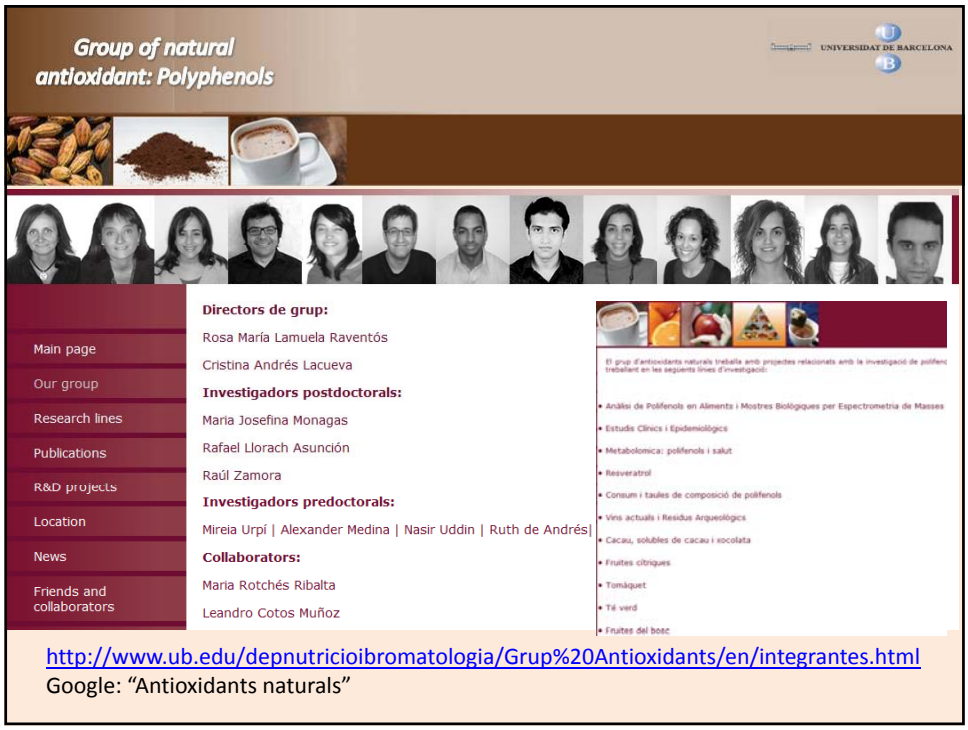

http://www.ub.edu/depnutricioibromatologia/Grup%20Antioxidants/en/integrantes.html
Google: "Antioxidants naturals"

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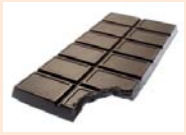
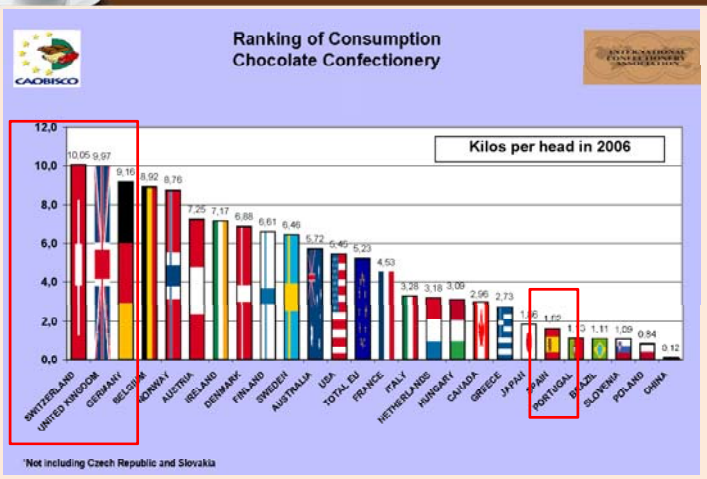
http://www.ub.edu/depnutricioibromatologia/Grup%20Antioxidants/en/integrantes.html

Group Leaders	Rosa Lamuela-Raventós	Cristina Andres-Lacueva	
Posdoctoral Scientist	Rafa Llorach	Mireia Urpi-Sarda	Sara Tulipani
	Raul Zamora-Ros	Giuseppe Di Lecce	Sara Arranz
PhD students	Miriam Martínez	Nasir Khan	Montse Rabassa
	Alex Medina	Maria Rotchés	Anna Vallverdú
	Anna Tresserra	Maria Boto	Rosa Vazquez
	Gemma Chiva		
Collaborators	Marta Perez	Paola Quifer	

Group of natural antioxidant: Polyphenols

CONSUMPTION OF CHOCOLATE

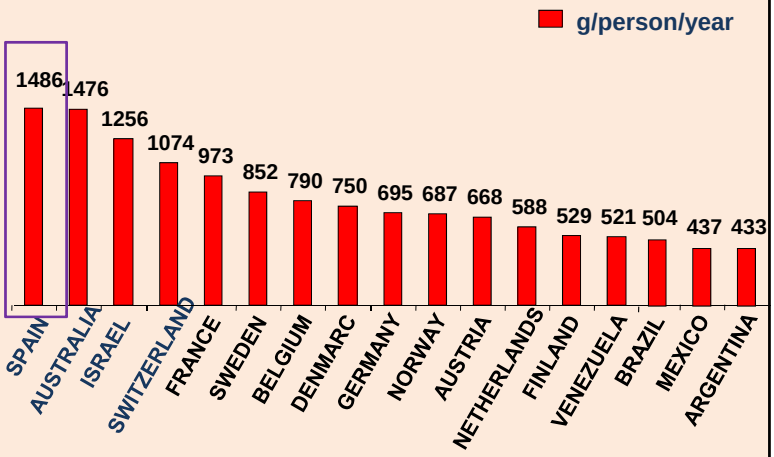




Reports of ACNielsen, Euromonitor International and Caobisco. Association of the Chocolate biscuit and confectionery industries of the EU. http://www.caobisco.com

Group of natural antioxidant: Polyphenols

CONSUMPTION OF COCOA POWDER

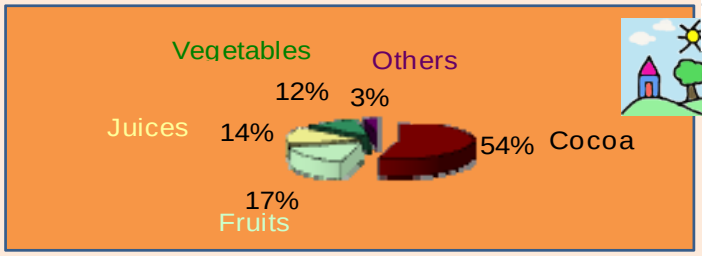


Source : Nielsen, Euromonitor, Procter & Gamble, Nestlé

Group of natural antioxidant: Polyphenols

CONSUMPTION OF FLAVONOIDS IN INFANTILE POPULATION

Percentage of flavonoids in Spanish infantile diet



TOTAL FLAVONOID CONSUMPTION: 24,2 mg/day

- 54% come from cocoa consumption (13,13 mg/day)

CONSUMPTION COCOA PRODUCTS: 33,82 g/day

Zamora-Ros et al.

Group of natural antioxidant: Polyphenols

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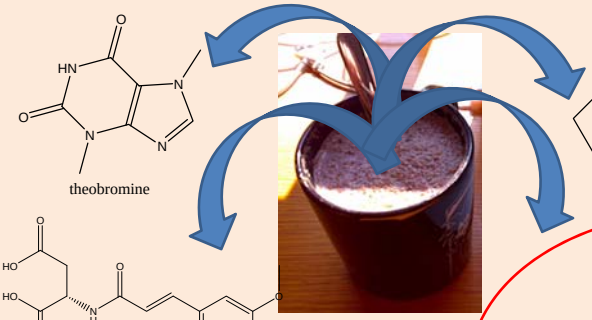
Cocoa Phytochemicals



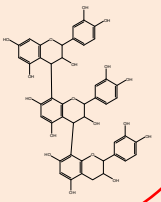
theobromine

diketopiperazine

N-phenylpropeonyl-L-aminoacids

POLYPHENOLS: FLAVONOIDS

flavan-3-ols



Procyanidins

Group of natural antioxidant: Polyphenols

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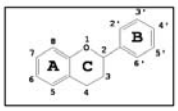
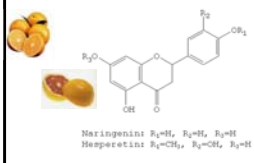
FLAVONOIDS

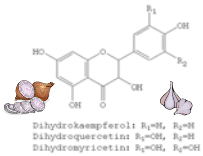


Flavanones



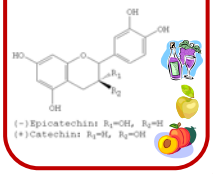
Naringenin: R_1=H, R_2=H, R_3=H
Hesperetin: R_1=CH_3, R_2=OH, R_3=H

Dihydroflavonols



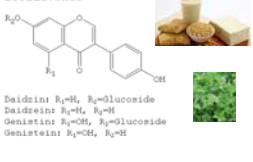
Dihydrokaempferol: R_1=H, R_2=H
Dihydroquercetin: R_1=OH, R_2=H
Dihydromyricetin: R_1=OH, R_2=OH

Flavan-3-ols



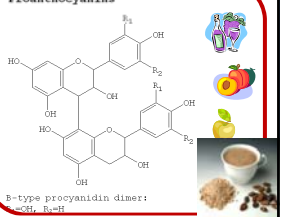
(-)Epicatechin: R_1=OH, R_2=H
(+)Catechin: R_1=H, R_2=OH

Isoflavones



Daidzin: R_1=H, R_2=Glucoside
Daidzein: R_1=H, R_2=H
Genistin: R_1=OH, R_2=Glucoside
Genistein: R_1=OH, R_2=H

Proanthocyanins



B-type procyanidin dimer:
R_1=OH, R_2=H

COCOA

5-10% Monomers (Epicatechin, catechin)
\> 90% Oligomers
Polymers (proanthocyanidins)

Andres-Lacueva et al. *J Agr Food Chem*. 2008

Proanthocyanidins may account for a major fraction of the total polyphenols ingested in Western diets ***(Scalbert, 2000)***

Andres-Lacueva, C.; Medina-Remon, A.; Llorach, R.; Urpi-Sarda, M. et al. 2009. Phenolic compounds. Chemistry and occurrence in fruits and vegetables.

Group of natural antioxidant: Polyphenols

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COCOA AND HEALTH

Circulation
American Heart Association. Learn and Live.
Contemporary Reviews in Cardiovascular Medicine

Cocoa and Cardiovascular Health

March 17, 2009

Roberto Corti, MD*; Andreas J. Flammer, MD*; Norman K. Hollenberg, MD, PhD; Thomas F. Lüscher, MD

Because of the limitations of the data available so far, future studies should provide detailed information about the chocolate product used; the exact content in polyphenols, especially flavanols; and most importantly, the flavanol plasma concentrations achieved. Furthermore, it has to be taken into account that cocoa contains many other potentially active substances, eg, theobromine or magnesium, substances not discussed in this review.

Finally, to definitively clarify the protective effects of cacao on cardiovascular health, larger studies with a placebo-controlled prospective design focusing initially on surrogate end points such as carotid atherosclerosis and eventually morbidity and mortality are needed.

Group of natural antioxidant: Polyphenols

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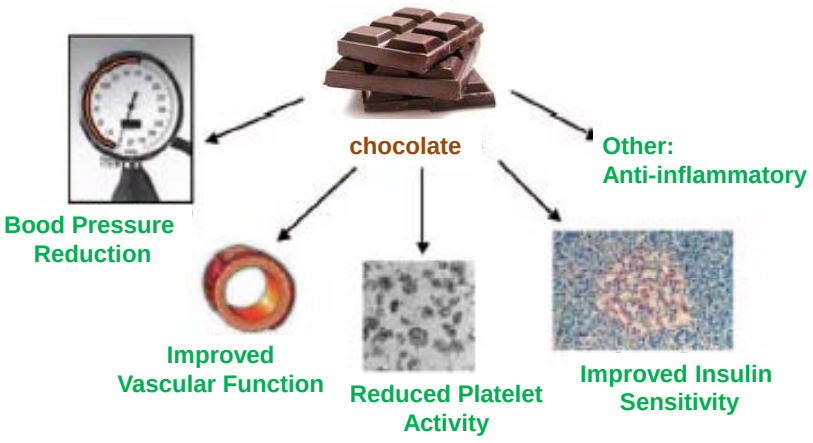
Health relevant effects of chocolate



Corti et al. (2009) Circulation

Group of natural antioxidant: Polyphenols

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PREVENTION CARDIOVASCULAR DISEASES

SUBJECTS	POLYPHENOL SOURCE	INTERPRETATION	REFERENCE
Human (N=45)	Solid Dark Chocolate bar (containing 22 g cocoa powder) or a cocoa-free placebo bar (containing 0 g cocoa powder).	Improved endothelial function and lowered blood pressure in overweight adults	Faridi Z et al. Am J Clin Nutr. 2008
Human (N=22)	40 g Flavonoid-rich Dark Chocolate	Significant reduction of serum oxidative stress.	Flammer AJ et al. Circulation. 2007
Human (N=44)	6.3 g/day of dark Chocolate containing 30 mg of Polyphenols or matching Polyphenol-free White Chocolate	Efficiently reduced blood pressure and improved formation of Vasodilative Nitric Oxide.	Taubert D et al. JAMA. 2007
Human (N=173)	Cocoa and Tea (Meta Analysis)	Consumption of foods rich in cocoa may reduce blood pressure, while tea intake appears to have no effect	Taubert D et al. Arch Intern Med. 2007

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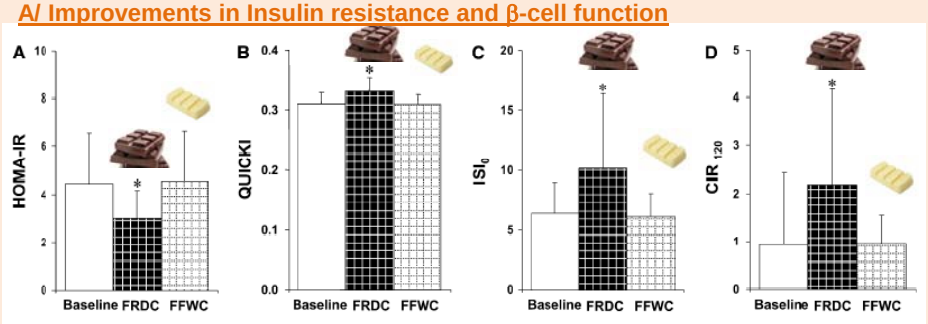
Reduction of Blood Pressure and Increase of Insulin Sensitivity in Glucose-Intolerant and Hypertensive Subjects

19 individuals with essential hypertension and impaired glucose tolerance
Age: 44 ± 8 years

15 days consumption of:
-100 g dark chocolate (polyphenol-rich)(147 mg flavan-3-ols)
- 100 g white chocolate (polyphenol-free)

A/ Improvements in Insulin resistance and β-cell function



Grassi et al., J Nutr (2008)

Group of natural antioxidant: Polyphenols

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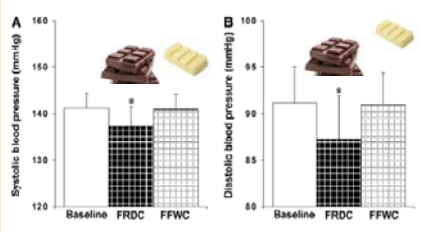
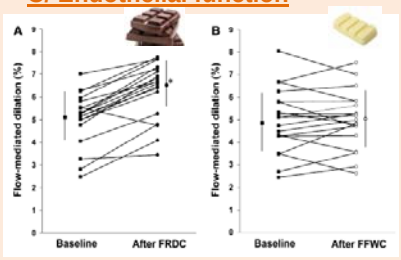
Reduction of Blood Pressure and Increase of Insulin Sensitivity in Glucose-Intolerant and Hypertensive Subjects

B/ Decrement of Blood pressure



C/ Endothelial function



No changes in glyceryl trinitrate-induced vasodilation

D/ Lipid profile

Baseline

Total cholesterol
LDL cholesterol

No changes HDL cholesterol and TG

E/ Other variables

No changes in:
-CRP
-Serum electrolytes
-Fibrinogen
-Homocysteine
-Uric acid

Grassi et al., J Nutr (2008)

Group of natural antioxidant: Polyphenols

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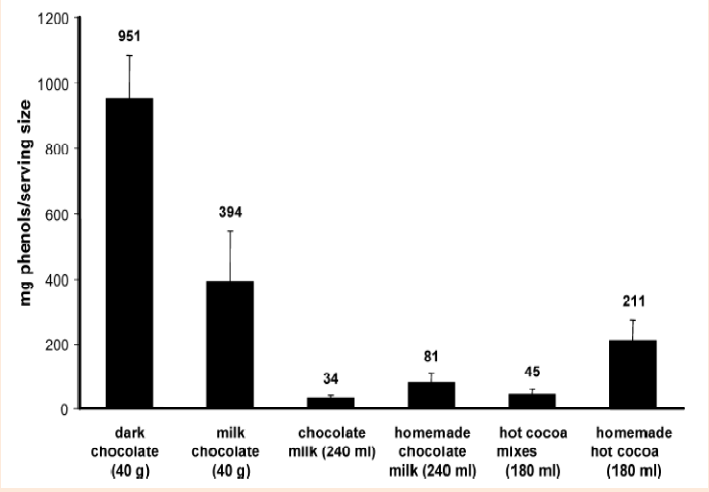
Polyphenols amount in different kind of chocolates



Visioli et al., Crit Rev Food Sci Nutr (2009)

Group of natural antioxidant: Polyphenols

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BIOAVAILABILITY OF POLYPHENOLS FROM COCOA POWDER AND THEIR BENEFICIAL EFFECT IN HEALTH

Subproject 1:	Subproject 2:
Bioavailability of cocoa powder polyphenols in humans (intestinal and colonic absorption). Milk effect	Scientific bases of phenol and Inflammatory biomarkers interaction in atherosclerosis
IP (1) : Dr. Cristina Andres-Lacueva	IP (2) : Dr. Ramón Estruch

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NUTREXPA

ColaCao

CLÍNIC BARCELONA Hospital Universitari

IDIBAPS

Lactalis – Leche de Galicia (President)

Group of natural antioxidant: Polyphenols

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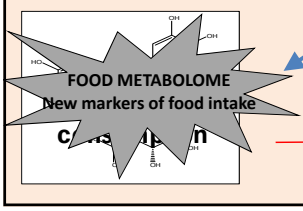
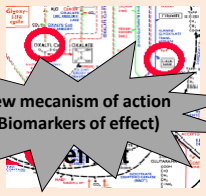
Hypothesis

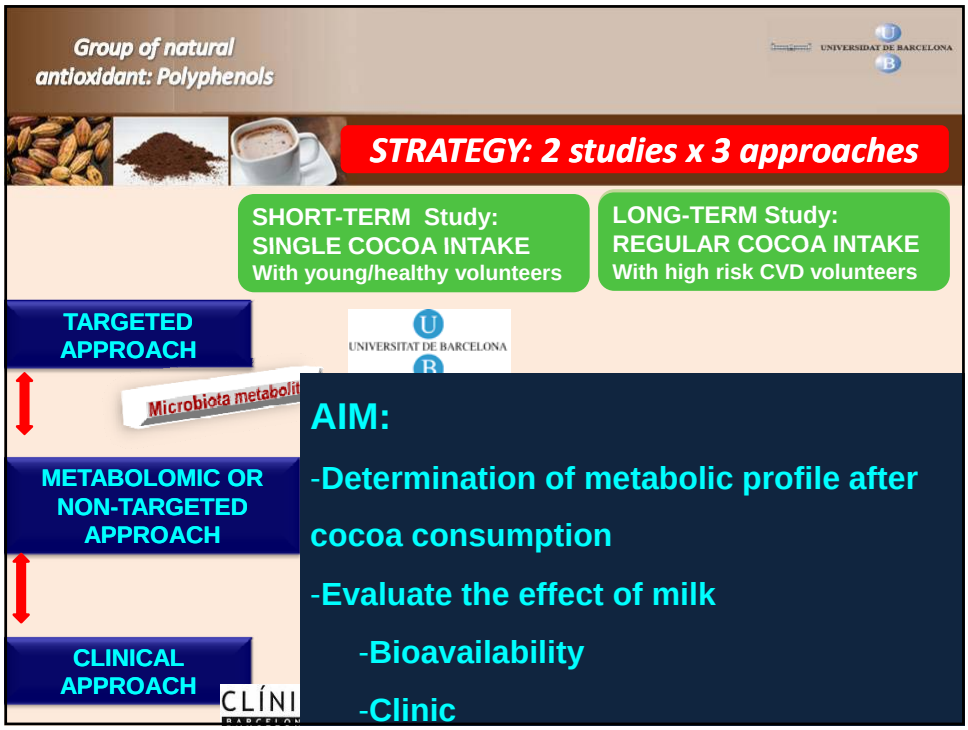
METABOLISM

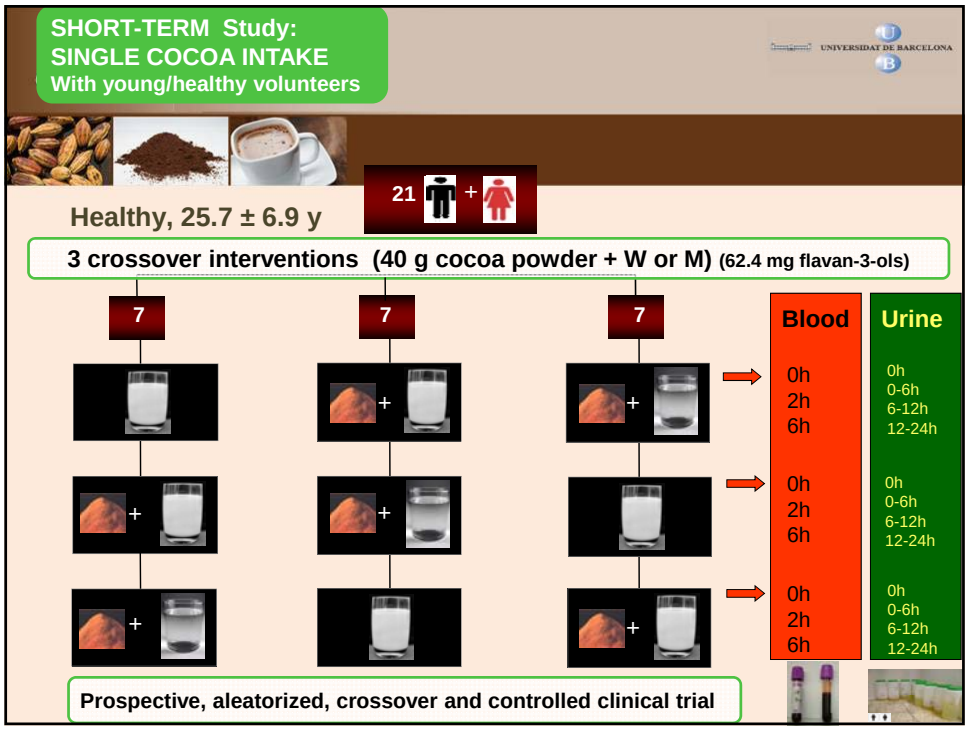


FOOD METABOLOME
New markers of food intake



New mecanism of action
(Biomarkers of effect)


Group of natural antioxidant: Polyphenols
STRATEGY: 2 studies x 3 approaches
SHORT-TERM Study:
SINGLE COCOA INTAKE
With young/healthy volunteers
LONG-TERM Study:
REGULAR COCOA INTAKE
With high risk CVD volunteers
TARGETED APPROACH
Microbiota metabolit
METABOLOMIC OR NON-TARGETED APPROACH
CLINICAL APPROACH
AIM:
-Determination of metabolic profile after cocoa consumption
-Evaluate the effect of milk
-Bioavailability
-Clinic


SHORT-TERM Study:
SINGLE COCOA INTAKE
With young/healthy volunteers
Healthy, 25.7 ± 6.9 y
21
3 crossover interventions (40 g cocoa powder + W or M) (62.4 mg flavan-3-ols)
7
7
7
Blood
0h
2h
6h
0h
2h
6h
0h
2h
6h
Urine
0h
0-6h
6-12h
12-24h
0h
0-6h
6-12h
12-24h
0h
0-6h
6-12h
12-24h
Prospective, aleatorized, crossover and controlled clinical trial

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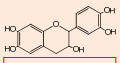
FLAVONOID METABOLISM

Cocoa Polyphenols
(FLAVAN-3-OL MONOMERS, OLIGOMERS AND POLYMERS)

Small intestine

COMT, UDPGT



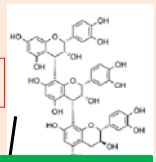
monomers

Phase II

-Glucuronides
-O-Methyl glucuronides
-O-Methyl derivatives

Bilis

Oligomers (DP>3) and polymers (DP>5)

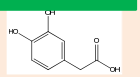


Colon

INTESTINAL MICROBIOTA

Microbiota metabolites

Valerolactones
Phenolic acids



TISSUES

Liver

COMT, UDPGT, SULT

Collaborations
-Dra Castell
-Dr Santos-Buelga

POSTERIOR METABOLISM
-Glucuronides
-Sulfates
-O-Methyl derivatives

Phase II

Kidney

URINE

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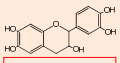
TARGETED PROFILING METHODOLOGY

Cocoa Polyphenols
(FLAVAN-3-OL MONOMERS, OLIGOMERS AND POLYMERS)

Small intestine

COMT, UDPGT

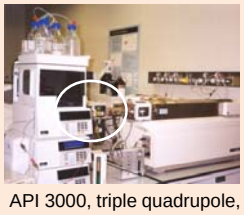


monomers

Phase II

-Glucuronides
-Sulfates
-O-Methyl derivatives

SOLID-PHASE EXTRACTION
HLB (30 mg)
(Waters)



API 3000, triple quadrupole, Applied Biosystems
PE Sciex (Ontario, Canada)

Support of Dr Olga Jauregui and Dr Isidre Casals

Urpí-Sarda et al. 2009. J Crom A

Group of natural antioxidant: Polyphenols

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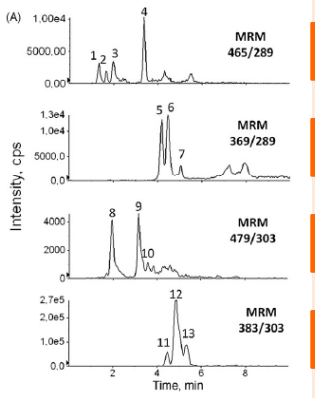
TARGETED PROFILING METHODOLOGY

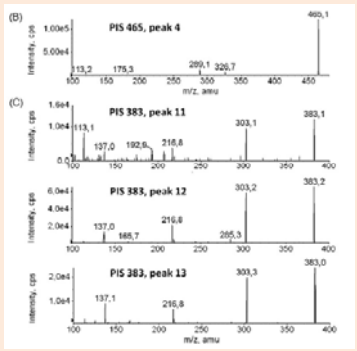
Phase II

Phase II Metabolites — **MRM transition**



- (Epi)catechin glucuronides
- Epicatechin sulfates
- Methyl-epicatechin glucuronides
- Methyl-epicatechin sulfates

Confirmation by PIS



Urpí-Sarda et al. 2009. J Crom A

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TARGETED PROFILING METHODOLOGY

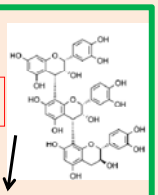
Enzymatic Hydrolysis β-glucuronidase / sulfatase

MCX (30 mg) (Waters)

API 3000, triple quadrupole, Applied Biosystems
PE Sciex (Ontario, Canada)

Microbiota metabolites

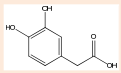
Oligomers (DP>3) and polymers (DP>5)



Colon

INTESTINAL MICROBIOTA

Phenolic acids
Valerolactones



Support of Dr Olga Jauregui and Dr Isidre Casals

Urpi-Sarda et al. 2009. *Anal Bioanal Chem*

Group of natural antioxidant: Polyphenols

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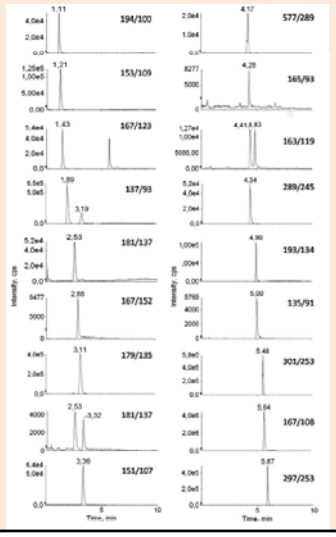
TARGETED PROFILING METHODOLOGY

Microbiota metabolites



Colonic Microbiota Metabolites	MRM transition
Hydroxyphenylvalerolactones	
5-(3',4'-dihydroxyphenyl)-γ-valerolactone	207/163
5-(3'-methoxy-4'-hydroxyphenyl)-γ-valerolactone	221/162
C_6C_3	
3,4-dihydroxyphenylpropionic acid	181/137
3-hydroxyphenylpropionic acid	165/93
m-coumaric acid	163/119
p-coumaric acid	163/119
caffeic acid	179/135
ferulic acid	193/134
C_6C_2	
3,4-dihydroxyphenylacetic acid	167/123
3-methoxy-4-hydroxyphenylacetic acid	181/137
3-hydroxyphenylacetic	151/107
phenylacetic acid	135/91
C_6C_1	
protocatechuic acid	153/109
vanillic acid	167/152
4-hydroxybenzoic acid	137/93
3-hydroxybenzoic acid	137/93
4-hidroxyhippuric acid	194/100
3-hidroxyhippuric acid	194/150
Flavanols	
(-)-epicatechin	289/245
procyanidin B2	577/289

Urpi-Sarda et al. 2009. *Anal Bioanal Chem*

SHORT-TERM Study: SINGLE COCOA INTAKE
With young/healthy volunteers

Microbiota metabolites

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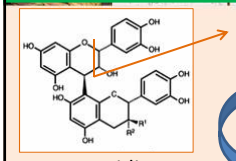
TARGETED METABOLIC PROFILING

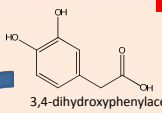
Microbiota metabolites

Negatively affected by milk intake

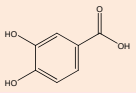


Procyanidins

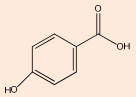
Appeldorn, MM; et al. J Agric Food Chem; 2009. 57, 1084-92



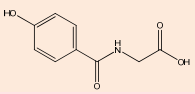
3,4-dihydroxyphenylacetic acid



Protocatechuic acid (PA)



4-Hydroxybenzoic acid (4-HBA)

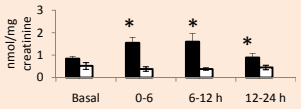


4-Hydroxyhippuric acid (4-HHA)

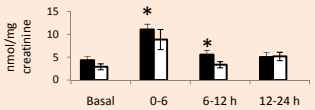
Cocoa with water (CW)
Cocoa with milk (CM)

Differences

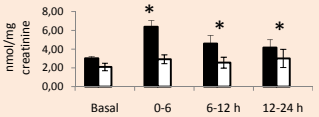
49-76%



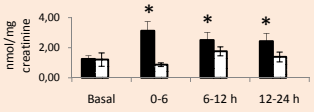
20-40%



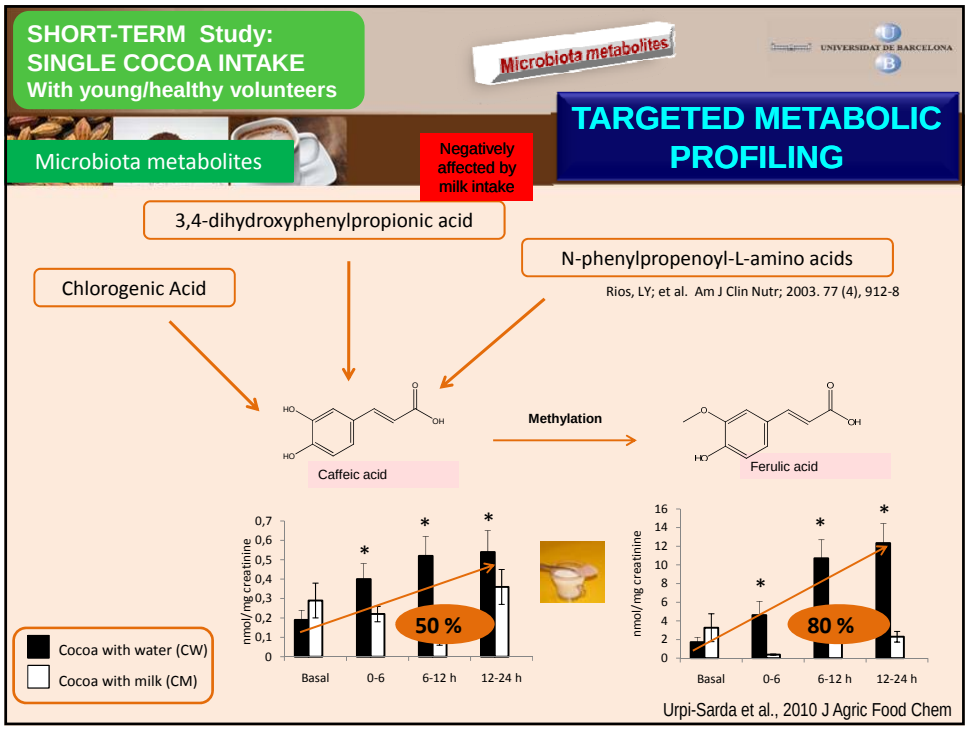
28-72%

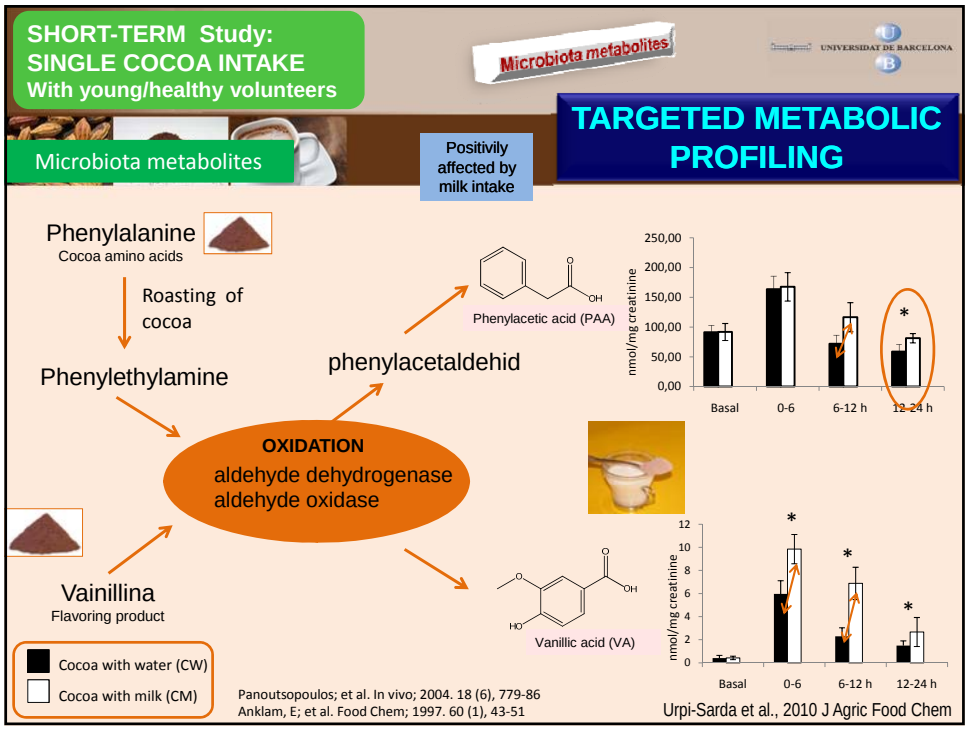


30-72%



Urpi-Sarda et al., 2010 J Agric Food Chem


SHORT-TERM Study:
SINGLE COCOA INTAKE
With young/healthy volunteers
Microbiota metabolites
TARGETED METABOLIC PROFILING
Microbiota metabolites
Negatively affected by milk intake
3,4-dihydroxyphenylpropionic acid
N-phenylpropenoyl-L-amino acids
Chlorogenic Acid
Rios, LY; et al. Am J Clin Nutr; 2003. 77 (4), 912-8
Methylation
Caffeic acid
Ferulic acid
nmol/mg creatinine
50 %
80 %
Basal
0-6
6-12 h
12-24 h
Cocoa with water (CW)
Cocoa with milk (CM)
Urpi-Sarda et al., 2010 J Agric Food Chem


SHORT-TERM Study:
SINGLE COCOA INTAKE
With young/healthy volunteers
Microbiota metabolites
TARGETED METABOLIC PROFILING
Microbiota metabolites
Positivily affected by milk intake
Phenylalanine
Cocoa amino acids
Roasting of cocoa
Phenylethylamine
phenylacetaldehid
Phenylacetic acid (PAA)
OXIDATION
aldehyde dehydrogenase
aldehyde oxidase
Vainillina
Flavoring product
Vanillic acid (VA)
nmol/mg creatinine
Basal
0-6
6-12 h
12-24 h
Cocoa with water (CW)
Cocoa with milk (CM)
Panoutsopoulos; et al. In vivo; 2004. 18 (6), 779-86
Anklam, E; et al. Food Chem; 1997. 60 (1), 43-51
Urpi-Sarda et al., 2010 J Agric Food Chem

SHORT-TERM Study:
SINGLE COCOA INTAKE
With young/healthy volunteers

Microbiota metabolites

Microbiota metabolites

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TARGETED METABOLIC PROFILING

+

Intake

15 studied compounds

7 phenolic acids ↓ excretion

3,4-Dihydroxyphenylacetic acid, protocatechuic acid, 4-hydroxybenzoic acid, 4-hydroxyhippuric acid, hippuric acid, caffeic acid and ferulic acid.

2 phenolic acids ↑ excretion

Vanillic acid and phenylacetic acid

= 6 phenolic acids absorption

Milk has a partial effect on the phenolic acid excretion profile after consumption of cocoa powder.

Group of natural antioxidant: Polyphenols

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Nutrición y "-Ómicas"

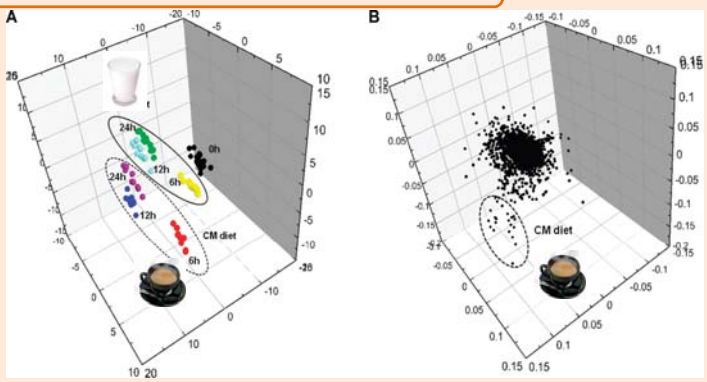
Proceso celular	Tecnología ómica	Descripción biológica
ADN		
Transcripción	Genómica (secuencia, regulación, epigenoma, SNPs)	Lo que podría pasar
ARNm		
Traducción	Transcriptómica (MICROARRAYS)	Lo que parece que esta ocurriendo
Proteínas		
Ocurrencia *Actividad*	Proteómica (identificación, función, modificaciones post-traducción)	Lo que hace que ocurra
Metabolitos	Metabolómica (perfil), Metabonómica (función)	Lo que ha ocurrido o está ocurriendo
Respuesta fenotípica		CONSECUENCIAS

SHORT-TERM Study:
SINGLE COCOA INTAKE
With young/healthy volunteers

METABOLOMIC OR NON-TARGETED APPROACH

URINARY METABOLOME MODIFICATIONS

OSC-PLS scores plot



OSC-PLS loading plot

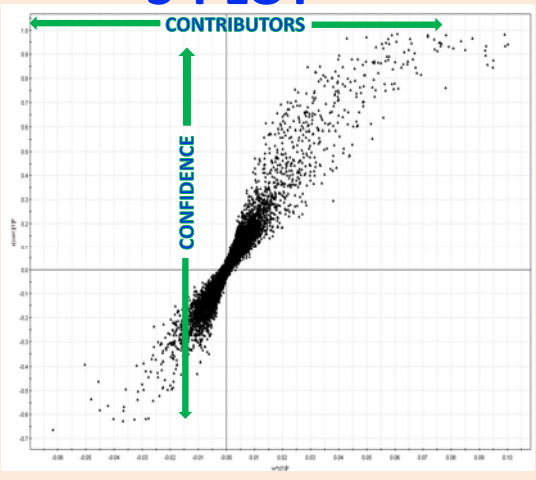
-Kinetic evolution during the 24 h after test-meal intake in the urine fingerprint of both diets, suggesting influence of circadian rhythm of urine
-Clear differences between nutritional interventions

Llorach et al., 2009 J. Proteome Res.
Llorach et al., 2010.J Pharm & Biomed An

SHORT-TERM Study:
SINGLE COCOA INTAKE
With young/healthy volunteers

METABOLOMIC OR NON-TARGETED APPROACH

S-PLOT



BIOMARKERS DETECTION AND IDENTIFICATION

Wiklund et al. Anal Chem. (2008)

SHORT-TERM Study:
SINGLE COCOA INTAKE
With young/healthy volunteers

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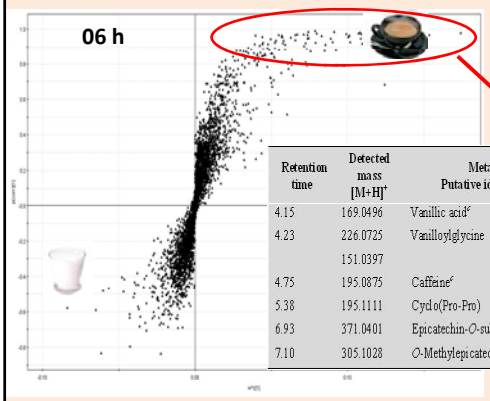
METABOLOMIC OR NON-TARGETED APPROACH



BIOMARKERS DETECTION AND IDENTIFICATION

Retention time	Detected mass $[M+H]^+$	Metabolite Putative identification	Assignation	Theoretical mass $[M+H]^+$	Mass difference (mDa)
4.15	169.0496	Vanillic acid*	$[M+H]^+$	169.0495	-0.1
4.23	226.0725	Vanilloylglycine	$[M+H]^+$	226.0709	-1.6
	151.0397		$[M+H\text{-glycine}]^+$	151.0395	-0.2
4.75	195.0875	Caffeine*	$[M+H]^+$	195.0876	0.1
5.38	195.1111	Cyclo(Pro-Pro)	$[M+H]^+$	195.1127	1.6
6.93	371.0401	Epicatechin-*O*-sulfate	$[M+H]^+$	371.0431	3.0
7.10	305.1028	*O*-Methylepicatechin	$[M+H]^+$	305.1019	-0.9

The higher contributors were related with the host phytochemicals metabolites (food metabolome)

Llorach et al., 2009 J. Proteome Res.

SHORT-TERM Study:
SINGLE COCOA INTAKE
With young/healthy volunteers

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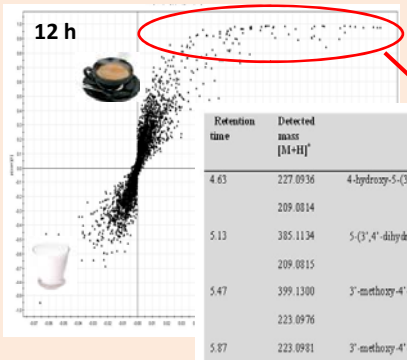
METABOLOMIC OR NON-TARGETED APPROACH

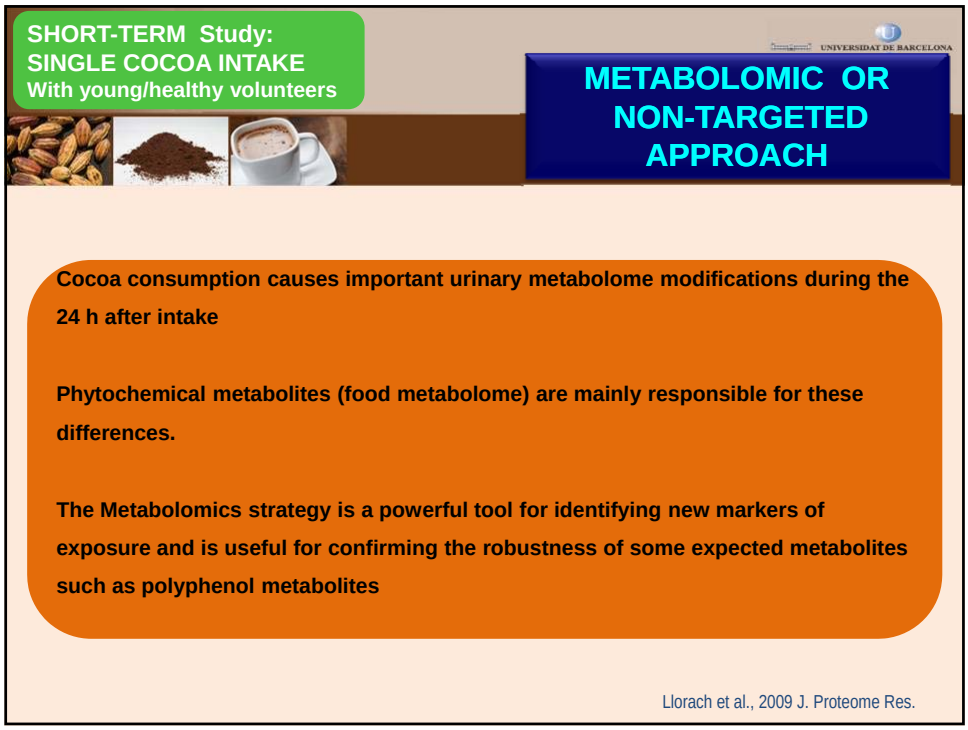


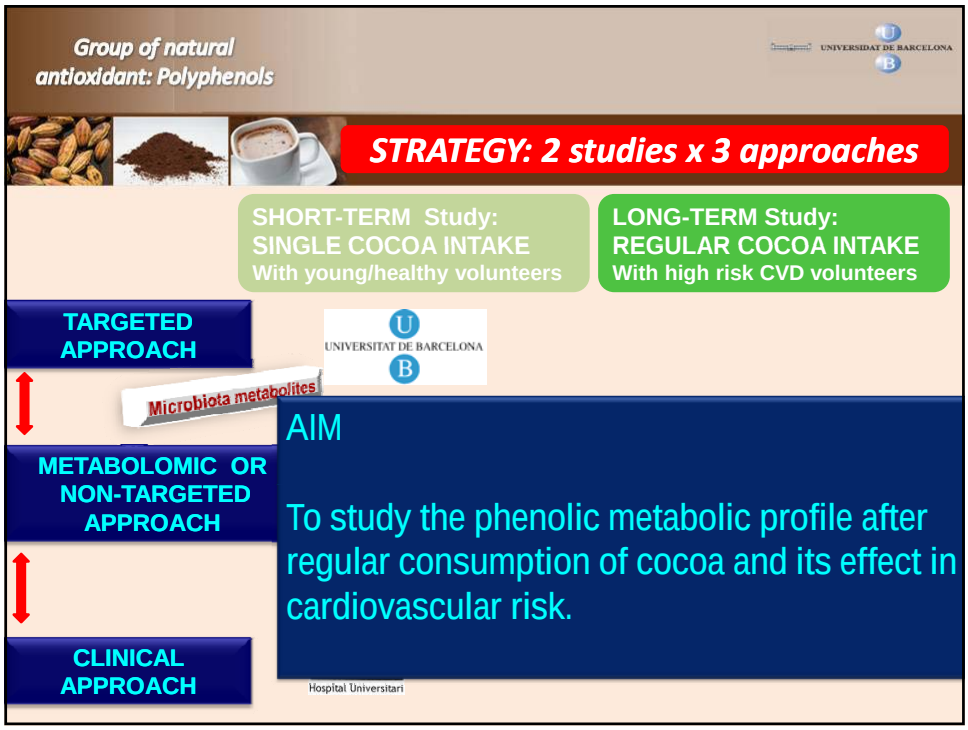
BIOMARKERS DETECTION AND IDENTIFICATION

Retention time	Detected mass $[M+H]^+$	Metabolite Putative identification	Assignation	Theoretical mass $[M+H]^+$	Mass difference (mDa)
4.63	227.0936	4-hydroxy-5-(3,4-dihydroxyphenyl)-valeric acid*	$[M+H]^+$	227.0913	-2.3
	209.0814		$[M+H\text{-}H_2O]^+$	209.0808	-0.6
5.13	385.1134	5-(3',4'-dihydroxyphenyl)-γ-valerolactone glucuronide*	$[M+H]^+$	385.1129	-0.5
	209.0815		$[M+H\text{-glcA}^*]$	209.0808	-0.7
5.47	399.1300	3'-methoxy-4'-hydroxyphenylvalerolactone glucuronide*	$[M+H]^+$	399.1285	-1.5
	223.0976		$[M+H\text{-glcA}]^+$	223.0964	-1.2
5.87	223.0981	3'-methoxy-4'-hydroxyphenylvalerolactone*	$[M+H]^+$	223.0964	-1.7
6.12	289.0386	5-(3',4'-dihydroxyphenyl)-γ-valerolactone-sulfate*	$[M+H]^+$	289.0376	-1.0
7.00	289.0404	5-(3',4'-dihydroxyphenyl)-γ-valerolactone-sulfate*	$[M+H]^+$	289.0376	-2.8

The higher contributors were related with the microbiota polyphenols metabolites (food metabolome)

Llorach et al., 2009 J. Proteome Res.


SHORT-TERM Study:
SINGLE COCOA INTAKE
With young/healthy volunteers
METABOLOMIC OR NON-TARGETED APPROACH
Cocoa consumption causes important urinary metabolome modifications during the 24 h after intake
Phytochemical metabolites (food metabolome) are mainly responsible for these differences.
The Metabolomics strategy is a powerful tool for identifying new markers of exposure and is useful for confirming the robustness of some expected metabolites such as polyphenol metabolites
Llorach et al., 2009 J. Proteome Res.


Group of natural antioxidant: Polyphenols
STRATEGY: 2 studies x 3 approaches
SHORT-TERM Study:
SINGLE COCOA INTAKE
With young/healthy volunteers
LONG-TERM Study:
REGULAR COCOA INTAKE
With high risk CVD volunteers
TARGETED APPROACH
Microbiota metabolites
METABOLOMIC OR NON-TARGETED APPROACH
CLINICAL APPROACH
AIM
To study the phenolic metabolic profile after regular consumption of cocoa and its effect in cardiovascular risk.
Hospital Universitari

LONG-TERM Study: REGULAR COCOA INTAKE With high risk CVD volunteers

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Crossover, randomized, controlled

1st Intervention

Wash-out

15 days

40 g/d solubles of cocoa with skimmed milk (93 mg flavan-3-ols)

Skimmed milk

28 days

2nd Intervention

Skimmed milk

40 g/d solubles of cocoa with skimmed milk (93 mg flavan-3-ols)

28 days

Inclusion criteria: risk factors

- Age (men ≥45 years; women ≥55 years)
- Smokers
- Diabetes mellitus (glycemia ≥126 mg/dl)
- Hypertension (BP ≥140/90 mmHg)
- HDL cholesterol (< 35 mg/dl)
- LDL cholesterol (≥ 160 mg/dL)
- Familiar history of cardiovascular disease
- Obesity (IMC ≥25)

Personal int

sugar-fr
93 mg fl

- ✓Control of diet and exercise
- ✓General analyses and nutritional valoration
- ✓Coagulation test
- ✓Serum lipids and lipoproteins
- ✓Immunologic studies
- ✓*In vitro* studies

LONG-TERM Study: REGULAR COCOA INTAKE With high risk CVD volunteers

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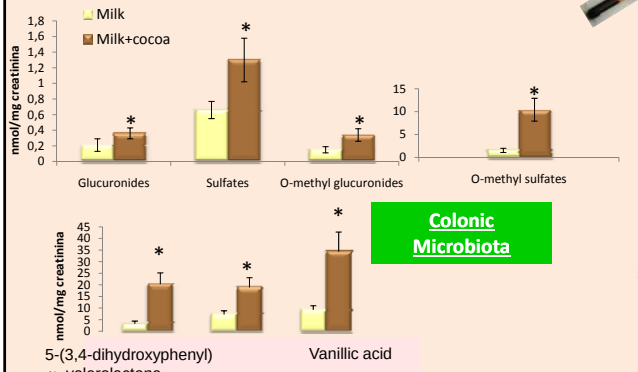
TARGETED METABOLIC PROFILING: COMPLIANCE

24h Urine Samples

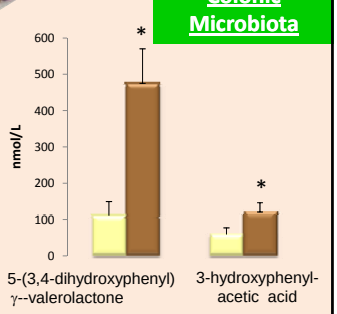
Phase II metabolites of epicatechin



Colonic Microbiota

Fasting Plasma Samples

Colonic Microbiota



Urpi-Sarda et al. 2009 J. Chrom. A

LONG-TERM Study:
REGULAR COCOA INTAKE
With high risk CVD volunteers

METABOLOMIC OR NON-TARGETED APPROACH

Compliance was assessed measuring epicatechin metabolites derived from phase II metabolism in 24-h urine samples.

Regular consumption of 40 g cocoa powder with milk per day resulted in a urinary excretion of 18.38 µmol/d of total phase II metabolites of epicatechin (global increment of 458%) in comparison with intake of milk (3.29 µmol/L).

Urpi-Sarda et al. 2009 J. Chrom. A

LONG-TERM Study:
REGULAR COCOA INTAKE
With high risk CVD volunteers

CLINICAL APPROACH

CLÍNIC BARCELONA Hospital Universitari

The American Journal of Clinical Nutrition *Am J Clin Nutr* 2009;90:1144–50.

Effect of cocoa powder on the modulation of inflammatory biomarkers in patients at high risk of cardiovascular disease[1–4]

Maria Monagas, Nasiruddin Khan, Cristina Andres-Lacueva, Rosa Casas, Mireia Urpí-Sardà, Rafael Llorach, Rosa Maria Lamuela-Raventós, and Ramón Estruch

LONG-TERM Study:
REGULAR COCOA INTAKE
With high risk CVD volunteers

CLINICAL APPROACH

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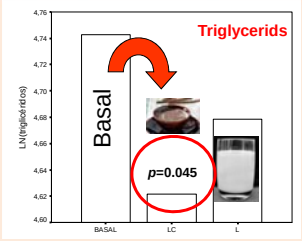
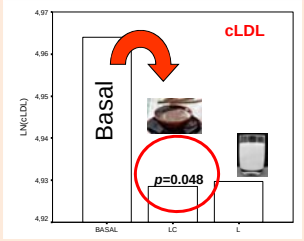
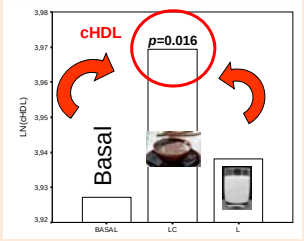
Serum Lipids and serum proteins







It was observed a significant diminution of triglycerides and cLDL after cocoa consumption with respect to basal status.

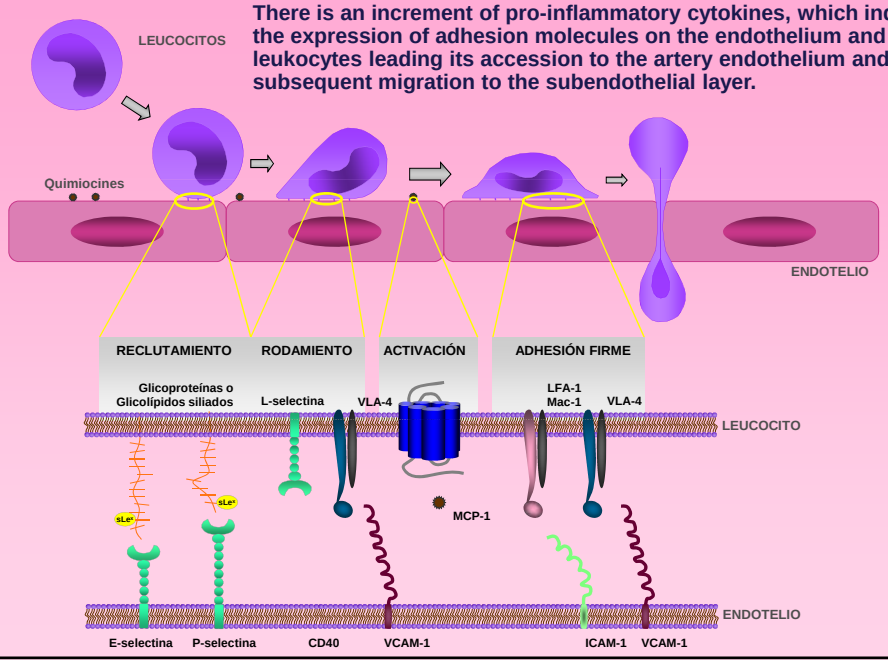
It was observed a significant increase of cHDL after cocoa consumption when compared with basal and with milk intake.

NO CHANGES IN:

-Total cholesterol
-Lipoproteins (including Apo A and Apo B)

Monagas et al., (2009)AJCN

Inflammation and initial phases of atherosclerosis



LONG-TERM Study: REGULAR COCOA INTAKE With high risk CVD volunteers

CLINICAL APPROACH

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Expression of adhesion molecules on the surface of T-lymphocytes and monocytes

RESULTS

Monagas et al., (2009)AJCN

	C+M intervention	M intervention	*t*-Paired test (C+M *vs.* M)			
				95% CI		
	Mean ± SD	Mean ± SD	Mean difference	Lower	Upper	*p*
T-lymphocytes (MFI)						
LFA-1	75.11 ± 10.81	79.60 ± 9.55	-4.49	-8.96	-0.02	0.049
Mac-1	59.43 ± 11.24	59.13 ± 11.05	0.30	-6.04	6.65	0.923
VLA-4	54.29 ± 2.45	54.15 ± 2.54	0.14	-0.83	1.11	0.772
SLex	127.81 ± 41.25	134.69 ± 31.81	-6.88	-24.77	11.01	0.440
CD40	57.50 ± 11.08	55.68 ± 10.56	1.82	-2.18	5.82	0.361
Monocytes (MFI)						
LFA-1	30.73 ± 5.20	29.95 ± 4.86	0.78	-0.78	2.34	0.314
Mac-1	35.73 ± 7.81	36.02 ± 6.91	-0.29	-3.10	2.51	0.834
VLA-4	22.99 ± 1.92	24.43 ± 2.72	-1.43	-2.79	-0.08	0.039
SLex	61.46 ± 17.10	61.67 ± 15.33	-0.21	-4.47	4.04	0.919
CD40	23.21 ± 2.17	24.49 ± 2.78	-1.28	-2.32	-0.25	0.017
CD36	23.86 ± 7.75	28.03 ± 6.80	-4.16	-8.00	-0.33	0.035

Significant decrease in the expression of VLA-4, CD40 and CD36 on the surface of monocytes after COCOA intake compared with MILK intake. **Monagas et al., (2009)AJCN**

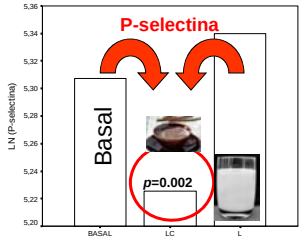
LONG-TERM Study: REGULAR COCOA INTAKE With high risk CVD volunteers

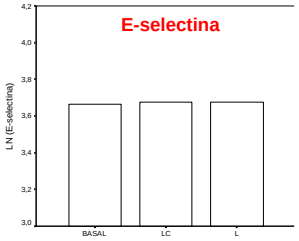
CLINICAL APPROACH

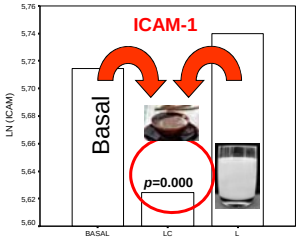
CLÍNIC BARCELONA Hospital Universitari

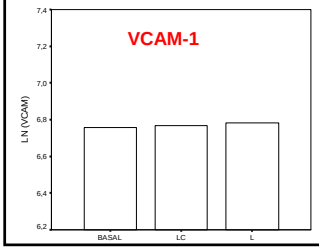
Serum concentrations of soluble adhesion molecules

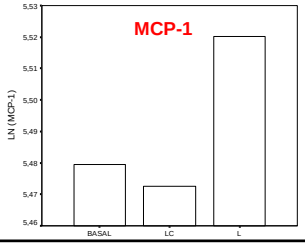
CHANGES IN CIRCULATING INFLAMMATORY MARKERS











It was observed a significant diminution of serum levels of P-Selectin and ICAM-1 after cocoa consumption.

Monagas et al., (2009)AJCN

LONG-TERM Study:
REGULAR COCOA INTAKE
With high risk CVD volunteers

CLINICAL APPROACH

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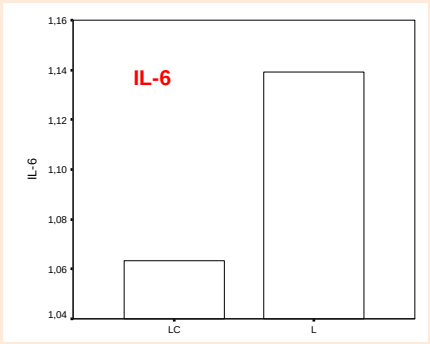
Serum concentrations of pro-inflammatory cytokines



NO CHANGES in hs-CRP

It was observed a diminution in IL-6 levels after cocoa consumption although without significant difference.

Monagas et al., (2009) AJCN

LONG-TERM Study:
REGULAR COCOA INTAKE
With high risk CVD volunteers

CLINICAL APPROACH

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CONTRADICTORY RESULTS IN LITERATURE

SUBJECTS (n)	INTAKE (flavanol/day)	REDUCTION	NO CHANGES	REFERENCE
Healthy (25)	36.9g dark chocolate + 30.9g cocoa powder for 6 weeks (651 mg/d)		IL-1, IL-6, CRP, TNF-α, P-selectin	Mathur et al. J Nutr (2002)
Individuals with CAD (30 male)	Flavanol-rich chocolate bar and cocoa beverage for 6-week (444 mg/d)		ICAM-1, P-selectin, VCAM-1, E-Selectin,	Farouque et al. Clin Sci (2006)
Hypercholesterol Subjects (49)	2 dark chocolate bars with sterol for 4 weeks (360mg/d)	cLDL, total chol	Hs-CRP, ICAM-1, sCD40L	Allen et al. J Nutr (2008)
Hypercholesterol Women (32)	Flavanol-rich cocoa beverage for 6-weeks (446 mg/d)	VCAM-1	E-Selectin, P-selectin	Wang-Polagruto et al J Cardiovasc Pharmacol (2006)
Healthy women (49)	Dark chocolate for 6-week	ICAM-1	VCAM-1,IL-6, hs-CRP	Kurlandsly et al. Nutr Res (2006)
Individuals with CV risk factors (42)	**Cocoa powder for 1-month (93 mg/d)**	**P-selectin ICAM-1,**	**VCAM-1, E-selectin, MCP-1, IL6, CRP**	**Monagas et al. Am J Clin Nutr (2009)**

In accordance to our study

LONG-TERM Study:
REGULAR COCOA INTAKE
With high risk CVD volunteers

CLINICAL APPROACH

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Our results suggest that regular consumption of nutritional doses of cocoa may have an effect on all initial phases of the atherosclerosis process in subjects at high-risk of coronary heart disease .

Monagas et al., (2009)AJCN

Thanks for your attention.

Courtesy of Llorach


see you in
Barcelona 5th ICPH 2011
October 17-20th