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Translating Science to Medicine

Biomarkers of Glucose Metabolism in Human Plasma and Saliva

Dr. Michael Gröschl

A Few General Considerations

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

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Scope of the Study

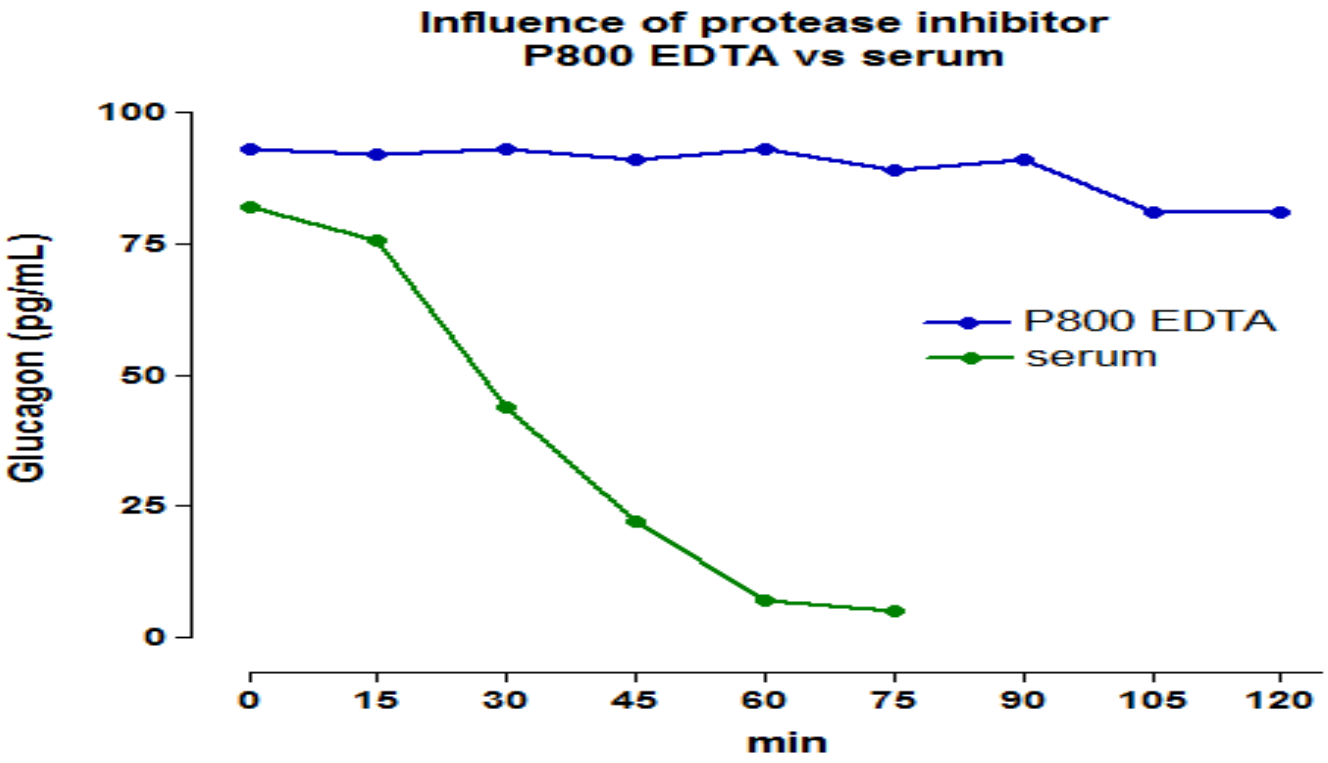
- Standardize the assessment of biomarkers with regards to study set-up, sample collection, treatment and storage
- Optimize the pre-analytical conditions for very labile parameters
- Combine a set of known markers in a well described physiolgical setting: Insulin, C-Peptide, Amylin, Glucagon, Leptin, Resistin, Adiponectin, Ghrelin, Cortisol during **O**ral **G**lucose **T**olerance 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

Yi J, Warunek D, Craft D PLoS One 29;10(7) 2015

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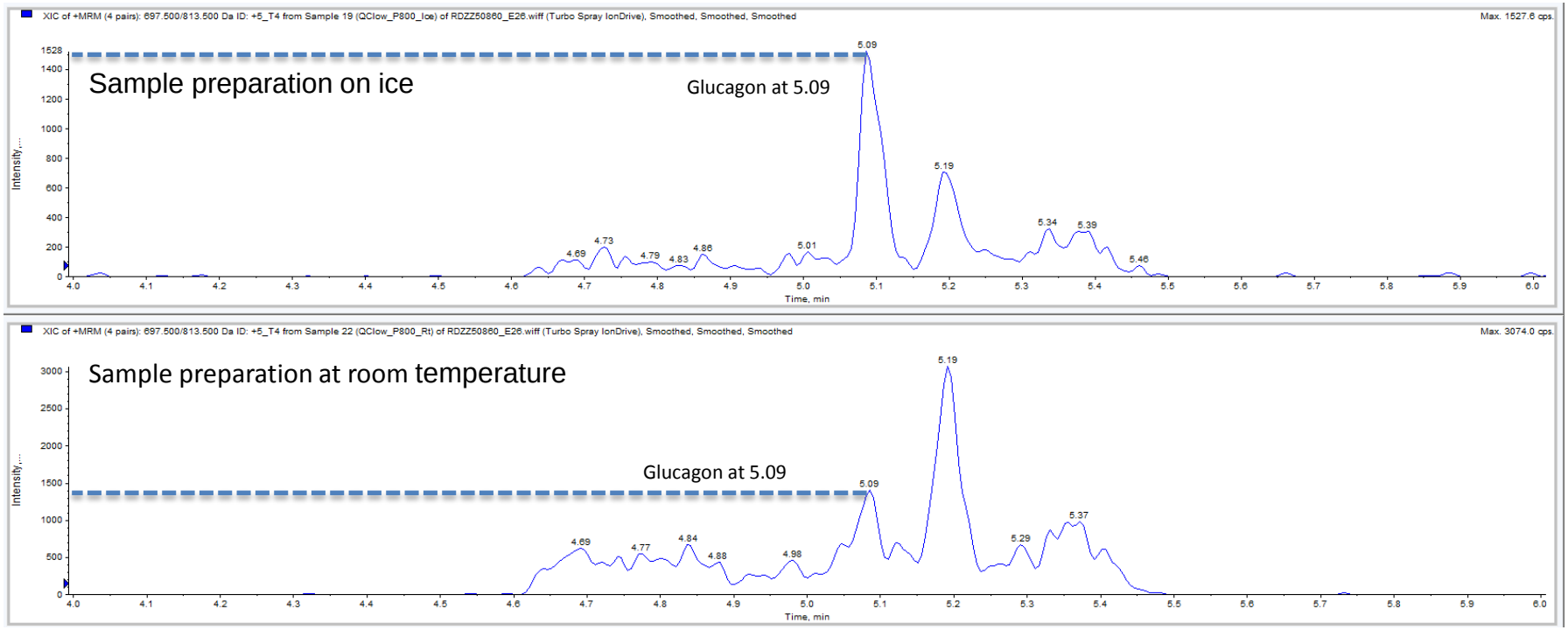
Impact of deviations from standard procedure

What happens if….

…. temperature sensitive analytes are handled too careless



Effect of temperature:
Glucagon response sligthly 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 temprature

Dan B, Celerion, unpublished data 2017

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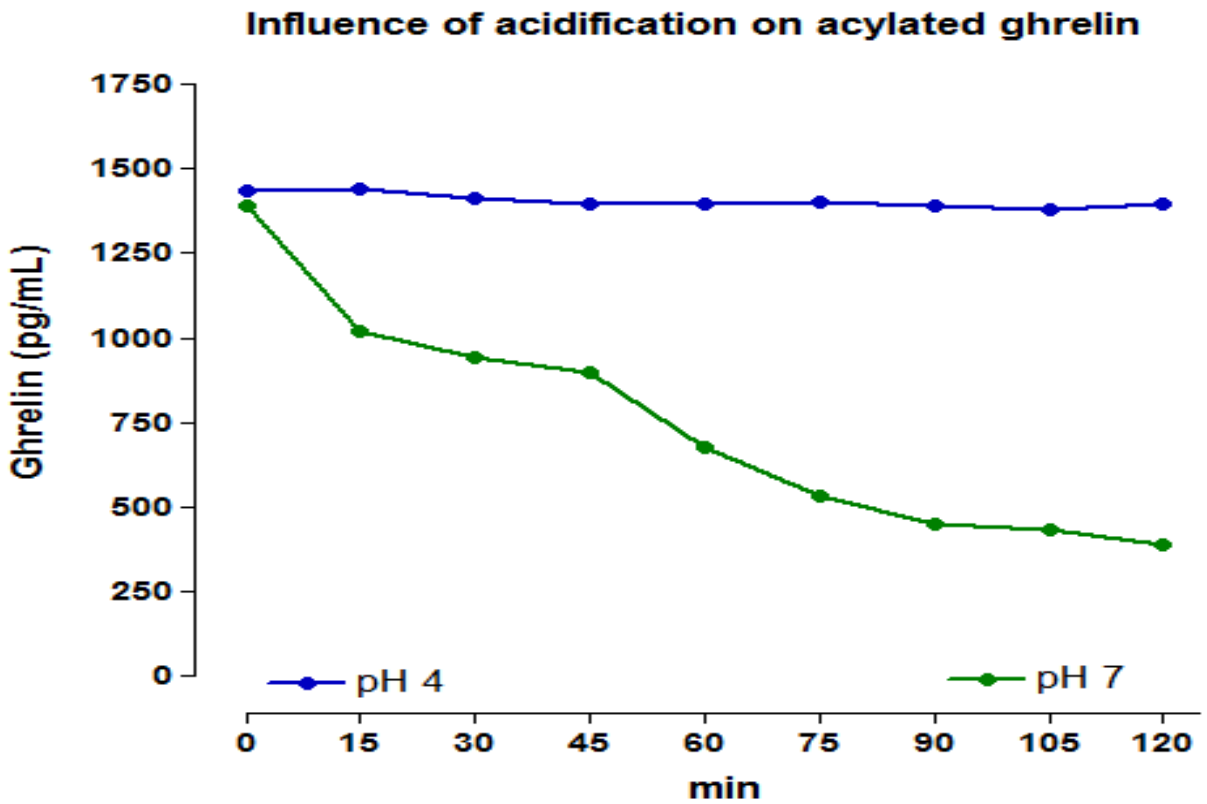
Impact of deviations from standard procedure

What happens if….

…. acidification is not applied for pH-sensitive parameters



NOTE: Sample collected at t_0, aliquots frozen (LN_2)every 15 minutes

Rauh M, Gröschl M. Clinical Chemistry 53:5 902–910 (2007)

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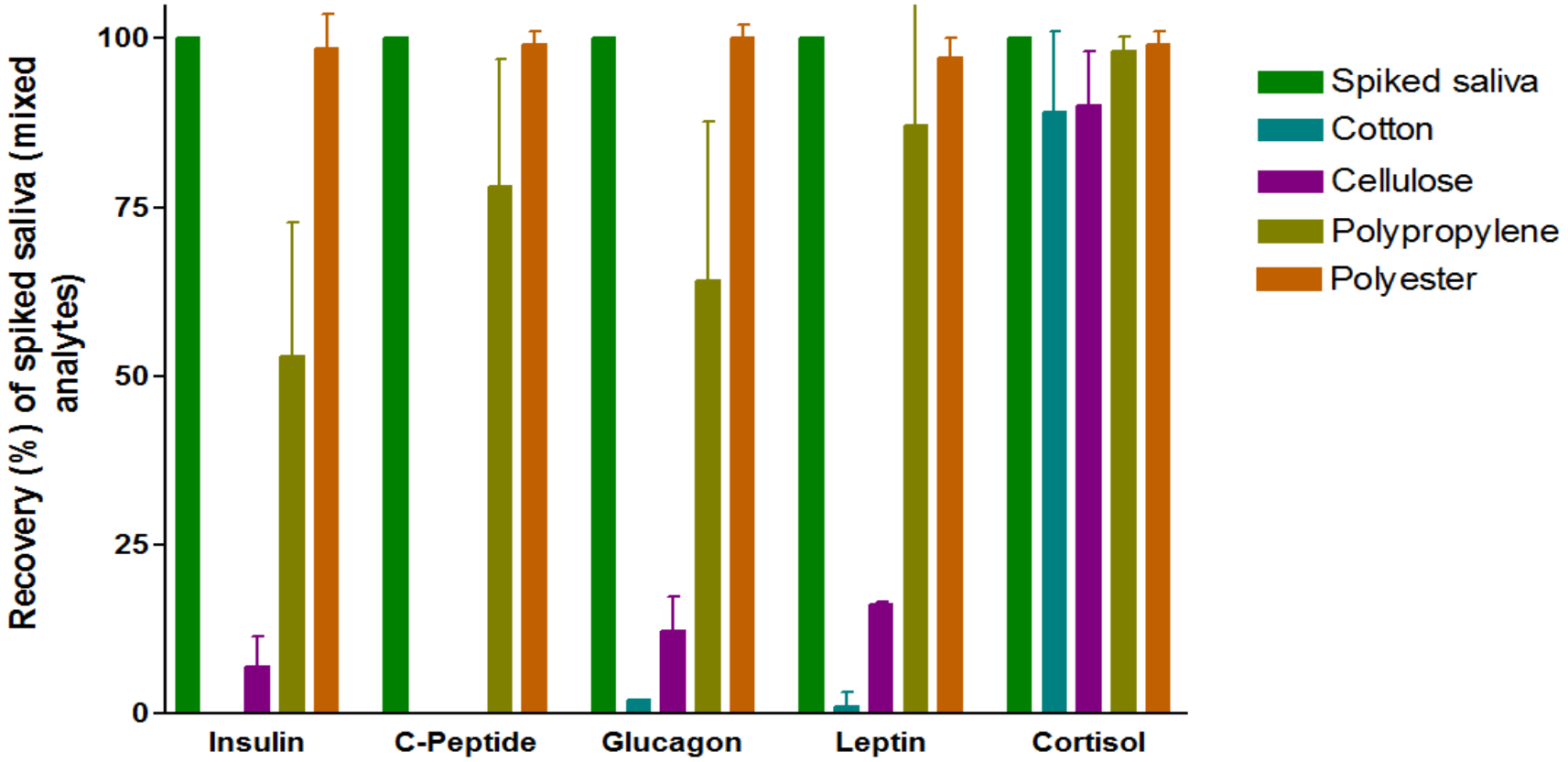
Impact of deviations from standard procedure

What happens if….

… salivary collection device causes loss by adsorption of the analyte



Gröschl M, Köhler H. J.Pharm.Biomed.Analysis 47: 478–486 (2008)

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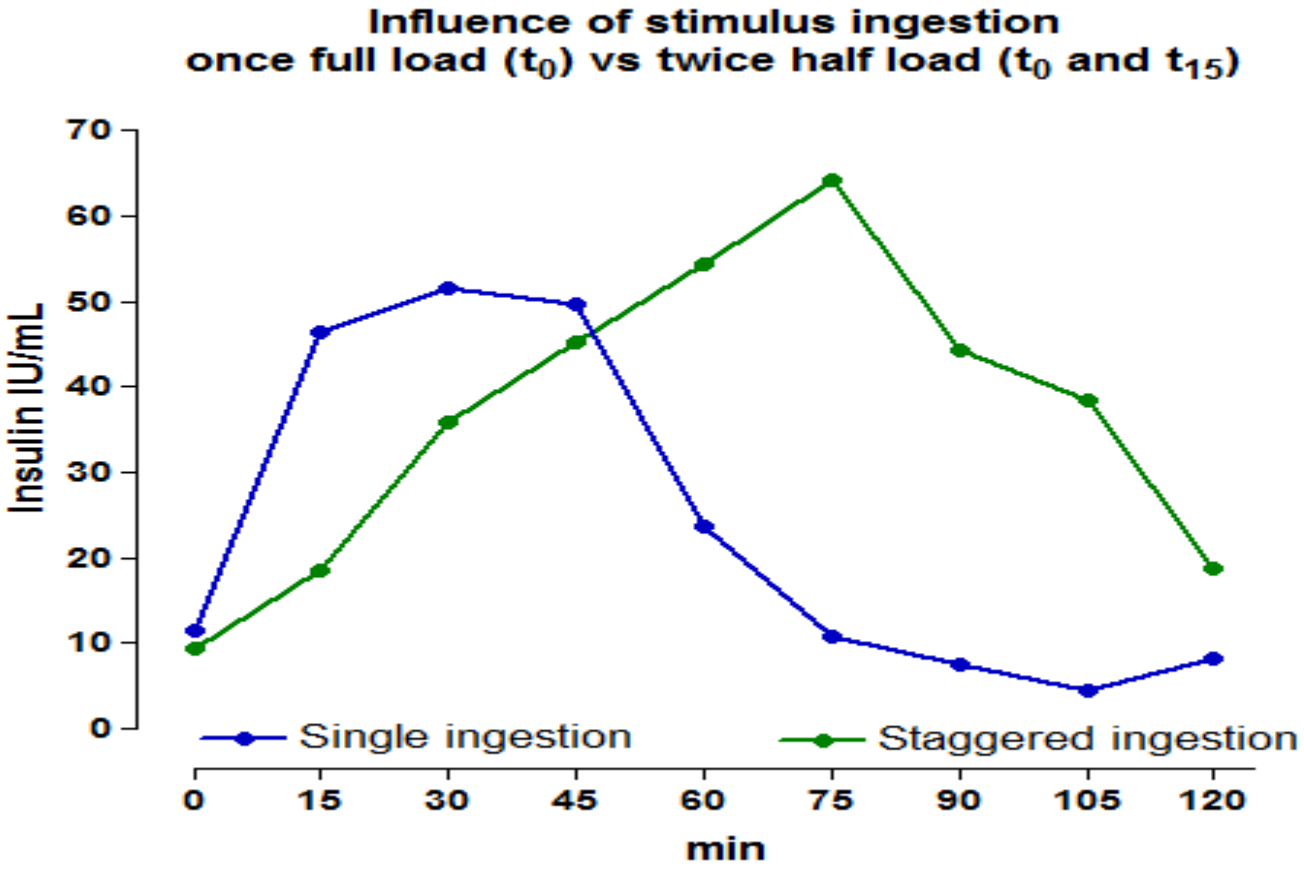
Impact of deviations from standard procedure

What happens if….

…. the stimulus is not provided as planned



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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)

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Sample collection and storage

stimulus

t0 – t15 – t30 – t45 – t60 – t75 – t90 – t105 – t120

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Sample Collection and Storage

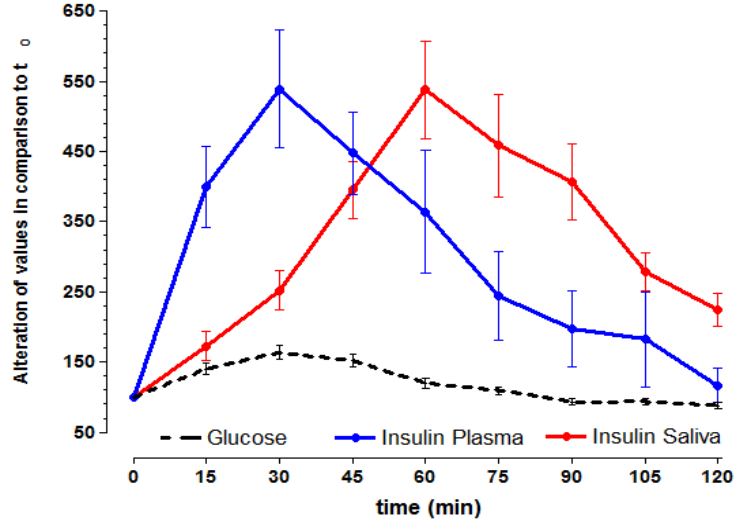
500 µL	Split 1 acidified	Ghrelin	Split 1 acidified	500 µL
500 µL	Split 2 acidified	Glucagon	Split 2 acidified	500 µL
500 µL	Split 3 normal	Insulin, C-Peptide, Amylin	Split 3 normal	500 µL
500 µL	Split 4 normal	Leptin, Resistin, Adiponectin	Split 4 normal	500 µL
500 µL	Split 5 normal	Cortisol	Split 5 normal	200 µL

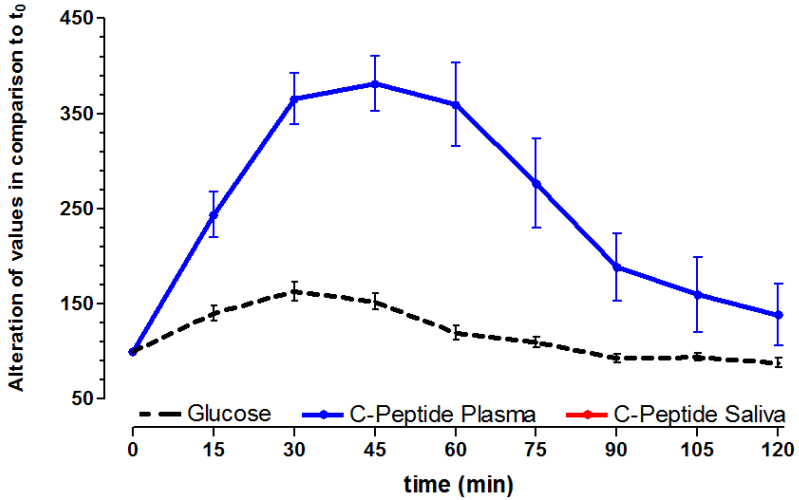
- 80°C until analysis

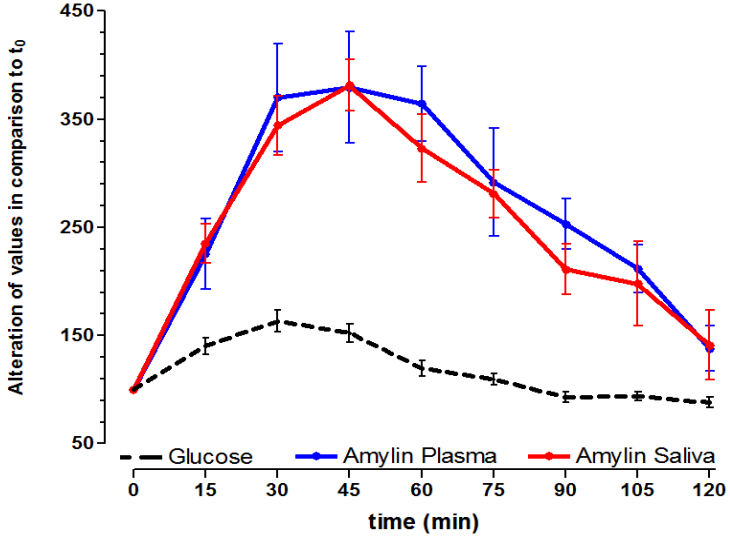
Study Results

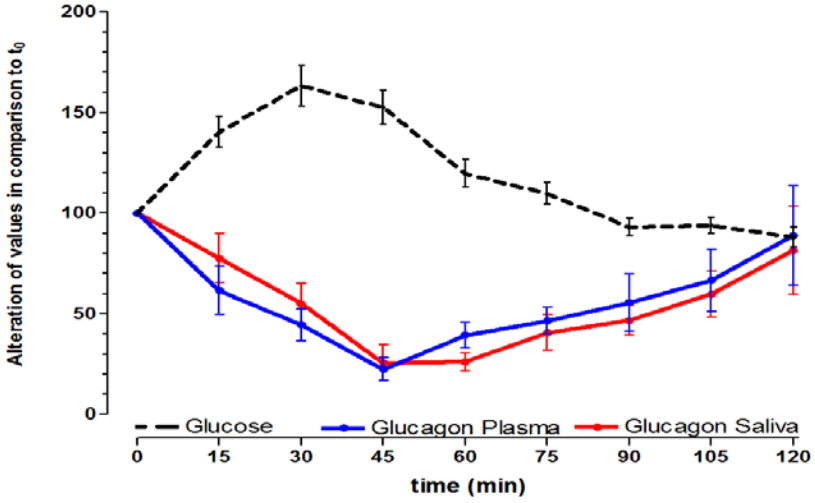
Response of pancreatic peptides to OGT

all values as mean ± SD, related to t0 = 100%



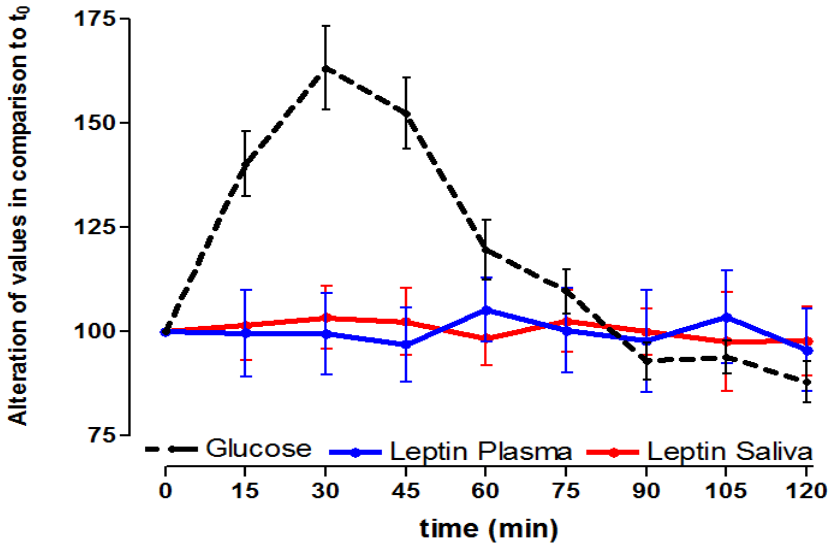


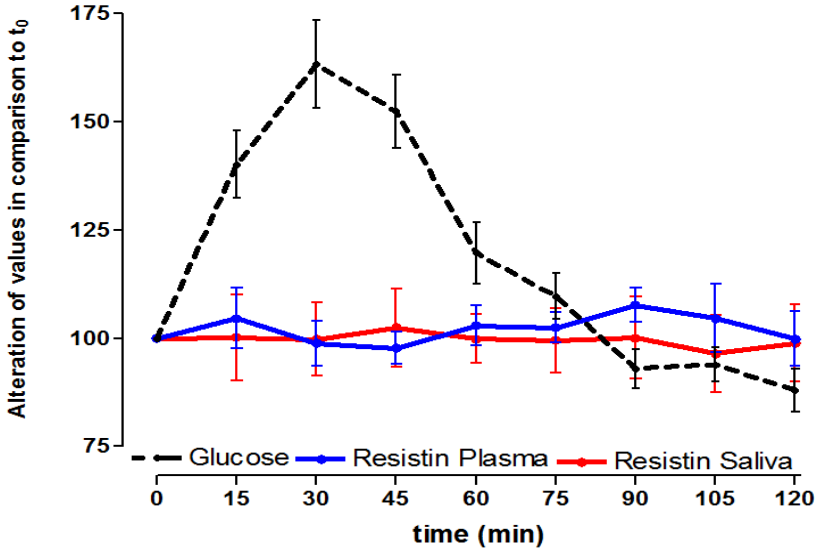


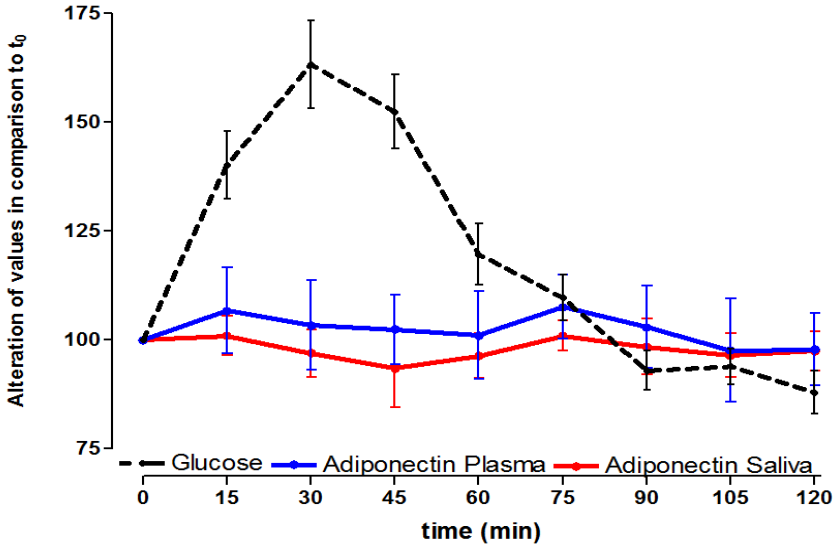


Response of adipocytic peptides to OGT

all values as mean ± SD, related to t0 = 100%







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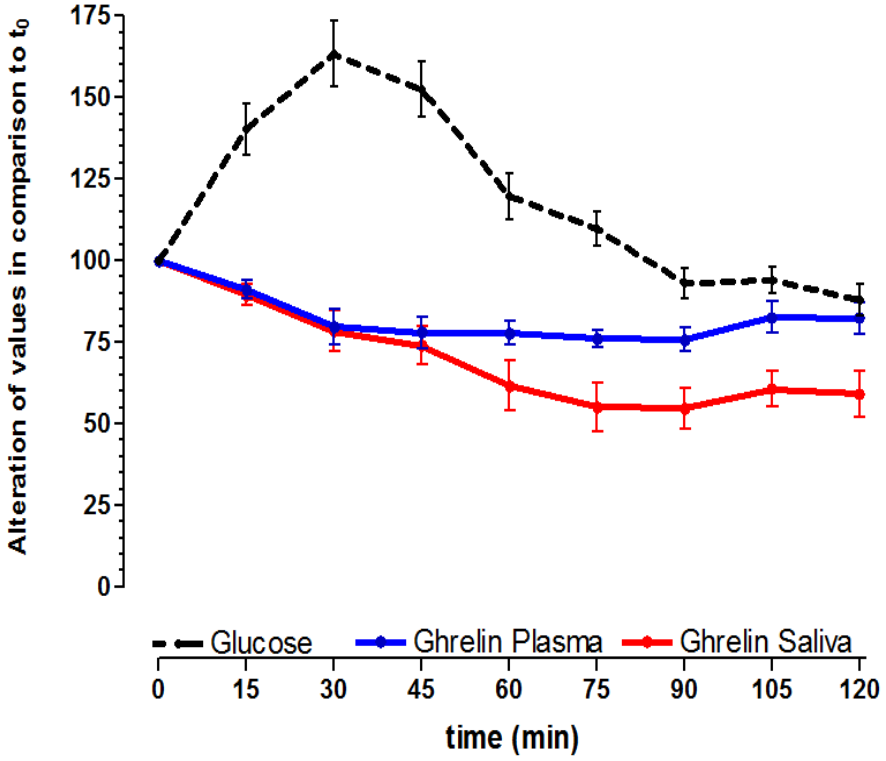
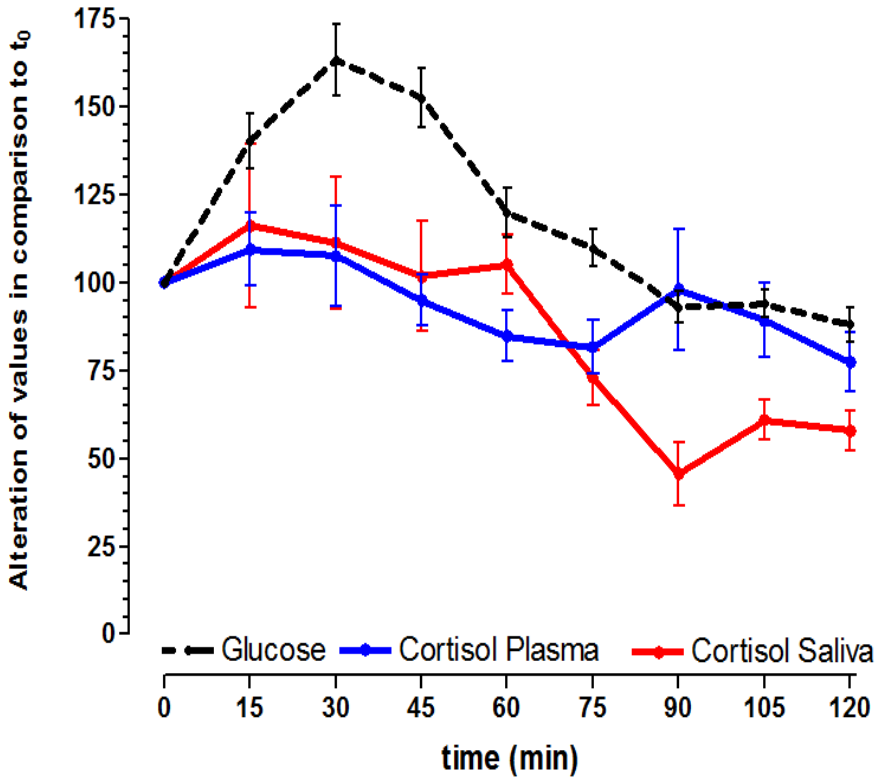
Response of gastric and adrenal hormones to OGT

all values as mean ± SD, related to t0 = 100%





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Conclusions

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