4^ Edizione

CORSO DI 2° LIVELLO
PER L'ORGANIZZAZIONE E LA GESTIONE
DI UN AMBULATORIO DEGLI STILI DI VITA

14 • 15 Maggio 2016
Frascati (RM)

# Nutraceutica e nutrigenomica: nuove frontiere di intervento dietetico-nutrizionale

**Roberta Masella**
**Istituto Superiore di Sanità**

SIMPeSV
Società Italiana di Medicina di Prevenzione e degli Stili di Vita

# Articolazione del Corso

- Parte generale-Definizioni, diagramma di flusso della ricerca in nutrigenomica
- Studi clinici-Studi di intervento
- Ricerca biomolecolare-Meccanismi di azione molecolare di componenti contenuti in alimenti funzionali

# Obiettivi del corso

Fornire strumenti utili ad

- **Aumentare la conoscenza** dei principi di base della nutraceutica e della nutrigenomica e del complesso processo di studio e ricerca necessario a supportare qualsiasi  nuova evidenza
- **Accrescere la capacità di giudicare** con competenza e senso critico i tanti messaggi, spesso privi di solide basi scientifiche, quotidianamente indirizzati ai cittadini così da indirizzare correttamente le loro scelte nutrizionali.

**Nutrizione** **Farmaceutica**

**Nutraceutica**

studio di alimenti che hanno una funzione benefica sulla salute umana.

British Journal of Clinical Pharmacology

Br J Clin Pharmacol (2016) •• ••–•• 1

# REVIEW-THEMED ISSUE

# Defining 'nutraceuticals': neither nutritious nor pharmaceutical

**Correspondence** Dr J. K. Aronson, Centre for Evidence Based Medicine, Nuffield Department of Primary Care Health Sciences, Radcliffe Observatory Quarter, Woodstock Road, Oxford OX26GG, UK. E-mail: jeffrey.aronson@phc.ox.ac.uk

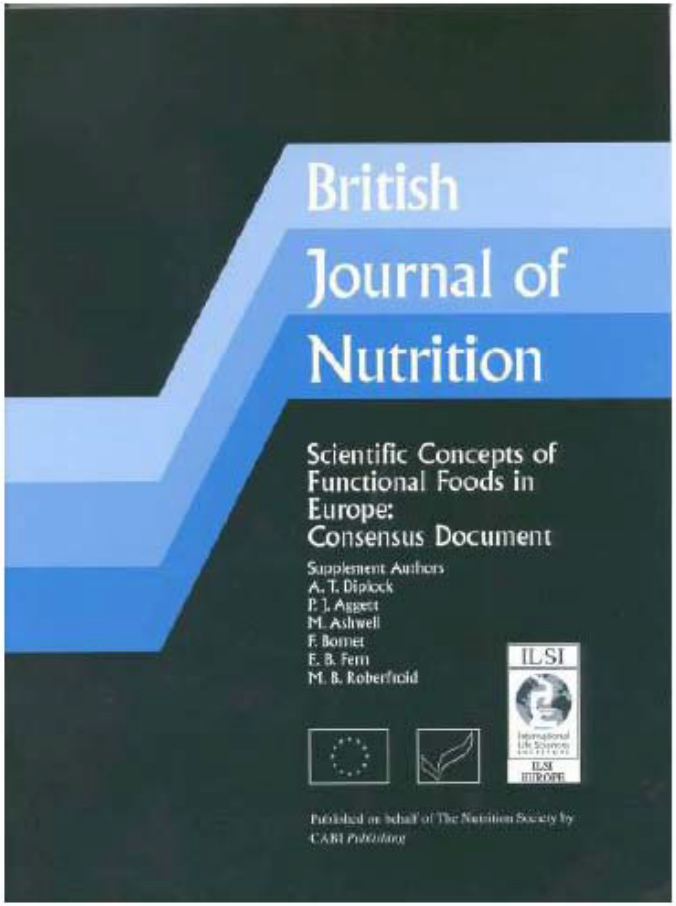
**Received** 13 February 2016; **accepted** 9 March 2016

Jeffrey K. Aronson

*Centre for Evidence Based Medicine, Nuffield Department of Primary Care Health Sciences, Radcliffe Observatory Quarter, Oxford OX26GG, UK*

**Keywords** designer foods, dietary regimens, dietary supplements, fortified foods, functional foods, nutraceuticals

# European Commission Concerted Action on Functional Food Science in Europe – FUFOSE coordinata dal International Life Science Institute (ILSI) Europe (1995-1998)




## Scientific Concepts of Functional Food in Europe: Consensus Document

A.T. Diplock, P.J. Aggett, M. Ashwell, F. Bornet, E.B. Fern & M.B. Roberfroid.

*Brit. J. Nutr. (1999) Vol. 81, S1-S27*

- Introduction
- Scientific basis
- Target functions
- Technological aspects
- Communication of health benefits (Health Claims)

# Un alimento funzionale:

- **Possiede effetti addizionali dovuti alla presenza di componenti, generalmente non-nutrienti, che interagiscono selettivamente con una o più funzioni fisiologiche dell'organismo (biomodulazione) in modo tale che risultino evidenti un miglioramento dello stato di salute e di benessere e/o una riduzione del rischio di malattia.**

- **E' un alimento, non una pillola, una capsula, o un integratore alimentare**

- **Esercita la sua funzione nelle quantità normalmente previste da una dieta equilibrata**

- **Deve dimostrare attraverso modelli e successivi trial clinici un effetto monitorabile sulla salute dell'uomo = Effetti funzionali scientificamente documentati e accettati**

**Nutraceutico**= componente bioattivo contenuto in un alimento funzionale con proprietà curative di comprovata efficacia

- micronutrienti (vitamine e acidi grassi)
- non nutrienti (fitocomposti e probiotici)

## Table 1

Functional foods classification, some sources, and examples of bioactive substances.

| Functional food | Bioactive component (nutraceutic) | | Source (s) |
|---|---|---|---|
| Micronutrients | Vitamins | Retinol (vitamin A)<br>α-tocopherol (vitamin E)<br>Calciferol (vitamin D3) | Walnuts, almonds, hazelnuts, spinach, fish oil |
| | Polyunsaturated fatty acids (PUFAs) | Omega 3 Fatty acids:<br>eicosapentaenoic acid (EPA)<br>docosahexaenoic acid (DHA) | Salmon, tuna and others fish oils |
| Nonnutrients Phytochemicals | Carotenoids | Beta-carotene<br>luteín, zeaxanthin<br>lycopene | Carrots, pumpkin, collards, kale, spinach, tomatoes, watermelon |
| | Phenolic acid derivatives | Caffeic acid<br>Ferulic acid<br>Gallic acid<br>Curcumin | Coffee, pears, apples, corn, curcumin, vanilla |
| | Flavonoids | Flavonols (quercetin)<br>Isoflavones<br>Coumarins<br>Anthocyanidines<br>Stilbenes (resveratrol) | Berries, cherries, red grapes, tea, cocoa, apples, citrus fruits, onion, broccoli, cranberries, strawb |
| | Sulfides/thiols | Diallyl sulfide<br>S-allyl cysteine sulfoxide<br>1,2-viniyldithiin | Garlic, onions, banana, cruciferous vegetables |
| | Dietary fiber (prebiotic) | Fructooligosaccharides<br>Neoglicans | Whole grains, onions, chicory, agave, some fruits |
| Probiotics | PUFAs induction | *Saccharomyces cerevisiae* (var. *boulardii*)<br>Bifidobacteria and<br>*Lactobacillus* genus | Certain yogurts and other cultured dairy and no-dairy applications |

**TABLE 1** Recent observational studies on adherence to the Mediterranean diet and cardiovascular disease[1]

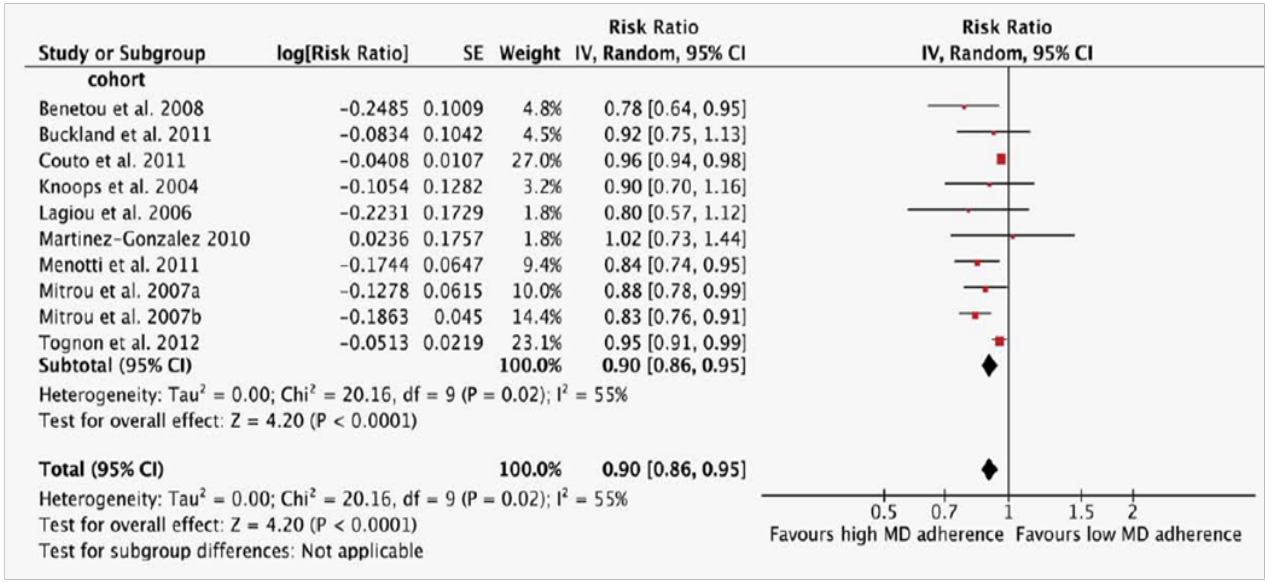
| Study | Country | Sample size | Outcome | Events | Comment |
|---|---|---|---|---|---|
| Dilis et al., 2012 (12) | Greece | 23,929 | CHD incidence and death from CHD | 636 CHD events and 240 CHD deaths | There was a stronger inverse association for mortality than for incidence. |
| Tognon et al., 2012 (13) | Sweden | 77,151 | CVD death | 680 deaths | The inverse association was only significant among women. Effect of the dietary pattern (only present among women) was smaller than in studies conducted in Mediterranean countries. |
| Gardener et al., 2011 (14) | United States | 2568 | Stroke, myocardial infarction, and CVD death | 518 events | In a multiethnic population, a dietary pattern resembling the Mediterranean diet was protective against the combined outcome of ischemic stroke, myocardial infarction, and vascular death. |
| Misirli et al., 2012 (15) | Greece | 23,601 | Stroke | 395 incident cases and 196 stroke deaths | Inverse trends were stronger with respect to ischemic rather than hemorrhagic stroke. |
| Hoevenaar-Blom et al., 2012 (16) | The Netherlands | 40,011 | Fatal and nonfatal CVD events | 4881 events, including 487 CVD deaths | There were significant inverse linear associations for fatal CVD, total CVD, myocardial infarction, and stroke. |
| Menotti et al., 2012 (17) | Italy | 1139 | CHD death | 162 CHD deaths | There was an inverse association between an index of adequacy to the Mediterranean diet and total mortality in a male cohort. |
| Tognon et al., 2013 (18) | Denmark | 1849 | Fatal and nonfatal CVD events | 755 CVD events and 223 CVD deaths | Higher adherence to a Mediterranean dietary score was inversely associated with CVD and myocardial infarction but not with stroke. |

[1] Description of observational studies recently published but not included in the 2010 meta-analysis by Sofi et al. (8). CHD, coronary heart disease; CVD, cardiovascular disease.

International Journal of Cancer
Early View, Article first published online: 11 MAR 2014
Abstract | Full Article (HTML) | Enhanced Article (HTML) | References
Supporting Information | Cited By
Wiley Online Library

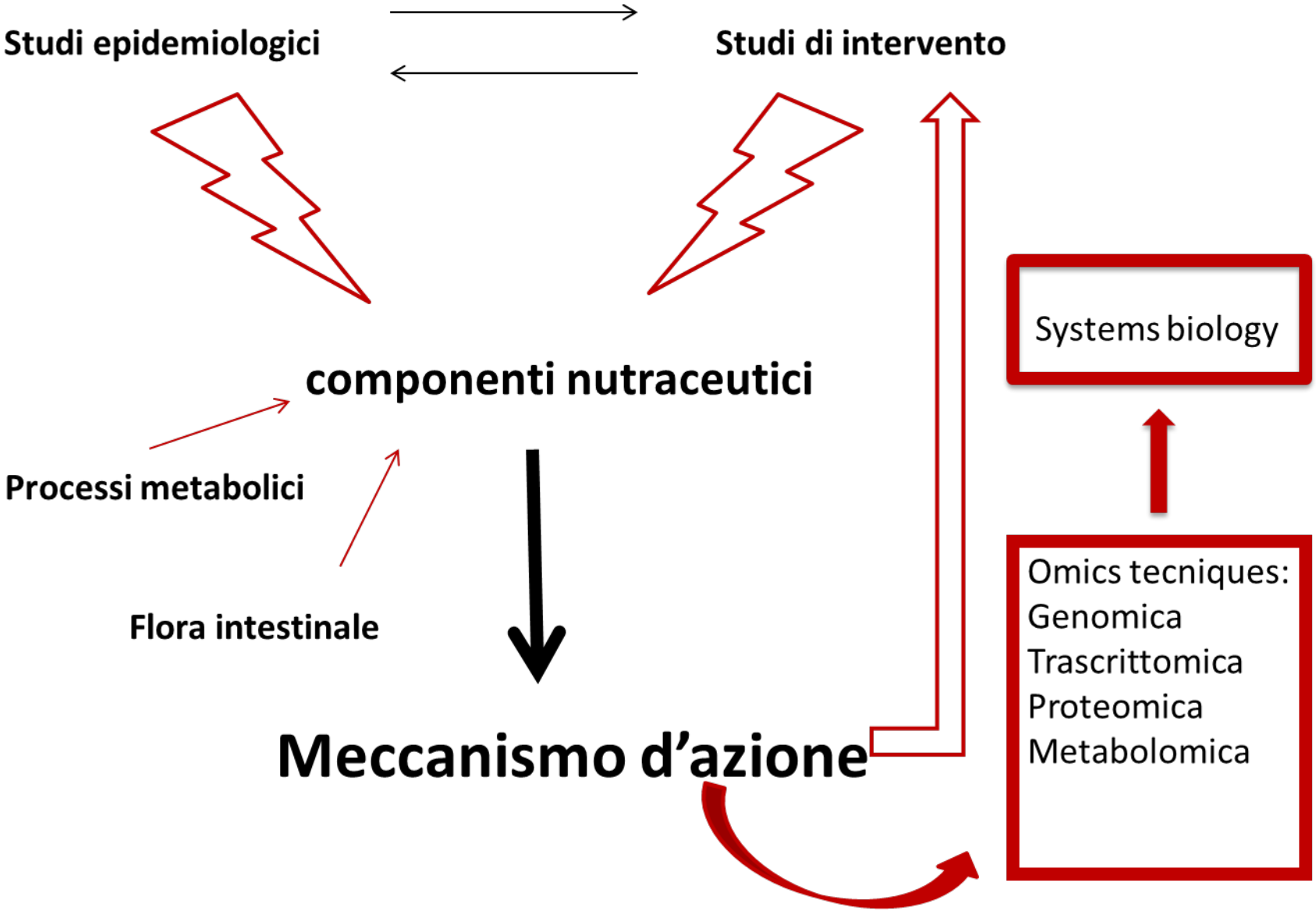
# Adherence to Mediterranean diet and risk of cancer: A systematic review and meta-analysis of observational studies

Lukas Schwingshackl and Georg Hoffmann




**What's new?**

Adherence to a "Mediterranean Diet" is associated with **significant improvements in health** status, including a **lower overall risk of cancer**, **especially colorectal and aerodigestive cancers**.


Studi epidemiologici
Studi di intervento
componenti nutraceutici
Processi metabolici
Flora intestinale
Meccanismo d'azione
Systems biology
Omics tecniques:
Genomica
Trascrittomica
Proteomica
Metabolomica

# Nutrigenomica

Studia i meccanismi con i quali gli alimenti funzionali possono influenzare

**l'espressione genica**

- **il transcrittoma → profilo degli RNA**
- **il proteoma → profilo delle proteine**
- **il metaboloma → profilo dei metaboliti**

**Obiettivo finale**: comprendere come il cibo interferisce con il codice genetico e come l'organismo risponde a queste interferenze modificando il fenotipo.

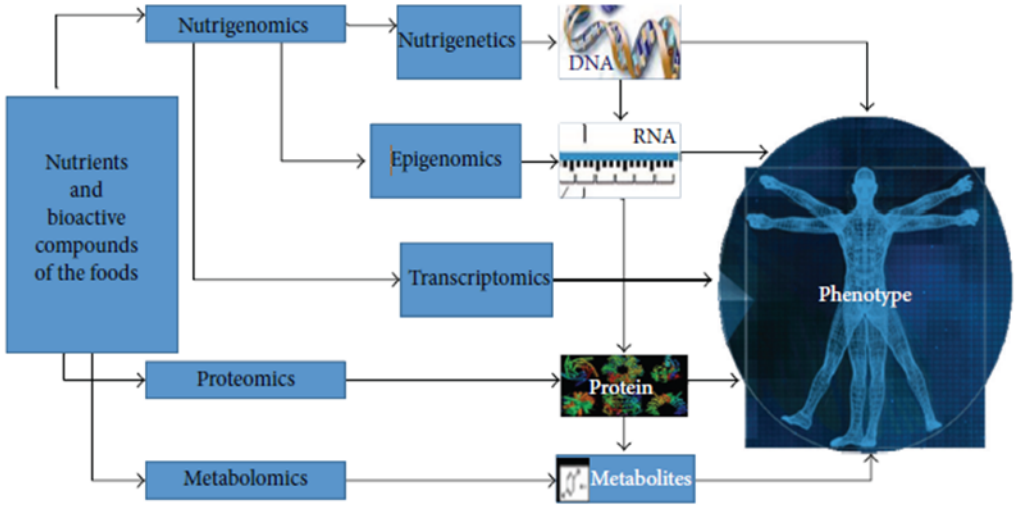
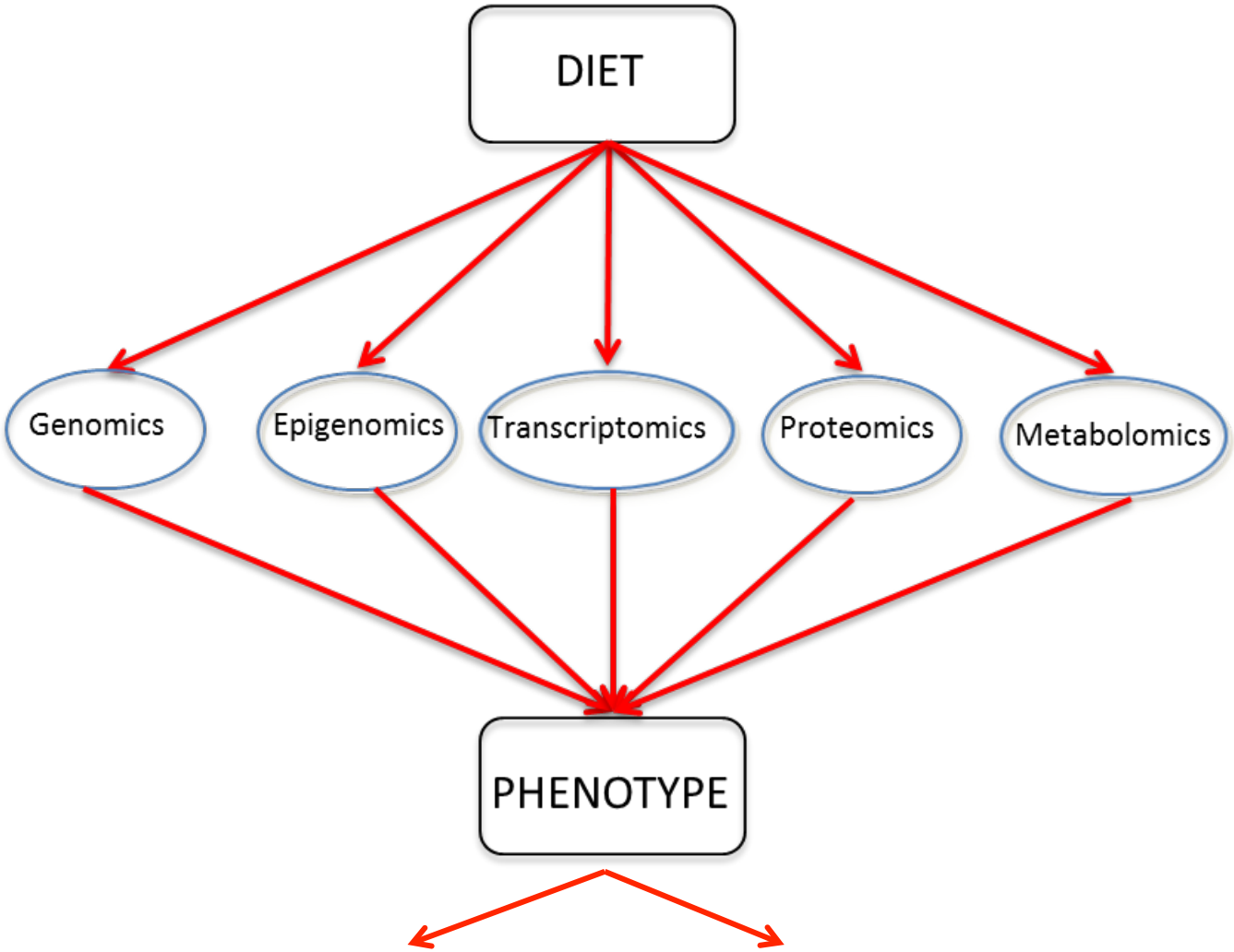
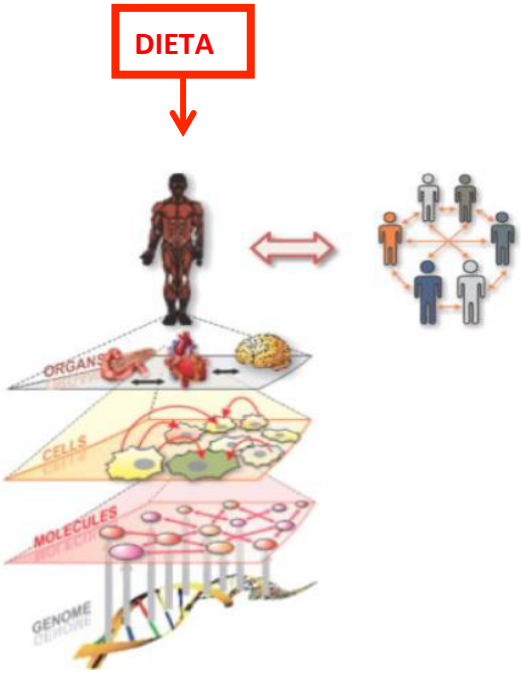



FIGURE 1: "Omics" sciences used in understanding the relationship between nutrition versus health versus disease (source: [4], with modifications; [9] with modifications).


DIET
Genomics
Epigenomics
Transcriptomics
Proteomics
Metabolomics
PHENOTYPE
Health
Disease




- **La dieta agisce sulle funzioni dell'organismo.**
- **L'individuo ha caratteristiche proprie che influenzano gli effetti della dieta**

→ **Nutrigenetica**

**Table 2. Selected Studies Showing Statistically Significant Gene-Diet Interactions in Determining Intermediate CVD Phenotyes**

| Reference | Phenotype | Description of the Gene-Diet Interaction |
|---|---|---|
| Lopez-Miranda et al[60]* | Postprandial LDL-C | The −75G/A *APOA1* SNP influenced the postprandial LDL-C response to MUFA. After consumption of a high MUFA diet, significant increases in LDL-C were noted in carriers of the A allle but not in G/G subjects. |
| Jansen et al[61]* | Postprandial LDL-C | Postprandial LDL-C response to dietary fat is influenced by the 347Ser mutation of *APOA4*. Carriers of the 347Ser allele presented a greater decrease in LDL-C when they were switched from the SFA to the NCEP type 1 diet than homozygous the 347Thr allele. |
| D'Angelo et al[62]* | Plasma homocysteine | The C677T SNP in the *MTHFR* gene interacted with folate and vitamin B12 levels in determining plasma homocysteine concentrations. |
| Campos et al[63] | VLDL and HDL-C | The *APOE* genotype interacted with saturated fat in determining VLDL and HDL-C concentrations (higher VLDL and lower HDL-C in E2 carriers with a high fat). |
| Luan et al[64] | BMI and fasting insulin | An interaction was found between the PUFA:saturated fat ratio and the Pro12Ala *PPARG* polymorphism for both BMI and fasting insulin. With a low ratio, the BMI in Ala carriers was greater than that in Pro homozygotes, but when the dietary ratio was high, the opposite was seen. |
| Corella et al[65] | Fasting plasma LDL-C concentrations | Alcohol intake interacted with the *APOE* SNP in determining LDL-C in men. In E2 subjects, LDL-C was significantly lower in drinkers than in nondrinkers but was significantly higher in drinkers than in nondrinkers in E4 subjects. |
| Leeson et al[66] | Endothelium-dependent, flow-mediated brachial artery dilatation (FMD) and endothelium-independent dilatation response | An endothelial nitric oxide synthase (eNOS) SNP (Glu298Asp) interacted with dietary omega-3 in determining endothelial responses. Omega-3 was positively related to FMD in Asp298 carriers but not in Glu298 homozygotes. |
| Ordovas et al[67] | HDL-C concentrations and HDL particle size | The −514C>T *LIPC* polymorphism interacted with dietary fat in determining HDL-related measures. T allele was associated with significantly greater HDL-C concentrations and large HDL size only in subjects consuming <30% of energy from fat. |

*Circ Cardiovasc Genet. 2009;2:637-651.)*

**Capire come la dieta ed i suoi componenti possono interferire con questi meccanismi è un elemento chiave per la definizione di strategie di prevenzione nutrizionali efficaci e sostenibili che mirino alla cosiddetta**

**NUTRIZIONE PERSONALIZZATA** → **AUMENTO DELL'EFFICACIA**

**NUTRIZIONE PERSONALIZZATA** → **DIMINUZIONE DEI COSTI**

# Studi Nutrigenomici

- **Correlare  modifiche dell'espressione genica a risultati sistemici**

- **Mettere insieme i risultati delle diverse tecniche «omiche» con lo studio classico dei biomarcatori**

**Visione olistica di come la dieta può influenzare i nostri geni**

**Evidence-based medicine** → **High level of scientific evidence**

↓

**Nutritional recommendation**

**Randomized, controlled double-bind, clinical intervention trials ( level I of evidence)**

**Large cohort studies (level II of evidence)**

Table 1. Summary of Dietary Recommendations to Participants in the Mediterranean-Diet Groups and the Control-Diet Group.

| Food | Goal |
|---|---|
| **Mediterranean diet** | |
| Recommended | |
| Olive oil* | ≥4 tbsp/day |
| Tree nuts and peanuts† | ≥3 servings/wk |
| Fresh fruits | ≥3 servings/day |
| Vegetables | ≥2 servings/day |
| Fish (especially fatty fish), seafood | ≥3 servings/wk |
| Legumes | ≥3 servings/wk |
| Sofrito‡ | ≥2 servings/wk |
| White meat | Instead of red meat |
| Wine with meals (optionally, only for habitual drinkers) | ≥7 glasses/wk |
| Discouraged | |
| Soda drinks | <1 drink/day |
| Commercial bakery goods, sweets, and pastries§ | <3 servings/wk |
| Spread fats | <1 serving/day |
| Red and processed meats | <1 serving/day |
| **Low-fat diet (control)** | |
| Recommended | |
| Low-fat dairy products | ≥3 servings/day |
| Bread, potatoes, pasta, rice | ≥3 servings/day |
| Fresh fruits | ≥3 servings/day |
| Vegetables | ≥2 servings/day |
| Lean fish and seafood | ≥3 servings/wk |
| Discouraged | |
| Vegetable oils (including olive oil) | ≤2 tbsp/day |
| Commercial bakery goods, sweets, and pastries§ | ≤1 serving/wk |
| Nuts and fried snacks | ≤1 serving /wk |
| Red and processed fatty meats | ≤1 serving/wk |
| Visible fat in meats and soups¶ | Always remove |
| Fatty fish, seafood canned in oil | ≤1 serving/wk |
| Spread fats | ≤1 serving/wk |
| Sofrito‡ | ≤2 servings/wk |

# PREDIMED trial (Prevención con Dieta Mediterránea)

Studio a bracci paralleli, multicentrico, randomizzato

7447 soggetti ( donne e uomini; 55-80 anni)

- No CVD al momento dell'arruolamento
- T2D o almeno 3 fattori di rischio (fumo, ipertensione, alto c-LDL, basso c-HDL, sovrappeso/obesità)

- Dieta mediterranea **+ EVOO** (1 lt/settimana)

- Dieta mediterranea + **noci/mandorle/nocciole** (30 gr/die)

- Dieta di controllo (**low fat**)

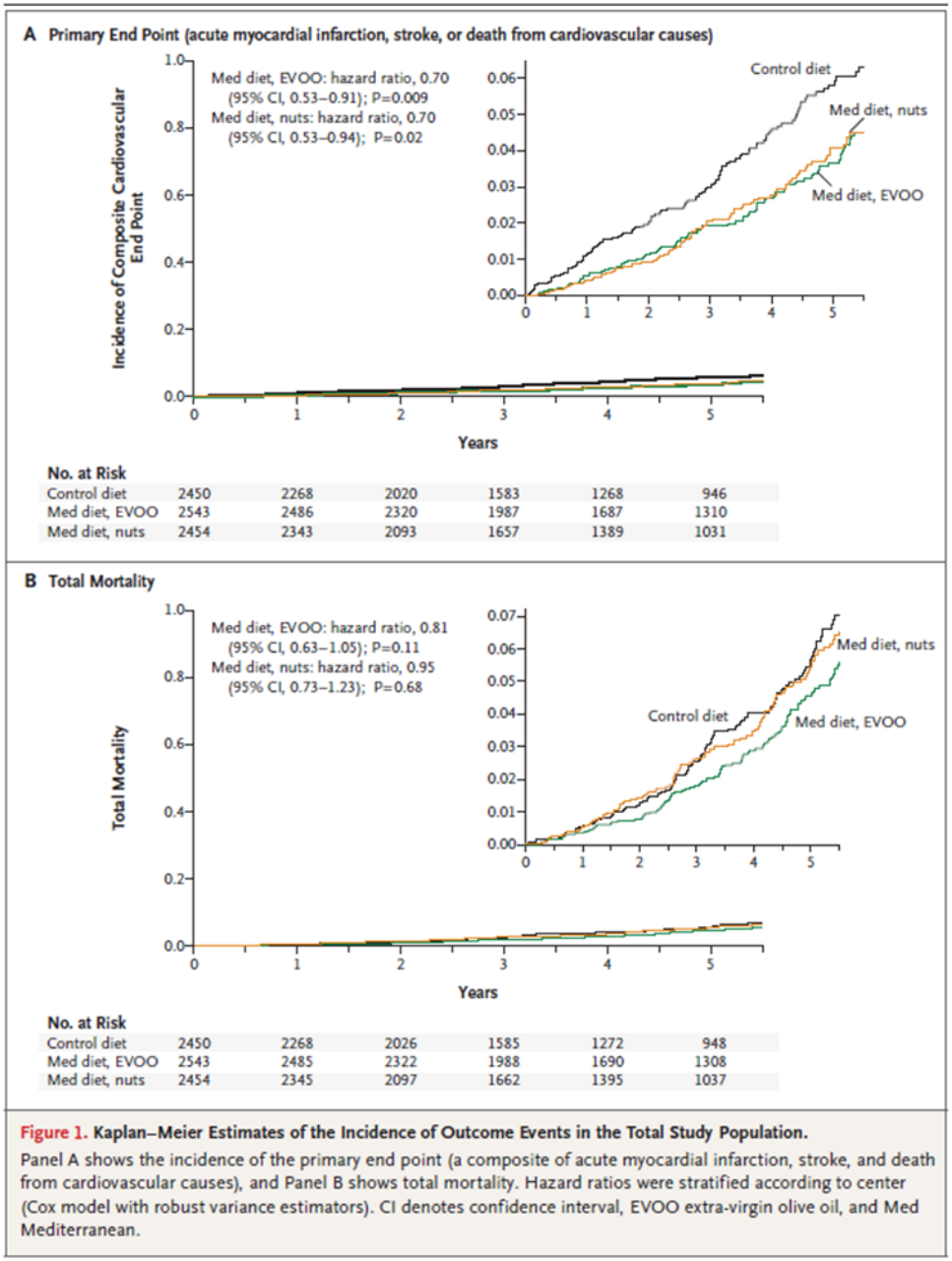



**Figure 1. Kaplan–Meier Estimates of the Incidence of Outcome Events in the Total Study Population.**

Panel A shows the incidence of the primary end point (a composite of acute myocardial infarction, stroke, and death from cardiovascular causes), and Panel B shows total mortality. Hazard ratios were stratified according to center (Cox model with robust variance estimators). CI denotes confidence interval, EVOO extra-virgin olive oil, and Med Mediterranean.

# In vivo transcriptomic profile after a Mediterranean diet in high–cardiovascular risk patients: a randomized controlled trial[1–3]

*Olga Castañer, Dolores Corella, Maria-Isabel Covas, José V Sorlí, Isaac Subirana, Gemma Flores-Mateo, Lara Nonell, Monica Bulló, Rafael de la Torre, Olga Portolés, and Montserrat Fitó for the PREDIMED study investigators*

*Am J Clin Nutr 2013;98:845–53*

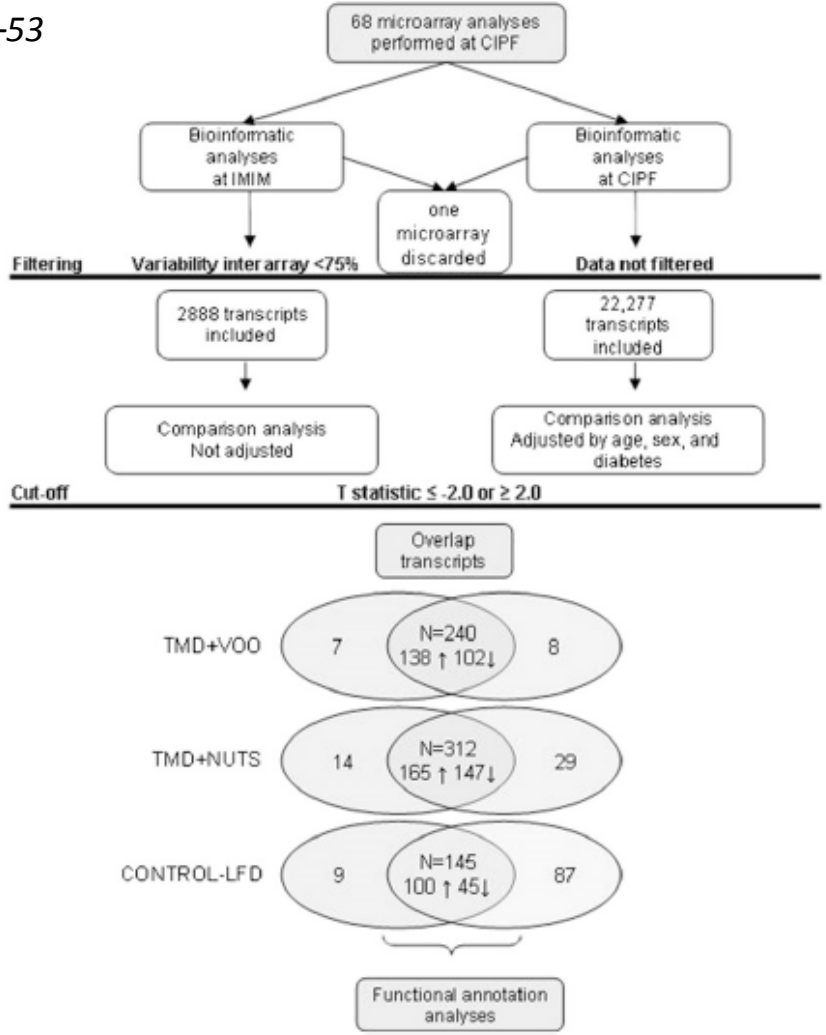
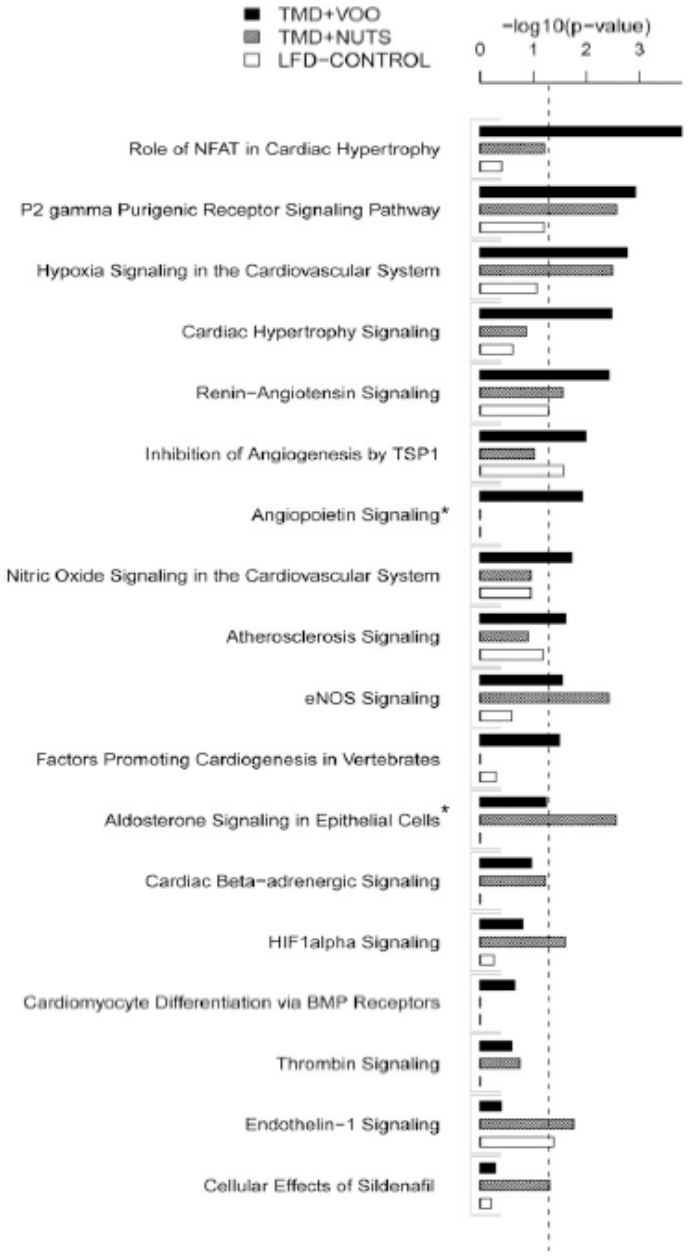



**FIGURE 1.** Flowchart of the 2 bioinformatics analysis approaches and procedures. CIPF, Príncipe Felipe Investigation Center; IMIM, Hospital del Mar Research Institute; LFD, low-fat diet; TMD+Nuts, traditional Mediterranean diet supplemented with nuts; TMD+VOO, traditional Mediterranean diet supplemented with virgin olive oil; ↓, downregulated; ↑, upregulated.

## TRANSCRIPTOMIC PROFILE AFTER A MEDITERRANEAN DIET




*Castagner et al. Am J Clin Nutr 2013;98:845–53*

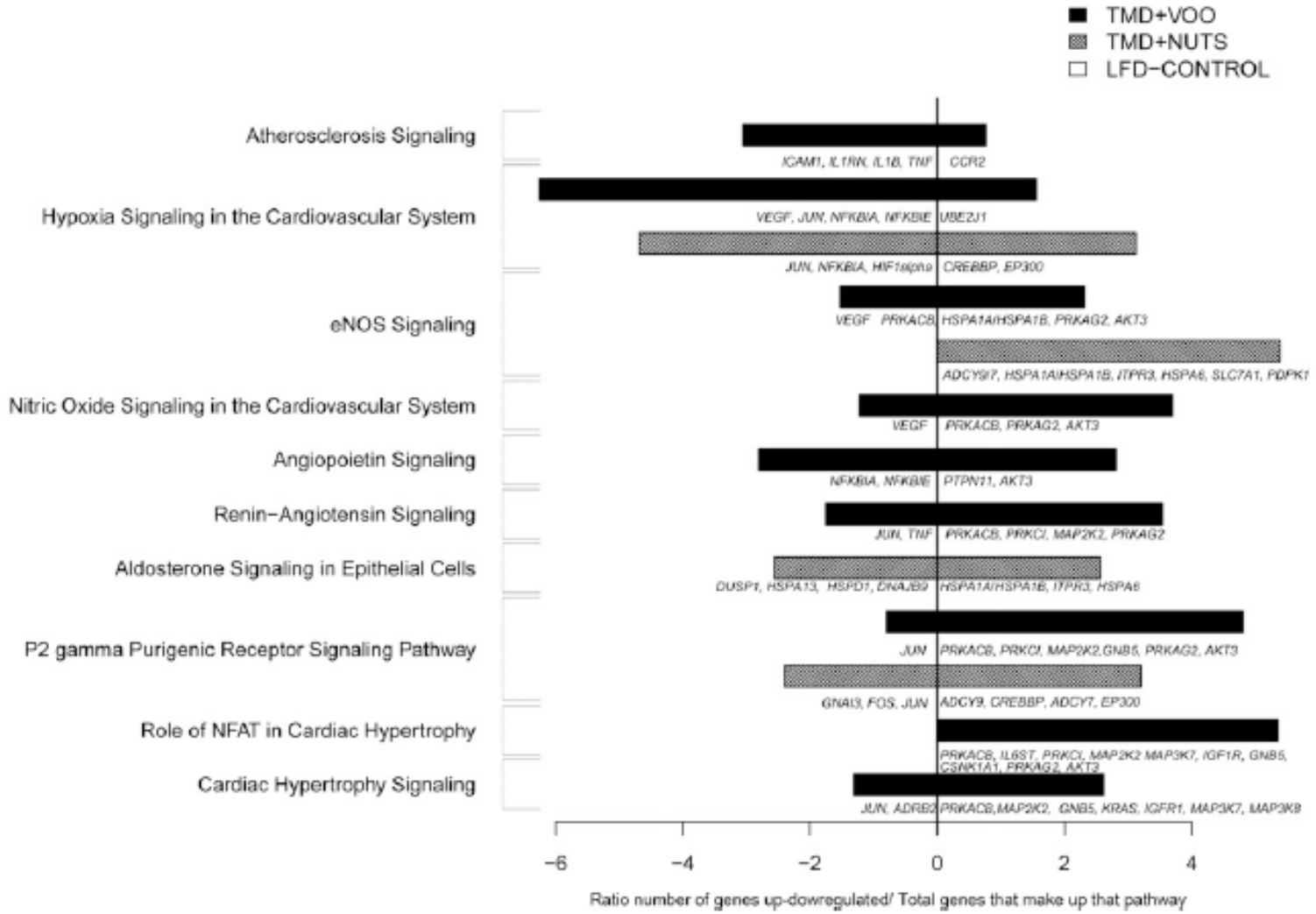


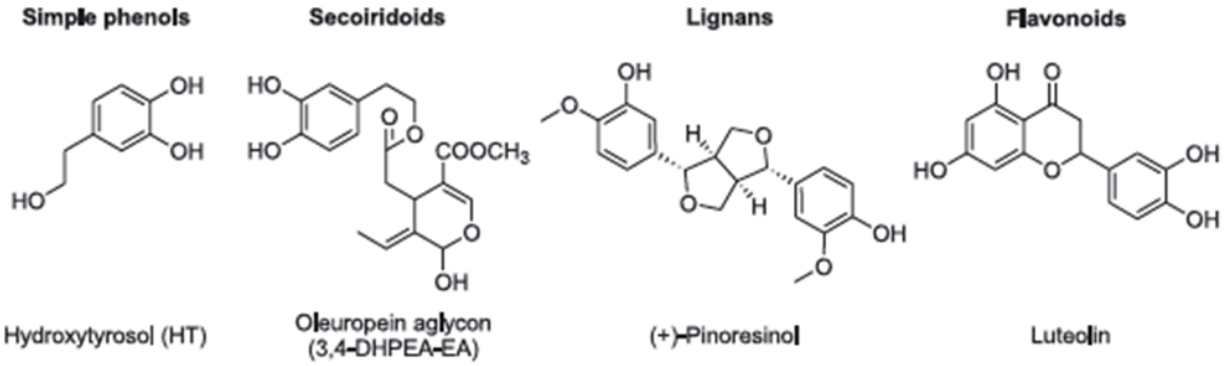

**FIGURE 4.** Cardiovascular canonical pathways significantly modulated after Benjamini-Hochberg correction and direction of the changes for the associated genes. TMD+VOO: $n = 11$; TMD+Nuts: $n = 11$; LFD, $n = 12$. eNOS, endothelial nitric oxide synthase; LFD, low-fat diet; NFAT, nuclear factor of activated T cells; TMD+Nuts, traditional Mediterranean diet supplemented with nuts; TMD+VOO, traditional Mediterranean diet supplemented with virgin olive oil.

Am J Clin Nutr 2013;98:845–53

- Elevato contenuto di MUFA
- Elevato contenuto di polifenoli

*Mol. Nutr. Food Res.* 2013, *57*, 760–771






Hydroxytyrosol (HT) | Oleuropein aglycon (3,4-DHPEA-EA) | (+)-Pinoresinol | Luteolin

**Figure 1.** Main classes of OOPC with representative compounds.

**Attività antiossidante**

# POLIFENOLI

- Stilbeni

- Acidi Fenolici
  - Acidi idrossicinnamici

  - Acidi idrossibenzoici

- Flavonoidi
  - Isoflavoni

  - Flavonoli

  - Antociani

  - Flavanoni, flavoni

  - Flavanoli
    - Catechine

    - Pro(anto)cianidine

# I Polifenoli

- **Sono assorbiti in quantità piuttosto bassa ed i loro livelli ematici sono molto più bassi di quelli di vitamine come ascorbato e tocoferoli**

- **Sono modificati durante i processi metabolici**

# Polifenoli

Antiossidanti e non solo….

## Attività biologiche

**Modulatori di** → **vie di segnale intracellulare**
**attività enzimatiche**
**recettoriale**

## Extra Virgin Olive Oil Biophenols Inhibit Cell-Mediated Oxidation of LDL by Increasing the mRNA Transcription of Glutathione-Related Enzymes[1]

R. Masella et al. J. Nutr 134: 785–791, 2004.

- Oleuropeina
- Acido protocatecuico

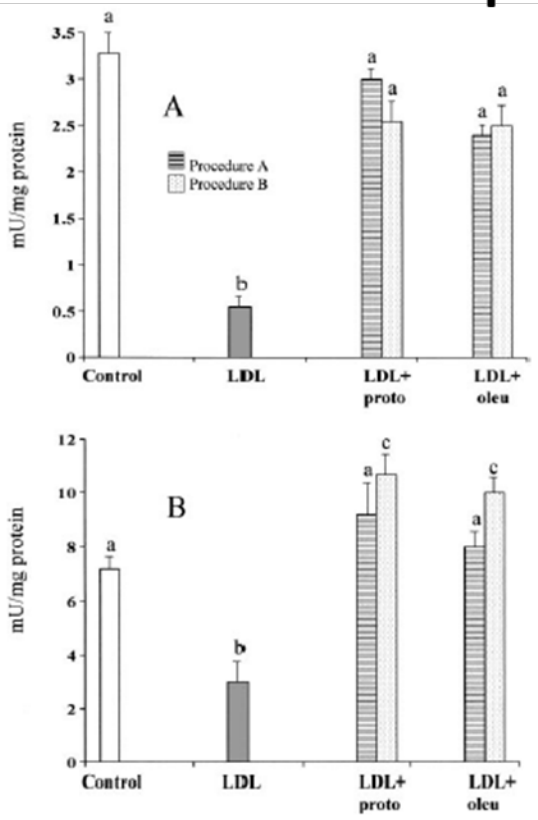
Macrofagi murini J774 A.1

I due polifenoli proteggono le LDL dall'ossidazione anche quando non sono presenti nel mezzo di coltura.

- **Diminuzione di radicali liberi prodotti**
- **Aumento del GSH**

**Polifenoli dell'olio di oliva innescano processi cellulari di difesa.**

# Espressione di enzimi di fase 2




GR

GPx

**FIGURE 2** Biophenols restore GR (*A*) and GPx (*B*) activities in J774 A.1 macrophage-like cells following both procedure A or procedure B. Activities were measured after a 24-h incubation with LDL (0.2 g protein/L). Values are means ± SEM, $n = 4$. Bars without a common letter differ, $P < 0.05$. LDL = cell exposed to LDL; LDL + proto = cell exposed to LDL and protocatechuic acid; LDL + oleu = cell exposed to LDL and oleuropein.

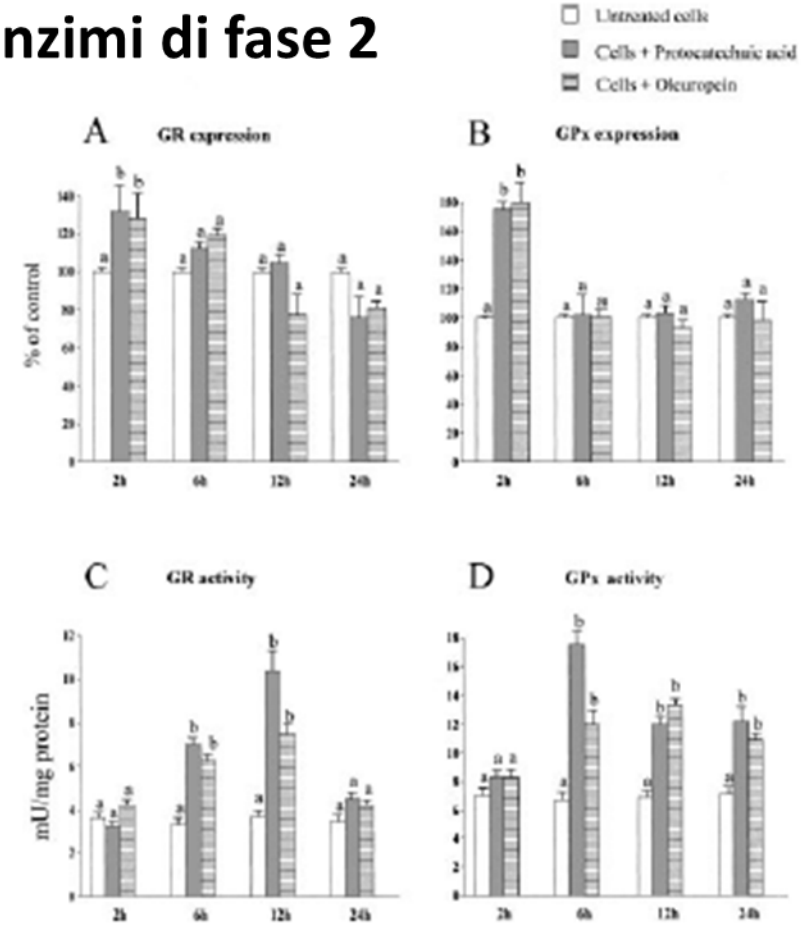
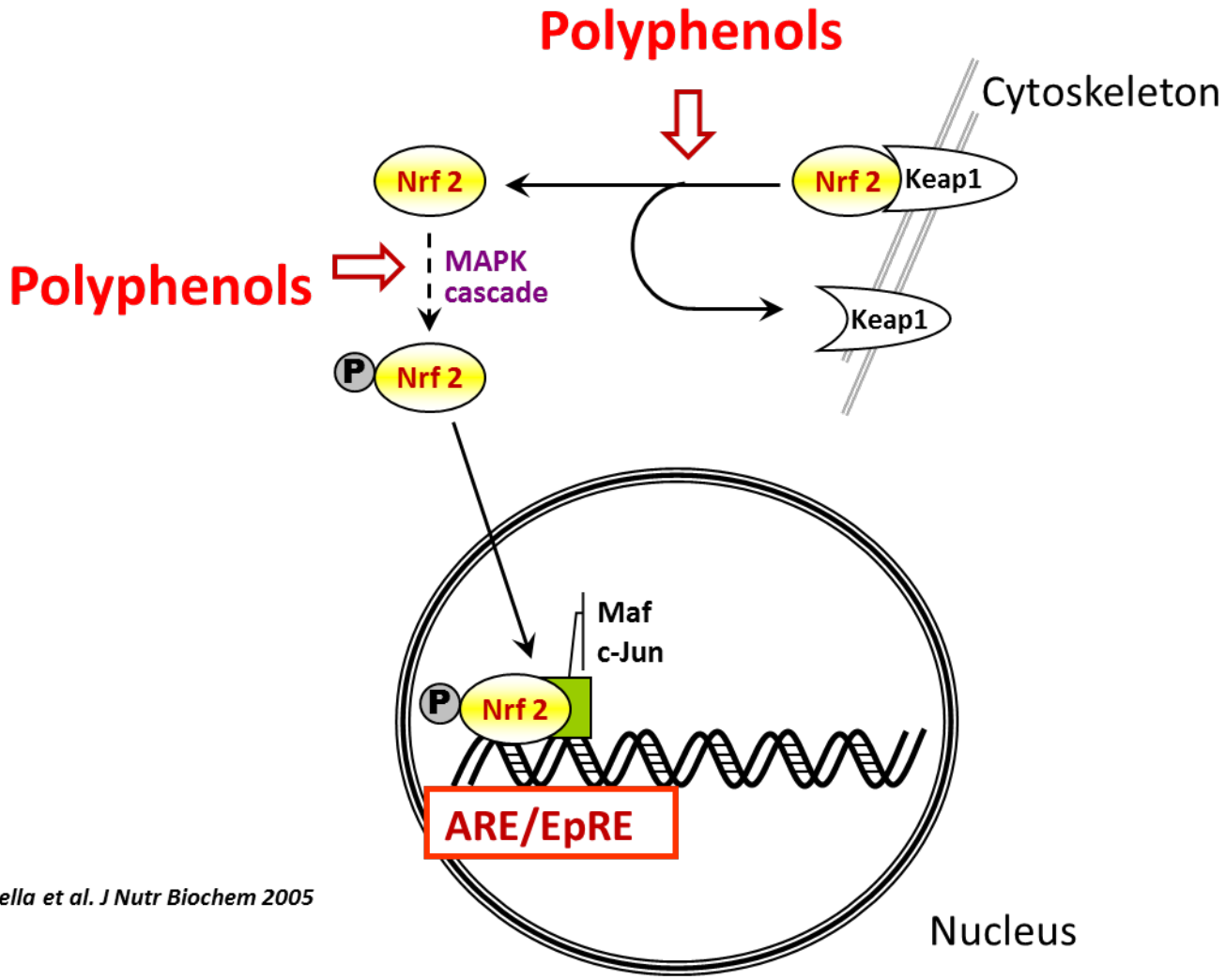
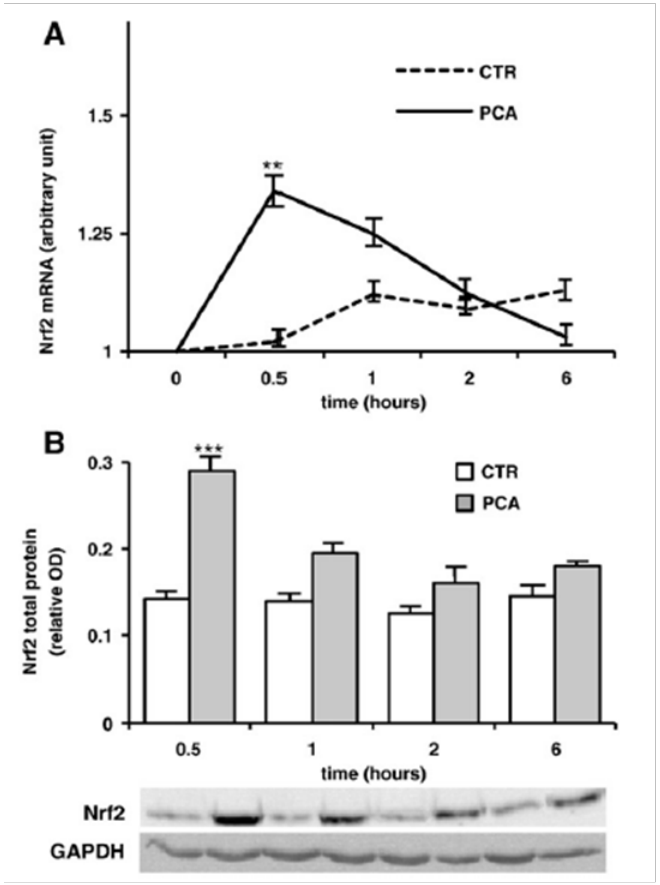


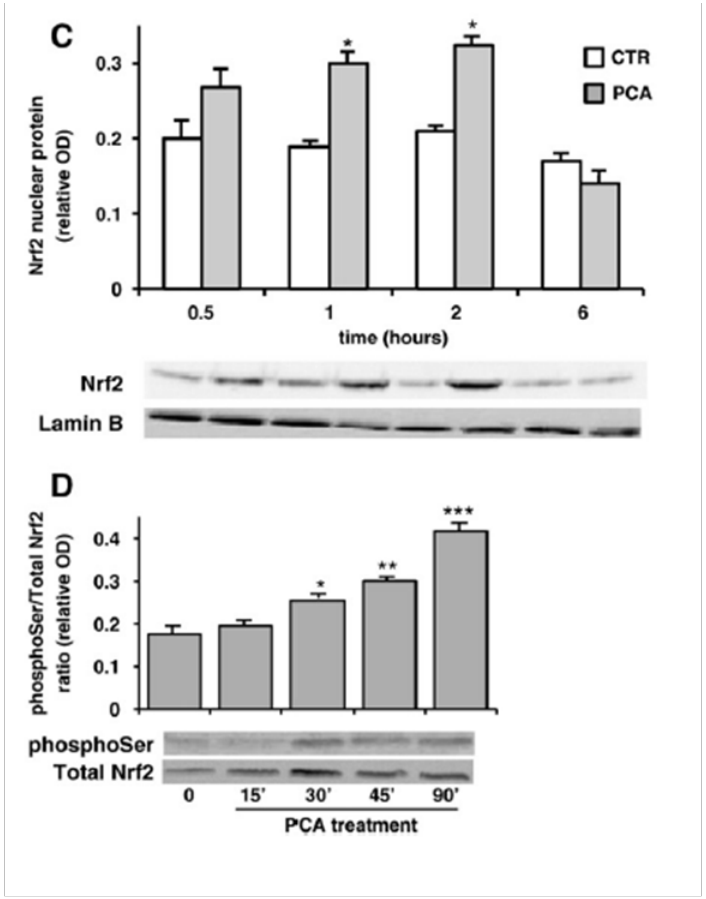

**FIGURE 4** Direct effect of the biophenols on DNA transcription of GSH-related enzymes in J774 A.1 cells incubated with protocatechuic acid and oleuropein following procedure B. (*A*) Semiquantitative RT-PCR time-course evaluation of mRNA for GR and GPx. (*B*) Time-course evaluation of GR and GPx activities. Values are means ± SEM, $n = 4$. Bars without a common letter differ, $P < 0.05$.

# Meccanismi di regolazione dell'espressione di enzimi di fase 2




*R. Masella et al. J Nutr Biochem 2005*




*R. Varì et al. J Nutr Biochem, 2011*

**Table 1.** Randomized, crossover, controlled studies on the antioxidant effect of sustained consumption of phenolic compounds from olive oil on *in vivo* markers of lipid and DNA oxidation

| | Olive oil intervention (time) | Daily olive oil dose | Subjects | Washout period | Oxidative markers | Effects |
|---|---|---|---|---|---|---|
| Vissiers *et al.* (2001) [79] | High-phenol vs Low-phenol (3 weeks) | 69 g ( in sauces, or baked products) | 46 healthy (31 women, 15 men) | 2 weeks without olives and olive oil | MDA, FRAP<br>LP, PC<br>LDL-resistance[a)] to oxidation | None |
| Moschandreas *et al.* (2002) [80] | High vs Low phenol (3 weeks) | 70 g raw | 25 healthy (14 women, 11 men) | 2 weeks without olives and olive oil | MDA, FRAP<br>LP, PC<br>LDL resistance[a)] to oxidation | None |
| Marrugat *et al.* (2004) [66] | Virgin vs Common vs Refined (3 weeks with refined olive oil for cooking) | 25 mL (22 g) raw | 30 healthy men | 2 weeks with refined olive oil for raw and cooking purposes | Plasma oxidized LDL<br>LDL resistance[a)] to oxidation<br>Antobodies against oxidized LDL<br>HDL-cholesterol | Decrease with olive oil phenolics<br><br>None<br><br>Increase after virgin olive oil |
| Weinbrenner *et al.* (2004) [68] | High vs Meium vs Low phenol (4 days with low phenolic olive oil for raw and cooking | 25 mL raw | 12 healthy men | 10 days: low phenol olive oil for raw and cooking; very-low antioxidant diet | Plasma oxidized LDL<br>MDA in urine<br>8oxodG in urine and lymphoctyes<br>$F_2$-isoprostanes<br>GSH-Px<br>HDL cholesterol | Decrease with olive oil phenolics<br><br><br>None<br>Increase with olive oil phenolics |
| Visioli *et al.* (2005) [81] | Virgin vs refined (raw) | 40 mL raw | 22 lipemic patients (12 men, 10 women) | 4 weeks with | Plasma antioxidant capacity<br>$F_2$-isoprostanes | Increase with olive oil phenolics<br>None |
| Fitó *et al.* (2005) [82] | Virgin vs Refined (raw) (3 weeks, refined olive oil for cooking) | 50 mL, raw | Coronary heart disease patiens (40 men) | 2 weeks withrefined olive oil for all purposes | Plasma oxidized LDL, LP<br>GSH-Px | Decrease with olive oil phenol ics<br>Increase with olive oil phenolics |
| Salvini *et al.* (2006) [103] | High vs Low (8 weeks) phenolics | *ad libitum* in substitution of other fats | 10 post-menopausal women | 2 weeks (usual diet) | Comet assay for DNA oxidation | Decrease with olive oil |
| Covas *et al.* (2006) [84] | Virgin vs Common vs Refined (3 weks) | 25 mL, raw | 200 healthy men | 2 weeks without olives and olive oil | Plasma oxidized LDL<br>Uninduced dienes<br>Hydroxy fatty acids<br>Antiboides against oxidizee LDL $F_2$-isoprostans | Decrease with olive oil phenolics<br><br><br>None |

ELSEVIER Journal of Nutritional Biochemistry 24 (2013) 1334–1339

# Olive oil polyphenols enhance the expression of cholesterol efflux related genes *in vivo* in humans. A randomized controlled trial

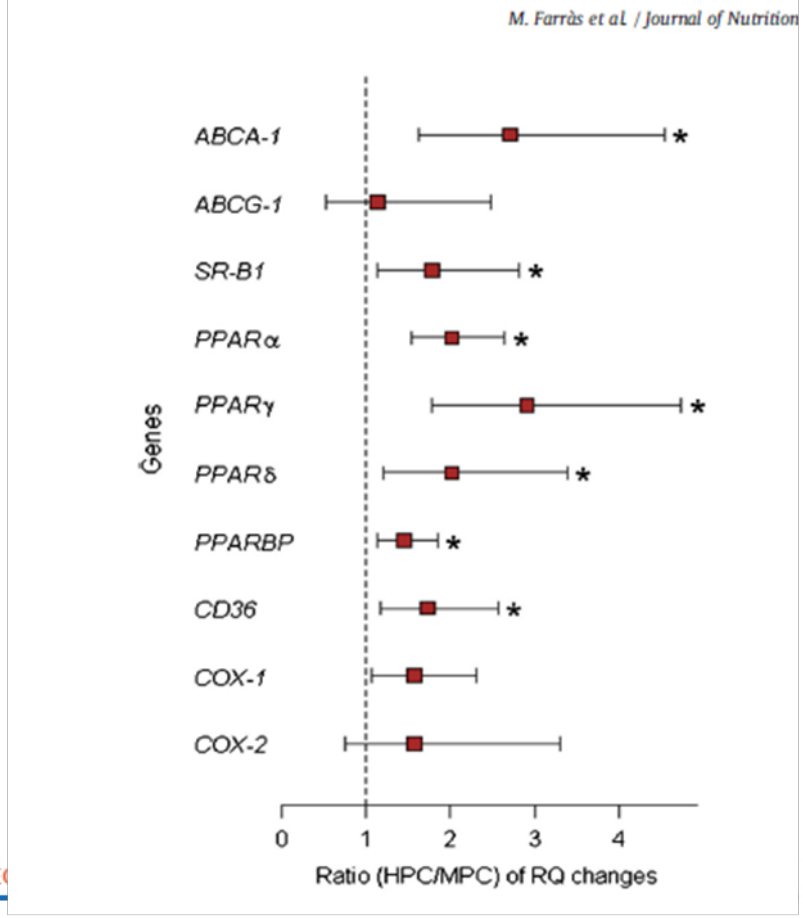
Marta Farràs[a,b], Rosa M. Valls[c], Sara Fernández-Castillejo[c], Montserrat Giralt[c], Rosa Solà[c], Isaac Subirana[d], María-José Motilva[e], Valentini Konstantinidou[c], María-Isabel Covas[a,*,1], Montserrat Fitó[a,*,1]

22 partecipanti ipertesi;
cross-over randomizzato; a doppio cieco.
30 ml di uno dei due olii di oliva :

- **MPC**= a medio contenuto di poilifenoli

- **HPC** = MPC arricchito con un estratto di polifenoli (7 mg /ml di olio)

Dopo 5 h **mRNA** dei geni responsabili del trasporto di colesterolo dalle cellule alle HDL nelle cellule bianche del sangue



# POLIFENOLI

- Stilbeni
- Acidi Fenolici
  - Acidi idrossicinnamici
  - Acidi idrossibenzoici
- Flavonoidi
  - Isoflavoni
  - Flavonoli
  - Antociani
  - Flavanoni, flavoni
  - Flavanoli
    - Catechine
    - Pro(anto)cianidine

# Dietary flavonoid intakes and risk of type 2 diabetes in US men and women[1–5]

*Nicole M Wedick, An Pan, Aedín Cassidy, Eric B Rimm, Laura Sampson, Bernard Rosner, Walter Willett, Frank B Hu, Qi Sun, and Rob M van Dam*

Am J Clin Nutr 2012;95:925–33.

**TABLE 2** (*Continued*)

| | Frequency of consumption | | | | | |
|---|---|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 | Q5 | *P*-trend |
| NHS II | | | | | | |
| Median value (mg/d) | 9.0 | 16.5 | 27.7 | 56.2 | 148.4 | |
| Cases/person-years | 784/274,916 | 510/279,651 | 510/281,582 | 575/279,240 | 705/277,424 | |
| Model 1[2] | 1.00 | 0.78 (0.70, 0.87) | 0.84 (0.75, 0.94) | 0.86 (0.78, 0.96) | 0.98 (0.88, 1.08) | 0.06 |
| Model 2[3] | 1.00 | 0.91 (0.81, 1.02) | 0.98 (0.87, 1.10) | 0.96 (0.86, 1.07) | 1.01 (0.91, 1.12) | 0.40 |
| HPFS | | | | | | |
| Median value (mg/d) | 9.0 | 16.7 | 25.4 | 43.9 | 103.9 | |
| Cases/person-years | 653/144,321 | 527/145,054 | 457/145,424 | 487/145,311 | 525/145,066 | |
| Model 1[2] | 1.00 | 0.84 (0.75, 0.94) | 0.76 (0.68, 0.86) | 0.82 (0.73, 0.93) | 0.85 (0.76, 0.96) | 0.25 |
| Model 2[3] | 1.00 | 0.90 (0.80, 1.02) | 0.85 (0.75, 0.96) | 0.91 (0.80, 1.02) | 0.88 (0.78, 0.99) | 0.22 |
| Pooled results[4] | | | | | | |
| Random-effects model | 1.00 | 0.92 (0.87, 0.98) | 0.91 (0.85, 0.98) | 0.94 (0.89, 0.99) | 0.91 (0.84, 1.00) | 0.32 |
| *P*-heterogeneity | — | 0.80 | 0.27 | 0.78 | 0.07 | 0.03 |
| Anthocyanins | | | | | | |
| NHS | | | | | | |
| Median value (mg/d) | 2.2 | 4.7 | 8.1 | 13.1 | 22.3 | |
| Cases/person-years | 1688/286,253 | 1513/303,189 | 1293/314,489 | 1251/314,333 | 1133/309,332 | |
| Model 1[2] | 1.00 | 0.87 (0.81, 0.93) | 0.75 (0.70, 0.81) | 0.75 (0.70, 0.80) | 0.69 (0.64, 0.74) | <0.001 |
| Model 2[3] | 1.00 | 0.93 (0.86, 0.99) | 0.84 (0.78, 0.91) | 0.85 (0.79, 0.92) | 0.83 (0.77, 0.90) | <0.001 |
| NHS II | | | | | | |
| Median value (mg/d) | 2.0 | 4.5 | 8.0 | 13.7 | 24.3 | |
| Cases/person-years | 898/270,677 | 702/277,111 | 513/281,465 | 515/281,334 | 456/282,225 | |
| Model 1[2] | 1.00 | 0.87 (0.79, 0.96) | 0.72 (0.64, 0.80) | 0.74 (0.66, 0.82) | 0.68 (0.61, 0.76) | <0.001 |
| Model 2[3] | 1.00 | 0.98 (0.88, 1.08) | 0.84 (0.75, 0.94) | 0.88 (0.79, 0.99) | 0.83 (0.73, 0.94) | 0.002 |
| HPFS | | | | | | |
| Median value (mg/d) | 2.3 | 4.9 | 8.3 | 14.0 | 24.2 | |
| Cases/person-years | 621/144,223 | 541/144,956 | 519/145,403 | 508/145,413 | 460/145,183 | |
| Model 1[2] | 1.00 | 0.90 (0.80, 1.01) | 0.88 (0.78, 0.99) | 0.87 (0.77, 0.98) | 0.80 (0.70, 0.90) | <0.001 |
| Model 2[3] | 1.00 | 0.95 (0.84, 1.06) | 0.96 (0.85, 1.08) | 0.95 (0.84, 1.07) | 0.93 (0.81, 1.05) | 0.34 |
| Pooled results[4] | | | | | | |
| Random-effects model | 1.00 | 0.94 (0.89, 0.99) | 0.87 (0.80, 0.94) | 0.88 (0.83, 0.94) | 0.85 (0.80, 0.91) | <0.001 |
| *P* for heterogeneity | — | 0.69 | 0.15 | 0.33 | 0.34 | 0.20 |
| Total flavonoids | | | | | | |

The Journal of Nutrition
Nutrition and Disease

J Nutr 2015;145:742–8.

# Purified Anthocyanin Supplementation Reduces Dyslipidemia, Enhances Antioxidant Capacity, and Prevents Insulin Resistance in Diabetic Patients[1–3]

**TABLE 2** Anthropometric data and lipid profiles of diabetic patients in the placebo and anthocyanin groups at baseline and after the 24-wk intervention[1]

| | Placebo | | Anthocyanin | | |
|---|---|---|---|---|---|
| | Baseline | 24 wk | Baseline | 24 wk | $P^2$ |
| Plasma anthocyanin (Cy3g and Dp3g), nmol/L | Not detectable | Not detectable | Not detectable | 9.37 ± 1.06* | <0.01 |
| Systolic blood pressure, mm Hg | 128 ± 10 | 129 ± 9 | 130 ± 13 | 126 ± 11 | 0.034 |
| Diastolic blood pressure, mm Hg | 81 ± 9 | 82 ± 7 | 82 ± 8 | 80 ± 10 | 0.16 |
| Serum total cholesterol, mmol/L | 5.03 ± 0.78 | 4.99 ± 0.86 | 5.07 ± 0.89 | 4.88 ± 0.94* | 0.041 |
| Serum LDL cholesterol, mmol/L | 3.19 ± 0.42 | 3.21 ± 0.48 | 3.17 ± 0.35 | 2.92 ± 0.54* | 0.030 |
| Serum HDL cholesterol, mmol/L | 0.98 ± 0.08 | 0.95 ± 0.07 | 1.03 ± 0.11 | 1.23 ± 0.12* | 0.012 |
| Serum TGs, mmol/L | 2.02 ± 0.36 | 1.96 ± 0.45 | 2.04 ± 0.41 | 1.57 ± 0.72** | <0.01 |
| Serum apo A-I, g/L | 1.35 ± 0.24 | 1.32 ± 0.36 | 1.33 ± 0.32 | 1.39 ± 0.43* | 0.13 |
| Serum apo B-48, mg/L | 0.95 ± 0.17 | 0.93 ± 0.22 | 0.97 ± 0.20 | 0.81 ± 0.27* | 0.017 |
| Serum apo B-100, g/L | 5.93 ± 1.44 | 5.85 ± 1.08 | 5.88 ± 1.37 | 5.66 ± 1.22 | 0.09 |
| Serum apo C-III, mg/L | 134 ± 15 | 136 ± 14 | 137 ± 18 | 122 ± 13* | <0.01 |
| Serum FFAs, mmol/L | 0.75 ± 0.19 | 0.77 ± 0.28 | 0.77 ± 0.16 | 0.73 ± 0.22 | 0.15 |

[1] Values are means ± SEMs, $n = 29$/group. No significant differences were found for any variable between the placebo and anthocyanin groups at baseline by unpaired Student's $t$ test. *,**Different from baseline: *$P < 0.05$, **$P < 0.01$. Cy3g, cyanidin-3-O-β-glucoside; Dp3g, delphinidin-3-O-β-glucoside.

[2] $P$ values for differences between placebo and anthocyanin groups after the 24-wk intervention.

**TABLE 3** Antioxidant capacity of diabetic patients in the placebo and anthocyanin groups at baseline and after the 24-wk intervention[1]

| | Placebo | | Anthocyanin | | |
|---|---|---|---|---|---|
| | Baseline | 24 wk | Baseline | 24 wk | $P^2$ |
| Plasma FRAP, mmol $Fe^{2+}$/L | 1.02 ± 0.13 | 1.04 ± 0.11 | 1.04 ± 0.08 | 1.35 ± 0.14* | 0.013 |
| Plasma TRAP, mmol/L | 1.09 ± 0.06 | 1.12 ± 0.08 | 1.07 ± 0.09 | 1.33 ± 0.10* | 0.017 |
| Plasma 8-iso-$PGF_{2\alpha}$, pmol/mL | 11.6 ± 2.78 | 11.4 ± 3.13 | 11.5 ± 3.55 | 8.73 ± 2.86** | <0.01 |
| Plasma 13-HODE, pmol/mL | 28.8 ± 4.87 | 27.9 ± 5.38 | 29.0 ± 6.25 | 20.7 ± 5.93** | <0.01 |
| Plasma carbonylated protein, nmol/mg | 0.68 ± 0.05 | 0.65 ± 0.03 | 0.67 ± 0.07 | 0.52 ± 0.03* | 0.022 |

[1] Values are means ± SEMs, $n = 29$/group. No significant differences were found for any variable between placebo and anthocyanin groups at baseline by unpaired Student's $t$ test. *,**Different from baseline: *$P < 0.05$, **$P < 0.01$. FRAP, ferric ion reducing antioxidant power; TRAP, total radical-trapping antioxidant parameter; 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin F2$\alpha$; 13-HODE, 13-hydroxyoctadecadienoic acid.
[2] $P$ values for differences between placebo and anthocyanin groups at 24 wk.

**TABLE 4** Serum adipokine and proinflammatory molecules in diabetic patients in the placebo and anthocyanin groups at baseline and after the 24-wk intervention[1]

| | Placebo | | Anthocyanin | | |
|---|---|---|---|---|---|
| | Baseline | 24 wk | Baseline | 24 wk | $P^2$ |
| BMI, kg/$m^2$ | 25.3 ± 2.5 | 25.4 ± 2.9 | 25.1 ± 2.7 | 25.0 ± 3.2 | 0.19 |
| Fat mass, % (body weight) | 35.2 ± 5.9 | 34.8 ± 5.3 | 35.4 ± 6.1 | 34.6 ± 6.5 | 0.13 |
| Fasting plasma glucose, mmol/L | 7.3 ± 1.7 | 7.1 ± 1.5 | 7.1 ± 2.2 | 6.5 ± 1.8* | 0.042 |
| Plasma insulin, mU/L | 11.6 ± 4.13 | 11.7 ± 3.76 | 11.9 ± 4.30 | 11.1 ± 3.98 | 0.14 |
| Plasma Hb $A_{1c}$, % | 6.6 ± 1.5 | 6.5 ± 1.4 | 6.5 ± 1.7 | 6.2 ± 1.9 | 0.06 |
| HOMA-IR | 3.76 ± 0.53 | 3.69 ± 0.64 | 3.74 ± 0.55 | 3.21 ± 0.76* | 0.035 |
| Serum adiponectin, μg/mL | 5.05 ± 0.79 | 5.09 ± 0.84 | 5.08 ± 0.92 | 6.28 ± 0.96** | <0.01 |
| Serum HMW adiponectin, μg/mL | 2.23 ± 0.56 | 2.16 ± 0.52 | 2.21 ± 0.67 | 3.26 ± 0.73** | <0.01 |
| HMW:total adiponectin ratio, % | 44.2 ± 6.52 | 42.6 ± 5.93 | 43.6 ± 6.79 | 51.9 ± 7.08* | 0.024 |
| Serum IL-6, pg/mL | 3.26 ± 0.57 | 3.18 ± 0.63 | 3.23 ± 0.49 | 2.21 ± 0.42** | 0.021 |
| Serum TNF-$\alpha$, pg/mL | 16.2 ± 2.35 | 15.9 ± 2.67 | 16.2 ± 2.58 | 14.8 ± 2.13* | 0.045 |
| Plasma $\beta$-hydroxybutyrate, mg/dL | 1.14 ± 0.37 | 1.18 ± 0.46 | 1.17 ± 0.42 | 1.68 ± 0.51** | 0.010 |

Nutrition, Metabolism and Cardiovascular Diseases

Volume 23, Issue 9, September 2013, Pages 843–849

# Anti-inflammatory effect of purified dietary anthocyanin in adults with hypercholesterolemia: A randomized controlled trial

Y. Zhu[a,b], W. Ling[a], H. Guo[c], F. Song[a], Q. Ye[a], T. Zou[d], D. Li[a], Y. Zhang[a,e], G. Li[a], Y. Xiao[a], F. Liu[a], Z. Li[a], Z. Shi[a], Y. Yang[a,]

150 soggetti, ipercolesterolemici,
320 mg/die antociani in capsule
+ dieta abituale

**Table 1** Changes in the lipids profile of the participants at baseline and at week 24 of the trial.[a]

| | Placebo ($n$ = 73) | | | Anthocyanin ($n$ = 73) | | | $P$-value[c] |
|---|---|---|---|---|---|---|---|
| | Basseline | 24 wk | Mean change, % (95%CI)[b] | Basseline | 24 wk | Mean change, % (95%CI) | |
| Total cholesterol (mmol/L) | 6.48 ± 0.84 | 6.25 ± 0.83 | −3.6 (−7.8–0.6) | 6.45 ± 1.02 | 6.18 ± 0.82 | −2.9 (−6.3–0.5) | 0.556 |
| HDL-cholesterol (mmol/L) | 1.24 ± 0.21 | 1.23 ± 0.20 | −0.9 (−5.2–3.4) | 1.22 ± 0.23 | 1.37 ± 0.22[d] | 14.0 (7.9–20.2)[e] | 0.036 |
| LDL-cholesterol (mmol/L) | 3.29 ± 0.47 | 3.30 ± 0.52 | 0.3 (−2.9–3.5) | 3.36 ± 0.58 | 3.01 ± 0.41[d] | −10.4 (−14.8 to −6.0)[e] | 0.030 |
| Triacylglycerol (mmol/L)[f] | 2.41 (1.47–2.70) | 2.34 (1.35–2.62) | −3.2 (−7.6–1.2) | 2.45 (1.53–2.74) | 2.35 (1.37–2.61) | −4.8 (−9.8–0.2) | 0.462 |

**Table 2** Changes in the inflammatory cytokines of the participants at baseline and at weeks 12 and 24 of the trial.[a]

| | Placebo ($n$ = 73) | | | | Anthocyanin ($n$ = 73) | | | | $P$-value[c] |
|---|---|---|---|---|---|---|---|---|---|
| | Basseline | 12 wk | 24 wk | Mean change, % (95%CI)[b] | Basseline | 12 wk | 24 wk | Mean change, % (95%CI)[b] | |
| hsCRP (mg/L)[d] | 2.26 (0.97–3.72) | 2.23 (1.08–3.76) | 2.19 (0.93–3.82) | −2.5 (−7.0–2.1) | 2.25 (1.06–4.25) | 1.95 (0.92–2.84)[e] | 1.74 (0.86–2.60)[e] | −21.6 (−37.5 to −5.7)[f] | 0.001 |
| sVCAM-1 (ng/mL) | 544.2 ± 107.8 | 546.3 ± 106.9 | 547.6 ± 109.5 | 0.4 (−4.6–5.4) | 542.9 ± 103.6 | 481.0 ± 91.8[e] | 478.7 ± 97.8[e] | −12.3 (−21.5 to −3.1)[f] | 0.005 |
| TNF-α (pg/mL) | 18.0 ± 6.0 | 19.1 ± 6.7 | 18.5 ± 5.4 | 2.8 (−3.4–9.1) | 18.7 ± 6.4 | 17.9 ± 5.1 | 18.4 ± 5.6 | −1.6 (−5.6–3.4) | 0.673 |
| IL-1β (pg/mL) | 4.77 ± 1.71 | 4.23 ± 0.91 | 4.71 ± 1.60 | −1.3 (−5.3–2.7) | 5.18 ± 2.11 | 4.62 ± 1.20[e] | 4.51 ± 1.60[e] | −12.8 (−24.4 to −1.2)[f] | 0.019 |

[a] hsCRP: high-sensitive C-reactive protein. sVCAM-1: soluble vascular adhesion molecule-1. TNF-α: tumor necrosis factor-alpha. The data, unless otherwise specified, were expressed as mean ± SD. No significant differences were found for any variable between the two groups at baseline via the unpaired Student's $t$ test.

[b] Calculated as (value at 24 wk − value at baseline)/value at baseline × 100.

[c] The effects of the intervention on these variables were tested by repeated-measures MANCOVA with the BMI and lipid profile (including HDL- and LDL-cholesterol, triacylglycerol and total cholesterol) values as covariates.

[d] Geometric mean; upper and lower quartiles in parentheses (all such values).

[e] $P < 0.05$ vs baseline, assessed by paired Student's $t$ tests.

[f] $P < 0.05$ vs percentage changes in the placebo group, assessed by unpaired Student's $t$ tests.

ORIGINAL ARTICLE

# Cyanidin-3-*O*-β-Glucoside and Protocatechuic Acid Exert Insulin-Like Effects by Upregulating PPARγ Activity in Human Omental Adipocytes

Beatrice Scazzocchio,[1] Rosaria Varì,[1] Carmelina Filesi,[1] Massimo D'Archivio,[1] Carmela Santangelo,[1] Claudio Giovannini,[1] Annunziata Iacovelli,[2] Gianfranco Silecchia,[3] Giovanni Li Volti,[4,5] Fabio Galvano,[4] and Roberta Masella[1]

*Diabetes, 2011; 60*

 DOI 10.1002/mnfr.201400816 *Mol. Nutr. Food Res.* 2015, *59*, 1472–1481

RESEARCH ARTICLE

# Protocatechuic acid activates key components of insulin signaling pathway mimicking insulin activity

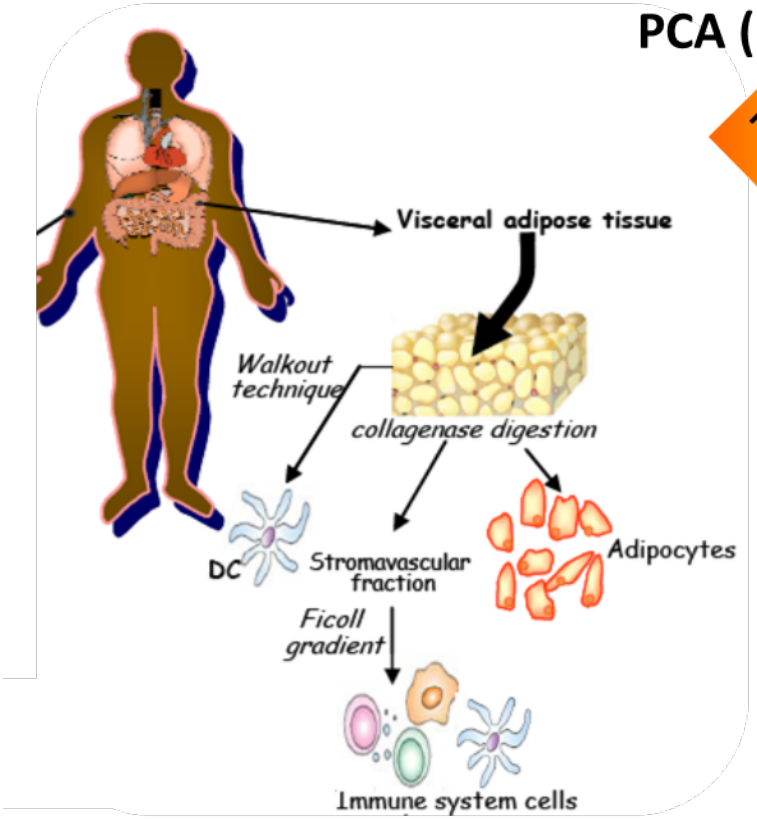
*Beatrice Scazzocchio[1], Rosaria Varì[1], Carmelina Filesi[1], Ilaria Del Gaudio[1], Massimo D'Archivio[1], Carmela Santangelo[1], Annunziata Iacovelli[2], Fabio Galvano[3], Francesca Romana Pluchinotta[4], Claudio Giovannini[1] and Roberta Masella[1]*

*Research Article*

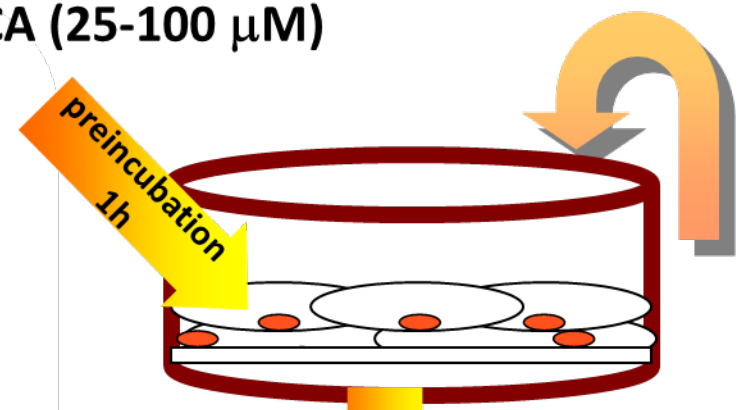
Oxidative Medicine and Cellular Longevity. Volume 2015, Article ID 351827

# Protocatechuic Acid Prevents oxLDL-Induced Apoptosis by Activating JNK/Nrf2 Survival Signals in Macrophages

Rosaria Varì,[1] Beatrice Scazzocchio,[1] Carmela Santangelo,[1] Carmelina Filesi,[1] Fabio Galvano,[2] Massimo D'Archivio,[1] Roberta Masella,[1] and Claudio Giovannini[1]




## PCA (25-100 μM)

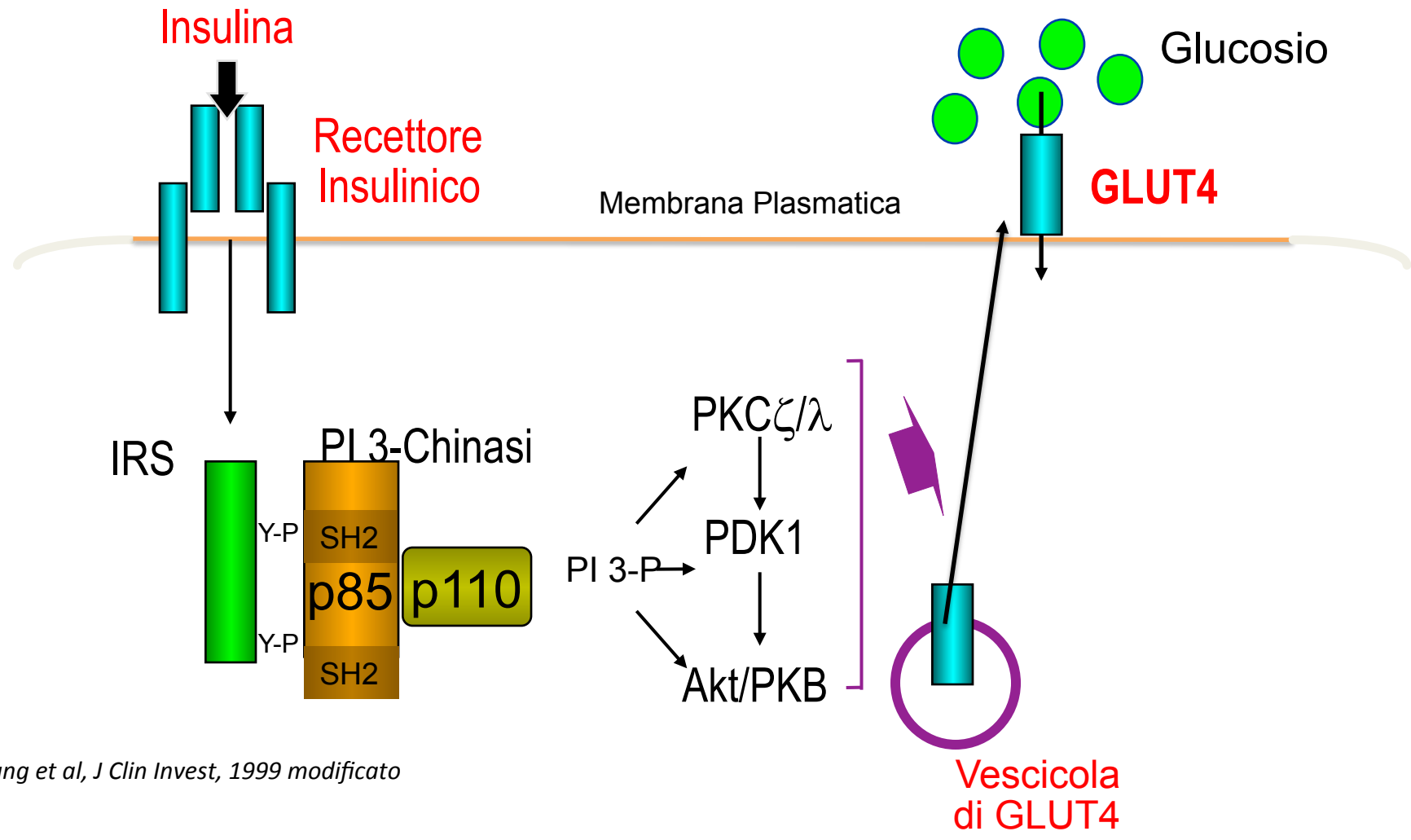



Incubation 1-18h

**Washing**
**(2 x PBS/0.4% BSA)**
**(2 x PBS)**

**Cells collecting** → **Western Blot RNA extraction**

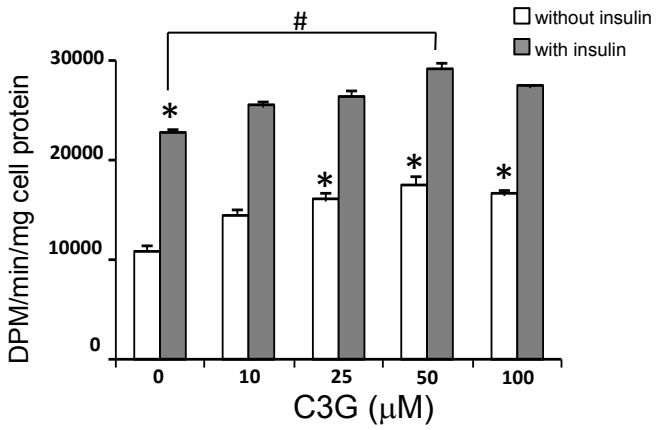
# Insulina e uptake del glucosio

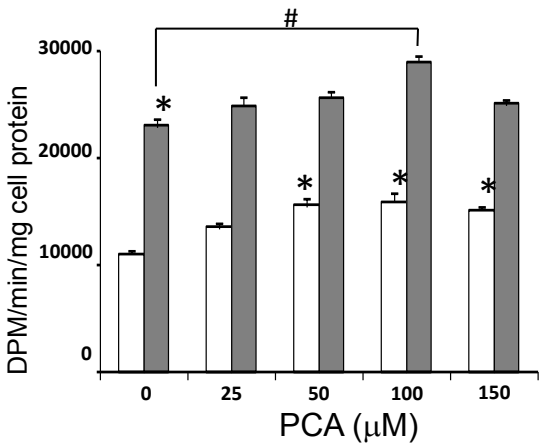



*Jiang et al, J Clin Invest, 1999 modificato*

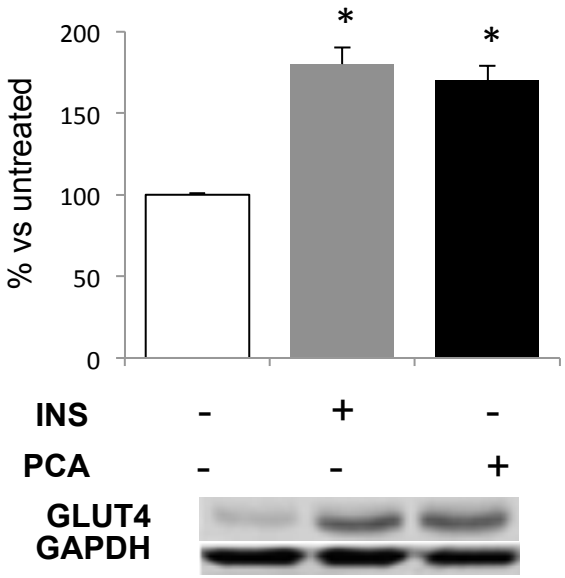
# C3G e PCA hanno un'azione insulino-mimetica

## Uptake del glucosio

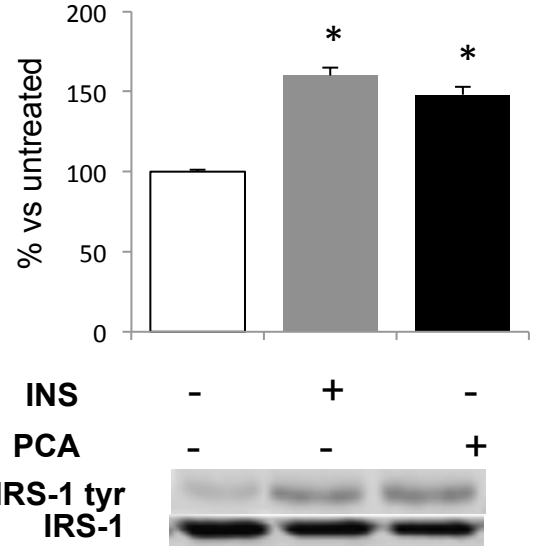



## Translocazione del GLUT4



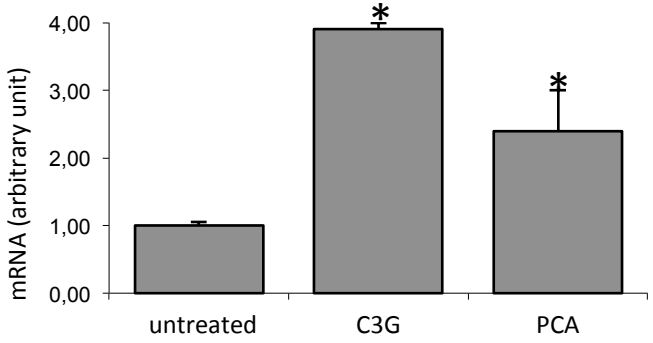

## IRS-1 attivato

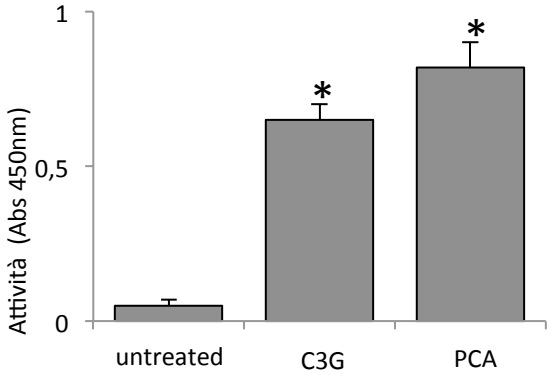



B Scazzocchio et al. Diabetes 60:2234–2244, 2011

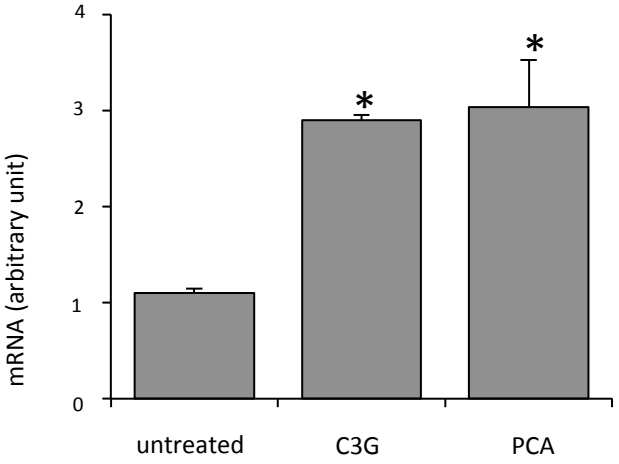
# L'attività insulino-mimetica di C3G e PCA è mediata dall'aumento dell'espressione e dell' attività di PPARY e di adiponectina

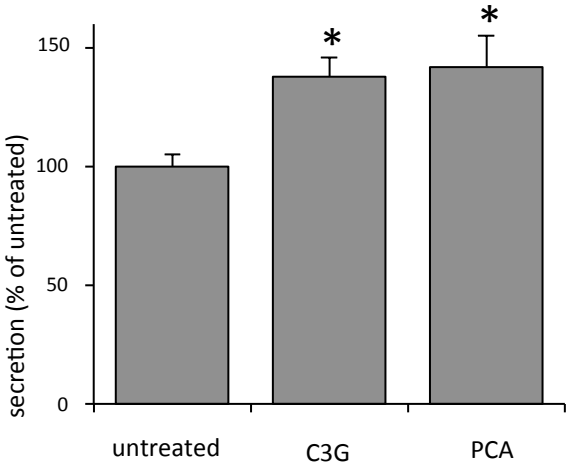
## PPARγ



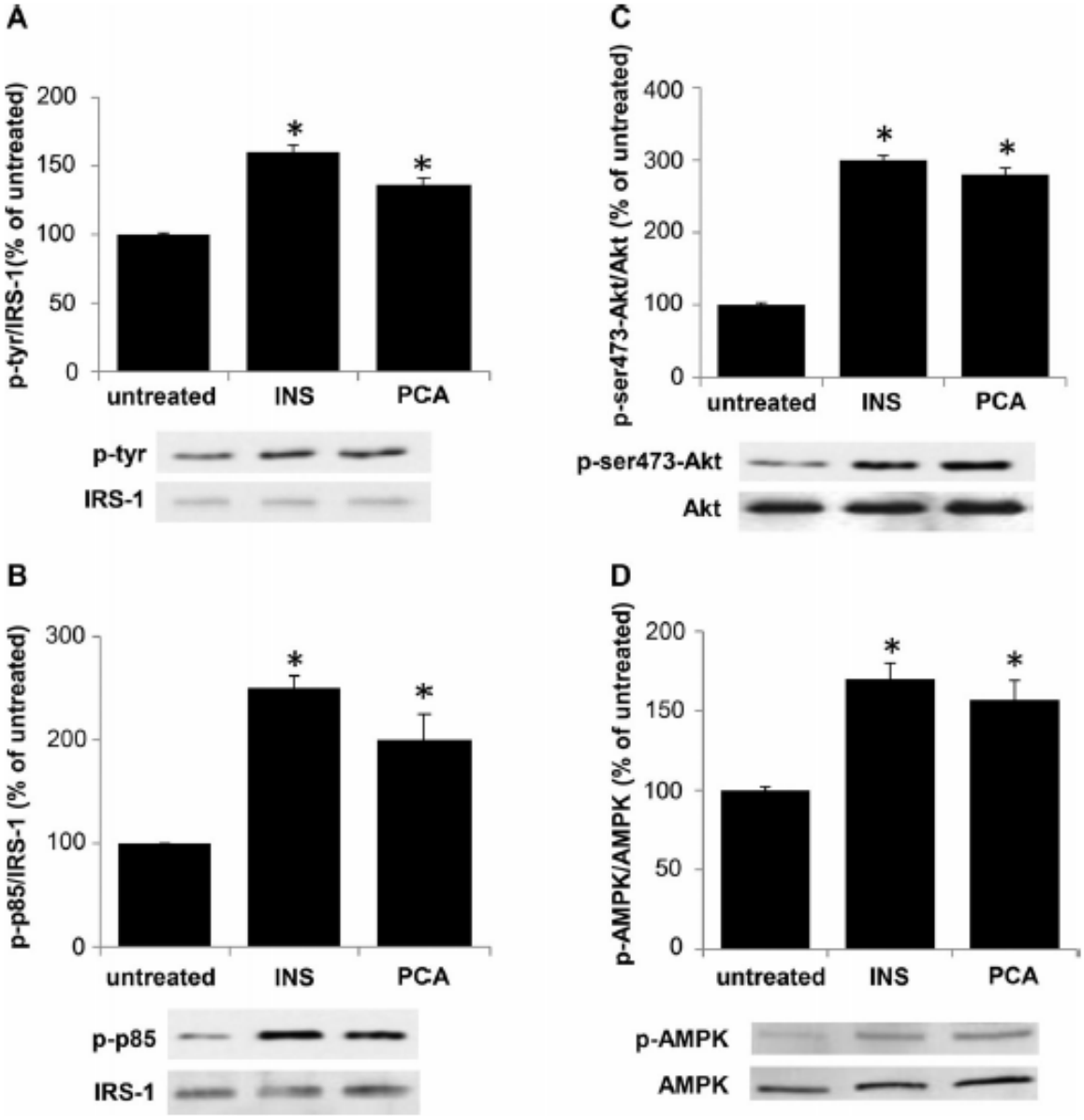

## ADIPONECTINA



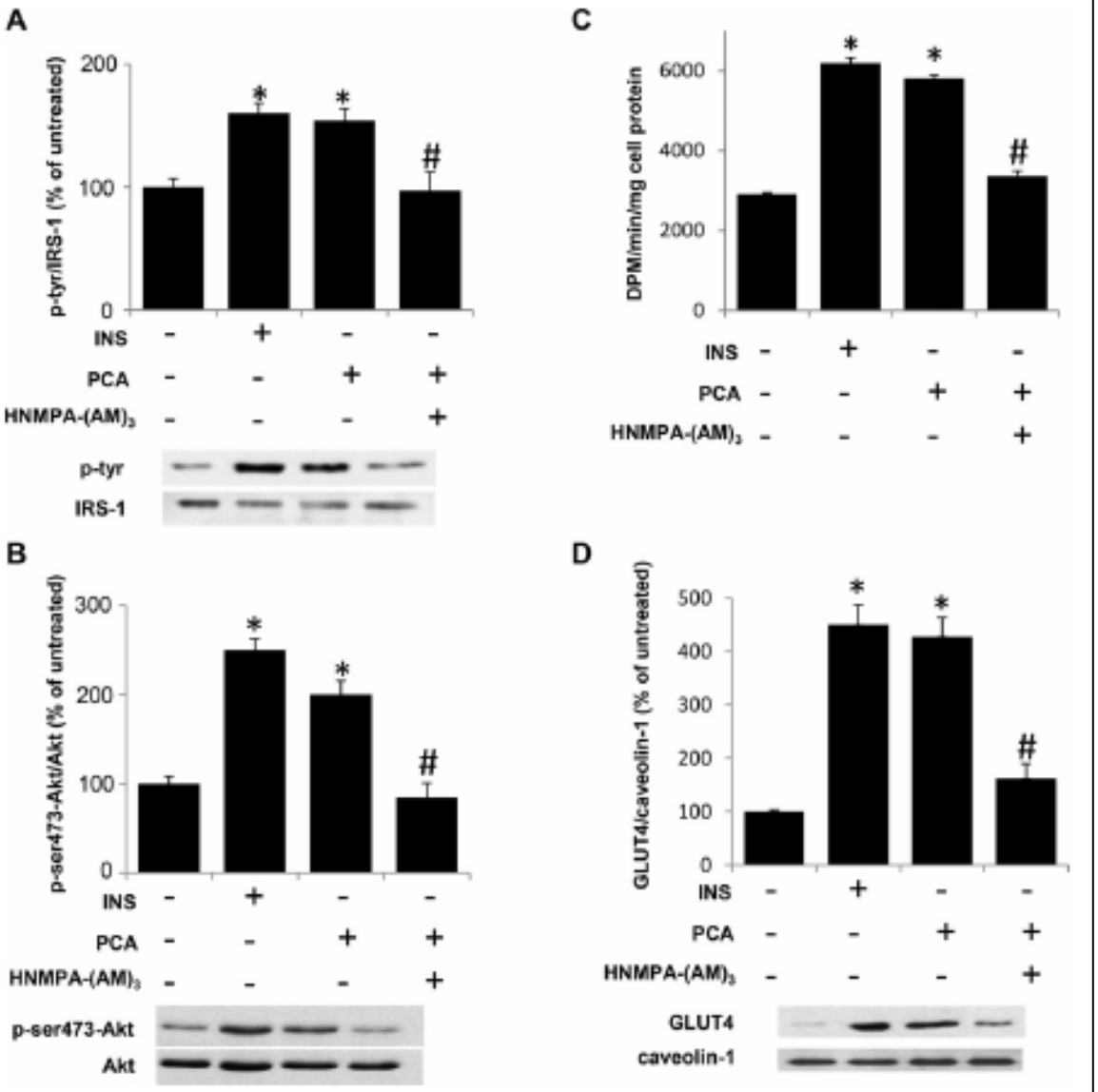

*B.Scazzocchio et al. Diabetes 60:2234–2244, 2011*

*Mol. Nutr. Food Res.* 2015, *59*, 1472–1481




# PCA attiva i componenti della via di segnale insulinico

*Mol. Nutr. Food Res.* 2015, *59*, 1472–1481




# PCA esercita i suoi effetti attraverso il recettore per l'insulina

# Grazie per l'attenzione