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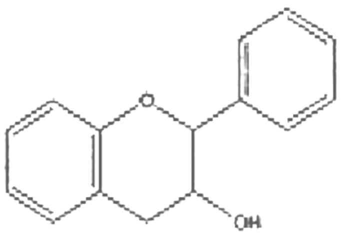
BIOAVAILABILITY OF FLAVONOIDS

XIII CHAIR J. de CLERCK
Louvain- la-Neuve
September 9, 2008



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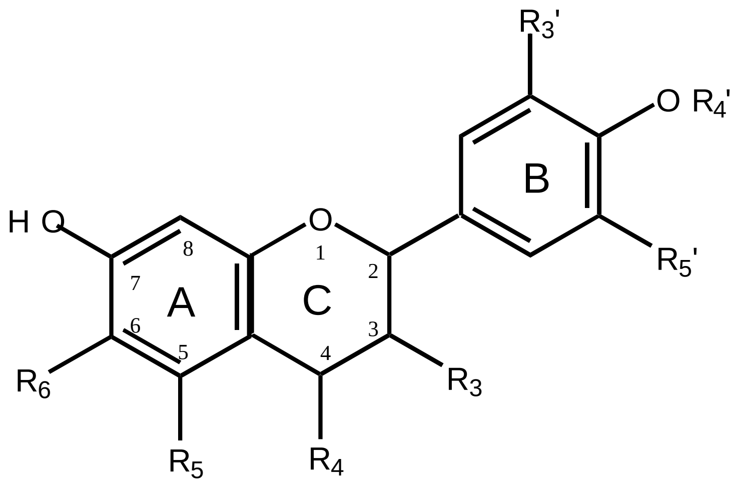
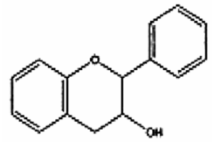
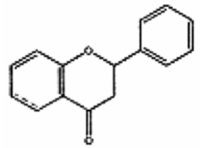
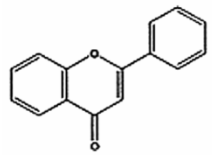
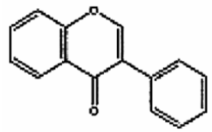
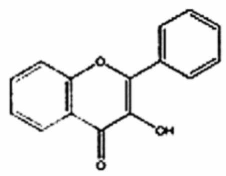
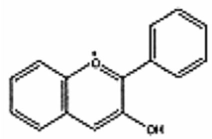
FLAVONOIDS

>9.000



Flavonoids (subgrup)	Chemical Structure	Dietetics Sources
Flavan-3-ols		Tea, red wine, beer, cocoa and grape.
Flavanones		Citric fruits.
Flavones		Spicies (e.g. parsley).
Isoflavones		Soy and legums.
Flavonols		Onions, apples, wine, blueberries, tea…
Anthocyans		Berries, red wine…

Approximate flavonoid content in the main sources of dietary consumption




Food (portion size)	mg/portion	Food (portion size)	mg/portion
Apple, with skin (150g)		**Black grape (150g)**	
Flavan-3-ols	13	Flavan-3-ols	35
Flavonols	6	Flavonols	5
Proanthocyanidins	147	Proanthocyanidins	93
Total	**166**	**Total**	**133**
Black tea (200ml)		**Green tea (200ml)**	
Flavan-3-ols	6	Flavan-3-ols	304
Flavonols	10	Flavonols	12
Thearubigins	116	Thearubigins	3
Total	**132**	**Total**	**319**
Black chocolate (40g)		**Beer (200ml)**	5
Flavan-3-ols	24	Flavan-3-ols	2
Flavonols		Flavonols	2,
Proanthocyanidins	165	**Total**	**9,8**
Total	**189**		
Red wine (100ml)		**Blackberries (50g)**	
Flavan-3-ols	10-20	Flavan-3-ols	10
Flavonols	10	Flavonols	<1
Proanthocyanidins	77-103	Proanthocyanidins	65
Anthocyanidins	9-45	Anthocyanidins	41
Total	**106-178**	**Total**	**116**




USDA 2003 and 2004; Beecher, 2003

Factors that affect the bioavailability of flavonoids

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- Modification of cleavage of one or more attached sugars
- Solubility, delivery, and food matrix
- Dose and adaption to dose
- Chemical changes that occur during processing or in the gastrointestinal tract
- Interactions and competitions with other components

Parameter	Flavonols	Flavanols	Flavanones and flavones	Phenolic acids
Modification or cleavage of one or more attached sugar	↑↑↑↑↑	-	↑↑↑↑↑	↑↑↑↑↑
Solubilization (e.g. ethanol, propylene glycol)	↑↑↑	↑	↑↑↑↑	?
Lipids and emulsifiers	↑↑↑	↑↑↑	?	?
Carbohydrate	?	↑↑↑	?	?
Other food matrix	↑	↑↑↑	↑↑↑	↑↑↑
Epimerization	-	↑↑↑	?	-
Other compounds	↑↑	↑↑	?	?
Dose response of absorption	Rutin linear, quercetin saturated in humans	EGCG linear when pure, EGCG saturated when in green tea in humans, epicatechin linear in rats	?	Ferulic acid linear in rat perfusion model

Relative effects from weak (↑) to strong (↑↑↑↑↑)
- not relevant
? not yet determined

Sholz and Williamson, Int. J. Vitam. Nutr. Res. 77, 2007, 224-235.

Components from diet that may affect flavonoids bioavailability

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Author	**Study**	**Polyphenol**	**Diet component**	**Effect**
Donovan *y col.*, 1999		Catechin	Alcohol	=
Yamashita *y col.*, 2002		Catechin	Tartaric acid	
Azuma *y col.*, 2002		Quercetin	Alcohol	
Visoli *y col.*, 2003	 	Hydroxityrosol	Fat	
Schramm *y col.*, 2003		Flavonols	Diet rich in carbohydrates	
Schramm *y col.*, 2003		Flavonols	Diet rich in lipids or proteins	=
Lesser *y col.*, 2004		Quercetin	Fat	
Lambert *y col.*, 2004		Epigallocatequin-3-gallate	Piperine	

Case: Bioavailability of flavonoids in cocoa

Why cocoa?

Flavonoid consumption

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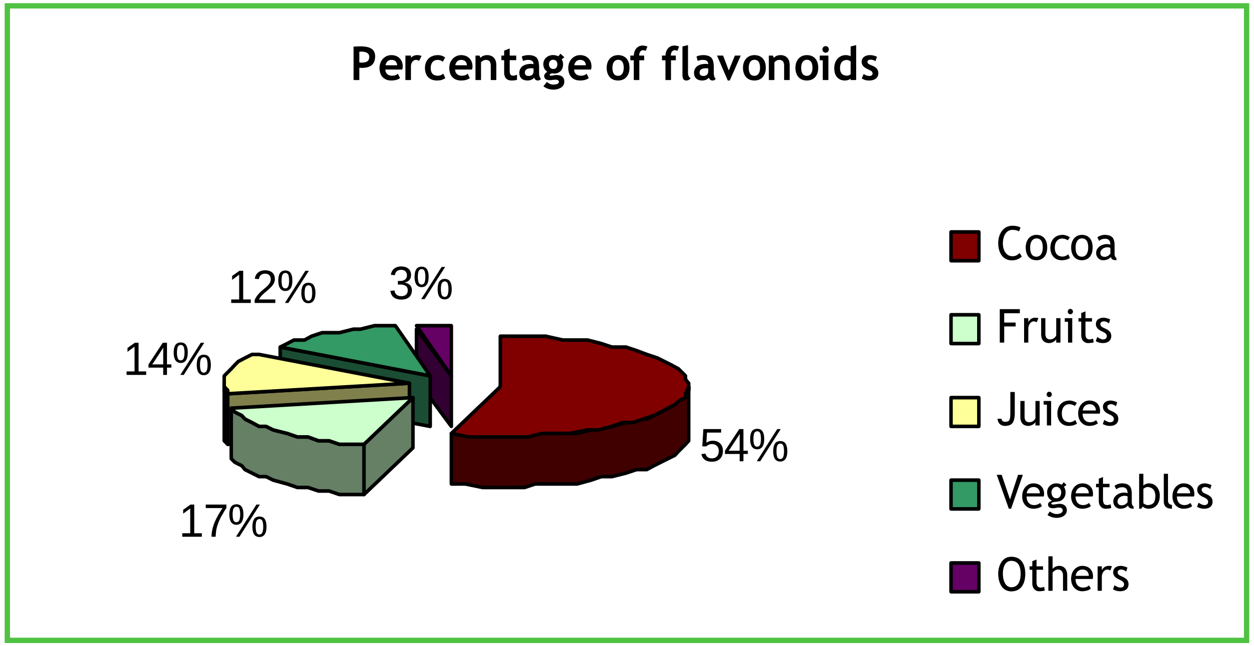

Children

24,2 mg/day

- Consumption of cocoa products g 33,82 g/day
- Flavonoid consumption g 24,2 mg/day

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

Zamora R, (2004) V Congreso Internacional de Barcelona sobre la dieta mediterránea

Controversy???

•*Serafini et al., Eur J. Clin. Nutr 1996*

YES decrease absorption

•*Van het Hof et al., Eur J. Clin. Nutr 1998*

NO decrease absorption

?

•*Serafini et al., Nature 2003*

YES decrease absorption

•*Shroeter et al., Nature 2003 and Schramm et al, 2003*

NO decrease absorption

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Does milk affect flavonoids bioavailability?

Study	Nº	Main conclusion
Serafini et al, 1996	10	When tea was consumed with milk their in vivo activity was totally inhibited
Van het Hof et al, 1998	12 (5 m and 7f)	Milk do not impair the bioavailability of tea catechins
Hollman et al, 2001	18	Addition of milk does not affect the absorption of flavonols from tea in man
Serafini et al, 2003	12 (5m and 7f)	Addition of milk, either during ingestion or in the manufacturing process, therefore inhibits the in vivo antioxidant activity on chocolate and the absorption into blood stream of epicatechina
Schroeter et al, 2003	12	The presence of milk in coca products does not counteract the absorption ans biological activity of monomeric flavanols from cocoa products.
Schramm et al, 2003	6 females	The intake of flavanol rich cocoa with milk had no significant effects on flavonol absorption

Cocoa flavonoid composition

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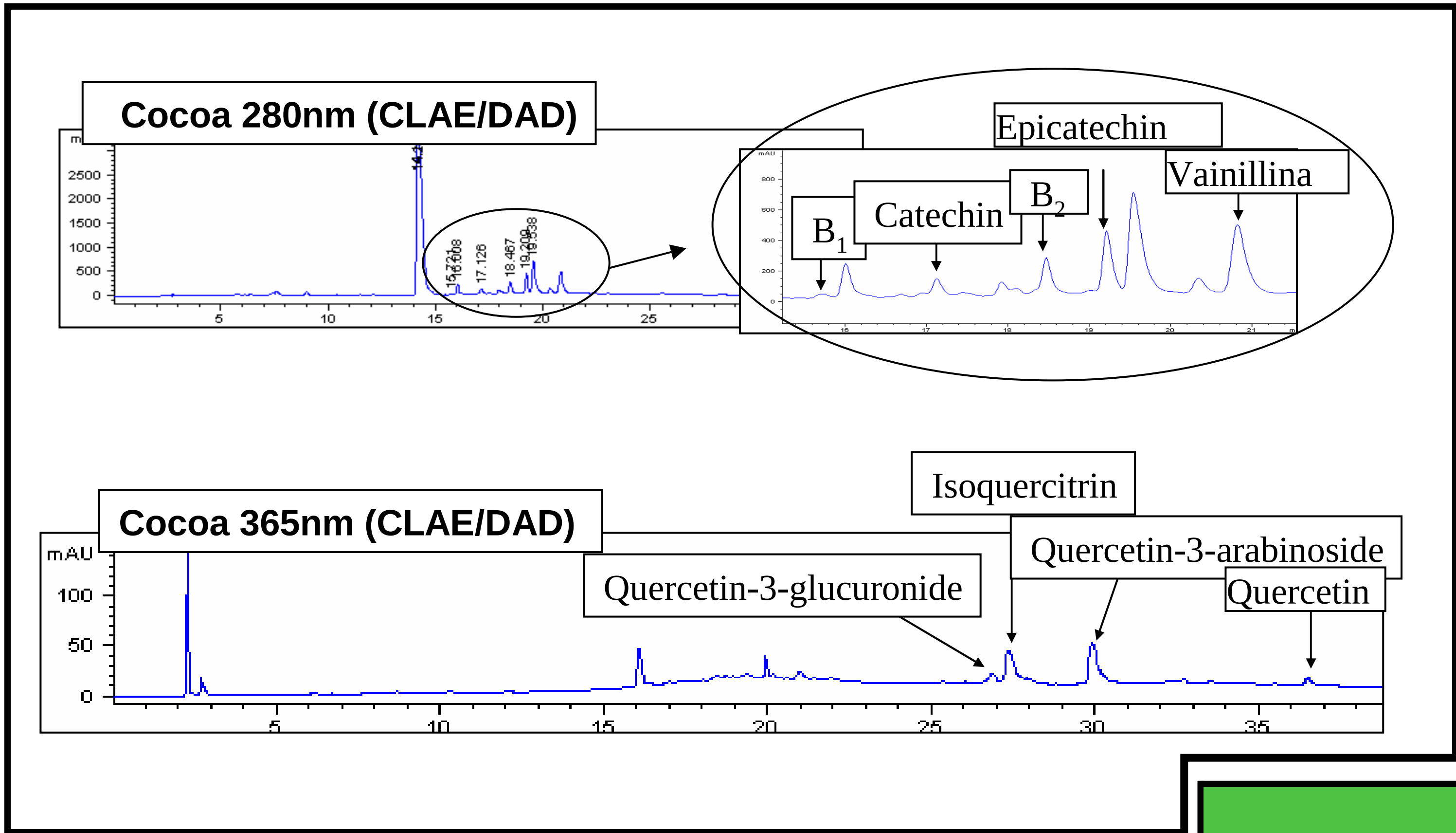

	mg/g
Epicatechin	1.41
Catechin	0.42
Procianid. B_2	1.29
Isoquercetin	0.03
Quercetin	0.01
Quer-arabinoside	0.05
Quer-glucuronide	0.01

Andrés-Lacueva et al LC·GC Europe December 2000

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STUDY DESIGN

prospective, randomized, opened, cross-over and controlled

Three different interventions one week among them

250 mL milk

31g

250 mL milk + 40g cacoa powder

250 mL water + 40g cocoa

40.6g

STUDY DESIGN

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Nutrient	Cocoa in milk	Cacoa in water
Carbohydrates (g/250mL)	30.75	58.4
Fat (g/250mL)	10.91	2.16
Proteins (g/250mL)	13.54	5.64
Energy (Kcal/250mL)	275.35	276.6

Interventions

Test	1st Intervention	2nd Intervention	3rd Intervention
Milk	Grup 1	Grupo 2	Grupo 3
Cocoa –milk	Grup 2	Grupo 3	Grupo 1
Cocoa-water	Grup 3	Grupo 1	Grupo 2

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STUDY DESIGN

Previous day →

- Free phenolics diet
- Without vitamin supplements
- 10-12h without any food

Study day →

- **0h**: Blood and urine samples
- Test food intake
- **2h**: Second blood extraction
- **4h**: Cheese Sandwich
- **6h**: Urines 6, 12 and 24 hours

PLASMA

At what time we perform the extraction?

- According to literature
- We perform a previous assay

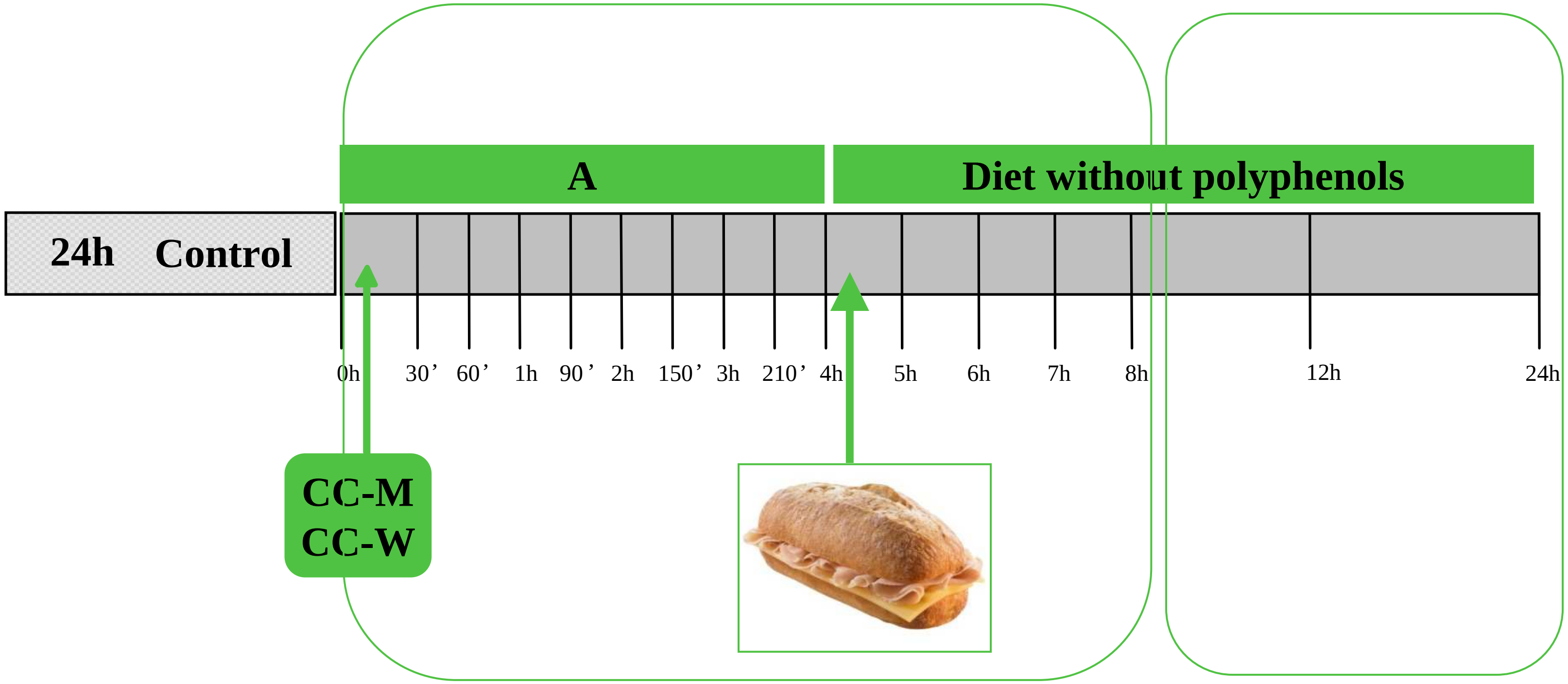
Plasma 2h or 6h?

Previous bioavaiability studies

Fuente	Ind	Dose (mg)	T (h)	C max (µmol/L)	Authors
Chocolate	8	82, 164 (-)-Ec	**2-2.6**	0.38, 0.7	Michelle *y col.*, 1999
Cocoa	5	220 (-)-Ec	**2**	4.92	Baba *y col.*, 2000
Chocolate	5	220 (-)-Ec	**2**	4.77	Baba *y col.*, 2000
Chocolate	20	46, 92, 138 (-)-Ec	**2**	0.13, 0.26, 0.36	Wang *y col.*, 2000
Chocolate	10	137 (-)-Ec	**2**	0.26	Rein *y col.*, 2000a
Cocoa drink	5	323mg CAT	**2**	5.9(-)-Ec + 0.16 CAT	Holt *y col.*, 2002a
Cocoa drink	5	256mg dímmer	**2**	0.041 B2	Holt *y col.*, 2002a
Cocoa	6	1.53 mg/kg	**2**	1-1.5	Schramm *y col.*, 2003

Plasma 2h or 6h?

Previous cinetic assay



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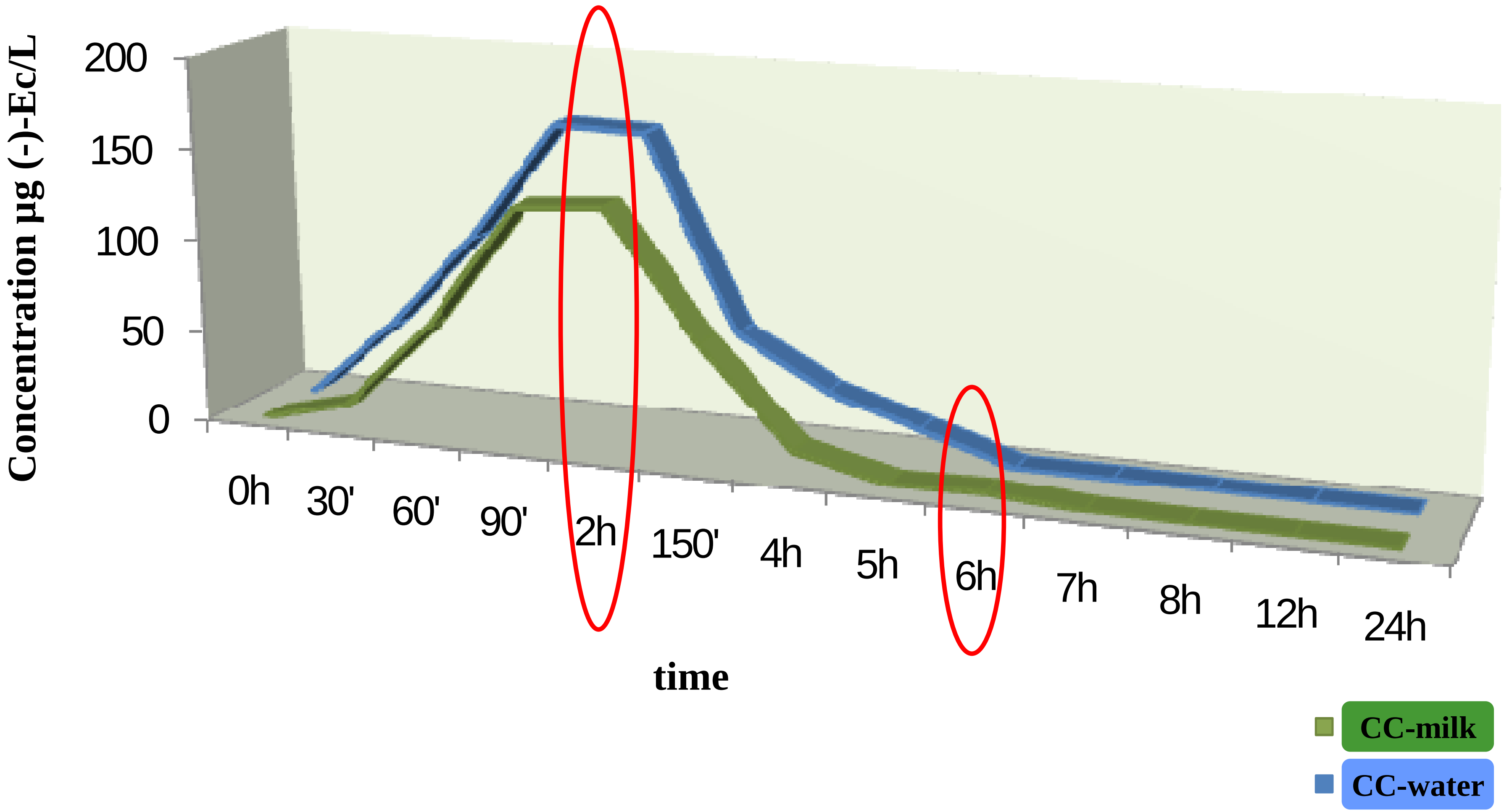
Plasma 2h or 6h?



STUDY DESIGN

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Restricted food	Restricted drinks	Food and drink allowed
Fruits, vegetables, marmalades, garlic , olives, olive oil, potatoes, nuts, almonds, peanuts, honey, legumes, soy and derivates, mushroom, whole-grain bread, cocoa, chocolate and any cakes and sweets that containing it. 	Tea, infusions, coffee, beer, wine, cava, cocoa beverages, cider, fruit and vegetable juice, colas, orangeade, lemonade and any soft drink fruit based. 	Milk and dairy products (cheeses, yoghurt, butter…), meat and derivative, poultry and derivatives, eggs, rice, pasta, white bread, fish, seafood, shellfish and cakes, cookies and sweets without chocolate or fruit. 

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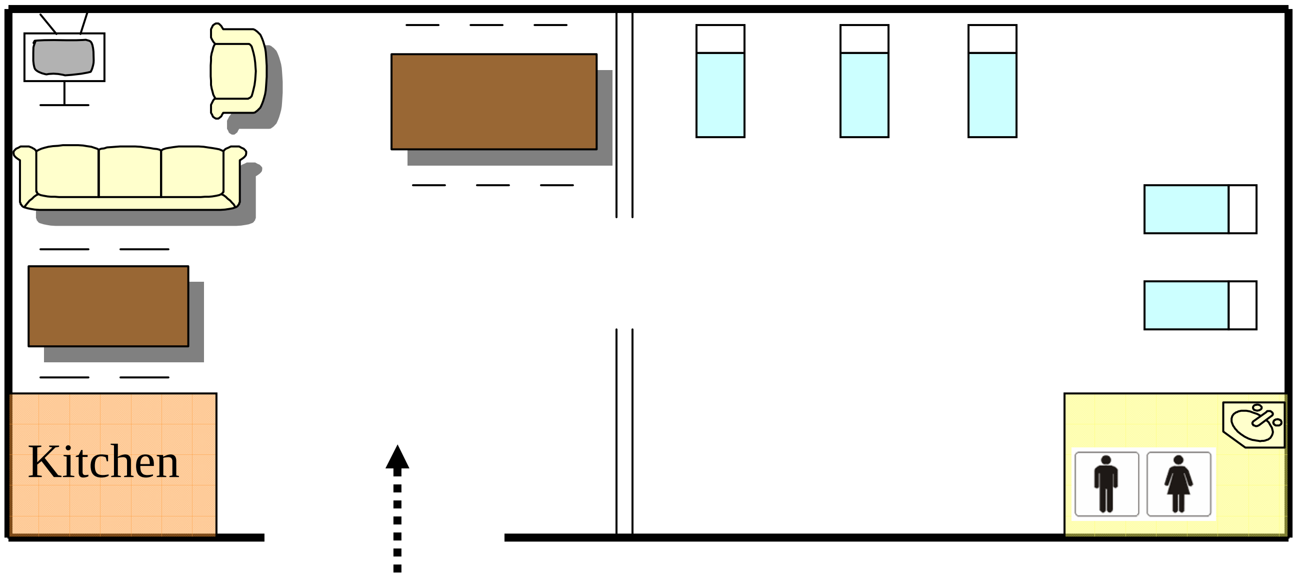
STUDY DESIGN

"Hospital Clínic" of Barcelona



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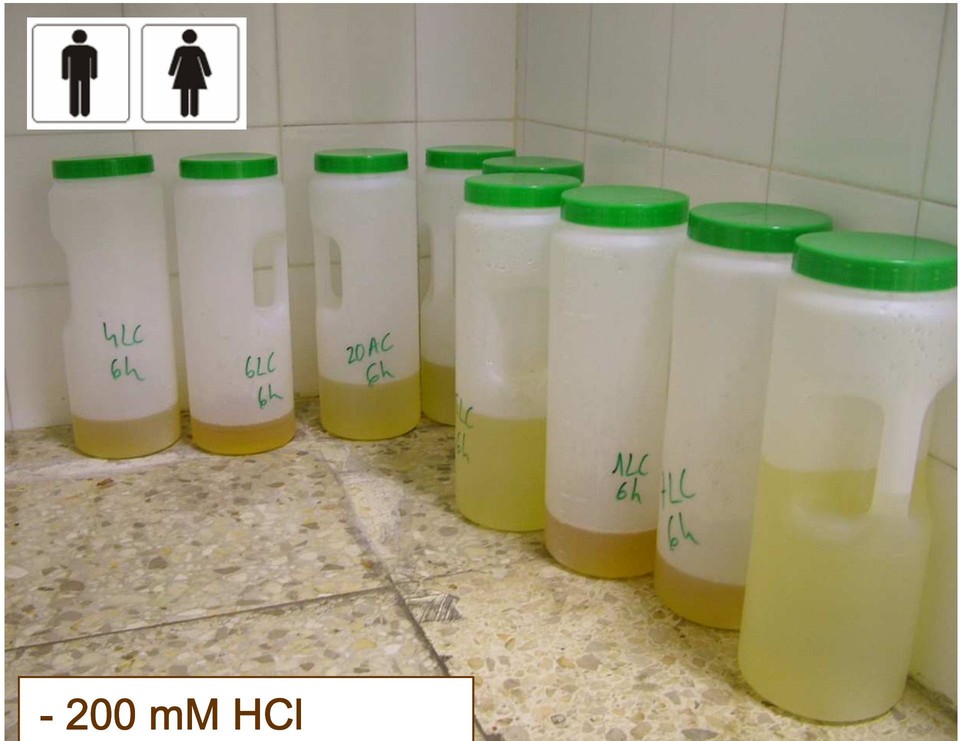
STUDY DESIGN



- 200 mM HCl
- Cong: - 80ºC

Urines of 0- 6,
6-12
12-24 hours

Plasma from 0 and 2 hours

- 80ºC

STUDY DESIGN

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Interventions

Intervention day	
Arriving at the hospital, determination of blood preasure	
1st blood extraction and collection of urine (before intake)	
Intervention	
0h	Test food intake
2h	2nd extraction for the determination of polyphenols
4h	Breakfast (white bread, butter and cheese)
6h	3rd extraction for determination polyphenols adhesion molecules
0-6h	Collection of all the urine during the 6h of intervention
6-24h	Collection of all the urine in 2 bottles of 1,5L after the stage at the hospital, one from 6 to 12h and the other from 12 to 24h.

PLASMA METHODOLOGY

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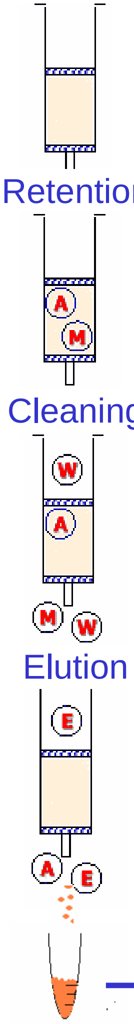

Activation

1 ml Methanol pure (HPLC)
1 ml 1.5M formic en water mQ

Retention

To load all the solution (A) — **Vortex 2 min** — (A) 1.5ml plasma, 555µl antioxidant solution, 30µl o-phosphoric

Cleaning

2 ml 1.5M formic in Water mQ
2 ml 5% methanol in water mQ

Elution

2 ml de Methanol 0.1% of formic

→ N_2 →

0.45µm →

Roura et al, JAFC 2005

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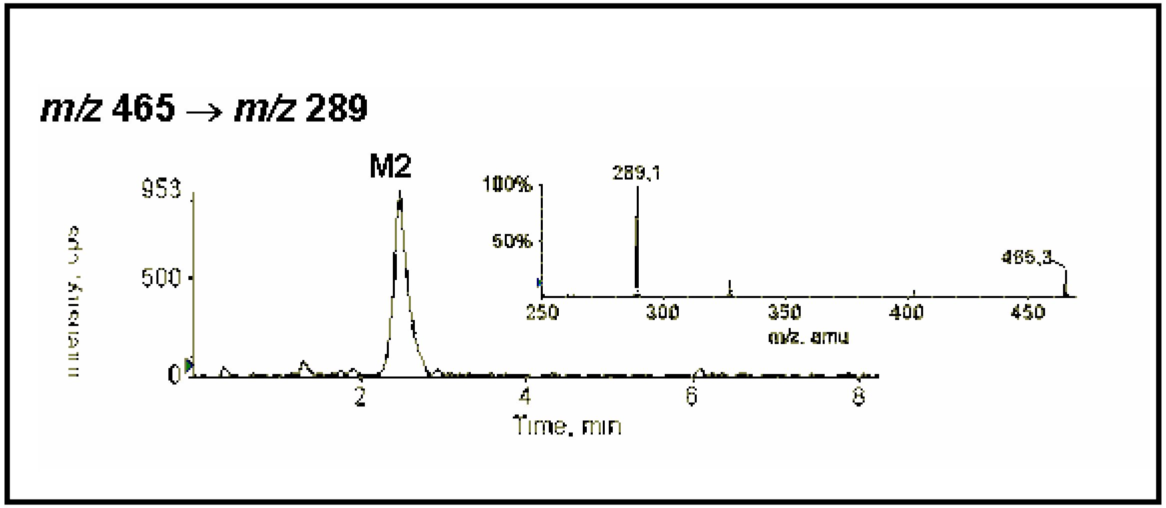
PLASMA METHODOLOGY

Metabolites by HPLC-MS/MS

Metabolits	**Mw**	***Trace***
(-)- Epicatechin ($C_{15}H_{14}O_6$)	290	m/z 289 → m/z 245
O-methyl epicatechin	304	m/z 303 → m/z 289
(-)-Epicatechin-*O*-*β*-D-glucuronide	466	m/z 465 → m/z 289
O-methyl epicatechin glucuronide	480	m/z 479 → m/z 289
Sulfated epicatechin	370	m/z 369 → m/z 289
Sulfated-*O*-methyl epicatechin	384	m/z 383 → m/z 289
Sulfate epicatechin-*O*-β-D-glucuronide	546	m/z 545 → m/z 289
Sulfate-*O*-methyl epicatechin glucoronide	560	m/z 559 → m/z 289

PLASMA RESULTS



Epicatechin-glucuronide

↓

the only metbolite present in plasma 2h after cocoa beverage intake

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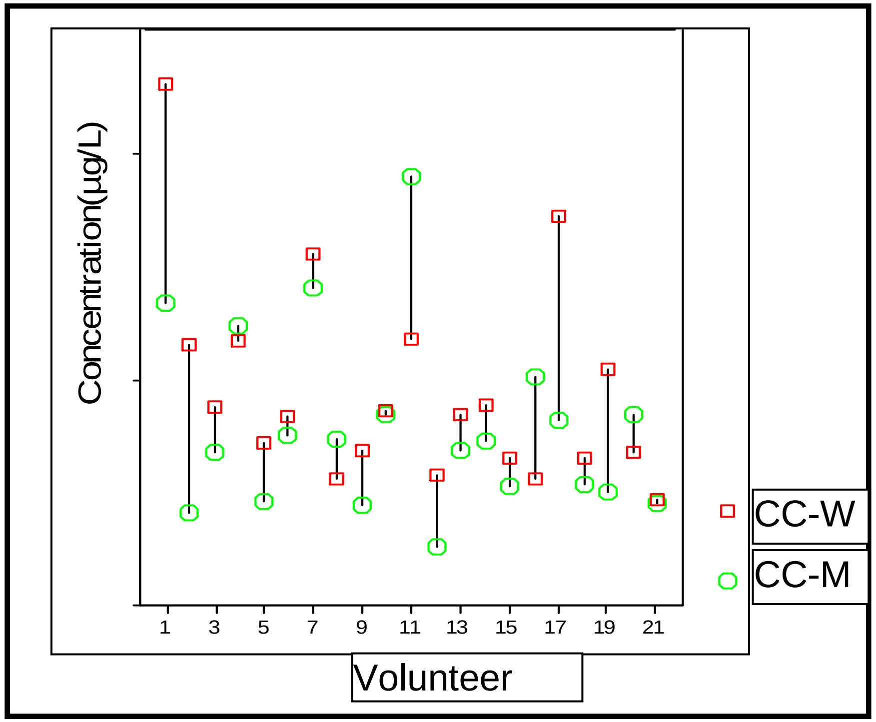
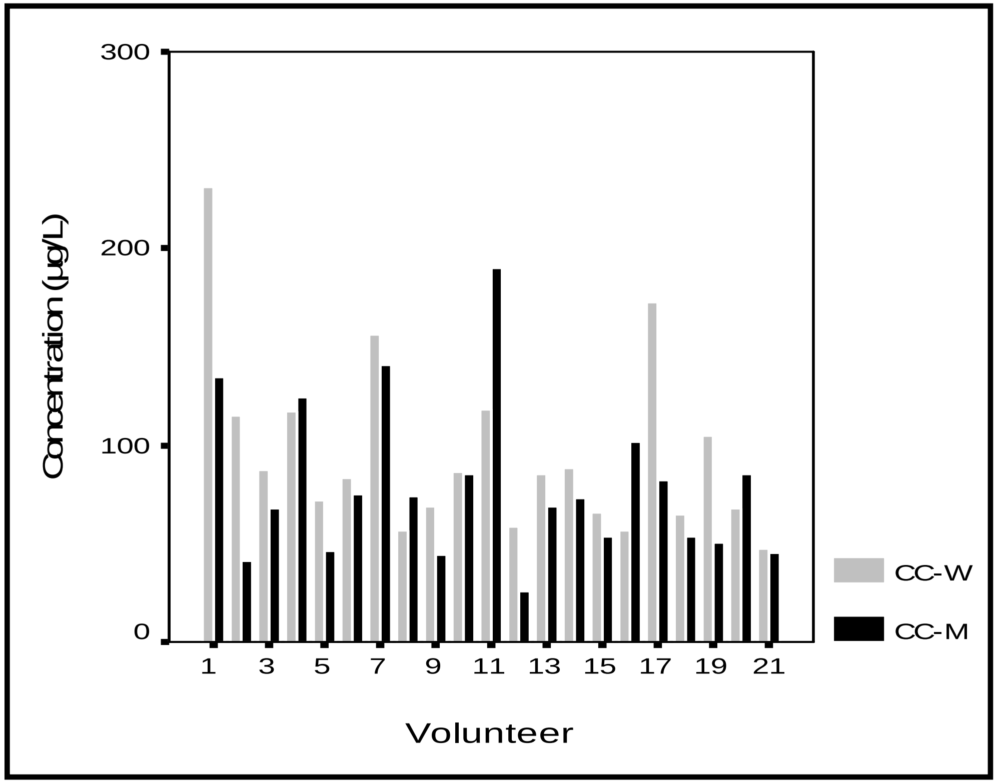
PLASMA RESULTS





(-)-EcG concentration (µg/L) in each volunteer 2h after the intake of the two cocoa beverages, milk based cocoa beverage (CC-M) and water based cocoa beverage (CC-W)

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PLASMA RESULTS

Treatmen	Min concentration (µg/L)	Max concent. (µg/L)	Average (SD)
CC-M	41,06	190,20	79,1µg.l-1 ± 40
CC-W	47,04	231,53	95,5µg.l-1 ± 45.1

**n=21*

CC-M: Cocoa beverage with whole milk

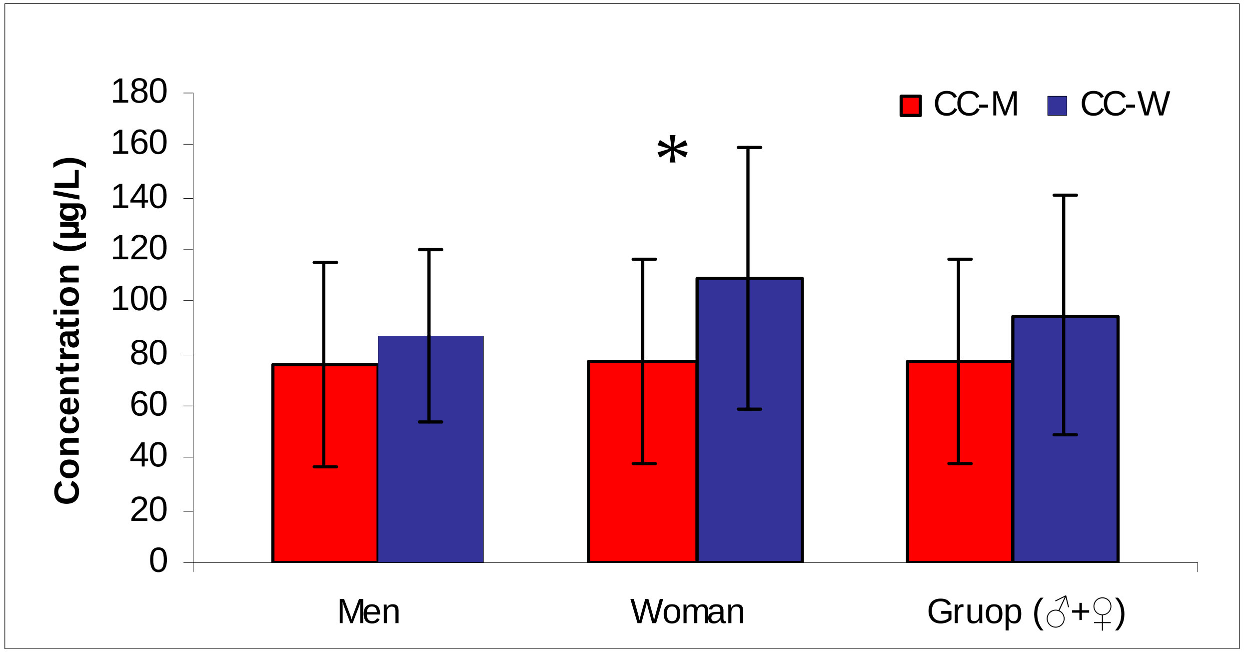
CC-W: Cocoa beverage with water

Minimum, maximum and mean (SD) (-)-EcG concentration found in each intervention group (CC-M and CC-W).

	CC-M (µg/L) mean (SD)	**CC-W** (µg/L) mean (SD)	***P*** *(T student)*
♀	78.02 (39.2)	108.9 (58.7)	*<0.05
♂	76.10 (41.7)	84.64 (34.7)	>0.05

Mean concentration (µg/L) (SD) of (-)-EcG 2 hours after the cocoa beverages intake by sexes and significance results of the *t students* test (♂: males, ♀: females).

PLASMA RESULTS



Representation of the mean (-)-EcG concentration and standard deviation for each intervention in the men group, the woman group and in all the participants.

* Significant difference ($P<0.05$) between cocoa beverages (with milk or water) (CC-M/CC-W).

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PLASMA RESULTS

P<0.05

P>0.05

P<0.05

P>0.05

CC-W	CC-M
86,67	84,85
231,53	134,42
115,54	41,06
87,81	67,98
117,53	124,05
118,01	190,20
58,13	25,77
84,98	68,65
88,50	72,60
65,75	53,20
72,26	46,43
56,13	101,59
172,97	82,33
65,04	53,80
104,50	50,20
83,47	75,09
67,91	85,00
155,92	140,85
47,05	44,97
56,52	73,77
68,44	44,56

n=9

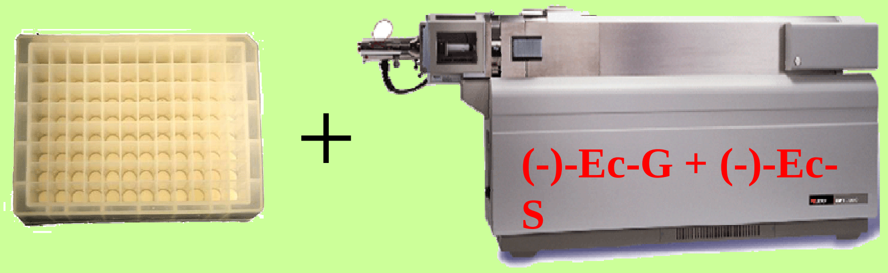
URINE METHODOLOGY

Samples from 0-6, 6-12 y 12-24h

Interventions: CC-water, CC-milk, control

n = 252

Metabolites: (-)-Ec-Glucuronide
(-)-Ec-Sulfate



Roura E y col. J Agric Food Chem. 2005; Aug 10; 53(16):6190-4.

URINE METHODOLOGY

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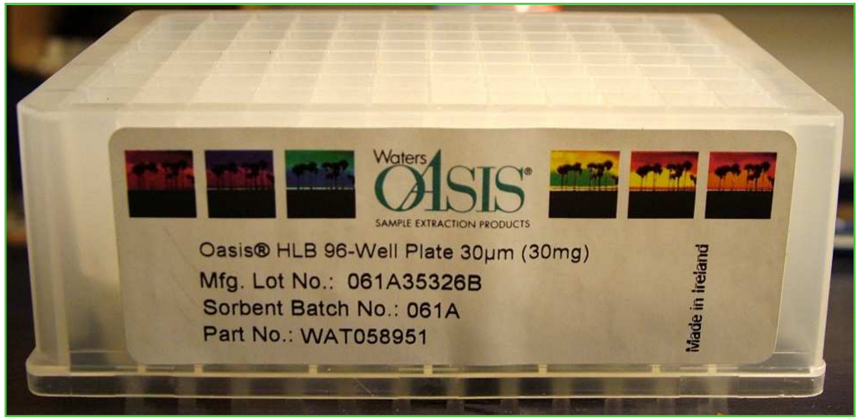
SPE in plates

n=12



n=96

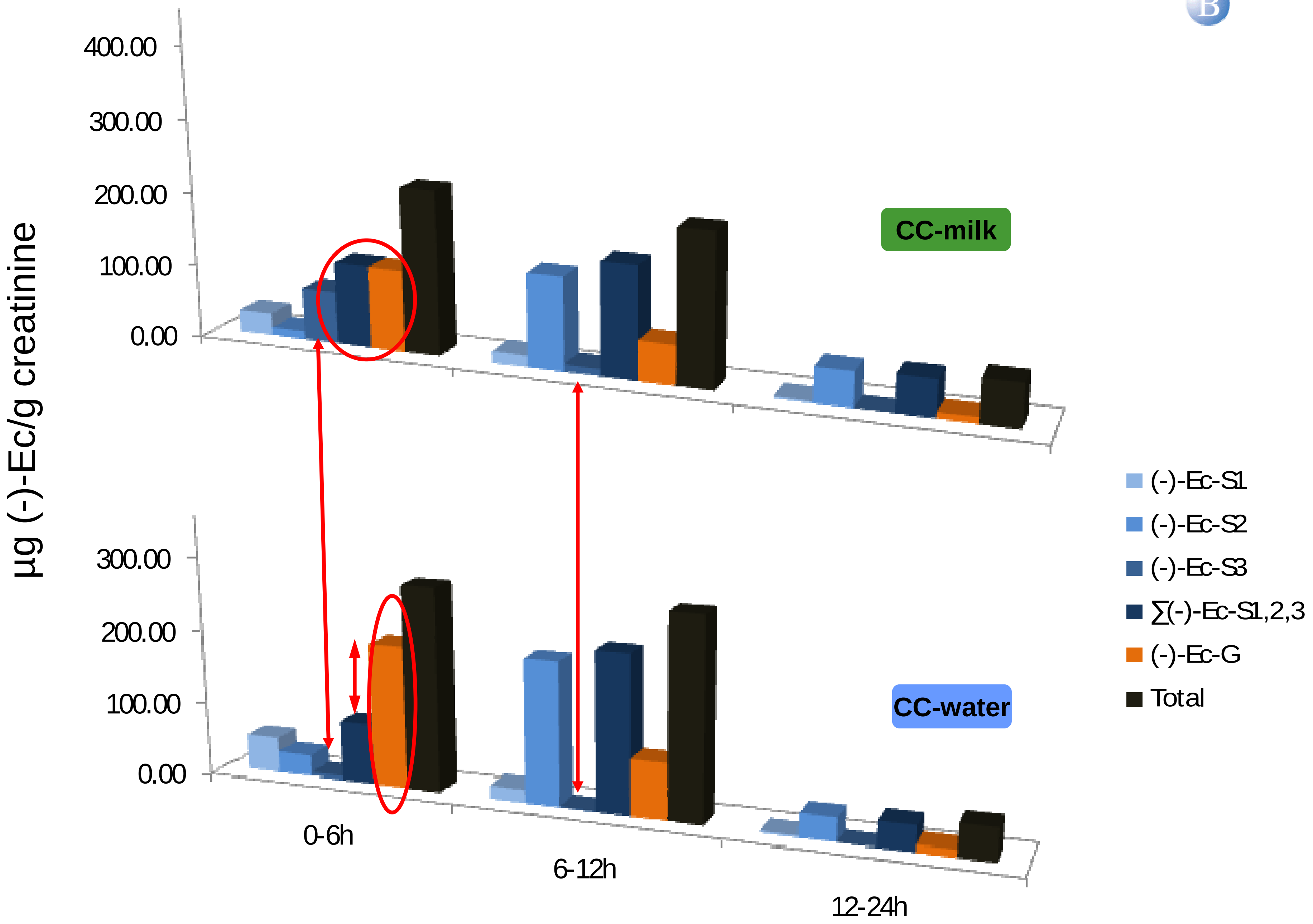
URINE RESULTS

Metabolites (µg (-)-Ec/g creatinine)

Drink	t *(h)*	Ec-S[1]	Ec-S[2]	Ec-S[3]	$\sum$Ec-S1, Ec-S2, Ec-S3	Ec-G	Total	(SD)
	0-6	30.86	10.61	72.45	113.92	112.79	226.7	(195.68)
CC-milk	***6-12***	15.74	128.47	9.86	154.07	58.9	207.41	(132.03)
	12-24	5.04	48.01	nd	50.65	9.25	58.92	(34.76)
	0-6	48.83	29.61	5.07	83.52	194.95	278.47	(252.47)
CC-water	***6-12***	18.57	195.29	1.30	215.16	64.20	272.60	(215.33)
	12-24	2.74	32.30	nd	37.10	9.41	44.45	(29.18)

P>0.05

URINE RESULTS



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CONCLUSIONS

Conclusions from plasma results

Milk did not affect flavonoid bioavailatility when the total number of subjects is considered.

- However differences are observed among genders.
- A high inter-subject variability, probably related to genetic polymorphism.
- A larger number of individuals and a crossover design must be used to account for the large inter-individual variation.

CONCLUSIONS

Conclusions from urine results

The consumption of cocoa soluble with milk does not reduce significantly the plasma concentration of (-)-Ec Glucuronide nor the total concentration of (-)-Ec metabolites excreted in urine.

There are differences in the profile of metabolites excreted in urine depending on whether cocoa soluble is taken with water or with milk. There are differences in the ratio sulfates/glucuronides formed and also in the position of sulfate.

When cocoa is consumed with milk, is favored the sulfatation route regarding glucuronidation in the first 6h excreted. Which different physiological effect may have is still unknown.

Roura E *y col. Br J Nutr. 2008 Feb 7;1-6*

Roura E *y col. Ann Nutr Metab.* 2007 Nov;51(6):493-8

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CONCLUSIONS

Bioavailability conclusions

We need more studies to arrive to any conclusion

- In humans with a high number of subjects.
- Each matrix is different and should be evaluated.
- Effect of microbiota has to be considered

Antioxidantes Naturales - Mozilla Firefox

Archivo Editar Ver Historial Marcadores Herramientas Ayuda

http://www.ub.es/depnutricioibromatologia/Grup Antioxidar

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Rosa Maria Lamuela Raventós, PhD

Titular professor of the Department of Nutrition and Food Science

Leader's group

Rosa has a pharmaceutical degree. Master of Nutrition and Food Technology, University of Barcelona Viticulture and Enology, UPC. She made her postdoc at UC Davis (California) with Andrew Waterhou Department of Viticulture and Enology, where she started to work with polyphenols and health. Wher back to University of Barcelona as an Assistant Professor, she created with Cristina Andrés the gro Antioxidants: Polyphenols

Email: lamuela@ub.edu

Telephone: +34 934034843

Fax: +34934035931

Barcelona city

Natural Antioxidants:
Polyphenols Research Group

Thank you!