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Group of Polyphenols
Nutrition and Food Science
Department
Pharmacy School

Department of Internal Medicine Hospital Clinic of Barcelona

Google: “Antioxidants naturals”
<table>
<thead>
<tr>
<th>Group Leaders</th>
<th>Rosa Lamuela-Raventós</th>
<th>Cristina Andres-Lacueva</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postdoctoral Scientist</td>
<td>Rafa Llorach</td>
<td>Mireia Urpi-Sarda</td>
</tr>
<tr>
<td></td>
<td>Raul Zamora-Ros</td>
<td>Giuseppe Di Lecce</td>
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<tr>
<td></td>
<td></td>
<td>Sara Tulipani</td>
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<td></td>
<td></td>
<td>Sara Arranz</td>
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<tr>
<td>PhD students</td>
<td>Miriam Martínez</td>
<td>Nasir Khan</td>
</tr>
<tr>
<td></td>
<td>Alex Medina</td>
<td>Maria Rotchés</td>
</tr>
<tr>
<td></td>
<td>Anna Tresserra</td>
<td>Maria Boto</td>
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<td></td>
<td>Gemma Chiva</td>
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<tr>
<td>Collaborators</td>
<td>Marta Perez</td>
<td>Paola Quifer</td>
</tr>
</tbody>
</table>

**Group of natural antioxidant: Polyphenols**

### CONSUMPTION OF CHOCOLATE

**Ranking of Consumption**

Chocolate Confectionery

CONSUMPTION OF COCOA POWDER

Source: Nielsen, Euromonitor, Procter & Gamble, Nestlé

CONSUMPTION OF FLAVONOIDS IN INFANTILE POPULATION

Percentage of flavonoids in Spanish infantile diet

- 54% come from cocoa consumption (13.13 mg/day)

TOTAL FLAVONOID CONSUMPTION: 24.2 mg/day

CONSUMPTION COCOA PRODUCTS: 33.82 g/day

Zamora-Ros et al.
**Group of natural antioxidant: Polyphenols**

**Cocoa Phytochemicals**

- Theobromine
- Diketopiperazine
- N-phenylpropanyl-L-aminoacids

**POLYPHENOLS: FLAVONOIDS**

- Flavan-3-ols
- Procyanidins

**COCOA**

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

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

Cocoa and Cardiovascular Health

Corti et al. (2009) Circulation

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, e.g., 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 endpoints such as carotid atherosclerosis and eventually morbidity and mortality are needed.

Health relevant effects of chocolate

- Blood Pressure Reduction
- Improved Vascular Function
- Reduced Platelet Activity
- Improved Insulin Sensitivity
- Other: Anti-inflammatory
**Group of natural antioxidant: Polyphenols**

### PREVENTION CARDIOVASCULAR DISEASES

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

**Group of natural antioxidant: Polyphenols**

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

B/ Decrement of Blood pressure

C/ Endothelial function

D/ Lipid profile

E/ Other variables

No changes in glyceryl trinitrate-induced vasodilation

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

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

Visioli et al., J Nutr (2008)

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

**Subproject 1:**
Bioavailability of cocoa powder polyphenols in humans (intestinal and colonic absorption). Milk effect

IP (1) : Dr. Cristina Andres-Lacueva

**Subproject 2:**
Scientific bases of phenol and inflammatory biomarkers interaction in atherosclerosis

IP (2) : Dr. Ramón Estruch

Hypothesis

**FOOD METABOLOME**
New markers of food intake

**New mechanism of action**
(Biomarkers of effect)
LONG-TERM Study: REGULAR COCOA INTAKE
With high risk CVD volunteers

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

METABOLIC 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

3 crossover interventions (40 g cocoa powder + W or M) (62.4 mg flavan-3-ols)

Prospective, aleatorized, crossover and controlled clinical trial
**Group of natural antioxidant: Polyphenols**

**FLAVONOID METABOLISM**

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

**TISSUES**

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

- Blads
  - Glucuronides
  - Sulfates
  - O-Methyl derivatives

- Colon
  - Microbiota metabolites
    - Valerolactones
    - Phenolic acids

- Small intestine
  - Phase II
  - Cont, UDPGT

- Liver
  - Posterior metabolism
  - Cont, UDPGT, SULT

- Kidney
  - Phase II

- Urine

**SOLID-PHASE EXTRACTION**

- 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
Phase II Metabolites

**ENZYMATIC HYDROLYSIS**

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

**MCX (30 mg) (Waters)**

**Confirmation by PIS**

**Microbiota metabolites**

- Oligomers (DP>3) and polymers (DP>5)
- Phenolic acids
- Valerolactones

**InTESTINAL MICROBIOTA**

Support of Dr Olga Jauregui and Dr Isidre Casals

TARGETED PROFILING METHODOLOGY

Microbiota metabolites

Colonic Microbiota Metabolites

<table>
<thead>
<tr>
<th>Compound</th>
<th>NMR transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>5,4-dihydroxyphenylactic acid</td>
<td>207/142</td>
</tr>
<tr>
<td>4-hydroxyphenylactic acid</td>
<td>345/237</td>
</tr>
<tr>
<td>4-hydroxyphenylacetic acid</td>
<td>162/160</td>
</tr>
<tr>
<td>guaiacol</td>
<td>169/120</td>
</tr>
<tr>
<td>4-quinolone</td>
<td>119/119</td>
</tr>
<tr>
<td>3-hydroxybenzaldehyde</td>
<td>158/134</td>
</tr>
<tr>
<td>3-phenylpropionic acid</td>
<td>147/138</td>
</tr>
<tr>
<td>4-hydroxybenzaldehyde</td>
<td>781/167</td>
</tr>
<tr>
<td>2-hydroxybenzoic acid</td>
<td>1,53/95</td>
</tr>
<tr>
<td>3-hydroxybenzoic acid</td>
<td>138/139</td>
</tr>
<tr>
<td>vanillic acid</td>
<td>1471/128</td>
</tr>
<tr>
<td>4-hydroxybenzoic acid</td>
<td>1,53/95</td>
</tr>
<tr>
<td>2-hydroxybenzoic acid</td>
<td>781/167</td>
</tr>
<tr>
<td>3-hydroxybenzoic acid</td>
<td>138/139</td>
</tr>
<tr>
<td>protocatechuic acid</td>
<td>22-72%</td>
</tr>
<tr>
<td>3,4-dihydroxyphenylactic acid</td>
<td>20-40%</td>
</tr>
<tr>
<td>4-hydroxybenzoic acid (4-HBA)</td>
<td>28-72%</td>
</tr>
<tr>
<td>4-hydroxycinnamic acid</td>
<td>30-72%</td>
</tr>
</tbody>
</table>


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

TARGETED METABOLIC PROFILING

Microbiota metabolites

Differences

5,4-dihydroxyphenylactic acid

3,4-dihydroxyphenylactic acid

Protopocatechuic acid (PA)

4-hydroxybenzoic acid (4-HBA)

4-hydroxycinnamic acid

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

TARGETED METABOLIC PROFILING

Microbiota metabolites

3,4-dihydroxyphenylpropionic acid
Negatively affected by milk intake

Chlorogenic Acid

N-phenylpropenoyl-L-amino acids

Methylation

Ferulic acid

Phenylalanine
Positive affected by milk intake

Oxidation

Phenylethylamine

Vanillina
Flavoring product

Panoutsopoulos; et al. In vivo; 2004. 18 (6), 779-86
Anklam, E; et al. Food Chem; 1997. 60 (1), 43-51
SHORT-TERM Study: SINGLE COCOA INTAKE
With young/healthy volunteers

TARGETED METABOLIC PROFILING

15 studied compounds

7 phenolic acids ↓ excretion
- 3,4-Dihydroxyphenylacetic acid
- Protocatechuic acid
- 4-hydroxybenzoic acid
- 4-hydroxyhippuric acid
- Hippuric acid
- Caffeic acid
- Ferulic acid

2 phenolic acids ↑ excretion
- Vanillic acid
- 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

Nutrición y “Ómicas”

Proceso celular
- ADN Transcripción
- ARN Traducción

Tecnología ómica
- ADN Genómica (secuencia, regulación, epigenoma, SNPs)
- ARNm Transcriptómica (MICROARRAYS)
- Proteínas Proteómica (identificación, función, modificaciones post-traducción)
- Metabolitos Metabolómica (perfil), Metabonómica (función)

Descripción biológica
- Lo que podría pasar
- Lo que parece que está ocurriendo
- Lo que hace que ocurra
- Lo que ha ocurrido o está ocurriendo

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

BIOMARKERS DETECTION AND IDENTIFICATION

S-PLOT

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

**METABOLOMIC OR NON-TARGETED APPROACH**

**BIOMARKERS DETECTION AND IDENTIFICATION**

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

Llorach et al., 2009 J. Proteome Res.

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

**METABOLOMIC OR NON-TARGETED APPROACH**

**BIOMARKERS DETECTION AND IDENTIFICATION**

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

Llorach et al., 2009 J. Proteome Res.
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

**AIM**

To study the phenolic metabolic profile after regular consumption of cocoa and its effect in cardiovascular risk.
LONG-TERM Study: REGULAR COCOA INTAKE
With high risk CVD volunteers

Crossover, randomized, controlled

1st Intervention
Wash-out
15 days
28 days

2nd Intervention
28 days

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

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)

CONTROL OF DIET AND EXERCISE
- General analyses and nutritional valoration
- Coagulation test
- Serum lipids and lipoproteins
- Immunologic studies
- In vitro studies

TARGETED METABOLIC PROFILING: COMPLIANCE

24h Urine Samples
Fasting Plasma Samples

Phase I metabolites of epicatechin

Colonic Microbiota

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

Effect of cocoa powder on the modulation of inflammatory biomarkers in patients at high risk of cardiovascular disease

María Monagas, Nasiruddin Khan, Cristina Andrey-Lacueva, Rosa Casas, Mireia Urpi-Sardà, Rafael Llorach, Rosa María Lamuela-Raventós, and Ramón Estruch
LONG-TERM Study: REGULAR COCOA INTAKE
With high risk CVD volunteers

CLINICAL APPROACH
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

There is an increment of pro-inflammatory cytokines, which induce the expression of adhesion molecules on the endothelium and in leukocytes leading its accession to the artery endothelium and their subsequent migration to the subendothelial layer.
Expression of adhesion molecules on the surface of T-lymphocytes and monocytes

CLINICAL APPROACH

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

RESULTS
Monagas et al., (2009) AJCN

<table>
<thead>
<tr>
<th>Expression of adhesion molecules on the surface of T-lymphocytes and monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>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</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T-Lymphocytes (MFI)</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>Mean difference</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFA-1</td>
<td>75.1 ± 10.81</td>
<td>70.6 ± 9.55</td>
<td>-4.49</td>
<td>-8.06 to -0.92</td>
<td>0.49</td>
</tr>
<tr>
<td>Mac-1</td>
<td>59.43 ± 11.24</td>
<td>59.13 ± 11.05</td>
<td>0.30</td>
<td>-6.04 to 6.65</td>
<td>0.923</td>
</tr>
<tr>
<td>VLA-4</td>
<td>54.29 ± 2.45</td>
<td>54.15 ± 2.54</td>
<td>0.14</td>
<td>-0.83 to 1.11</td>
<td>0.772</td>
</tr>
<tr>
<td>SLeX</td>
<td>127.81 ± 41.25</td>
<td>134.69 ± 31.81</td>
<td>-6.88</td>
<td>-24.77 to 11.01</td>
<td>0.440</td>
</tr>
<tr>
<td>CD40</td>
<td>57.59 ± 11.08</td>
<td>55.68 ± 10.56</td>
<td>1.82</td>
<td>-2.18 to 5.82</td>
<td>0.361</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Monocytes (MFI)</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>Mean difference</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFA-1</td>
<td>30.73 ± 5.20</td>
<td>29.95 ± 4.86</td>
<td>0.78</td>
<td>-0.78 to 2.34</td>
<td>0.314</td>
</tr>
<tr>
<td>Mac-1</td>
<td>35.73 ± 7.81</td>
<td>36.02 ± 6.91</td>
<td>0.29</td>
<td>-3.10 to 2.51</td>
<td>0.834</td>
</tr>
<tr>
<td>VLA-4</td>
<td>22.99 ± 1.92</td>
<td>24.43 ± 2.72</td>
<td>-1.43</td>
<td>-2.79 to -0.08</td>
<td>0.039</td>
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<tr>
<td>SLeX</td>
<td>61.46 ± 17.10</td>
<td>61.67 ± 15.33</td>
<td>0.21</td>
<td>-4.47 to 4.04</td>
<td>0.919</td>
</tr>
<tr>
<td>CD40</td>
<td>23.21 ± 2.17</td>
<td>24.49 ± 2.28</td>
<td>-1.28</td>
<td>-2.32 to -0.25</td>
<td>0.017</td>
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<tr>
<td>ICAM-1</td>
<td>23.86 ± 2.75</td>
<td>23.03 ± 6.80</td>
<td>-1.46</td>
<td>-8.00 to 0.33</td>
<td>0.353</td>
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<tr>
<td>VCAM-1</td>
<td>23.86 ± 2.75</td>
<td>23.03 ± 6.80</td>
<td>-1.46</td>
<td>-8.00 to 0.33</td>
<td>0.353</td>
</tr>
<tr>
<td>MCP-1</td>
<td>23.86 ± 2.75</td>
<td>23.03 ± 6.80</td>
<td>-1.46</td>
<td>-8.00 to 0.33</td>
<td>0.353</td>
</tr>
</tbody>
</table>

It was observed a significant diminution of serum levels of P-Selectin and ICAM-1 after cocoa consumption. Monagas et al., (2009) AJCN

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
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

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.
see you in

Barcelona 5th ICPH 2011
October 17-20th