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Determination of digestive enzymes activities in human duodenal fluids

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Introduction

Context

The improvement of *in vitro* digestion models is essential and depends, among others, on a better knowledge of the enzymatic activities in the human digestive tract. Within the framework of a collaboration between INFOGEST and UNGAP, a COST Action on oral drug absorption, access to intestinal fluids was possible.

Objectives

Determination of enzymes activities in duodenum effluents : trypsin, chymotrypsin and lipase and pH measurements

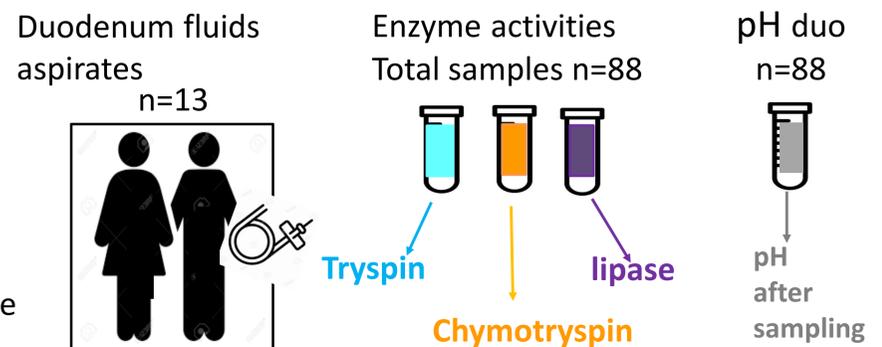


New set of data for enzymes activities in human duodenum

Materials and methods

- 13 healthy adult volunteers were used for the determination of digestive enzyme activities
- Meal : * 240 ml of **water (fasted)**
Or * 400 ml of **Ensure Plus vanilla** +240 mL of water (**fed**)
- Kinetics of sampling : from -60 min before ingestion to 110 min after water or meal ingestion
- Samples storage: samples were mixed with glycerol (1:1) and inhibitors before storage at -20°C
- Statistical analysis: paired t-test

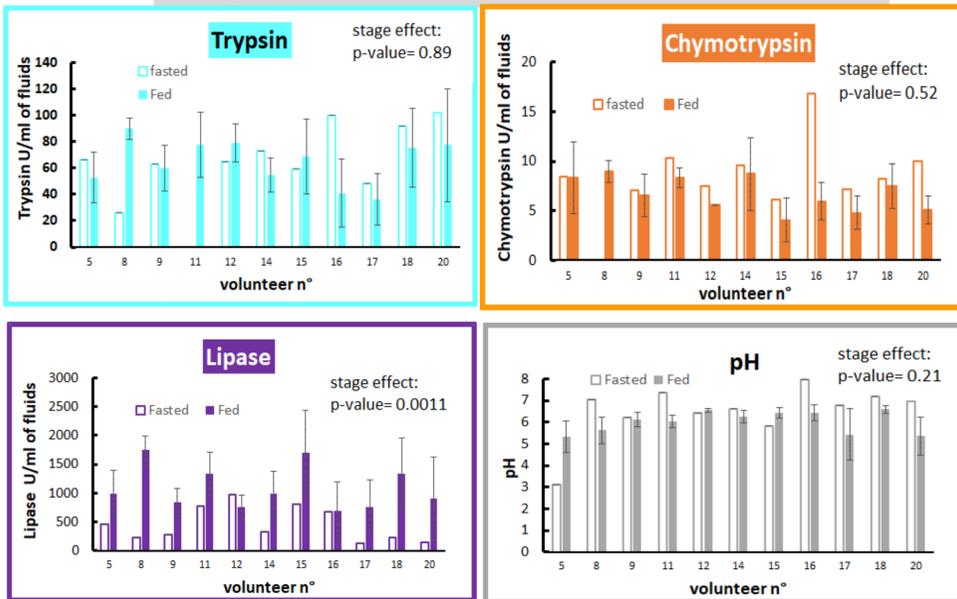
Analysis



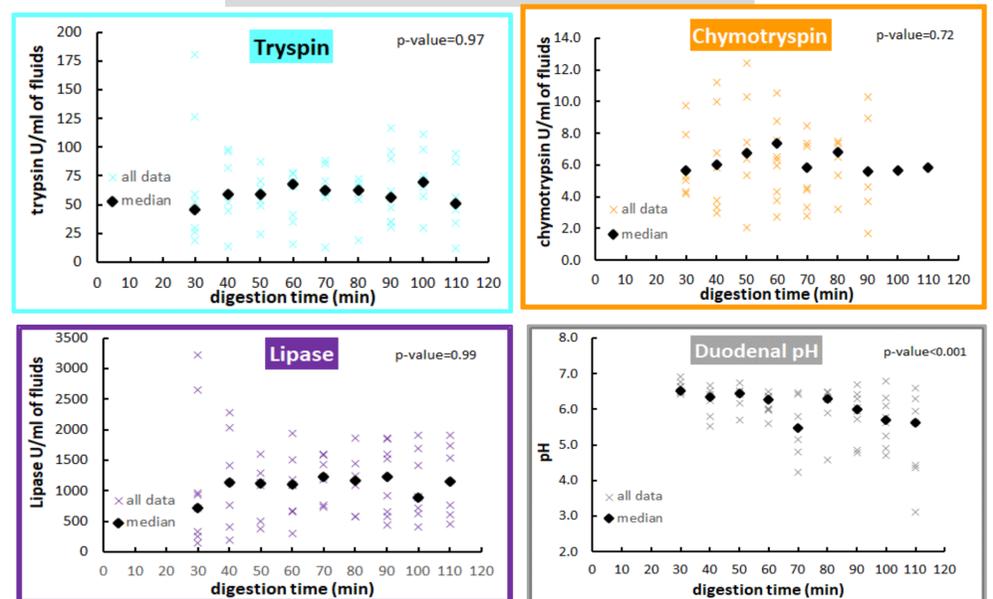
Enzymatic assays : Brodkord et al;2019 Supplementary Material except that trypsin and chymotrypsin activities were determined at 37°C instead of 25°C, for lipase no modification (37°C).

Results

Stage effect: fasted vs fed-state



Post-prandial time effect



- Stage effect on lipase activity
- Average trypsin: mean_{fasted} = 63.2 ± 30.6 vs mean_{fed} = 64.9 ± 17.2 U/ml
- Average chymotrypsin: mean_{fasted} = 9.9 ± 3.9 vs mean_{fed} = 6.7 ± 1.7 U/ml
- Average lipase: mean_{fasted} = 457 ± 299 vs mean_{fed} = 1093 ± 376 U/ml
- Average pH: pH_{fasted} = 6.5 ± 1.3 vs pH_{fed} = 6.0 ± 0.5
- Fasted: 1 time point / volunteer
- Fed 2 to 9 time points /volunteer

- No effect of post-prandial time on enzyme activities / effect on pH
- Average: mean_{trypsin activity} = 62.1 ± 29.9 U/ml / mean_{chymotrypsin} = 6.1 ± 2.6
- Average: mean_{lipase activity} = 1129 ± 612 U/ml / pH_{average} = 6.1 ± 2.6
- Ratio_{trypsin/chymotrypsin} = 10.2
- 25 vs 37°C trypsin & chymotrypsin temperature assay : a 1.8 factor

| | Trypsin (U/ml of intestinal content) | Lipase (U/ml of intestinal content) | assay temperature (°C) |
|--------------------------|--------------------------------------|-------------------------------------|------------------------|
| Infogest digestion model | 100 | 2000 | 25 |
| In vivo (fed state) | 34.5 | | 25 |
| In vivo (fed state) | 62.1 | 1129 | 37 |

Care should be taken to use similar conditions for enzymatic assays to compare the data.

Conclusions

There is currently little data in the literature on enzyme levels in the GI tract, so this new data set is of great importance. These results highlight the need for additional in vivo fluid characterisation and would help the scientific community to adapt and improve *in vitro* digestion models.