Lipid protection by fruit and vegetables and their polyphenols during gastric digestion in minipigs

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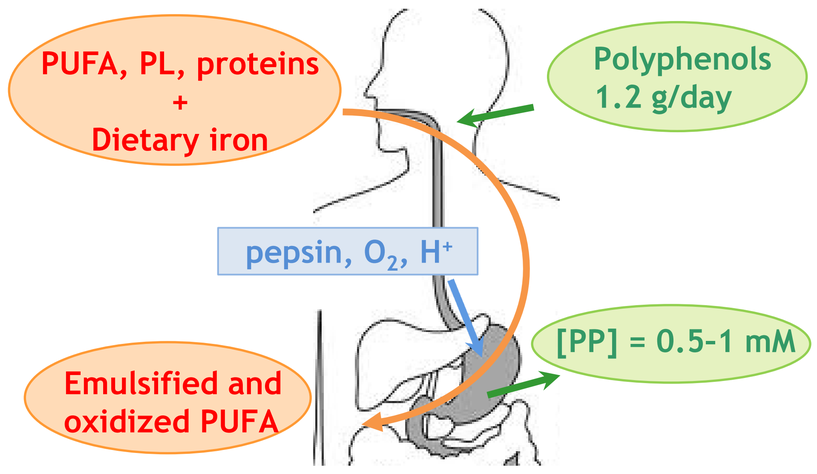
Introduction

There are compelling evidences that dietary lipid oxidation products play a key role in the development of cardiovascular diseases.[1] Lipid oxidation products can be generated *in vivo* and the gastric tract has been proposed as a major site for diet-related oxidative stress.[2] Indeed, after food intake, dietary iron may trigger lipid oxidation as demonstrated in emulsion systems modelling the gastric content.[3] On the other hand, cardiovascular diseases are inversely associated with the intake of flavonoids, a class of polyphenols largely distributed in fruit and vegetables.[4]

Objectives : Assessing 1) lipid oxidation in the gastric tract of minipigs fed with a standard Western diet, 2) the protective capacities of fruit and vegetables (F&V) and the corresponding polyphenol extract (PE).



Materials & Methods

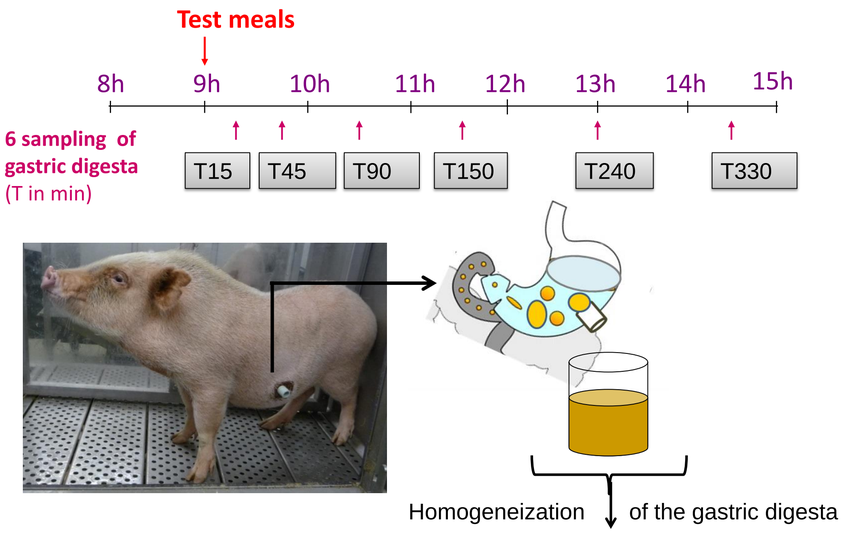
Animals and Test meals

Six female Göttingen minipigs (20-25 kg) were surgically fitted with a cannula on the greater curvature of the stomach. The fasted minipigs were fed three different meals containing principally sunflower oil, cooked ground beef meat and egg phospholipids (= Beef meal).

1) Beef Meal

2) Beef meal added with cubed apple, plum and artichoke (F&V meal)

3) Beef meal added with a polyphenol extract from F&V (PE meal) containing :
- 154 mg of monomeric phenolic compounds (caffeoylquinic acids contributes for ¾, w/w)
- 79 mg of oligomeric flavanols



- In vivo gastric pH
- Heme and non-heme iron forms
- Total lipids
- Lipid oxidation products (conj. dienes, TBARS)

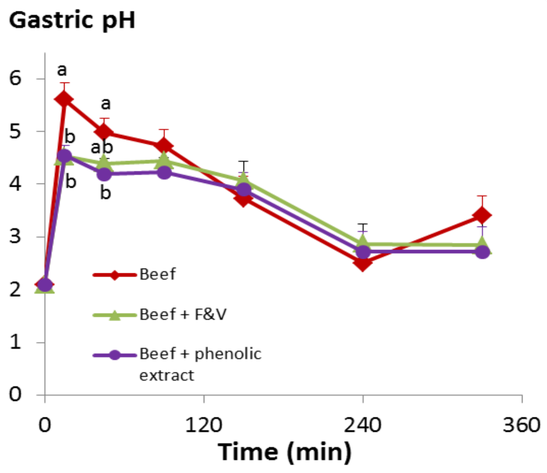
Analyses

- ✓ *Total iron* : wet mineralization then ICP-MS
- ✓ *Free iron and Fe(II)* : ferrozine assay in the presence and absence of ascorbate, respectively.
- ✓ *[Heme iron]* = [total iron] – [free iron].
- ✓ *Total lipids* : according to the Folch procedure.
- ✓ *Lipid-derived conjugated dienes* (CD) : determined spectophotometrically at 234 nm, ε= 24 000 $M^{-1}cm^{-1}$.
- ✓ *TBARS* : reaction of the digesta with 2-thiobarbituric acid, calibration with tetraethoxypropane (in µg of equiv. malondialdehyde (MDA) per g of lipids)

Statistics : one-way ANOVA for repeated measures (Tukey post-hoc test for statistical effects). Data are mean ± SEM.

Results & Discussion

Gastric pH

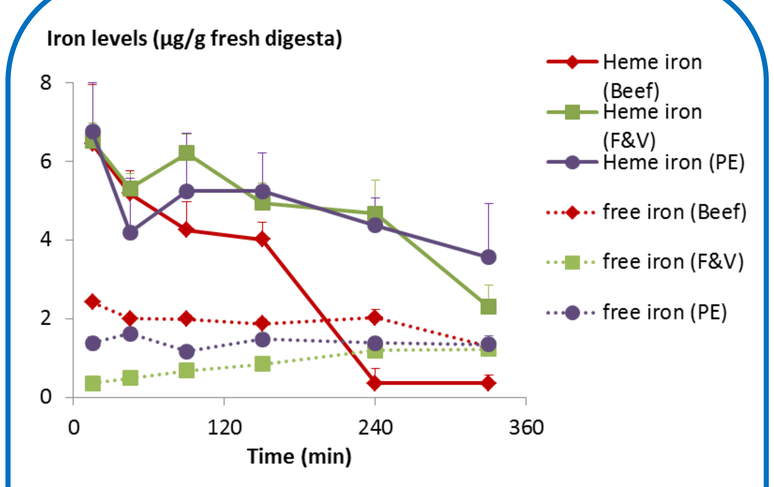


At T15 min after meal ingestion :
- ✓ Beef meal : the gastric pH increased sharply from 2.1 (fasting state) to 5.6.
- ✓ F&V and PE meals : this pH was found to be 4.5 outlining a significant effect of meal ($p < 0.05$).

The pH variations recorded are similar to those observed in humans after the ingestion of a liquid meal enriched with F&V purees [5].

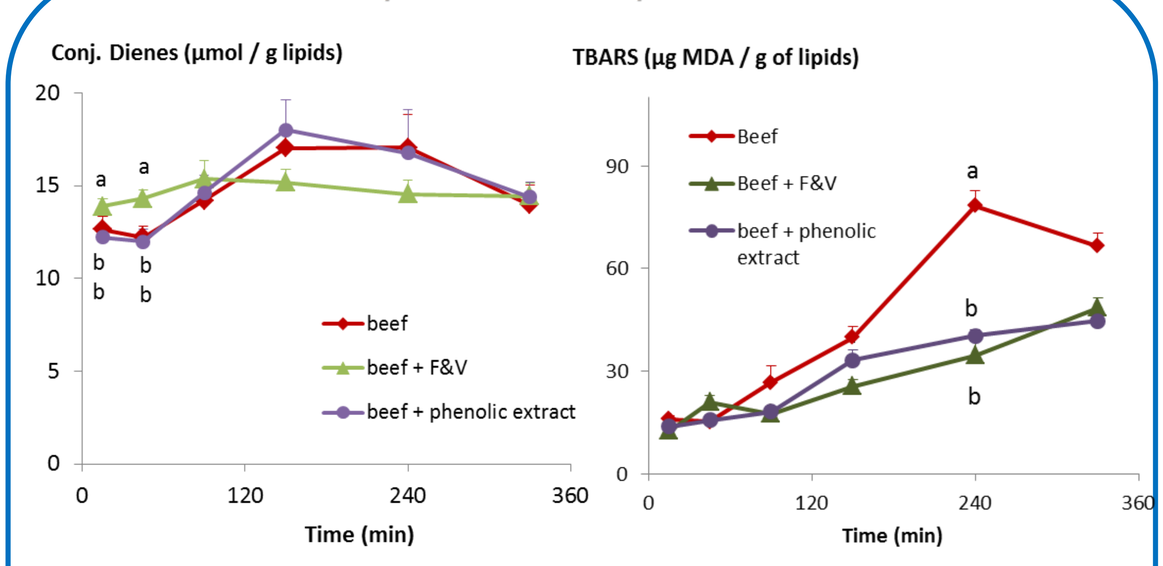
➔ Minipig is a relevant model for gastric digestion.

Iron forms



- ✓ [heme iron] >> [free iron]
  - as in raw beef meat
  - As in the initial meals indicating that steam cooking did not induce iron release.
  - Free iron is dominated by the Fe(III) form.
- ✓ Decrease in heme iron which became undetectable after 240 min (beef meal).
  - Slower decreases for the F&V and the PE meals.
  - Free iron constant or even accumulating in agreement with the suggested conversion of heme iron into free iron at pH<4.

Lipid oxidation products



- ✓ Bell-shaped kinetics indicating faster rates of CD formation than CD decomposition between 15 and 150 min.
- ✓ Significantly higher initial CD content for the F&V meal (+10%) although no further increase during the course of digestion.
- ✓ No inhibition of CD accumulation by the phenolic extract.
- ✓ TBARS levels increased regularly during gastric digestion in agreement with the continuous degradation of the primary lipid oxidation products.
- ✓ Both F&V and the phenolic extract proved to be highly protective of lipids (TBARS accumulation / by a 2.5 to 3-fold factor).

Conclusion

The present study clearly demonstrates :
- ➢ the occurrence of *in gastro* oxidation of dietary polyunsaturated lipids in the presence of meat iron.
- ➢ that F&V and their phenolic compounds can play a protective role.

Proposed mechanism : phenolic compounds, displaying the 1,2-dihydroxyphenyl moiety that is critical to their antioxidant capacity, may reduce hypervalent heme iron forms and chelate free iron forms, both species initiating lipid oxidation.

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