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
- 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

Prevention of Diabetes
- Increased insulin sensitivity
- Increased glucose tolerance
- Improved pancreatic function
- Increased mitochondrial function
- Increased muscle glucose uptake

Increased blood volume
Increased insulin sensitivity
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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

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

Exercise
Prevalence of Obesity Among Male Adults in the United States

CDC: Prevalence of Obesity Among Adults in the United States (2015-2016)
Effects of Maternal and Paternal Exercise on Offspring Metabolic Health
Increased physical activity of parents will improve the metabolic phenotype of offspring.
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.
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

- Pre-Conception (2 wks)
  - Sedentary

- Breeding (3 days)
  - Training
  - Male breeders were sedentary and chow fed

- Gestation (3 wks)
  - Sedentary

Chow

- Training done by voluntary wheel running

High-Fat

- 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

GTT Area Under the Curve

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
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)

**Chow Diet**
- (21% kcal from fat)
  - Sedentary (3wks)
  - Trained (3wks)

**High-Fat Diet**
- (60% kcal from fat)
  - 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.
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
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 of 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 mouse per litter, n=6 each group
The combination of both paternal and maternal exercise may have even greater beneficial effects on offspring metabolic health.
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.
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

Basal Hepatocyte Glucose Production

Stanford et al, Diabetes 2017
Maternal Exercise Effects Glucose Production in Isolated Hepatocytes of Offspring

Basal Hepatocyte Glucose Production

Stanford et al, Diabetes 2017
Hepatic gene expression?
Glucose production
Improved glucose tolerance
Offspring
Liver collection
Dams
Hepatic gene expression?
Maternal Exercise in Mice Results in Improved Offspring Liver Gene Profile

Offspring Liver Metabolic Gene Expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chow</th>
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Pyruvate metabolism  
Krebs cycle  
Fatty acid metabolism

Stanford et al. Diabetes 2015; Stanford et al, Diabetes 2017
Effects of Maternal Exercise on Offspring Liver Function

- Improved glucose tolerance
- Glucose production
- Improved gene expression
  - Pyruvate metabolism
  - Krebs cycle activity
  - Fatty acid metabolism
At what stage in the offspring development do the changes in hepatic gene expression occur?
Maternal Exercise Affects Hepatic Gene Expression in Young Offspring

Embryo (E13.5) → Neonate (Day 0) → Post-Weaned (4 weeks) → Adult (52 weeks)

Liver of embryo (E13.5)

<table>
<thead>
<tr>
<th>Gluconeogenesis</th>
<th>Pyruvate metabolism</th>
<th>Krebs cycle activity</th>
<th>Fatty acid transport and oxidation</th>
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<tr>
<td><em>G6pc</em></td>
<td>Chow Fbp1</td>
<td>Chow Pgc1a</td>
<td>Chow Pck1</td>
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Arbitrary unit
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

DNA de-methylation

Transcription Activation

5-methylcytosine (5-mC)

5-hydroxymethylcytosine (5-hmC)
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

Pyruvate metabolism
High Fat Diet

Summary

Dams → HFD → Offspring → Decreased glucose tolerance

Liver collection → Epigenetic change → Hypermethylation → Aberrant heterochromatin → Worsened gene expression → Glucose production
Maternal Exercise Reverses the Detrimental Effects of a High Fat Diet on Offspring Liver Methylation
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)

Tet1  Tet2  Tet3

5-hydroxymethylcytosine (5-hmC)

Does maternal exercise training regulate Tet activity in offspring liver?
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 αKG are Increased in E13.5 Livers of Offspring of Trained Dams

![Diagram of metabolic pathways involving Tet, Idh, and αKG]

- **Liver of E13.5**
  - **Sed**
  - **Train**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sed</th>
<th>Train</th>
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<tbody>
<tr>
<td>Tet1</td>
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<td>Tet2</td>
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<td>Idh1</td>
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<td>Idh2</td>
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<td>αTubulin</td>
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</table>

**Graph:**
- **aKG (nmol/mg)**
  - **Sed**
  - **Train**
  - *Note: Significance indicated by asterisk.*
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 αKG in Hepatoblasts from E13.5 Liver

E13.5 embryo from sedentary dam

AICAR (100 µM) or Metformin (100 µM) for 24h incubation

Primary hepatoblasts

Bar graph showing expression levels of Tet1, Tet2, Idh1, and Idh2 in control, AICAR, and Metformin treatments. ** indicates statistical significance.
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, 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)

E13.5 embryo from sedentary dam

Sedentary dams (pregnant)
Training dams (pregnant)
Training females (non pregnant)

Serum collection

10% serum stimulation for 24h

Improved hepatic profile in E13.5 offspring liver confirmed
Exercise Trained Dam-Derived Serum Stimulation Increases Hepatic Gene Expressions in Embryonic Hepatoblasts

- Pyruvate metabolism
- Krebs cycle activity
- Fatty acid transport and oxidation
Exercise Trained Dam-Derived Serum Stimulation Increases Hepatic Gene Expressions in Embryonic Hepatoblasts

- Pyruvate metabolism
- Krebs cycle activity
- Fatty acid transport and oxidation
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**

**Sedentary dam (pregnant)**

**Training dam (pregnant)**

**Serum (3 μl)**

- Improved hepatic profile in E13.5 offspring liver confirmed

- 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

<table>
<thead>
<tr>
<th></th>
<th>Placenta</th>
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![Image]
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

Primary hepatoblasts

recombinant protein X (20 ng/ml)
Protein X Treatment Decreases CpG Methylation of Metabolic Genes in Hepatoblasts

E13.5 embryo from Chow diet-fed dam

recombinant protein X (20 ng/ml)

Primary hepatoblasts

![Graph showing % Methylation for different genes with Con and ProX conditions.](image-url)
Protein X Stimulation induces AMPK Phosphorylation in Hepatoblasts

E13.5 embryo from sedentary dam

Liver collection → Mechanical disruption → Collagenase digestion → Cell sorting by E-cadherin antibody

Protein X (20 ng/ml) → AICAR (100 uM) → Metformin (100 uM)

Primary hepatoblasts

<table>
<thead>
<tr>
<th>min</th>
<th>Protein X (20 ng/ml)</th>
<th>Metformin</th>
<th>AICAR</th>
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- pAMPK
- AMPKα1
- AMPKα2
- αTubulin
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

E13.5 embryo from sedentary dam

Hepatoblast isolation

AMPKa1, AMPKa2 & LKB1 siRNA or Compound C +/- Protein X stimulation

Primary hepatoblasts

AMPKα1-siRNA

AMPKα1
α Tubulin

AMPKα2-siRNA

AMPKα2
α Tubulin

LKB1-siRNA

LKB1
α Tubulin

Control si  AMPKα1 si  AMPKα2 si  LKB1 si  Compound C

Tet1

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

E13.5 embryo from sedentary dam

AMPKa1, AMPKa2 & LKB1 siRNA or Compound C with Protein X stimulation

Hepatoblast isolation

Primary hepatoblasts

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| AMPKα2-siRNA | |
|--------------|-
| AMPKα2       | |
| α-Tubulin    | |

**Graph:**

- Control si
- AMPKα1 si
- AMPKα2 si
- LKB1 si
- Compound C

Arbitrary unit

- Tet1
- Tet2
- Idh1
- Idh2

- *p < 0.05
- **p < 0.01
- $p < 0.001
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

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