Why do we expect flavonoids to function as antioxidants *in vivo*?

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FLAVONOIDS:
FOCUS OF MUCH CURRENT NUTRITIONAL AND THERAPEUTIC INTEREST

- **CARDIOPROTECTION**

Role for flavonoid-rich dietary components in reduction in risk of cardiovascular disease

- **NEUROPROTECTION**

Anthocyanin-rich fruit associated with protection against age-related decline in cognitive function

- **CHEMOPREVENTION**

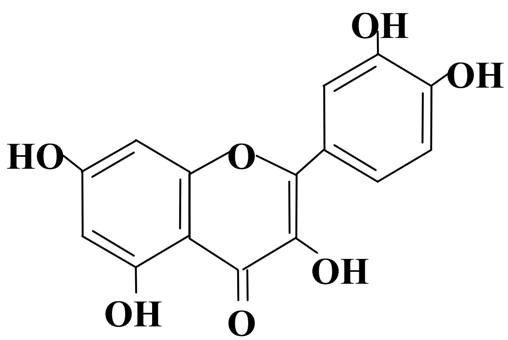
Flavonoids: naturally occurring low molecular wt phenols consisting of 2 benzene rings linked via a heterocyclic pyrone or pyran ring -> patterns and substitutions comprising the sub-classes:

- **Anthocyanin** - berries
- **Flavanone** - citrus
- **Flavanol** - red wine, teas, chocolate, fruit
- **Flavonol** - fruit, vegetables
- **Hydroxycinnamates** - most fruit & some vegetables

Flavonol

***e.g.* quercetin**

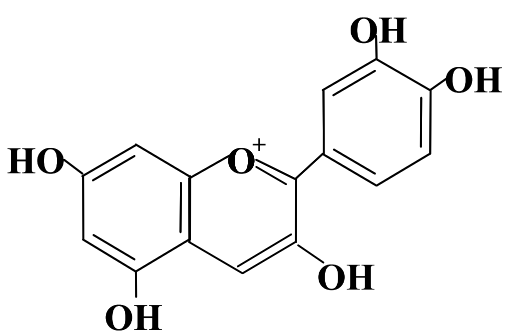
onion, cranberry, red apple
many fruit and vegetables



Anthocyanidin

***e.g.* cyanidin**

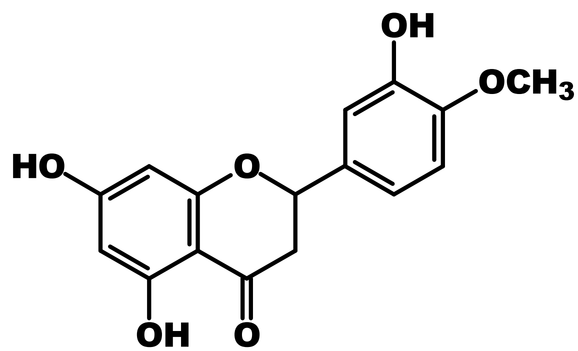
major constituents of dark
red fruit berries e.g. raspberries



Flavanone

e.g. hesperetin

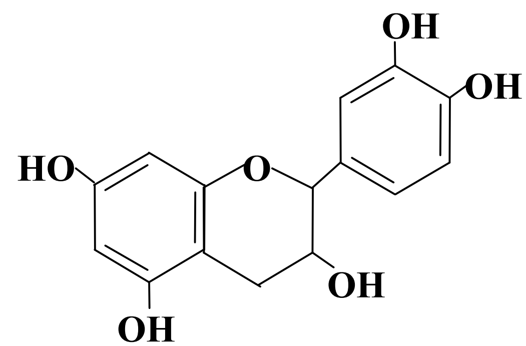
Citrus fruit, orange



Flavanol

***e.g.* epicatechin**

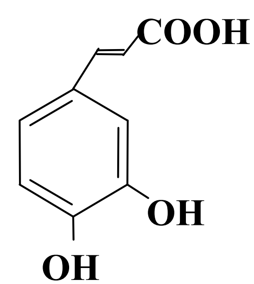
red wine, green tea,
as procyanidins in apple, chocolate



Hydroxycinnamate

***e.g.* caffeic acid**

most fruit especially tomato, apple
some vegetables e.g. egg plant
grains



SMALL DIFFERENCES IN STRUCTURE
→ LARGE CHANGES IN BIOLOGICAL ACTIVITIES

Number and specific positions of OH groups / nature of substitutions determine whether flavonoids function as:

antioxidant, anti-inflammatory, cytotoxic or antimutagenic agents in vitro or in vivo.

- **Antioxidant/pro-oxidant activities**
- **Enzyme induction / inhibition**
- **Cell proliferation / growth inhibition**
- **Lipophilicity / polarity - cellular access**

PROTECTIVE PROPERTIES OF FLAVONOIDS AGAINST OXIDATIVE STRESS ARE STRUCTURE-DEPENDENT

- **Scavengers of reactive oxygen species-**

H-donating abilities

- **Transition metal chelators – catechol requirement?**
- **Scavengers of reactive nitrogen species**

nitric oxide, peroxynitrite etc – nitration or oxidation?

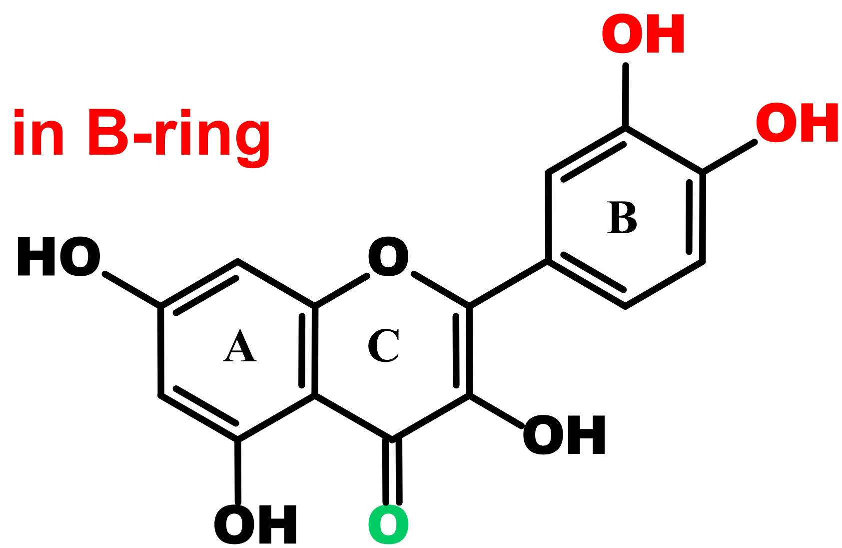
- **Non-antioxidant mechanisms - modulation of signaling pathways, gene expression**

STRUCTURAL REQUIREMENTS FOR H-DONATING ANTIOXIDANT ACTIVITY:

ortho-dihydroxy substitution in B-ring

2,3-unsaturation in C-ring

4-carbonyl group



QUERCETIN

Bors *et al.* 1990; Rice-Evans *et al.* 1996;

SCREENING FLAVONOIDS FOR ANTIOXIDANT ACTIVITY: INFLUENCE OF B-RING STRUCTURE

	Reduction potentials E_7	Antioxidant activity TEAC
CATECHOLS		
quercetin	0.33	4.7
epicatechin	0.57	2.4
MONOHYDROXY B-RING		
kaempferol	0.75	1.3
hesperetin	0.72	0.9
ALKYLPEROXYL RADICAL	1.06	
VITAMIN C	0.25	

Jovanovic *et al.* 1998; Rice-Evans *et al.* 1996

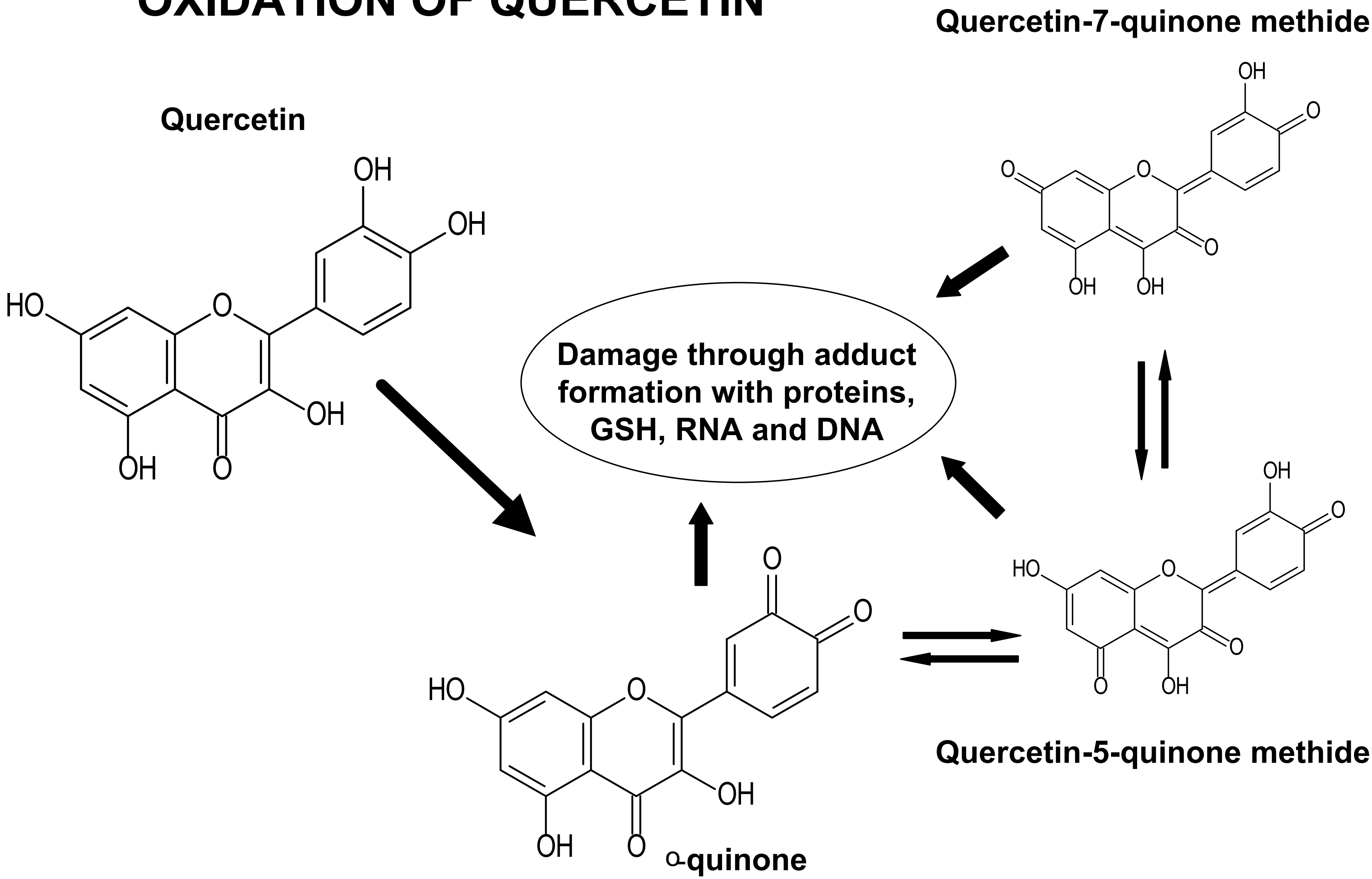
STRUCTURAL DETERMINANTS OF CYTOTOXICITY

- **Ease of oxidation –**

  catechol *vs* monophenolic

- **lipophilicity**

OXIDATION OF QUERCETIN



STRUCTURAL DEPENDENCE OF PEROXIDATIVE METABOLISM OF FLAVONOIDS – *monophenolic B-ring*

FlavOH + ferryl radical → FlavO$^{\blacksquare}$ Phenoxyl radical

FlavO$^{\blacksquare}$ + GSH → GS$^{\blacksquare}$ Thiyl radical

GS$^{\blacksquare}$ + O_2 → Reactive oxygen species

→ GSSG

Galati *et al.* 2002

WHAT'S HAPPENING IN VIVO?

STRUCTURAL CHANGES ON ABSORPTION

Influence of conjugation and metabolism on structural parameters governing biological properties

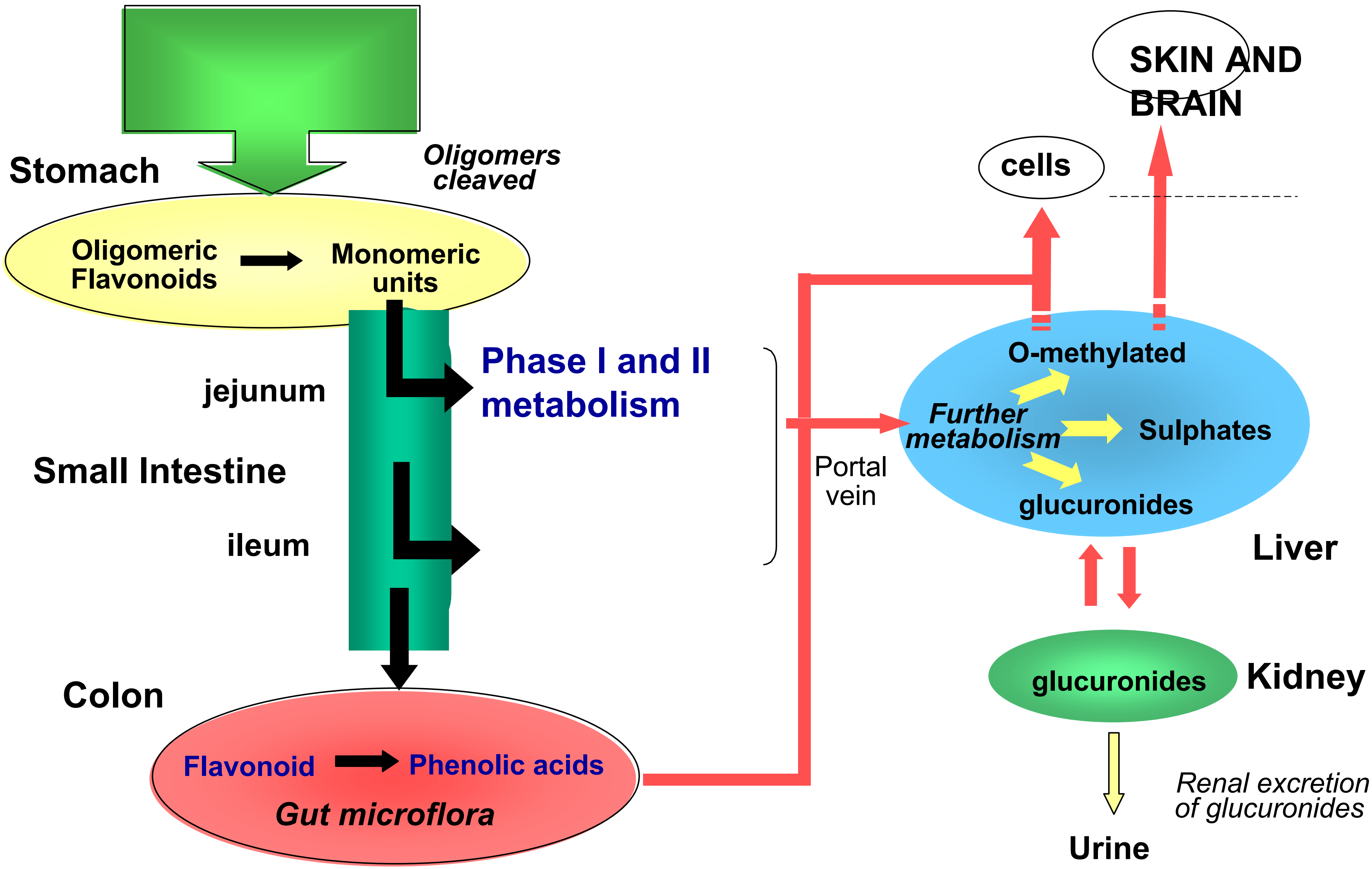
MAJOR METABOLIZING ENZYMES:
small intestine / liver / colon

- Glucosidases
- UDP-glucuronosyl transferases
- Catechol-O-methyl transferases
- Sulfotransferases
- Hydrolases
- Esterases
- Cytochrome P450s

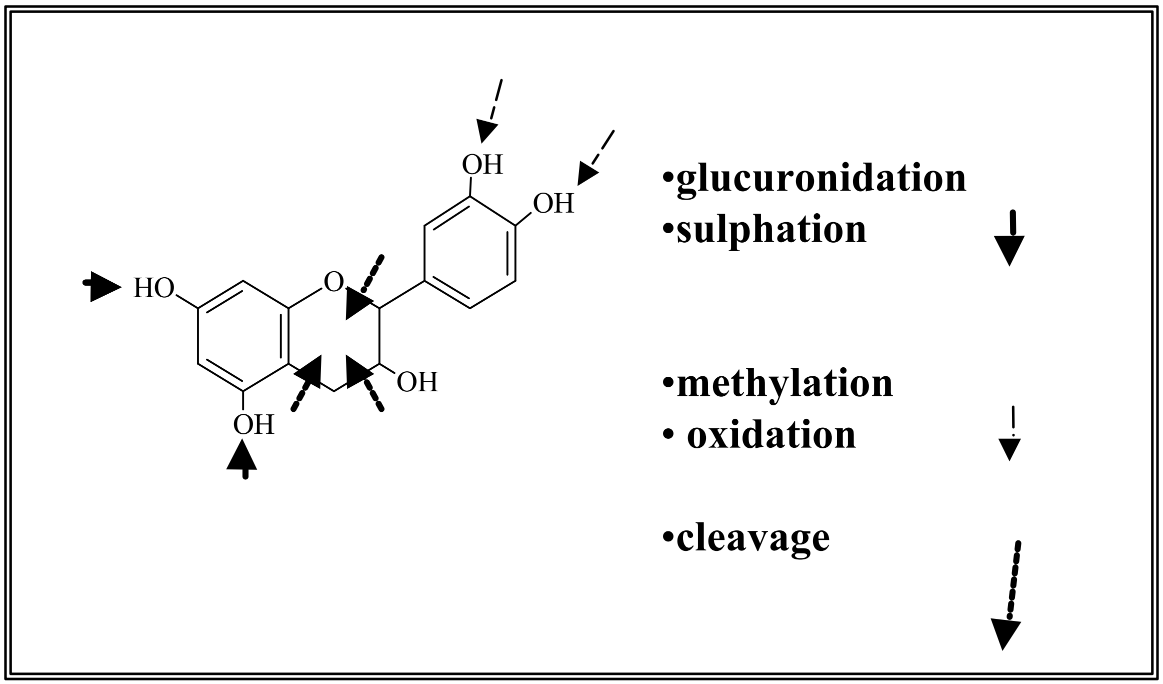
OTHERS:

- Glutathione-S transferases
- Quinone reductases

Absorption and Biotransformation of Dietary Flavonoids *In Vivo*

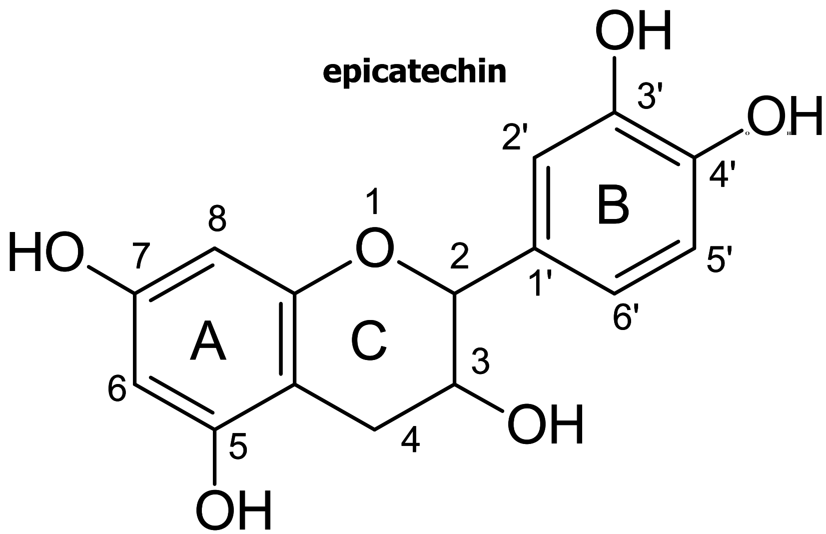


POTENTIAL MOLECULAR SITES OF METABOLIC MODIFICATION

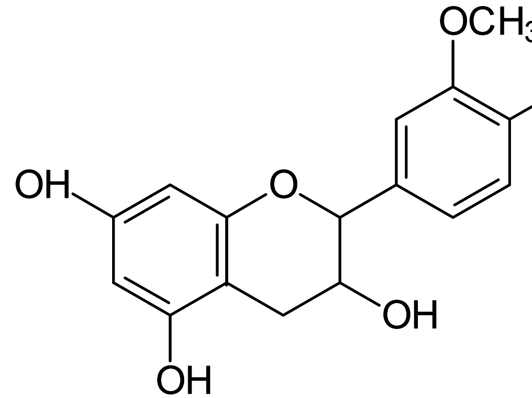


EFFECTS OF METABOLISM ON FLAVONOID STRUCTURES – IMPLICATIONS FOR BIOLOGICAL PROPERTIES

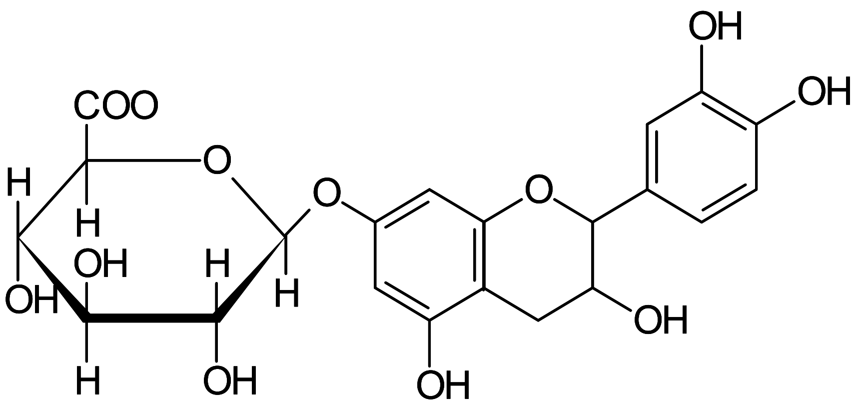
epicatechin



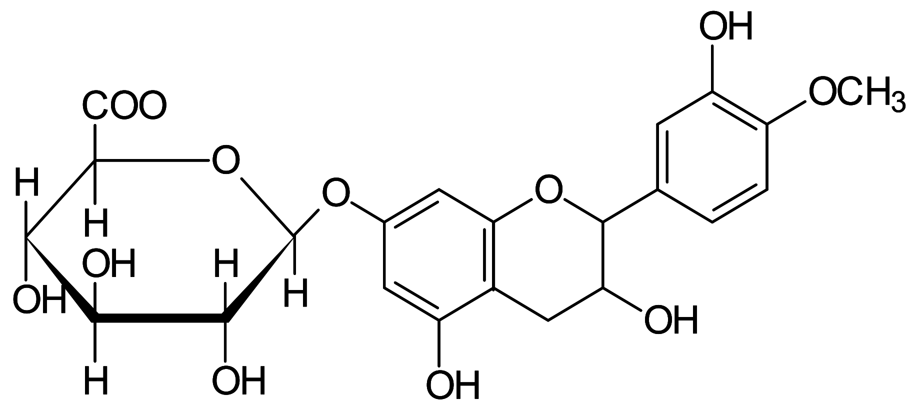
3‘-O-methyl-epicatechin



epicatechin-7-b-D-glucuronide



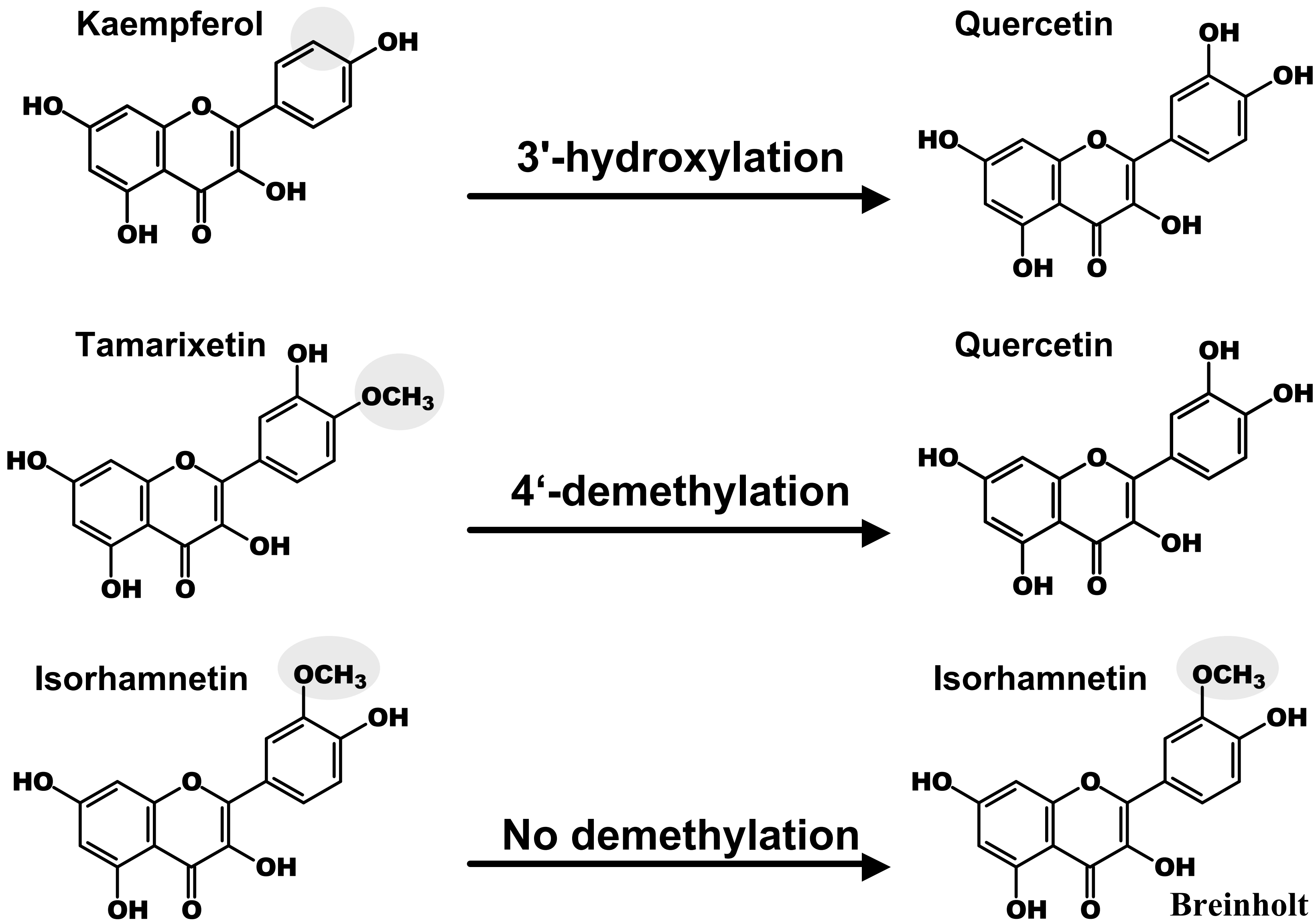
4‘-O-methyl-epicatechin-7-b-D-glucuronide



STRUCTURAL FACTORS INFLUENCING INTRACELLULAR ANTIOXIDANT PROPERTIES

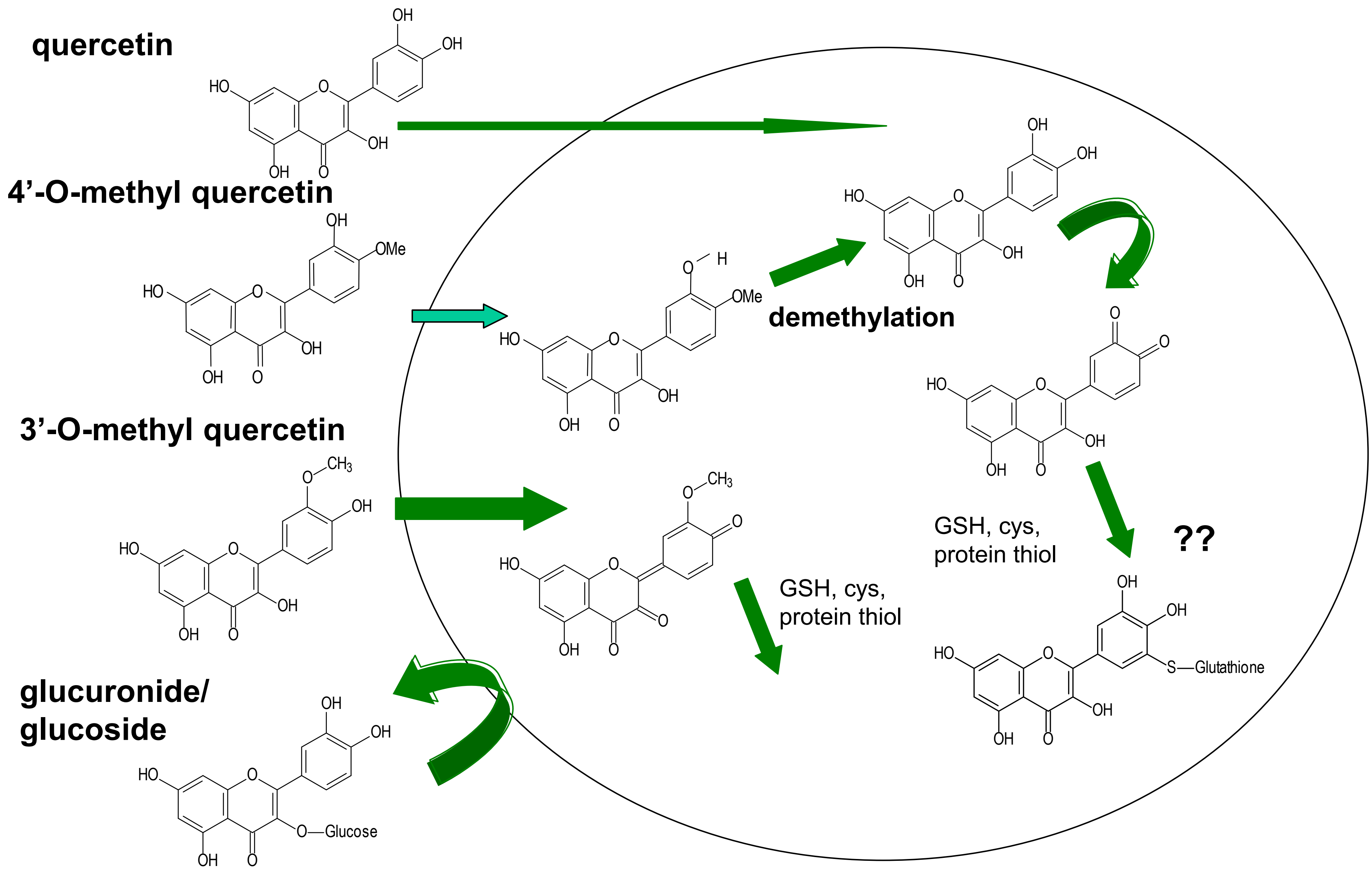
- **Reduction potentials of resulting conjugates**
- **Cellular access and partition coefficients**
- **Intracellular/extracellular metabolism and structural modifications**

FLAVONOIDS CAN BE EXTENSIVELY METABOLISED BY cytP450s
-> metabolites with modified biological activities–human liver microsomes II

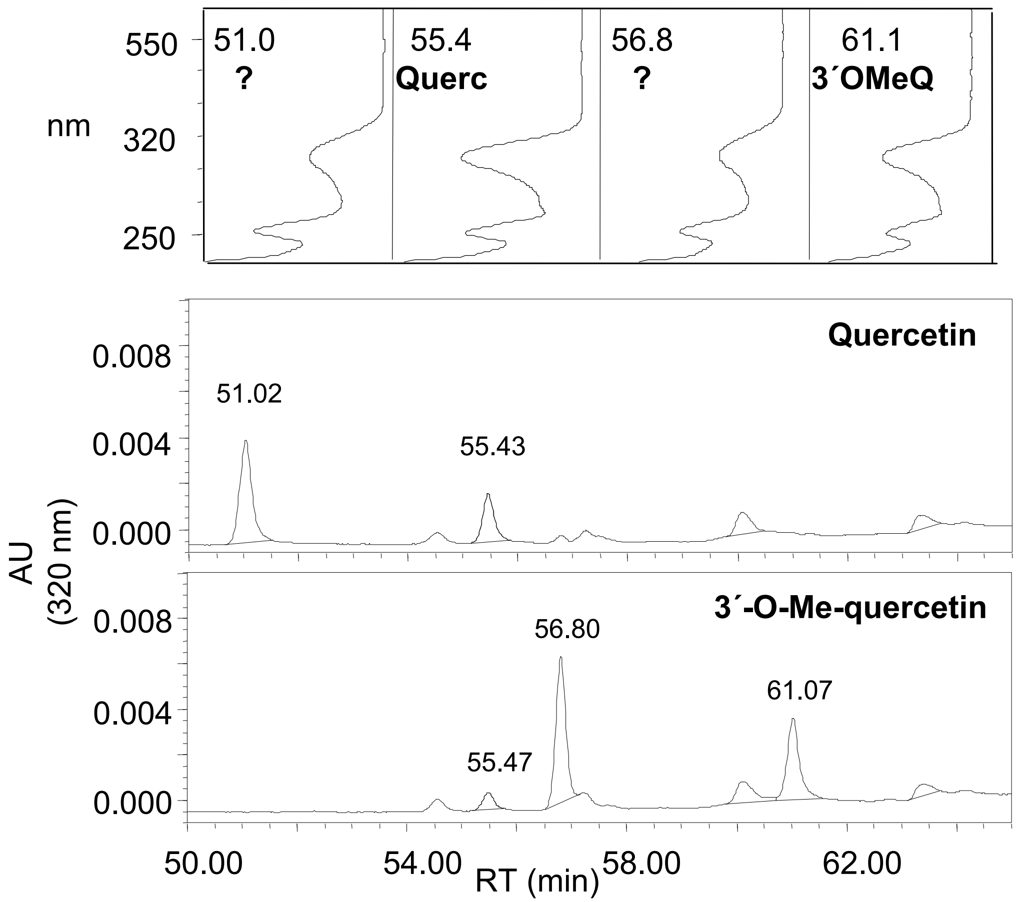


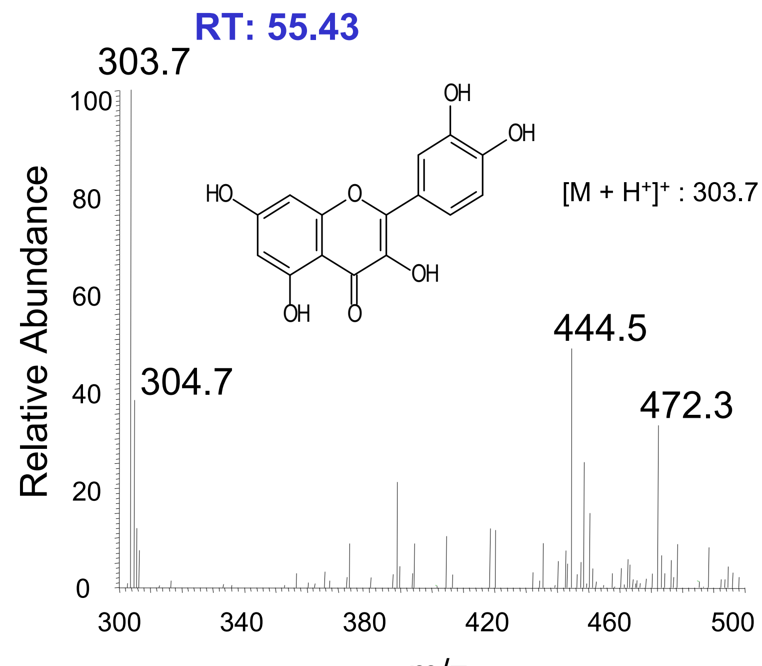
Breinholt *et al.* 2002

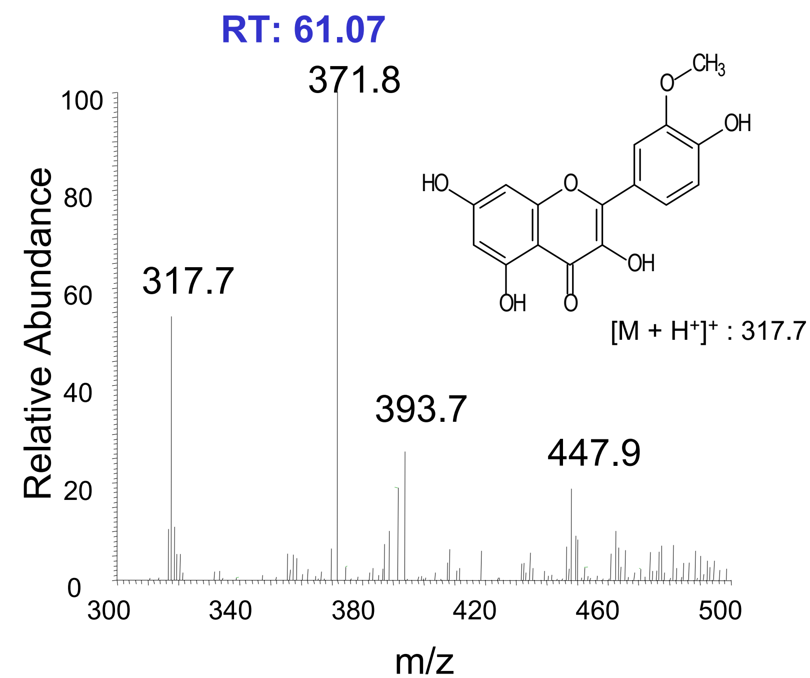
STRUCTURAL CONSEQUENCES OF INTRACELLULAR METABOLISM



Quercetin





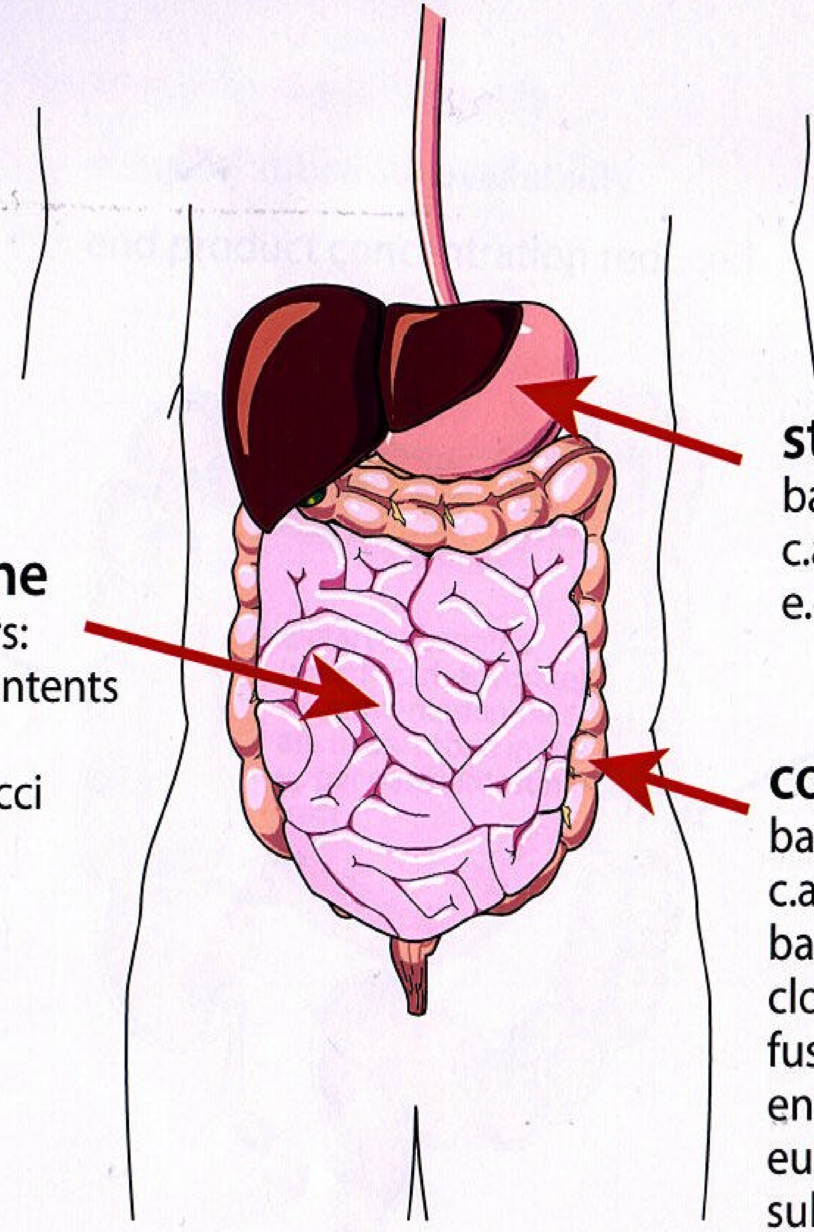


Spencer *et al.* 2002

COLONIC BIOTRANSFORMATION

WHAT'S HAPPENING IN THE COLON?

Majority of ingested flavonoids undergo colonic metabolism



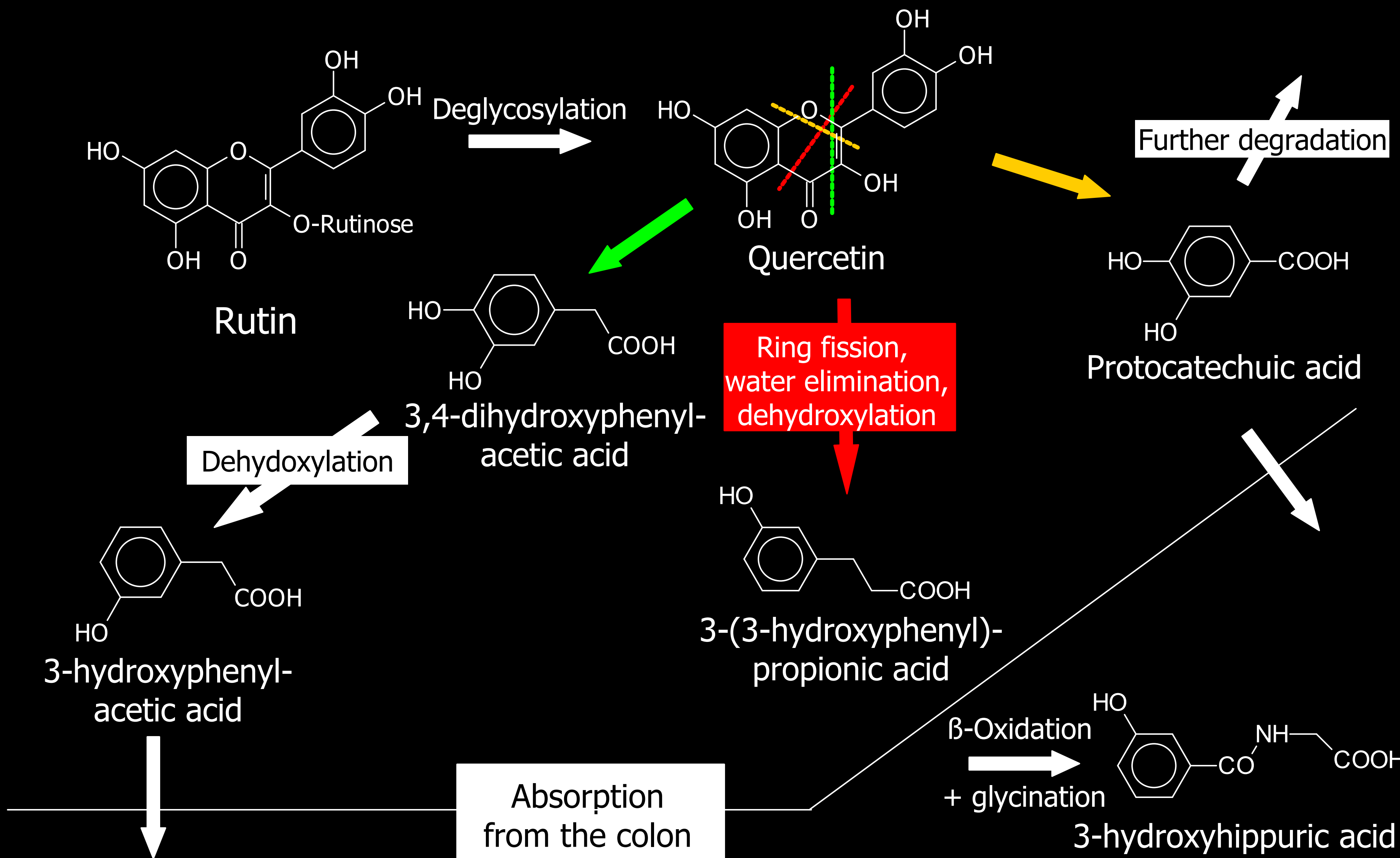
stomach
bacterial numbers:
c.a. 10^3/ml contents
e.g. *Helicobacter pylori*

small intestine
bacterial numbers:
c.a. 10^4-10^6/ml contents
e.g. lactobacilli,
Gram positive cocci

colon
bacterial numbers:
c.a. 10^{12}/g contents
bacteroides, bifidobacteria,
clostridia, peptostreptococci,
fusobacteria, lactobacilli,
enterobacteria, enterococci,
eubacteria, methanogens,
sulphate reducers etc

G. Gibson, University of Reading UK

Pathway of the colonic degradation of rutin - implications for properties of in vivo metabolites



MAJOR COLONIC METABOLITES

- 3,4-dihydroxyphenyl acetic acid
- 3-(3-hydroxyphenyl)propionic acid
- 3-(4-hydroxyphenyl)propionic acid
- Hydroxybenzoates

SO DO WE EXPECT FLAVONOIDS TO BE ANTIOXIDANTS IN VIVO?

IT DEPENDS:

- ***on what we mean by 'antioxidation'***

- ***on the extent and structural consequences of conjugation and metabolism***

BIOAVAILABILITY AND METABOLISM OF FLAVONOIDS

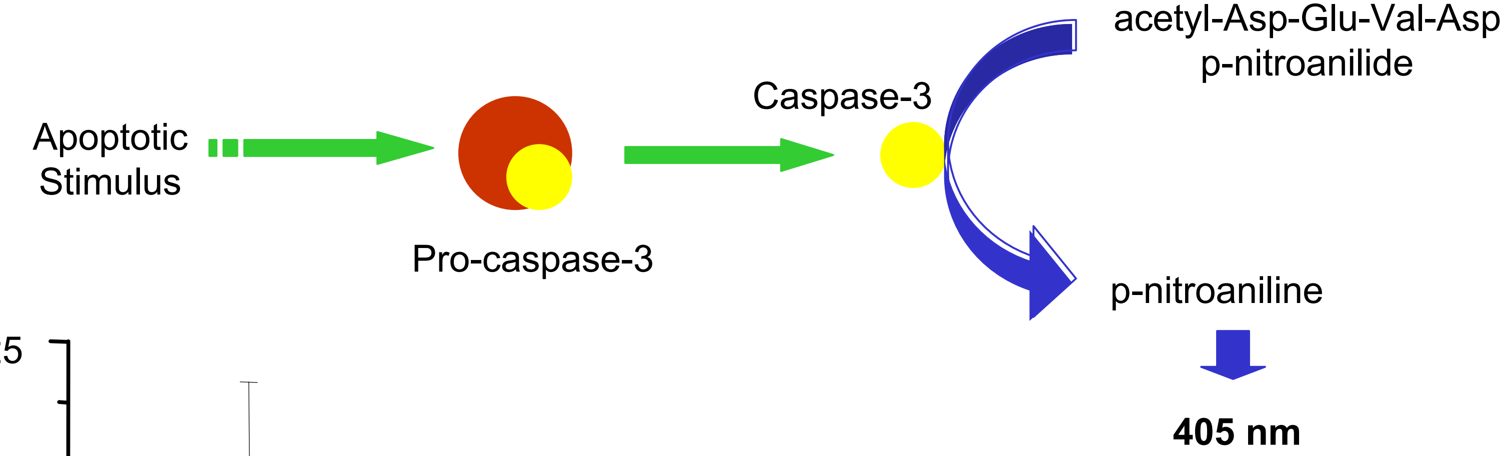
- **Less bioavailable than ascorbate and tocopherols**
- **MODIFIED by metabolism on absorption**
- **Less extensively absorbed and circulating levels *in vivo* much lower**

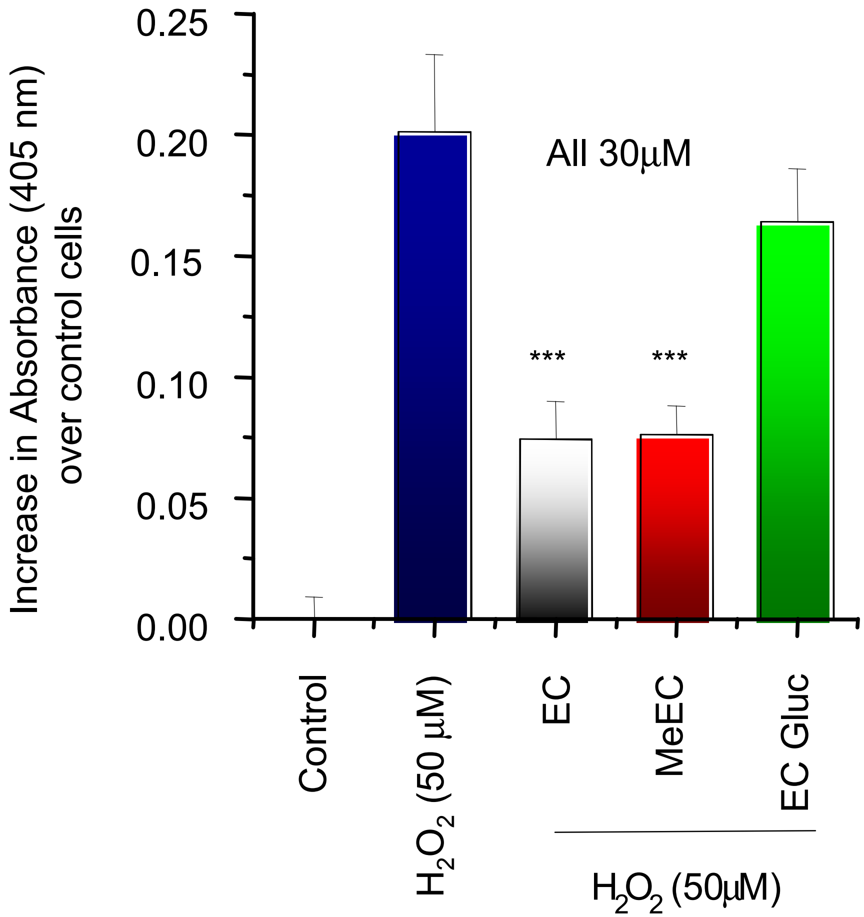
PLASMA LEVELS OF FLAVONOID CONJUGATES

Flavan-3-ol: **Wine catechins**	**100 nM**	**METHYL + SULPHATE +GLUCURONIDE**
Procyanidin: **Chocolate/cocoa**	**4 uM; 0.26 uM; 0.7 uM**	**EPICATECHIN SULPHATE + GLUCURONIDE**
Flavanone – grapefruit/orange	**< 4 uM**	**NARINGENIN /HESPERETIN GLUCURONIDE**
Anthocyanin – berry juices	**100 nM; 147 nM**	**ANTHOCYANIN GLYCOSIDES**

Donovan *et al*., Keen *et al*., Baba *et al*., Ameer et al, Miyazawa *et al.*

IN VIVO METABOLITE FORMS *VERSUS* CELLULAR OXIDATIVE STRESS





▪**METHYLATED METABOLITE**

Lower H-donating potential – *modified catechol group*

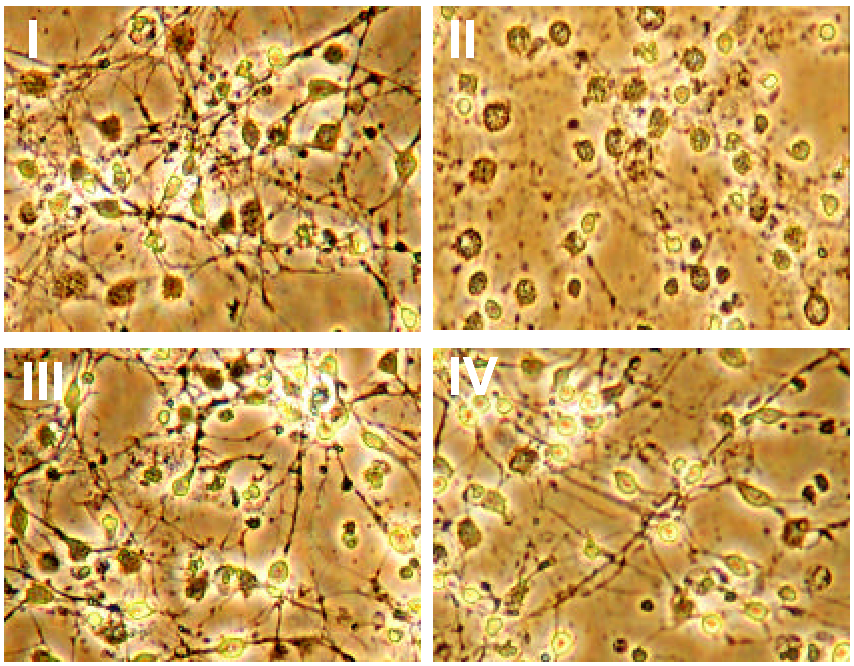
Similar protective effects against oxidative stress-induced cell death

▪**GLUCURONIDE METABOLITE**

Marginally lower H-donating potential

No protective effects against oxidative stress-induced cell death- *inaccessibility or substituted A-ring?* SPENCER *et al.* 2001

PROTECTION OF NEURONS FROM OXIDATIVE STRESS-INDUCED CELL DEATH BY EPICATECHIN



I Control neurons
II Neurons exposed to oxidative stress
III Control neurons treated with epicatechin
IV Neurons pretreated with epicatechin prior to oxidative stress

Schroeter *et al.* 2000

CONCLUSIONS:

- ? **BIOACTIVITY OF FLAVONOIDS *in vivo* MAY NOT DEPEND ON THEIR ACTIVITIES AS DIRECT SCAVENGERS OF REACTIVE OXYGEN OR NITROGEN SPECIES *PER SE***

- ? **BUT RATHER ON THE INFLUENCE OF THEIR *IN VIVO* FORMS ON THE MODULATION OF ENZYME / PROTEIN FUNCTIONS, INTRACELLULAR CELL SIGNALLING AND RECEPTOR ACTIVITIES**