Tracing the fate of dietary fat: metabolic studies in humans

Barbara Fielding
Sir David P Cuthbertson (1900-1989)
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Tracing the fate of dietary fat

Background

Postprandial studies
• After two sequential meals
• After fructose ingestion
• Tissue specific measurements (arterio-venous difference)

Fatty acid composition of adipose tissue depots
Why study postprandial fat metabolism?

Nutritional support of patients should be based upon a thorough understanding of the metabolic response to food.
Why study postprandial fat metabolism?

- Postprandial lipaemia
- CVD
- Oxidative stress
- Inflammation
- Ectopic fat accumulation
Overnight fasting

VLDL very low density lipoprotein
NEFA non-esterified fatty acids
FA fatty acids
TG triacylglycerol/triglyceride
LPL lipoprotein lipase
Chylomicrons (via lymphatics)

VLDL very low density lipoprotein
NEFA non-esterified fatty acids
FA fatty acids
TG triacylglycerol/triglyceride
LPL lipoprotein lipase
Typical postprandial metabolite concentrations
Typical postprandial metabolite concentrations

- Plasma TG (μmol/L)
- Plasma glucose (mmol/L)

Time (min)
Typical postprandial metabolite concentrations

Plasma TG (µmol/L)

Plasma glucose (mmol/L)

Plasma insulin (pmol)
Typical postprandial metabolite concentrations
Tracing the fate of dietary fat

Background

Postprandial studies
- After two sequential meals
- After acute fructose ingestion
- Tissue specific measurements
  - high carbohydrate diet

Fatty acid composition of adipose tissue depots
Two-meal study

safflower oil
(68% 18:2 n-6)

olive oil /avocado (75%
18:1 n-9)

Fielding et al 1996 AJCN
Meal fatty acids in chylomicron TG (n=7)

Meal containing olive oil (18:1 n-9)

18:1 n-9

Fielding et al 1996 AJCN
Meal fatty acids in chylomicron TG (n=7)

Meal containing olive oil (18:1 n-9)

18:2 n-6

18:1 n-9

Fielding et al 1996 AJCN
Meal fatty acids plasma NEFA (n=7)

Meal containing olive oil (18:1 n-9)

Fielding et al 1996 AJCN
Meal fatty acids plasma NEFA (n=7)

Meal containing olive oil (18:1 n-9)

18:2 n-6
18:1 n-9

Fielding et al 1996 AJCN
Appearance of meal fatty acids into the plasma NEFA pool

Fielding et al 1996 AJCN
Hypothesis

• Dietary fat resides in the enterocytes even after 5h.

• Subsequent meals release the stored TG, causing a peak in plasma TG and NEFA.

Tracing fatty acids…
## Stable isotope tracers

<table>
<thead>
<tr>
<th>Common form</th>
<th>Stable isotope</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>$^2$H (0.02%)</td>
</tr>
<tr>
<td>$^{12}$C</td>
<td>$^{13}$C (1.1%)</td>
</tr>
<tr>
<td>$^{14}$N</td>
<td>$^{15}$N (0.37%)</td>
</tr>
<tr>
<td>$^{16}$O</td>
<td>$^{18}$O (0.04%)</td>
</tr>
</tbody>
</table>
Setting up mass spectrometry at OCDEM

March 2003

November 2003
Tracing the fate of dietary fat

Background

Postprandial studies
• After 2 sequential meals
• After fructose ingestion
• Tissue specific measurements (arterio-venous difference)

Fatty acid composition of adipose tissue depots
The effect of glucose and fructose on postprandial adipose tissue fatty acid metabolism

<table>
<thead>
<tr>
<th>M/F</th>
<th>8/6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43 (15.3)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.3 (0.9)</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>5.0 (0.1)</td>
</tr>
<tr>
<td>Plasma TG (µmol/l)</td>
<td>1240 (600)</td>
</tr>
</tbody>
</table>

Chong et al 2007 AJCN
Test ‘meal’

Lemon drink
• Fructose/glucose powder

Chocolate drink
• Palm oil
• Safflower oil
• $[^2H_2]$palmitic acid
• Cocoa powder
• Emulsifier

Chong et al 2007 AJCN
Plasma glucose concentrations

Time (min)
-60 0 60 120 180 240 300 360
Plasma glucose (mmol/l)
3 4 5 6 7 8 9

Glucose
Fructose

Chong et al 2007 AJCN
Plasma insulin concentrations

Chong et al 2007 AJCN
Plasma NEFA concentrations

Suppression of lipolysis

Glucose

Fructose

Time (min)

Plasma non-esterified fatty acids (μmol/l)

Chong et al 2007 AJCN
Appearance of tracer from meal $[^{2}\text{H}_2]\text{palmitate}$ in plasma TG

Chong et al 2007 AJCN
Appearance of $[^2\text{H}_2]\text{palmitate}$ in plasma NEFA

![Graph showing the appearance of $[^2\text{H}_2]\text{palmitate}$ in plasma NEFA over time. The graph includes data points for glucose and fructose, with error bars indicating variability. The graph shows a significant increase in $[^2\text{H}_2]\text{palmitate}$ levels with time, particularly for fructose, with P<0.05 compared to glucose.](image)

Chong et al 2007 AJCN
Components of plasma NEFA after ingestion of glucose or fructose

Data from Chong et al 2007 AJCN
Components of plasma NEFA after ingestion of glucose or fructose

Data from Chong et al 2007 AJCN
Conclusions

• In the postprandial period, as much as 40 % of the postprandial NEFA pool are derived directly from the spillover of meal fatty acids which are hydrolysed in adipose tissue but spillover into the plasma.

• Lower postprandial NEFA concentrations after fructose ingestion are due to a lower contribution of meal fatty acids.
Tracing the fate of dietary fat

Background

Postprandial studies
• After 2 sequential meals
• After fructose ingestion
• Tissue specific measurements (arterio-venous difference)

Fatty acid composition of adipose tissue depots
The effect of a short-term high-carbohydrate (HC) v high-fat (HF) diet

* Participants consumed either a HC or HF isoenergetic diet for 3 days.
3-day diet composition

<table>
<thead>
<tr>
<th>Diet</th>
<th>High CHO</th>
<th>High fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fats: CHO (% energy)</td>
<td>10:75</td>
<td>40:45</td>
</tr>
<tr>
<td>Starch: sugar (% energy)</td>
<td>30:70</td>
<td>30:70</td>
</tr>
<tr>
<td>Protein (% energy)</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

Fat was provided in the form of a fat spread (lard, palm oil, olive oil, corn oil)
Chylomicrons (via lymphatics)

[U-\(^{13}\)C]palmitate drink

Exogenous pathway

\(^{13}\)C TG

\(^{13}\)C NEFA

LPL

FA

TG

NEFA

Endogenous pathway

\(^{2}\)H\(_2\) VLDL

\([^{2}\)H\(_2\)]\)palmitate iv infusion

\(^{13}\)C NEFA

FA

TG

LPL
Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43 ± 3</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>6/2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73 ± 4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>116 ± 3</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>76 ± 2</td>
</tr>
</tbody>
</table>

Roberts et al 2008 AJCN
Study day protocol (same procedure after each dietary regime)

Test meal 40g fat ([U-\textsuperscript{13}C]palmitic acid), 40g carbohydrate

exogenous (dietary)

Fatty acid infusion ([\textsuperscript{2}H\textsubscript{2}]palmitic acid)

endogenous fatty acid metabolism

Blood sampling time points

Roberts et al 2008 AJCN
Measurements of arteriovenous difference

[U-13C]palmitate drink

Vein draining the forearm (muscle)

Forearm blood flow

Vein draining adipose tissue

[2H2]palmitate infusion

AT blood flow

(Femoral) artery
Metabolic response to the same test meal

- High CHO diet
- High fat diet (same test meal)

Roberts et al 2008 AJCN
De novo lipogenesis and SCD action

Dietary sugar → Pyruvate → Acetyl CoA → Malonyl CoA → Palmitic acid (16:0) → Palmitoleic acid (16:1 n-7)

ACC = acetyl-CoA carboxylase  
FAS = fatty acid synthase  
SCD = stearoyl-CoA desaturase
Isotopic postprandial desaturation index

(\[^{2}\text{H}16:1\ n-7/\[^{2}\text{H}]16:0\])

- High CHO diet
- High fat diet (same test meal)

Chong, Hodson et al 2008 AJCN
Metabolic response to the same test meal

- High CHO diet
- High fat diet (same test meal)

Roberts et al 2008 AJCN
Lower whole body and forearm muscle (meal) fatty acid oxidation after high CHO diet

Whole body dietary fat oxidation

- High CHO diet
- High fat diet (same test meal)

Roberts et al 2008 AJCN
Lower whole body and forearm muscle (meal) fatty acid oxidation after high CHO diet

- **Whole body dietary fat oxidation**

- **Forearm $^{13}$CO$_2$ release (µmol 100g$^{-1}$ min$^{-1}$)**

  - High CHO diet
  - High fat diet (same test meal)

Roberts et al 2008 AJCN
Conclusions

After a 3-day high CHO diet (compared with a high Fat diet)

• FAs were repartitioned away from oxidation, towards esterification (TG)
• Carbohydrates were partitioned towards de novo lipogenesis
• Fasting plasma TG concentrations were increased
• Skeletal muscle failed to upregulate TG clearance
Using the same model......


McQuaid SE, Humphreys SM, Hodson L, Fielding BA, Karpe F, Frayn KN. Femoral adipose tissue may accumulate the fat that has been recycled as VLDL and nonesterified fatty acids. *Diabetes* 2010;59:2465-2473
Tracing the fate of dietary fat

Background

Postprandial studies
• After 2 sequential meals
• After acute fructose ingestion
• Tissue specific measurements
  - high carbohydrate diet

Fatty acid composition of adipose tissue depots
Adipose tissue fatty acid composition

To what extent does adipose tissue composition reflect dietary intake?

Does adipose tissue composition differ between subcutaneous fat depots?
Adipose tissue fatty acids represent long term dietary intake, although monounsaturated fatty acids tend to be over-represented.

<table>
<thead>
<tr>
<th>Food</th>
<th>% 16:1 n-7 (g/100g fatty acids)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>0.3 %</td>
</tr>
<tr>
<td>Chocolate</td>
<td>0.6 %</td>
</tr>
<tr>
<td>Butter</td>
<td>1 %</td>
</tr>
<tr>
<td>Olive oil</td>
<td>1.4 %</td>
</tr>
<tr>
<td>Lard</td>
<td>2.5 %</td>
</tr>
<tr>
<td>Trout/tuna</td>
<td>9/4.2 %</td>
</tr>
<tr>
<td>Macadamia nuts</td>
<td>14 %</td>
</tr>
</tbody>
</table>

Hodson et al 2008 Prog Lipid Res
DEXA scans of women showing body fat distribution

The risk of myocardial infarction decreases with increasing hip circumference

Methods (simultaneous measurement of gene expression and adipose tissue fatty acid composition)

Abdominal

Gluteofemoral

50 µl

Fatty acid composition measured by GC *

mRNA content of transcripts relevant for FA metabolism analysed by QPCR

*Hodson et al submitted
Subject characteristics
(n=37, 19M, 18F aged 30-50)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>28.2</td>
<td>5.8</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Insulin (IU/L)</td>
<td>12.8</td>
<td>7.7</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.4</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Dietary glucose/fructose

\[\text{ACLY} \rightarrow \text{Acetyl CoA}\]

\[\text{ACC1} \rightarrow \text{Malonyl CoA}\]

\[\text{FAS} \rightarrow \text{Palmitic acid (16:0)}\]

\[\text{SCD} \rightarrow \text{Myristoleic acid (14:1 n-5)}\]

\[\text{SCD} \rightarrow \text{Palmitoleic acid (16:1 n-7)}\]

\[\text{ELOVL6} \rightarrow \text{Cis-vaccenic acid (18:1 n-7)}\]

\[\text{ACLY} = \text{ATP citrate lyase}\]

\[\text{ACC} = \text{acetyl-CoA carboxylase}\]

\[\text{FAS} = \text{fatty acid synthase}\]

\[\text{SCD} = \text{stearoyl-CoA desaturase}\]

\[\text{ELOVL} = \text{elongation of very long-chain fatty acids}\]
Abdominal Gluteofemoral

14:0

\[ P < 0.001 \]

14:1 n-5

\[ P < 0.001 \]
16:0

\[ P < 0.001 \]

16:1 n-7

\[ P < 0.001 \]
Abdominal Gluteofemoral % Composition

18:0

P<0.001

18:1 n-9

P<0.001
16:1n-7

Gluteofemoral vs Abdominal

\[ r = 0.80 \]
\[ P = < 0.001 \]

16:1n-7/16:0 ratio (SCD index)

Gluteofemoral vs Abdominal

\[ r = 0.753 \]
\[ P = < 0.001 \]

18:1 n-7/16:1 n-7 (elongation index)

Gluteofemoral vs Abdominal

\[ r = 0.68 \]
\[ P = < 0.001 \]

SCD indices

14:1 n-5/14:0

Gluteofemoral vs Abdominal

\[ r = 0.945 \]
\[ P = < 0.001 \]
Can site-specific differences in the gene expression of key enzymes involved in *de novo* lipogenesis (DNL) explain these differences in fatty acid composition?
Depot-specific expression of genes involved in fatty acid metabolism

FASN; fatty acid synthase, ACAC; acetyl CoA carboxylase, ACLY; ATP citrate lyase, SCD; stearoyl CoA desaturase, ELOVL6: enzyme for the elongation of very long chain fatty acids
Conclusions

Enriched in saturated FA; upregulation of genes involved in FA synthesis and elongation. Lower enrichment of MUFA

Enriched in MUFA; upregulation of SCD gene expression

Work ongoing…..
Present

Hepatic fatty acid partitioning and body fat distribution in women – with Dr Leanne Hodson

Future

Senior Research Fellow
Postgraduate Medical School

Margot Umpleby Diabetes & Metabolic Medicine
Acknowledgements

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BMSS

MRC CORD