EARLY NUTRITION, METABOLIC EFFECTS AND LONG-TERM HEALTH

Olaf Uhl
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EARLY PROGRAMMING
OBESITY PANDEMIC

- Increase of mean BMI from 1975 to 2014:
  - 21.7 to 24.2 kg/m² in men
  - 22.1 to 24.4 kg/m² in women
- Prevalence of obesity reached 10.8% in men and 14.9% in women in 2014
- In 2014 more obese than underweight
- Diabetes caused 1.5 million deaths in 2012
- additional 2.2 million deaths by cardiovascular and other diseases

Ng et al., Lancet, 2016, 387(10026):1377-96.
METABOLIC PROFILING

Genotype → Phenotype

Genomics → Epigenetics → Proteomics → Metabolomics

Genes → Diet → Physiological condition → Stress → Exposure

Carbohydrates → Triacylglycerols → Phospholipids → Fatty acids → Amino acids

Diseases

http://www.kegg.jp/
EARLY PROGRAMMING HYPOTHESES

Fetal overnutrition:
e.g. maternal obesity, high pregnancy weight gain, diet in pregnancy, and gestational diabetes

Environment
Lifestyle
Genes

Postnatal overnutrition:
e.g. overfeeding, short breastfeeding, and excessive protein supply

Adiposity/diabetes
Visceral adiposity, metabolic syndrome, insulin resistance, hypertension, coronary heart disease, stroke, and asthma

Fuel-mediated in utero hypothesis
Mismatch hypothesis

Fetal undernutrition and postnatal overnutrition:
e.g. maternal malnutrition and placental dysfunction

Accelerated postnatal growth hypothesis

CLINICAL TARGETED METABOLOMICS
CLINICAL TARGETED METABOLOMICS

- Metabolomics = determination of metabolites:
  - substrates, intermediates or products of biological processes
  - small molecules (<1.5 KDa)
- Metabolome = Complete set of metabolites within a biological sample

Untargeted Metabolomics
- Registers all ions within a certain mass range

Targeted Metabolomics
- Measure of ions from known metabolites
  - Absolute quantification (µmol/L)
  - Specific:
    - Chromatographic separation
    - Molecular fragmentation
  - Sensitive
CLINICAL TARGETED METABOLOMICS

Phospholipids → Lipid intake

Non-esterified fatty acids → Adipose tissue

Acylcarnitines → β-Oxidation → Amino acid oxidation

Amino acids → Proteins

Organic acids → Citric acid cycle
SAMPLE PREPARATION

50µL Plasma

+450 µL Methanol (IS)

PL
NEFA
TCA
AA
CARN
CLINICAL TARGETED METABOLOMICS

- Phospholipids (PL)
- Acylcarnitines (CARN)
- Nonesterified fatty acids (NEFA)
- Tricarboxylic acids (TCA)

4000 QTrap

API 2000

- Amino Acids (AA)
TRIPLE QUADRUPOLE MASS SPECTROMETRY

Nitrogen Collision Cell

1 mass transition
CHROMATOGRAPHIC SEPERATION

Leucine
- 131 g/mol
- Fragment: 86 g/mol

Isoleucine
- 131 g/mol
- Fragment: 86 g/mol
QUALITY CONTROL SAMPLES

- Monitor the performance of a method
- Providing information about analytical precision

Best choice:
1. Pool of all samples
2. Surrogate, comparable to samples
   - Matrix (e.g. liver QC for liver samples)
   - Amount (adult plasma QC for adult plasma)
3. QC for internal analytical control, without any meaning to samples

--> Measurement of QC aliquots throughout the analytical measurement: 6 aliquots per 81 samples = Batch
PRECISION

Inter-batch Precision → precision with time over all batches

Intra-batch Precision → precision within one batch
RESULTS FROM COHORT STUDIES AND INTERVENTIONAL TRIALS
RESULTS FROM COHORT STUDIES AND INTERVENTIONAL TRIALS
UCI COHORT

Accepted Article Preview: Published ahead of advance online publication

Association of maternal pre-pregnancy BMI with metabolomic profile across gestation

C Hellmuth, K L Lindsay, O Uhl, C Buss, P D Wadhwa, B Koletzko, S Entringer
UCI COHORT

- Pregnant women were recruited at the University of California Irvine (UCI)

- Plasma samples of 160 pregnant women at:
  - 10 - 12 weeks of gestation
  - 20 - 22 weeks of gestation
  - 30 - 31 weeks of gestation

- Influence of maternal metabolism by:
  - Stress, ethnicity, pre-pregnancy BMI, diet, gestational weight gain

Hellmuth et al., Int J Obesity, accepted
UCI COHORT

- No significane association of AHEI-P index (quality) and total energy intake (quantity)
- Pre-pregnancy BMI ~ NEFA in trimester 2
- Pre-pregnancy BMI ~ DGLA species:
  - LPC 20:3
  - NEFA 20:3
  - PC(30:3)
  - PC(32:3)
  - PC(38:3)
- weak associations between maternal metabolites and birthweight (NEFA+, LPC-)

Hellmuth et al., Int J Obesity, accepted
Effects of obesity and gestational diabetes mellitus on placental phospholipids

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\textsuperscript{e}Department of Pediatrics, School of Medicine, University of Granada, Granada, Spain
PREOBE

• Pregnant women were recruited at the 20th week of gestation at the University of Granada

• subjects were divided in different groups:
  • Controls: BMI < 25 kg/m², normal OGTT
  • Obese: BMI > 30 kg/m², normal OGTT
  • GDM: BMI < 25 kg/m², abnormal OGTT

• Placenta tissue was analyzed for phospholipids from 63 subjects
GPL PATTERN OF GDM AND OBESE IN COMPARISON TO CONTROLS

Uhl et al., Diab Res Clin Prac, 2015
Dietary Protein Intake Affects Amino Acid and Acylcarnitine Metabolism in Infants Aged 6 Months


Division of Metabolic and Nutritional Medicine (F.F.K., U.H., M.W., V.G., H.D., W.P., P.R., B.K., C.H.), Dr von Hauner Children’s Hospital, 80337 Munich, Germany; Centre Hospitalier Chrétien St Vincent (A.X.), 4000 Liège-Rocourt, Belgium; Department of Paediatrics (C.C.), University Children’s Hospital Queen Fabiola, Université Libre de Bruxelles, 1020 Brussels, Belgium; Paediatrics Research Unit (N.F., J.E.), Universitat Rovira i Virgili, 43201 Reus, Spain; Department of Paediatrics (E.V.), San Paolo Hospital, University of Milan, 20142 Milano, Italy; and Neonatal Intensive Care Unit (P.S., D.G.), Children’s Memorial Health Institute, 04-736 Warsaw, Poland
European Childhood Obesity Project
5 Countries: Belgium, Germany, Italy, Poland, and Spain

Randomized formula groups

Birth
8 wk
6 mo

Higher Protein

Lower Protein

Observational Group
Breastfed

Kirchberg et al., J Clin Endocrinol Metab, 2015
- Higher protein content is associated with higher weight
- Difference in BMI up to the age of 6 years
- ↑Protein → ↑Insulin/ ↑IGF-I → ↑Adipogenic activity?
P values from univariate linear mixed models with random intercept for study center

Kirchberg et al., J Clin Endocrinol Metab, 2015
Lipidomics reveals associations of phospholipids with obesity and insulin resistance in young adults

Sebastian Rauschert 1, Olaf Uhl 1, Berthold Koletzko 1, Franca Kirchberg 1, Trevor A. Mori 2, Rae-Chi Huang 3, Lawrence J. Bellin 2, Christian Hellmuth 1*, Wendy H. Oddy 3*

1 Ludwig Maximilians University of Munich, Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children’s Hospital, Munich, Germany; 2 School of Medicine and Pharmacology, Royal Perth Hospital Unit, The University of Western Australia, Perth, Western Australia, Australia 6000; 3 Telethon Kids Institute, The University of Western Australia, Perth, Western Australia, Australia 6000
RAINE COHORT

The Western Australian Pregnancy Cohort (RAINE) Study
• Prospective longitudinal pregnancy cohort study to examine effects of multiple ultrasound imaging
• Started 1989 → ongoing
• 1172 Blood samples of the 20y follow up
• Metabolites determined:
  • Lyso-Phospholipids
  • Phospholipids
  • Sphingomyelins
  • Non-Esterified fatty acids
Multiple linear regression model
Outcome: HOMA-IR, Waist Circumference
Confounder: HDL-cholesterol, LDL-cholesterol, smoking, alcohol consumption, dietary patterns, physical and sedentary behavior, biological sex

Associations with insulin resistance:

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<tr>
<th>Analyte</th>
<th>Standardized Estimate</th>
<th>Bonferroni CI</th>
<th>Bonferroni p-value</th>
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P values of multiple regression model for waist circumference

Rauschert et al., J Clin Endocrinol Metab. 2016
ONGOING PROJECTS
ONGOING PROJECTS

Challenge of human studies:

- Individual study design
- Various primary outcomes
- Different meta-data

Influences

- Raine: WC ~ SM, PC(PUFA), 1/LPC
- IR ~ LPC(14:0)
- CHOP: HP → BCAA, Carn C3, C4, C5
- PREOBE: Ob = GDM ~ DHA, AA, 1/20:3
- UCI: pBMI ~ NEFA, 20:3

Physiological outcomes

- CHOP: rapid growth ~ LPC(14:0)
ONGOING PROJECTS

- Baboon animal model
  - CHOP
  - GINI/LISA
  - Ulm
- Prevent CD
- UCI
- Rolo
- UPBEAT
- RAIN
- BeMiM
- Obeldicks
- NiGo Health
- Proteus
- GenR
THANKS FOR YOUR ATTENTION