Fat in all the wrong places: Lipotoxicity and Insulin Resistance

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Obesity (BMI > 30): Prevalence

Organisation for Economic Co-operation and Development, 2004
Lipotoxicity and Insulin Resistance

↑ Triglyceride Accumulation
Lipotoxicity and Insulin Resistance

↑ Triglyceride Accumulation

Energy Input → Insulin Resistance

Energy Output
What is known:

• Fat accumulation in liver or skeletal muscle is associated with insulin resistance

What is NOT known:

• Causal relationship between this fat accumulation and insulin resistance

• Contribution coming from different tissues in inducing insulin resistance
Will Fat Accumulation in the Liver or Skeletal Muscle Cause Insulin Resistance?
Triacylglycerol Biosynthesis

DGAT: acyl CoA:diacylglycerol acyltransferase
Experimental Approach

- **Diacylglycerol**
  - FACoA
  - DGAT1
  - DGAT2
  - Triglycerides

**LiVLE6 (hAPOE) promoter**
- DGAT
- TG

**MCK promoter**
- DGAT
- TG
Experimental Approach

**Diacylglycerol**

**FACoA**

**DGAT1**

**DGAT2**

**Triglycerides**

LiVLE6 (hAPOE) promoter

DGAT

2 DGAT2 overexpressing mouse lines (Liv-DGAT2-low 2 x, Liv-DGAT2-high 3.5 x)

1 DGAT1 overexpressing mouse line
Does overexpression of DGAT2 in Liver lead to hepatic steatosis?

.....and insulin resistance?
DGAT2 mRNA Level Is Increased in the Liver of Liv-DGAT2 Mice

Mean ± SE, 12-14 week old male mice on chow diet, n=6-9 *P<0.05, **P<0.005, ***P<0.001 vs WT
DGAT2 mRNA Level Is Increased in the Liver of Liv-DGAT2 Mice

Mean ± SE, 12-14 week old male mice on chow diet, n=6-9 *P<0.05, **P<0.005, ***P<0.001 vs WT
Liv-DGAT2 Mice Have Hepatic Steatosis

WT  Liv-DGAT2-low  Liv-DGAT2-high

Liver  Sk.Muscle

Mean ± SE, 12-15 week old male mice on chow diet, n=6-10, *P<0.001
Liv-DGAT2 Mice Have Hepatic Steatosis

Mean ± SE, 12-15 week old male mice on chow diet, n=6-10, *P<0.05 **P<0.001
Liv-DGAT2 Mice Have Hepatic Steatosis

Diacylglycerol

FACoA

DGAT1

DGAT2

Triglycerides

Mean ± SE, 12-15 week old male mice on chow diet, n=7-10, *P<0.01, **P<0.001
Summary: Liv-\textit{DGAT2} transgenic mice

- Overexpression of \textit{DGAT2} in liver leads to Hepatic steatosis

Liv-DGAT2-low (x2 fold DGAT2 mRNA)

Liv-DGAT2-high (X3.5 fold DGAT2 mRNA)

Hepatic steatosis: Lipids (Triglycerides, ceramide, Diacylglycerol, unsaturated AcylCoA)
Are Liv-$DGAT2$ Mice Insulin Resistant?
No Change in Glucose and Insulin Tolerance in Liv-DGAT2-low Mice

Glucose Tolerance Test (1g/kg BW)

<table>
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<tr>
<th>Time (min)</th>
<th>WT</th>
<th>Liv-DGAT2-low</th>
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<td>120</td>
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Insulin Tolerance Test (1U/kg BW)

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<tr>
<th>Time (min)</th>
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Mean ± SE, 10-12 week old male mice on chow diet, n=12
Liv-\textit{DGAT2}-low Mice Are not Insulin Resistant

Mean ± SE, 20 week old male mice on chow diet, \( n=7 \)
Insulin infusion rate: 2 mU/kg/min
Insulin Signaling in the Liver

IR → IRS-1, IRS-2 → PI3K → PKB/AKT → ↑ Glycogen, ↓ Glucose production and release

PI-P3, PDK1 → PKCζ/λ → ↑ SREBP-1c, ↑ FAS, etc, ↑ Lipid synthesis
No Changes in Insulin Signaling Pathway in Liv-DGAT2-low Mice

Mean ± SE, 15-17 week old male mice on chow diet, n=5
...is there just not enough fat in the liver to get insulin resistance?
No Change in Glucose and Insulin Tolerance in Liv-DGAT2-high Mice

Glucose Tolerance Test
(1g/kg BW)

Insulin Tolerance Test
(1U/kg BW)

Mean ± SE, 10-12 week old male mice on chow diet, n=12
Summary: Liv-DGAT2 transgenic mice

- Overexpression of DGAT2 in liver leads to Hepatic steatosis

  Liv-DGAT2-low (x2 fold DGAT2 mRNA)

  Liv-DGAT2-high (X3.5 fold DGAT2 mRNA)

  Hepatic steatosis: ↑Lipids (TG, ceramide, DG, unsaturated AcylCoA)

- No Insulin resistance in Liv-DGAT2 mice

  Hepatic steatosis → Insulin resistance
...is this effect specific to this particular mouse model?
Liv-DGAT1 Mice Have Hepatic Steatosis

Mean ± SE, 12-15 week old male mice on chow diet, n=6-10, *P<0.05, **P<0.001
No Change in Glucose and Insulin Tolerance in Liv-\textit{DGAT1} Mice

Mean ± SE, 10-12 week old male mice on chow diet, n=12
...if lipids in the liver do not trigger insulin resistance, what does?
No Inflammatory Marker Changes in Liver of Liv-DGAT Mice

Optical Density (Arbitrary Units)

pJNK/JNK

pNFkB/NFkB

0 1 2

WT Liv-DGAT1 Liv-DGAT2-low Liv-DGAT2-high

12-15 week old male mice on chow diet, n=3-6, *P<0.05, **P<0.01
No Inflammatory Marker Changes in Liver of Liv-DGAT Mice

12-15 week old male mice on chow diet, n=3-6, *P<0.05, **P<0.01
No Inflammatory Marker Changes in Liver of Liv-DGAT Mice

12-15 week old male mice on chow diet, n=3-6, *P<0.05, **P<0.01
Summary: Liv-\textit{DGAT} transgenic mice

- Overexpression of \textit{DGAT1} or \textit{DGAT2} in liver leads to Hepatic steatosis

  \uparrow \text{Lipids (TG, ceramide, DG, unsaturated AcylCoA)}

- No Insulin resistance in Liv-\textit{DGAT} mice

  Hepatic steatosis \rightarrow \text{Insulin resistance}

- No Inflammation in Liv-\textit{DGAT} mice

  \text{No changes in inflammatory markers}
Experimental Approach

- Diacylglycerol
- FACoA
- DGAT1
- DGAT2
- Triglycerides

MCK promoter

- DGAT
- TG
Oxidative and Glycolytic Skeletal Muscle

Oxidative muscle
(Type I, slow-twitch, red)
- High TG content
- More insulin sensitive
- ↑TG after endurance training

Glycolytic muscle
(Type II, fast-twitch, white)
- Less TG content
- Less insulin sensitive
Increased DGAT2 Expression in Glycolytic Muscle in MCK-DGAT2 Mice

Oxidative muscle: Soleus
Glycolytic muscle: White gastroc

Mean ± SE, 12 week old male mice on chow diet, n=8-9
* p<0.001 vs WT, † p<0.001 vs WT oxidative muscle.
Triglyceride Content Is Increased in Glycolytic Muscle in MCK-DGAT2 Mice

Oxidative muscle: Soleus + red gastroc
Glycolytic muscle: White gastroc

Mean ± SE, 12 week old male mice on chow diet, * p<0.05, n=8-9
Diacylglycerol and Ceramide Levels in Muscle in MCK-DGAT2 Mice

Mean ± SE, 3 month old male mice on chow diet, n=5-9, *p < 0.05 vs WT

With M. Watt
Unsaturated FACoAs Are Increased in Glycolytic Muscle in MCK-DGAT2 Mice

Mean ± SE, 3 month old male mice on chow diet, n=5-7,
*p < 0.05 vs WT glycolytic muscle, **p < 0.01 vs WT glycolytic muscle
Glucose and Insulin Tolerance Are Impaired in MCK-DGAT2 Mice

Mean ± SE, 3 month old male mice on chow diet, * p<0.05, n=10-12 for GTT, 6-9 for ITT
**In Vivo Muscle Glucose Uptake in MCK-DGAT2 Mice**

Mean ± SE, 3 month old male mice on chow diet, insulin 1U/kg
n=8-9 for oxidative muscle and 6-7 for glycolytic muscle.
Insulin Signaling in Skeletal Muscle
PKB Activity Is Impaired in Muscle in MCK-DGAT2 Mice

Mean ± SE, 15 week old male mice on chow diet, insulin 1U/kg, n=3-5
*p < 0.05 vs basal, ** p<0.01 vs basal, † p<0.05 vs WT insulin-stimulated glycolytic muscle

With RV. Farese, Sr
PKC λ/ζ Activity Is Impaired in Muscle in MCK-DGAT2 Mice

Mean ± SE, 15 week old male mice on chow diet, insulin 1U/kg, n=8-11
*p < 0.05 vs basal, ** p<0.01 vs basal

With RV. Farese, Sr
Summary: fat accumulation in glycolytic muscle leads to insulin resistance

- DGAT2 overexpression in glycolytic muscle results in:
  - Lipids (TG, ceramide, unsaturated AcylCoA)
  - Insulin resistance in MCK-DGAT2 mice

- Increased lipid deposition -> Increased insulin resistance
- Decreased whole body glucose tolerance
Conclusions

• Uncoupled fatty liver and lipid accumulation in glycolitic muscle from other obesity related effects.

• Increased lipid content in the liver do not cause hepatic insulin resistance.

• Other mechanisms, possibly inflammation, may be involved in inducing insulin resistance.
Conclusions

• Deposition of lipids in glycolytic muscle may contribute to the development of insulin resistance and type 2 diabetes.

• It is important to consider oxidative and glycolytic muscle separately in research studies.
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