Biomarkers of glucose metabolism in human plasma and saliva

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Biomarkers are an ancestral domain of clinical laboratories, being analysed either as
• disease markers (e.g. PSA for prostate cancer)
• therapy control markers (e.g. 17OHP for congenital adrenal hyperplasia)

Analysis occurs (mostly) with commercial kits, being at the most qualified, but rarely validated

Evaluation of results is based on in-house reference values (based on literature, sometimes expanded by in-house collection and analysis)

Evaluation of method is based on inter-laboratory / round-robin tests

In regulated bioanalysis, biomarkers need to be addressed differently:
• Method development
• Method qualification
• Method validation
Scope of the study

• Standardize the assessment of biomarkers with regards to study set-up, sample collection, treatment and storage

• Optimize the pre-analytical conditions for very labile parameters

• Combine a set of known markers in a well described physiological setting: Insulin, C-Peptide, Amylin, Glucagon, Leptin, Resistin, Adiponectin, Ghrelin, Cortisol during Oral Glucose Tolerance test

• The aim was not to deliver new scientific data, but to evaluate what parameters need to/can be standardized
Preanalytical precautions
Impact of deviations from standard procedure

What happens if....

.... protease inhibitors are not added for unstable parameters

NOTE: Sample collected at $t_0$, aliquots frozen (LN$_2$) every 15 minutes

Impact of deviations from standard procedure

What happens if….

.... temperature sensitive analytes are handled too careless

**Sample preparation on ice**

Glucagon at 5.09

**Sample preparation at room temperature**

Glucagon at 5.09

**Effect of temperature:**
Glucagon response slightly decreases
Increased background noise and increase of the peak eluting at 5.19 min, making a good integration, quantification of the sample more difficult at room temperature

*Dan B, Celerion, unpublished data 2017*
Impact of deviations from standard procedure

What happens if…. acidification is not applied for pH-sensitive parameters

NOTE: Sample collected at \( t_0 \), aliquots frozen (LN\(_2\)) every 15 minutes

\[ \text{Influence of acidification on acylated ghrelin} \]

Impact of deviations from standard procedure

What happens if....

... salivary collection device causes loss by adsorption of the analyte

Impact of deviations from standard procedure

What happens if....

.... the stimulus is not provided as planned

Influence of stimulus ingestion
once full load (t₀) vs twice half load (t₀ and t₁₅)

Insulin IU/mL

Single ingestion  Staggered ingestion

0  15  30  45  60  75  90  105  120

0  10  20  30  40  50  60  70

min
Study design
Study setup

Biomarkers of interest and methodology

**ELISA:** Insulin, C-Peptide, Amylin, Leptin, Adiponectin, Resistin  
**LCMS:** Ghrelin, Glucagon, Cortisol  
**Enzymatic:** Glucose

**Stimulus** ACCU-CHEK Dextrose 300 mL (75 g Glucose)

**Collection devices**  
- Permanent catheter B.Braun Vasofix Safety  
- P800 Blood Collection System for Plasma Metabolic Biomarker Preservation (BD)  
- Salivettes (Sarstedt)

12 healthy volunteers (m/f; 22-30 y)
Sample collection and storage

stimulus

t0 – t15 – t30 – t45 – t60 – t75 – t90 – t105 – t120
Sample collection and storage

500 µL
Split 1 acidified
Ghrelin
Split 1 acidified
500 µL

500 µL
Split 2 acidified
Glucagon
Split 2 acidified
500 µL

500 µL
Split 3 normal
Insulin, C-Peptide, Amylin
Split 3 normal
500 µL

500 µL
Split 4 normal
Leptin, Resistin, Adiponectin
Split 4 normal
500 µL

500 µL
Split 5 normal
Cortisol
Split 5 normal
200 µL

- 80°C until analysis
Study results
Response of pancreatic peptides to OGT

all values as mean ± SD, related to t0 = 100%
Response of adipocytic peptides to OGT

all values as mean ± SD, related to t0 = 100%
Response of gastric and adrenal hormones to OGT

call values as mean ± SD, related to t0 = 100%
Conclusions
A strict standardisation of

- Stimulus (amount and administration)
- sample collection (collection device, protease inhibitors, acidification)
- sample aliquoting (aliquot volume)
- sample treatment (temperature)

is mandatory to deliver robust and meaningful data in biomarker studies

Saliva is suitable for the non-invasive assessment of most biomarkers
Thank you for your attention