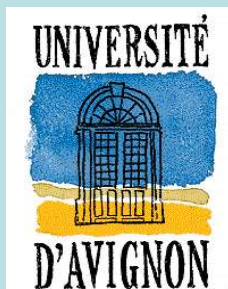


ADEBIOTECH - STRESS OX (31 Mai & 1^{er} Juin 2016)

Biodisponibilité des polyphénols chez l'humain et sa nécessaire prise en compte dans la modélisation de leurs effets sur la santé



Olivier DANGLES
olivier.dangles@univ-avignon.fr



UMR408 Université d'Avignon - INRA
Sécurité & Qualité des Produits d'Origine Végétale



Polyphénols: Abondance Alimentaire

Quantité ingérée quotidiennement par personne (en équiv. aglycone)

Base de données anglaise: 450 - 600 mg

Principales sources de polyphénols = café & thé noir

Clifford & Brown (2006)

Base de données française: \approx 800 mg

Perez-Jimenez et al. (2011)

Pommes et **pommes de terre** fournissent environ 50% du total des polyphénols ingérés via les fruits & légumes en France



P. Brat et al., *J. Nutr.* 2006, 136, 2368-2373



Site Web: <http://www.phenol-explorer.eu/>

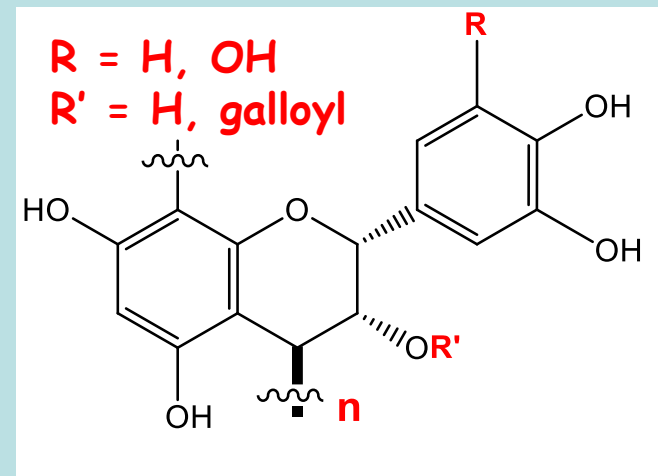
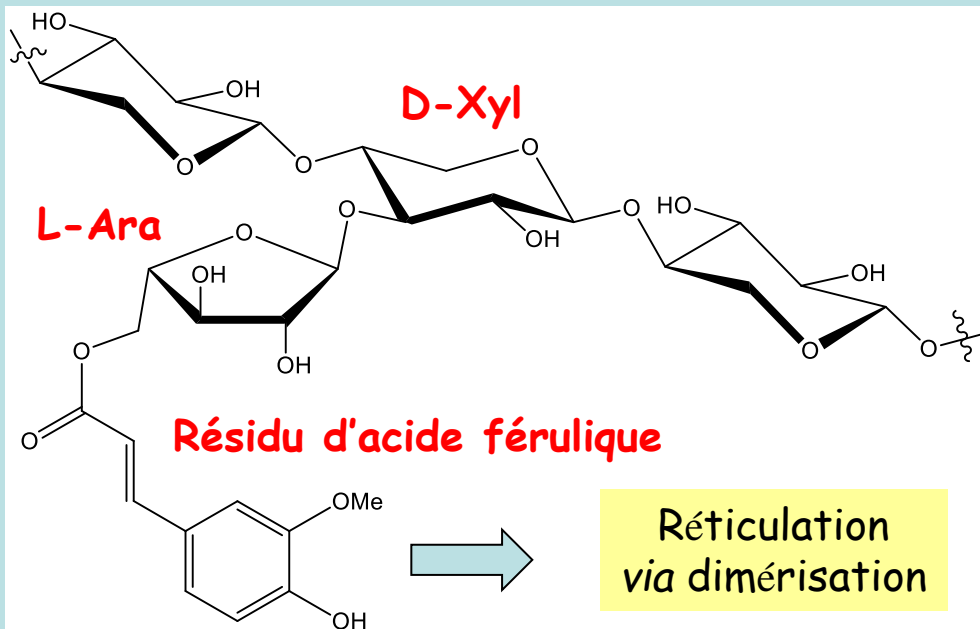
Polyphénols des pommes, pêches & nectarines

Polyphénols extractibles

Obtenus par extraction avec des mélanges méthanol/acétone/eau
18.8 - 28 mg / 100 g de fruit frais

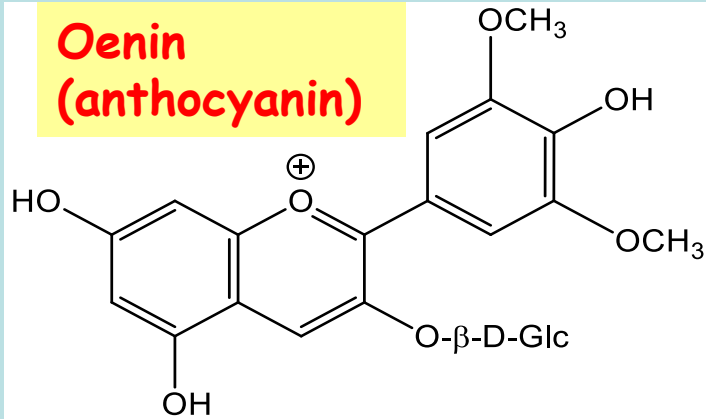
Polyphénols non extractibles: féruloyl-arabinoxylanes (parois végétales)
+ tannins liés aux fibres & protéines

Obtenus par hydrolyse acide des résidus d'extraction
112 - 126 mg / 100 g de fruit frais

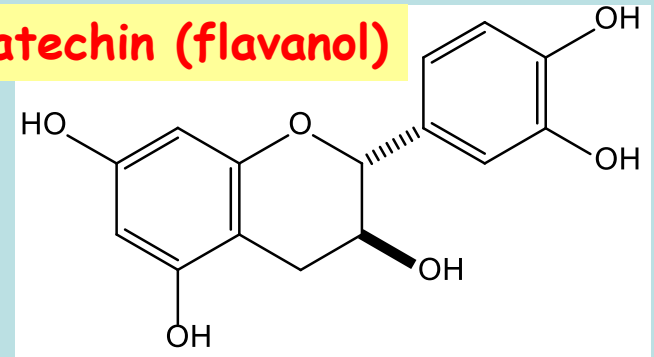


Examples of common plant & food antioxidants

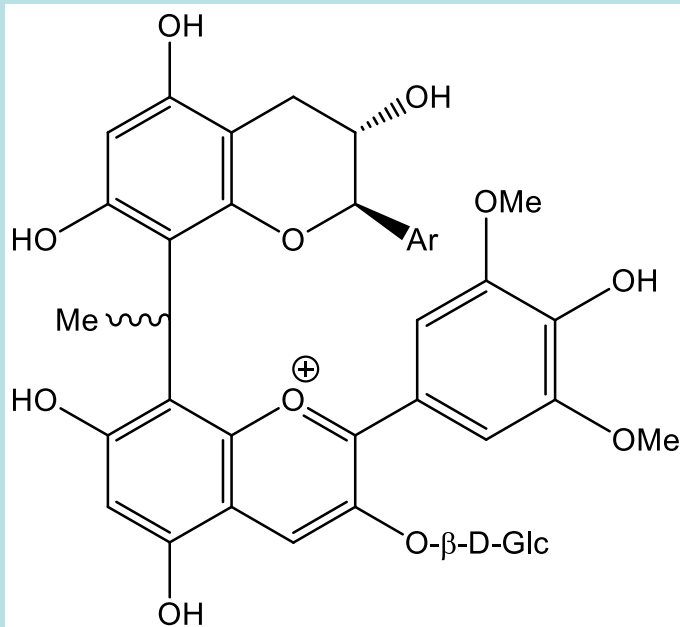
**Oenin
(anthocyanin)**



Catechin (flavanol)



Winemaking & storage



M. Duenas et al., *J. Agric. Food Chem.* 2006, 54, 189

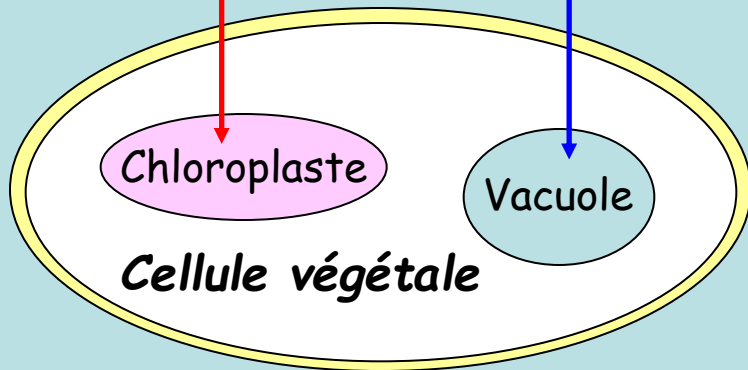
Etape 1: Bioaccessibilité la libération dans le tractus digestif

Aliment cru

Procédés industriels
Pratiques culinaires

Caroténoïdes

Polyphénols



Aliment transformé

Polyphénols Caroténoïdes
+ composés dérivés
Nouvelles interactions avec lipides,
protéines, sucres complexes

Mastication

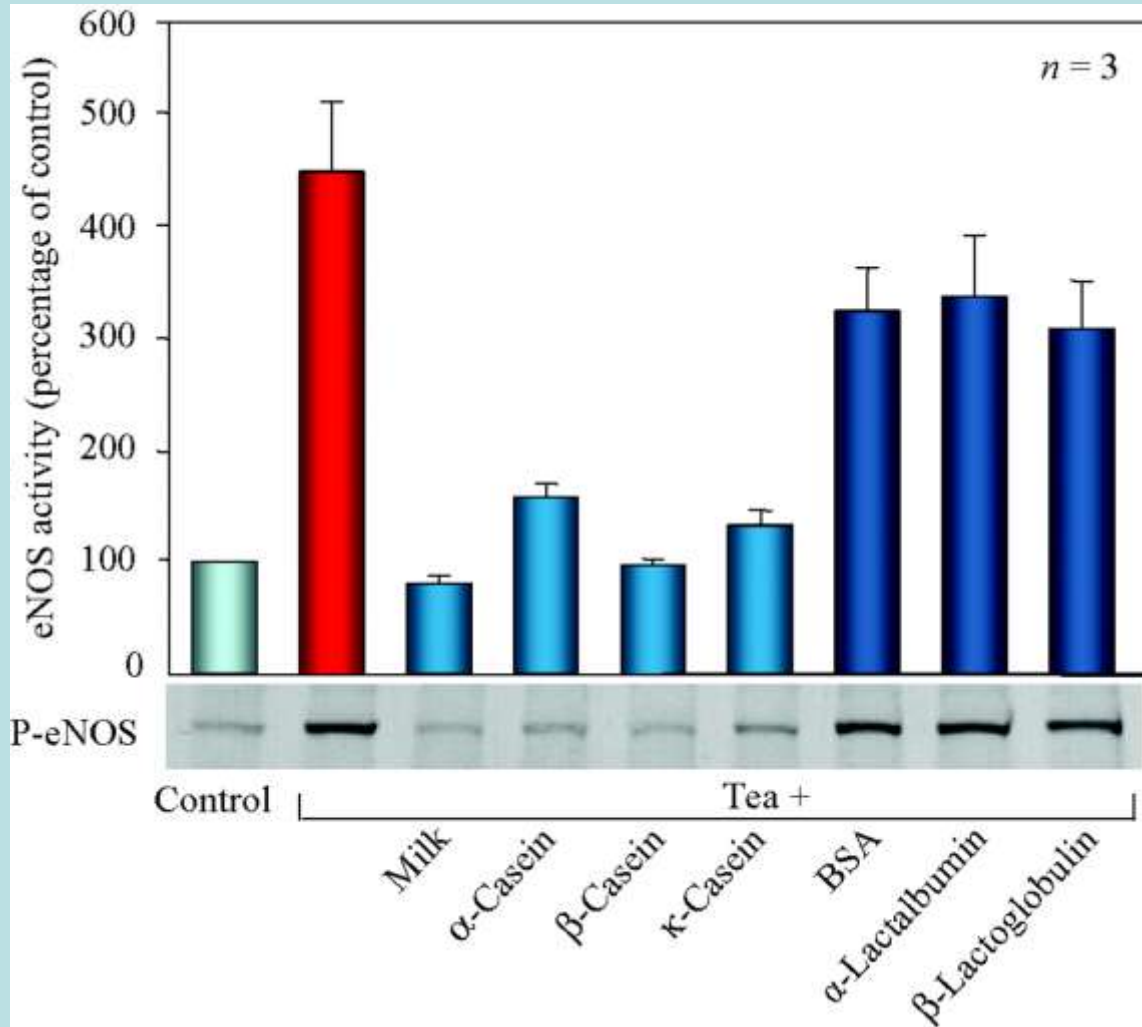
Autres composants du bol alimentaire
Sécrétions humaines (enzymes, bile...)
Broyage, hydrolyse, transfert...

Consommation

Consommation

Polyphénols & **Caroténoïdes** plus ou moins libres dans le tractus digestif
donc +/- disponibles pour franchir la barrière des cellules intestinales

Effet du lait et des protéines du lait sur la production de NO induite par le thé noir dans les cellules endothéliales



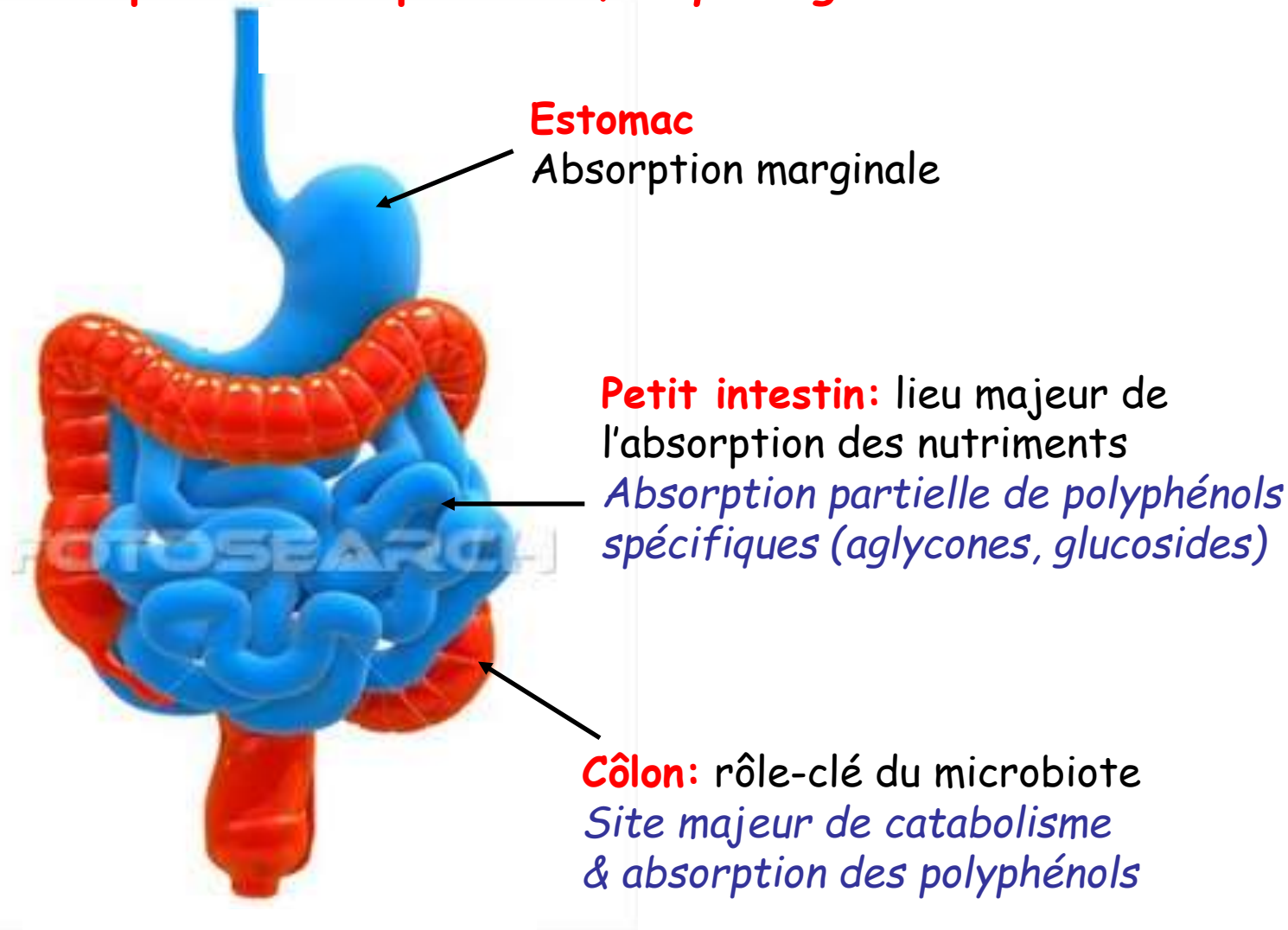
Consommation de thé noir

⇒ Vasodilatation

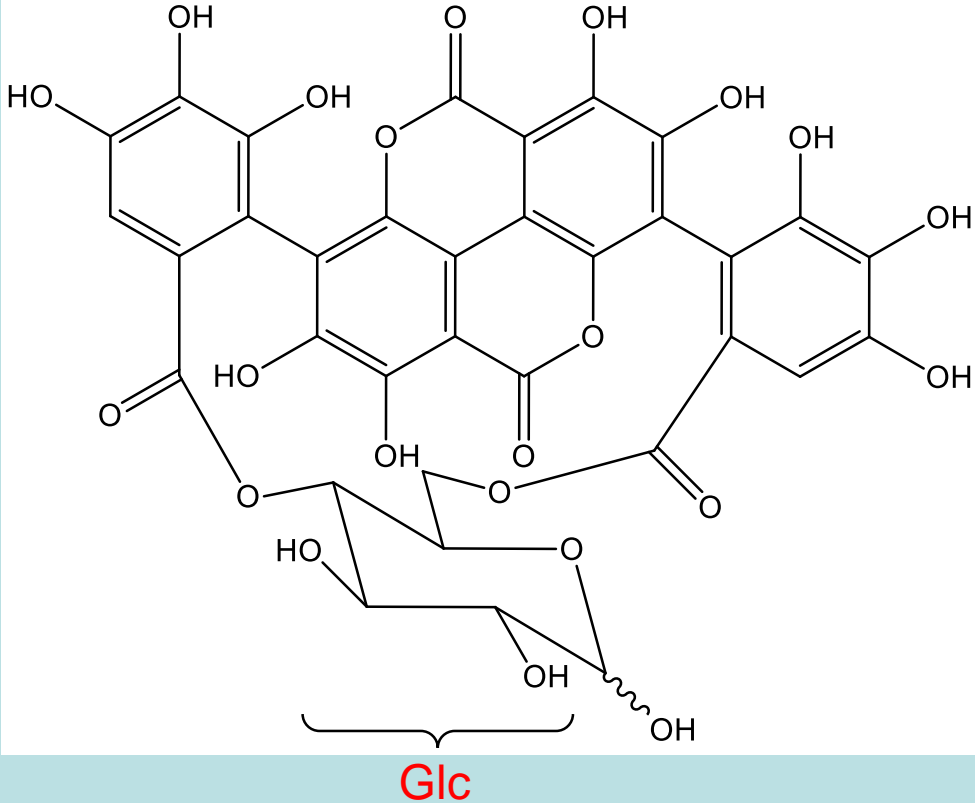
Effet aboli par consommation simultanée de lait

Formation probable de complexes polyphénol - caséine peu solubles

Etape 2: Biodisponibilité, le passage de la barrière intestinale

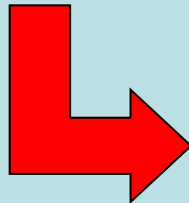


Après absorption, forte conjugaison par les enzymes de l'intestin & du foie

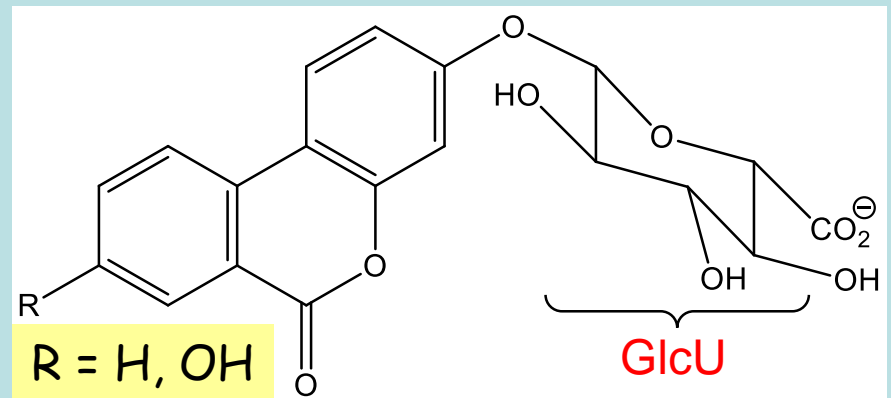


Seeram et al., J. Nutr., 2006, 136, 2481-2485.

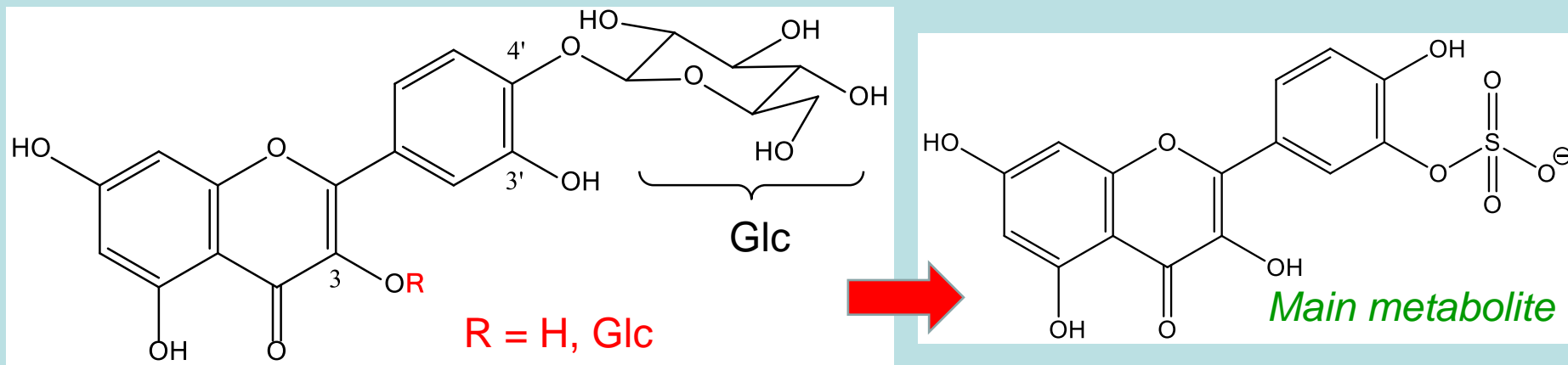
Punicalin, one of the major ellagitannins in pomegranate juice



*Catabolism (microbiota)
+ Conjugation (intestinal & hepatic cells)*



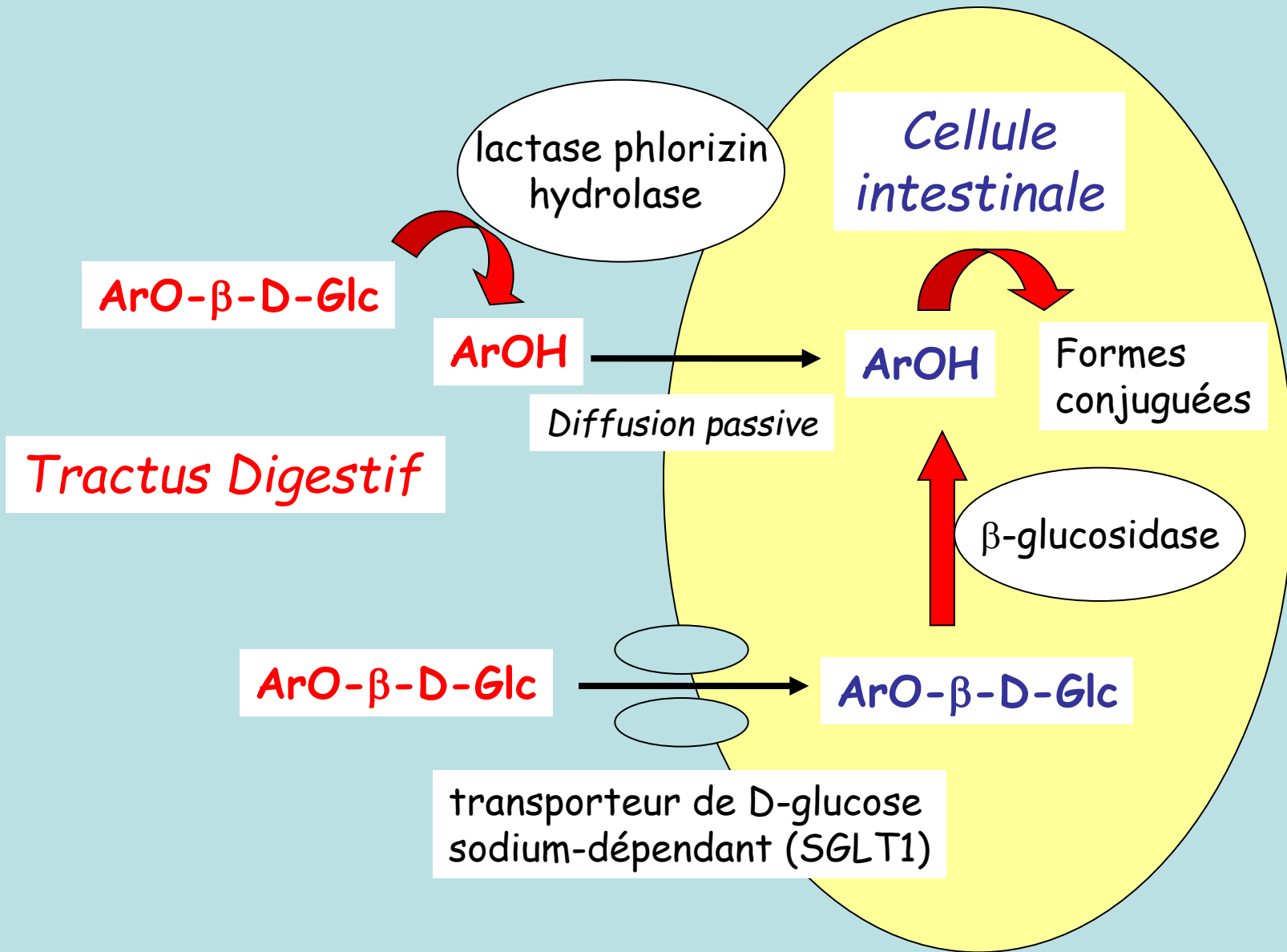
Urolithin A & B 3-O-Glucuronides are the main metabolites in plasma & urine



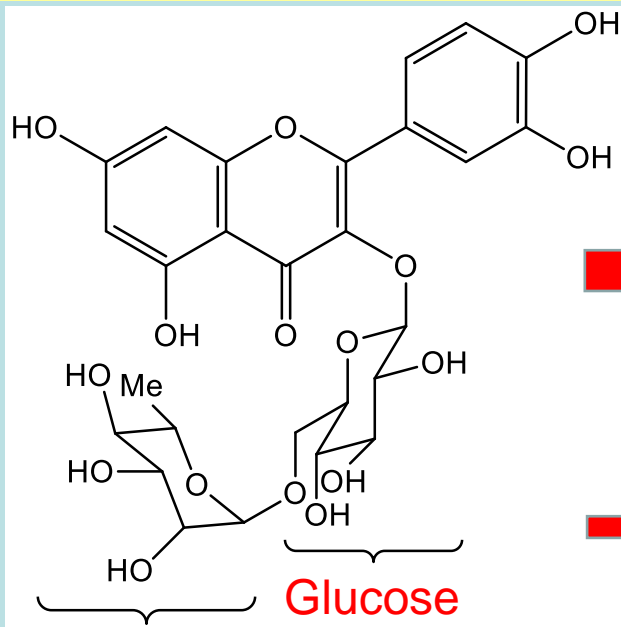
Pharmacokinetic Analysis of Quercetin Metabolites in the Plasma of Volunteers After the Consumption of 270 g of Fried Onions Containing 275 μmol of Flavonol Glucosides ($n = 6$)

Metabolites	C_{max} (nM)	t_{max} (h)	$t_{1/2}$ (h)
3'-O-Sulfonylquercetin	665 (\pm 82)	0.75 (\pm 0.12)	1.71
Quercetin-3-O-glucuronide	351 (\pm 27)	0.60 (\pm 0.10)	2.33
3'-O-Methylquercetin-3-O-glucuronide	112 (\pm 18)	0.60 (\pm 0.10)	5.34
Quercetin-O-diglucuronide	62 (\pm 12)	0.80 (\pm 0.12)	1.76
O-Sulfonylquercetin-O-glucuronide	123 (\pm 26)	2.5 (\pm 0.22)	4.54

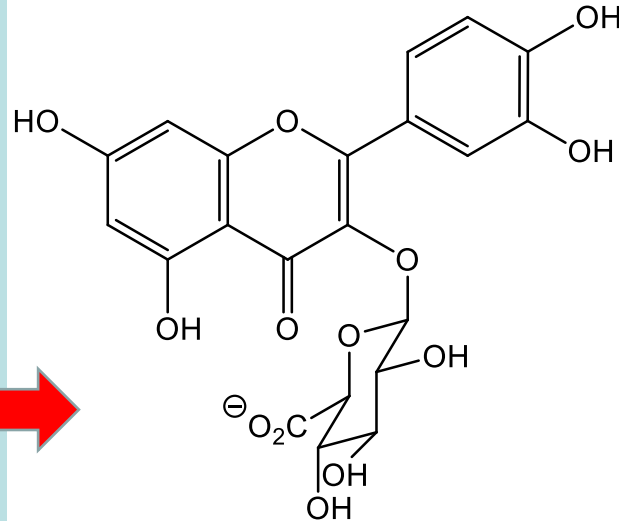
% of excretion after 24h = 4.7% of total intake



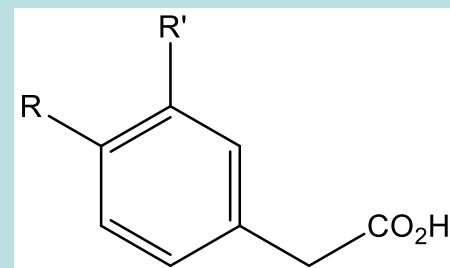
Quercetin-3-O-Rutinoside (rutin)



L-Rhamnose



R = OH, R' = OH
R = OH, R' = OMe
R = H, R' = OH

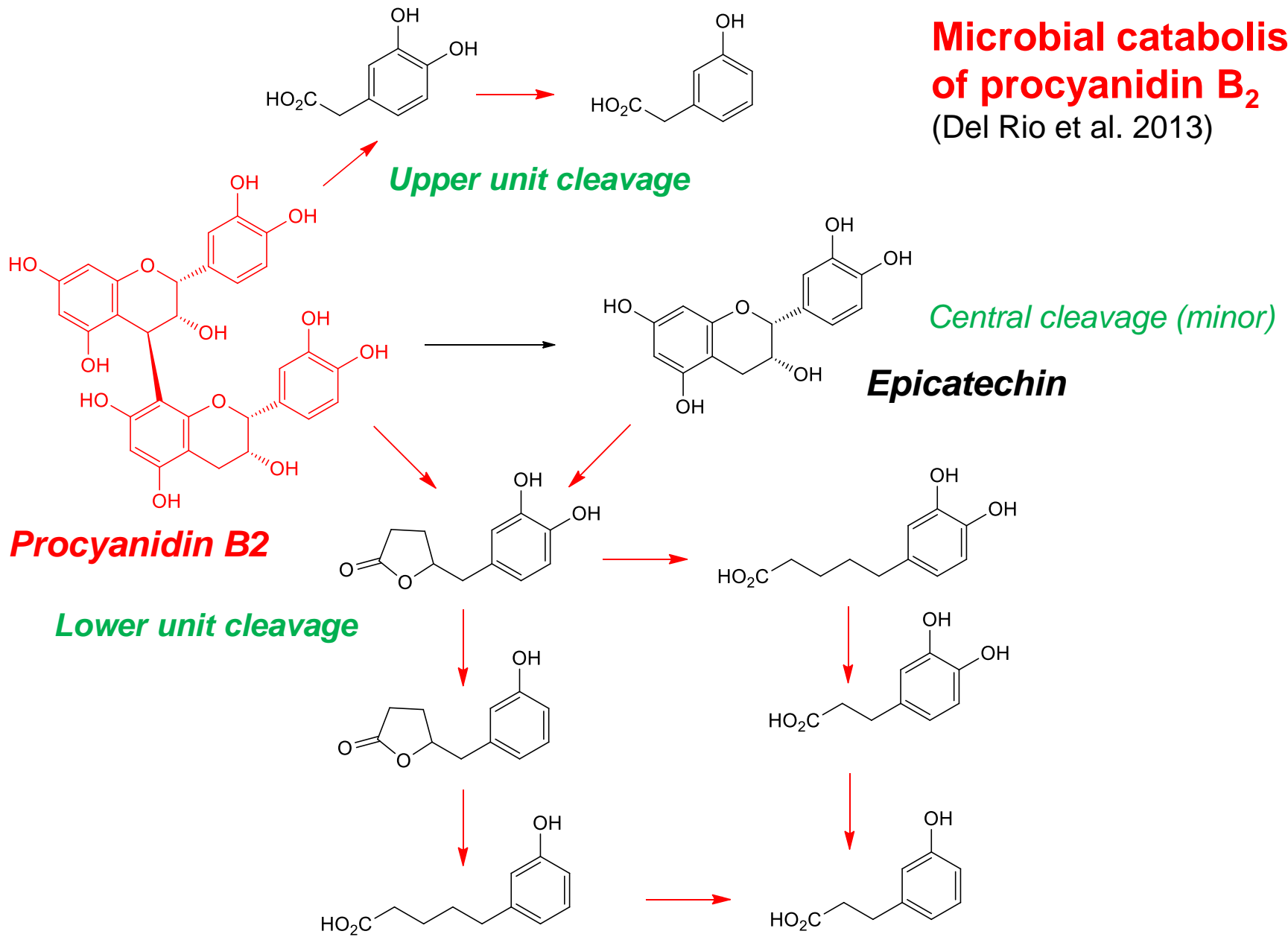


Bacterial catabolites (microbiota)
~ 22% of ingested dose

Pharmacokinetic Analysis of **Quercetin Metabolites** in the Plasma of Volunteers After the Consumption of 250 ml of **Tomato Juice** Containing 176 μmol of **Quercetin-3-O-Rutinoside** ($n = 6$)

Metabolites	C_{max} (nM)	t_{max} (h)
Quercetin-3-O-glucuronide	12 (\pm 2)	4.7 (\pm 0.3)
3'-O-Methylquercetin-3-O-glucuronide	4.3 (\pm 1.5)	5.4 (\pm 0.2)

**Microbial catabolism
of procyanidin B₂**
(Del Rio et al. 2013)



**The bioavailability of catechins (monomers) is high:
Total recovery of catechin metabolites in urine ≈ 70%**

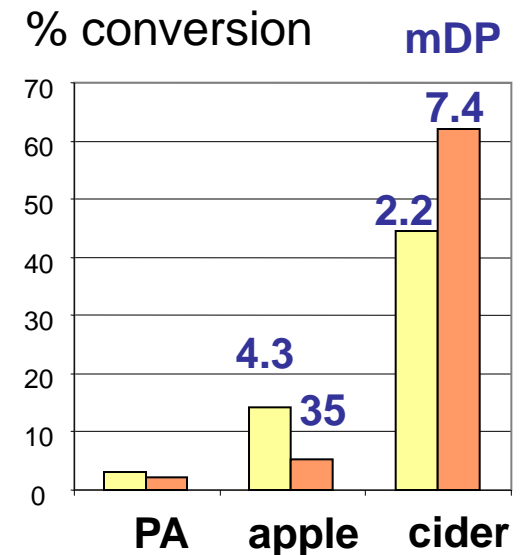
In vitro catabolism of apple procyanidins by the colonic microbiota: extracts / apple powder / cider

$$\text{CR} = \text{conversion rate} = \Sigma [\text{metabolites}] / [\text{tannins}]$$

- Monomers (catechins): CR = 70 - 90%
- *Proanthocyanidins (PAs): CR = 2 - 3% only*
- CR (isolated PAs) < CR(apple) << CR(cider)
- CR lower in apple when DPM ↗
- Max CR reached after 2h for PAs / 8h for apple

Inhibition of microbiota activity by PAs

Less inhibition in the presence of the food matrix



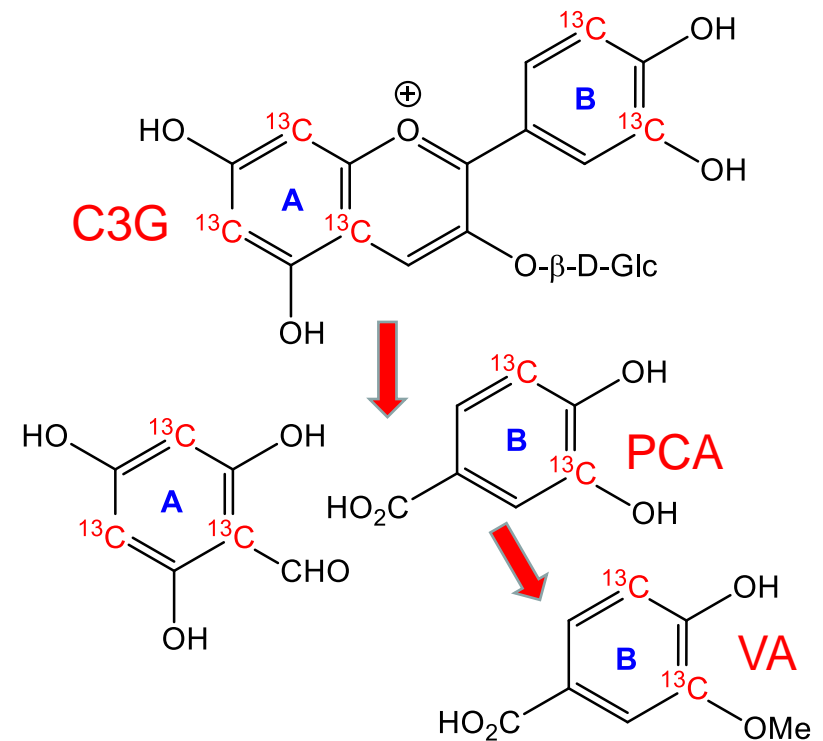
Apple variety

Marie Meynard

Avrolles

Compounds, number of ¹³ C	Location
Parent anthocyanins	
Cyanidin-3-glucoside (C3G) 5	Urine, serum, faeces
Cyanidin-glucuronide (x2) 5	Urine
Peonidin-3-glucoside 5	Urine
Methyl-cyanidin-glucuronides (x3) 5	Urine
Metabolites	
Protocatechuic acid (PCA) 2	Urine, serum, faeces
Phloroglucinaldehyde 3	Urine, serum, faeces
3-Hydroxybenzoic acid 2	Urine, faeces
4-Hydroxybenzoic acid 2	Urine, faeces
Benzoic acid-4-glucuronide 2	Urine, serum, faeces
PCA-3-glucuronide 2	Urine, serum, faeces
PCA-4-glucuronide 2	Urine, serum, faeces
PCA-3-sulfate 2	Urine, serum, faeces
PCA-4-sulfate 2	Urine, serum, faeces
Vanillic acid (VA) 2	Urine, serum, faeces
IsoVA 2	Urine, serum, faeces
IsoVA-3-glucuronide 2	Urine, serum, faeces
VA-4-glucuronide 2	Urine, serum, faeces
IsoVA-3-sulfate 2	Urine, serum, faeces
VA-4-sulfate 2	Urine, serum, faeces
4-Hydroxyphenylacetic acid 2	Urine, faeces
3,4-Dihydroxyphenylacetic acid 2	Urine, faeces
4-Hydroxybenzaldehyde 2	Urine, serum, faeces
3,4-Dihydroxybenzaldehyde 2	Urine, faeces
Caffeic acid 2	Faeces
Ferulic acid 2 & isomers 3	Urine, serum, faeces
Hippuric acid 2	Urine, serum, faeces
4-Methoxybenzaldehyde 2	Faeces
2-Hydroxy-4-methoxybenzoic acid 2	Urine, faeces
Methyl vanillate 2	Faeces
Methyl-3,4-dihydroxybenzoate 2	Urine, serum, faeces

HPLC-MS/MS identification (MRM) of ¹³C-labelled cyanidin-3-glucoside (C3G) and its metabolites in the serum, urine and faeces of healthy volunteers (n = 8) after the consumption of 500 mg of ¹³C-labelled C3G



De Ferrars *et al.*, *Br. J. Pharmacol.* 2014, 171, 3268–3282

Serum pharmacokinetic profiles of C3G, its degradation products and derived metabolites in humans after the consumption of 500 mg ¹³C-labelled C3G^a

Metabolite	n ^b	C _{max} (nM)	t _{max} (h)	t _{1/2} (h)	AUC ₀₋₄₈ (nM h)
Parent anthocyanins					
Cyanidin-3-glucoside	5	141 ± 70	1.8 ± 0.2	0.4	279 ± 170
Degradation products					
Protocatechuic acid (PCA)	8	146 ± 74	3.3 ± 0.7	9.9 ± 3.4	1377 ± 760
Phloroglucinaldehyde	4	582 ± 536	2.8 ± 1.1	NQ	7882 ± 7768
Metabolites					
Benzoic acid-4-glucuronide	7	74 ± 20	10.9 ± 3.4	17.1 ± 3.0	1467 ± 489
Methyl-3,4-dihydroxybenzoate	8	12 ± 5	8.4 ± 5.7	21.6 ± 5.9	171 ± 70
PCA-3-glucuronide	5	11 ± 3	2.7 ± 1.0	18.0 ± 15.6	60 ± 38
PCA-4-glucuronide	8	68 ± 61	3.8 ± 0.8	19.4 ± 3.1	618 ± 489
PCA-sulfates ^c	8	157 ± 116	11.4 ± 3.8	31.9 ± 19.1	1180 ± 349
Vanillic acid (VA)	2	1845 ± 838	12.5 ± 11.5	6.4	23 319 ± 20 650
VA-4-glucuronide	8	24 ± 4	4.8 ± 0.4	NQ	74 ± 11
IsoVA-3-glucuronide	8	35 ± 5	4.3 ± 0.6	1.6 ± 0.2	103 ± 13
VA-sulfates ^c	4	430 ± 299	30.1 ± 11.4	NQ	10 689 ± 7751
4-Hydroxybenzaldehyde	7	667 ± 653	5.6 ± 3.1	17.9 ± 8.8	663 ± 505
Ferulic acid	7	827 ± 371	8.2 ± 4.1	21.4 ± 7.8	17 422 ± 11 054
Hippuric acid	8	1962 ± 1389	15.7 ± 4.1	95.6 ± 77.8	46 568 ± 30 311
Ferulic acid isomers ^d	6	87 ± 38	13.3 ± 7.9	96.5 ± 73.4	1816 ± 1054

^aValues are expressed as mean ± SEM.

^bMetabolite detected in *n* = number of participants.

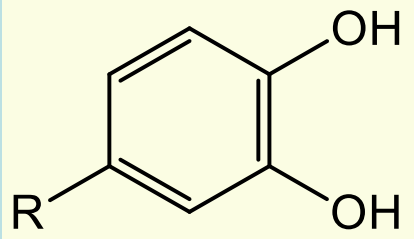
^cPCA-sulfate and VA-sulfate isomers could not be separated, values presented are cumulative concentration of both.

^dAlternative isomers of ferulic acid include 2-hydroxy-4-methoxycinnamic acid or 4-hydroxy-2-methoxycinnamic acid

Etape 3: Bioactivité des Polyphénols

Les polyphénols sont capables de réduire rapidement les espèces oxygénées réactives dans la plante, l'aliment & le tractus digestif

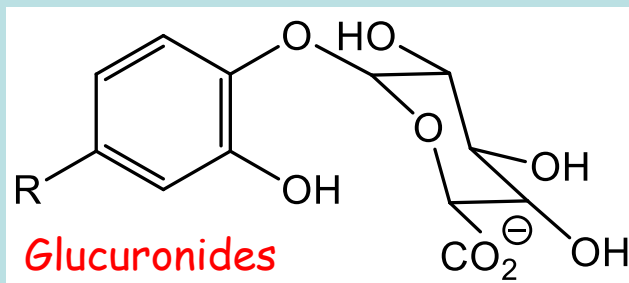
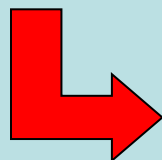
Review: O. Dangles, *Current Organic Chemistry* 2012, 16, 692-714



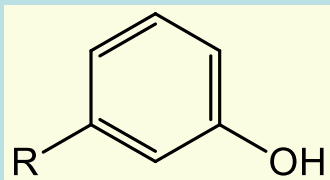
Forme native: noyau catéchol \Rightarrow antioxydant
Forte conc. dans le tractus digestif (~ 1 mM)

Mais sont peu à même de perpétuer cette action antioxydante au-delà (tissus)

Metabolisme
chez l'humain



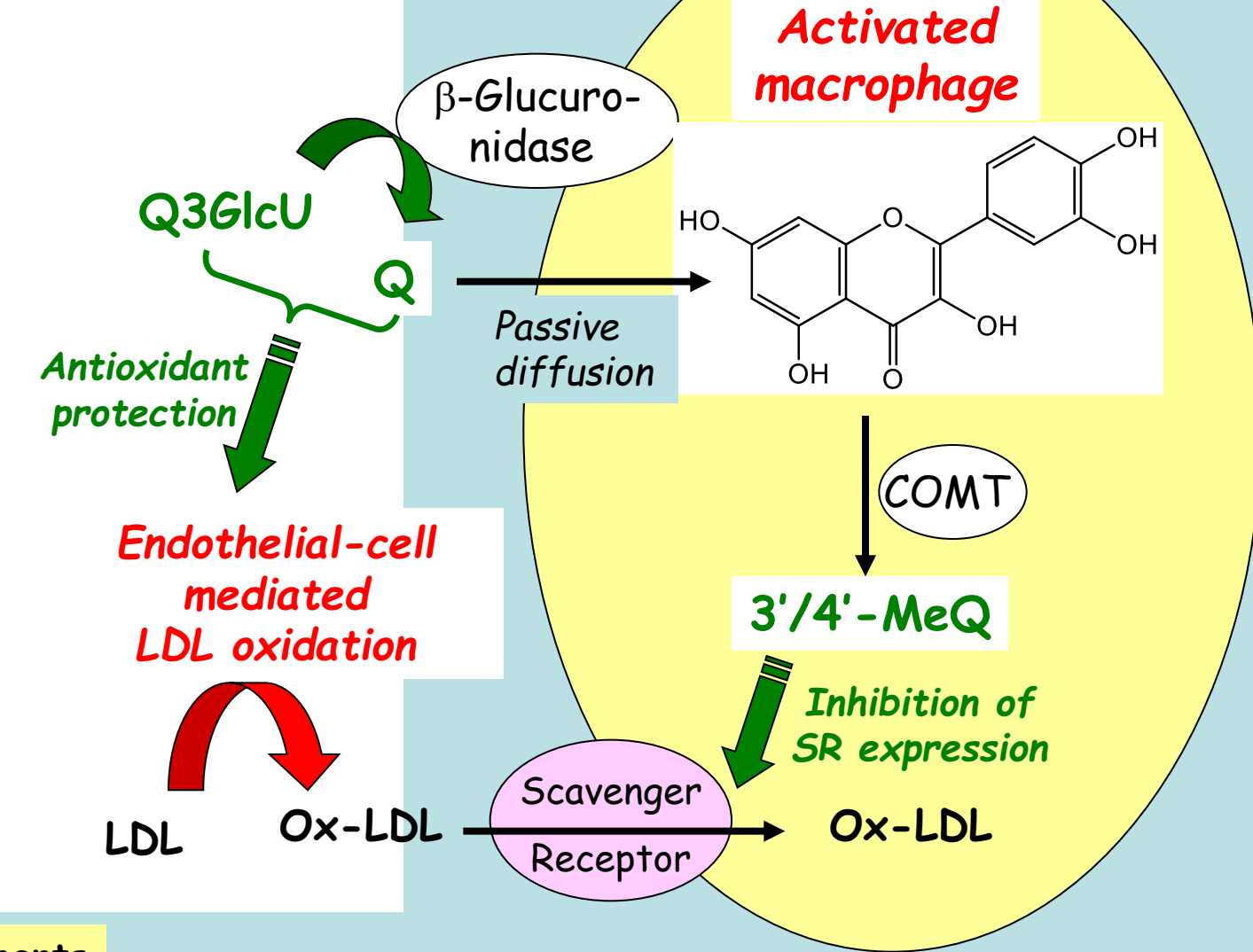
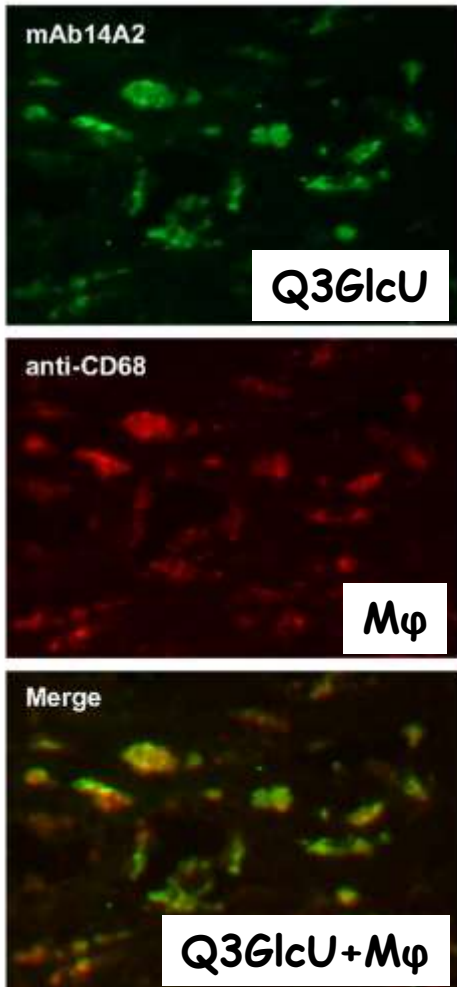
Cellules intestinales &
hépatiques: conjugaison



Microbiote (côlon): nombreuses voies,
y compris la désoxygenation

Une distribution de métabolites circulants (faibles conc., < 1 μ M)
Potentiellement bioactifs mais faiblement antioxydants

Q3GlcU specifically accumulated in human atherosclerotic lesions in association with macrophages



Immunostaining experiments

Antioxidant Activity of Polyphenols & Carotenoids in the GI tract

Dietary Bolus

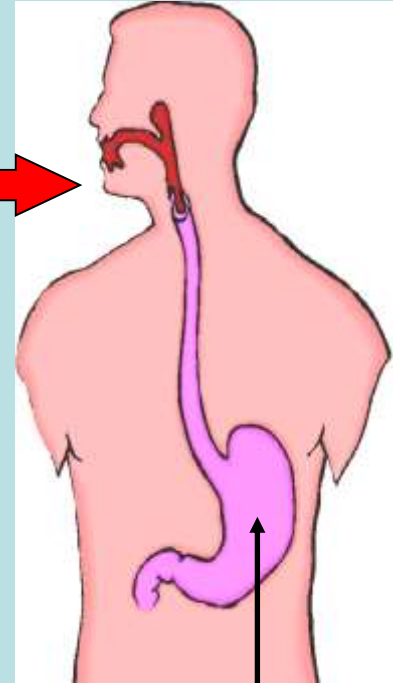
- **Oxidizable Nutrients:** polyunsaturated lipids, proteins...
- **Electron-rich (reducing) micronutrients:**
Polyphenols, carotenoids, vitamins C & E

• **Initiators of oxidative processes**

Iron (heme & non-heme): ca. 30 μg / g of beef (heme > 80%)

Copper: up to 10 μg / g in vegetables & fruit

Hydroperoxides: typical PV of fresh oils = 1 - 4 μmol / g



The stomach is a potential site for dietary lipid autoxidation

Kanner & Lapidot, *Free Radical Biol. Med.* 2001, 31, 1388-1395

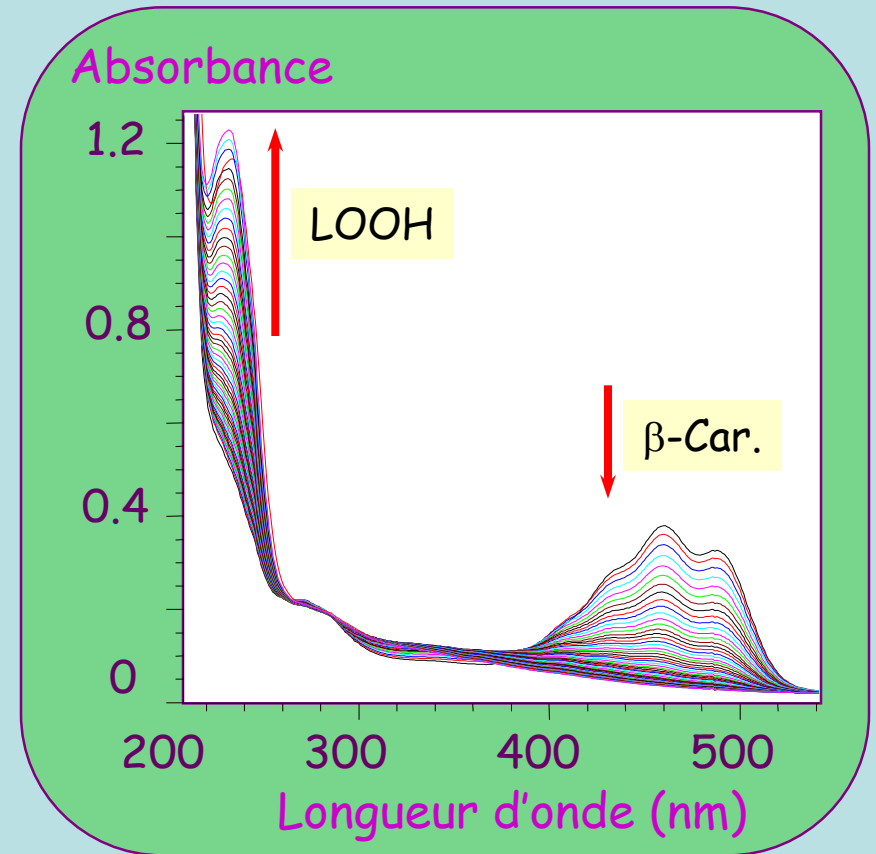
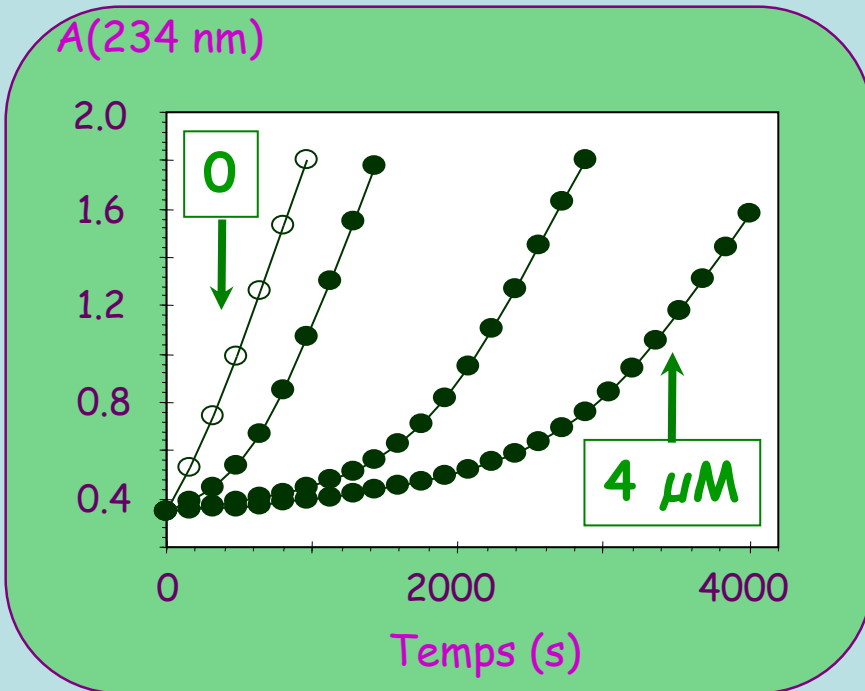
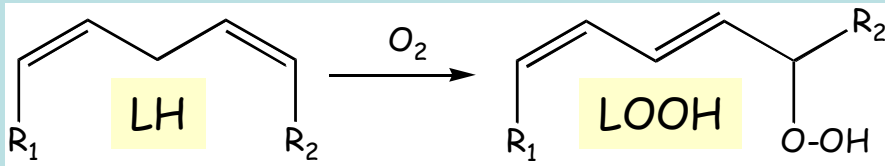
O₂, pH 6 → 2

**Loss of essential lipids, formation of reactive lipid oxidation products
⇒ modifications of dietary proteins, cardiovascular risk?**

Possible inhibition by dietary antioxidants such as polyphenols

Inhibition de la peroxydation lipidique induite par la metmyoglobine

Acide linoléique (0.7 mM) + β -Carotène (0-4 μ M) + Metmyoglobine (100 nM)
(37°C, pH 5.8, 2 mM Tween 20)



Inhibition de la peroxydation de l'acide linoléique induite par la metmyoglobine

Acide linoléique (0.7 mM) + Metmyoglobine (100 nM)
(37°C, pH 5.8, 2 mM Tween 20)

Antioxydant	IC ₅₀ / μM
Quercétine	0.33
Rutine	0.63
Acide caféique	0.49
Acide chlorogénique	0.51
Oenine	0.27
α-Tocophérol (vit. E)	0.28
β-Carotène	1.52

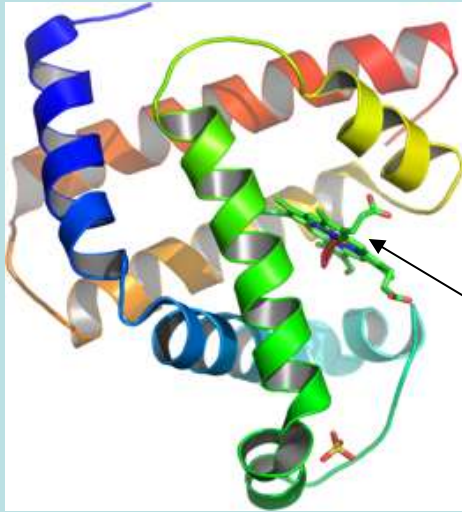
Bonne efficacité des polyphénols communs de l'alimentation

IC₅₀ = conc. d'antioxydant permettant de doubler le temps requis pour accumuler une conc. fixée de LOOH

N.B.: rutine = quercétine 3-β-D-Glc-1,6-α-L-Rha, oenine = malvidine-3-β-D-Glc

Peroxydation lipidique induite par le fer héminique & mécanismes d'inhibition

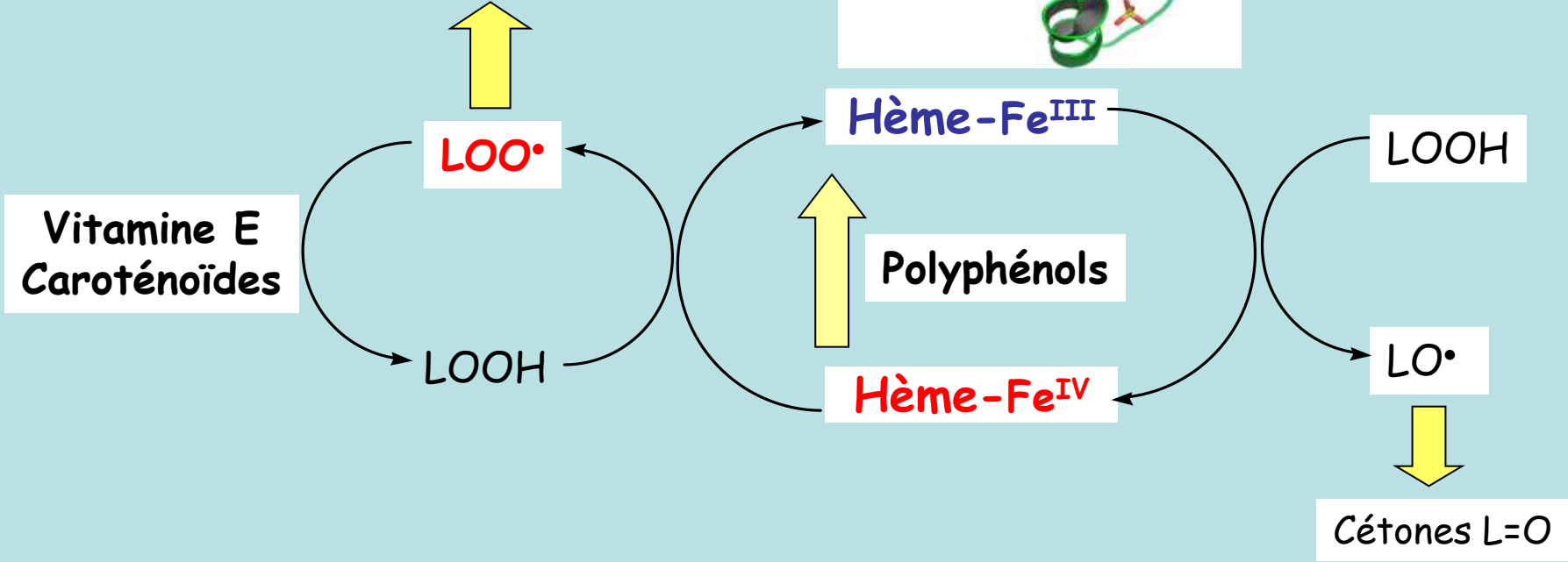
Lipide polyinsaturé LH
+ traces d'hydroperoxydes lipidiques LOOH



Fer héminique
Metmyoglobine
(viande rouge)

Cofacteur
(hème)

Propagation de l'oxydation



E. Vulcain, P. Goupy, C. Caris-Veyrat, O. Dangles, *Free Radical Res.* 2005, 39, 547-563.
P. Goupy, E. Vulcain, C. Caris-Veyrat, O. Dangles, *Free Radical Biol. Med.* 2007, 43, 933-946.
B. Lorrain, C. Dufour, O. Dangles, *Free Radical Biol. Med.* 2010, 48, 1162-1172.

Extension à des Emulsions Huile (tournesol) dans Eau

Interface: BSA



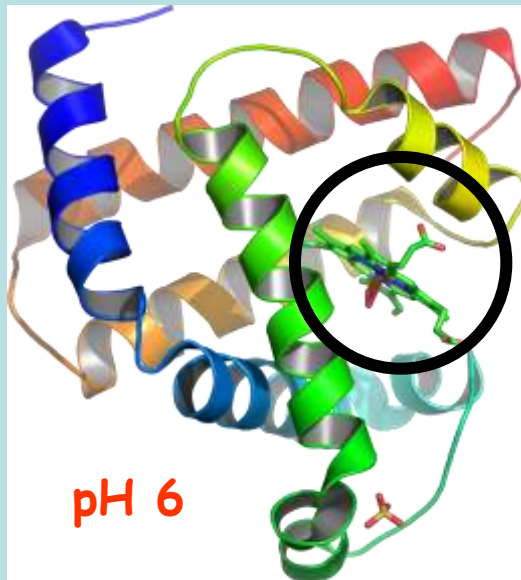
$$d_{[3,2]} = 2.4 \mu\text{m}$$

Interface: phospholipides d'oeuf (PL)

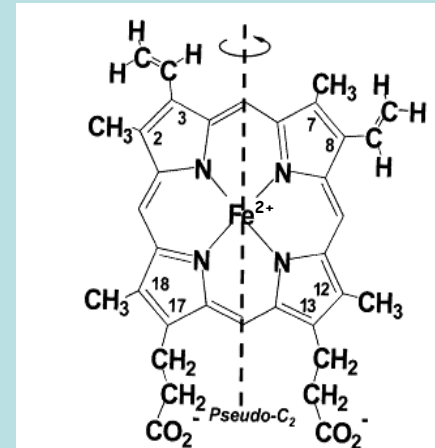


$$d_{[3,2]} = 2.8 \mu\text{m}$$

Initiateur = metmyoglobine



Metmyoglobine



Hématine

pH 4

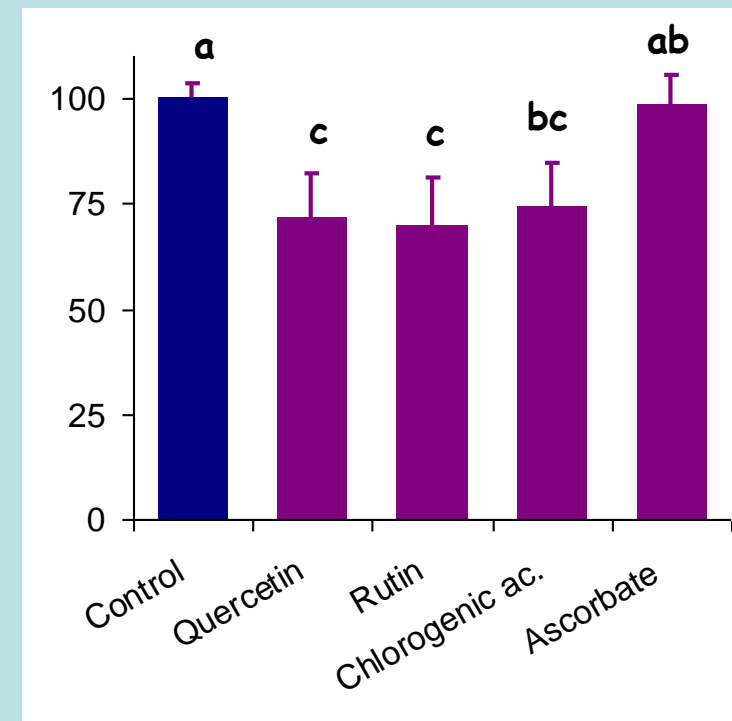
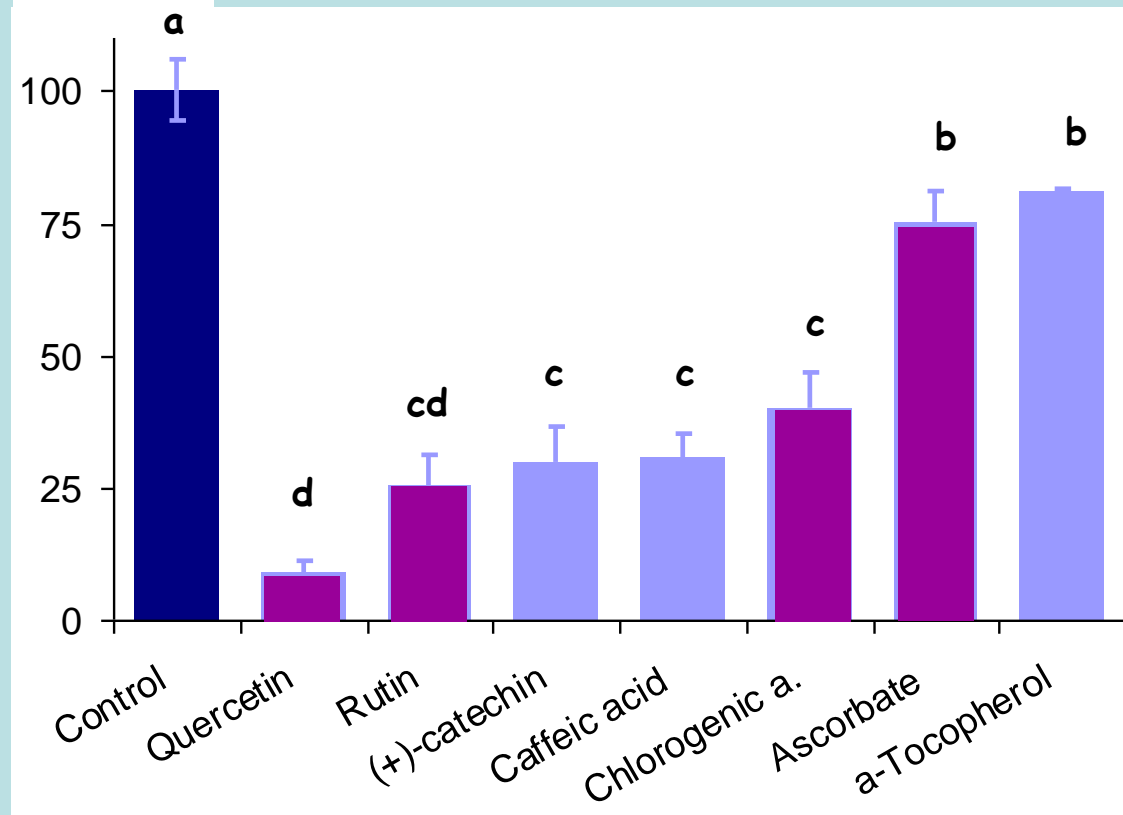
+ Globine libre

Peroxydation d'émulsions d'huile de tournesol Interface BSA (10 g huile / 100 mL, 20 μ M metmyoglobine)

pH 6

pH 4

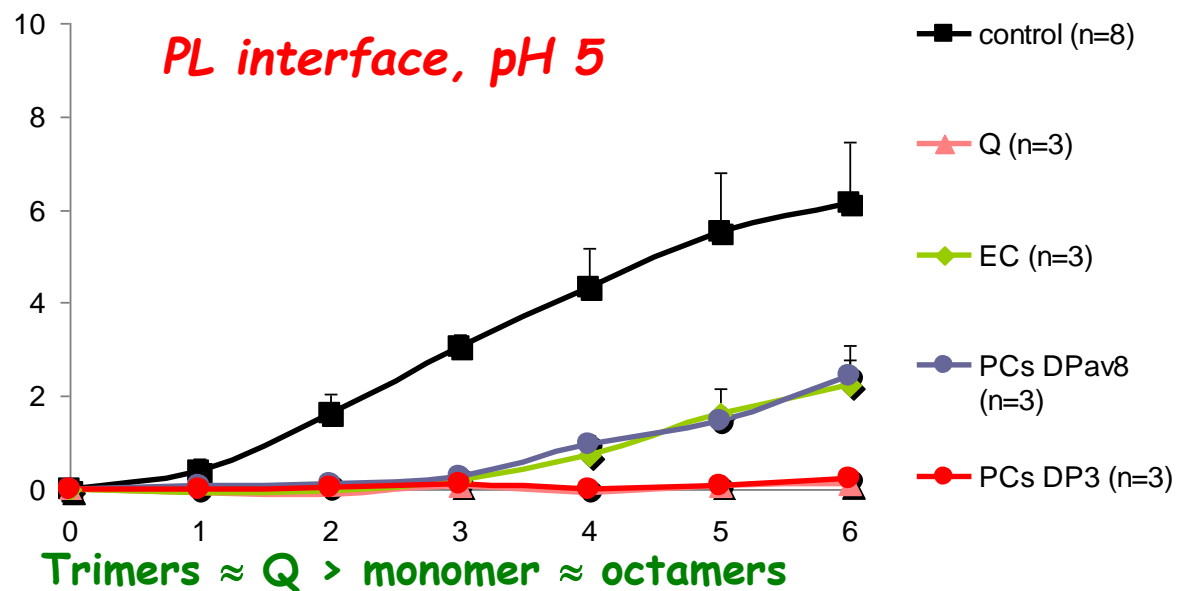
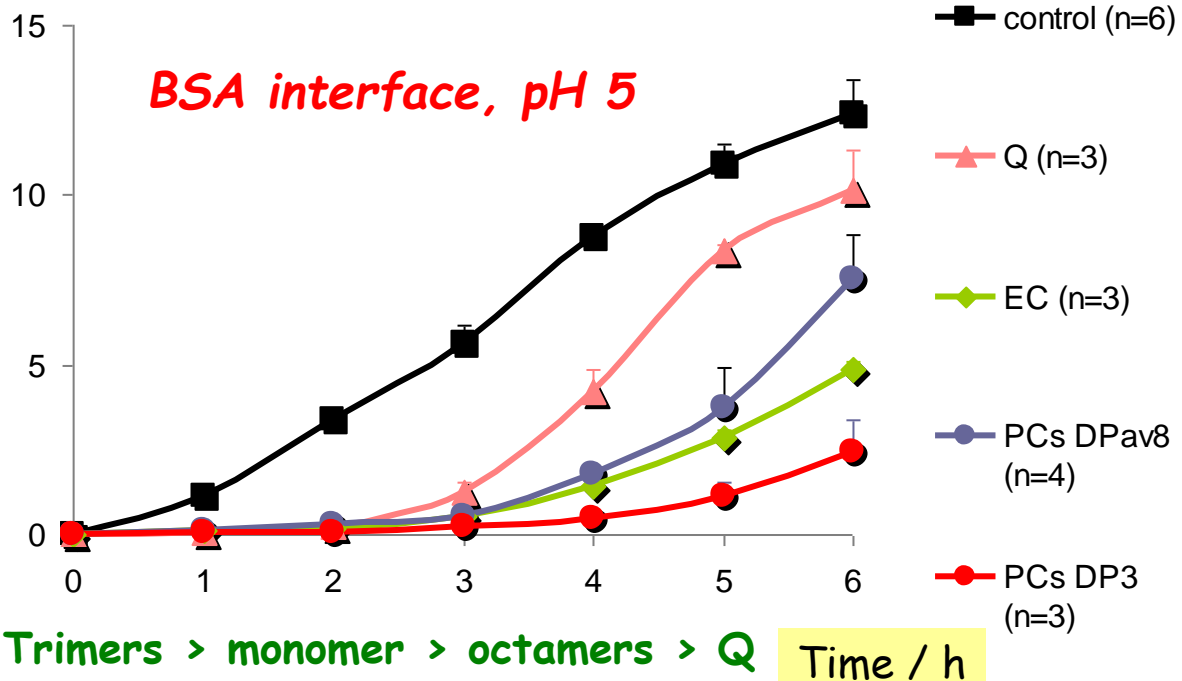
Rel. CDs



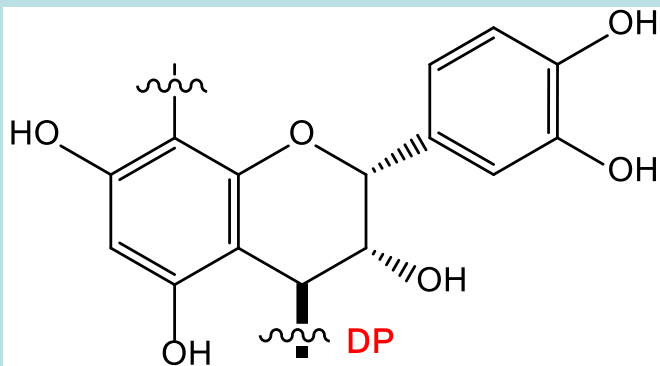
*Idem avec les émulsions stabilisées par les phospholipides:
protection dans la 1ere phase de la digestion gastrique*

[Antioxydant] = 100 μ M
Tukey-Kramer p < 0.05

Conjugated dienes / mM



Condensed tannins, a major class of dietary polyphenols, are potent antioxidants



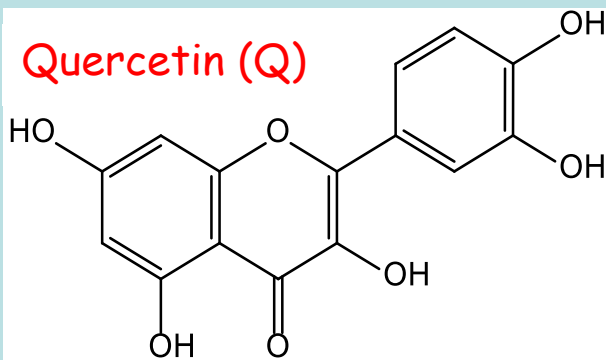
Apple procyanidins (PCs)

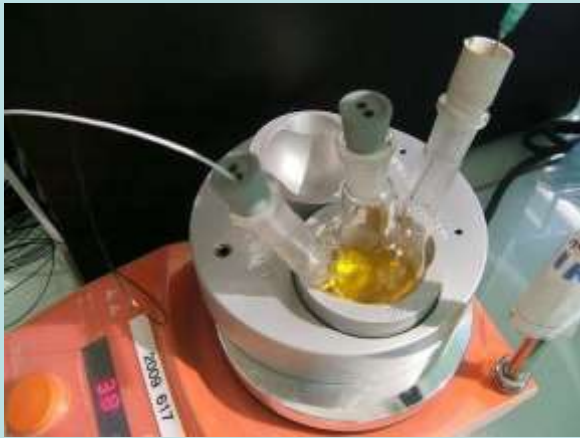
DP1: monomer (EC)

DP3: mixture of trimers

DPav8: mixture of oligomers with averaged DP = 8

Quercetin (Q)

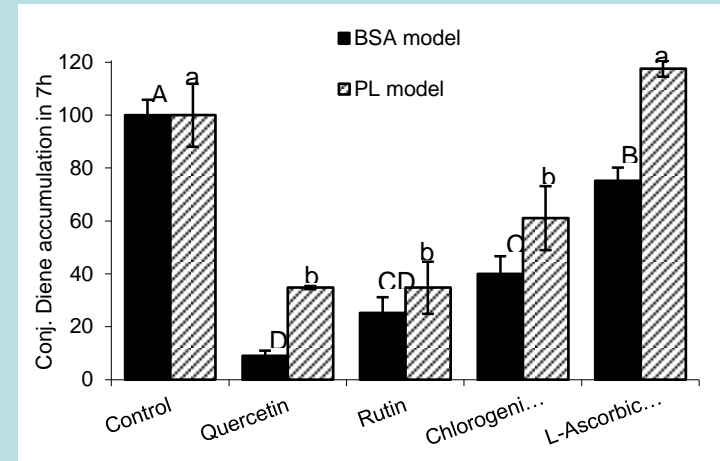




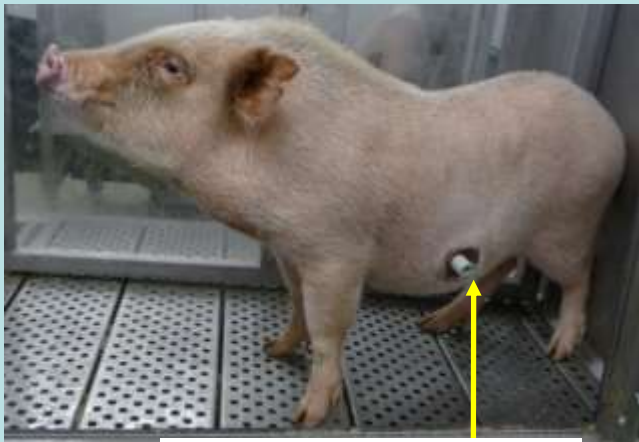
In vitro models



o/w emulsion
+ Heme/non-heme iron

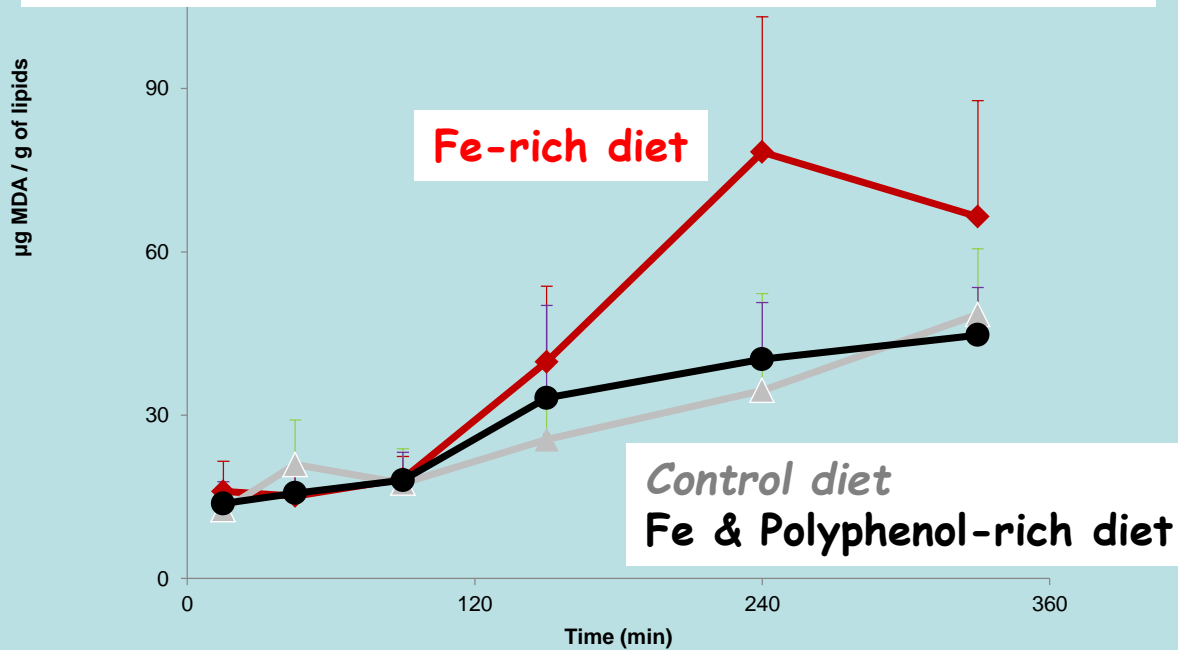


In vivo validation The minipig model



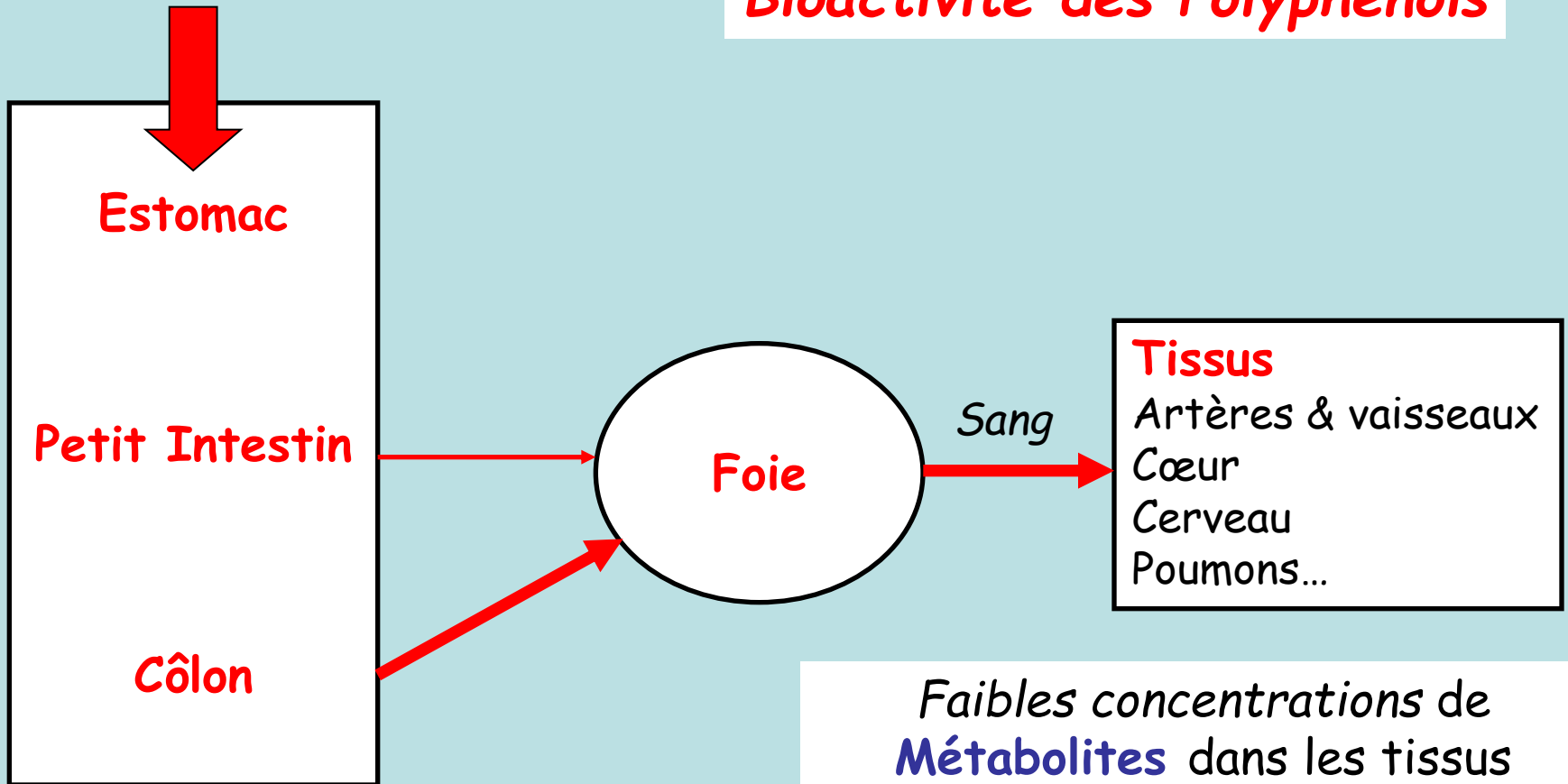
Intragastric canula

INRA program LIPROPHEN, coord.: C. Dufour



Produits Végétaux

Bioactivité des Polyphénols



Fortes concentrations de **Polyphénols** dans le tractus digestif
⇒ **Action antioxydante directe** (capture d'espèces oxydantes)

Faibles concentrations de **Métabolites** dans les tissus
⇒ **Action anti-inflammatoire**
Action antioxydante indirecte (ex.: stimulation de la biosynthèse des enzymes antioxydantes, inhibition des enzymes pro-oxydantes)