Why Moms and Dads Should Exercise: Molecular Discoveries of the Beneficial Effects of Parental Exercise on Offspring Health

Katarina T. Borer Lectureship in Exercise Endocrinology and Metabolism
School of Kinesiology, University of Michigan
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Laurie J. Goodyear, Ph.D.
Section on Integrative Physiology and Metabolism
Joslin Diabetes Center and Harvard Medical School

Benefits of Exercise on Health

Improved liver function

Increased lung capacity

Increased blood volume

Improved lipid profile

Improved sleep

Reduced fat stores

Lower blood pressure

Decreased rates of cancer
(Colon, breast, ovarian, prostrate)

Decreased depression, anxiety

Decreased Alzheimer's

Decreased appetite

Decreased stroke

Increased flexibility

Increased bone density

Improved cardiac function

Increased muscle strength

<u>Prevention of Diabetes</u>

Increased insulin sensitivity

Increased glucose tolerance

Improved pancreatic function

Increased mitochondrial function

Increased muscle glucose uptake

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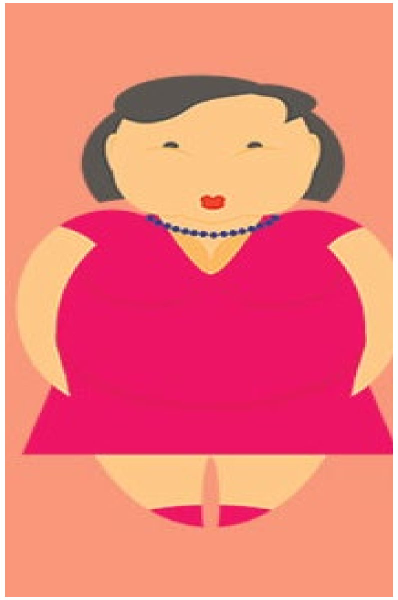
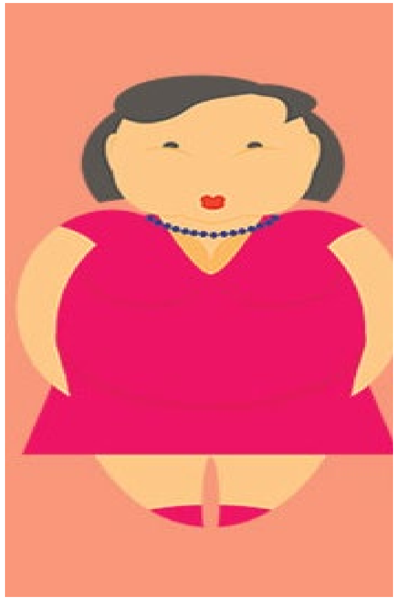
What are the underlying molecular mechanisms mediating the beneficial effects of exercise on health?

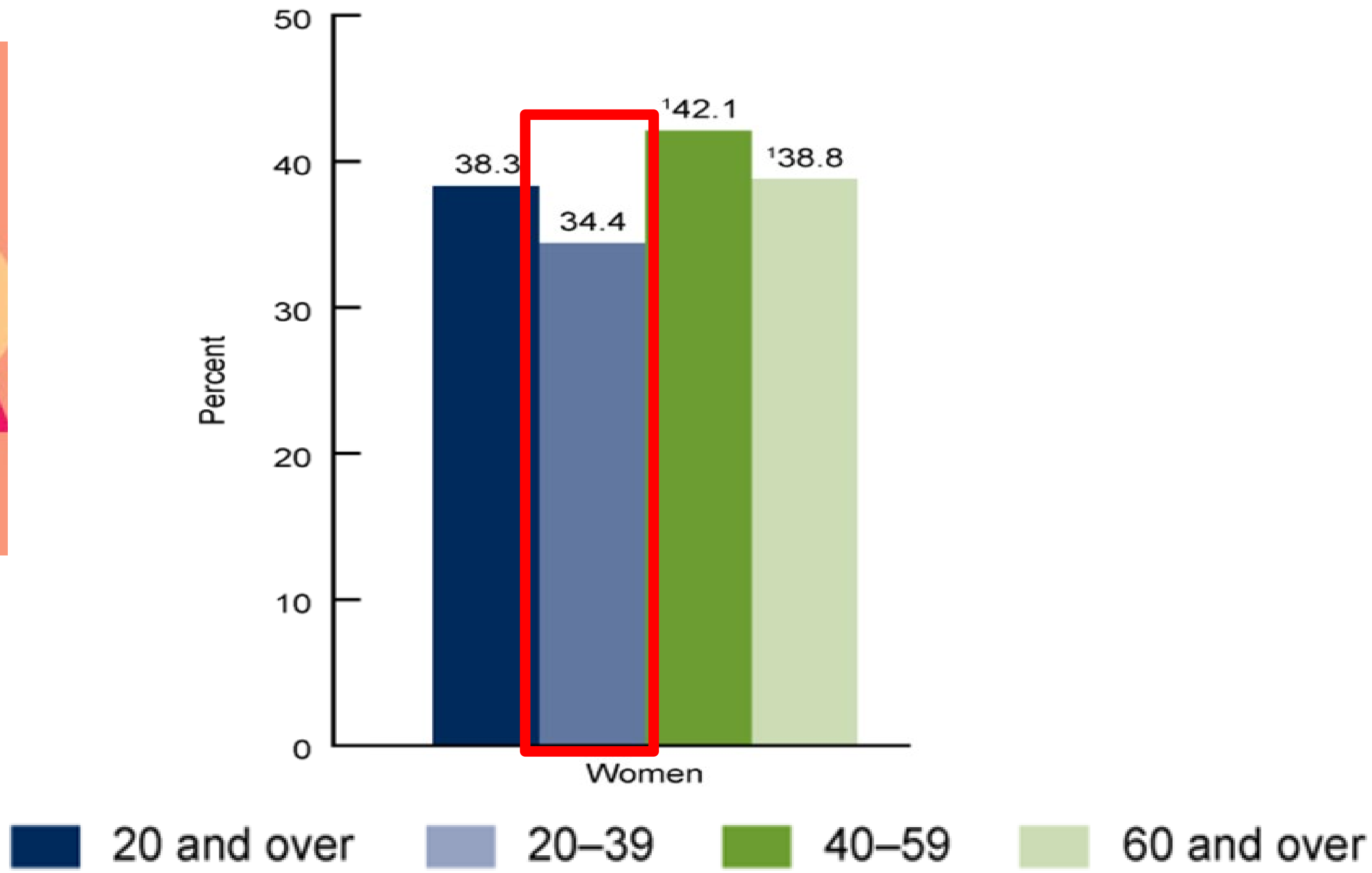
Improved pancreatic function
Increased mitochondrial function
Increased muscle glucose uptake

Obesity Has Detrimental Effects on Health



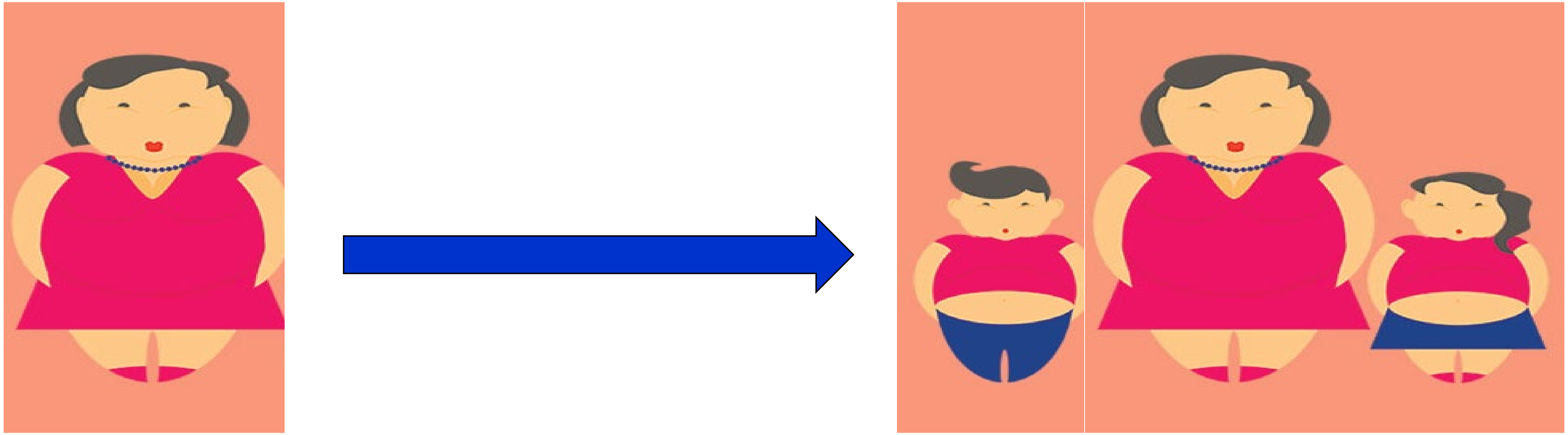
High Rates of Obesity in Women of Child Bearing Age





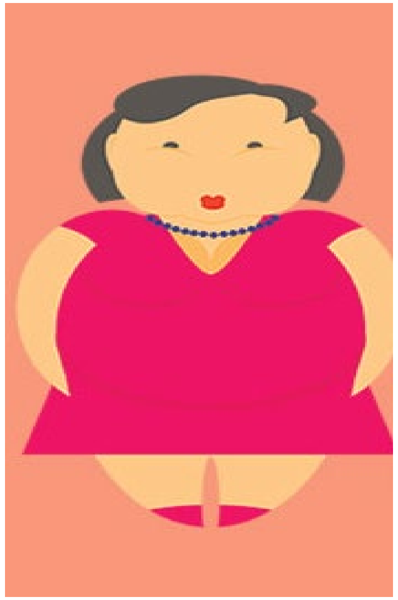
CDC: Prevalence of Obesity Among Adults in the United States (2015-2016)

Maternal Obesity and Type 2 Diabetes Can Have Detrimental Effects on Offspring Health

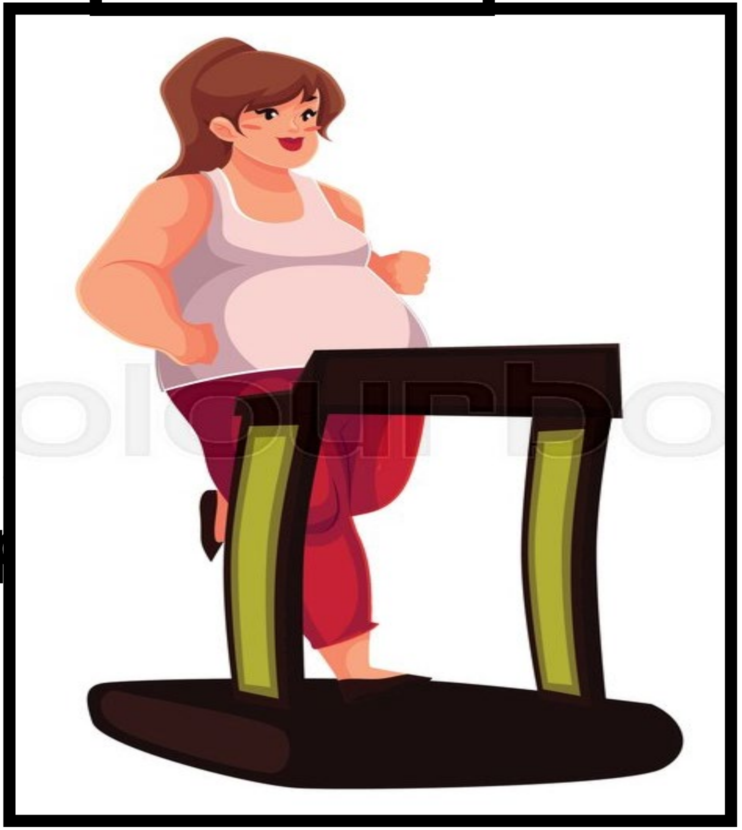


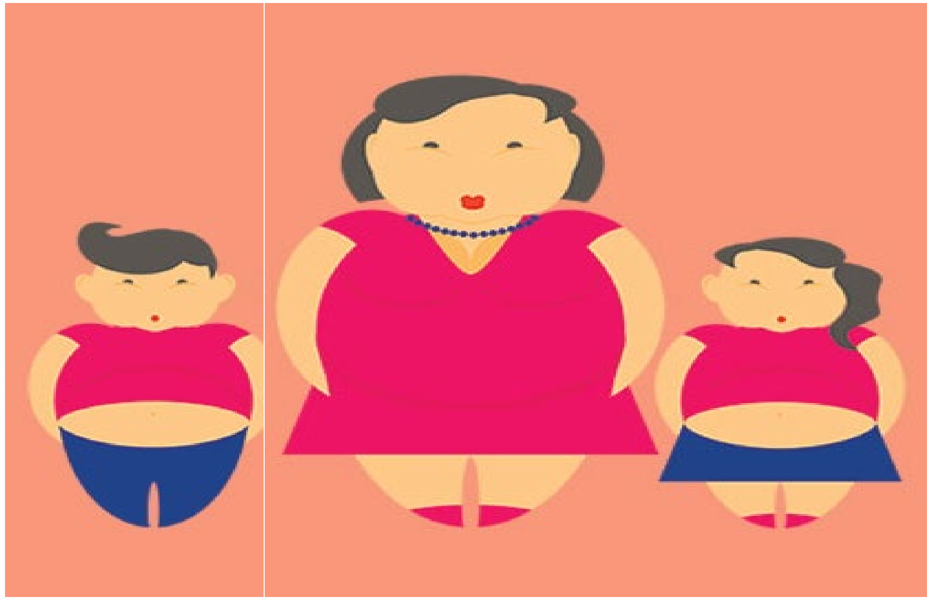
- Is there a means to reduce the transmission of increased risk of metabolic disease to offspring?

Maternal Obesity and Type 2 Diabetes Can Have Detrimental Effects on Offspring Health



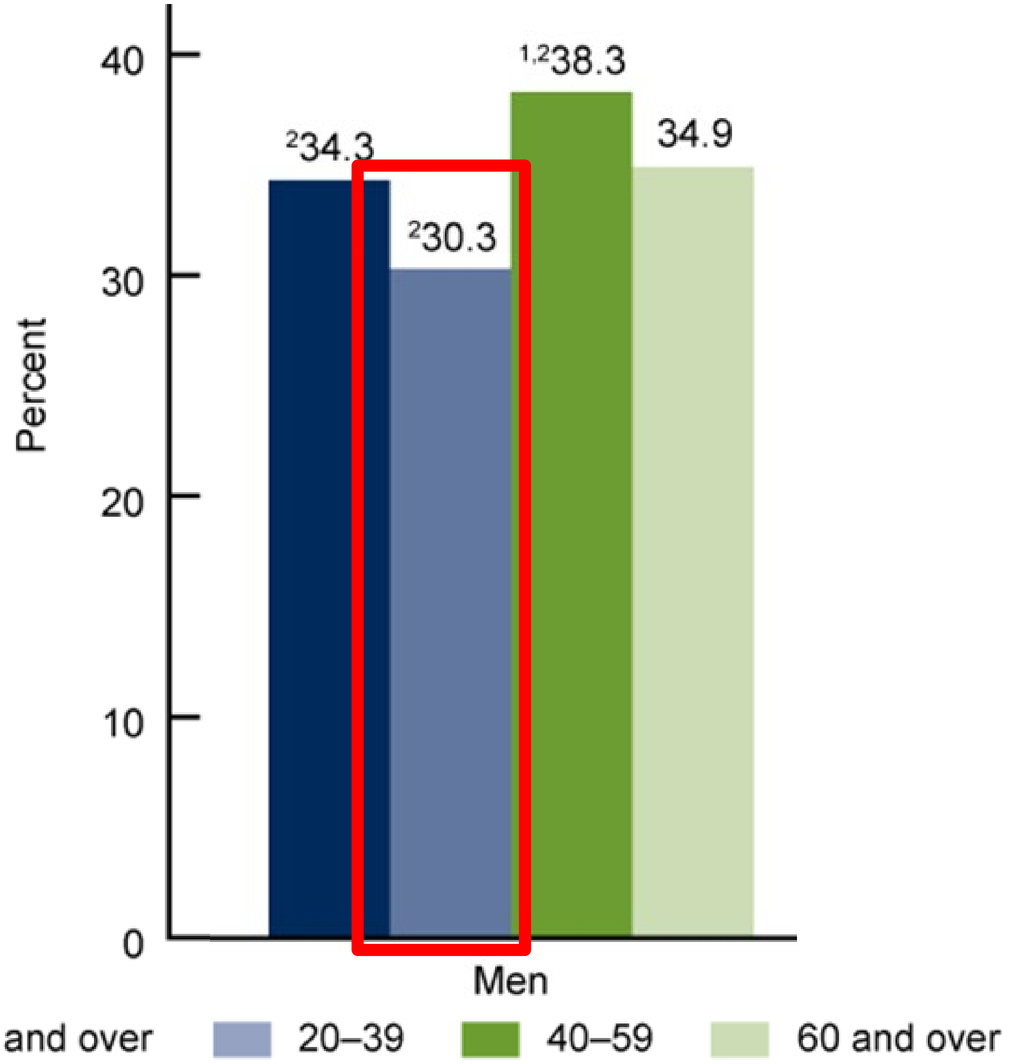
Exercise

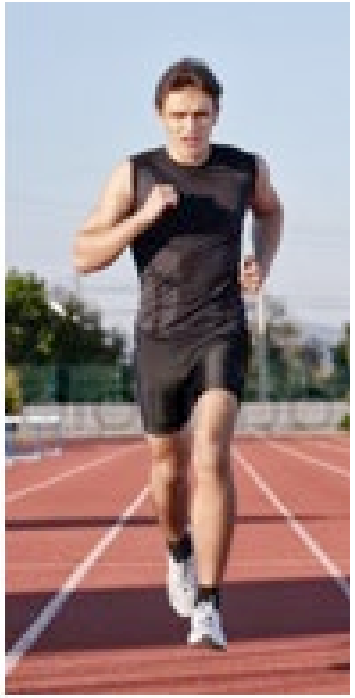




- Is there a me nsmission of risk of metabolic disease to offs

Prevalence of Obesity Among Male Adults in the United States





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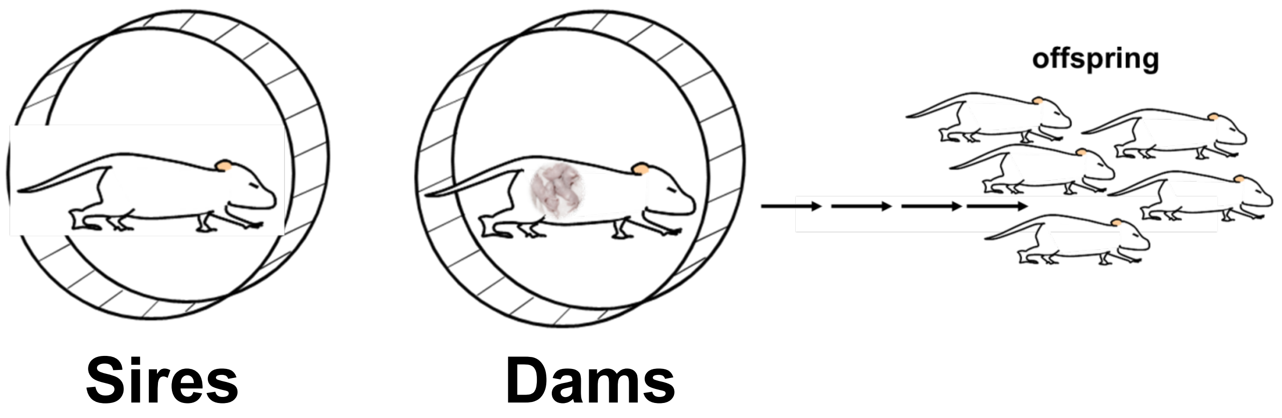
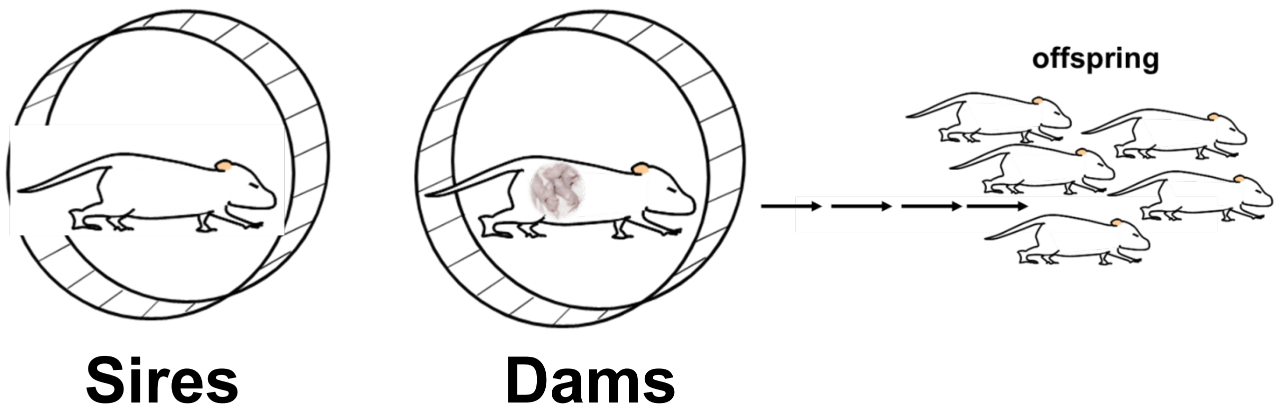
Effects of Maternal and Paternal Exercise on Offspring Metabolic Health

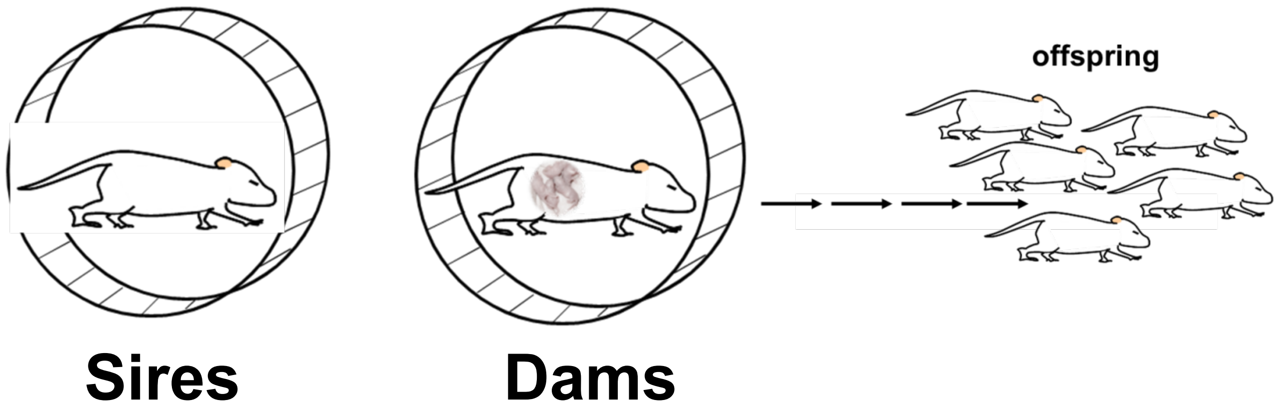


Hypothesis



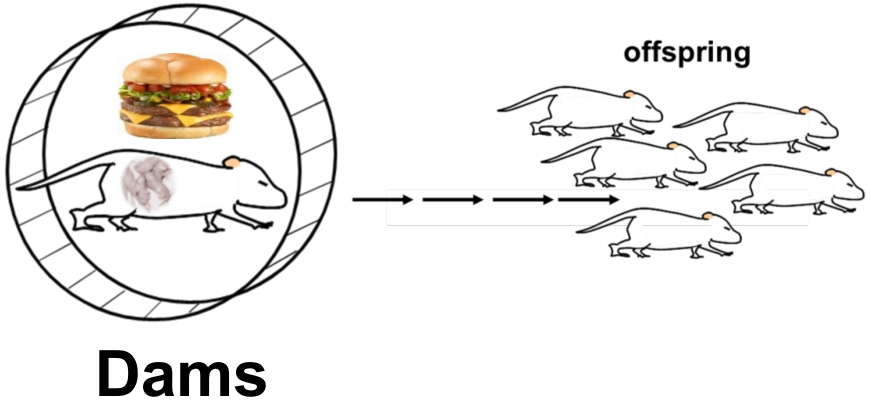
➤ **Increased physical activity of parents will improve the metabolic phenotype of offspring.**

Outline of Data Presentation



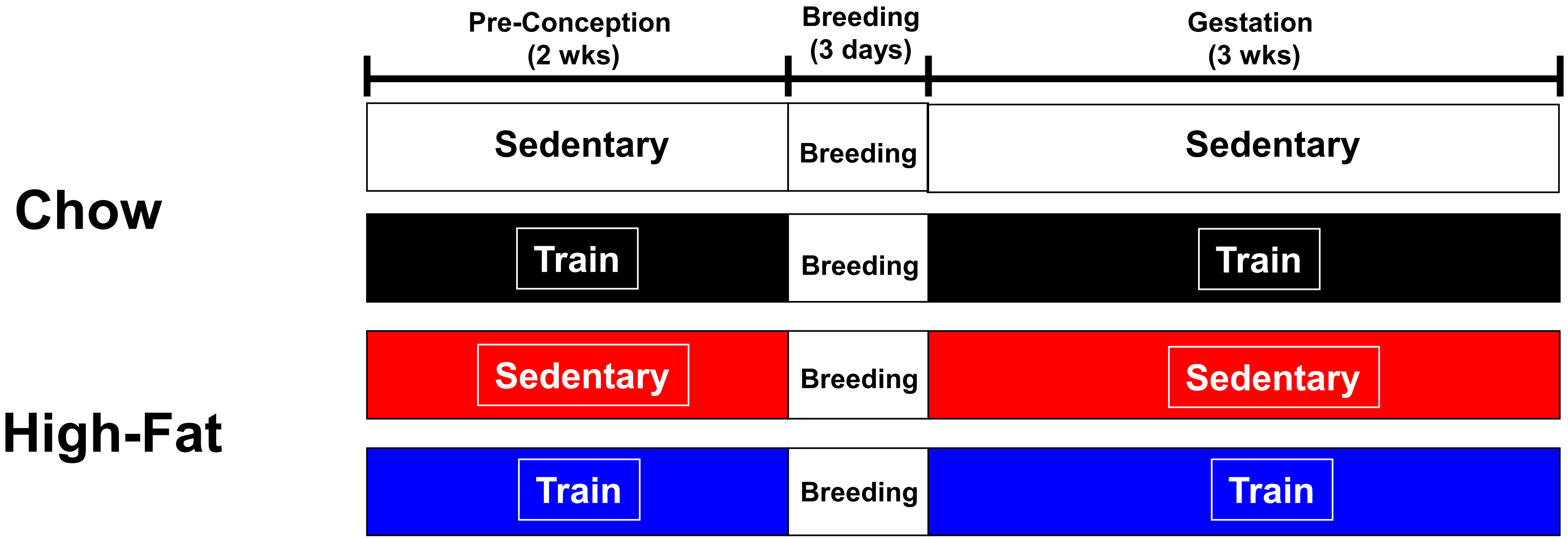
- ➢ **Maternal, Paternal and Maternal + Paternal Exercise: effects on offspring glucose tolerance.**
- ➢ **Maternal Exercise: mechanisms and epigenetic regulation of offspring.**
- ➢ **Maternal Exercise: identification of a novel protein that can improve offspring health.**

Effects of Maternal Exercise Training on Offspring



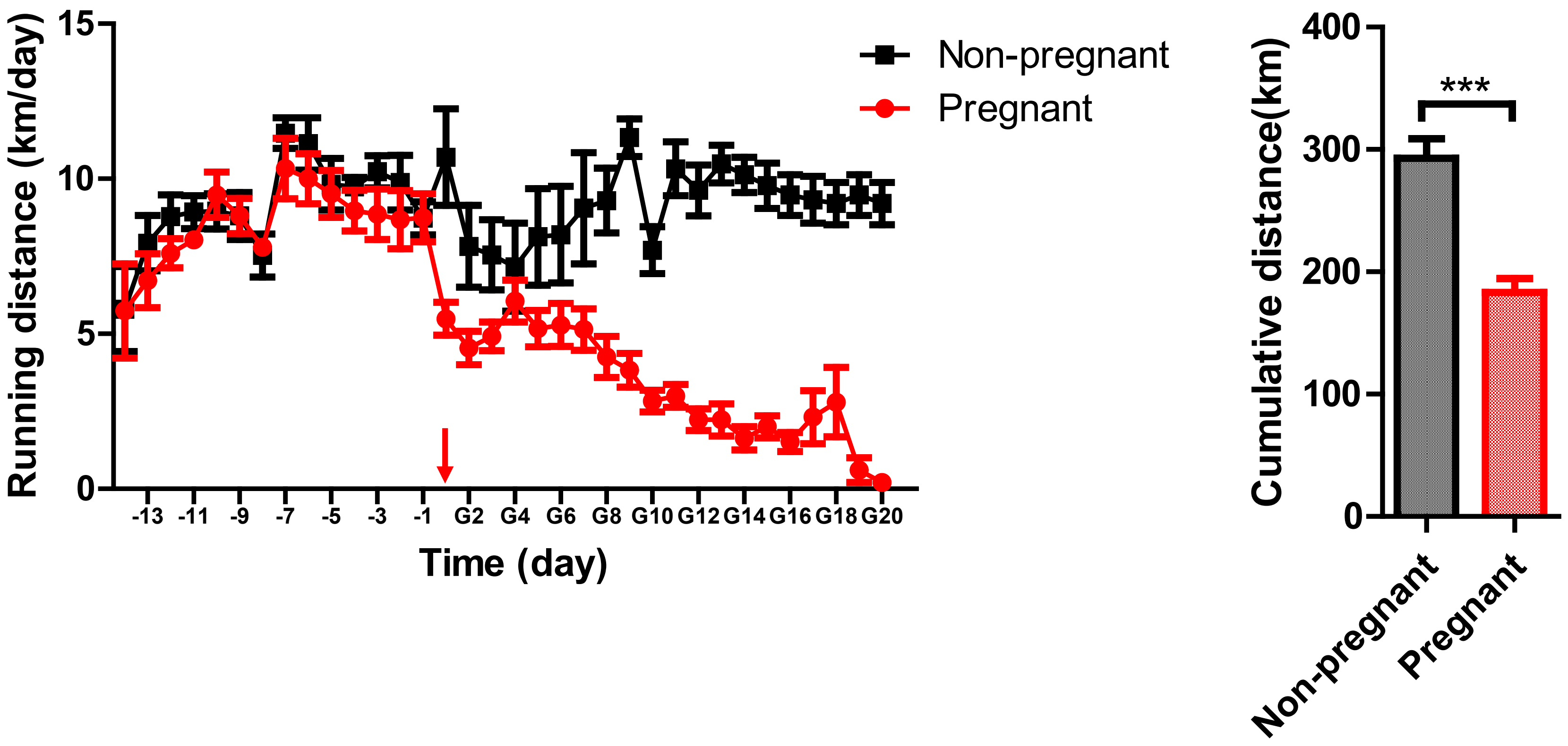
- What is the effect of maternal exercise on glucose tolerance and insulin concentrations?
- Can maternal exercise prevent the detrimental effects of a high fat diet on offspring metabolic health?

Experimental Design – Maternal Exercise Studies



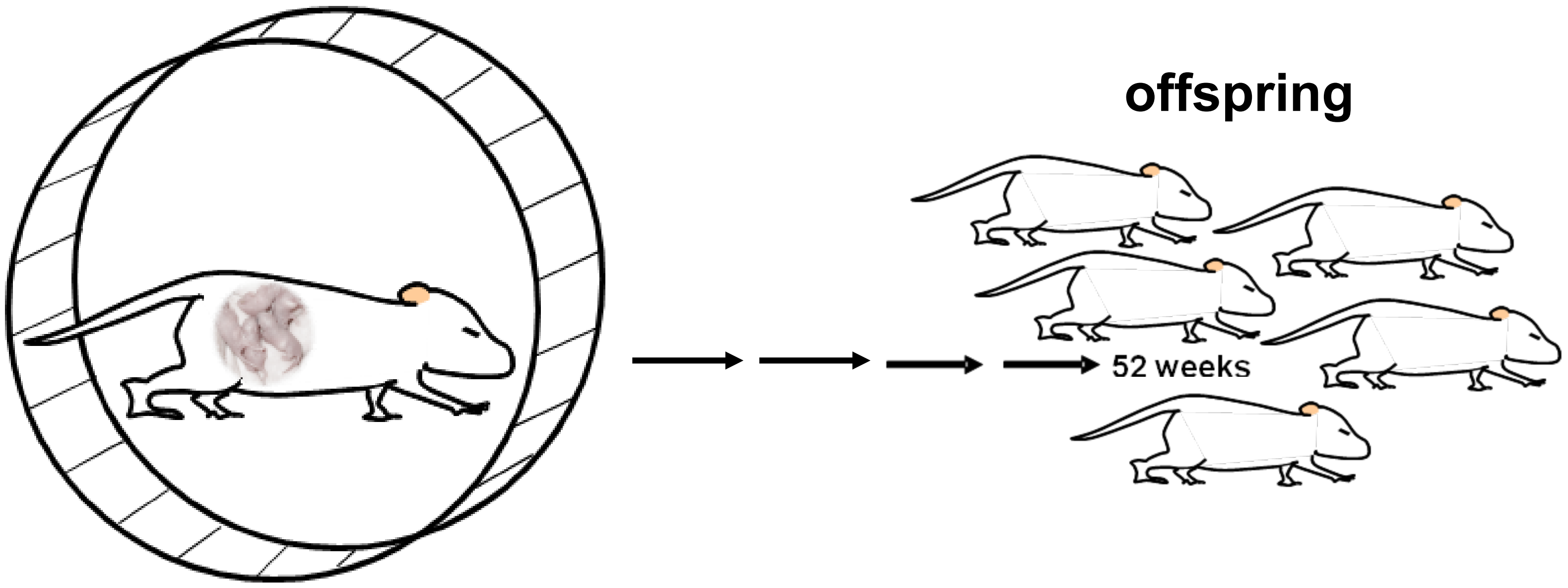
- Training done by voluntary wheel running
- Male breeders were sedentary and chow fed

Running Distance of Non-Pregnant and Pregnant Females



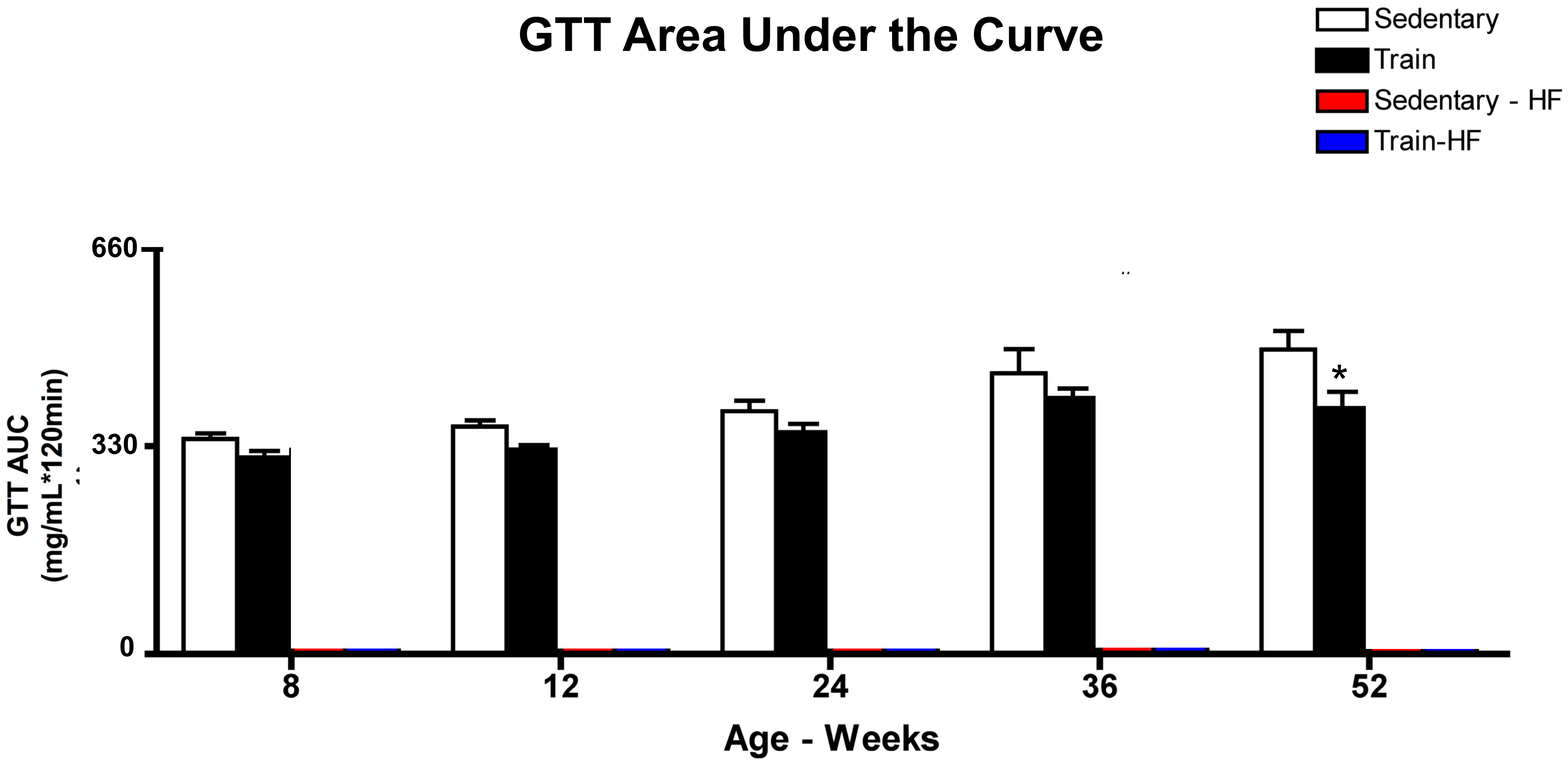
Data represent mean±SEM. ***P<0.001, n=18-20 each group

Experimental Design – Effects on Offspring Metabolic Health



- **Offspring were sedentary and chow-fed**
- **Offspring metabolic health monitored from youth until middle age (52 weeks)**
- **Glucose tolerance test area under the curve (AUC), insulin concentrations**

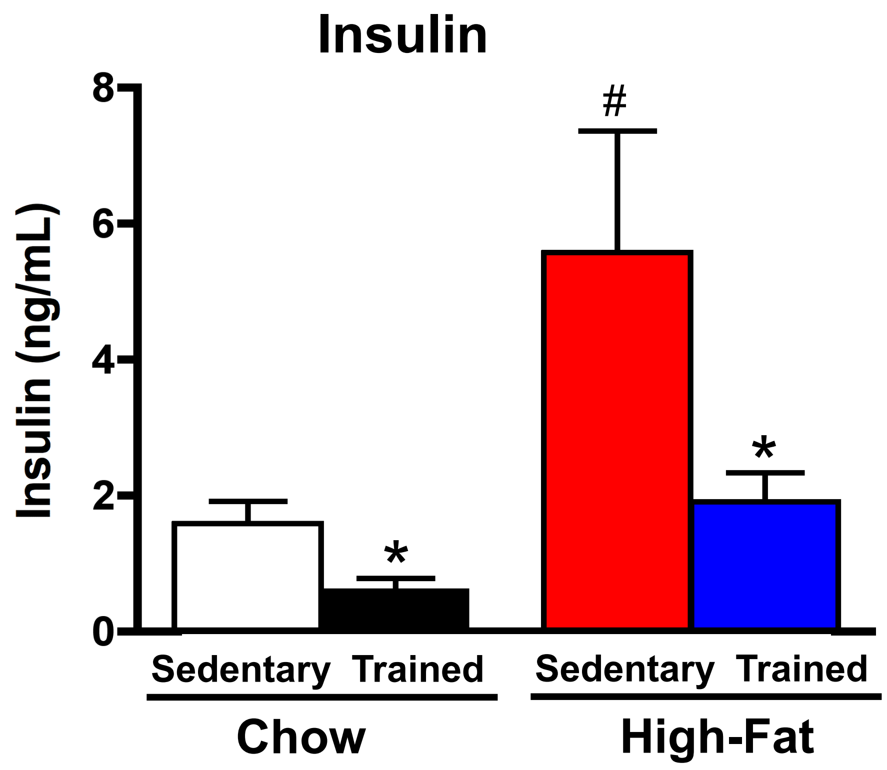
Maternal Exercise Improves Glucose Tolerance in Adult Male Offspring

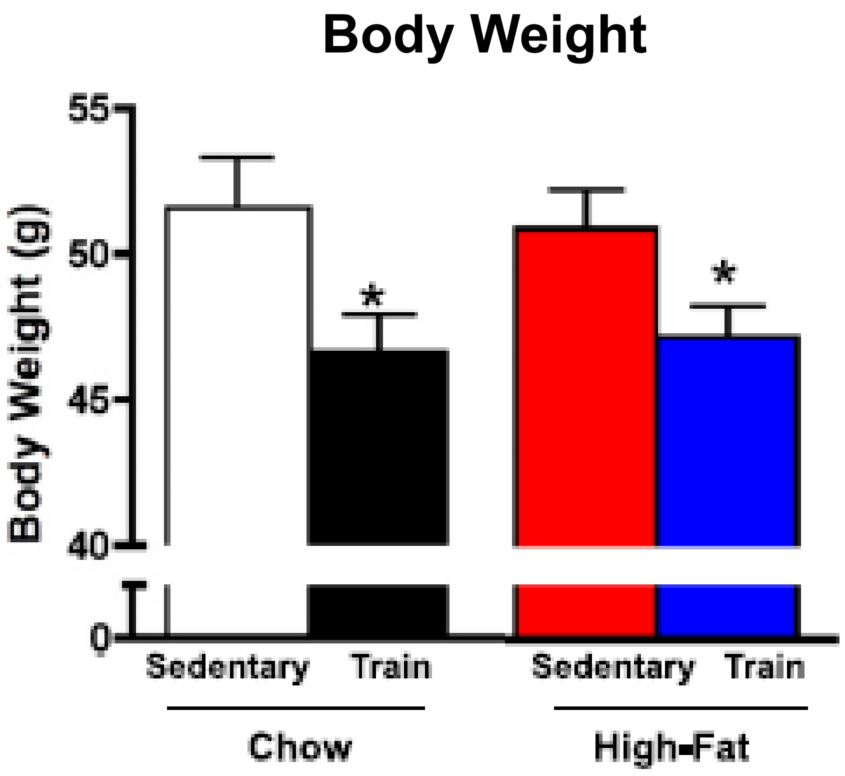


Stanford et al, Diabetes 2015

Maternal Exercise Decreases Offspring Insulin Concentrations and Body Weight

52 week old Male Offspring

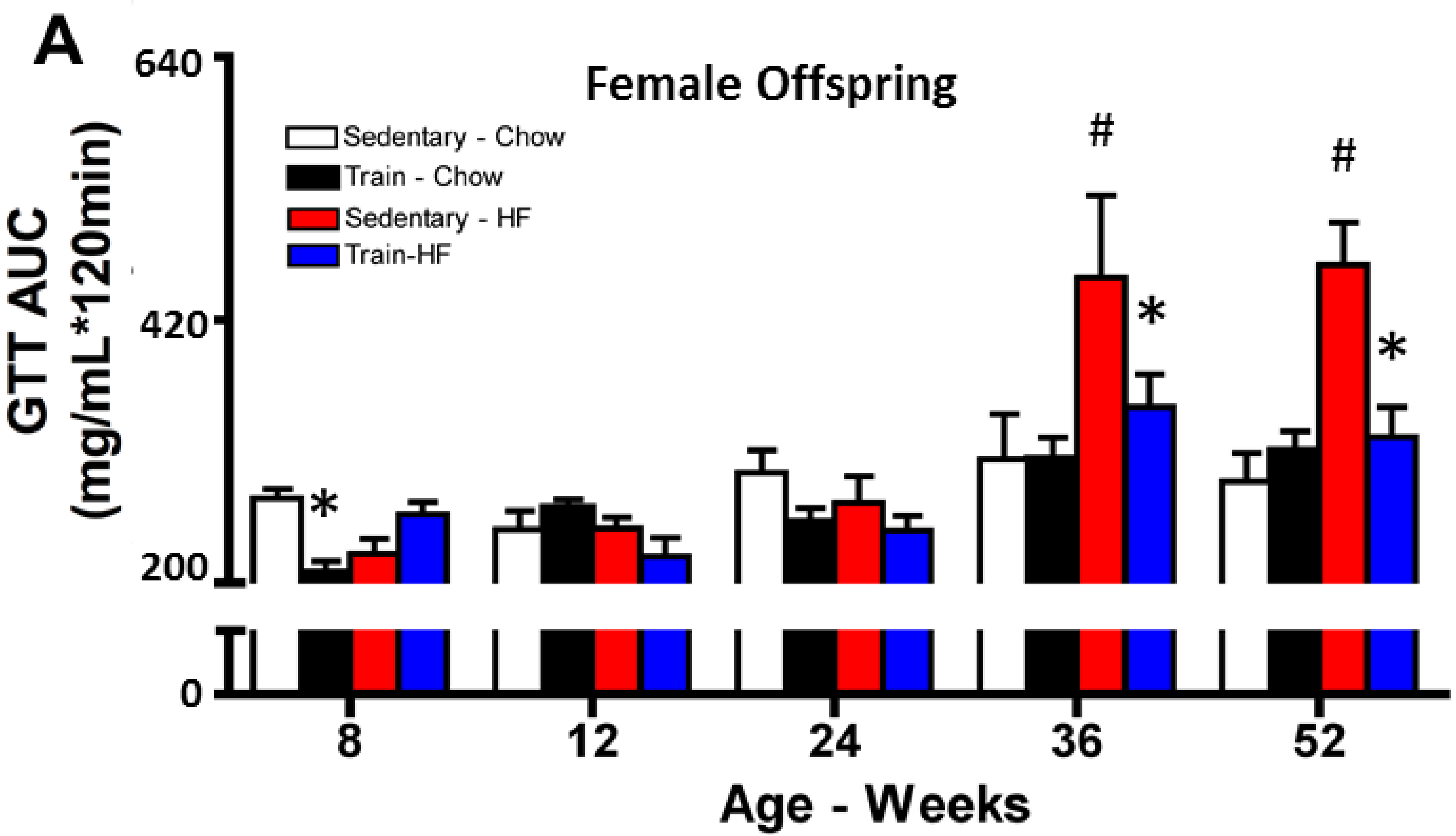




Stanford et al, Diabetes 2015

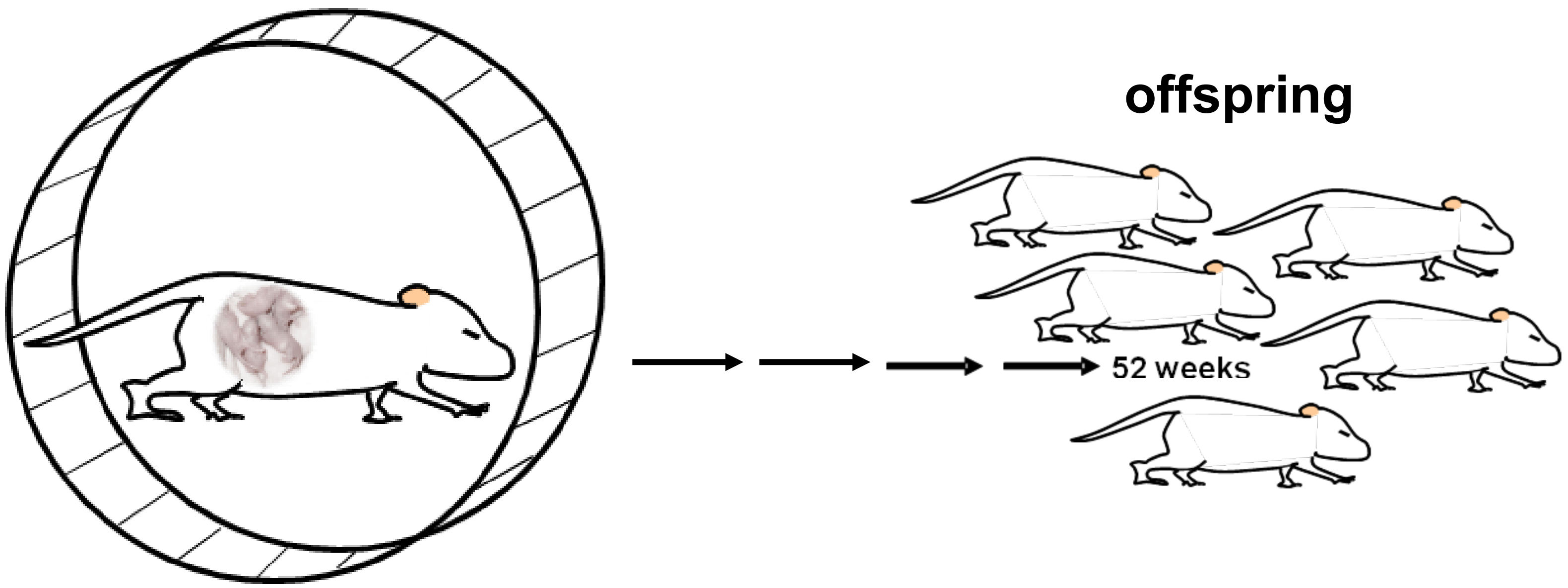
Maternal Exercise Improves Glucose Tolerance in Female Offspring

GTT Area Under the Curve



Stanford et al, Diabetes 2017

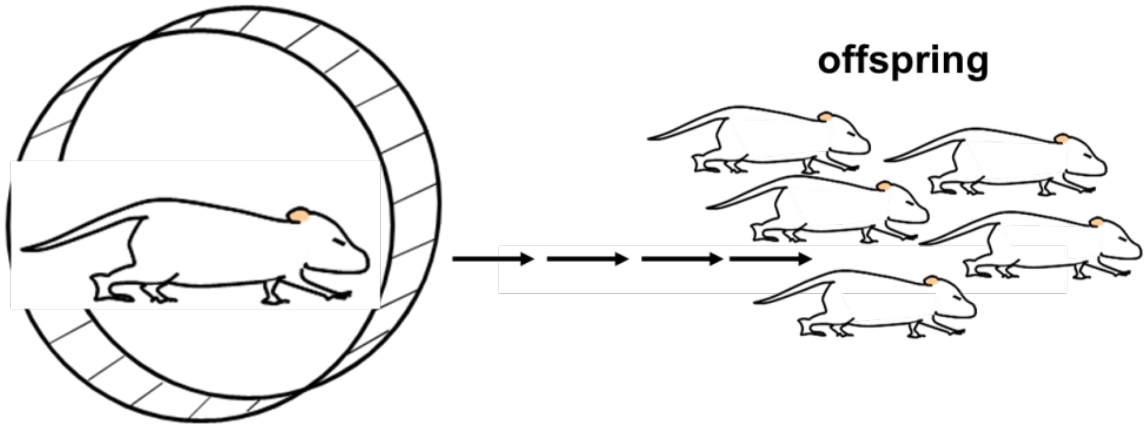
Summary



- Maternal exercise before and during pregnancy improves the metabolic health of male and female offspring.

- Maternal exercise prevents the deleterious effects of high fat feeding on the metabolic health of male and female offspring.

Effects of Paternal Exercise on Offspring Metabolic Health



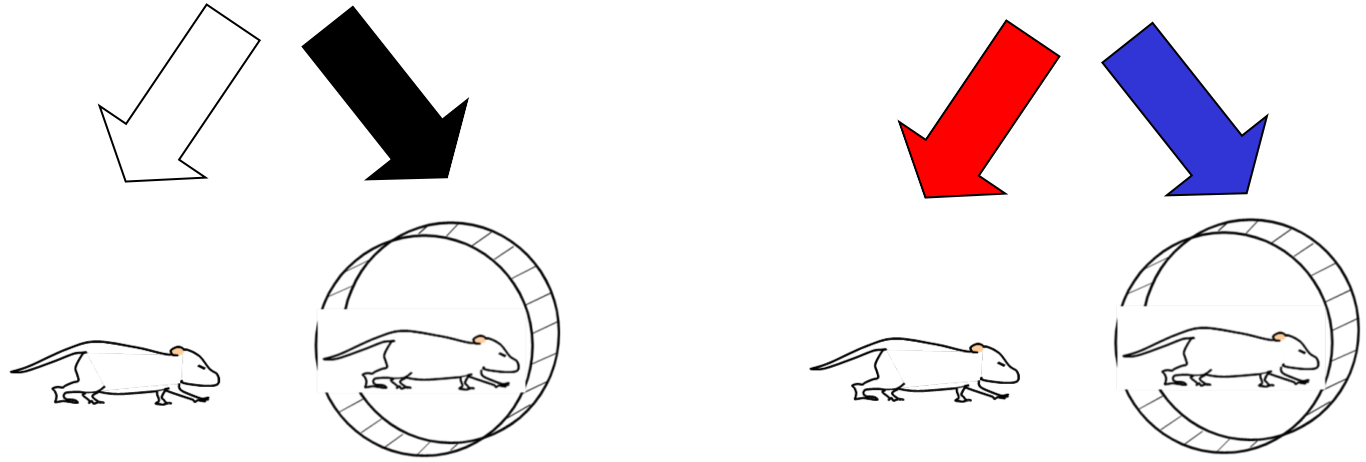
Sires

Paternal Experimental Design

- C57BL/6 male mice (7 wks old)

Chow Diet
(21% kcal from fat)

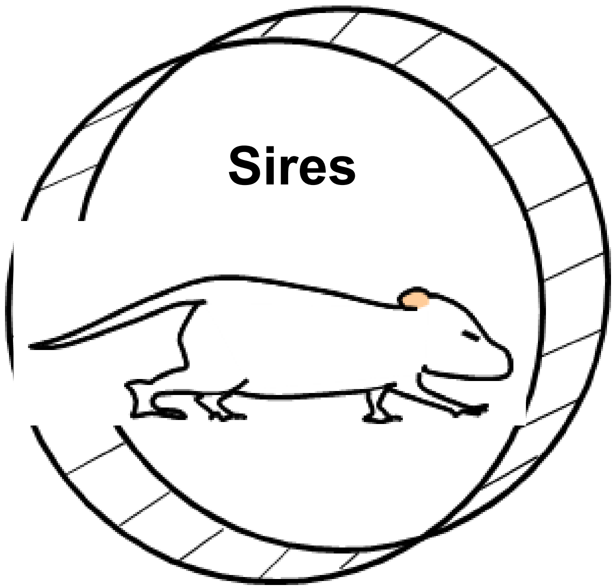
High-Fat Diet
(60% kcal from fat)



Sedentary (3wks) | Trained (3wks) | Sedentary (3wks) | Trained (3wks)

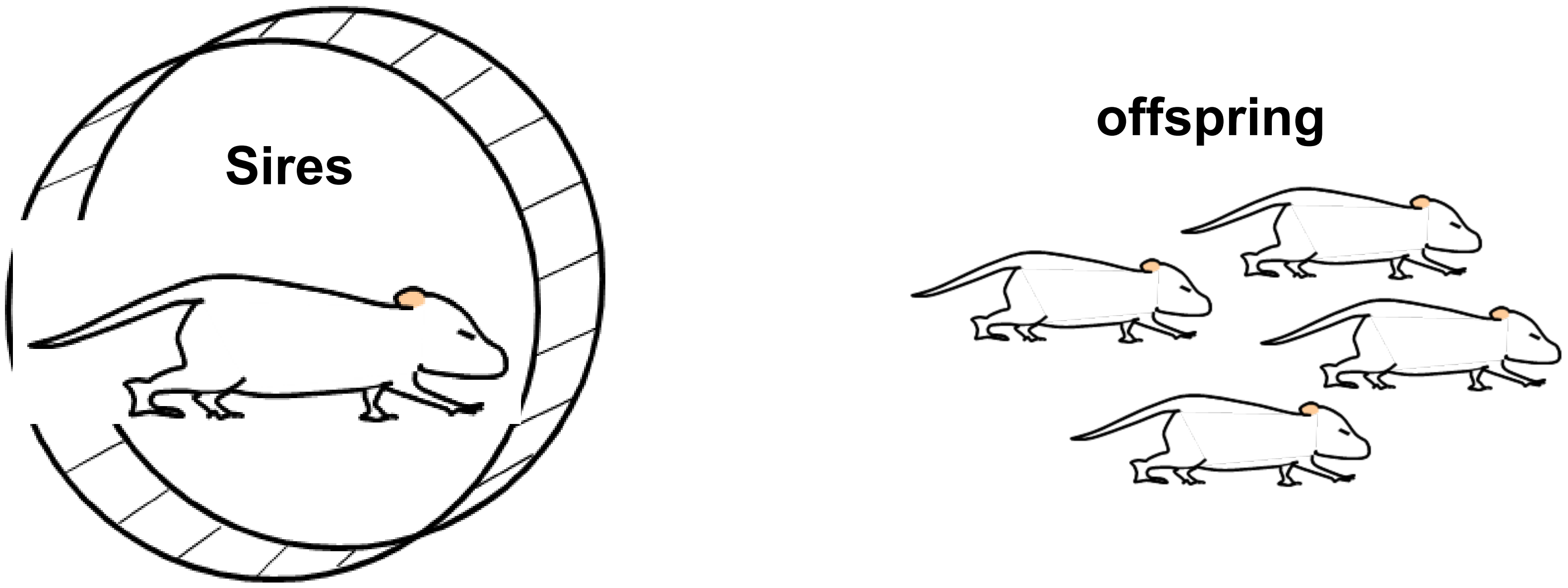
Bred with chow-fed, sedentary, female C57Bl/6 mice (8 wks old)

Effects of High Fat Diet and Exercise Training on the Male Breeders



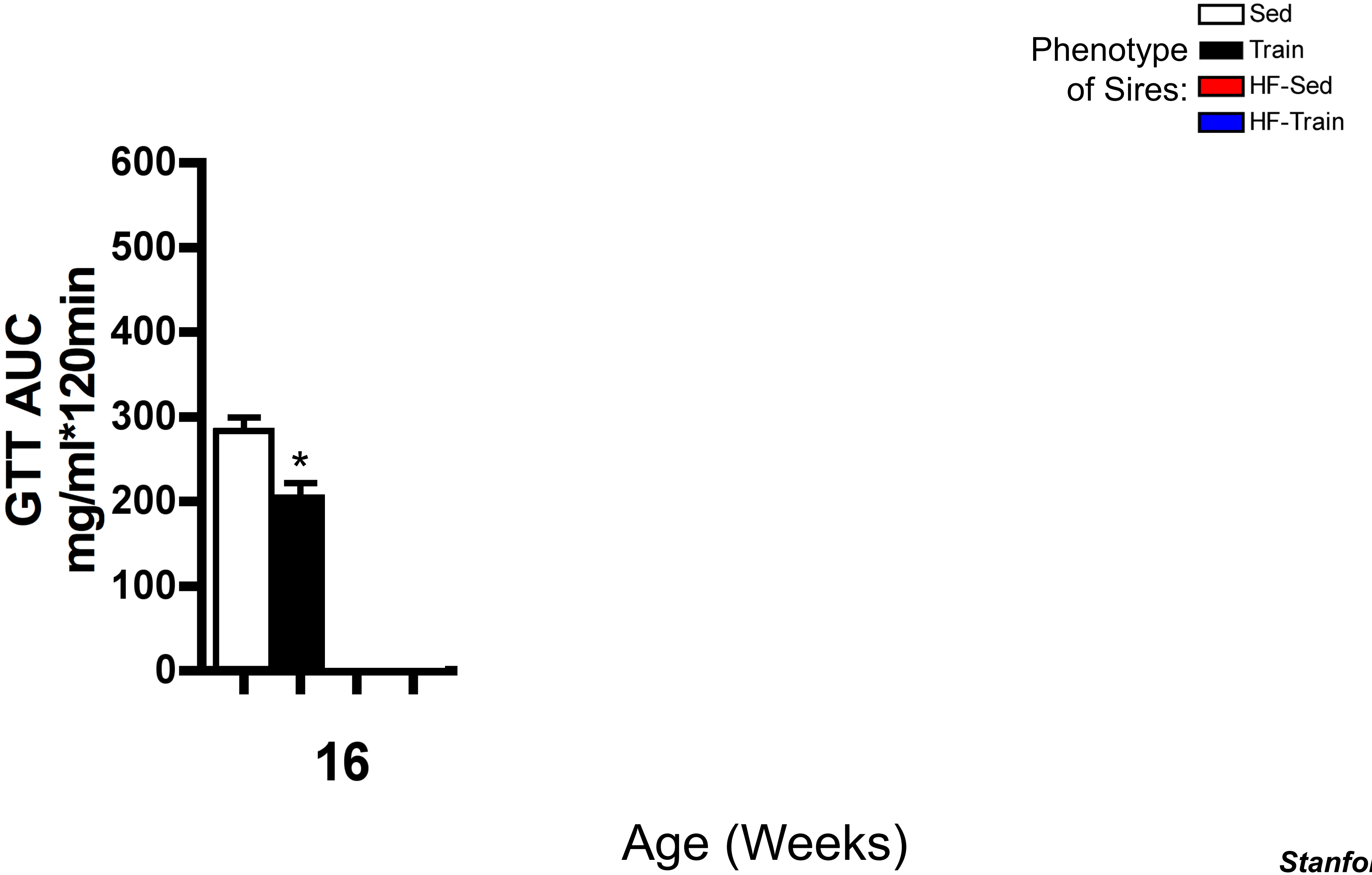
- Running distance 5-6 km/day; no difference between chow and fat-fed mice.

Experimental Design – Effects on Offspring Metabolic Health



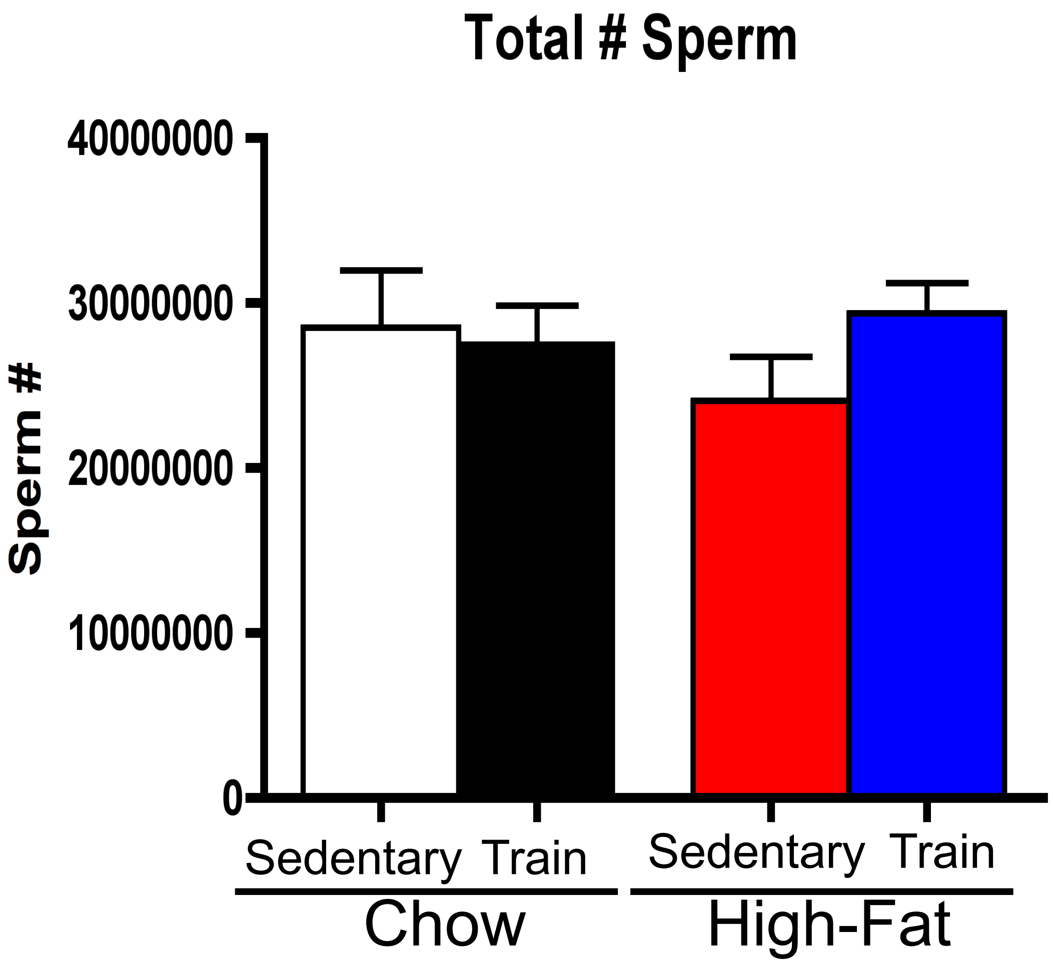
- **Offspring were sedentary and chow-fed**
- **Offspring metabolic health monitored from youth until middle age (52 weeks)**

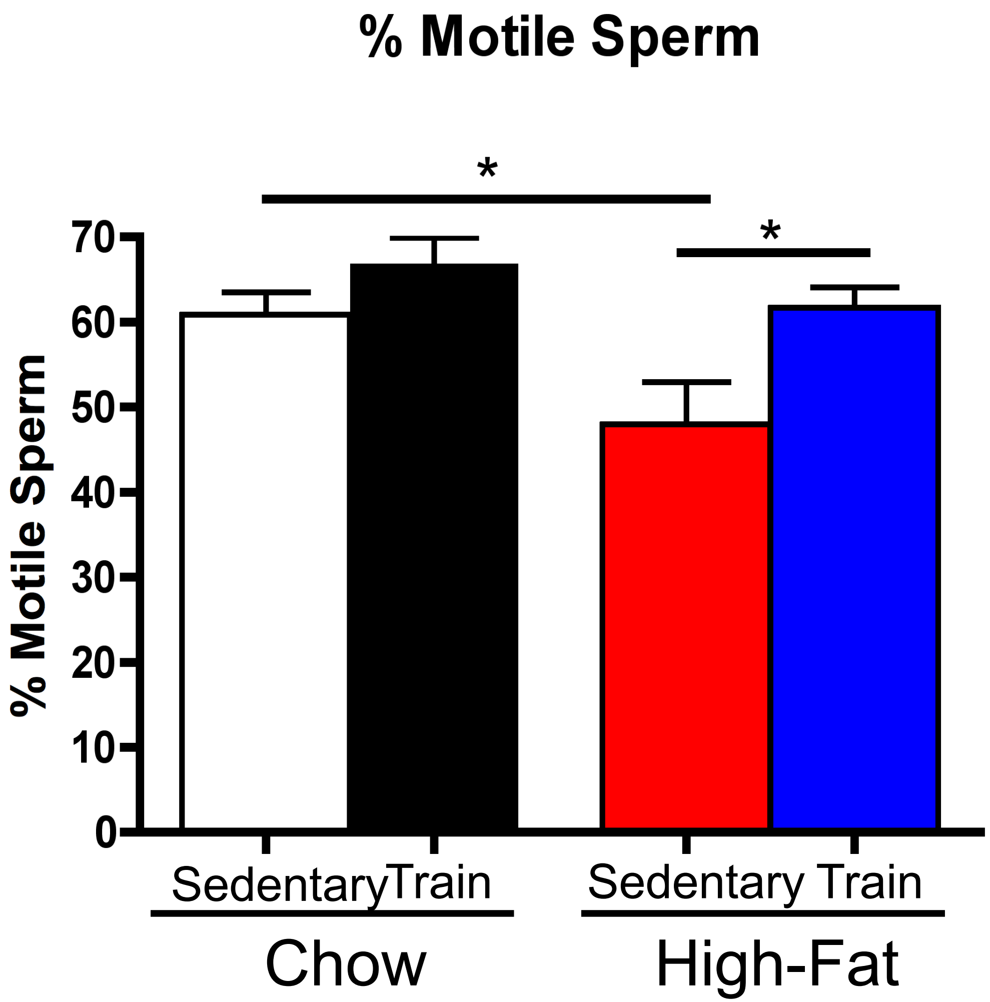
Paternal Exercise Improves Glucose Tolerance in Male Offspring



Stanford et al, Diabetes 2018

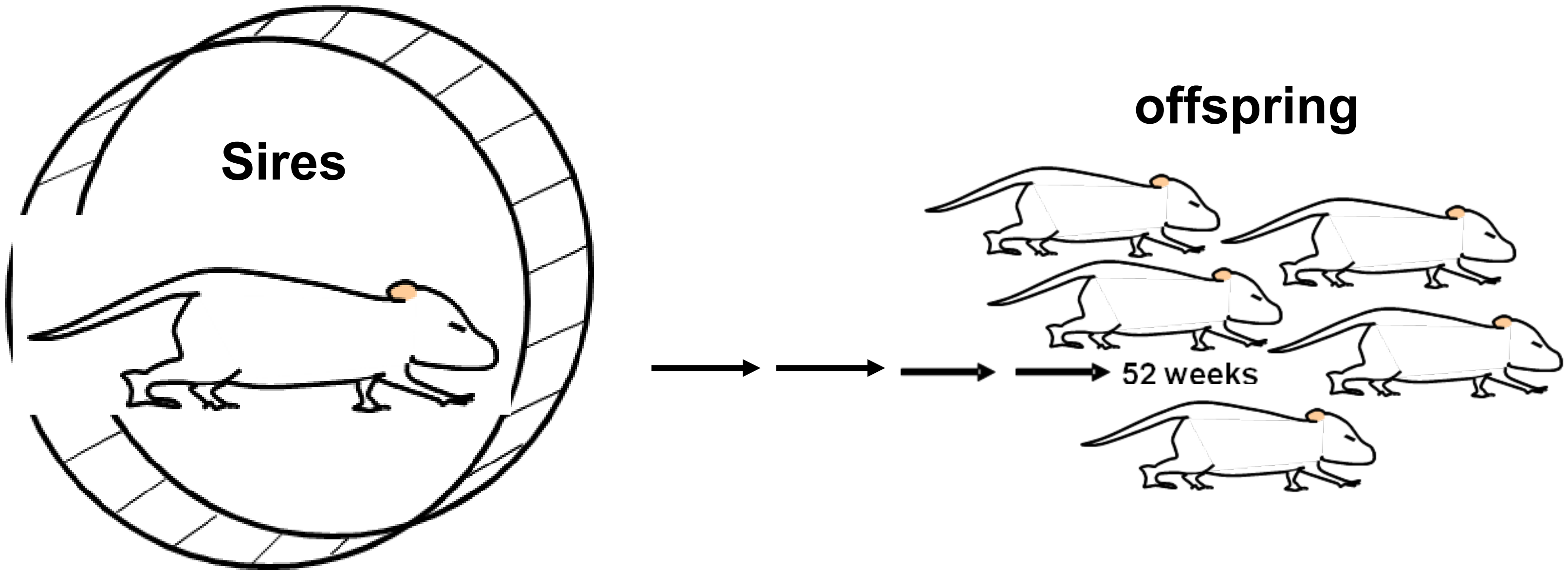
Exercise Training Prevents the Decrease in Sperm Motility with High Fat Feeding





Stanford et al, Diabetes 2018

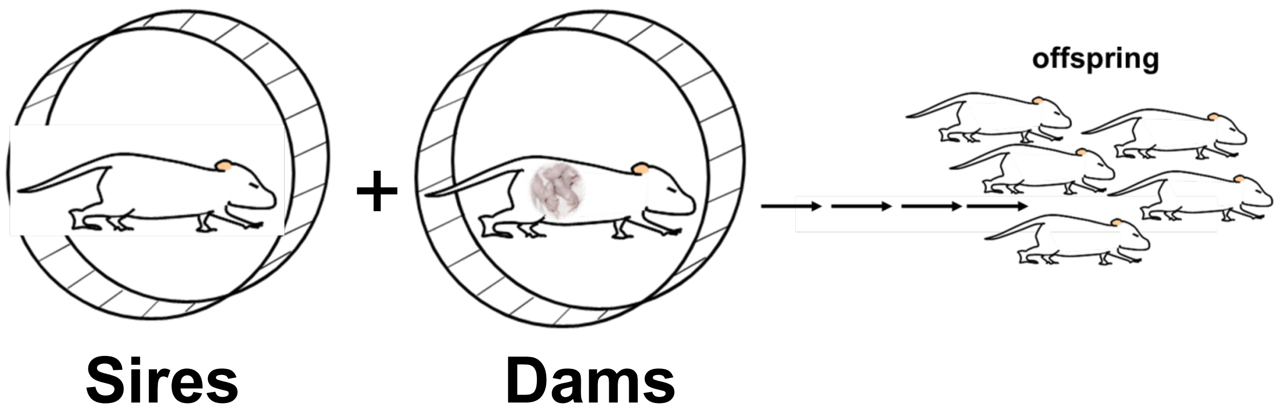
Summary



- Paternal exercise for 3 weeks prior to breeding improves the metabolic health of male and female offspring, and reverses the detrimental effects of sire high-fat feeding.

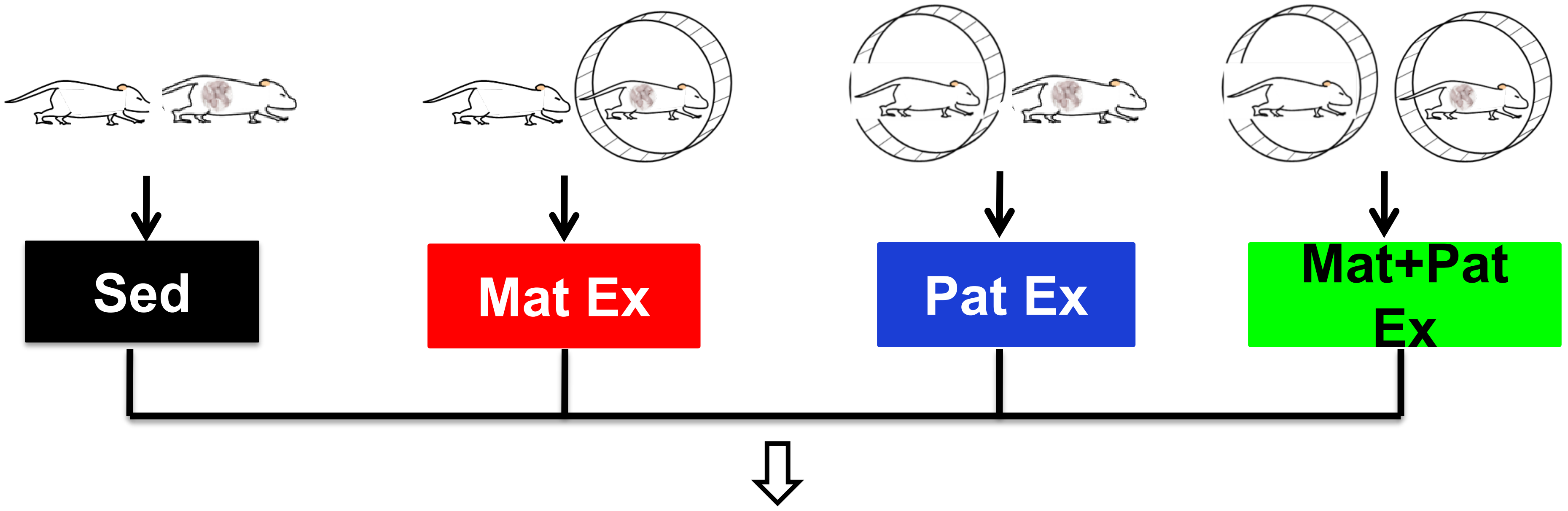
- Paternal exercise alters the motility and the non-coding RNA profile of sperm; how these may alter offspring health is under investigation.

Effects a Combined Paternal and Maternal Exercise on Offspring Glucose Tolerance



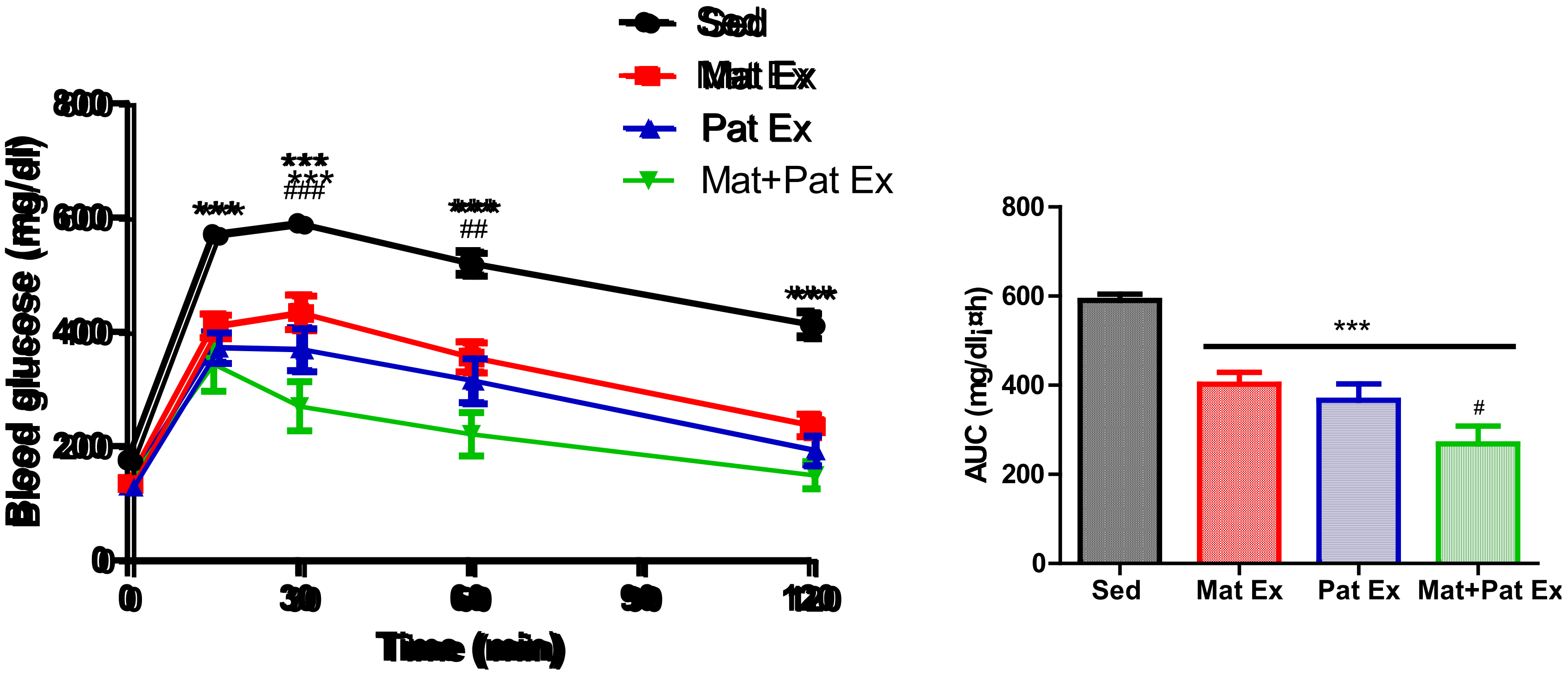
Experimental Design

- All parents fed a high-fat diet (60% kcal from fat)



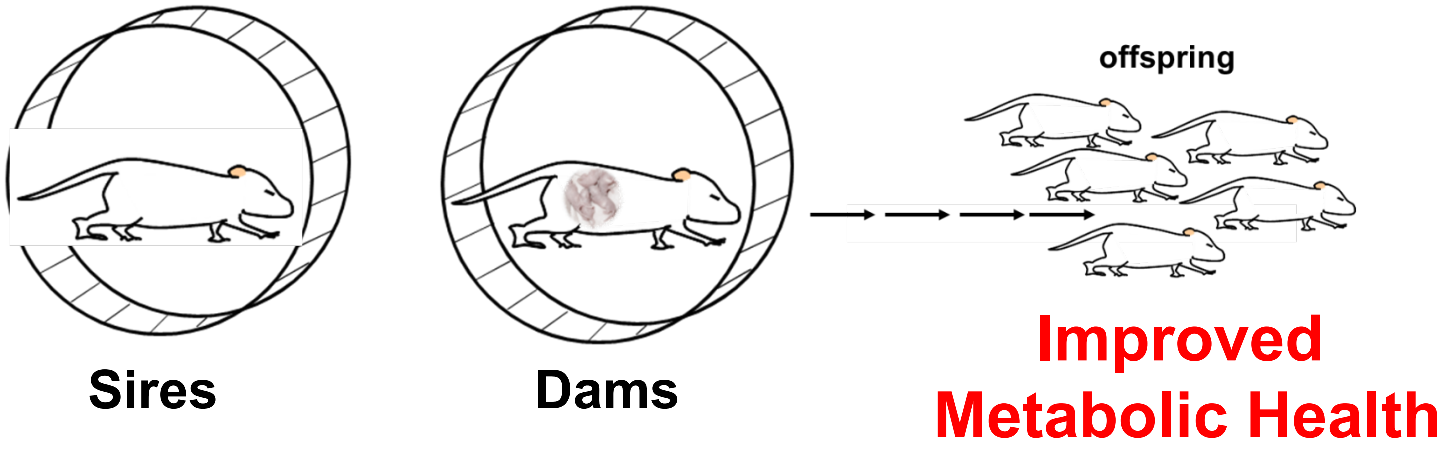
- Offspring were sedentary and chow-fed
- Offspring glucose tolerance at 52 weeks of age

Improved Glucose Tolerance in Male Offspring from Maternal and Paternal Exercise at 52 Weeks of Age



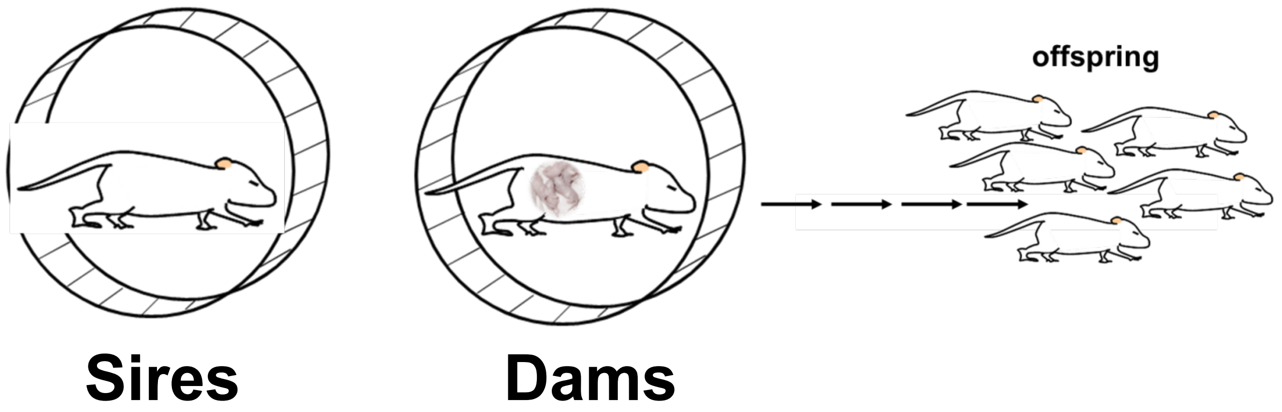
Data represent mean ± SEM
***P<0.001, #P<0.05 , ##P<0.01 ###P<0.001
one mice per litter, n=6 each group

Summary



- The combination of both paternal and maternal exercise may have even greater beneficial effects on offspring metabolic health.

Maternal and Paternal Exercise Training



- Maternal, Paternal and Maternal + Paternal Exercise: effects on offspring glucose tolerance.
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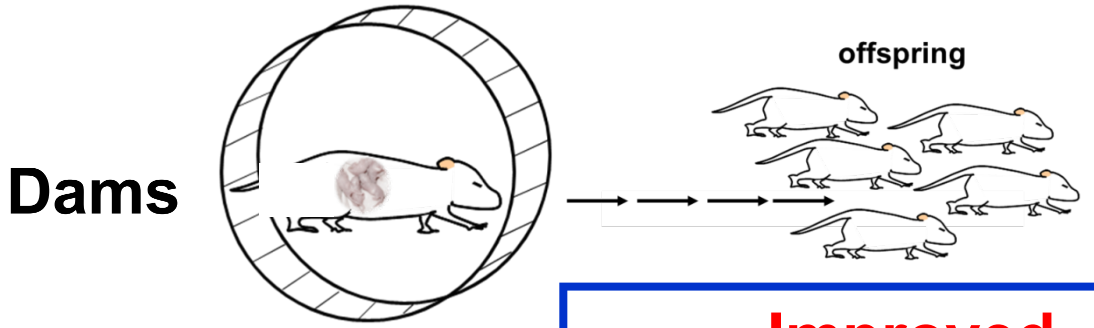
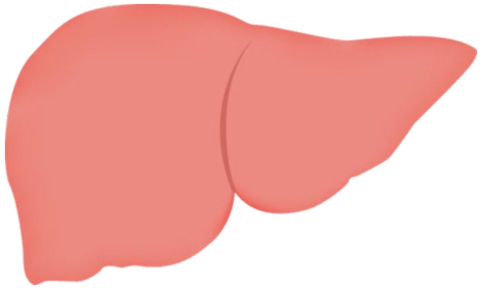
- Maternal Exercise: Identification of a novel protein that can improve offspring health.

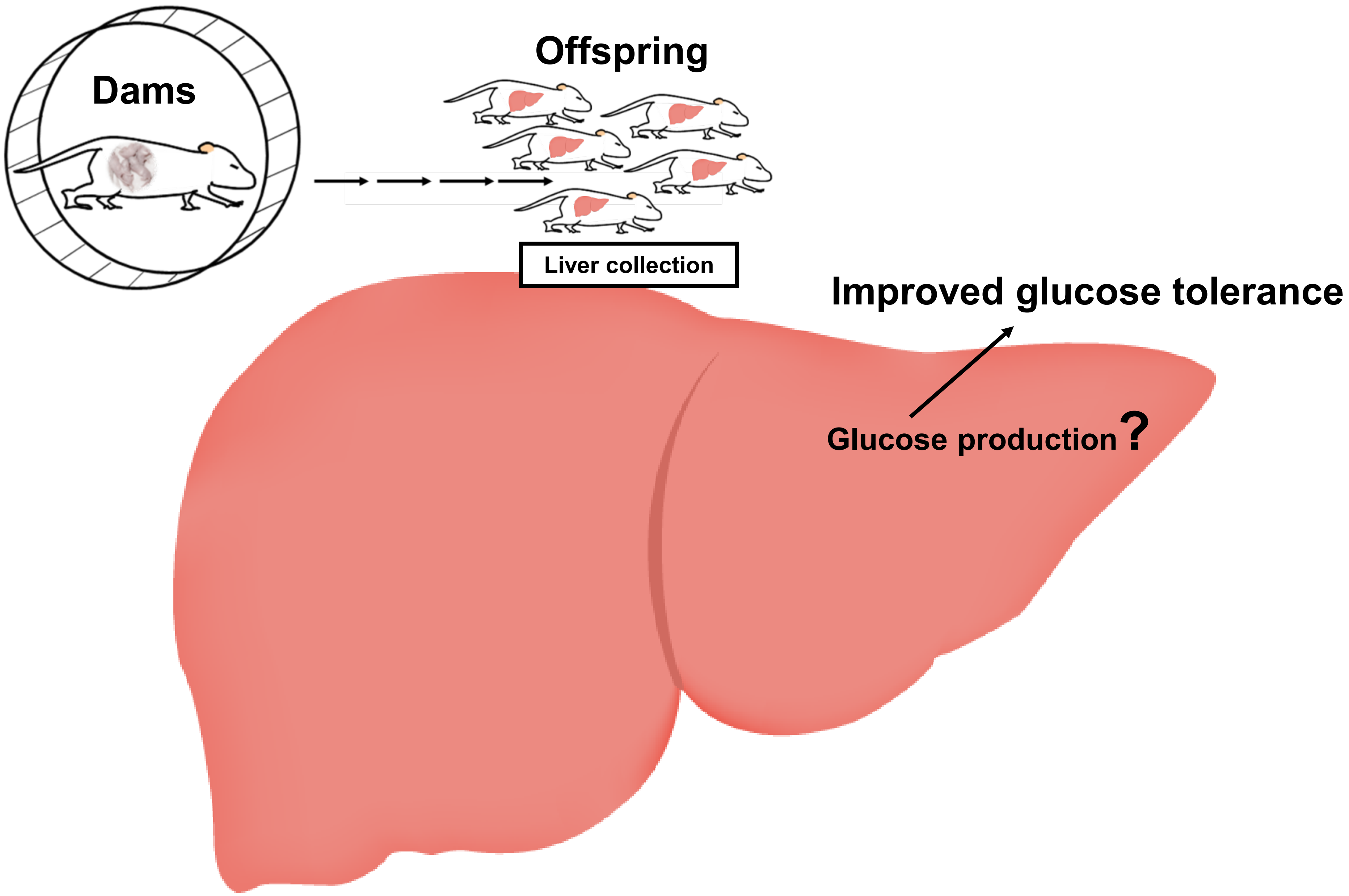
Maternal Exercise Training



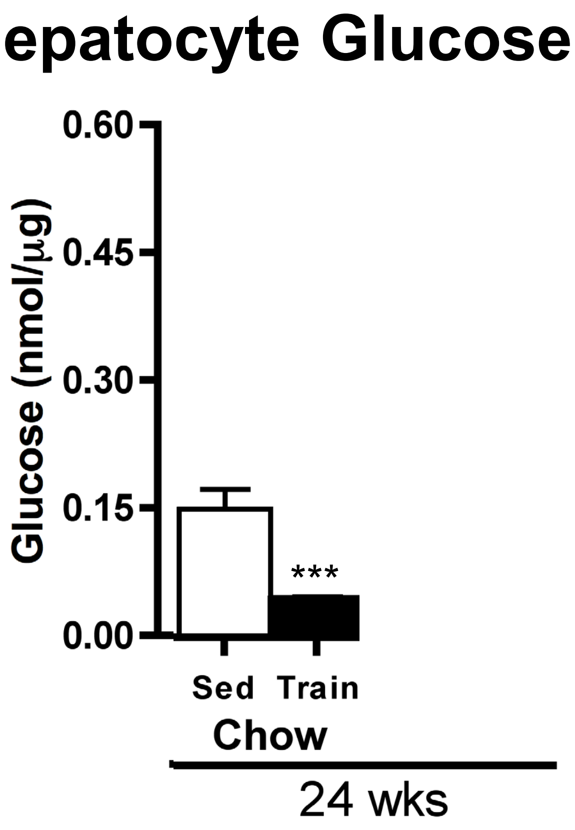
Improved
Glucose Homeostasis

- What tissue(s) are responsible for the improved glucose tolerance in the offspring of mothers that exercised?



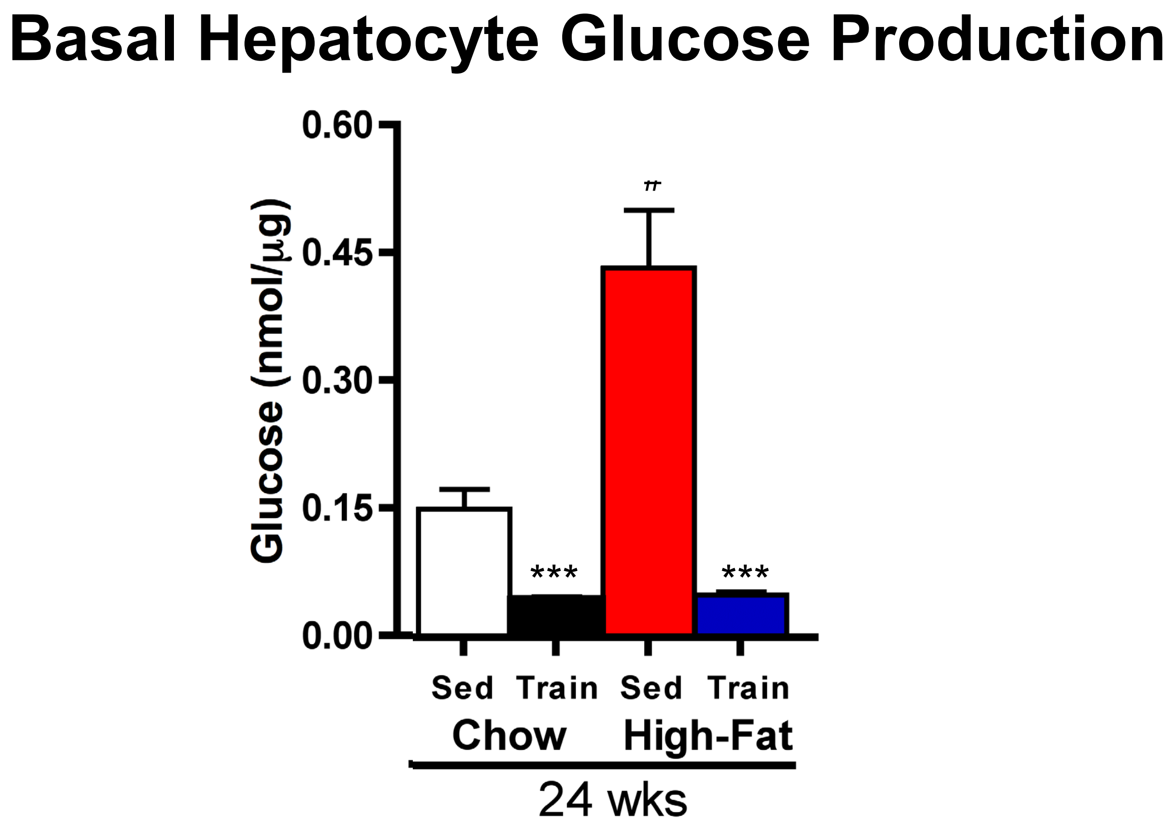

Dams
Offspring
Liver collection
Improved glucose tolerance
Glucose production?

Maternal Exercise Effects Glucose Production in Isolated Hepatocytes of Offspring

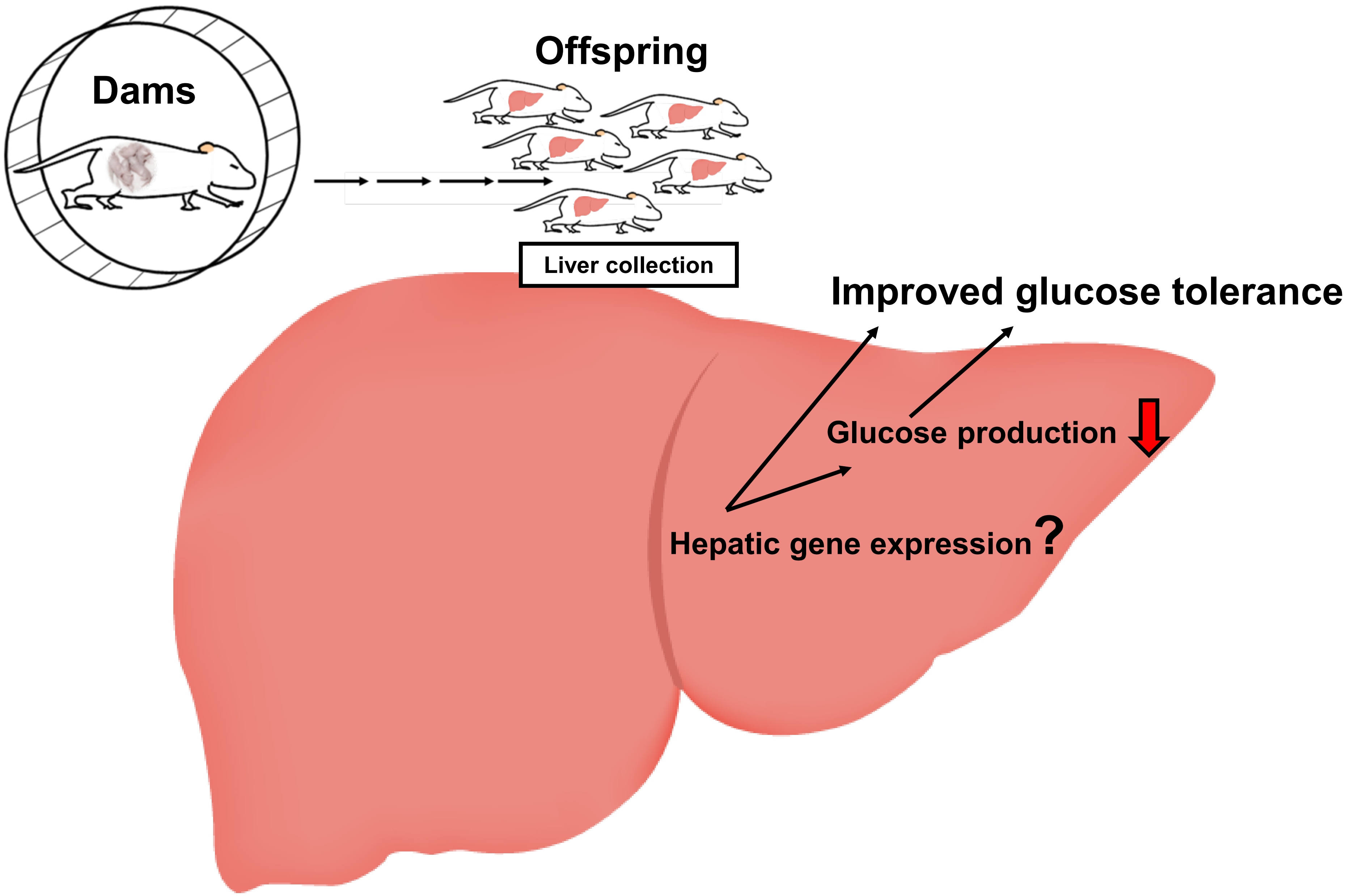


Stanford et al, Diabetes 2017

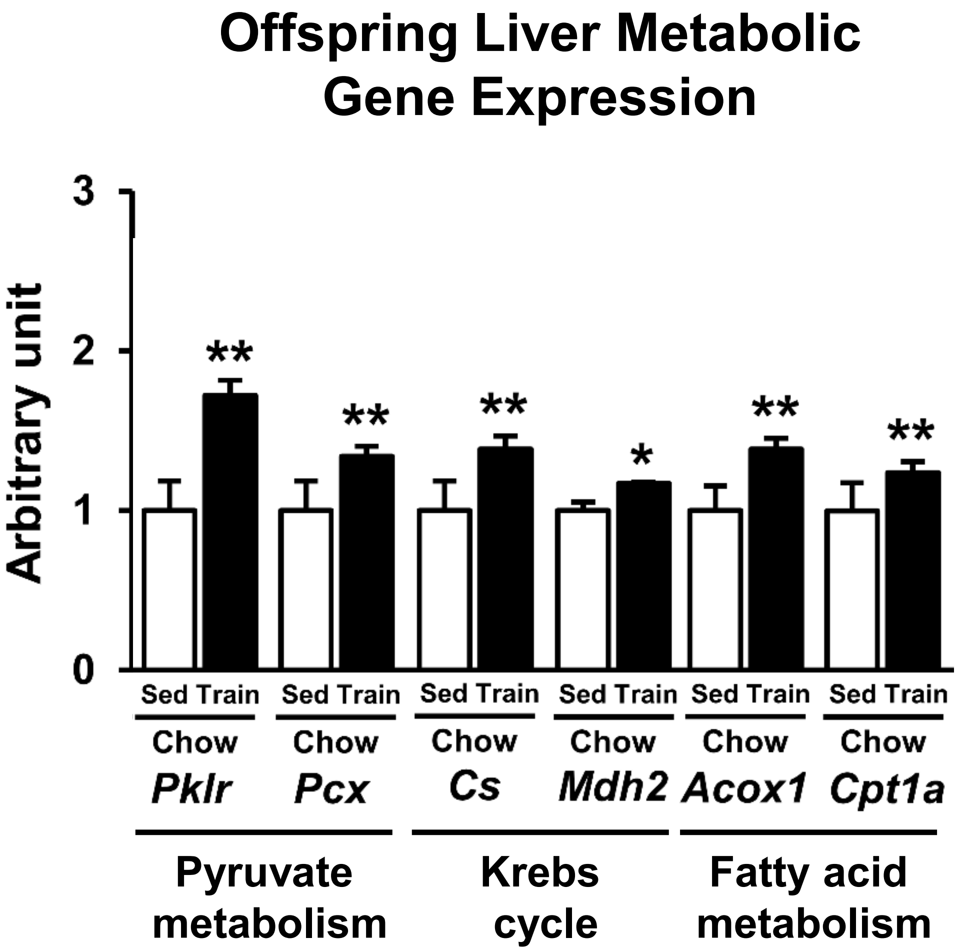
Maternal Exercise Effects Glucose Production in Isolated Hepatocytes of Offspring



Stanford et al, Diabetes 2017

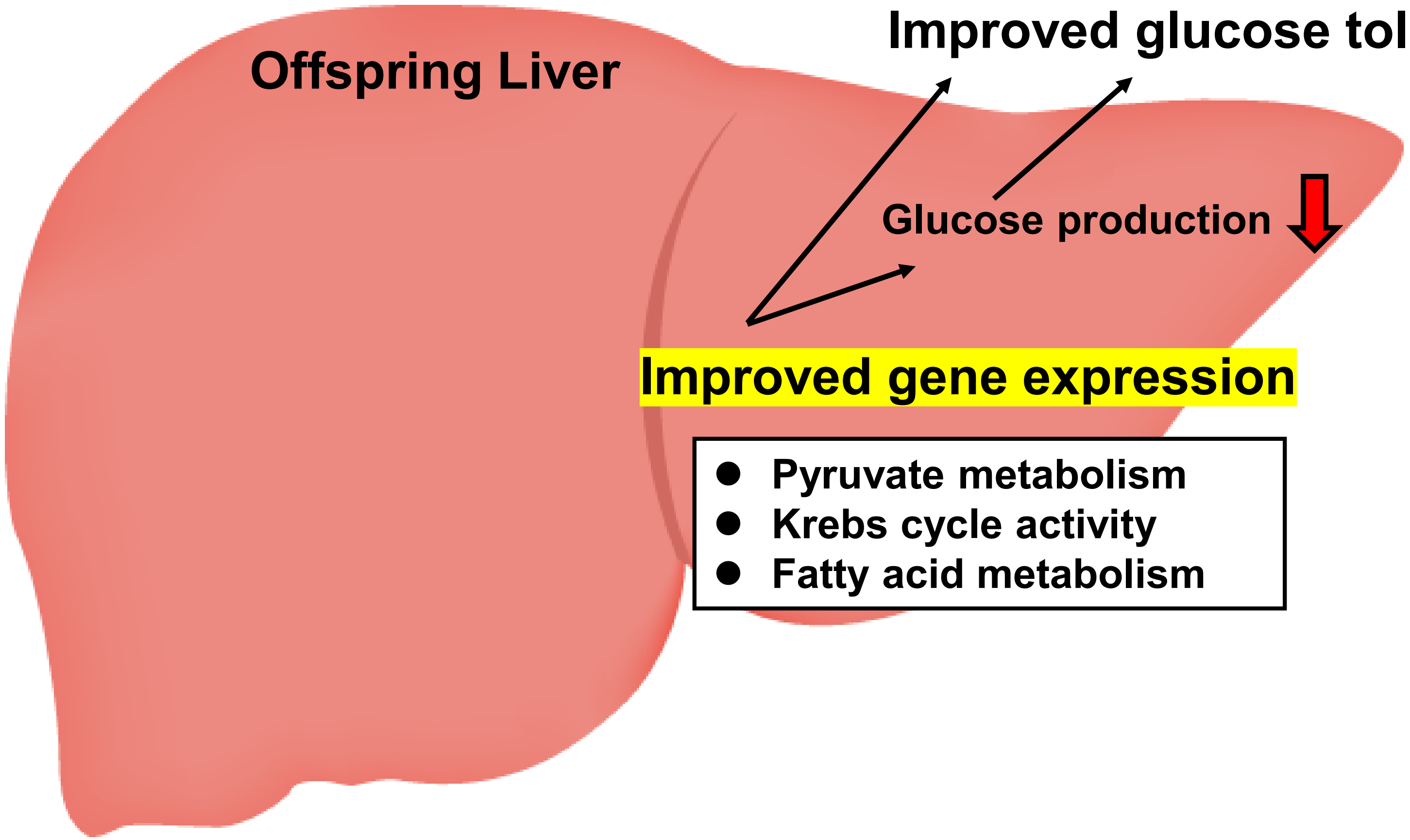

Dams
Offspring
Liver collection
Improved glucose tolerance
Glucose production
Hepatic gene expression?

Maternal Exercise in Mice Results in Improved Offspring Liver Gene Profile

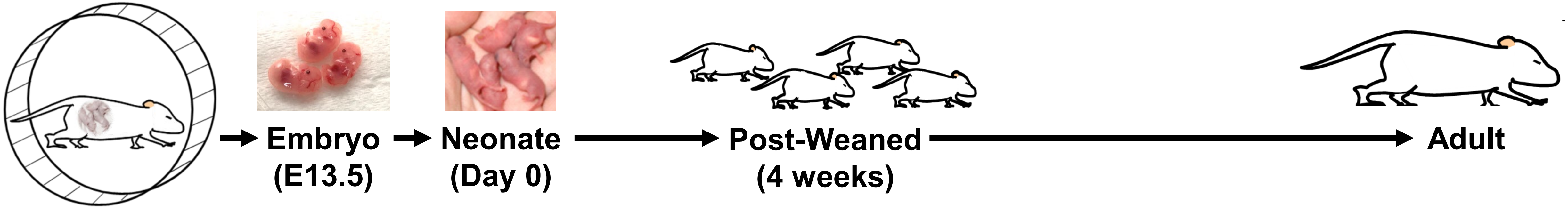


Stanford et al. *Diabetes* 2015 ; Stanford et al, *Diabetes* 2017

Effects of Maternal Exercise on Offspring Liver Function

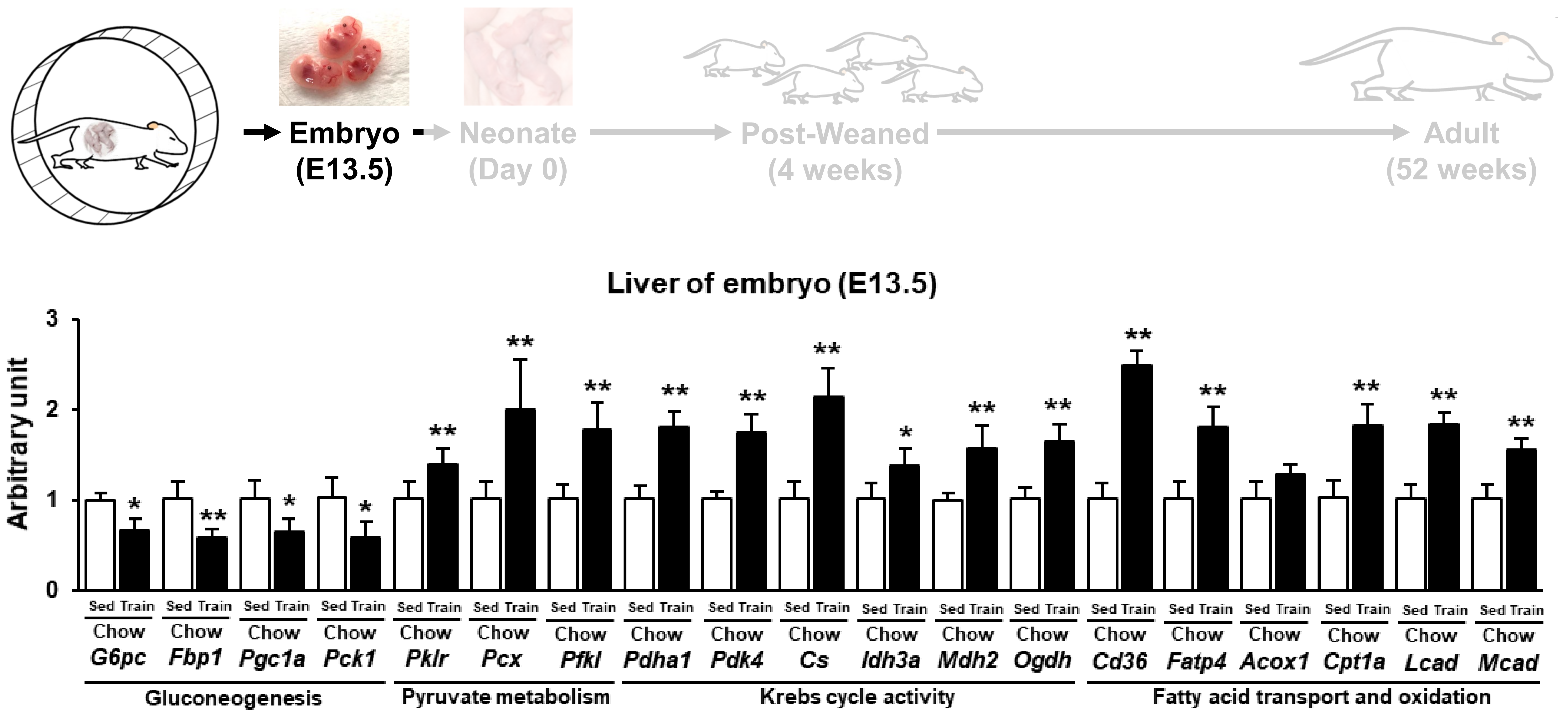


Maternal Exercise Training

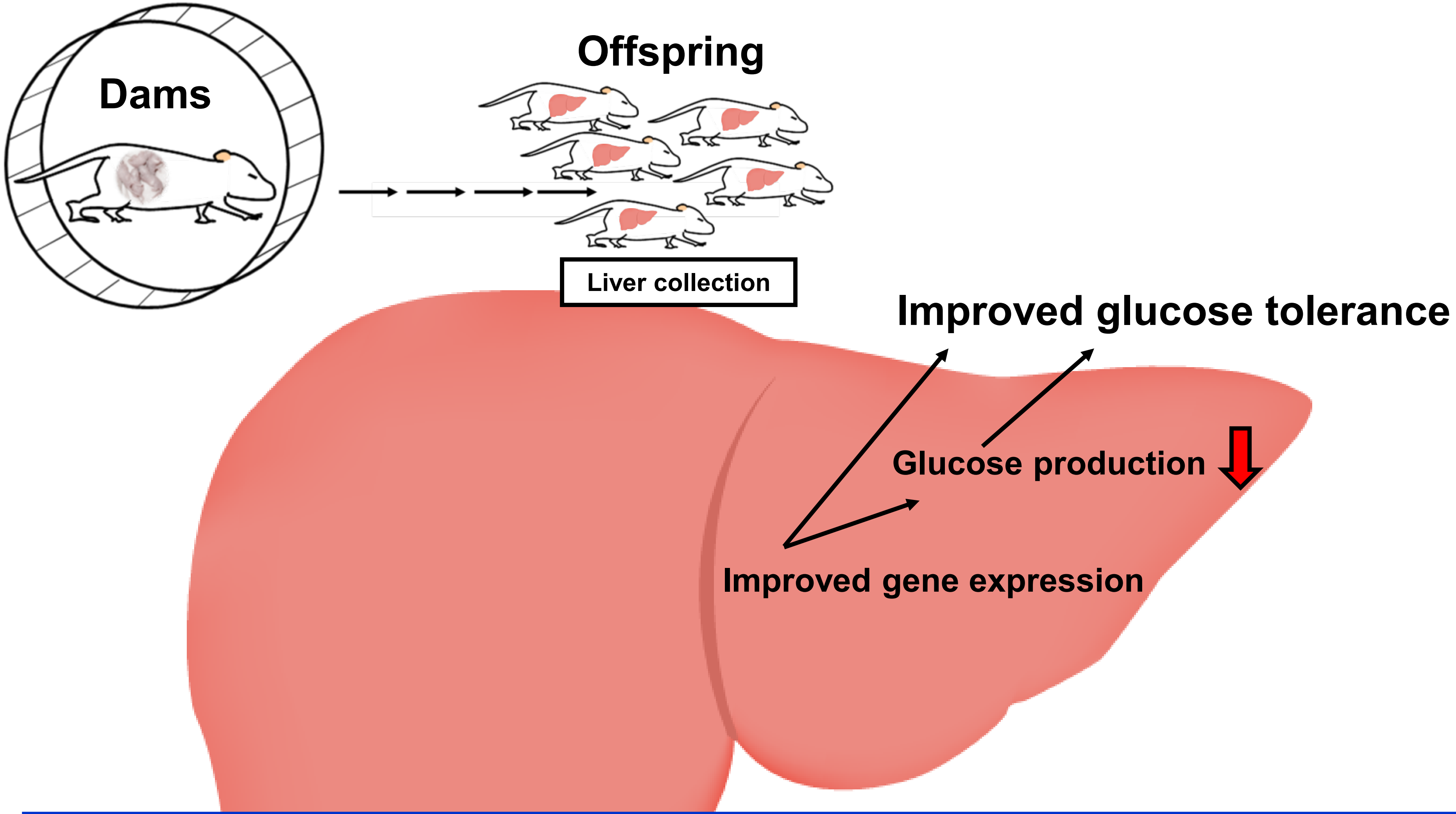


- **At what stage in the offspring development do the changes in hepatic gene expression occur?**

Maternal Exercise Affects Hepatic Gene Expression in Young Offspring



Summary

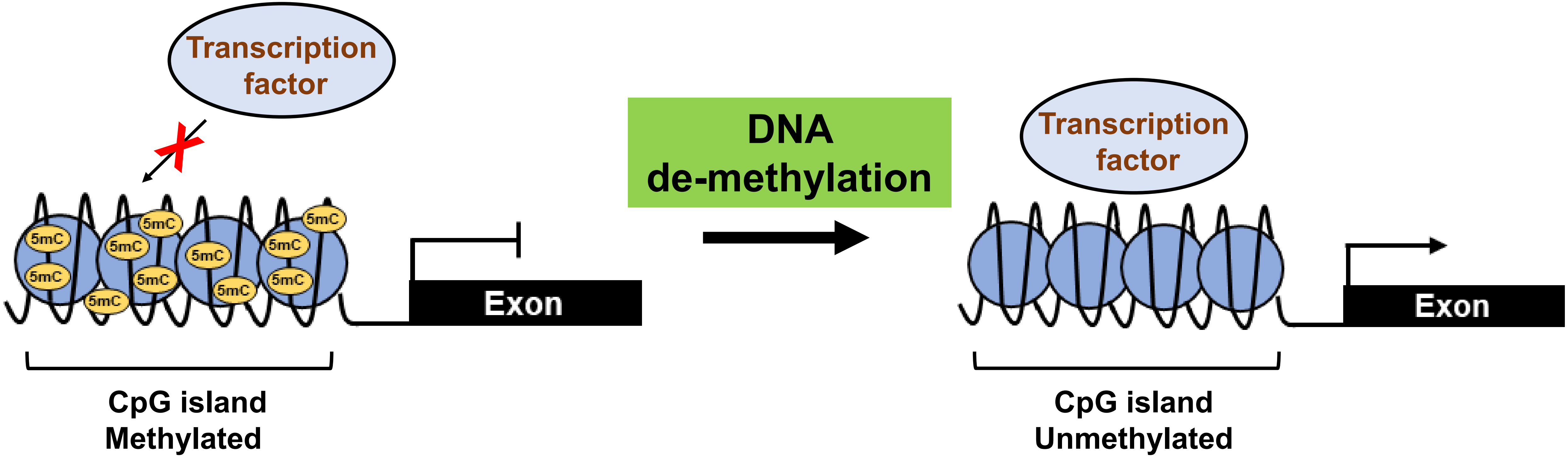


➢Changes in offspring gene expression occur in early development, suggesting epigenetic changes.

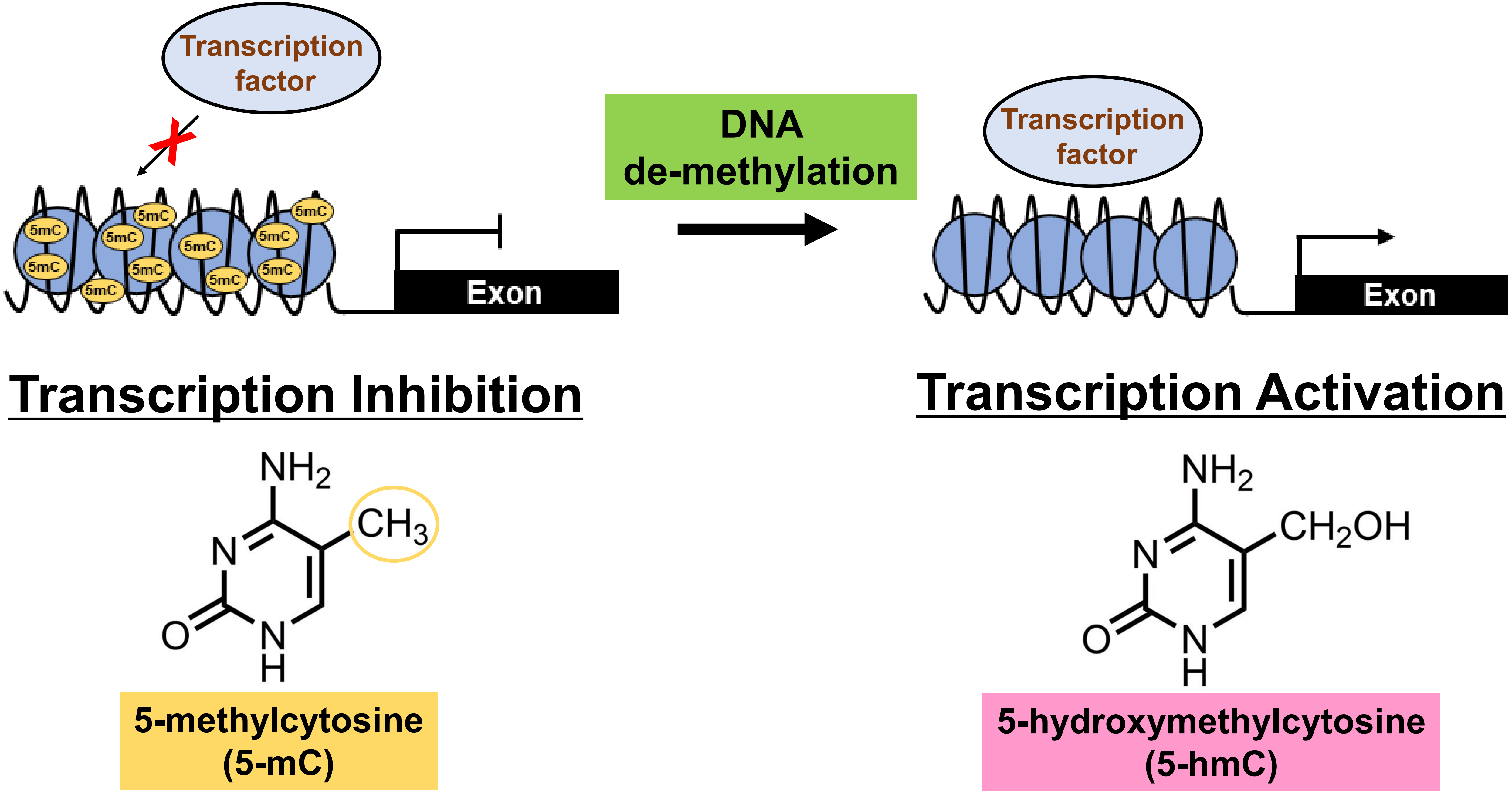
Epigenetics

Heritable Changes in Gene Function that DO NOT Involve Changes in the DNA Sequence

- **DNA cytosine methylation**
- **Histone post-translational modifications in chromatin**
- **Regulation by non-coding RNAs**

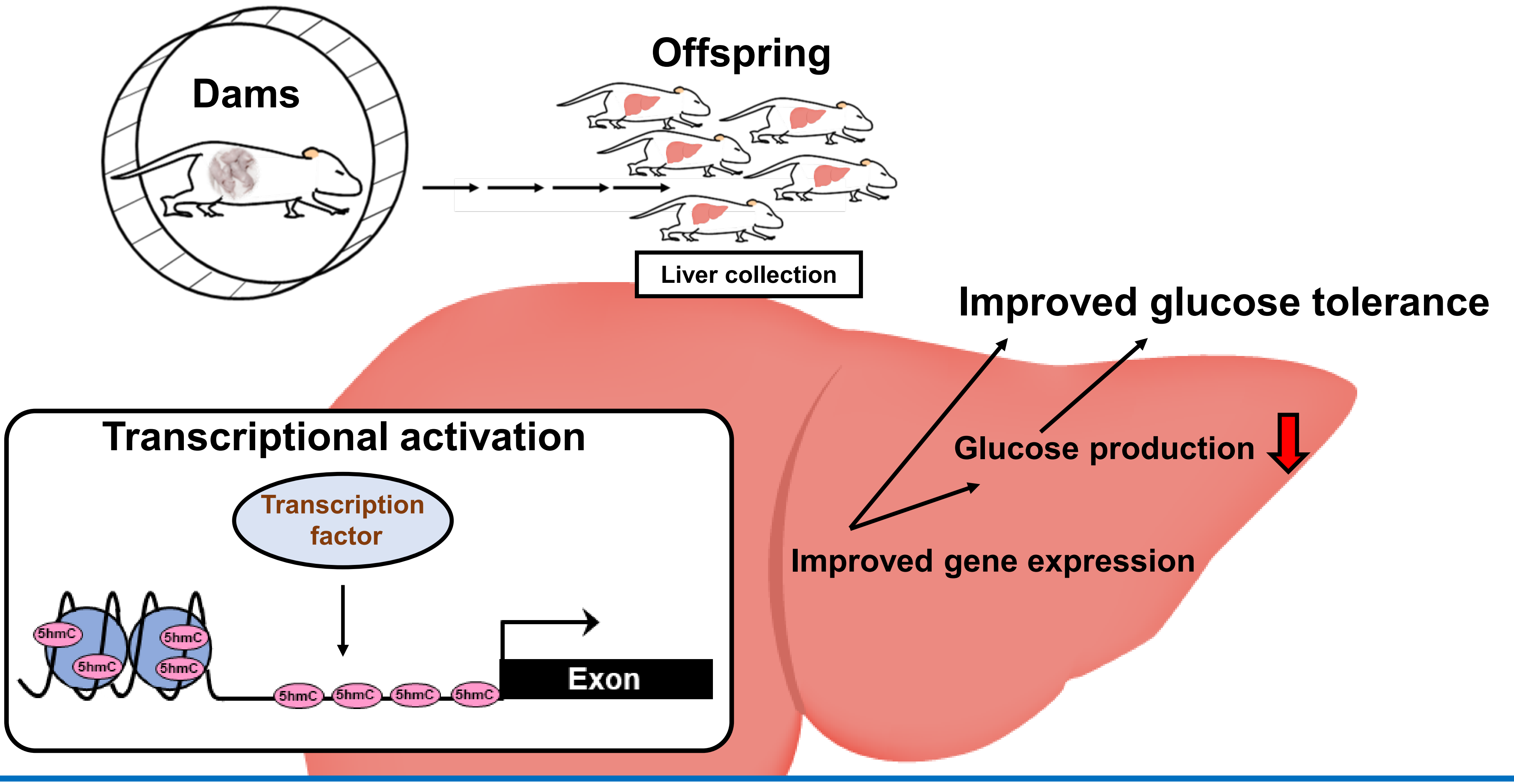


Juan et al. *Annu Rev Nutr* (2015), partly modified



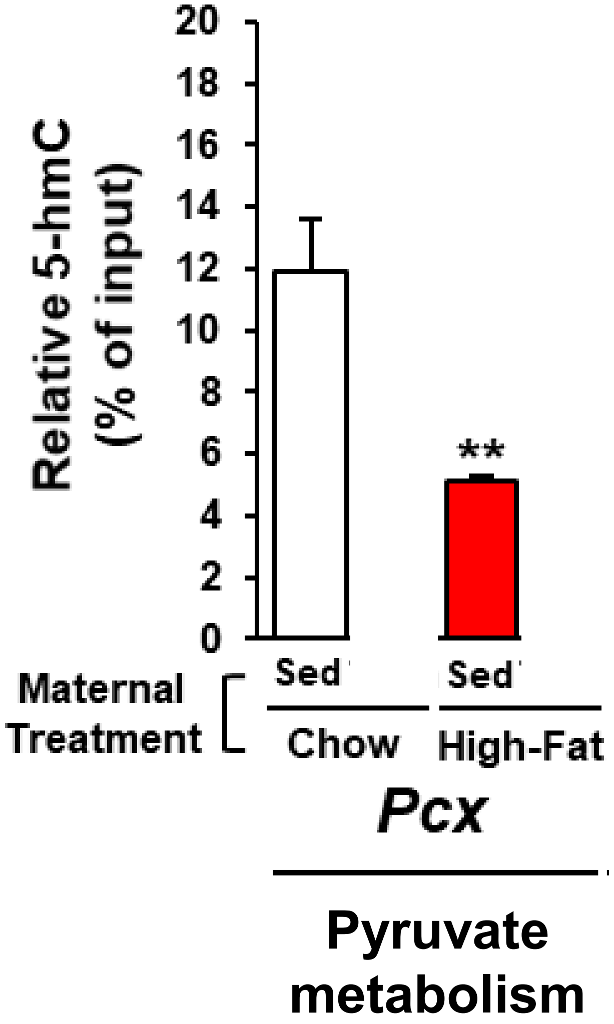
Transcription factor
5mC
Exon
DNA de-methylation
Transcription factor
Exon
Transcription Inhibition
Transcription Activation
NH2
CH3
N
O
N
H
NH2
CH2OH
N
O
N
H
5-methylcytosine (5-mC)
5-hydroxymethylcytosine (5-hmC)

Increased Hepatic Gene Transcription May Be Caused by DNA Demethylation

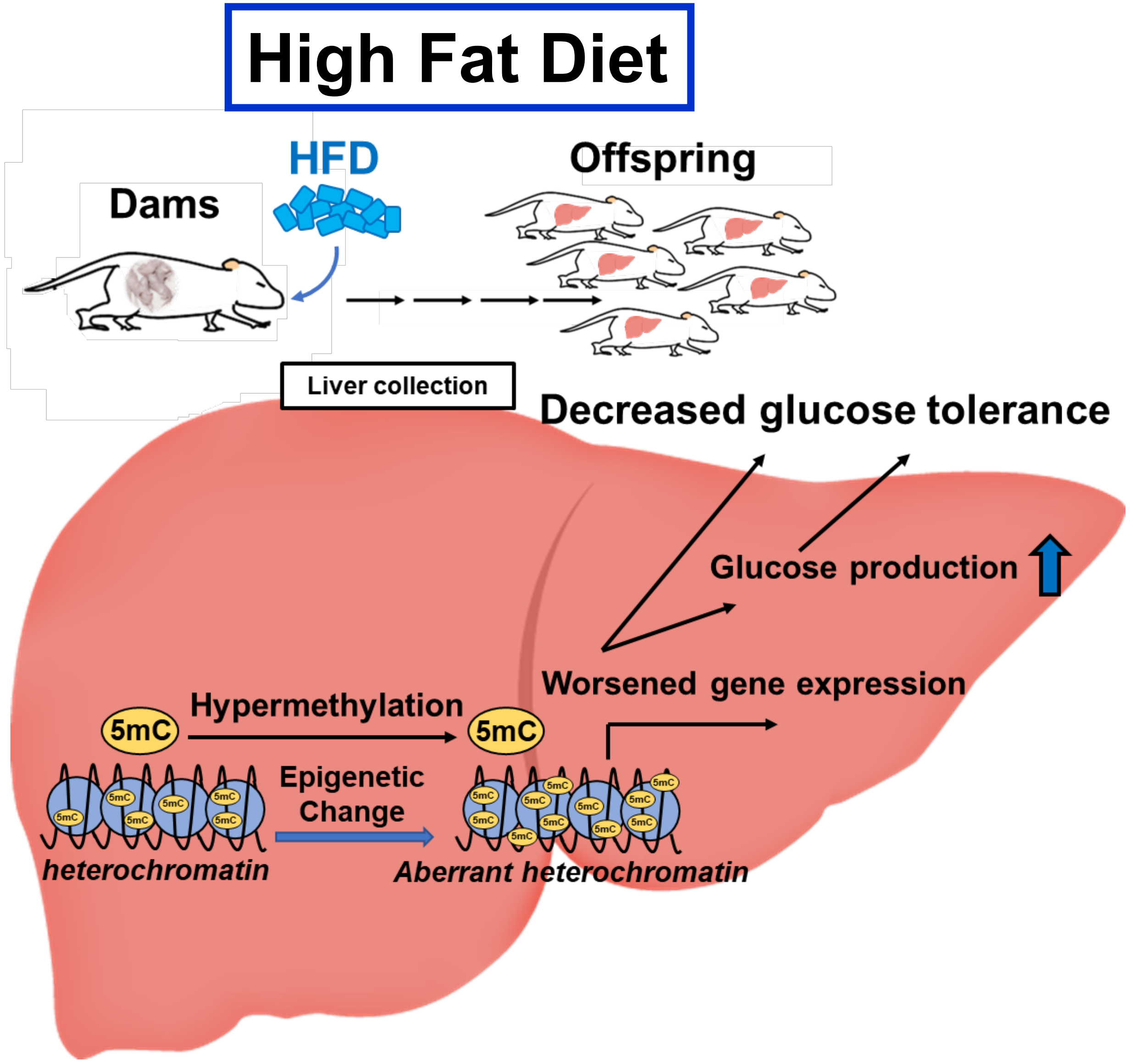


- **Does high-fat feeding decrease 5-hmC and does maternal exercise increase 5-hmC in liver of offspring?**

Maternal Exercise Increases 5-hmC Generation at the Promoters of Metabolic Genes in Offspring Liver

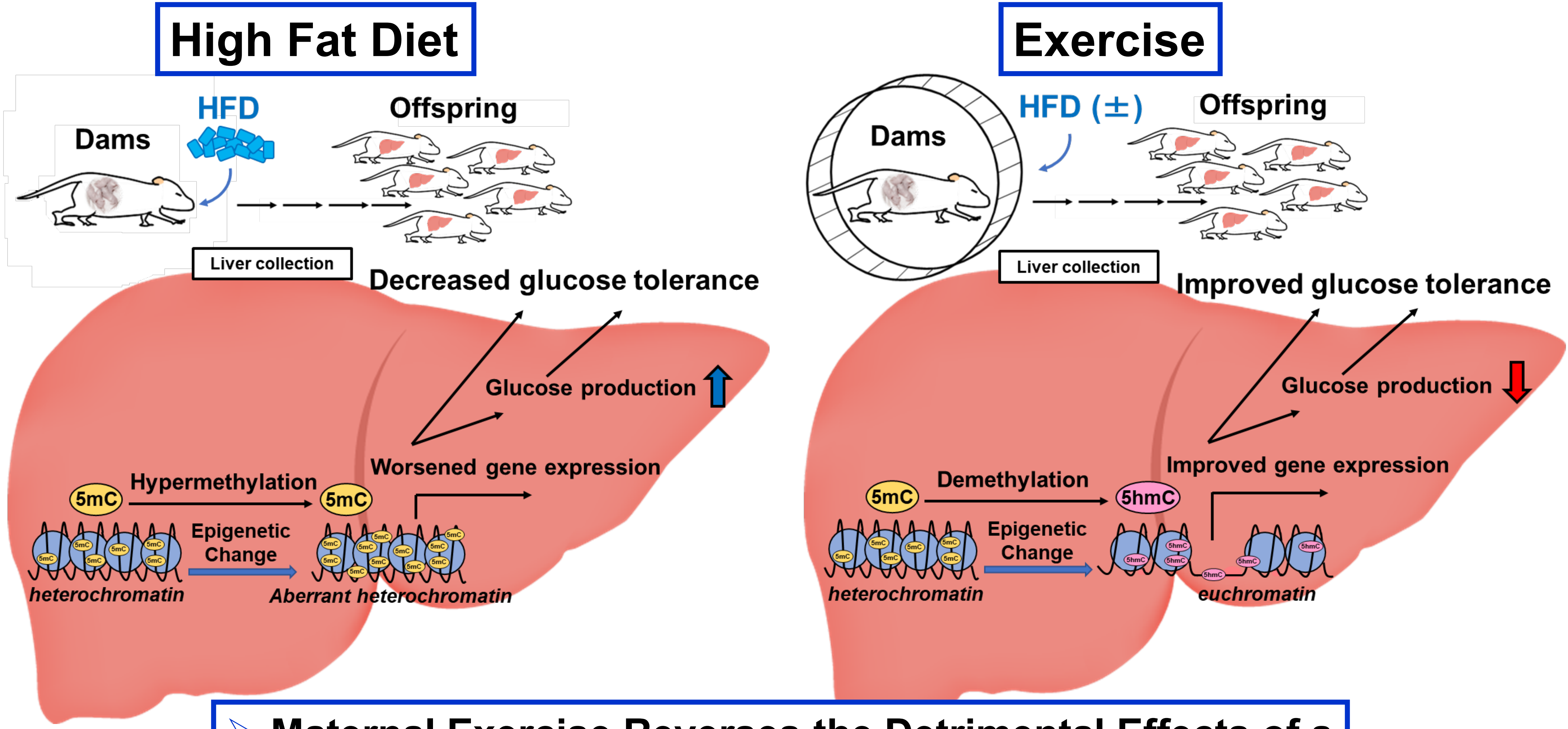


Summary

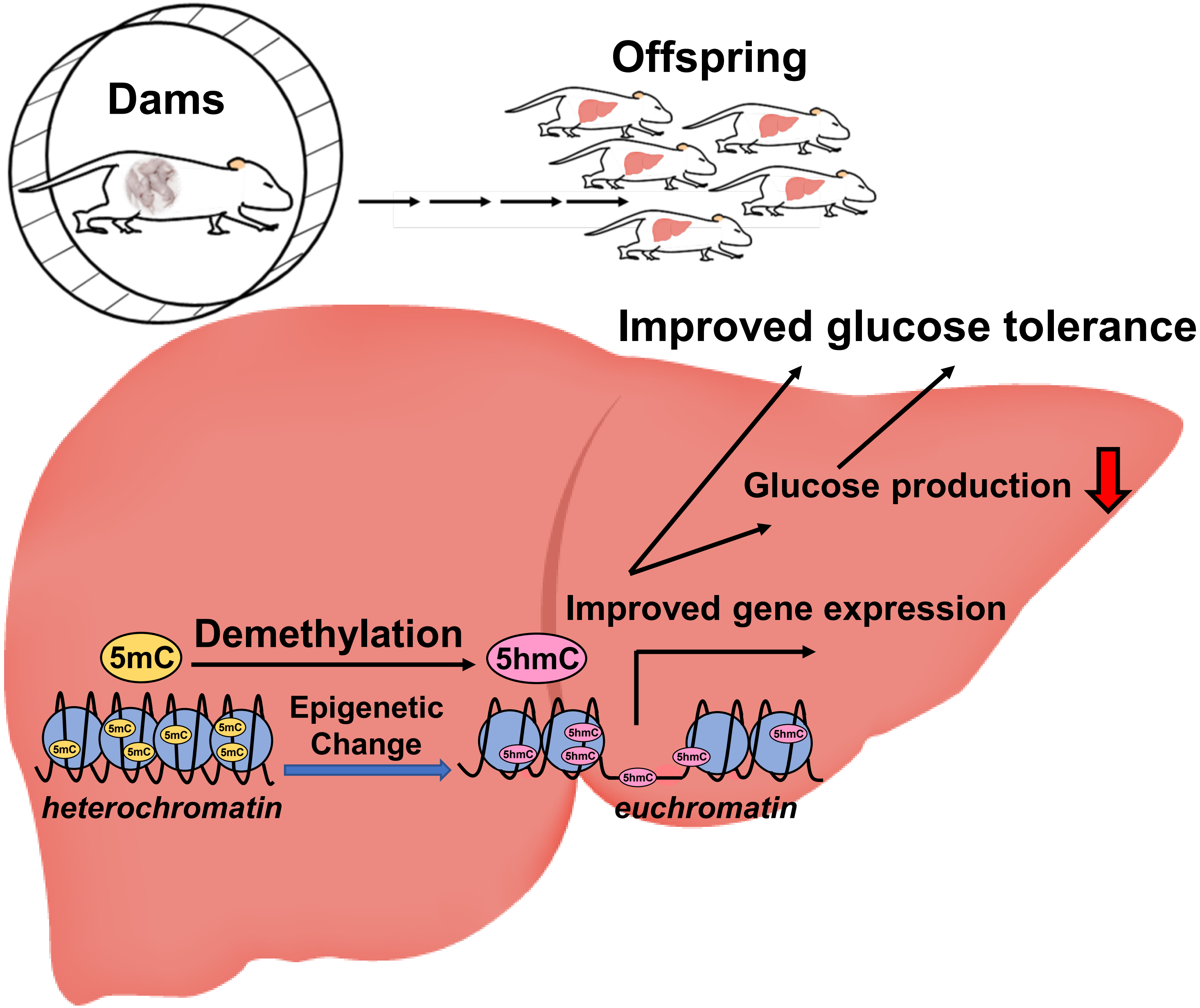


High Fat Diet
HFD
Dams
Offspring
Liver collection
Decreased glucose tolerance
Glucose production
Worsened gene expression
Hypermethylation
5mC
5mC
Epigenetic
Change
heterochromatin
Aberrant heterochromatin

Summary

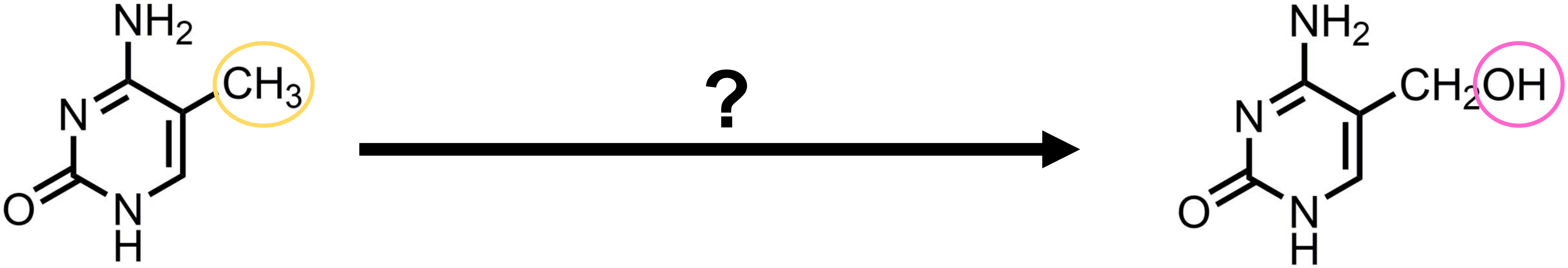


➢ **Maternal Exercise Reverses the Detrimental Effects of a High Fat Diet on Offspring Liver Methylome**



What are the molecular signals regulating demethylation?

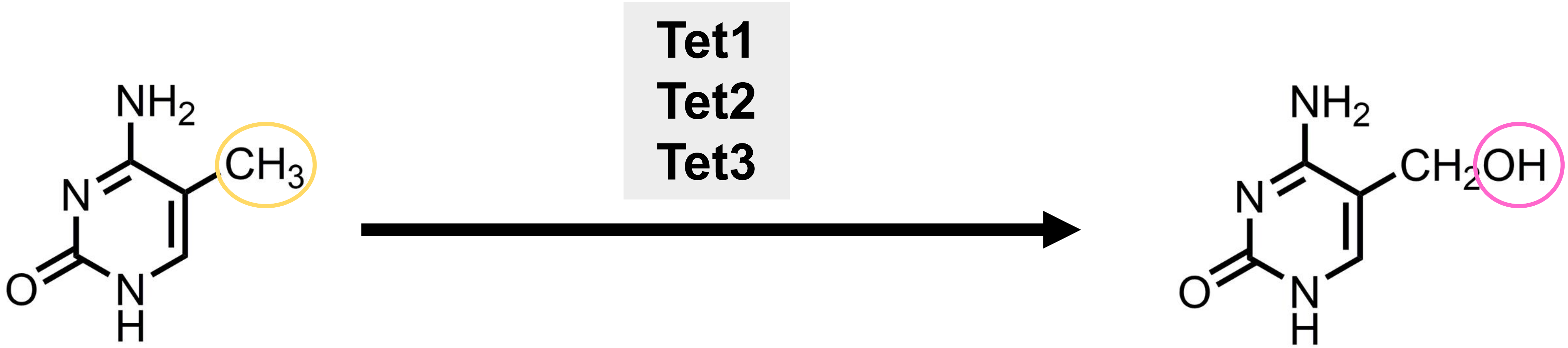
Regulation of Methylation



5-methylcytosine (5-mC)

5-hydroxymethylcytosine (5-hmC)

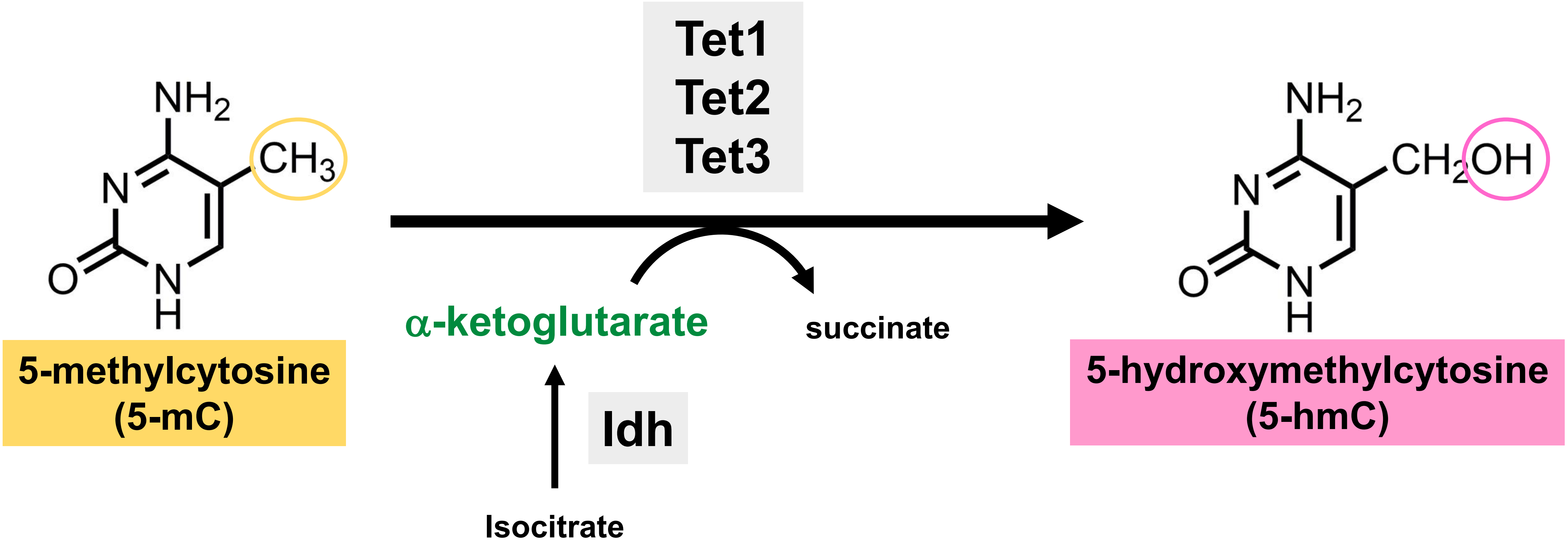
Regulation of Methylation by Ten-Eleven Translocation (Tet) Enzymes



5-methylcytosine (5-mC) → **5-hydroxymethylcytosine (5-hmC)**

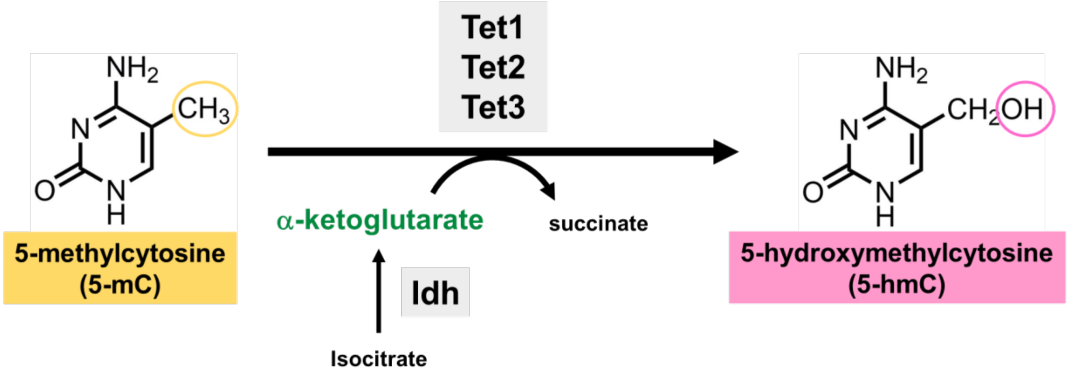
- **Does maternal exercise training regulate Tet activity in offspring liver?**

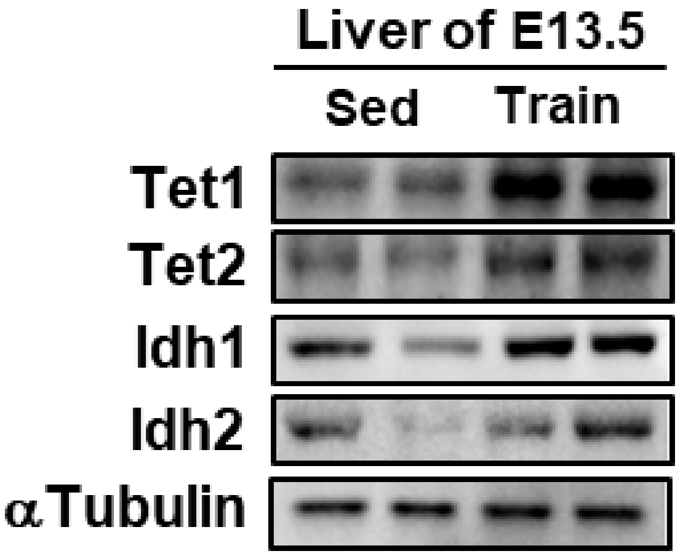
Regulation of Tet Activity



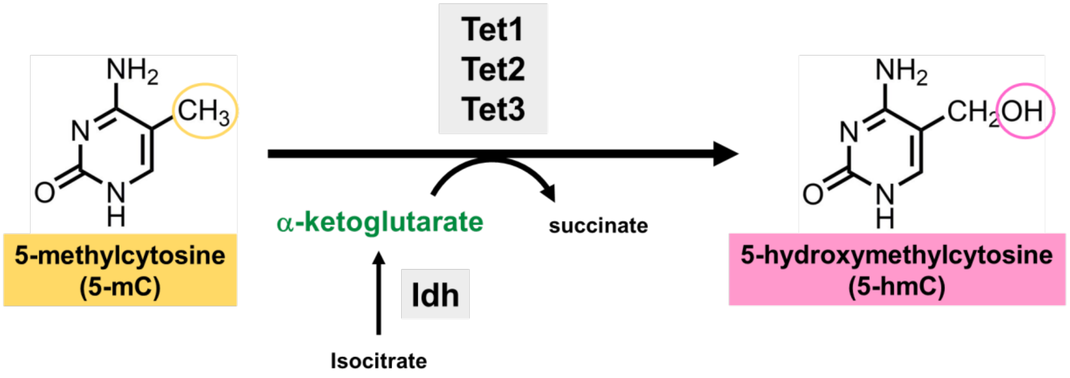
- Does maternal exercise training regulate Idh and α-ketoglutarate in offspring liver?

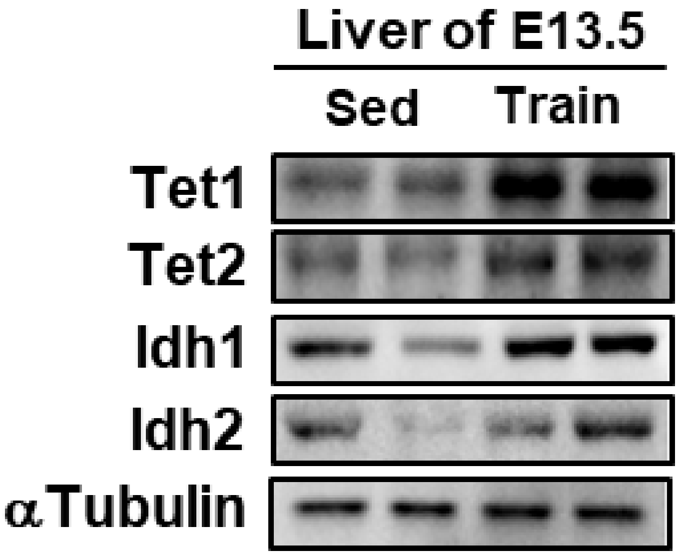
Tet, Idh, and αKG are Increased in E13.5 Livers of Offspring of Trained Dams

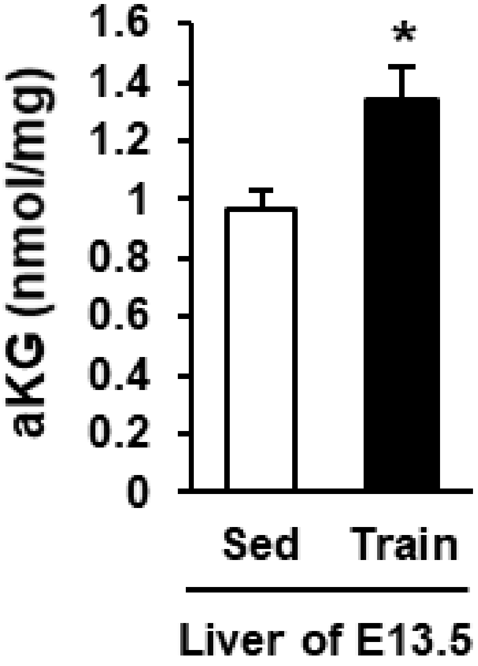




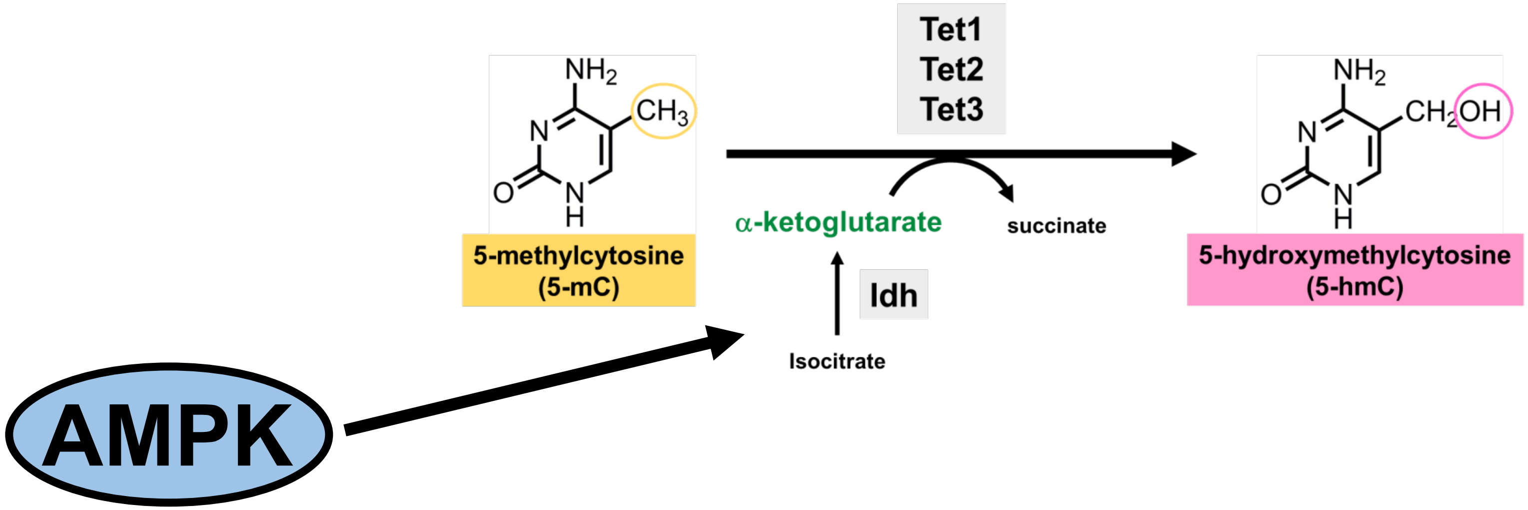
Tet, Idh, and αKG are Increased in E13.5 Livers of Offspring of Trained Dams





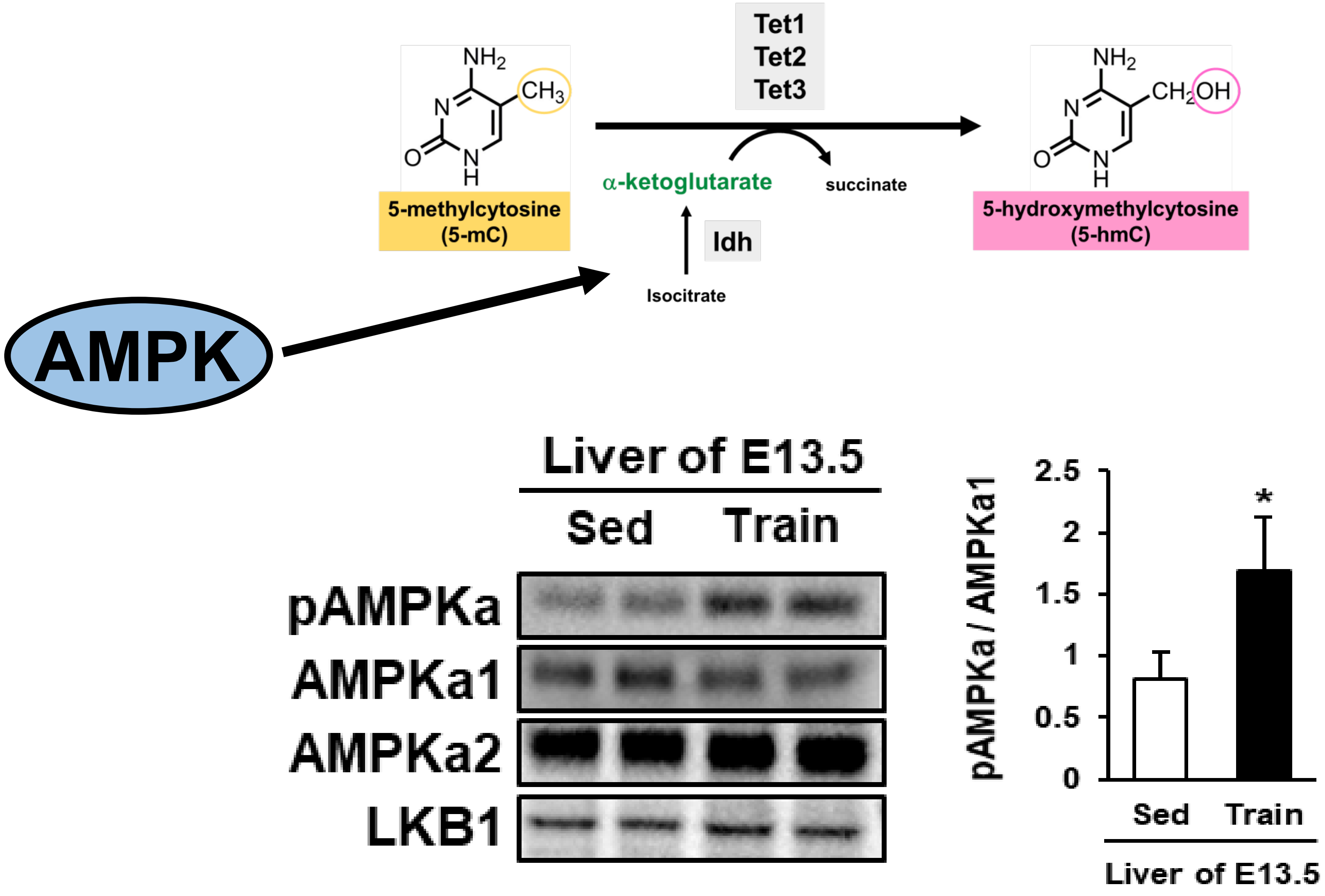


Tet, Idh, and αKG are Increased in E13.5 Livers of Offspring of Trained Dams

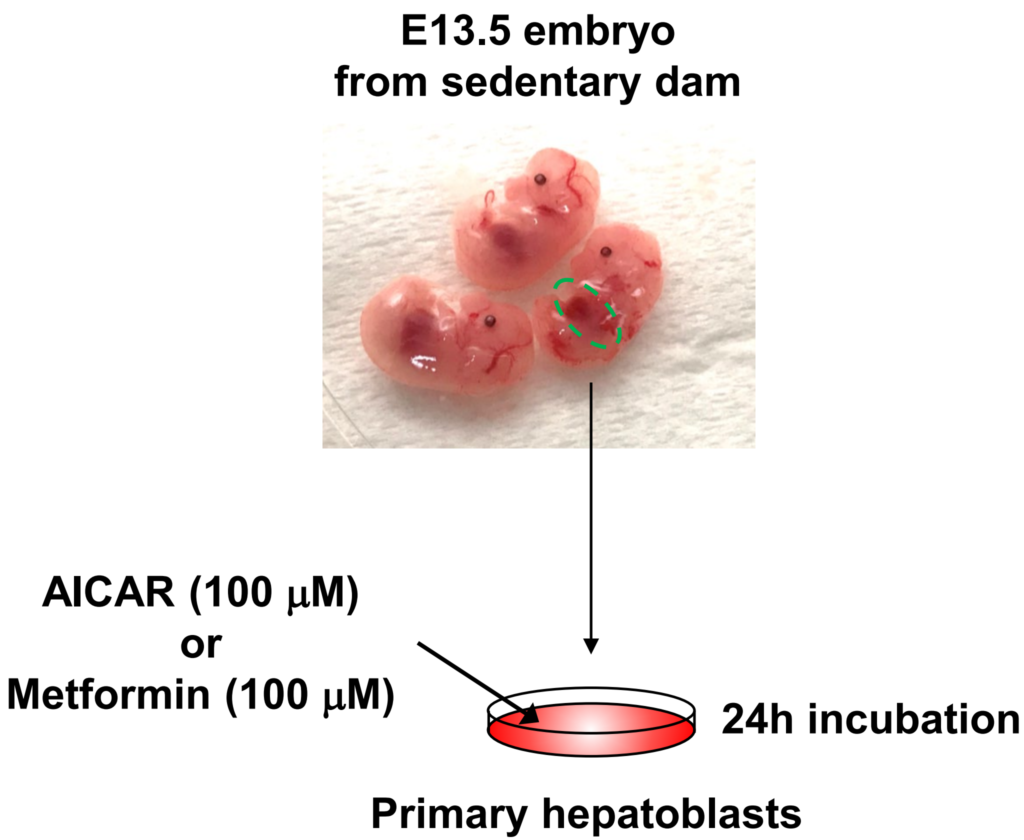


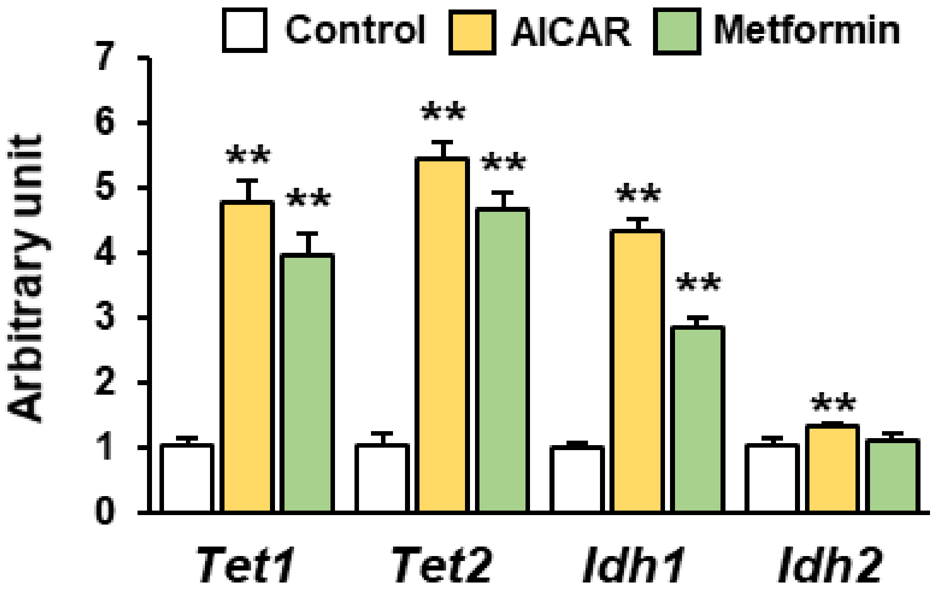
- What signals the increase in Tet, Idh, and αKG in offspring liver in response to maternal exercise training?

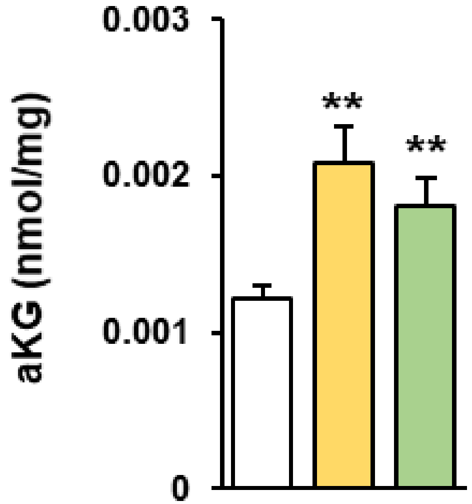
AMPK Phosphorylation is Increased in E13.5 Livers of Offspring of Trained Dams



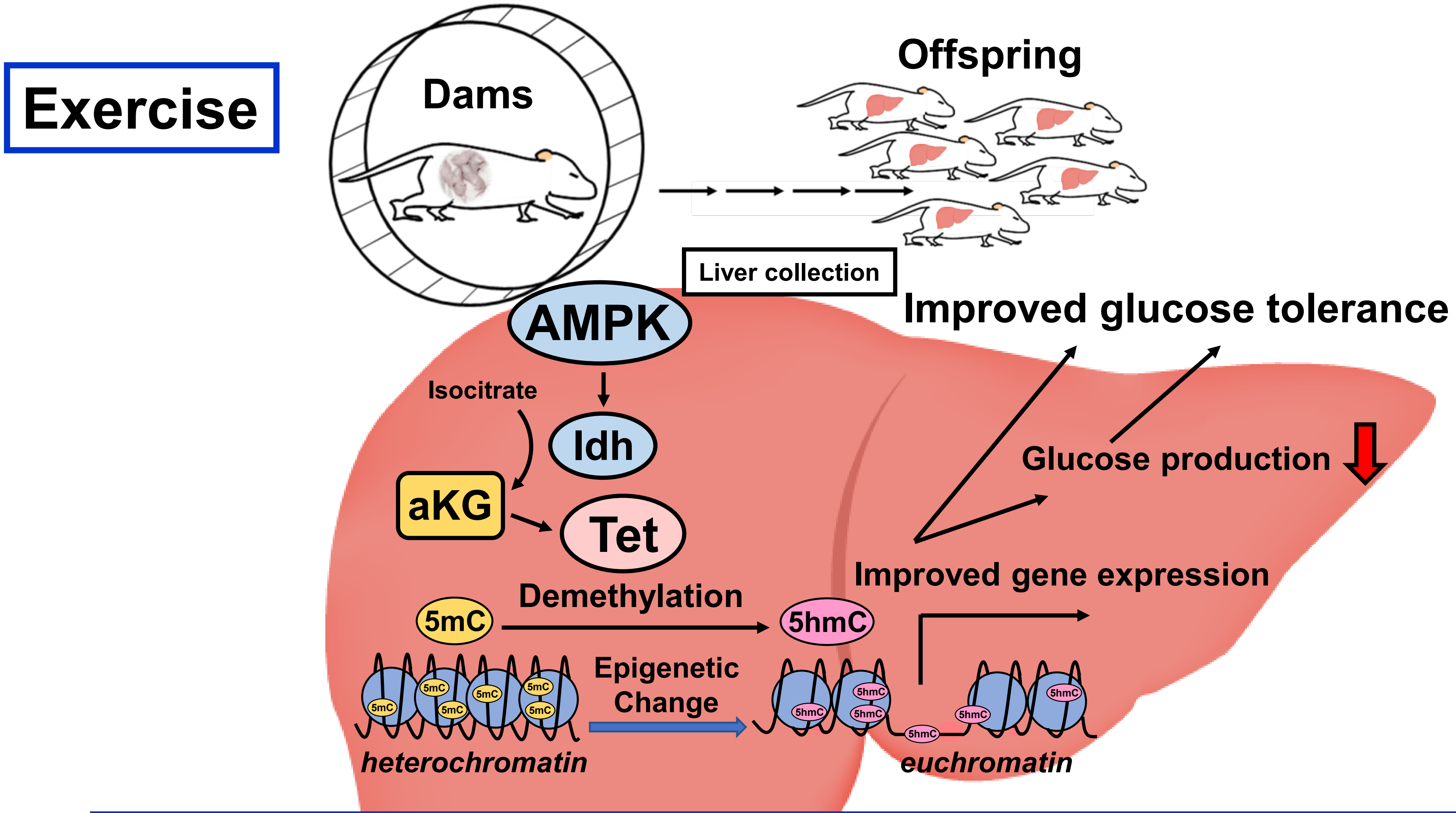
AMPK Activators Increase Tet and Idh mRNA Expression and αKG in Hepatoblasts from E13.5 Liver





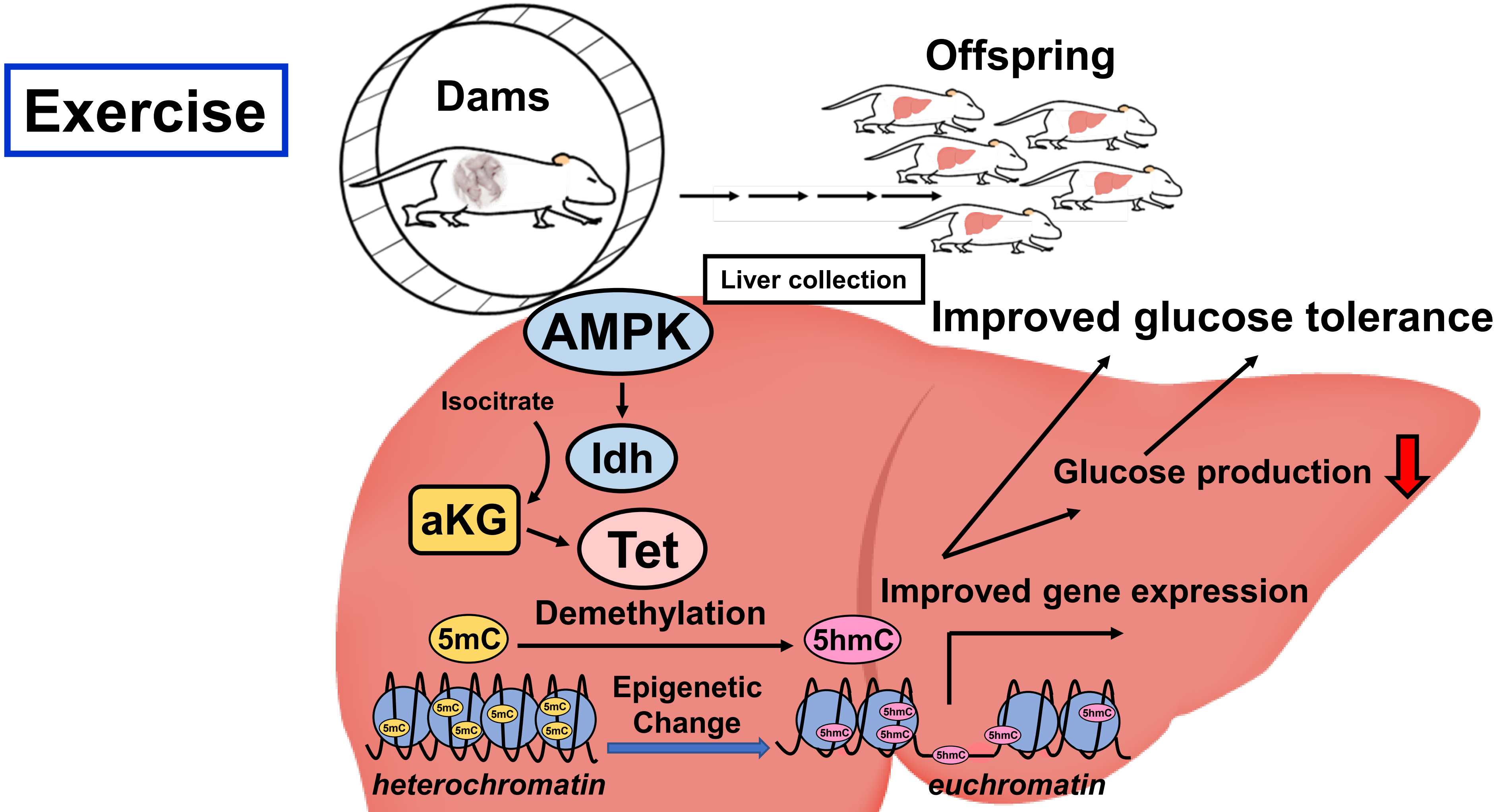


Summary



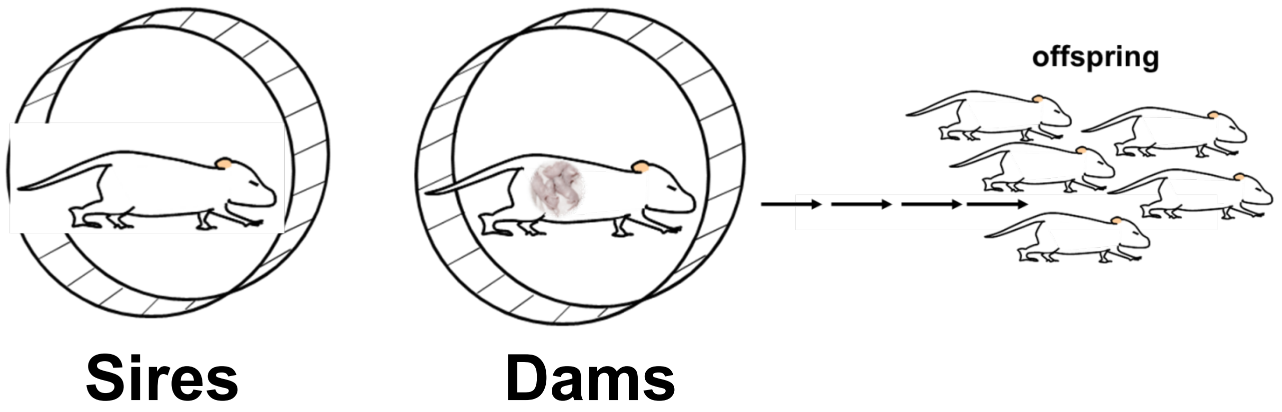
- **Maternal Exercise Activates an AMPK-Idh-aKG-Tet Signaling in Offspring Liver.**

Important Question



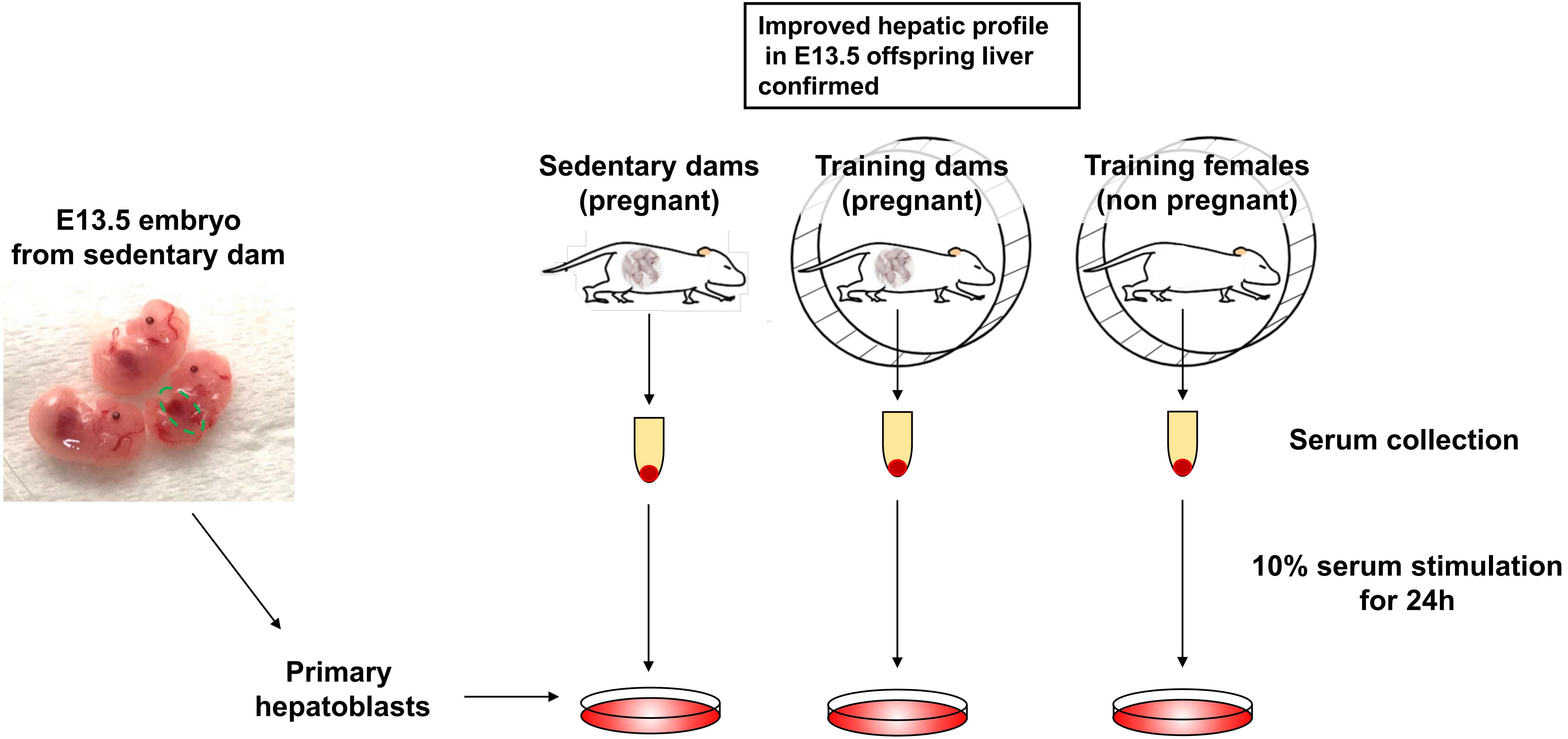
- How does the exercising mother stimulate AMPK-Tet signaling leading to DNA demethylation in offspring liver?

Maternal and Paternal Exercise Training

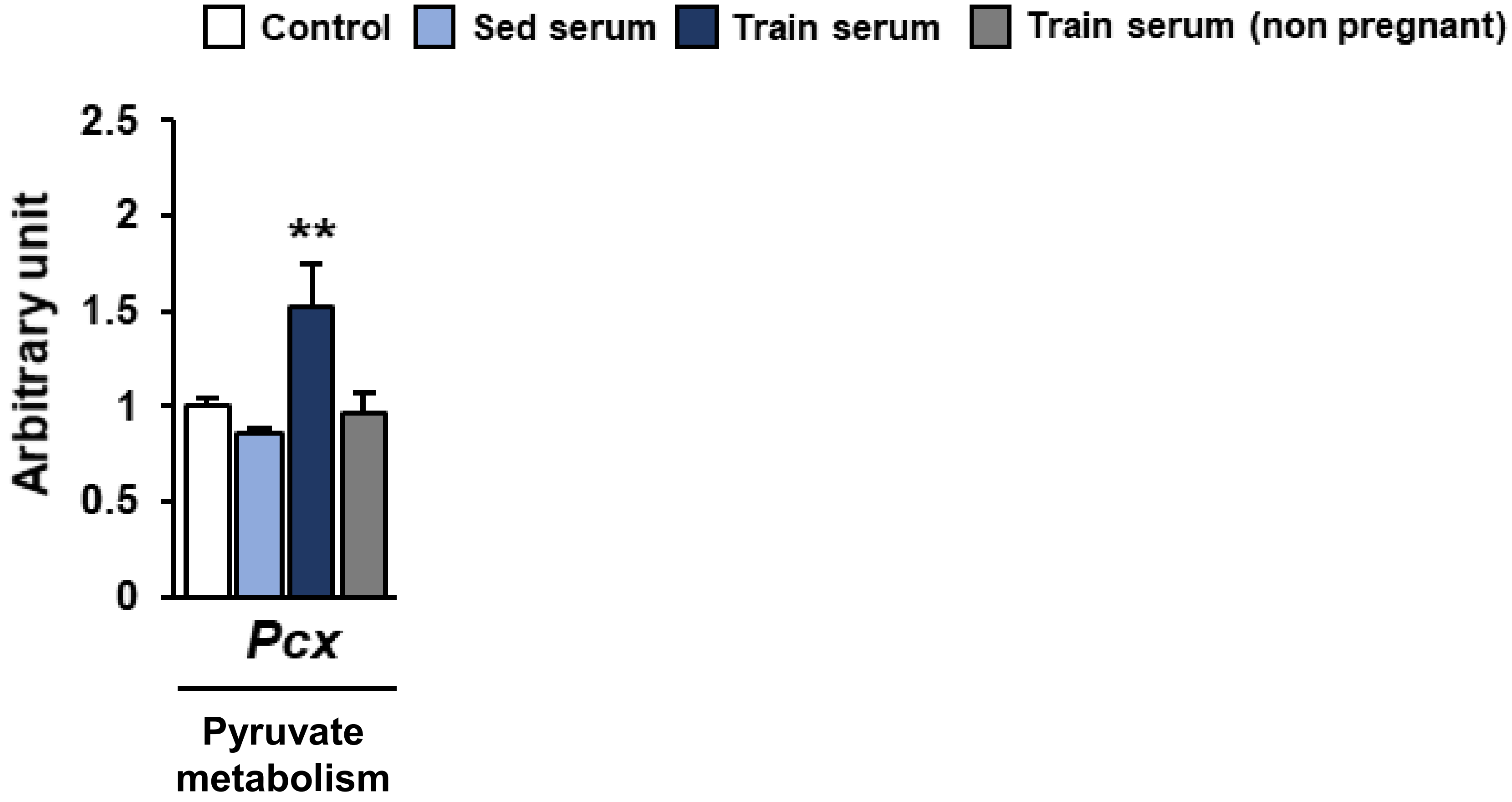


- ➢ **Maternal, Paternal and Maternal + Paternal Exercise: effects on offspring glucose tolerance.**
- ➢ **Maternal Exercise: mechanisms and epigenetic regulation of offspring.**
- ➢ **Maternal Exercise: Identification of a novel protein that can improve offspring health.**

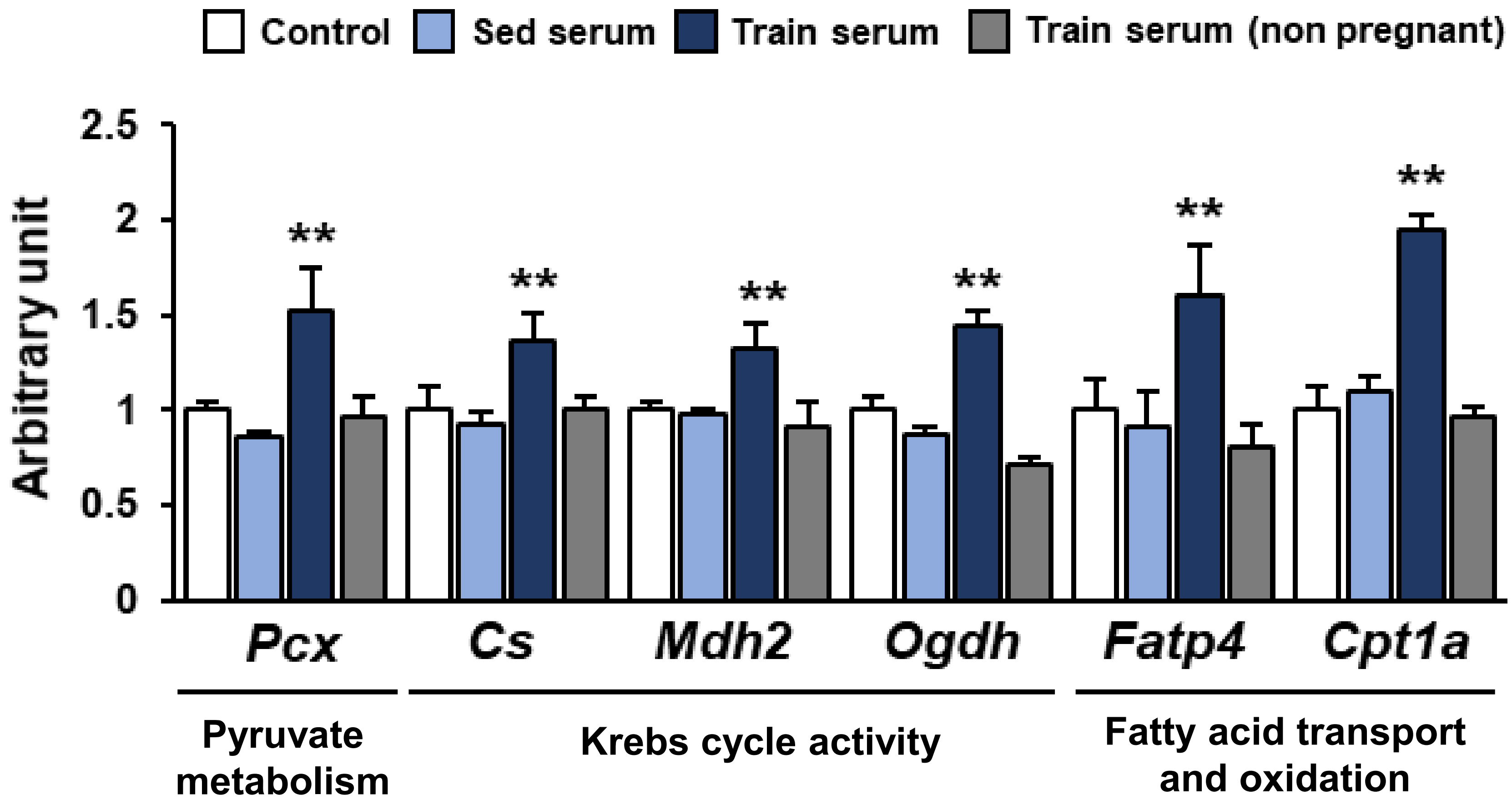
Dam-Derived Serum Stimulation of Primary Hepatoblasts
(E13.5 Embryos)



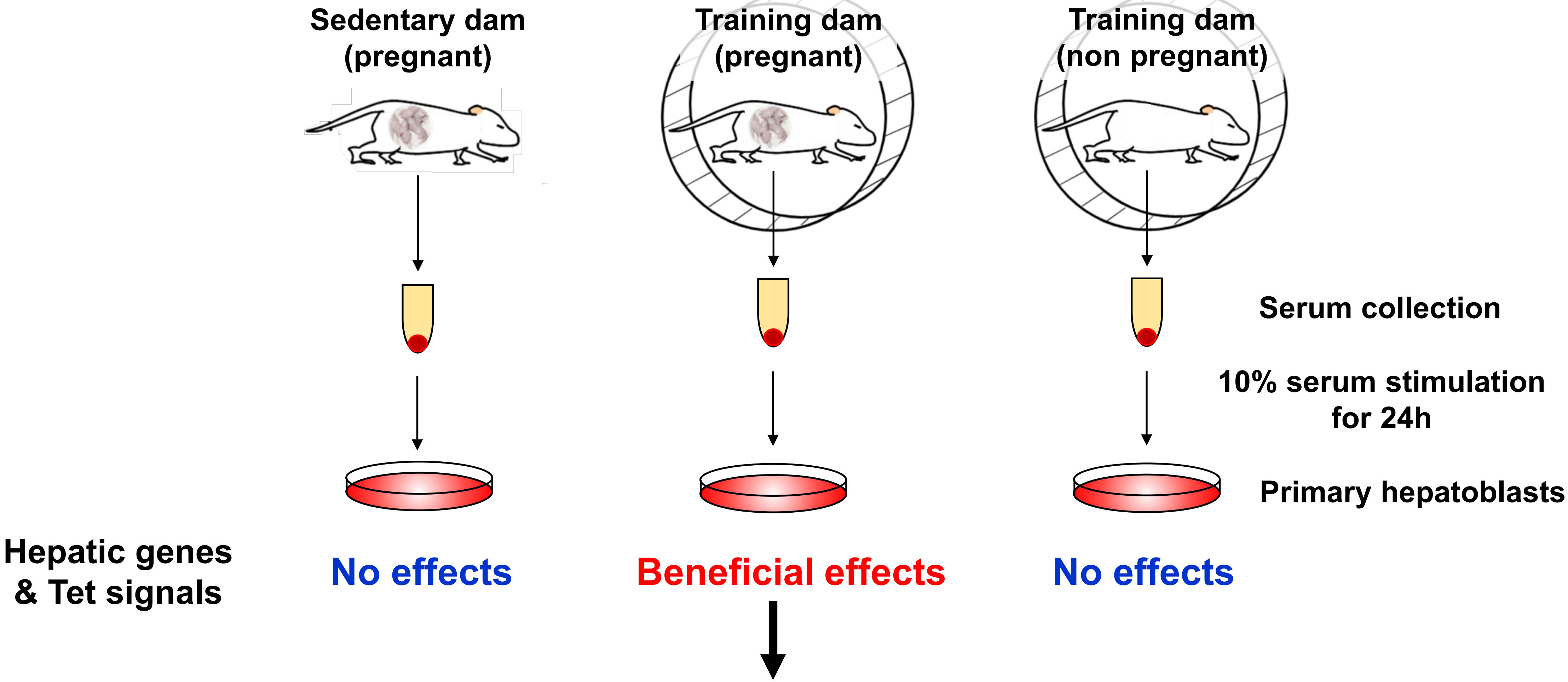
Exercise Trained Dam-Derived Serum Stimulation Increases Hepatic Gene Expressions in Embryonic Hepatoblasts



Exercise Trained Dam-Derived Serum Stimulation Increases Hepatic Gene Expressions in Embryonic Hepatoblasts

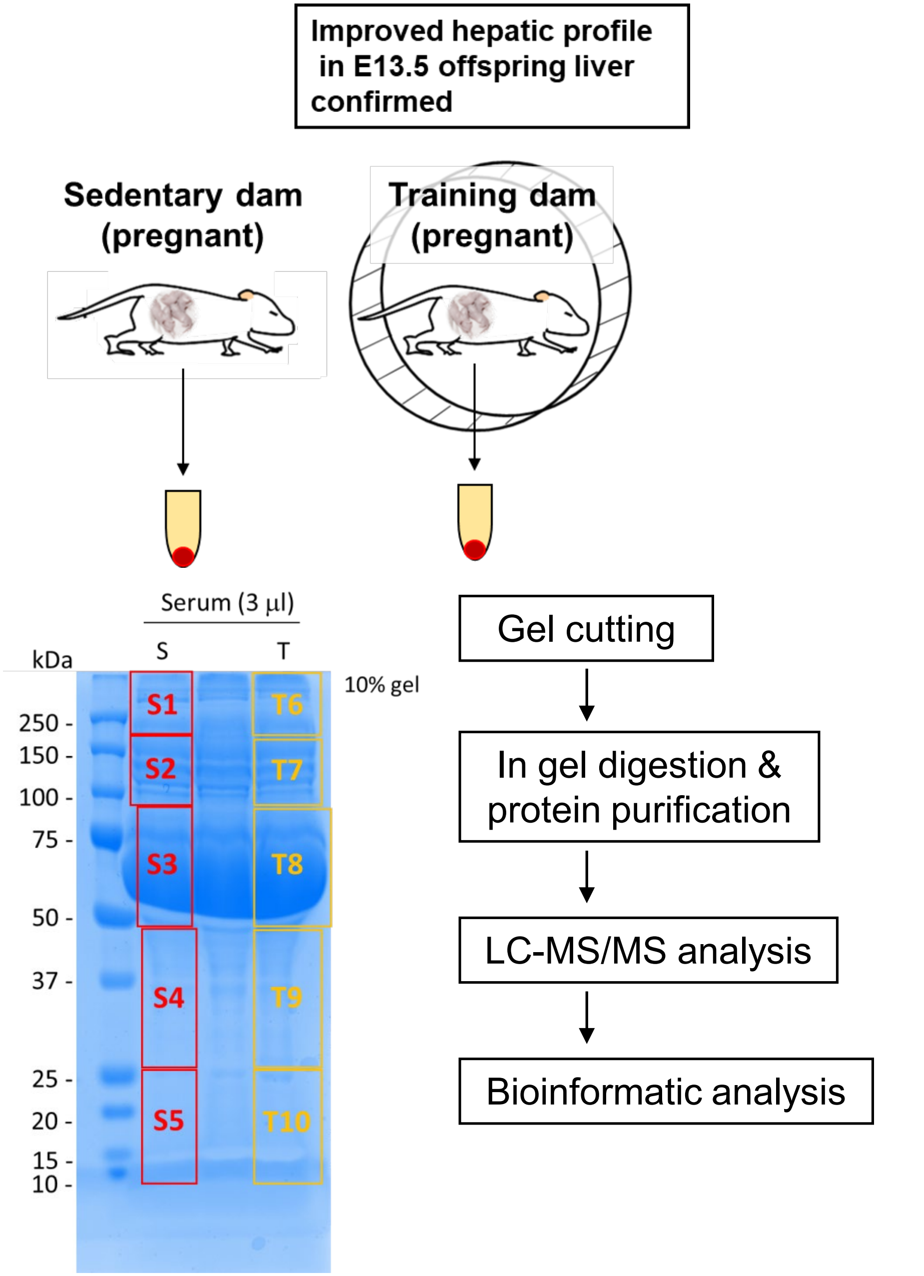


Beneficial Factors for Liver Metabolism of Offspring in Exercise Trained Dam-Derived Serum

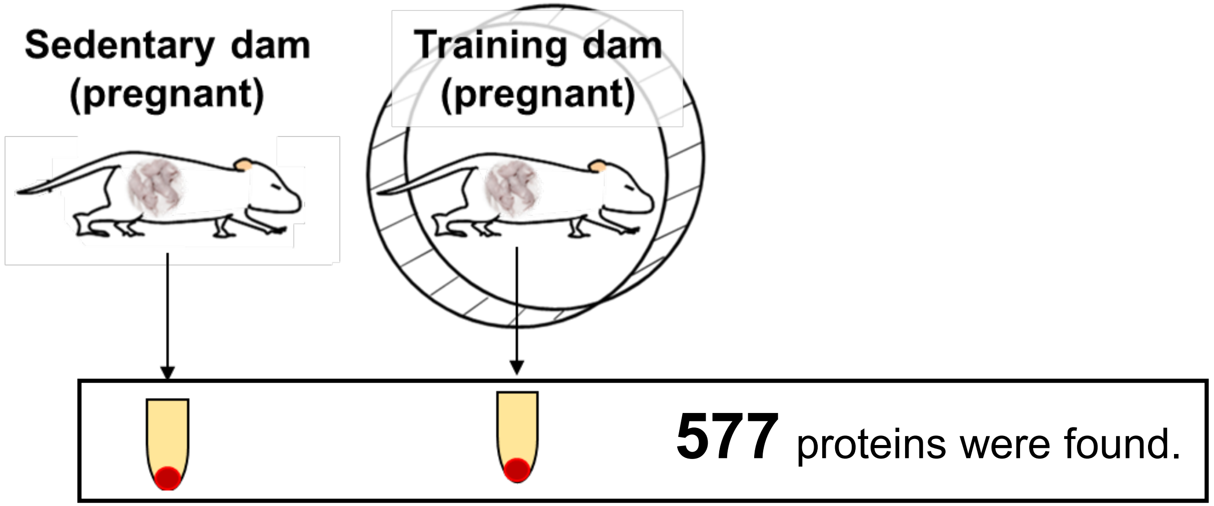


- What is the factor(s) in the serum of exercise trained dams that cause the beneficial effects on hepatoblasts?

LC-MS/MS analysis in Dam-Derived Serum

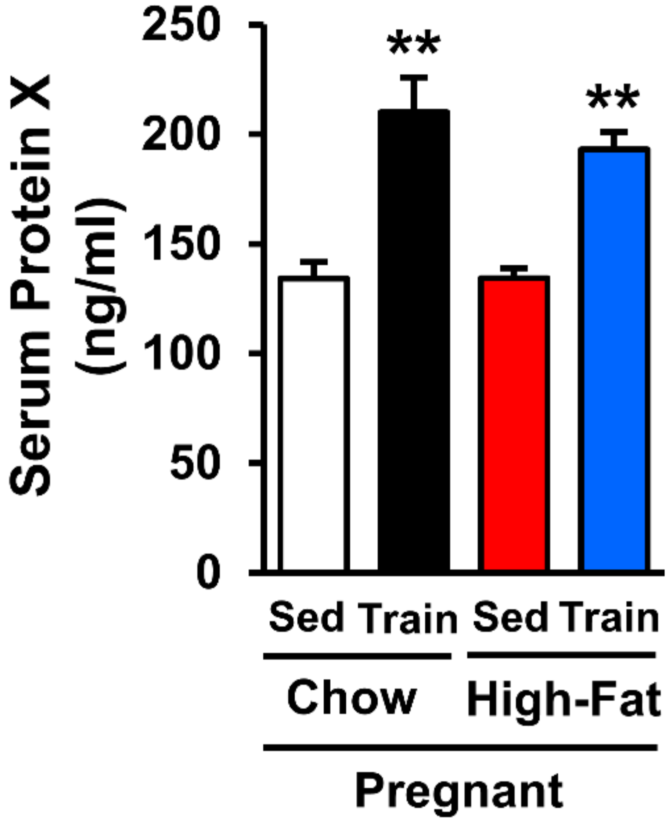


Gel cutting
↓
In gel digestion & protein purification
↓
LC-MS/MS analysis
↓
Bioinformatic analysis

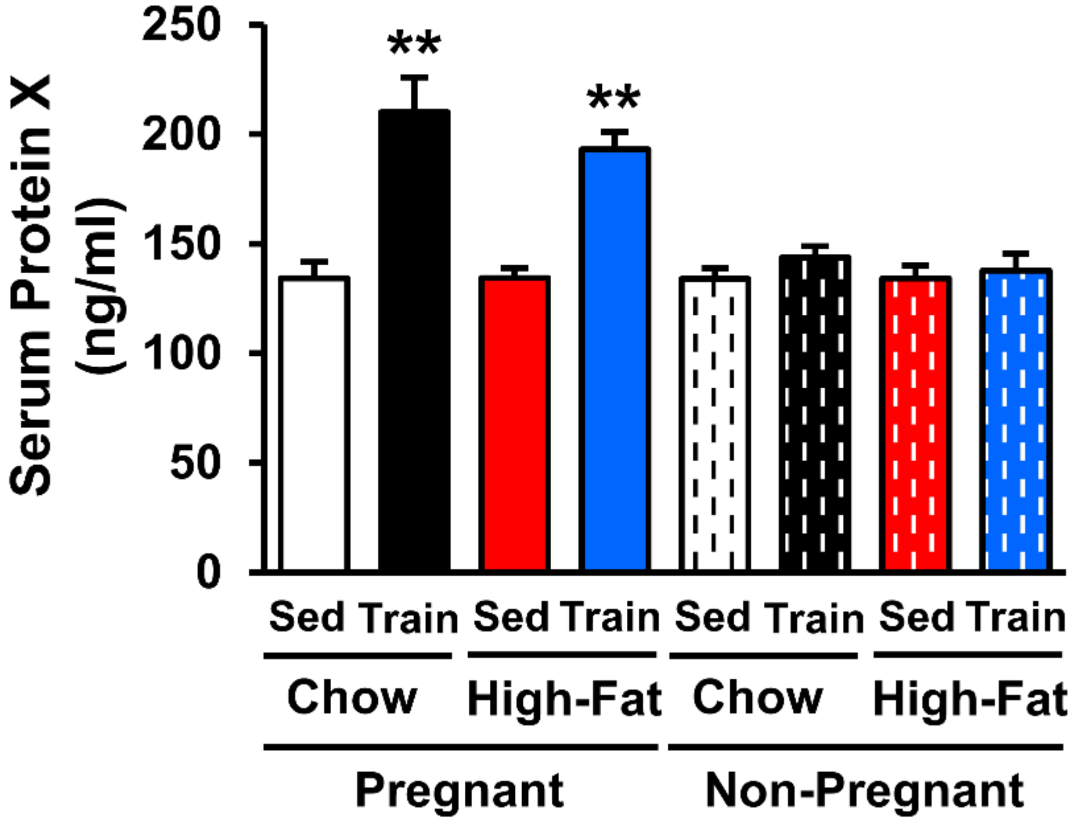


577 proteins were found.
↓
78 proteins were increased (>1.5 fold)
↓
19 proteins were secretory cytokines
↓
4 proteins were expressed in placenta
↓
1 protein has been reported to increase AMPK phosphorylation

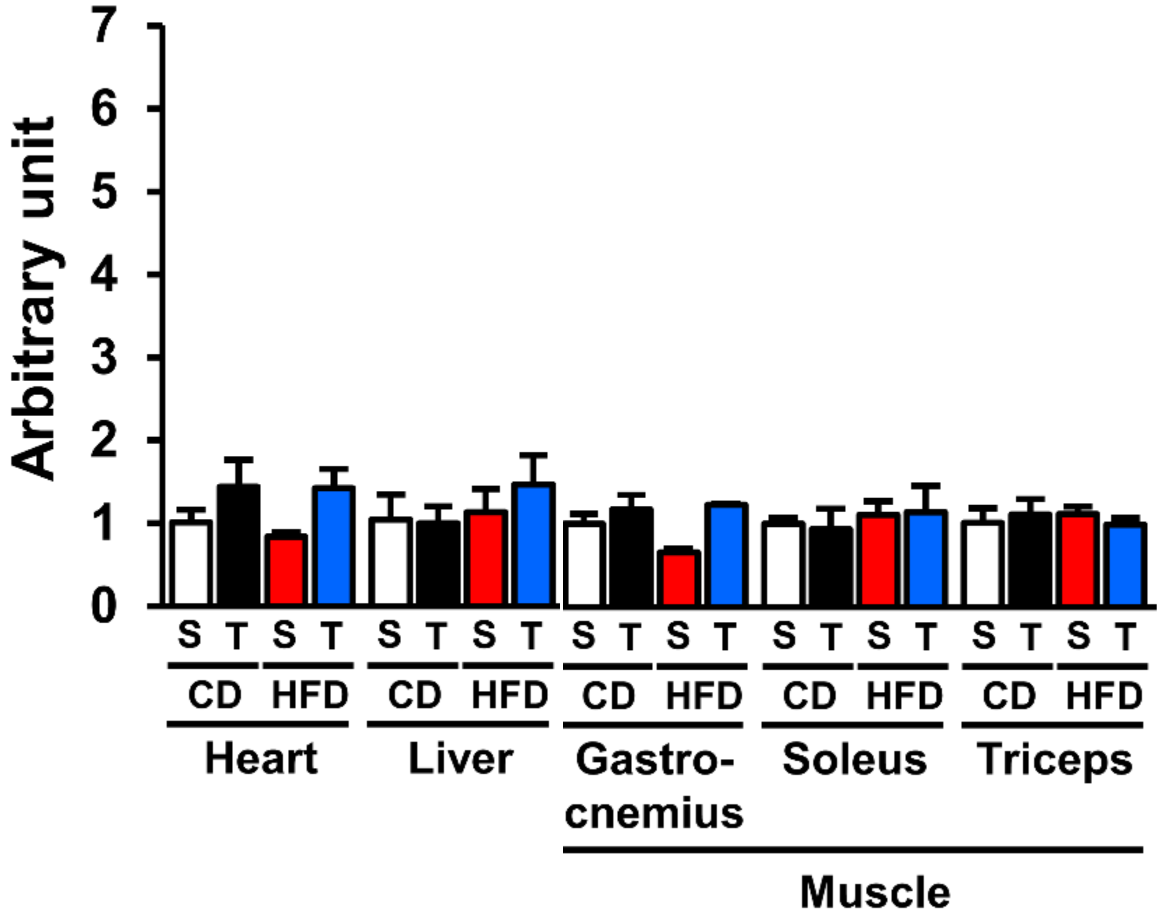
Protein X is Increased in the Serum of Trained and Pregnant Dams



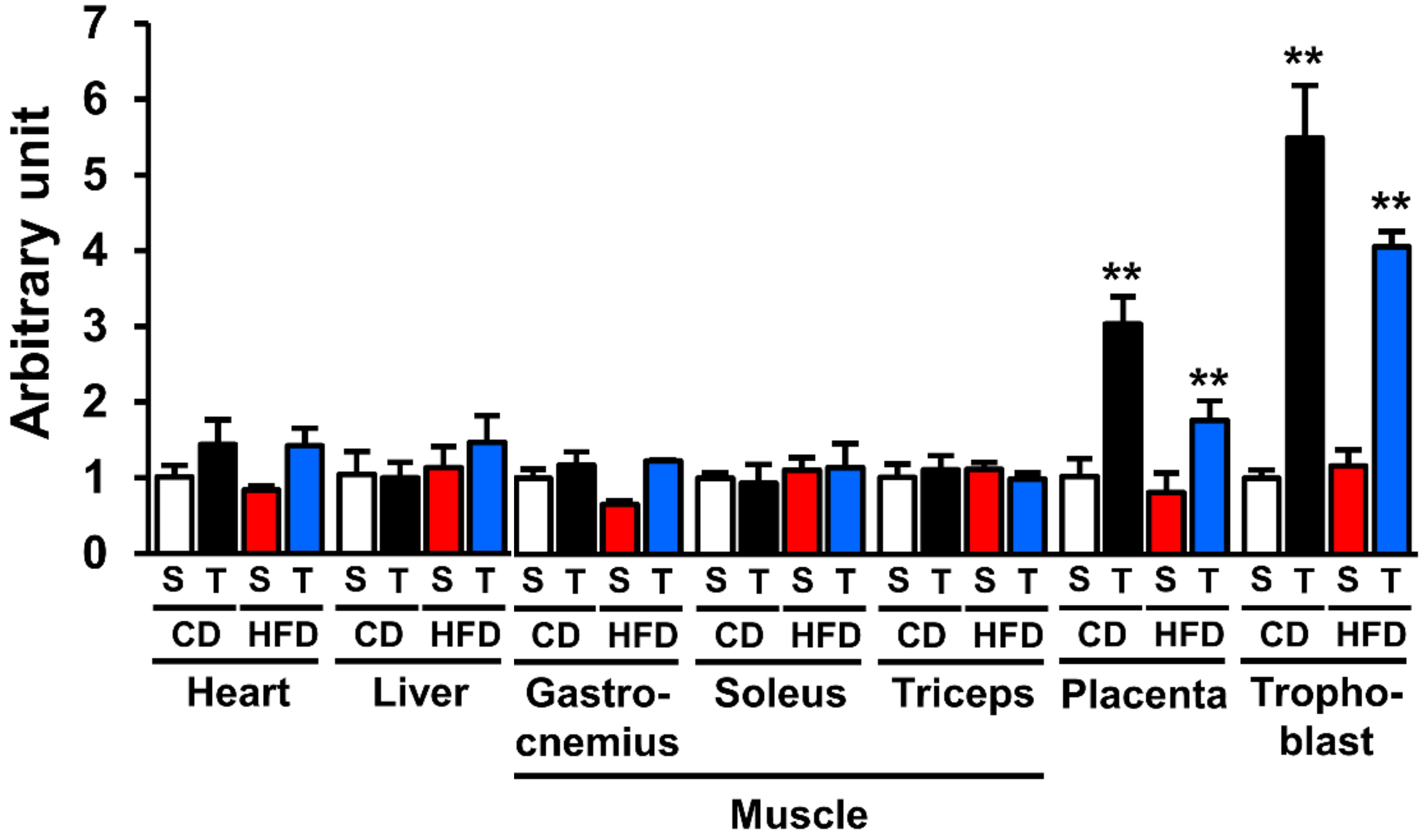
Protein X is Not Increased in the Serum of Trained and Pregnant Dams



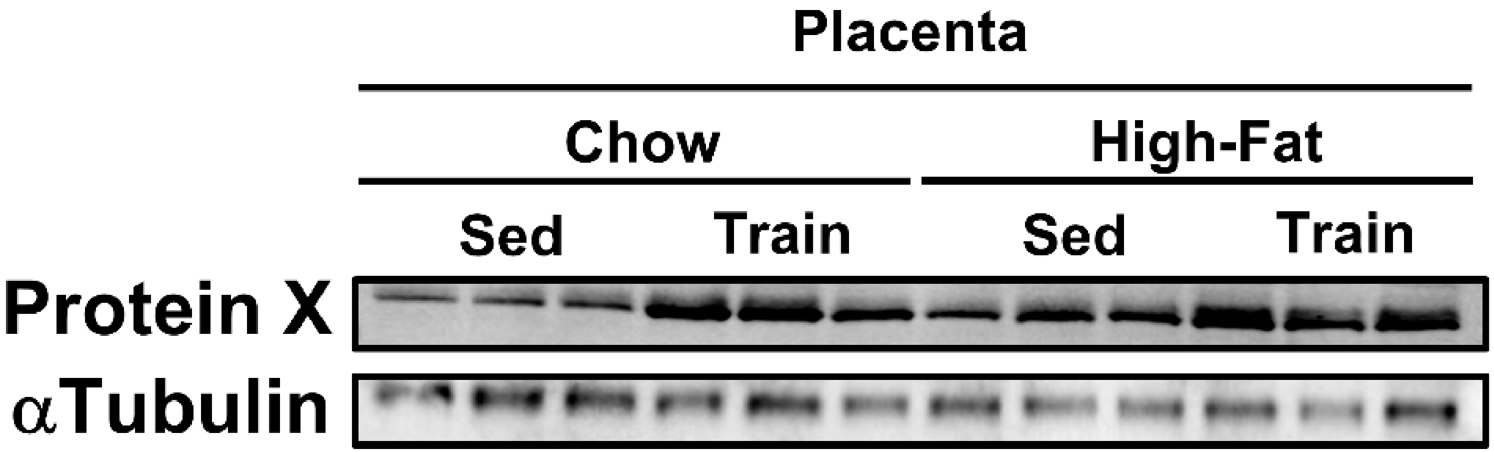
Protein X mRNA Expression is Increased in the Placenta and Trophoblast from Trained Dams



Protein X mRNA Expression is Increased in the Placenta and Trophoblast from Trained Dams

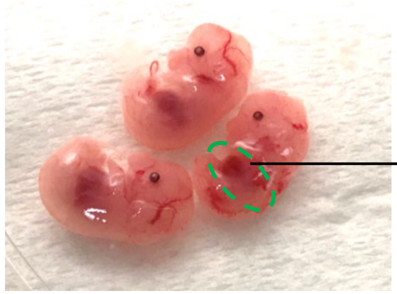


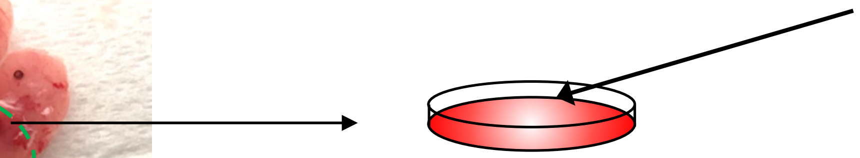
Protein X Expression is Increased in the Placenta from Trained Dams

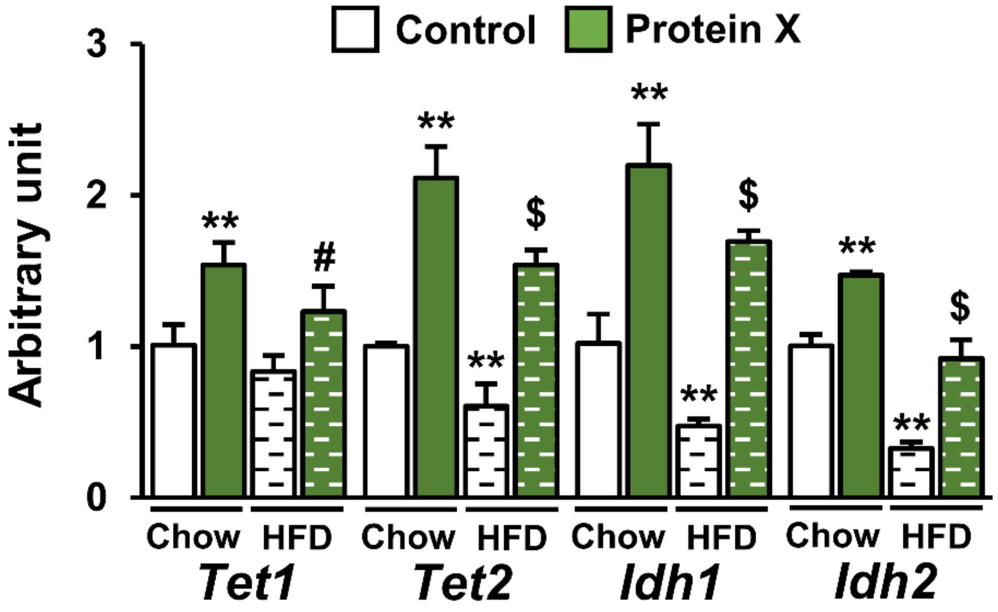


Protein X Has Promotive Effects on Tet Signaling Axis in Hepatoblasts from both Chow Diet and HFD-Fed Dams

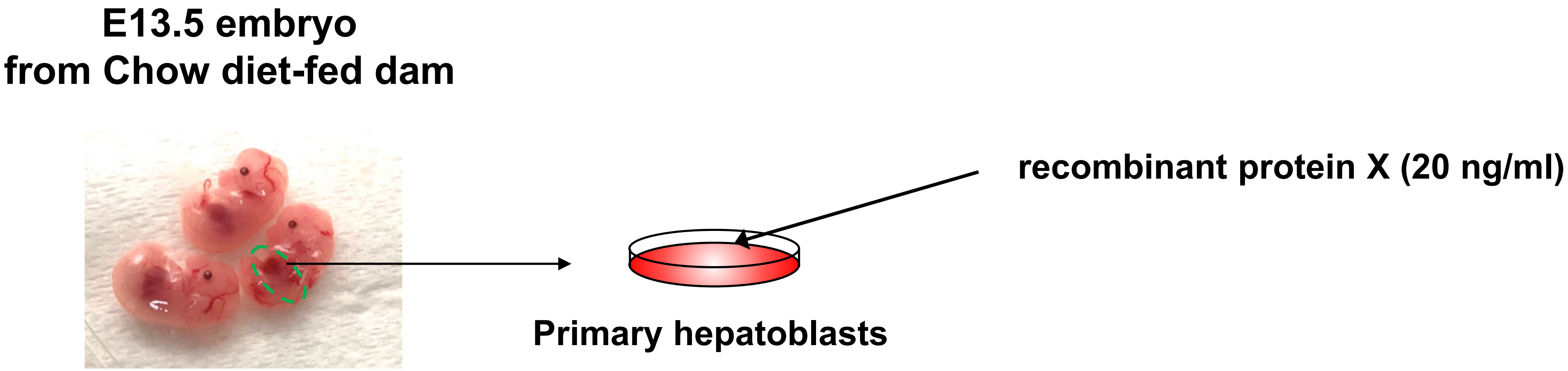
E13.5 embryo
from Chow diet or HFD-fed dam

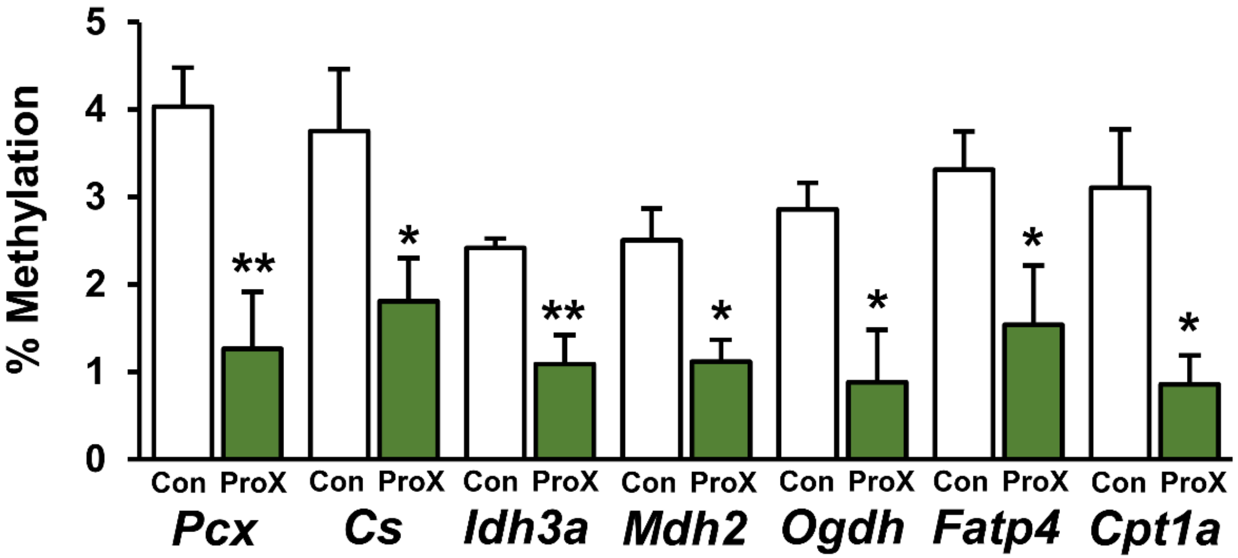




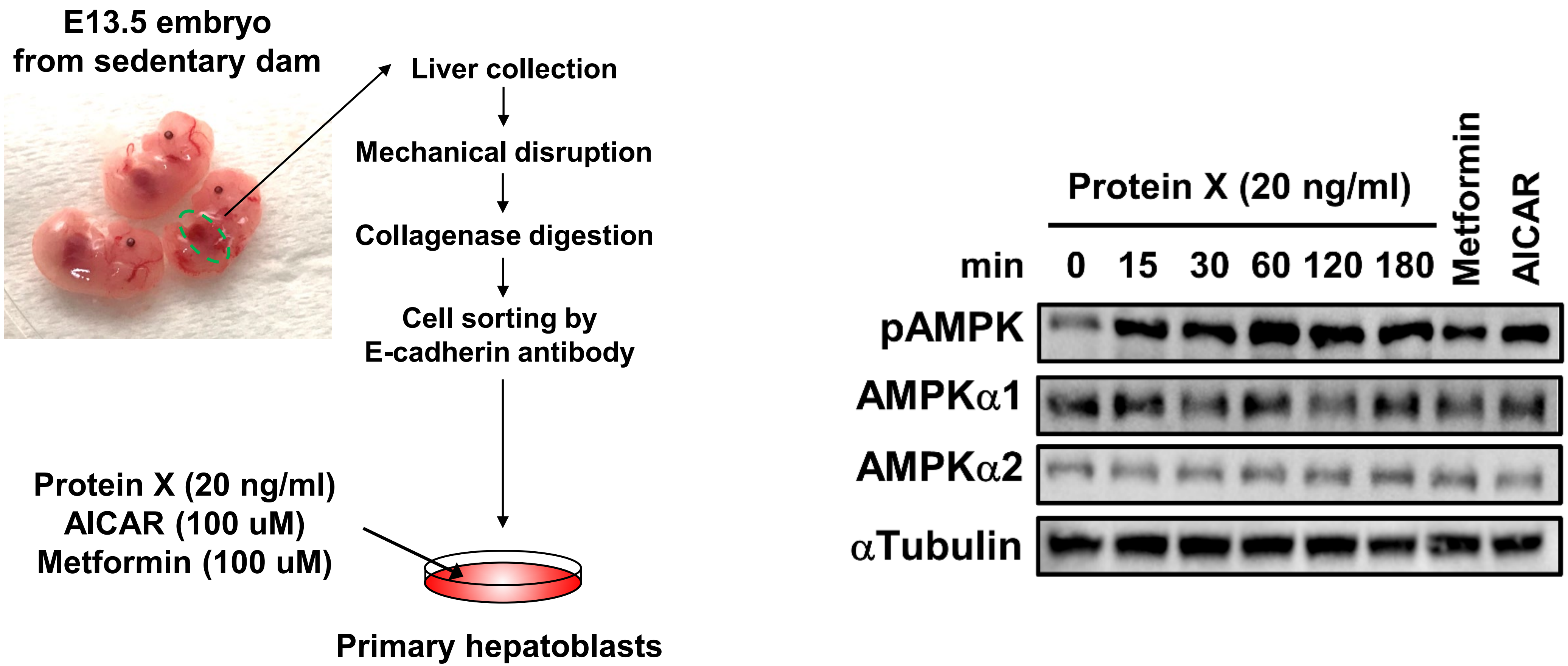


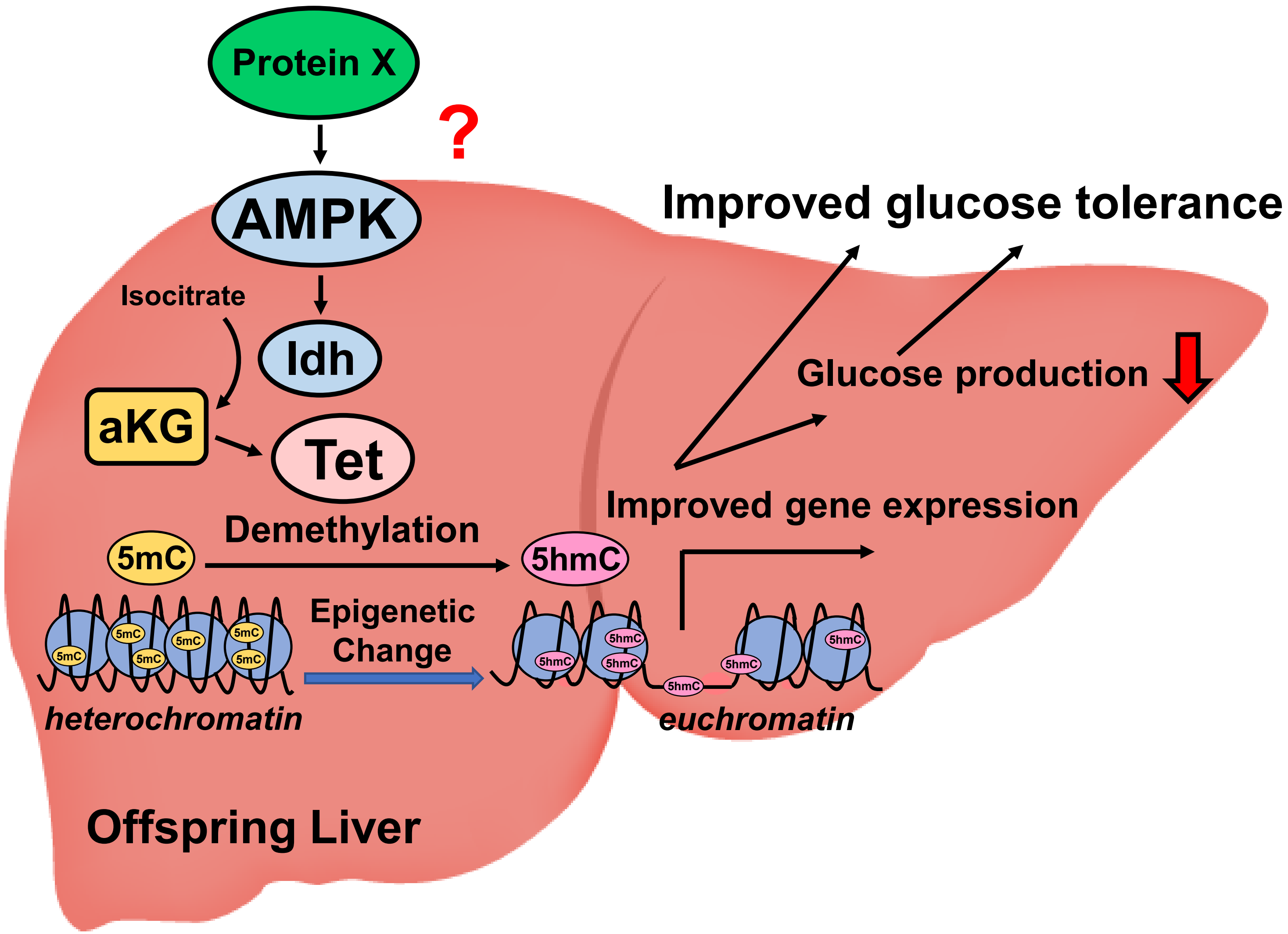
Protein X Treatment Decreases CpG Methylation of Metabolic Genes in Hepatoblasts





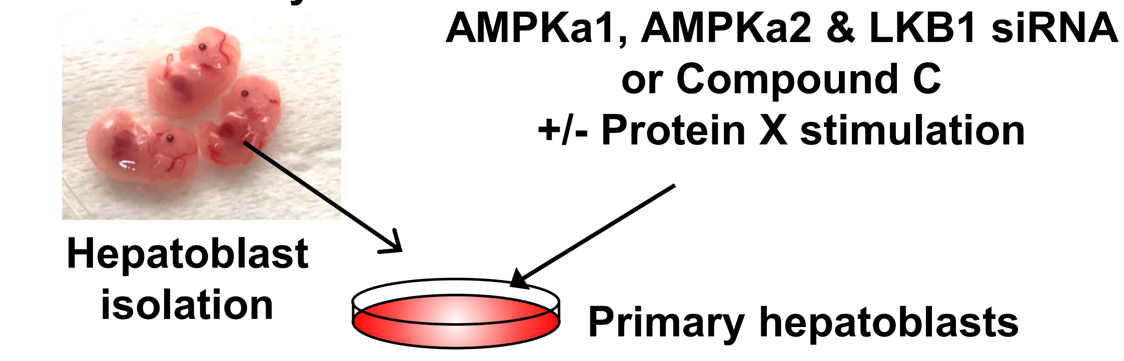
Protein X Stimulation induces AMPK Phosphorylation in Hepatoblasts

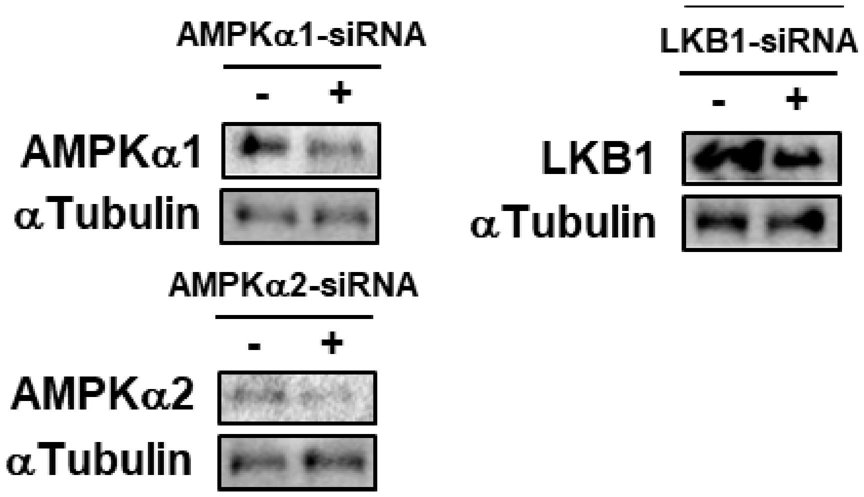




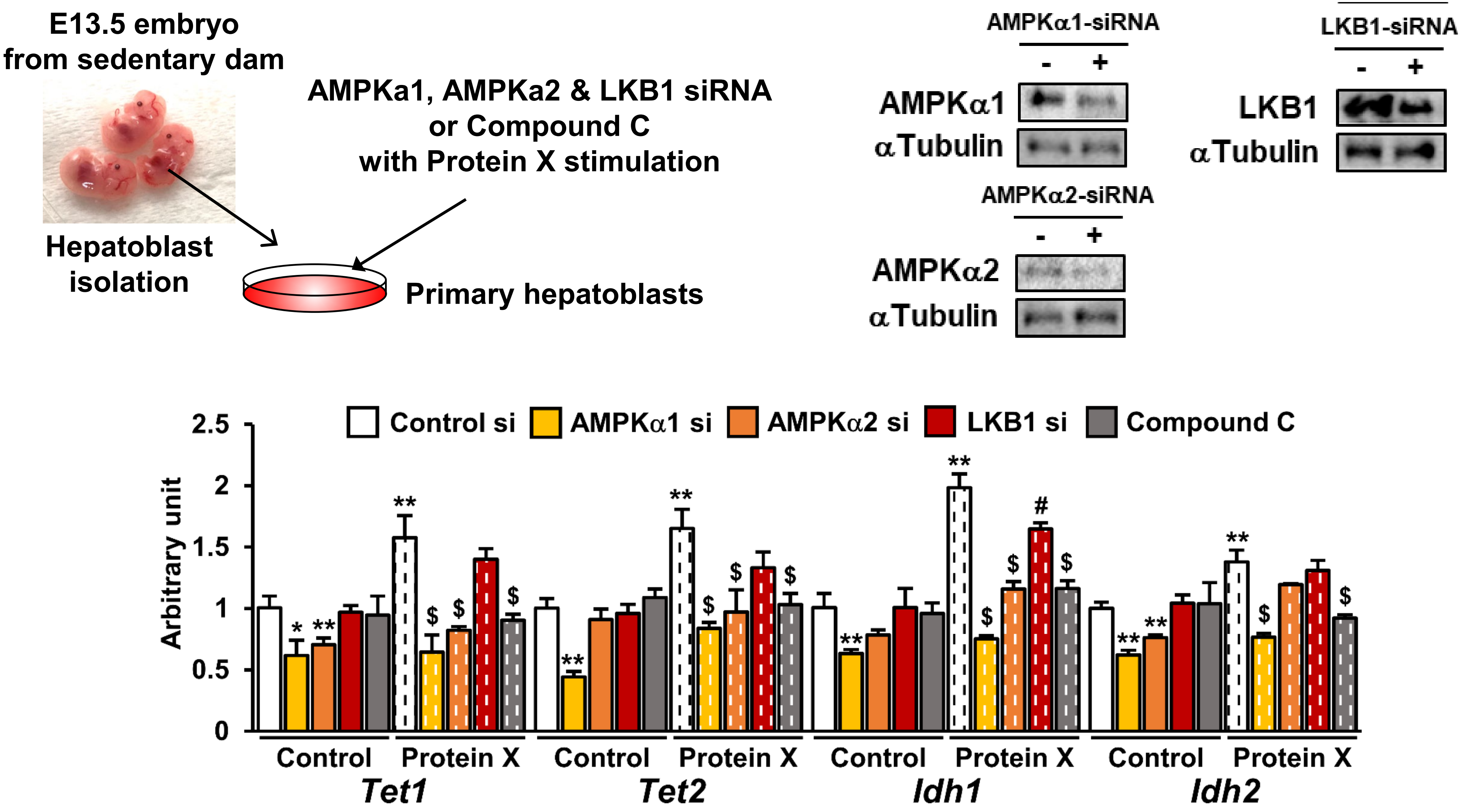
- Does Protein X signal epigenetic changes via AMPK?

The Effects of Protein X on Tet Signaling Axis is Inhibited by AMPKα Knockdown and Inhibition in Hepatoblasts

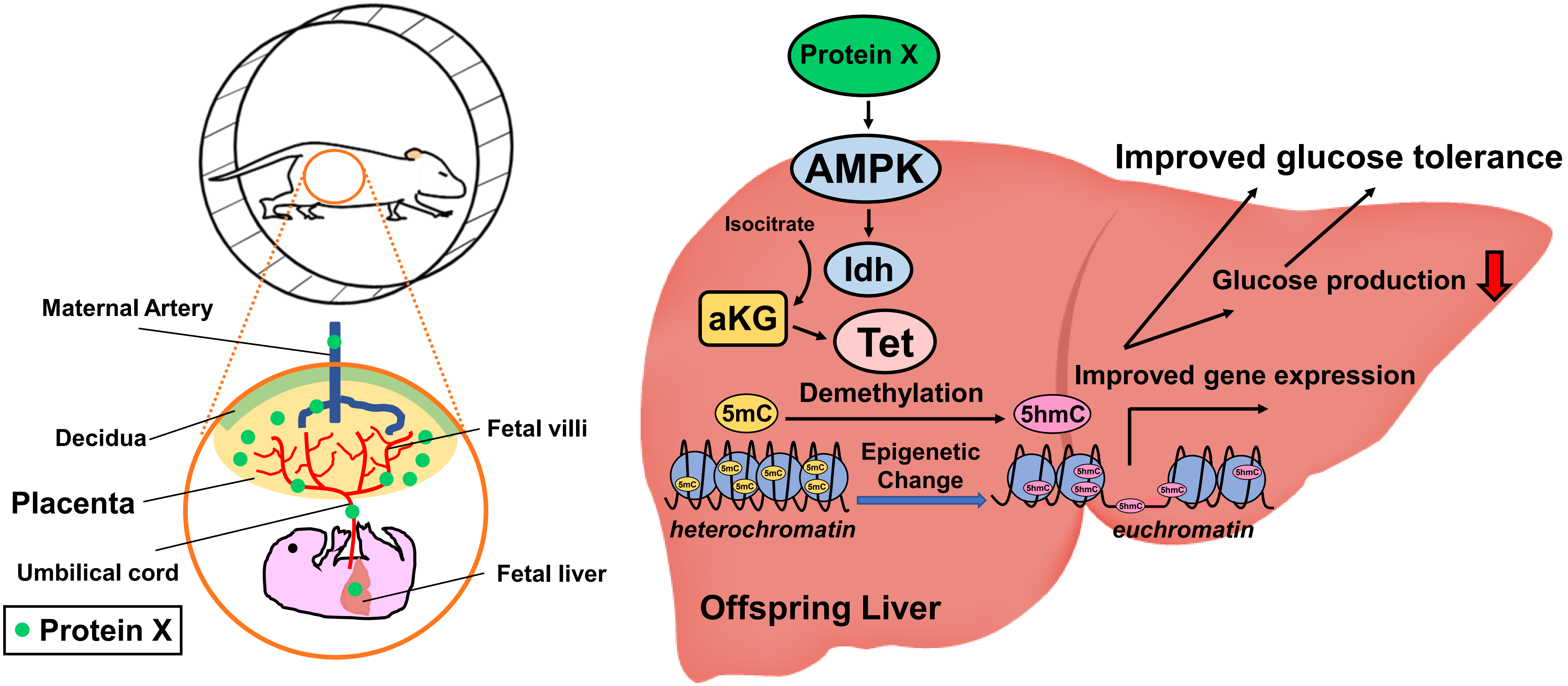




The Effects of Protein X on Tet Signaling Axis is Inhibited by AMPKα Knockdown and Inhibition in Hepatoblasts

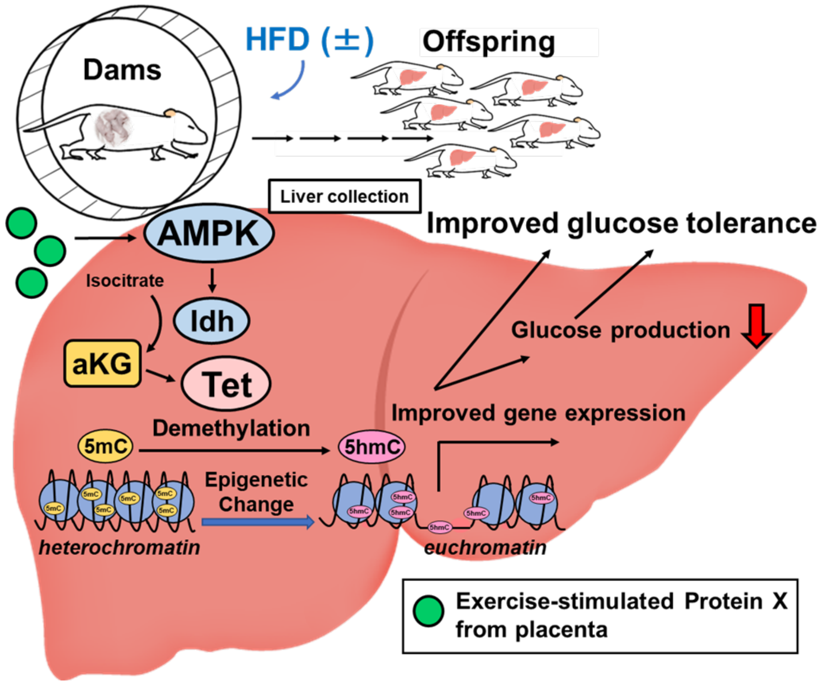


Summary



- Placenta-Derived Protein X is a Maternal Exercise-Stimulated Factor Regulating the Tet Signaling Axis and Hepatic Gene Expression

Next Steps: Animal Studies



- **Placenta-specific Protein X knockout mice**
- **Effects of Protein X on other organs (e.g. pancreas, muscle, brain, etc.)**

Next Steps: Human Studies

Parental Exercise

Offspring

Improved Metabolic Health

- Do highly active pregnant women have higher levels of protein X?
- Could protein X be used to improve the metabolic health of offspring?
- Studies of the effects of exercise on sperm in healthy and diabetic men.

Conclusion

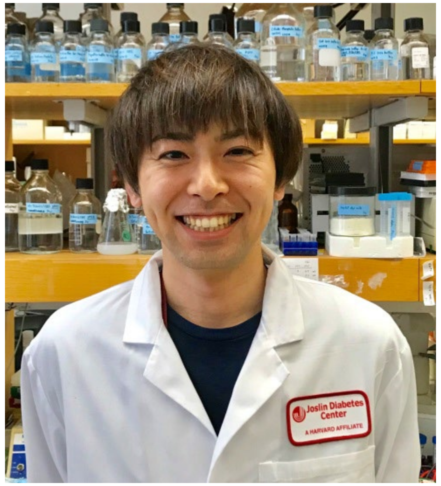
Parental Exercise

Offspring

Improved Metabolic Health

- **These findings, if translated to humans, will have enormous implications for the prevention of obesity and type 2 diabetes.**

Kristin Stanford



Joji Kusuyama

Ana Wagner

Jia Zhang
Hirokazu Takahashi
Noah Prince
Nathan Makarewicz
Pasquale Nigro
Christiano Alves
Jan-Willem Middelbeek
Emily Miele
Brent Albertson
Susana Llopis
Maria Vamvini
Laura Simpson
Royce Conlin
Maya Yosub
Michael Hirshman

Collaborators:
Mary Elizabeth Patti
(Joslin)

Ollie Rando
(Univ. Massachusetts)

Kallie Longval
Allen Clermont
(Joslin Animal Physiology
Core Laboratory)

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