New technologies to maximise output from nutrition studies

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UCD, Belfield, Dublin 4, Ireland

www.ucd.ie/foodandhealth
Disclosure Slide

CEO- NuGO- The Nutrigenomics Organisation

Secretary- Irish Section Nutrition Society
Outline

- Nutrigenomics
- Metabolomics - Food Intake/ Mechanistic
- Individual responses
- TakeAways
Areas where nutrigenomics can help

- Study compliance
- Surrogate endpoints
- Mechanisms
- Inter-individual responses
- Personalised Nutrition
1. Biomarkers of food intake

2. Metabolic phenotyping / Precision Nutrition

3. Alterations following interventions

4. Study of diet related diseases
Metabolomics in nutrition

- Food Intake
  - Exogenous metabolome
  - Food Intake Biomarkers
  - Metabolic Phenotype
  - Altered pathways
  - Endogenous metabolome
Background

Food Intake Biomarkers
The need for Dietary Biomarkers

Limitations:

• Measurements over short periods of time may be unrepresentative
• Recall of eating behaviour can be difficult
• Errors in reporting intakes
• Recording process alters dietary habits
• Difficult to validate

Food diaries, FFQ, 24 hr recalls
**Approach**

- Controlled Interventions
  - Exact intakes

- Response
  - Calibration curves

- Determination of intake
  - Independent studies

**metabolomics**
Food Intake Biomarkers - Study 1

- Identify biomarkers of legume intake
- Confirmation of biomarkers
Legume biomarker discovery

Foods, sample collection and analysis

TEST FOOD A
Carrots 141g

TEST FOOD B
Peas 138g

N=11

CONTROL FOOD A
Turnips 141g

CONTROL FOOD B
Couscous 138g

Feature extraction and alignment

Feature finding

84161 (-ve) 73734 (+ve)

Mass profiler

1004 (-ve) 819 (+ve)

SIMCA/Metaboanalyst

Statistical analysis

Peas F vs 4h

R²X, 0.41; Q², 0.4
**Excretion kinetics**

Accurate mass (MS1 scan)

LC-MS/MS

**Markers identified**

2-Isopropylmalic acid (Level 1)

Asparaginyl valine (level 2)

N-Carbamoyl-2-amino-2-(4-hydroxyphenyl)acetic acid (level 2)
How do we use the biomarkers?
- Measure of adherence to the dietary intervention
- Objective measures of dietary intake
- Relationships with health parameters
Determine biomarker concentration → Determine food intake g/day
Food Intake Biomarkers - Study 2

Fasting & 24 hr samples → Metabolomic analysis → Proline betaine quantification

Estimation of dietary intakes

Proline Betaine (mmol/L) vs. Orange Intake (grams)

Proline Betaine (mmol/L)

0 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00

0 50 100 150 200 250 300 350 400 450 500 550 600

Orange Intake (grams)
Comparison to self-reported intake

4 day semi-weighed food diaries

Biomarker Data

Calibration curves

Estimate intake – g/day

Habitual intake – g/day
Calibration curves

NANS

Biomarker calculated citrus intake

Reported citrus intake
Validation; NANS

565 NANS Participants

Reported Citrus Intake

Proline Betaine Quantification

Predicted Citrus Intake (g/day)
Dose response

• Controlled intervention

• 2/3 data – to develop calibration curves

• 1/3 data – comparison of estimated intake with actual intake
# Orange juice intake prediction (g)

<table>
<thead>
<tr>
<th>NutriTech ID</th>
<th>Week</th>
<th>Actual Orange juice intake (g)</th>
<th>Orange juice intake predicted (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT018</td>
<td>1</td>
<td>250</td>
<td>237</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>220</td>
<td>287</td>
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<tr>
<td></td>
<td>3</td>
<td>50</td>
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<tr>
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<td>279</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>50</td>
<td>76</td>
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</table>
## Association between actual & predicted intakes

<table>
<thead>
<tr>
<th>Predicted orange juice intake</th>
<th>Actual orange juice intake (r)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h urine samples (normalized to osmolality)</td>
<td>0.859</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting urine samples (normalized to osmolality)</td>
<td>0.919</td>
<td>&lt;0.001</td>
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</tbody>
</table>
Prediction of citrus intakes in population study

Proline Betaine Quantification in fasting urine → Predicted Citrus Intake (g/day)
<table>
<thead>
<tr>
<th>NANS ID</th>
<th>Recorded Citrus Intake (g/day)</th>
<th>Predicted Citrus Intake (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>239</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>271</td>
<td>0</td>
<td>0</td>
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<tr>
<td>270</td>
<td>85</td>
<td>86</td>
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<td>304</td>
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<td>593</td>
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<td>714</td>
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<td>24</td>
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<td>667</td>
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<td>212</td>
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<td>1953</td>
<td>72</td>
<td>81</td>
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<tr>
<td>2552</td>
<td>130</td>
<td>129</td>
</tr>
</tbody>
</table>
Agreement; reported & predicted intakes
Study Design

- Incorporation of biomarkers into study design
  - Checking and monitoring adherence

- Ex: Predimed: urinary hydroxytyrosol, the main phenolic compound in extra-virgin olive oil
- Plasma alpha-linolenic acid - as a measure of adherence to walnut consumption
Exploring the Links between Diet and Health in an Irish Cohort: A Lipidomic Approach

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Mechanistic work

intervention

Metabolomics
Proteomics
Transcriptomics
Epigenetics

Metabolomics
Proteomics
Transcriptomics
Epigenetics
Mechanistic work

One-carbon metabolism and related pathways
Individual variability

- Estimated that only 40% of individuals respond to an intervention
- High variability within responses
- Need new approaches to capture this
Role of dairy proteins in glycaemic management

- Dairy proteins
- Individual response
- Predictive response
Randomised crossover study examining the effects of protein drinks on glycaemic control:

- Intact protein
- Casein hydrolysate A
- Casein hydrolysate B

- The protein drink was consumed twice daily for three days, directly before ingestion of study breakfast and evening meals

- 20 participants, aged 40-65y, BMI 25-35 kg/m², free of prescription medication
FreeStyle Libre Glucose Monitor

- Participants wore monitor for 14 days
- Glucose reading every 15 minutes

Drink 1

Drink 2

Drink 3
Response at breakfast

![Graph showing glucose response at breakfast](image)

- Intact sodium caseinate
- Casein hydrolysate A
- Casein hydrolysate B
Individual level analysis

Individual response to the same meals

Study design allowed N-of-1 analysis
Individual level analysis

- Only 3 individuals would benefit from drink A
- Only 3 individuals would benefit from drink B
- Need a predictive model to identify those that would benefit
<table>
<thead>
<tr>
<th></th>
<th>Responders</th>
<th>Non-responders</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.39 ± 0.34</td>
<td>5.31 ± 0.84</td>
<td>0.039</td>
</tr>
<tr>
<td>Serine (nmol/mL)</td>
<td>83.87 ± 10.08</td>
<td>112.12 ± 22.58</td>
<td>0.039</td>
</tr>
<tr>
<td>SM C16:0 (µM)</td>
<td>106.67 ± 7.77</td>
<td>124.93 ± 11.71</td>
<td>0.017</td>
</tr>
<tr>
<td>PC aa C30:1 (µM)</td>
<td>36.8 ± 2.76</td>
<td>42.42 ± 3.86</td>
<td>0.038</td>
</tr>
<tr>
<td>PC aa C30:2 (µM)</td>
<td>2.21 ± 0.10</td>
<td>3.18 ± 0.69</td>
<td>0.002</td>
</tr>
<tr>
<td>N-C12:0(OH) Cer (µM)</td>
<td>0.008 ± 0.003</td>
<td>0.005 ± 0.002</td>
<td>0.039</td>
</tr>
<tr>
<td>N-C28:0 Cer (µM)</td>
<td>0.029 ± 0.001</td>
<td>0.031 ± 0.001</td>
<td>0.010</td>
</tr>
</tbody>
</table>
## Individual Responses
Responses highly reproducible within an individual

<table>
<thead>
<tr>
<th></th>
<th>ICC</th>
<th>95% CI</th>
<th>RI(^1)</th>
<th>Intra-CV (%)(^2)</th>
<th>Inter-CV (%)(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakfast</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact caseinate</td>
<td>0.892</td>
<td>0.782 - 0.954</td>
<td>0.997</td>
<td>5.66 ± 3.51</td>
<td>19.41 ± 0.49</td>
</tr>
<tr>
<td>Casein hydrolysate A</td>
<td>0.804</td>
<td>0.614 - 0.919</td>
<td>0.992</td>
<td>5.20 ± 4.41</td>
<td>14.82 ± 1.50</td>
</tr>
<tr>
<td>Casein hydrolysate B</td>
<td>0.764</td>
<td>0.548 - 0.901</td>
<td>0.990</td>
<td>7.65 ± 5.46</td>
<td>17.70 ± 3.82</td>
</tr>
</tbody>
</table>

1. RI: Reliability Index
2. CV: Coefficient of Variation
- Very reproducible responses within an individual
- High variability between individuals
- Need to consider individual responses- multiple measurements can facilitate analysis
Key TakeAways

- Incorporation of omics into study design can enhance studies
- Mechanistic information
- Compliance/adherence- biomarkers
- Precision Nutrition- capturing the individual response
Nutrition, Biomarkers and Health team
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Past members
All past members

Collaborators
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NANS: A Flynn, M Gibney, A Nugent, B McNulty, J Walton
FoodBall Partners
Food4me Partners

http://www.ucd.ie/nutrimarkers