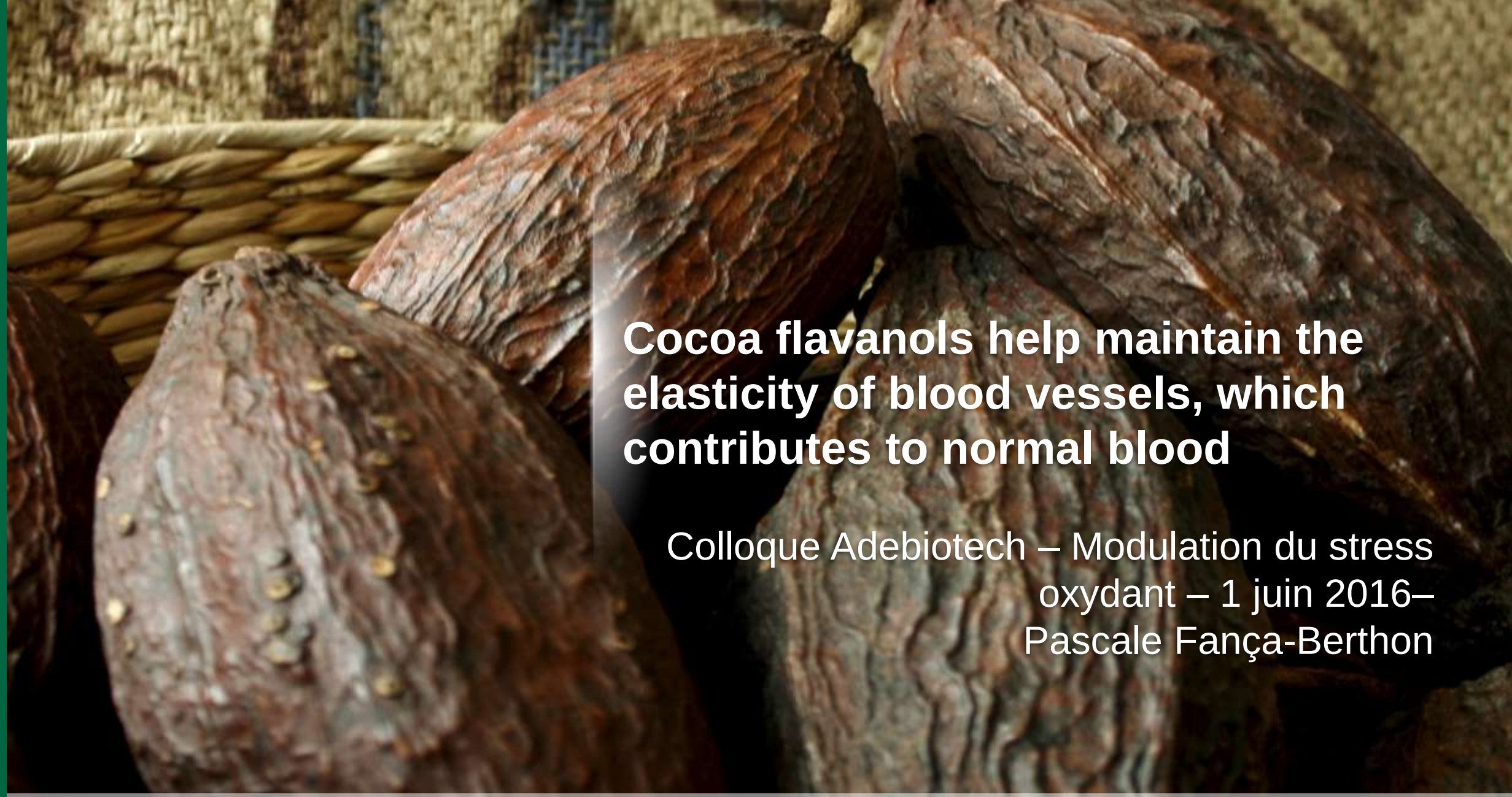

Cocoa flavanols help maintain the elasticity of blood vessels, which contributes to normal blood

Colloque Adebiotech – Modulation du stress oxydant – 1 juin 2016–
Pascale Fança-Berthon

Nutrition & Health

MAINTENANCE OF ENDOTHELIAL FUNCTION AND ITS RELATION TO CARDIOVASCULAR HEALTH

Cardiovascular Disease (CVD)

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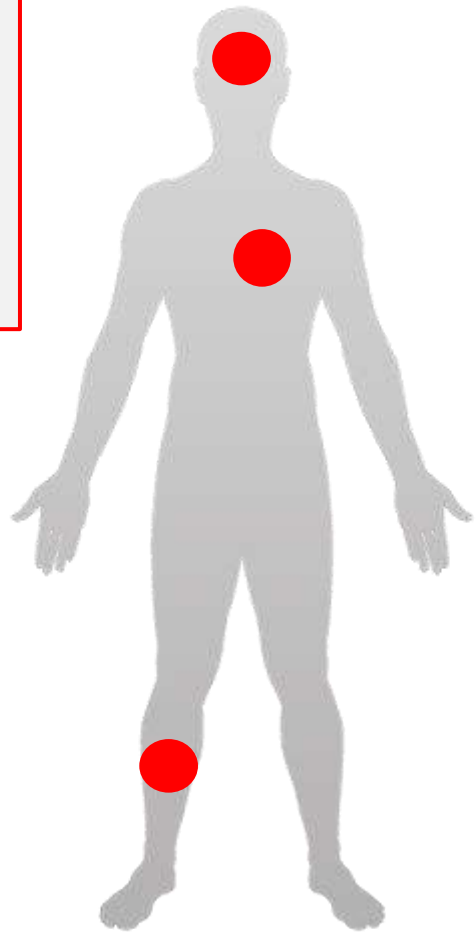
CEREBROVASCULAR DISEASE

Ruptured plaques in the brain's arteries causes strokes with the potential for permanent brain damage.

Temporary blockages in an artery can also cause transient ischemic attacks (TIAs), which are warning signs of stroke; however, there is no brain injury.



CORONARY ARTERY DISEASE

Stable plaques in the heart's arteries cause angina (chest pain on exertion). Sudden plaque rupture and clotting causes heart muscle to die. This is a heart attack, or myocardial infarction.

PERIPHERAL ARTERY DISEASE

Narrowing in the arteries of the legs caused by plaque causes poor circulation.

This causes pain on walking and poor wound healing. Severe disease may lead to amputations.

What causes CVD? The Atherosclerosis process

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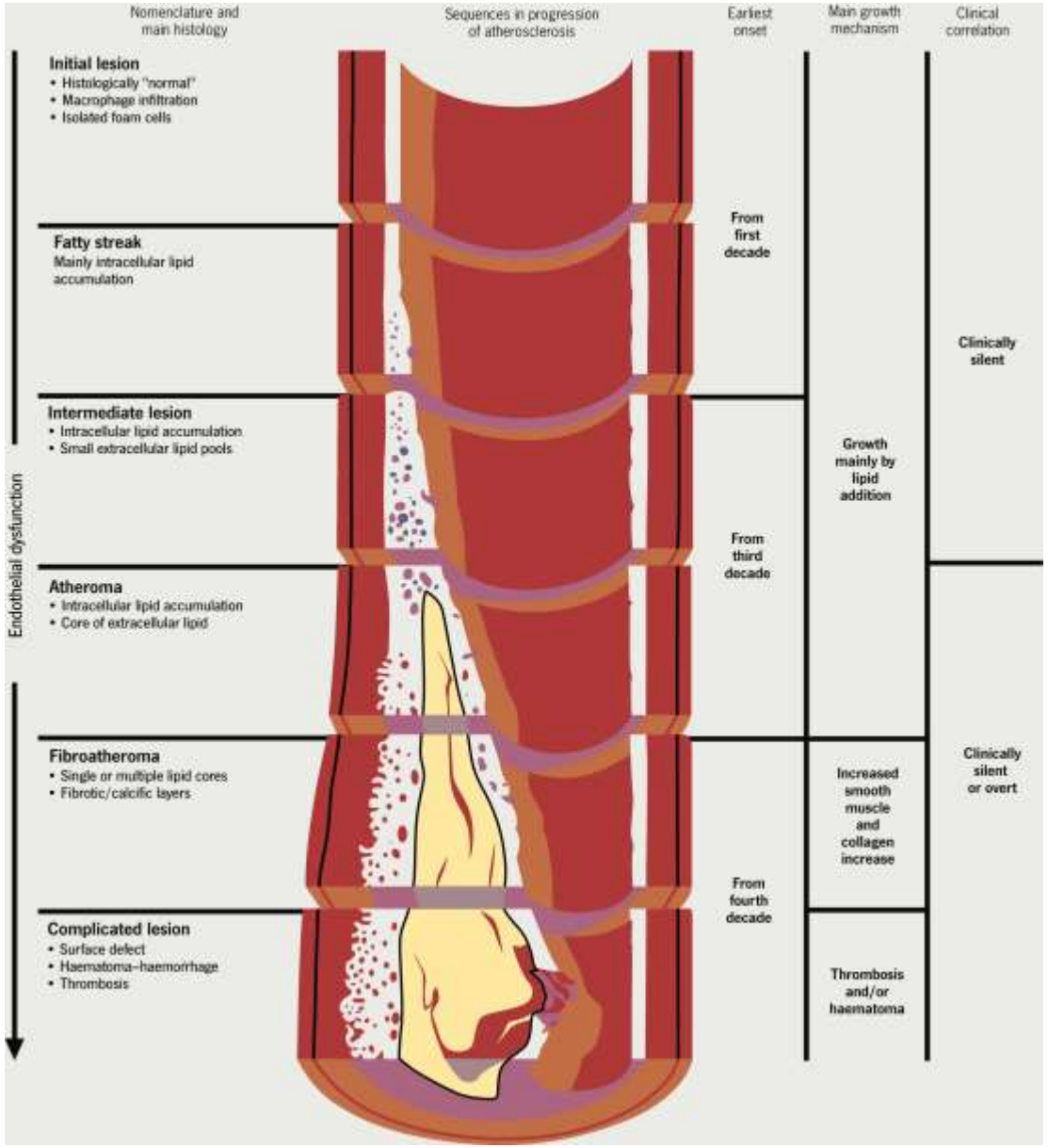
Atherosclerosis causes arteries to narrow, weaken and be less flexible. It's the term for the process of fatty buildup in the inner lining of an artery. The buildup that results is called plaque and reduces the amount of blood and oxygen that is delivered to vital organs.



The British Journal of Cardiology, 2016

CVD consequences

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50%+

Cardiovascular disease (CVD) causes more than half of all deaths in Europe.[1]

4 million

Number of deaths per year in Europe caused by cardiovascular disease (CVD) (1.9 million deaths in the EU). [2]

#1

CVD is the main cause of death in women in all countries of Europe and is the main cause of death in men in all but 6 countries. [2]

€196 billion

Overall estimated to yearly cost of CVD to the EU economy. [2]

1. WHO 2. European Society of Cardiology

Cardiovascular Risk Factors

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BUT the major causes of chronic diseases are known, and if these risk factors were eliminated, at least 80% of all heart disease, stroke and type 2 diabetes would be prevented.[1]

The major risk factors associated with cardiovascular health and coronary heart disease are:

- Physical inactivity
- Poor diet
- Alcohol abuse
- Smoking
- Obesity
- Diabetes
- **Hypertension (high blood pressure)**
- **High cholesterol**

1. WHO

Endothelial Function and CV health

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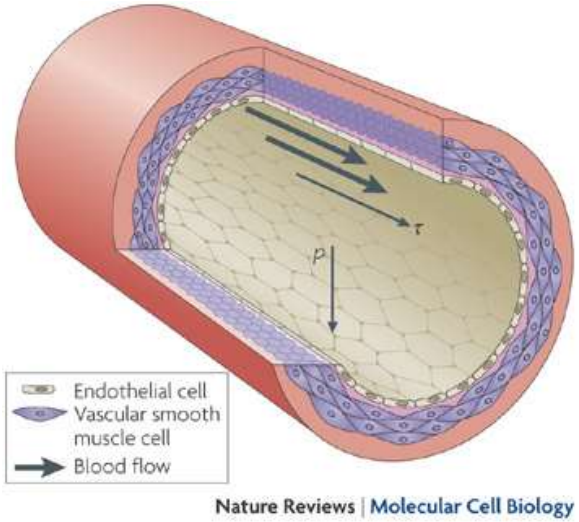
The endothelium are the cells that line the entire circulatory system from the heart to the smallest capillaries. It plays multiple physiological roles:

- **Regulates smooth muscle tone => vasoconstriction/vasodilation**
- Hemostasis (= keep the blood within the vessel)
- Controls thrombosis => prothrombosis/antithrombosis
- Controls proliferation and inflammation
- Inhibits leucocyte and platelet cell adhesion



Endothelial cells secrete numerous vasoactive substances:

- **Vasodilators (NO**, prostacyclin, endothelial derived hyperpolarizing factor)
- Vasoconstrictor (Endothelin-1, angiotensin II and thromboxane)

Endothelial dysfunction and CVD

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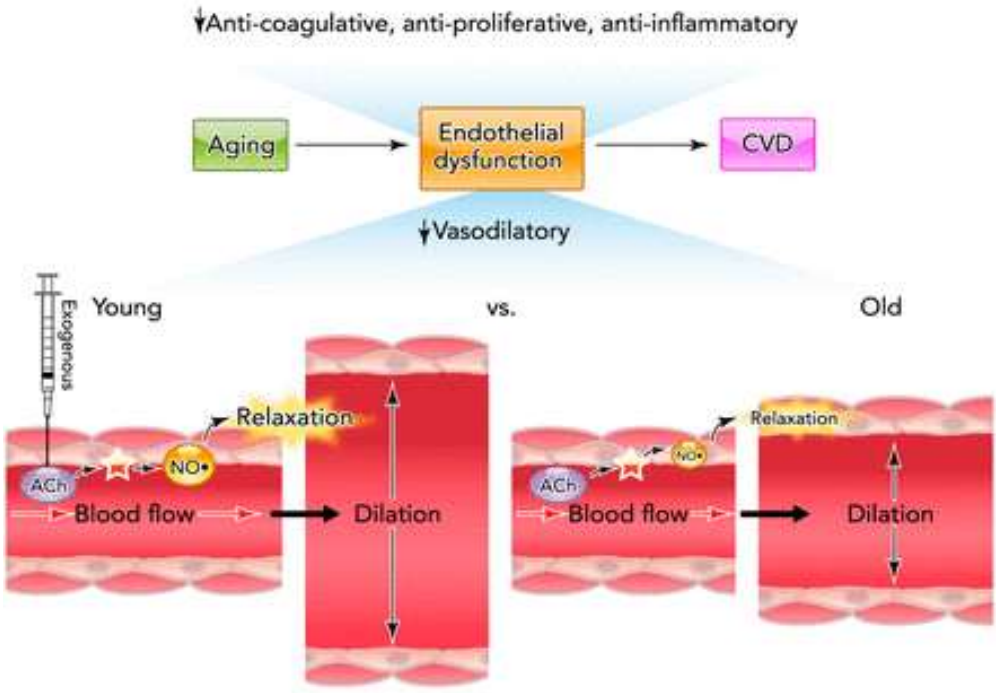
Endothelial dysfunction

- can be defined as any phenotype in which this normal functional state is altered
- recognized as the earliest identifiable event in the process of atherosclerosis
- is associated with aging



Seals et al, *Physiology*, 2014

Endothelial dysfunction and CVD

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CLASSICAL CARDIOVASCULAR RISK FACTORS
Diabetes mellitus
Dyslipidemia
Active and passive smoking
Arterial hypertension
Aging

NOVEL RISK FACTORS
Physical inactivity
Postprandial state
Hyperhomocysteinemia
Obesity
Inflammation

Intrinsic vulnerability, genetic and environmental factors

Endothelial dysfunction

Impaired vasodilation

Proinflammatory state

Arterial wall proliferation

Prothrombotic state

Atherogenesis / plaque progression

Plaque activation / rupture

Thrombosis / vasospasm

Decreased blood flow / ischemia

Cardiovascular disease

Endothelial dysfunction could be seen as the results of the effects of all CV risk factors on vascular health

(Heiss et al, *Antioxidants & redox signaling,* 2015)

How can we assess endothelial function? ED-FMD

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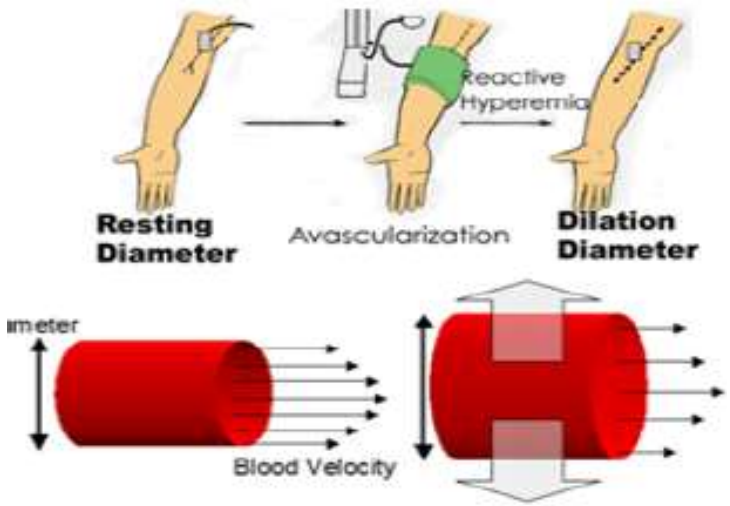
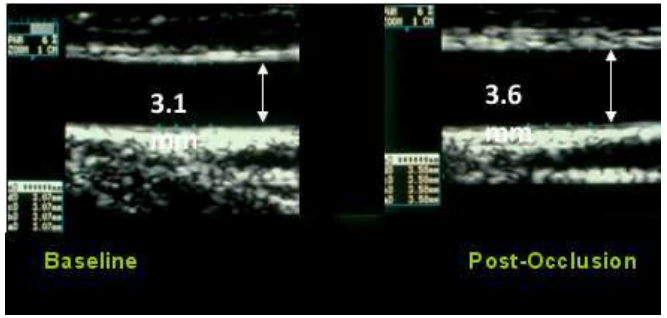
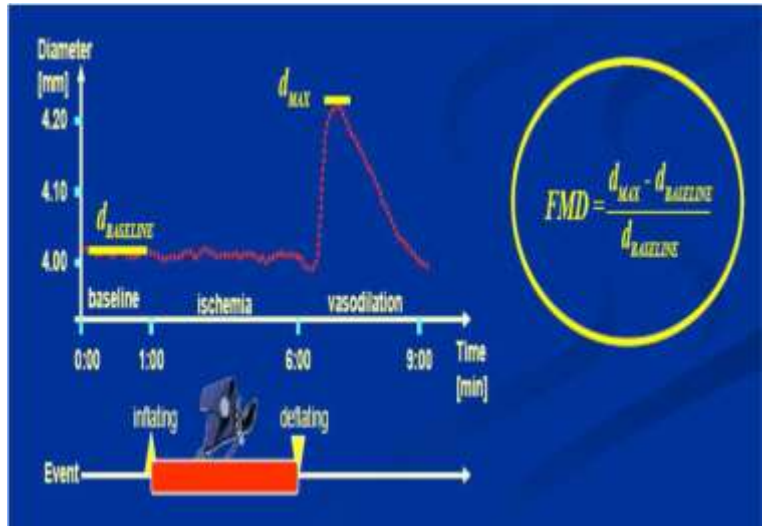
- Endothelial Dependent – Flow Mediated Dilation (ED-FMD) is a non-invasive method to measure vascular endothelial function
- used since 1992 (Celermajer et al.) and widely used (correlate well with invasive method)
- principle: induction of reactive hyperemia (increased blood flow following a period of transient brachial artery occlusion - 5 min) → induction of shear stress → increase in arterial diameter (= vasodilation) that is compared to baseline diameter
- mainly NO dependent (related to NO bioavailability)







(Flammer et al, *Circulation*, 2012)

FMD is predictor of future CVD

Significant inverse association between brachial FMD and future cardiovascular risk events

Increase in FMD of 1% is associated with a decrease in CVD risk

- **from 8%** (Ras et al, 2013; meta-analysis of **23 studies** with **14 753 subjects**)
- to **12%** (Matsuzawa et al, 2015; meta-analysis of **35 studies with 17 280 subjects**; see figure)

1 SD worsening in endothelial function is associated with doubled cardiovascular risk (Matsuzawa et al, 2015)

Dietary strategies aiming to improve endothelial function in healthy individuals are therefore important targets for the primary prevention of CVD.

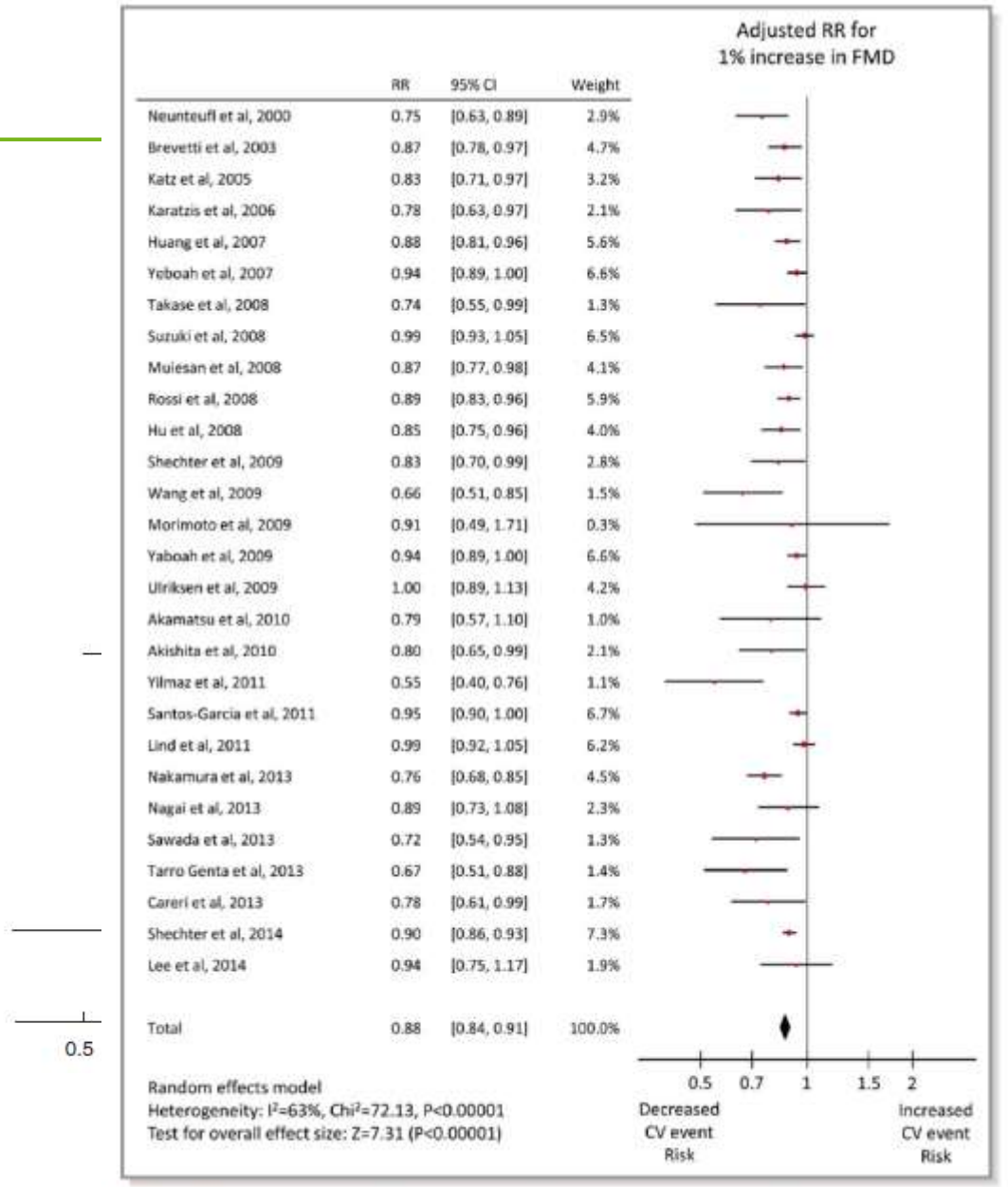


Figure 4. Forest plot of adjusted risk ratio of FMD for cardiovascular events. CV indicates cardiovascular; FMD, flow-mediated dilation; RR, risk ratio.

(Matsuzawa et al, *J Am Heart Assoc*, 2015)

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THE 13.5 CLAIM
POSITIVE OPINIONS

THE 13.5 CLAIM ON COCOA FLAVANOLS

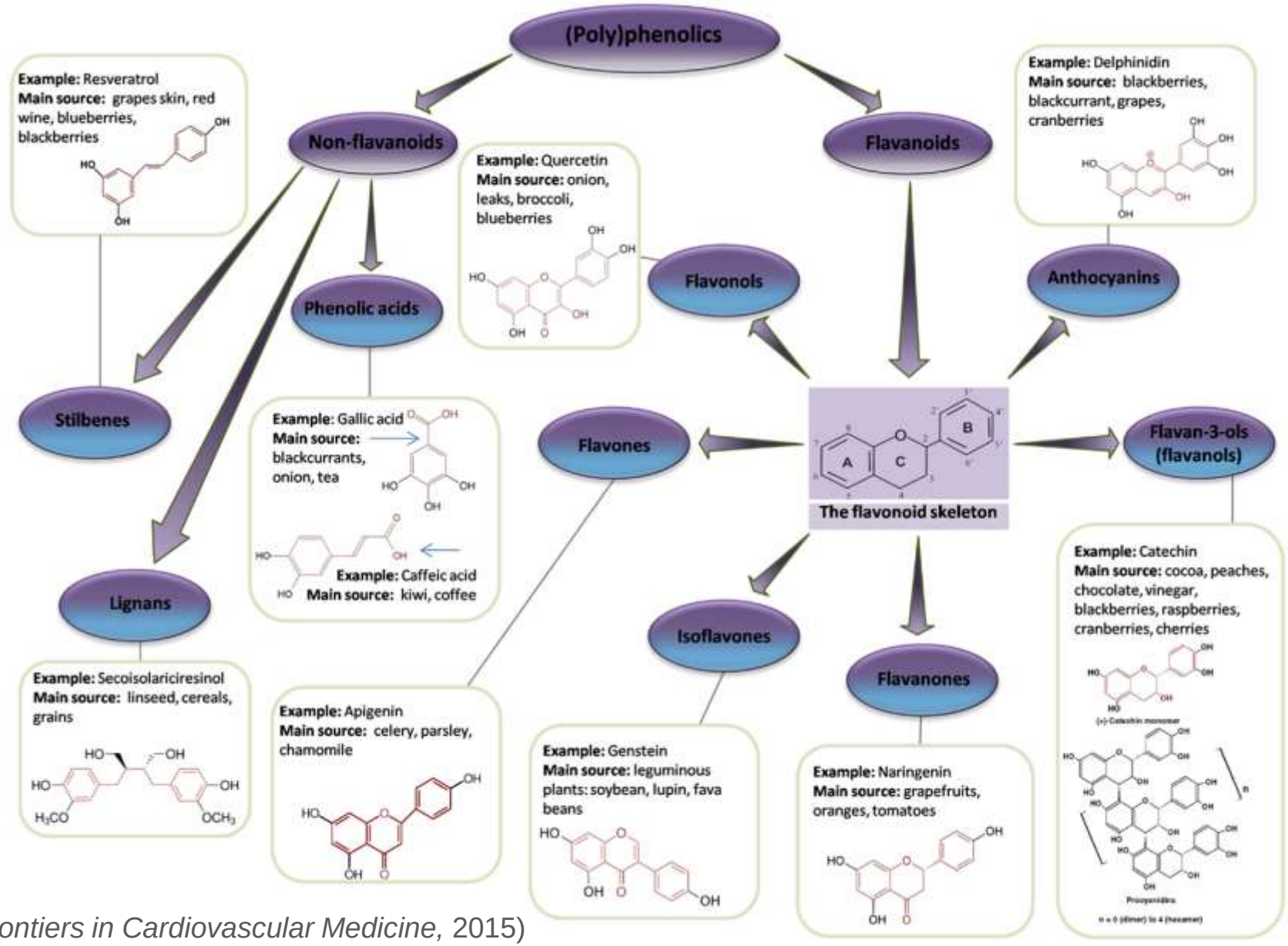
NATUREX

“Cocoa flavanols help maintain the elasticity of blood vessels, which contributes to normal blood flow”

- ✔ Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 200 mg of cocoa flavanols.
- ✔ The claim can be used only for cocoa beverages (with cocoa powder) or for dark chocolate which provide at least a daily intake of 200 mg of cocoa flavanols with a degree of polymerisation 1-10

Commission Regulation (EU) No 2015/539

What are cocoa flavanols?



Monomeric flavanols (DP1)

Polymeric flavanols (DP2 to DP10)

(Goszcz et al, *Frontiers in Cardiovascular Medicine,* 2015)

The Health claim dossier

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- 25 potentially pertinent publications – 4 published studies were used and discussed in the health claim application

EFSA concluded that:	EFSA considered that the following studies provided supportive evidence:
• 1 study showed an effect on fasting ED-FMD after 12 weeks consumption of cocoa flavanols (Davison 2008) • 1 study showed a dose dependent effect on fasting ED-FMD after one week of consumption (Grassi 2015) • 2 additional studies (without proper control) were considered as supporting data (Grassi 2005 and 2008)	• Patients with coronary artery disease (Balzer 2008 and Heiss 2010) • Acute studies (Heiss 2007, Balzer 2008, Heiss 2003, Heiss 2005, Heiss 2007) • Acute study/obese participants (Berry 2010) • Acute healthy older adults (Monahan 2011)

Davison, *International Journal of Obesity* 2008

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NATUREX

Design: Randomized, double blind, placebo controlled, parallel.

Population: 49 Overweight and Obese adults

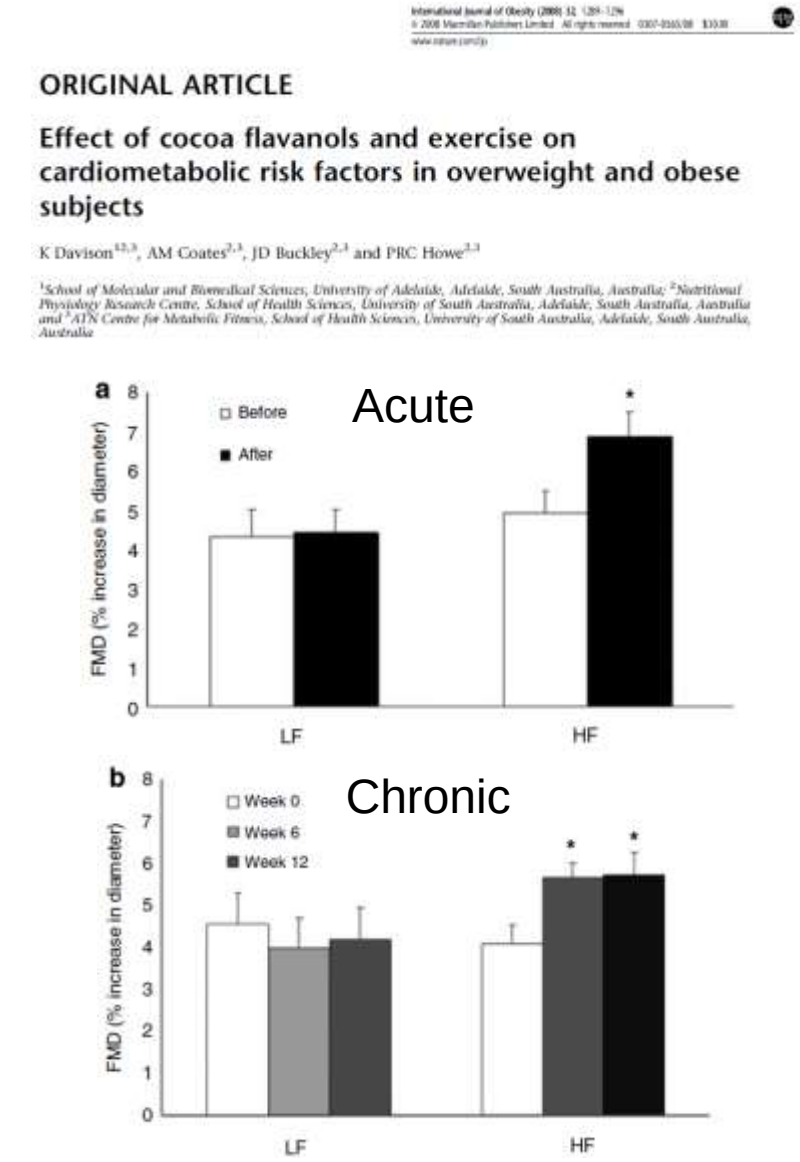
Site: Nutritional Physiology Research Centre at the University of South Australia

Dose: 902 mg flavanols + exercise, 902 mg flavanols w/o exercise, 36 mg flavanols + exercise, 36 mg flavanols w/o exercise

Duration: 12 weeks

Results: Compared to LF, HF increased FMD acutely (2 h post-dose) by 2.4% (P<0.01) and chronically (over 12 weeks; P<0.01) by 1.6%

Element	Remarks
EFSA Opinion	Key Study
Improvements in FMD	**Acute and Chronic, Significant and Clinically Relevant**
Improvements in Blood Pressure	Significant ($p < 0.05$)



ORIGINAL ARTICLE

Effect of cocoa flavanols and exercise on cardiometabolic risk factors in overweight and obese subjects

K Davison[1,2,3], AM Coates[2,3], JD Buckley[2,3] and PRC Howe[2,3]

[1]School of Molecular and Biomedical Sciences, University of Adelaide, Adelaide, South Australia, Australia; [2]Nutritional Physiology Research Centre, School of Health Sciences, University of South Australia, Adelaide, South Australia, Australia and [3]ATN Centre for Metabolic Fitness, School of Health Sciences, University of South Australia, Adelaide, South Australia, Australia

Grassi, *Journal of Hypertension* 2015

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Design: Randomized, double blind, controlled, cross-over.

Population: 20 healthy volunteers

Dose: 0, 80, 200, 500 and 800mg cocoa flavanols/day in five periods lasting 1 week each

Duration: 1 week (treatment)

Results: Cocoa dose-dependently increased FMD from 6.2% (control) to 7.3, 7.6, 8.1 and 8.2% after the different flavanols doses, respectively (P<0.0001).

Element	Remarks
EFSA Opinion	Key Study - proprietary
Improvements in FMD	**Significant and Clinically Relevant with Clear Dose Response**
Improvements in Blood Pressure	Significant ($p < 0.05$), with Clear Dose Response
Arterial stiffness	Dose response
Endothelin-1 (vascoconstrictor)	Dose response

Cocoa consumption dose-dependently improves flow-mediated dilation and arterial stiffness decreasing blood pressure in healthy individuals

Davide Grassi[a], Giovambattista Desideri[a], Stefano Necozione[a], Paolo di Giosia[a], Remo Barnabei[a], Leen Allegaert[b], Herwig Bernaert[b], and Claudio Ferri[a]

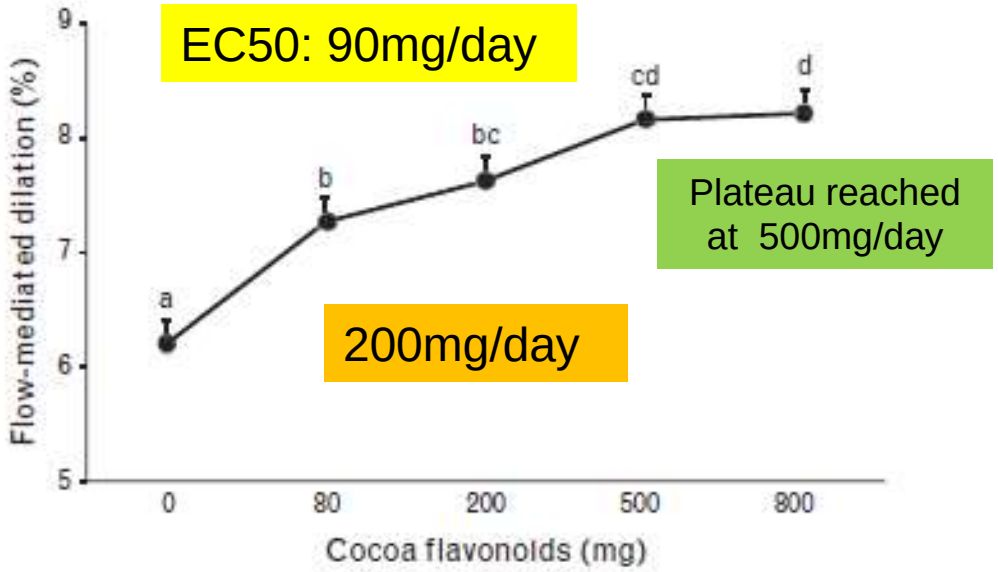

FIGURE 2 Effects of cocoa on endothelium-dependent flow-mediated dilation in 20 healthy volunteers. Data are expressed as least square means with standard error of the mean. Data points with different superscripts are significantly different. Differences are considered significant when *P* value is less than 0.05.

Grassi, *Hypertension* 2005

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Design: Randomized, double blind, controlled, cross-over.

Population: 20 essentially hypertensive and 15 control

Dose: 100 g per day Dark Chocolate (containing 88 mg flavanols) or 90 g per day flavanol-free white chocolate

Duration: 15 days (treatment)

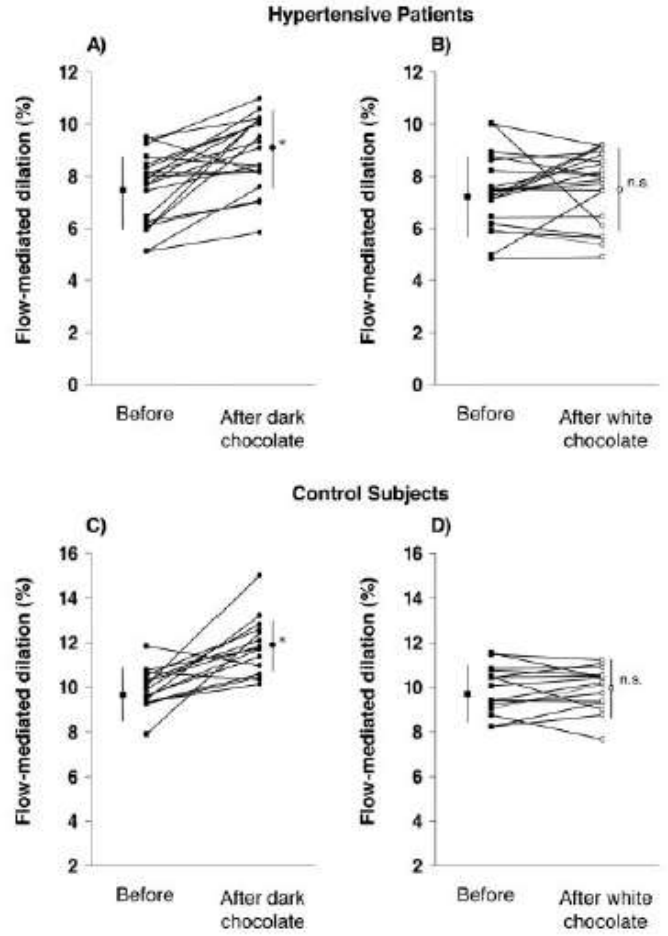
Results: Hypertensives, FMD increased to almost normal values in the Dark Chocolate group vs. White Chocolate group (p<0.0001)

Control, FMD increased in Dark Chocolate group vs. White Chocolate group (p<0.0001)

Element	Remarks
EFSA Opinion	Supporting Study (Control)
Improvements in FMD	**Significant and Clinically Relevant (p > 0.0001)**
Improvements in Blood Pressure	BP vs. Baseline (p > 0.0001) SBP: -11.0 +/- 6.3 mm Hg (p<0.0001) DBP: -6.2 +/- 4.2 mm Hg Ambulatory BP vs. Baseline (p > 0.0001) SBP: -11.9 +/- 7.7 mm Hg (p<0.0001) DBP: -8.5 +/- 5.0 mm Hg (p<0.0001)
LDL Cholesterol	Significant (p > 0.05)
Insulin sensitivity	Improvments in QUICKI and ISI

Cocoa Reduces Blood Pressure and Insulin Resistance and Improves Endothelium-Dependent Vasodilation in Hypertensives

Davide Grassi, Stefano Necozione, Cristina Lippi, Giuseppe Croce, Letizia Valeri, Paolo Pasqualetti, Giovambattista Desideri, Jeffrey B. Blumberg, Claudio Ferri



Grassi, *Journal of Nutrition* 2008

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NATUREX

Design: Randomized, double blind, controlled, cross-over.

Population: 19 Hypertensives with Impaired Glucose Tolerance

Site: The Division of Internal Medicine and Centre of Hypertension and Cardiovascular Prevention Outpatient Unit

Dose: 100 mg flavonol rich dark chocolate (FRDC) bar and 100 mg flavonol free white chocolate (FFWC) bar

Duration: 1 week (treatment)

Results: FRDC ingestion significantly increased FMD ($p<0.05$). FFWC did not affect vascular reactivity.

Element	Remarks
EFSA Opinion	Supporting Study (Control)
Improvements in FMD	**Significant and Clinically Relevant ($p > 0.05$)**
Improvements in Blood Pressure	BP: ($p < 0.0001$) SBP -3.82 +/- 2.40 mm Hg DBP -3.92 +/-1.98 mm Hg Ambulatory BP SBP -4.52 +/- 3.94 mm Hg DBP -4.17 +/- 3.29 mm Hg
Cholesterol	-6.5% ($p < 0.0001$)
LDL Cholesterol	-7.5% ($p < 0.0001$)

Blood Pressure Is Reduced and Insulin Sensitivity Increased in Glucose-Intolerant, Hypertensive Subjects after 15 Days of Consuming High-Polyphenol Dark Chocolate[1–3]

Davide Grassi,[4]* Giovambattista Desideri,[4] Stefano Necozione,[4] Cristina Lippi,[4] Raffaele Casale,[4] Giuliana Properzi,[4] Jeffrey B. Blumberg,[5] and Claudio Ferri[4]

[4]Department of Internal Medicine and Public Health, University of L'Aquila, 67100 L'Aquila, Italy and [5]Antioxidants Research Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA 02111

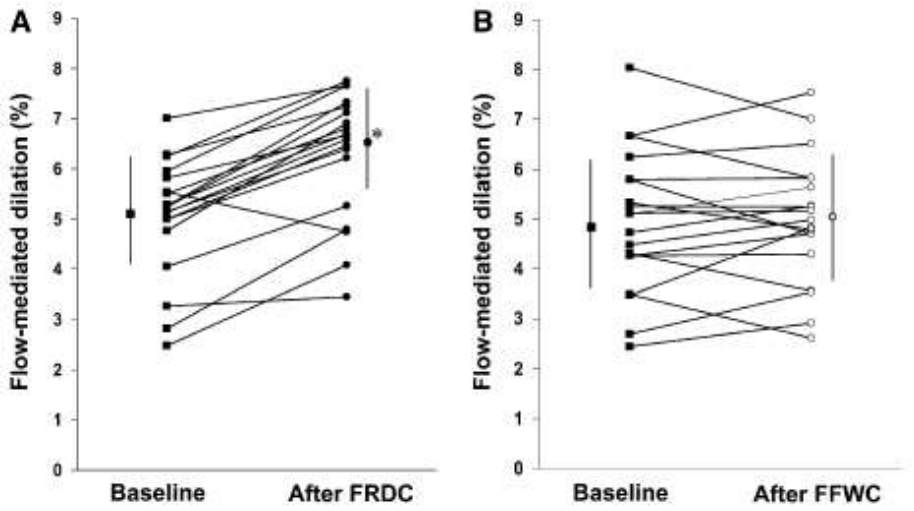
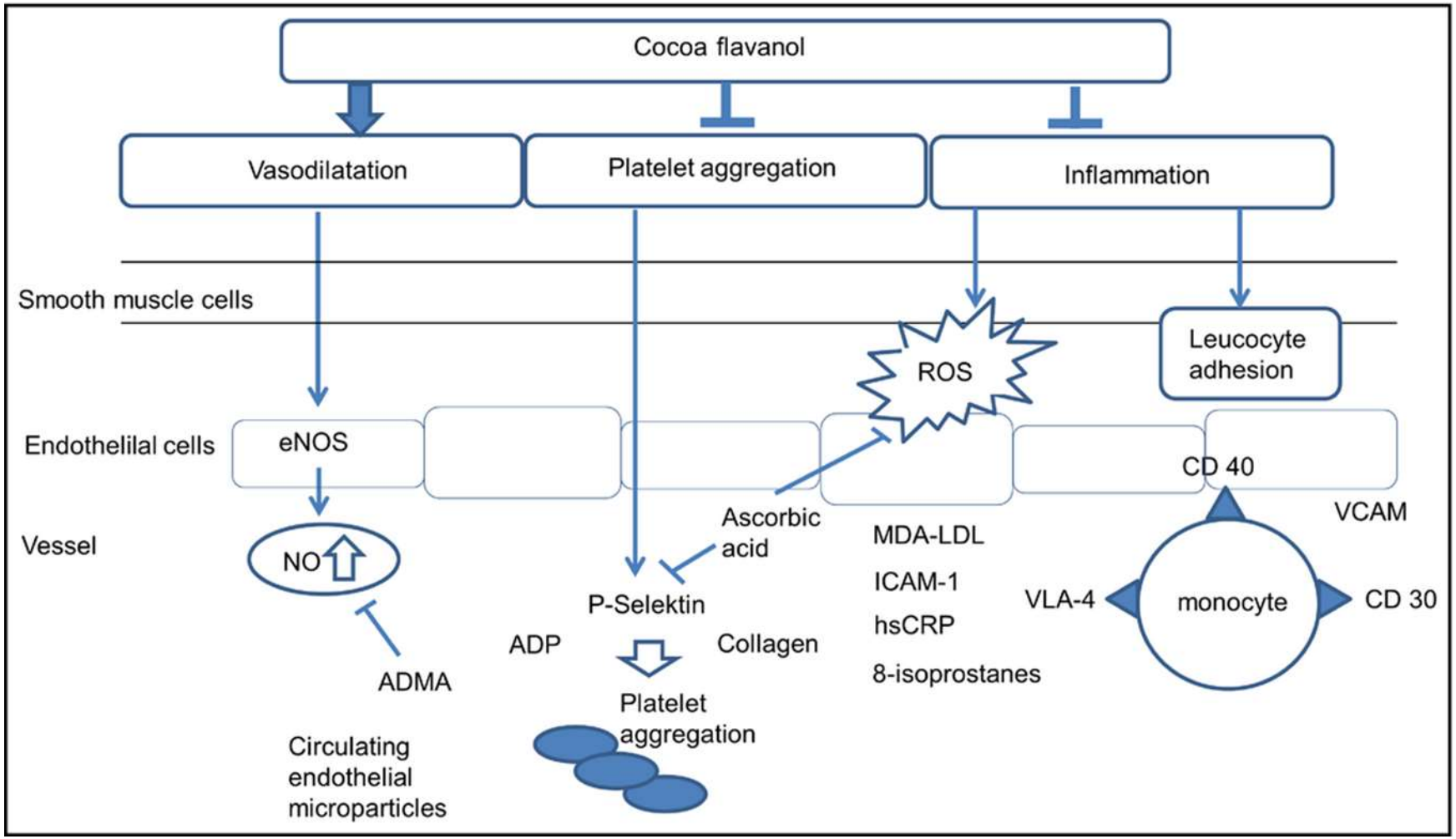


FIGURE 3 Effect of FRDC (*A*) and FFWC (*B*) on FMD in IGT EH patients. Data are means ± SD, $n = 19$. *Different from baseline and FFWC, $P < 0.05$.

Underlying mechanisms of the vasoprotective effects



(Jumar and Schmieder, *The Journal of Clinical Hypertension*, 2015)

HIGH-FLAVANOL COCOA EXTRACT: EXTENSION OF THE 13.5 CLAIM

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“Cocoa flavanols help maintain the elasticity of blood vessels, which contributes to normal blood flow”

efsa

SCIENTIFIC OPINION

Scientific Opinion on the modification of the authorisation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13(5) of Regulation (EC) No 1924/2006[1] following a request in accordance with Article 19 of Regulation (EC) No 1924/2006

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

- ✔ Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 200 mg of cocoa flavanols.
- ✔ The claim can be used only for cocoa beverages (with cocoa powder) or for dark chocolate which provide at least a daily intake of 200 mg of cocoa flavanols with a degree of polymerisation 1-10.
- ✔ The claim can be used only for capsules or tablets containing **high-flavanol cocoa extract which provide at least a daily intake of 200 mg of cocoa flavanols** with a degree of polymerisation 1-10.

Commission Regulation (EU) No 2015/539

ADME of flavanols

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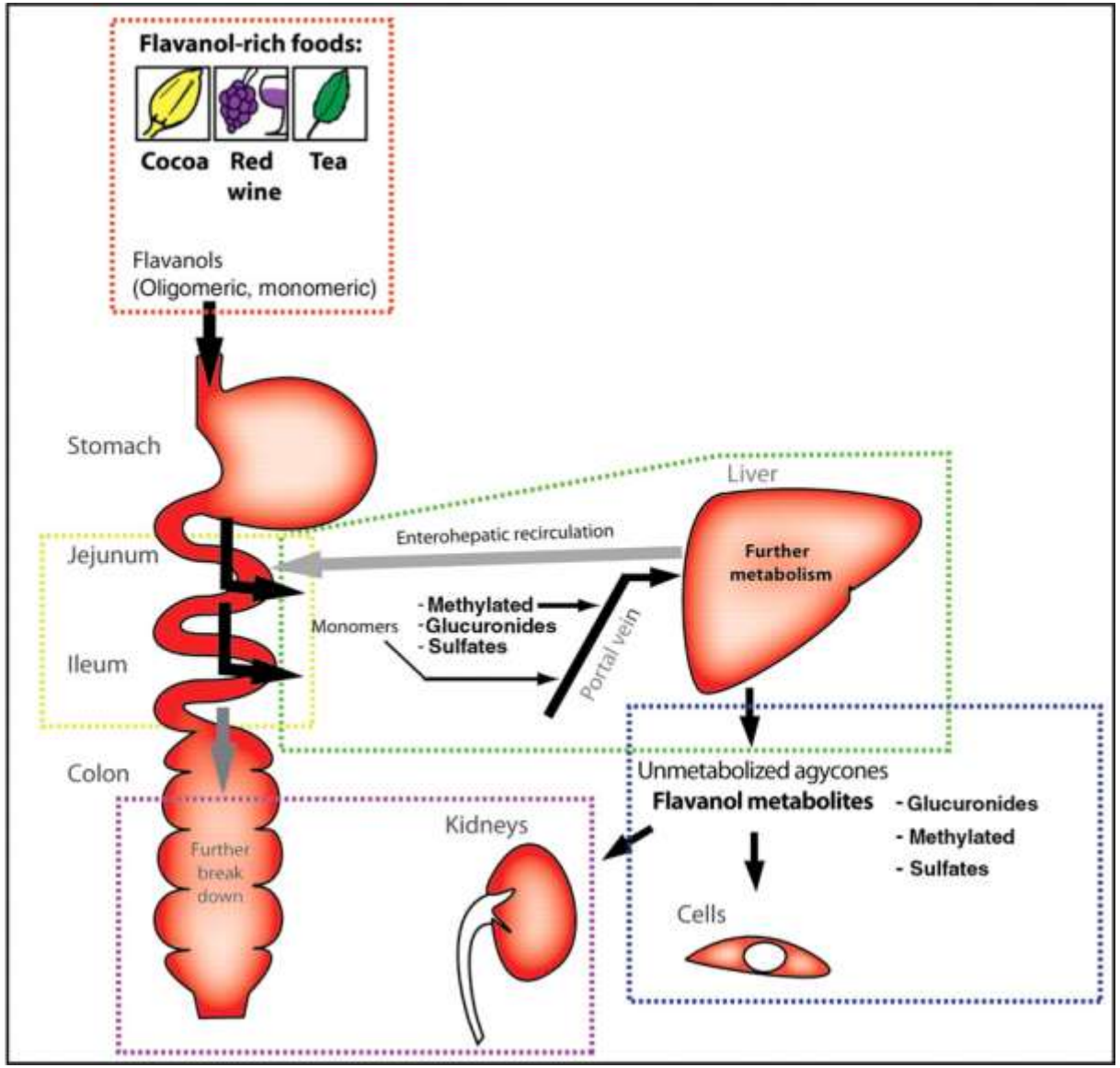
Composition

Absorption
Distribution
Metabolism

Excretion



The acute effects of cocoa flavanols are mainly driven by monomers (epicatechin and catechin) and their metabolites

(Heiss et al, *European Heart Journal,* 2010)

High-Flavanol cocoa extract pharmacokinetic study

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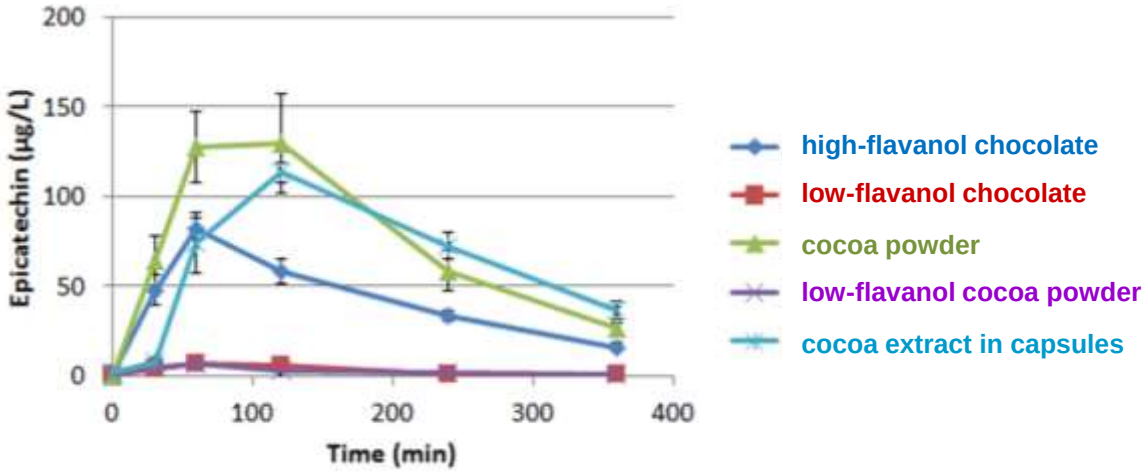
Design: Randomized, partially blinded cross-over (5-day washout period)

Population: 6 healthy participants (3 men / 3 women)

Matrix & Dose: High-flavanol chocolate (460 mg flavanols), low-flavanol chocolate (60 mg flavanols), cocoa powder (459 mg flavanols), low-flavanol cocoa powder (27 mg flavanols), cocoa extract in capsules (449 mg flavanols)

Duration: one dose acute test + blood sampling before and during 6h after consumption (30, 60, 120, 240 and 360 min)

Endpoints: PK parameters on monomeric flavanols (epicatechin) – Cmax, Tmax, AUC



Formulation	C_{MAX} ($\mu g.L^{-1}$)	T_{MAX} (min)	AUC ($\mu g.L^{-1}.h$)
High-flavanol chocolate	**81.9 ± 5.7[a]**	**60.0 ± 0.0**	**15317.2 ± 1200.1[a]**
low-flavanol chocolate	7.7 ± 2.0[b]	108.0 ± 35.0	1186.5 ± 303.3[b]
cocoa powder	**151.1 ± 25.8[c]**	**110.0 ± 28.6**	**27946.0 ± 4120.4[c]**
low-flavanol cocoa powder	7.4 ± 1.2[b]	50.0 ± 6.3	959.6 ± 180.6[b]
cocoa extract (caps)	**119.6 ± 4.8[ac]**	**100.0 ± 12.6**	**24614.1 ± 851.1[c]**

Cocoa flavanols consumed as a high-flavanol cocoa extract in capsules are as bioavailable as those contained in other matrices (dark chocolate and cocoa powder)

High-Flavanol cocoa extract patented process

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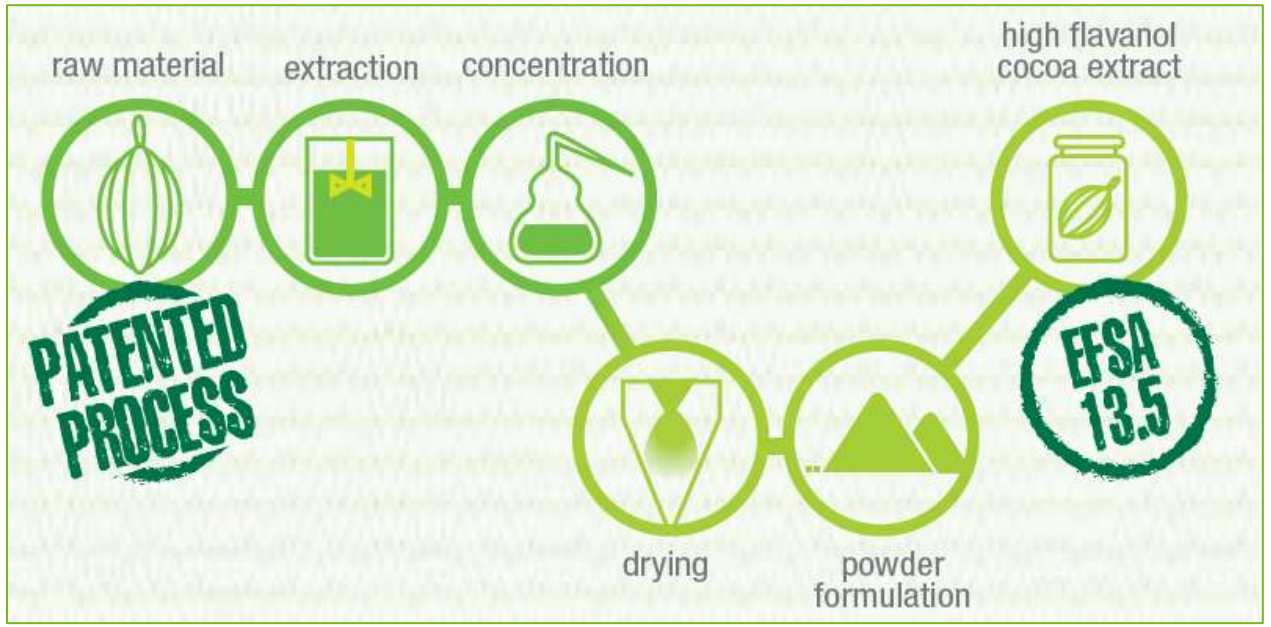
- Unique patent-protected method for obtaining the high flavanol cocoa extract:
  → standardization to 29+% cocoa flavanols (AOAC method)
  → 23% of monomeric flavanols/total flavanols



- Convenient for nutraceuticals applications (effective quantity in caps)
- Advantage in comparison to classical cocoa matrices (low fat, low sugar) and regarding quantity of flavanols that could reasonably be consumed per day

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HIGH-FLAVANOL COCOA EXTRACT: BEHIND THE CLAIM

The case of blood pressure reduction

Moderate but relevant effect of cocoa flavanols on BP

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The up to date data

Cochrane review (Ried et al, 2012):

- 20 acute or short term chronic studies (mean 4.4 weeks, range 2-8 weeks, n=19, and one trial of 18 weeks)
- 856 adults with or without hypertension (mean systolic and diastolic blood pressure across control groups from 110 to 154 mm Hg and from 66 to 91.6 mm Hg respectively)
- daily intake : 30-1080 mg of flavanols (mean=545.5 mg) / 7-236 mg monomers (mean = 119 mg)

Mean difference SBP (95%CI): -2.77 (-4.72, -0.82) mm Hg, p=0.005, n=20;
Mean difference DBP (95%CI): - 2.20 (-3.46, -0.93) mm Hg, p=0.006, n=19

Meta-analysis systematic review (Hooper et al, 2012)

- 42 acute or short term chronic studies (mean 4.4 weeks, range 2-8 weeks, n=19, and one trial of 18 weeks)
- 1297 adults with or without hypertension
- Significant effect at dose ≥ 50 mg/day

Outcome	Epicatechin dose	Mean effect (95% CI)[2]	No. of studies[3] (no. of participants)	I^2	*P* value for difference between subgroups
SBP, chronic (mm Hg)	≤50	0.10 (−2.20, 2.41)	6 (299)	33	0.002
	>50–100	−4.48 (−6.32, −2.63)	5 (161)	90	
	>100	−4.58 (−5.95, −3.21)	3 (110)	0	
DBP, chronic (mm Hg)	≤50	−0.38 (−1.97, 1.20)	6 (299)	7	0.001
	>50–100	−4.25 (−5.66, −2.85)	5 (161)	38	
	>100	−3.62 (−5.50, −1.74)	2 (78)	66	

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How relevant are those effects?

On a population basis, a BP decrease of 3 mm Hg has already been recognized relevant by the FDA (antihypertensive drugs superiority)

A reduction of 2-3 mm Hg was one of the prioritary nutritionnal objective of the first (2001-2005) and second (2006-2010) French National Nutrition & Health Program

In terms of Public Health, the effects could be quite relevant!

Take home messages

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- ✔ Endothelial dysfunction plays a major role in atherosclerotic cardiovascular disorders
- ✔ The maintenance of a normal endothelial function could be part of primary and secondary prevention of CVD
- ✔ 200mg daily cocoa flavanols have a recognized (EFSA approved; 13.5 proprietary claim) positive impact on endothelial function, vessels elasticity, and blood flow, assessed by ED-FMD
- ✔ The high flavanol cocoa extract obtained from an unique patented extraction process enables 200mg flavanols intake in small and convenient nutraceutical applications
- ✔ Cocoa flavanols are not only effective on endothelial function but also on other CV risk factors: blood pressure, glucose tolerance, insulin sensitivity, inflammation

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R&D team

Emilie Fromentin

Leila Falcao

Thanks to

Vitafoods Europe

BARRY CALLEBAUT

Leen Allegaert

Herwig Bernaert

Claudine Vandemeulebroucke

Paul Arendsen

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