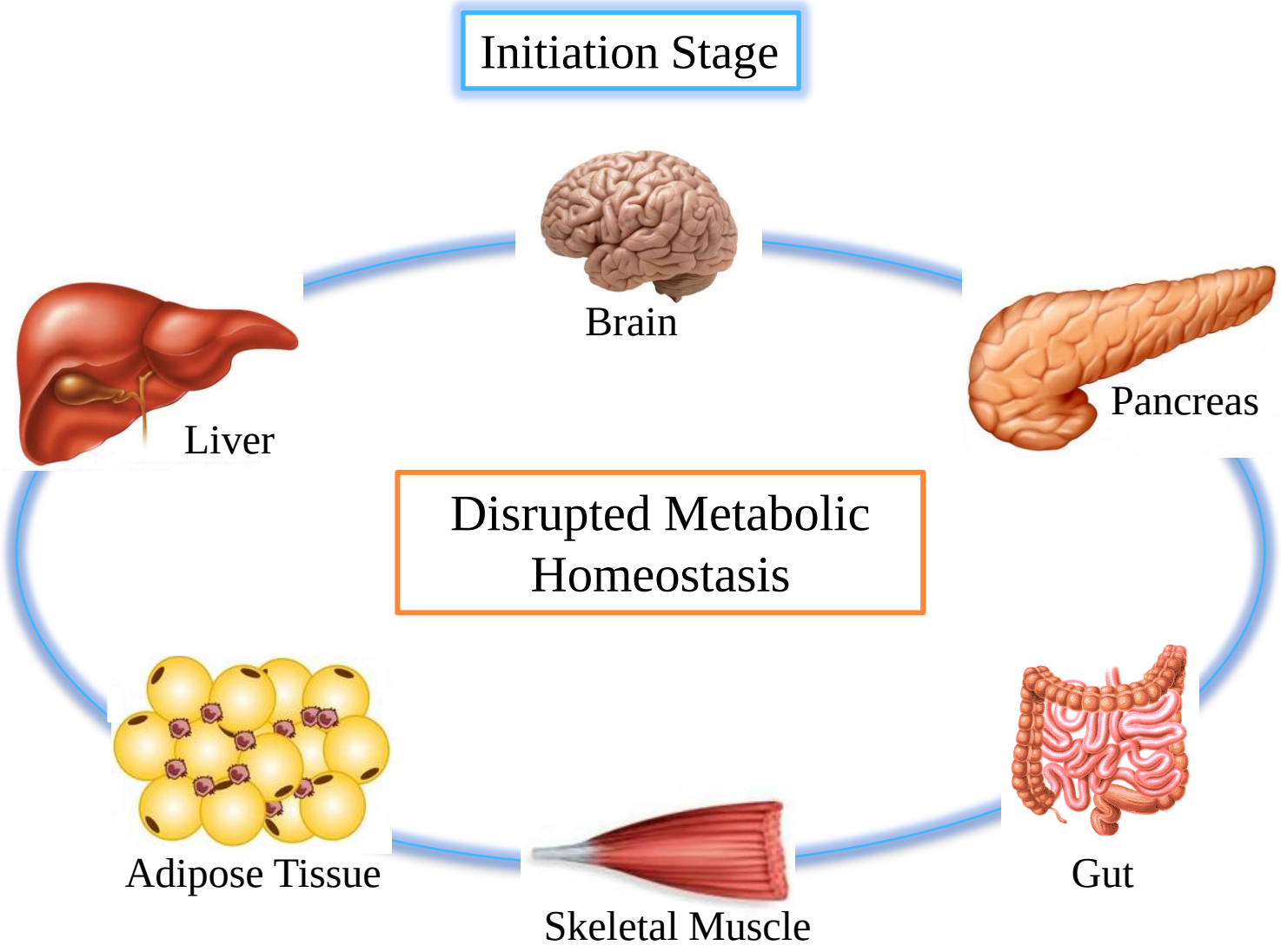
UNCOVERING CIRCULATING FACTORS LINKING PREDIABETES TO DIABETES

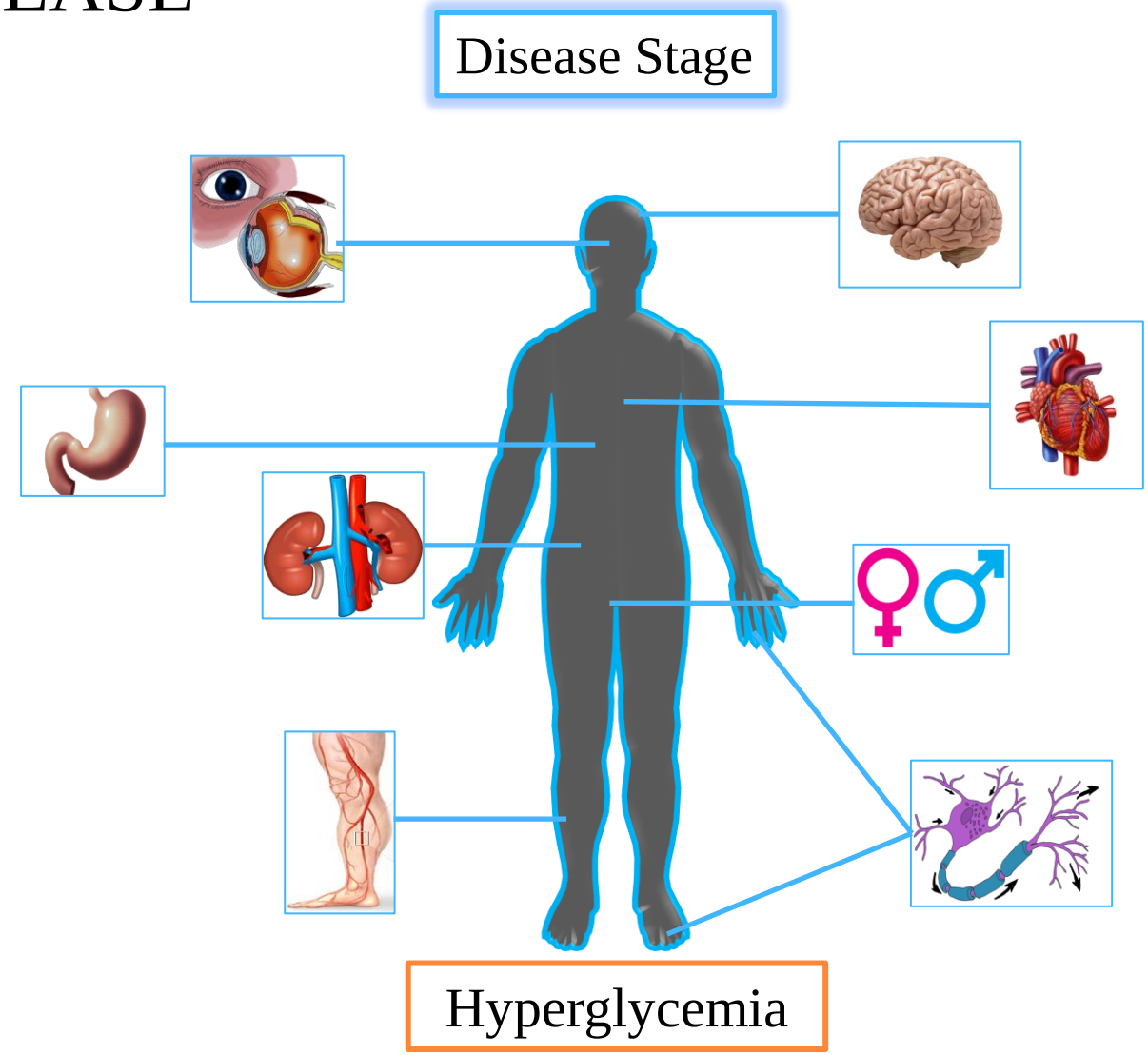
Ying Liu PhD

Mar.07/2017

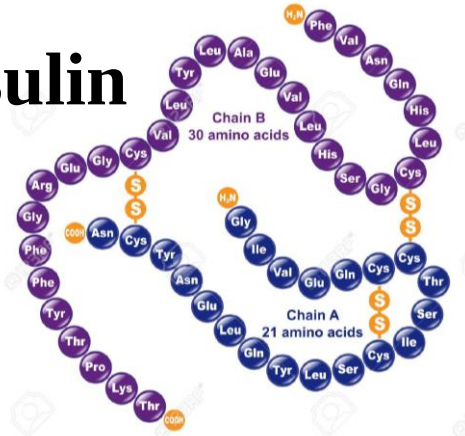
Diabetes Mellitus: A multi-organ disease



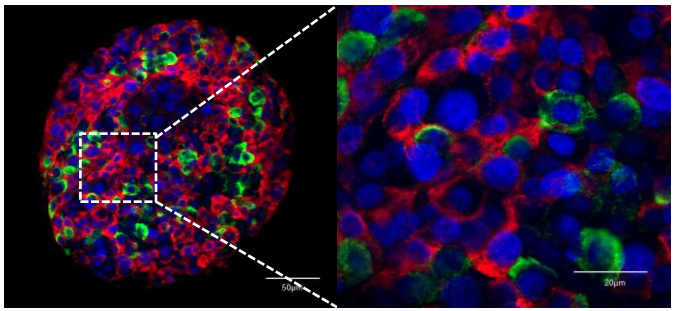
Diabetes Mellitus: A multi-organ disease



Diabetes Mellitus

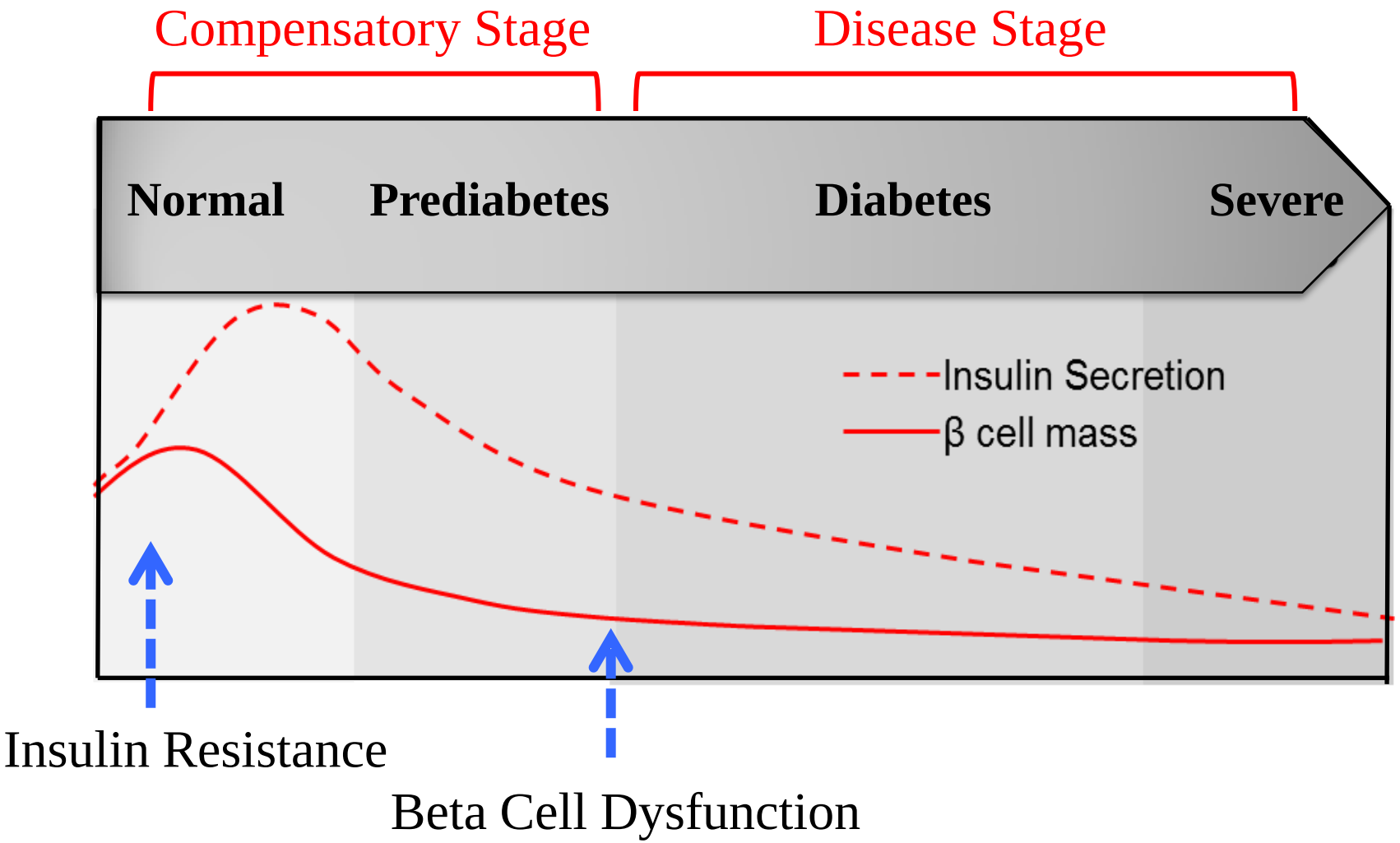


- Type 1 Diabetes: beta cell destruction
- Type 2 Diabetes: beta cell failure in respond to peripheral metabolic demands
- Gestational Diabetes: Insufficient beta cell function in pregnancy



Red: Insulin (β cells)
Green: Glucagon (α cells)
Blue: Nucleus

Diabetes Mellitus: A Progressive Disease



What can we do???

Early Detection

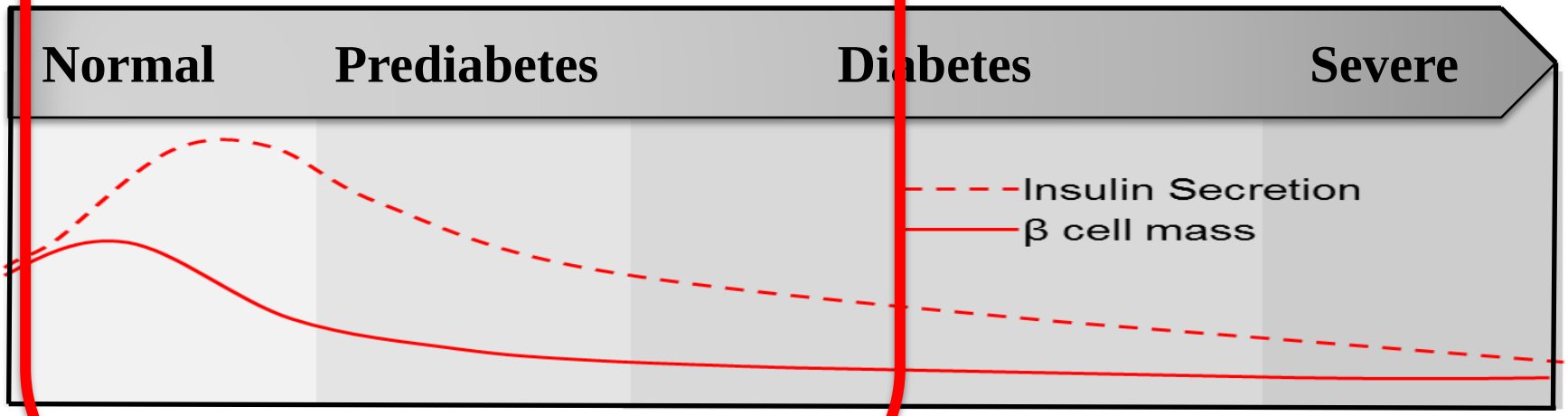
Prevent disease progression

Therapeutic Targets

Prevent loss of or restore β cell function

β cell Replacement

Therapy to restore β cell function



WHAT CAN WE DO???

Early Detection

Therapeutic Targets

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
75g OGTT:

- fasting > 5.1mmol/L
- 1hr > 10.0 mmol/L
- 2hr > 8.5 mmol/L

NGT: 24
GDM: 24

Cohort 2

T2D: Shanghai/China
BMI, Age, Sex Matched

Prospective Cohort
(2007,2008 ➔ 2011,2012)

Pre-diabetes Diagnosis

- 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

Normal: 50
Pre-diabetes: 75
Diabetes: 71

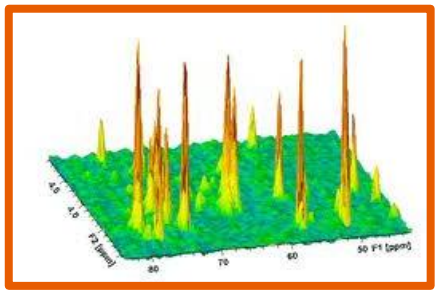
Newly Diagnosed Diabetes, No Drug Intervention

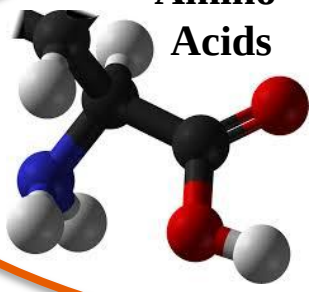
GDM, Prediabetes and Diabetes conditions were determined based on ADA criteria (American Diabetes, 2014)

Discover novel metabolite

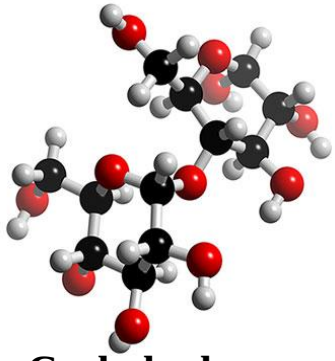
Metabolomics: Advancing Technology for Biological Discovery

Blood Sample

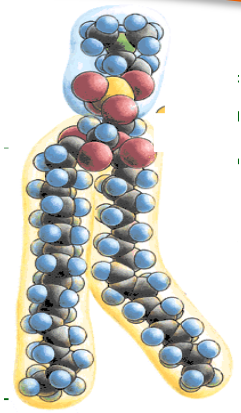




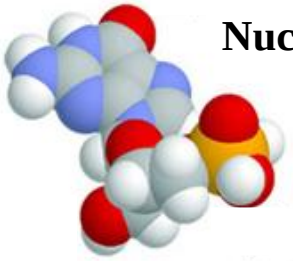
Amino Acids



Carbohydrate



Lipids



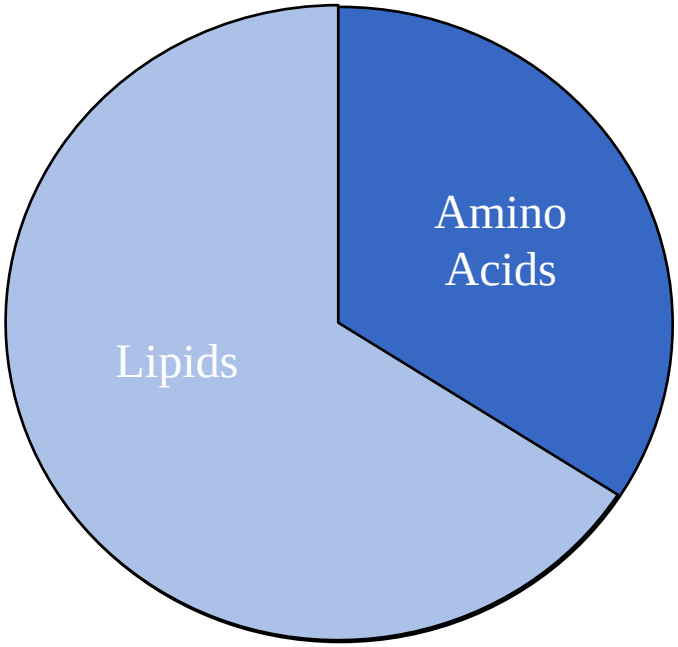
Nucleotides

Discover novel metabolite

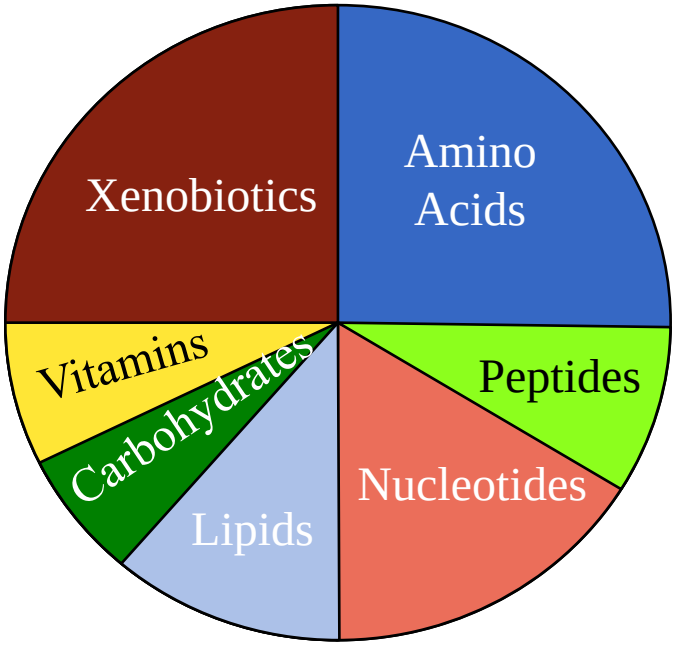
Cohort 1: Toronto – GDM & Cohort 2: Shanghai – T2D

342 metabolites

Up-regulation



Down-regulation

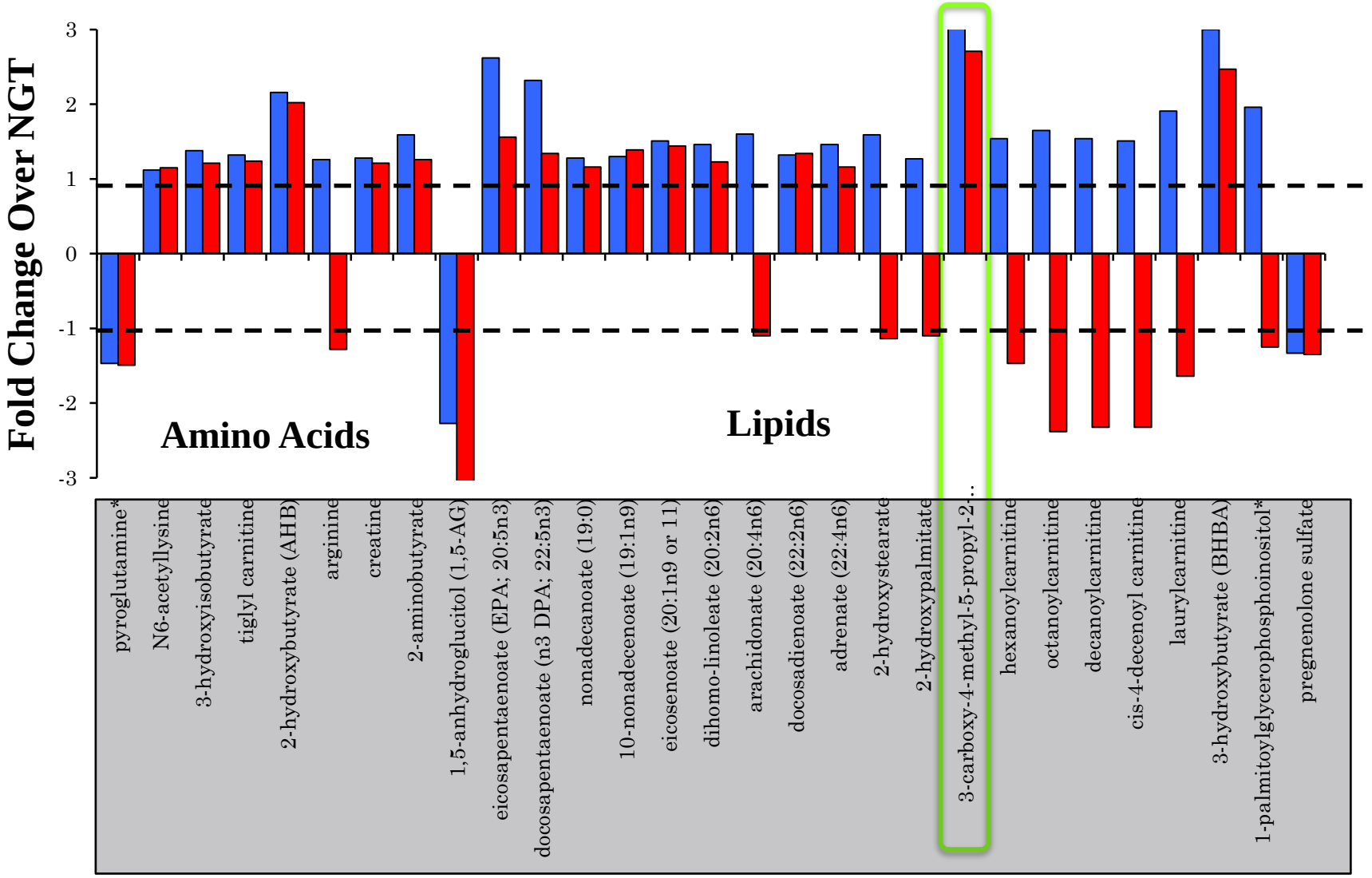


Prentice et. al., Cell Metabolism, 2014

DISCOVER NOVEL METABOLITE

Cohort 1: Toronto - GDM

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[a]

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

[a]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).

Spiteller Gerhard, Furan fatty acids: occurrence, synthesis, and reactions. Are furan fatty acids responsible for the cardioprotective effects of a fish diet?; Lipids; 2005 Aug;40(8):755-71.

CMPF IN UREMIC PATIENTS

Clinica Chimica Acta, 173 (1988) 127–138
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]

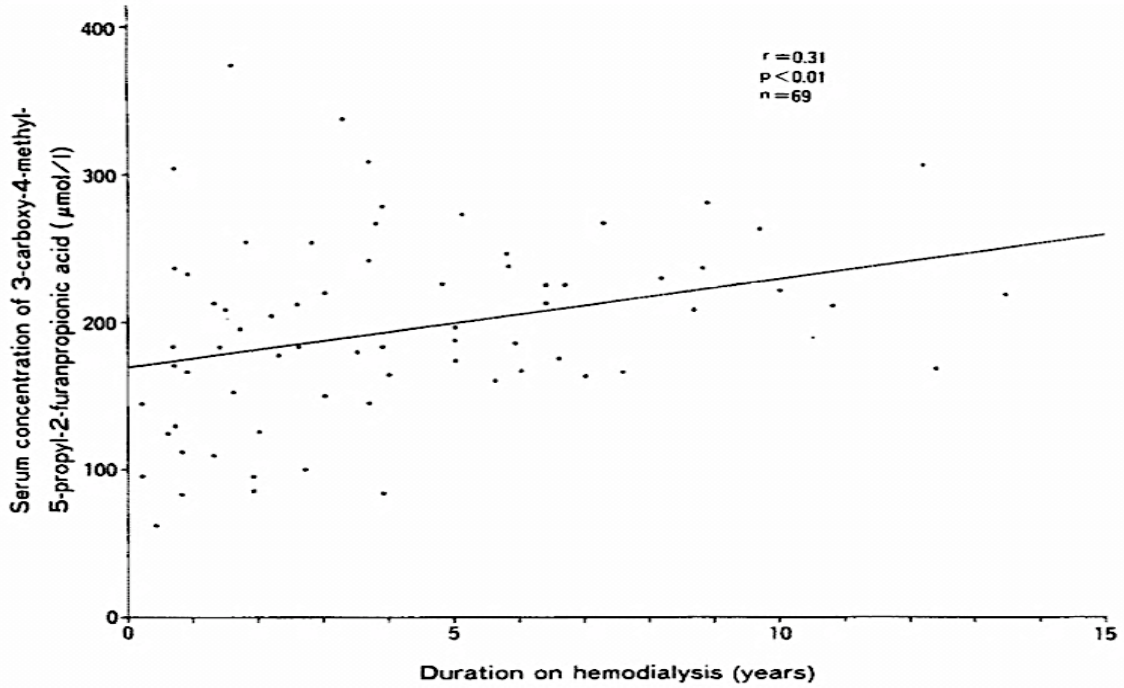


Fig. 4. Correlation between serum level of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid and duration on hemodialysis.

Uremia Patients
↕
Impaired Insulin Secretion

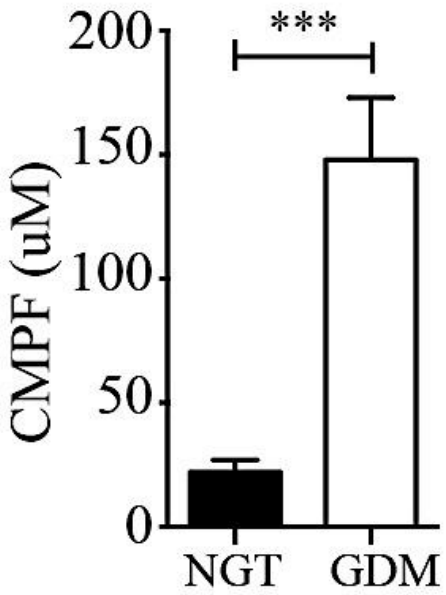
CMPF ⟷ Diabetes

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

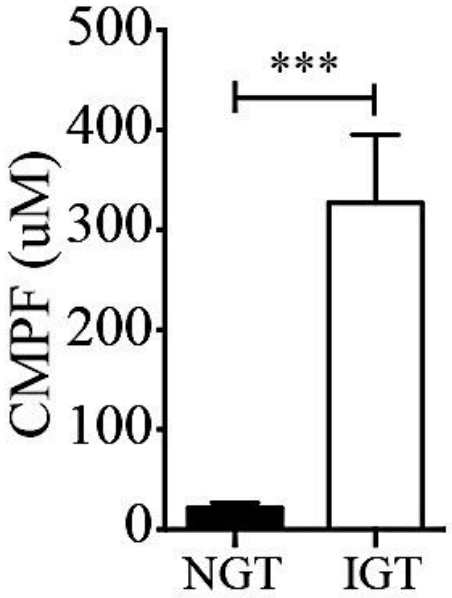
Discover novel metabolite

Cohort 1: Toronto - GDM

Pregnant



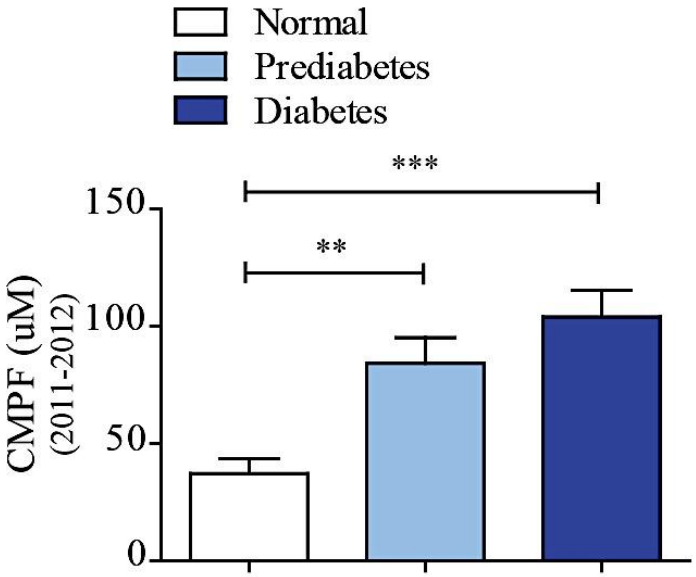
Postpartum



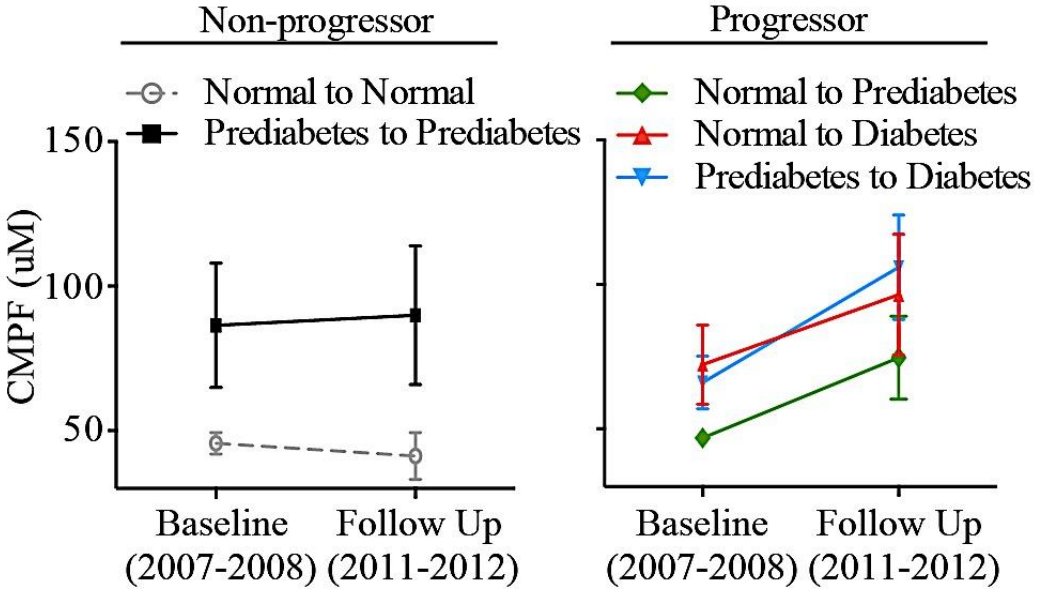
Prentice et. al., Cell Metabolism, 2014

DISCOVER NOVEL METABOLITE

Cohort 2: Shanghai – T2D



Prospective Cohort
(2007,2008 → 2011,2012)



Liu et. al., Cell Reports, 2016

DISCOVER NOVEL METABOLITE

Cohort 2: Shanghai – T2D

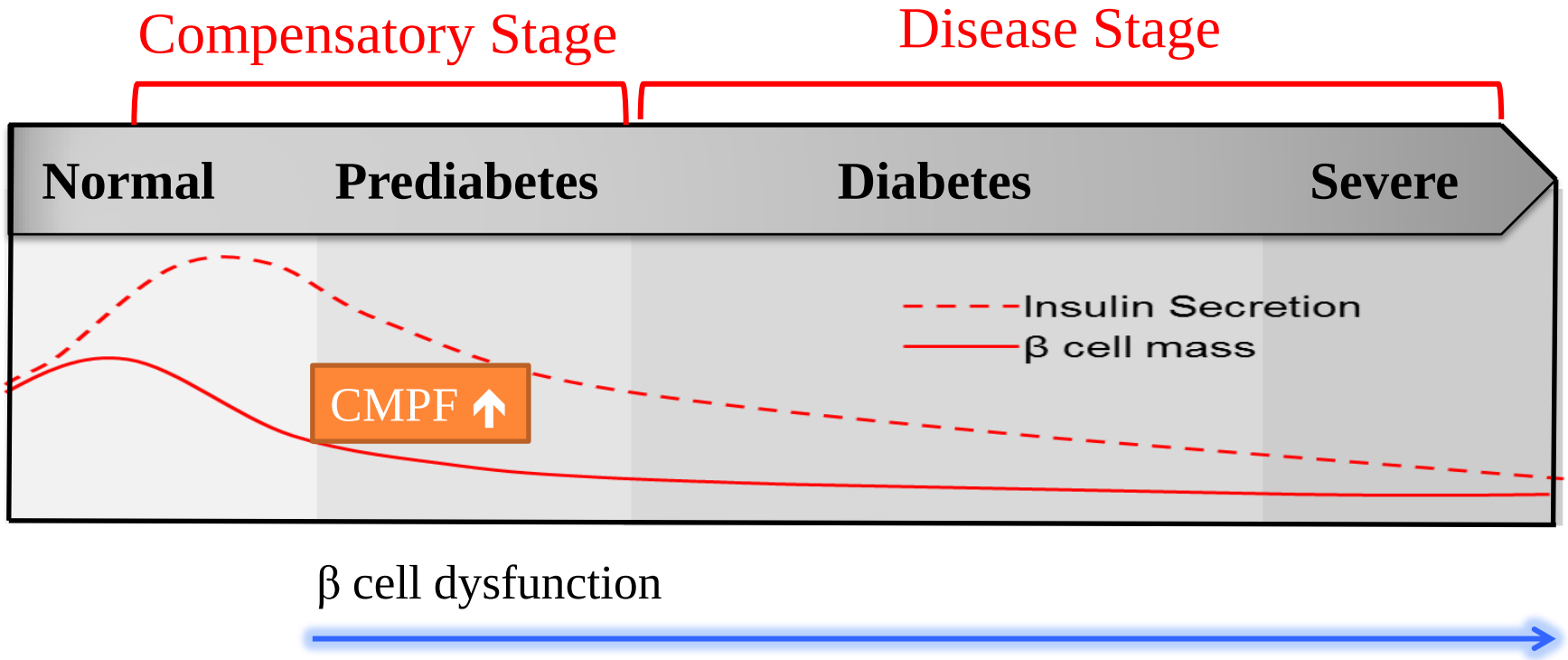
Relation between the change in CMPF levels and the risk of future diabetes development, with adjustment for Age, Sex, BMI and estimated glomerular filtration rate

Change in CMPF levels during 4-5 years follow up period	Development of diabetes compared to the maintenance of a non-diabetic state	
	Likelihood Ratio	P-value
1st quartile	Base line	-----
2nd quartile	1.74	0.188
3rd quartile	5.41	**0.02**
4th quartile	7.35	**0.00669**
Continuous	9.57	**0.00198**

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

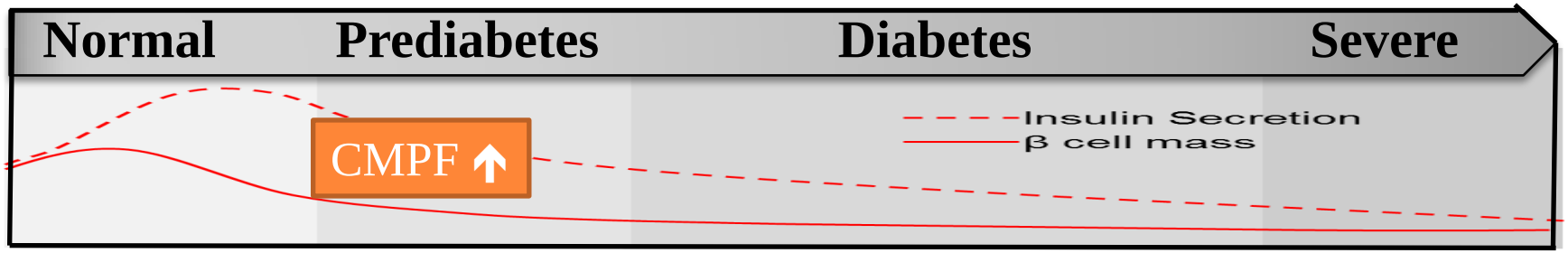
Liu et. al., Cell Reports, 2016

Diabetes Mellitus: A Progressive Disease



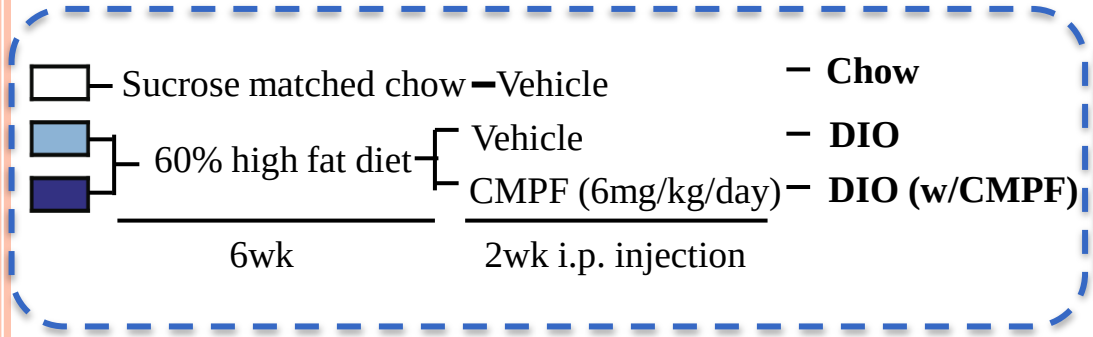
What role does CMPF play during the progression of diabetes?

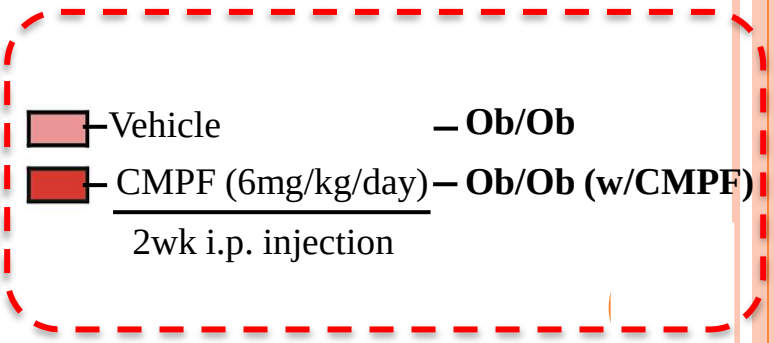
CMPF AND BETA CELL FUNCTION



Diet Predisposed Model (DIO)

Genetic Predisposed Model (Ob/Ob)

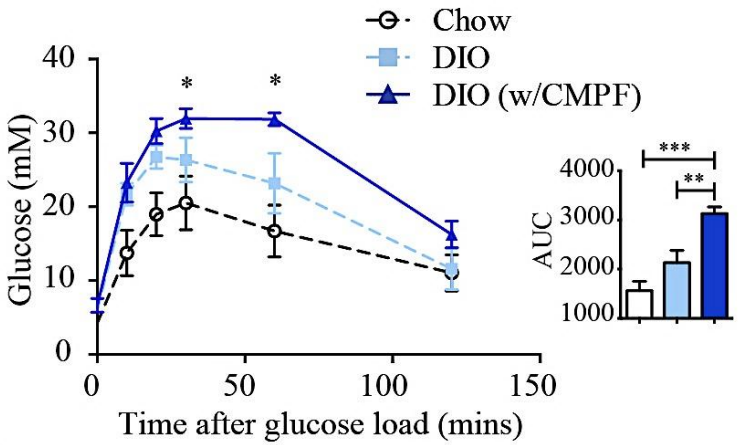


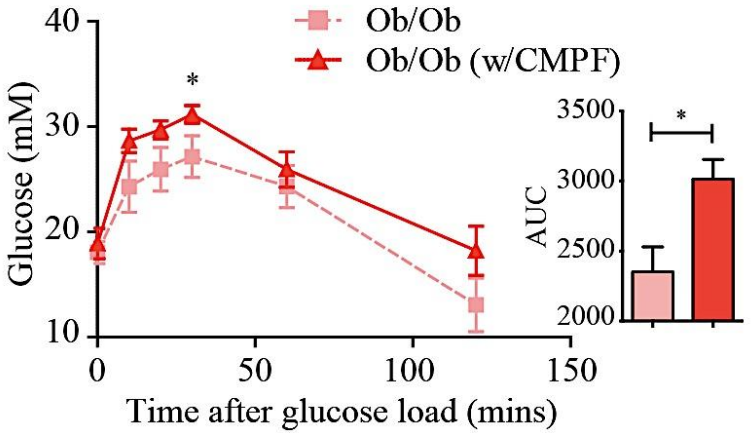


Liu et. al., Cell Reports, 2016

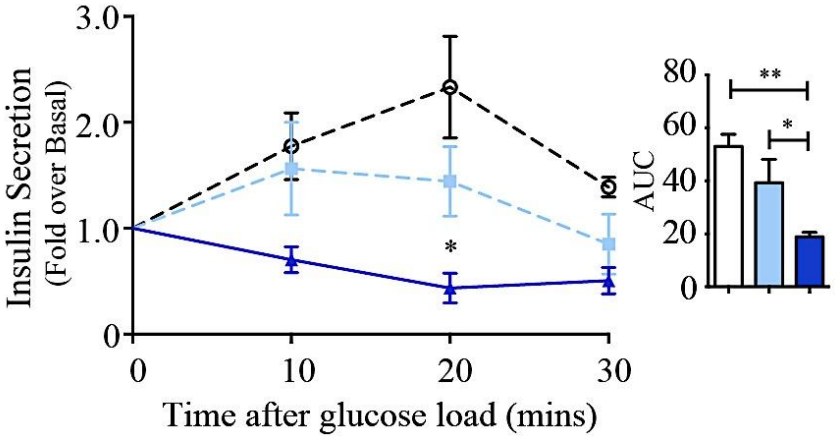
CMPF AND BETA CELL FUNCTION

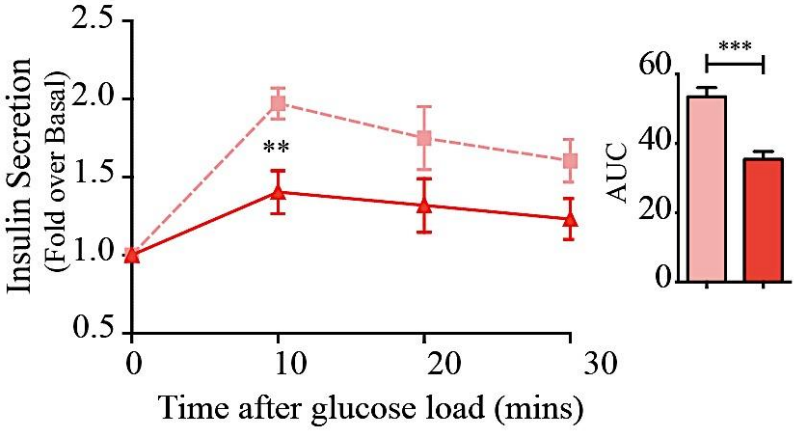
Glucose Tolerance Test





Insulin Secretion *in-vivo*

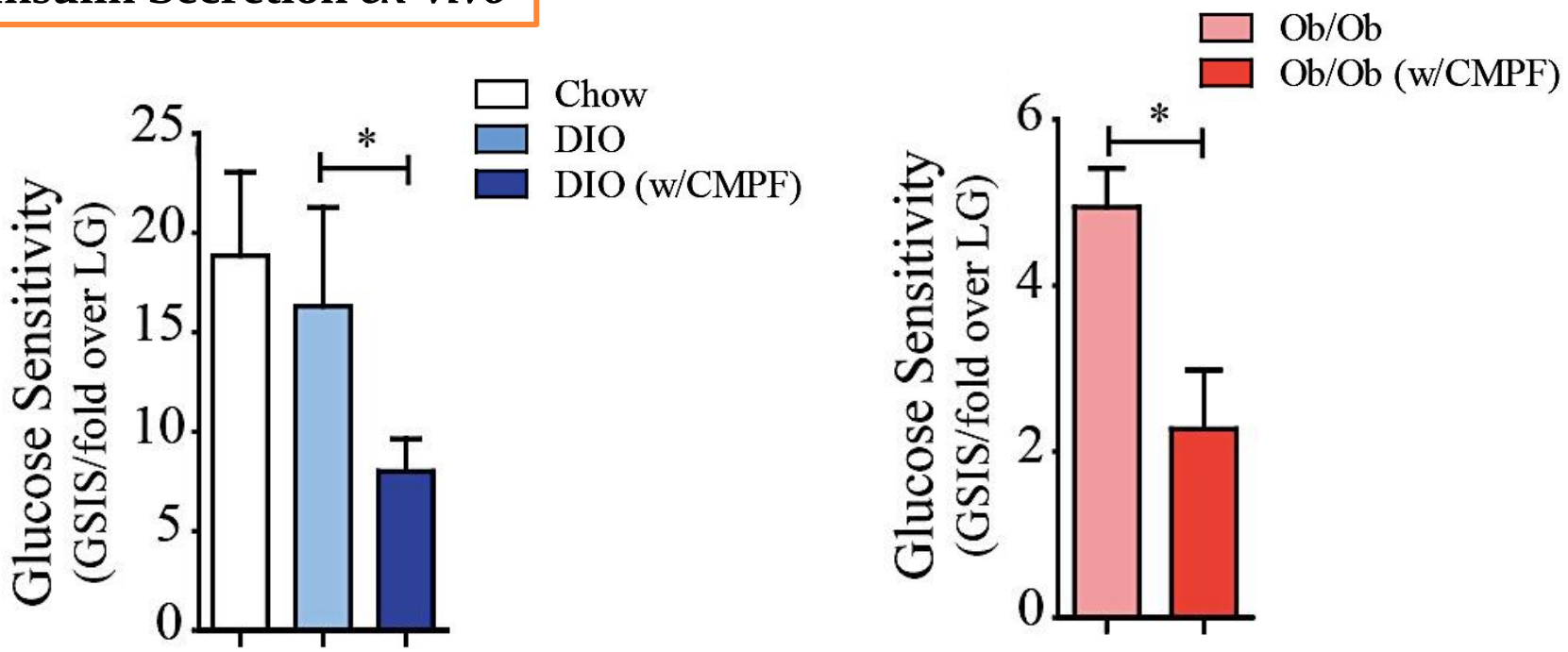




Liu et. al., Cell Reports, 2016

CMPF AND BETA CELL FUNCTION

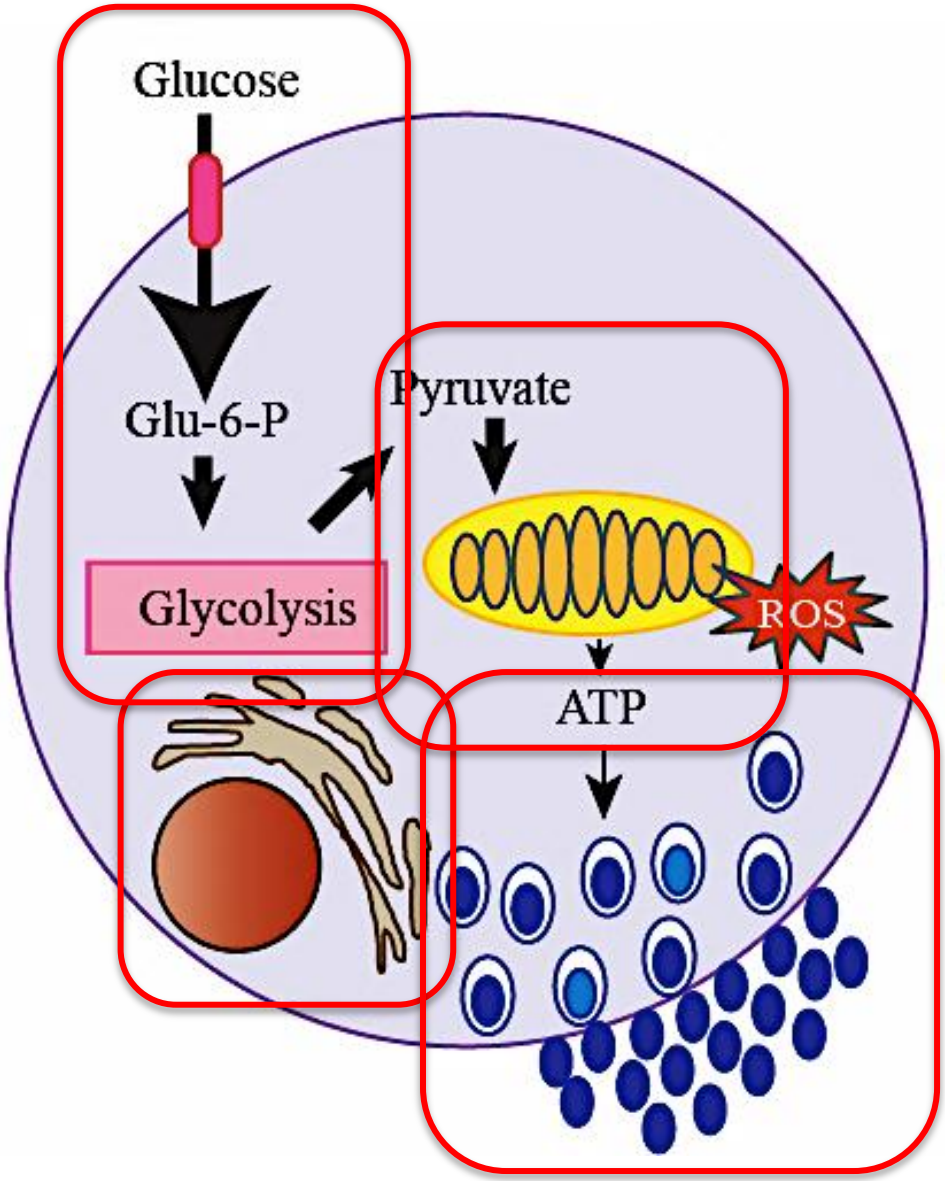
Insulin Secretion *ex-vivo*



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

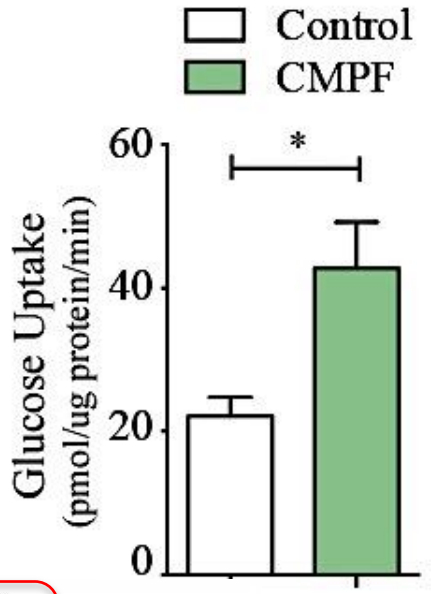
Exocytosis x

↑

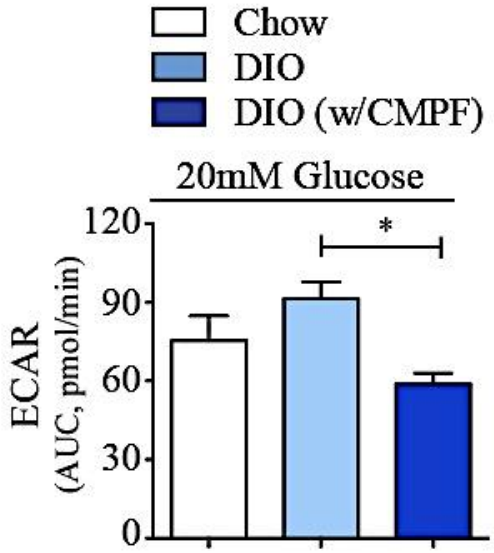
CMPF

CMPF: GLUCOSE SENSING (GLYCOLYSIS)

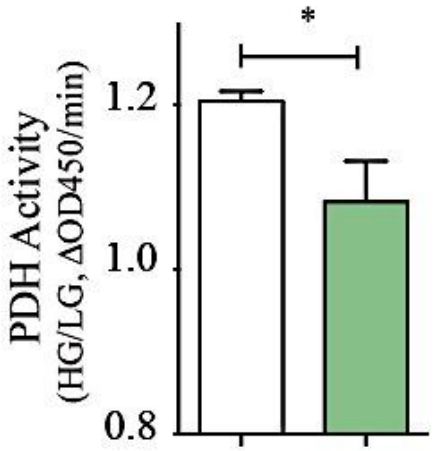
Glucose Uptake

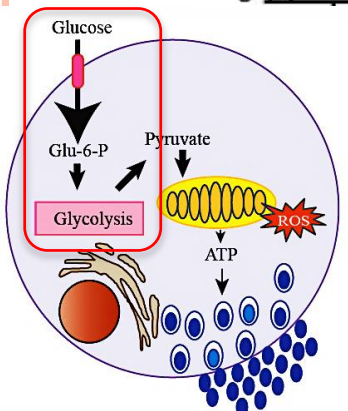


Glycolysis



Pyruvate Dehydrogenase (PDH)



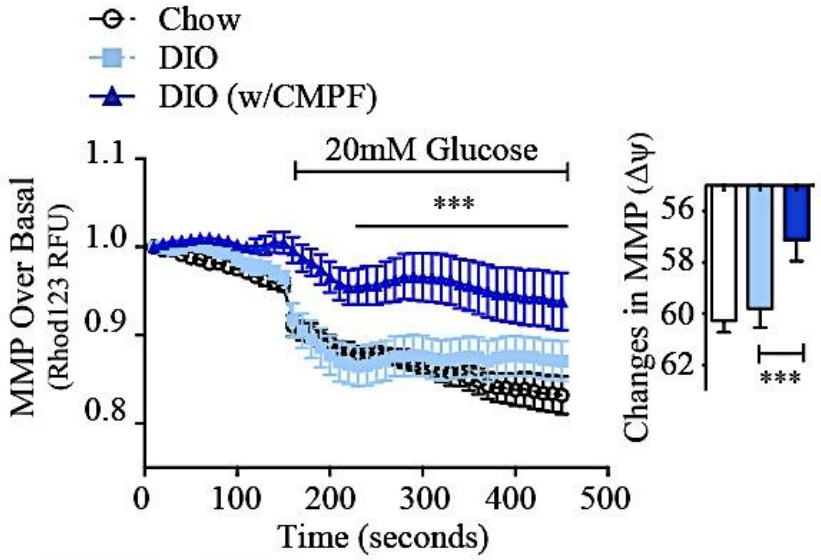


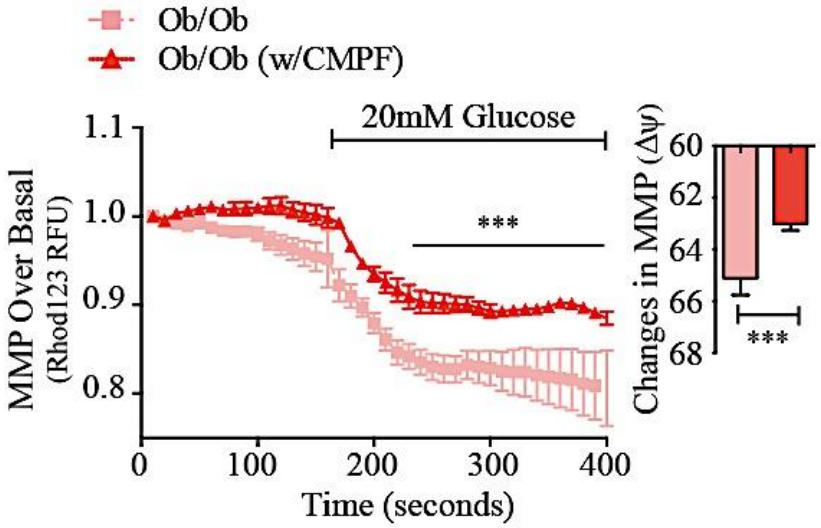
CMPF Impairs Glycolysis

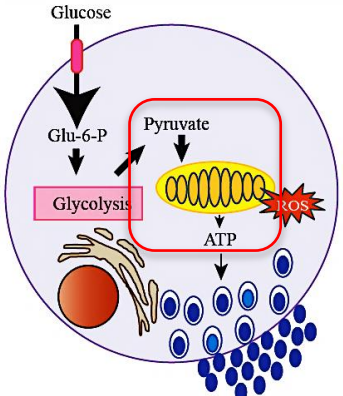
Liu et. al., Cell Reports, 2016

CMPF: MITOCHONDRIAL METABOLISM

Mitochondrial Membrane Potential (MMP)



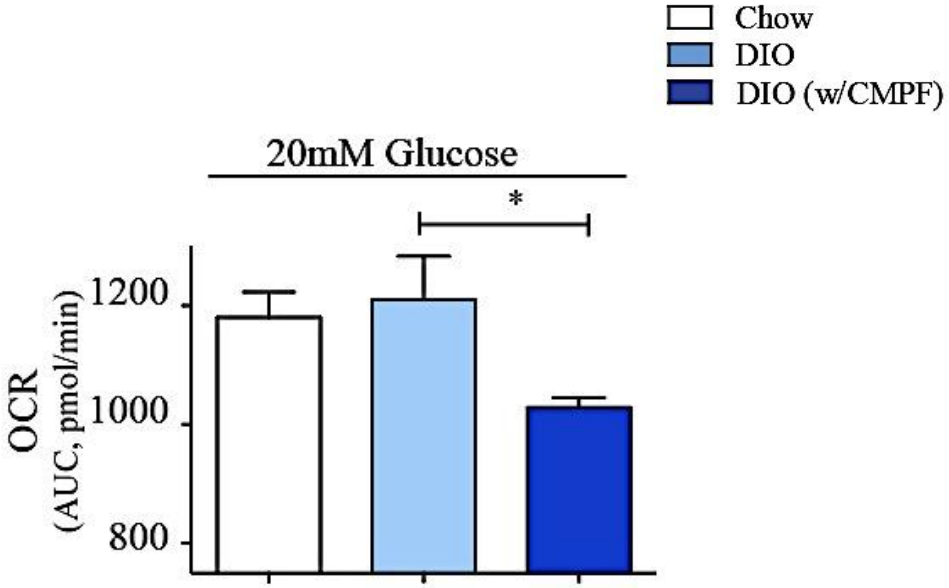




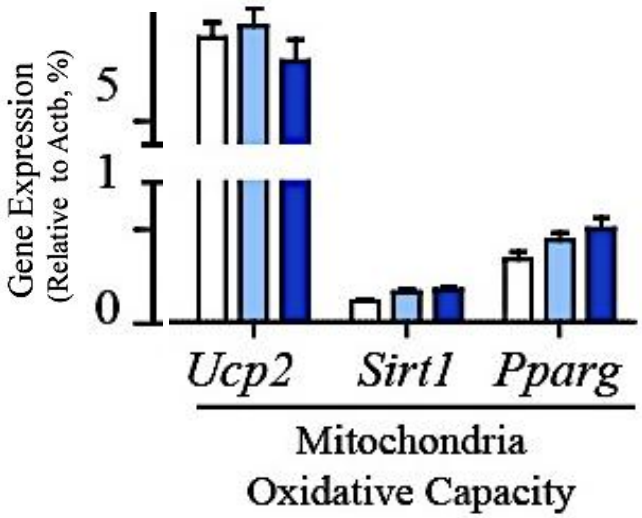
Liu et. al., Cell Reports, 2016

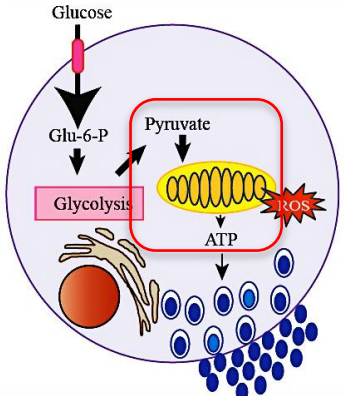
CMPF: MITOCHONDRIAL METABOLISM

Glucose Oxidation



Mitochondrial Biogenesis



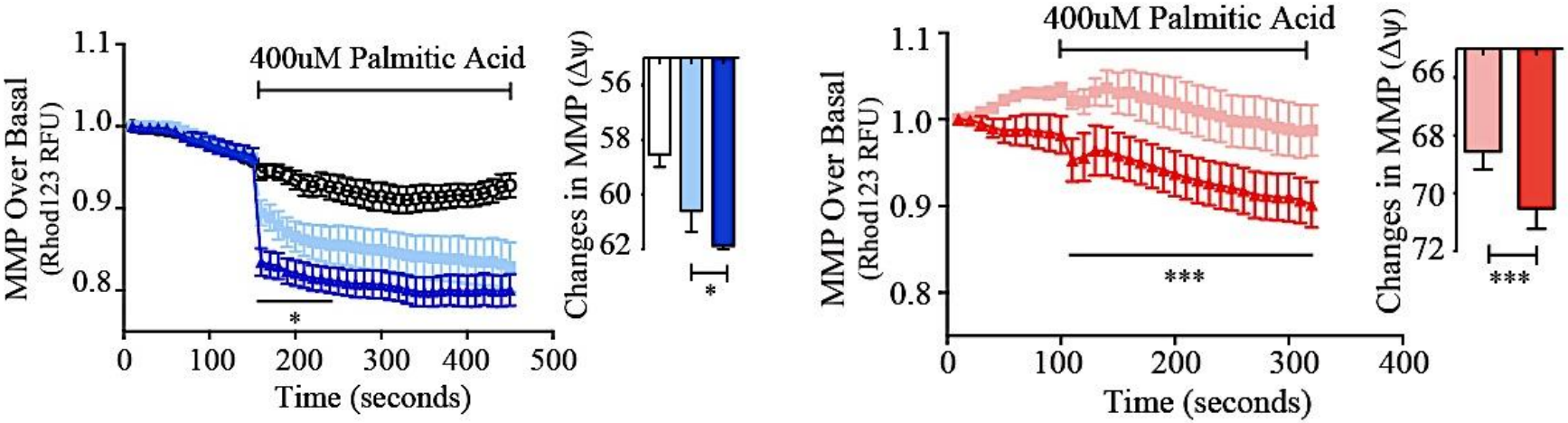


CMPF Impairs Glucose Metabolism without affect Mitochondrial Biogenesis

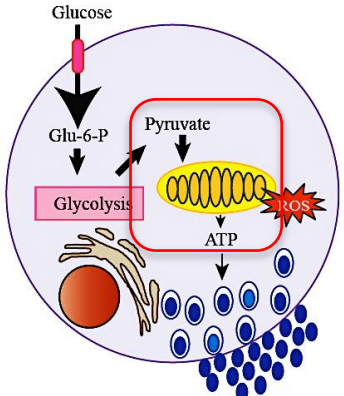
Liu et. al., Cell Reports, 2016

CMPF: MITOCHONDRIAL METABOLISM

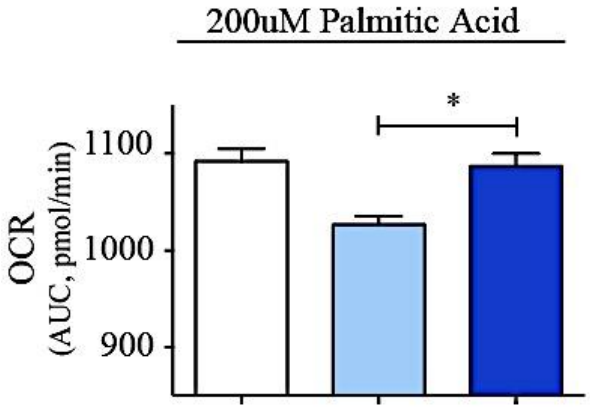
Mitochondrial Membrane Potential (MMP)



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



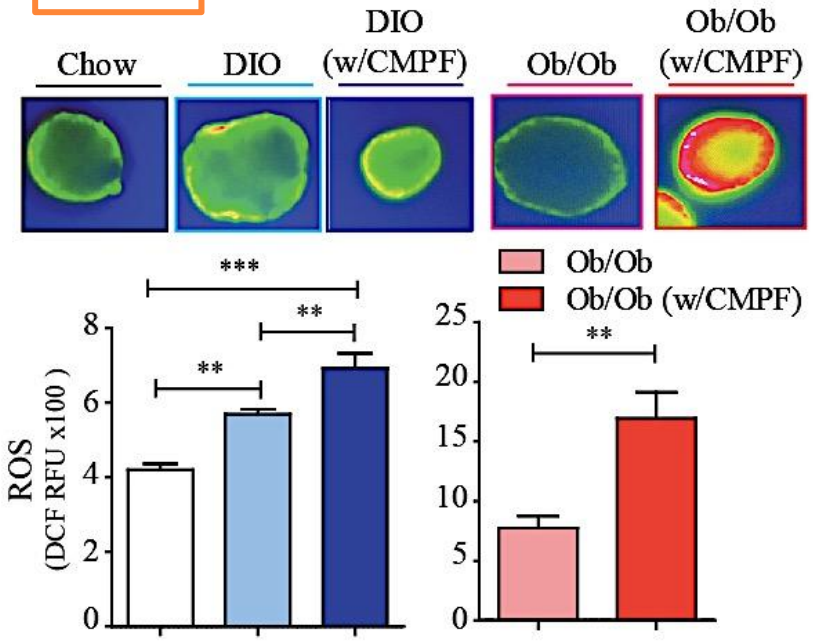
Fatty Acid Oxidation



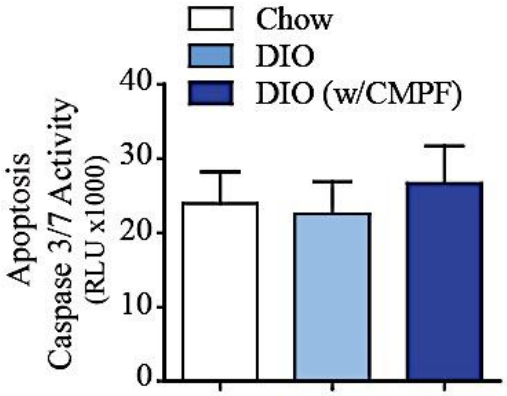
Liu et. al., Cell Reports, 2016

CMPF: Metabolic Remodeling

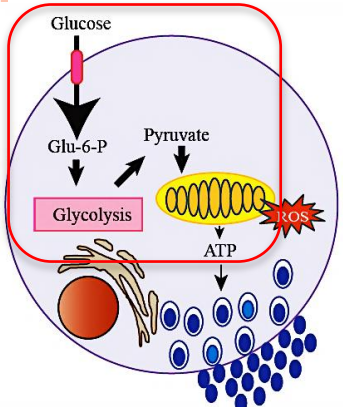
ROS



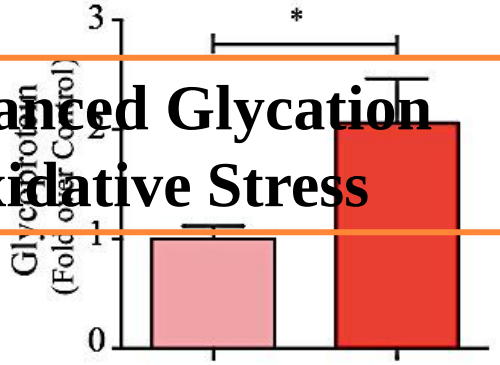
Apoptosis



Advanced Glycation End-products (AGEs)

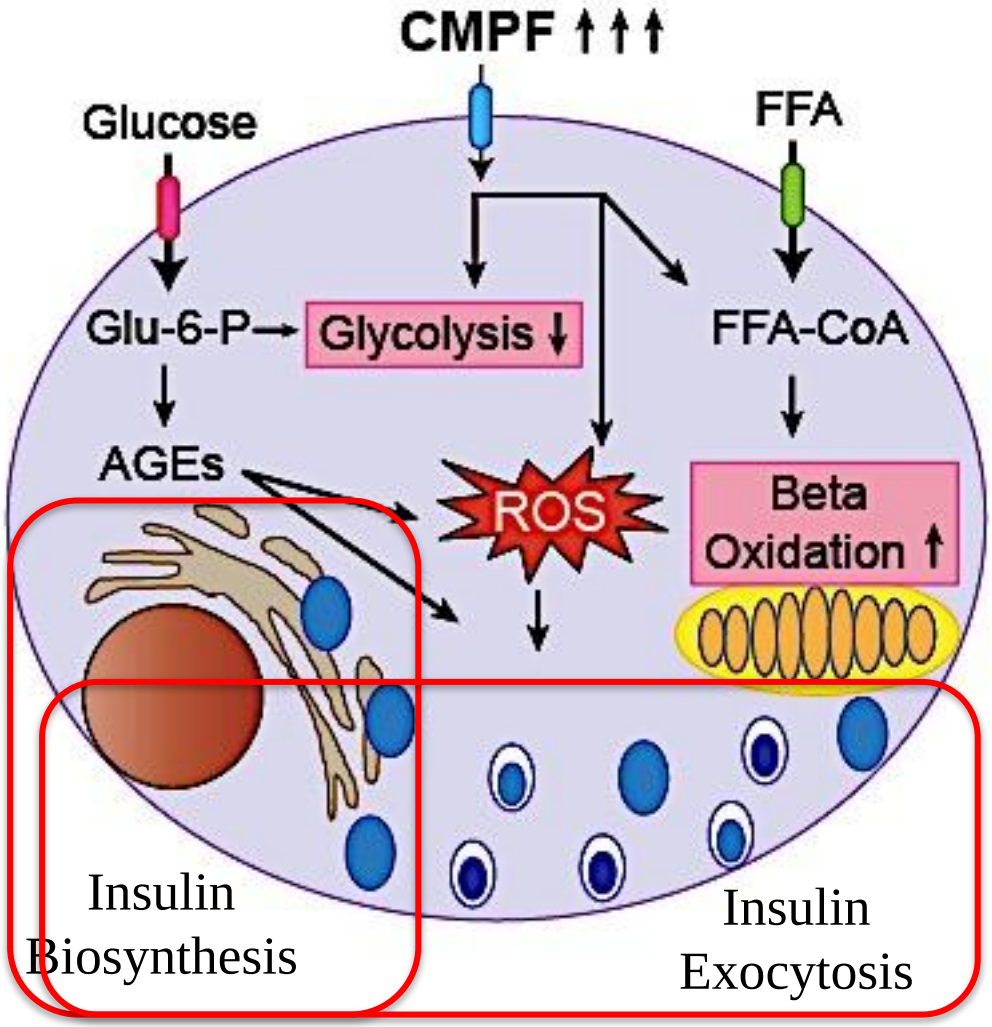


CMPF Increases Advanced Glycation End-Products and Oxidative Stress

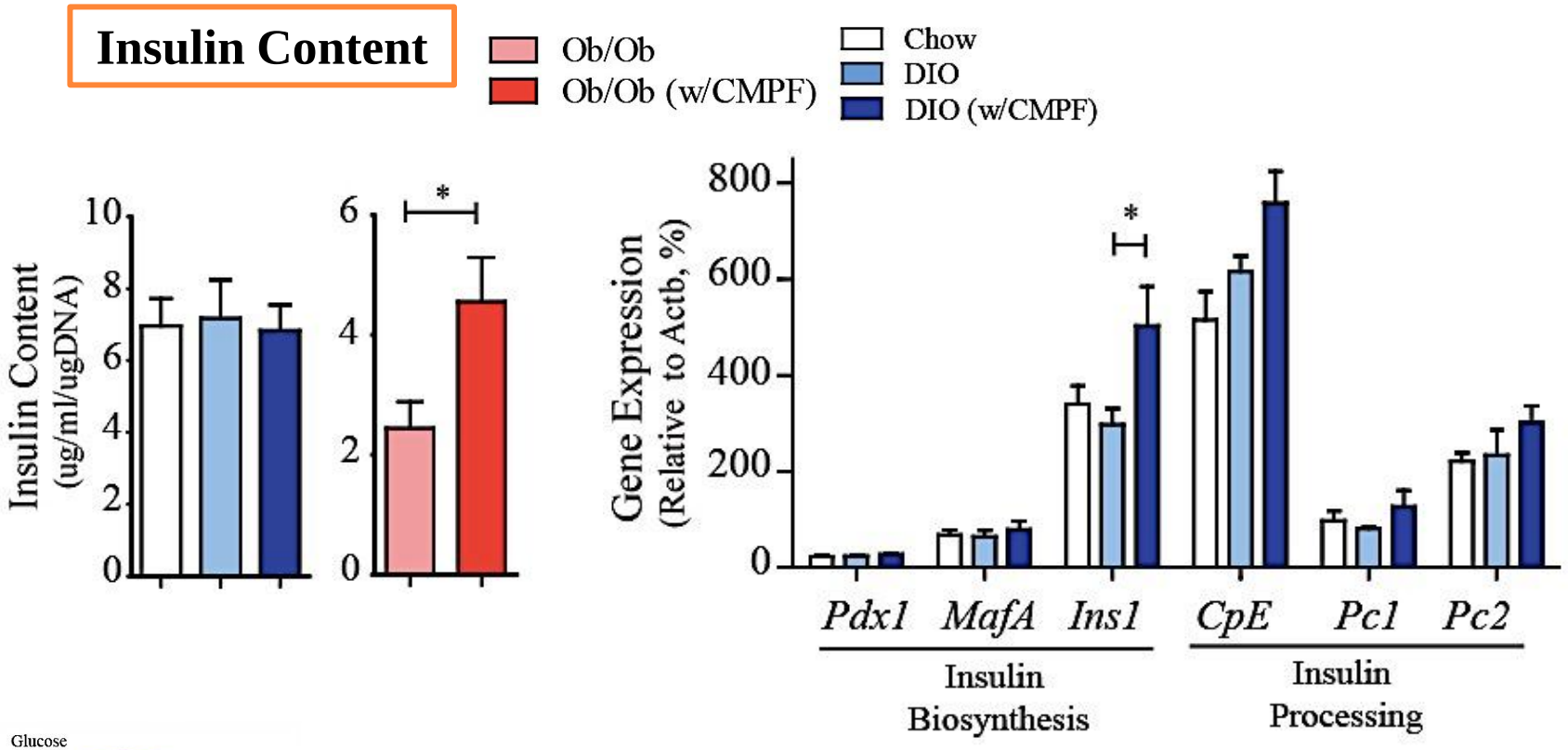


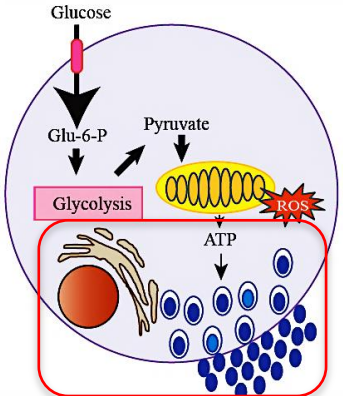
Liu et. al., Cell Reports, 2016

CMPF: Insulin secretion in beta cell



CMPF: INSULIN BIOSYNTHESIS & EXOCYTOSIS

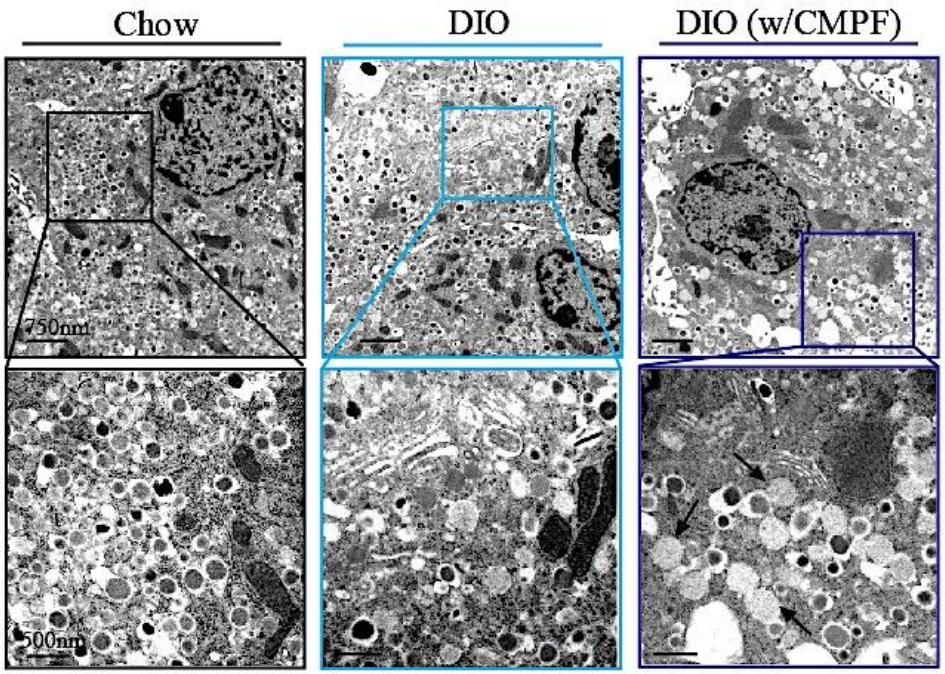


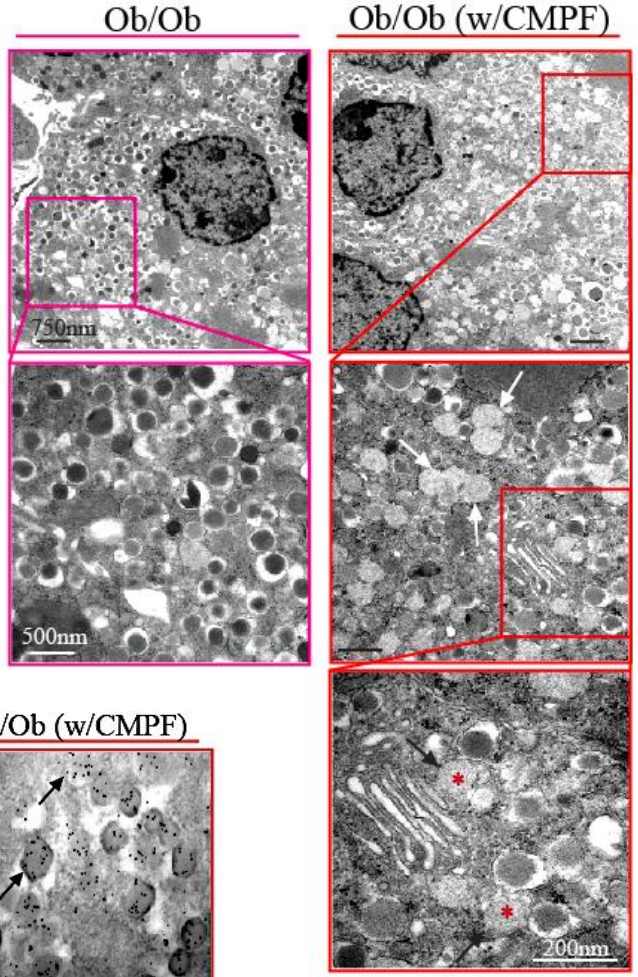


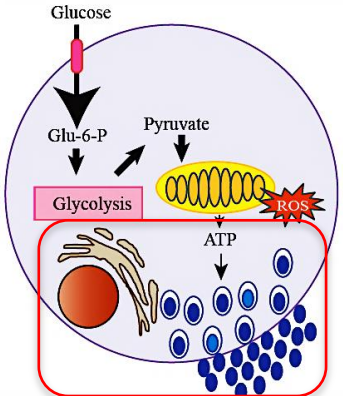
Liu et. al., Cell Reports, 2016

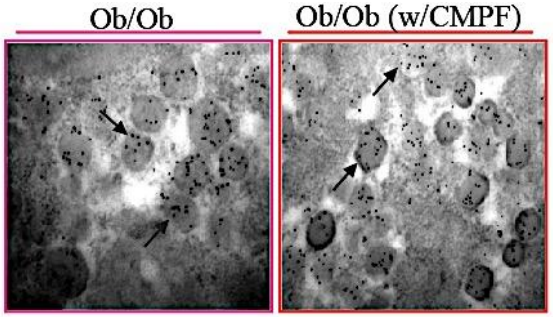
CMPF: Insulin biosynthesis & exocytosis

Transmitted Electron Microscopy (TEM)







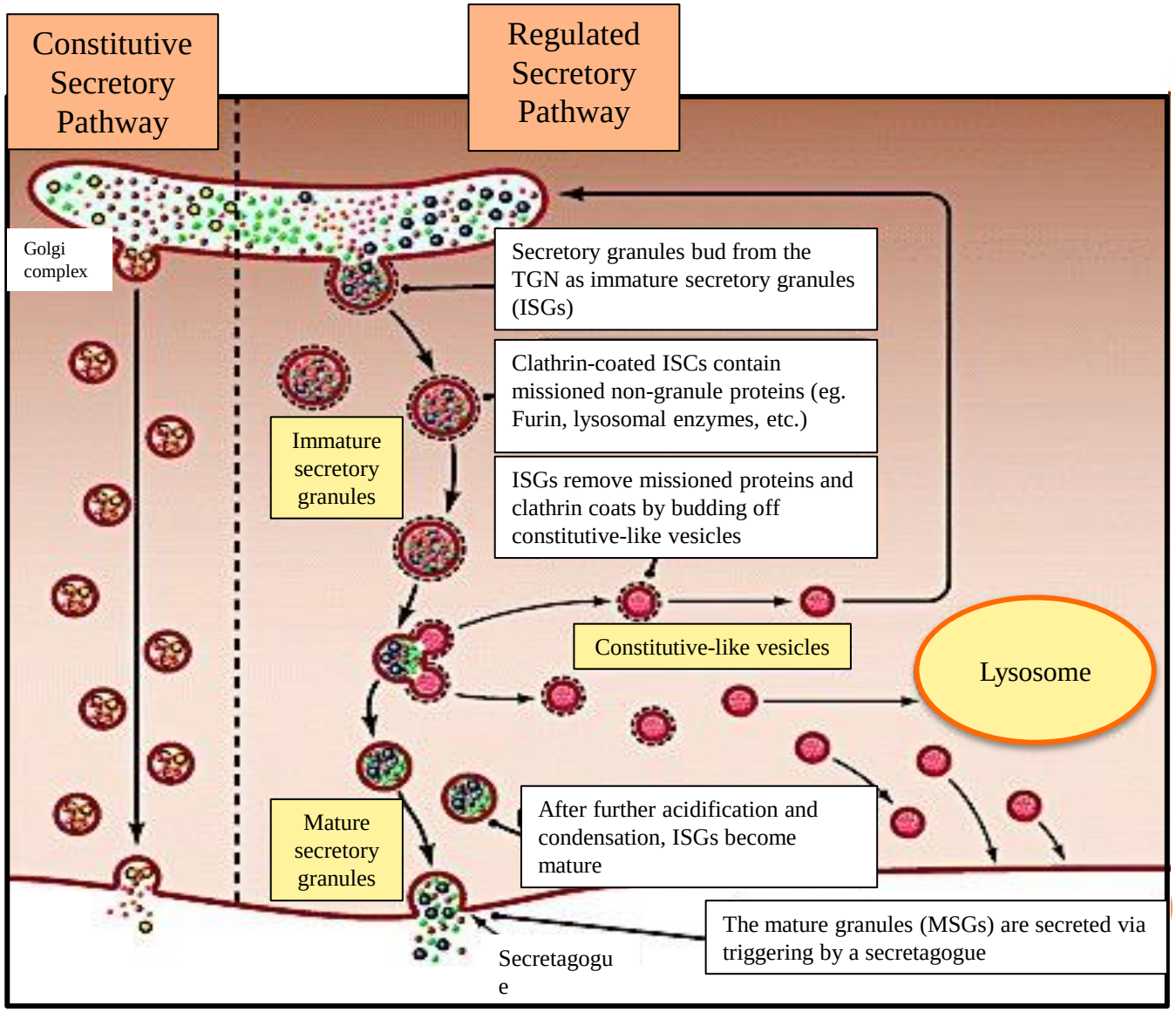


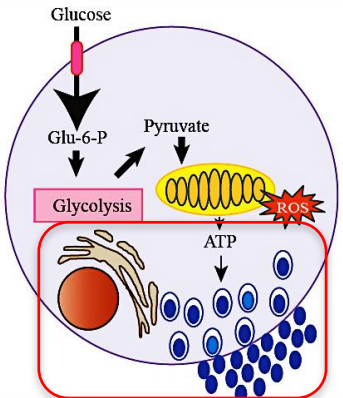
Immuno-Gold Insulin Staining

Liu et. al., Cell Reports, 2016

CMPF: INSULIN BIOSYNTHESIS & EXOCYTOSIS

The constitutive secretory pathway present in all cell types does not require stimulation for exocytosis

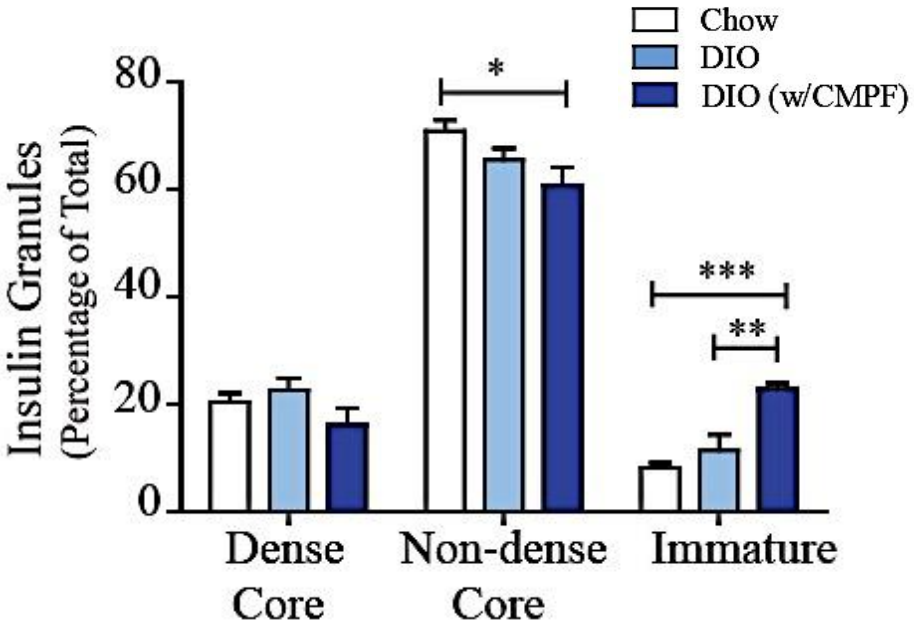


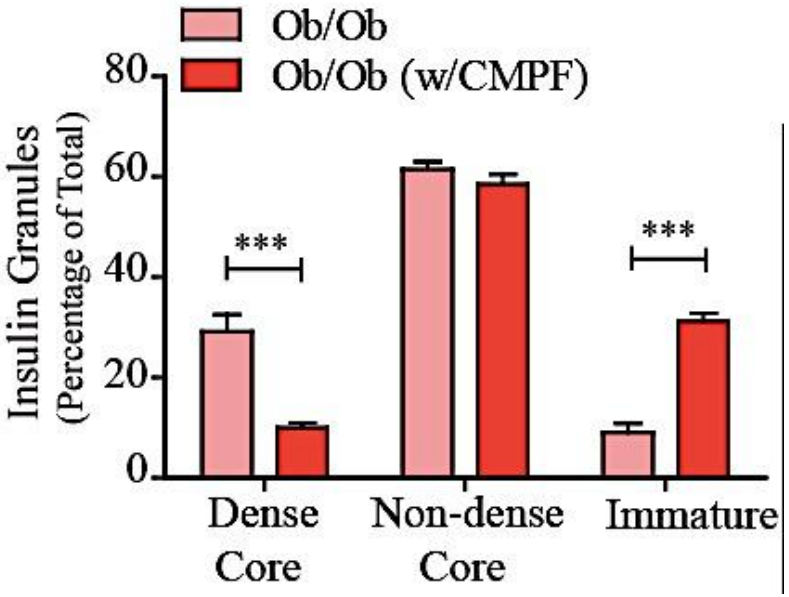


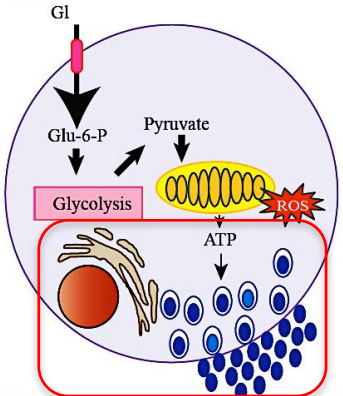
Liu et. al., Cell Reports, 2016

CMPF: Insulin biosynthesis & exocytosis

Transmitted Electronic Microscopy (TEM)





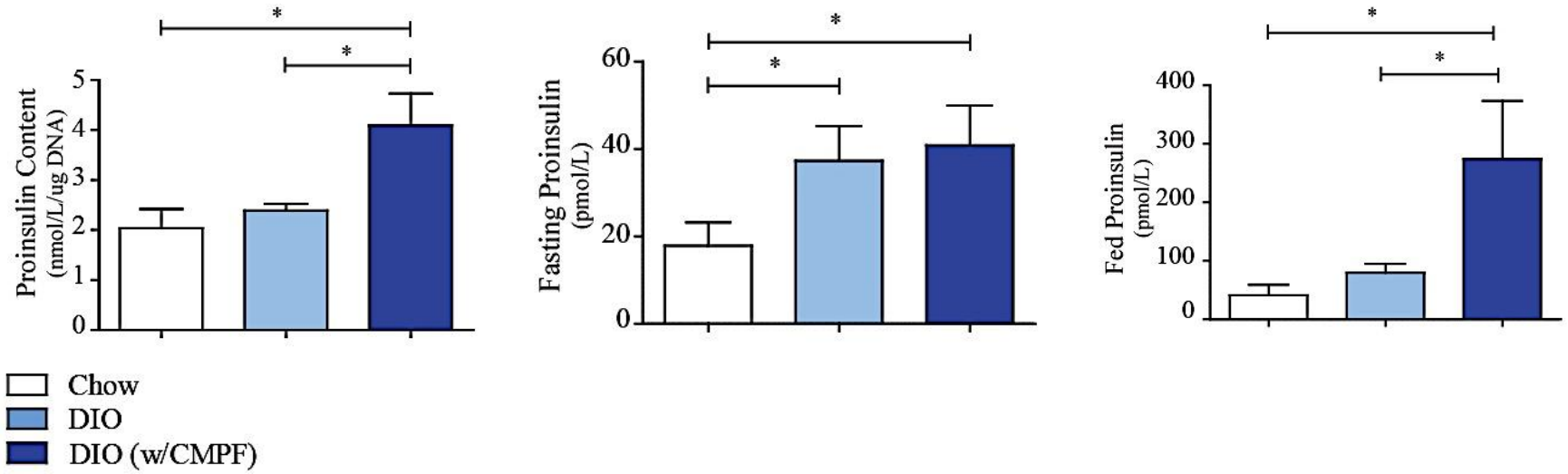


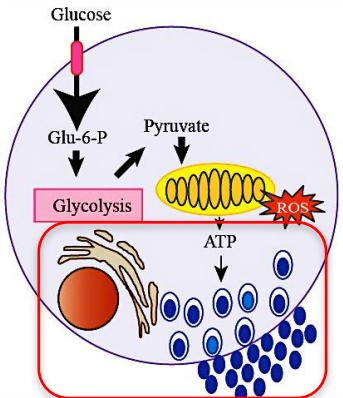
Liu et. al., Cell Reports, 2016

CMPF: Insulin biosynthesis & exocytosis

Cellular

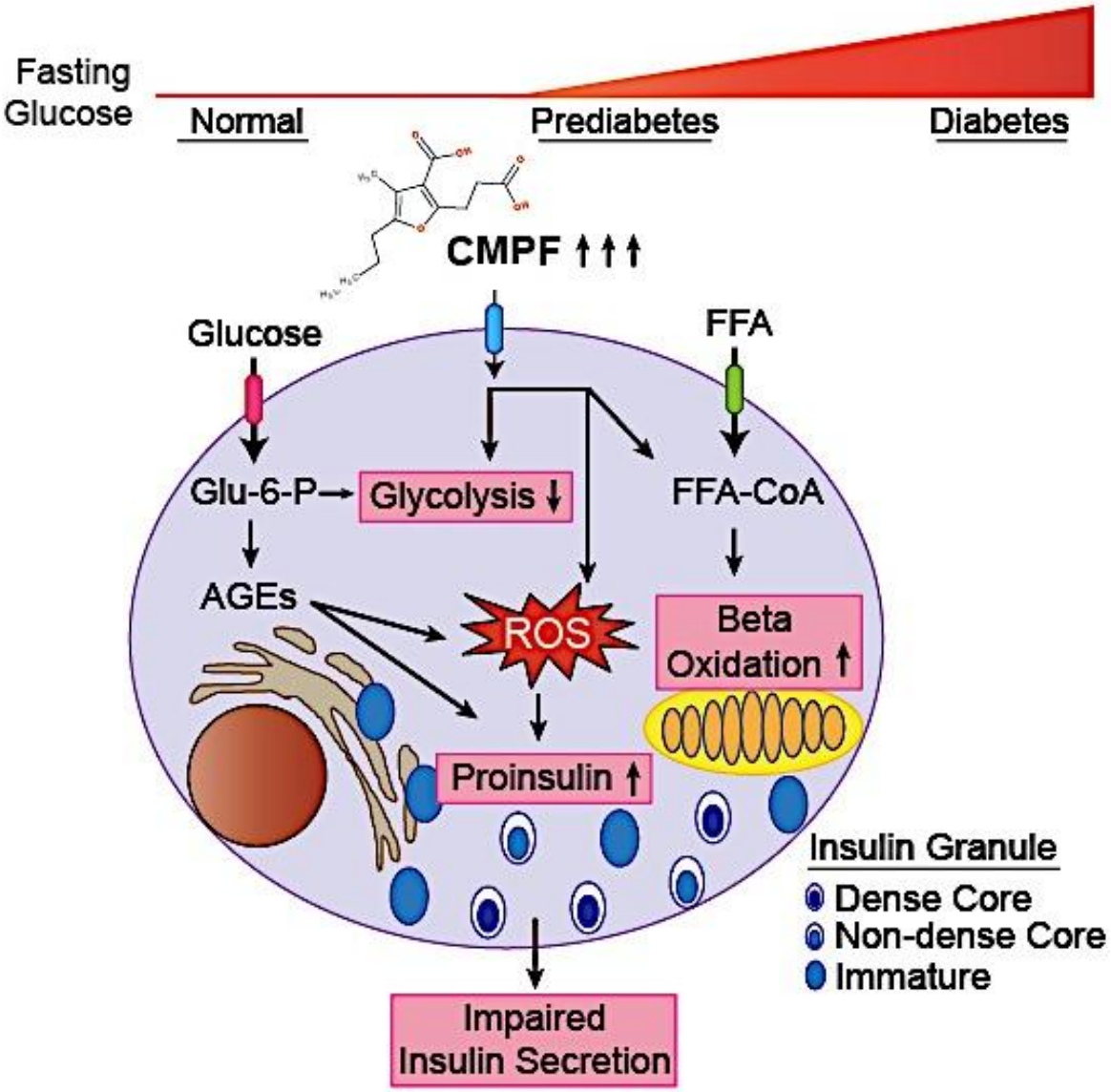
Exocytosis: Secretion





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

Liu et. al., Cell Reports, 2016

ACKNOWLODGEMENTS

University of Toronto
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Dr. Kacey Prentice
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Andrea Eversley
Dr. Feihan Dai

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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???

Early Detection

Therapeutic Targets

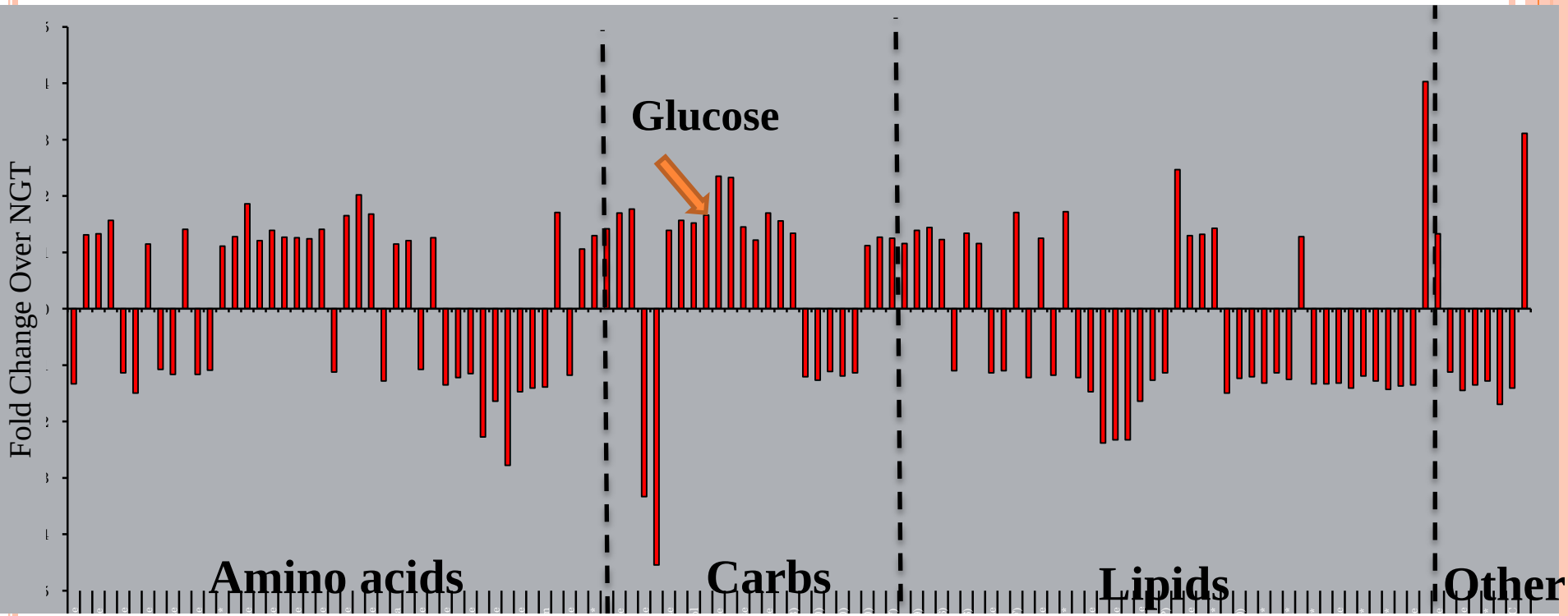
1. DISCOVER ✔ CMPF

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



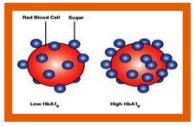
Metabolomic Signature: it is more than just hyperglycemia

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

Establish Signature Panels to Predict and Detect Diabetes

Identify metabolites associated with GDM/T2D

↓

Develop an SRM method for quantification of each metabolite

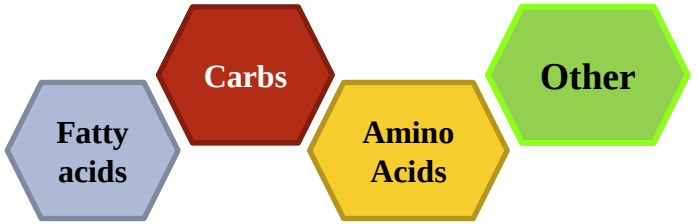
↓

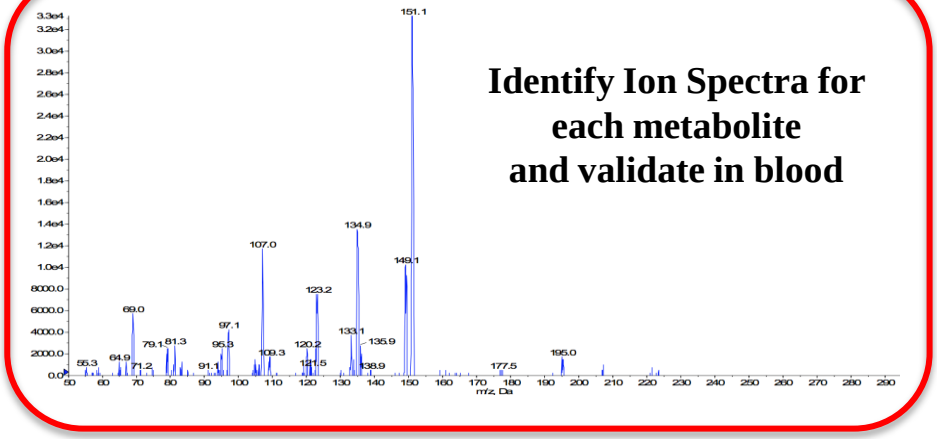
Validate/quantify each metabolite in test plasma

↓

Assay Prospective Cohort

181 metabolites chosen for testing

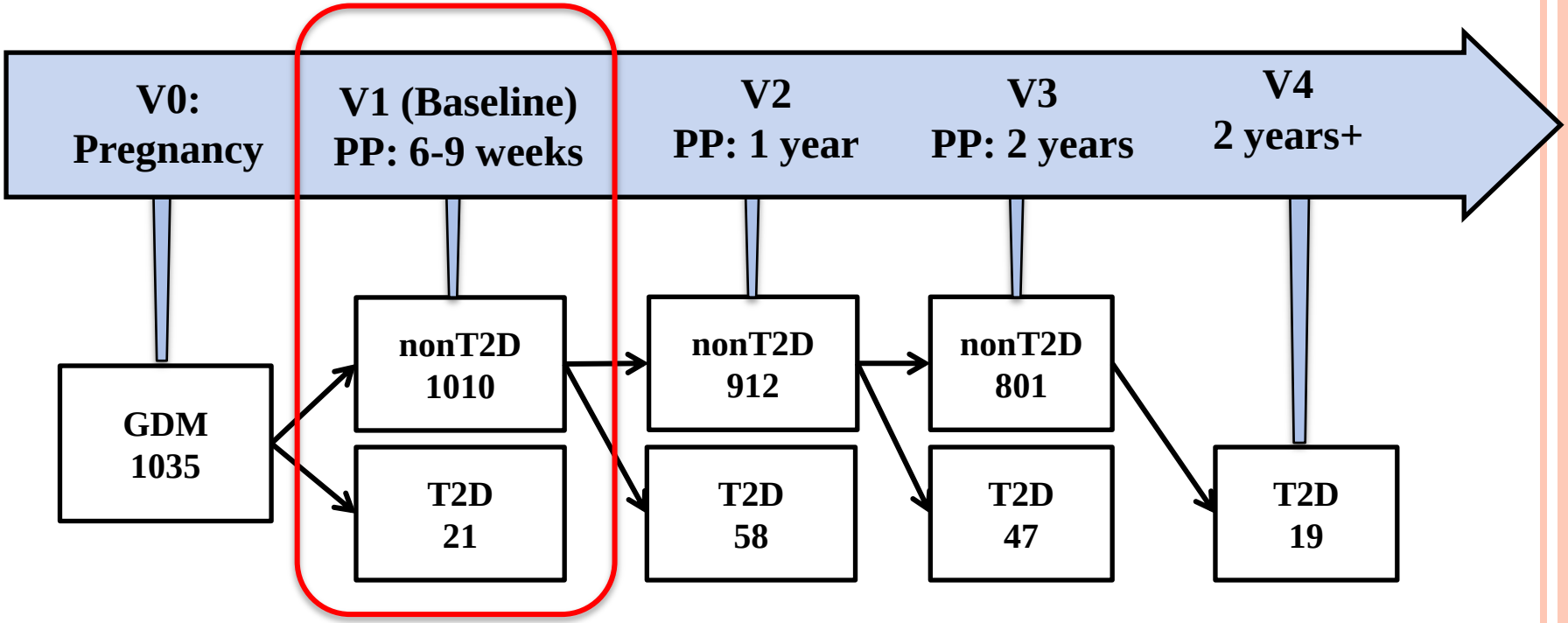




Allalou et. al., Diabetes, 2016

Establish Signature Panels to Predict and Detect Diabetes

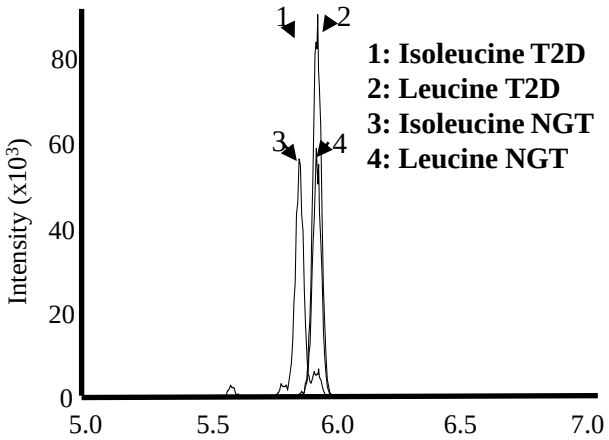
SWIFT Prospective GDM Cohort



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



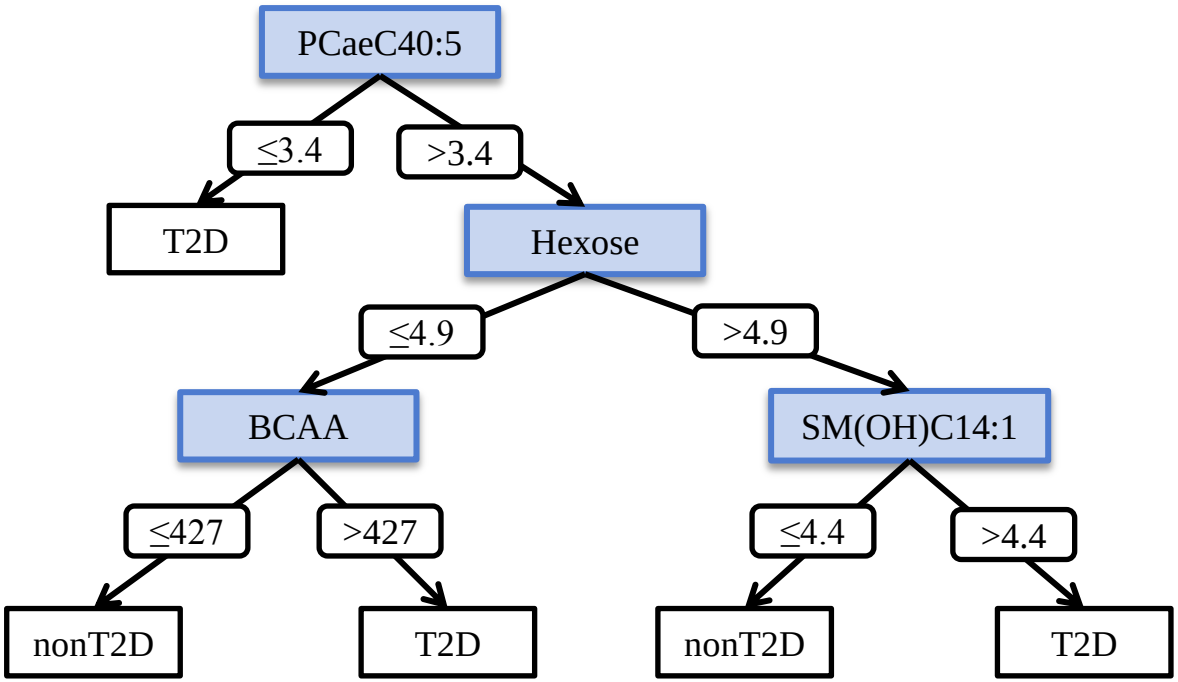
	Metabolite	Class	Fold Change	P value
	Metabolites			
1	Hexose	Carbs	1.097	<0.000001
2	SMC20:2	SMs	0.819	<0.001
3	Tyr	AAs	1.121	<0.001
4	Val	AAs	1.094	<0.01
5	SMC18:1	SMs	0.891	<0.01
6	Leu	AAs	1.098	<0.01
7	2-AAA	AAs	1.20	<0.01
8	Ile	AAs	1.095	<0.01
9	SMC24:1	SMs	0.913	<0.05
10	Trp	AAs	1.057	<0.05
11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

Over 20 metabolites associated with future T2D status

Allalou et. al., Diabetes, 2016

Establish Signature Panels to Predict and Detect Diabetes

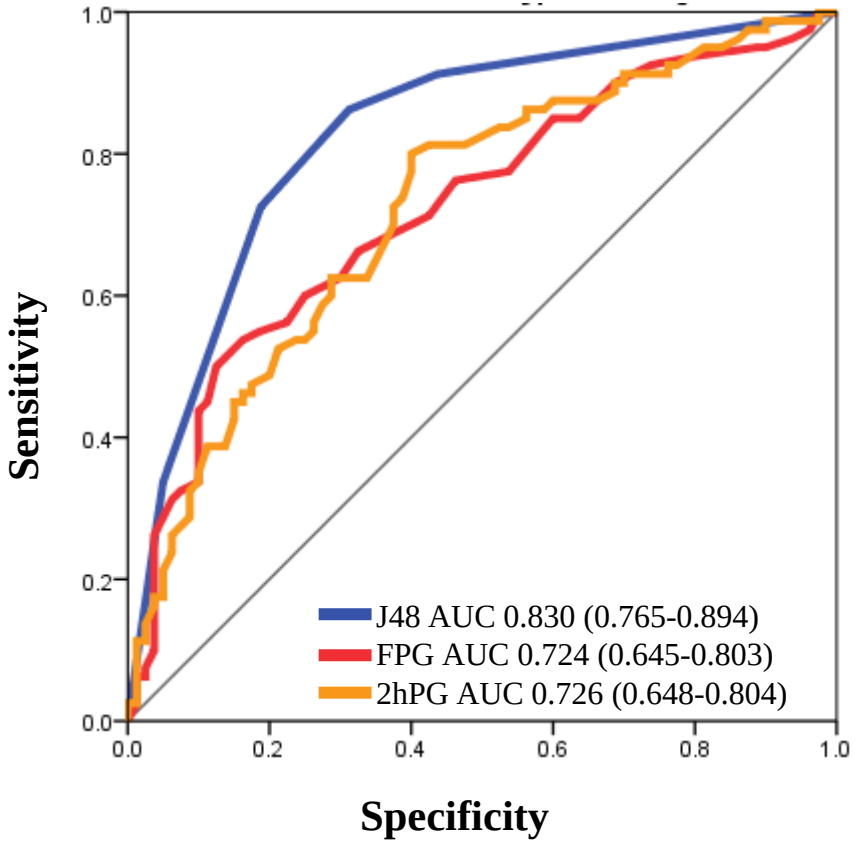
Machine Learning: Decision Tree Algorithm



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???

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2. PREDICT & DETECT

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✔ Predictive Signature Panel

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We need more prospective cohort to test our signature metabolite panel for future diabetes prediction!!!

INTERESTED???