13th NuGOweek
PHENOTYPES AND PREVENTION -
THE INTERPLAY OF GENES, LIFE-STYLE FACTORS
AND GUT ENVIRONMENT
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University of Copenhagen - Faculty of Science
Copenhagen, Denmark

HEALTHY COGNITIVE AGEING:
Associations between nut intake biomarkers assessed by metabolomics and cognitive decline. The InCHIANTI study

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Aging

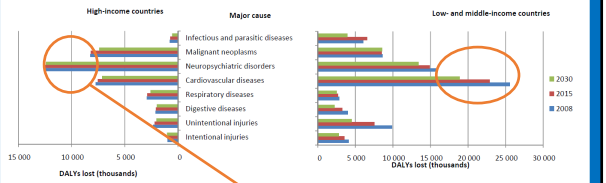
As a progressive time-dependent deterioration of physiological integrity, leading to impaired function and increased vulnerability to death.

➢ **Individual aging differs greatly**

EPIDEMIOLOGY OF AGING

Disability

✓ As the most powerful markers in predicting adverse outcomes.



*DALY: disability-adjusted life-year.

Neurodegenerative diseases like dementia will become more frequent.
A decline in cognitive function is one of the major symptoms of dementia and represents a significant public health problem worldwide

World Health Organization

Source: Global burden of disease: 2004 update, 2008

NUTRITION/DIET STRATEGY FOR SLOWING EFFECTS OF AGING

Healthy lifestyle behaviors: e.g, **healthy dietary pattern**

JRC SCIENCE AND POLICY REPORTS
The Role of Nutrition in Active and Healthy Ageing

Aging → **Physiological dysfunction** (e.g., impaired physical and cognitive function, frailty syndrome) → **Age-related chronic diseases & disability** → **Mortality**

Recent epidemiological studies suggest that healthy eating habits can help to prevent or delay the age-related cognitive decline

(European Comission - Joint Research Centre, 2014)

Introduction

HEALTHY DIETARY PATTERNS

PLANT-RICH DIETS

Mediterranean diet

DASH diet

Healthy eating index

Vegetarian

PHYTOCHEMICALS-POLYPHENOLS

(Dietary Guidelines Advisory Committee, 2015; European Comission - Joint Research Centre, 2014)

POLYPHENOL-RICH DIETS & HEALTH

- ✓ Mortality
- ✓ CVD
- ✓ Type 2 diabetes
- ✓ Obesity
- ✓ Cancer
- ✓ Neurodegenerative diseases
- ✓ Functional limitations and disability

POLYPHENOLS AND HEALTH

PRECLINICAL STUDIES

N° of publications: 37856

N° of publications: 73922

Anti-oxidant
Anti-inflammatory
Anti-tumoral
Cardioprotection
Neuroprotection
Anti-diabetes
Anti-obesity
Anti-aging
Other effects

PubMed (Dec 2015)

EPIDEMIOLOGICAL STUDIES

N° of publications: 713

Limited evidence

Aging
Cardiovascular diseases
Type 2 diabetes
Some cancers
Obesity
Metabolic syndrome
Osteoporosis

Polyphenols and human health: prevention of disease and mechanisms of action.

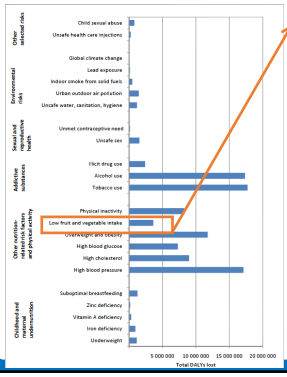
Dietary flavonoids and the development of type 2 diabetes and cardiovascular diseases: review of recent findings.

Measuring exposure to the polyphenol metabolome in observational epidemiologic studies: current tools and applications and their limits.

(Rabassa M. *et al. Trillas*, 2012; Rabassa M. *et al. Nova Science Publishers*, 2012)

EPIDEMIOLOGY OF AGING

The main risk factors are known for disability



It is particulary important for older adults to be aware of fruit and vegetable intake because they tend to eat smaller quantities of food overall, which can lead to deficiency of important vitamins, minerals and phytochemical compounds, such as polyphenols.

Polyphenols and cognitive function

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Epidemiological Studies

Total and Specific Polyphenol Intakes in Midlife Are Associated with Cognitive Function Measured 13 Years Later[1–3]

Emmanuelle Kesse-Guyot,[4]* Léopold Fezeu,[4] Valentina A. Andreeva,[4] Mathilde Touvier,[4] Augustin Scalbert,[5] Serge Hercberg,[4,6] and Pilar Galan[4]

2574 middle-aged adults (SU.VI.MAX 2 cohort)

13-y of follow-up (Prospective study)

Cognitive function

4 neuropsychological tests:
-phonemic and semantic fluency
-RI-48 Cuell Recall Test
-Trail Making test
-Forward and Backward Digit Span

24-h dietary record (6 records/y)

Total and class-specific polyphenol intake

J. Nutr. 142: 76–83, 2012.

Polyphenols and cognitive function

JN THE JOURNAL OF NUTRITION

Epidemiological Studies

TABLE 3 Energy-adjusted associations between quartiles of total and class-specific polyphenol intakes and language and verbal memory (cognitive factor 1) (SU.VI.MAX and SU.VI.MAX 2 studies, 1994–2007)[1]

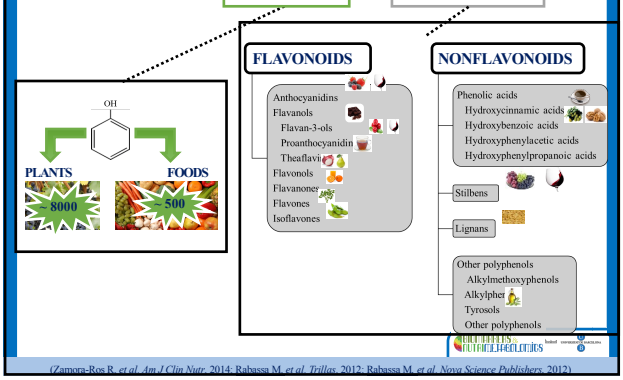
TABLE 4 Energy-adjusted associations between quartiles of total and class-specific polyphenol intakes and executive functioning (cognitive factor 2) (SU.VI. MAX and SU.VI.MAX 2 studies, 1994–2007)[1]

Conclusion

High intake of specific polyphenols, including flavonoids and phenolic acids, may help to preserve verbal memory, which is a salient vulnerable domain in pathological brain aging.

J. Nutr. 142: 76–83, 2012.

POLYPHENOLS: OCCURRENCE AND CLASSIFICATION

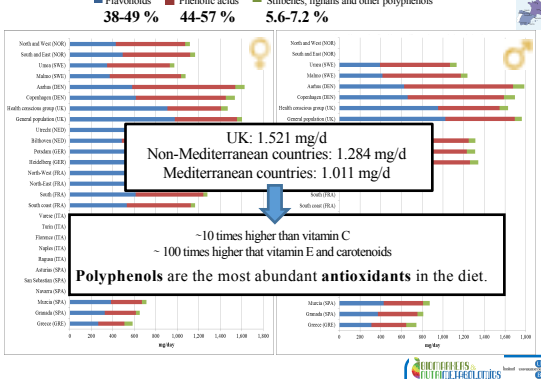


(Zamora-Ros R. *et al. Am J Clin Nutr*, 2014; Rabassa M. *et al. Trillas*, 2012; Rabassa M. *et al. Nova Science Publishers*, 2012)

ESTIMATION OF TOTAL POLYPHENOLS INTAKE

epic

Flavonoids 38-49 % | Phenolic acids 44-57 % | Stilbenes, lignans and other polyphenols 5.6-7.2 %



UK: 1.521 mg/d
Non-Mediterranean countries: 1.284 mg/d
Mediterranean countries: 1.011 mg/d

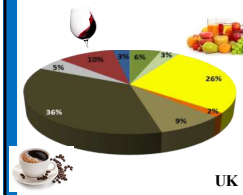
~10 times higher than vitamin C
~ 100 times higher that vitamin E and carotenoids

Polyphenols are the most abundant **antioxidants** in the diet.

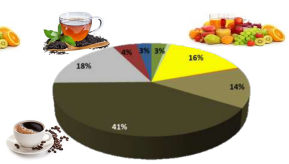
(Zamora-Ros R. *et al. Eur J Nutr*, 2015; Rabassa M. *et al. Trillas*, 2012; Rabassa M. *et al. Nova Science Publishers*, 2012)

MAIN FOOD SOURCES OF TOTAL POLYPHENOLS INTAKE

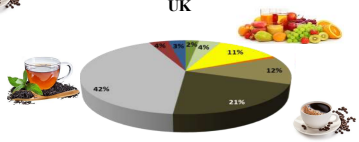
Mediterranean countries



Non-Mediterranean countries



UK



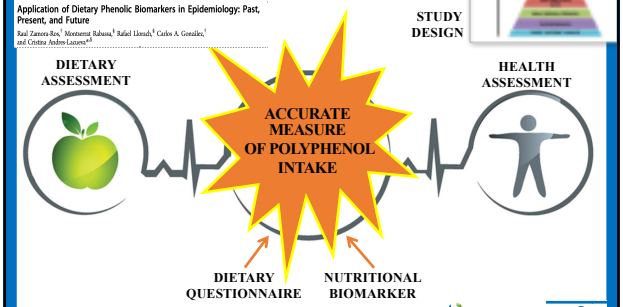
- Vegetables and potatoes
- Legumes
- Fruits and fruit juices
- **Nuts and seeds**
- Cereals and cereal products
- Coffee
- Tea
- Wine
- Other

(Zamora-Ros R, *et al. Eur J Nutr,* 2015)

DIET & HEALTH

Application of Dietary Phenolic Biomarkers in Epidemiology: Past, Present, and Future

Raul Zamora-Ros,[†] Montserrat Rabassa,[‡] Rafael Llorach,[†] Carlos A. González,[†] and Cristina Andres-Lacueva[*,†]



STUDY DESIGN

(Trespalacios MP, […], Rabassa M, *et al. Nova Science Publishers*, 2012; Zamora-Ros R, Rabassa M, *et al. J Agric Food Chem*, 2012; Rabassa M*, Zamora-Ros R*, *et al. Ann Ny Acad Sci*, 2015)

EPIDEMIOLOGICAL ASSESSMENT OF POLYPHENOLS INTAKE

DIETARY QUESTIONNAIRE

- Simple methodology
- Low cost
- A lot of information

NUTRITIONAL BIOMARKER

- objective:
- y dependent
- Independent of the variability of foods
- No limitation by food composition tables
- Bioavailability considered

COMBINE MEASURES

(Trespalacios MP, […], Rabassa M, *et al. Nova Science Publishers*, 2012; Zamora-Ros R, Rabassa M, *et al. J Agric Food Chem*, 2012; Rabassa M*, Zamora-Ros R*, *et al. Ann Ny Acad Sci*. 2015)

JN THE JOURNAL OF NUTRITION

The Journal of Nutrition
Nutritional Epidemiology

High Concentrations of a Urinary Biomarker of Polyphenol Intake Are Associated with Decreased Mortality in Older Adults[1,2]

Raul Zamora-Ros,[3,4] Montserrat Rabassa,[3] Antonio Cherubini,[5,6]* Mireia Urpí-Sardà,[3] Stefania Bandinelli,[7] Luigi Ferrucci,[8] and Cristina Andres-Lacueva[3]

J. Nutr. 143: 1445–1450, 2013.

Aim

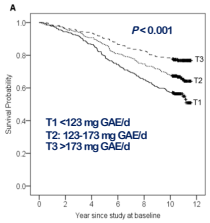
We evaluated the relationship between total polyphenol intake, measured by a dietary questionnaire (TDP) or a nutritional biomarker (TUP), and all-cause mortality over a 12-y follow-up period among older adults in the Invecchiare in Chianti (InChianti) study.

JN THE JOURNAL OF NUTRITION

High Concentrations of a Urinary Biomarker of Polyphenol Intake Are Associated with Decreased Mortality in Older Adults[1,2]

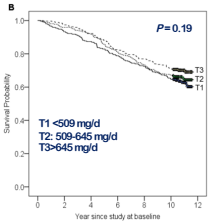
Nutritional biomarker

Total urinary polyphenols (TUP)



Dietary marker

Total dietary polyphenols (TDP)



274 participants (34%) died

Causes of death
CVD: 24%
Cancer: 41%
Other causes: 27%
N.A.: 8%

30% reduction [HR = 0.70 (95% CI: 0.49-0.99); P-*trend* = 0.045]

[HR = 1.22 (95% CI: 0.85-1.63); P-*trend* = 0.33]

JN THE JOURNAL OF NUTRITION

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Conclusion

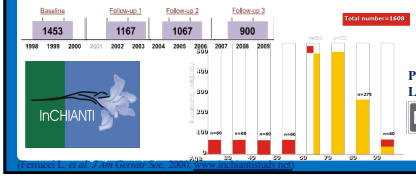
We conclude that participants who consume a diet rich in foods containing polyphenols reduce their risk of all-cause mortality by 30% compared to those who consume diets low in polyphenols.

STUDY DESIGN

INVECCHIARE NEL CHIANTI

Two areas:
Greve in Chianti (rural)
Bagno a Ripoli (urban)

- MAIN OBJECTIVE: To evaluate factors affecting loss of mobility in later life.
- Prospective cohort with 12 years of follow-up.
- A representative population-based study of older persons living in the Chianti geographic area (Tuscany, Italy).
- Age between 20-102 years.
- N=1155 (≥65years; 45% men)



Principal Investigators:
Luigi Ferrucci, Stefania Bandinelli

NIH National Institute on Aging

(Ferrucci L. *et al. J Am Geriatr Soc*, 2000)

InChianti data collection

Home Interview

Informed Consent

Cognitive Status
Depression

Functional Status
Ecological Assessment
Compensatory Strategies
Environmental Factors
Social Factors

Life Time History of
Alcohol
Smoking
Physical Activity
Falls

Foot Problems

Current Diet (EPIC)

Blood & 24h Urine

Muscle Metabolism
Insulin, IGF-1, IGF-2
24h Creatinine, 3-Meth-Hyst.
24h Cortisol

Genetic Polymorphisms
Apo-E, IL-1, IL-6, Myostatin

Calcium Metabolism
24h Ca++ Excretion, Vit. D
PTH , Bone Alkaline Phosph.

Inflammation-Infections
TNF-a, IL-1-2-4-6-6R-8-10
Lymph. CD3-CD4-CD8-CD19
Helicobacter Pylori
Chlamydia Pneumoniae

Ultrasound
Carotids
Lower Extremity (v+a)

Leg CT Scan

ECG, NCV

Medical Exam
Disease Diagnoses
Disease Severity
Chronic Inflammation
Neurological Exam
Visual Analysis of Gait
Vision and Hearing
Joint Pain

Performance Tests
EPESE Battery
Climbing Stairs
Walking
Usual, Fast, Narrow Path
Obstacles + Sung asses
Visual Fed, Talking
Pick Object
400 m + Weight Load

Muscle Strength
Isometric (10 groups)
Lower Extremity Power

www.inchiantistudy.net

TOTAL POLYPHENOLS & COGNITIVE DECLINE

N = 652 dementia-free adults (45% men, ≥65y)
Follow-up: 3y
Cases → MMSE: 203 (31.1%), TMT-A: 103 (20.2%), TMT-B: 221 (52.2%)

Journal of the AMERICAN GERIATRICS SOCIETY

Low Levels of a Urinary Biomarker of Dietary Polyphenol Are Associated with Substantial Cognitive Decline over a 3-Year Period in Older Adults: The Invecchiare in Chianti Study

Montserrat Rabassa, MSc, Antonio Cherubini, MD, PhD,† Raul Zamora-Ros, PhD,‡ Mireia Urpi-Sarda, DPharm, PhD,* Stefania Bandinelli, MD,§ Luigi Ferrucci, MD, PhD,‖ and Cristina Andres-Lacueva, DPharm, PhD**

(Rabassa M, *et al. J Am Geriatr Soc*, 2015)

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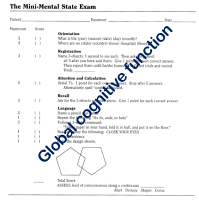
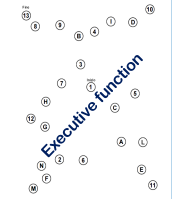
Aim

We investigated the association of total urinary polyphenols (TUP) and total dietary polyphenols (TDP) with cognitive decline over a 3-year follow-up in a older participants free of dementia at baseline who were enrolled in the InChianti study.

MMSE | TMT A | TMT B

MMSE	TMT A	TMT B
no. of cases: 203 (31.1%)	no. of cases: 103 (20.2%)	no. of cases: 221 (52.2%)





Odds Ratio [OR] comparing extreme tertiles (95% confidence interval [CI])

46% reduction — itional biom — 46% reduction — nary polyphenols (TUP)

0.54 (0.34-0.86); *P*-trend = 0.01 | 0.54 (0.29-0.98); *P*-trend = 0.04 | 0.94 (0.55-1.61)

Dietary marker: Total dietary polyphenols (TDP)

1.06 (0.68-1.66) | 0.63 (0.32-1.25) | 1.16 (0.63-2.13)

Rabassa M, *et al.* JAGS. 2014 (Submitted)

Low Levels of a Urinary Biomarker of Dietary Polyphenol Are Associated with Substantial Cognitive Decline over a 3-Year Period in Older Adults: The Invecchiare in Chianti Study

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Conclusion

High concentrations of polyphenols, a nutritional biomarker of polyphenol intake, were associated with a lower risk of cognitive decline in the older population studied over a 3-year period, suggesting a protective effect against cognitive impairment.

Polyphenol content of nuts (mg/100g)

Walnuts

Anthocyanidins (cyanidin)	2.71
Proanthocyanidins	60.32
Isoflavones	0.05
Lignans	0.15
Hydroxybenzoic acids	**449.37**
- **Ellagic acid**	**252.50**
- **Valoneic acid dilactone**	**196.87**

Almonds

Anthocyanidins (cyanidin)	2.46
Flavan-3-ols	4.47
Proanthocyanidins	**176.25**
Flavonols	3.39
Flavanones	0.68
Isoflavones	0.03
Coumestans	0.02
Lignans	0.13
Hydroxybenzoic acids	0.44
- Protocatechuic acid	0.27
- Vanillic acid	0.17

Hazelnuts

Anthocyanidins (cyanidin)	6.71
Flavan-3-ols	5.25
Proanthocyanidins	**490.83**
Flavanones	0.68
Isoflavones	0.04
Lignans	0.07

Peanuts

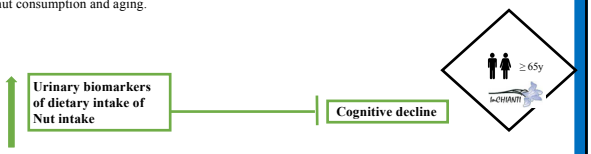
Proanthocyanidins	30.03
Isoflavones	0.27
Stilbenes	0.09

US Department of Agriculture (Flavonoids, Proanthocyanidins and isoflavones)
and Phenol-Explorer Databases
Rothwell et al. Nutr Res Rev 2013, 24, 244-275

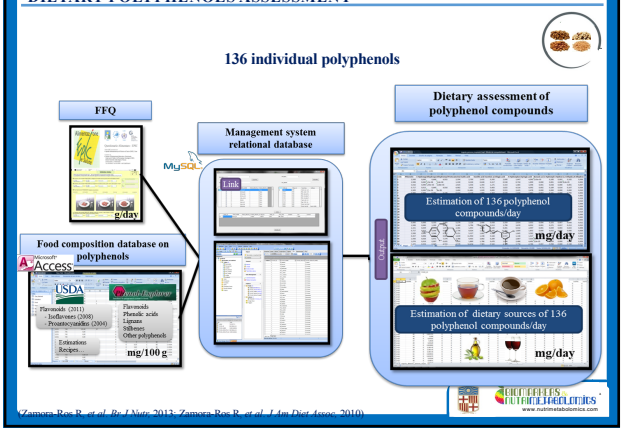
HYPOTHESIS

High urinary concentrations associated with NUT consumption, as biomarkers for nut intake could be associated with a lower cognitive decline, among the Italian population over 65 years within the InCHIANTI study.

The use of biomarkers of dietary exposure assessment will provide more reliable associations between nut consumption and aging.



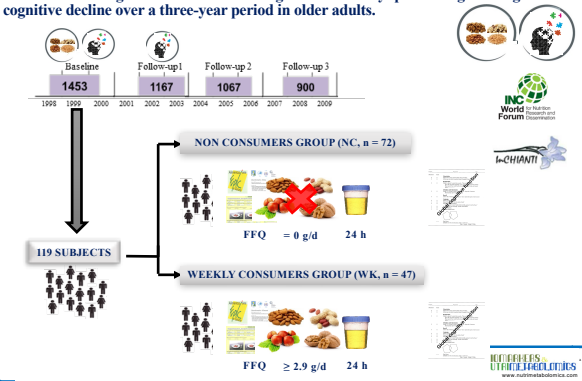
DIETARY POLYPHENOLS ASSESSMENT



(Zamora-Ros R. *et al. Br J Nutr*, 2013; Zamora-Ros R. *et al. J Am Diet Assoc*, 2010)

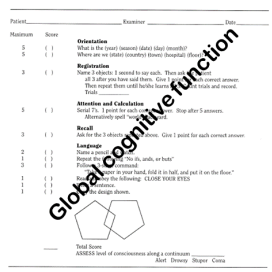
PROJECT'S RESEARCH DESIGN

AIM: To investigate whether a diet high in nuts may protect against age-related cognitive decline over a three-year period in older adults.



COGNITIVE FUNCTION ASSESSMENT

MMSE



It is an 11-question test that evaluates five areas of cognitive function:
- Orientation
- Registration
- Attention and calculation
- Recall
- Language.

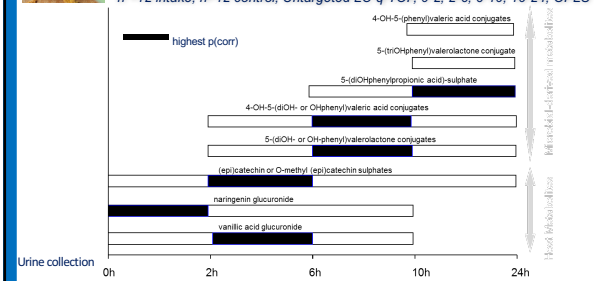
The total score range from 0 to 30.

→ Decline was defined as a decrease of ≥ 2 points.

(Llewellyn DJ, *et al. Arch Intern Med*, 2010; Mitchell AJ, *et al. J Psychiatr Res*, 2009; Folstein MF, *et al. J Psychiatr Res*, 1975)

Potential biomarkers of almond skin intake

n =12 intake; n=12 control, Untargeted LC-q-TOF, 0-2, 2-6, 6-10, 10-24, OPLS



Microbiota metabolites responsible of the metabolome differences after 6 h single intake of almond polyphenols

These results reinforce that metabolomics produced new biomarker of food intake. This insight contribute to build-up the food metabolome concept.

(Llorach R. *et al.*, *Proteomics*, 2009)

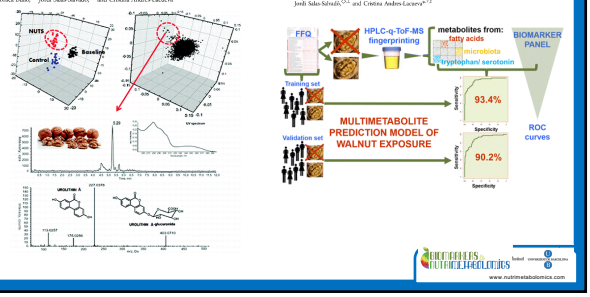
NUTS & WALNUTS

INTERVENTION STUDY | OBSERVATIONAL STUDY

Journal of proteome research

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

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



RESULTS: Identified Metabolites

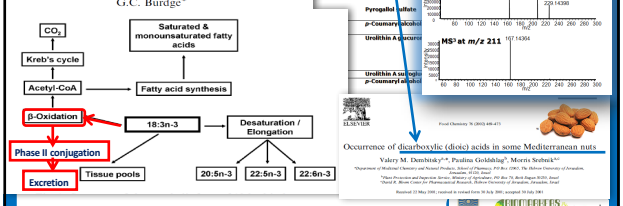
Metabolomics Unveils Urinary Changes in Subjects with Metabolic Syndrome following 12-Week Nut Consumption

Fatty acids metabolites

✓ Products of β-oxidative and phase II metabolism of PUFA

Metabolism of α-linolenic acid in humans

G.C. Burdge

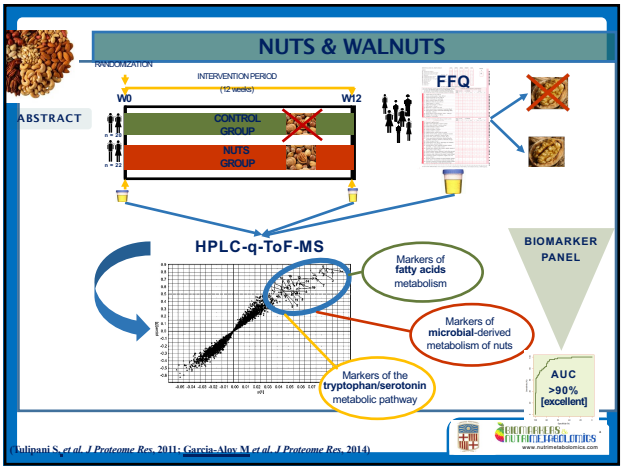


Dodecanedioic acid

Occurrence of dicarboxylic (dioic) acids in some Mediterranean nuts

NUTS & WALNUTS

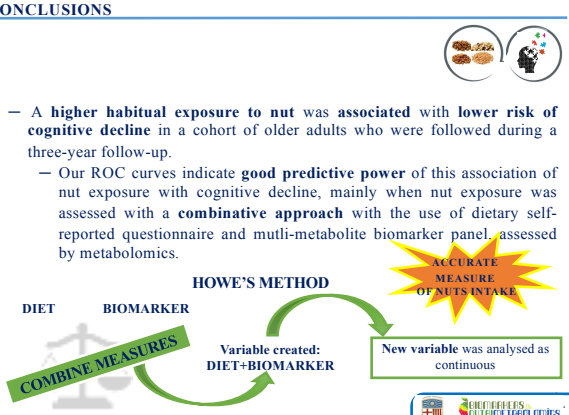
ABSTRACT



Tulipani S. *et al. J Proteome Res.* 2011; Garcia-Aloy M *et al. J Proteome Res.* 2014)

CONCLUSIONS

- A **higher habitual exposure to nut** was **associated** with **lower risk of cognitive decline** in a cohort of older adults who were followed during a three-year follow-up.
  - Our ROC curves indicate **good predictive power** of this association of nut exposure with cognitive decline, mainly when nut exposure was assessed with a **combinative approach** with the use of dietary self-reported questionnaire and mutli-metabolite biomarker panel, assessed by metabolomics.



Conclusion

- Our findings in the InCHIANTI study provide evidence on the protective effects of total polyphenols against cognitive decline and overall mortality.
- Our results also demonstrate the importance of assessing the intake of polyphenols, whenever possible, using nutritional biomarkers and not only by dietary questionnaires.

Biomarkers and Nutritional & Food Metabolomics

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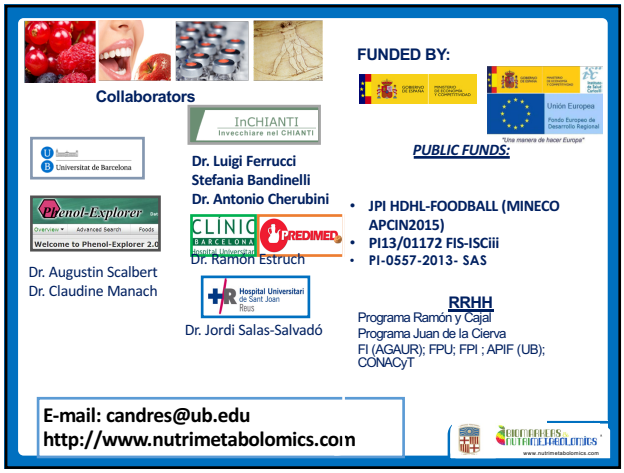

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Thank you for the attention

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