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

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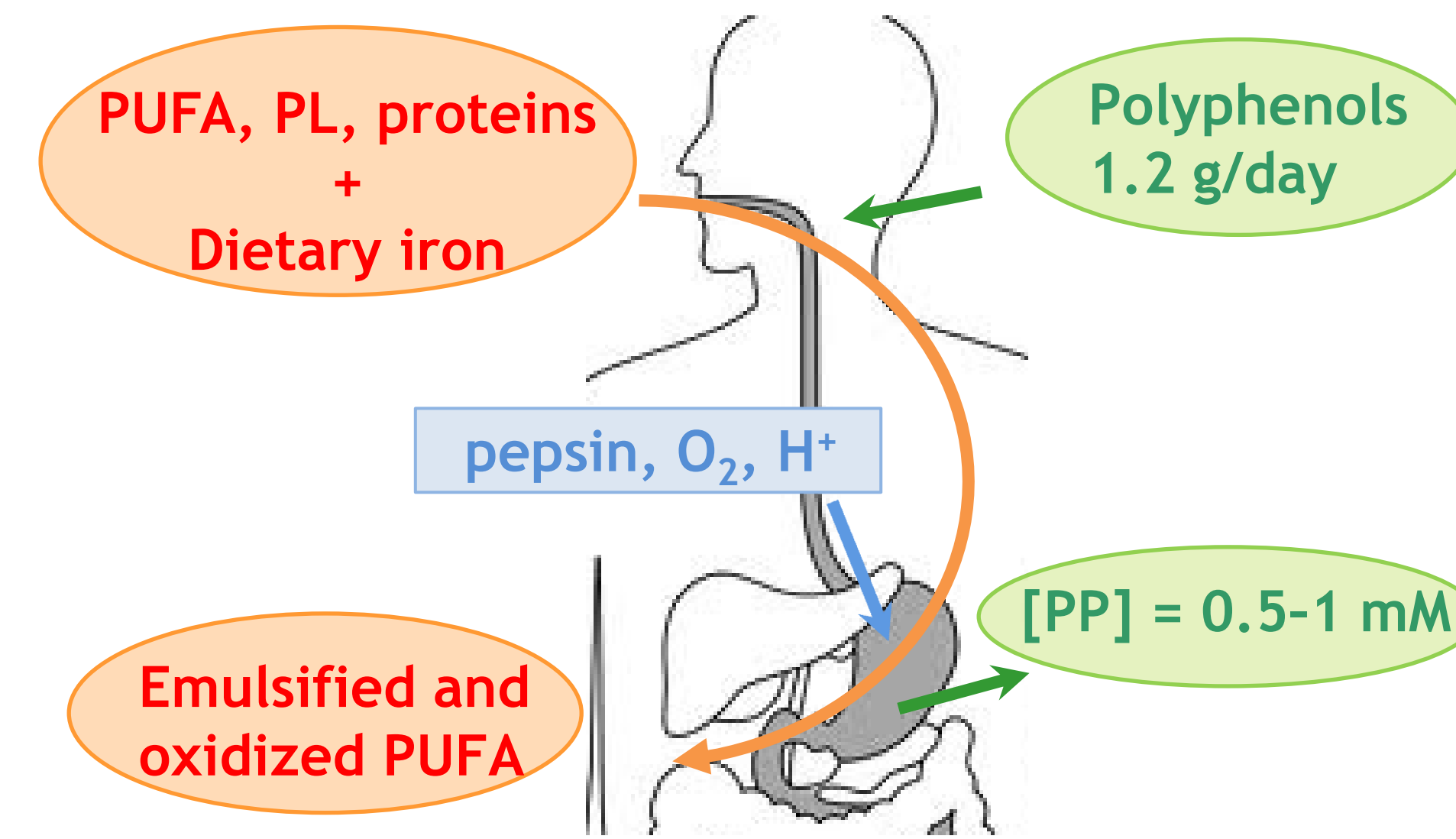
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Introduction

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

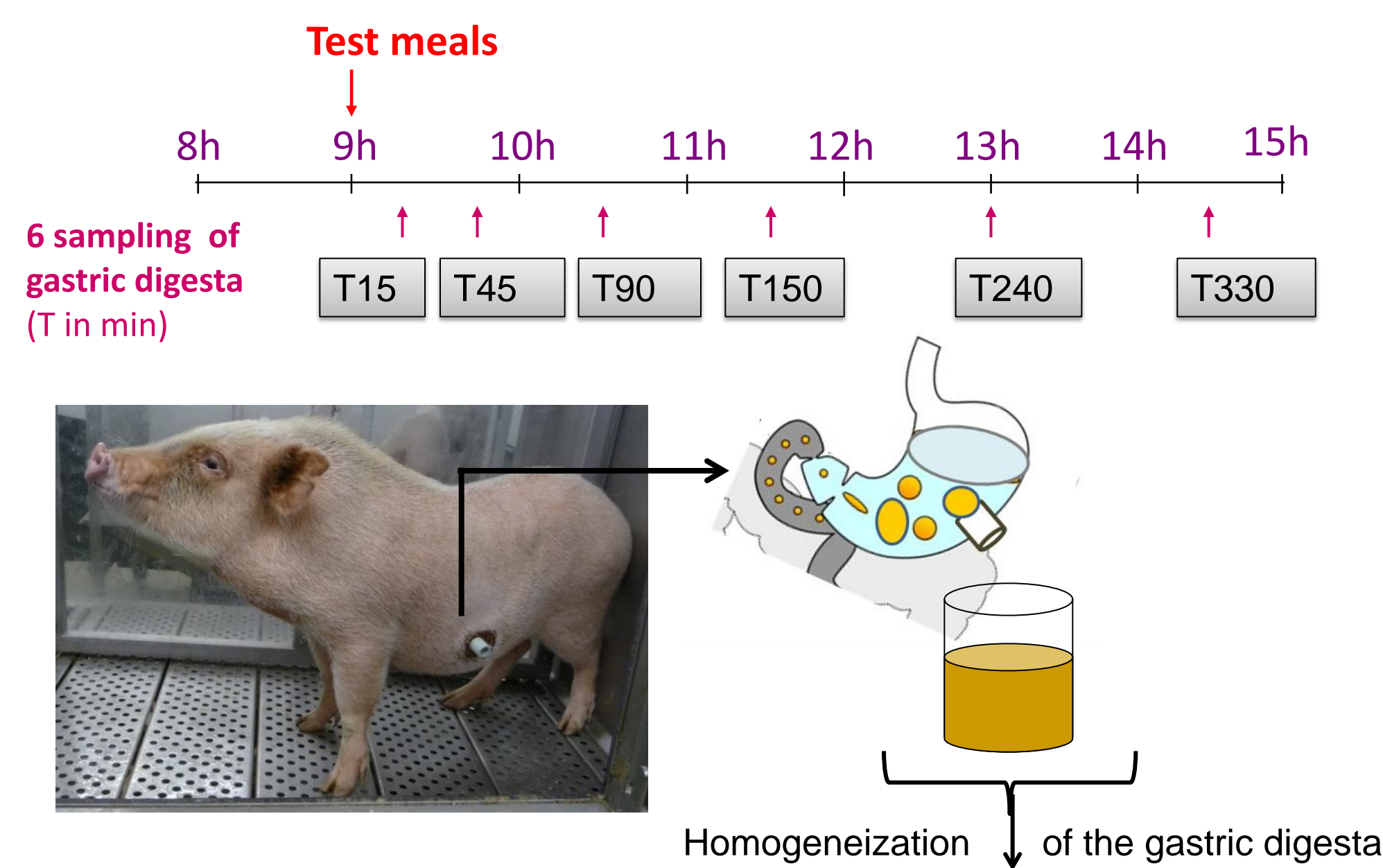
Six female Göttingen minipigs (20-25 kg) were surgically fitted with a cannula on the greater curvature of the stomach. The fasted minipigs were fed three different meals containing principally sunflower oil, cooked ground beef meat and egg phospholipids (= Beef meal).

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 3/4, w/w)
- 79 mg of oligomeric flavanols



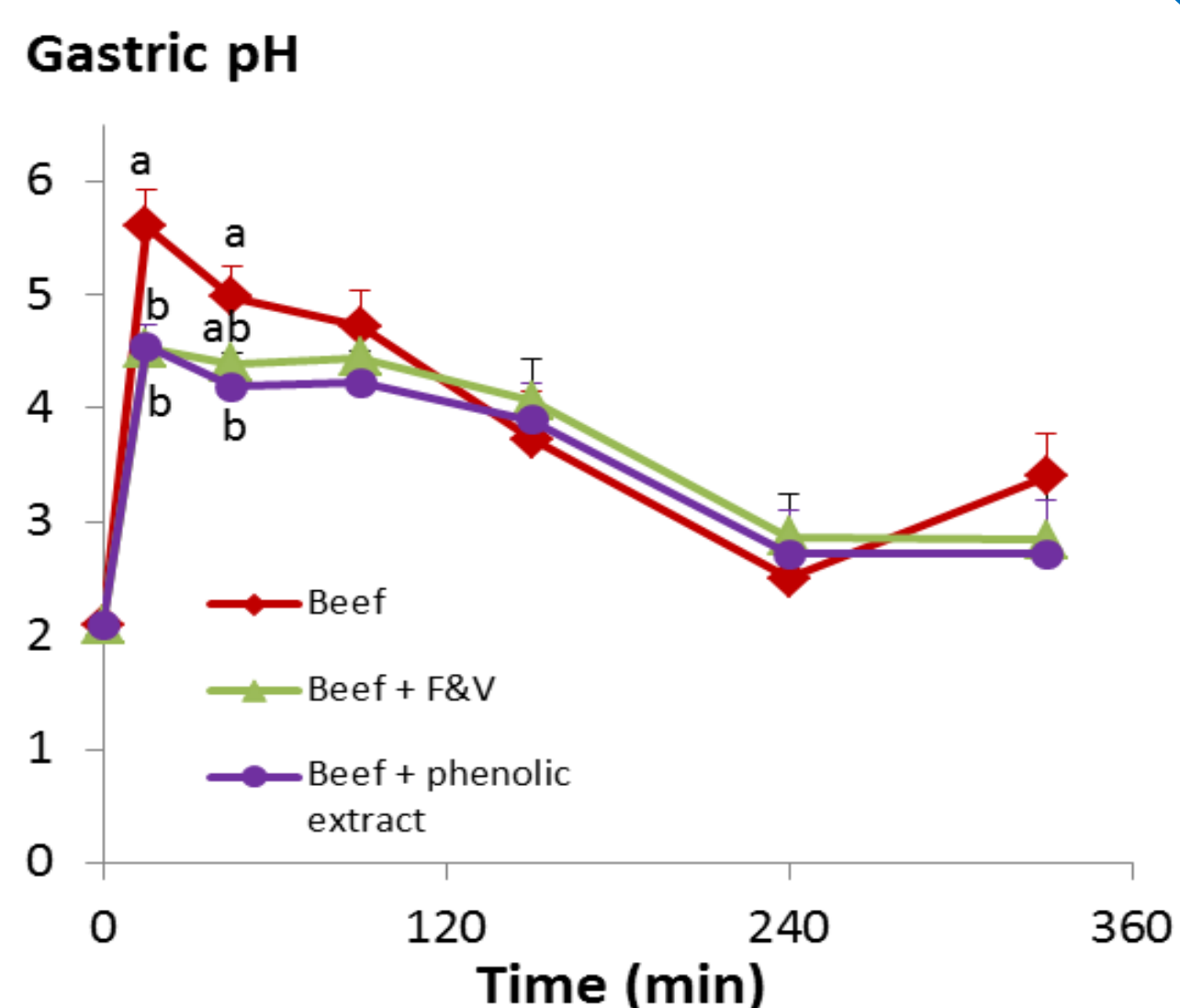
- > In vivo gastric pH
- > Heme and non-heme iron forms
- > Total lipids
- > Lipid oxidation products (conj. dienes, TBARS)

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 spectrophotometrically at 234 nm, $\epsilon = 24\ 000\ M^{-1}cm^{-1}$.
 - ✓ **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 \pm SEM.

Results & Discussion

Gastric pH



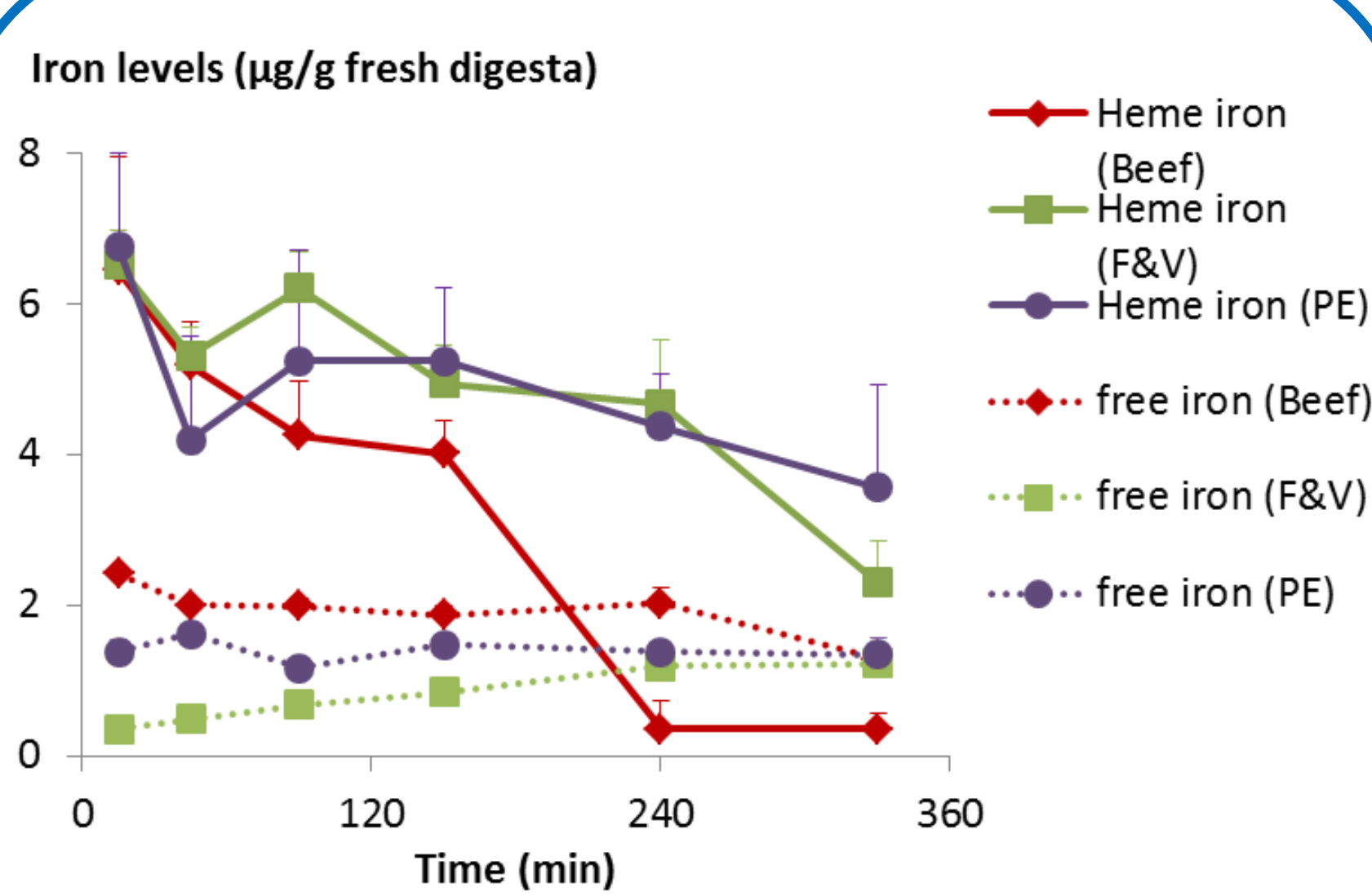
At T15 min after meal ingestion :

- ✓ **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].

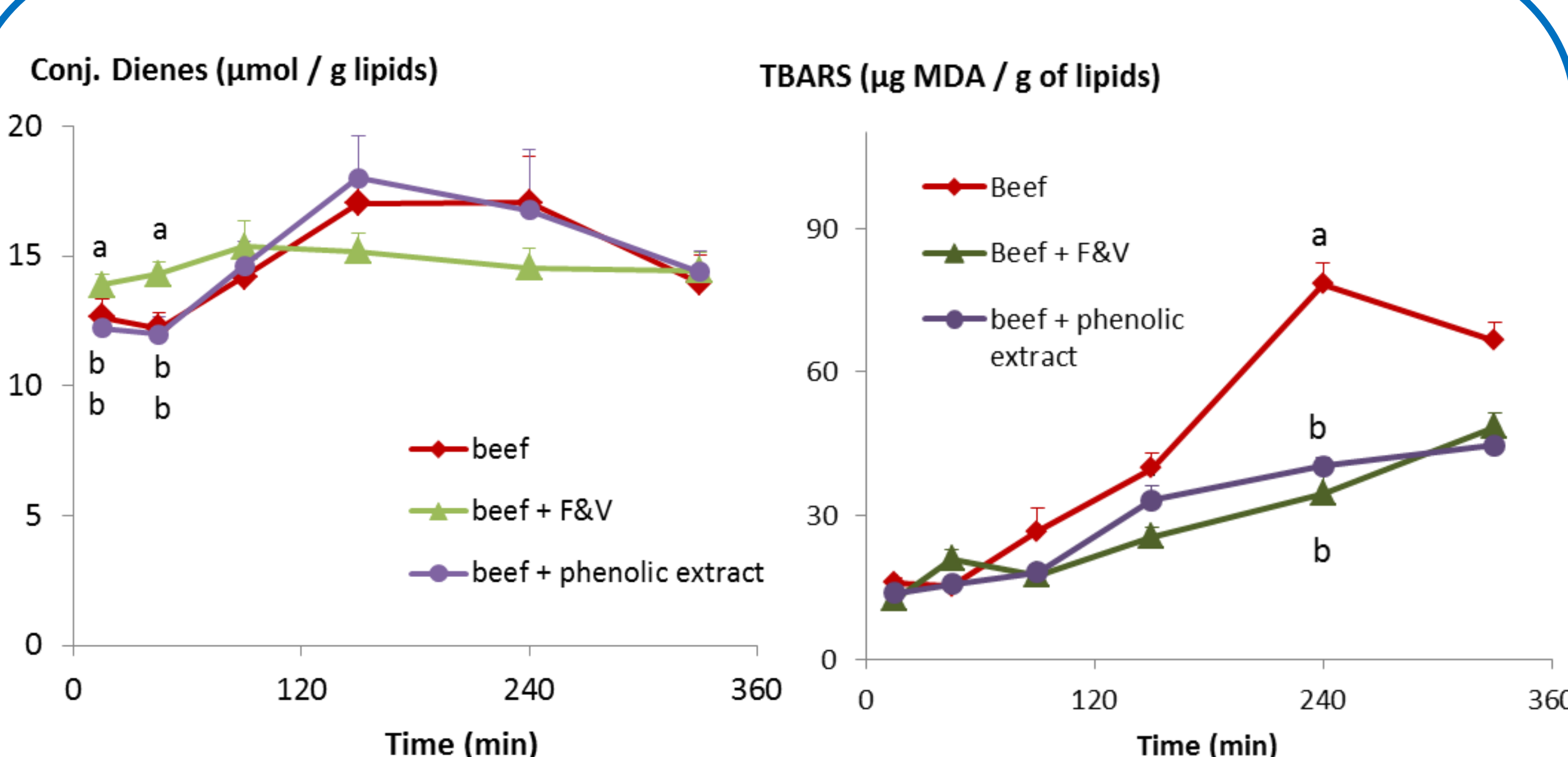
→ Minipig is a relevant model for gastric digestion.

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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