Olive Oil as Medicine

How does olive oil and its natural phytochemicals work as a "nutraceutical" in our body as an anti-inflammatory and promoter of vascular health

Prokopios Magiatis
Olive storage 16th century BC.

19th-20th century

TODAY
In Greece the last 5 years there is a long and vivid discussion among scientists with very different opinions expressed through the media about the characterization of olive oil.

Is it a food or a drug or something else?

Some scientists insist that olive oil should be considered only as a food - basic ingredient of the Mediterranean diet and should stay away from medical applications.
Το λάδι «φάρμακο» για καρκίνο, καρδιαγγειακά, Πάρκινσον, Αλτσχάιμερ

Μπορεί το εξτρα παρθένο ελαιόλαδο να λειτουργήσει ως φάρμακο;

Το λάδι δεν είναι φάρμακο
Με όλες αυτές τις άγνωστες παραμέτρους καινές ακόμη δεν μπορεί να μιλήσει για την ανάπτυξη φαρμάκων από τα συστατικά του ελαιόλαδου. Προς το παρόν κυκλοφορούν στην αγορά αρκετά συμπληρώματα διατροφής με βάση την ελιά και τα προϊόντα της, άλλα αναγράφοντας επιπεδόμενους ισχυρισμούς υγείας και άλλα όχι, και μα η νομοθεσία στη συγκεκριμένη κατηγορία είναι αρκετά χαλαρή και ο έλεγχος από τις αρμόδιες αρχές γίνεται μόνο κατόπιν καταγγελίας και με ρυθμό... χελώνας. Παράλληλα υπάρχουν στην αγορά και...
What is the opinion of the market?
Where is the truth?

- Olive oil is not yet recognized as a normal medicine or as a traditional herbal medicine with therapeutic use by EMA or FDA.
- It is recognized by EFSA and by FDA as a food with qualified health claims (under specific conditions related to chemical composition).
- In this frame it can be used as ingredient of food supplements distributed through the Pharmacy stores.
- However there are numerous experimental studies that demonstrate the therapeutic properties of specific types of olive oil or of specific ingredients.
**BACKGROUND**

**Europe-EFSA**

Food supplements are concentrated sources of nutrients (or other substances) with a nutritional or physiological effect that can be marketed in “dose” form, such as pills, tablets, capsules, liquids in measured doses, etc.

**USA-FDA** Dietary supplements. A dietary supplement is either intended to provide nutrients in order to increase the quantity of their consumption, or to provide non-nutrient chemicals which are claimed to have a biologically beneficial effect.

Dietary supplements should not be used to treat any disease or as preventive healthcare.

Health claims on food labels and in food marketing are claims by manufacturers of food products that their food will reduce the risk of developing a disease or condition.
Qualified health claims of Olive oil

**FDA:**
- Limited and not conclusive scientific evidence suggests that eating about 2 tablespoons (23 grams) of olive oil daily may reduce the risk of coronary heart disease due to the monounsaturated fat in olive oil. To achieve this possible benefit, olive oil is to replace a similar amount of saturated fat and not increase the total number of calories you eat in a day.

**EFSA:**
- Replacing saturated fats in the diet with unsaturated fats contributes to the maintenance of normal blood cholesterol levels. Oleic acid is an unsaturated fat.
- Vitamin E contributes to the protection of cells from oxidative stress.
- Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress.

The claim may be used only for olive oil which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) per 20 g of olive oil. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 g of olive oil.
Example of olive oil with health claim

The olive oil polyphenols contribute to the protection of blood lipids from oxidative stress. According to the 432/2012 EU regulation, the beneficial effect is obtained with a daily consumption of 20g of olive oil. Οι πολυφαινόλες ελαιολάδου συμβάλλουν στην προστασία των λυστιδίων του αίματος από το οξειδωτικό στρες. Σύμφωνα με τον κανονισμό 432/2012 της Ευρωπαϊκής Ένωσης, τα ευεργετικά αποτελέσματα εξασφαλίζονται με την ημερήσια κατανάλωση 20g ελαιολάδου.
Food or drug?

- Olive oil is not a simple food or a simple source of lipids.

- It contains healthy monounsaturated lipids BUT it also contains unique minor chemical compounds with delicate structures that can enter in the human body through the usual diet and have an impact on health.

- The only known edible source of these specific chemicals is the olive fruit and the olive oil.

- Olive oil is in the intermediate between food and drug providing an excellent example of Hippocratic medicine.
Olive- olive oil

Dioscorides and after him all the ancient doctors insist that the best health effects come from the fresh olive oil from unripe olives or from specific varieties **but not from all olive oils**

Numerous applications are reported including headache, toothache

**Obvious indications of antinflammatory activity**

*Dioscorides Pedanius Med., De materia medica.*

ἔλαιον πρός τὴν ἐν υγιείᾳ χρήσιν ἄριστον τὸ ωμοτριβές, ὥσκαι ὁ μφάκινον καλοῦσι.
Why Dioscorides discriminates olive oils according to their impact on health?

Based on a modern pharmacologic view we would expect that the active ingredients in olive oil are not present in all oils or they exist in different concentrations.

Quite common phenomenon in phytochemistry and phytotherapy.

Olive harvest. Pot of the 6th century BC.
A possible answer is: Oleocanthal

- Oleocanthal (decarboxymethyl ligstroside aglycone) is responsible for the pungency of fresh olive oil coming from unripe olives (irritation of oropharyngeal region).
- It disappears from the oil as the fruit matures.
- Oleocanthal possesses antiinflammatory activity similar to Ibuprofen.
Oleocanthal is produced during the malaxation of the olive paste.

- Same olives, same day, same olive mill. The phenolic profile (with significant impact on taste) changes dramatically during malaxation.
Oleocanthal is absent or significantly reduced in olive oils from ripe olives

Same olive grove, same variety, same olive mill

Magiatis et al JAFC 2012
Oleocanthal

- COX1 and COX2 inhibitor
- Protection from Alzheimer’s disease in vivo
- Treatment of rheumatoid arthritis
- Against tumor promotion

Chronic exposure to low doses of anti-inflammatory agents like oleocanthal offers protection against cardiovascular diseases and aging.
Oleacein has a similar structure with oleocanthal. It is a derivative of hydroxytyrosol and it is the most powerful antioxidant constituent of olive oil and also an inhibitor of LOX.
**Oleuropein aglycon (monoaldehyde form)**

- Powerful antioxidant
- Protection against Alzheimer in animals (July 2013)

**Ligstroside aglycon (monoaldehyde form)**

- Antioxidant activity
- Can block metastasis
Oleokoronal-Oleomissional

- New compounds isolated for the first time in olive oil and in nature

- The olive oil after thousands of years of human use it continues to surprise us with its secrets

- Yet unknown taste and bioactivity

Magiatis et al OLIVAE 2015
The EU regulation: a closer look

- 5 mg of hydroxytyrosol and derivatives (oleuropein complex and tyrosol) per day offer protection against LDL oxidation.

- Oleocanthal and oleacein are the two most abundant forms of conjugated hydroxytyrosol and tyrosol in most olive oils, together with oleuropein aglycon and ligstroside aglycon.

- A few years ago we published a reliable method to measure all the compounds mentioned in the regulation in one experiment and provide the necessary data for the health claims.

  Magiatis et al JAFC 2014
The significance of ox LDL

The wording of the health claim is unfortunately not very "attractive"
In the period 2009-2016 my research team has developed a methodology for fast olive oil analysis by NMR for its phenolic ingredients and has performed a screening of ~3000 olive oil samples creating the first database and maps related to olive oil quality. Most producers in Greece already know us because we have offered certifications concerning the olive oil health claim to hundreds of companies.
TYPICAL EXAMPLE OF $^1$H-NMR OF THE ALDEHYDIC PROTONS REGION OF OLIVE OIL EXTRACT AT 600 MHZ
1D vs 2D NMR

Like a 2D bar code!!
The problems

- All the tyrosol and hydroxytyrosol derivatives found in olive oil present several technical difficulties in their chemical analysis and the existing data are not reliable.

- They are sensitive to polar solvents (water or methanol) or silica based stationery phases used for chromatographic quantitation (HPLC-UV or LCMS) and special care must be taken to obtain reliable results.

- The HPLC method of the International Olive Council measures all phenols together as tyrosol equivalents and this causes a dramatic underestimation of the phenolic content and especially the oleocanthal and oleacein content.
WE KNOW SO LITTLE ABOUT OLIVE OIL....
24 months at room temperature

24 months

12 months

Fresh

Oleocanthal

Oleacein

????
New ingredient

- Oxidized derivative of oleocanthal
- Yet unknown taste and biological activity
- It is a tyrosol ester and should be measured for the EU health claim

Oleocanthalic acid

\[ \text{Oxidized derivative of oleocanthal} \]

\[ \text{Yet unknown taste and biological activity} \]

\[ \text{It is a tyrosol ester and should be measured for the EU health claim} \]

\[ \text{Oleocanthalic acid} \]
Recent experimental and new clinical data

Beyond the EU health claim
LDL/HDL—Total phenol content

EUROLIVE
n=200 (men) healthy
336 mg/kg vs 164 mg/kg vs 2.7 mg/kg; 25 ml/day crossover; 3 weeks each oil

Linear increase in HDL
- 2.7 mg/kg = 0.9 mg/dl
- 164 mg/kg = 1.2 mg/dl
- 336 mg/kg = 1.7 mg/dl.

Compared to baseline levels:
- 366 mg/kg decreased LDL 6 mg/dl
- 2.7 mg/dl increased LDL 6 mg/dl

Castaner, O. AJCN. 2012, 95:1238

Three more human trials with unknown phenol content obtained either positive or neutral effects on HDL or LDL.
Impact of olive oil phenolics on animal model

The lack of consistency among human trials related to cholesterol impact is caused by the lack of knowledge of the chemistry of olive oil and especially the phenolic ingredients.

To investigate the role of total and specific phenolics of olive oil we designed an experiment with mice following high fat diet: 30% animal fat and 10% oil for 90 days. The 50th day the mice were injected with streptozotocin to develop diabetes.

A series of biochemical parameters were monitored.

Each animal group was fed with a different type of oil.
The high levels of oleocanthal in oils 3 & 5 were correlated with lower levels of total and LDL cholesterol in comparison with sunflower oil 1 and the olive oil without phenols 2 at day 90.

Every EVOO has different impact on cholesterol depending on the phenolic profile.
Inflammation

- Spain
  n = 24 (women)
  564 mg/kg vs refined olive oil; 60 ml/day crossover; 8 weeks each oil
  Extra virgin olive oil with 564 mg/kg compared to baseline:
  decreased c-reactive protein (CRP) 1.9 mg/L

- Spain
  n = 28; stable coronary disease 161 mg/kg v 14.67 mg/kg; 50 ml crossover; 3 weeks each oil
  Extra virgin olive oil:

"the level of systemic inflammation as measured by circulating levels of C-reactive protein (CRP) has been linked to prognosis in patients with atherosclerotic disease, congestive heart failure, atrial fibrillation, myocarditis, aortic valve disease and heart transplantation."
Blood pressure

- **Spain**
  extra virgin olive oil vs sunflower oil; 60 grams/day
crossover study; 4 weeks each oil
  Extra virgin olive oil: **decrease in systolic BP**, no change for diastolic

- **Italy**
  extra virgin olive oil vs sunflower oil; 30 to 40 grams/day
crossover study; 6 months each oil
  Extra virgin olive oil: **decrease in systolic and diastolic blood pressure**
Ferrara, LA. Arch Intern Med. 2000, 160:837

- **USA**
  extra virgin olive oil (46 g/day) vs mix of corn and soybean oil, and butter
(49 g/day): 3 months each dietary fat source
  Extra virgin olive oil: **decrease in systolic blood pressure**, no difference
diastolic
Rozati, M. Nutr Metab. 2015, 12:28

Phenolic content unknown!!
Blood pressure / phenolic content

Spain
161 mg/kg vs refined; 50 ml/day; crossover; 3 weeks each oil
High phenolic EVOO: lowered systolic BP; no change in diastolic
Fito, M. Athero. 2005; 181:149.

Spain
564 mg/kg vs refined; 60 ml/day crossover; 8 weeks each oil
High phenolic EVOO: lowered systolic and diastolic

EUROLIVE
366 mg/kg vs 2.7 mg/kg; 25 ml/day; crossover; 3 weeks each oil
High phenolic EVOO: decrease in diastolic BP no change for systolic BP
Castaner, O. AJCN. 2012, 95:1238
All tested olive oil showed positive impact on blood pressure (minimum 25 ml/day).

Daily use of extra virgin olive oil may decrease or even avoid the need for hypertensive medications.

Three studies correlate the total phenolic content with the antihypertensive activity.

Are there specific phenols or other related compounds that can explain the mechanism?
It is a derivative of oleuropein and ligstroside aglycon.
It is not a phenol.
It has never been previously reported to be present in olive oil.
It has antihypertensive activity patented in the 60's.
Most high phenolic olive oils contain high quantities of elenolide although nobody had ever studied because it is decomposed during usual chromatography.
Effects of Extra Virgin Olive Oil Oleocanthal Content on Platelet Reactivity in Healthy Adults
Platelets – Promoters of Cardiovascular Disease

Borissoff et al., NEJM 364: 1746-60, 2011
Signaling Cascade Associated with Platelet Aggregation

To compare the impact of the acute intake of oleocanthal-rich versus oleocanthal-poor olive oils with equivalent total phenolic content on platelet reactivity in healthy adults
Extra Virgin Olive Oil Composition

Oil A: provided < 20 mg/kg oleocanthal; < 10 mg/kg oleacein

All oils provided similar amounts of total phenols (213 - 295 mg/kg caffeic acid equivalents) and had similar lipid profile.
Oleocanthal and Oleacein-Rich EVOO Intake reduces Collagen-Induced Platelet Aggregation Relative to control EVOO

Blue: Super market control oil
Red: California Arbequina (High oleacein 12 mg dose)
Green: Kalamata oil (High oleocanthal 12 mg dose)
Purple: Ibuprofen (400 mg dose)

Collagen Stimulated (n=9)
The changes in 12-LOX and COX products were more strongly correlated with Platelet Aggregation Effects in Oil Treated Subjects.
All Subjects responded to Ibuprofen (I) 4 of 9 Responded to Oils B & C

The responder groups showed similar effects with pharmaceutical dose of ibuprofen in the case of oleocanthal rich EVOO (C)
For the first time we were able to demonstrate in humans an activity related to oleoanthal

Oleoanthal and Oleacein-rich extra virgin olive oils acutely reduce platelet aggregation.

The variance in platelet response to EVOO intake was strongly correlated with changes in 12-LOX and COX products.

Taken together the data suggests that changes in platelet aggregation and the cardiovascular protection after a single intake of EVOO is dependent on the precise phenolic composition of the oil.

Oleocanthal and Alzheimer

How Extra Virgin Olive Oil Can Protect from Alzheimer's Disease

Olive-oil-derived oleocanthal enhances β-amyloid clearance as a potential neuroprotective mechanism against Alzheimer's disease: in vitro and in vivo studies.

Abuznait AH, Qosa H, Busnena BA, El Sayed KA, Kaddouni A.
Enhancement of Aβ amyloid clearance from brain

- P-glycoprotein (P-gp) and LDL lipoprotein receptor related protein-1 (LRP1) is the main transport proteins of Aβ, in the blood-brain barrier (BBB).

- A study with $^{125}$I-Aβ 40 showed that administration of oleocanthal in C57BL/6 wild-type mice enhanced the clearance of $^{125}$I-Aβ 40 by the brain from 62.0 ± 3.0% for control rats to 79.9 ± 1.6% for mice with oleocanthal.

- The study lasted two weeks and the animals were treated intraperitoneally with a dose of 10 mg/kg twice daily.

- An increased P-gp expression and LRP1 at the microvasculature of the brain was observed confirming the role of these proteins to enhance clearance $^{125}$I-Aβ 40 by oleocanthal.

- Moreover, the results showed a significant increase in degradation of $^{125}$I-Aβ 40 as a result of upregulation of degrading enzymes Ab nepriylsini (NEP) and insulin degrading enzyme (IDE).
Oleocanthal reduces total Aβ and Aβ plaques

4 weeks treatment starting at the age of 4 months reduces Aβ brain load

Oleocanthal reduces Aβ deposit on brain vasculature

The same results have been obtained after administration of oleocanthal rich olive oil through the diet of the animals!!

Oleuropein aglycon and Alzheimer

Double transgenic mice TgCRND8 (overexpressing two mutations in the human amyloid precursor protein), ages 1, 5, and 4 months, and age-matched wild type control mice. Were used

Oleuropein aglycone was administered for 8 weeks in their diet at a dose 50 mg / kg food.

The administration of oleuropein aglycone significantly improved cognitive performance in young / middle-aged TgCRND8 mice compared to age-matched mice with unsupplemented diet. Brain tissue immunofluorescence analysis in transgenic mice administered the oleuropein aglycone showed significantly reduced levels of beta amyloid and plaques that appeared were less and less compact.

Animal model: Experimental autoimmune encephalitis (EAE) induction

- 10 mg/kg/day, i.p injection

In collaboration with:
Dr. Marisa Nieto
IBGM-CSIC/
Universidad de Valladolid
Spain
Animal model: Experimental autoimmune encephalitis (EAE) induction

- 10 mg/kg/day, i.p injection

- The olive oil phenols oleacein and oleocanthal can enter into the brain and exhibit a clear and strong protective activity

- Oleocanthal & Oleacein were isolated from E-LA-WON olive oil
It was recently found that Oleocanthal causes cancer cells to break down and die very quickly; within 30 minutes, instead of the 16 to 24 hours it takes for programmed apoptotic cell death.

The proposed mechanism is that Oleocanthal disrupts the lysosome membrane.

Breslin P. et al Molecular & Cellular Oncology, 2015
Investigation of the cytotoxic activity in the university of Athens

Dose response of pure oleocanthal and oleacein on HeLa cell death

The OOE extract containing equimolar quantities of OLEO and OLEA presents higher activity than each pure compound at the same concentration.

Isolated from Volvi Estate olive oil
After NMR screening of >3000 different olive oils (variety + geographic origin + harvest time + processing) we identified in September 2017 in Cyprus an olive oil from Kalamata variety with 3762 mg/Kg oleocanthal.

25 gr olive oil per day can offer 94 mg of oleocanthal.
ARISTOIL TARGETS

- Our target is to increase the economic value of olive oil for the benefit of a very large number of producers and NOT for the benefit of a small number of pharmaceutical companies.
- Educate and assist the producers/millers to make olive oil with increased health protecting properties.
- Offer certification of the healthy properties of every olive oil sample in the Mediterranean through a non-profit certification center.
TARGETS

Our target is not to reduce the cost of production (to make it comparable with seed oils)

BUT

To transform (a part of) olive oil to a certified functional food with higher price

=>to make olive oil production a viable and sustainable endeavor
THE OLYMPIA HEALTH & NUTRITION AWARDS
The consumers concerned about the health protecting properties of olive oil need to know that the method of production of high-phenolics olive oils requires special care and the producers must be rewarded for this additional effort. High phenolics oils are produced at lower yield and this should be balanced by a higher price.
Connection of price with phenolic content

- The phenolic content should be related with the price because

- A 250 mg/Kg oil requires consumption of 20 g to offer the necessary amounts of phenolics

- A 2500 mg/Kg oil requires only 2 g to offer the same amount of healthy phenolics with less calories

- A reasonable connection must be adopted by the market
Health claims=better price?

The olive oils that contain high quantities of hydroxytyrosol and its derivatives will be able to claim health protecting activities and consequently to achieve a better price.

Patients with cardiovascular, metabolic or neurodegenerative diseases = an new consumer target group for olive oil

However the consumers must be educated to accept and value the taste of these oils.

It will also be a difficult task for the producers to achieve balanced taste and high concentration of specific polyphenols.

We must encourage millers to move towards this difficult task.
Taste awards AND health awards = ALLIES

- In fact the best olive oils in the world will be those combining excellent organoleptic properties and excellent phenolic content
Clinical studies in progress

Today three clinical trials with specially selected Greek oils are in progress for:
- multiple myeloma
- Alzheimer
- Platelet aggregation

Not generally rich in polyphenols but rich in specific polyphenols (oleocanthal or oleacein) and in particular the oleocanthal that seems to characterize most Greek varieties.

The studied oils are precisely characterized in the same way with the officially recognized herbal medicines.
Our ultimate target is to collect sufficient clinical data to transform the high-phenolic or high oleocanthal olive oils from healthy foods and food supplements to herbal medicines.

To demonstrate the effectiveness and safety of the pure active ingredients like oleocanthal in humans and provide real new drugs coming from olive oil.
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