Ectopic Triglyceride Accumulation in Early Pathogenesis of Type 2 Diabetes: In vivo MRS studies

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Obesity rates are on the rise and doubled in last 3 decades.

More than a third of US population is obese and additional third is overweight.
Adipose tissue is a fantastic endocrine organ. It is busy secreting variety of hormones.

On the cellular level, in “young” adipose cell TG is dispersed in the cytosol in form of TG droplets.

Adipose tissue

Mature adipose cell is a single large fat droplet.

Adipose tissue stores energy/fat in times of feast for the release in time of famine.

If famine extends for too long adipocytes fill up to their capacity and do not store fat properly.
In lean animals tissues maintain small amount of fat that is needed simply for cell housekeeping.

In obese animals levels of ectopic fat are high.
Lipotoxicity Hypothesis

Chronic high levels of ectopic fat lead to organ steatosis and lipotoxic dysfunction.

Testing Lipotoxicity Hypothesis

- It was impossible to test lipotoxicity in human directly, in vivo.
- Now, the non-invasive method is available that allows to quantify fat in non-adipose tissues in vivo.
- The method is called localized magnetic resonance spectroscopy (MRS).
- MRS has been successfully implemented to quantify ectopic fat accumulation in human skeletal muscle and the liver.
- We extended the application of MRS to the heart and pancreas.
Steps of MRS *in vivo* Experiment

1) High resolution image for a roadmap
2) Selection of a volume for testing (voxel) using magnetic field gradients

“virtual biopsy”
Steps of MRS in vivo Experiment

3) Data collection.

For example: MRS resonance from skeletal muscle contains strong signals from water and fat.
In 1993 Fritz Schick first demonstrated that a spectrum from fat in a skeletal muscle had two lines. This observation set ground for the MRS as the new tool to measure cytosolic fat in vivo.
Molecules of triglyceride experience different electromagnetic environments in cytosol and in adipose tissue, aqueous vs fatty. For this reasons they resonate at slightly different frequencies due to in BMS.
Model of Fat Compartments in Muscle

Szczepaniak et al MRM 2002

Intralipid™

Soybean oil

Intralipid™ & Soy oil
Model of Fat Compartments in Muscle

Intralipid™

\[
R_2 = \underset{\text{R2}}{\text{C=O}} \quad \text{O} \quad \text{CH}_2 \quad \underset{\text{R1}}{\text{O-C-R1}} \quad \text{CH} \\
\text{CH}_2 \quad \underset{\text{R3}}{\text{O-C-R3}} \quad \text{O}
\]

\[
R_3 = \text{CH}_2 - \text{CH}_2 - (\text{CH}_2)_{n-2} - \text{CH}_2 - \text{CH}\equiv\text{CH} - \text{CH}_2 - \text{CH}\equiv\text{CH} - \text{CH}_2 - (\text{CH}_2)_{n-1.2} - \text{CH}_3
\]

\[
R_1 = \text{CH}_2 - \text{CH}_2 - (\text{CH}_2)_{n-2} - \text{CH}_3
\]
Model of Fat Compartments in Muscle

Intralipid™

Soybean oil

Intralipid™ & Soybean oil

Various CH₂

(CH₂)ₙ

CH₃
Congenital Lipodystrophy as an Experiment of Nature

Szczepaniak et al AJP 1999
TG in Soleus *in vivo*

Control

$t2d$

Intramuscular TG

Adipose TG

Car Cr

0.5 1.5 2.5 3.5

Intramuscular TG

Adipose TG

Car Cr

0.5 1.5 2.5 3.5
Intra-Myocellular TG Content vs Insulin Sensitivity

Glucose Utilization (mg/m²/min) x 10^{-2}

Intra-muscular TG (umol/g)

NGT  CGL  t2d

McGarry, Diabetes 2002
MRS of intramuscular TG is well established and validated;

Numerous cross sectional studies confirmed our findings in skeletal muscle;

intramuscular TG vs: caloric fat intake; Intralipid infusion; fasting; exercise; gender; obesity levels etc, etc …
How about ectopic fat in other tissues?

Intra Hepatic TG
Liver Biopsy – The Gold STD

- Invasive Procedure
- Complications
  - 1 - 3% hospitalized
- Size 50 -100 mg
- Biased
  - grades and stages
  - biochemical assays
Liver Spectroscopy in vivo

Hepatic Water

Hepatic Tissue Metabolites

\(-(\text{CH}_2)_n\)-

\(-\text{CH}_3\)

Hepatic TG

3 cm

Szczepaniak AJP 2004
Reproducibility: MRS of hepatic fat

Hepatic TG (f/w %)
measurement #1

Hepatic TG (f/w %)
measurement #2

$r^2 = 0.9816$

CV = 8.5 %

Szczepaniak AJP 2004
Dallas Heart Study

n = 2349
median = 4.88%

distribution

(%)
Dallas Heart Study: Healthy sub-sample

Low-Risk Subjects  n = 394
median = 2.07 %

95th percentile = 5.56 %

Szczepaniak AJP 2004
Dallas Heart Study:
Healthy sub-sample

Low-Risk Subjects  n = 394
median = 2.07 %

95th percentile = 5.56 %

hepatic TG (%)
Fatty Heart
Obesity-Related Heart Disease: 
Framingham Risk Factor Mediation

Traditional View

- Elevated BP
- Hyperglycemia
- Atherosclerosis
- Remodeling of heart & vessels
- Plaque progression
- MI
- Stroke
- Death

Adapted from McGavock, Ann Int Med 2006
Mycardial Lipotoxicity

Impaired Fat Storage → Triglyceride Spills Over to Ectopic Sites → Cardiac Steatosis & Dysfunction

Adapted from J McGavock et al., Circulation 2007
Rodent Models

ZDF rats with a loss-of-function mutation in tissue leptin receptors

Transgenic models of cardiomyocyte- restricted TG accumulation

Transgenic Mice Resistant to Obesity
Rodent Models

Wild Type

Obese or Transgenic

Zhou et al., PNAS 2000;97(4):1784-9
Lee PNAS. 2004;101:13624-9
Rodent Models

Transgenic model of cardiomyocyte-restricted TG accumulation

<table>
<thead>
<tr>
<th>Heart TG (mg/g)</th>
<th>0</th>
<th>1.5</th>
<th>3.0</th>
</tr>
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</table>

Wild-type

Transgenics

Le *PNAS*. 2004;101:13624-9
Rodent Models

Transgenic model of cardiomyocyte-restricted TG accumulation

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<th>Heart TG (mg/g)</th>
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<td>Wild-type</td>
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<tr>
<td>Transgenics</td>
<td></td>
</tr>
<tr>
<td>leptin</td>
<td></td>
</tr>
<tr>
<td>or</td>
<td></td>
</tr>
<tr>
<td>TZD</td>
<td></td>
</tr>
<tr>
<td>Rx</td>
<td></td>
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Zhou et al., PNAS 2000;97(4):1784-9
Lee PNAS. 2004;101:13624-9
Translation

- Is heart of obese human fatty?
- Is fatty heart dysfunctional?
- Is cardiac MRS in vivo possible?
- Is myocardial steatosis reversible / preventable?
- Is myocardial dysfunction reversible / preventable?
Rodent Models - MRS

Lean Rat

Fatty Zucker Rat

Myocardial Triglyceride

-(CH₂)ₙ-

-CH₃

Tissue Metabolites

4.0 3.0 2.0 1.0 ppm

4.0 3.0 2.0 1.0 ppm

Szczepaniak MRM 2002
Is heart of obese human fatty?

Old pathologists in XVII and XVIII centuries:

“Myocardial fatty degeneration with fatty droplets within sarcoplasm.”
Is heart of obese human fatty?

Postmortem biopsies of human myocardium

Overweight
BMI 27

Obese
BMI 42

Unger and Orci, FASEB J., 2001
Is fatty heart dysfunctional?

Intra-myocardial lipid accumulation in the failing human heart: Biopsy studies in heart transplant patients

Sharma et al; FASEB J. 2004 18(14):1692-700
Is cardiac MRS in vivo possible?
Respiratory Motion
Cardiac Motion
Motion Compensation

1. Respiratory gating and cardiac triggering

2. Motion Tracking

Szczepaniak et al, MRM 2003
Van der Meer et al, Radiology 2007
Cardiac Imaging

Subcutaneous fat
Chest muscles
RV
LV
RA
LA
Cardiac MRS

Reproducibility

Measurement #2
90 days
Fat/Water (%)

Measurement #1 baseline
Fat/Water (%)

CV = 5%

Reingold et al, AJP 2004
Healthy Humans (N=15)

<table>
<thead>
<tr>
<th>BMI</th>
<th>lean</th>
<th>overweight</th>
<th>obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>lean Myocardial Triglyceride (f/w%)</td>
<td>1</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>overweight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>obese</td>
<td></td>
<td></td>
<td></td>
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$r^2 = 0.55$

Body Mass Index (kg/m²)

Healthy Humans (N=15)

BMI

| lean | overweight | obese |

LV mass (g)

Body Mass Index (kg/m²)

Myocardial Triglyceride (F/W %)

$r^2 = 0.27$

$r^2 = 0.61$

Healthy Humans (N=15)

BMI

lean | overweight | obese

Concentricity

LV mass/volume (g/cm³)

Myocardial Wall Contractility

Myocardial Triglyceride (F/W %)

Physiological Intervention: (single meal)

Serum TG (mg/dl)

Myocardial TG (F/W%)

Fasting

Postprandial

Reingold et al, AJP 2004
Physiological Intervention:
48-hour fast (hypocaloric diet)

Serum TG (mg/dl)

Myocardial TG (f/w%)

Baseline 48 Hrs fast

Reingold et al, AJP 2004
Consistent with Animal Data

Genes for fatty acid oxidation and gluconeogenesis ↑

Genes for glycolysis ↓

Natural History of Fatty Heart

Cross-sectional study (N=134)

OGTT

Cardiac MRS - steatosis

Cardiac MRI - function

Hepatic fat MRS
Abdominal (visceral & subcutaneous) adiposity MRI

McGavock et al., Circulation 2007
## Natural History of Fatty Heart

<table>
<thead>
<tr>
<th></th>
<th>Lean (n = 15)</th>
<th>Obese (n= 21)</th>
<th>IGT (n= 20)</th>
<th>T2D (n= 78)</th>
</tr>
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<tbody>
<tr>
<td><strong>Male (%)</strong></td>
<td>47</td>
<td>48</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td><strong>Ethnicity(%)</strong></td>
<td>47/33/20</td>
<td>19/57/24</td>
<td>25/25/50</td>
<td>26/40/34</td>
</tr>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>35 ± 13</td>
<td>36 ± 12</td>
<td>49 ± 9</td>
<td>47 ± 10</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>23 ± 2</td>
<td>32 ± 5⁺</td>
<td>31 ± 6</td>
<td>34 ± 7</td>
</tr>
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McGavock et al., Circulation 2007
Natural History of Fatty Heart

McGavock et al., Circulation 2007

Glucose

Insulin

Myocardial TG

 Lean         Obese       IGT       T2D

normal values

McGavock et al., Circulation 2007
**Lipotoxicity**

*(Ann Intern Med 2006, 144; 517-524)*

- Increased adipose tissue mass → High Ectopic Fat Levels → Dilated Cardiomyopathy

**Hepatic TG**

![Graph showing hepatic triglyceride levels](image)

**Myocardial TG**

![Graph showing myocardial triglyceride levels](image)

**Peak Filling Rate**

![Graph showing peak filling rate](image)

McGavock et al., Circulation 2007
Surrogates?

McGavock et al., Circulation 2007
Surrogates?

Myocardial Triglyceride
fat/water (%)

Serum Triglyceride (mg/dL)

McGavock et al., Circulation 2008
Reduction of Myocardial Steatosis

Lipodystrophy
5 Months of Leptin Rx

Simha et al 2002
Reduction of Myocardial Steatosis

Type 2 Diabetic (n=32)

Randomized

Insulin

Insulin + TZD

Myocardial TG

*
No Change in Myocardial Function

- Are functional changes irreversible?
- Was intervention long enough?
- Was reduction of mTG large enough to yield functional improvements?
- Is the method for Fx evaluation sensitive enough?
- Is it better to treat or to prevent myocardial steatosis and lipotoxicity?

![Graphs showing Myocardial TG and Peak Filling Rate](image-url)
Is Uncomplicated Obesity observed in laboratory animals?


Beta-cell lipotoxicity in the pathogenesis of NIDDM of obese rats

Longitudinal study; 6% fat diet

Obese male ZDF
Lean male ZL
Obese female ZFF

Is ZFF rat a model of uncomplicated obesity?

ZFF rats can also develop diabetes – but it takes significant overfeeding (24% fat) over longer time (+20 weeks).
Uncomplicated Obesity in Humans?
Jean Vauge 50 years ago first described the heterogeneity in obese patients population with predominant Obesity phenotypes:

- an upper body, central abdominal – ANDROID
- a lower body, gluteal - femoral - GYNOID
Distribution of fat matters

Today we know:

• Upper body obesity predisposes to cardiovascular and metabolic complications

• Fat gets to “wrong places” if adipose tissue does not work.
Obesity Pathways

Abdominal obesity and metabolic syndrome
Jean-Pierre Després and Isabelle Lemieux

Healthy energy storage within adipose tissue or uncomplicated obesity with no metabolic disorders.

Impaired fat storage – steatosis - lipotoxicity
**Obese IR vs Obese IS**


**BMI (kg/m²)**

- **> 25**
- **≤ 25, >30**
- **≤ 30**
- **≤ 30**

**Categories:**
- Lean
- Overweight
- Obese IS
- Obese IR
Intima-Media Thickness of the carotid artery, IMT, an early marker of atherosclerosis.

10% of the study population and 25% of the obese subjects had a uncomplicated obesity.
Our data: **obese IS vs obese IR**

<table>
<thead>
<tr>
<th>BMI</th>
<th>OGGT G 0</th>
<th>OGGT G 120</th>
<th>heart TG</th>
<th>Liver TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>78</td>
<td>59</td>
<td>0.3</td>
<td>0.68</td>
</tr>
<tr>
<td>36</td>
<td>104</td>
<td>142</td>
<td>1.3</td>
<td>7.71</td>
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19
Uncomplicated Obesity √
but .... How long it lasts?
## 10 years window of Uncomplicated Obesity

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*McGavock et al., Circulation 2006*
~ 10 years window of Uncomplicated Obesity and it is over
Conclusions

**Obese IS individuals:**
- Have no metabolic complications
- Have adipose obesity without spillover to ectopic sites.

**Obese IR-IGT individuals:**
- Have non-functional adipose tissue and excess of dietary TG spills over to ectopic sites causing steatosis, lipotoxicity and MS complications.

**Does Uncomplicated Obesity last for ever? NO**
- It is only a matter of a sedentary lifestyle that lasts uninterrupted for too long.
- If obese IS individuals feast without a brake and don’t burn fat they eventually develop IR, IGT and associated metabolic complications.
# Acknowledgements

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