Understanding Metabolic Adaptation to Changes in Dietary Patterns in Humans

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“It wasn’t really insulin. You don’t have diabetes yet. It was just a warning shot.”
What do we study?

- Skeletal muscle substrate metabolism
- Context of obesity and insulin resistance

Models
- Cell culture (primary myotubes)
- Human interventions
Oxidative substrate metabolism

Fatty Acids
- Beta Oxidation
- Acetyl-CoA
- ETC
- ADP

Carbohydrates
- Glycolysis
- Pyruvate
- ATP

TCA cycle
- NADH
- FADH²
- CO₂

Metabolism processes:
- Carbohydrates: Glycolysis
- Fatty Acids: Beta Oxidation
- ATP: ADP
- NAD: FAD
Why study skeletal muscle in this context?

- Comprises significant proportion of body mass
- Major site of glucose (fed state) and FFA (fasted state) metabolism
- Dynamic changes in metabolism
- Altered substrate handling associated with obesity, insulin resistance, and diabetes
Skeletal muscle fiber type composition and obesity

Glycolytic:oxidative ratio and insulin resistance

$r = -0.60$
$p < 0.01$

Glycolytic:oxidative ratio and insulin resistance

More oxidative → More glycolytic

In vitro muscle studies
Fasting humans

Palmitate Oxidation
(nmole/CO₂.g/h)

Lean

Obese

Fatty acids are preferentially stored vs. oxidized with obesity.

Hulver et al. Cell Metabolism. 2005
A Metabolically flexible

Robust preference for fat oxidation in muscle

Suppressed glucose oxidation

Lean, aerobically fit individual

B Metabolically inflexible

Blunted preference for fat oxidation in muscle

Less suppression of glucose oxidation

Obese, aerobically unfit individual
Metabolic flexibility

Adapted from Kelley et al. AJP, 1999
Across the leg studies
Fasting humans

**During fasting**

A. Metabolically flexible
- Robust preference for fat oxidation in muscle
- Suppressed glucose oxidation
- Lean, aerobically fit individual

B. Metabolically inflexible
- Blunted preference for fat oxidation in muscle
- Less suppression of glucose oxidation
- Obese, aerobically unfit individual

**During insulin-stimulated conditions**

C. Metabolically flexible
- Suppression of fat oxidation
- Robust stimulation of glucose oxidation
- Lean, aerobically fit individual

D. Metabolically inflexible
- Blunted suppression of fat oxidation
- Less stimulation of glucose oxidation
- Obese, aerobically unfit individual
Metabolic flexibility

Adapted from Kelley et al. AJP, 1999
Metabolic flexibility

Adapted from Kelley et al. AJP, 1999
Across the leg studies
Fasting humans

Respiratory Quotient (RQ)

Figure 1

a.

Adapted from Galgani et al., 2008

Metabolically flexible

Metabolically inflexible

Adapted from Bergouignan et al., 2012
Respiratory Quotient (RQ)

Figure 1

a. Metabolically flexible
b. More flexible
Less flexible
Metabolically inflexible

Adapted from Bergouignan et al., 2012

Adapted from Galgani et al., 2008
Primary interest

Understanding the signals that contribute to altered oxidative substrate preference and metabolic inflexibility
Controlled feeding studies in humans

Model for understanding metabolic adaptation?
Fat and carbohydrate balances during adaptation to a high-fat diet

Steven R Smith, Lilian de Jonge, Jeffery J Zachwieja, Heli Roy, Tuong Nguyen, Jennifer C Rood, Marlene M Windhauser, and George A Bray

Changes in fat oxidation in response to a high-fat diet\textsuperscript{1,2}

Patrick Schrauwen, Wouter D van Marken Lichtenbelt, Wim HM Saris, and Klaas R Westerterp

Study Design

The study design includes a pre-enrollment screening followed by a 2-week lead-in diet (controlled feeding), lasting from Days 1-14. This is followed by a high-fat meal (HFM) challenge on Day 15, then a 5-day high-fat diet (HFD) from Days 16-20. Another HFM challenge is scheduled on Day 21. Throughout the study, various assessments are conducted:

- 12-hour overnight fast
- Muscle biopsy
- High-fat meal
- Blood draws
- Muscle biopsy

These assessments are performed at specific time points: -30 min, 0 hour, 10 min, 1 hour, 2 hour, 3 hour, and 4 hour.
## Diet Composition

<table>
<thead>
<tr>
<th>Diet Condition</th>
<th>Energy (kcal/day)</th>
<th>Protein (%)</th>
<th>CHO (%)</th>
<th>Fat (%)</th>
<th>SFA (%kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitual</td>
<td>2318 ± 104</td>
<td>16.9</td>
<td>44.3</td>
<td>35.9</td>
<td>13.1</td>
</tr>
<tr>
<td>2 week lead-in (control)</td>
<td>2768 ± 66</td>
<td>15.2</td>
<td>53.9</td>
<td>30.9</td>
<td>9.4</td>
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<td>High Fat Diet</td>
<td>2735 ± 73</td>
<td>15.3</td>
<td>30.9</td>
<td>53.8</td>
<td>24.5</td>
</tr>
<tr>
<td>HF Meal Challenge</td>
<td>820 kcal/meal</td>
<td>12 (24g/meal)</td>
<td>25 (52g/meal)</td>
<td>63 (58g/meal)</td>
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Acute high fat feeding in Humans

Endotoxin

Anderson et al, Obesity, 2015
Acute high fat feeding in Humans

Endotoxin

Anderson et al, Obesity, 2015
Acute high fat feeding in Humans

Endotoxin

Cani, P. et al, 2007
Early lessons

- Controlled feeding is a must
- Assessing tissue metabolism under fasting conditions provides limited information
  - Incorporated meal challenge
Acute high fat feeding in Humans
Importance of control feeding

Metabolic Flexibility
(pyruvate oxidation:pyruvate oxidation + FFA)
Human participants

- Young males (age ~22yrs)
- Healthy, non-obese (BMI ~23kg/m²)
- Sedentary (< 2 planned exercise session, 20 min or less)
  - n=13 - 25
- Exclusion criteria
  - BMI >25kg/m²
  - Family history of Diabetes
  - Any known cardiovascular condition
  - Smokers
  - Moderate to heavy alcohol drinkers
  - Medications known to affect lipid/CHO metabolism
  - Those with habitual high fat diet (>35%, 24 hr recall, food records)
# Acute high fat feeding in Humans

## Subject characteristics

<table>
<thead>
<tr>
<th>Variable (n=13)</th>
<th>Pre HFD</th>
<th>Post HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>22.2 ± 0.4</td>
<td>--</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.77 ± 0.02</td>
<td>--</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.1 ± 0.9</td>
<td>23.0 ± 0.8</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>72.09 ± 3.2</td>
<td>71.98 ± 2.9</td>
</tr>
<tr>
<td>Body Fat Mass (kg)</td>
<td>16.57 ± 2.1</td>
<td>16.28 ± 2.0</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>22.03 ± 1.7</td>
<td>21.44 ± 1.7</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>54.15 ± 1.7</td>
<td>54.51 ± 1.9</td>
</tr>
</tbody>
</table>
Acute high fat feeding in Humans
Whole body insulin sensitivity - IVGTT
Acute high fat feeding in Humans
Glucose oxidation

![Graph showing glucose oxidation before and after high fat feeding (HFD). The graph indicates a significant increase in glucose oxidation post-HFD compared to pre-HFD.](image)

- **Pre HFD**
- **Post HFD**

**P < 0.05**

Glucose Oxidation (nmol/mg/hr)
Acute high fat feeding in Humans
Glucose oxidation

P<0.05
Acute high fat feeding in Humans
Glucose oxidation

* Interaction, p<0.05
Acute high fat feeding in Humans
Fatty acid oxidation

* $p < 0.05$

<table>
<thead>
<tr>
<th></th>
<th>Pre HFD</th>
<th>Post HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CO_2$ (nmol/mg/hr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid soluble metabolites (nmol/mg/hr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$CO_2 + ASM$ (nmol/mg/hr)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Pre Meal Challenge

$p < 0.05$
Acute high fat feeding in Humans
Fatty acid oxidation

* p<0.05
Acute high fat feeding in Humans
Fatty acid oxidation

* Interaction, p=0.011
Acute high fat feeding in Humans
Metabolic flexibility

[Diagram showing bar chart with Pre HFD and Post HFD values for Pyr ox/Pyr Ox + FA, indicating changes in metabolic flexibility before and after high-fat diet feeding.]
Acute high fat feeding in Humans
Metabolic flexibility

P<0.05

[Bar chart showing comparison between Pre HFD and Post HFD for Pyr ox/Pyr Ox + FA, with Pre Meal and Post Meal Challenge bars highlighted.]

Pre Meal Challenge
Post Meal Challenge
Acute high fat feeding in Humans
Metabolic flexibility

P<0.05

Pyr ox/Pyr Ox + FA

Pre HFD Post HFD

Pre Meal Challenge
Post Meal Challenge
Changes in fat oxidation in response to a high-fat diet$^{1,2}$

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Fat and carbohydrate balances during adaptation to a high-fat diet

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Highly variable response.

What drives this?
How do we get there? Does it matter?
Respiratory Quotient (RQ)

**Metabolically flexible**

**Metabolically inflexible**

Adapted from Bergouignan et al., 2012

---

**a.**

**b.**

More flexible

Less flexible

**C.**

Less flexible

More flexible

Adapted from Galgani et al., 2008
What now?
Better understand post-translational modifiers
Post-translational targets

- O-GlcNAcylation
- Phosphoproteome
- Acetylome
O-GlcNAcylation

Hardiville et al., 2016 (2)
Red denotes proteins susceptible to O-GlcNAcylation

Ma et al. 2016 (30)
O-GlcNAc and Phosphorylation

Hardiville et al., 2016 (2)
Phosphorylation

Zhao et al., 2014 (10)
Acetylation
## Acetylation

### Figure 9

<table>
<thead>
<tr>
<th>Pyruvate Oxidation</th>
<th>Fold change (DIO/Control)</th>
<th>P&lt;0.05</th>
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</thead>
<tbody>
<tr>
<td>Pyruvate dehydrogenase E1 component subunit beta</td>
<td>1.35</td>
<td>*</td>
</tr>
<tr>
<td><strong>TCA Cycle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>1.71</td>
<td>*</td>
</tr>
<tr>
<td>Aconitate hydratase</td>
<td>1.29</td>
<td>*</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase [NADP]</td>
<td>1.49</td>
<td>*</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase [NAD] subunit</td>
<td>1.33</td>
<td>*</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase [NAD] subunit alpha</td>
<td>1.44</td>
<td>*</td>
</tr>
<tr>
<td>Succinyl-CoA ligase [ADP-forming] subunit beta</td>
<td>1.45</td>
<td>*</td>
</tr>
<tr>
<td>Succinyl-CoA ligase [ADP/GDP-forming] subunit alpha</td>
<td>1.49</td>
<td>*</td>
</tr>
<tr>
<td>Succinate dehydrogenase [ubiquinone] flavoprotein subunit</td>
<td>1.21</td>
<td>*</td>
</tr>
<tr>
<td>Succinate dehydrogenase [ubiquinone] flavoprotein subunit</td>
<td>1.21</td>
<td>*</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>1.22</td>
<td>*</td>
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<tr>
<td><strong>Electron Transport Chain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9</td>
<td>1.45</td>
<td>*</td>
</tr>
<tr>
<td>Cytochrome b-c1 complex subunit 7</td>
<td>1.34</td>
<td>*</td>
</tr>
<tr>
<td>Cytochrome c oxidase subunit 4 isoform 1</td>
<td>1.41</td>
<td>*</td>
</tr>
<tr>
<td>Cytochrome c oxidase subunit 5B</td>
<td>1.26</td>
<td>*</td>
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<tr>
<td>Electron transfer flavoprotein-ubiquinone oxidoreductase</td>
<td>1.54</td>
<td>*</td>
</tr>
<tr>
<td>Electron transfer flavoprotein subunit beta</td>
<td>1.81</td>
<td>*</td>
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<tr>
<td>ATP synthase subunit alpha</td>
<td>1.34</td>
<td>*</td>
</tr>
<tr>
<td>ATP synthase subunit beta</td>
<td>1.39</td>
<td>*</td>
</tr>
<tr>
<td>ATP synthase subunit d</td>
<td>1.51</td>
<td>*</td>
</tr>
<tr>
<td>ATP synthase subunit epsilon</td>
<td>1.64</td>
<td>*</td>
</tr>
<tr>
<td>ATP synthase subunit g</td>
<td>1.22</td>
<td>*</td>
</tr>
<tr>
<td>ATP synthase subunit g</td>
<td>1.22</td>
<td>*</td>
</tr>
<tr>
<td>ATP synthase subunit O</td>
<td>1.63</td>
<td>*</td>
</tr>
<tr>
<td>ATP synthase F(0) complex subunit B1</td>
<td>1.39</td>
<td>*</td>
</tr>
</tbody>
</table>

In progress........ Developing one of the largest mass spec data sets to date with human clinical samples.
$1,200,000

24 unmodified peptide samples
24 phosphopeptide samples
24 acetylated peptide samples
72 total samples
each run in triplicate

216 MS runs requiring at least 3 hours per run
27 days of MS time

Over 10,000,000 MS/MS spectra

Over 300 GB data
Weeks of data processing time required

Over 100,000 peptides from approximately 1,000 proteins identified and quantified.
Mass spec results

- Phosphoproteome ONLY
  - 1,016,484 MS/MS spectra obtained for the 24 samples
  - This led to 65,980 peptide/spectrum matches.
  - These were matched to 3065 peptides which mapped to 633 proteins.
Mass spec analysis – Perseus

Stay tuned....
Energy metabolism

Dogma tells us…
this is a "pull" system, meaning
driven by energy demand,
not a “push” system driven by
energy input
Energy metabolism

Thus, the only way to increase metabolic rate is to increase energy demand.
Control of Energy metabolism

- Acetyl-CoA
- TCA cycle
- NADH
- FADH$_2$
- ETC
- O-GlcNAcylation
- Phosphorylation
- Acetylation

CHO, FAT, AA

+ or -

ATP
Phosphorylation

Zhao et al., 2014 (10)
Control of Energy metabolism

Insulin-stimulated Phosphorylation

TCA cycle

NADH

FADH$_2$

ETC

Acetyl-CoA

CHO FAT AA

Acetyl-CoA

ATP

ATP

ATP
Control of Energy metabolism

![Diagram showing the control of energy metabolism with key components including CHO, FAT, AA, Acetyl-CoA, TCA cycle, NADH, FADH$_2$, ATP, ADP, and ETC.]
Control of Energy metabolism

Insulin-stimulated Phosphorylation

TCA cycle

Acetyl-CoA

NADH

FADH$_2$

ETC

ADP

ATP

CHO FAT AA

Acetyl-CoA

Acetyl-CoA

Acetyl-CoA
What if we short circuit this?
What if we short circuit this?
What if we short circuit this?

Insulin-stimulated Phosphorylation

![Diagram showing CHO, FAT, AA, Acetyl-CoA, TCA cycle, NADH, FADH₂, ETC, and ATP.]
What if we short circuit this?

Insulin-stimulated Phosphorylation

Begin to slow

ETC

FADH₂

NADH

TCA cycle

Acetyl-CoA

FAT

CHO

AA

Acetyl-CoA

Acetyl-CoA

ATP

ATP

ATP
What if we short circuit this?
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Questions?
Chronic high fat feeding in Humans
Study design

- Controlled feeding
- 2 week lead-in
- 4 weeks of high fat overfeeding
- 1000 kcal over energy balance
  - 55% fat (25% SF), 30% CHO, 15% protein

<table>
<thead>
<tr>
<th>Diet condition</th>
<th>Energy (kcal day^{-1})</th>
<th>Protein (g day^{-1})</th>
<th>CHO (g day^{-1})</th>
<th>Fat (g day^{-1})</th>
<th>SFA (g day^{-1})</th>
<th>Fiber (g day^{-1})</th>
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<tbody>
<tr>
<td>Control</td>
<td>2,903 ± 78</td>
<td>108 ± 3.0</td>
<td>408 ± 11.0*</td>
<td>98 ± 4.0</td>
<td>28 ± 1.0</td>
<td>18 ± 1.0*</td>
</tr>
<tr>
<td>High fat</td>
<td>3,947 ± 79*</td>
<td>125 ± 3.0*</td>
<td>282 ± 6.0</td>
<td>256 ± 5.0*</td>
<td>140 ± 2.0*</td>
<td>14 ± 1.0</td>
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Chronic high fat feeding in Humans
Variation in response
Chronic high fat feeding in Humans
Variation in response

How do these groups differ?
Chronic high fat feeding in Humans
Variation in response

- Weight gain (kg)

- Fatty Acid Oxidation (% change)

- Pyruvate Oxidation (% change)
Chronic high fat feeding in Humans
Variation in response

- **Complex I**:
  - Gained less weight: 
  - Gained more weight: 

- **Complex III**:  
  - Gained less weight:  
  - Gained more weight: 

- **Reverse Complex I**:  
  - Gained less weight:  
  - Gained more weight: