Identificación y propiedades de los principales polifenoles y con caracteristicas farmacológicas

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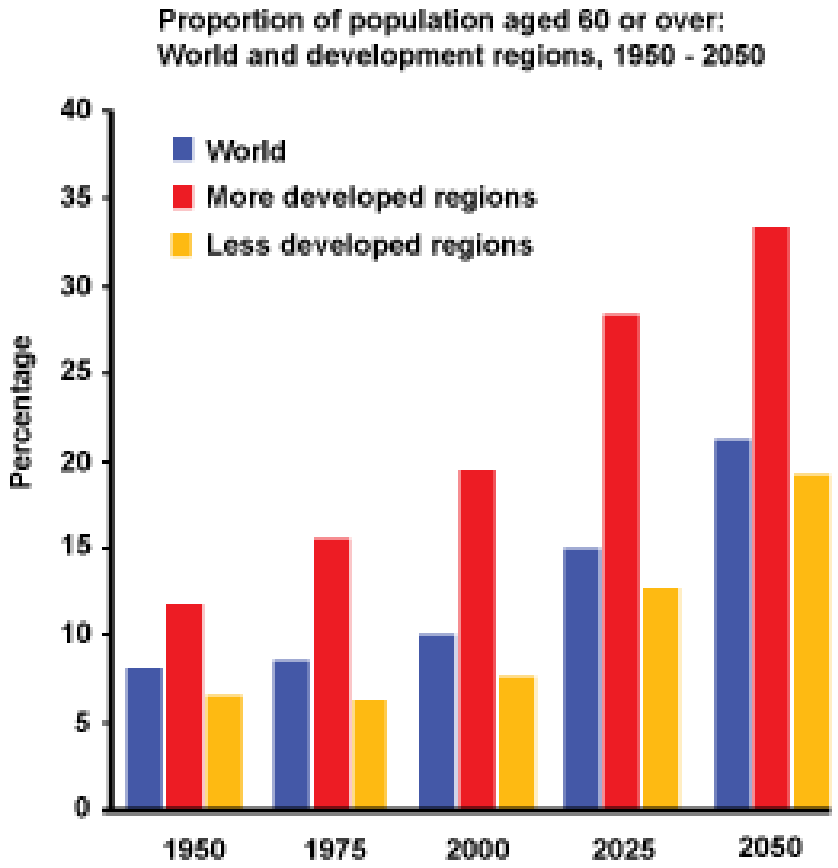
confidential data

Las enfermedades cardiovasculares son responsables del mayor número de muertes , (17,7 millones por año), seguido de cánceres (8,8 millones), enfermedades respiratorias (3,9 millones) y diabetes (1,6 millones).

INTRODUCTION



• The World Health Organization is calling for urgent action to make sure all people reach old age in the best possible health.

• The U.N. health agency says even in the poorest countries, elderly people are not dying from infectious diseases or gastroenteritis. Rather, they are dying from HEART DISEASES, stroke, cancer, diabetes, and chronic lung disease.

INTRODUCTION

• The World Health Organization recommends several key actions to strengthen healthy and active aging. It urges governments to promote good healthy behaviors throughout life and to provide basic primary health care to detect chronic diseases early so they can be treated.

30% of the population is overweight and 11% suffer obesity

62% of the population don't eats vegetables every day.

65% of young people from 15 to 18 years drink soda with sugar every day.

Factores clásicos de riesgo cardiovascular:

- Edad y sexo
- Tabaquismo
- Hipertensión
- Obesidad
- Hypercolesterol
- Desiquilibrio alimenticio
- Sedentarismo
- Consumo excesivo de alcohol

Factores de riesgo cardiovascular no clásicos

- Hiperhomocistanemia
- Factores psicosociales y medio-ambientales (epigenetismo)
- Factores hormonales (anticonceptivos orales)
- La concentración de la CRP (1,5 mg/L)
- El estrés oxidante
- La disfunción endotelial

Definición del estres oxidante
(Sies 1985)

« **Desequilibrio entres los** oxidantes (EROs incluídos los radicales libres, peróxido de hidrógeno, oxígeno singleto, ácido hipocloroso) **y los antioxidantes en favor de los primeros**, estos que conducen a los daños celulares irreversibles »

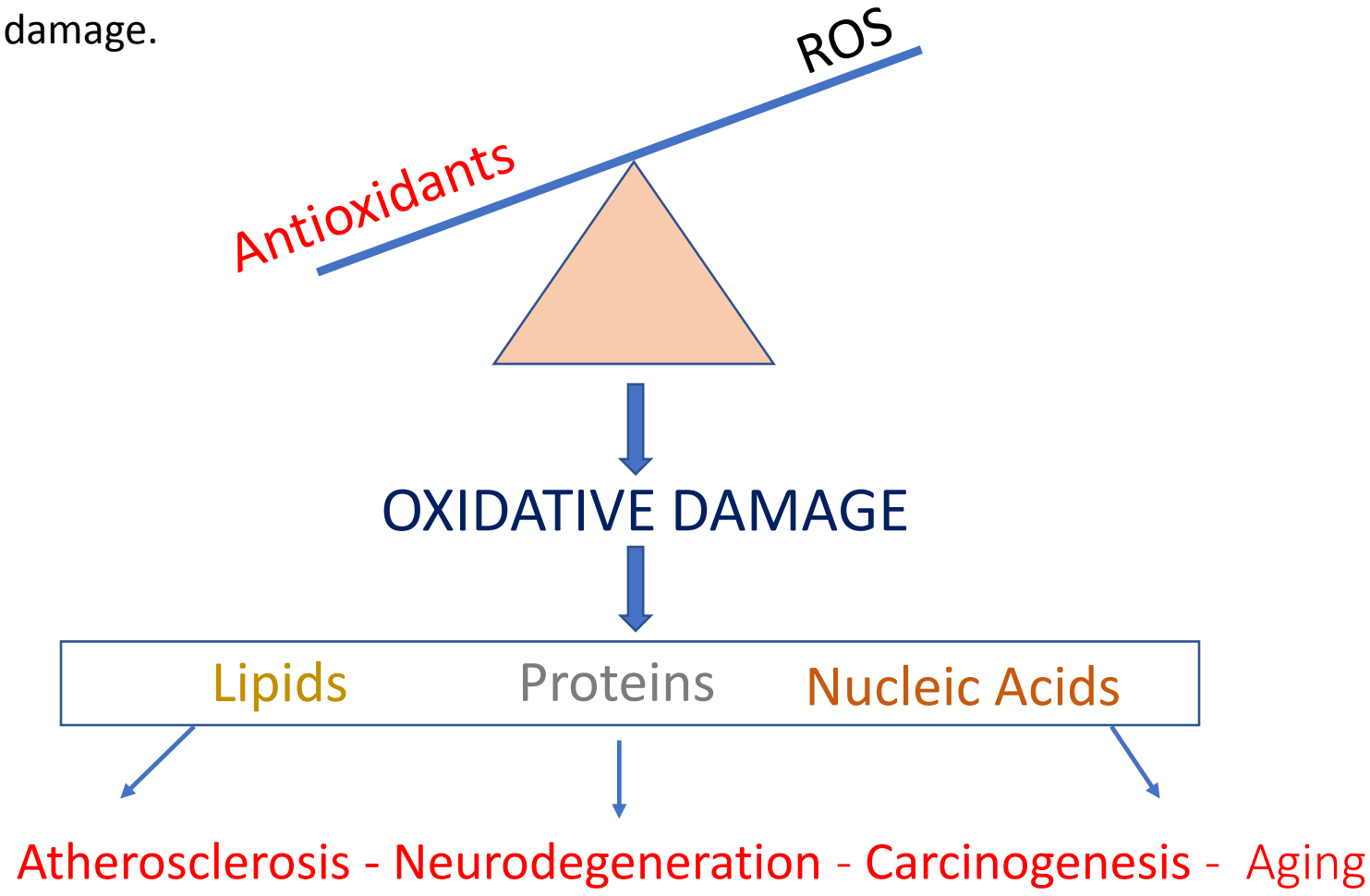
Definición actualizada del estres oxidante
(Jones et Sies, 2007)

« **Desequilibrio entre** los oxidantes en favor de los primeros, estos que conducen a una ruptura de la señalización redox y a los daños celulares »

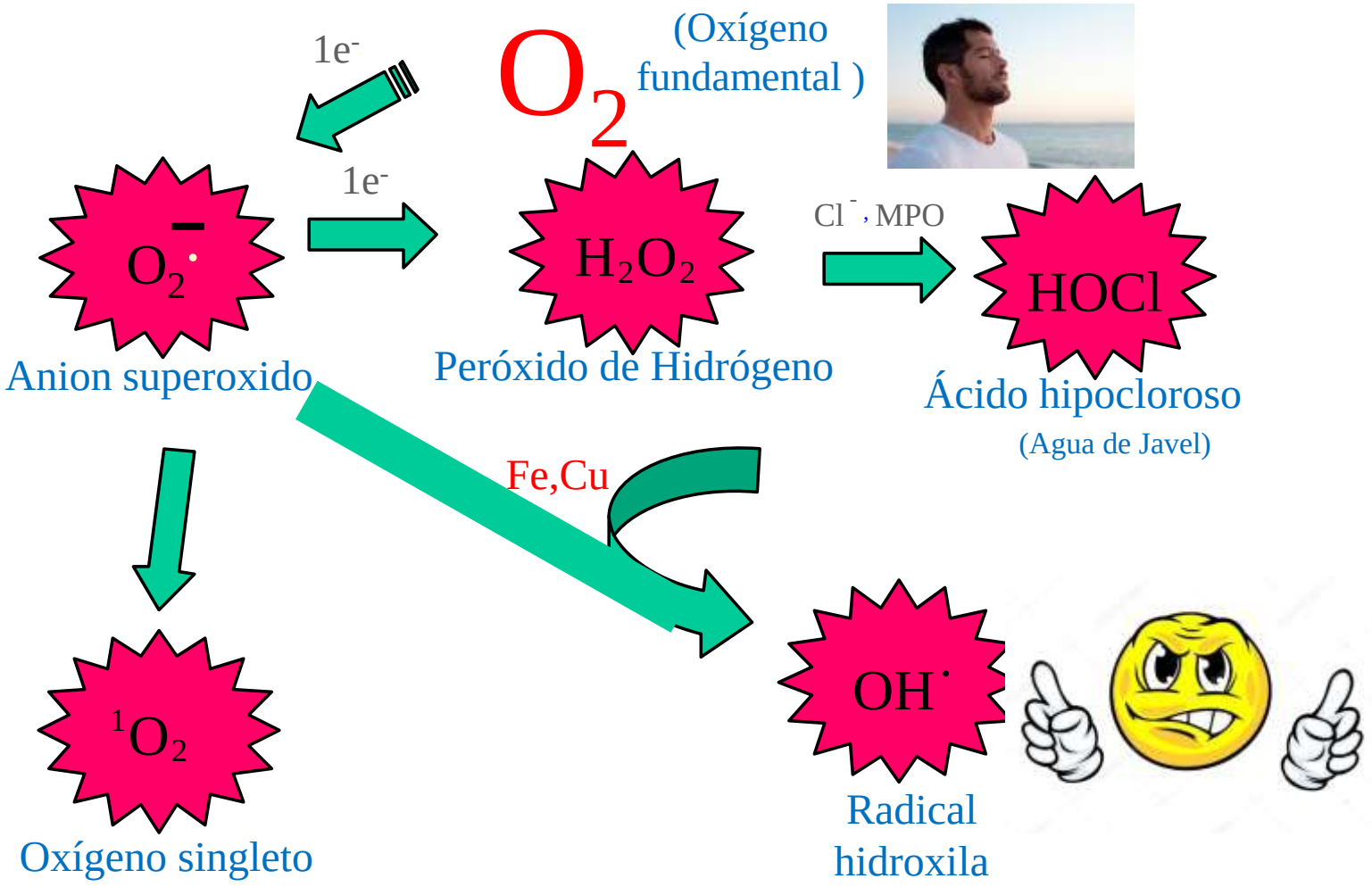
Definición que incluye efectos tóxicos y fisiologicos de las EROs

Oxidative Stress (OS)

As an imbalance between oxidants (e.g. free radical species derived from oxygen) and antioxidants in favour of oxidants, leading to a disruption of redox signalling and/or molecular damage.



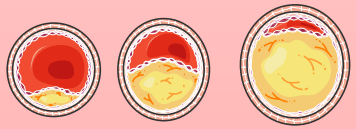
Jones DP, 2006. *Trachootham D, et. Al, 2009. Andersen JK, 2004. Paravicini TM, 2006. Haigis MC, 2010.*



Las especies reactivas de Oxigeno (EROs)

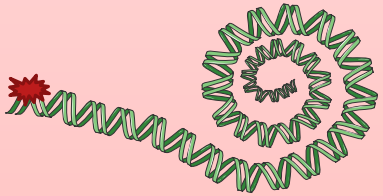
Producción de derivados tóxicos oxigenados en exceso en nuestro organismo (radicales libres y agua oxigenada)

LIPIDIOS

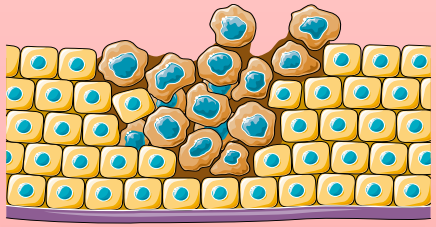


Arteroclerosis, enfermedades cardiovaculares

Lipidios oxidados (ROOH, LDL oxidadas)

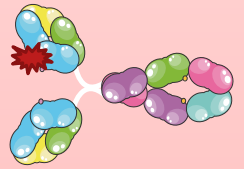


ADN

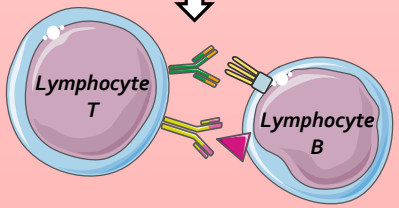


Cáncer

ADN oxidado (8OHdG)



PROTEINAS



Enfermeddes auto-inmunes

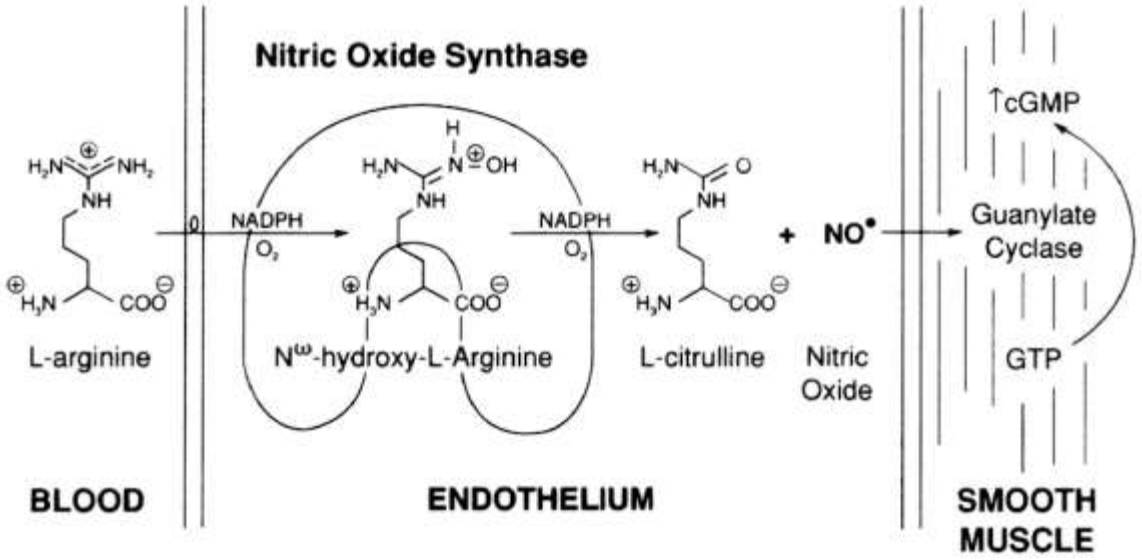
Enfermedad de **Alzheimer…**

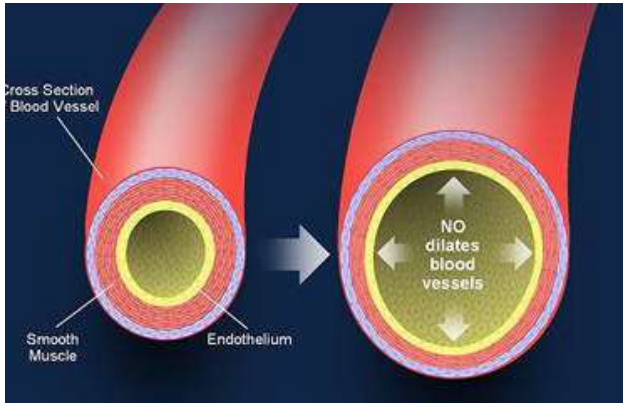
Proteinas oxidadas (carbonilas)

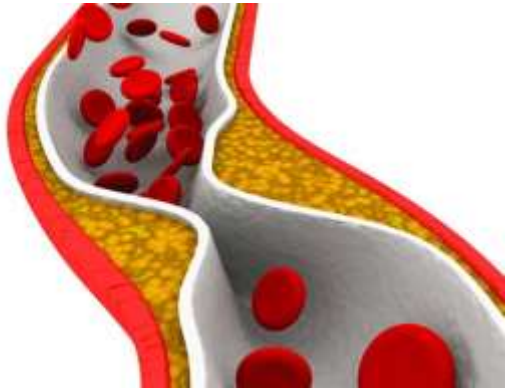
INTRODUCTION

- Endothelium is the bioactive inner layer of the blood vessels, which serves as an important locus on control of vascular and thus other organ function regulating vascular tone permeability .

- It produces components of extracellular matrix such a NO.



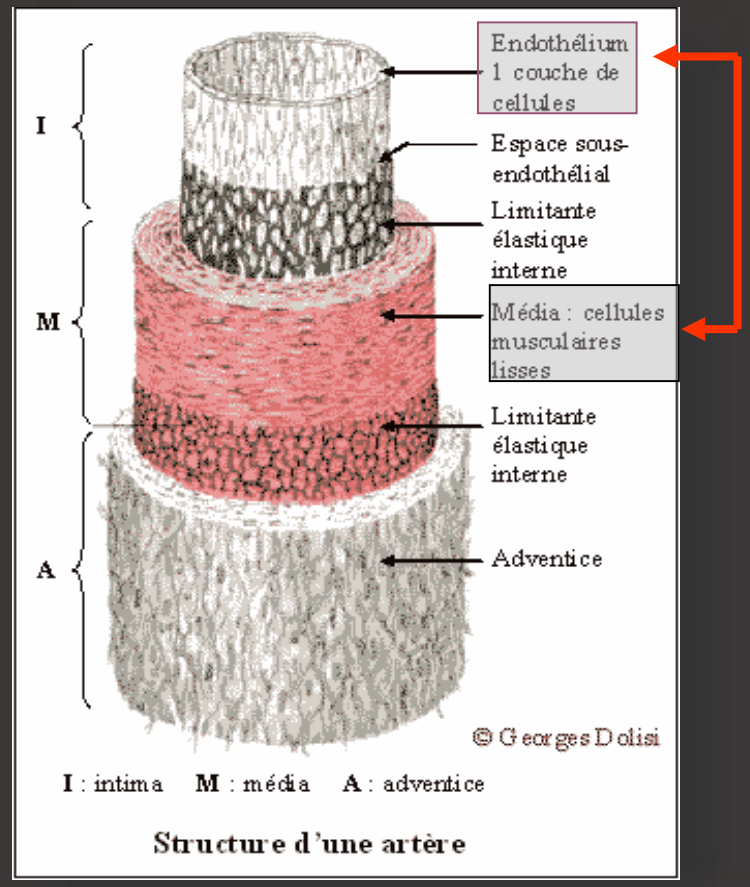




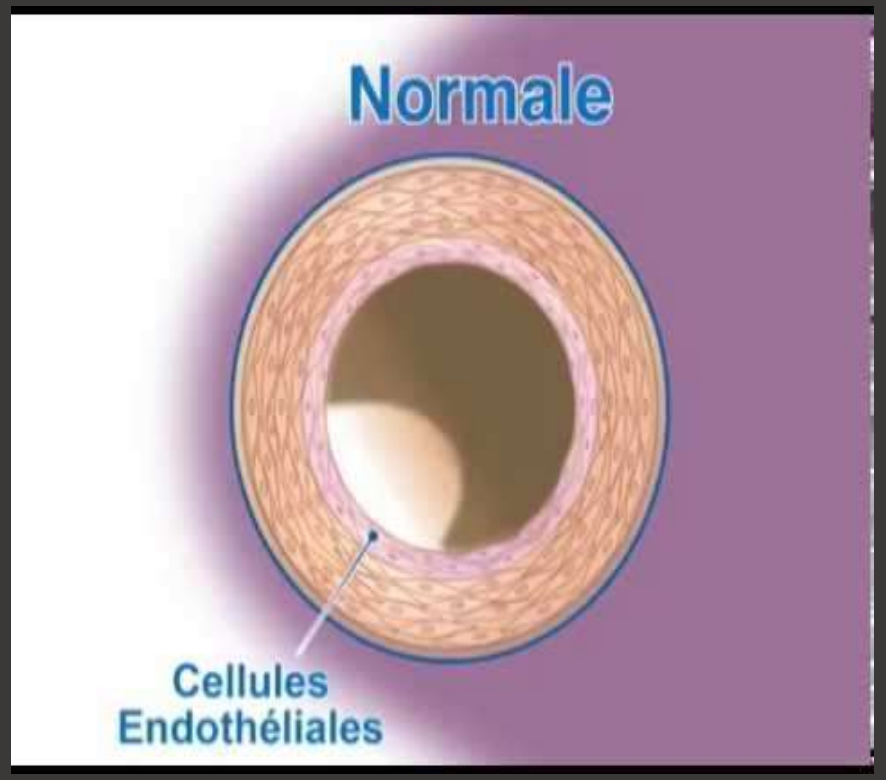
- Endothelium dysfunction is a nearly event in atherosclerotic disease.

La disfunción endotelial

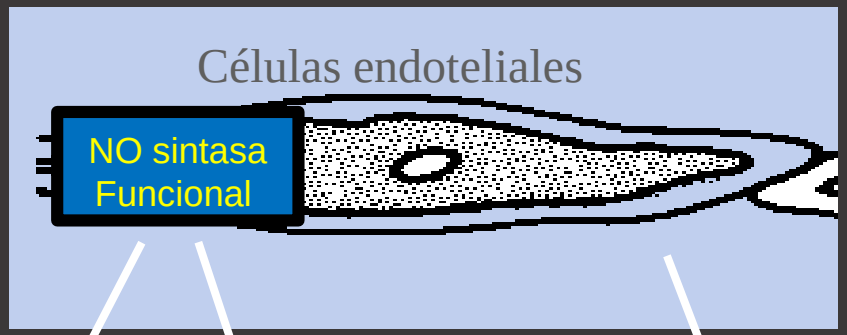
Las células endoteliales, que están junto a la pared interna de todos los vasos sanguineos, regulan la presión arterial.



Structure d'une artère



Buena función endotelial



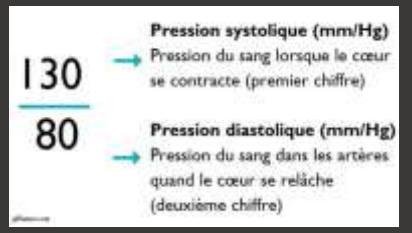
prostacyclina

$NO^{\cdot}$

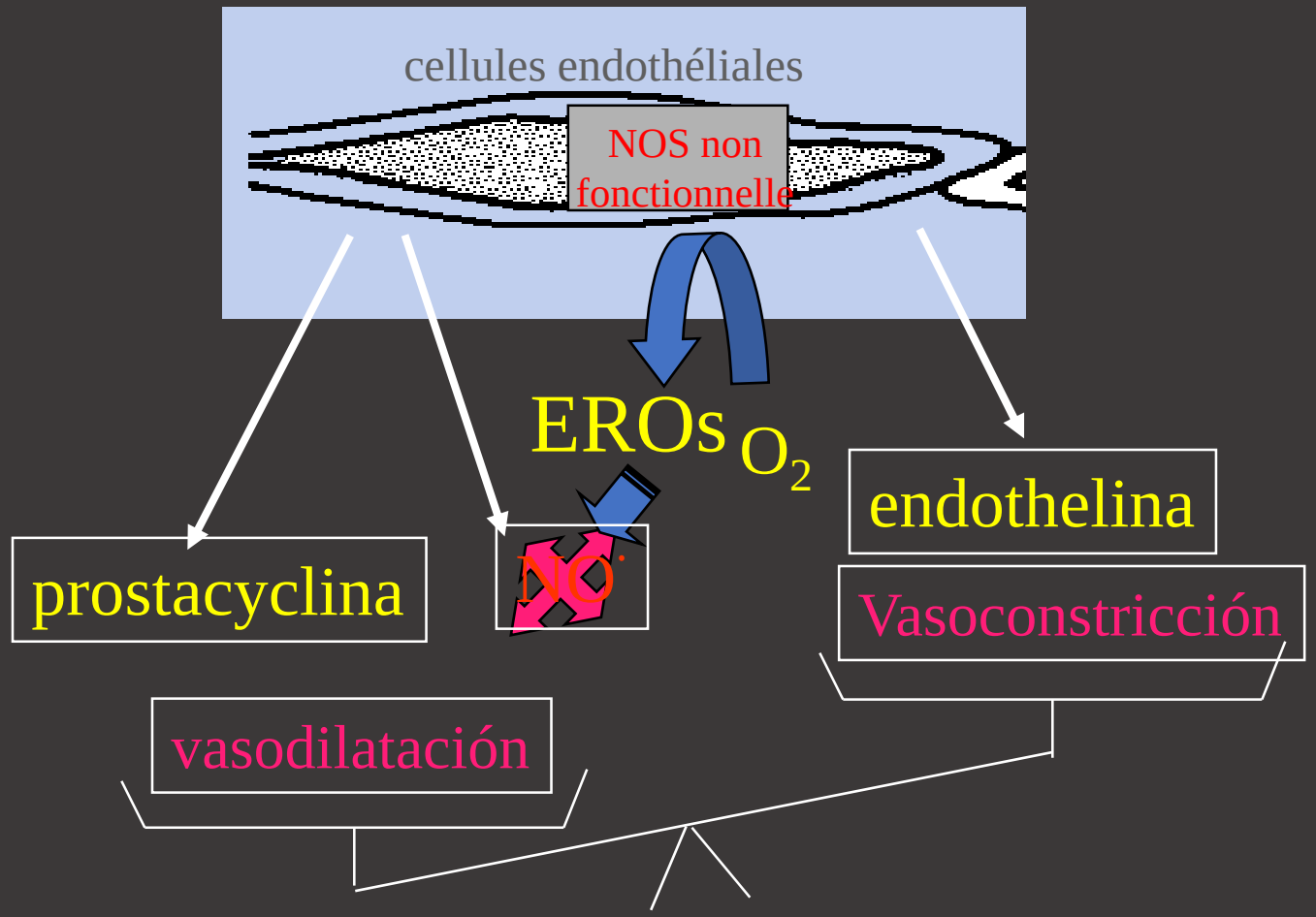
endothelina

vasodilatación = vasoconstrición

Buena presión arterial (120/80 mm Hg)



Disfunción endotelial



Hipertensión arterial: factor de riesgo cardiovascular (140 mm/90 – 100 mm)

Red compleja de antioxidantes

Endógenos

Enzimas antioxidantes

(Dismutasa del superoxido, Superoxidasa de la glutationa, paraoxonasa, etc..)

Únicamente por la alimentación

Pequeños antioxidantes

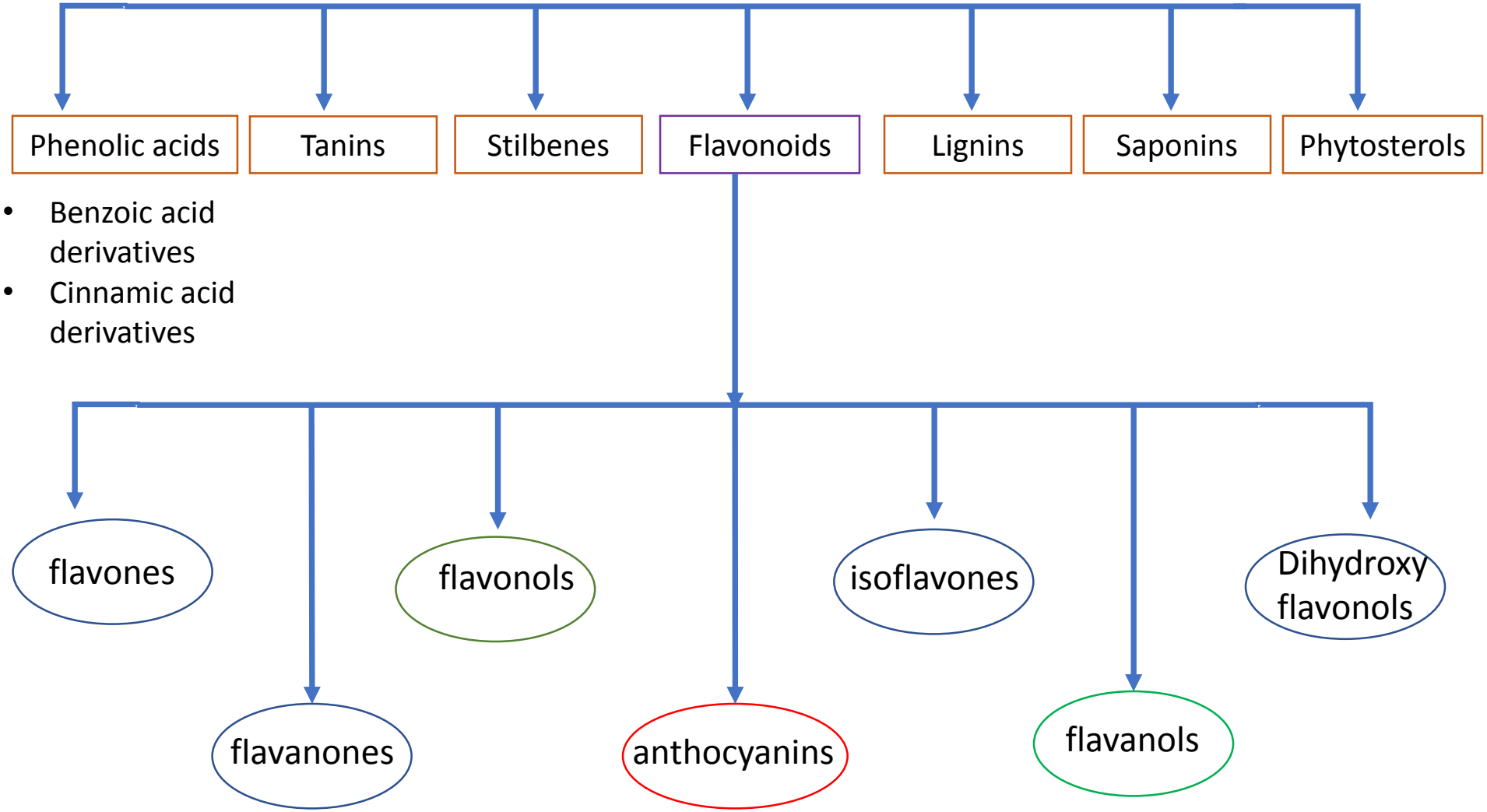
(vitaminas C y E, carotenoides, glutationa, ubiquinona, polifenoles (flavonoides), …)

oligoelementos necesarios para la actividad de ciertas enzimas antioxidantes

(selenio, cobre, zinc, ...)

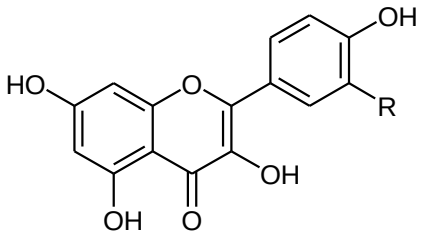
Todos los estudios de observación indican
que una alimentación pobre en antioxidantes
naturales (vitamines C et E,
carotenoides, polifenoles)
provocan mayor riesgo de desarrollar
enfermedades cardiovasculares y cánceres.

Phenolic Compounds



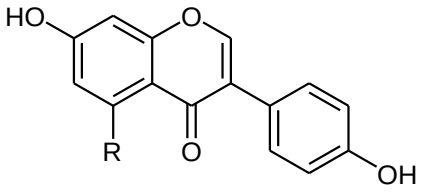
Flavonoids

Flavonol



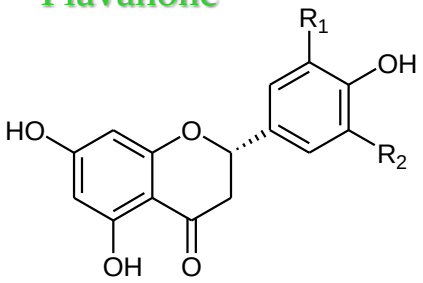
R = H kaempferol *(broccoli)*
R = OH quercetin *(apples)*

Isoflavone

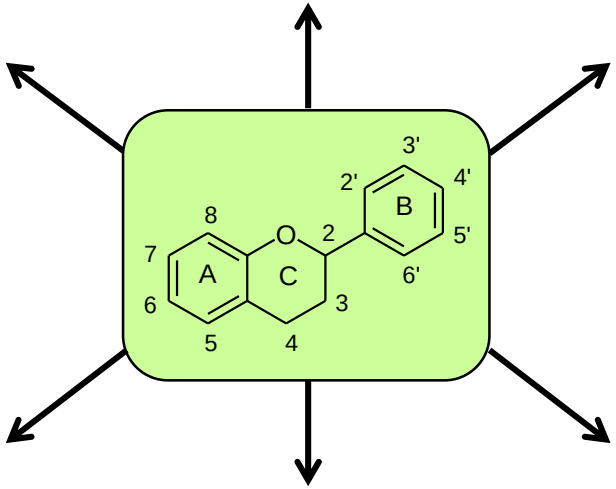


R = H daidzein *(soy)*
R = OH genistein *(soy)*

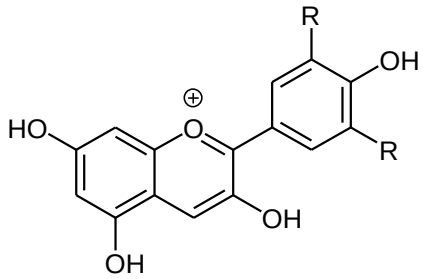
Flavanone



R1 = OCH_3, R2 = OH hesperitin *(citrus fruit)*
R1 = R2 = H naringenin *(grapefruit)*

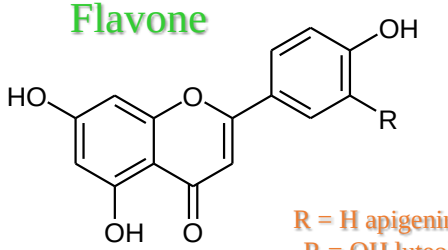


Anthocyanidin



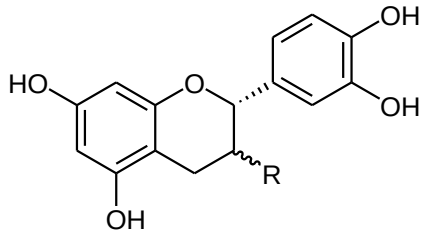
R = OCH_3 malvidin *(red wine)*
R = OH delphinidin *(bilberries)*

Flavone

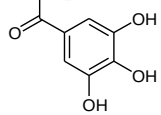


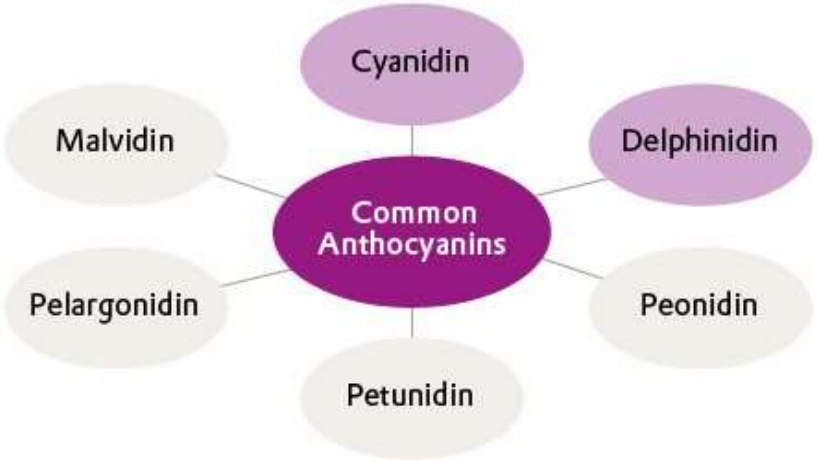
R = H apigenin *(parsley)*
R = OH luteolin *(sage)*

Flavan-3-ol



R—OH catechin *(dark chocolate)*
R epicatechin gallate *(green tea)*





- *Ribes nigrum* (Blackcurrant) is well-known to be a plant enriched in antioxidant compounds like vitamin C and flavonoids, more particularly anthocyanins.
- The new trend is to implement adequate diets of these compounds to prevent cardiovascular diseases.

Excellent regulators of arterial blood pressure

Polifenoles:
Definición General

-Conjuto de moleculas que llevan funciones alcoholicas sobre los ciclos aromaticos

-Estos son metabolitos secundarios de vegetales:

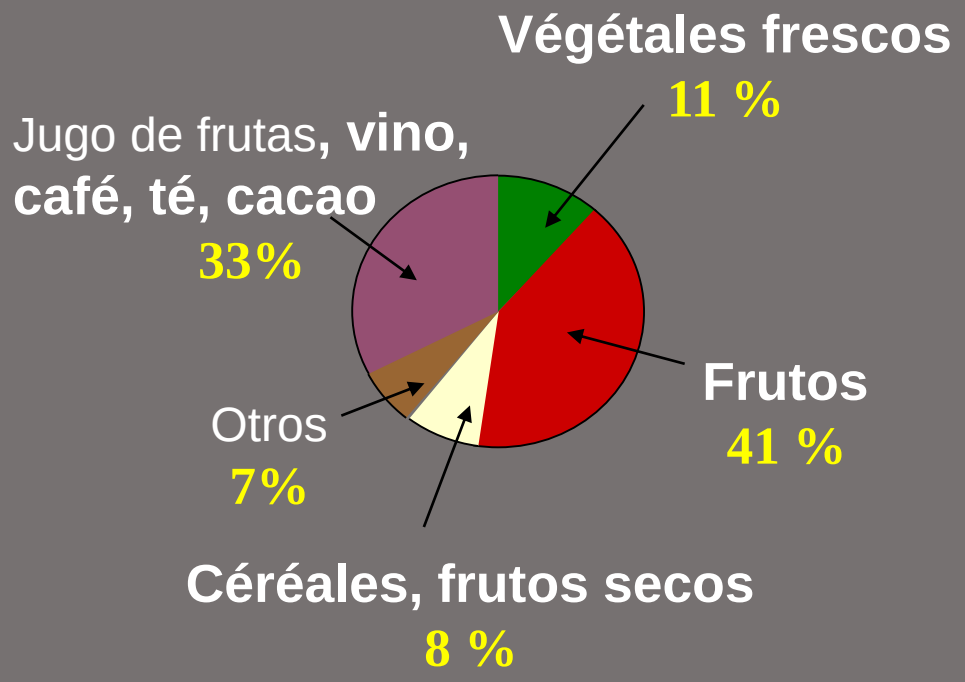
- Que participan en las reacciones de defensa de los végétales frente a diversos tipos de estrés bioticos o abioticos (patogenos, herbivoros, rayos UV, gel ….)

-Que no representan caracter tóxico y son consumidos en la matriz vegetal

- Poseen propiedades organolépticas

Fuentes alimenticias de polifenoles

Aporte diario recomendado: 1000 mg



FLAVONOLES
(quercétine, kaemphérol, myricétine, isorhamnétine)




FLAVONAS
(lutéoline, apigénine)


FLAVANONAS
(héspérétine, naringénine, ériodictyol)



CATEQUINAS (proanthocyanidines)
(catéchines, épicatéchine, epigallocatéchine)



ANTOCIANINAS
(cyanidine, delphinidine, malvidine, péonidine, petunidine, pelargonidine)



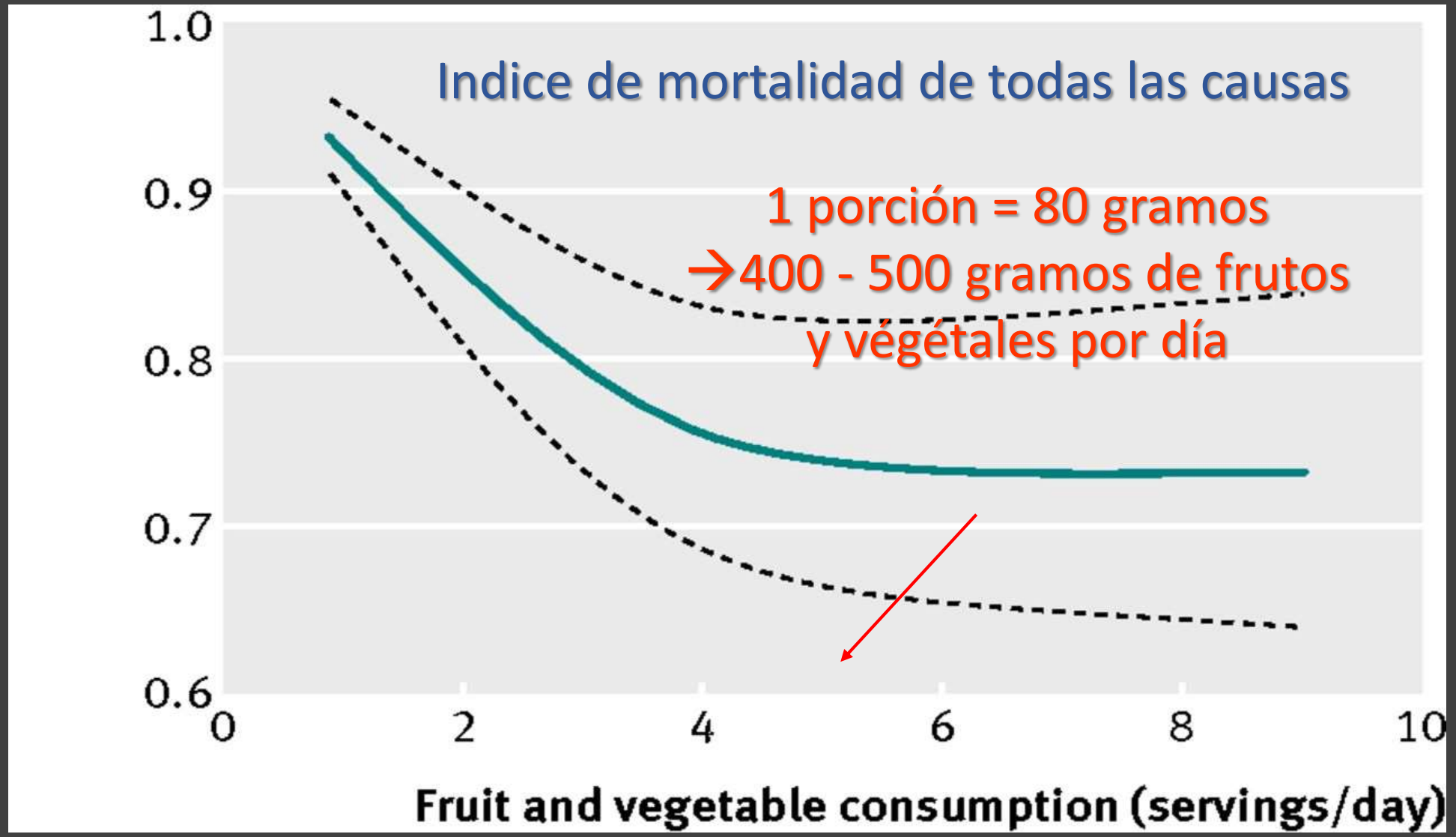






ISOFLAVONAS
(daidzéine, génistéine, glycitéine)

les fruits en Equateur

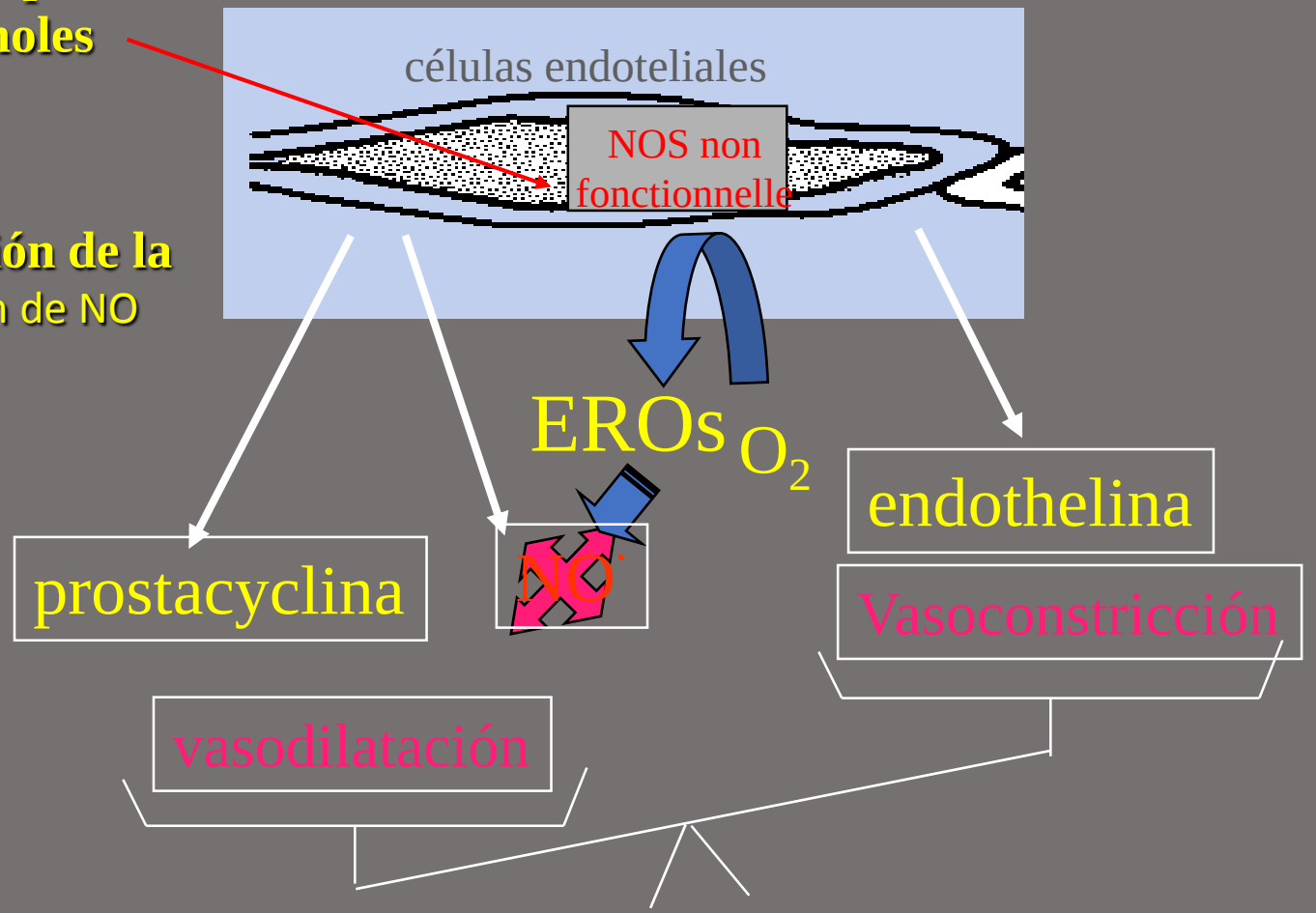


Wang et al. BMJ 2014;349:g4490

Disfunción endotelial

Super expresión de la NO sintasa por los polifenoles

Restauración de la producción de NO

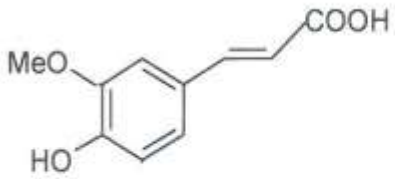


Hipertensión arterial: factor de riesgo cardiovascular (140 mm/90 – 100 mm)

Polyphénols du cacao

Acides phénoliques

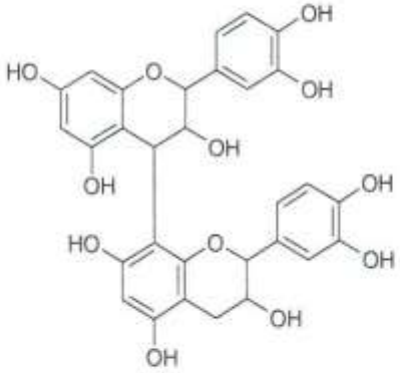
Acide férulique *(25 ppm)*



Acide caféique,
Acide vanillique,
Acide syringique,
....

Antioxydant, précurseur d'arôme

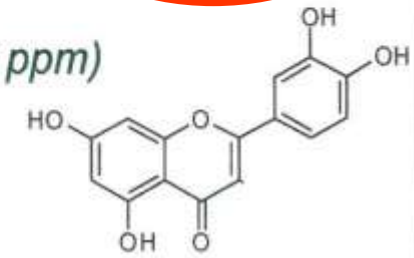
Flavonoïdes et Anthocyane



Flavanols:
catéchine, épicatéchine,
dimères, trimères
-> Oligomères

*Total: 97000 ppm***

Flavonols: Quercetine *(25 ppm)*



Flavones:
Lutéoline, Apigénine

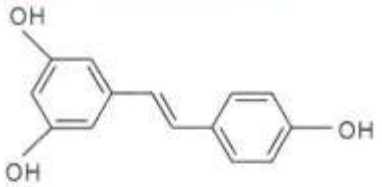
Flavanones: Naringénine

Anthocyanidines: Cyanidine-glycoside

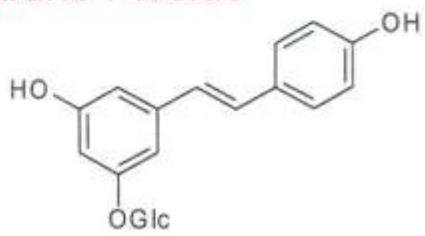
Antioxydant, Astringent, couleur, propriétés cardioprotectrices,

Stilbènes*

trans-Resvératrol



trans-Picéide



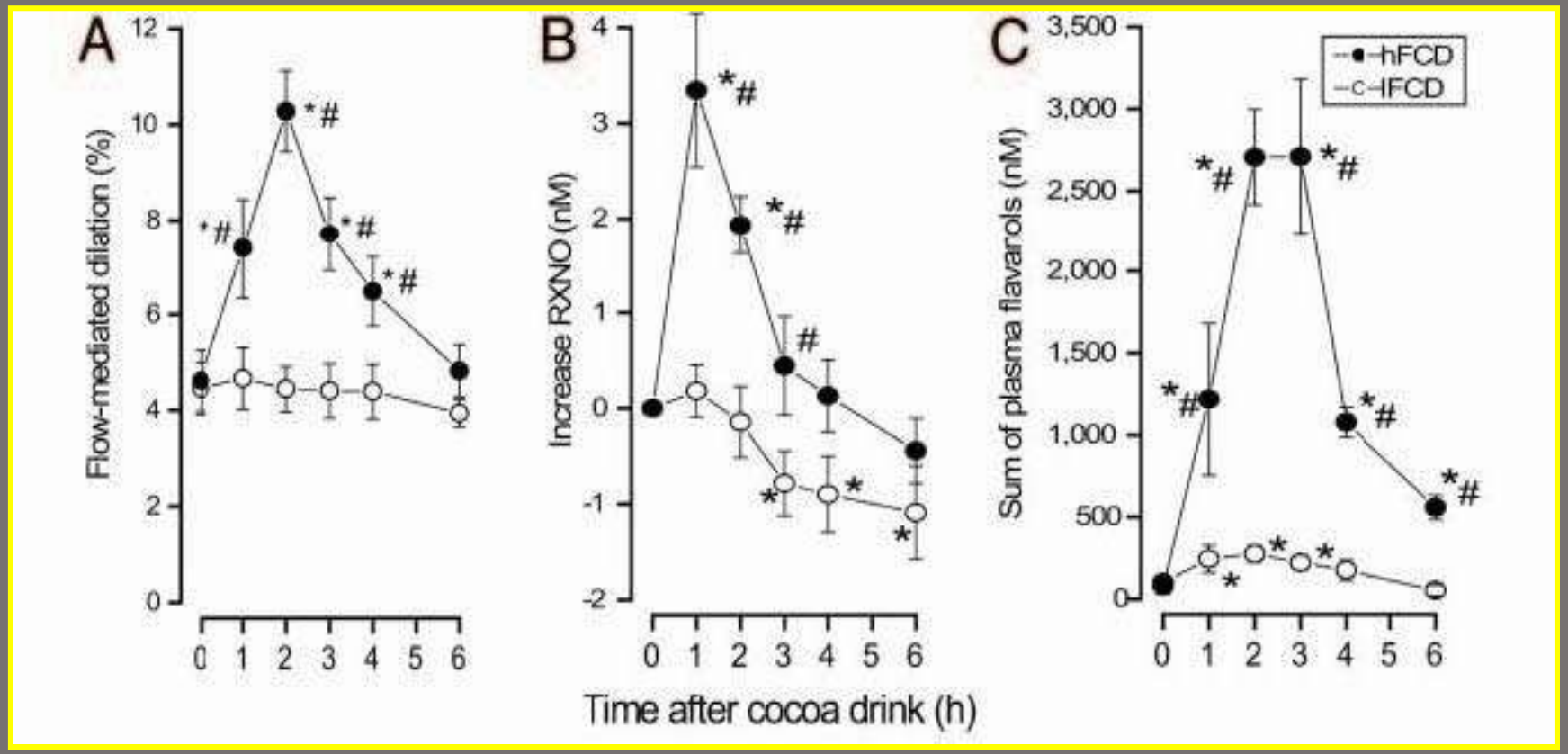
Propriétés cardioprotectrices, anti-inflammatoires et anti-cancéreuses

* Counet, Callemien, Collin, *Food Chem.*, 98 (2006), 649-657.
** L. Gu, *et al*, *J. Agric. Food Chem.*, 50 (2002), 4852-4860.

Ingestion of Flavanol-rich Cocoa Enhanced Flow-mediated Vasodilatation in Healthy Adults

Healthy male adults:

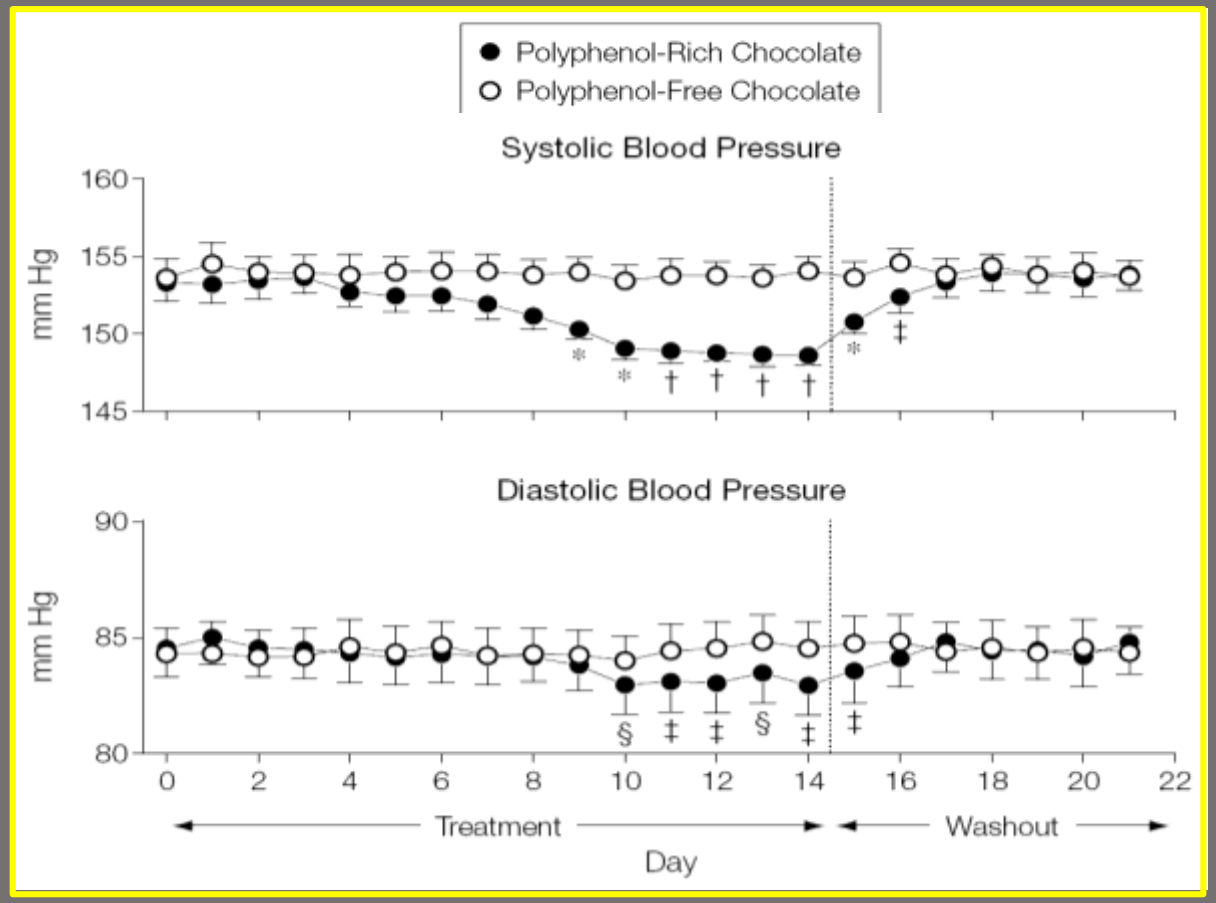
High-flavanol cocoa drink (hFCD) · Low-flavanol cocoa drink (lFCD)



Schroeter et al. PNAS 2006; 103: 1024-1029

Intake of Polyphenol-rich Dark Chocolate lowers Blood Pressure in Subjects with Mild Isolated Hypertension

Daily intake of: - 10 g dark chocolate for 14 days (polyphenol-rich chocolate)
- 9 g white chocolate for 14 days (polyphenol-free chocolate



Taubert et al. *JAMA* 2003; **290**:1029-1030


Chambre Syndicale Nationale des Chocolatiers
LA FABRICATION DU CHOCOLAT
1 CACAOYER
2 CABOSSE
3 ECABOSSAGE
D'un coup de gourdin, on ouvre les cabosses. On y trouve les fèves de cacao enveloppées d'une pulpe blanche : le mucilage.
fèves de cacao avec le mucilage
fèves de cacao fermentées
4 FERMENTATION
Le mucilage s'écoule, les fèves se modifient, les précurseurs d'arôme se développent.
5 SÉCHAGE
Séchées au soleil ou dans des séchoirs artificiels durant deux semaines, les fèves de cacao prennent leur couleur brune.
grains de cacao
6 CONCASSAGE
A partir de la fève on sépare le grain de cacao de la coque.
7 TORRÉFACTION
Les grains de cacao sont grillés.
grains de cacao
Conservación de polifenoles originales?????
8 BROYAGE
A partir du broyage des grains de cacao on obtient la pâte de cacao.
9 PRESSAGE
A partir de la pâte de cacao, le pressage sépare la partie liquide : le beurre de cacao et la partie solide : le tourteau.
partie solide = tourteau
beurre de cacao
filtrage
9 bis BLUTAGE
Le tourteau est passé à travers un tamis pour obtenir la poudre de cacao.
Poudre de cacao
*Lait : pour chocolat au lait
10 MALAXAGE
Pour obtenir le chocolat, on mélange dans le pétrin les différents ingrédients.
11 BROYAGE
Pour obtenir une pâte fine.
12 CONCHAGE
Pour rendre le chocolat plus lisse et affiner le goût.
C'est ici que le savoir-faire du chocolatier se révèle
13 TEMPÉRAGE
C''est le tempérage qui permet une bonne cristallisation du chocolat en lui donnant un aspect brillant, une texture croquante et fondante.
14 MÉLANGE
Le chocolat est associé selon les goûts aux noisettes, amandes, raisins, fruits confits, céréales, riz soufflé...
15 MOULAGE OU ENROBAGE
Tablettes, Moulages, Bonbons de chocolat, Barres, Billes de chocolat, Pâtes à tartiner.
LE CHOCOLAT NOIR
chocolat noir = pâte de cacao + beurre de cacao + sucre
LE CHOCOLAT AU LAIT
chocolat au lait = pâte de cacao + beurre de cacao + lait en poudre + sucre
LE CHOCOLAT BLANC
chocolat blanc = beurre de cacao + lait en poudre + sucre

Determination of antioxidant capacity using CHEMILUMINESCENCE methodology

Test is performed on whole blood, which contains red blood cells, white blood cells and platelets. For this test, we focus on white blood cells or leucocytes, which have a nuclear core unlike red blood cells and platelets.

There are several types of white blood cells: neutrophils, basophils, eosinophils, monocytes etc. The low oxygen consumption of neutrophils at rest is favorable for this test.

Activation of these neutrophils is caused by a stimulant called phorbol myristate acetate (PMA), that increases their oxygen consumption by 400%.

ROS, and especially superoxide anion, will react with lucigenin.

CHEMILUMINESCE

- 200µL of blood sample
- 50µL of Lucigenin

15 min of Incubation at 37 °C, after Activation of Photon Counting photomultiplier Tube(PMT)

- 10µL of fruit or vegetable juice diluted 10x in (PBS).
- 10µL of Phorbol-12Mirystate,13Acetate (PMA) diluted 10x in Phosphate Buffer Saline (PBS).

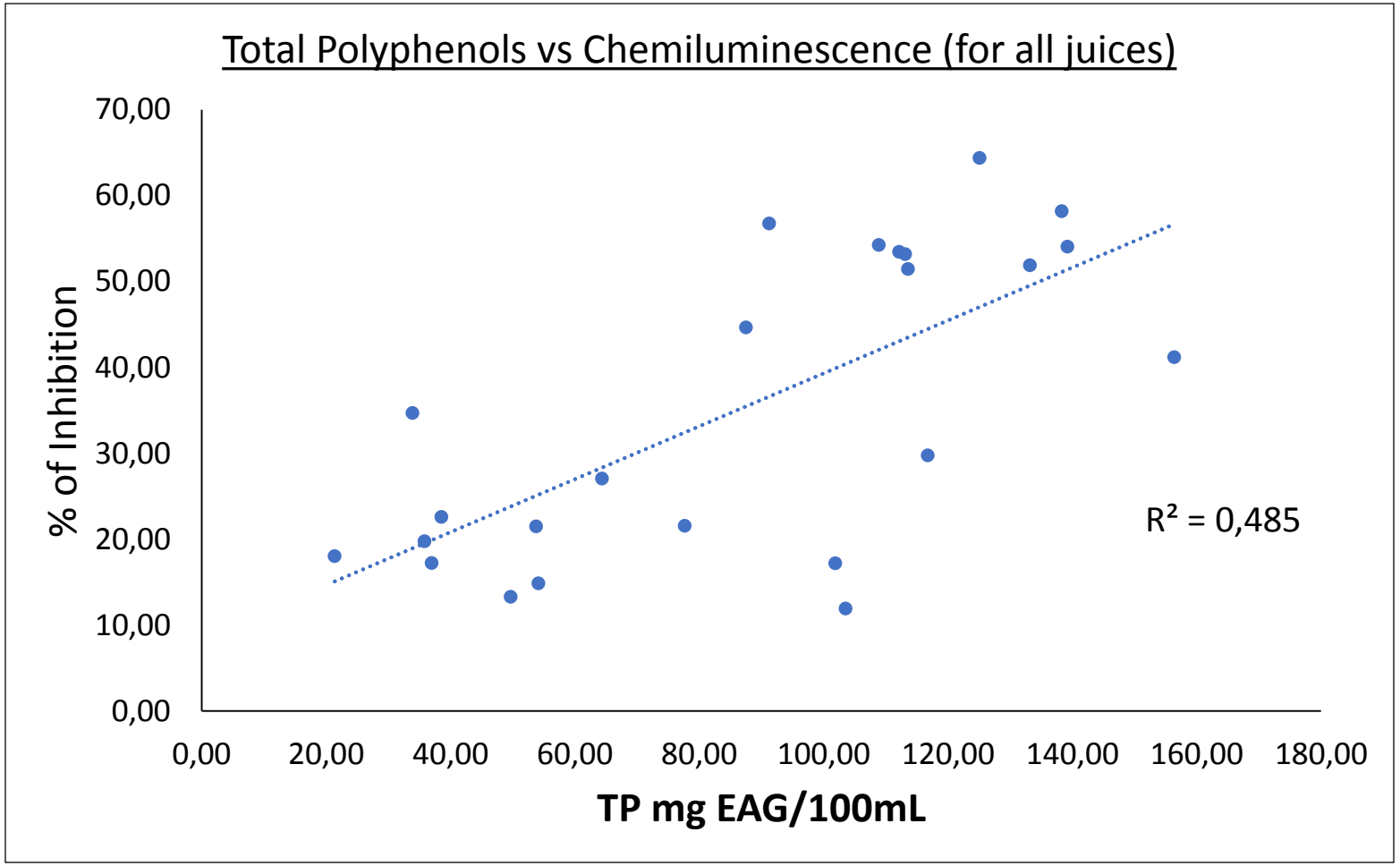


Figure 2. Correlation between Total Polyphenols and Chemiluminescence.

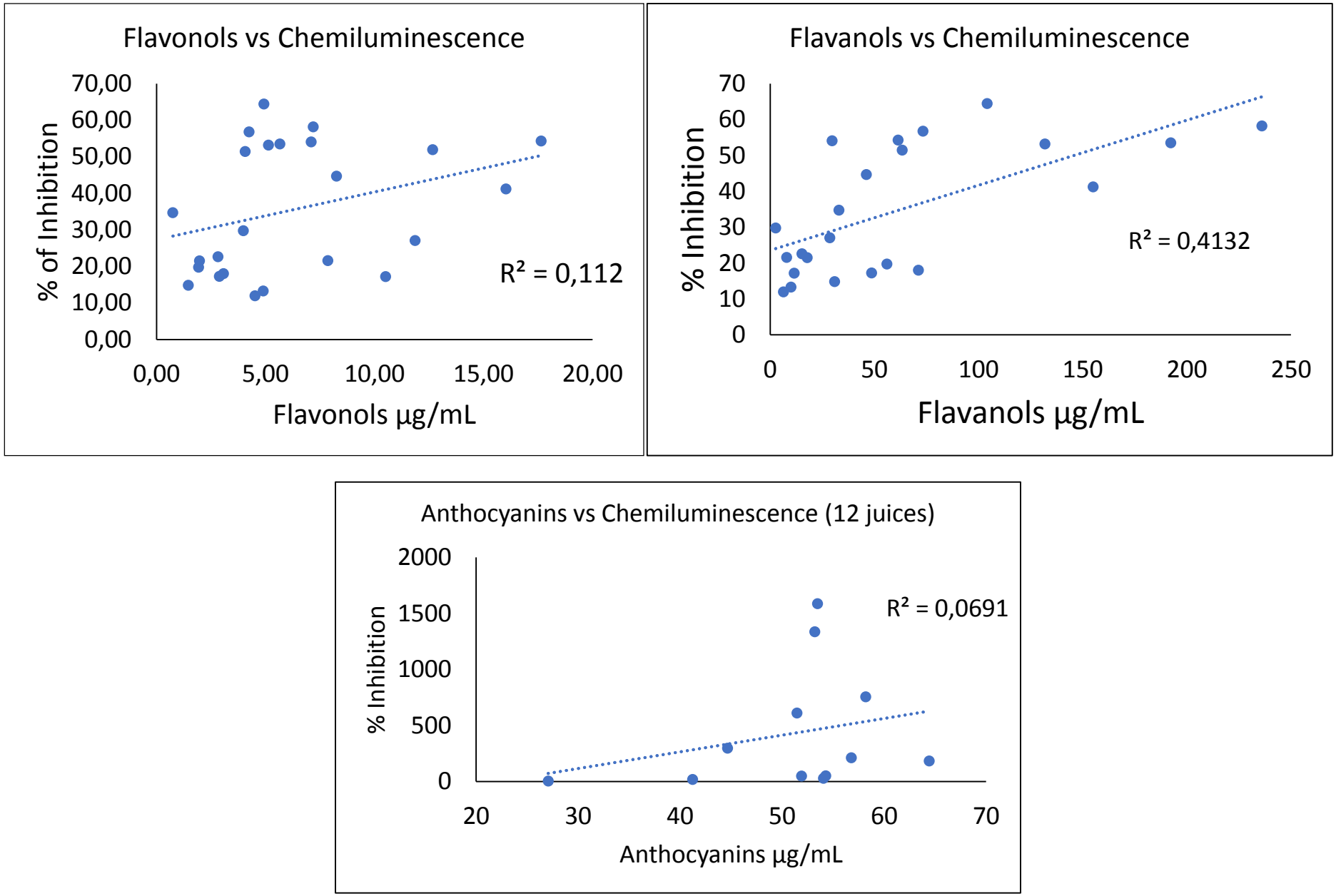
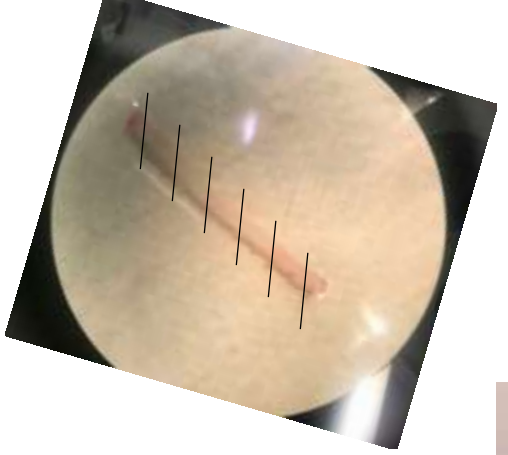


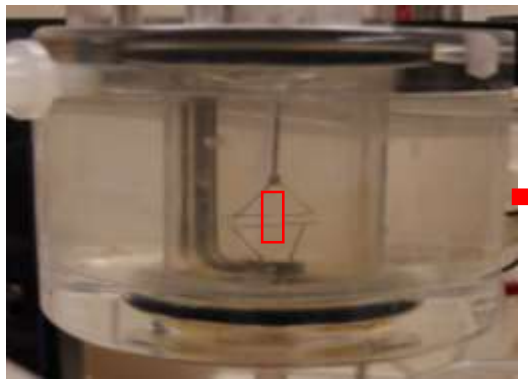
Figure 3. Correlation between subclasses of flavonoids and Chemiluminescence.

	Pearson r	P value
Total Polyphenols vs Chemiluminescence (n=24)	**0.6962**	**0.05**
Flavanols vs Chemiluminescence (n=24)	0.3526	0.09
Flavonols vs Chemiluminescence (n=24)	0.3346	0.1
Anthocyanins vs Chemiluminescence (n=12)	0.2837	0.37
Gallocatechin (GC) vs Chemiluminescence (n=24)	0.1652	0.44
Epigallocatechine (EGC) vs Chemiluminsecence (24)	0.2462	0.25
Epigallocatechine gallate (EGCG) vs Chemiluminescence (24)	0.6518	0.0005
Epicatechine gallate (ECG) vs Chemiluminescence (24)	0.2008	0.35
Catechine (C) vs Chemiluminesence (24)	0.4119	0.04
Epicatechine (EC) vs Chemiluminescence (24)	0.1485	0.49
Cyanidine rutinoside (CR) vs Chemiluminescence (n=12)	0.2193	0.50
Delphinidine rutinoside (DR) vs Chemiluminescence (n=12)	0.4247	0.17
Cyanidine glucoside (CG) vs Chemiluminescence (n=12)	0.2292	0.47
Peonidine glucoside (PG) vs Cemiluminescence (n=9)	0.7852	0.01
Delphinidine glucoside (DG) vs Chemiluminescence (n=12)	0.1733	0.60
Myrcetine vs Chemiluminescence (n=24)	0.2795	0.19
Quercetine vs Chemiluminescence (n=24)	0.4718	0.01
Kaempferol vs Chemiluminescence (n=24)	0.1903	0.37

Table V. Correlation between subclasses of flavonoids and Chemiluminescence.

Vasorelaxation of aorta

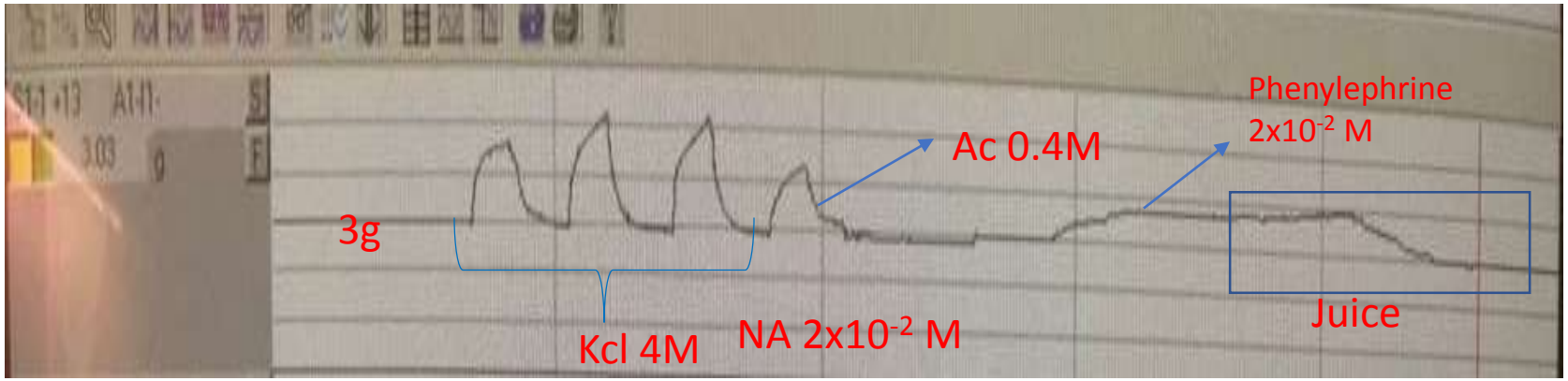




Thoracic aorta of laboratory rats is removed carefully, cleaned of adhering fat and connective tissue, and cut into 8 rings (2-3mm length).

The rings were then mounted in 20 mL organ bath filled with Krebs solution, maintained at 40°C and continuously bubbled with 95% of O2 and 5% of CO2. Rings are equilibrated for a period of 120min before initiating experimental protocols.

VASORELAXATION PROCEDURE



VASORELAXATION

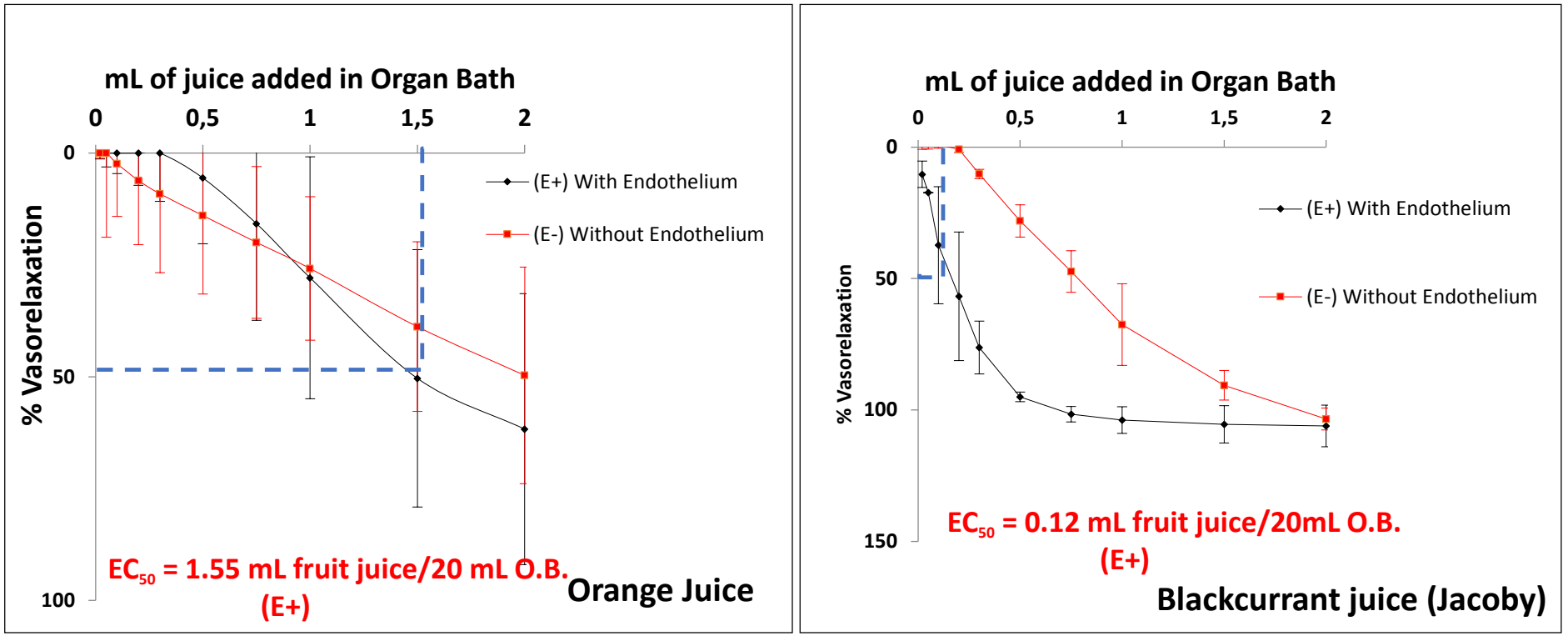
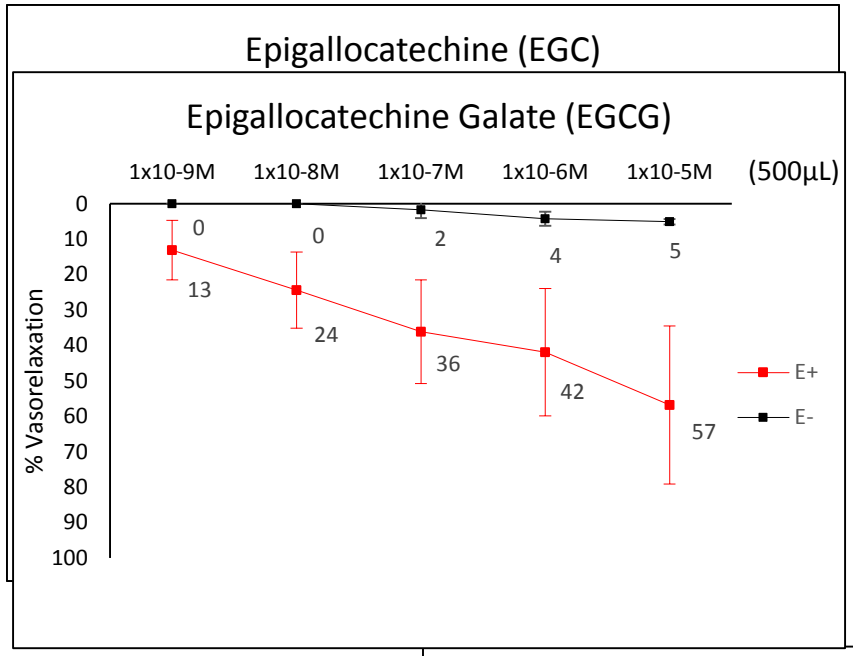
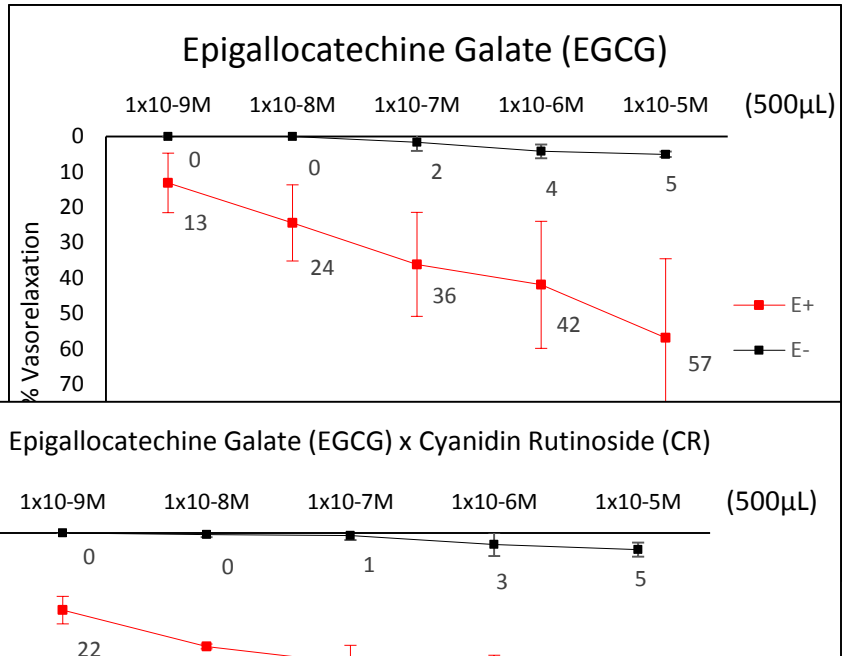
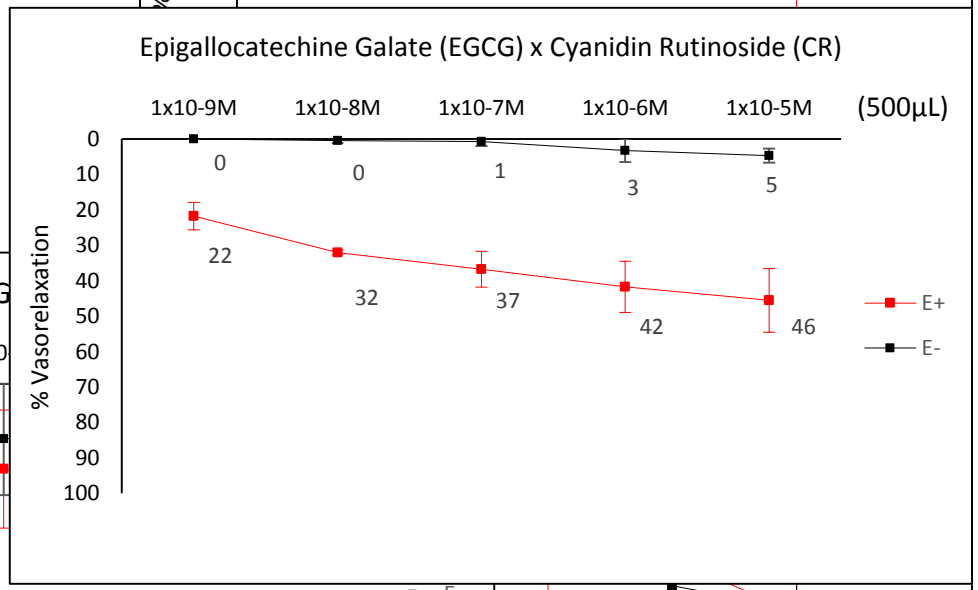
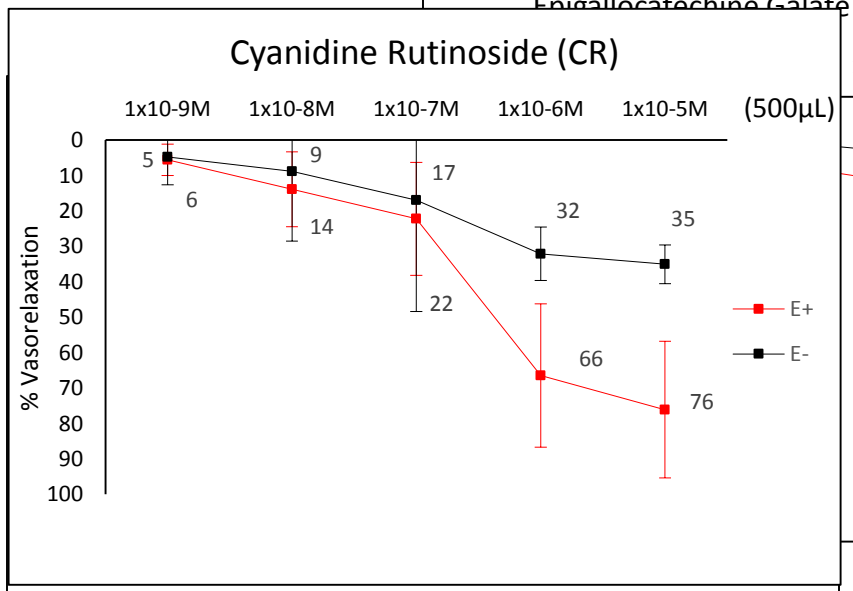


Figure 3. Examples of thoracic aorta vasorelaxation kinetics without and with endothelium in presence of fruits and vegetable juices. Lowest EC_{50} means highest efficiency of juice on vasorelaxation thoracic aorta.


Epigallocatechine (EGC)
Epigallocatechine Galate (EGCG)
1x10-9M
1x10-8M
1x10-7M
1x10-6M
1x10-5M
(500µL)
% Vasorelaxation
0
10
20
30
40
50
60
70
80
90
100
0
0
2
4
5
13
24
36
42
57
E+
E-


Epigallocatechine Galate (EGCG)
1x10-9M
1x10-8M
1x10-7M
1x10-6M
1x10-5M
(500µL)
% Vasorelaxation
0
10
20
30
40
50
60
70
0
0
2
4
5
13
24
36
42
57
E+
E-


Epigallocatechine Galate (EGCG) x Cyanidin Rutinoside (CR)
1x10-9M
1x10-8M
1x10-7M
1x10-6M
1x10-5M
(500µL)
% Vasorelaxation
0
10
20
30
40
50
60
70
80
90
100
0
0
1
3
5
22
32
37
42
46
E+
E-


Cyanidine Rutinoside (CR)
1x10-9M
1x10-8M
1x10-7M
1x10-6M
1x10-5M
(500µL)
% Vasorelaxation
0
10
20
30
40
50
60
70
80
90
100
5
6
9
14
17
22
32
66
35
76
E+
E-

Thank you
for your attention