INFLUENCE OF EARLY NUTRITION ON METABOLIC PROCESSES

Investigated by targeted LC/MS based metabolomics
Christian Hellmuth
02/04/2014
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METABOLOMICS

FUNCTIONAL INSIGHTS IN MOLECULAR CHANGES

Genotype → Transcriptomics → Proteomics → Genomics

Phenotype

Metabolomics
METABOLOMICS?

- Determination of Metabolites

- Metabolites
  - Small molecules (<1000 Dalton)
  - Substrates, intermediates and products of biological processes

- Metabolome
  - Complete set of metabolites found within a biological sample

- Understanding of biochemical changes & differences
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BENEFITS OF THE NEXT „OMICS” FIELD

- Dynamic response of living systems to stimuli and genetics¹

- Information on what is actual happening²

BENEFITS OF THE NEXT „OMICS“ THING

- Insights into underlying molecular pathology\(^1\)
- Definition of biomarkers in biofluids\(^1\)
- Is the interplay between functional subunits changed by nutritional changes?
- Can we gain new functional insights?

\(^1\) Heazell, Dune et al. Placenta. 2011 Mar;32 Suppl 2:S119-24
HOW TO ANALYSE THE METABOLOME?

First: THINK
BEFORE I DETERMINE THE METABOLOME!

- What do I want to know?
  - Background of my clinical trial
  - Hypothesis for metabolic differences/response

- What do I have?
  - Sample number, material, volume
  - Sampling conditions

- Which of the possible analytical strategies are available?
  - Targeted vs. untargeted metabolomics
  - Analytical equipment – GC, LC, MS, NMR
EARLY PROGRAMMING EFFECTS REFLECTED IN THE METABOLOME

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EARLY PROGRAMMING

Genotype

Conception

- Maternal nutrition
- Maternal stress
- Placental development
- Placental transfer

Birth

- Infant nutrition

6 Month

Later Life

- Obesity
- Diabetes
- CVD
- Allergy
- Cognition
- Behaviour
- ...

Environment and Nutrition

Programmings

Metabolomics

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DIVISION OF METABOLIC AND NUTRITIONAL MEDICINE
DR. VON HAUNER CHILDREN’S HOSPITAL
EARLY PROTEIN HYPOTHESIS

Protein Intake → Metabolomics → Insulin, IGF1 levels

Metabolomics → Insulinogenic amino acids

Metabolomics → Changes in energetic efficiency

Weight gain 0-24 month → Metabolomics → Changes in fat oxidation - acylcarnitines

Metabolomics → Changes in lipid profile - phospholipids

Adipogenic activity → Later obesity risk

EUROPEAN CHILDHOOD OBESITY PROJECT

**Intervention groups**
- Lower protein formula (LP)
- Higher protein formula (HP)

**Observational group**
- Breastfed (BF)

**Randomization**
- Blood sampling at 6 month of age

- 263
- 265
- 163

Reference:
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**CHOP – WEIGHT GAIN**


EARLY PROTEIN HYPOTHESIS

Higher Protein Intake ✔

Elevated Insulin, IGF1 levels ✔

Increased weight gain 0-24 month ✔

EUROPEAN CHILDHOOD OBESITY PROJECT

- Amino acids determined by LC-MS/MS\(^1\)
- Polar lipids and glucose analysis by FIA-MS/MS\(^2\)

- 21 Amino acids (AA)
- 41 Acylcarnitines (Carn.a)
- 14 Sphingomyelins (SM)
- 76 Phosphatidylcholins (PC)
- 15 Lysosphatidylcholins (LPC)

\(^1\) Harder, Peissner et al. J Chromatogr B. 2011 Mar 1;879(7-8):495-504.
\(^2\) Absolute IDQ p 150 kit
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CHOP – HP vs. LP

Branched-chain amino acids

<table>
<thead>
<tr>
<th>log$_{10}$(P)</th>
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<tbody>
<tr>
<td>60</td>
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<td>40</td>
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</table>

Mean LP < Mean HP

Mean LP > Mean HP

- Essential AA
- BCAA
- Nonessential AA
- Free Carnitine
- Short Acylcarne
- Medium Acylcarne
- Long Acylcarne
BRANCHED-CHAIN AMINO ACIDS (BCAA)

- Leucine, Isoleucine, Valine
- Elevated in HP group
- Dietary BCAA escape first-pass liver metabolism
- Oxidized in skeletal muscle
FIRST PASS METABOLISM

Dietary protein

Amino acids
20% BCAA

GIT

Portal vein

Liver

Amino acid metabolism

Systemic circulation

Skeletal muscle

BCAA Oxidation

Amino acids > 50% BCAA

BRANCHED-CHAIN AMINO ACIDS (BCAA)

- Leucine, Isoleucine, Valine
- Elevated in HP group
- Dietary BCAA escape first-pass liver metabolism
- Oxidized in skeletal muscle
BCAA OXIDATION

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CHOP – LP VS. HP

Branched-chain amino acids

Short-chain acylcarnitines

Mean LP < Mean HP

Mean LP > Mean HP
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BCAA OXIDATION

Valine
\[ \text{Val} \rightarrow 5.0.\text{oxo} \]

Leucine
\[ \text{Leu} \rightarrow 6.0.\text{oxo} \]

Isoleucine
\[ \text{Ile} \rightarrow 6.0.\text{oxo} \]

BCKD

Rate limiting

4.0

4.1

4.0.\text{OH}

4.0.\text{oxo}

NAD\(^+\)

3.0

NADH/H\(^+\)

5.0

5.1

5.0.\text{OH}

5.0.\text{oxo}

3.0 2.0


Non-hepatic

BCKD

Adipose Tissue

Heart

Intestine

Kidney

Liver

Skeletal Muscle

Brain

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LEUCINE OXIDATION

- Physiological level - linear stimulation of degradation pathway
  - Regulation of BCAA level
- Excess BCAA ➔ BCAA exceed degradation pathway
BCAA EFFECTS

- Hydrophobic substrates for proteins\(^1\)
- Stimulation of insulin secretion\(^2\)
- Inhibition of glucagon secretion\(^2\)
- Reducing protein breakdown\(^2\)
- Enhancing protein synthesis (mTOR)\(^3\)
- Brain-uptake of glutamate\(^1\)

1 Brosnan, Brosnan J Nutr. 2006 Jan;136(1 Suppl):207S-11S
IMPACT OF BCAA ON FAT METABOLISM

- Leucine slows-down beta-oxidation\(^1,2\)
- Leucine decreases lipolysis\(^2\)
- Leucine increases lipogenesis\(^2\)

- Leucine, Isoleucin and Valin deprivation reduce fat mass\(^3\)

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BETA-OXIDATION

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<td>Carn C18:1</td>
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Even-chain acylcarnitines

Key:
- Essential AA
- BCAA
- Nonessential AA
- Free Carnitine
- Short Acylcarne
- Medium Acylcarne
- Long Acylcarne

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EARLY PROTEIN HYPOTHESIS

Protein Intake ✔

Elevation of insulinogenic amino acids (BCAA) ✔

Insulin, IGF1 levels ✔

Reduction of beta-oxidation substrates (long-chain acylcarnitines) ✔

Weight gain 0-24 month ✔

Adipogenic activity

TAKE HOME MESSAGE

- Metabolomics provides insights into pathways and metabolic changes
- Higher Protein intake results especially in elevated BCAA levels
- Excess BCAA levels overcome BCAA degradation
- BCAA may effect beta-oxidation and fat storage
- Reduced long-chain acylcarnitines reflect reduced beta-oxidation
THANK YOU FOR YOUR ATTENTION

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www.project-earlynutrition.eu