The Impact of Glucotoxicity on Hepatic Transcriptomic, Metabolomics and Paternal Intergenerational Transmission of Diabetes

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My current projects in Patti Laboratory

Project 1 : Dissecting the impact of glucotoxicity resolution on hepatic metabolism, transcriptome and epigenome

Project 2: Paternal intergenerational transmission of metabolic risk through non-genetic mechanisms

Project 3: The role of vagal subneuronal populations in the control of metabolism

Other Role at Joslin :
Fellow Council Social Chair

Joslin Fellows Council

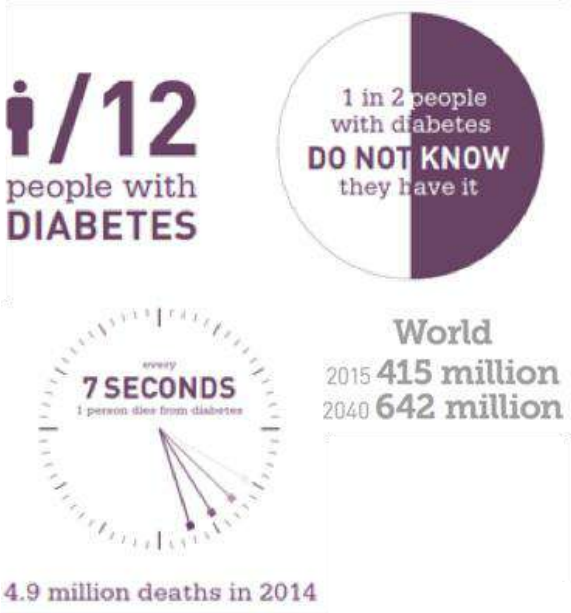
Halloween Party

New Year Party

Post Doc Appreciation Week

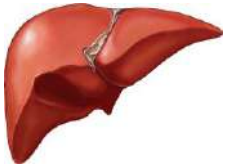
Type 2 Diabetes - Epidemiology

- 415 million people with type 2 diabetes worldwide
- 5 million Thais have type 2 diabetes (~8.9% prevalence)
- Diabetes costs 4 billion baht annually to the Thai national health care system



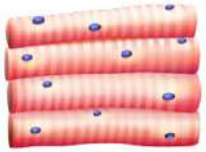
International Diabetes Federation. IDF Diabetes Atlas, 7th edn. Brussels, Belgium: International Diabetes Federation, 2015.

Type 2 Diabetes



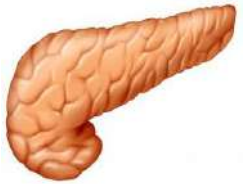
Genetic predisposition

↑ Insulin resistance



↑↓ Liver glucose production

↓ Insulin secretion



Hyperglycaemia

Glucotoxicity

Environment:
Nutrient overload
Physical inactivity

My interest in hyperglycemia

Kanchana Ngaosuwan MD
My first mentor who sparked my interest in hyperglycemia as a medical student

Clinical inertia

- **Clinical inertia:** failure to intensify treatment in timely manner.
- Patients with HbA_{1C}>9% for more than 3 months without treatment intensification
- **Initial Aim**
  - Identify modifiable risk factors associated with failure in treatment intensification

Osataphan S et al J of Diabetes 2017

Serendipity Finding

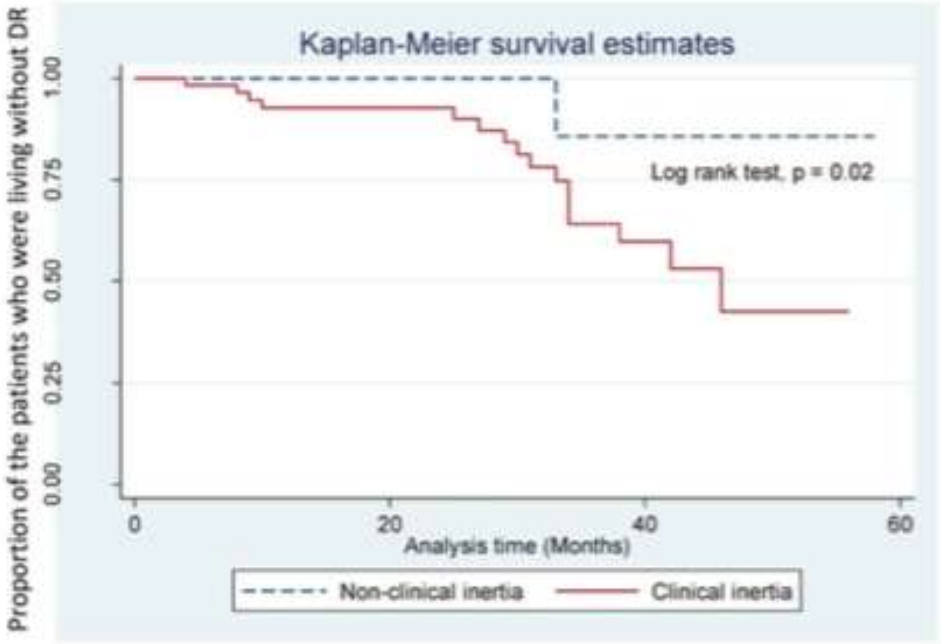


Figure 2 Kaplan–Meier curves for the occurrence of new or progressive diabetic retinopathy between the clinical inertia and non-inertia groups.

1. Incidence rate ratio to new or progression of DR was higher in the clinical inertia group odds ratio (OR) of 4.78 over median follow up time of 2.5 years

2. Prolonged hyperglycemia during clinical inertia causes new or rapid progression of diabetes retinopathy and diabetic complication

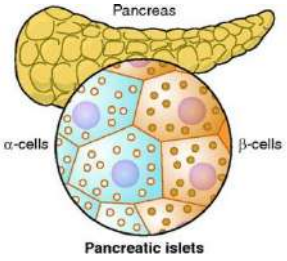
Osataphan S et al J of Diabetes 2017

Hyperglycemia and glucotoxicity

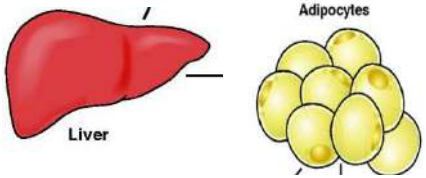
-> Characteristic of diabetes mellitus

Progression and micro and macro vascular complication

Beta cell dysfunction and apoptosis

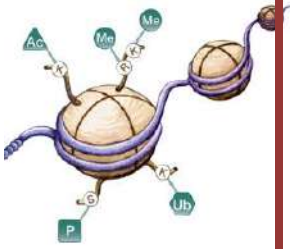


Further propagate insulin resistance in metabolically active tissue

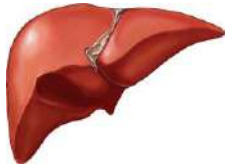


Hyper insulinemia

Epigenetic alteration -> metabolic memory (legacy effect)



Type 2 diabetes

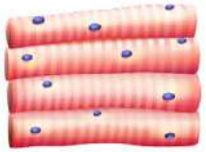


SGLT2 inhibitors

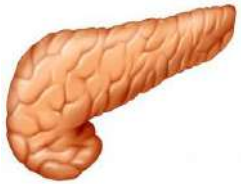
Genetic predisposition

↑ Liver glucose production

↑ Insulin resistance



↓ Insulin secretion



Hyperglycaemia

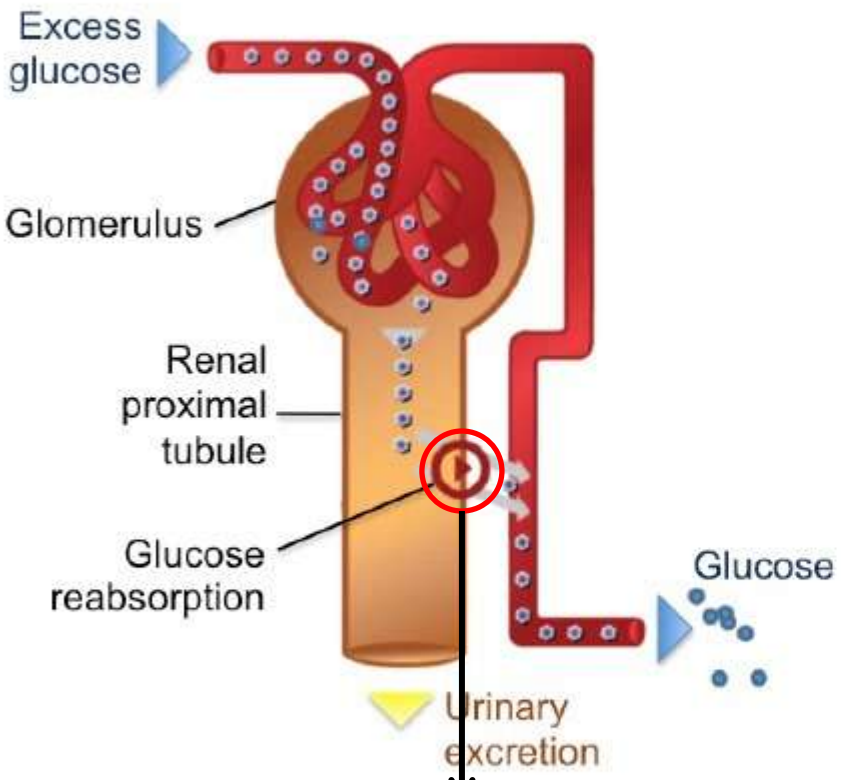
Glucotoxicity

Environment:
Nutrient overload
Physical inactivity

Further insulin resistance
Beta cell toxicity
Vascular complications
Epigenetic reprogramming

Renal SGLT2 Inhibition

Patients with type 2 diabetes

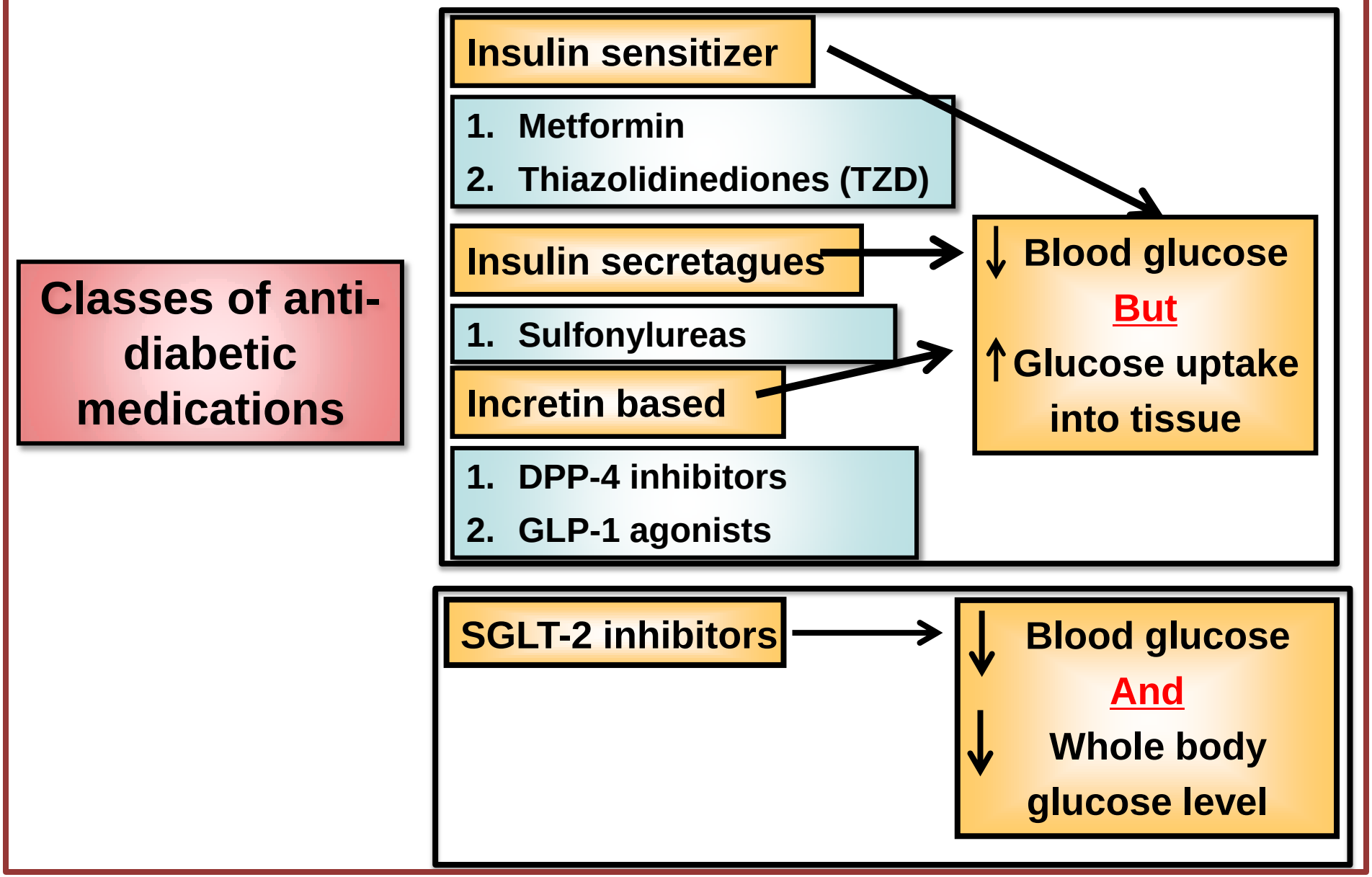


Sodium glucose cotransporter2 (SGLT2)

Adapted from:

1. Chao EC & Henry RR. Nature Reviews Drug Discovery 2010;9:551-559.
2. DeFronzo RA, et al. Diab Obes Metab 2012;14:5-14.
3. Washburn WN. J Med Chem 2009;52:1785-1794.

SGLT-2 inhibitors as a tool to study glucotoxicity



SGLT-2 inhibitors

- **Reduction in whole body glucose**
- **Resolution of glucotoxicity**

Aims

1. To dissect the molecular mechanism mediating beneficial effect of glucotoxicity resolution at tissue and metabolic level
2. To determine the impact of sustained increase in glucose and its reversal on epigenetic regulation

EXPERIMENTAL DESIGN

4 weeks

C57BL/6
6 wk-old

0

High fat diet (HFD)
60% kcal fat

High Fat Diet (60%)

4 or 8 weeks

HFD + Canagliflozin

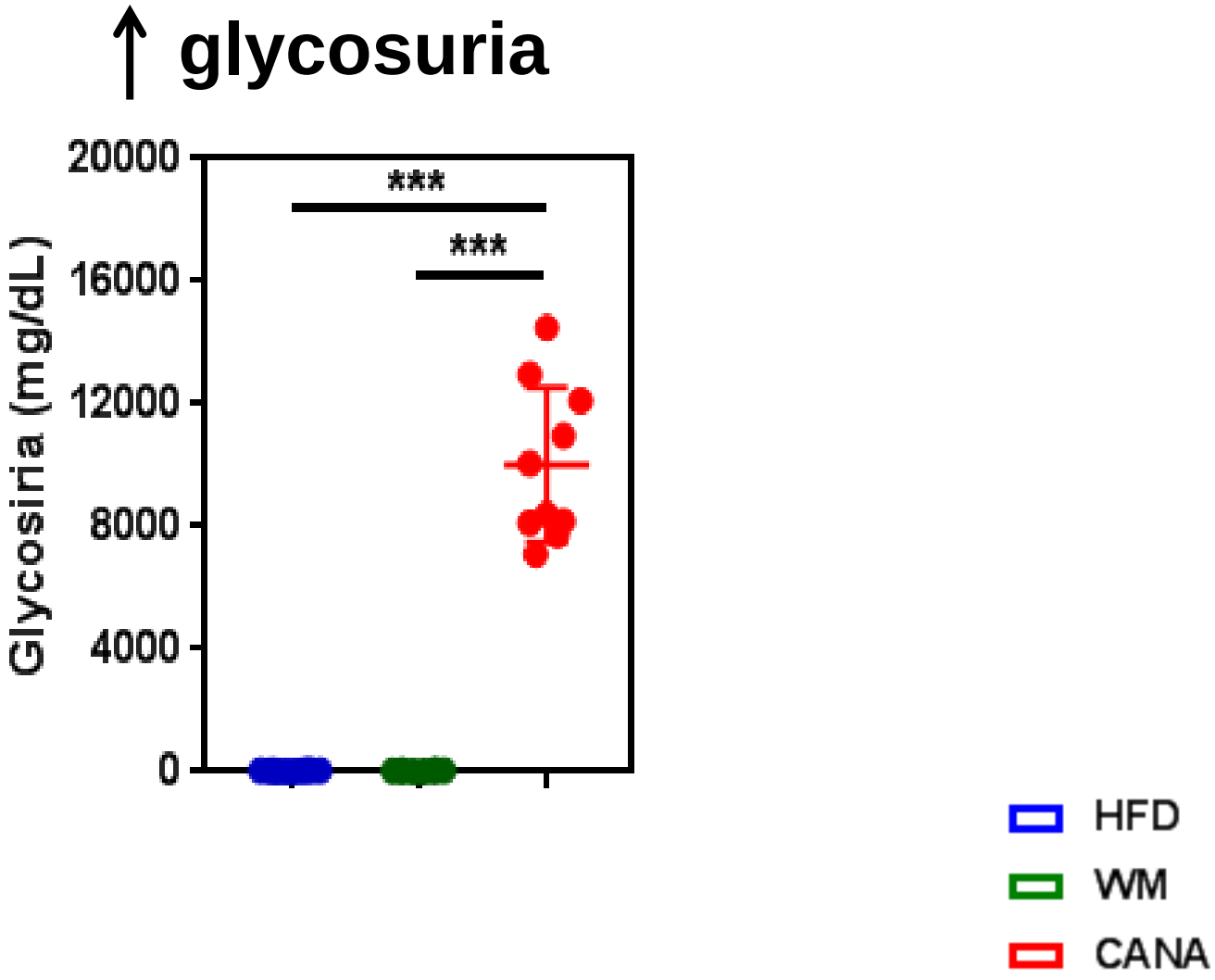
HFD weight-matched to HFD + CANA (15-30% caloric restriction)

Systemic, tissue metabolism assessment

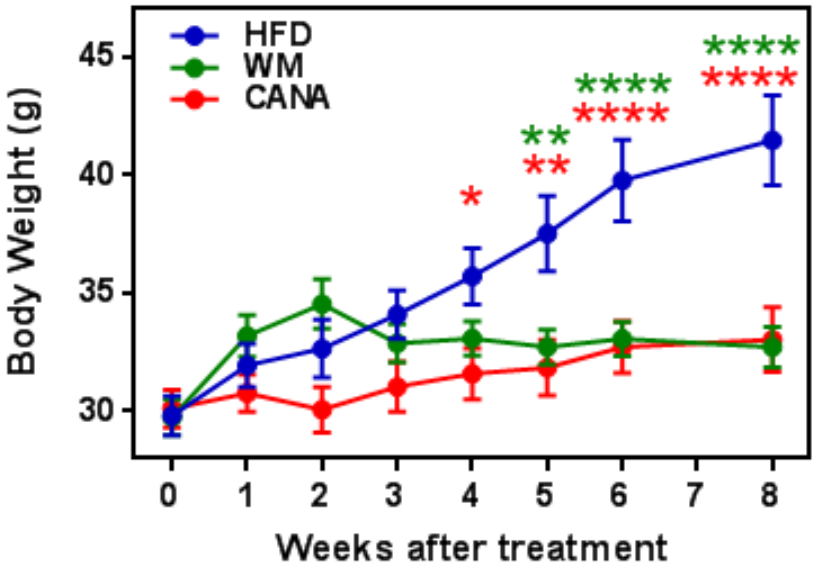
canagliflozin: 30 mg/kg/d, admixed in diet

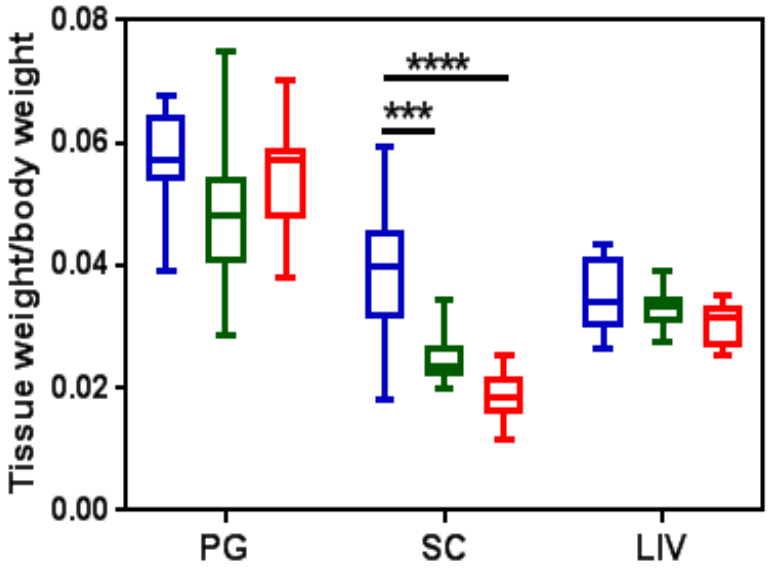
Courtesy to Chiara Macchi

SGLT2i induces glycosuria



Body weight and fat mass after SGLT2i treatment





1. CANA treated mouse do not gain as much weight as control HFD

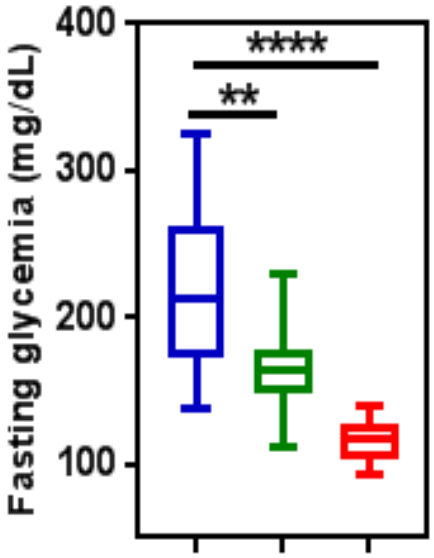
2. CANA causes reduction in subcutaneous adipose tissue

HFD
WM
CANA

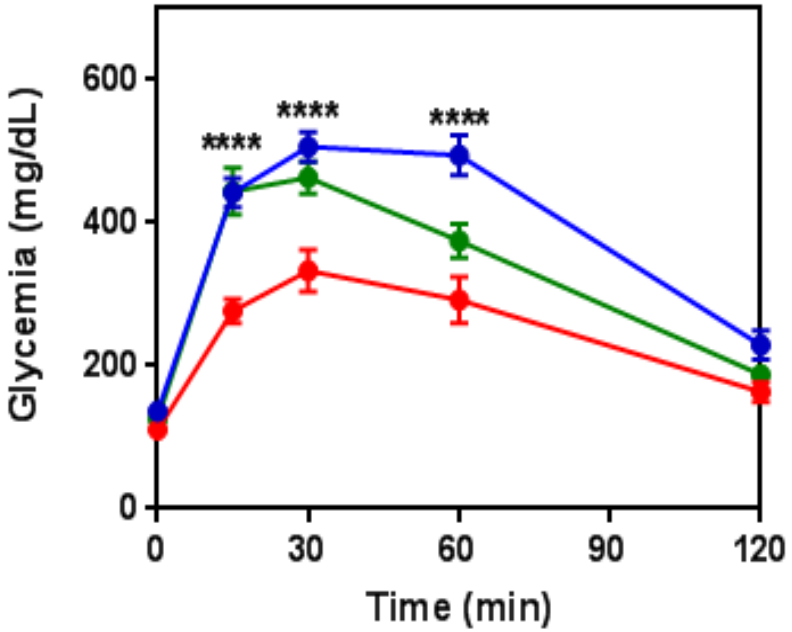
PG: Perigonadal fat, SC: subcutaneous fat, BAT: brown adipose tissue
*p=<0.05, ** p<0.01; ***p<0.001; *1 way-ANOVA,n=8-11*

SGLT2i improves glucose tolerance beyond weight loss

↓ **Fasting blood glucose**



Improve glucose tolerance



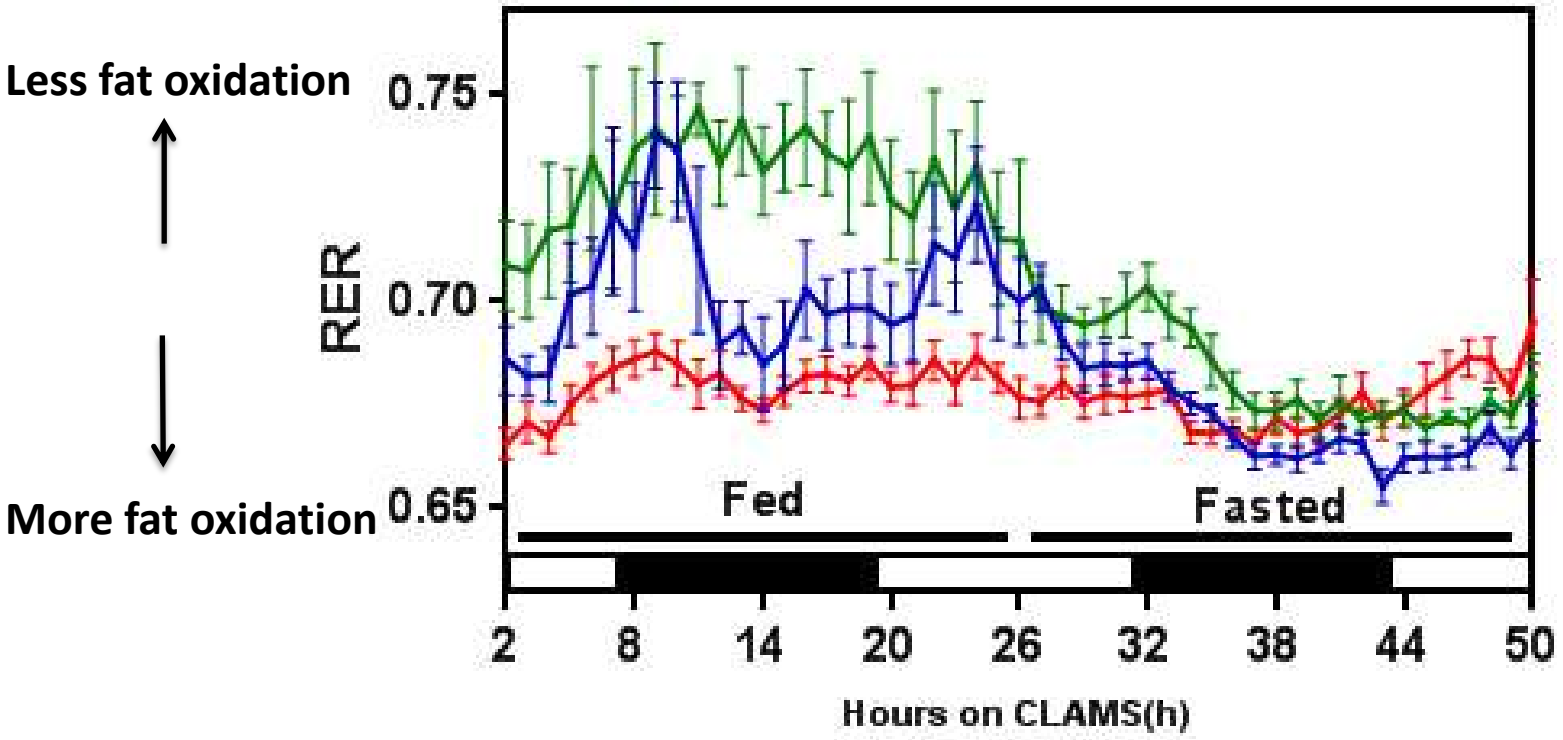
*p=<0.05, ** p<0.01; ***p<0.001; n=8-11/group *1 way-ANOVA*

Comprehensive Laboratory Animal Monitoring System

VO2 : Oxygen consumption
VCO2 : Carbon dioxide production
RER : Respiratory exchange ratio (Fuel utilization)

Heat production
Activity
Feeding
Drinking

SGLT2i alters fuel utilization with preference for fatty acid oxidation



RER interpretation

1.0 : Glucose Oxidation

0.7 : Fat Oxidation

<0.7 : Ketone utilization

HFD

WM

CANA

Experimental Design

C57BL/6
6 wk-old

4 weeks

0

High fat diet (HFD)
60% kcal fat

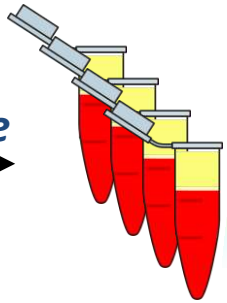
8 weeks

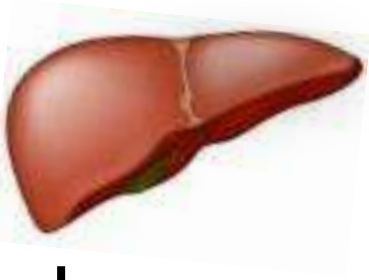
HFD

HFD + Canagliflozin

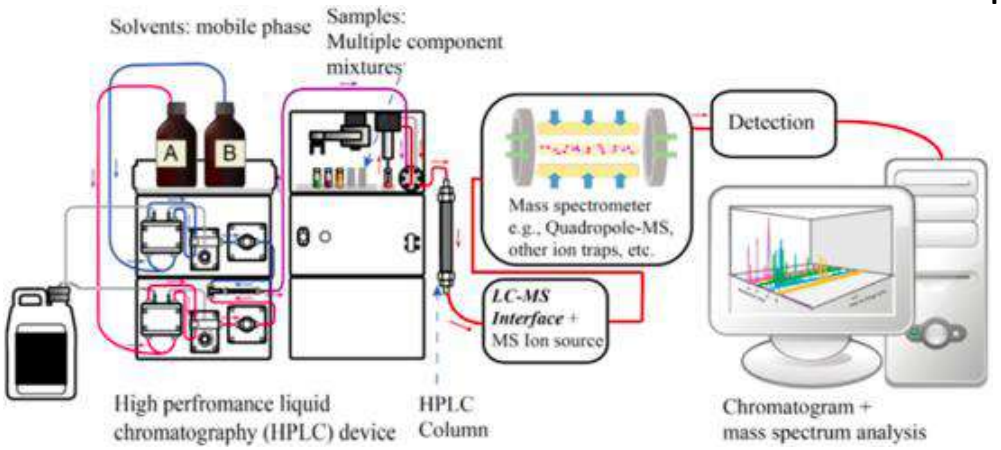
HFD weight-matched to HFD + CANA

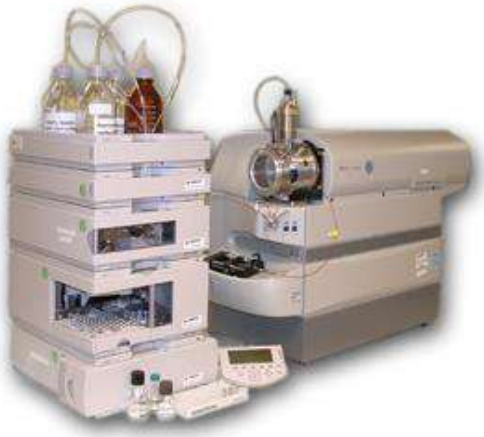
Sacrifice





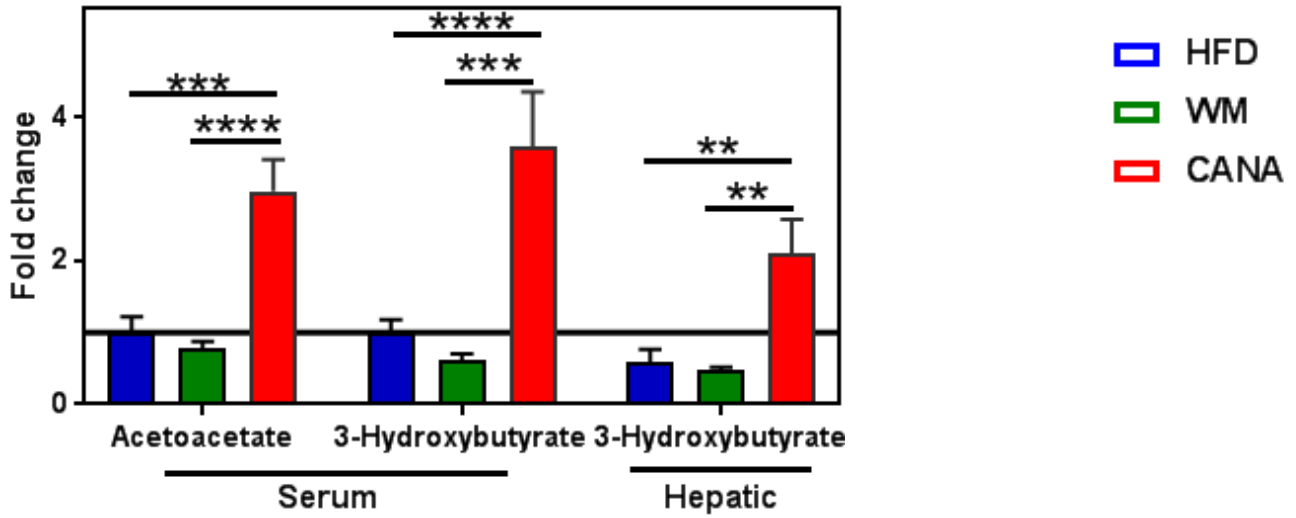
Metabolomics/lipidomics using
Liquid Chromatography/Mass Spectometry



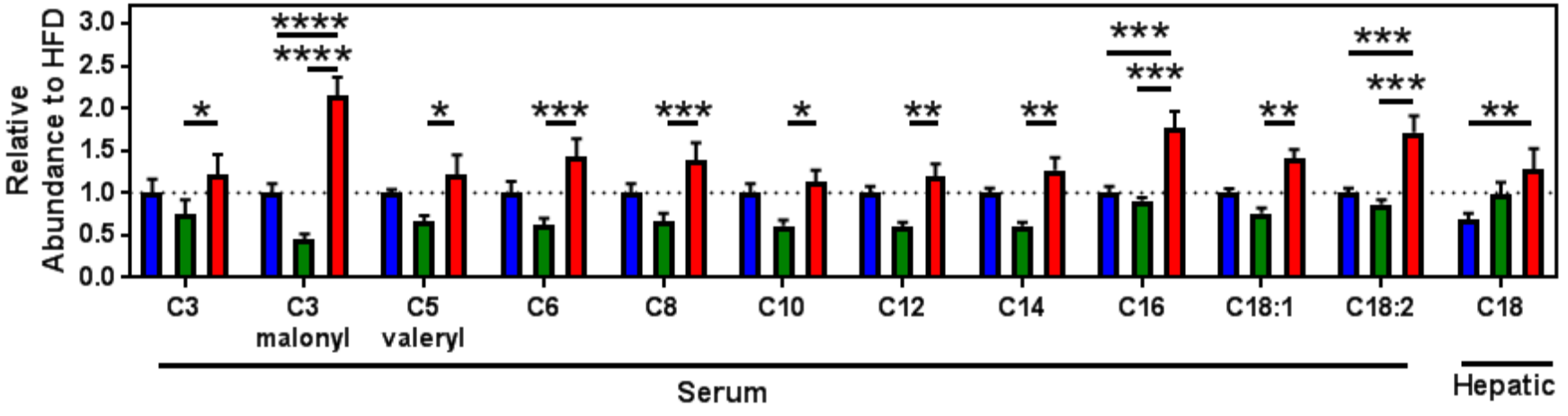


In collaboration with Gerszten Lab, BIDMC

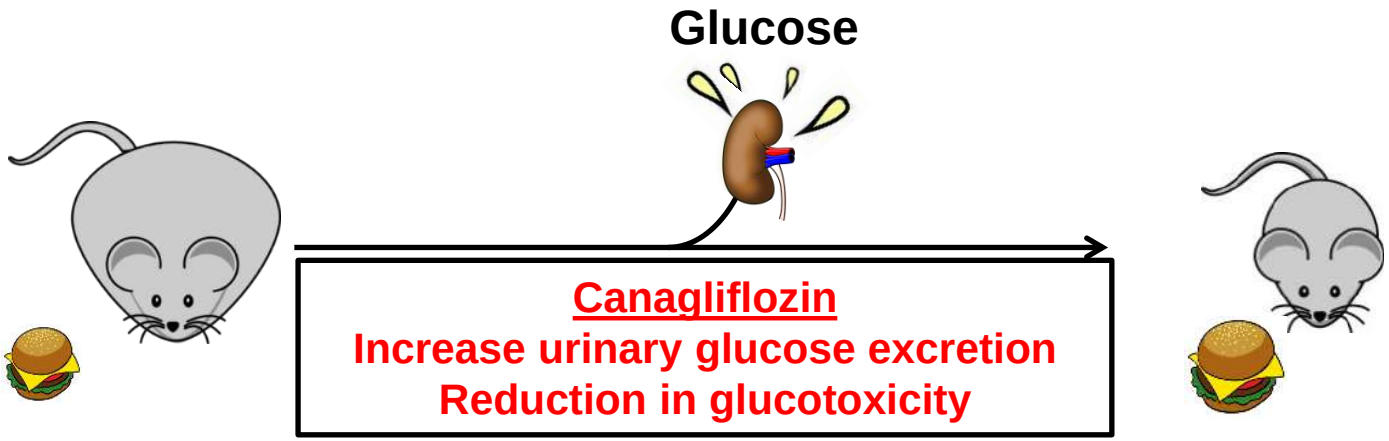
Metabolomics revealed increased in by-products of fatty acid oxidation and ketogenesis



Acylcarnitine Species

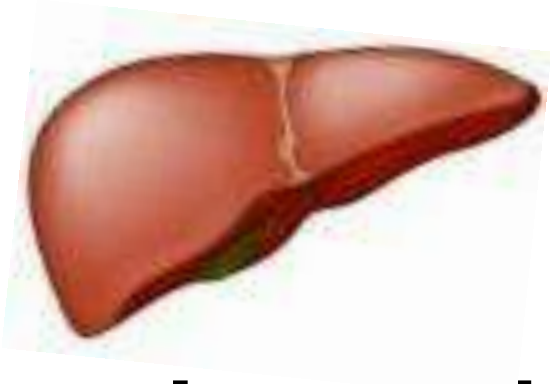


Summary 1 : Whole body metabolism

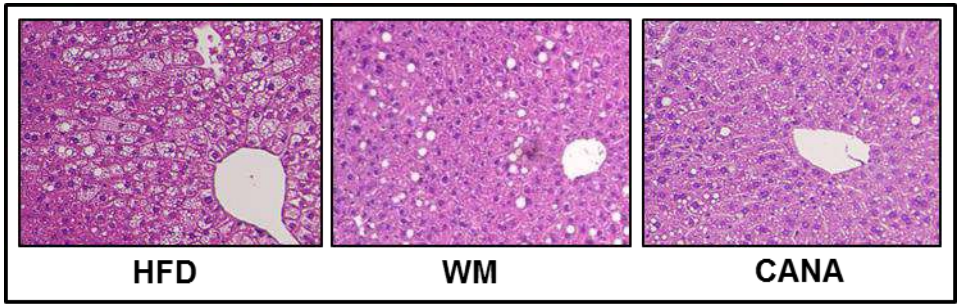


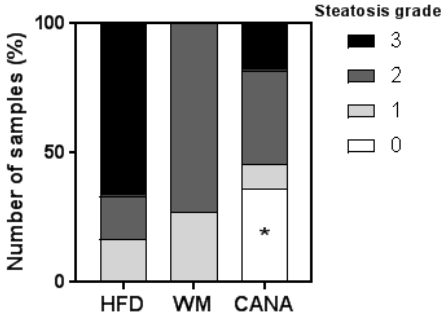
Additional improvement in metabolism beyond weight loss (comparison with weight-matched control)

- Improved glucose tolerance
- Shift energy preference to fatty acid oxidation
- ↑ Ketone bodies



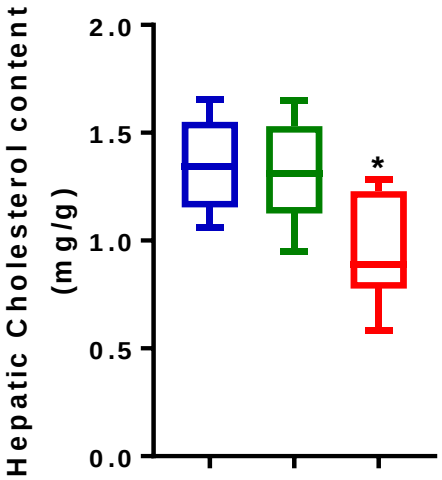
Question 2 : What is the effect of SGLT-2 inhibitors effect on liver metabolism and gene transcription


HFD
WM
CANA

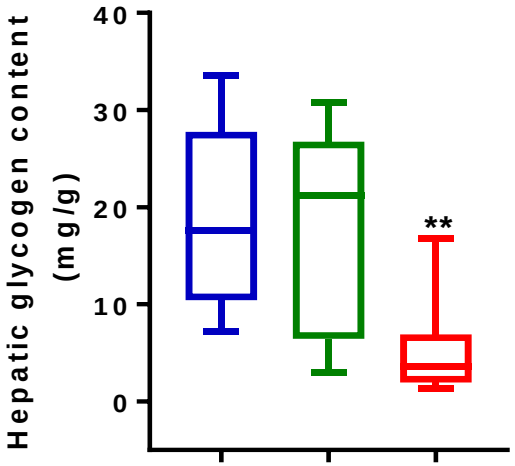

Steatosis grade
3
2
1
0
Number of samples (%)
100
50
0
HFD
WM
CANA
*

SGLT2i reduces anabolic storage in the liver (cholesterol and glycogen)

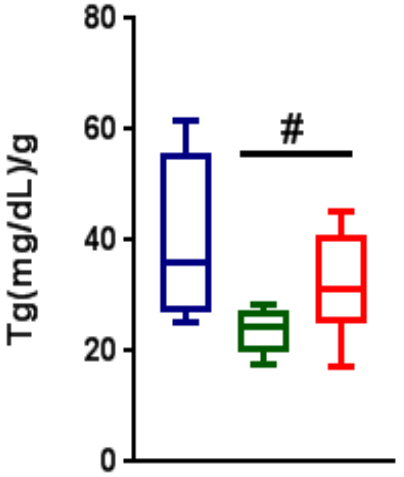
Liver Cholesterol Content



Liver Glycogen Content



Liver Triglyceride Content

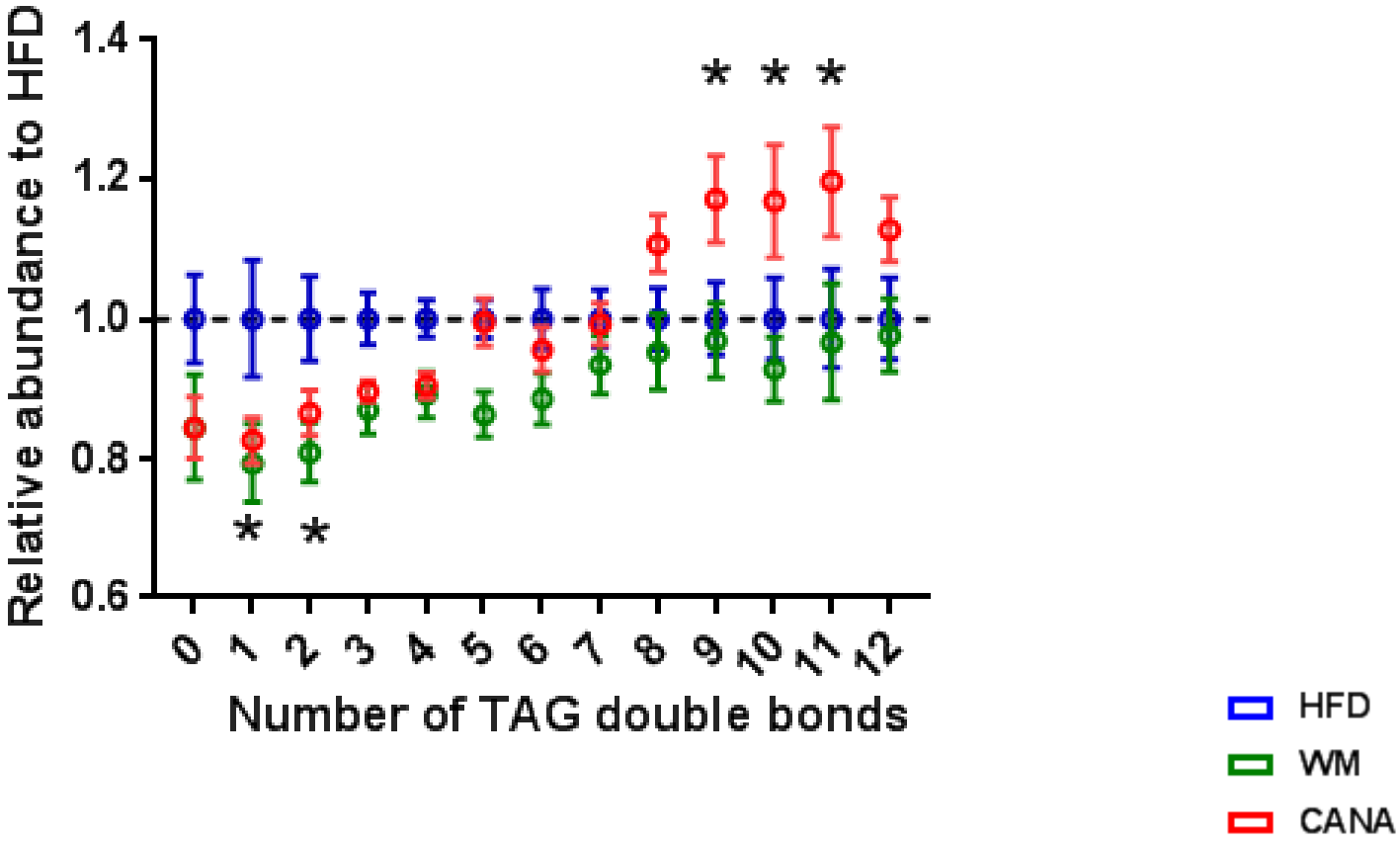


1. Significant ↓ hepatic cholesterol and glycogen
2. Surprisingly no change in total hepatic TG

HFD
WM
CANA

Animal sacrificed after O/N fast after 4 of CANA treatment
(n=8-12/group) *p=<0.05, ** p<0.01; ***p<0.001; *1 way-ANOVA*

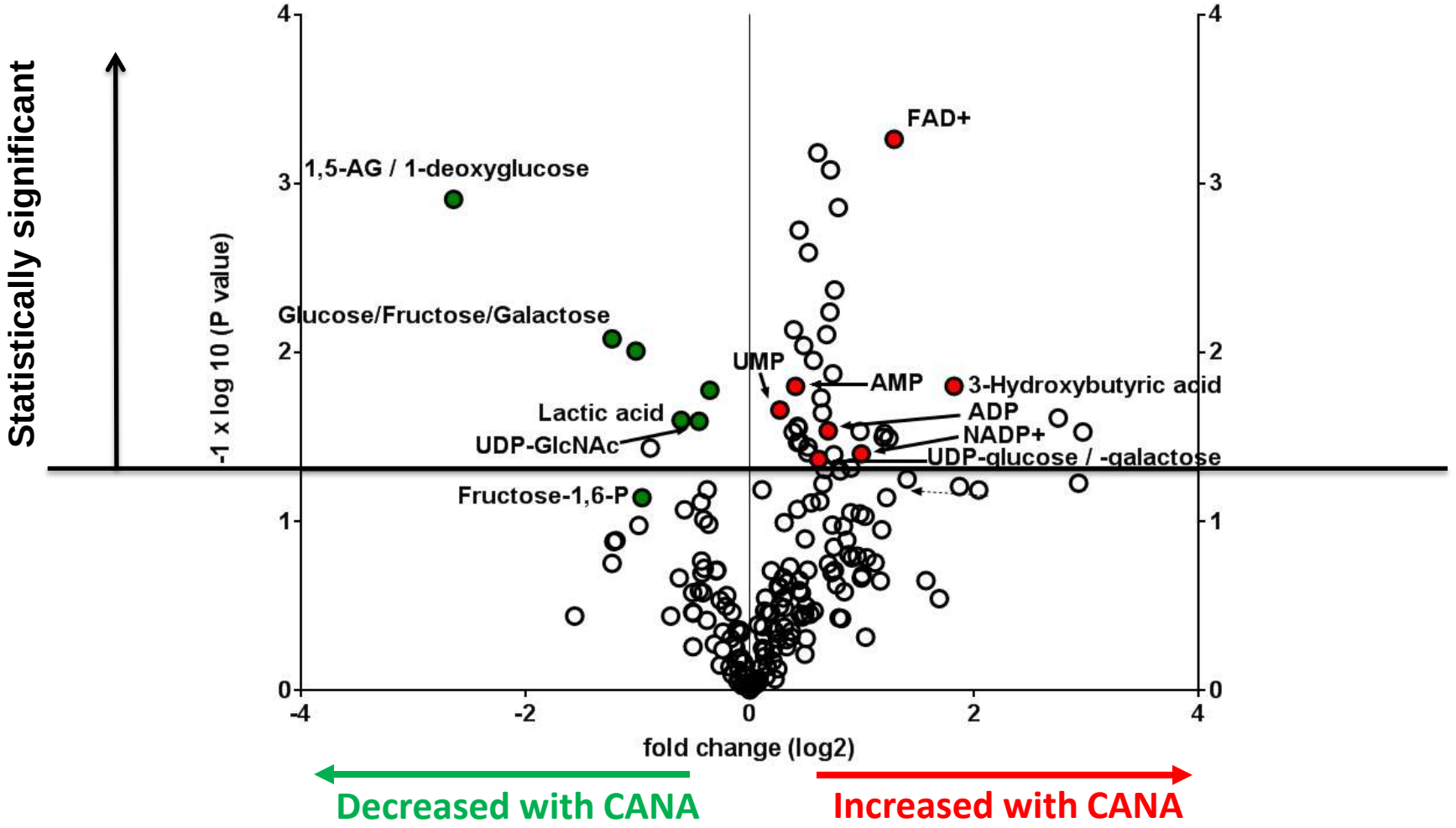
Lipidomics analysis revealed decrease in monounsaturated triglycerides and increase in polyunsaturated triglycerides



LC/MS platform- lipidomics – Gerszten Lab
Animal sacrificed after O/N fast after CANA treatment
(n=8group) *p=<0.05, ** p<0.01; ***p<0.001; *1 way-ANOVA*

Metabolomics analysis reveals ↑ in hepatic ketone bodies and intermediates reflecting catabolism

Volcano plot : metabolite comparison between CANA vs HFD



Animal sacrificed after O/N fast after 4 weeks of CANA treatment (n=8/group); LC/MS profiling of metabolites; collaboration with Gerzten Lab

EXPERIMENTAL DESIGN

C57BL/6
6 wk-old

0

4 weeks

High fat diet (HFD)
60% kcal fat

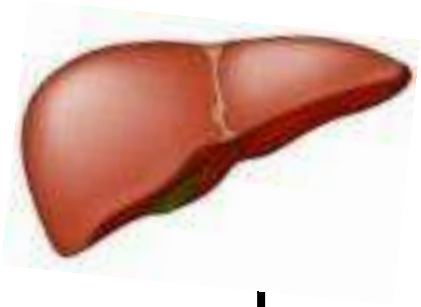
8 weeks

HFD

HFD + Canagliflozin

HFD weight-matched to HFD + CANA

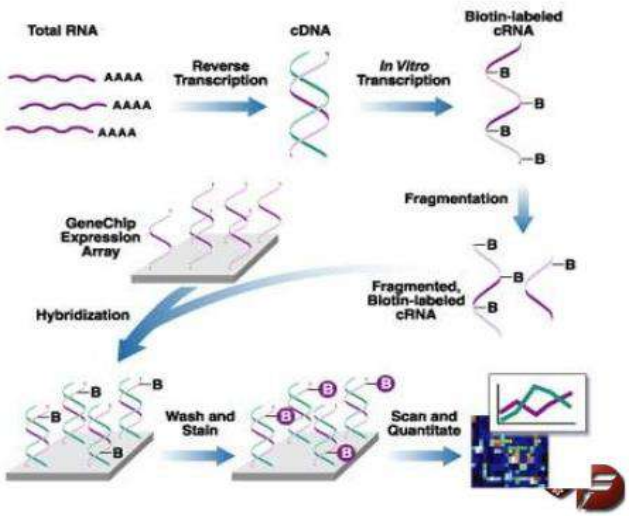
Sacrifice



Trizol based RNA extraction

scanner

Affymetrix
GeneChip

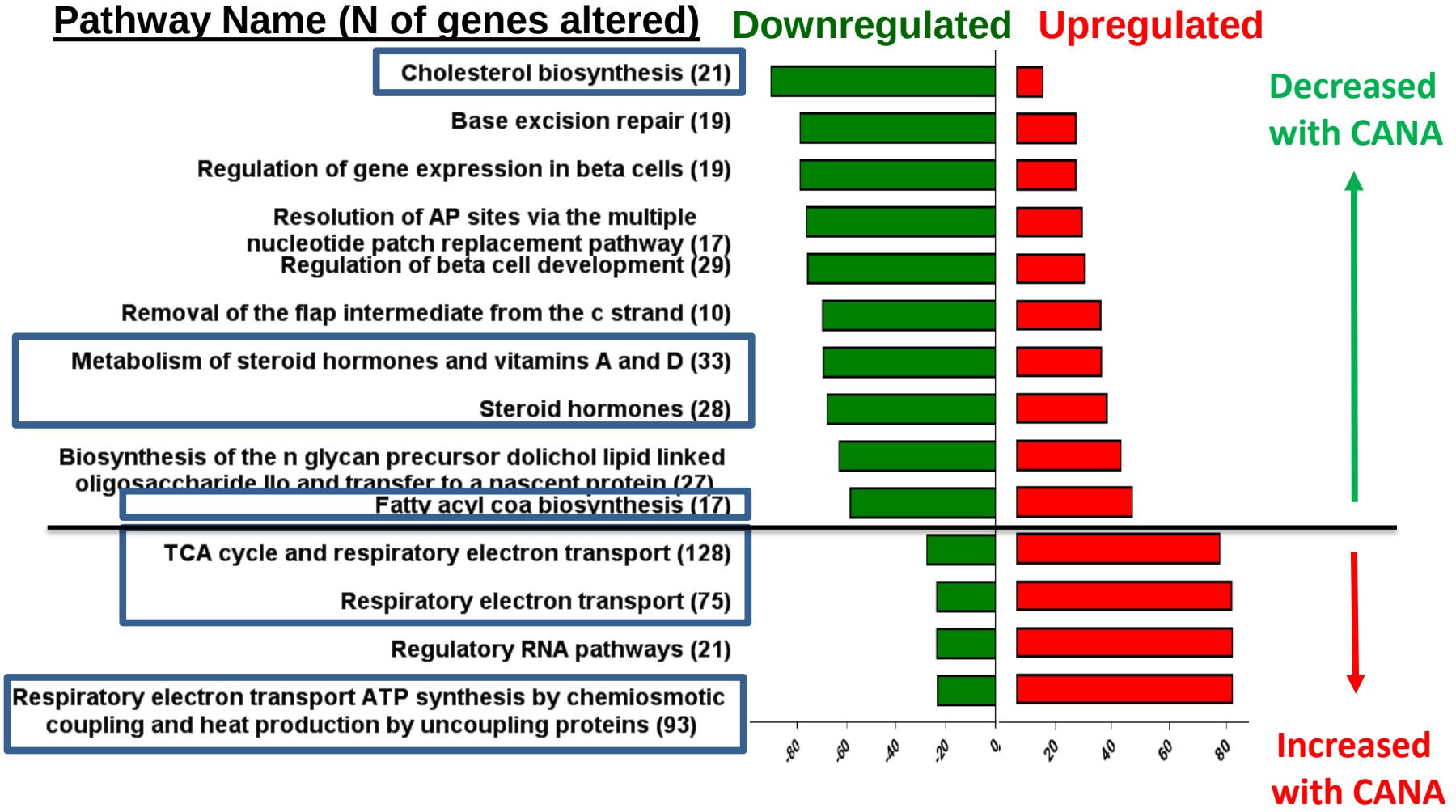


canagliflozin: 30 mg/kg/d, admixed in diet

Thank you to Grace Daher, Genomics Core, JDC

Pathway Analysis of Hepatic Transcriptome

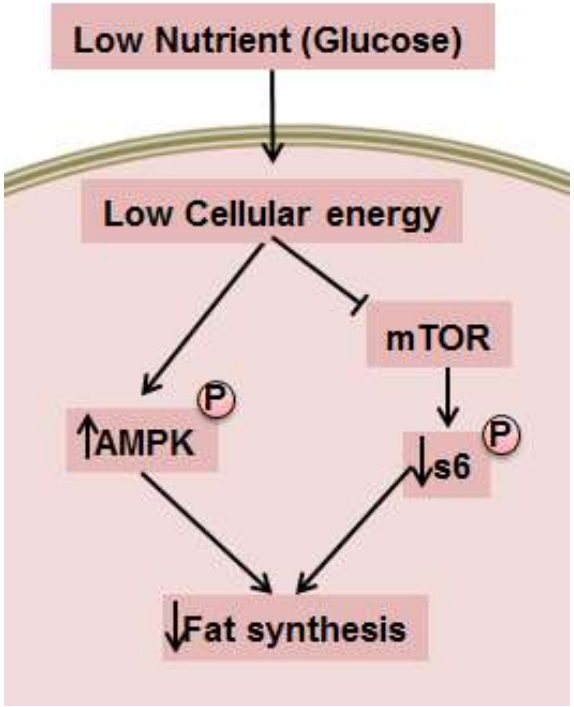
14 Pathways Differentially Regulated in [CANA vs. CR]



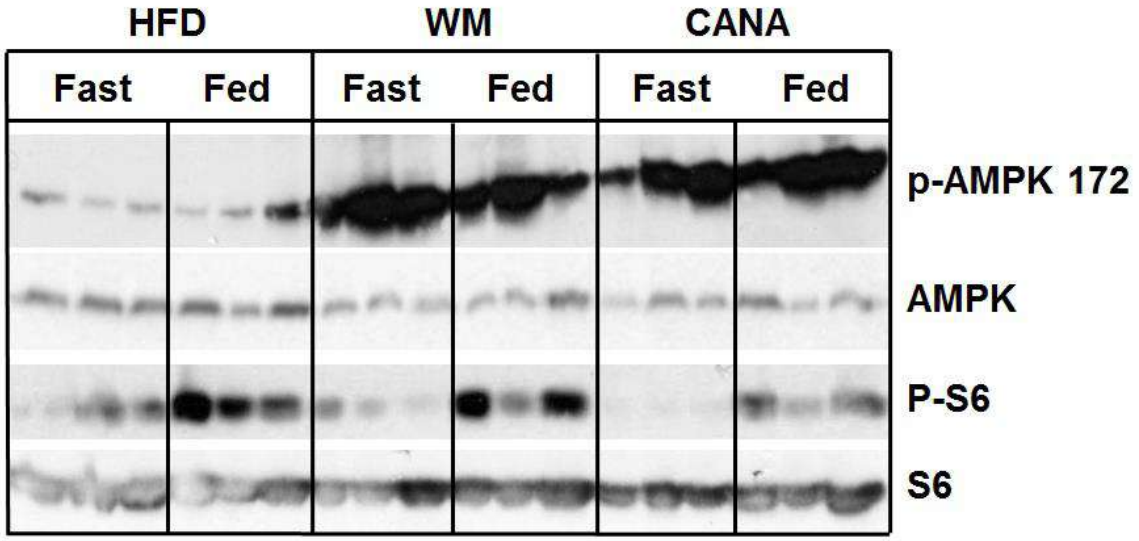
Reactome pathway, p<0.05

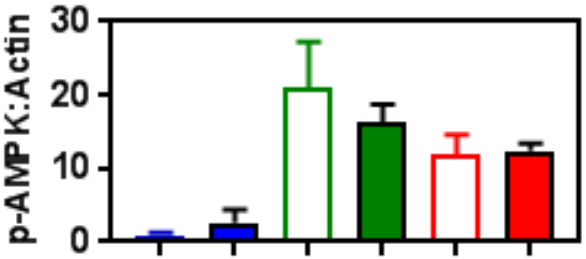
Thanks to Jonathan and Hui Bioinformatic core, JDC

SGLT2i changes the way cell senses energy

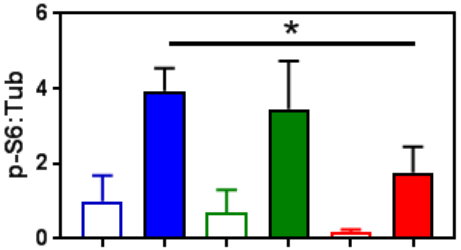


Cellular nutrient sensors sensed a lower energy state

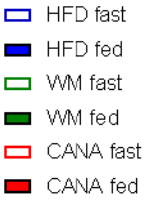




Increased AMPK Activation

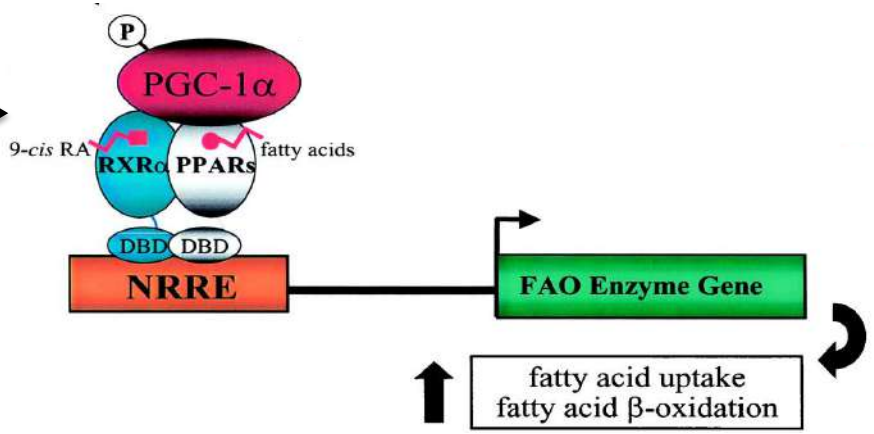


Reduced Tor Pathway Activation

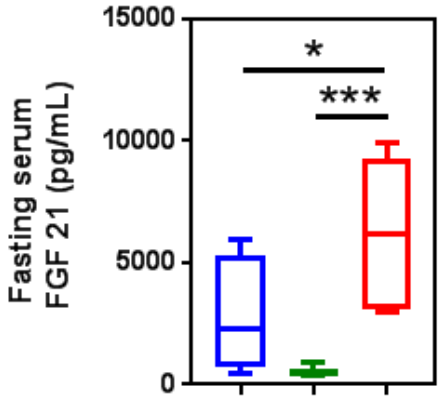


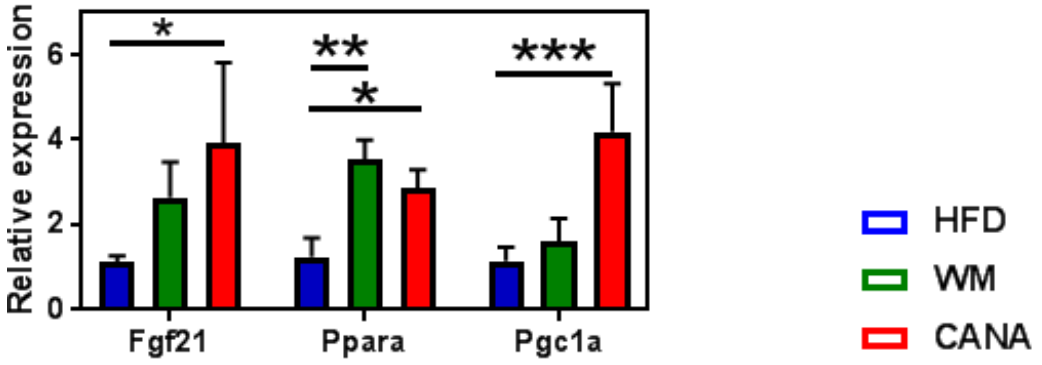
Increases in Serum FGF21 and Hepatic mRNA

Hepatic derived hormone fibroblast growth factor 21 (FG21)

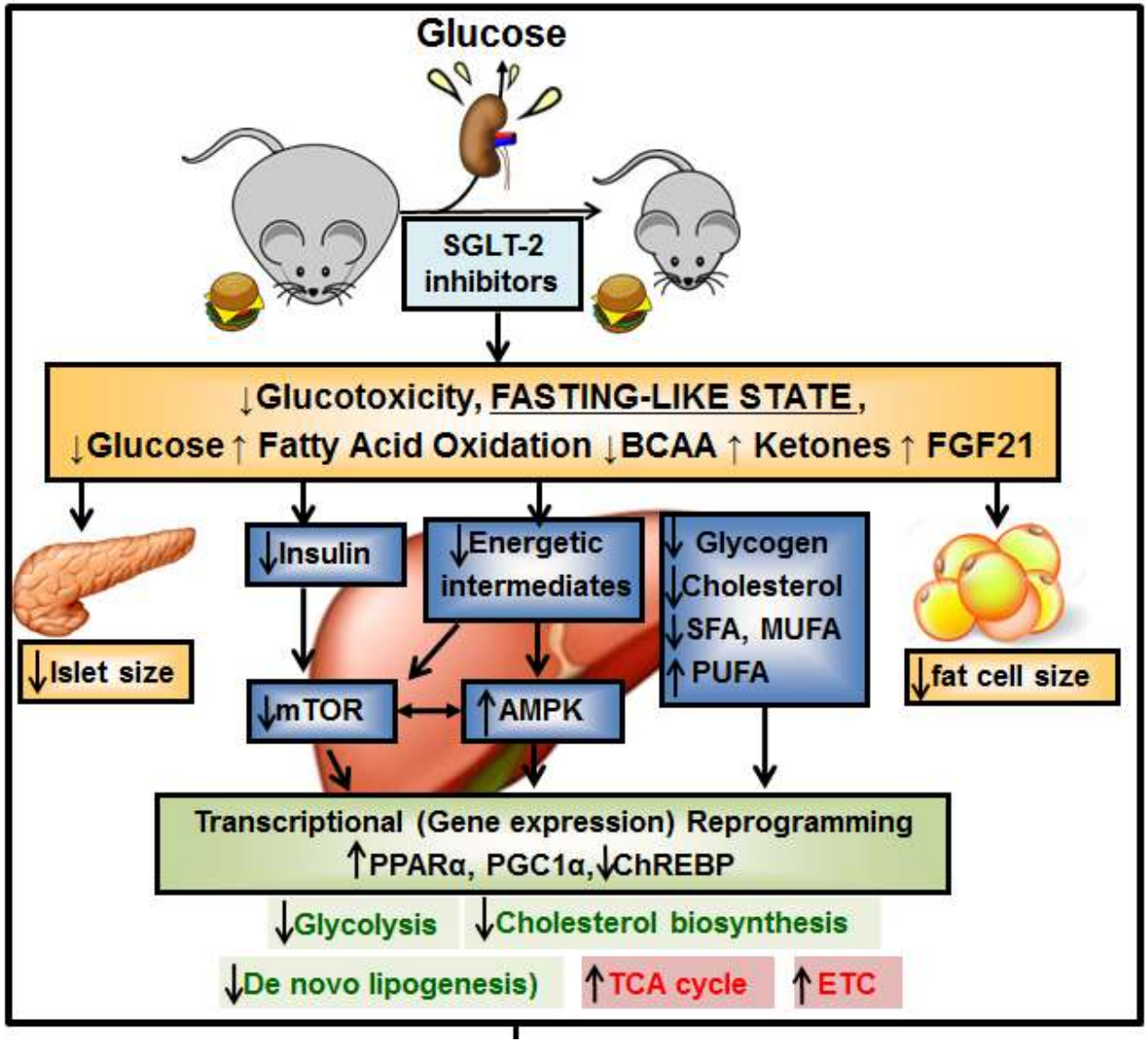


↑ Serum FGF21 ↑ hepatic mRNA FGF21, Ppara and pgc1a





Potential molecular mediators



Epigenetic mediates Gene-environment interaction by altering chromatin accessibility and gene expression

SGLT-2 inhibitor: Remove glucose from cell via kidney

Gene

Epigenetic

Cellular nutrient excess including glucose and fat

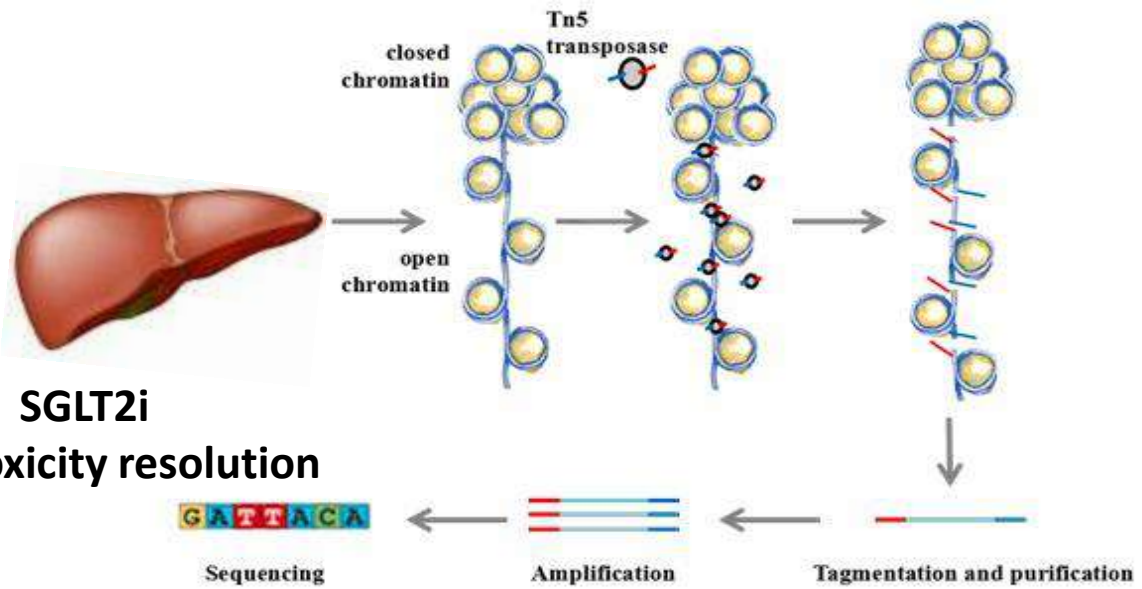
Environment

Insulin resistance
Type 2 Diabetes

High fat diet
Physical inactivity
Prenatal nutritional states
Paternal metabolism

Epigenetic: facilitate gene environment interaction and changes how gene are turn on and off

On going work: Hepatic Chromatin Accessibility: Assay of Transposase Accessible Chromatin with High Throughput Sequencing (ATAC-seq)



SGLT2i
Glucotoxicity resolution

In collaboration with BIDMC Functional Genomics and Bioinformatics core, Evan Rosen MD PhD, Linus Tsai MD PhD.

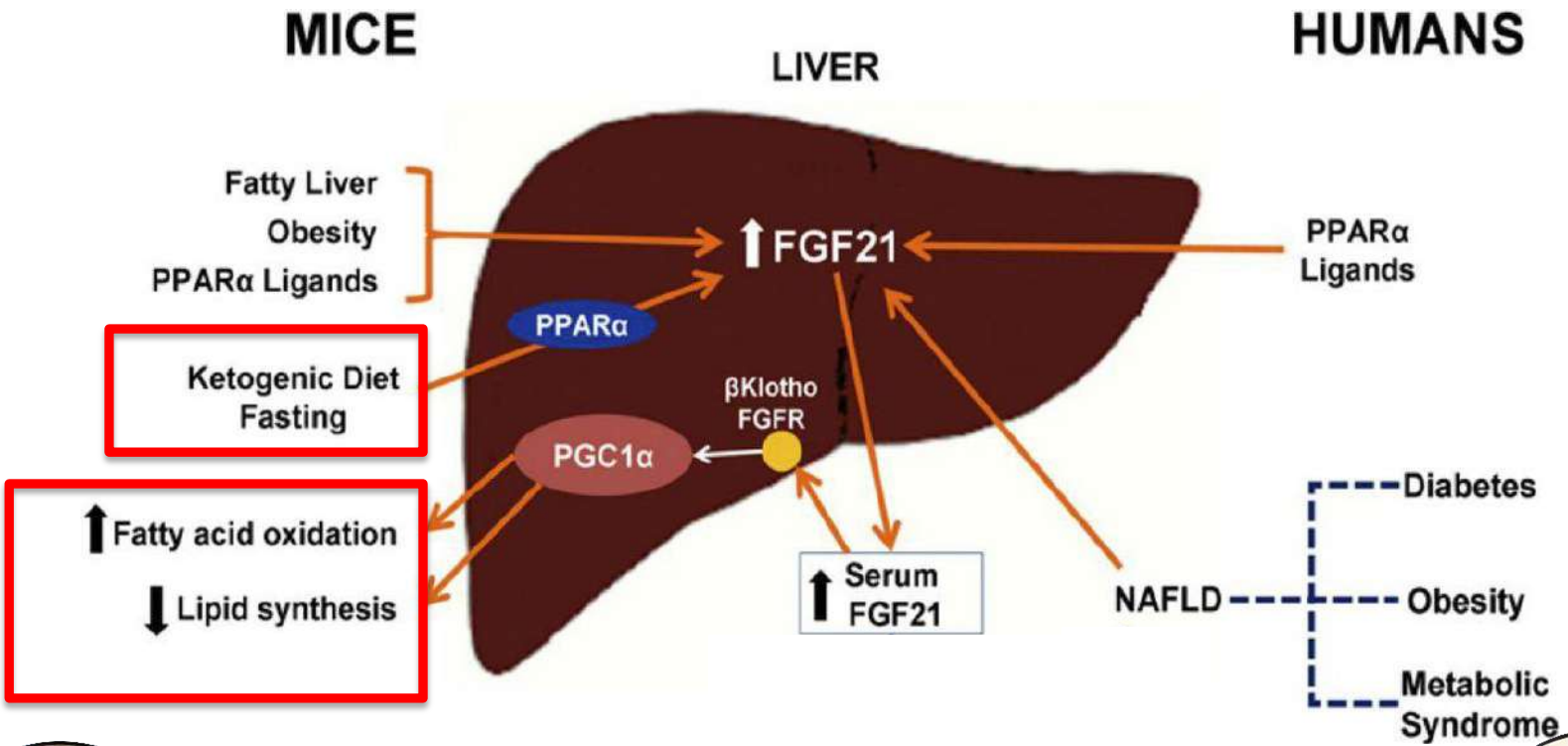
BNORC
Boston Nutrition Obesity Research Center

Beth Israel Deaconess Medical Center
HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

Nature Methods volume 10, pages 1213–1218 (2013)

On going work : requirement of FGF21 in mediating the beneficial effect of SGLT2i

Beth Israel Deaconess Medical Center
HARVARD MEDICAL SCHOOL TEACHING HOSPITAL



In collaboration with Eleftheria Maratos-Flier MD and Garima Singhal PhD at BIDMC
Treating FGF21 Knockout mouse with CANA (in progress)

Adapted from AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00527.2015

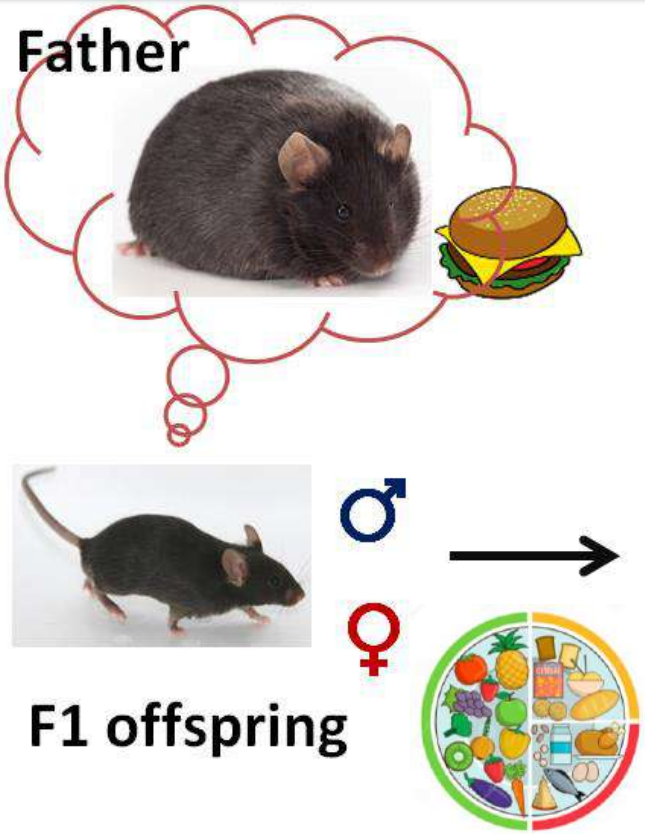
Project 2: Paternal Intergenerational transmission of metabolic risk through epigenetic

Metabolic inheritance : You are what your father eats

Paternal (father) “acquired” traits e.g obesity can be transmitted to offspring and increase offspring risk of diabetes independent of genetic code

Nature. 2010 Oct 21;467(7318):963-6.
Cell. 2010 Dec 23;143(7):1084-96.

Known data on the effect of HFD in father on offspring



In both male and female offspring

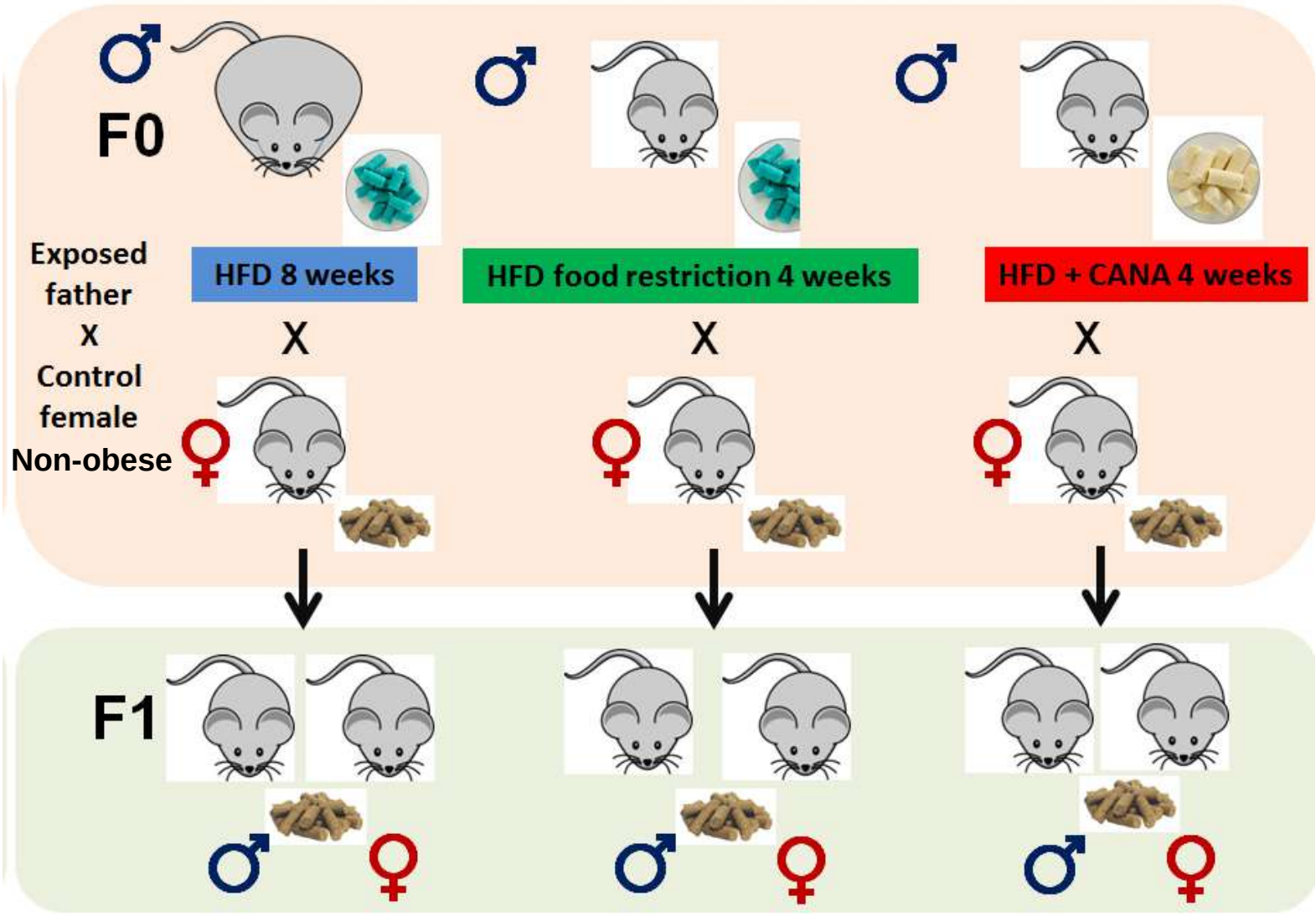
1. Increase body weight
2. Worsen glucose tolerance
3. Worsen insulin resistance
4. Sensitised offspring to future HFD exposure

Even when offspring have balanced diet throughout their life and were never exposed to high fat diet

Research question : Could improvement in father metabolism such as reduction in glucotoxicity reduce offspring diabetes risk?

FASEB J. 2013 Oct;27(10):4226-43
Mol Metab. 2015 Dec 25;5(3):184-97.
Sci Rep. 2016 Aug 23;6:31857

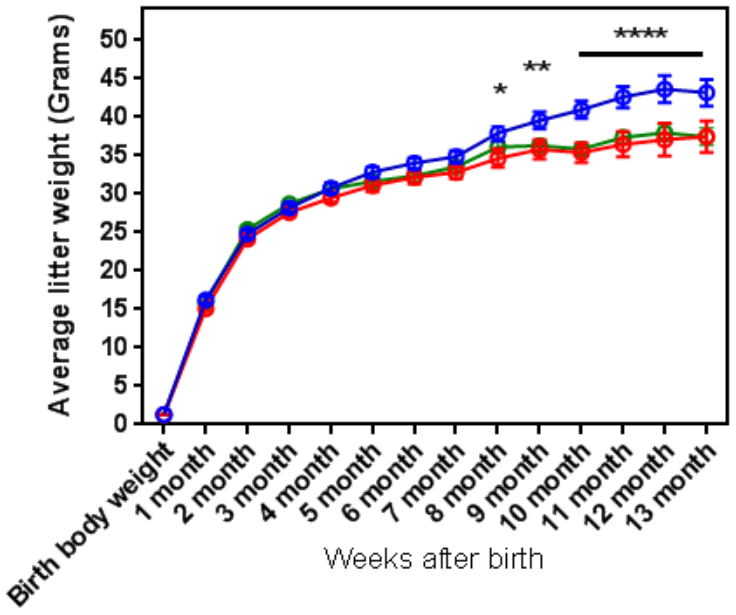
Experimental design



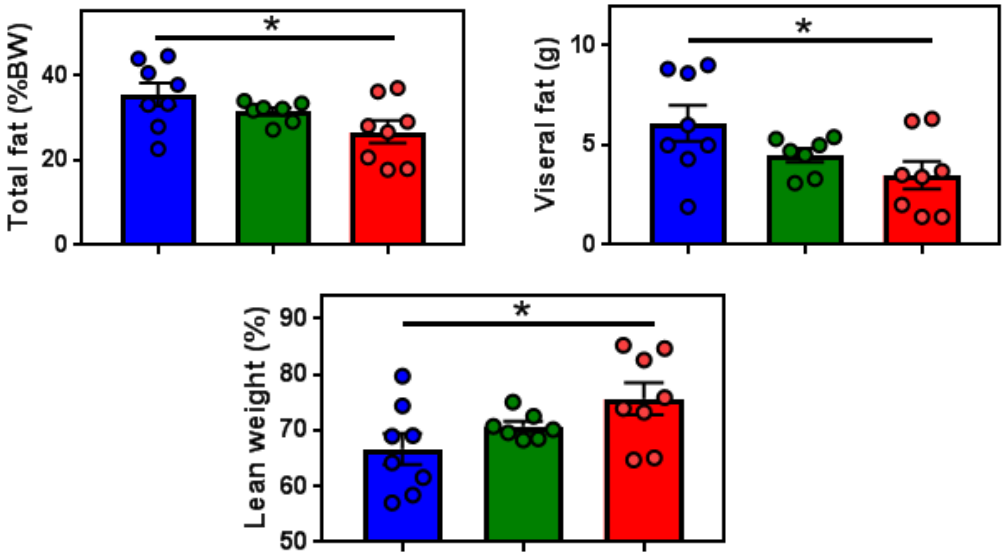
Offspring only eat balanced diet throughout their life and were never exposed to drug or high fat diet

Yanin Tangjaroenpaisan
3rd year Medical Student
Srinakharinwirot- Nottingham Joint Medical Program
Joslin Summer Student 2017

SGLT2i treatment in father causes a reduction in body weight and fat mass later in life in male offspring



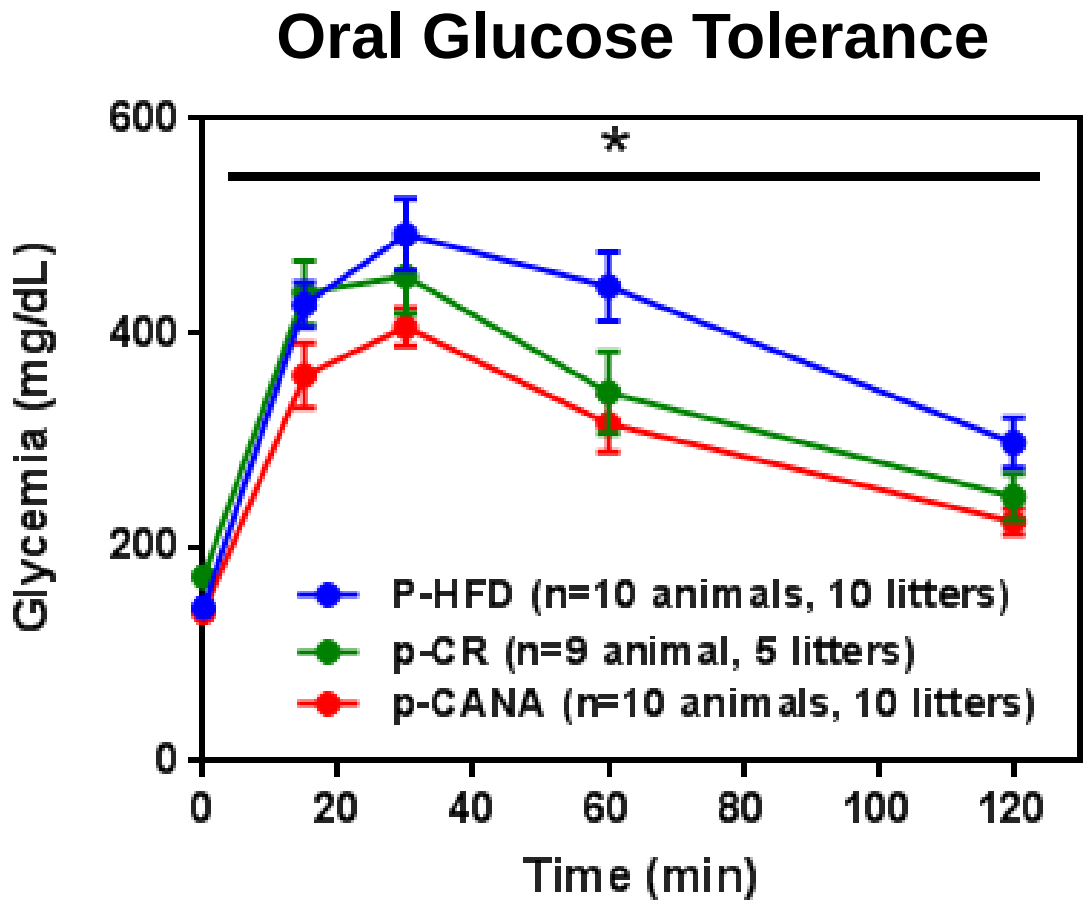
Lower body weight later in life



Reduction in fat and increase in lean mass later in life as measured by Dual X-ray Absorptiometry

- P-HFD (n=8 litters)
- p-CR (n=7 animals, 5 litters)
- p-CANA (n=8 litters)

Improved glucose tolerance in offspring with better paternal metabolism



Conclusion and implication

- Improving father's metabolism during conception can have beneficial effect on offspring metabolism later in life
- Implications: Paternal glycemic control during conception could reduce diabetes risk transmission

Current Work

- Analyze the Transcriptome and epigenome in Father's sperm & offspring somatic tissue

Upcoming work – Clinical Study

Joslin Why WAIT program – Weight Achievement & Intensive Treatment

- 12-week multidisciplinary program for weight control and intensive diabetes management – up to 20 pounds weight loss
- We will collect sperm before and after weight loss to analyzed alteration in epigenetic marks

NIDDK
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

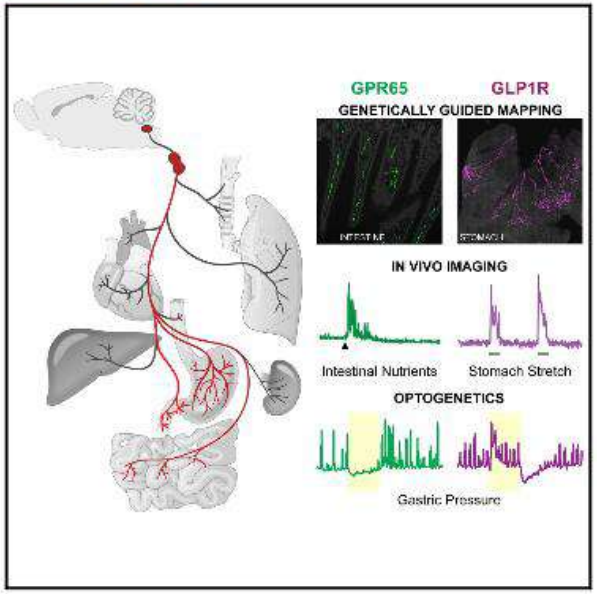
Project 3 The role of vagal subneuronal population in the control of metabolism

Article

Cell

Sensory Neurons that Detect Stretch and Nutrients in the Digestive System

Graphical Abstract



Authors

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Correspondence

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In Brief

Two types of neurons sending signals from the gut to the brain control digestion. One densely innervates intestinal villi and detects food, while another targets stomach and intestinal muscle and senses stretch.

In Collaboration with Racheal Burst PhD, Stephen Liberles PhD (PI), Department of Cell Biology, Harvard Medical School

Recently funded by Joslin Pilot and Feasibility Program.

Cell. 2016 Jun 30;166(1):209-21.

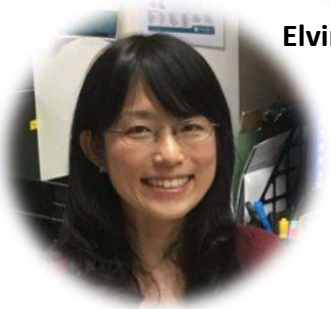
Mary-Elizabeth Patti MD FACP

PRINCE MAHIDOL AWARD FOUNDATION

Patti Lab member


Elvira Isganaitis MD MPH


Chiara Macchi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Ruchanee Ausavarungniran

Thanomrat watcharachaipong

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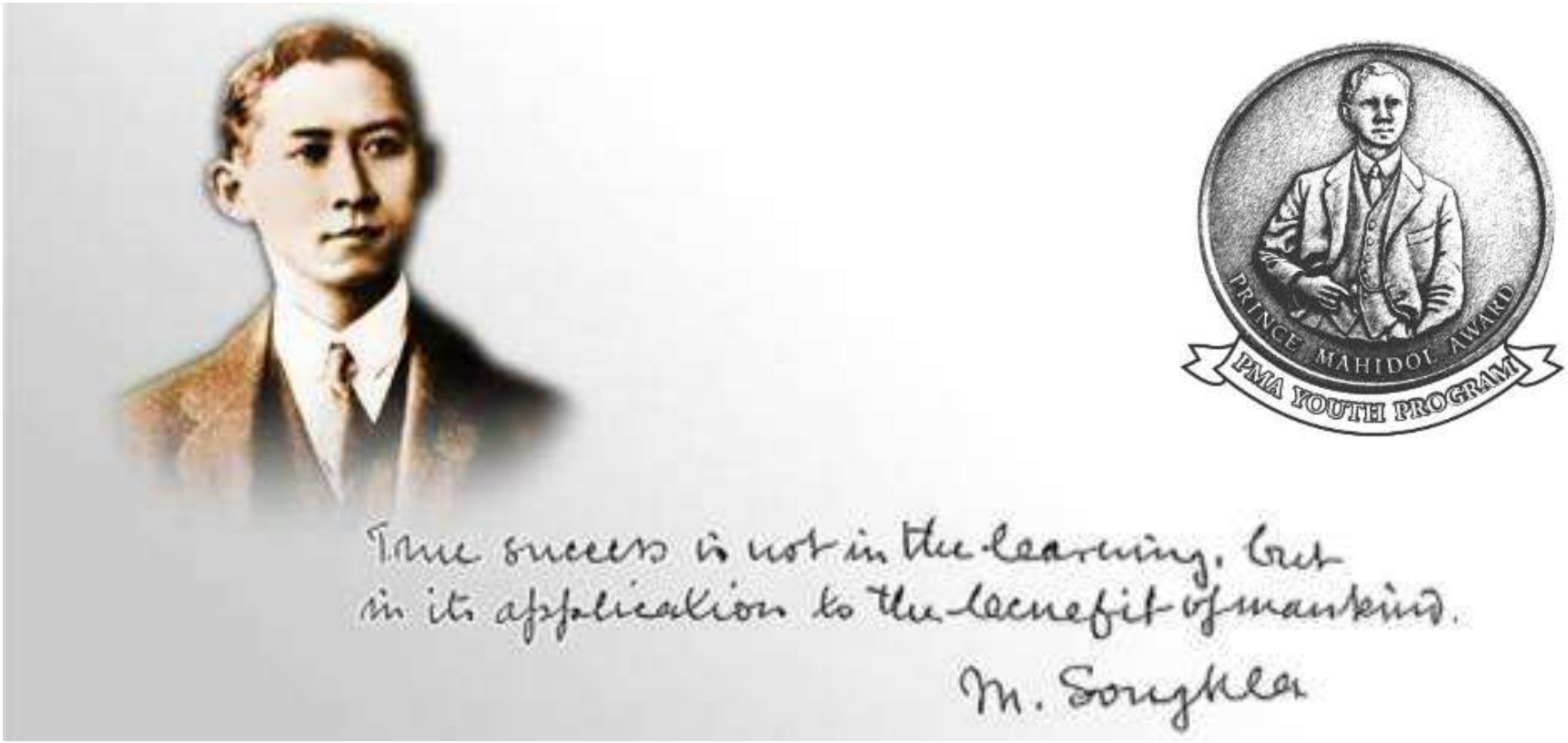

I am especially grateful for the Prince Mahidol Award Youth Program for funding my fellowship

Thank you for your attention

Q&A session