3° CONGRESSO NAZIONALE SIMPeSV / 70° Congresso FIMMG

DALLA MEDICINA DI PREVENZIONE ALL'AMBULATORIO DEGLI STILI DI VITA

Nutraceutica, Alimenti Funzionali e Integratori: Risorsa e Strumento della Medicina

NUTRACEUTICA E NUTRIGENOMICA

Roberta Masella
Istituto Superiore di Sanità
Roma

6 – 11 ottobre 2014
Santa Margherita di Pula (CA) - Forte Village

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

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Scuola Nazionale di Medicina degli Stili di Vita

Nutrizione **Farmaceutica**

Nutraceutica

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

“ Un alimento può essere definito ‘ funzionale’ se è dimostrata in maniera soddisfacente la sua capacità di influenzare positivamente una o più funzioni fondamentali dell’organismo, al di là degli effetti strettamente nutrizionali, in modo che determini un miglioramento dello stato di salute e di benessere e/o una riduzione del rischio di malattia.
Gli alimenti funzionali devono rimanere alimenti e devono dimostrare i loro effetti in concentrazioni comparabili a quelle normalmente assunte con la dieta:
non sono pillole né capsule ma parti di un normale regime dietetico.»

The European Commission's Concerted Action on Functional Food Science in Europe (FuFoSE), coordinated by International Life Science Institute (ILSI) Europe

« Alimenti nei quali alcuni componenti sono stati aggiunti, eliminati o modificati»

Scientific concept of functional foods in Europe Consensus document
The British Journal of Nutrition, 81 (1999), pp. S1–S27

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

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

Alimenti funzionali

Table 1

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

Functional food	Bioactive component (nutraceutic)		Source (s)
Micronutrients	Vitamins	Retinol (vitamin A) α-tocopherol (vitamin E) Calciferol (vitamin D3)	Walnuts, almonds, hazelnuts, spinach, fish oil
	Polyunsaturated fatty acids (PUFAs)	Omega 3 Fatty acids: eicosapentaenoic acid (EPA) docosahexaenoic acid (DHA)	Salmon, tuna and others fish oils
Nonnutrients Phytochemicals	Carotenoids	Beta-carotene luteín, zeaxanthin lycopene	Carrots, pumpkin, collards, kale, spinach, tomatoes, watermelon
	Phenolic acid derivatives	Caffeic acid Ferulic acid Gallic acid Curcumin	Coffee, pears, apples, corn, curcumin, vanilla
	Flavonoids	Flavonols (quercetin) Isoflavones Coumarins Anthocyanidines Stilbenes (resveratrol)	Berries, cherries, red grapes, tea, cocoa, apples, citrus fruits, onion, broccoli, cranberries, strawberries, soybeans
	Sulfides/thiols	Diallyl sulfide S-allyl cysteine sulfoxide 1,2-viniyldithiin	Garlic, onions, banana, cruciferous vegetables
	Dietary fiber (prebiotic)	Fructooligosaccharides Neoglicans	Whole grains, onions, chicory, agave, some fruits
Probiotics	PUFAs induction	*Saccharomyces cerevisiae* (var. *boulardii*) Bifidobacteria and *Lactobacillus* genus *Escherichia coli* strain Nissle1917 (EcN) Compound VSL3	Certain yogurts and other cultured dairy and no-dairy applications

- Increased consumption of fruit and vegetables for the primary prevention of cardiovascular diseases. **Cochrane Database Syst Rev. 2013.**
- Health benefits of fruit and vegetables. **Adv Nutr. 2012;** 3:506-16
- Population-level changes to promote cardiovascular health(on behalf of the PEP section of the EACPR). **Eur J Prev Cardiol. 2013**; 20(3):409-21
- Intake of fruit, berries, and vegetables and risk of type 2 diabetes in Finnish men: the Kuopio Ischaemic Heart Disease Risk Factor Study. **Am J Clin Nutr. 2014** doi: 10.3945/ajcn.113.069641.
- Systematic review and meta-analysis of school-based interventions to improve daily fruit and vegetable intake in children aged 5 to 12 y. **Am J Clin Nutr. 2012**;96(4):889-901.
- Health effects of mixed fruit and vegetable concentrates: a systematic review of the clinical investigations. **J Am Coll Nutr. 2011**; 30(5):285-94.
- Conformity to traditional Mediterranean diet and cancer incidence: the Greek EPIC cohort. **Br J Cancer 2008**; 99:191-5

Public Health Nutr. 2013 Nov 29:1-14. [Epub ahead of print]

Mediterranean diet and health status: an updated meta-analysis and a proposal for a literature-based adherence score.

Sofi F[1], Macchi C[2], Abbate R[1], Gensini GF[1], Casini A[1].

Author information

Abstract

OBJECTIVE: To update previous meta-analyses of cohort studies that investigated the association between the Mediterranean diet and health status and to utilize data coming from all of the cohort studies for proposing a literature-based adherence score to the Mediterranean diet.

DESIGN: We conducted a comprehensive literature search through all electronic databases up to June 2013.

SETTING: Cohort prospective studies investigating adherence to the Mediterranean diet and health outcomes. Cut-off values of food groups used to compute the adherence score were obtained.

SUBJECTS: The updated search was performed in an overall population of 4 172 412 subjects, with eighteen recent studies that were not present in the previous meta-analyses.

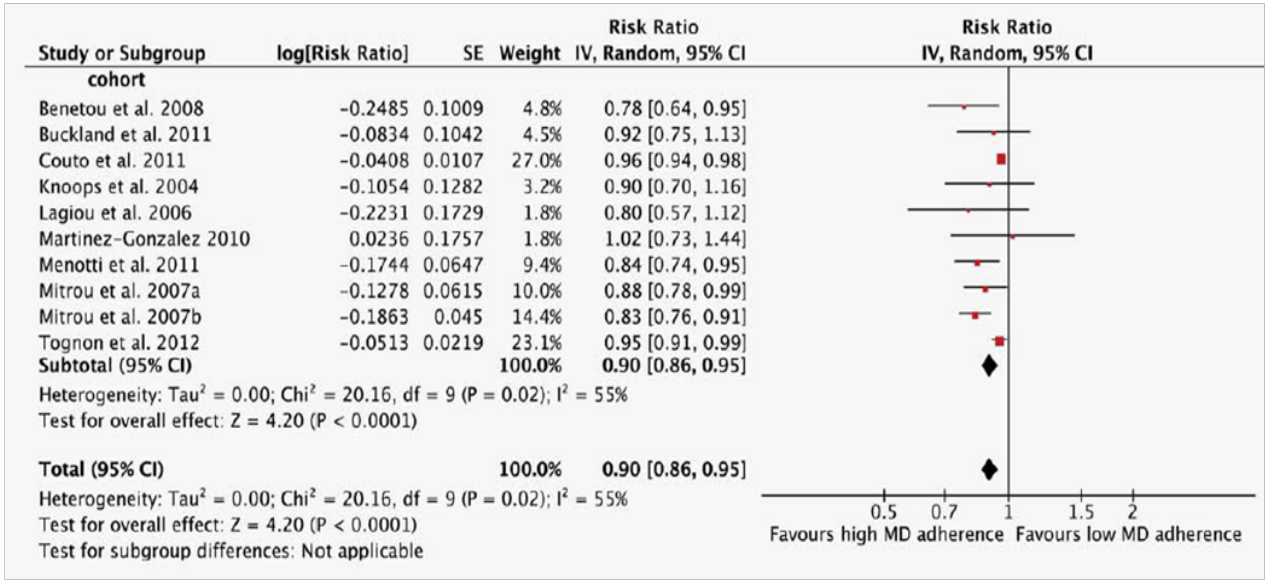
RESULTS: A 2-point increase in adherence score to the Mediterranean diet was reported to determine an 8 % reduction of overall mortality (relative risk = 0·92; 95 % CI 0·91, 0·93), a 10 % reduced risk of CVD (relative risk = 0·90; 95 % CI 0·87, 0·92) and a 4 % reduction of neoplastic disease (relative risk = 0·96; 95 % CI 0·95, 0·97). We utilized data coming from all cohort studies available in the literature for proposing a literature-based adherence score. Such a score ranges from 0 (minimal adherence) to 18 (maximal adherence) points and includes three different categories of consumption for each food group composing the Mediterranean diet.

CONCLUSIONS: The Mediterranean diet was found to be a healthy dietary pattern in terms of morbidity and mortality. By using data from the cohort studies we proposed a literature-based adherence score that can represent an easy tool for the estimation of adherence to the Mediterranean diet also at the individual level.

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



Study or Subgroup	log[Risk Ratio]	SE	Weight	Risk Ratio IV, Random, 95% CI
cohort				
Benetou et al. 2008	-0.2485	0.1009	4.8%	0.78 [0.64, 0.95]
Buckland et al. 2011	-0.0834	0.1042	4.5%	0.92 [0.75, 1.13]
Couto et al. 2011	-0.0408	0.0107	27.0%	0.96 [0.94, 0.98]
Knoops et al. 2004	-0.1054	0.1282	3.2%	0.90 [0.70, 1.16]
Lagiou et al. 2006	-0.2231	0.1729	1.8%	0.80 [0.57, 1.12]
Martinez-Gonzalez 2010	0.0236	0.1757	1.8%	1.02 [0.73, 1.44]
Menotti et al. 2011	-0.1744	0.0647	9.4%	0.84 [0.74, 0.95]
Mitrou et al. 2007a	-0.1278	0.0615	10.0%	0.88 [0.78, 0.99]
Mitrou et al. 2007b	-0.1863	0.045	14.4%	0.83 [0.76, 0.91]
Tognon et al. 2012	-0.0513	0.0219	23.1%	0.95 [0.91, 0.99]
Subtotal (95% CI)			**100.0%**	**0.90 [0.86, 0.95]**
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 20.16$, df = 9 (P = 0.02); $I^2 = 55\%$				
Test for overall effect: Z = 4.20 (P < 0.0001)				
Total (95% CI)			**100.0%**	**0.90 [0.86, 0.95]**
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 20.16$, df = 9 (P = 0.02); $I^2 = 55\%$				
Test for overall effect: Z = 4.20 (P < 0.0001)				
Test for subgroup differences: Not applicable				

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**.

J Alzheimers Dis. 2014;39(2):271-82. doi: 10.3233/JAD-130830.

Association of mediterranean diet with mild cognitive impairment and Alzheimer's disease: a systematic review and meta-analysis.

Singh B[1], Parsaik AK[2], Mielke MM[3], Erwin PJ[4], Knopman DS[2], Petersen RC[5], Roberts RO[5].

Author information

Abstract

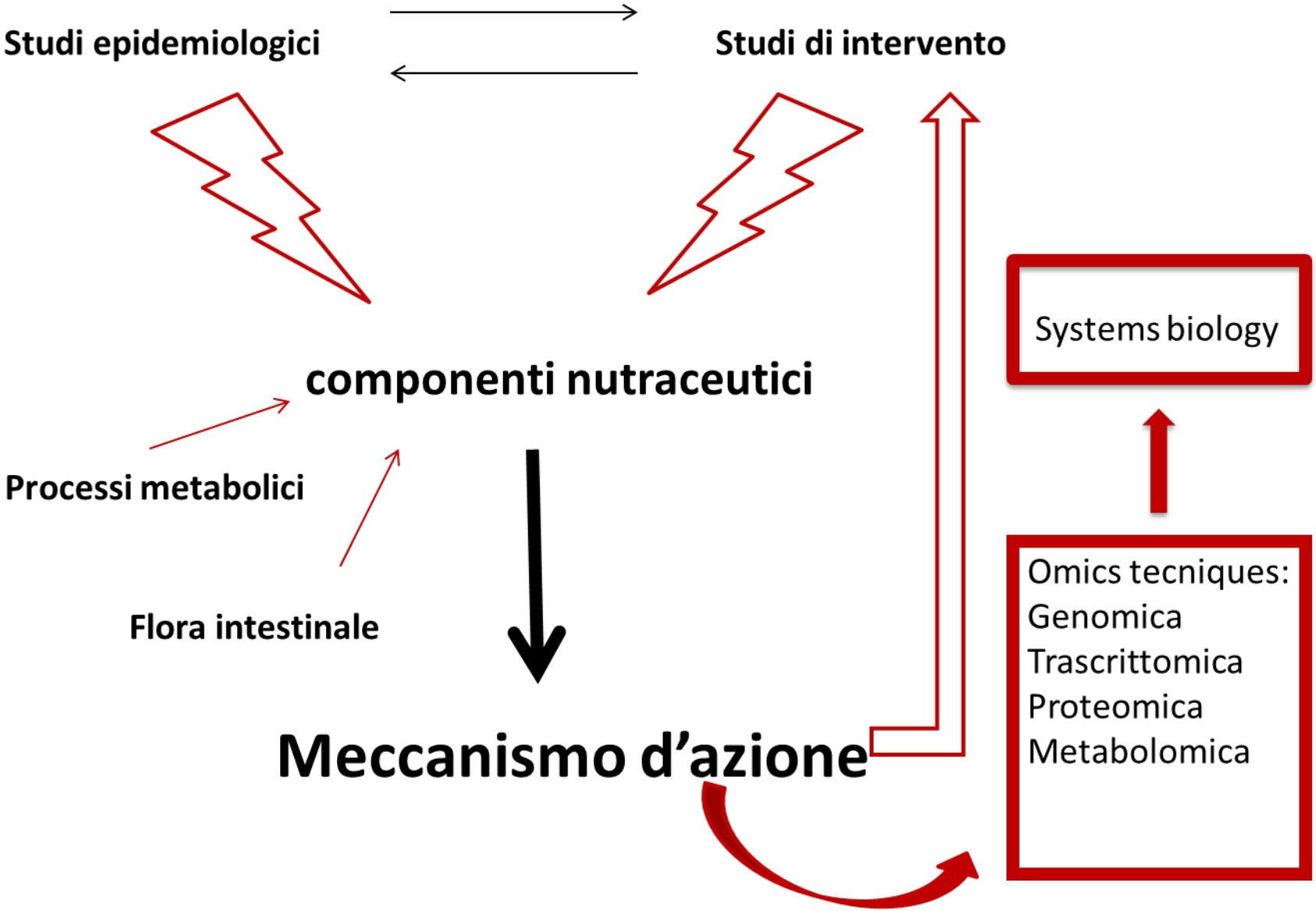
BACKGROUND/OBJECTIVE: To conduct a systematic review of all studies to determine whether there is an association between the Mediterranean diet (MeDi) and cognitive impairment.

METHODS: We conducted a comprehensive search of the major databases and hand-searched proceedings of major neurology, psychiatry, and dementia conferences through November 2012. Prospective cohort studies examining the MeDi with longitudinal follow-up of at least 1 year and reporting cognitive outcomes (mild cognitive impairment [MCI] or Alzheimer's disease [AD]) were included. The effect size was estimated as hazard-ratio (HR) with 95% confidence intervals (CIs) using the random-effects model. Heterogeneity was assessed using Cochran's Q-test and I2-statistic.

RESULTS: Out of the 664 studies screened, five studies met eligibility criteria. Higher adherence to the MeDi was associated with reduced risk of MCI and AD. The subjects in the highest MeDi tertile had 33% less risk (adjusted HR = 0.67; 95% CI, 0.55-0.81; $p < 0.0001$) of cognitive impairment (MCI or AD) as compared to the lowest MeDi score tertile. Among cognitively normal individuals, higher adherence to the MeDi was associated with a reduced risk of MCI (HR = 0.73; 95% CI, 0.56-0.96; $p = 0.02$) and AD (HR = 0.64; 95% CI, 0.46-0.89; $p = 0.007$). There was no significant heterogeneity in the analyses.

CONCLUSIONS: While the overall number of studies is small, pooled results suggest that a higher adherence to the MeDi is associated with a reduced risk of developing MCI and AD, and a reduced risk of progressing from MCI to AD. Further prospective-cohort studies with longer follow-up and randomized controlled trials are warranted to consolidate the evidence. Systematic review registration number: PROSPERO 2013: CRD42013003868.

KEYWORDS: Alzheimer's disease; Mediterranean diet; meta-analysis; mild cognitive impairment; systematic review


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

- Può l'espressione genica in risposta a processi metabolici influenzare lo stato di salute di un individuo?

- La relazione tra risposta metabolica e espressione genica può essere il risultato delle interazioni fra genotipo e nutrienti/ambiente?

- La comprensione di queste interazioni può portare alla prescrizione di diete specifiche per ogni individuo?

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.

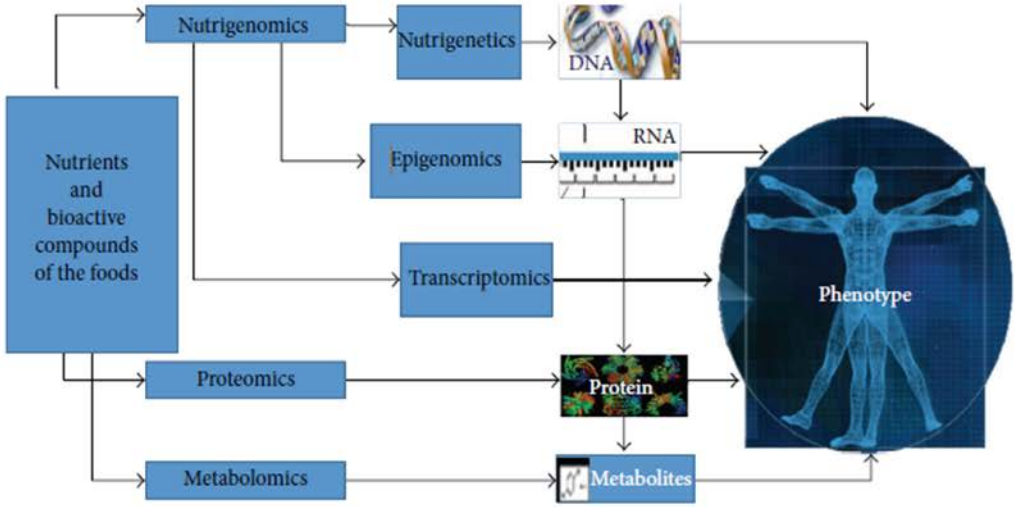
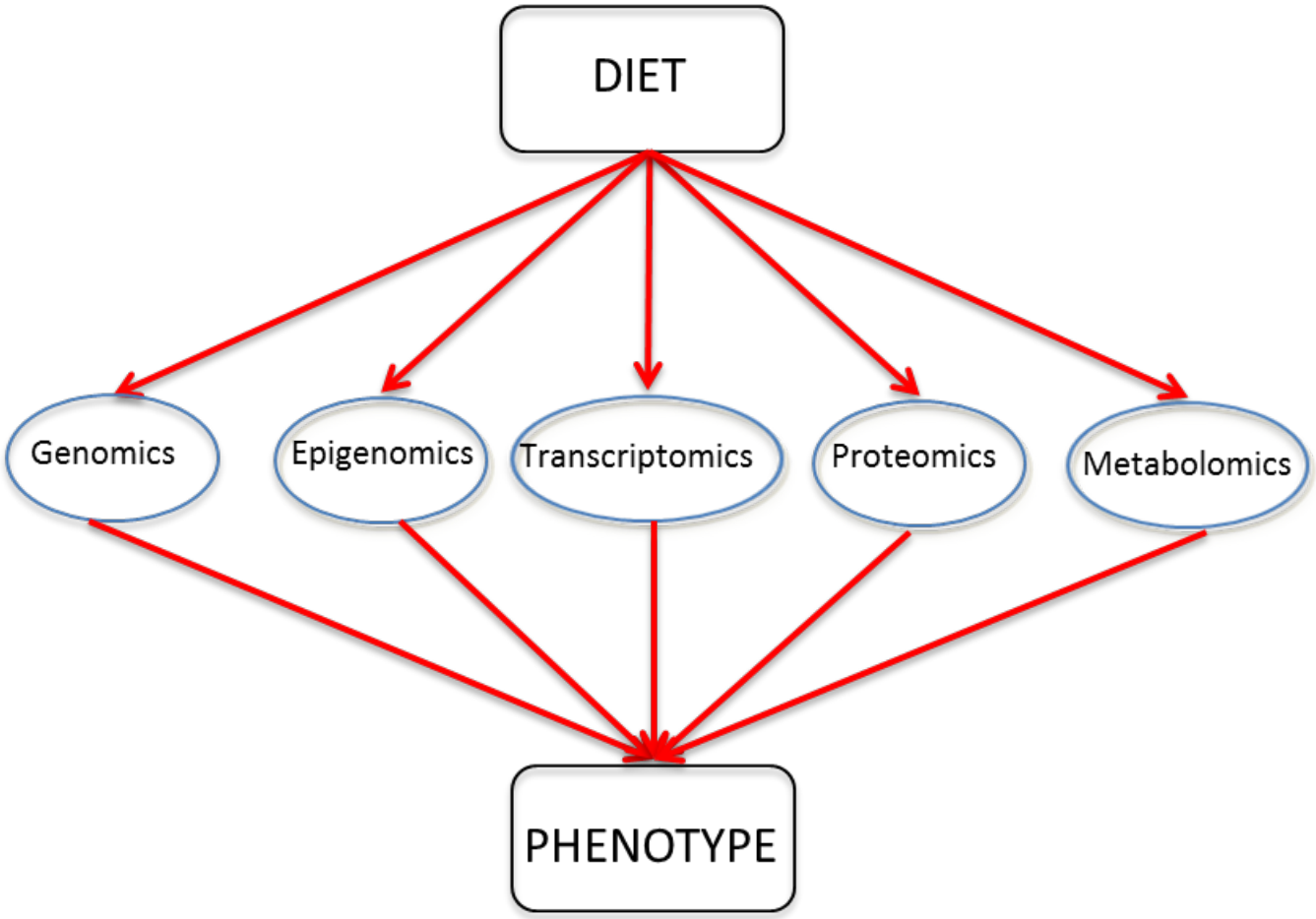


FIGURE 1: "Omics" sciences used in understanding the relationship between nutrition versus health versus disease (source: [4], with modifications; [9] with modifications).


DIET
Genomics
Epigenomics
Transcriptomics
Proteomics
Metabolomics
PHENOTYPE

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)

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 APRIL 4, 2013 VOL. 368 NO. 14

Primary Prevention of Cardiovascular Disease with a Mediterranean Diet

Ramón Estruch, M.D., Ph.D., Emilio Ros, M.D., Ph.D., Jordi Salas-Salvadó, M.D., Ph.D., Maria-Isabel Covas, D.Pharm., Ph.D., Dolores Corella, D.Pharm., Ph.D., Fernando Arós, M.D., Ph.D., Enrique Gómez-Gracia, M.D., Ph.D., Valentina Ruiz-Gutiérrez, Ph.D., Miquel Fiol, M.D., Ph.D., José Lapetra, M.D., Ph.D., Rosa Maria Lamuela-Raventos, D.Pharm., Ph.D., Lluís Serra-Majem, M.D., Ph.D., Xavier Pintó, M.D., Ph.D., Josep Basora, M.D., Ph.D., Miguel Angel Muñoz, M.D., Ph.D., José V. Sorlí, M.D., Ph.D., José Alfredo Martínez, D.Pharm, M.D., Ph.D., and Miguel Angel Martínez-González, M.D., Ph.D., for the PREDIMED Study Investigators*

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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**)

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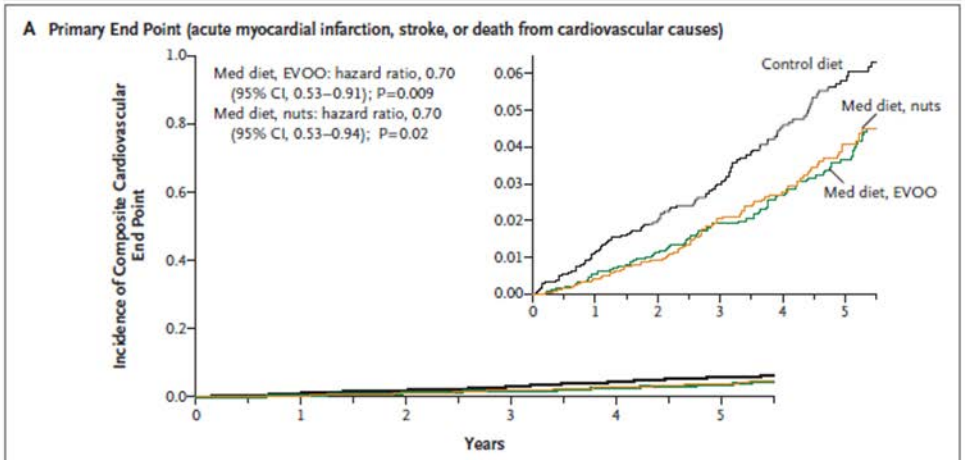
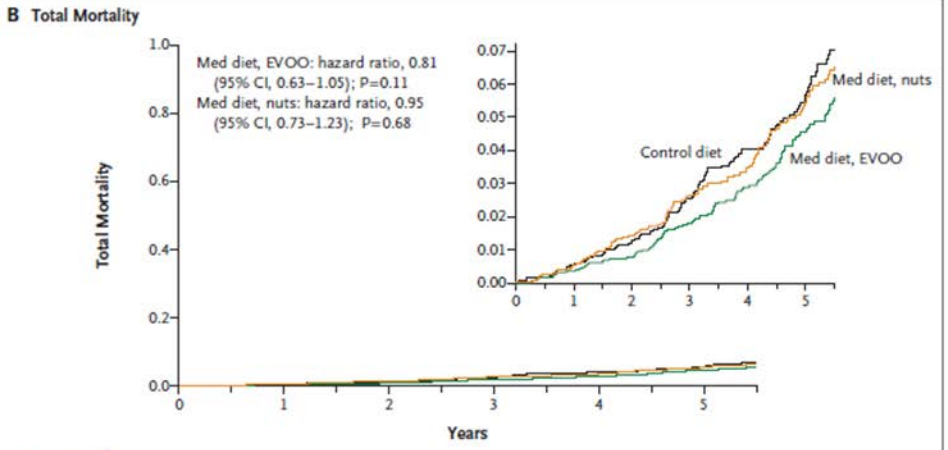


No. at Risk

Control diet	2450	2268	2020	1583	1268	946
Med diet, EVOO	2543	2486	2320	1987	1687	1310
Med diet, nuts	2454	2343	2093	1657	1389	1031



No. at Risk

Control diet	2450	2268	2026	1585	1272	948
Med diet, EVOO	2543	2485	2322	1988	1690	1308
Med diet, nuts	2454	2345	2097	1662	1395	1037

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.

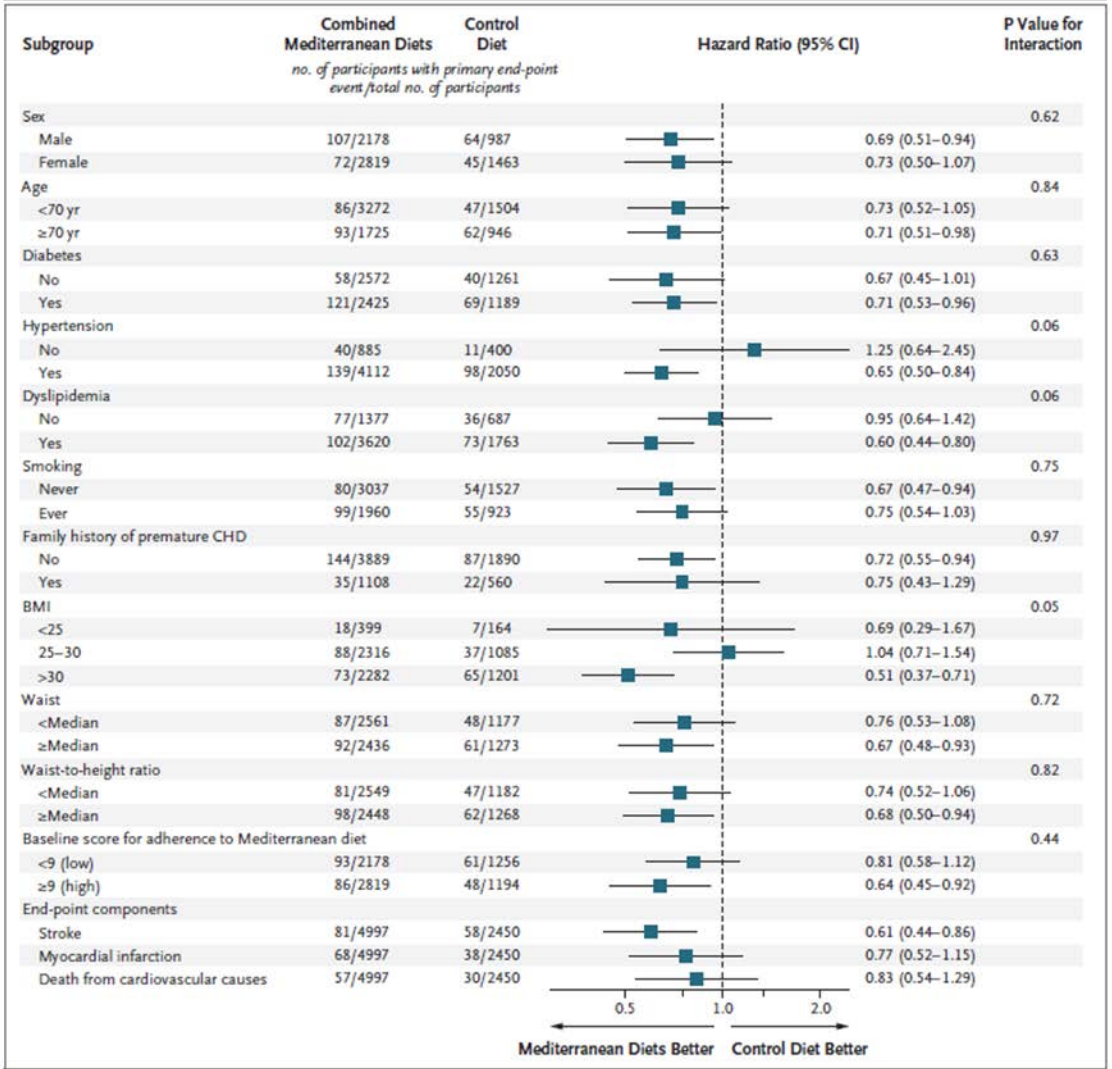


Figure 2. Results of Subgroup Analyses.

Shown are adjusted hazard ratios for the primary end point within specific subgroups. Squares denote hazard ratios; horizontal lines represent 95% confidence intervals. Hazard ratios indicate the relative risk in both intervention groups merged together (vs. the control group) within each stratum. Hazard ratios were stratified according to recruiting center and were adjusted for sex, age (continuous variable), family history of premature coronary heart disease (CHD) (yes or no), smoking (never smoked, former smoker, or current smoker), body-mass index (BMI) (continuous variable), waist-to-height ratio (continuous variable), hypertension at baseline (yes or no), dyslipidemia at baseline (yes or no), and diabetes at baseline (yes or no). Scores for adherence to the Mediterranean diet range from 0 to 14, with higher scores indicating greater adherence.

BMC Med. 2014 May 13;12(1):78. doi: 10.1186/1741-7015-12-78.

Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED Study.

Guasch-Ferré M, Hu FB, Martínez-González MA, Fitó M, Bulló M, Estruch R, Ros E, Corella D, Recondo J, Gómez-Gracia E, Fiol M, Lapetra J, Serra-Majem L, Muñoz MA, Pintó X, Lamuela-Raventós RM, Basora J, Buil-Cosiales P, Sorlí JV, Ruiz-Gutiérrez V, Martínez JA, Salas-Salvadó J[1].

Author information

Abstract

BACKGROUND: It is unknown whether individuals at high cardiovascular risk sustain a benefit in cardiovascular disease from increased olive oil consumption. The aim was to assess the association between total olive oil intake, its varieties (extra virgin and common olive oil) and the risk of cardiovascular disease and mortality in a Mediterranean population at high cardiovascular risk.

METHODS: We included 7,216 men and women at high cardiovascular risk, aged 55 to 80 years, from the PREvención con DIeta MEDiterránea (PREDIMED) study, a multicenter, randomized, controlled, clinical trial. Participants were randomized to one of three interventions: Mediterranean Diets supplemented with nuts or extra-virgin olive oil, or a control low-fat diet. The present analysis was conducted as an observational prospective cohort study. The median follow-up was 4.8 years. Cardiovascular disease (stroke, myocardial infarction and cardiovascular death) and mortality were ascertained by medical records and National Death Index. Olive oil consumption was evaluated with validated food frequency questionnaires. Multivariate Cox proportional hazards and generalized estimating equations were used to assess the association between baseline and yearly repeated measurements of olive oil intake, cardiovascular disease and mortality.

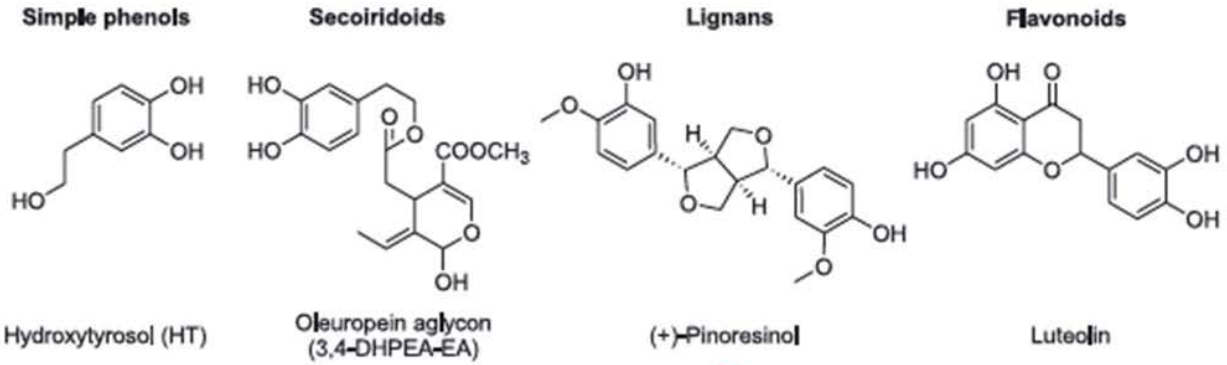
RESULTS: During follow-up, 277 cardiovascular events and 323 deaths occurred. Participants in the highest energy-adjusted tertile of baseline total olive oil and extra-virgin olive oil consumption had 35% (HR: 0.65; 95% CI: 0.47 to 0.89) and 39% (HR: 0.61; 95% CI: 0.44 to 0.85) cardiovascular disease risk reduction, respectively, compared to the reference. Higher baseline total olive oil consumption was associated with 48% (HR: 0.52; 95% CI: 0.29 to 0.93) reduced risk of cardiovascular mortality. For each 10 g/d increase in extra-virgin olive oil consumption, cardiovascular disease and mortality risk decreased by 10% and 7%, respectively. No significant associations were found for cancer and all-cause mortality. The associations between cardiovascular events and extra virgin olive oil intake were significant in the Mediterranean diet intervention groups and not in the control group.

CONCLUSIONS: Olive oil consumption, specifically the extra-virgin variety, is associated with reduced risks of cardiovascular disease and mortality in individuals at high cardiovascular risk.

TRIAL REGISTRATION: This study was registered at controlled-trials.com (http://www.controlled-trials.com/ISRCTN35739639). International Standard Randomized Controlled Trial Number (ISRCTN): 35739639. Registration date: 5 October 2005.

- Elevato contenuto di MUFA
- Elevato contenuto di polifenoli

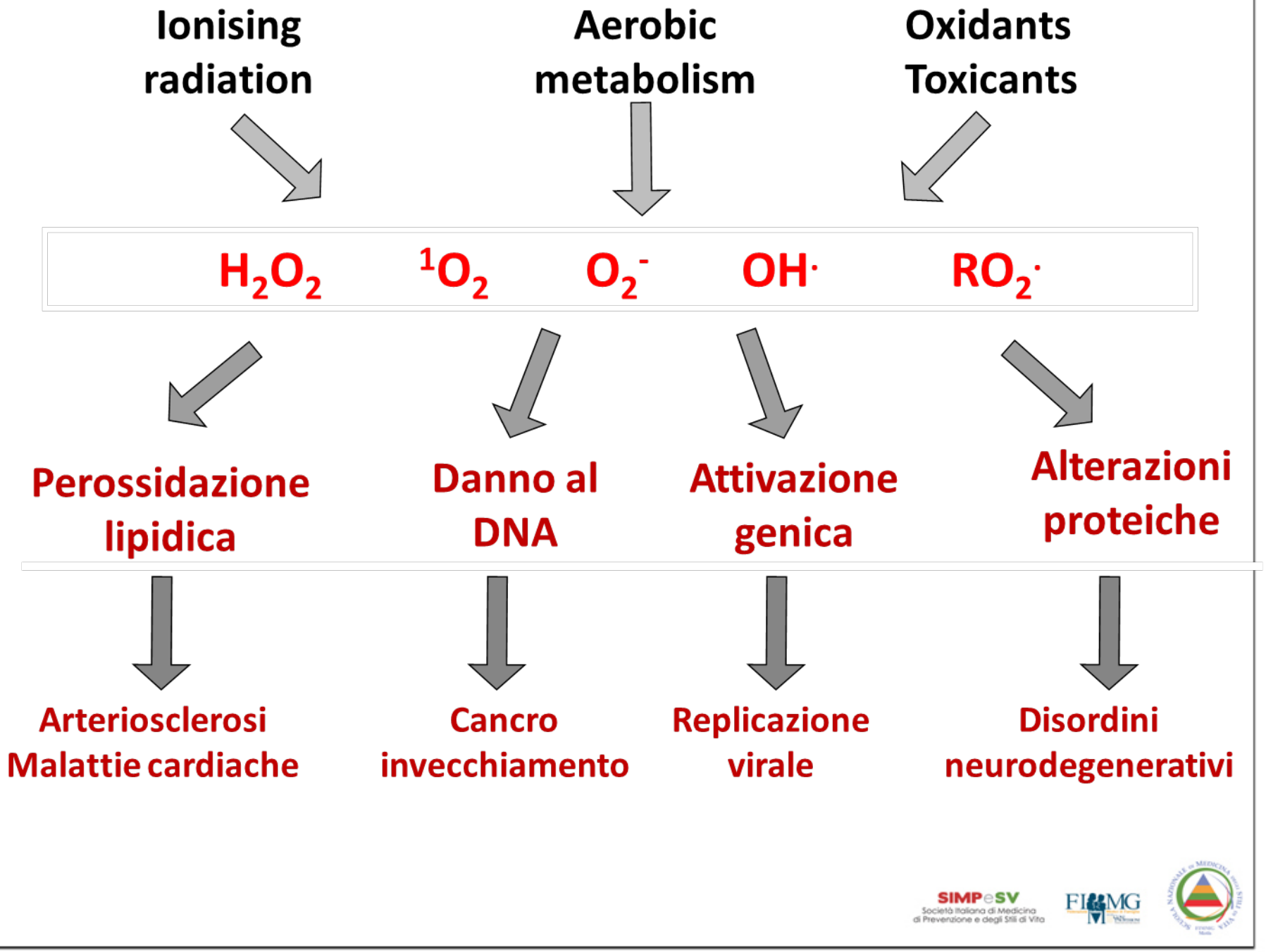
Mol. Nutr. Food Res. 2013, *57*, 760–771



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

Figure 1. Main classes of OOPC with representative compounds.

Attività antiossidante


Ionising radiation
Aerobic metabolism
Oxidants Toxicants
H_2O_2
$^1O_2$
O_2^-
$OH^\cdot$
$RO_2^\cdot$
Perossidazione lipidica
Danno al DNA
Attivazione genica
Alterazioni proteiche
Arteriosclerosi Malattie cardiache
Cancro invecchiamento
Replicazione virale
Disordini neurodegenerativi

Difese antiossidanti esogene

Vitamine antiossidanti

polifenoli

- Stilbeni

- Acidi Fenolici
  - Acidi idrossicinnamici

  - Acidi idrossibenzoici

- Flavonoidi
  - Isoflavoni

  - Flavonoli

  - Antociani

  - Flavanoni, flavoni

  - Flavanoli
    - Catechine

    - Pro(anto)cianidine

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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

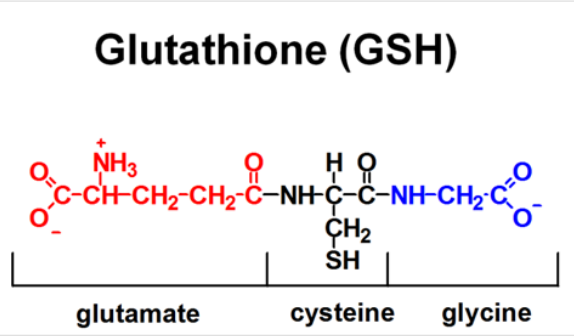
Macrofagi murini J774 A.1

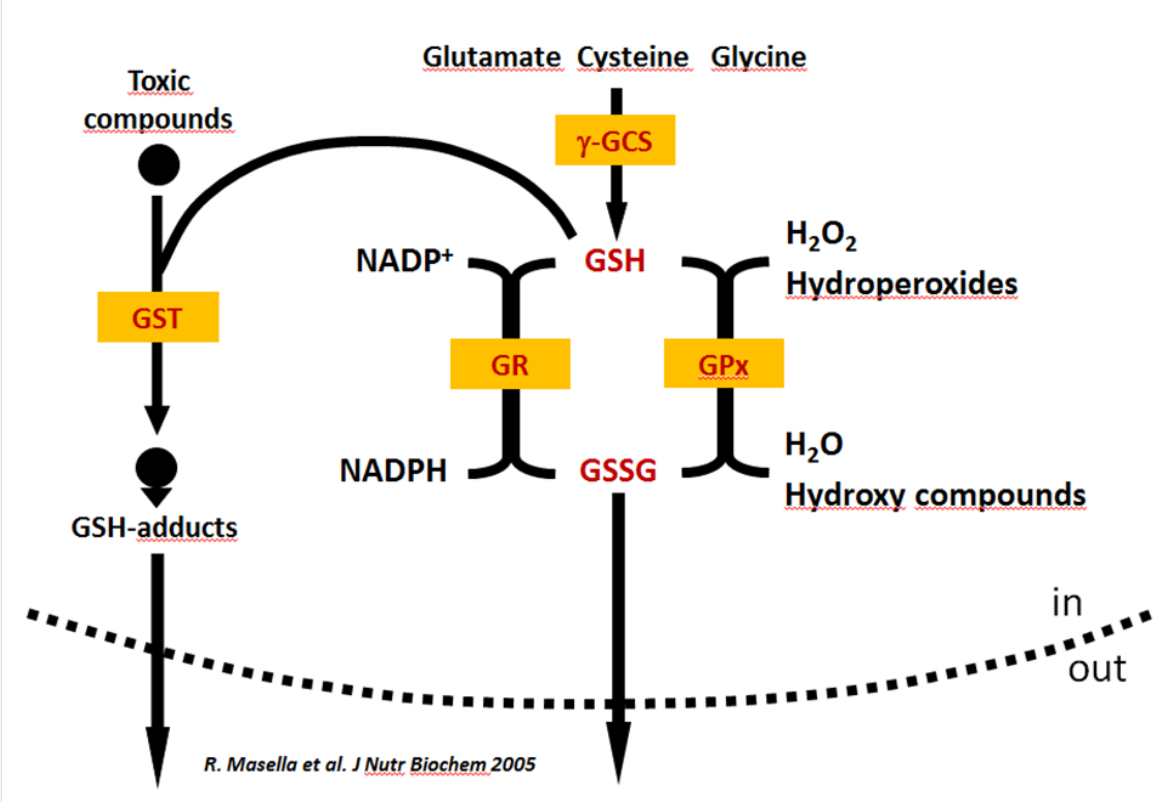
I due polifenoli proteggono le LDL dall'ossidazione anche quando non sono presenti nel mezzo di coltura.

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

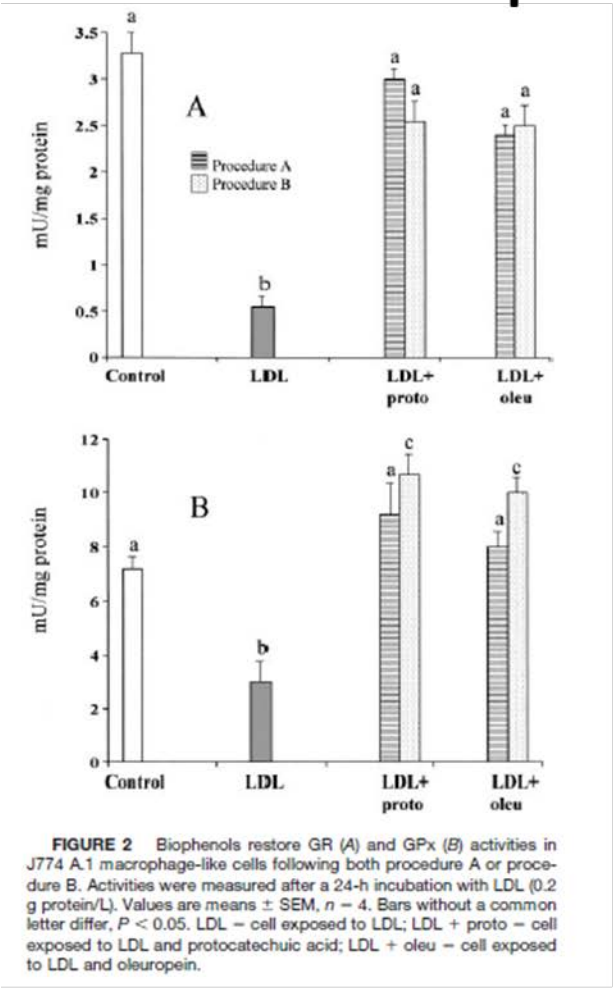
Polifenoli dell'olio di oliva innescano processi cellulari di difesa.

Difese antiossidanti endogene





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.

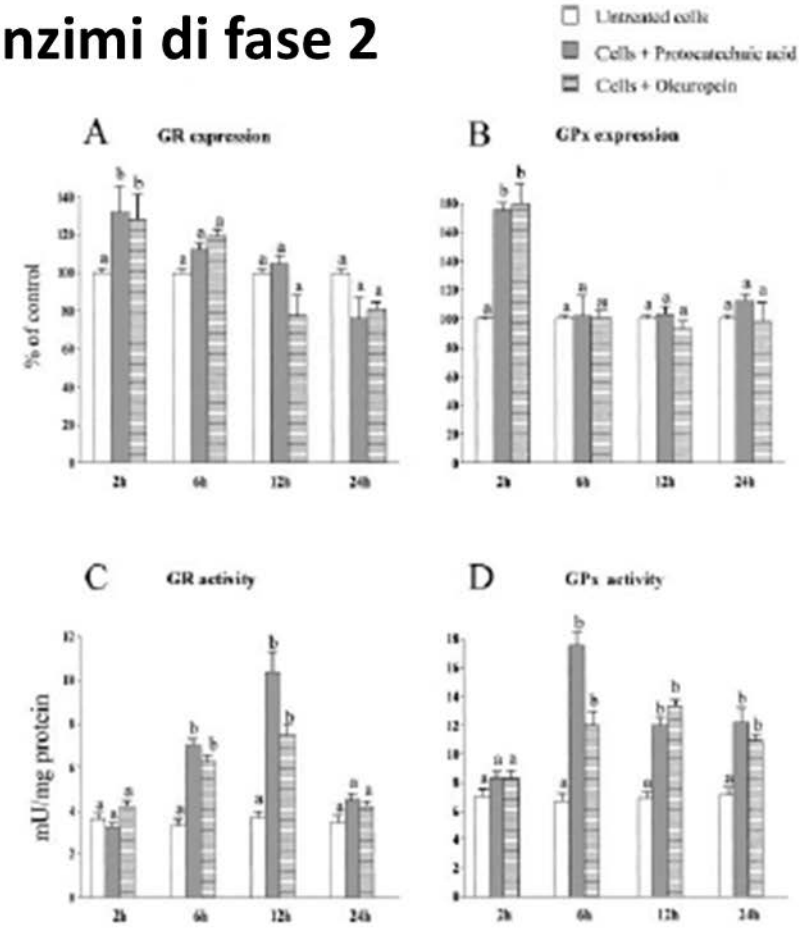
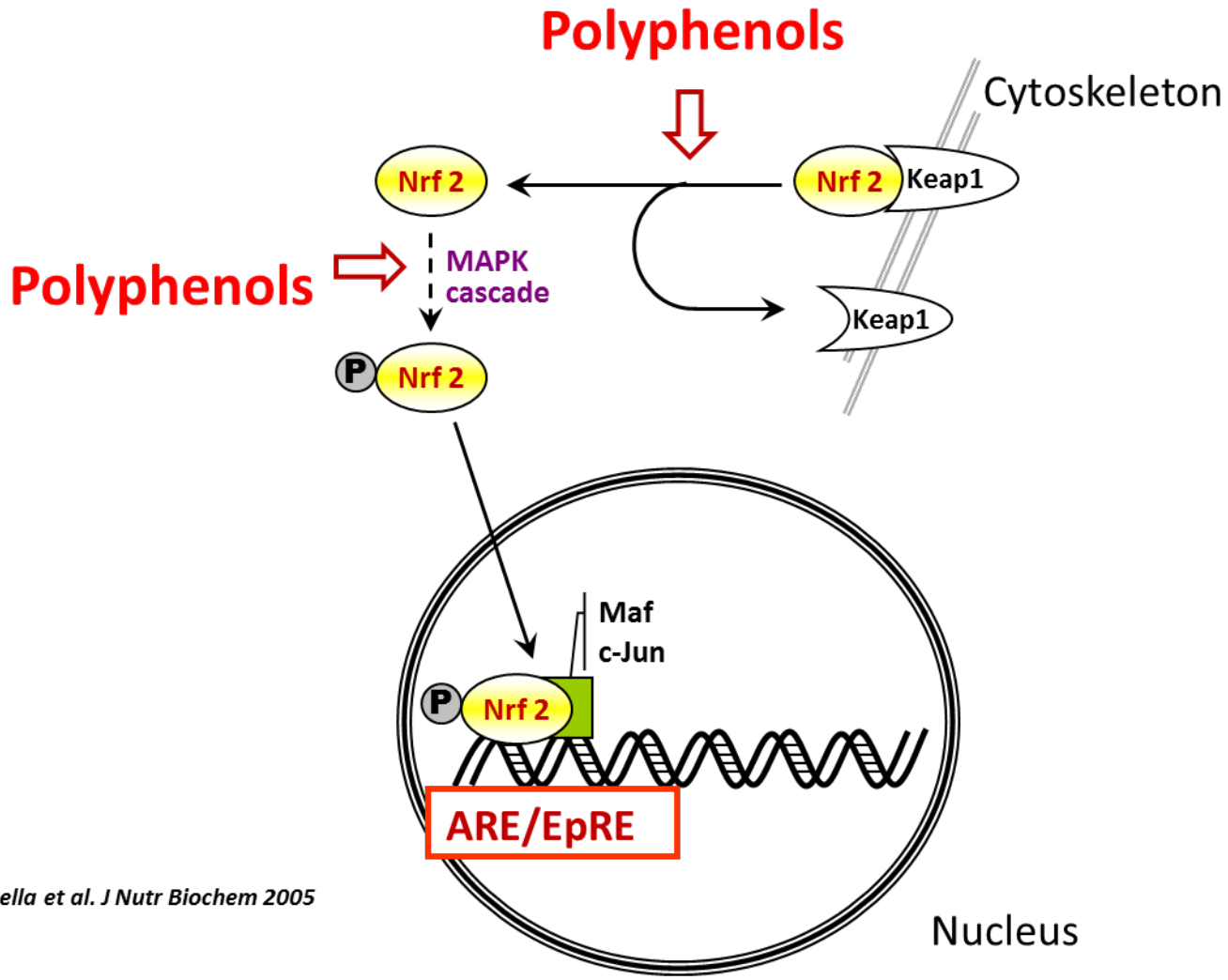
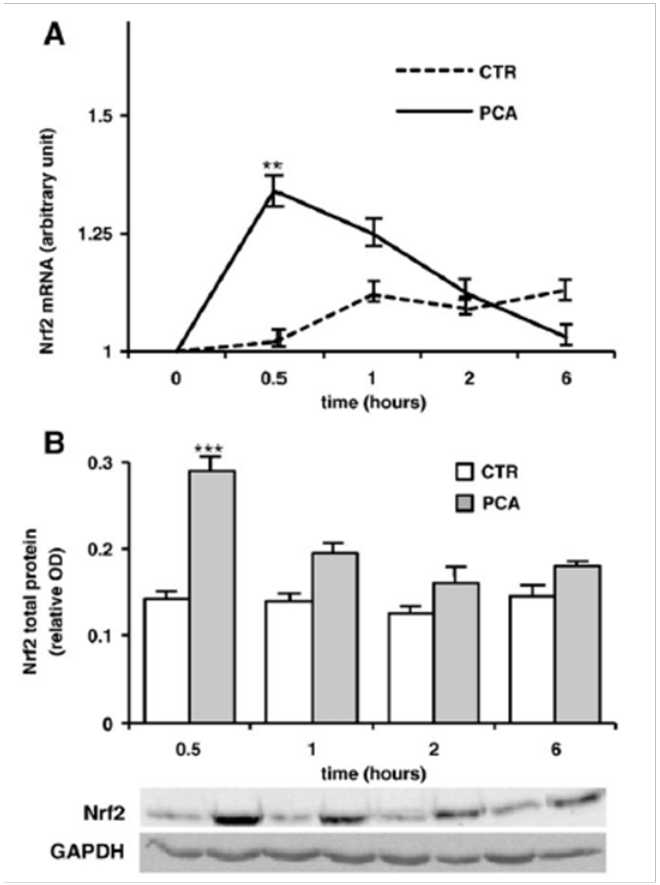


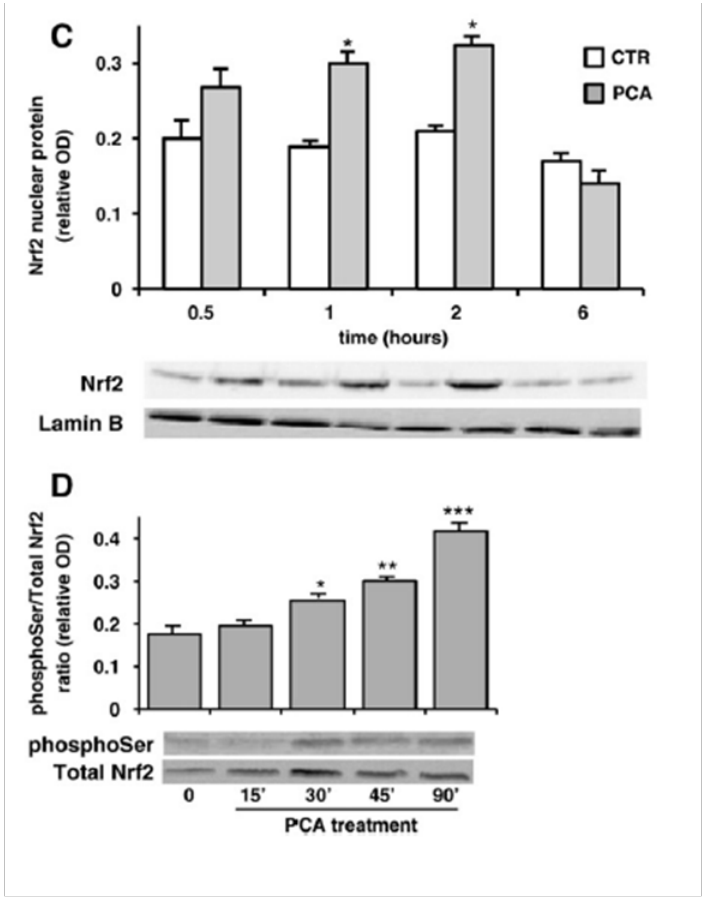
FIGURE 4 Direct effect of the biophenols on DNA transcription of GSH-related enzymes in J774 A.1 cells incubated with protocatechuic acid and oleuropein following procedure B. (*A*) Semiquantitative RT-PCR time-course evaluation of mRNA for GR and GPx. (*B*) Time-course evaluation of GR and GPx activities. 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

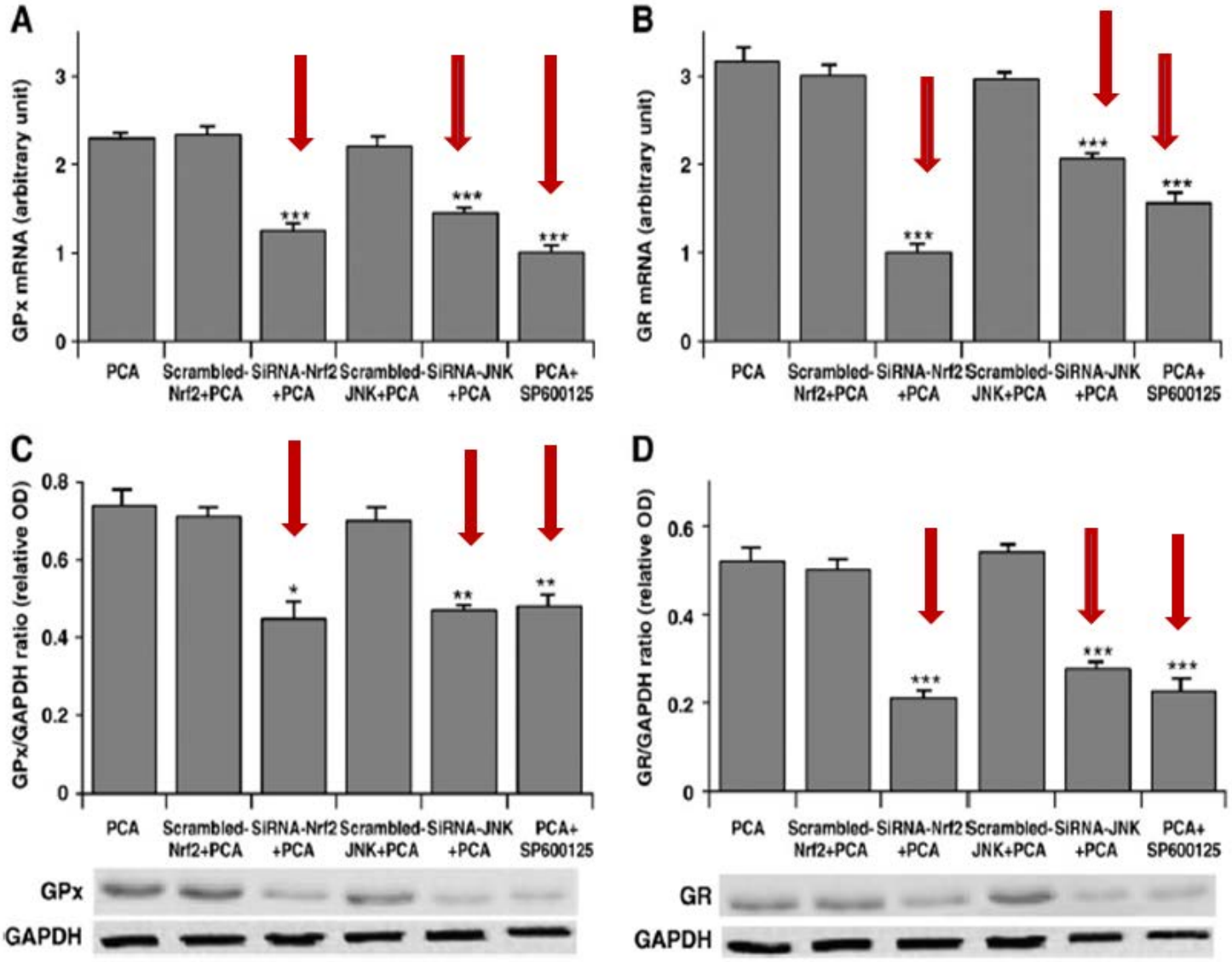

A
GPx mRNA (arbitrary unit)
B
GR mRNA (arbitrary unit)
C
GPx/GAPDH ratio (relative OD)
D
GR/GAPDH ratio (relative OD)
PCA
Scrambled-Nrf2+PCA
SiRNA-Nrf2+PCA
Scrambled-JNK+PCA
SiRNA-JNK+PCA
PCA+SP600125
GPx
GAPDH
GR
GAPDH

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 LP, PC 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 LP, PC 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 LDL resistance[a)] to oxidation Antobodies against oxidized LDL HDL-cholesterol	Decrease with olive oil phenolics None 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 MDA in urine 8oxodG in urine and lymphoctyes F_2-isoprostanes GSH-Px HDL cholesterol	Decrease with olive oil phenolics None 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 F_2-isoprostanes	Increase with olive oil phenolics 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 withre-fined olive oil for all purposes	Plasma oxidized LDL, LP GSH-Px	Decrease with olive oil phenol ics 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-meno-pausal 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 Uninduced dienes Hydroxy fatty acids Antiboides against oxidizee LDL F_2-isoprostans	Decrease with olive oil phenolics 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

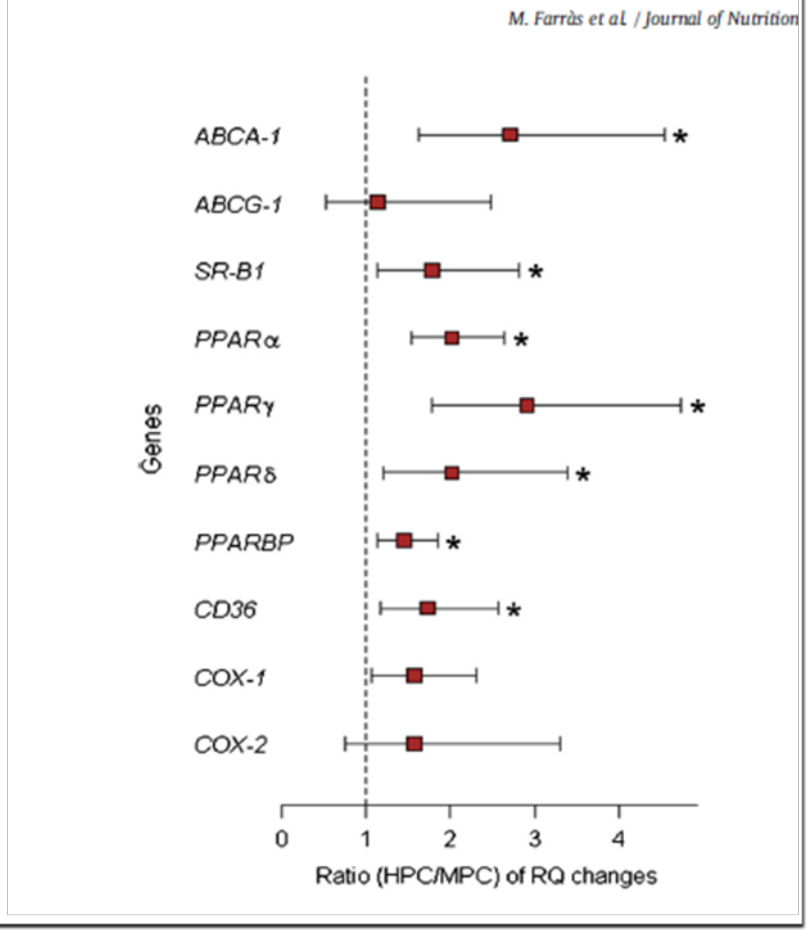
Marta Farràs[a,b], Rosa M. Valls[c], Sara Fernández-Castillejo[c], Montserrat Giralt[c], Rosa Solà[c], Isaac Subirana[d], María-José Motilva[e], Valentini Konstantinidou[c], María-Isabel Covas[a,*,1], Montserrat Fitó[a,*,1]

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



The FASEB Journal • Research Communication **24, 2546 –2557 (2010)**

In vivo nutrigenomic effects of virgin olive oil polyphenols within the frame of the Mediterranean diet: a randomized controlled trial

90 Soggetti sani

- TMDVOO extra vergine di oliva ricco in polifenoli
- TMDWOO extravergine di oliva deprivato di polifenoli
- Controllo dieta abituale

cellule bianche del sangue periferico

TABLE 4. *Change in expression of atherosclerosis-related genes after 3 mo of intervention*

Gene symbol	Gene name	Control, n = 20	TMD-global, n = 36	P value
Cholesterol, lipid transport, and metabolism				
ABCA1	ATP-binding cassette, subfamily A, member 1	0.320 ± 0.231	0.051 ± 0.159	0.334
ABCG1	ATP-binding cassette, subfamily G, member 1	0.146 ± 0.127	0.064 ± 0.092	0.608
ANXA1	Annexin A1	0.259 ± 0.229	−0.444 ± 0.161	0.160
ARHGAP15	Rho GTPase activating protein 15	0.448 ± 0.175	−0.040 ± 0.126	0.043
ARHGAP19	Rho GTPase activating protein 19	0.400 ± 0.151	0.134 ± 0.112	0.166
ARHGEF6	Rac/Cdc42 guanine nucleotide exchange factor 6	0.460 ± 0.144	0.157 ± 0.106	0.099
CD36	CD36 molecule (thrombospondin receptor)	0.197 ± 0.170	−0.009 ± 0.126	0.342
CETP	Cholesteryl ester transfer protein, plasma	−0.262 ± 0.331	−0.058 ± 0.257	0.631
MSR1	Macrophage scavenger receptor 1	0.542 ± 0.222	0.253 ± 0.157	0.301
PLA2G4B	Phospholipase A2, group IVB	0.148 ± 0.156	0.082 ± 0.109	0.735
SCARB1	Scavenger receptor class B, member 1	−0.025 ± 0.078	0.085 ± 0.056	0.261
Inflammation				
IFNg	Interferon, γ	1.048 ± 0.464	−0.109 ± 0.330	0.049
IL10	Interleukin 10	0.915 ± 0.360	0.609 ± 0.270	0.506
CHUK	Conserved helix-loop-helix ubiquitous kinase	0.325 ± 0.192	0.036 ± 0.140	0.236
ADAM17	ADAM metallopeptidase domain 17 (tumor necrosis factor, α, converting enzyme)	0.290 ± 0.153	0.008 ± 0.112	0.148
ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif, 1	0.166 ± 0.208	−0.120 ± 0.150	0.277
IFNA1	Interferon, α 1	0.726 ± 0.356	0.001 ± 0.258	0.117
TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10	0.195 ± 0.219	−0.195 ± 0.156	0.157
TNFSF12_13	Tumor necrosis factor (ligand) superfamily, member 12-member 13	−0.021 ± 0.102	0.133 ± 0.075	0.235
IL6	Interleukin 6	−0.017 ± 0.588	0.356 ± 0.401	0.612
IL7R	Interleukin 7 receptor	0.580 ± 0.182	0.095 ± 0.132	0.037
USP48	Ubiquitin specific peptidase 48	0.380 ± 0.179	0.203 ± 0.131	0.431
MPO	Myeloperoxidase	−0.159 ± 0.121	−0.013 ± 0.090	0.343
RGS2	Regulator of G-protein signaling 2, 24 kDa	0.439 ± 0.268	0.289 ± 0.196	0.656
NFKB2	Nuclear factor of κ light polypeptide gene enhancer in B-cells 2	−0.098 ± 0.082	0.008 ± 0.063	0.315
Nuclear receptors and fatty acids receptors				
NR1H2	Nuclear receptor subfamily 1, group H, member 2	−0.081 ± 0.070	−0.003 ± 0.050	0.369
NRIH3	Nuclear receptor subfamily 1, group H, member 3	0.166 ± 0.108	0.034 ± 0.077	0.331
PPARA	Peroxisome proliferator-activated receptor α	0.088 ± 0.123	0.068 ± 0.092	0.897
PPARBP	PPAR binding protein	0.341 ± 0.160	0.022 ± 0.105	0.084
PPARG	Peroxisome proliferator-activated receptor γ	0.002 ± 0.242	0.235 ± 0.175	0.463
PPARD	Peroxisome proliferator-activated receptor δ	0.066 ± 0.128	0.010 ± 0.096	0.732
Oxidative stress				
LIAS	Lipoic acid synthetase	0.228 ± 0.197	0.188 ± 0.148	0.874
PTGS1	Prostaglandin-endoperoxide synthase 1	−0.176 ± 0.171	−0.170 ± 0.117	0.978
PTGS2	Prostaglandin-endoperoxide synthase 2	0.170 ± 0.545	−0.231 ± 0.379	0.557
OLR1	Oxidized low-density lipoprotein (lectin-like) receptor 1	0.521 ± 0.948	0.113 ± 0.580	0.724
OSBP	Oxysterol binding protein	0.219 ± 0.130	0.035 ± 0.093	0.260
ADRB2	Adrenergic, β-2, receptor, surface	0.225 ± 0.135	−0.138 ± 0.098	0.036
OGT	*O*-linked *N*-acetylglucosamine (GlcNAc) transferase	0.373 ± 0.235	0.014 ± 0.162	0.218
ALDH1A1	Aldehyde dehydrogenase 1 family, member A1	−0.101 ± 0.187	−0.116 ± 0.135	0.949
DNA repair				
CCNG1	Cyclin G1	0.396 ± 0.192	0.004 ± 0.139	0.106
POLK	Polymerase (DNA directed) κ	0.595 ± 0.275	−0.115 ± 0.204	0.045
TP53	Tumor protein p53	−0.071 ± 0.077	−0.048 ± 0.056	0.812
DCLRE1C	DNA cross-link repair 1C	0.406 ± 0.169	0.052 ± 0.123	0.100
ERCC5	Excision repair cross-complementing rodent repair deficiency, complementation group 5	0.401 ± 0.227	0.049 ± 0.169	0.221
XRCC5	X-ray repair complementing defective repair in Chinese hamster cells 5 (double-strand-break rejoining; Ku autoantigen, 80 kDa)	0.267 ± 0.152	0.000 ± 0.111	0.166

Gene expression changes, adjusted by age and sex, are presented as means ± SE of the RQ $\log_2$ ratio (posttreatment *vs.* basal values).

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journal homepage: www.elsevier.com/locate/atherosclerosis

Effect of Mediterranean diet on the expression of pro-atherogenic genes in a population at high cardiovascular risk

Vicenta Llorente-Cortés[a], Ramón Estruch[b,c], Mari Pau Mena[b,c], Emilio Ros[b,d], Miguel Angel Martínez González[e], Montserrat Fitó[b,f], Rosa María Lamuela-Raventós[b,g], Lina Badimon[a,b,*]

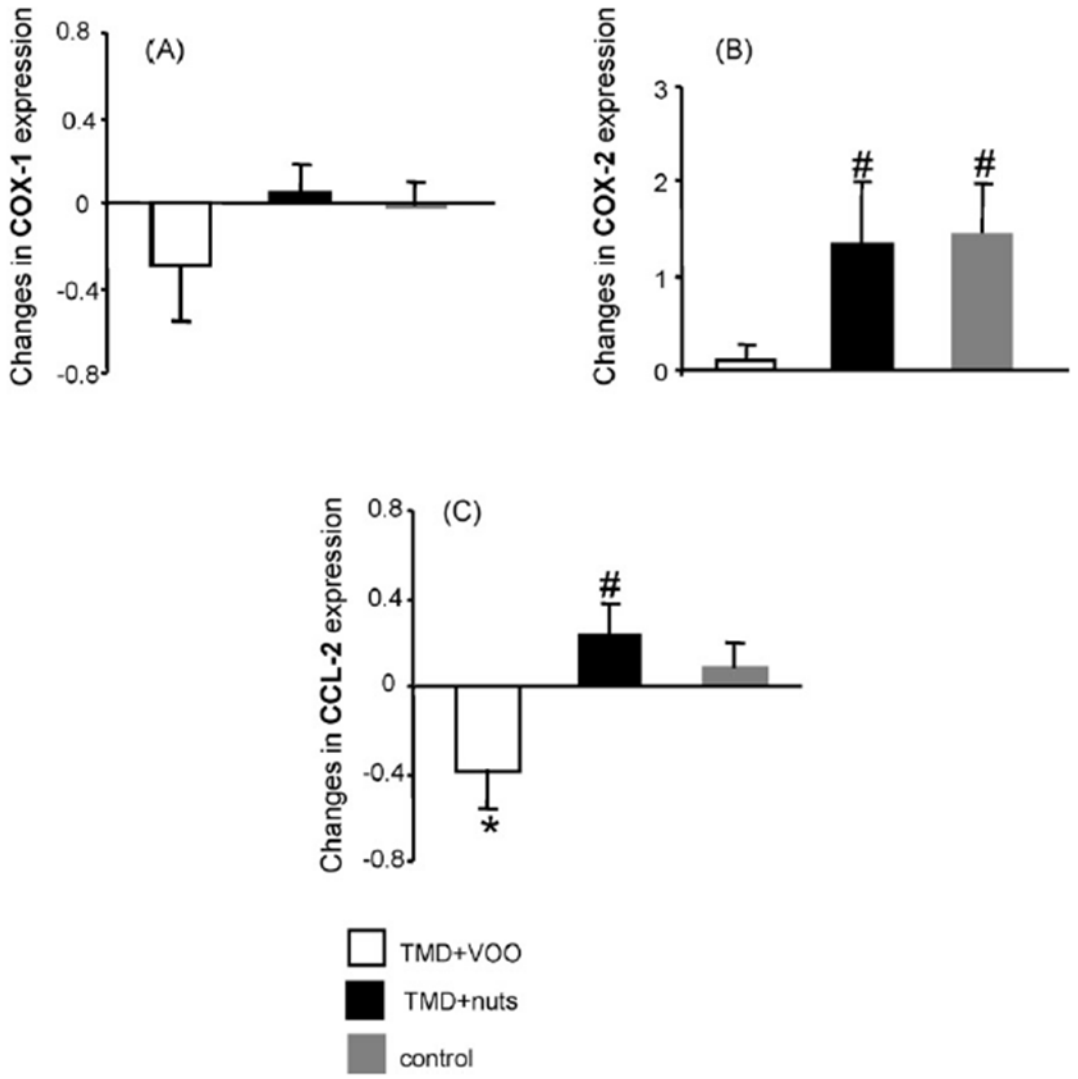
49 soggetti con almeno 2 fattori di rischio per CHD; 3 mesi di intervento: MD + 1L/sett EVOO o 30 gr/die noci/ mandorle/nocciole

Table 2
Changes in weight, adiposity, blood pressure, and other cardiovascular-risk factors[a].

Variable	TMD + VOO	TMD + nuts	Control	*P* time[b]	*P* group[c]	*P* interaction[d]
Weight, kg						
Baseline	73.6 ± 11.6[e]	76.9 ± 6.6	74.9 ± 13.1	0.488	0.761	0.373
Final	74.0 ± 11.1	76.0 ± 6.3	74.6 ± 13.5			
BMI, kg/m^2						
Baseline	28.8 ± 2.7	27.7 ± 2.5	29.9 ± 5.5	0.567	0.175	0.539
Final	28.8 ± 2.5	27.4 ± 2.3	29.9 ± 5.4			
Waist, cm						
Baseline	102 ± 10	101 ± 5	105 ± 16	0.007	0.706	0.533
Final	101 ± 8	98 ± 6[f]	100 ± 10			
Systolic BP, mmHg						
Baseline	153 ± 10	149 ± 18	161 ± 17	0.043	0.006	0.145[g,h]
Final	147 ± 11[f]	142 ± 15[i]	161 ± 11			
Diastolic BP, mmHg						
Baseline	82 ± 9	83 ± 8	87 ± 12	0.021	0.153	0.743
Final	80 ± 9	80 ± 8[f]	86 ± 11			
Glucose, mg/dL						
Baseline	156 ± 59	144 ± 47	156 ± 59	0.018	0.418	0.011[g]
Final	132 ± 40[i]	128 ± 36[f]	165 ± 79			
Cholesterol, mg/dL						
Baseline	231 ± 31	218 ± 23	205 ± 28	0.014	0.472	0.050[g]
Final	208 ± 40[i]	205 ± 18	209 ± 43			
LDL-cholesterol, mg/dL						
Baseline	148 ± 28	143 ± 29	125 ± 29	0.003	0.202	0.207
Final	129 ± 41[i]	135 ± 19	121 ± 31			
HDL-cholesterol, mg/dL						
Baseline	52.3 ± 12.9	48.1 ± 11.1	48.5 ± 9.9	0.154	0.252	0.201
Final	56.2 ± 14.1[f]	48.6 ± 10.0	48.4 ± 10.5			
Triglycerides, mg/dL						
Baseline	147 ± 67	127 ± 78	145 ± 68	0.215	0.275	0.405
Final	126 ± 50	106 ± 38	152 ± 84			
Cholesterol/HDL ratio						
Baseline	4.6 ± 1.0	4.7 ± 1.2	4.1 ± 0.68	0.004	0.682	0.041[g]
Final	3.9 ± 1.1[i]	4.3 ± 0.8[f]	4.1 ± 0.73			

Table 3
Changes in inflammatory, lipoprotein receptor and thrombotic gene expression[a].

Variable	TMD + VOO	TMD + nuts	Control	*P* time[b]	*P* group[c]	*P* interaction[d]
COX-1						
Baseline	1.16 ± 1.03[e]	1.02 ± 0.94	0.73 ± 0.48	0.289	0.676	0.057
Final	0.80 ± 0.68	1.04 ± 6.87	0.82 ± 0.49			
COX-2						
Baseline	1.09 ± 0.81	1.18 ± 0.87	0.88 ± 0.93	0.003	0.532	0.612
Final	1.75 ± 1.29	2.67 ± 1.67[f]	2.20 ± 1.97[g]			
MCP-1						
Baseline	1.07 ± 1.11	0.52 ± 0.56	0.33 ± 0.40	1.000	0.178	0.013
Final	0.67 ± 0.75[f]	0.71 ± 0.36	0.54 ± 0.42			
LDLR						
Baseline	0.68 ± 0.46	0.68 ± 0.51	0.54 ± 0.47	0.001	0.787	0.880
Final	0.92 ± 0.54	0.97 ± 0.44[f]	0.90 ± 0.59[f]			
LRP1						
Baseline	0.96 ± 0.60	0.75 ± 0.51	0.52 ± 0.61	0.001	0.303	0.017[g]
Final	1.06 ± 0.65	1.09 ± 0.51[f]	0.90 ± 0.70[f]			
CD36						
Baseline	0.93 ± 0.57	0.70 ± 0.53	0.61 ± 0.48	0.011	0.210	0.047[h]
Final	0.95 ± 0.49	1.08 ± 0.51[f]	0.69 ± 0.42			
TF						
Baseline	0.68 ± 0.43	0.63 ± 0.55	0.52 ± 0.65	0.064	0.675	0.946
Final	0.86 ± 0.68	0.88 ± 0.72	0.69 ± 0.58			
TFPI						
Baseline	0.99 ± 0.60	0.61 ± 0.47	0.72 ± 0.56	0.789	0.392	0.048[i]
Final	0.80 ± 0.63	0.85 ± 0.50[j]	0.74 ± 0.42			



Camargo *et al. BMC Genomics* 2010, **11**:253
http://www.biomedcentral.com/1471-2164/11/253

BMC Genomics

RESEARCH ARTICLE Open Access

Gene expression changes in mononuclear cells in patients with metabolic syndrome after acute intake of phenol-rich virgin olive oil

Conclusion: This study shows that intake of virgin olive oil based breakfast, which is rich in phenol compounds is able to repress *in vivo* expression of several pro-inflammatory genes, thereby switching activity of peripheral blood mononuclear cells to a less deleterious inflammatory profile. These results provide at least a partial molecular basis for

20 soggetti ; 40 gr olio con pane; dopo 4 ore prelievo;

98 geni modulati da EVOO ad alto contenuto di polifenoli rispetto a EVOO a basso contenuto di polifenoli: **19 iper-espressi**, **79 ipo-espressi**

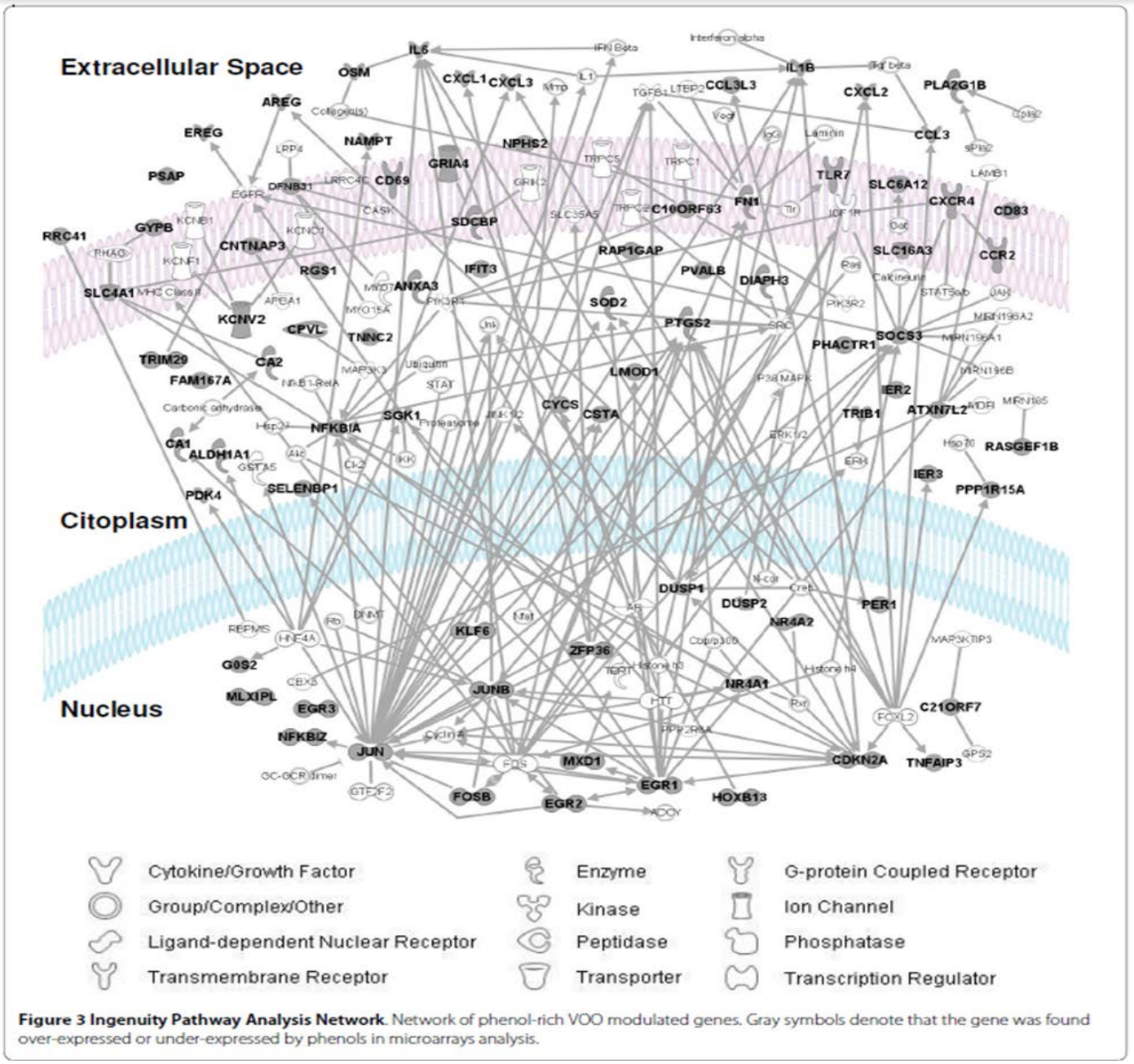


Figure 3 Ingenuity Pathway Analysis Network. Network of phenol-rich VOO modulated genes. Gray symbols denote that the gene was found over-expressed or under-expressed by phenols in microarrays analysis.

Alimenti funzionali ed Health Claims

- Gli health claims sugli alimenti devono essere basati sull'**evidenza scientifica** insieme alla conoscenza dei meccanismi molecolari responsabili dell'effetto salutistico.

- Studi controllati nell'uomo hanno dimostrato **l'effetto protettivo sull'ossidazione delle LDL**, gli **effetti anti-infiammatori**, ed in parte quelli **anti-trombotici.**

- Soltanto l'effetto protettivo di olio extra vergine di oliva capace di fornire almeno **5 mg/die di idrossitirosolo** sulla **ossidazione delle LDL** **è stato accettato come health claim da EFSA.**

- Per il resto gli studi sperimentali **non offrono sufficiente evidenza scientifica** al più alto livello possibile dei reali benefici per la salute

Grazie per l'attenzione