SFR TERSYS: *Journée d'Echanges sur le Stress Oxydant*

Jeudi 29 juin 2017

Biodisponibilité & Effets Antioxydants des Polyphénols des Fruits & Légumes

Olivier DANGLES

UMR408 *Sécurité & Qualité des Produits d'Origine Végétale*

Diet rich in
Plant Products

Epidemiology ↓

With moderation

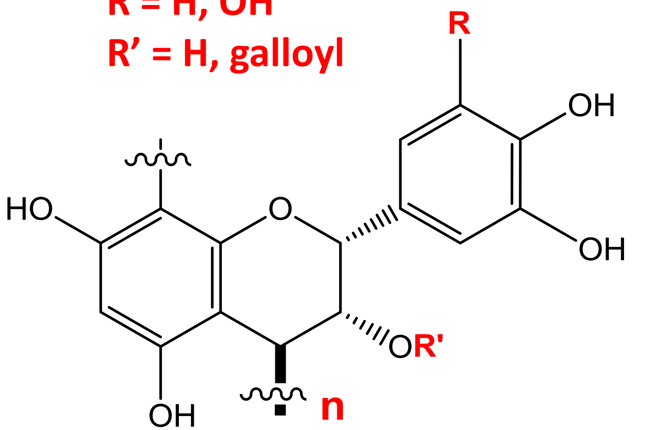
Apparent protection against cardiovascular disease,
possibly also against some cancers & age-related disorders

Non-essential micronutrients, mainly plant phenols & carotenoids, probably play a role in this protection

The main classes of dietary plant phenols

Flavanols

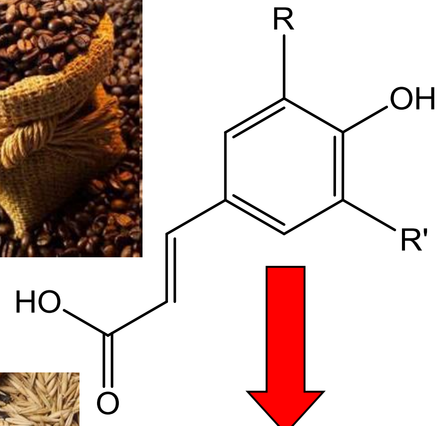
R = H, OH
R' = H, galloyl



n = 1 (monomers)

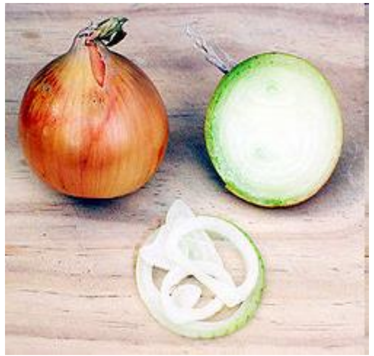
Oligomeric proanthocyanidins
(tannins)
n may be > 100
(macromolecules)

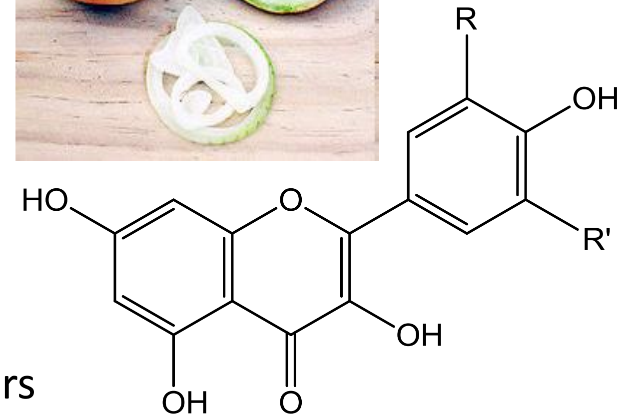
Hydroxycinnamic acids & derivatives

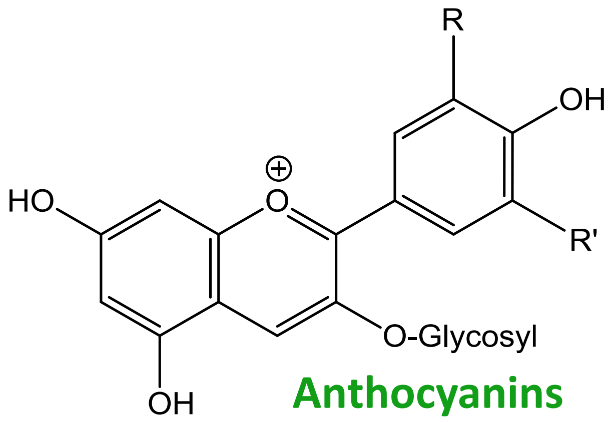


Covalently bound to fibers (hemicellulose) in cereals

Flavonols & glycosides







Anthocyanins
(*pigments*)

EPP: extractable polyphenols (typically titrated), mean consumption ≈ 1 g / day
NEPP: non-extractable polyphenols (tannins, hemicellulose-bound), *titrated after harsh acid solvolysis of residue*

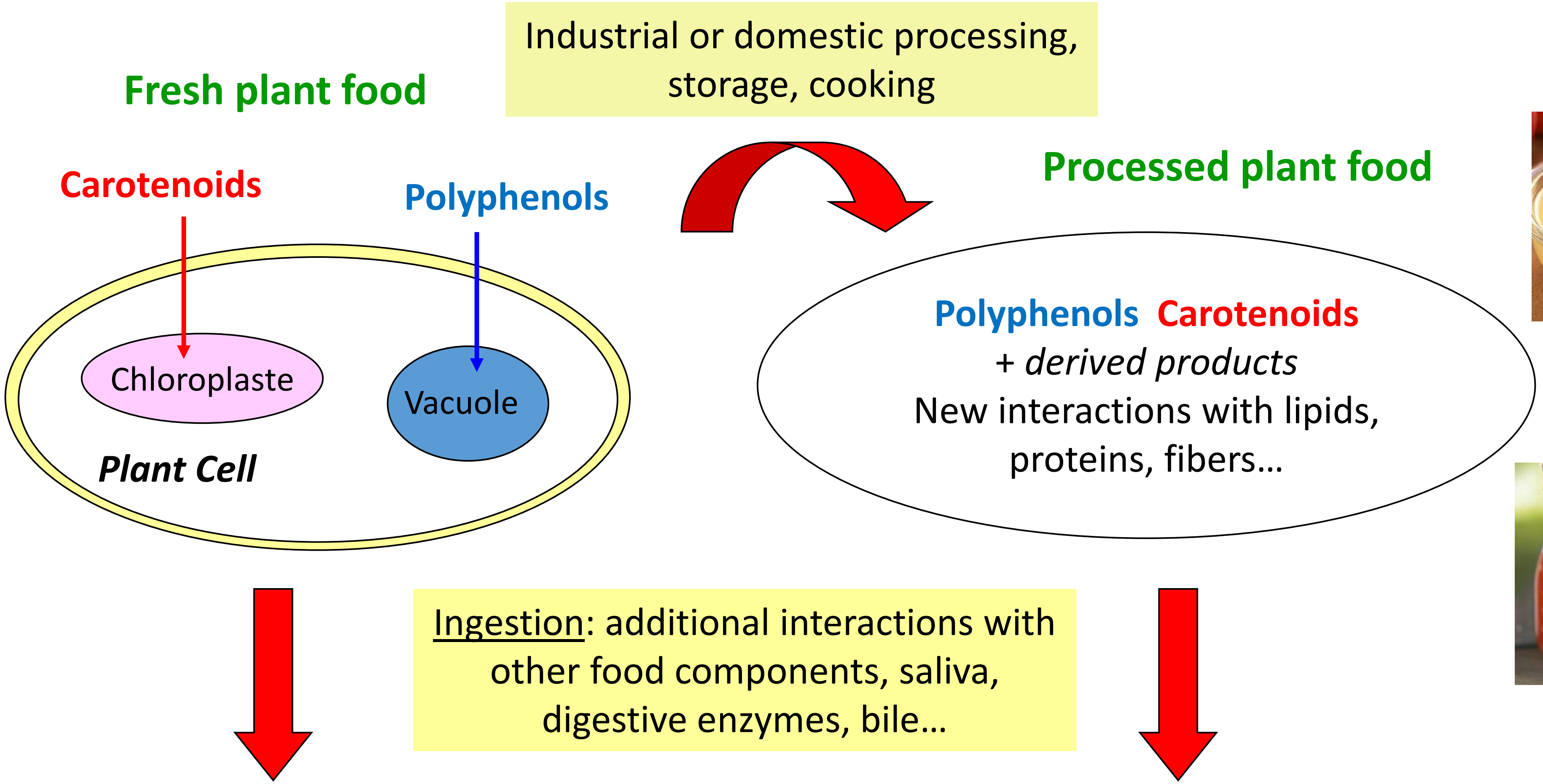
Sample	**EPP** (mg / 100 g dry material)	**Total** (mg / 100 g dry material)	**% NEPP**
Apple	609 ± 56	1747 ± 61	65
Asparagus	939 ± 14	1244 ± 48	24
Banana	297 ± 28	3581 ± 218	92
Broccoli	917 ± 38	2043 ± 71	55
Brussels sprout	857 ± 37	1579 ± 37	46
Grape	1870 ± 192	2484 ± 197	25
Lettuce	689 ± 73	2140 ± 111	58
Onion	455 ± 24	699 ± 35	35
Orange	786 ± 67	1911 ± 90	59
Peach	741 ± 53	1293 ± 65	43
Pear	376 ± 39	2219 ± 57	83
Pepper	1834 ± 187	2091 ± 189	12
Tomato	252 ± 14	632 ± 25	60

Non-extractable polyphenols prevail in many common fruits & vegetables
⇒ Polyphenol consumption is underestimated

J. Pérez-Jiménez & F. Saura-Calixto
Food Research International **2015**, 74, 315–323

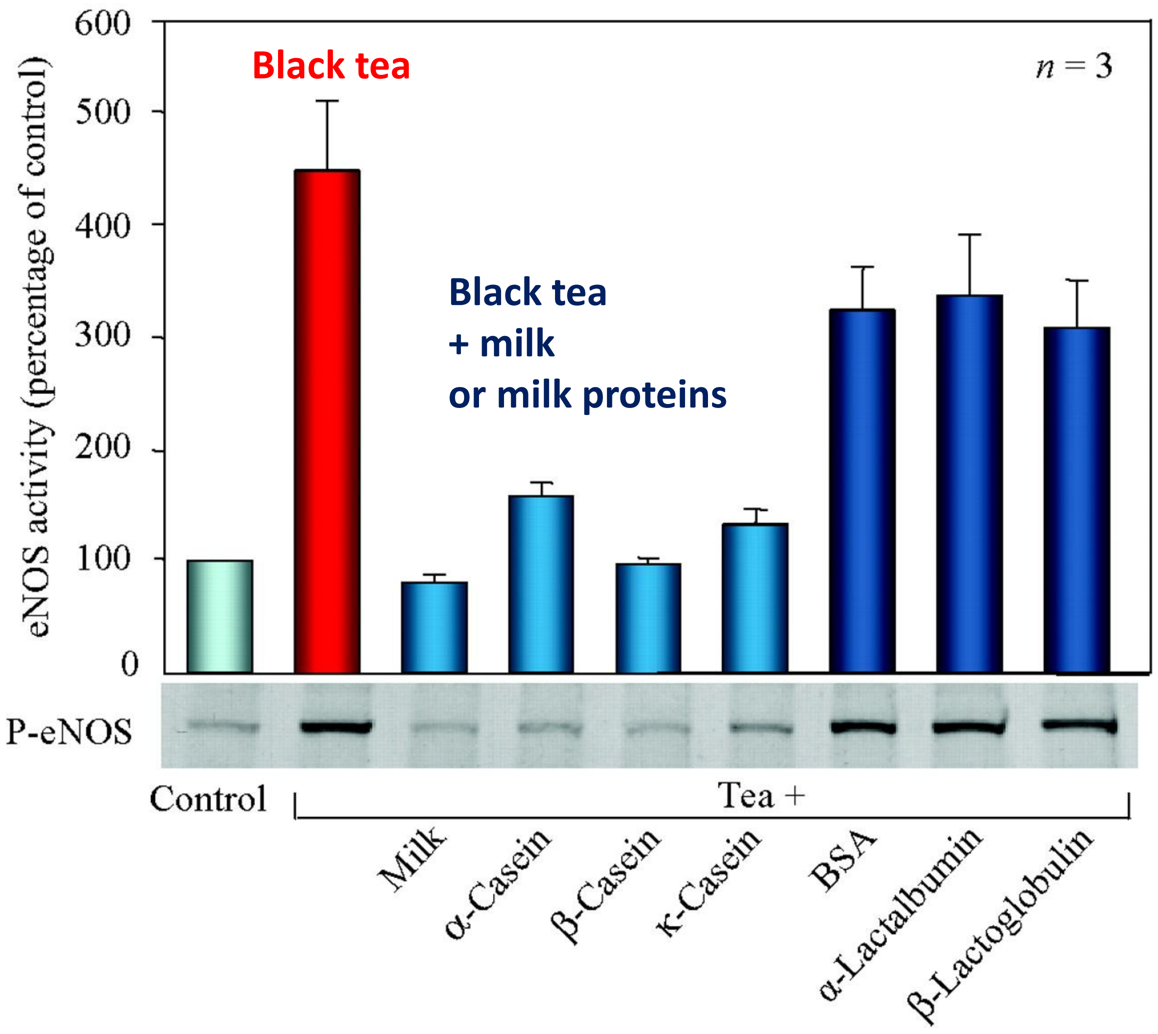
Fate & Activity of Micronutrients in Humans

Step 1: Bioaccessibility, i.e. their *release in the GI tract*



Polyphenols & Carotenoids more or less available in the GI tract
for activity (catabolism, antioxidant protection…) & intestinal absorption

Effect of milk & milk proteins on black tea-induced NO production in endothelial cells



Black tea consumption

⇒ Vasodilation

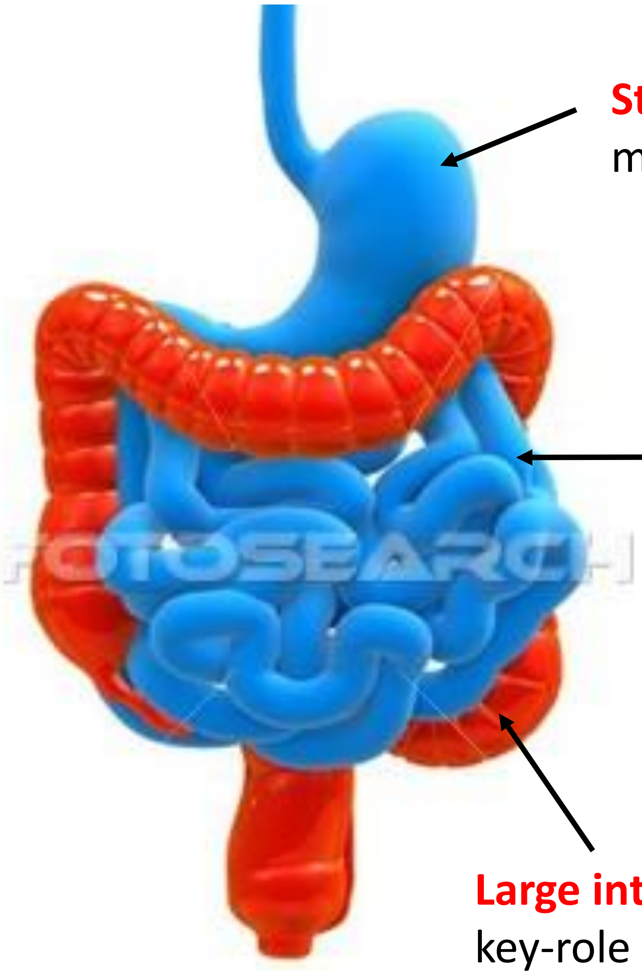
Effect abolished by simultaneous consumption of milk

Likely formation of insoluble polyphenol – casein complexes

Lorenz M et al., *Eur. Heart J.*, 2007, 28, 219-223

Step 2: critical to the overall bioavailability

The crossing of the intestinal barrier

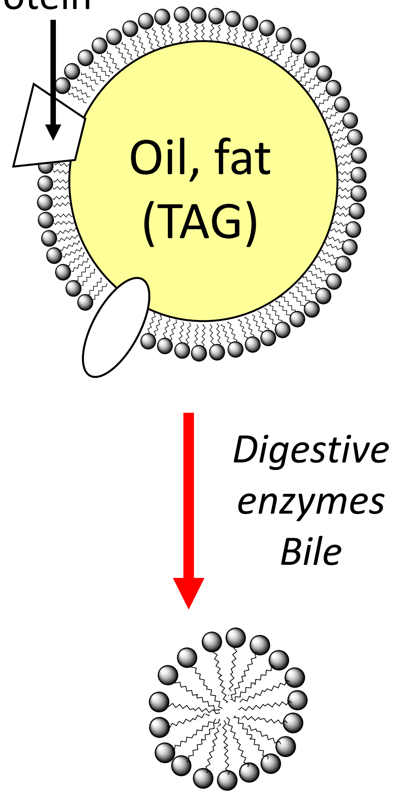


Stomach:
marginal absorption

Small intestine: major site for nutrient absorption
Partial absorption of specific polyphenols (aglycones, glucosides)

Partial absorption of carotenoids in association with lipids

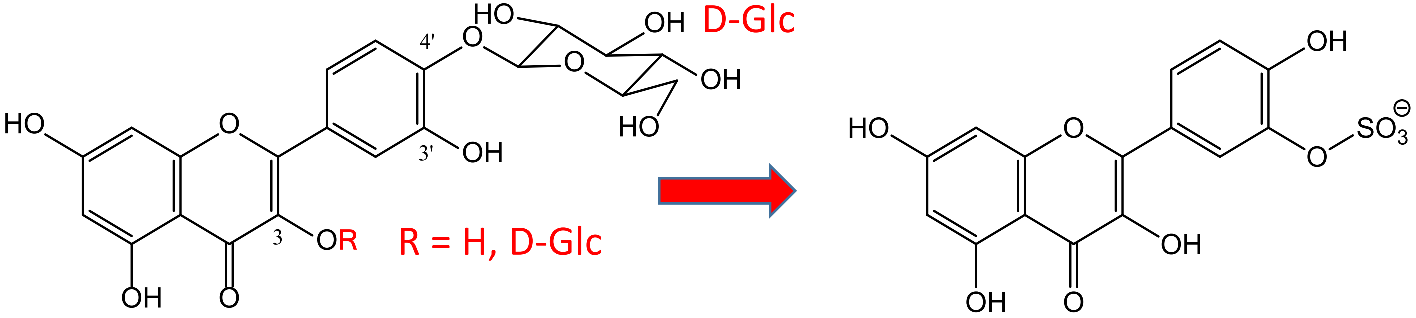
Large intestine (colon):
key-role of microbiota
Major site of polyphenol catabolism & absorption



Pharmacokinetic Analysis of Quercetin Metabolites in the Plasma of Volunteers (n = 6)

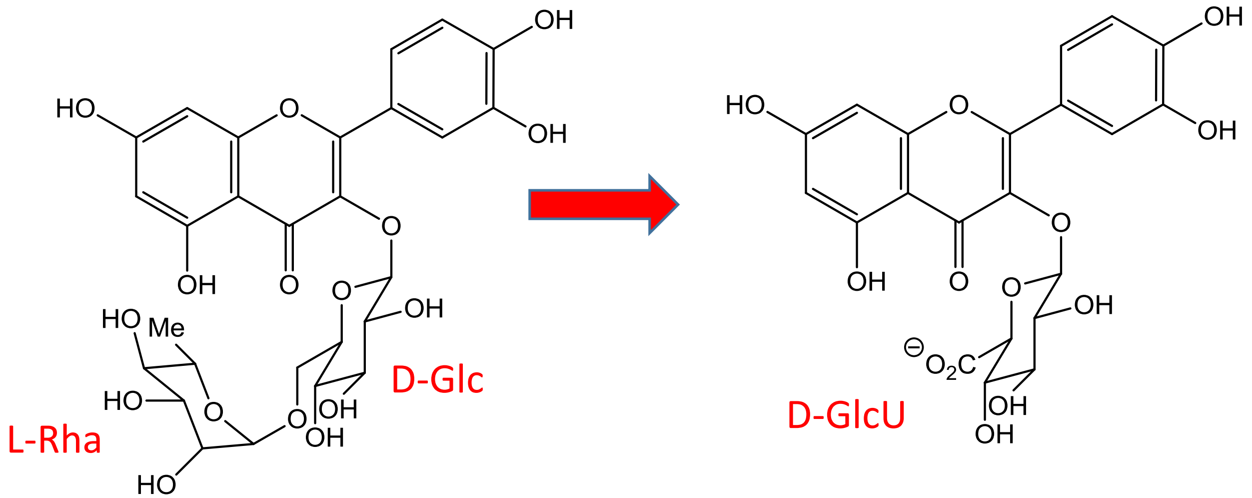
Food	Major Metabolites	C_{max} (nM)	t_{max} (h)
Fried Onions (270 g) ⇒ Quercetin Glucosides (275 µmol)	3’-O-Sulfoquercetin	665 (± 82)	0.75 (± 0.12)
	Quercetin-3-O-glucuronide	351 (± 27)	0.60 (± 0.10)
Tomato Juice (250 mL) ⇒ Quercetin 3-O-Rutinoside (176 µmol)	Quercetin-3-O-glucuronide	12 (± 2)	4.7 (± 0.3)
	3’-O-Methylquercetin-3-O-glucuronide	4.3 (± 1.5)	5.4 (± 0.2)

Onion flavonols: fast absorption from small intestine (uptake by intestinal β-glucosidase or Na-dpt Glc transporter)

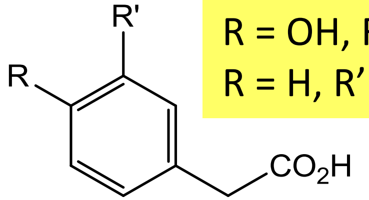


Deglycosidation + conjugation

Tomato flavonols: slow absorption from colon

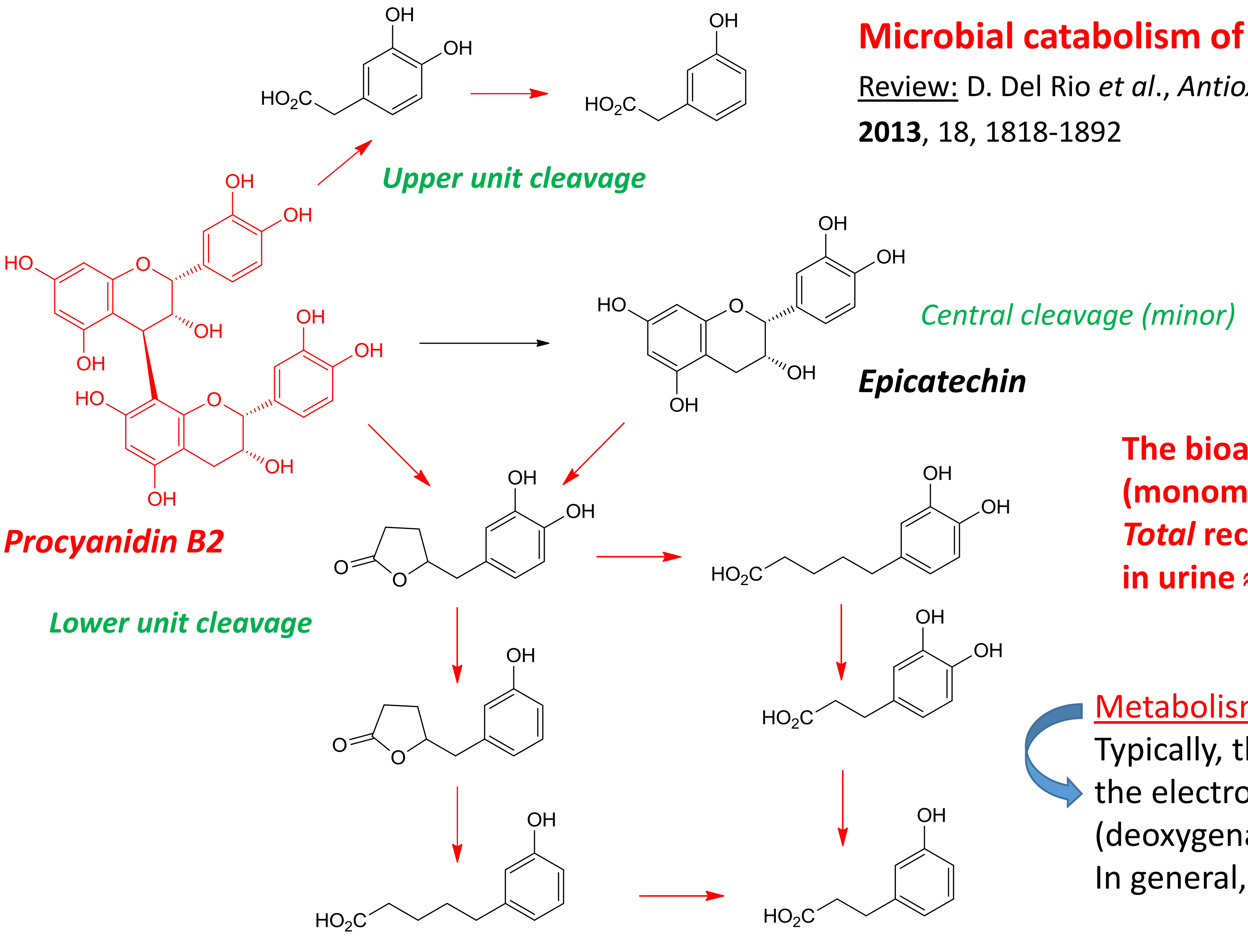


Review: D. Del Rio *et al.*, *Antioxidants & Redox Signaling* **2013**, 18, 1818-1892



R = OH, R’ = OH
R = OH, R’ = OMe
R = H, R’ = OH

Bacterial catabolites (microbiota)
~22% of ingested dose



Microbial catabolism of procyanidin B_2

Review: D. Del Rio *et al.*, *Antioxidants & Redox Signaling* **2013**, 18, 1818-1892

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

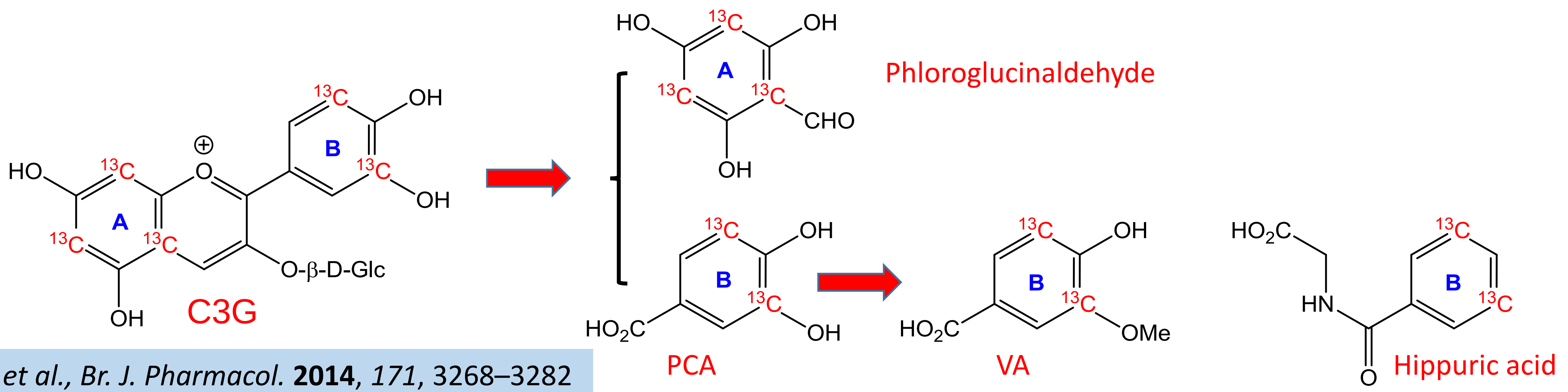
Metabolism

Typically, the catechol ring, which is critical to the electron-donating activity, is lost (deoxygenation, conjugation)

In general, *metabolites are poor ROS-scavengers*

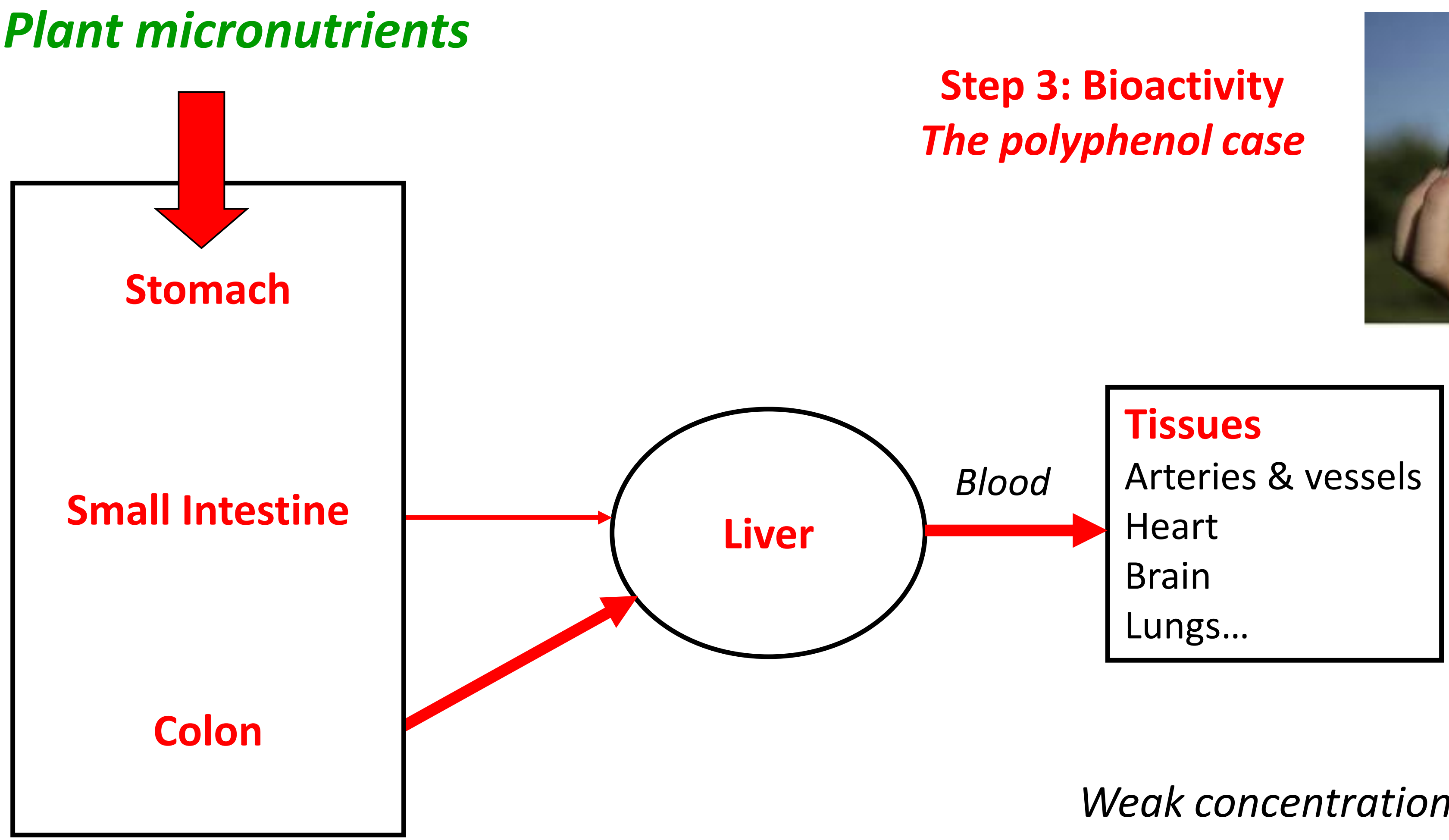
Serum pharmacokinetic profiles of cyanidin 3-glucoside (C3G) and its metabolites in humans after the consumption of 500 mg ^{13}C-labelled C3G

Compound	*n*	C_{max} (nM)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{0\text{-}48}$ (nM h)
Cyanidin-3-glucoside	**5**	**141 ± 70**	**1.8 ± 0.2**	**0.4**	**279 ± 170**
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
PCA-sulfates	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-sulfates	4	430 ± 299	30.1 ± 11.4	NQ	10 689 ± 7751
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**



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

Step 3: Bioactivity
The polyphenol case



High concentrations of
Polyphenols
⇒ ***Possible direct antioxidant action*** (ROS scavenging)

Weak concentrations of
Metabolites
⇒ **Specific cell effects**
e.g., anti-inflammatory
& indirect antioxidant protection

Postprandial oxidative stress in the GI tract & dietary antioxidants

Dietary Bolus

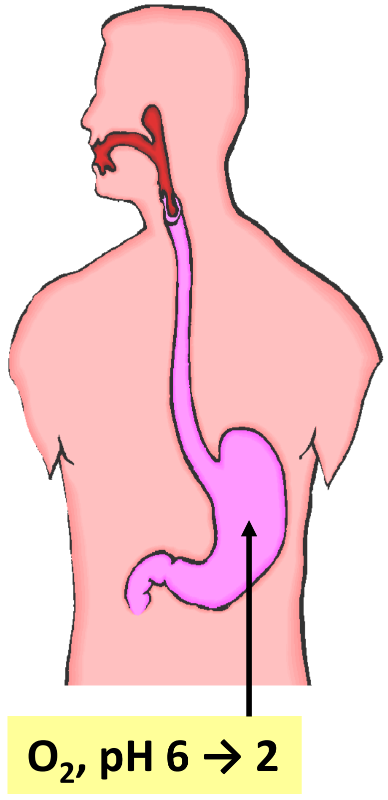
- **Oxidizable Nutrients:** polyunsaturated lipids, proteins…

- **Initiators of oxidative processes**

Iron (heme & non-heme): ca. 30 µg / g of beef (heme > 80%)
Hydroperoxides: typical PV of fresh oils = 1 – 4 µmol / g

The stomach is a potential site for dietary lipid autoxidation

- Loss of essential lipids
- Formation of reactive lipid oxidation products (hydroperoxides, aldehydes, epoxides) *via* even more reactive intermediates (lipid oxyl & peroxyl radicals)
- Modifications of dietary proteins (altering their digestibility & nutritional value)
- Cardiovascular risk?

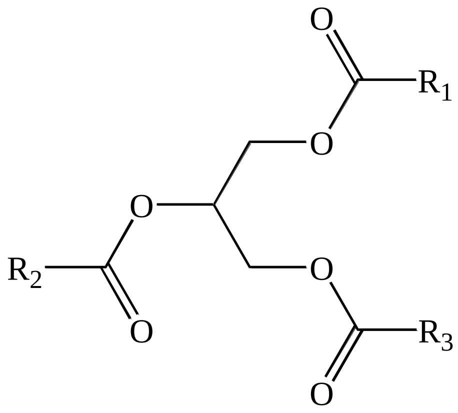


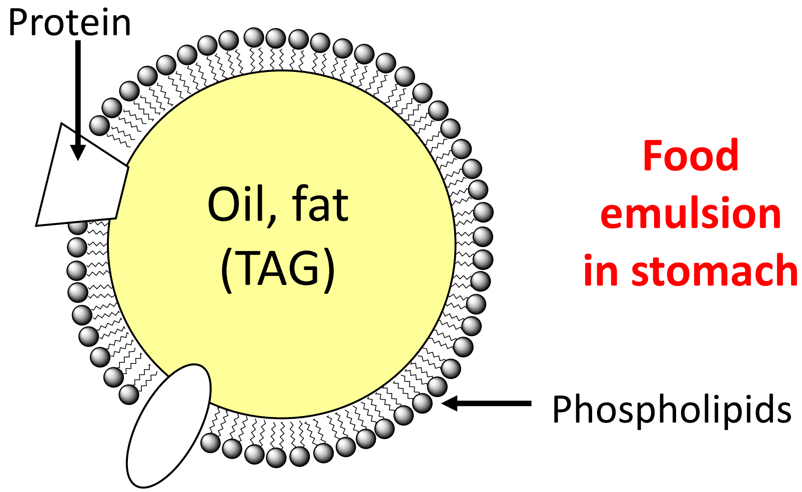
Possible inhibition by dietary antioxidants, such as polyphenols (> 1g / day / person)

Lipid-rich foods

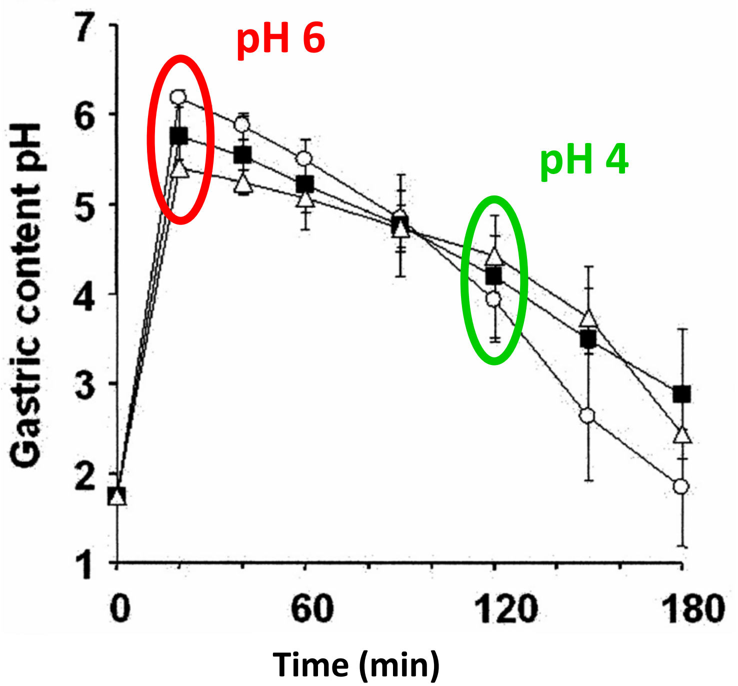
e.g., milk & dairy products
oils & sauces, fish

Triacylglycerols (TAG)
ca. 90%





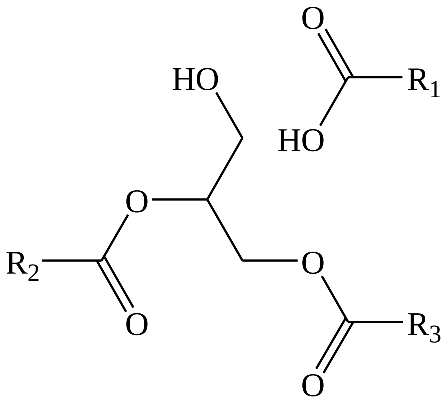
Food emulsion in stomach

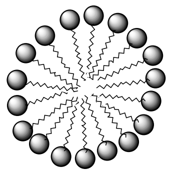


Tyssandier *et al.*, 2003

Gastric lipase

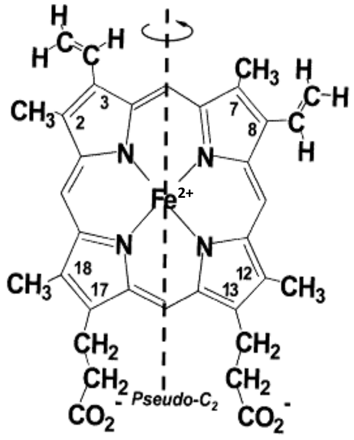
- pH activity range: 3 – 7, max. activity at pH ca. 5.4
- hydrolysis of a single ester group at sn1 or sn3
- *5 - 30% of lipid digestion*





Micelles
(DAG, free FA, phospholipids)

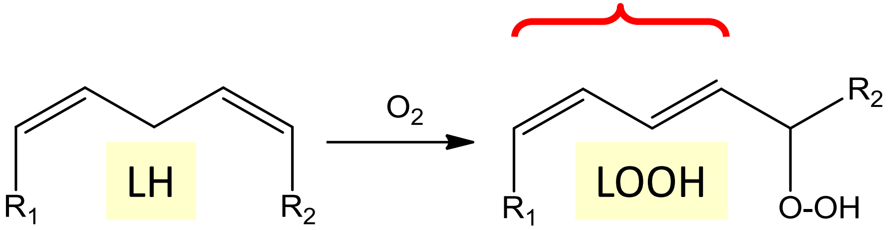
Inhibition of the metmyoglobin-induced lipid peroxidation

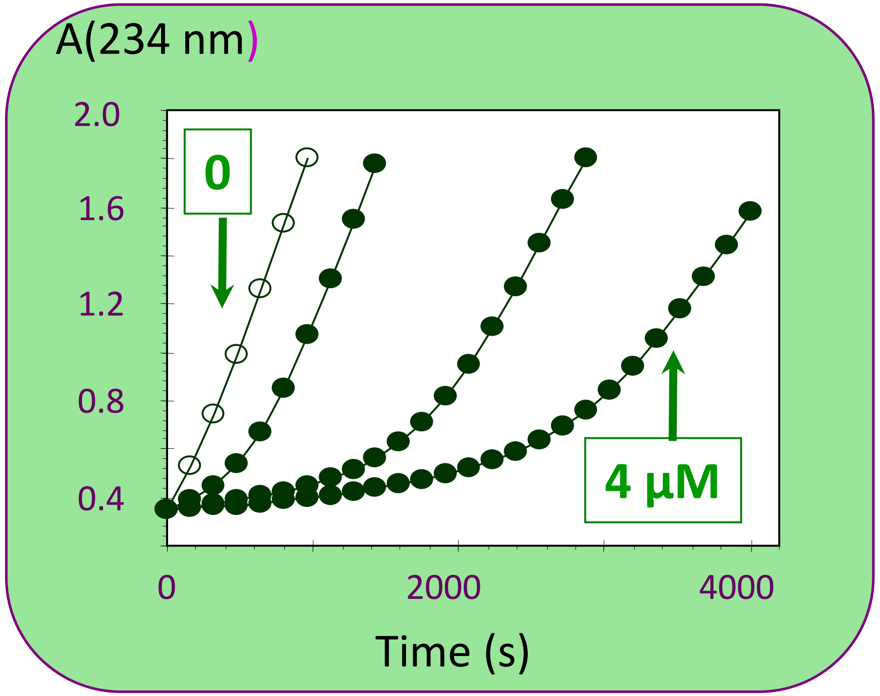


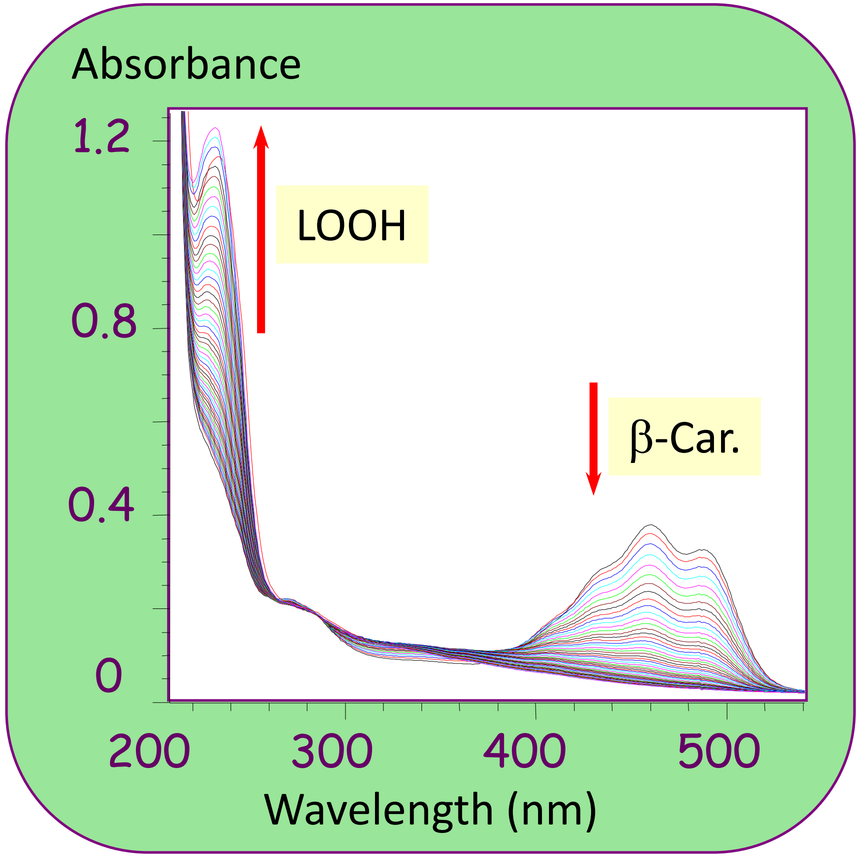
Hematin (Fe^{III}) + Globin (protein) = Metmyoglobin ($MbFe^{III}$)

Linoleic acid (0.7 mM) + β-Carotene (0 - 4 µM) + Metmyoglobin (100 nM)
(37°C, pH 5.8, 2 mM Tween 20)

Conjugated diene (detection at 234 nm)





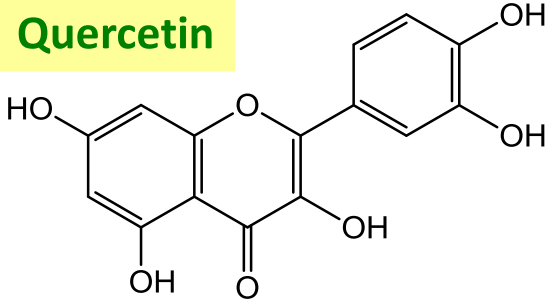


Inhibition of the metmyoglobin-induced peroxidation of linoleic acid

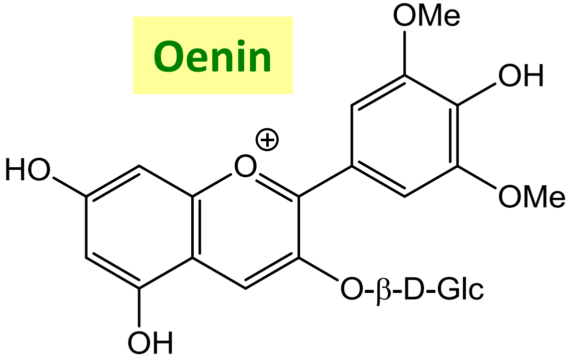
Linoleic acid (0.7 mM) + Metmyoglobin (100 nM)
(37°C, pH 5.8, 2 mM Tween 20)

Antioxidant	IC_{50} / mM a)
Quercetin	**0.33**
Rutin	0.63
Caffeic acid	0.49
Chlorogenic acid	0.51
Oenin	**0.27**
α-Tocopherol	**0.28**
β-Carotene	1.52

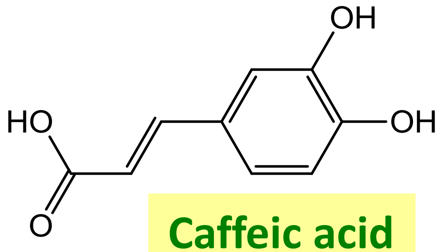
Quercetin



Oenin



Some common polyphenols are as effective as α-tocopherol



Caffeic acid

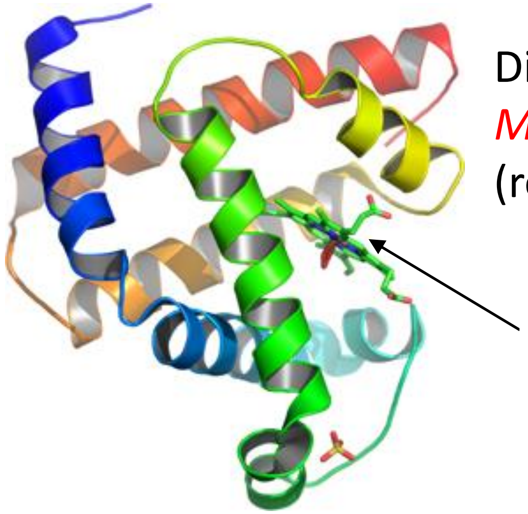
a) IC_{50} = antioxidant conc. that doubles the period needed to accumulate a fixed LOOH conc

NB: rutin = quercetin-3-β-D-Glc-1,6-α-L-Rha, chlorogenic acid = 5-caffeoylquinic acid

Heme-induced lipid peroxidation and its inhibition by antioxidants

Polyunsaturated lipid LH

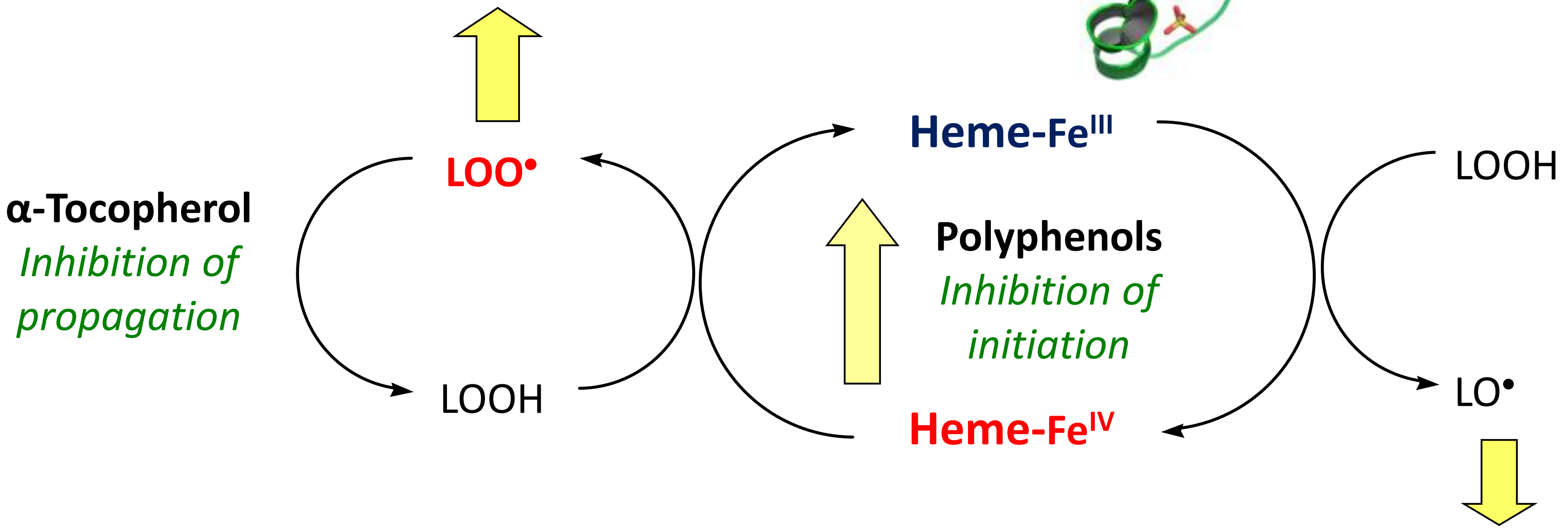
+ traces of lipid hydroperoxides LOOH



Dietary Iron
Metmyoglobin
(red meat)

Cofactor
(heme)

Propagation of oxidation

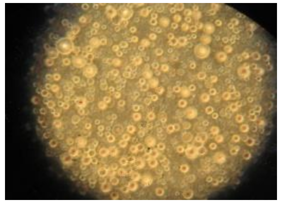


Ketodienes L=O

Polyphenols: *residual activity of oxidation products for a prolonged protection*

Extension to Sunflower Oil-in-Water Emulsions

Interface: BSA

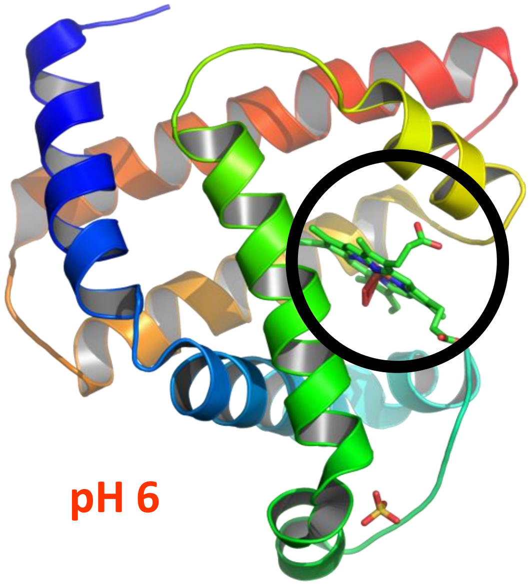


$d_{[3,2]}$ = 2.4 µm

Interface: egg phospholipids (PL)

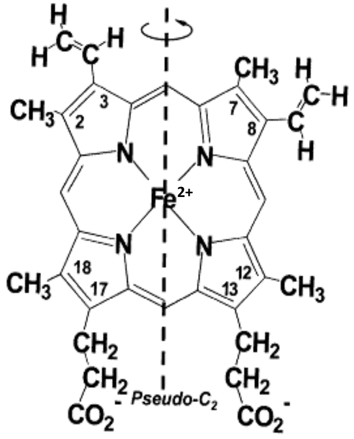
$d_{[3,2]}$ = 2.8 µm

Initiator = metmyoglobin *or* free iron (Fe^{II}, Fe^{III}-Ascorbate)



pH 6

Metmyoglobin ($MbFe^{III}$)



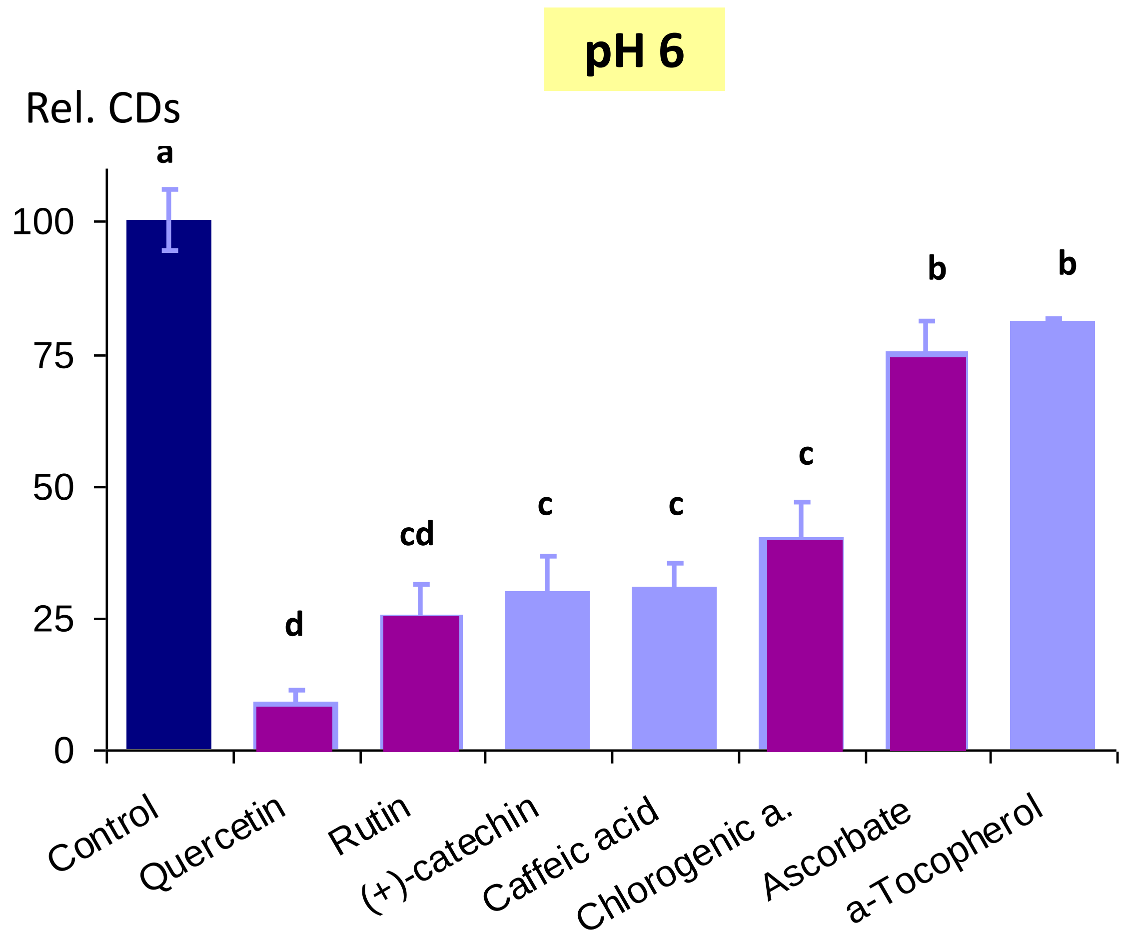
Hematin (Fe^{III})

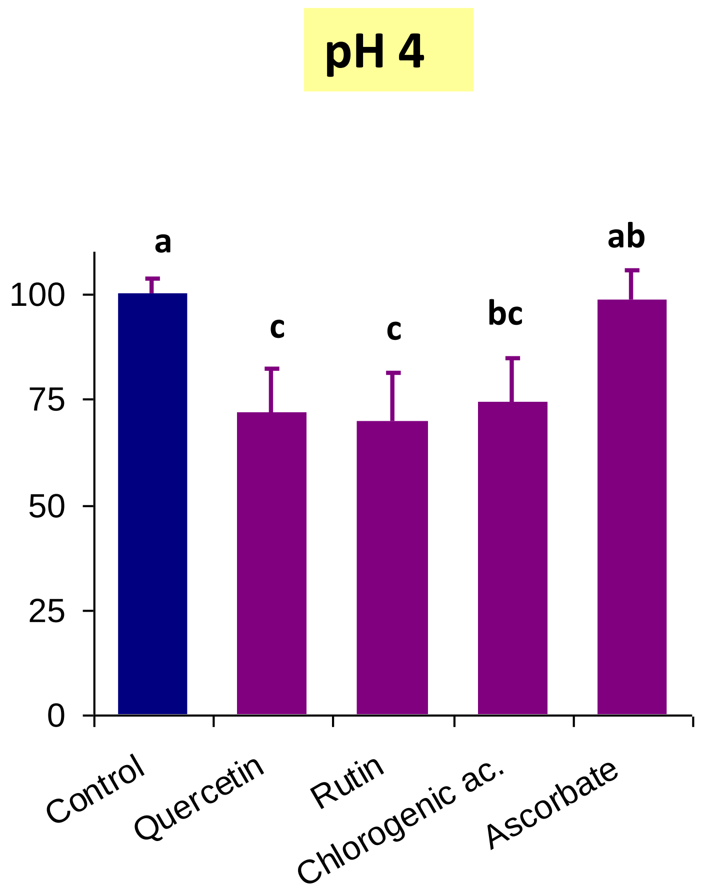
pH 4

+ free globin

Metmyoglobin-induced peroxidation of sunflower oil emulsions

BSA interface (10 g oil / 100 mL, 20 µM metmyoglobin)





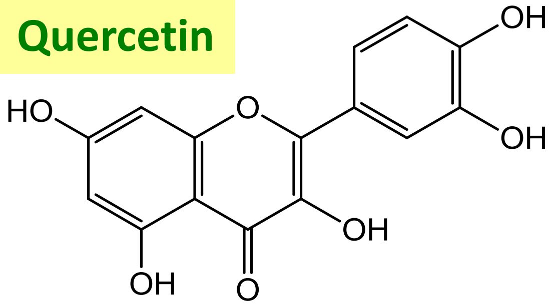
Efficient protection by polyphenols in the early phase of digestion only (pH 5 - 6)

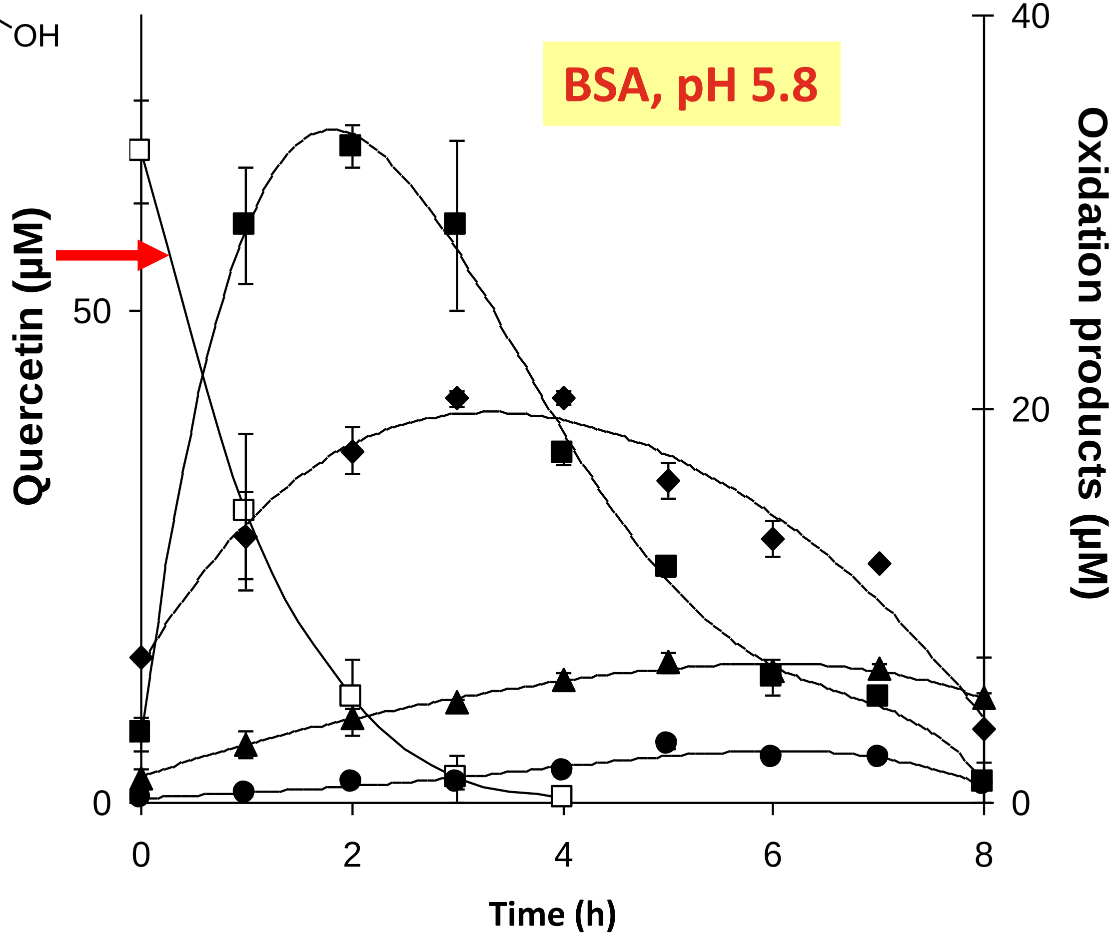
[Antioxidant] = 100 µM

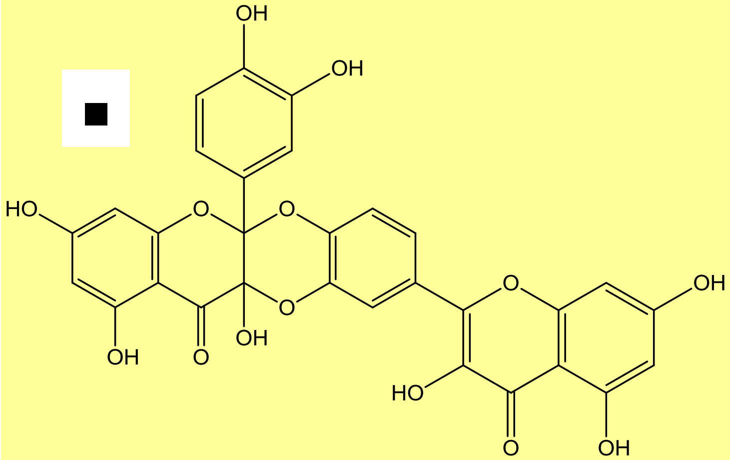
Tukey-Kramer $p < 0.05$

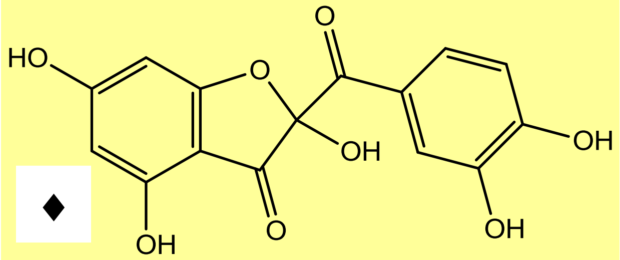
Fate of quercetin during inhibition

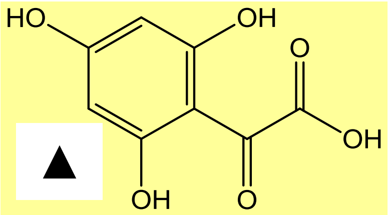
Quercetin

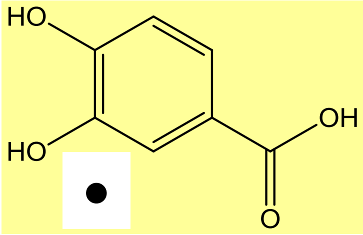












Quercetin consumed in 3 – 4h

More than 90% protection of oil over 8h

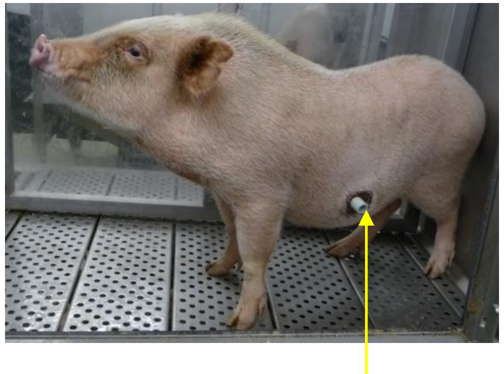
Quercetin oxidation products prolong the antioxidant protection

In vivo validation in gastric digestion in minipig

Western type diet
- sunflower oil
- egg phospholipids
- Beef meat
- starch, pectins, cellulose

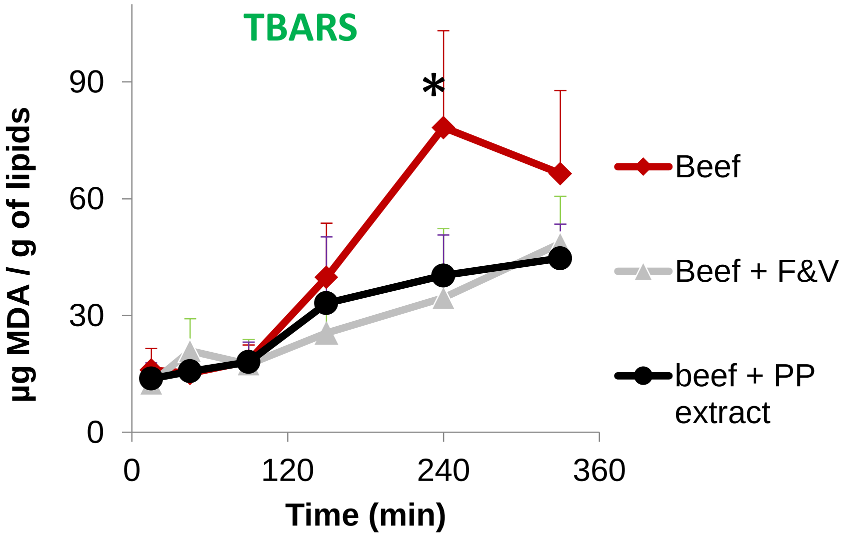

Control meal

+ Fruit & vegetables
(apple, plum & artichoke)
+ Polyphenol extract



Gastric canula

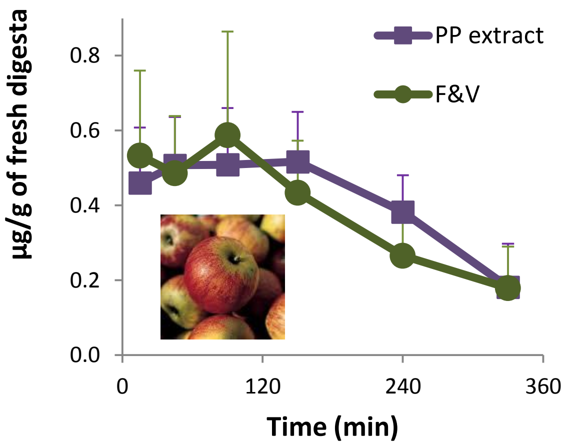
❑ F&V and polyphenols inhibit lipid oxidation in gastric digestion



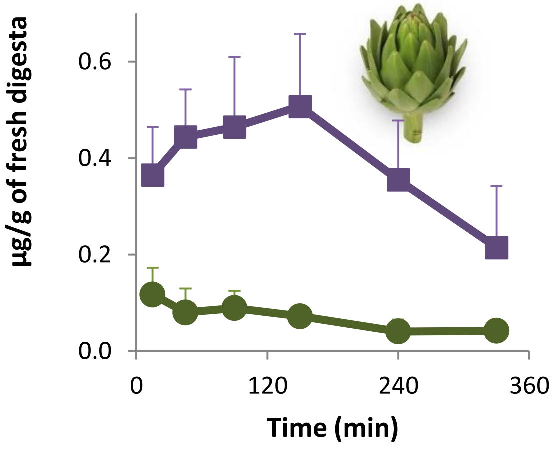
No apparent influence of the F&V matrix on the overall antioxidant activiy

The F&V matrix may influence the bioaccessibility of individual phenols

4-*p*-Coumaroylquinic acid



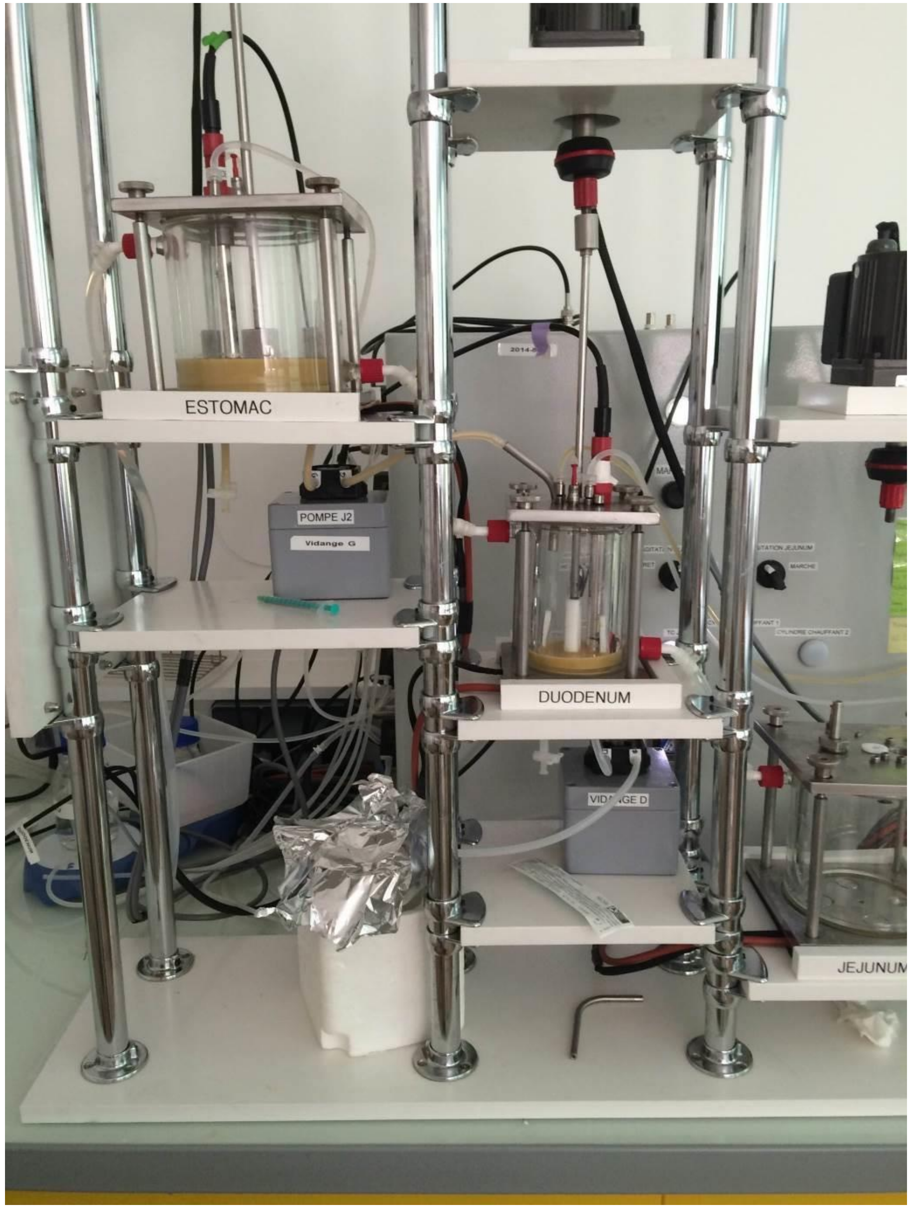
4-Caffeoylquinic acid



M. Loonis, C. Dufour
Coll. INRA-UNH, INRA-Quapa

Gobert et al., *Food & Function*, 2014
Delosière et al., *Food & Function*, 2016

More to come from improved in vitro models taking into account the dynamic of pH change & enzyme secretion along the gastro-intestinal digestion…



Dynamic digestion system (DIDGI)

Acknowledgments

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Patrick BOREL (Carotenoid bioavailability)

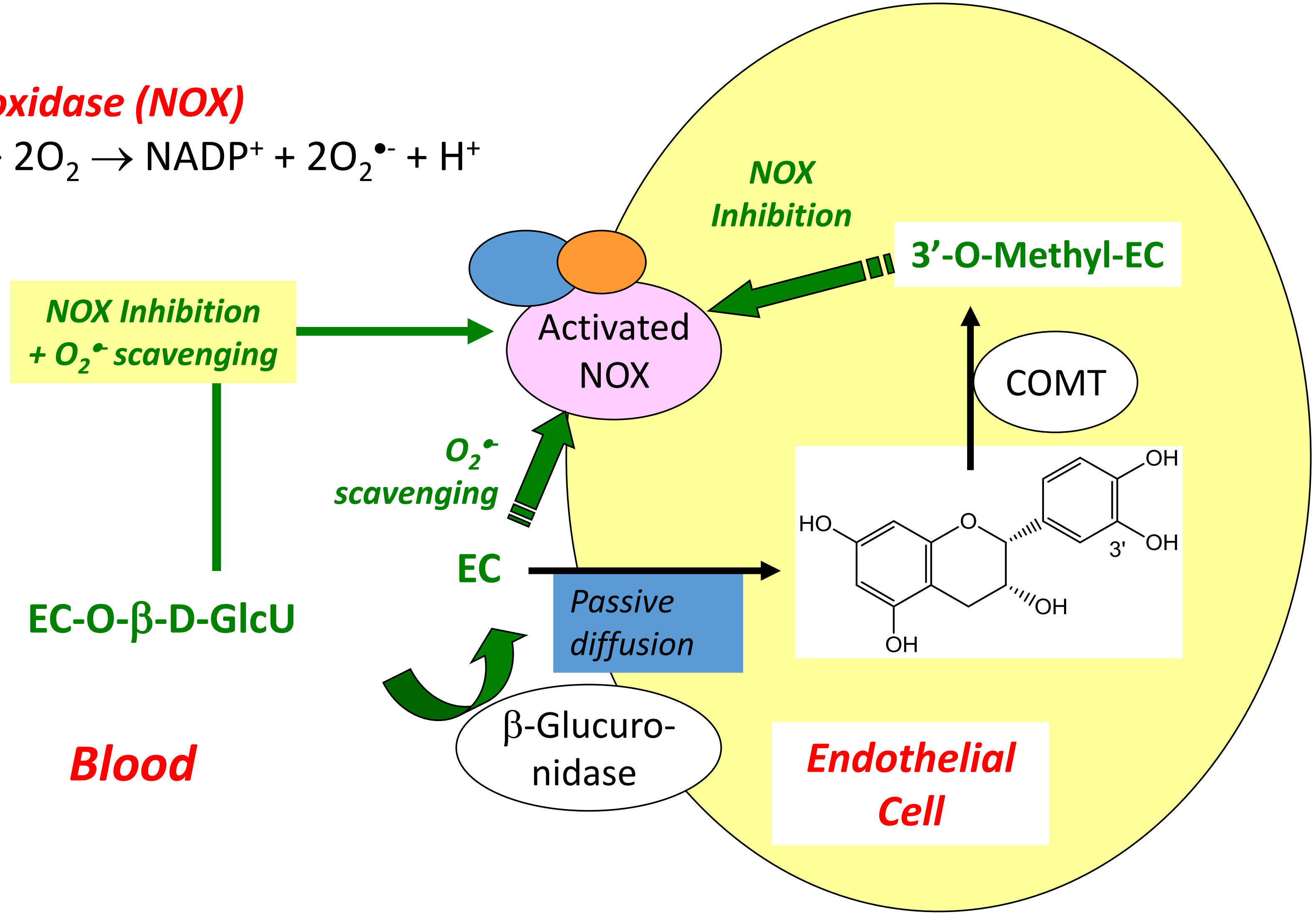
ANNEXE: EXEMPLES DE MECANISMES *CELLULAIRES* EXPRIMANT LES EFFETS ANTIOXYDANTS & ANTI-INFLAMMATOIRES DES POLYPHENOLS

Un impératif: valider les mécanismes avec les métabolites

Inhibition of NADPH oxidase by epicatechin (EC) glucuronides in Vascular Endothelial Cells

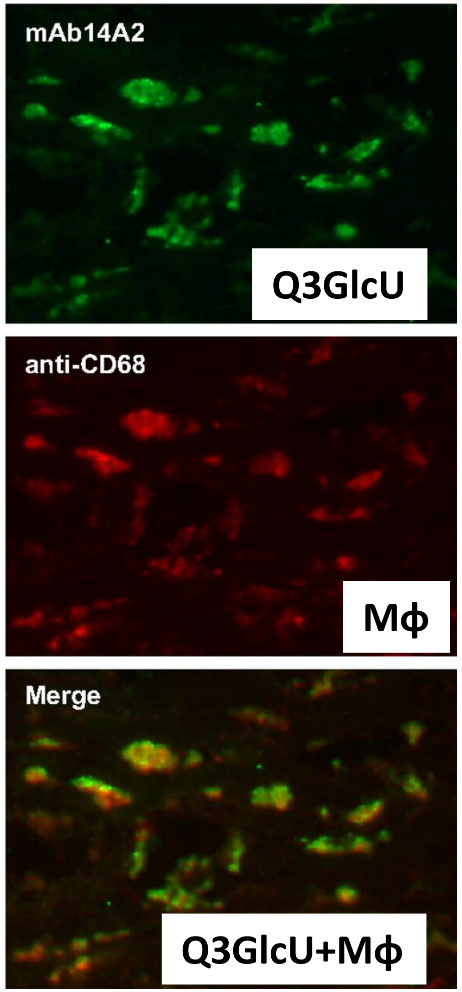
NADPH oxidase (NOX)

$$NADPH + 2O_2 \rightarrow NADP^+ + 2O_2^{\bullet -} + H^+$$

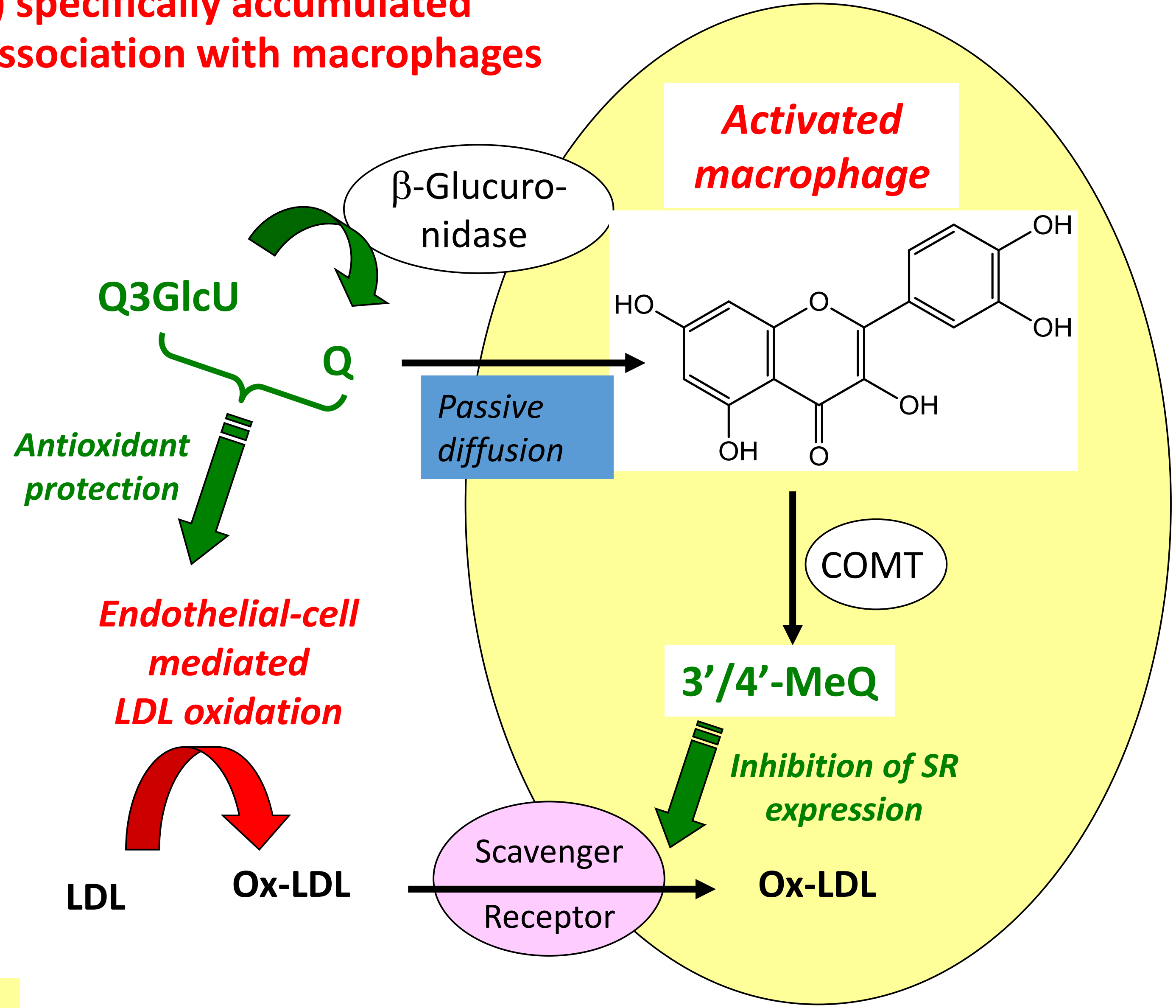


Steffen Y. *et al*. *Arch. Biochem. Biophys*. 2008, *469*, 209

Quercetin 3-glucuronide (Q3GlcU) specifically accumulated in human atherosclerotic lesions, in association with macrophages



Immunostaining experiments



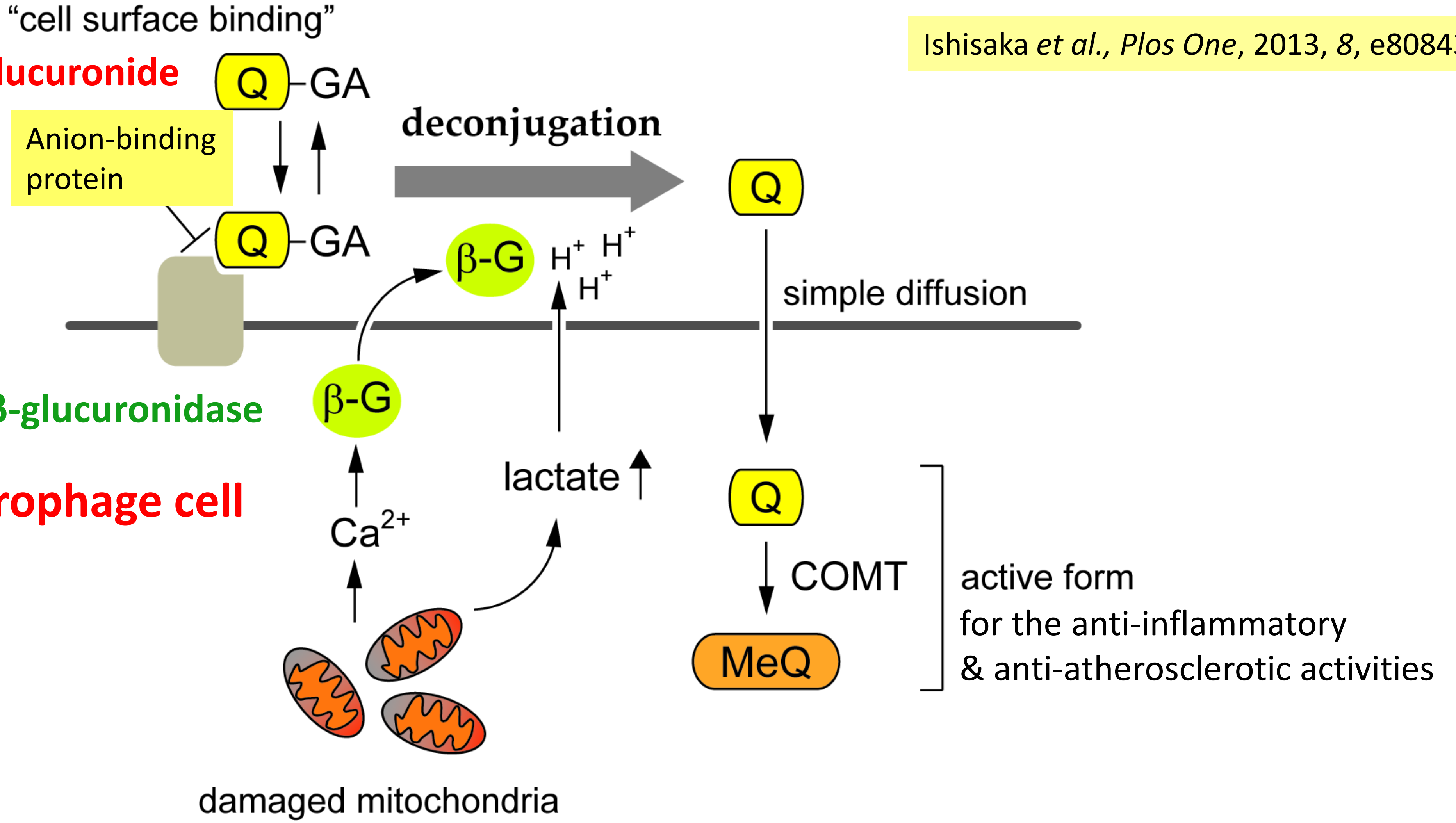
Kawai Y *et al.*, *J. Biol. Chem.*, 2008, 283, 9424-9434

Ishisaka *et al., Plos One*, 2013, *8*, e80843

QGA: quercetin 3-glucuronide

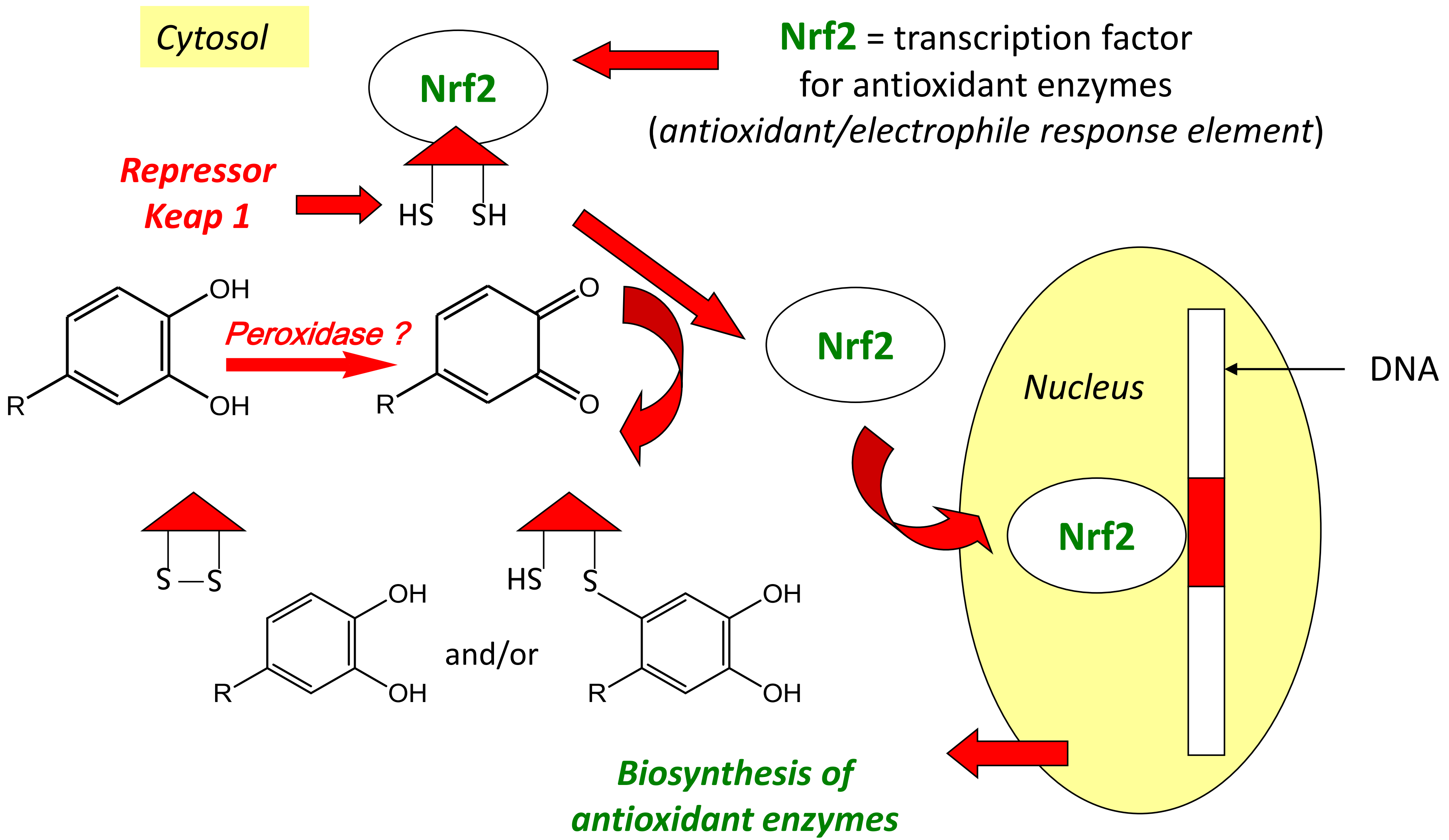
β-G: β-glucuronidase

Macrophage cell



Mitochondria dysfunction ⇒ increased acidity (lactate secretion) & intracellular Ca^{2+} concentration
⇒ Increased **β-glucuronidase** activity ⇒ release of **free quercetin**

Polyphenols can activate the antioxidant defense system in cells



Moskaug J. Ø. *et al. Mechanisms of Ageing and Development* **2004**, *125*, 315
Dinkova-Kostova A.T. et al. *Chem. Res. Toxicol*. **2005**, *18*, 1779