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METABOLIC PROFILING OF BLOOD PLASMA IN THE FIELDS OF EARLY NUTRITION

The Power of Programming 2014 - New Investigator Forum

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1. METABOLIC PROFILING

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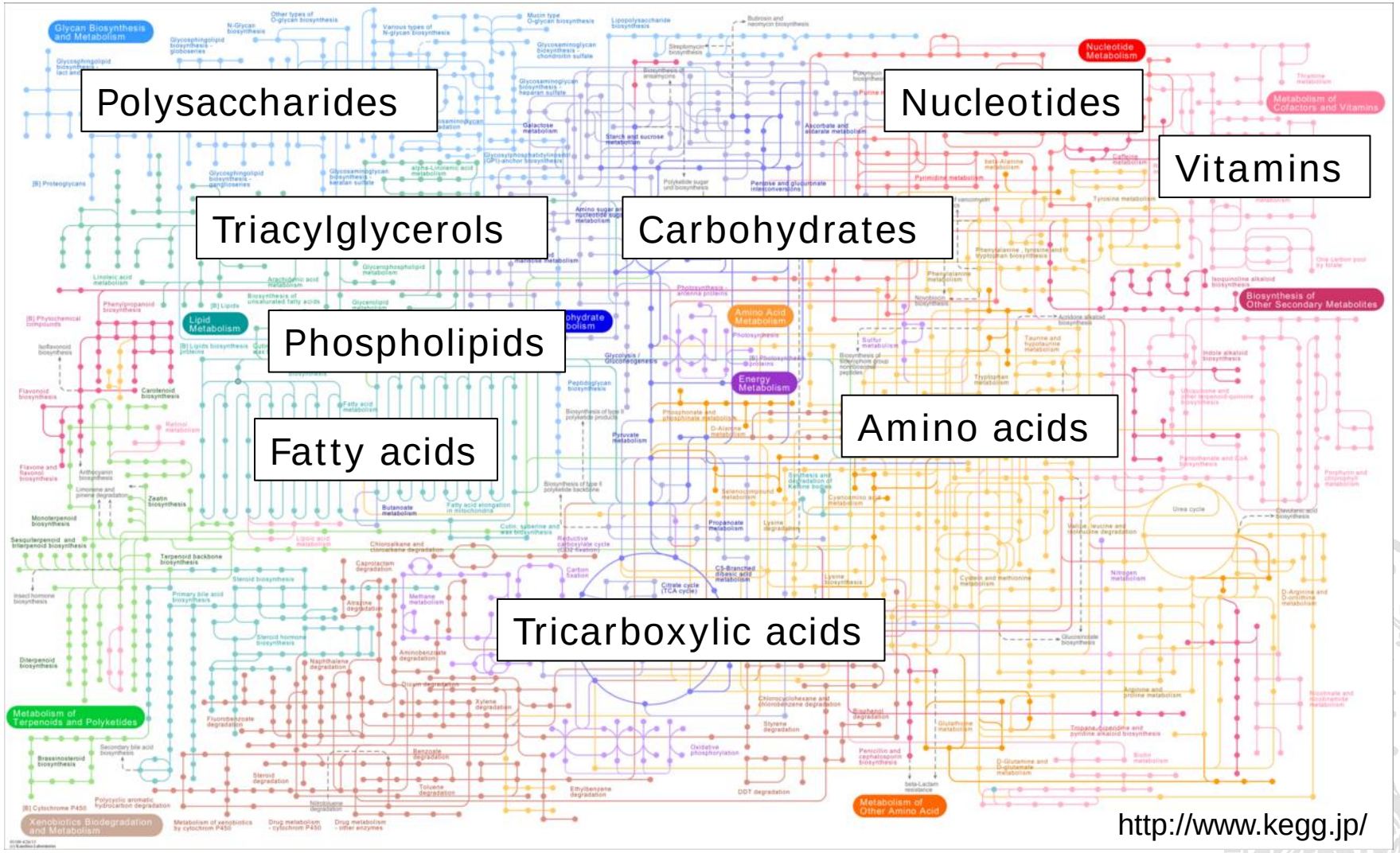
- Analysis of low molecular weight (<1 kDa) intermediates of a biological system[1]
- High variable metabolic pattern between individuals, but relatively constant for a given individual[2]
- Changes in specific metabolic patterns reflect changes in pathways and processes

[1] Oliver, Trends Biotechnol, 1998
[2] Williams, University of texas, 1951

1. METABOLIC PROFILING



2. SAMPLE MATRIX

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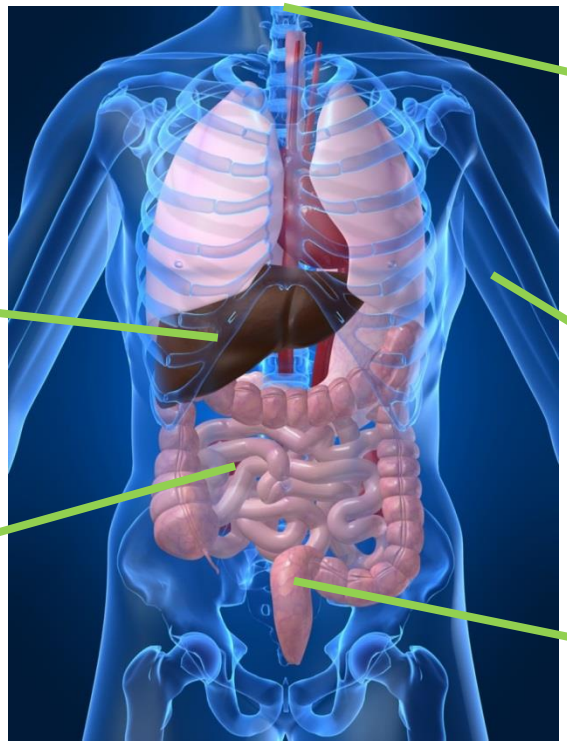


Mouth:
Enzymatic cleavage
→Saliva (water soluble metabolites only)

Liver:
Lipid Synthesis
Gluconeogenesis
→Biopsy

Muscles:
Lipid oxidation
Protein metabolism
→Biopsy

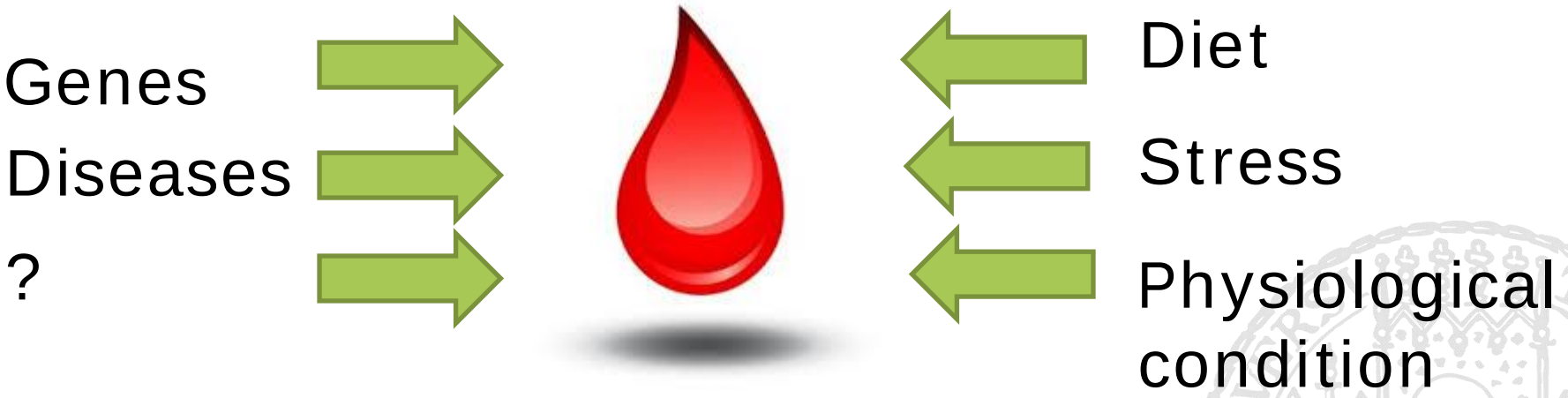
Kidney:
Excretion
→Urine (water soluble metabolites only)

Intestine:
Digestion, Absorption
→Biopsy

Blood: -Most appreciated
-Covers all kinds of metabolites
-Agreeable invasive sampling
→Biomarker determination

2. SAMPLE MATRIX

Effects on the current metabolic blood plasma profile of a living organism:



3. TECHNIQUES

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Untargeted Metabolomics

- No specific hypothesis necessary
- Up to 80,000 peaks with about 40,000 stoichiometric formulas per sample with ICR-FT/MS[1]
- Common techniques:
  - High-resolution mass spectrometry (e.g. Ion Cyclotron Resonance Fourier Transform Mass Spectrometry, Time-of-Flight Mass Spectrometry)
  - Nuclear magnetic resonance spectroscopy (^{1}H NMR)

- Advantages:
  - Description of the whole complexity of a biological system →fingerprint
  - Widest possible range of compounds (also unknowns or unexpected structures)

- Disadvantages:
  - Only relative changes possible
  - High risk of contamination →elaborate and expensive sample handling (e.g. high-purity solvents)
  - Very complex data handling →unknown metabolites need further structural clarification
  - No single technology available to analyse the entire metabolome

[1] Forcisi, J Chrom A, 2013

3. TECHNIQUES

Targeted Metabolomics

- Used to answer questions about specific metabolic pathways
- Based on specific hypothesis
- Common technique:
  - Triple quadrupol mass spectrometry (MS/MS)

- Advantages:
  - Absolute quantification
  - Sensitive (about 1 µmol/L)
  - Specific (molecular fragmentation)

- Disadvantages:
  - Number of metabolites is limited (predefined metabolites)
  - Internal (isotopic) standards necessary

3. TECHNIQUES

In-house LC-MS/MS based targeted methods:

- Polar lipid species
  - Peissner, under preparation
- Amino acids
  - Harder, *J Chromatogr B* 2011. 879(7-8): p.495-504
- Non-esterified fatty acids
  - Hellmuth, *Anal Chem* 2012. 84(3): p.1483-90
- Glycerophospholipid species
  - Uhl, *J Chromatogr B* 2011. **879**(30): p. 3556-64
- Sulfur containing amino acids
  - Hellmuth, *J Chromatogr B* 2011. **879**(1):83-9
- Creatinine
  - Niesser, *Ann Nutr Metab* 2012. 61(4):314-21
- Fatty acid determination by gas chromatography
  - Glaser, *PLoS One* 2010. **5**(8):e12045
- Vitamin A & E

3. TECHNIQUES

Data handling

- Detection of outliers:

  → graphical methods, statistical tests, leverage values

- Test for normal distribution

- Linkage to clinical data sets:

  →anthropometry, cognitive function, diet, epigenetics…

- Multivariate statistics:

  →PCA, PLS, random forrest, …

4. EARLY NUTRITION

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- Pre-pregnancy status
  - BMI
  - Diabetes
- PREOBE, Raine

- Pregnancy
  - Maternal malnutrition
  - Impaired placental transfer
- Baboon animal model NiGO-Health, LISA, PREOBE, UCI cohort

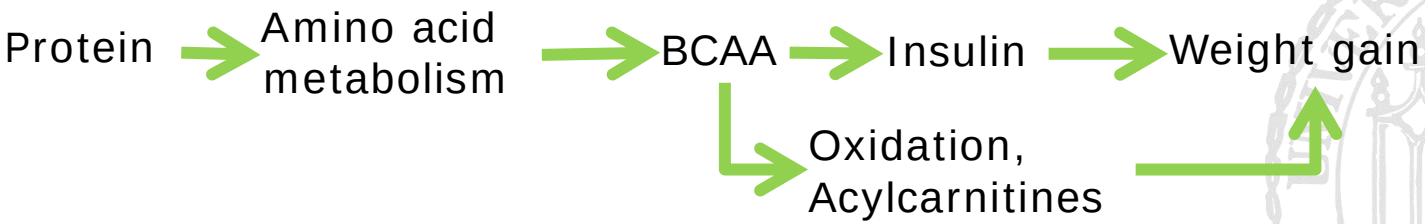
- Infant feeding
  - Duration of breastfeeding
  - Breast milk composition
  - Infant formula composition
- CHOP, Raine, Prevent CD, HUMIS, ProtEUs

4. EARLY NUTRITION

CHOP

- Randomised infant feeding trial
- Different protein contents of infant formulas
- Blood plasma samples at 0.5, 5.5, 8 and 11 years of age
- Metabolic profile:
  - Amino acids, polar lipid species, sulphur containing amino acids

Early protein hypothesis:



THANK YOU FOR YOUR ATTENTION

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