

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



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# 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, prostate)



**Decreased depression, anxiety**  
**Decreased Alzheimer's**  
**Decreased appetite**  
**Decreased stroke**  
**Increased flexibility**  
**Increased bone density**  
**Improved cardiac function**  
**Increased muscle strength**

## Prevention of Diabetes

**Increased insulin sensitivity**  
**Increased glucose tolerance**  
**Improved pancreatic function**  
**Increased mitochondrial function**  
**Increased muscle glucose uptake**

# Benefits of Exercise on Health

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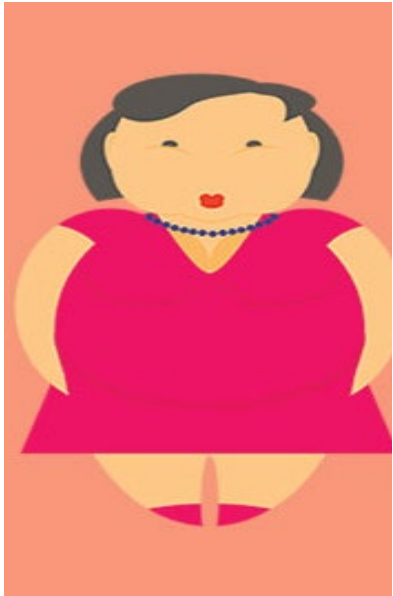


**Decreased depression, anxiety**  
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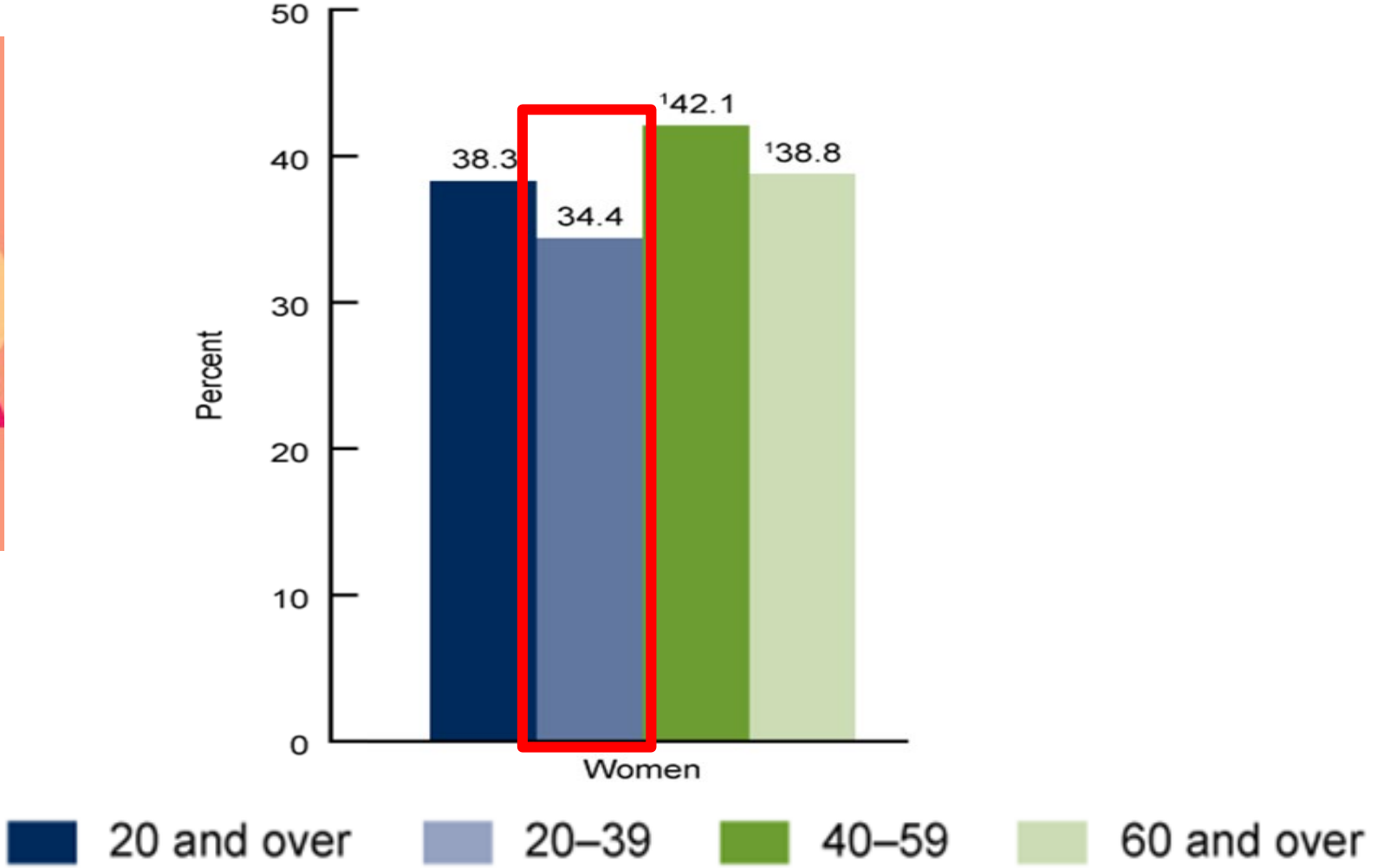
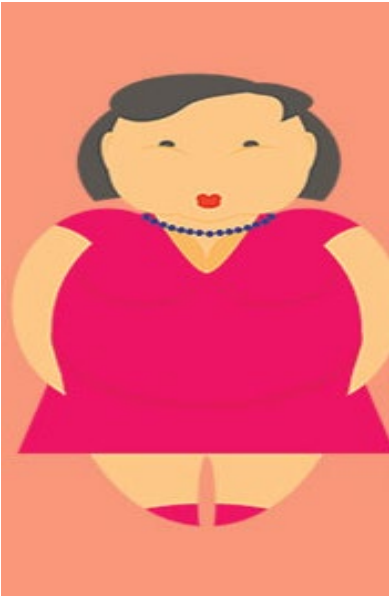
**What are the underlying molecular mechanisms mediating the beneficial effects of exercise on health?**

**Improved pancreatic function**  
**Increased mitochondrial function**  
**Increased muscle glucose uptake**

# Obesity Has Detrimental Effects on Health

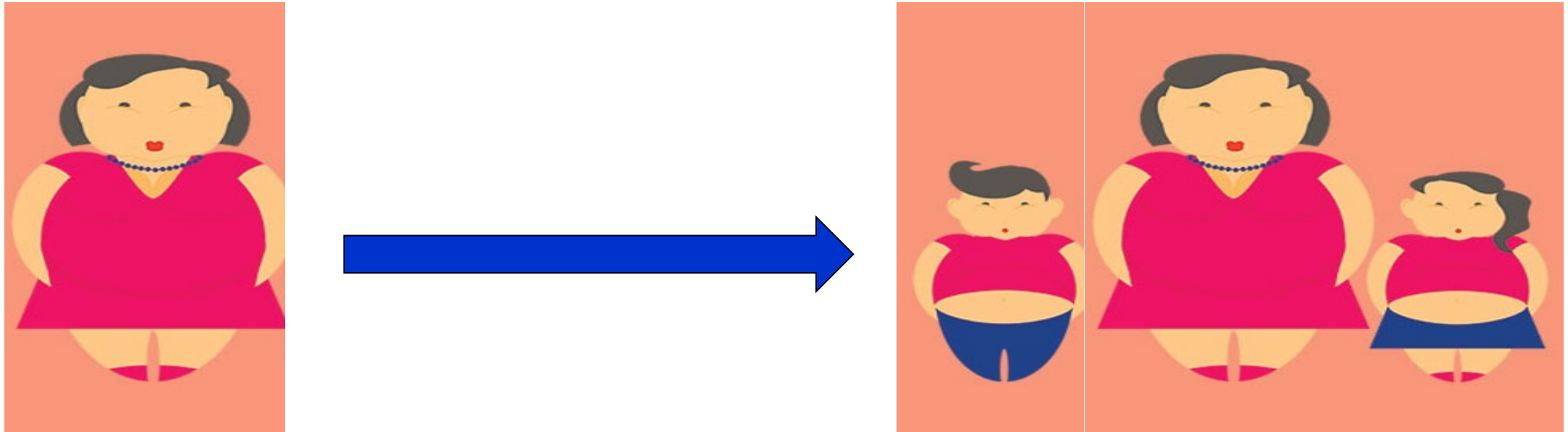


# High Rates of Obesity in Women of Child Bearing Age



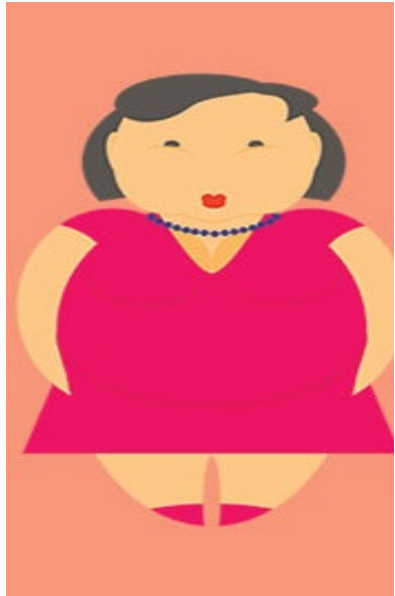
CDC: Prevalence of Obesity Among Adults in the United States (2015-2016)

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

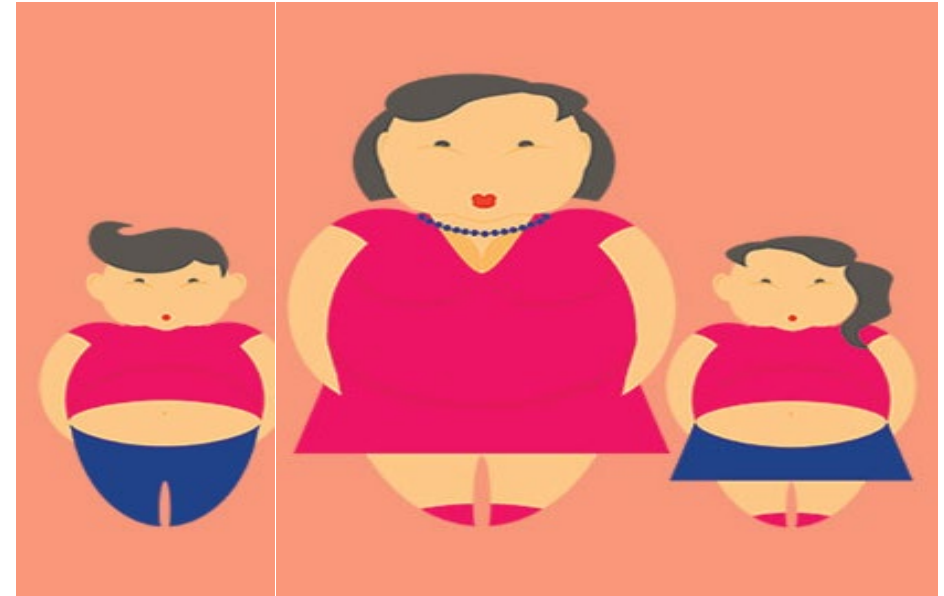
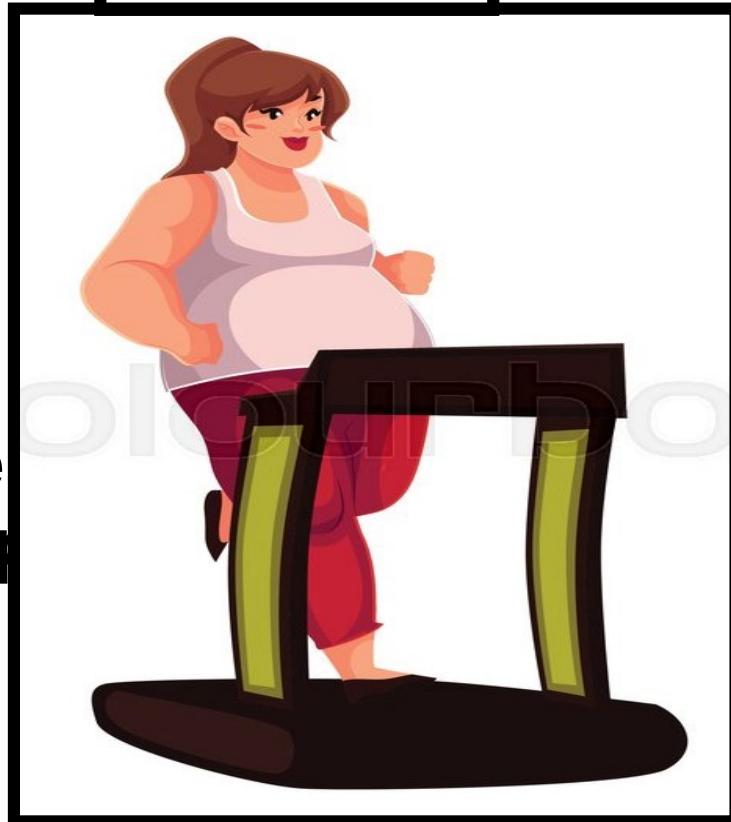


➤ Is there a means to reduce the transmission of increased risk of metabolic disease to offspring?

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



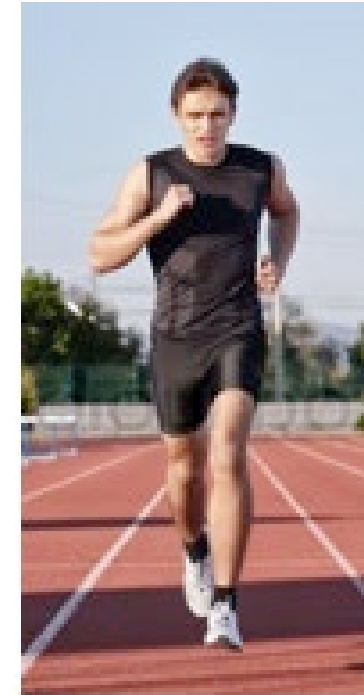
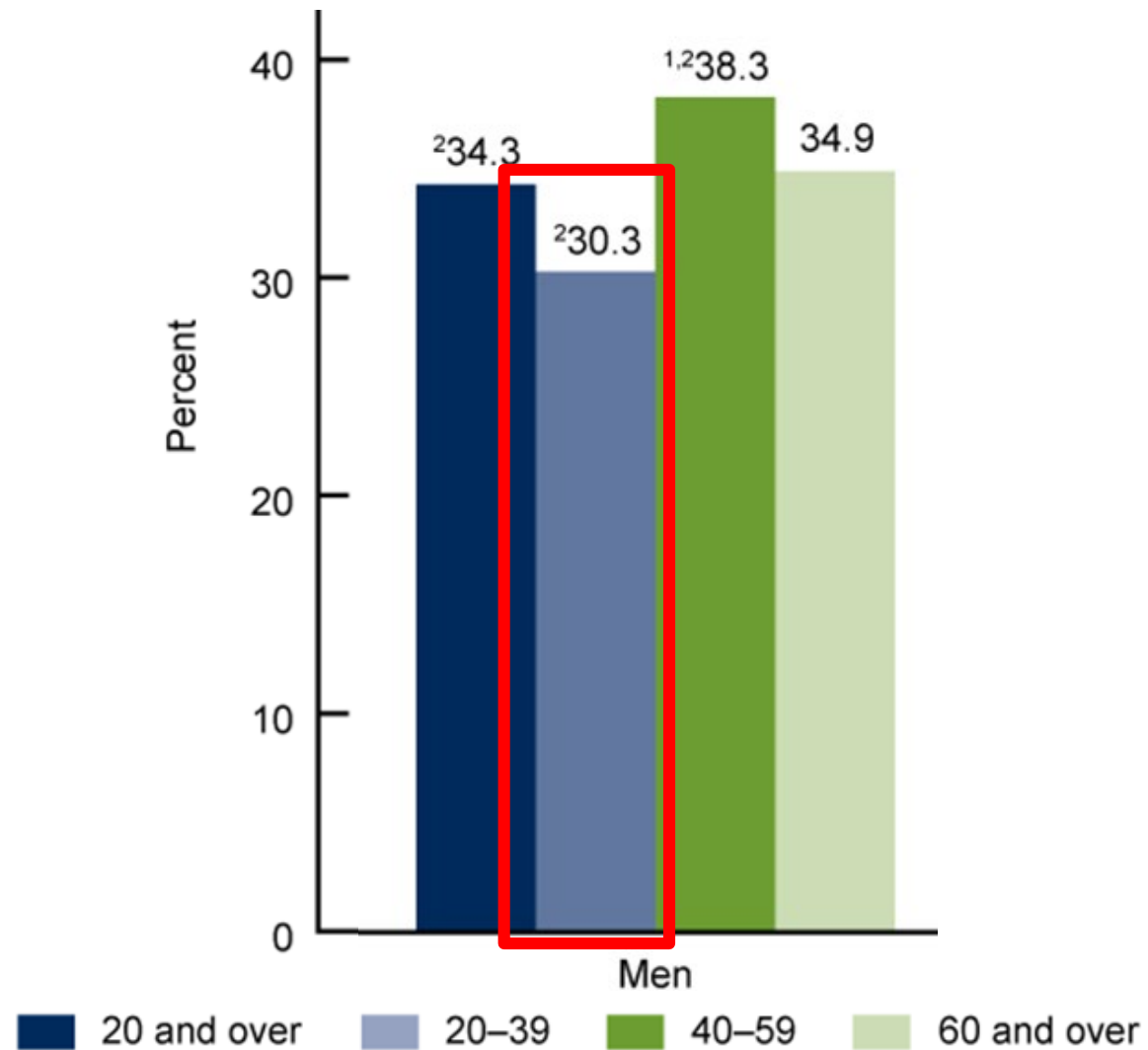
**Exercise**



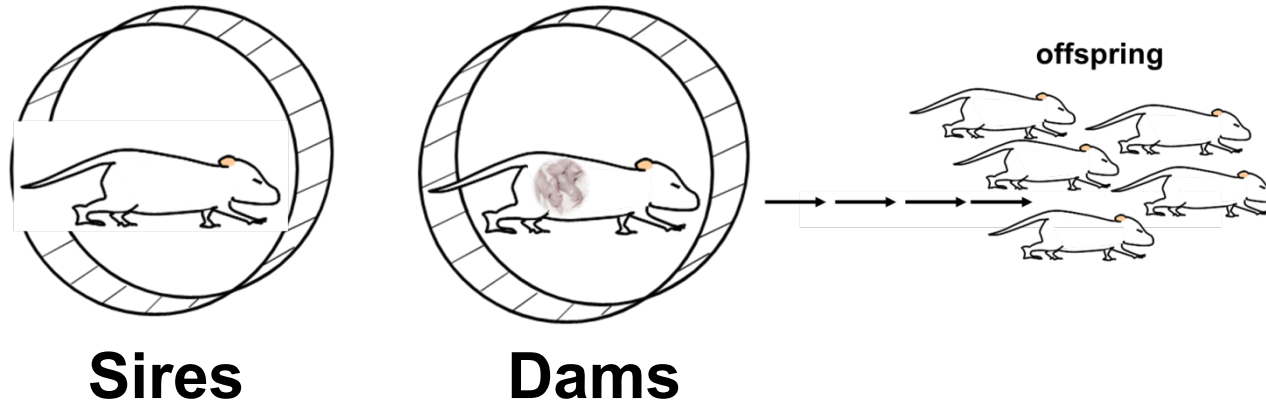
➤ Is there a mechanism of transmission of risk of metabolic disease to offspring?

Transmission of risk of metabolic

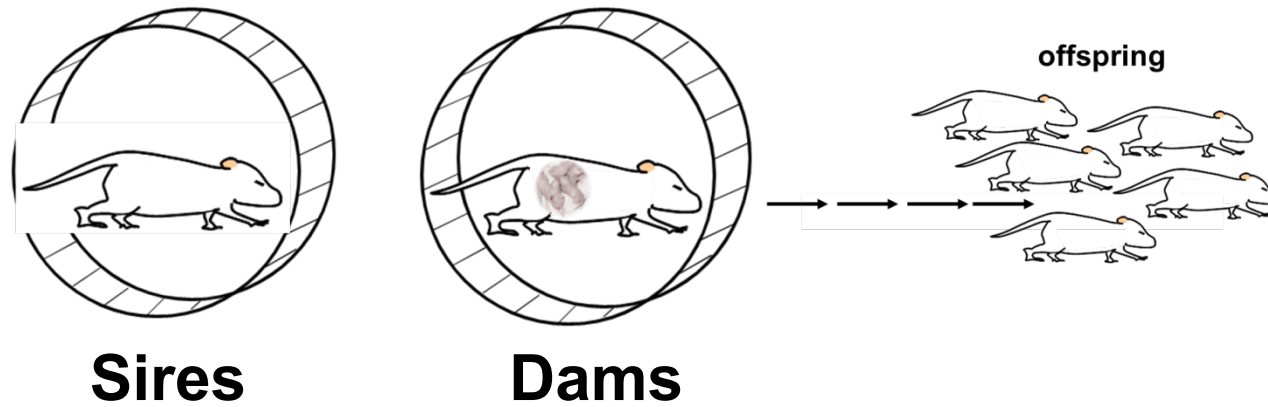
# Prevalence of Obesity Among Male Adults in the United States



# Effects of Maternal and Paternal Exercise on Offspring Metabolic Health

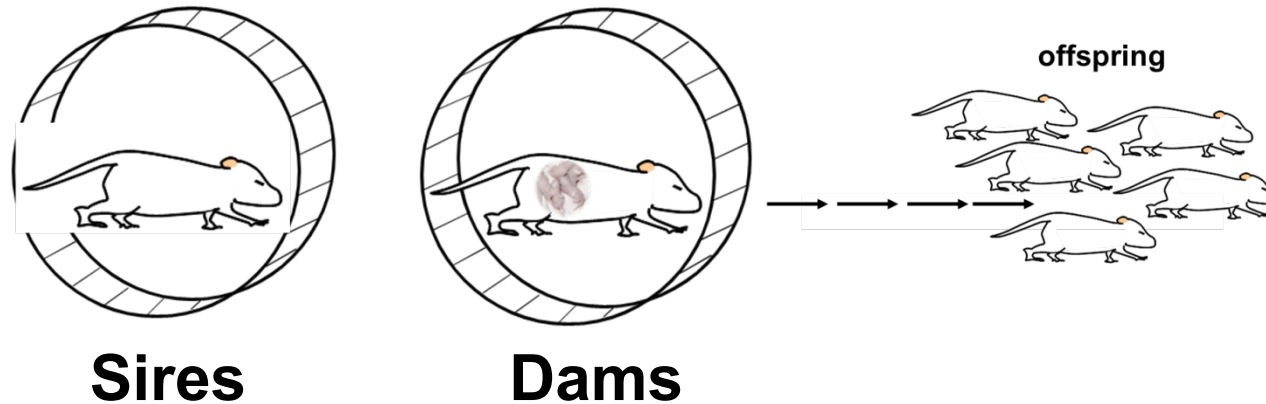


# Hypothesis



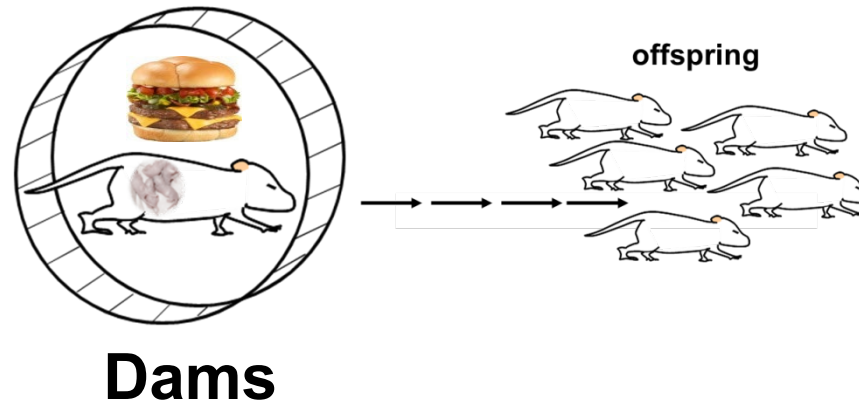
- **Increased physical activity of parents will improve the metabolic phenotype of offspring.**

# Outline of Data Presentation



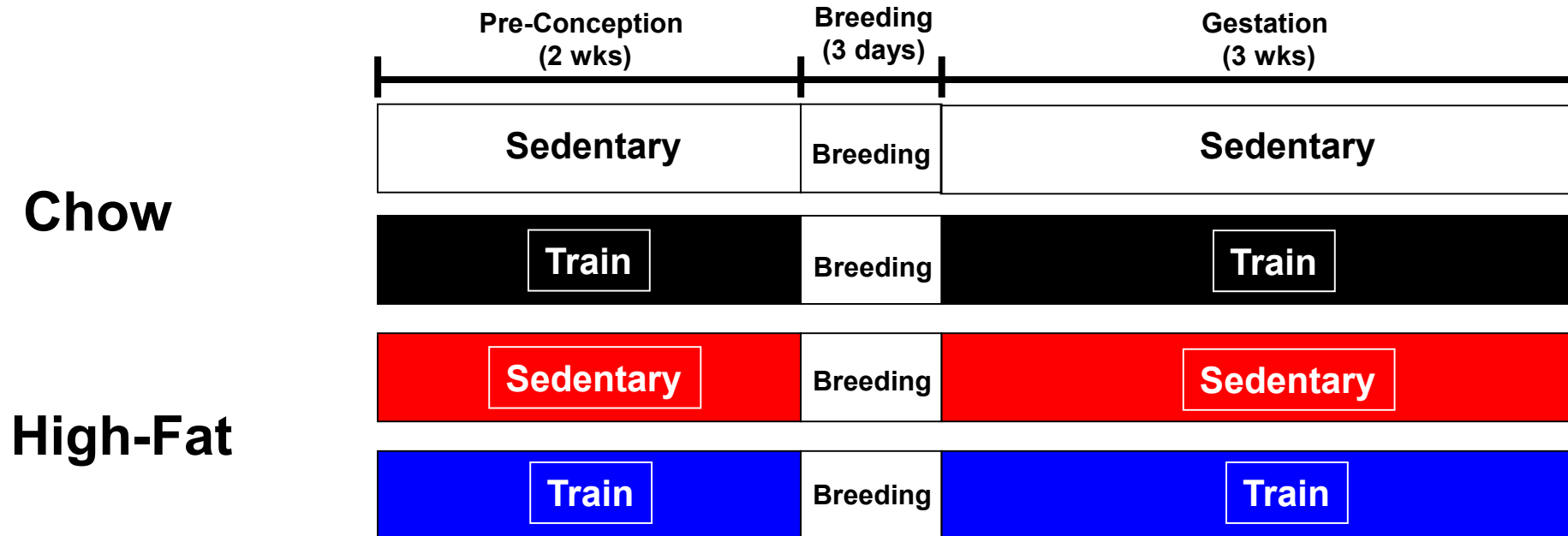
- **Maternal, Paternal and Maternal + Paternal Exercise: effects on offspring glucose tolerance.**
- **Maternal Exercise: mechanisms and epigenetic regulation of offspring.**
- **Maternal Exercise: identification of a novel protein that can improve offspring health.**

# Effects of Maternal Exercise Training on Offspring



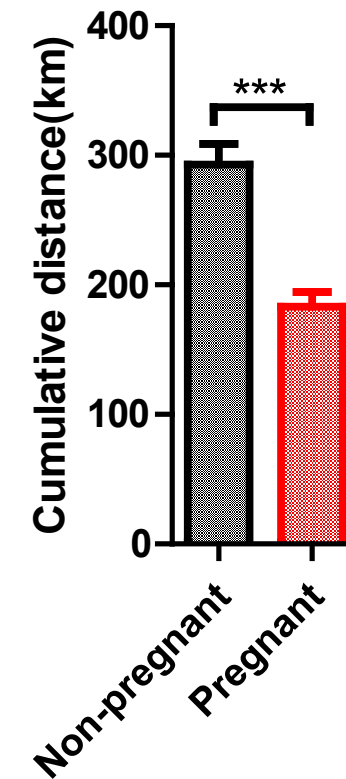
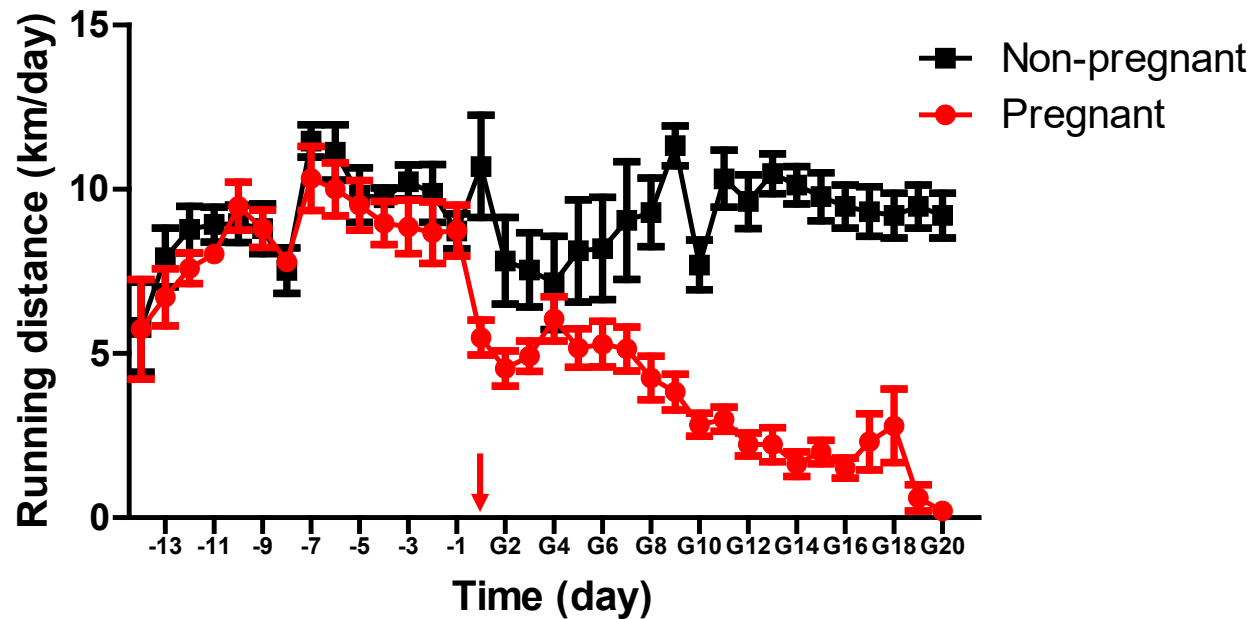
- **What is the effect of maternal exercise on glucose tolerance and insulin concentrations?**
- **Can maternal exercise prevent the detrimental effects of a high fat diet on offspring metabolic health?**

# Experimental Design – Maternal Exercise Studies



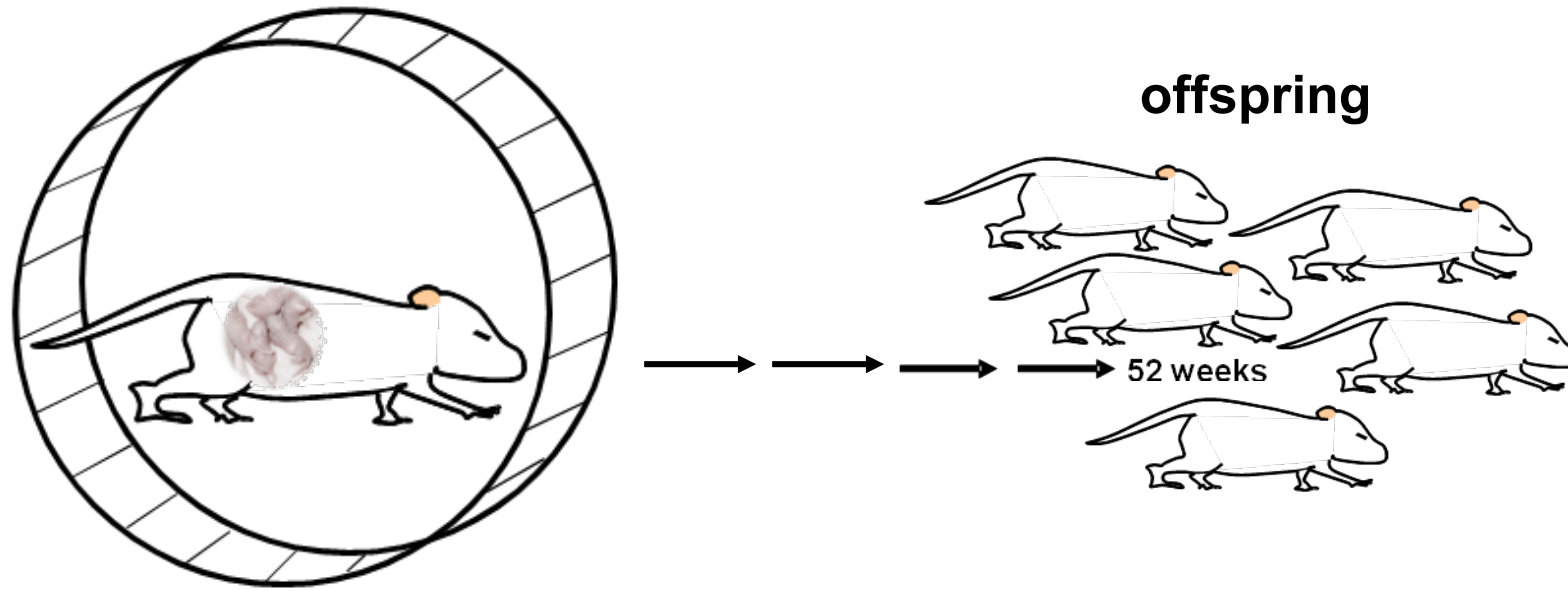
- Training done by voluntary wheel running
- Male breeders were sedentary and chow fed

# Running Distance of Non-Pregnant and Pregnant Females



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

# Experimental Design – Effects on Offspring Metabolic Health



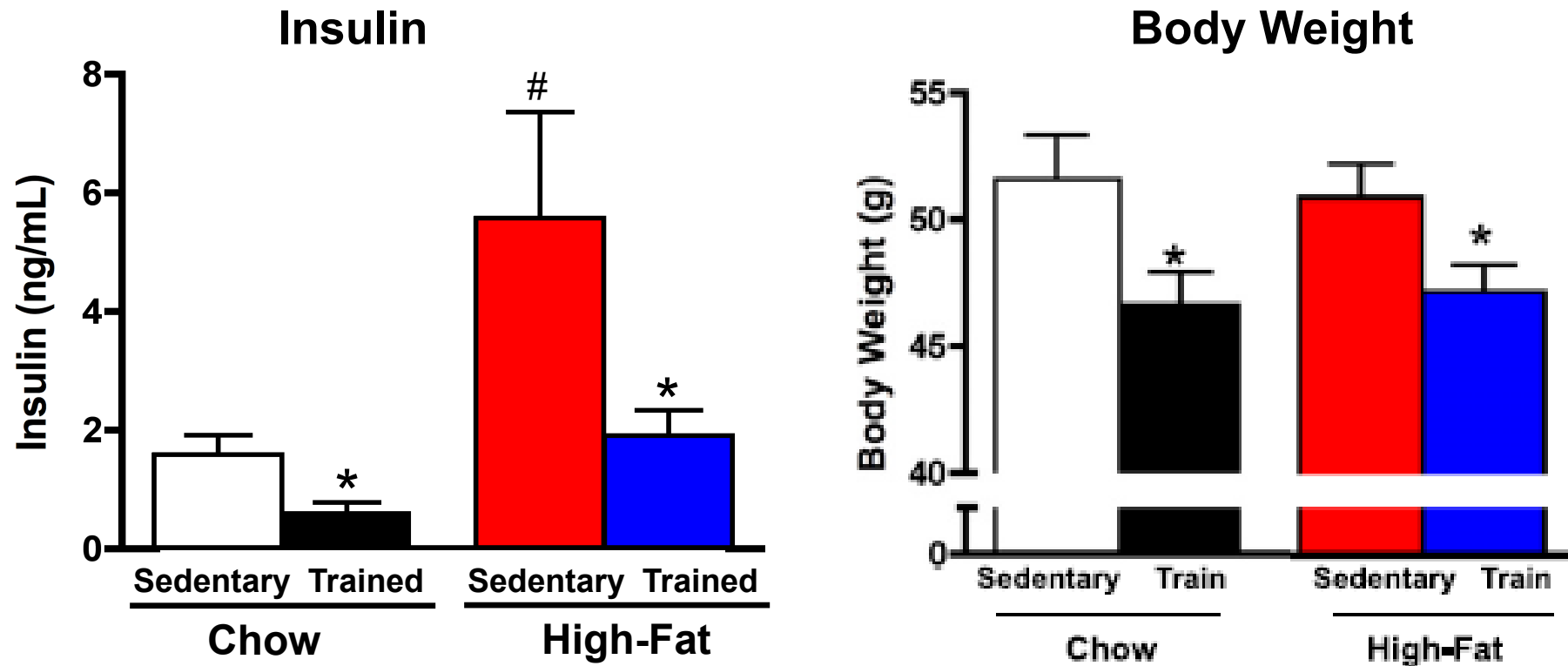
- Offspring were sedentary and chow-fed
- Offspring metabolic health monitored from youth until middle age (52 weeks)
- Glucose tolerance test area under the curve (AUC), insulin concentrations

# Maternal Exercise Improves Glucose Tolerance in Adult Male Offspring



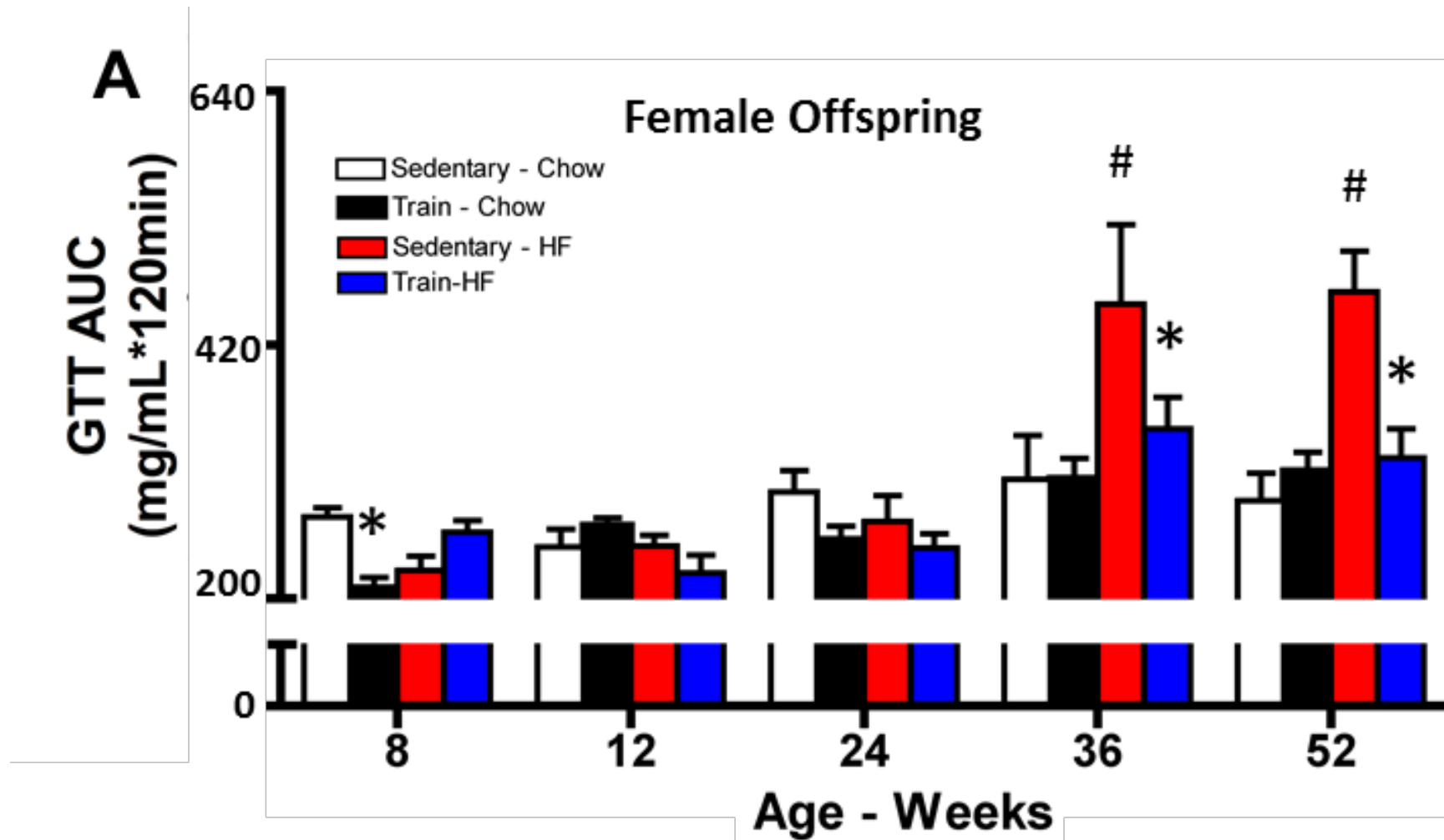
# Maternal Exercise Decreases Offspring Insulin Concentrations and Body Weight

52 week old Male Offspring

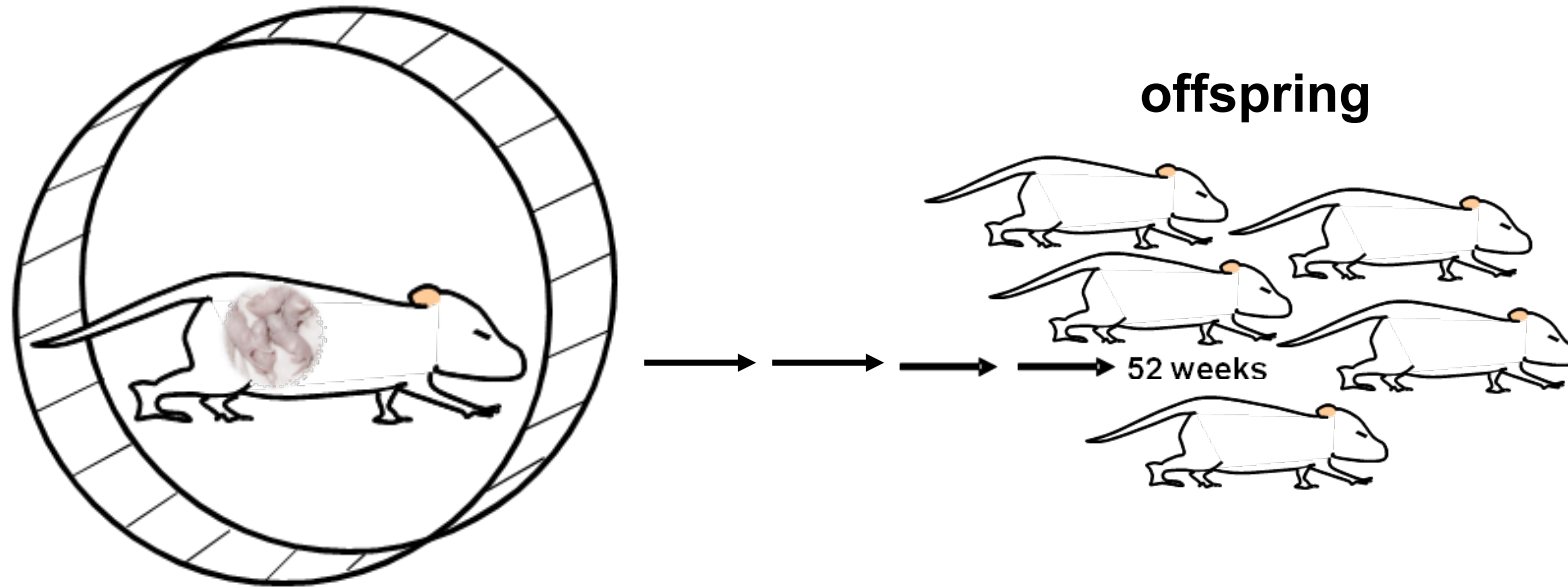


# Maternal Exercise Improves Glucose Tolerance in Female Offspring

GTT Area Under the Curve

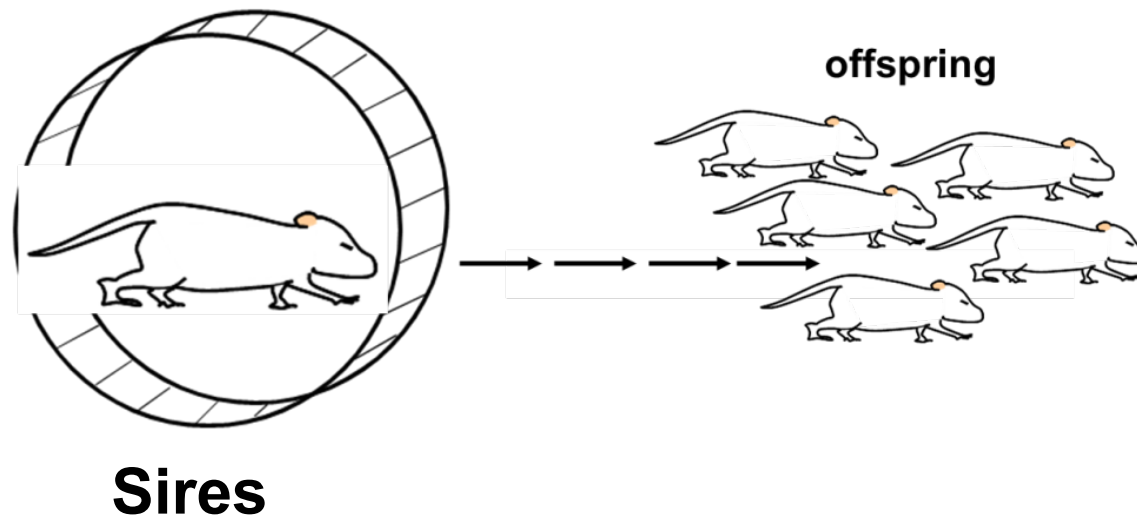


# Summary



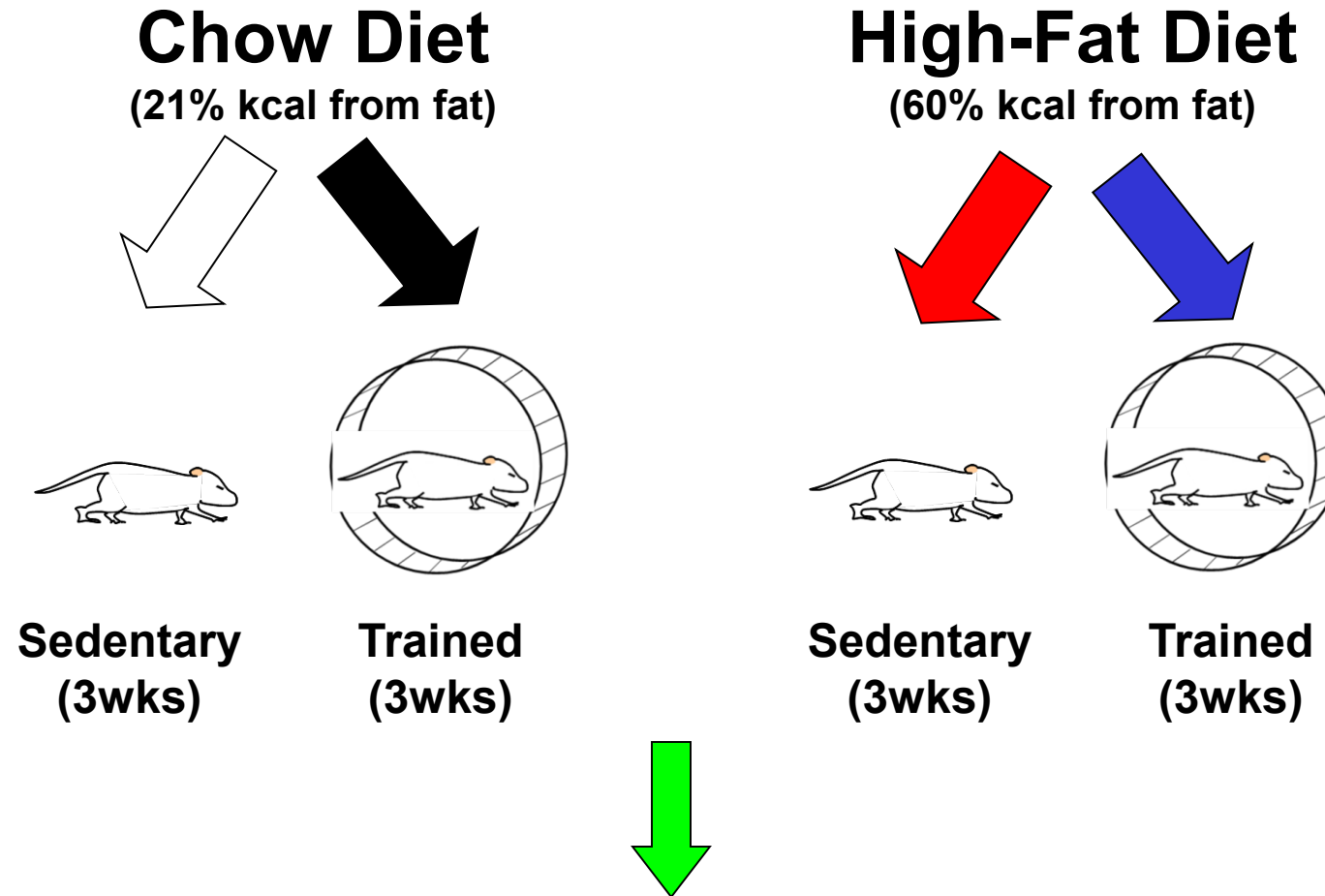
- **Maternal exercise before and during pregnancy improves the metabolic health of male and female offspring.**
- **Maternal exercise prevents the deleterious effects of high fat feeding on the metabolic health of male and female offspring.**

# Effects of Paternal Exercise on Offspring Metabolic Health



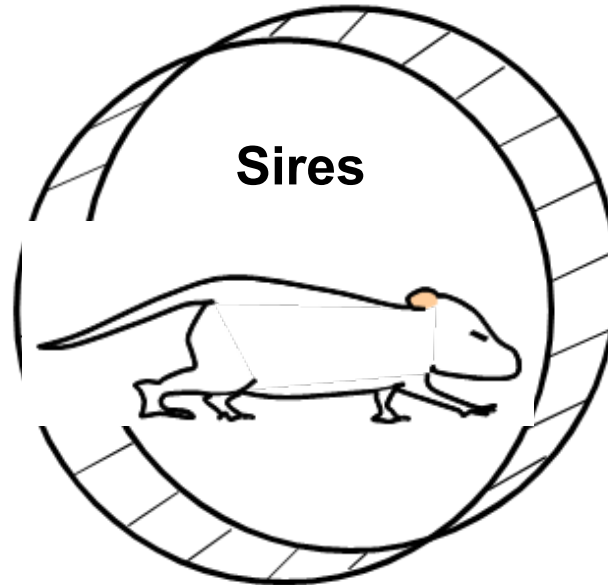
# Paternal Experimental Design

➤ C57BL/6 male mice (7 wks old)



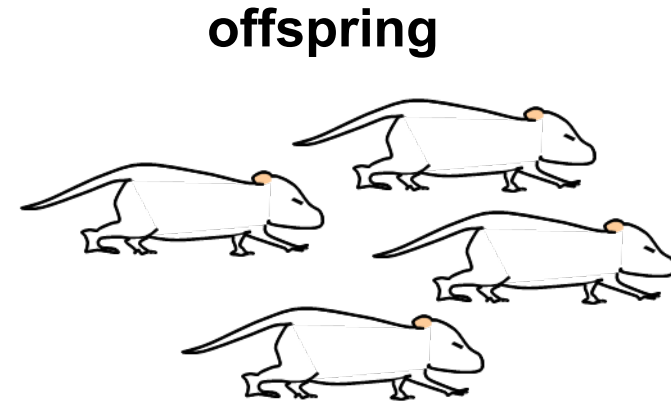
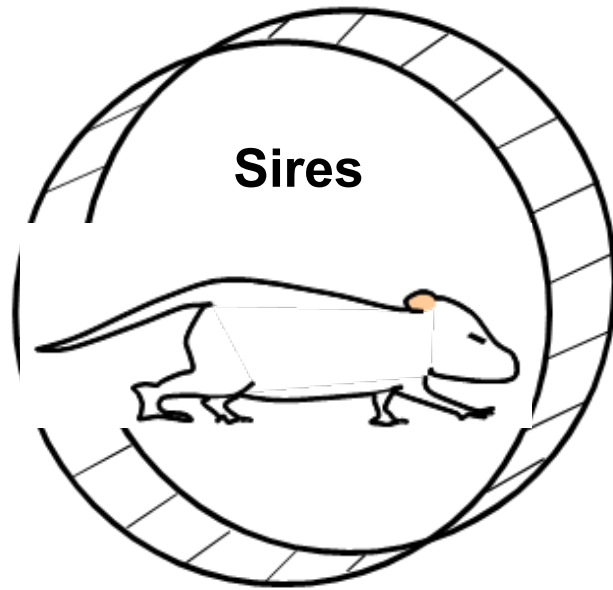
Bred with chow-fed, sedentary, female C57Bl/6 mice (8 wks old)

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



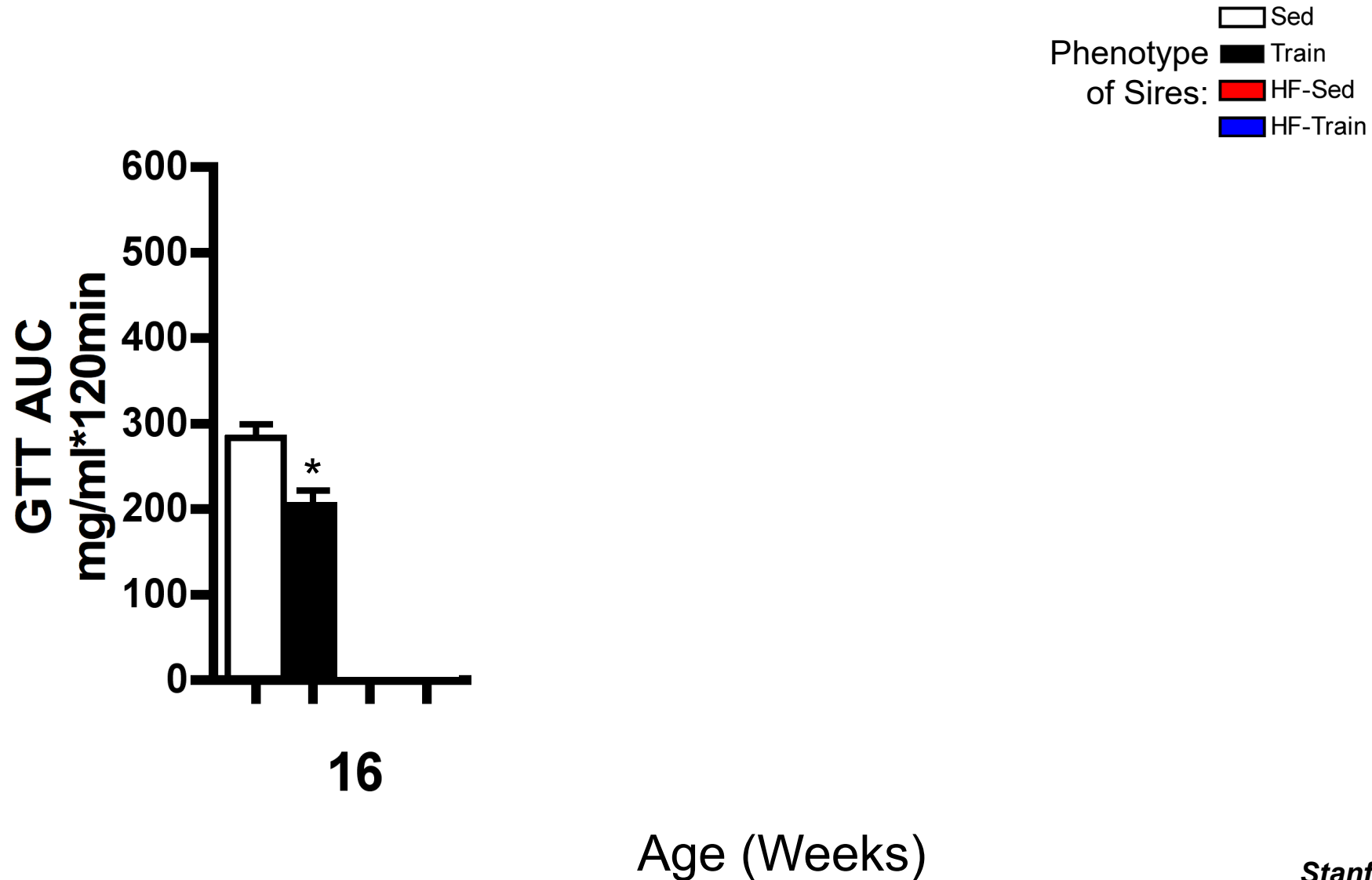
- **Running distance 5-6 km/day; no difference between chow and fat-fed mice.**

# Experimental Design – Effects on Offspring Metabolic Health

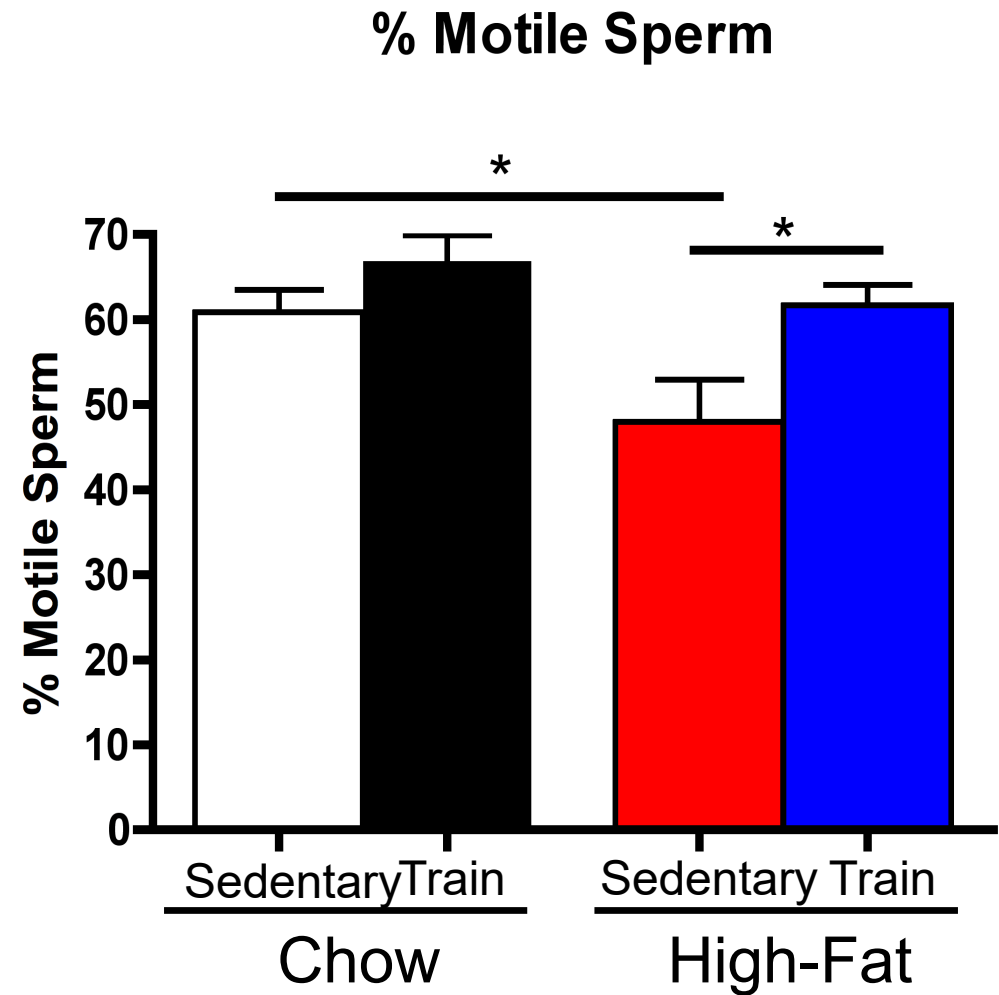
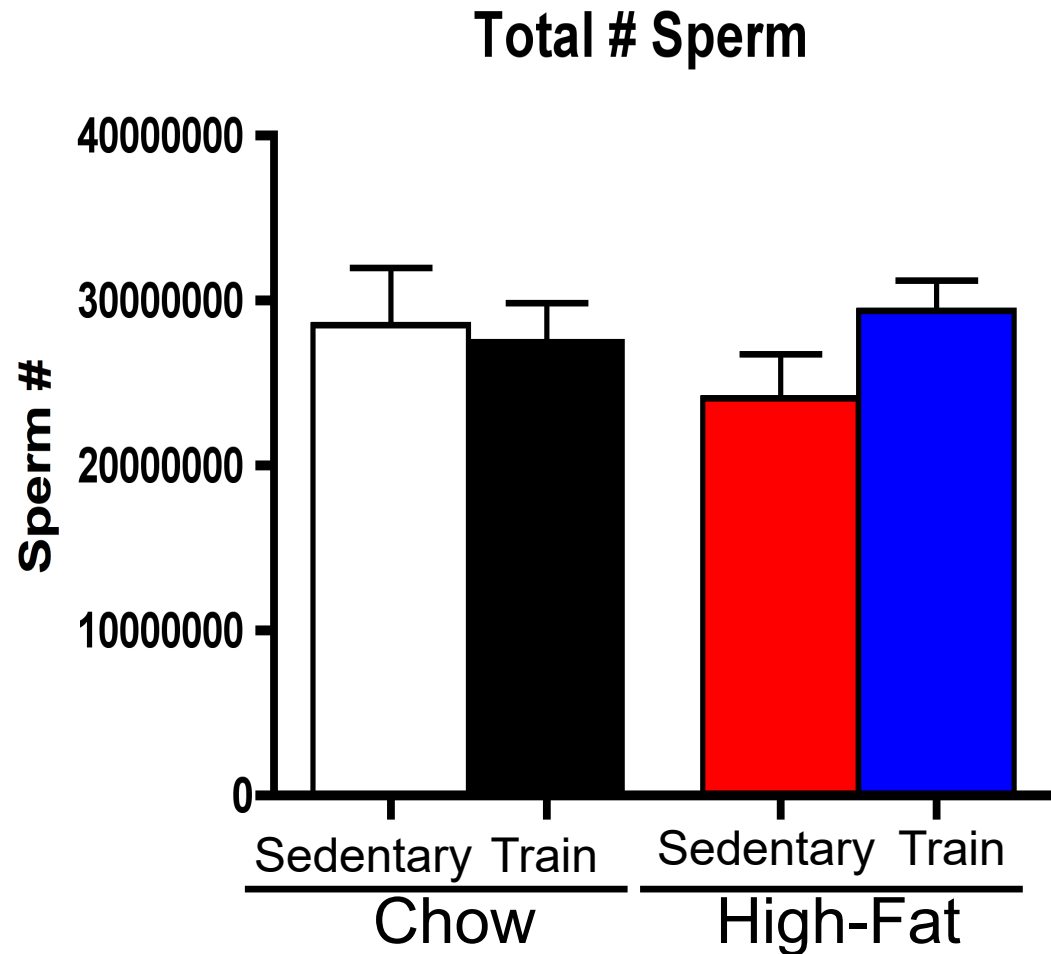


- **Offspring were sedentary and chow-fed**
- **Offspring metabolic health monitored from youth until middle age (52 weeks)**

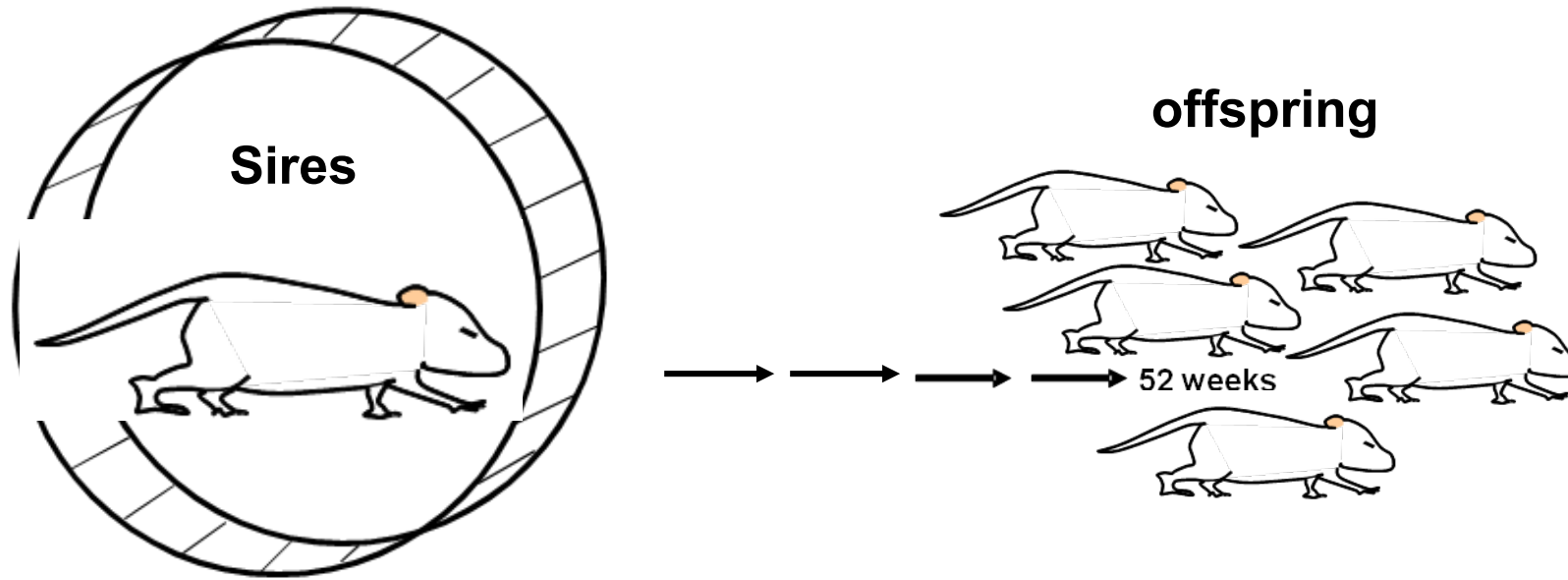
# Paternal Exercise Improves Glucose Tolerance in Male Offspring



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

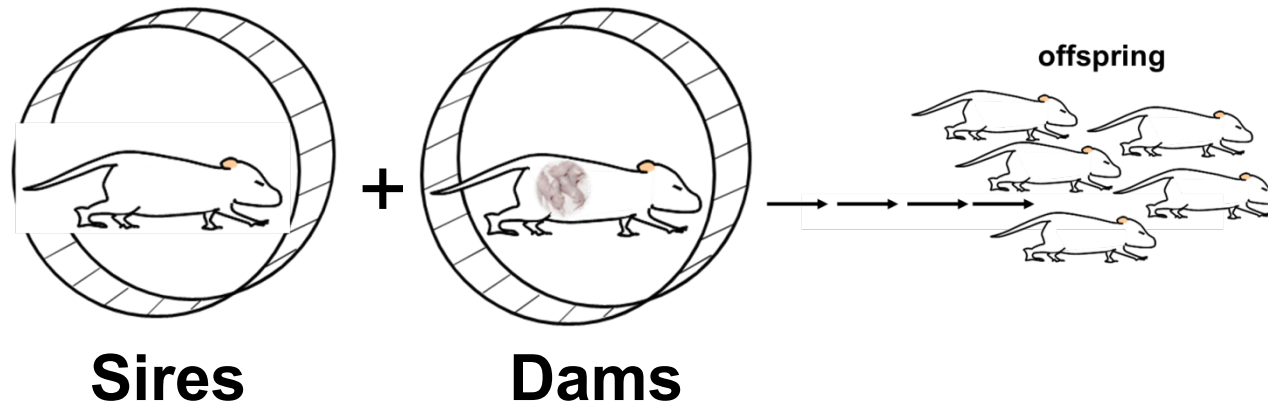


# Summary



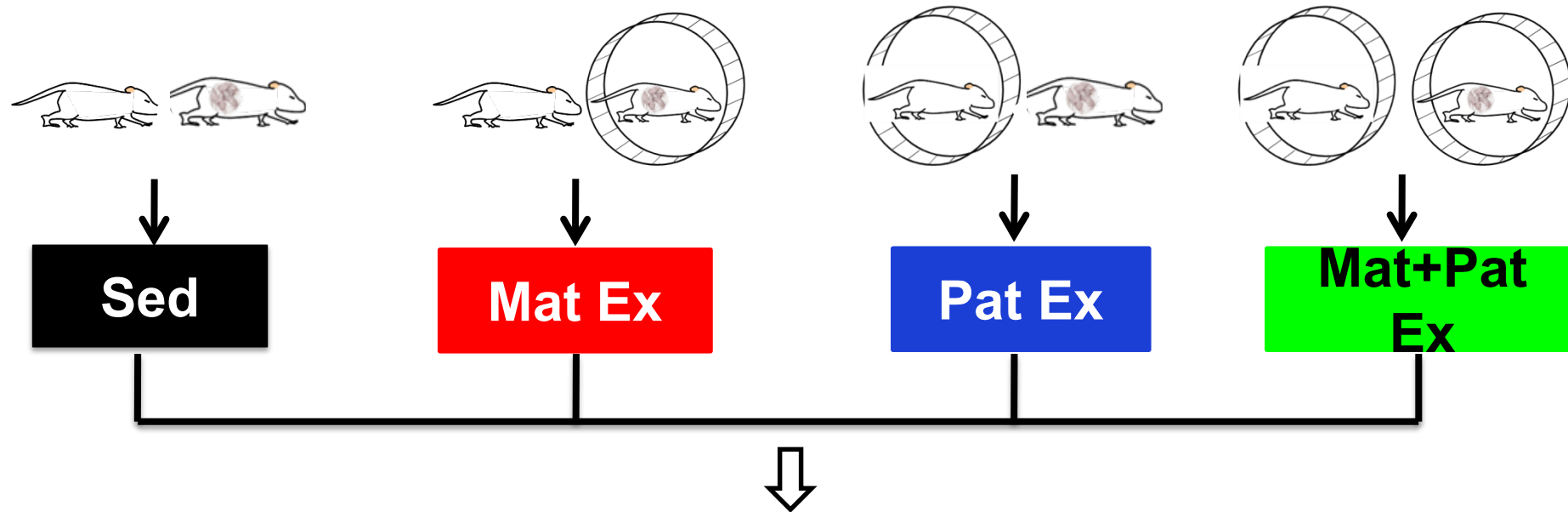
- **Paternal exercise for 3 weeks prior to breeding improves the metabolic health of male and female offspring, and reverses the detrimental effects of sire high-fat feeding.**
- **Paternal exercise alters the motility and the non-coding RNA profile of sperm; how these may alter offspring health is under investigation.**

# Effects a Combined Paternal and Maternal Exercise on Offspring Glucose Tolerance



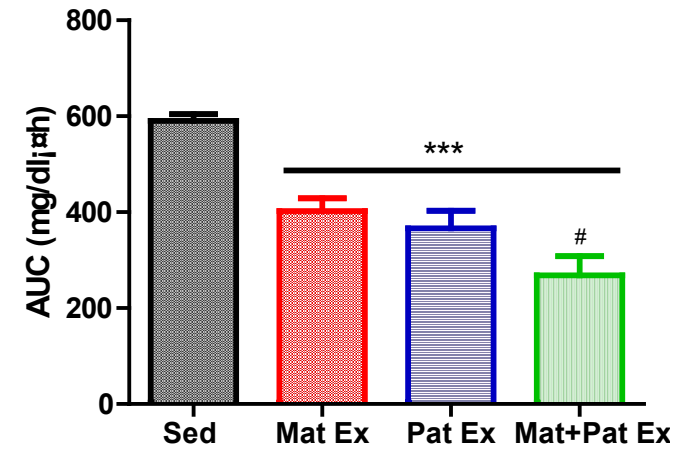
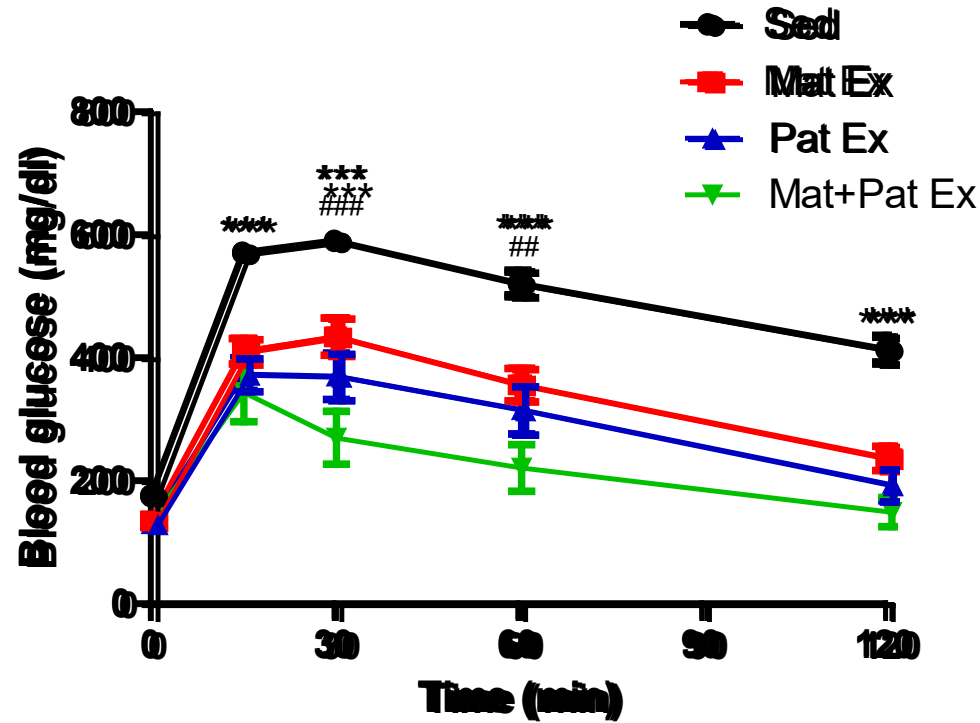
# Experimental Design

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



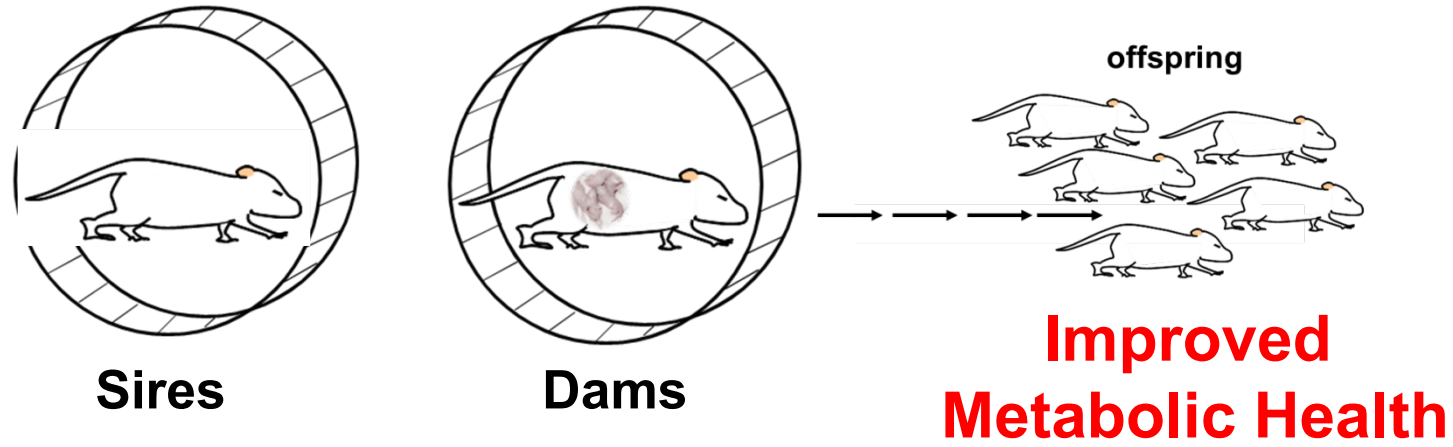
- Offspring were sedentary and chow-fed
- Offspring glucose tolerance at 52 weeks of age

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



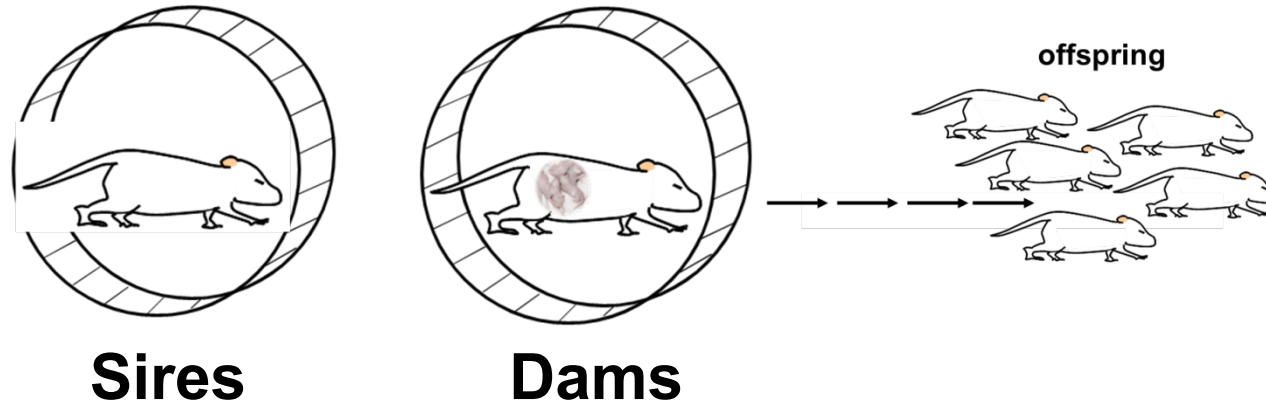
Data represent mean  $\pm$  SEM  
\*\*\*P<0.001, #P<0.05, ##P<0.01, ###P<0.001  
one mice per litter, n=6 each group

# Summary



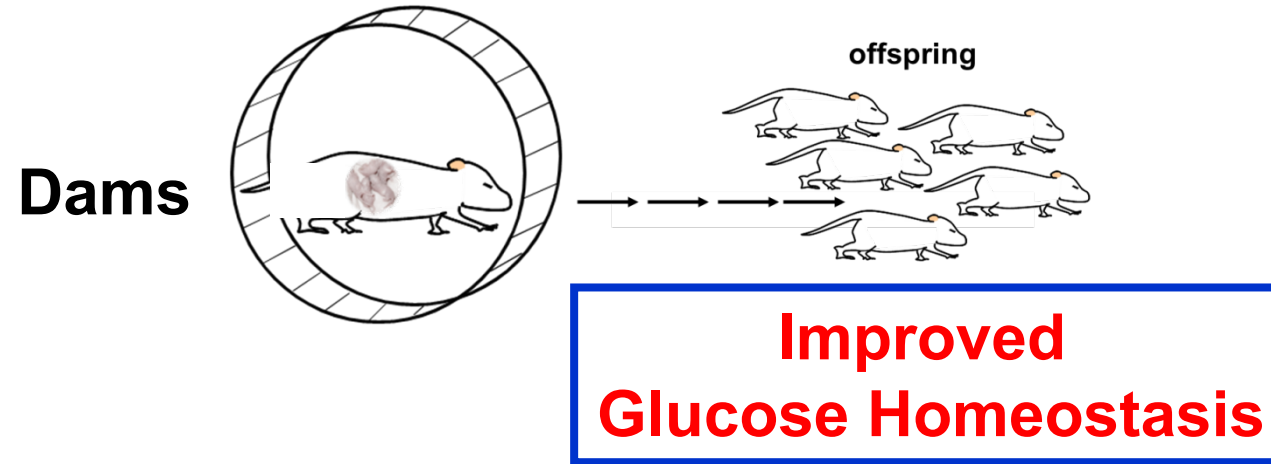
- The combination of both paternal and maternal exercise may have even greater beneficial effects on offspring metabolic health.

# Maternal and Paternal Exercise Training

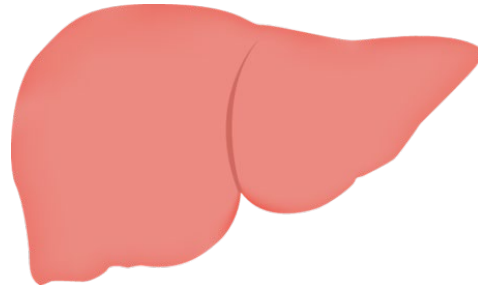


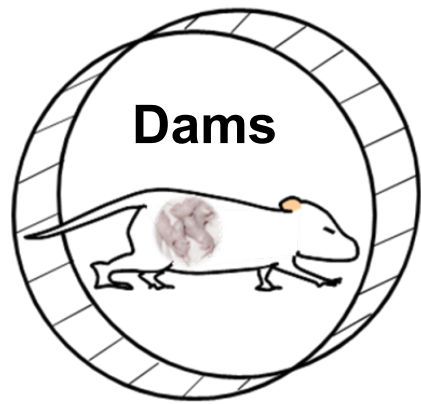
- **Maternal, Paternal and Maternal + Paternal Exercise: effects on offspring glucose tolerance.**
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# Maternal Exercise Training

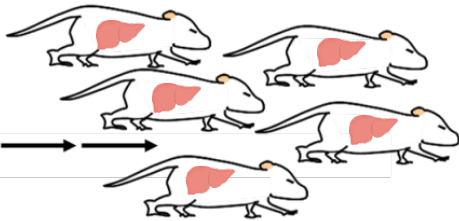


- **What tissue(s) are responsible for the improved glucose tolerance in the offspring of mothers that exercised?**





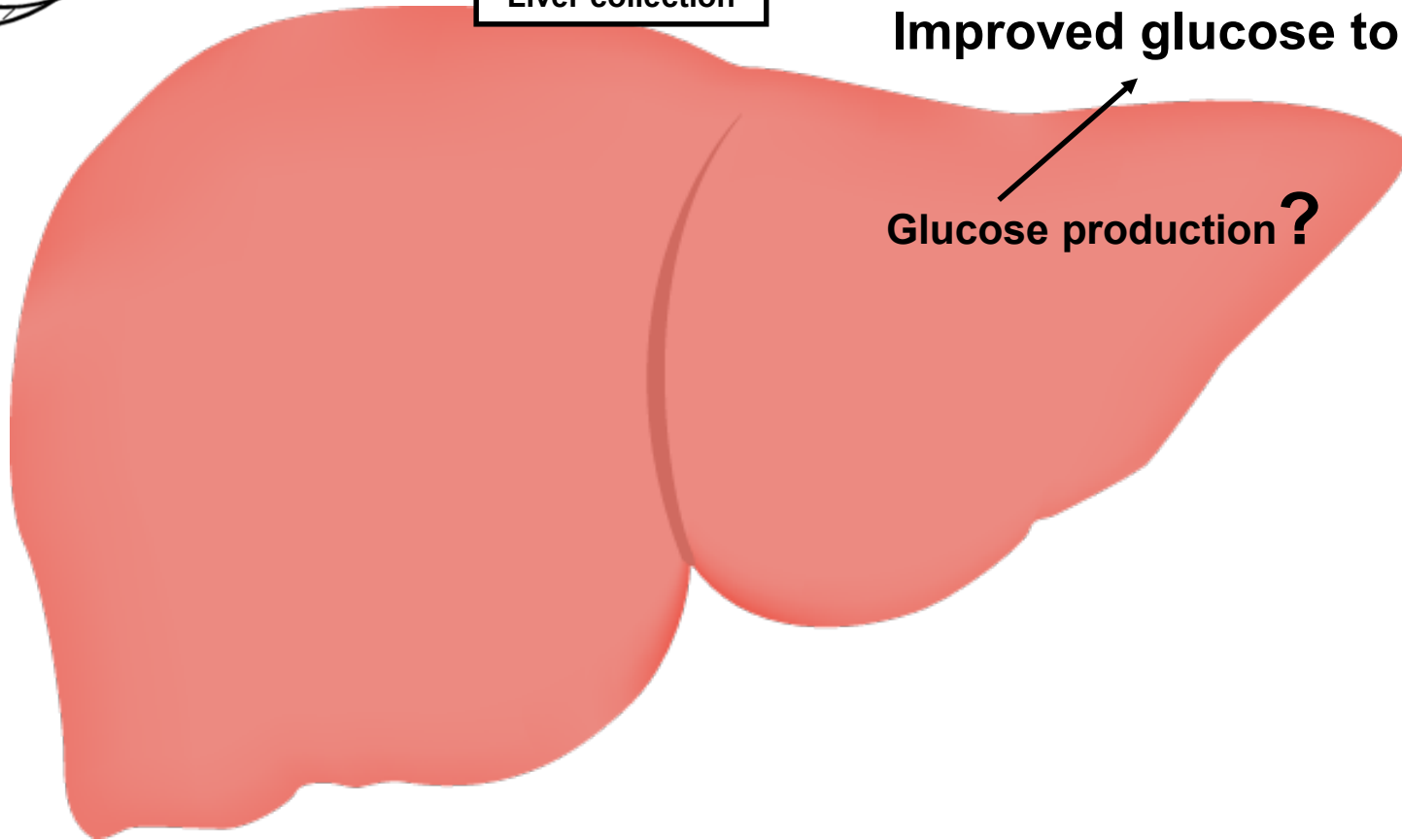
**Offspring**



**Liver collection**

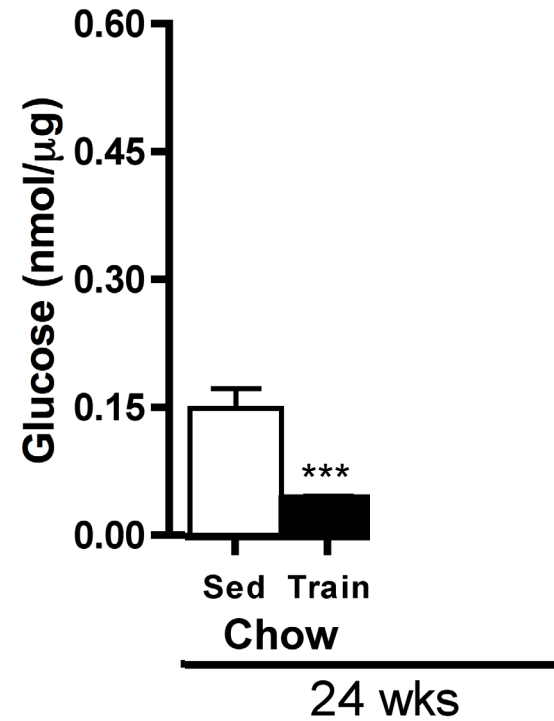
**Improved glucose tolerance**

**Glucose production ?**



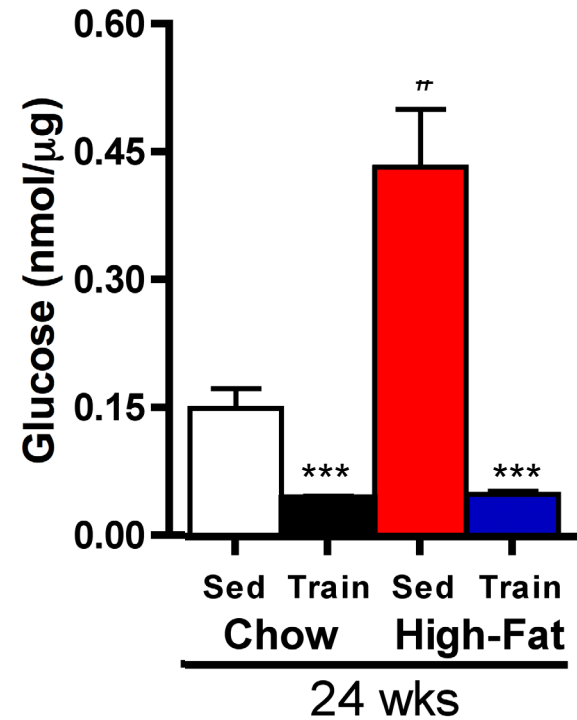
# Maternal Exercise Effects Glucose Production in Isolated Hepatocytes of Offspring

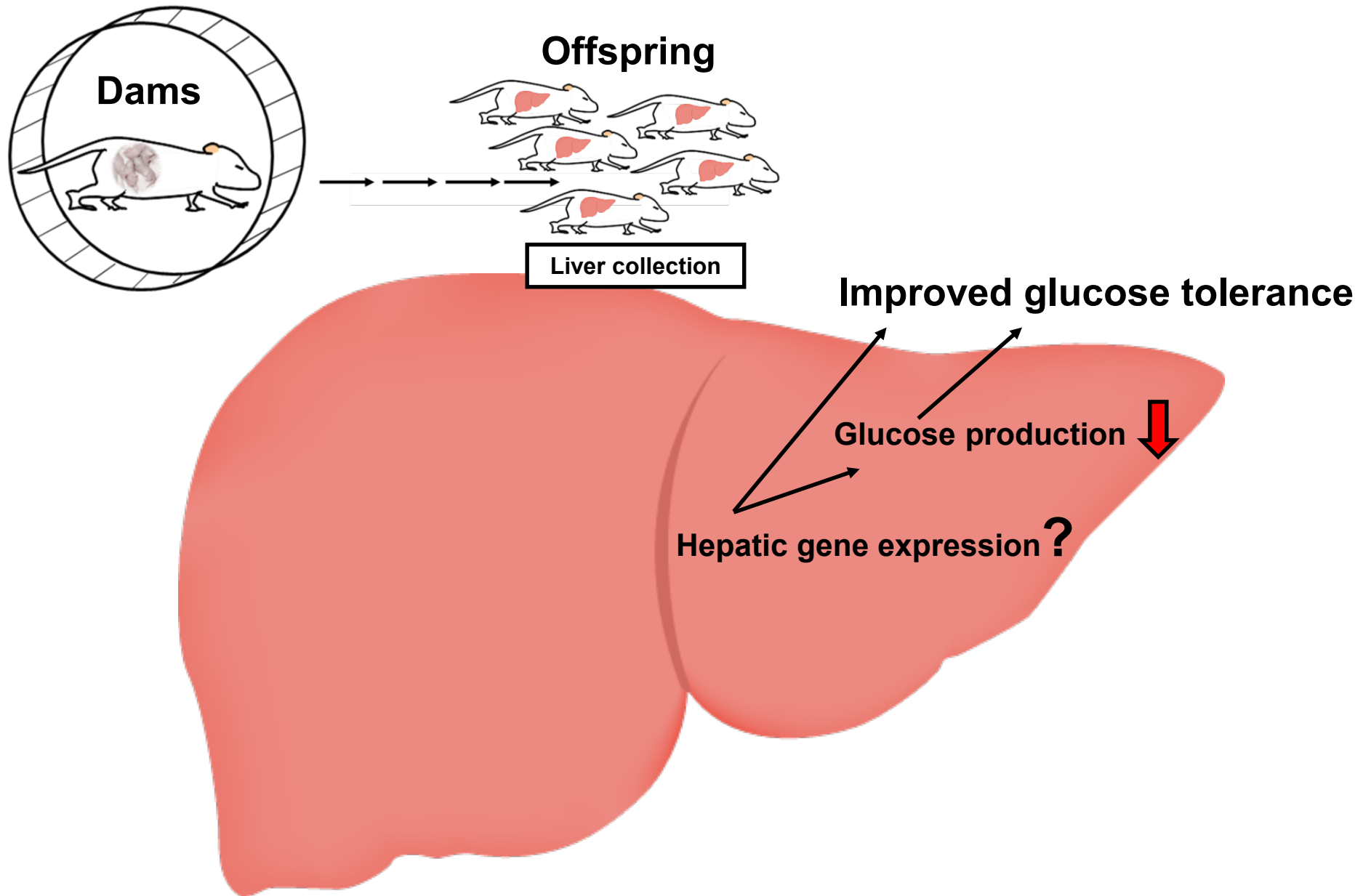
## Basal Hepatocyte Glucose Production



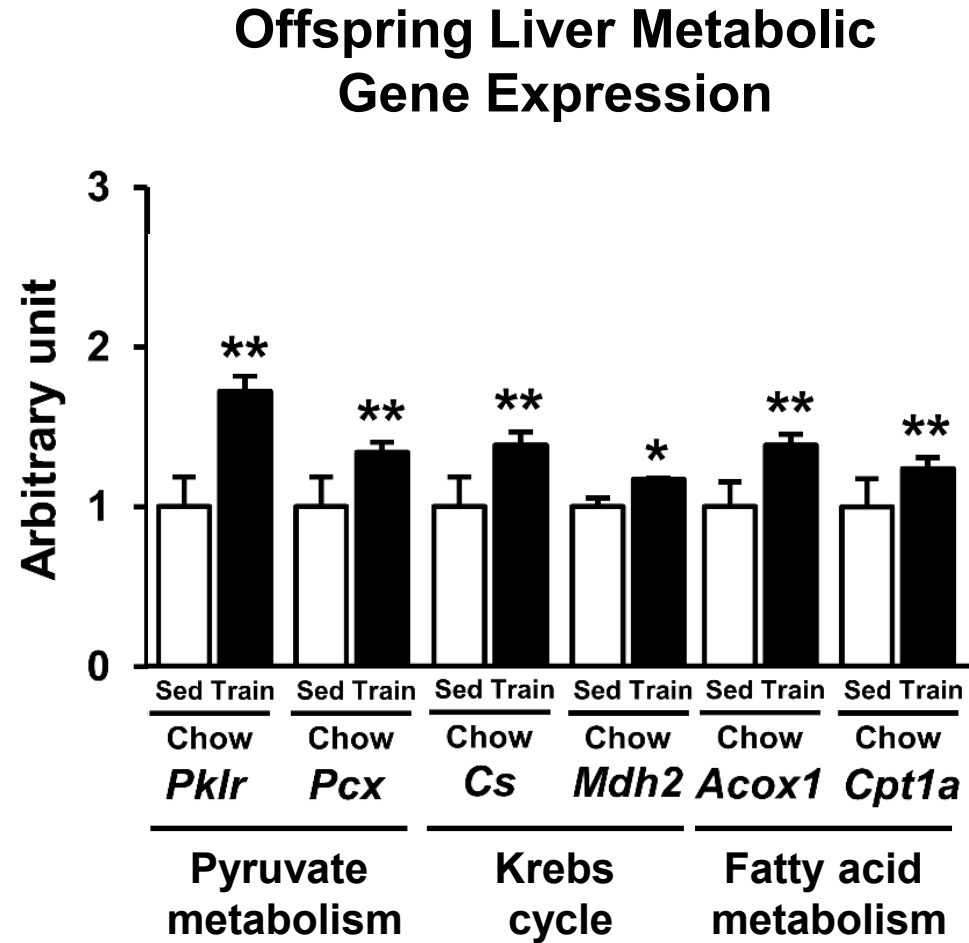
# Maternal Exercise Effects Glucose Production in Isolated Hepatocytes of Offspring

## Basal Hepatocyte Glucose Production

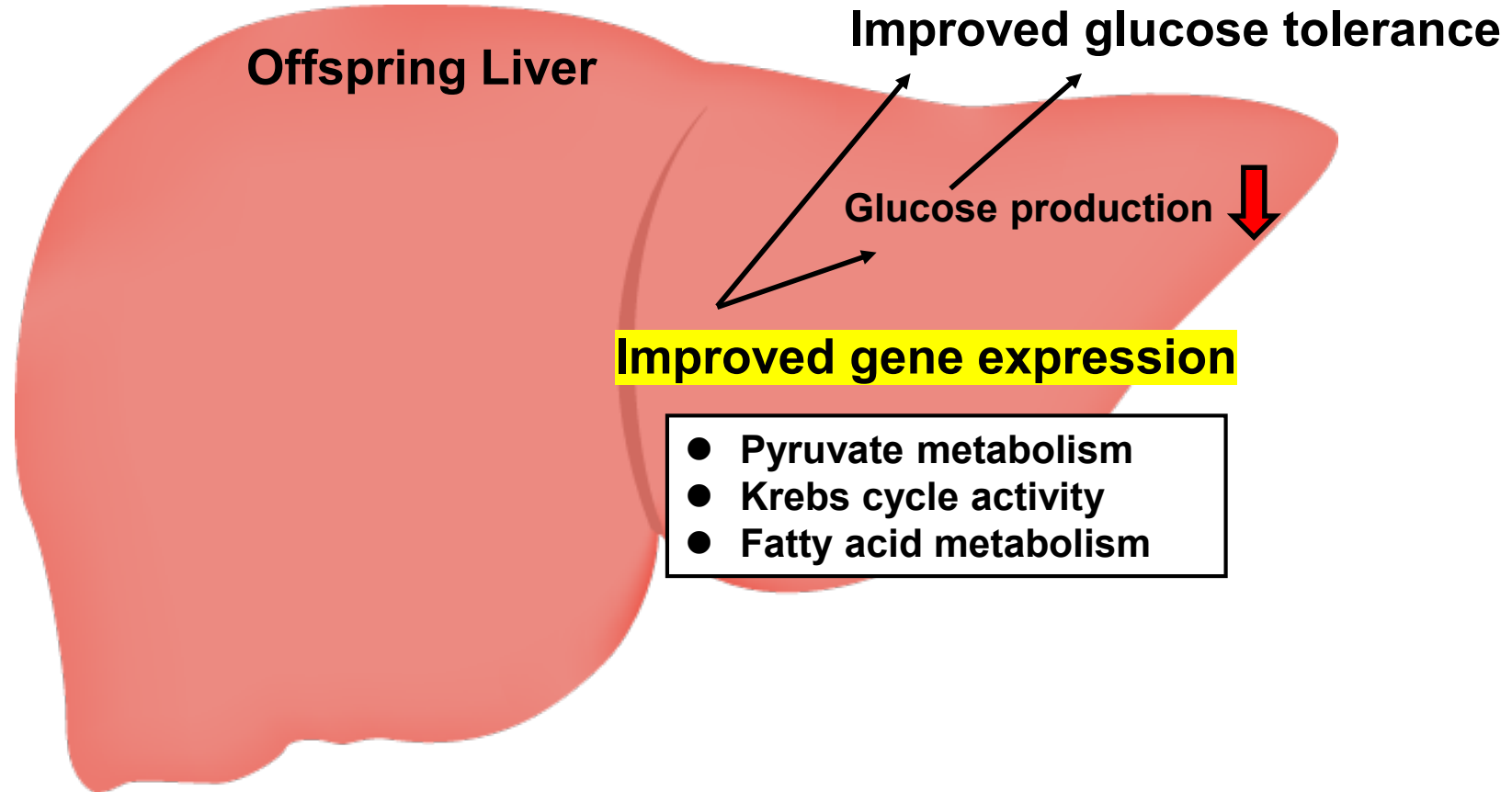




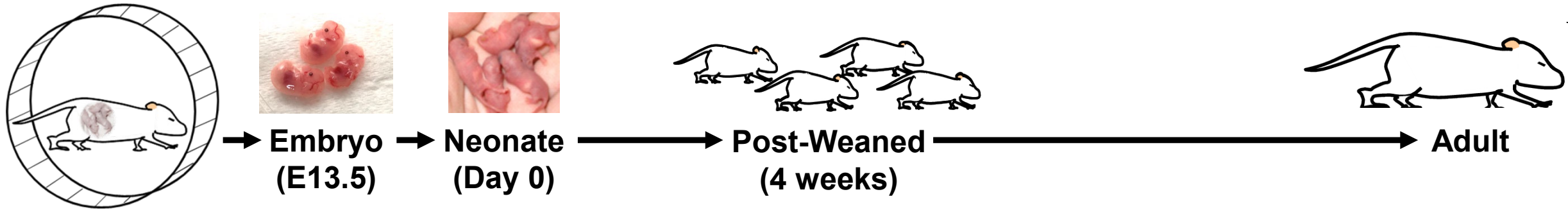
# Maternal Exercise in Mice Results in Improved Offspring Liver Gene Profile



# Effects of Maternal Exercise on Offspring Liver Function

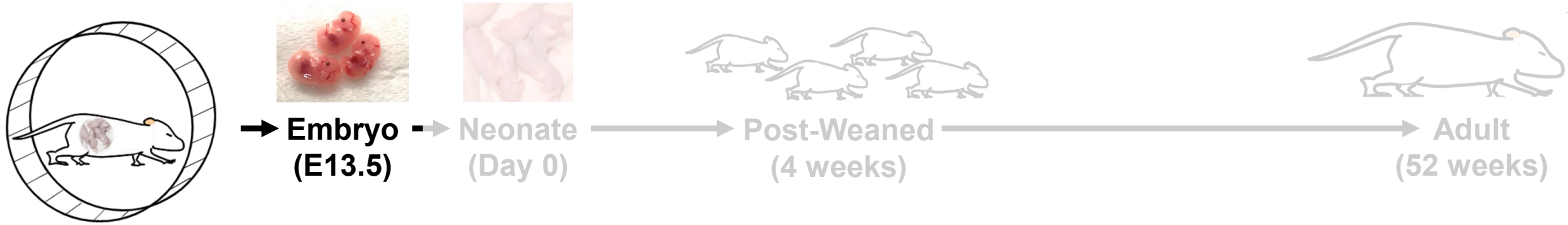


# Maternal Exercise Training

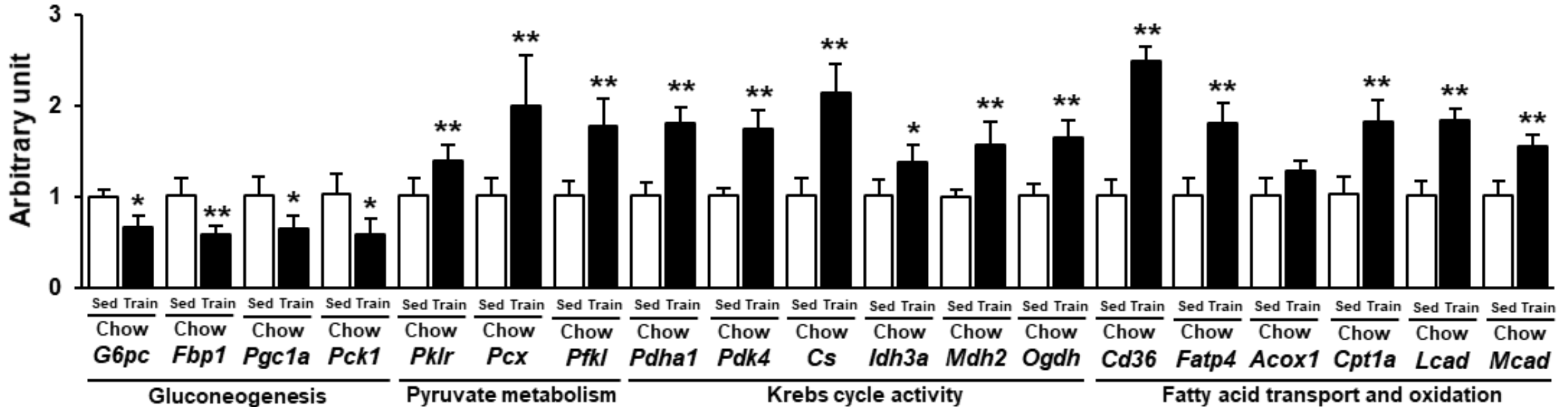


- **At what stage in the offspring development do the changes in hepatic gene expression occur?**

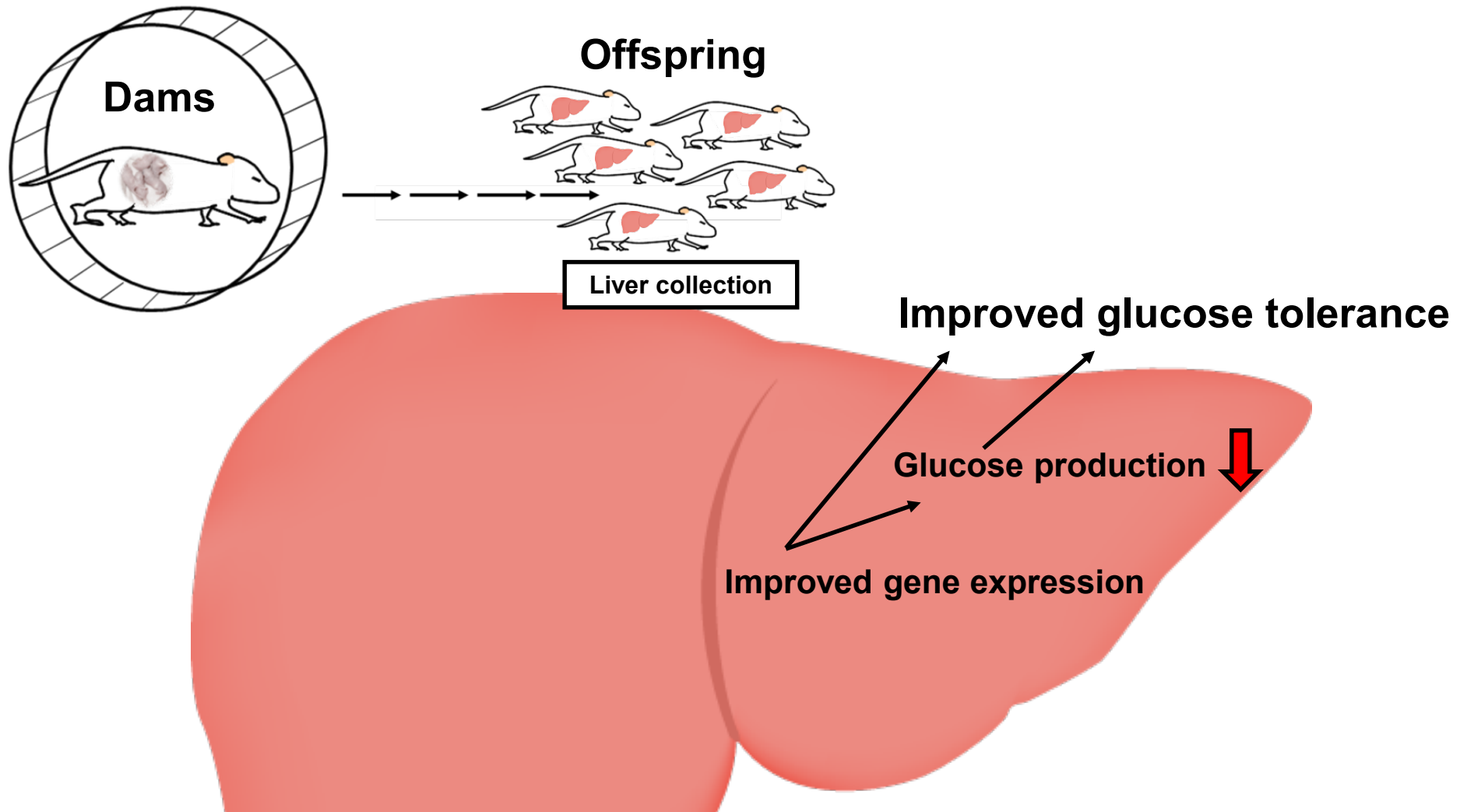
# Maternal Exercise Affects Hepatic Gene Expression in Young Offspring



Liver of embryo (E13.5)



# Summary

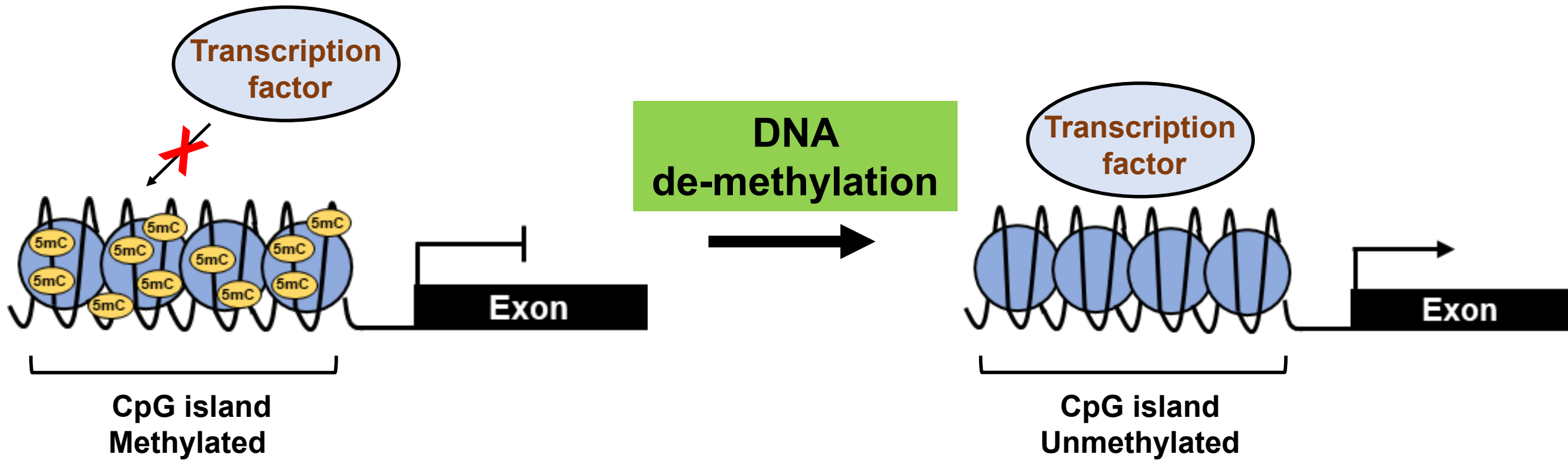


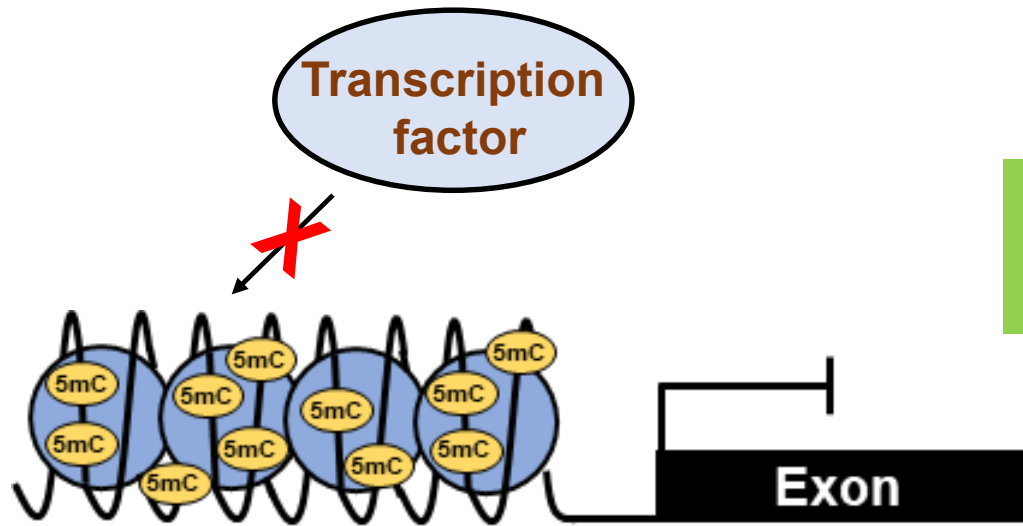
➤ **Changes in offspring gene expression occur in early development, suggesting epigenetic changes.**

# Epigenetics

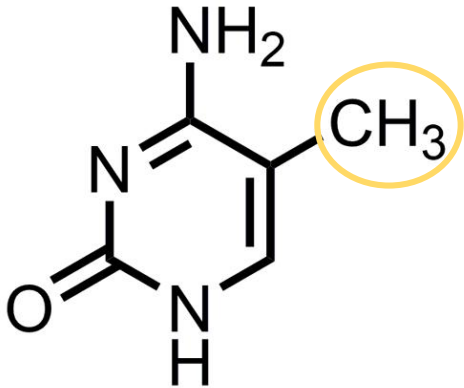
Heritable Changes in Gene Function that DO NOT Involve Changes in the DNA Sequence

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



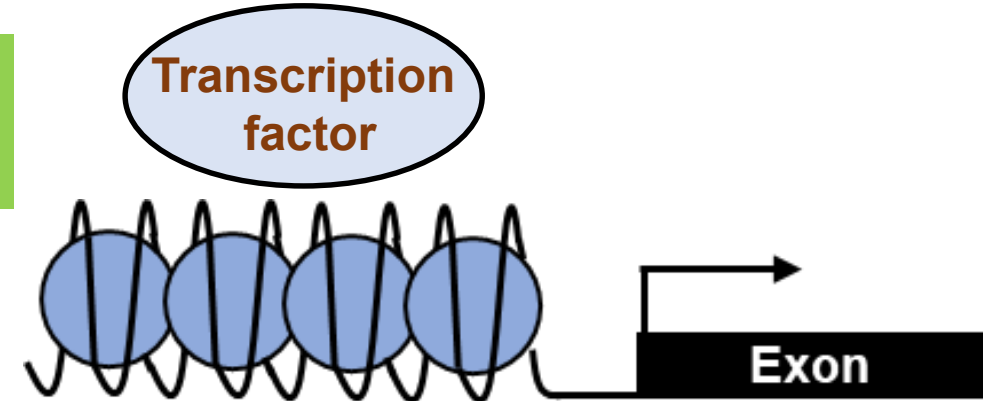


## Transcription Inhibition

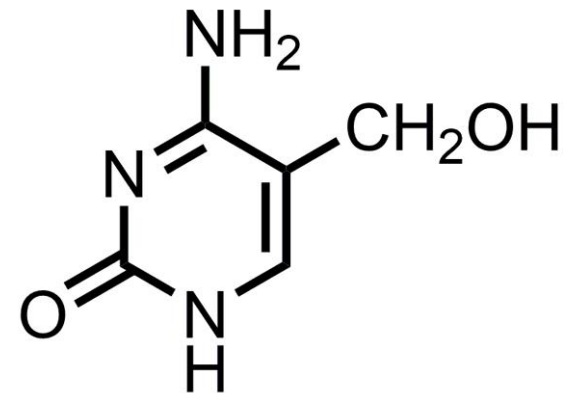


5-methylcytosine  
(5-mC)

DNA  
de-methylation

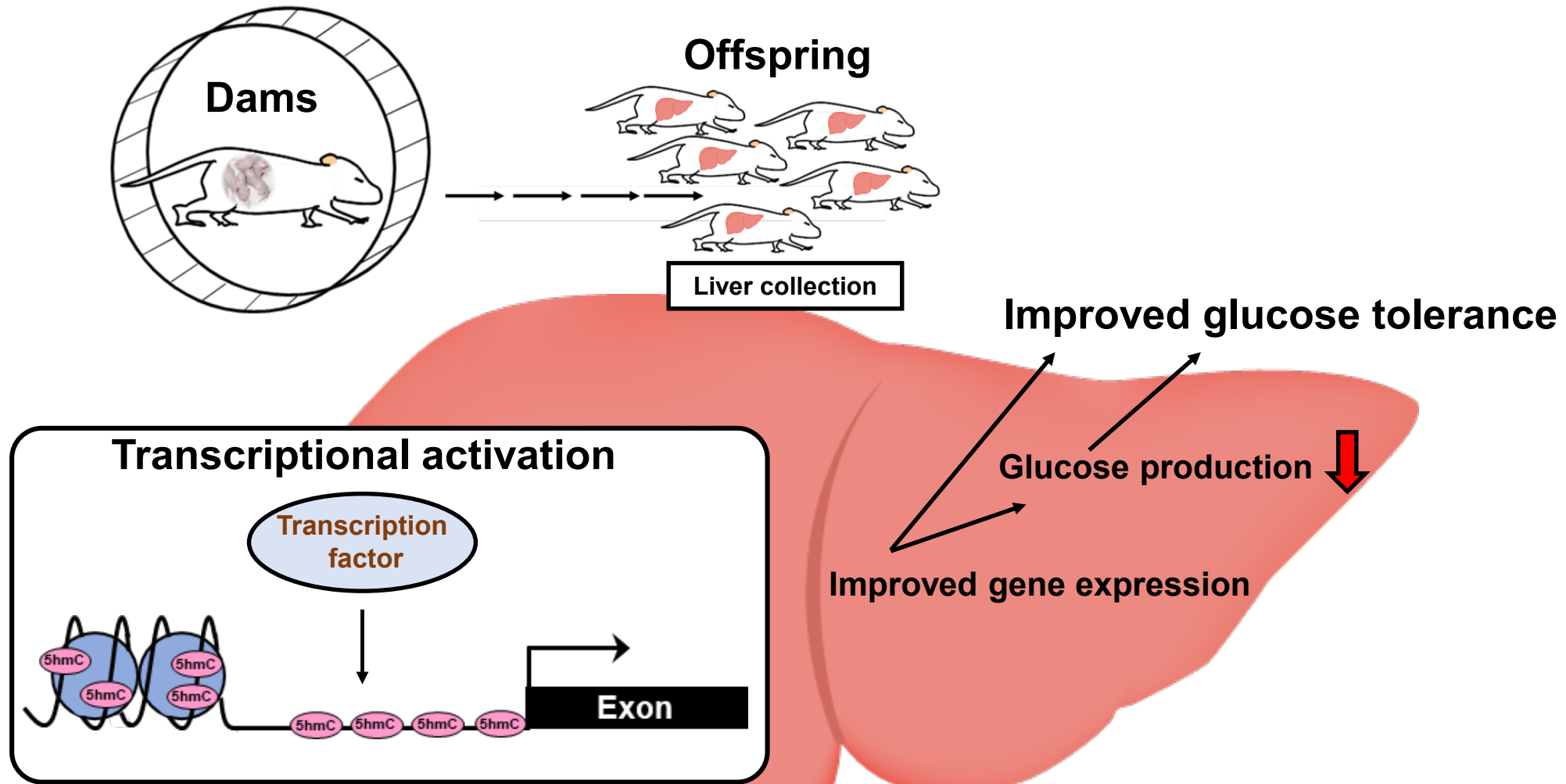


## Transcription Activation



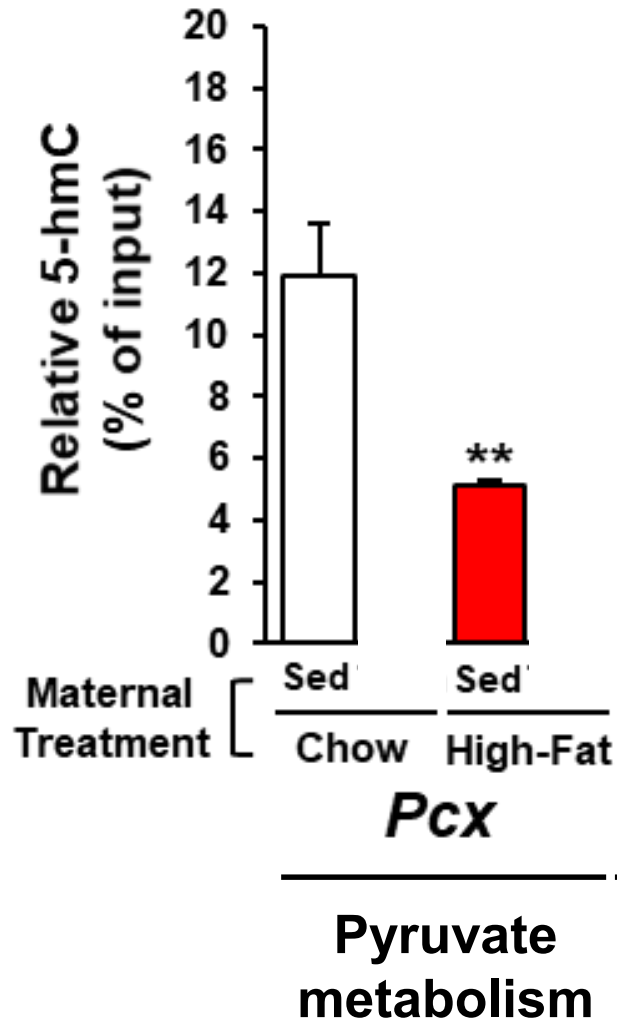
5-hydroxymethylcytosine  
(5-hmC)

# Increased Hepatic Gene Transcription May Be Caused by DNA Demethylation



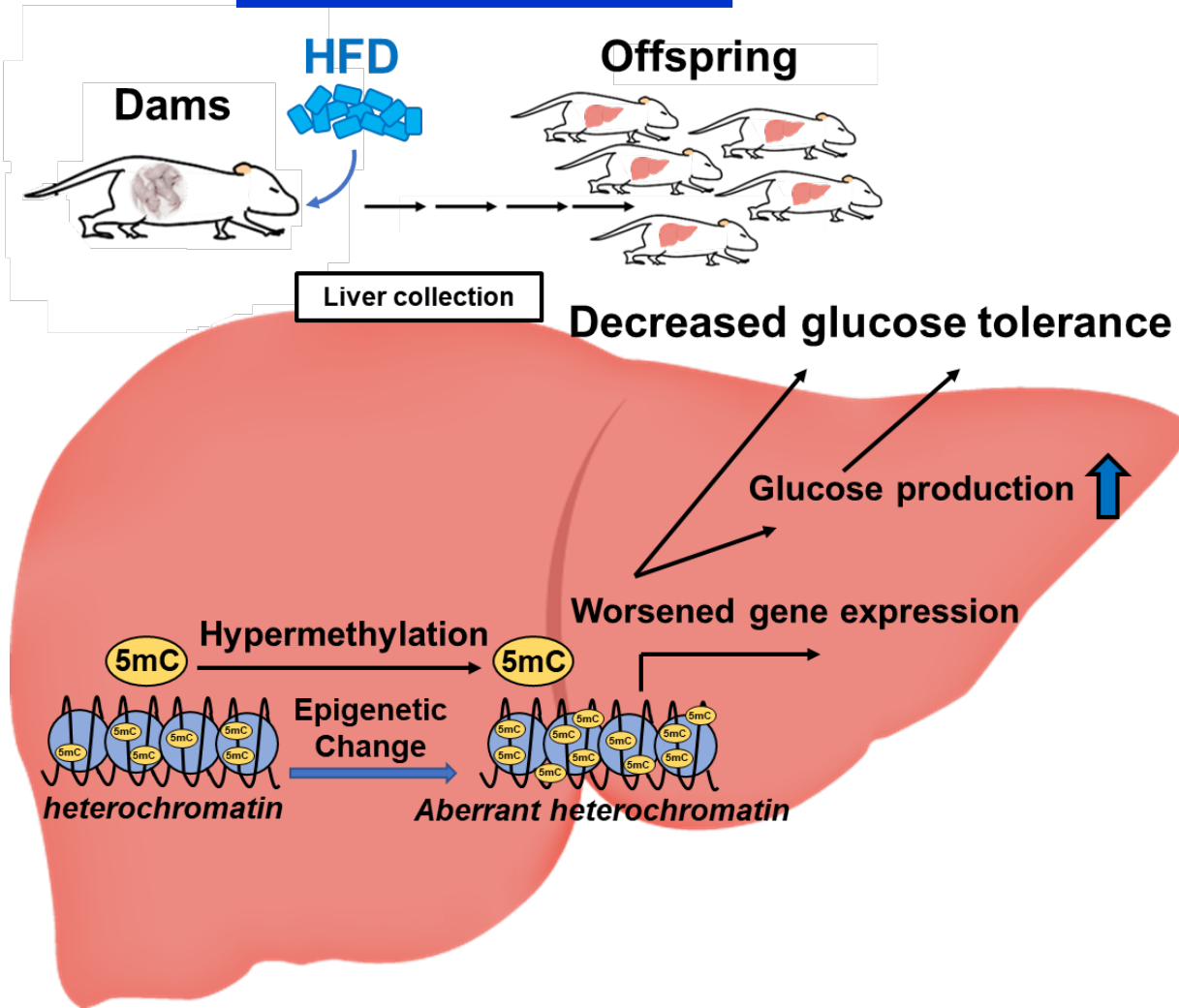
➤ Does high-fat feeding decrease 5-hmC and does maternal exercise increase 5-hmC in liver of offspring?

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



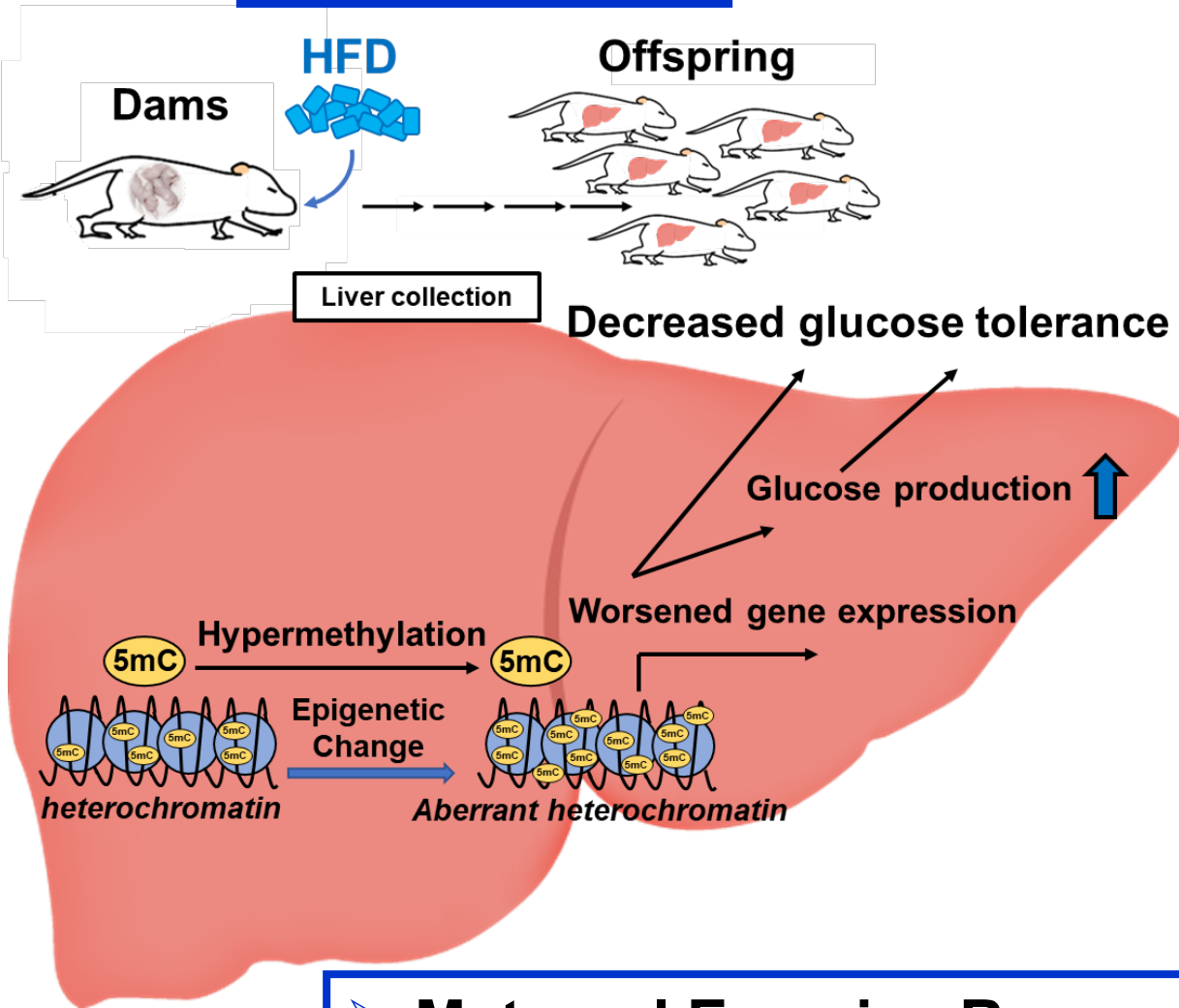
# Summary

## High Fat Diet

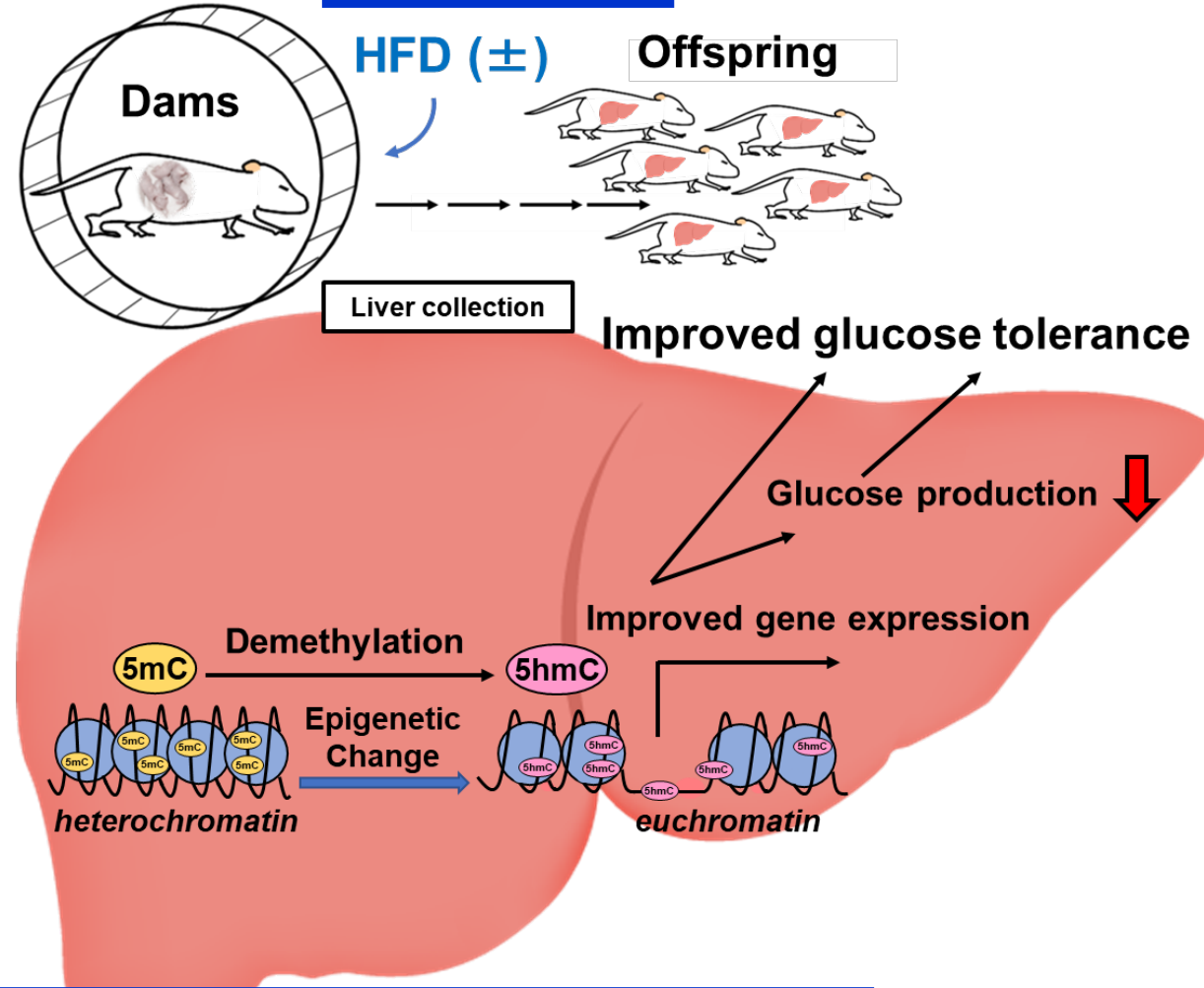


# Summary

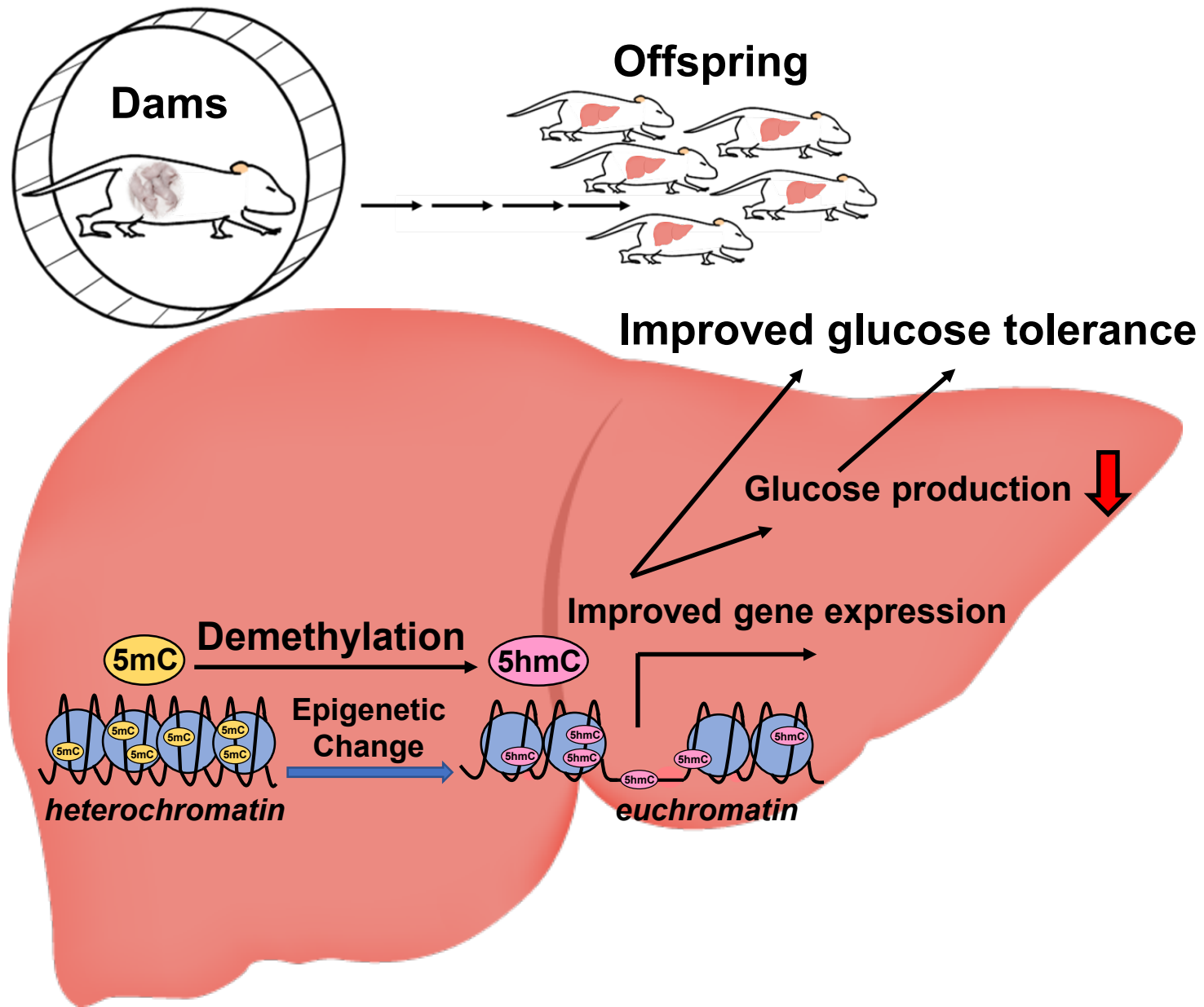
## High Fat Diet



## Exercise

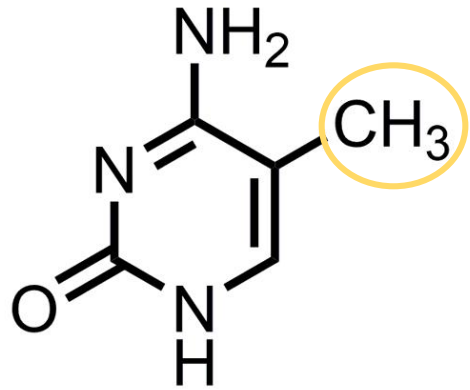


➤ **Maternal Exercise Reverses the Detrimental Effects of a High Fat Diet on Offspring Liver Methylome**

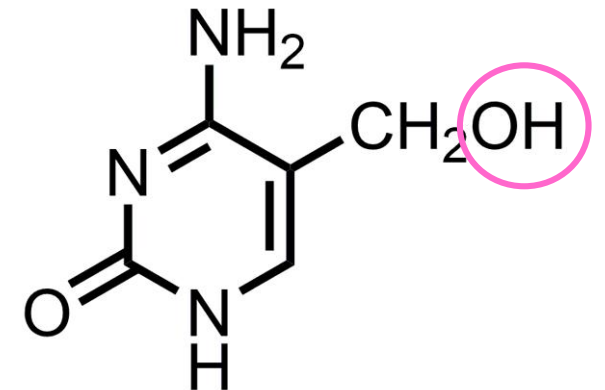
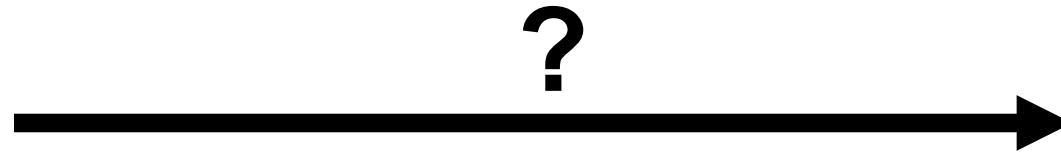


**What are the molecular signals regulating demethylation?**

# Regulation of Methylation



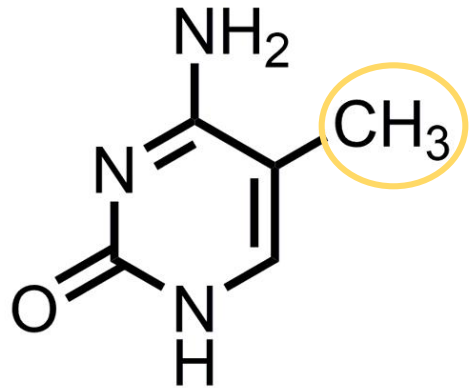
**5-methylcytosine  
(5-mC)**



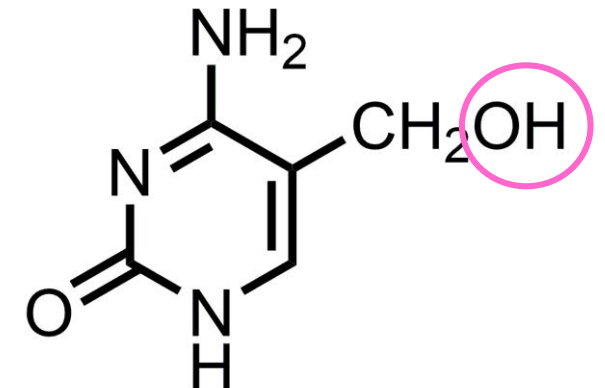
**5-hydroxymethylcytosine  
(5-hmC)**

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

Tet1  
Tet2  
Tet3



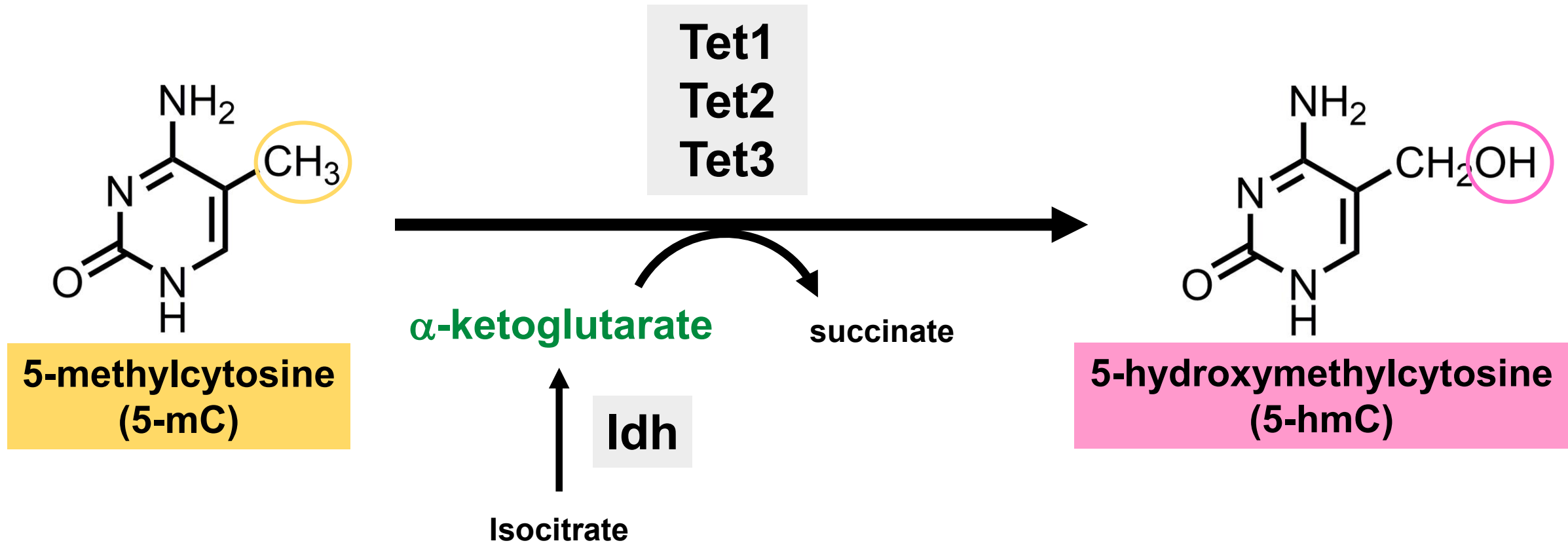
5-methylcytosine  
(5-mC)



5-hydroxymethylcytosine  
(5-hmC)

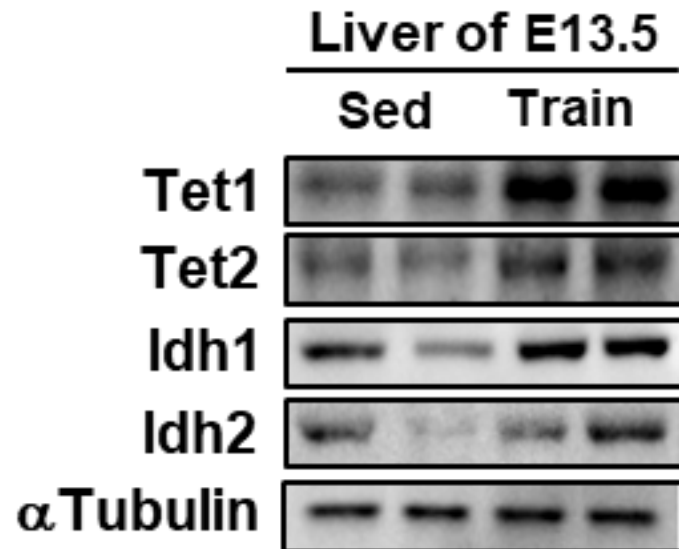
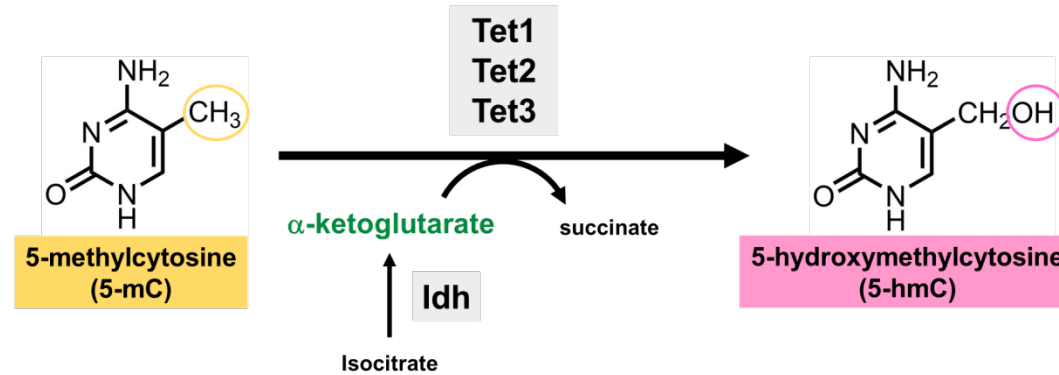
- Does maternal exercise training regulate Tet activity in offspring liver?

# Regulation of Tet Activity

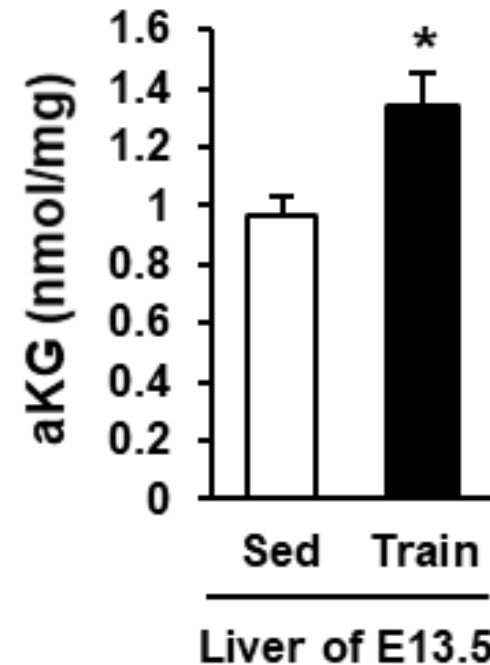
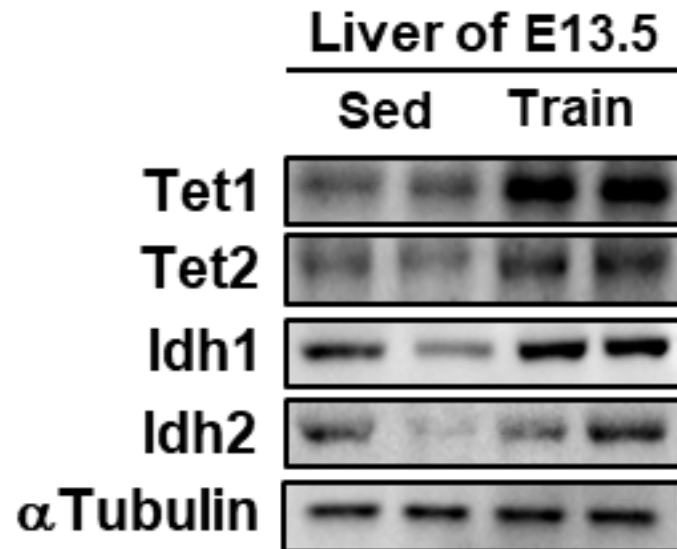
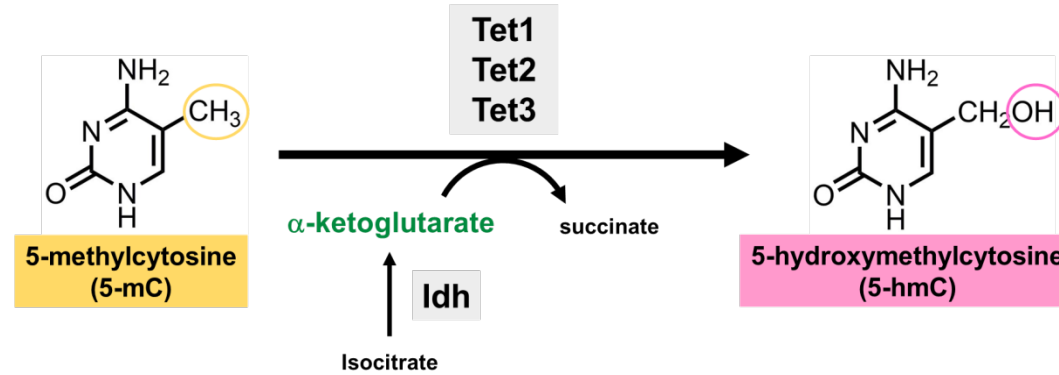


- Does maternal exercise training regulate Idh and α-ketoglutarate in offspring liver?

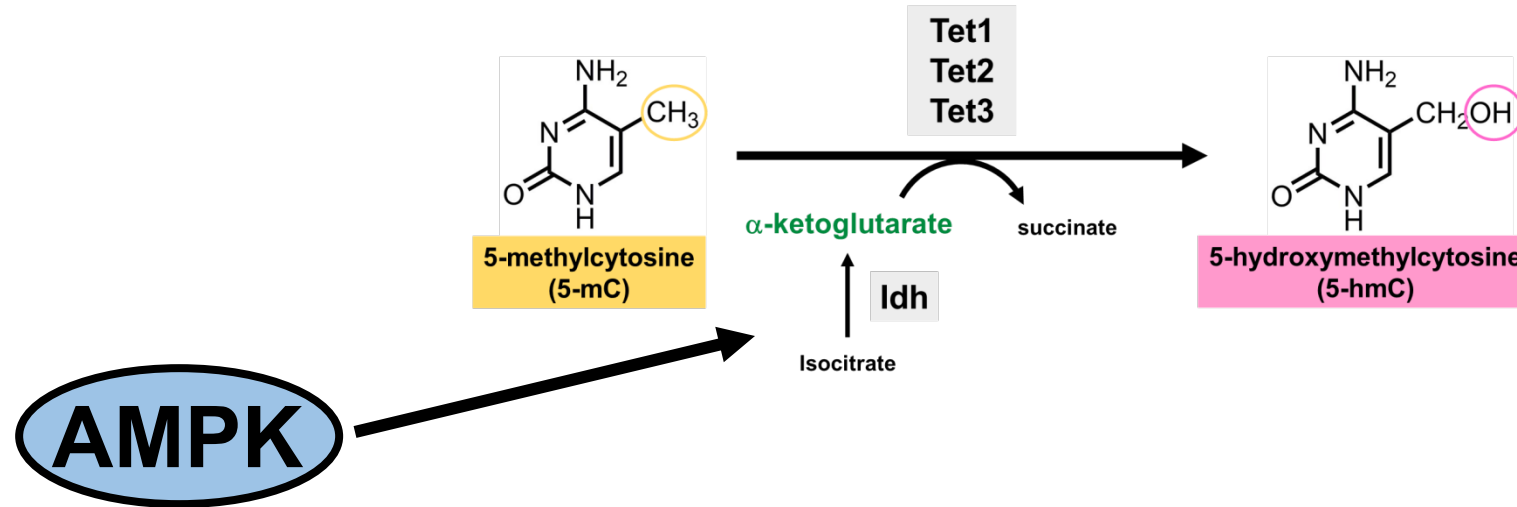
# Tet, Idh, and $\alpha$ KG are Increased in E13.5 Livers of Offspring of Trained Dams



# Tet, Idh, and $\alpha$ KG are Increased in E13.5 Livers of Offspring of Trained Dams

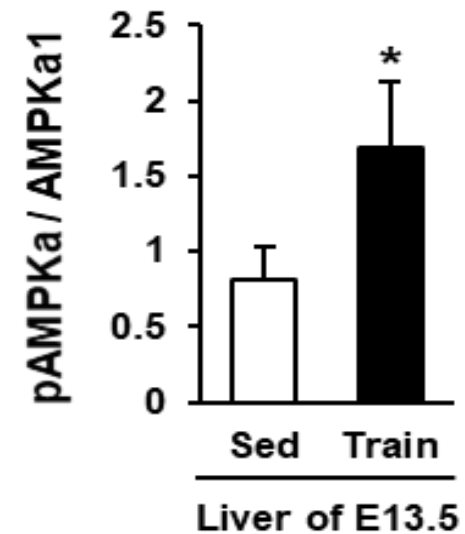
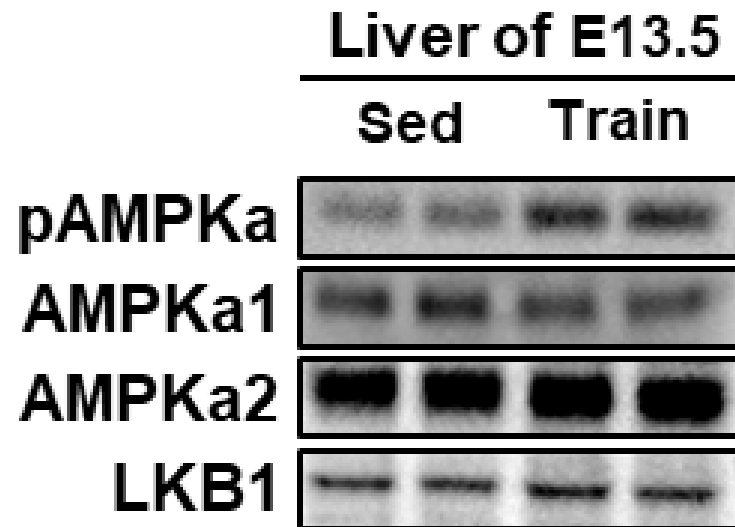
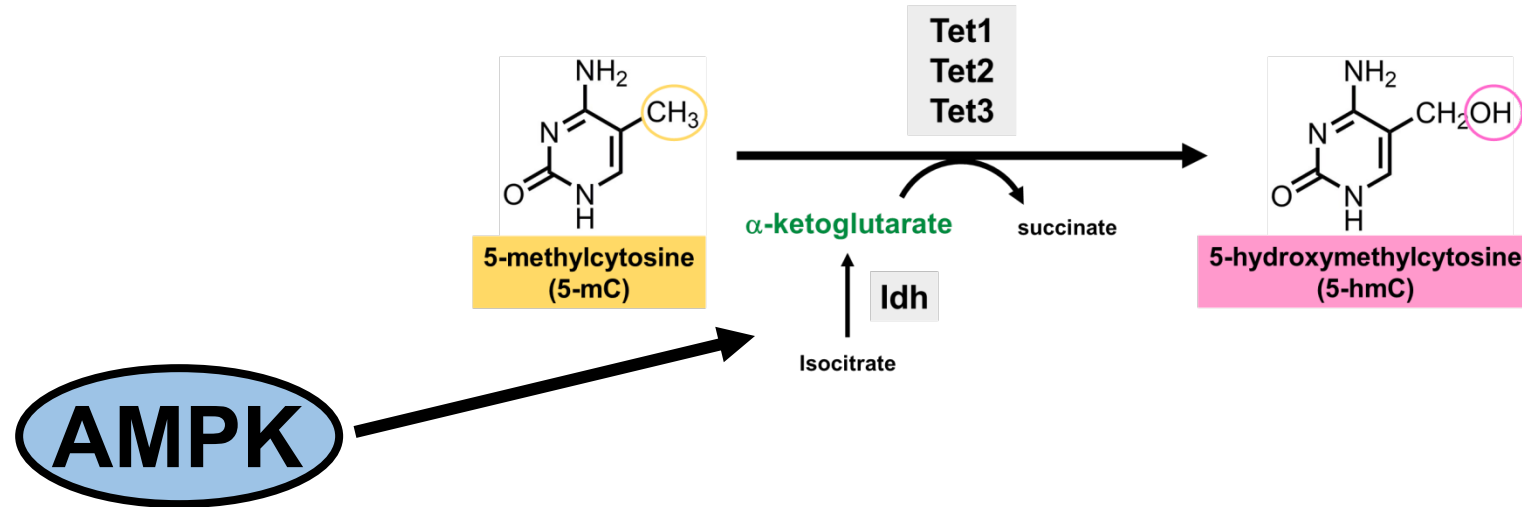


# Tet, Idh, and $\alpha$ KG are Increased in E13.5 Livers of Offspring of Trained Dams



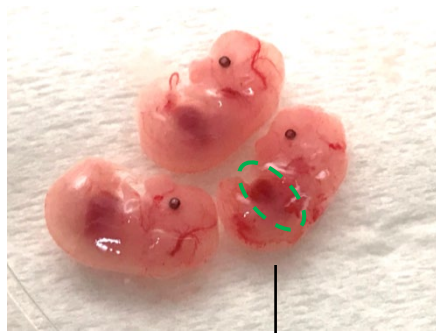
- What signals the increase in Tet, Idh, and  $\alpha$ KG in offspring liver in response to maternal exercise training?

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

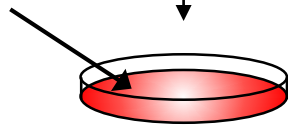


# AMPK Activators Increase Tet and Idh mRNA Expression and $\alpha$ KG in Hepatoblasts from E13.5 Liver

E13.5 embryo  
from sedentary dam

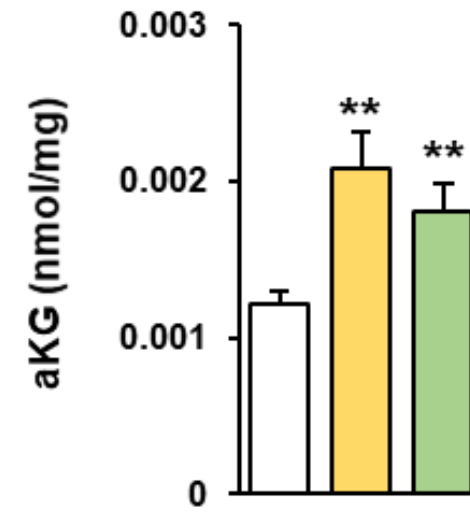
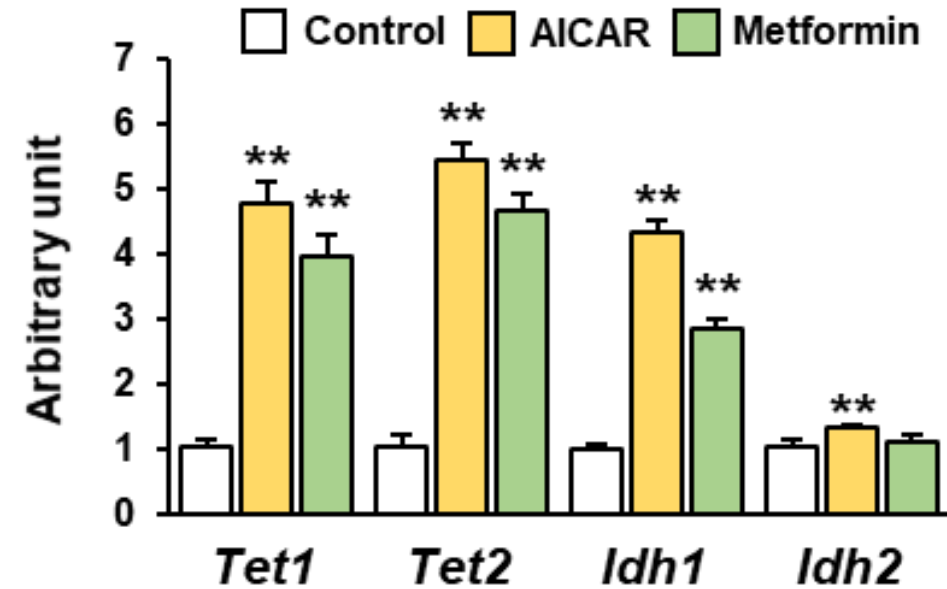


AICAR (100  $\mu$ M)  
or  
Metformin (100  $\mu$ M)



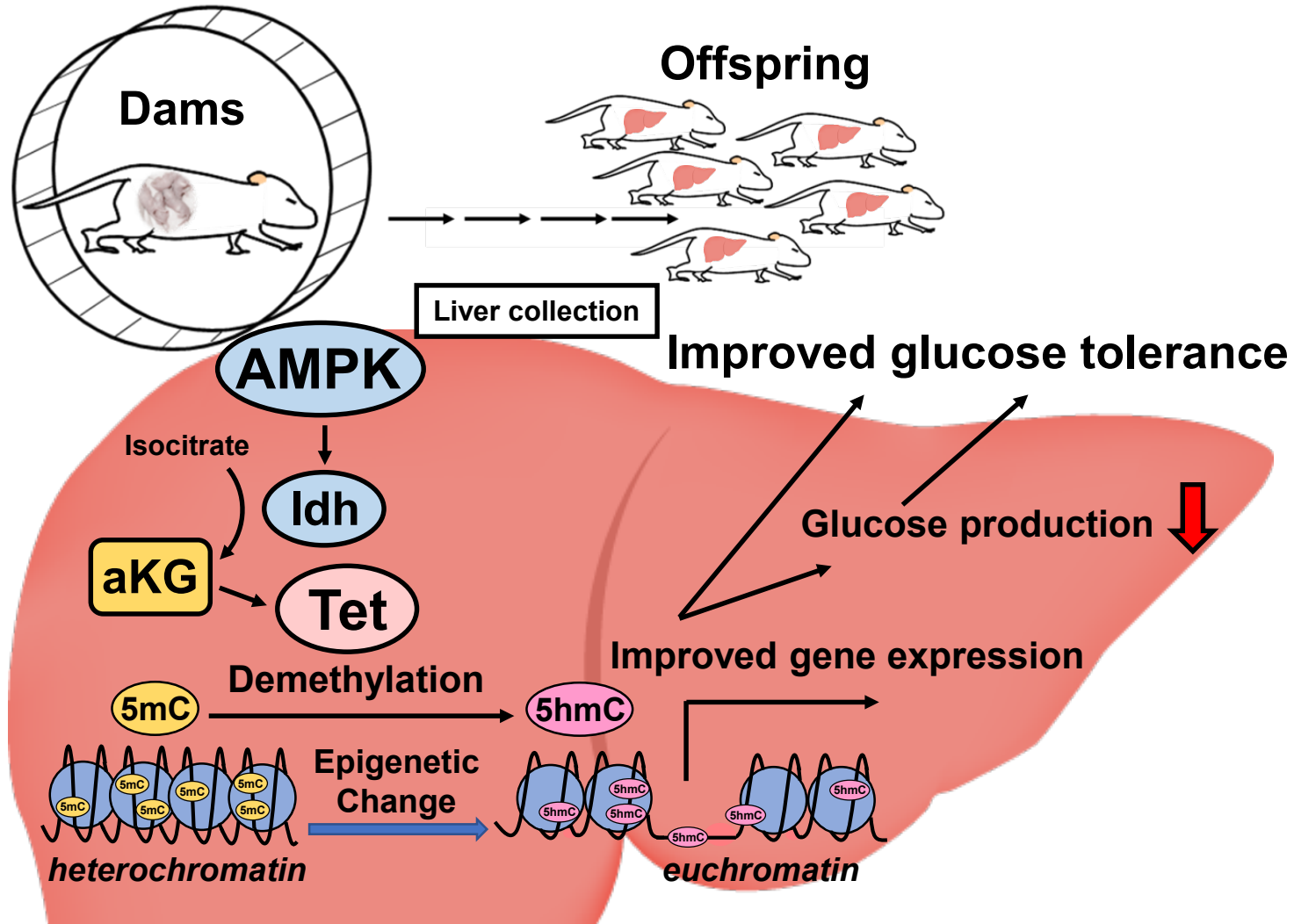
24h incubation

Primary hepatoblasts



# Summary

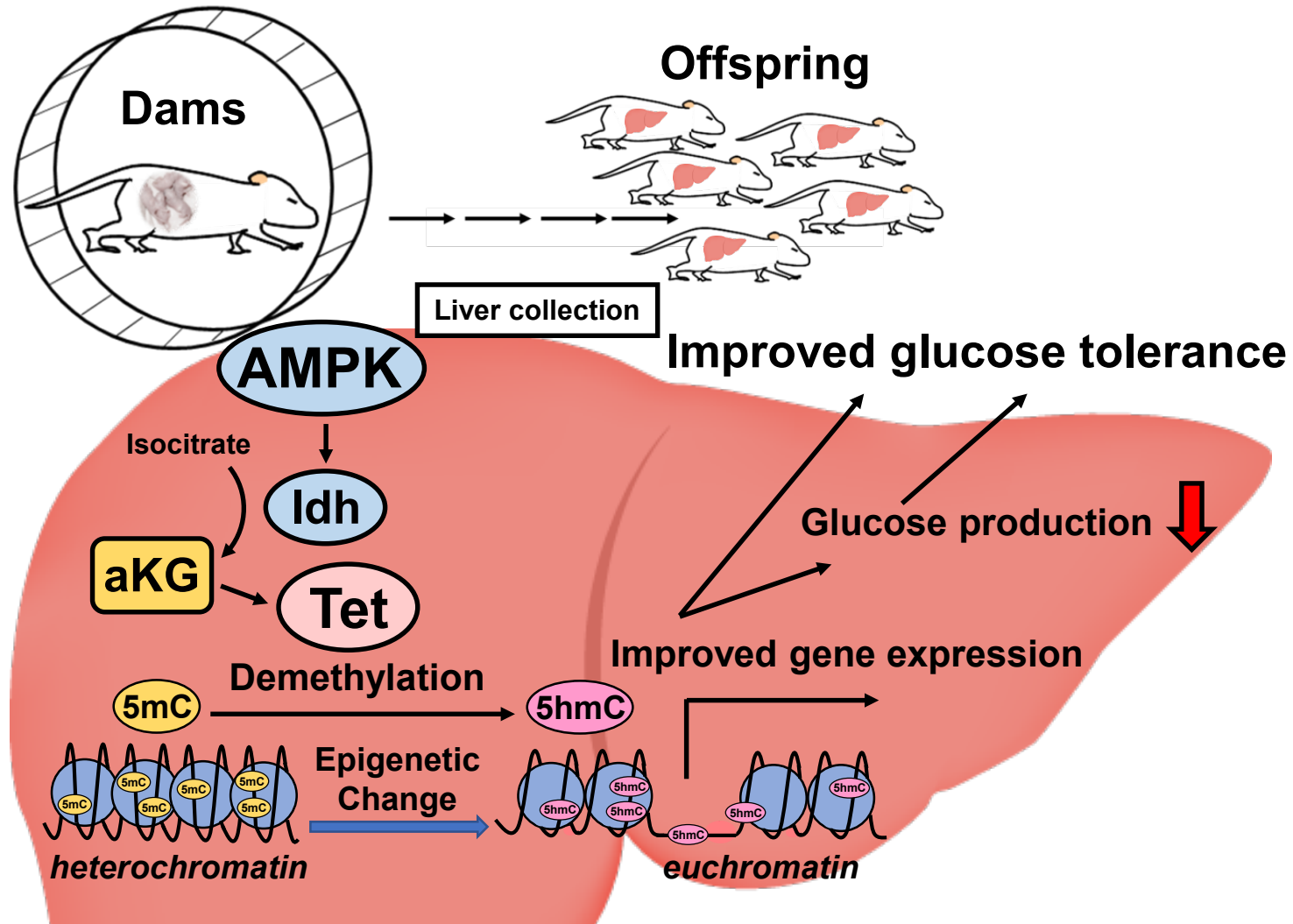
**Exercise**



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

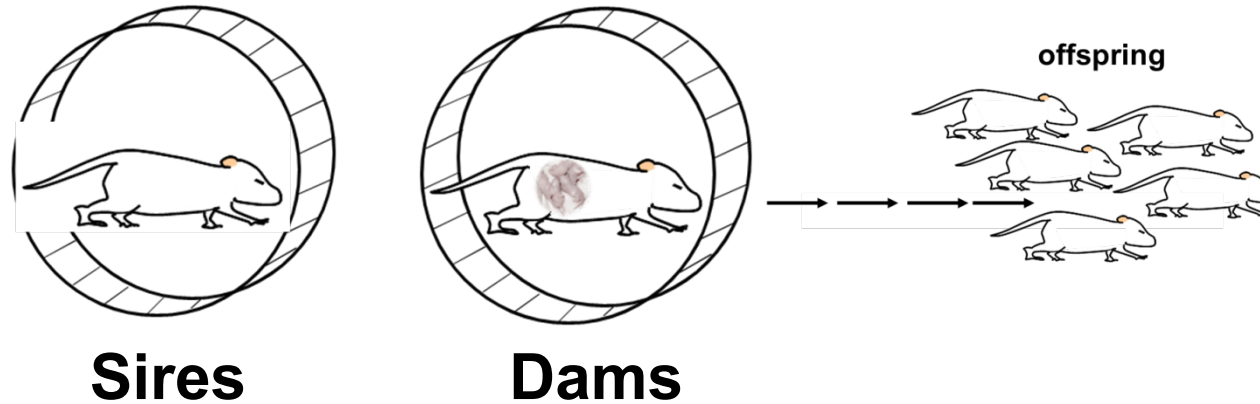
# Important Question

## Exercise



- 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)

Improved hepatic profile  
in E13.5 offspring liver  
confirmed

E13.5 embryo  
from sedentary dam



Primary  
hepatoblasts

Sedentary dams  
(pregnant)



Training dams  
(pregnant)



Training females  
(non pregnant)

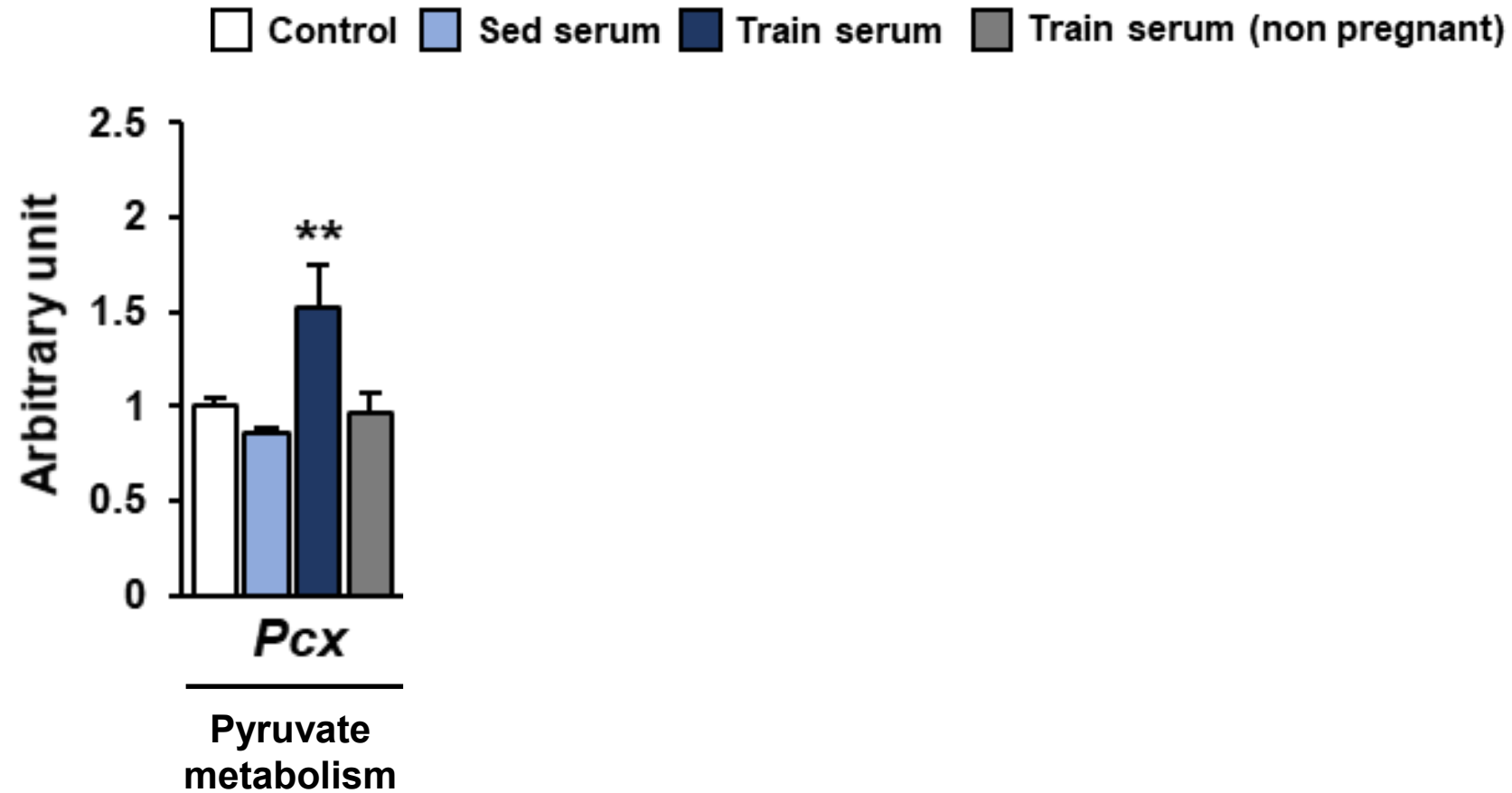


Serum collection

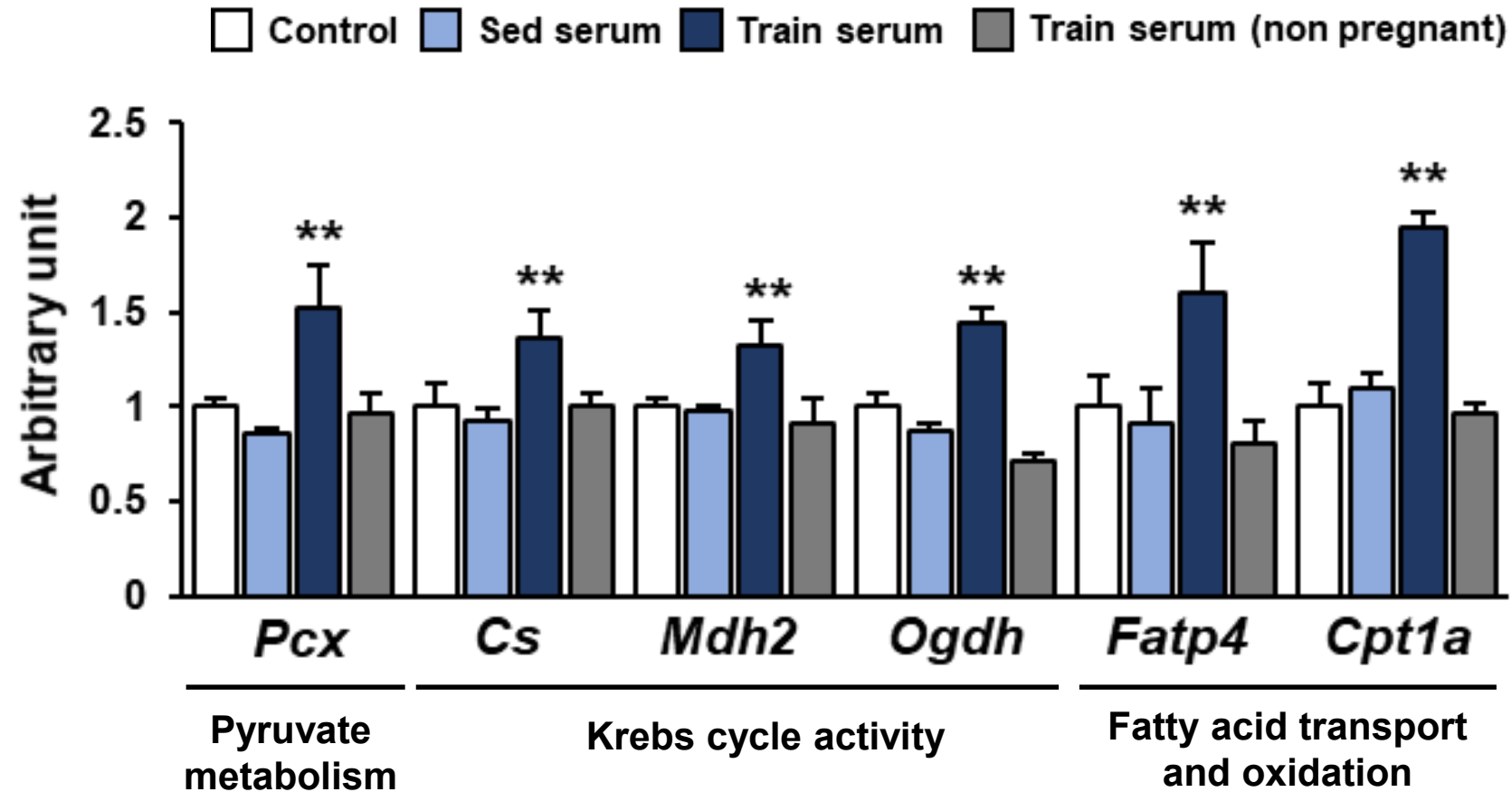
10% serum stimulation  
for 24h



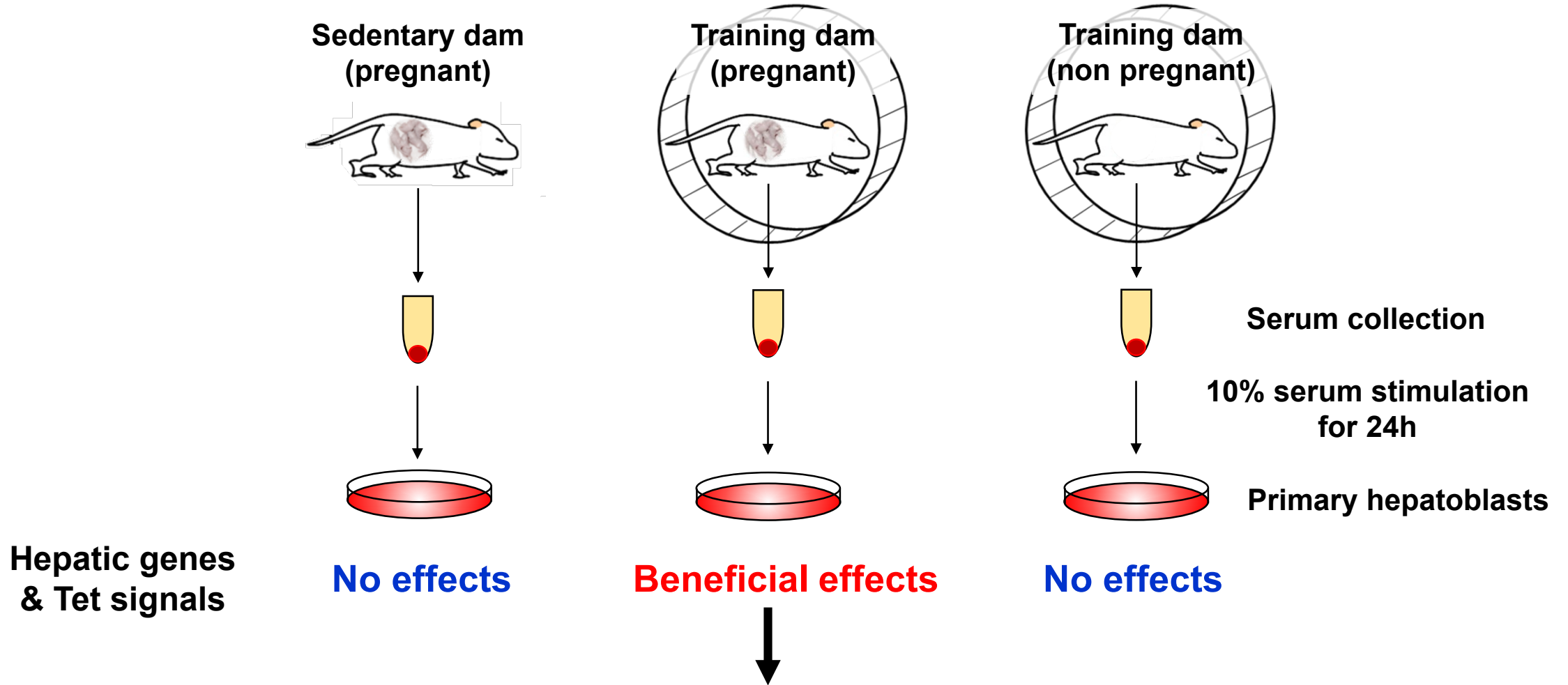
# Exercise Trained Dam-Derived Serum Stimulation Increases Hepatic Gene Expressions in Embryonic Hepatoblasts



# 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

Improved hepatic profile  
in E13.5 offspring liver  
confirmed

Sedentary dam  
(pregnant)



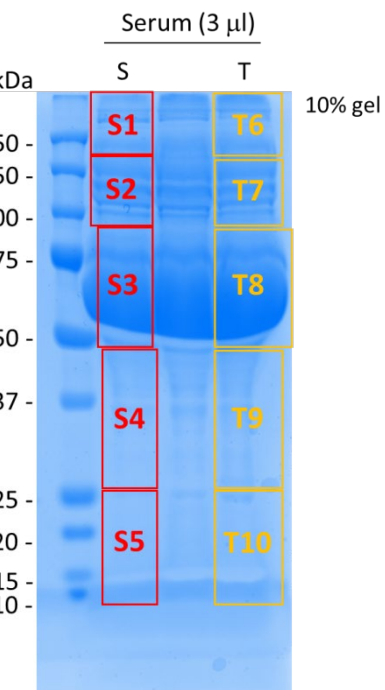
Training dam  
(pregnant)



Sedentary dam  
(pregnant)



Training dam  
(pregnant)



Gel cutting

In gel digestion &  
protein purification

LC-MS/MS analysis

Bioinformatic analysis

577 proteins were found.

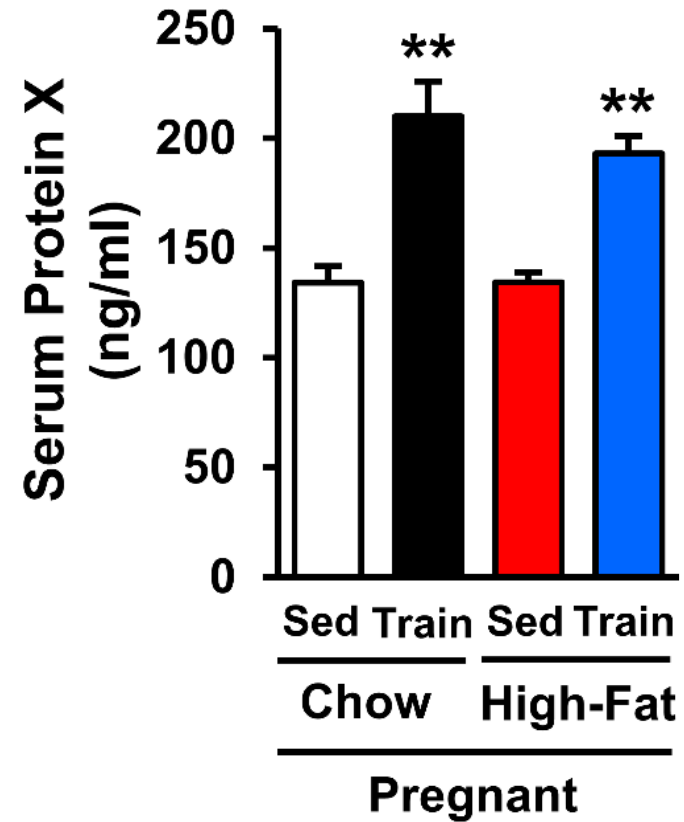
78 proteins were increased (>1.5 fold)

19 proteins were secretory cytokines

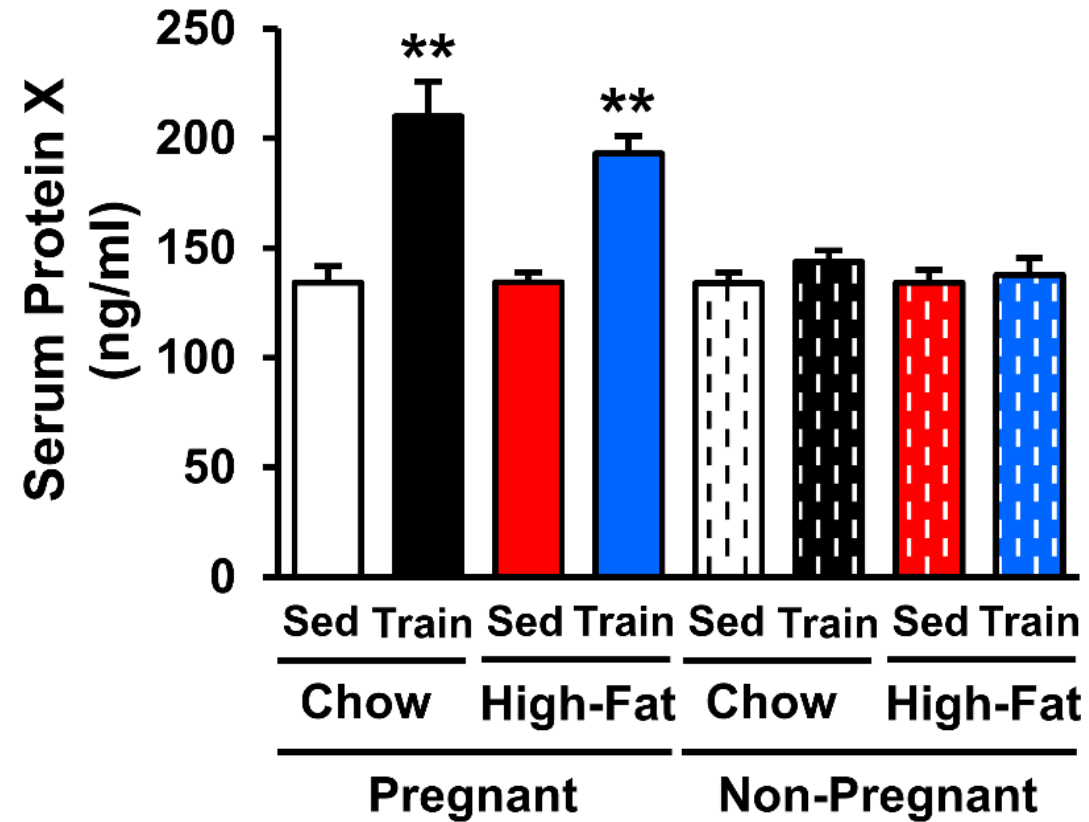
4 proteins were expressed in placenta

1 protein has been reported to increase AMPK phosphorylation

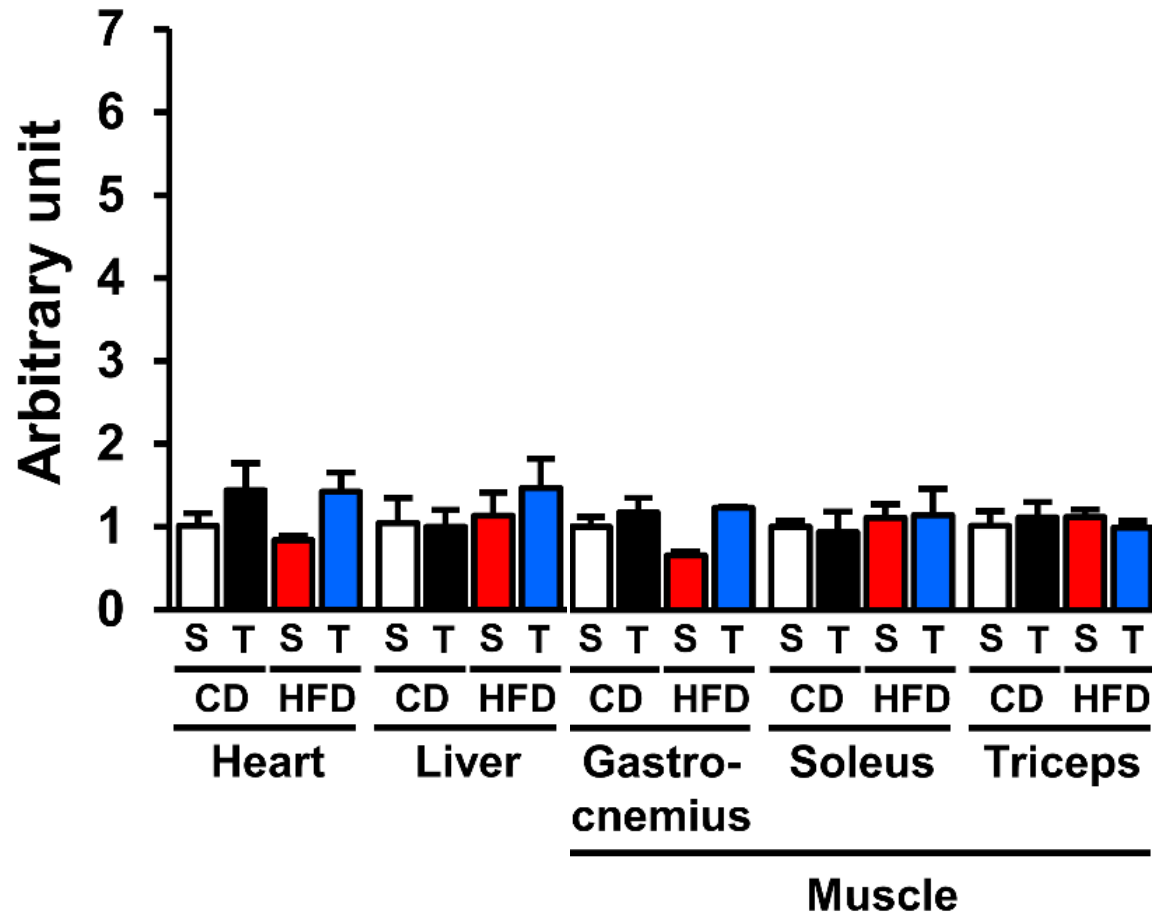
# Protein X is Increased in the Serum of Trained and Pregnant Dams



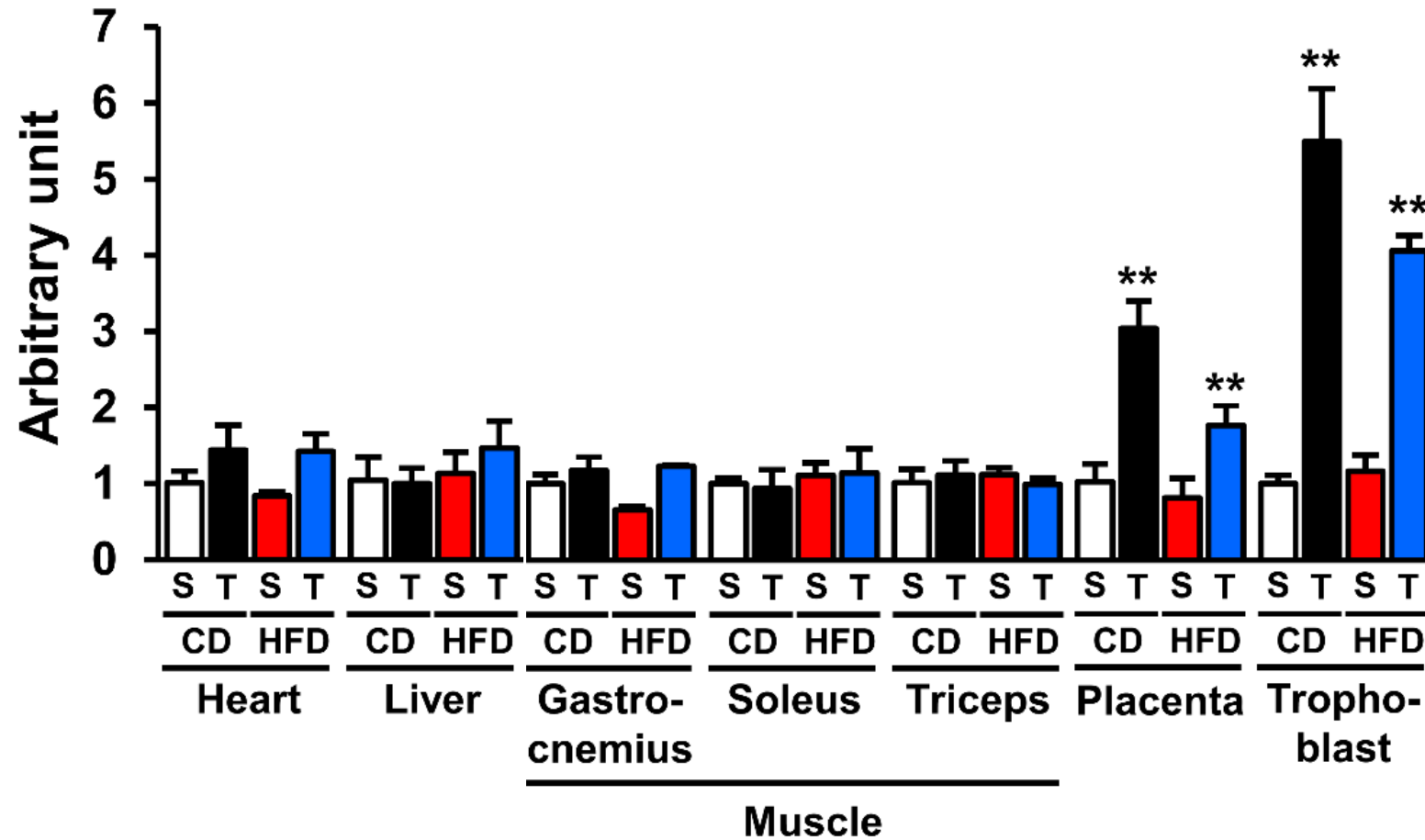
# Protein X is Not Increased in the Serum of Trained and Pregnant Dams



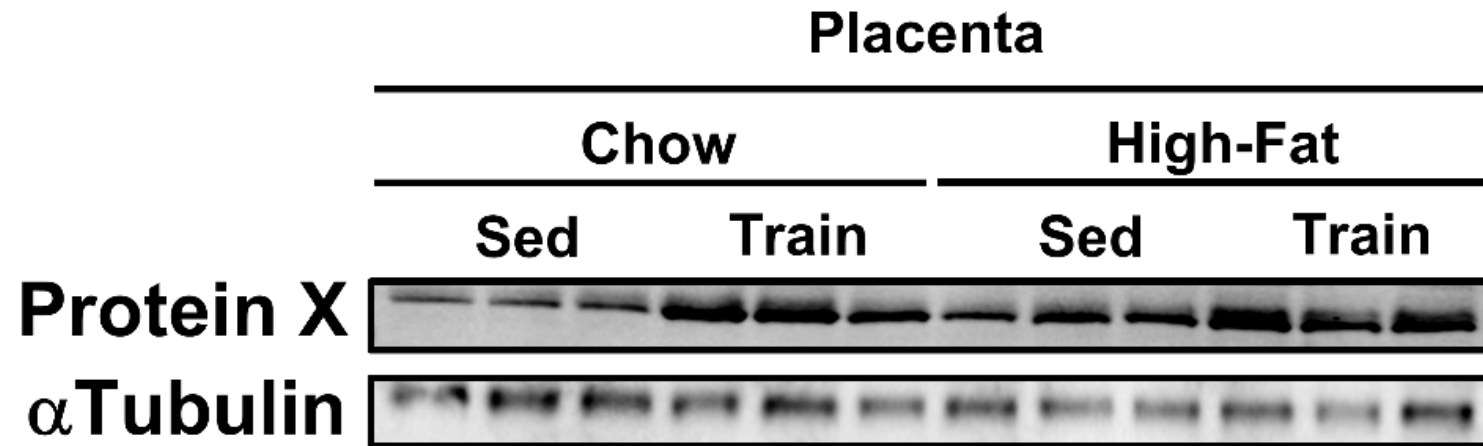
# Protein X mRNA Expression is Increased in the Placenta and Trophoblast from Trained Dams



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

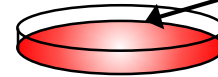
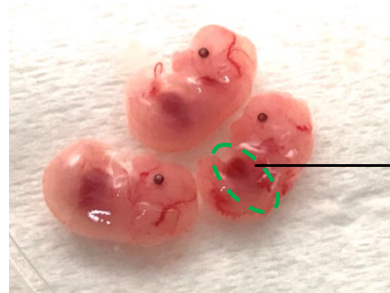


# Protein X Expression is Increased in the Placenta from Trained Dams



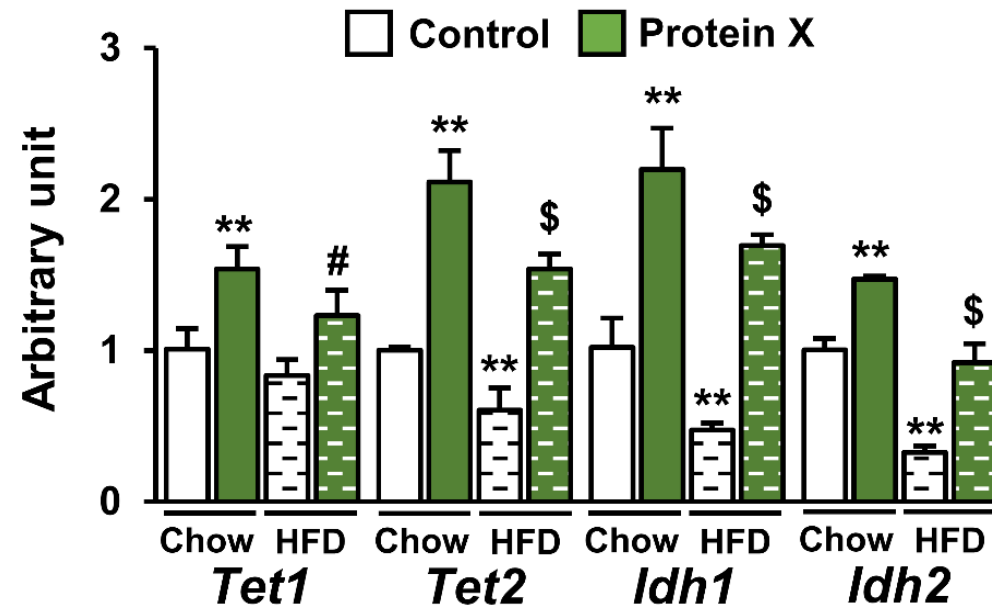
# Protein X Has Promotive Effects on Tet Signaling Axis in Hepatoblasts from both Chow Diet and HFD-Fed Dams

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



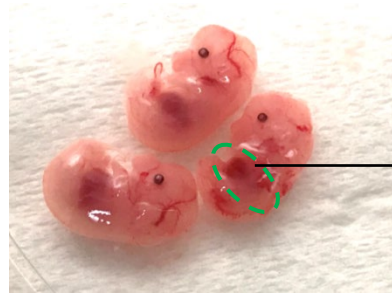
recombinant protein X (20 ng/ml)

Primary hepatoblasts



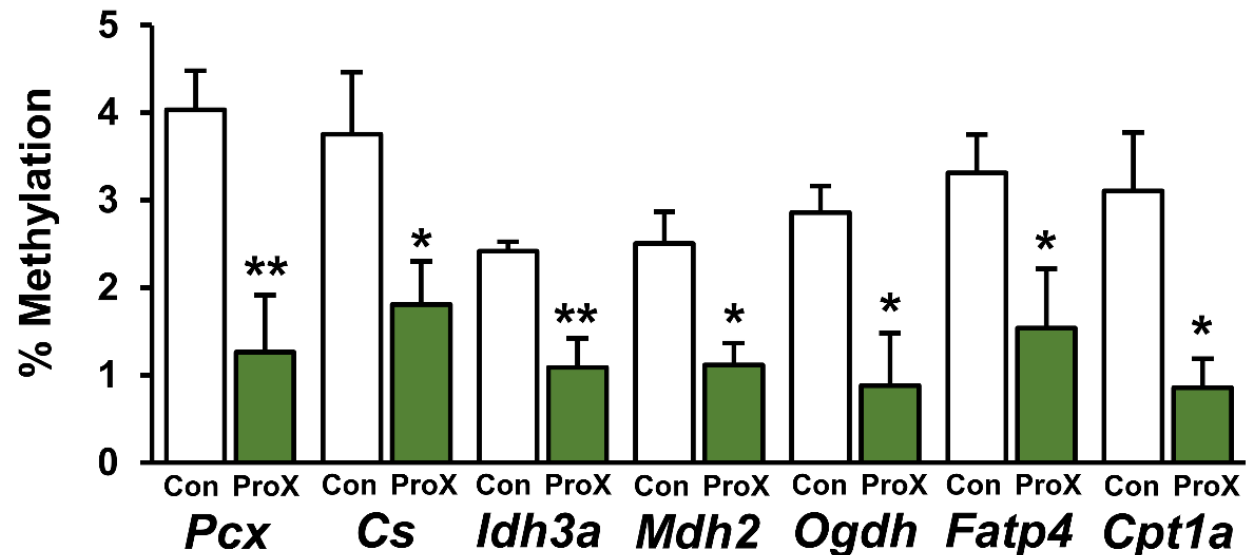
# Protein X Treatment Decreases CpG Methylation of Metabolic Genes in Hepatoblasts

E13.5 embryo  
from Chow diet-fed dam

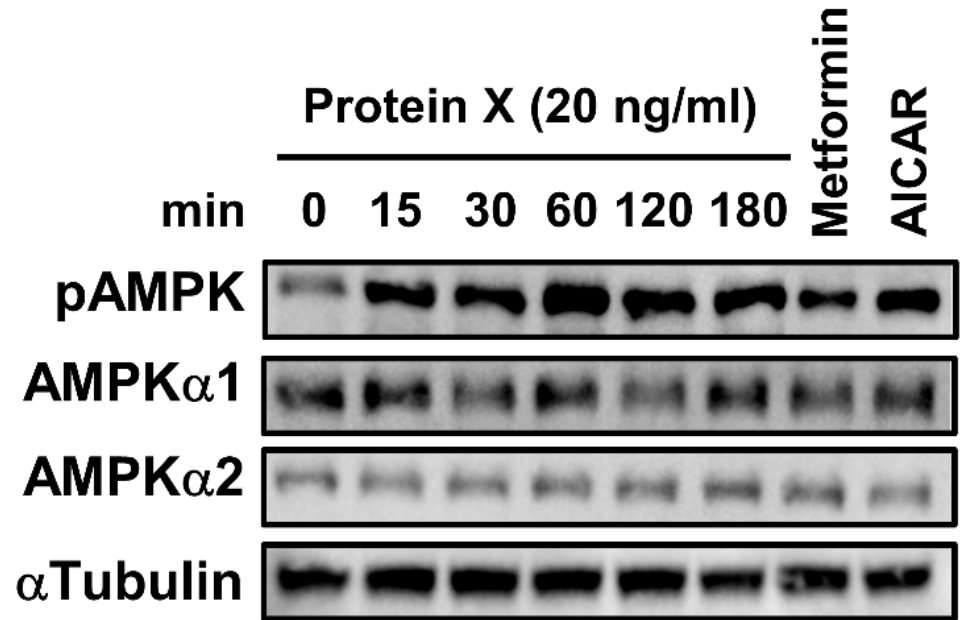
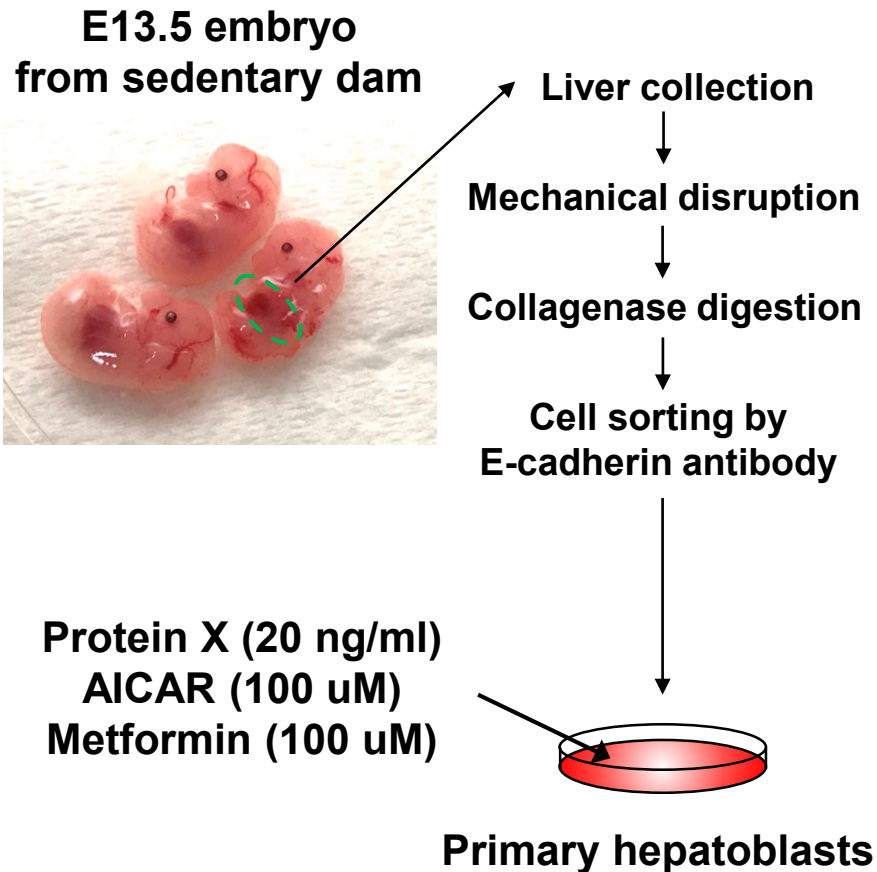


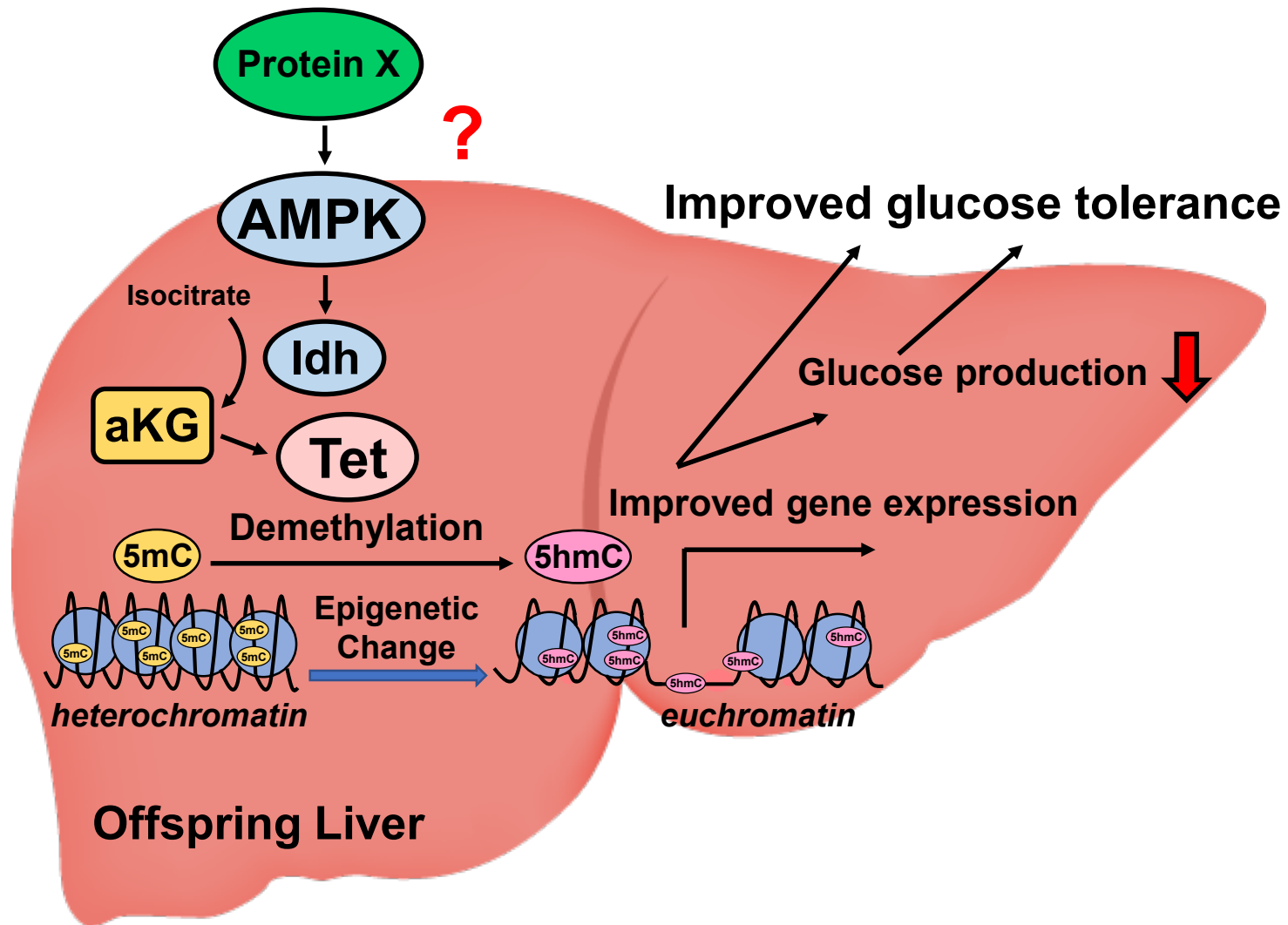
Primary hepatoblasts

recombinant protein X (20 ng/ml)



# Protein X Stimulation induces AMPK Phosphorylation in Hepatoblasts

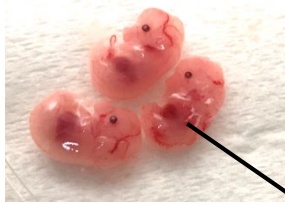




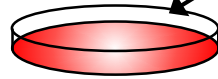
➤ Does Protein X signal epigenetic changes via AMPK?

# The Effects of Protein X on Tet Signaling Axis is Inhibited by AMPK $\alpha$ Knockdown and Inhibition in Hepatoblasts

E13.5 embryo  
from sedentary dam

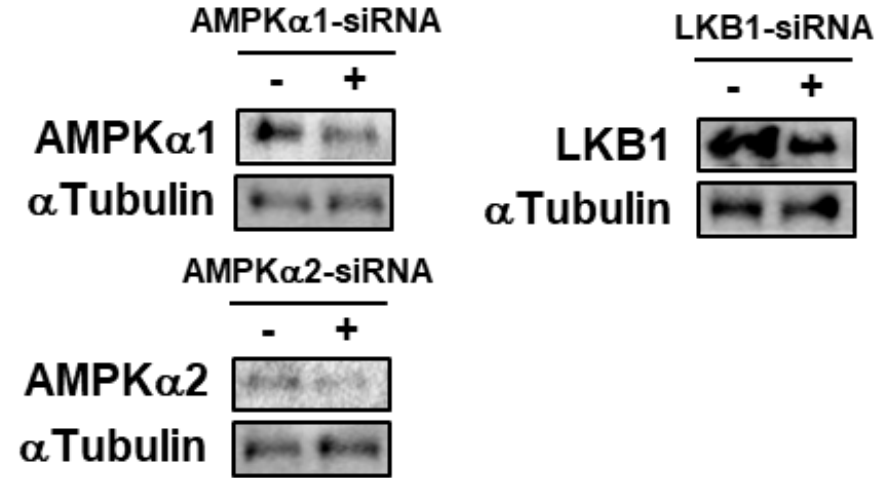


Hepatoblast  
isolation



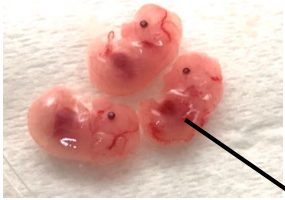
AMPK $\alpha$ 1, AMPK $\alpha$ 2 & LKB1 siRNA  
or Compound C  
+/- Protein X stimulation

Primary hepatoblasts

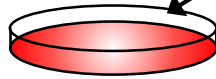


# The Effects of Protein X on Tet Signaling Axis is Inhibited by AMPK $\alpha$ Knockdown and Inhibition in Hepatoblasts

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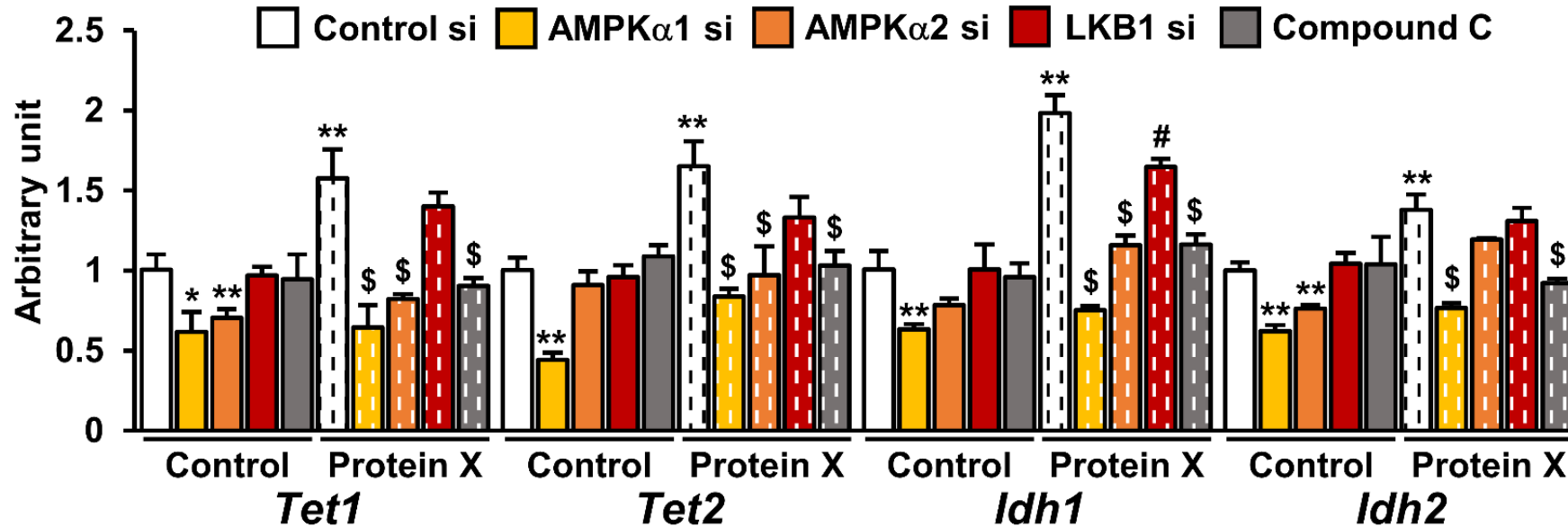
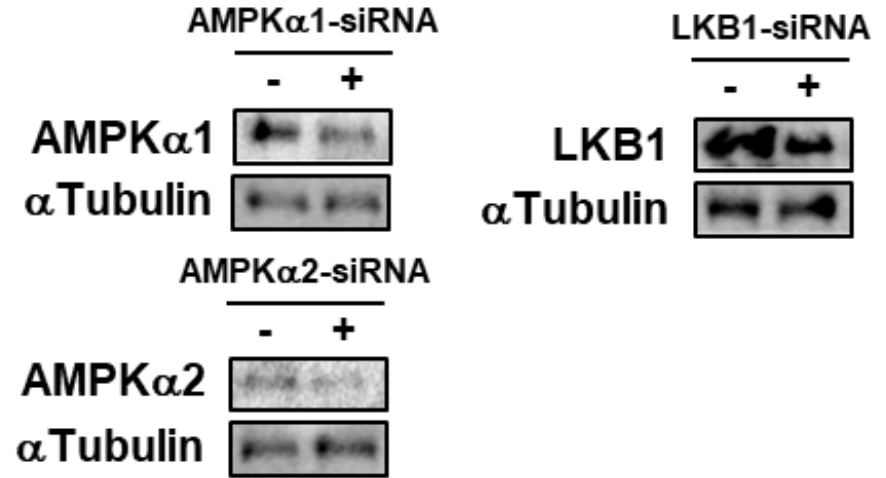


Hepatoblast  
isolation

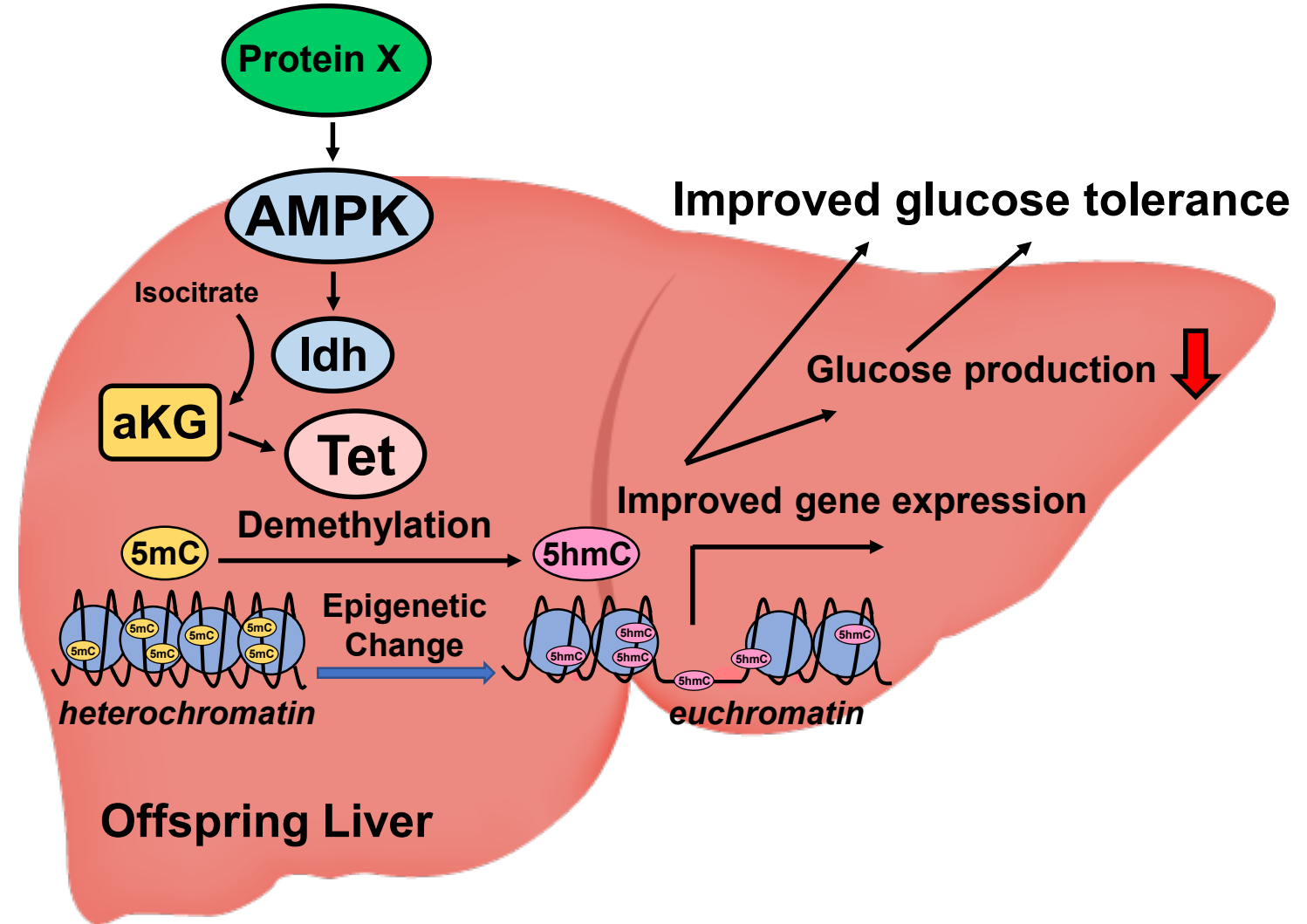
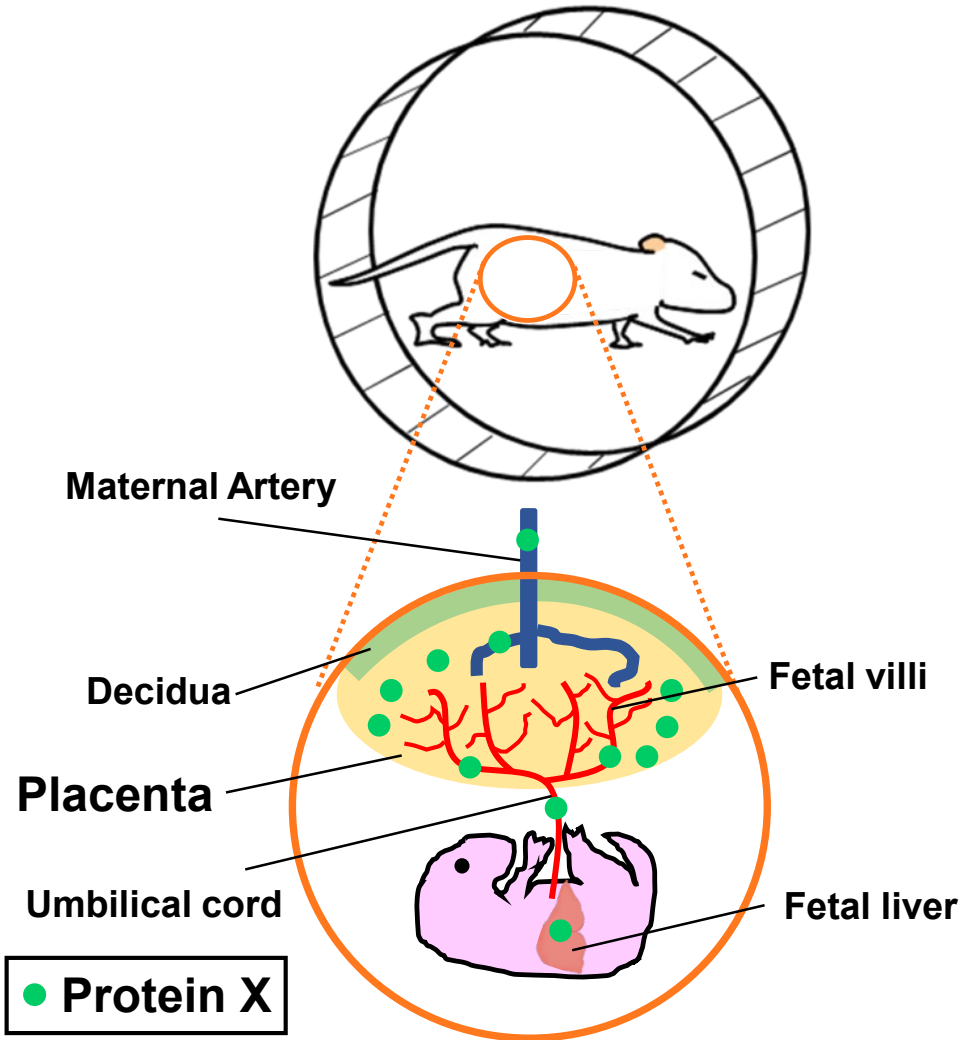


Primary hepatoblasts

AMPK $\alpha$ 1, AMPK $\alpha$ 2 & LKB1 siRNA  
or Compound C  
with Protein X stimulation

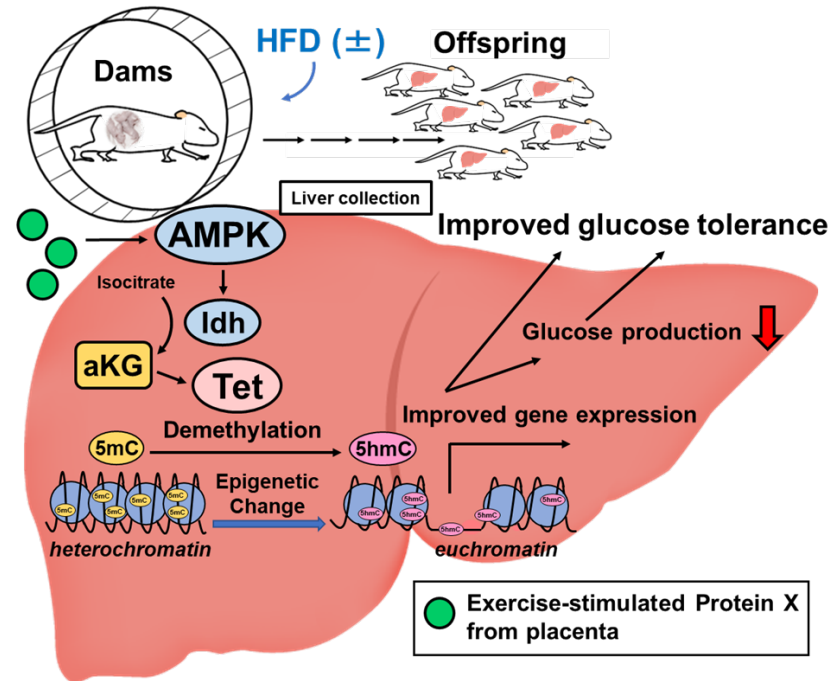


# Summary



➤ **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

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Parental Exercise



Offspring

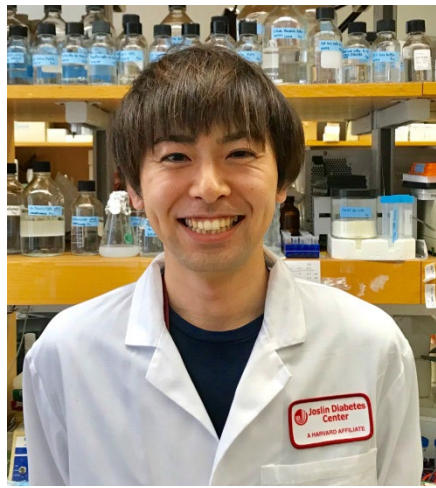


**Improved  
Metabolic Health**

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



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