THE ROLE OF FOOD METABOLOME IN BIOMARKER DISCOVERY: EVIDENCE FROM CLINICAL AND OBSERVATIONAL STUDIES

M Garcia-Aloy*, R Llorach, M Urpi-Sarda,
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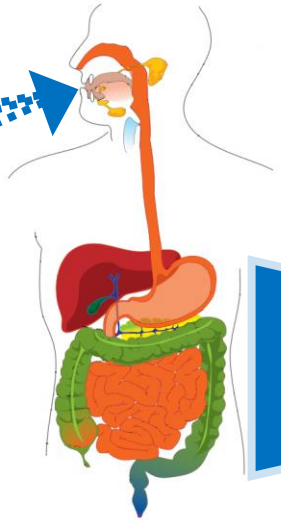
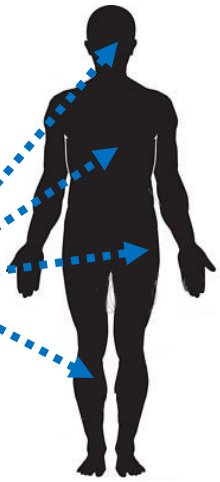
BIOMARKERS & NUTRIMETABOLOMICS

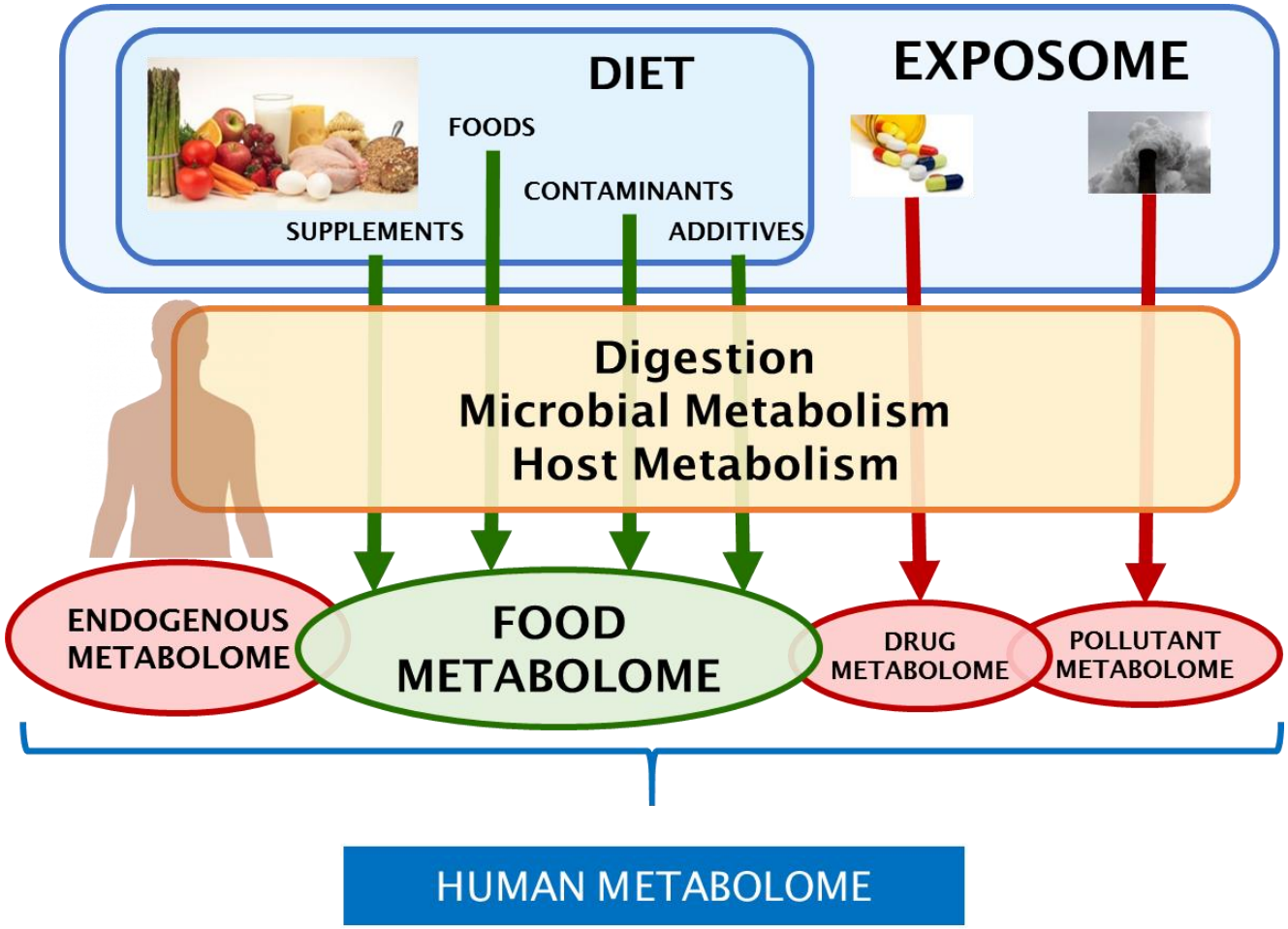
diet



health / disease



NUTRIMETABOLOMICS



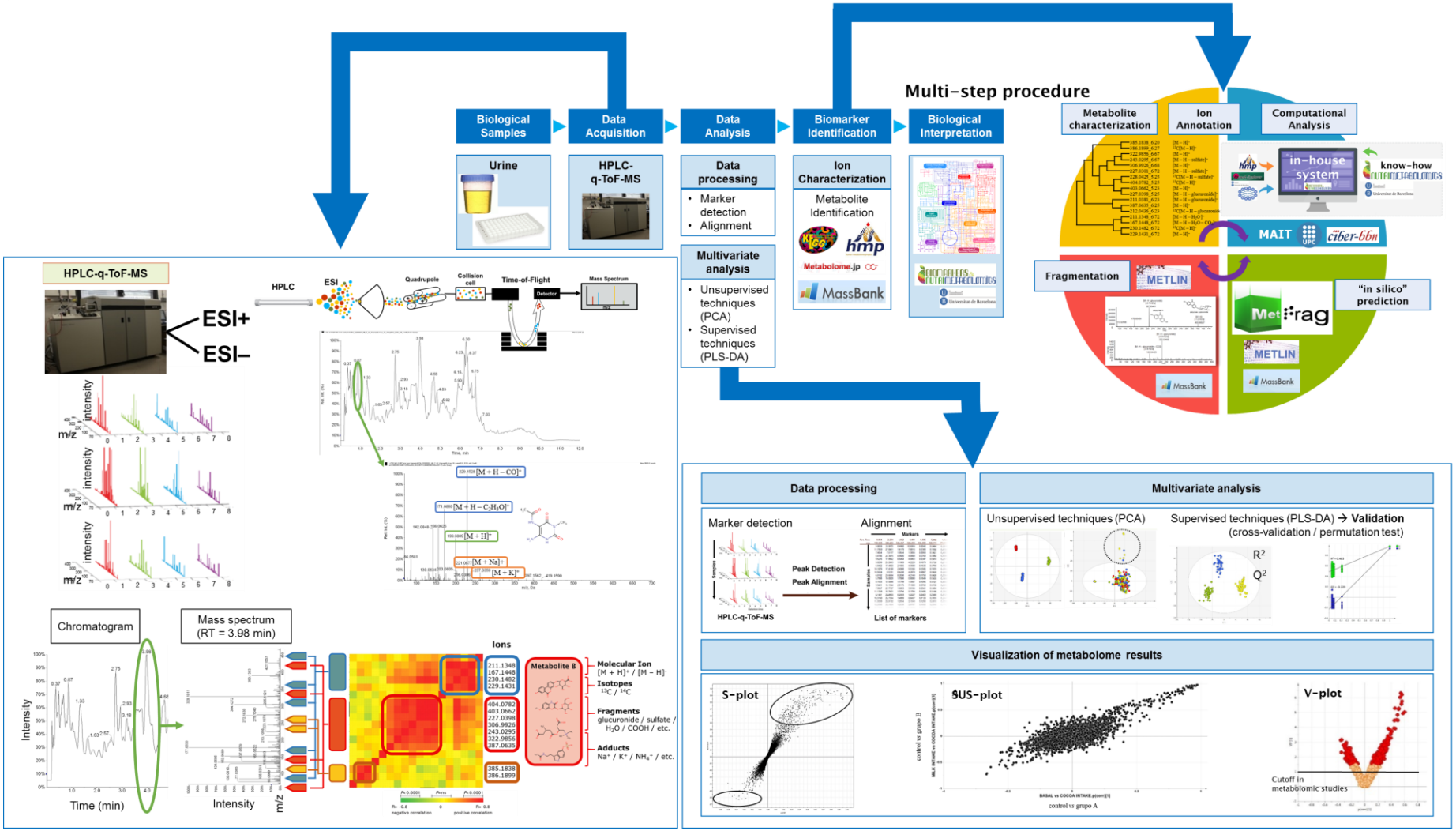
The American Journal of Clinical Nutrition
Official publication of the American Society for Nutrition

The food metabolome: a window over dietary exposure[1–3]

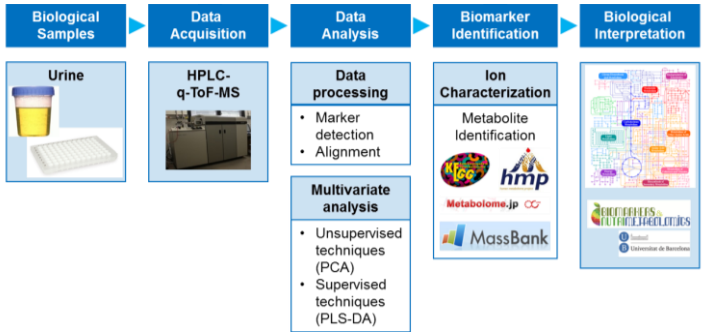
Augustin Scalbert, Lorraine Brennan, Claudine Manach, Cristina Andres-Lacueva, Lars O Dragsted, John Draper, Stephen M Rappaport, Justin JJ van der Hooft, and David S Wishart

The main aim of this work was to contribute to the identification of biomarkers related to food ingestion (biomarkers of intake), as well as their potential association with health (biomarkers of effect) through the application of an untargeted HPLC-q-ToF-MS metabolomic approach in nutritional studies with different designs.

HPLC-q-ToF-MS UNTARGETED METABOLOMIC ANALYSIS



HPLC-q-ToF-MS UNTARGETED METABOLOMIC ANALYSIS




Contents lists available at ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis
journal homepage: www.elsevier.com/locate/jpba

Methodological aspects for metabolome visualization and characterization
A metabolomic evaluation of the 24 h evolution of human urine after cocoa powder consumption

R. Llorach-Asunción[a], O. Jauregui[b], M. Urpi-Sarda[a], C. Andres-Lacueva[a,*]


analytical chemistry
Technical Note
pubs.acs.org/ac

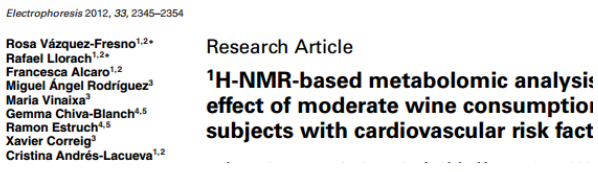
Peak Aggregation as an Innovative Strategy for Improving the Predictive Power of LC-MS Metabolomic Profiles

Francesc Fernández-Albert,[*,†,‡,§] Rafael Llorach,[*,‡] Cristina Andres-Lacueva,[‡] and Alexandre Perera-Lluna[†,§]


analytical chemistry
Article
pubs.acs.org/ac

Comparative Analysis of Sample Preparation Methods To Handle the Complexity of the Blood Fluid Metabolome: When Less Is More

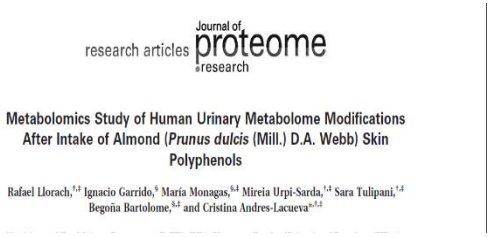
Sara Tulipani, Rafael Llorach, Mireia Urpi-Sarda, and Cristina Andres-Lacueva*


Electrophoresis 2012, *33*, 2345–2354

Rosa Vázquez-Fresno[1,2*]
Rafael Llorach[1,2*]
Francesca Alcaro[1,2]
Miguel Ángel Rodríguez[3]
María Vinaixa[3]
Gemma Chiva-Blanch[4,5]
Ramon Estruch[4,5]
Xavier Correig[3]
Cristina Andrés-Lacueva[1,2]

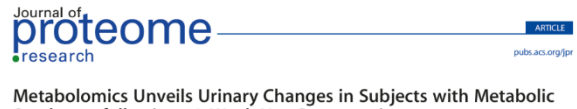
Research Article

[1]H-NMR-based metabolomic analysis effect of moderate wine consumption subjects with cardiovascular risk fact


research articles Journal of proteome research

Metabolomics Study of Human Urinary Metabolome Modifications After Intake of Almond (*Prunus dulcis* (Mill.) D.A. Webb) Skin Polyphenols

Rafael Llorach,[†,‡] Ignacio Garrido,[§] María Monagas,[§,‡] Mireia Urpi-Sarda,[†,‡] Sara Tulipani,[†,‡] Begoña Bartolome,[§,‡] and Cristina Andres-Lacueva*[,†,‡]


Journal of proteome research
ARTICLE
pubs.acs.org/jpr

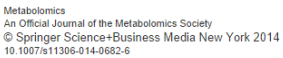
Metabolomics Unveils Urinary Changes in Subjects with Metabolic Syndrome following 12-Week Nut Consumption

Sara Tulipani,[†,‡] Rafael Llorach,[†,‡] Olga Jáuregui,[‡,§] Patricia López-Uriarte,[∥,⊥] Mar Garcia-Aloy,[†,‡] Mònica Bullo,[∥,⊥] Jordi Salas-Salvadó,[∥,⊥] and Cristina Andrés-Lacueva*[,†,‡]


research articles Journal of proteome research

An LC-MS-Based Metabolomics Approach for Exploring Urinary Metabolome Modifications after Cocoa Consumption

Rafael Llorach,[†,⊥] Mireia Urpi-Sarda,[†,⊥] Olga Jauregui,[‡,⊥] Maria Monagas,[§] and Cristina Andres-Lacueva*[,†,⊥]


Metabolomics
An Official Journal of the Metabolomics Society
© Springer Science+Business Media New York 2014
10.1007/s11306-014-0682-6

Original Article

Nutrimetabolomics fingerprinting to identify biomarkers of bread exposure in a free-living population from the PREDIMED study cohort


JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
Review
pubs.acs.org/JAFC

Nutrimetabolomic Strategies To Develop New Biomarkers of Intake and Health Effects

Rafael Llorach,[⊥] Mar Garcia-Aloy,[⊥] Sara Tulipani, Rosa Vazquez-Fresno, and Cristina Andres-Lacueva*

Nutrition and Food Science Department, XaRTA, INSA, Pharmacy Faculty, University of Barcelona, Avinguda Joan XXIII s/n, 08028 Barcelona, Spain, and INGENIO–CONSOLIDER Program, Fun-C-Food CSD2007-063, Ministry of Science and Innovation, Spain

NUTS & WALNUTS

Nutritional Intervention

Journal of proteome research
ARTICLE
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Metabolomics Unveils Urinary Changes in Subjects with Metabolic Syndrome following 12-Week Nut Consumption

Sara Tulipani,[†,‡] Rafael Llorach,[†,‡] Olga Jáuregui,[‡,§] Patricia López-Uriarte,[∥,⊥] Mar Garcia-Aloy,[†,‡] Mònica Bulló,[∥,⊥] Jordi Salas-Salvadó,[∥,○] and Cristina Andrés-Lacueva*[,†,‡]

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
Article
pubs.acs.org/JAFC

Urolithins Are the Main Urinary Microbial-Derived Phenolic Metabolites Discriminating a Moderate Consumption of Nuts in Free-Living Subjects with Diagnosed Metabolic Syndrome

Sara Tulipani,[†,§] Mireia Urpi-Sarda,[#,⊗] Rocío García-Villalba,[§,⊥] Montserrat Rabassa,[†,§] Patricia López-Uriarte,[△,⊗] Mònica Bulló,[△,⊗] Olga Jáuregui,[†,§] Francisco Tomás-Barberán,[§,⊥] Jordi Salas-Salvadó,[△,⊗] Juan Carlos Espín,[§,⊥] and Cristina Andrés-Lacueva*[,†,§]

Consumption Stratification

Journal of proteome research
Article
pubs.acs.org/jpr

Novel Multimetabolite Prediction of Walnut Consumption by a Urinary Biomarker Model in a Free-Living Population: the PREDIMED Study

Mar Garcia-Aloy,[†,‡] Rafael Llorach,[*,†,‡] Mireia Urpi-Sarda,[†,‡] Sara Tulipani,[†,‡,§] Ramon Estruch,[∥,⊥] Miguel A. Martínez-González,[#,⊥] Dolores Corella,[¶,⊥] Montserrat Fitó,[□,⊥] Emilio Ros,[△,⊥] Jordi Salas-Salvadó,[○,⊥] and Cristina Andres-Lacueva*[,†,‡]

Metabolomic analysis

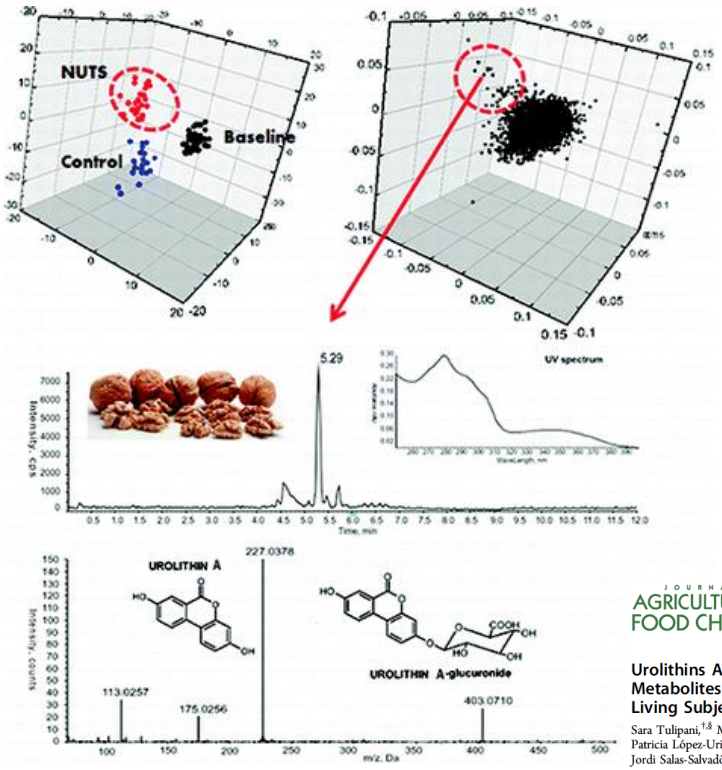
NUTS & WALNUTS

INTERVENTION STUDY

Journal of proteome research ARTICLE pubs.acs.org/jpr

Metabolomics Unveils Urinary Changes in Subjects with Metabolic Syndrome following 12-Week Nut Consumption

Sara Tulipani,[†,‡] Rafael Llorach,[†,‡] Olga Jáuregui,[†,§] Patricia López-Uriarte,[∥,⊥] Mar Garcia-Aloy,[†,‡] Mònica Bullo,[∥,⊥] Jordi Salas-Salvadó,[∥,⊥] and Cristina Andrés-Lacueva*[,†,‡]



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY Article pubs.acs.org/JAFC

Urolithins Are the Main Urinary Microbial-Derived Phenolic Metabolites Discriminating a Moderate Consumption of Nuts in Free-Living Subjects with Diagnosed Metabolic Syndrome

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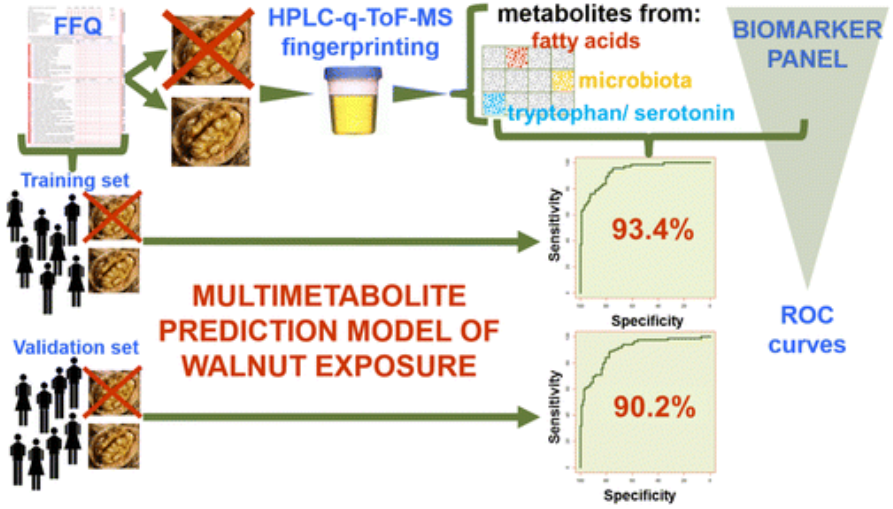
OBSERVATIONAL STUDY

Journal of proteome research Article pubs.acs.org/jpr

Novel Multimetabolite Prediction of Walnut Consumption by a Urinary Biomarker Model in a Free-Living Population: the PREDIMED Study

Mar Garcia-Aloy,[†,⁂] Rafael Llorach,[*,†,⁂] Mireia Urpi-Sarda,[†,⁂] Sara Tulipani,[†,⁂,§] Ramon Estruch,[∥,⊥] Miguel A. Martínez-González,[#,⊥] Dolores Corella,[▼,⊥] Montserrat Fitó,[□,⊥] Emilio Ros,[△,⊥] Jordi Salas-Salvadó,[◇,⊥] and Cristina Andres-Lacueva*[,†,‡]



BIOMARKERS & NUTRIMETABOLOMICS

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NUTS & WALNUTS

INTERVENTION STUDY

RANDOMIZATION

INTERVENTION PERIOD

(12 weeks)

W0 W12

n = 20

CONTROL GROUP

n = 22

NUTS GROUP

OBSERVATIONAL STUDY

AGL2009-13906-C02-01

PREDIMED COHORT (n=7447)

Subsample 1 (n=275) [cross-sectional analysis]

Subsample 2 (n=327) [cross-sectional analysis]

WALNUT consumption stratification

Excluded (n=80)

Excluded (n=141)

Non-consumers (n=128)

Habitual consumers (n=67)

Non-consumers (n=104)

Habitual consumers (n=82)

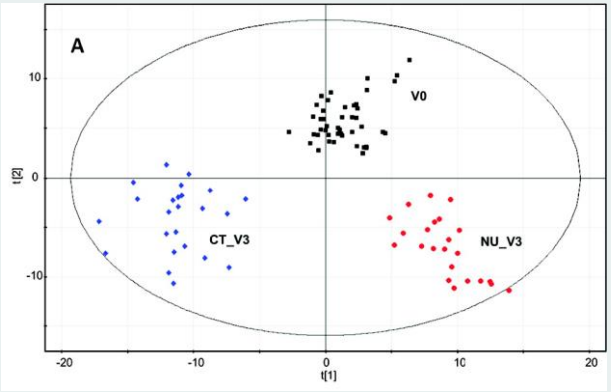
NUTS & WALNUTS

INTERVENTION STUDY

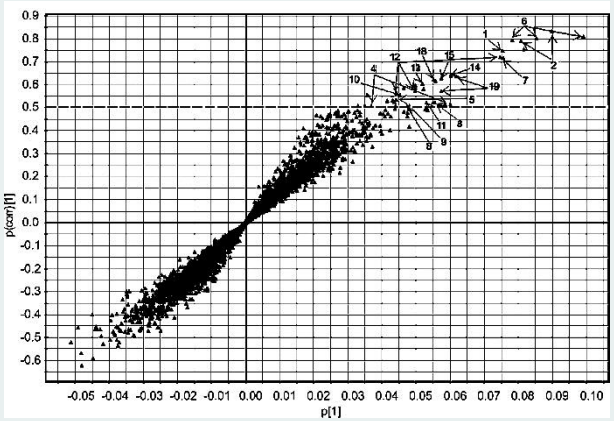
OBSERVATIONAL STUDY

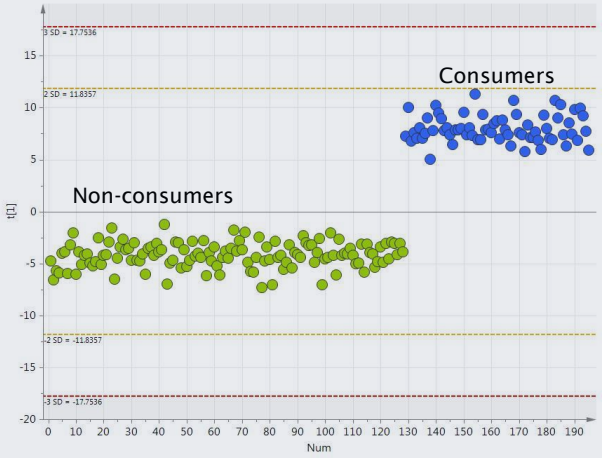
PREDIMED Prevención con Dieta Mediterránea

Multivariate analysis: OSC-PLS-DA

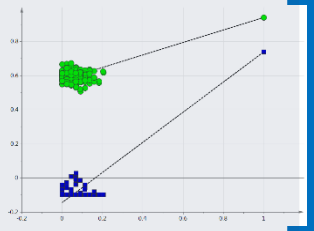


S-plot

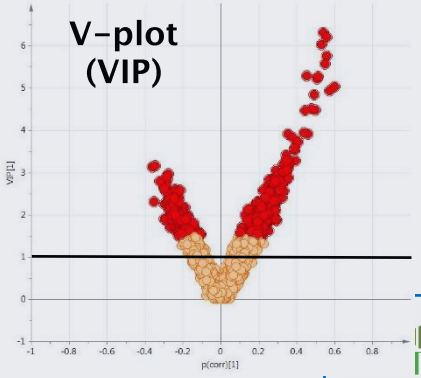




Permutation test



V-plot (VIP)



MARKERS & TRIMETABOLOMICS

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NUTS & WALNUTS

INTERVENTION STUDY

RT (min)	DETECTED MASS (m/z)	ASSIGNATION	IDENTIFICATION
4.80	257.0085	$[M - H]^-$	10-Hydroxy-decene-4,6-diynoic acid sulfate
	177.0545	$[M - H - \text{sulfate}]^-$	
6.25	385.1844	$[M - H]^-$	Tridecadienoic/tridecynoic acid glucuronide
	386.1880	$^{13}C[M - H]^-$	
	387.2011	$[M + H]^+$	
	211.1688	$[M + H - GlcA]^+$	
	193.1576	$[M + H - GlcA - H_2O]^+$	
6.72	229.1403	$[M - H]^-$	Dodecanedioic acid
	230.1441	$^{13}C[M - H]^-$	
	211.1314	$[M - H - H_2O]^-$	
	167.1433	$[M - H - H_2O - CO_2]^-$	
2.55	204.9827	$[M - H]^-$	Pyrogallol sulfate
	233.0118	$[HSO_3 - H]^-$	
5.10	325.0890	$[M - H]^-$	*p*-Coumaryl alcohol glucuronide
	326.0987	$^{13}C[M - H]^-$	
5.28	403.0627	$[M - H]^-$	Urolithin A glucuronide
	404.0654	$^{13}C[M - H]^-$	
	227.0357	$[M - H - GlcA]^-$	
	405.0817	$[M + H]^+$	
	229.0495	$[M + H - GlcA]^+$	
5.30	483.0195	$[M - H]^-$	Urolithin A sulfoglucuronide
6.55	229.0197	$[M - H]^-$	*p*-Coumaryl alcohol sulfate
	230.0221	$^{13}C[M - H]^-$	
	149.0615	$[M - H - \text{sulfate}]^-$	
	150.0646	$^{13}C[M - H - \text{sulfate}]^-$	
6.75	306.9885	$[M - H]^-$	Urolithin A sulfate
4.30	297.0560	$[M - H]^-$	*N*-Acetylserotonin sulfate
4.62	190.0505	$[M - H]^-$	Hydroxyindoleacetic acid
	146.0614	$[M - H - CO_2]^-$	
	192.0648	$[M + H]^+$	
	174.0539	$[M + H - H_2O]^+$	
	146.0592	$[M + H - CH_2O_2]^+$	

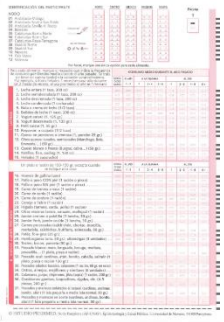
OBSERVATIONAL STUDY

RT (min)	DETECTED MASS (m/z)	ASSIGNATION	IDENTIFICATION
4.62	257.0149	$[M - H]^-$	10-Hydroxy-decene-4,6-diynoic acid sulfate
6.20	385.1838	$[M - H]^-$	Tridecadienoic/tridecynoic acid glucuronide
	386.1899	$^{13}C[M - H]^-$	
	387.1995	$[M + H]^+$	
	388.2035	$^{13}C[M + H]^+$	
	211.1668	$[M + H - GlcA]^+$	
5.22	419.0618	$[M - H]^-$	Urolithin C glucuronide
5.25	403.0662	$[M - H]^-$	Urolithin A glucuronide
	404.0677	$^{13}C[M - H]^-$	
	227.0398	$[M - H - GlcA]^-$	
	228.0425	$^{13}C[M - H - GlcA]^-$	
	405.0830	$[M + H]^+$	
	422.1100	$[M + NH_4]^+$	
	229.0490	$[M + H - GlcA]^+$	
5.35	483.0227	$[M - H]^-$	Urolithin A sulfoglucuronide
6.25	387.0770	$[M - H]^-$	Urolithin B glucuronide
	211.0381	$[M - H - GlcA]^-$	
	212.0436	$^{13}C[M - H - GlcA]^-$	
	389.0864	$[M + H]^+$	
	213.0534	$[M + H - GlcA]^+$	
6.34	473.1491	$[M - H]^-$	Enterolactone glucuronide
	474.1525	$^{13}C[M - H]^-$	
	297.1127	$[M - H - GlcA]^-$	
	492.1842	$[M + NH_4]^+$	
6.67	243.0295	$[M - H - \text{sulfate}]^-$	Urolithin C sulfate
6.72	306.9915	$[M - H]^-$	Urolithin A sulfate
	227.0348	$[M - H - \text{sulfate}]^-$	
3.23	336.0751	$[M - H]^-$	3-Indolecarboxylic acid glucuronide
	338.0854	$[M + H]^+$	
3.83	270.0081	$[M - H]^-$	Hydroxyindoleacetic acid sulfate
4.20	297.0561	$[M - H]^-$	*N*-Acetylserotonin sulfate

COCOA

DIETARY INTERVENTION

OBSERVATIONAL EPIDEMIOLOGY



Universitat de Barcelona

BIOMARKF

Acute Intervention Study

research articles Journal of proteome research

An LC-MS-Based Metabolomics Approach for Exploring Urinary Metabolome Modifications after Cocoa Consumption

Rafael Llorach,[†,‡] Mireia Urpi-Sarda,[†,‡] Olga Jauregui,[†,‡] Maria Monagas,[§] and Cristina Andres-Lacueva*[,†,‡]

Department of Nutrition and Food Science, XaRTA-INSA, Pharmacy Faculty, University of Barcelona, Barcelona, Spain, Scientific and Technical Services, University of Barcelona, Barcelona, Spain, Instituto de Fermentaciones Industriales (CSIC), Madrid, Spain, and INGENIO−CONSOLIDER program Fun-C-Food, CSD2007-063, Barcelona, Spain

Journal of Proteome Research *2009, 8, 5060–5068*

Chronic Intervention Study

962 DOI 10.1002/mnfr.201200736 *Mol. Nutr. Food Res.* 2013, *57*, 962–973

RESEARCH ARTICLE

Metabolomic fingerprint in patients at high risk of cardiovascular disease by cocoa intervention

Rafael Llorach[1,2], Mireia Urpi-Sarda[1], Sara Tulipani[1,2,3], Mar Garcia-Aloy[1,2], Maria Monagas[2,4] and Cristina Andres-Lacueva[1,2]

[1] Biomarkers and Nutritional & Food Metabolomics Research Group, Department of Nutrition and Food Science, XaRTA, INSA, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain
[2] INGENIO-CONSOLIDER Program, Fun-C-Food CSD2007-063, Ministry of Science and Innovation, Spain
[3] Research Laboratory, IMABIS Foundation, Virgen de la Victoria Clinical Hospital, Malaga, Spain
[4] Instituto de Investigación en Ciencias de la Alimentación (CIAL), CSIC-UAM. C/ Nicolás Cabrera 9, Campus de Cantoblanco, Madrid, Spain

Observational Study

DOI 10.1002/mnfr.201400434 *Mol. Nutr. Food Res.* 2015, *59*, 212–220

RESEARCH ARTICLE

A metabolomics-driven approach to predict cocoa product consumption by designing a multimetabolite biomarker model in free-living subjects from the PREDIMED study

Mar Garcia-Aloy[1,2], Rafael Llorach[1,2], Mireia Urpi-Sarda[1,2], Olga Jáuregui[2,3], Dolores Corella[4,5], Miguel Ruiz-Canela[5,6], Jordi Salas-Salvadó[5,7], Montserrat Fitó[5,8], Emilio Ros[5,9], Ramon Estruch[5,10] and Cristina Andres-Lacueva[1,2]*

[1] Biomarkers & Nutrimetabolomic Lab, Nutrition and Food Science Department, XaRTA, INSA, Campus Torribera, Pharmacy Faculty, University of Barcelona, Spain**

Prediméd Prevención con Dieta Mediterránea

Metabolomic analysis

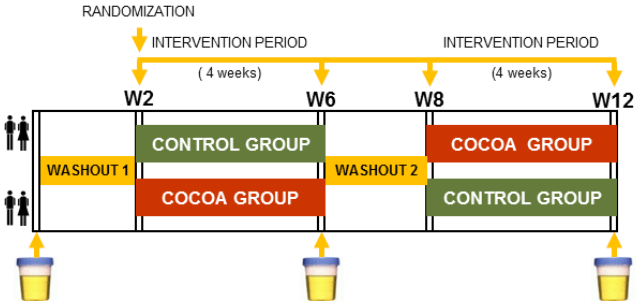
BIOMARKERS & NUTRIMETABOLOMICS — UNIVERSITAT DE BARCELONA

www.nutrimetabolomics.com

COCOA

ACUTE INTERVENTION

CHRONIC INTERVENTION



OBSERVATIONAL STUDY

Consumption data

Data Acquisition → **Data Analysis** → **Biomarker Identification** → **Biological Interpretation**

HPLC-q-ToF-MS

Data processing

- Marker detection
- Alignment

Multivariate analysis

- Unsupervised techniques (PCA)
- Supervised techniques (PLS-DA)

Ion Characterization

Metabolite Identification

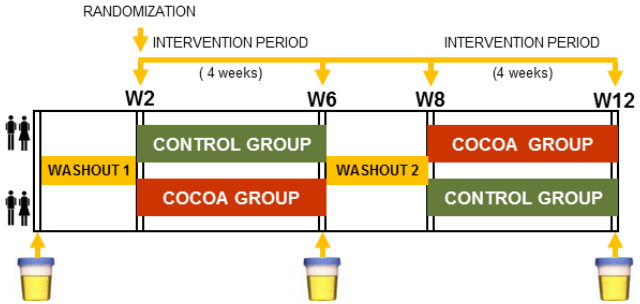
KNApSAcK · hmp · Metabolome.jp · MassBank

- **Theobromine** metabolism
- **Polyphenol** metabolism
- Cocoa **taste and flavour**
- **Endogenous** markers

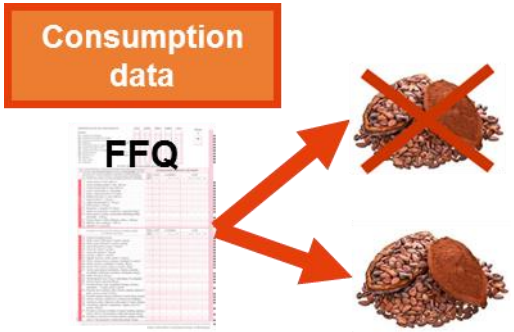
COCOA

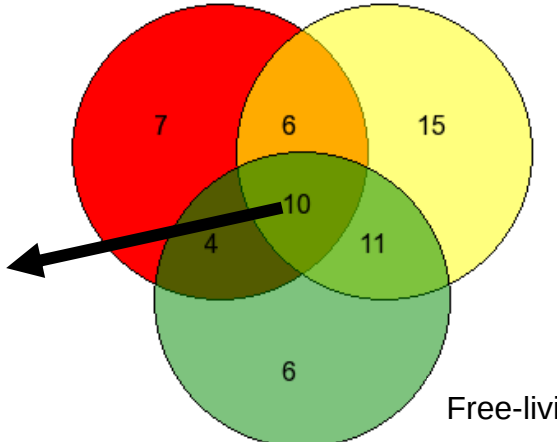
ACUTE INTERVENTION

CHRONIC INTERVENTION



OBSERVATIONAL STUDY





Theobromine Metabolism

- AMMU
- AMMU *isomer*
- 3-Methyluric acid
- 7-Metilxanthine
- 3-Metilxanthine
- 3,7-Dimethyluric acid
- Theobromine

Polyphenol metabolites produced by microbiota

- Methoxyhydroxyphenylvalerolactone
- 5-(3',4'-Dihydroxiphenyl)-valerolactone glucuronide
- 5-(3',4'- Dihydroxiphenyl)-valerolactone sulfate

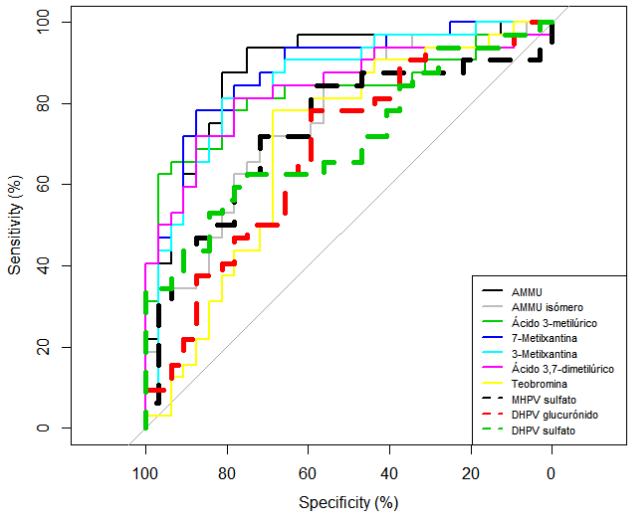
COCOA

OBSERVATIONAL STUDY

PREDIMED Prevención con Dieta Mediterránea

	AUCs
AMMU	88.18% (79.47%-96.90%)
AMMU isomer	76.66% (65.05%-88.27%)
3-Methyluric acid	82.23% (71.23%-93.22%)
7-Metilxanthine	88.28% (80.09%-96.48%)
3-Metilxanthine	85.16% (75.59%-94.72%)
3,7-Dimethyluric acid	83.59% (73.28%-93.91%)
Theobromine	69.82% (56.45%-83.20%)
Methoxyhydroxyphenylvalerolactone	73.44% (60.63%-86.24%)
5-(3',4'-Dihydroxiphenyl)-valerolactone GlcA	68.26% (55.02%-81.51%)
5-(3',4'- Dihydroxiphenyl)-valerolactone sulfate	71.09% (58.27%-83.92%)

ROC



90%-100% = excellent; **80%-90% = good**; 70%-80% = fair; 60%-70% = poor; y 50%-60% = fail

COCOA

MULTIMETABOLITE COMBINED MODELS

PREDIMED Prevención con Dieta Mediterránea

STEPWISE LOGISTIC REGRESSION

TRAINING SET

	Coefficient	Standard Error	*p*
AMMU			
AMMU isomer			
3-Methyluric acid			
7-Metilxanthine	5,563	1,899	0,003
3-Metilxanthine			
3,7-Dimethyluric acid			
Theobromine			
Methoxyhydroxyphenylvalerolactone			
5-(3',4'-Dihydroxiphenyl)-valerolactone GlcA	4,081	1,559	0,009
5-(3',4'- Dihydroxiphenyl)-valerolactone sulfate			

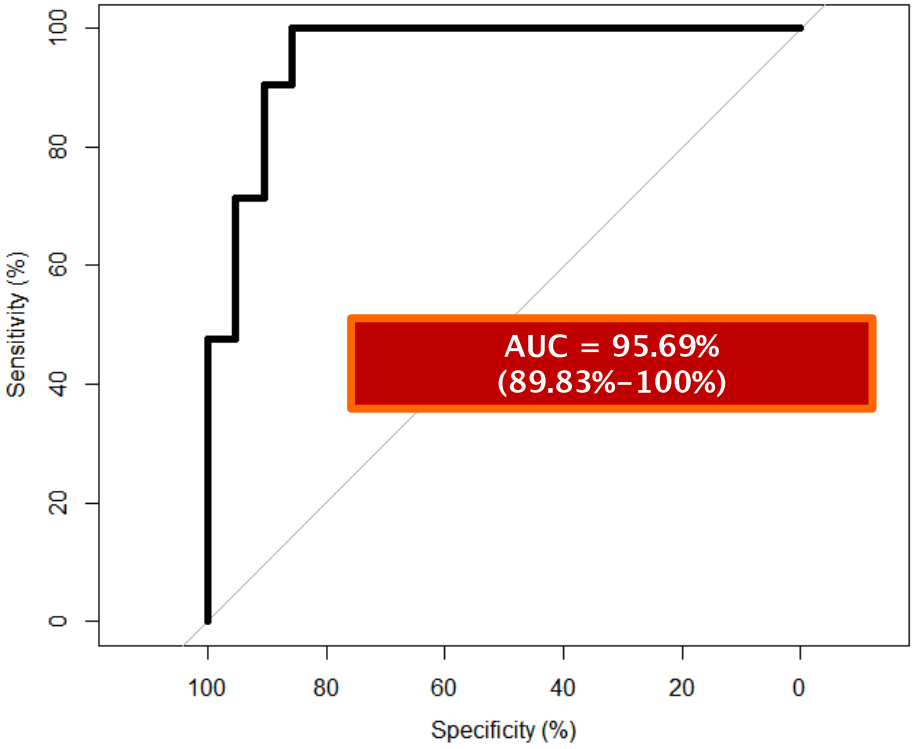
COCOA

MULTIMETABOLITE COMBINED MODELS

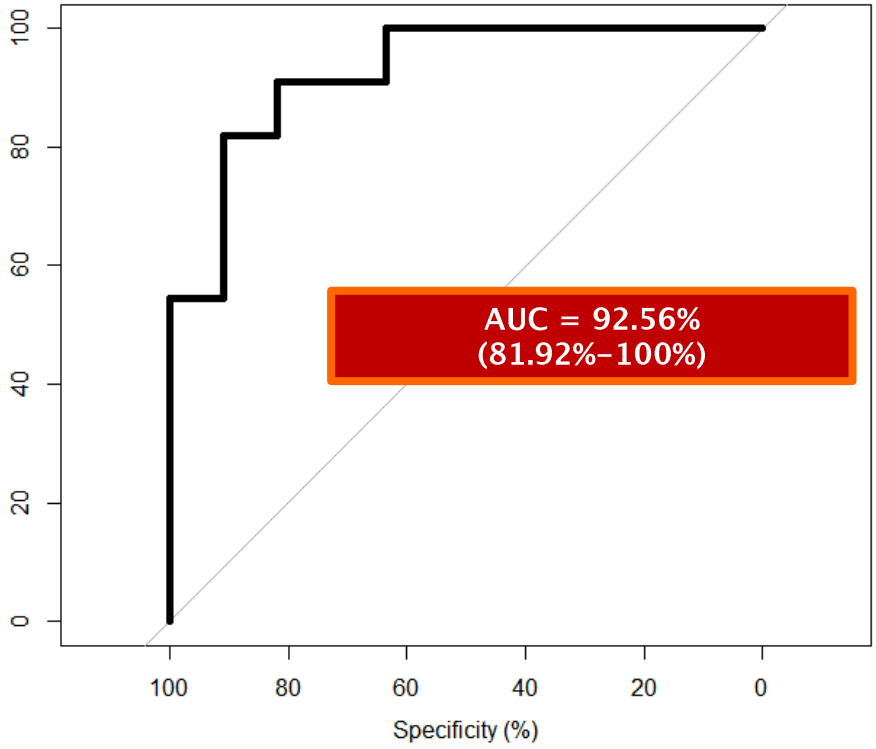
PREDIMED Prevención con Dieta Mediterránea

AUCs

TRAINING SET



VALIDATION SET



90%–100% = excellent; 80%-90% = good; 70%-80% = fair; 60%-70% = poor; y 50%-60% = fail

- ✓ Diet-related differences in urinary metabolome are associated with food digestion, microbiota metabolism and endogenous metabolism.
- ✓ Discriminating metabolites of metabolic fingerprint were replicated among studies with different design.
- ✓ Multi-metabolite models are a more accurate measurement of food intake as nutritional biomarkers than individual compounds.
- ✓ Non-targeted metabolomics approach allows to access unexplored pathways that are affected by diet.

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