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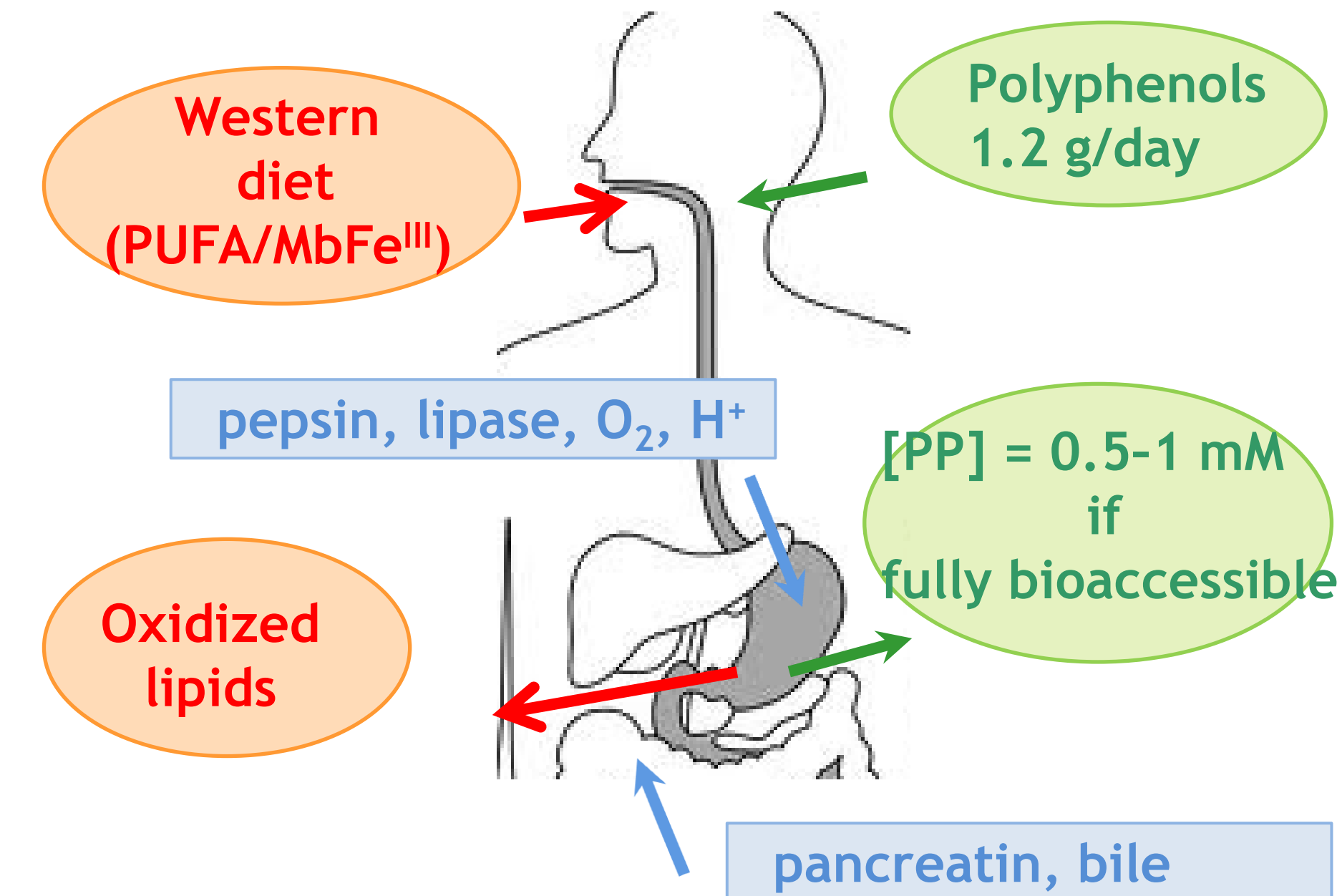
Geëtan Boléa, Pascale Goupy, Christian Ginies, Claire Dufour*

INRA, Avignon University UMR408 Safety and Quality of Plant Products, F-84914 Avignon, France.
Claire.dufour@inra.fr

Introduction

Atherosclerosis is frequently associated with ischemic pathologies and one of the main risk factors is the Western diet. This diet is rich in ω -6 polyunsaturated fatty acids (ω -6 PUFA) which are sensitive to oxidation and lead to the formation of genotoxic and cytotoxic aldehydes. Indeed, the modification of low density lipoproteins by coupling with 4-hydroxy-2-nonenal (4-HNE), a specific lipid oxidation product of ω -6 PUFA, is a key step in the development of atherosclerosis [1,2]. By contrast, the consumption of either fruit and vegetables or flavonoids, the main class of dietary polyphenols (PP), is inversely associated with the development of coronary artery disease [3,4].

Objectives : This study aims at assessing lipid oxidation during the digestion of a Western type meal and the antioxidant capacity of apple polyphenols in different matrix forms. Two *in vitro* gastrointestinal digestion systems are compared: a static one with conditions derived from the COST Infogest [5] and the dynamic system DIDGI® developed at INRA.



Methodology

Static *in Vitro* Digestion Model (COST Infogest)

Gastric Phase

Sunflower oil-in-water (10%) emulsion stabilized by egg yolk phospholipids in Simulated Gastric Fluid pH 5
+
Pepsin (1000 U/ml) / CaCl₂ (0,75 mM)
±
Epicatechin or apple puree / extract (100 μ M epicatechin equiv.)
+
Myoglobin (20 μ M) \rightarrow T0
↓
Adjustment at pH 5 (1 M HCl)
1 h, 37 °C, 280 rpm (sampling 0, 30 60 min)
↓
Adjustment at pH 3 (1 M HCl)
1 h, 37 °C, 280 rpm (sampling 90, 120 min)

Intestinal Phase

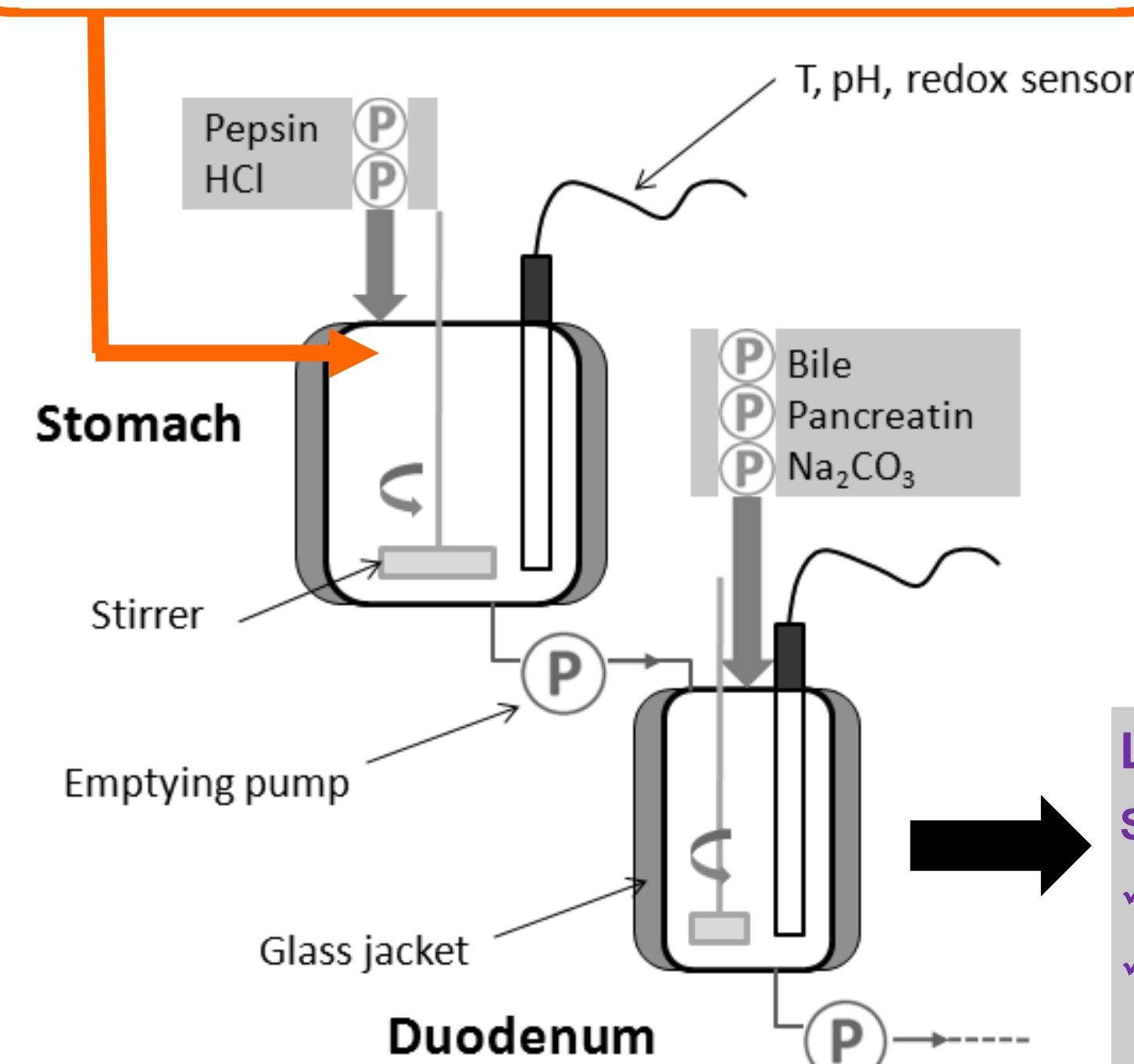
Gastric phase: 1:1 dilution with Simulated Intestinal Fluid pH 7
+
Pancreatin (100 U trypsin/ml)
+
Bile (10 mM) / CaCl₂ (0,675 mM)
↓
Adjustment pH 6.5 (1 M HCl)
2 h, 37 °C, 280 rpm
Sampling 0, 30, 60, 90, 120 min
↓
Evaluation of lipid oxidation
Sample dilution with iPrOH then centrifugation
✓ Conjugated dienes (Abs 234 nm)
✓ 4-hydroxy-2-nonenal (4-HNE) after coupling with DNPH (UPLC-MS)



Composition (w/w)
6% epicatechin
77% oligomeric procyanidins
19% chlorogenic acid
1.4% flavonols
2.6% phloretin glycosides

Dynamic *in Vitro* Digestion Model (INRA DIDGI®, Storm Software)

9% sunflower oil-in-water emulsion (200 g) stabilized by egg yolk phospholipids or 10% emulsion (180 g) + apple puree (20 g) + Myoglobin

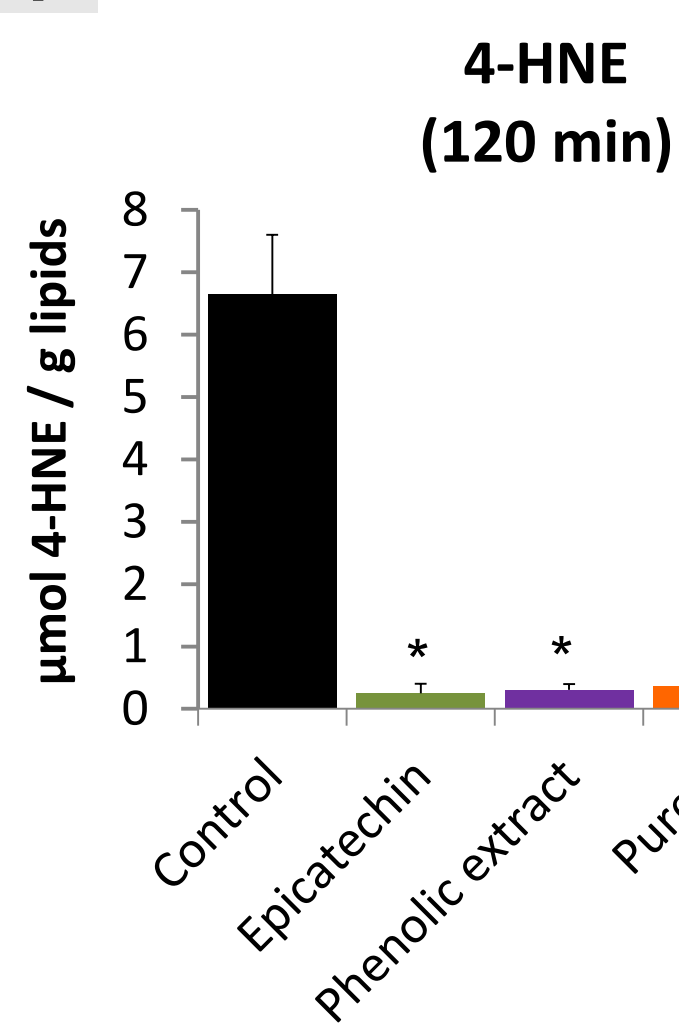
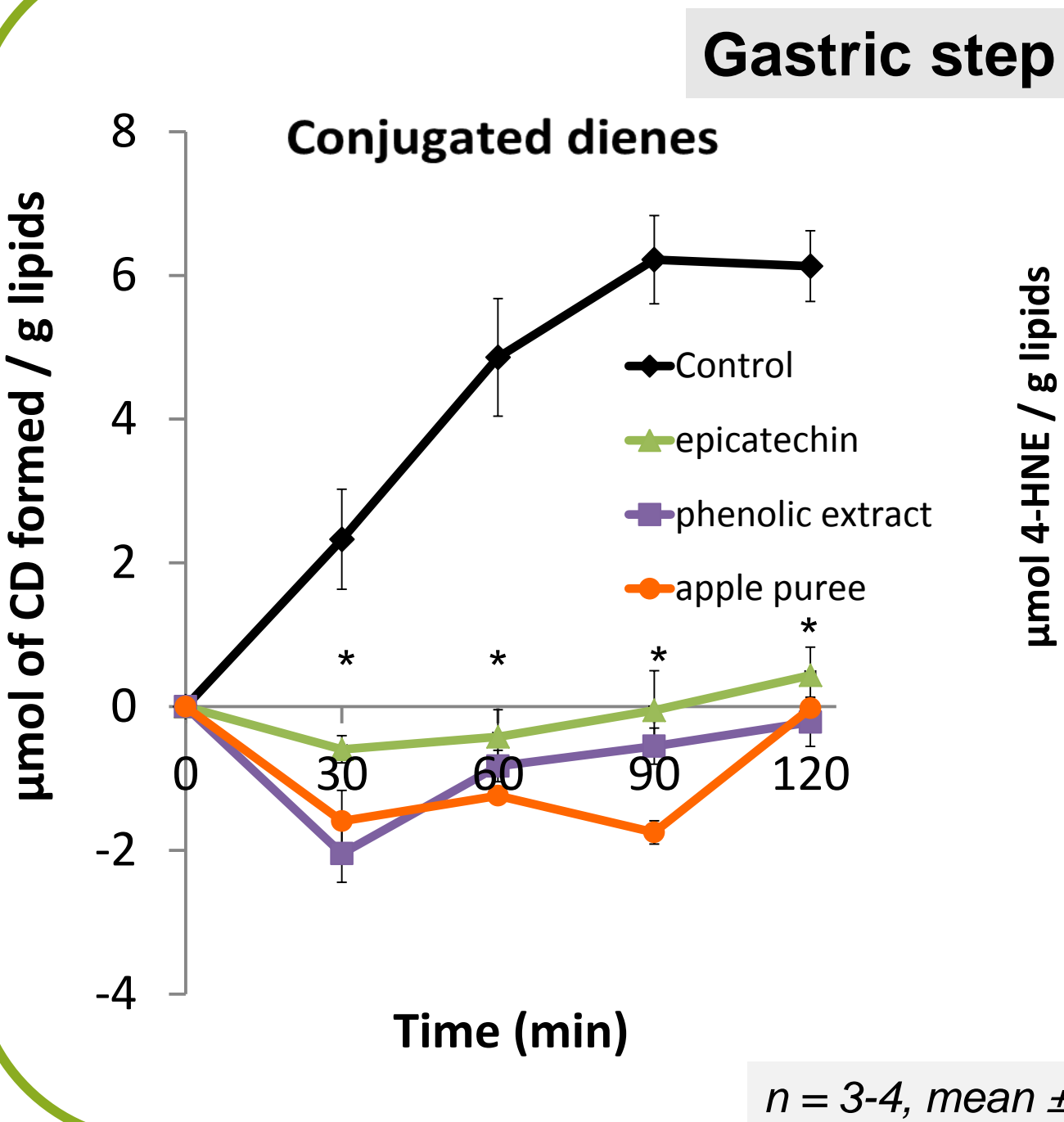


STEPS	Conditions
Gastric step	
Pepsin (Sigma P6887)	3 g/L - 0,5 mL/min
Myoglobin (MbFe ^{III})	62,5 μ M initial
HCl	0,1 M
beta	1
T 1/2	60 min
pH	5 \rightarrow 2 over 120 min
Duodenal Step	
Pancreatin (Sigma P7545)	4 g/L - 0,25 mL/min
Bile extract (Sigma B8631)	55g/L - 0,4 mL/min
NaHCO ₃	0,5 M
Volume max.	50 mL
pH	6,5

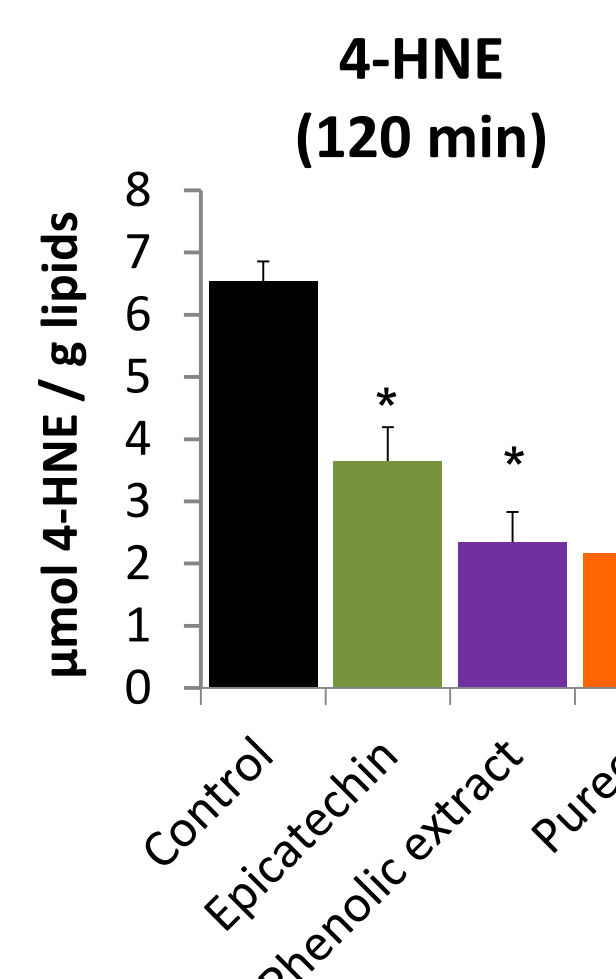
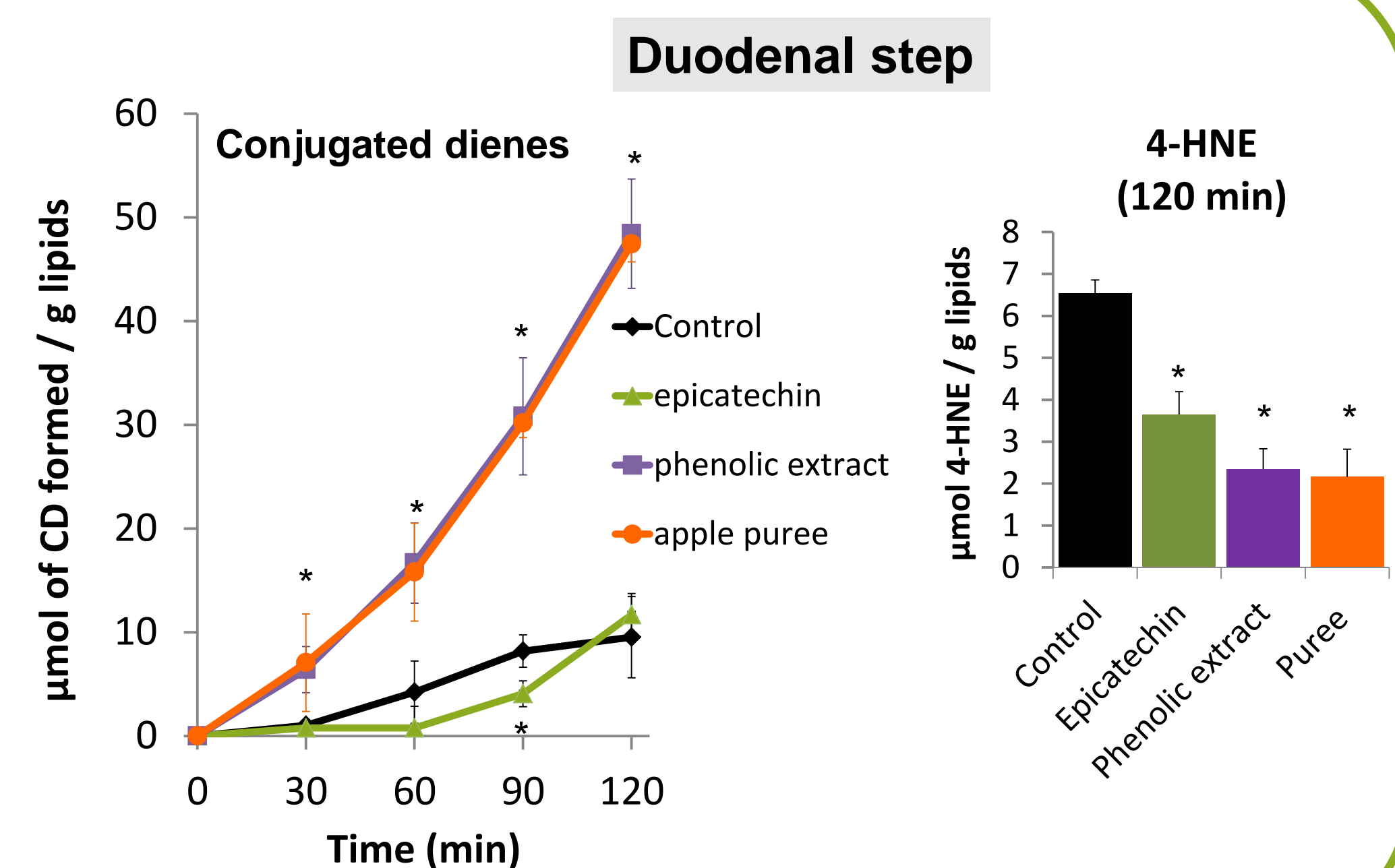
Lipid oxidation / phenolic compounds
Sampling in stomach and duodenum
✓ Conjugated dienes (Abs 234 nm)
✓ 4-hydroxy-2-nonenal / phenolic compounds (UPLC/DAD/MS)

Results & Discussion

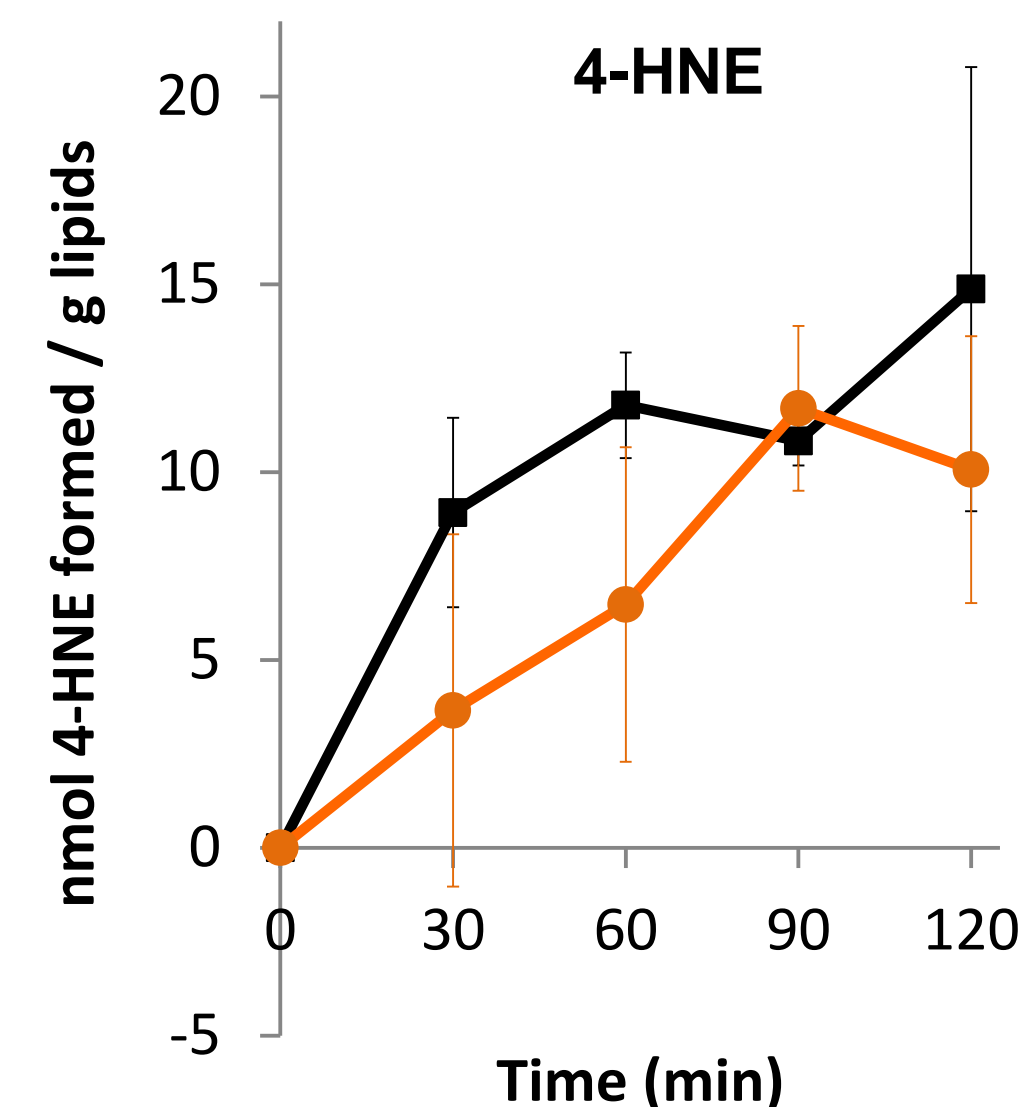
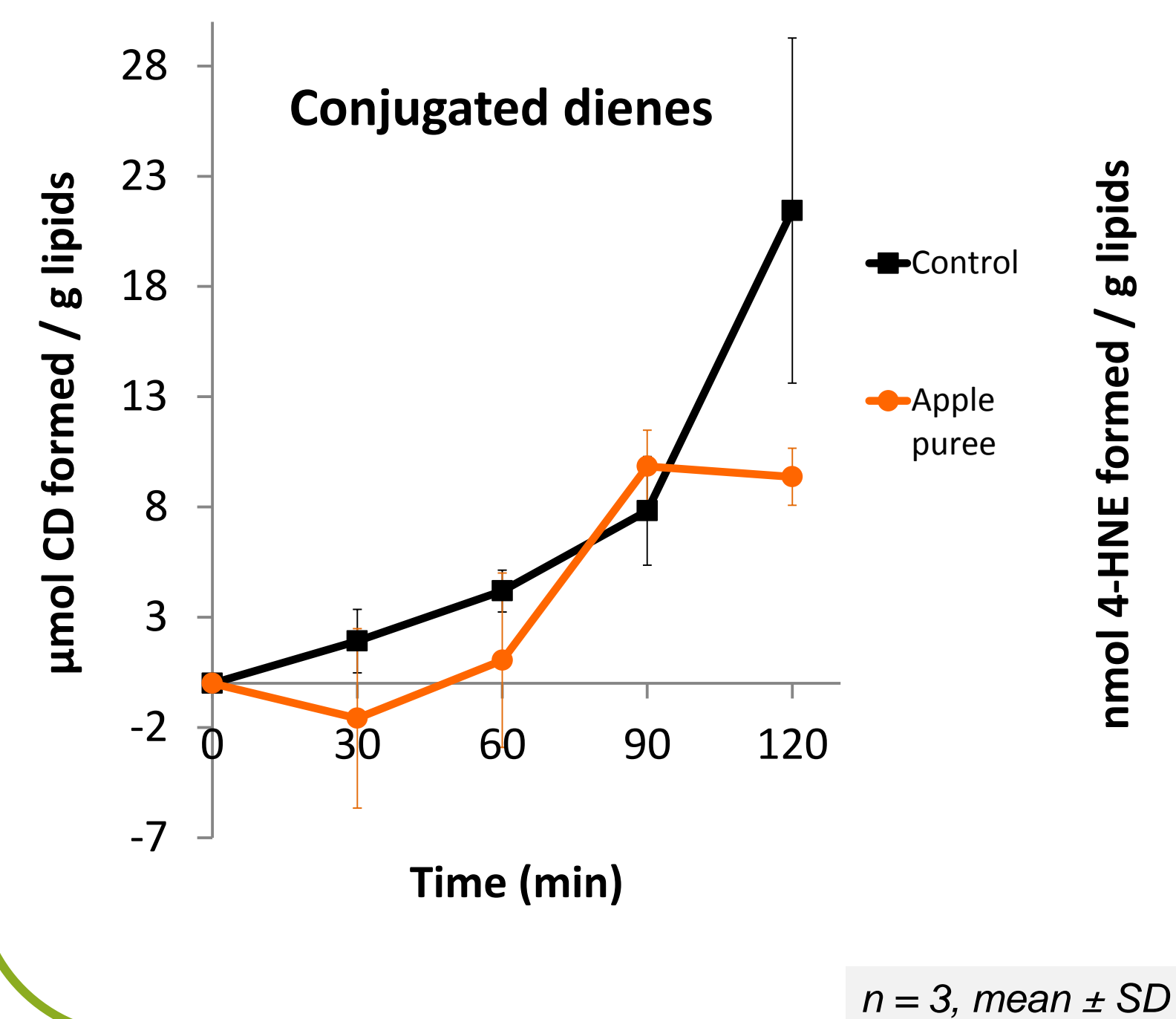
Static *in Vitro* Digestion



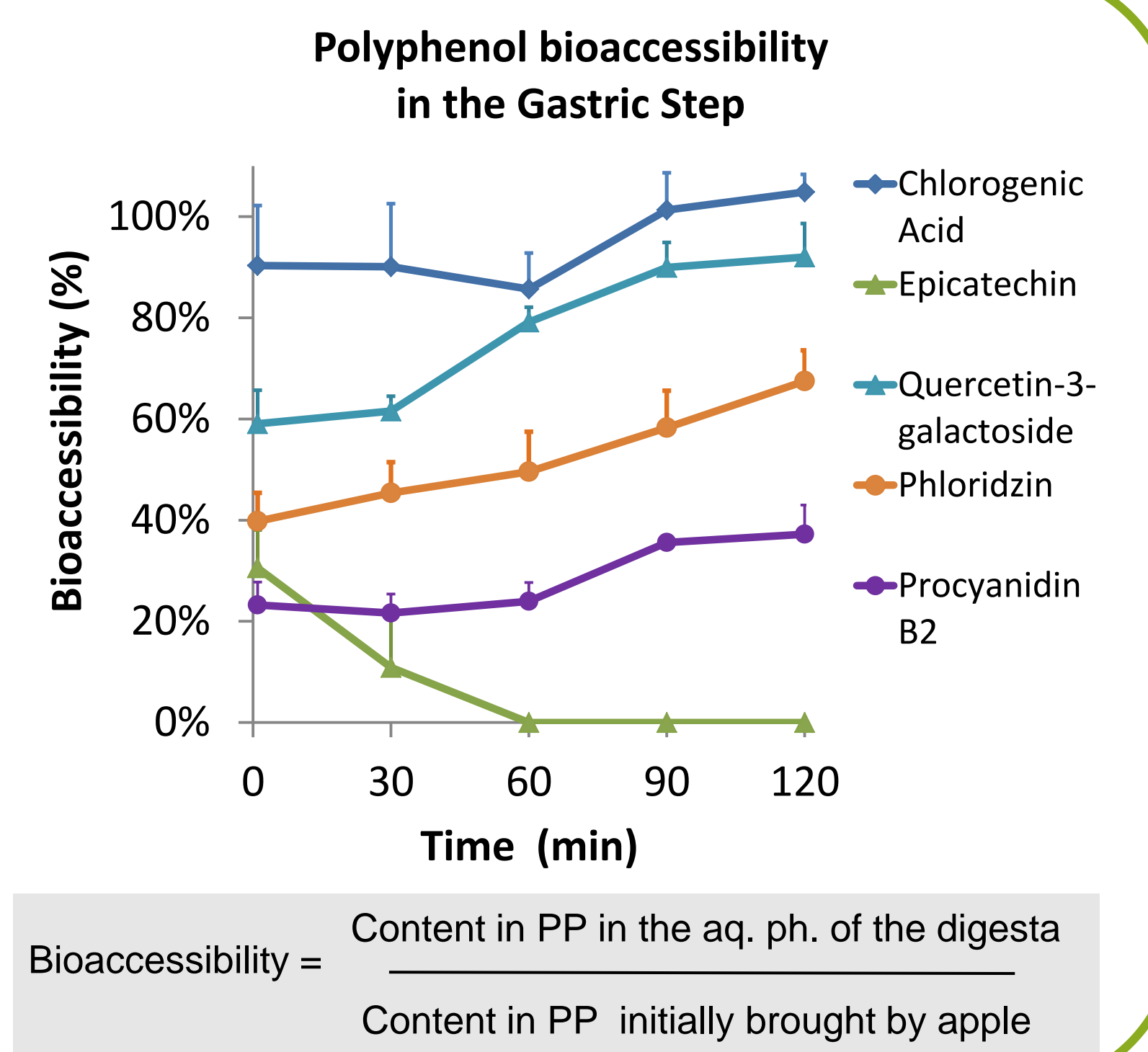
- ✓ Metmyoglobin-initiated lipid oxidation is evidenced during both steps of *in vitro* gastrointestinal digestion through the accumulation of lipid-derived conjugated dienes (CD) and 4-HNE.
- ✓ Epicatechin and apple puree or extract totally inhibited the accumulation of CD and 4-HNE at a dietary level (100 μ M epicatechin equivalents).
- ✓ Opposite results are observed in the duodenal step suggesting that apple products could produce compounds absorbing at 234 nm during proteolysis or lipolysis (to be checked).



Dynamic *in Vitro* Digestion - Gastric Step



- ✓ Metmyoglobin-initiated lipid oxidation is also evidenced during dynamic gastric digestion. Apple puree (1,2 mM total PP) appears to slow down the formation of CD and 4-HNE in a similar way during the first 60 min, although not statistically owing to the heterogeneity of digesta (more replicates needed).
- ✓ Polyphenol bioaccessibility is found to be ca. total for chlorogenic acid and increasing but partial for quercetin and phloretin glycosides. Recovery of procyanidins B1 and B2 as well as epicatechin is low to null owing to binding to proteins and low solubility.



CONCLUSION

MbFe^{III}-initiated lipid oxidation of emulsions stabilized by phospholipids has been demonstrated to occur during gastric digestion in static and dynamic systems as observed earlier *in vivo* in minipigs for a Western type diet [6]. Polyphenols brought by apple proved to be protective, limiting the formation of toxic lipid-oxidation products.

References

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