Magnetic resonance examinations on lipid metabolism in insulin resistance and beyond

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Learning Objectives

• How can Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS) elucidate lipid metabolism in vivo?

• Pertinent studies on insulin resistance and diabetes type 2 using MRI and MRS

• Future developments and limitations of MRI/MRS for studies of lipid metabolism in vivo
Outline of the talk

- Insulin resistance => lipid vs. glucose metabolism
- Why such an emphasis on Magnetic Resonance Imaging and (multinuclear) Spectroscopy?
- Different organs affected
- Lipid compartments, body composition, visceral fat
- Lipid composition of adipose tissue and hepatic fat
- Intra- (IMCL) vs. extramyocellular (EMCL) lipids
- Lipids in liver (IHCL), heart (ICCL), pancreas (IPCL)
- Developments and limitations of MRI/MRS
Terminology

Metabolic syndrome

Obesity

Insulin resistance

Diabetes

Epidemiology

Pathophysiology and Clinics
Age-Adjusted Prevalence of Obesity and Diagnosed Diabetes Among U.S. Adults

- **Obesity (BMI≥30 kg/m^2)**
  - Missing Data
  - 14.0%–17.9%
  - 18.0%–21.9%
  - 22.0%–25.9%
  - ≥26.0%

- **Diabetes**
  - Missing data
  - 4.5%–5.9%
  - 6.0%–7.4%
  - 7.5%–8.9%
  - ≥9.0%

http://www.cdc.gov/diabetes/statistics
Cardiovascular Risk of Metabolic Syndrome / Insulin Resistance
Magnetic Resonance: Non invasive access to the organs

That you may already know….

e.g. SIEMENS TIM TRIO
3.0 Tesla = 128 MHz $^1$H
“Contrast mechanism” in MR
MR Spectrum

- OH
- -OOC-CH-CH$_3$

- e.g. Lactate

signal intensity $\Rightarrow$ concentrations

chemical shift $\Rightarrow$ chemical identity
Multinuclear MR imaging/spectroscopy

- Glucose transport
- Glucose metabolism
  - Glucose-1-Phosphate
  - Glucose-6-Phosphate
  - Glycolysis
  - Glycogen synthesis
  - Glycogen phosphorylase
- UDP-Glucose to Glycogen
- Acetyl-CoA to TCA Cycle
- Acetyl carnitine
- Phospholipid metabolism
- Adipose tissue
- IMCL, IHCL, ICCL
- Beta-oxidation
- Lactate
- Pyruvate
- ATP/PCr
- Hyperpolarized metabolites
- Glutamate (labeled)
Metabolic Syndrome / Insulin Resistance
Pathophysiology, Different Organs

Functional MRI of skeletal muscle: Whole Body Assessment of Adipose Tissue Compartments during a Lifestyle Intervention

Lipid Composition: $^1$H spectra

Lipid composition: Visceral adipose tissue

Lipid composition: $^{13}$C spectra

Effects of diet

Lipid composition of neonates: Development with age

Lipid composition of membranes: Hepatic phospholipid metabolism

55y, female, fibrosis

49y, male, cirrhosis

Metabolic Syndrome / Insulin Resistance
Pathophysiology, Different Organs

Fat-selective MRI of skeletal muscle: Spectral-spatial-selective pulses and correlation with metabolic data

$^1$H MR spectrum of skeletal muscle

Arbitrary vertical scales

Deoxy-myoglobin

Carnosine

Creatine

TMA

Taurine

Acetyl-Carnitine

IMCL

EMCL

Lactate

78 ppm

8

7 ppm

4

3

2 ppm
Two types of lipids:


soleus muscle

yellow bone marrow
Origin of the two lipid compartments: Rotation of skeletal muscle in the magnetic field $B_0$

M. tibialis anterior
32 yr old woman
PRESS, 20ms TE

Myocellular lipids:
EMCL (extra) is affected by orientation,
IMCL (intra) is not

Boesch, Slotboom, Hoppeler, Kreis.
Adipose tissue vs. intramyocellular lipids [EMCL vs. IMCL]

subcutaneous fat

muscular fasciae

Intramyocellular Lipids = IMCL

Extramyocellular Lipids = EMCL
EMCL vs. IMCL signals with varying adipose tissue


subcutaneous fat

muscular fasciae

muscle tissue
Comparison MRS, biochemical analysis and morphometry

MRS: IMCL [mmol/kg ww]

Biochemistry: triglycerides [mmol/kg ww]

r = 0.934

r = 0.413

r = 0.475

Morphometry: Vv (lit, f) [mmol/kg ww]

Same subject examined 48 h and 1 week after exhaustive exercise

Howald, Boesch, Kreis et al.
## Comparison MRS, biochemical analysis and morphometry

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Correlation coefficient r</th>
<th>Slope</th>
<th>Intercept [mmol/kg ww]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^1)H-MRS vs. EM morphometry</td>
<td>0.934</td>
<td>0.467 ± 0.059, <em>p &lt; 0.001</em></td>
<td>0.367 ± 0.305, n.s.</td>
</tr>
<tr>
<td>(^1)H-MRS vs. biochemical assay</td>
<td>0.413</td>
<td>0.196 ± 0.153, n.s.</td>
<td>1.414 ± 0.859, n.s.</td>
</tr>
<tr>
<td>EM morphometry vs. biochemical assay</td>
<td>0.475</td>
<td>0.445 ± 0.292, n.s.</td>
<td>2.352 ± 1.639, n.s.</td>
</tr>
</tbody>
</table>

Lipoproteins and phospholipids
$^1$H-MRS: Diffusion in IMCL Droplets

Classical view vs. Hypothesis

$^1$H-MRS: Diffusion in IMCL Droplets

Magnetic field gradient

Free

Restricted or Hindered
EMCL vs. IMCL: 
*Decay due to diffusion*

Depletion and replenishment of intramyocellular lipids
**1H-MRS: Intramyocellular Lipids (IMCL)**

**Other Influences: Exercise and Diet**

<table>
<thead>
<tr>
<th>Days Post Marathon</th>
<th>M.vastus medialis</th>
<th>M.vastus intermedius</th>
<th>M.tibialis anterior</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.0</td>
<td>4.0</td>
<td>2.0</td>
</tr>
<tr>
<td>1</td>
<td>6.0</td>
<td>3.0</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>4.0</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Diets**

- **Low lipid diet** (6% fat)
- **High lipid diet** (63% fat)

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1H-MRS: Intramyocellular Lipids (IMCL) 
Relation with Insulin Sensitivity

Obese diabetes patients show higher IMCL levels (TML = Total muscle lipids)

Higher IMCL related to lower insulin sensitivity

Higher IMCL levels in insulin resistant patients


1H-MRS: Intramyocellular Lipids (IMCL)

Relation with Insulin Sensitivity

Resting IMCL [mmol/kg ww]

- Green diamonds: endurance runners
- Red diamonds: sedentary subjects

HOMA = homeostasis model assessment
= (fasting GLU x fasting INS) / 22.5

1/HOMA : T>UT p<0.05
1H-MRS in tibialis ant m.

Three-dimensional relationship between VO$_2$$_{\text{max}}$, IMCL in tibialis anterior muscle and insulin sensitivity.

Intramyocellular lipids (IMCL)

Cause or consequence?

Petersen KF, Shulman GI.
*Etiology of insulin resistance.*

Thamer, Machann, Bachmann, Haap, Dahl, Wietek, Tschritter, Niess, Brechtel, Fritsche, Claussen, Jacob, Schick, Haring, Stumvoll.
\textbf{\textsuperscript{1}H-MRS: Intramyocellular Lipids (IMCL)}

Relation with Insulin Sensitivity

- Insulin resistant
- Sedentary
- Athletes

- "Bad" IMCL?
- "Good" IMCL?
Intramyocellular lipids (IMCL)

Cause or consequence?

Fig. 2 Correlation of IMCL replenishment and insulin sensitivity

Effect of fructose overconsumption in offspring T2D and controls

Vegan diet: IMCL and HOMA

Table 3: MRI assessment of muscle triacylglycerol storage and lipoprotein triacylglycerols in vegans and omnivores

<table>
<thead>
<tr>
<th></th>
<th>Vegan (n = 21)</th>
<th>Omnivore (n = 25)</th>
<th>95% CI for difference (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus (IMCL/Cr_{tot})</td>
<td>11.7 (6.1–24.6)</td>
<td>16.9 (2.7–44.7)*</td>
<td>–13.20 to –3.29 (0.01)</td>
</tr>
<tr>
<td>Tibialis (IMCL/Cr_{tot})</td>
<td>6.5 (2.5–10.1)</td>
<td>5.9 (2.7–35.7)</td>
<td>–5.82 to 0.59 (0.4)</td>
</tr>
<tr>
<td>Gastrocnemius (IMCL/Cr_{tot})</td>
<td>10.7 (4.5–201.8)</td>
<td>10.3 (4.7–44.7)</td>
<td>–9.68 to 26.4 (0.4)</td>
</tr>
<tr>
<td>Lipoprotein TG content</td>
<td>1.74 (1.62–1.86)</td>
<td>2.81 (2.28–2.79)</td>
<td>0.47–1.66 (0.0001)</td>
</tr>
<tr>
<td>Unsaturation-index</td>
<td>0.26 (0.23–0.28)</td>
<td>0.17 (0.14–0.18)</td>
<td>–0.15 to –0.015 (0.018)</td>
</tr>
</tbody>
</table>

Table 4: Biochemistry results for vegans and omnivores

<table>
<thead>
<tr>
<th></th>
<th>Vegan (n = 21)</th>
<th>Omnivore (n = 25)</th>
<th>95% CI for difference (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>3.73 ± 0.72</td>
<td>4.18 ± 0.94</td>
<td>–0.96 to 0.06 (0.08)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.22 ± 0.25</td>
<td>1.32 ± 0.41</td>
<td>–0.32 to 0.09 (0.3)</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>2.25 ± 0.60</td>
<td>2.30 ± 0.96</td>
<td>–0.53 to 0.44 (0.9)</td>
</tr>
<tr>
<td>Cholesterol/HDL-cholesterol ratio</td>
<td>3.15 ± 0.69</td>
<td>3.50 ± 1.59</td>
<td>–1.10 to 0.41 (0.4)</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/l)</td>
<td>0.56 (0.07–2.12)</td>
<td>1.18 (0.53–1.91)*</td>
<td>–0.93 to –0.37 (&lt; 0.001)</td>
</tr>
<tr>
<td>NEFA (mmol/l)</td>
<td>606 ± 231</td>
<td>551 ± 164</td>
<td>–62.5 to 173.0 (0.3)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>3.99 (3.03–4.75)</td>
<td>4.03 (3.67–5.10)*</td>
<td>–0.73 to –0.09 (0.05)</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>45.0 ± 12.59</td>
<td>41.7 ± 17.64</td>
<td>–5.98 to 12.56 (0.5)</td>
</tr>
<tr>
<td>HOMA (%S)</td>
<td>116 (83.1–270.0)</td>
<td>138 (64.3–416.0)</td>
<td>–58.63 to 16.92 (0.3)</td>
</tr>
<tr>
<td>HOMA (%B)</td>
<td>141 ± 38.1</td>
<td>109 ± 35.2*</td>
<td>10.3–53.9 (0.005)</td>
</tr>
</tbody>
</table>

IMCL content, expressed as percentage of the water resonance. White bars, before cycling test; black bars, after cycling test. *, Significant differences.

“The Increase in Intramyocellular Lipid Content Is a Very Early Response to Training”

\(^1\)H-MRS: Intramyocellular Lipids (IMCL)

Influence of free fatty acids

Examples of 1H-spectra of one subject at different time points for TA during hyperinsulinemia + lipid infusion (a), and during hyperinsulinemia alone (c).

$^{13}$C-MRS: Glycogen depletion and recovery

- 30 min
- 2 h
- 6 h

Creatine

Glycogen

$1.5 \text{ h } 70\% \text{ VO}_{2\text{max}}$ in intervals

Rest

Recovery & nutrition

IMCL and Glycogen: Influence of hyperglycemia

IMCL and Glycogen: Influence of hyperglycemia
Substrate selection by the body

Exercise leads to:
- Utilization of IMCL
- Production of acetylcarnitine (AcCar)

Acetylcarnitine after heavy workload

Metabolites

Acetylcarnitine

S [au]

workload
Acetylcarnitine as marker for Acetyl-CoA from increased fat oxidation?

![Graph showing the change in acetylcarnitine from Euglycemia to Hyperglycemia.]

\[ \frac{\text{Acetylcarnitine}}{\text{Acetyl-CoA}} \approx 10^3 \]

**1H-MRS: Acetylcarnitine**

- Car: involved in transport of fatty acids into mitochondria
- Car: buffers excess acetyl-CoA by producing AcCar

From Carlin JI et al., J Appl Physiol 1990;69:42.
Acetylcarnitine

How is the excess acetyl-CoA produced?

CARBOHYDRATE OXIDATION
- Glucose / Glycogen
- Pyruvate
- Acetylcaritnine
- Acetyl-CoA
- TCA cycle

FAT OXIDATION
- Free fatty acids / IMCL
- Carnitine
- PDC
- CPT-I

Mitochondrion

**13C-MRS:**

Mitochondrial Activity, TCA cycle

- $[2-^{13}C]$ acetate infusion
- $^{13}$C incorporation into glutamate C4 (33.6 - 35.0 ppm)
- Modeling (Cwave software) -> TCA cycle flux

Metabolic Syndrome / Insulin Resistance
Pathophysiology, Different Organs

Effects of a short-term overfeeding with fructose or glucose in healthy young males

Eleven subjects, 7 days diet:

- weight maintenance, control diet
- High Fructose Diet (3·5 g fructose/kg fat-free mass per day)
- High Glucose Diet (3·5 g glucose/kg fat free mass per day)

Effect of fructose overconsumption in offspring T2D and controls

Effect of exercise: Average $\Delta IMCL/IMCL_{pre}$ and $\Delta IHCL/IHCL_{pre}$

**Skeletal muscle:**

$\Delta IMCL$  
$p = 0.006$

$\Delta IHCL$

**Liver:**

$\Delta IHCL$  
$p = 0.026$
Effect of exercise on IMCL and IHCL

Liver → IHCL ↑ → FFA → FFA → Working muscle

Glucose → IMCL ↓ → Glycogen

Adipose tissue
Lipid Composition: Liver vs. subcutaneous/visceral fat

Metabolic Syndrome / Insulin Resistance
Pathophysiology, Different Organs

Intracardiomyocellular lipids: ICCL

Intracardiomyocellular lipids: ICCL Diurnal changes and long-term variations

Ith M, Stettler C, Xu J, Boesch C, Kreis R.
Cardiac lipids show diurnal changes and long-term variations in healthy human subjects.
NMR Biomed. 2014; minor revision.
IMCL, IHCL, ICCL: Effects of exercise

Myocardial fat in metabolic syndrome

Cardiac Steatosis in DM2
Dallas Heart Study

Myocardial fat in dilated cardiomyopathy

Metabolic Syndrome / Insulin Resistance

Pathophysiology, Different Organs

Pancreatic fat: Multiecho

Pancreatic fat – measured by MRS

Pancreatic fat – Fat selective imaging

Heni M, Machann J, Staiger H, Schwenzer NF, Peter A, Schick F, Claussen CD, Stefan N, Haring HU, Fritsche A.
Pancreatic fat is negatively associated with insulin secretion in individuals with impaired fasting glucose and/or impaired glucose tolerance: a nuclear magnetic resonance study. Diabetes Metab.Res.Rev. 2010; 26: 200-205.
Summary and Outlook

> Advantages / disadvantages

> Development

> Methodological competitors
## Magnetic resonance imaging and (multinuclear) spectroscopy vs. other methods

### PRO’s

- Non invasive ↔ Biopsy
- Non ionizing ↔ PET, CT
- Repeatable ↔ External tracers, biopsy
- Localized ↔ Body fluids, breathing air

### CON’s

- Insensitive ↔ PET, PET/CT, PET/MR etc.
- Not beside, not mobile ↔ US, blood sample, etc
- Expenses, availability ↔ US, blood sample, etc
MRI and MRS:

Future developments

> Stronger magnetic fields (7 Tesla, 9.4 Tesla, …)
  — Increased sensitivity (signal-to-noise-ratio)
  — Better spectral resolution (MR spectroscopy)
  — Increased effects (e.g. signals of brain activation)

> New acquisition techniques (e.g. coil arrays, sampling scheme)
  — Faster acquisition
  — Increased sensitivity

> Hyperpolarized substances
  — Increased sensitivity
  — Better spatial resolution (Metabolite images)

> Availability
  — Neurosciences (fact)
  — Physiology, sports medicine, endocrinology (wishful thinking?)
Advantages of ultra-high magnetic fields
Examples at 7 Tesla


$^{13}$C-MRS: Hyperpolarized substances

$[^{13}C1]$-pyruvate metabolism in human prostate cancer


Images of lactate/pyruvate indicate cancer

Studies of metabolism in insulin resistance limited by time restrictions of hyperpolarization - nonetheless enormous potential
Take home message

- Insulin resistance: prominent application of multinuclear MRI/MRS
- MR is non-invasive, non-ionizing, localized, and repeatable
- Multiple organs accessible
- Insulin resistance involves lipid and carbohydrate metabolism
- Body composition, fat compartments
- Lipid composition
- IMCL, IHCL, ICCL, IPCL
- Further development, stronger magnetic fields, techniques
- Availability for physiologists, endocrinologists, sports medicine, etc.
Thank you....