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REVIEW ARTICLE

THE MEDITERRANEAN DIET, PART I: THE ANTICANCER EFFECT OF OLIVE OIL

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ABSTRACT

For good scientific reasons it has been argued that the Mediterranean diet has beneficial anticarcinogenic effects, evidenced by a relatively long cancer-free survival. Olive oil plays an important culinary role in the different regions of the Mediterranean basin, providing a legitimate reason to hypothesize that its constituents could exert a beneficial effect in cancer prevention. Indeed, many experimental studies have shown that both the monounsaturated oleic acid, the major lipid in olive oil, and minor olive oil components exert an anticancer effect, particularly on breast cancer and colon cancer cells. A recent meta-analysis by Sofi et al. (2008) provided solid evidence that adherence to the Mediterranean diet is positively correlated with a diminished cancer risk and there is a growing understanding that olive oil components act in an integrated manner with other molecules present in the diet. Data from epidemiological studies consistently show a beneficial effect of olive oil consumption on reducing breast cancer, but –up until now— not for colon cancer. Olive oil and olive oil phenolics have been positively associated with reduced oxidative DNA damage in humans. These encouraging findings need further confirmation in human interventional trials or large cohort studies in order to determine how this translates into decreased cancer incidence.

INTRODUCTION

Traditionally, the countries around the Mediterranean basin adhere to the so-called Mediterranean diet, rich in fruit, nuts, vegetables, whole-wheat bread, fish and olive oil, whereas only moderate amounts of alcohol, mostly red wine with meals, are consumed. This diet is essentially different from the diets in western and northern European countries. Thus, the relatively lower cancer occurrence in the European Mediterranean countries has been linked to their dietary habits. Rough estimations on the basis of over 100 case-control studies performed in the last decades of the previous century make it plausible that up to 25% of colorectal cancers and 15% of breast cancers could be prevented if the populations of the highly developed Western countries would shift to the traditional Mediterranean diet (1). Recent data from a general Greek population with a sample size of 25,623 participants (10,582 men and 15,041 women) and a median follow-up of 7.9 years provided solid evidence that adherence to the traditional Greek Mediterranean diet is associated with a 12% reduction in overall cancer incidence (2). Analyzing the impact of a particular dietary variable on cancer development, the highest reduction was observed for the ratio of monounsaturated to saturated lipids, for vegetables and for olive oil as such, with hazard ratios of 0.91, 0.96 and 0.97, respectively.

Although the Mediterranean area has different cultural and religious traditions, for many centuries olive oil has had a central and appreciated culinary position, as evidenced from historical documents. The people in ancient Egypt, Greece and Rome were well aware of the beneficial effects of olive oil. Already, Pliny the Elder (Gaius Plinius Secundis, AD 23-79) stated that: "Italy has an excellent olive oil…the best in the Mediterranean" (3). It has been reported that the Hebrew king David had guards watching over the olive trees (4) and there is evidence for olive oil production at the Carmel Coast in Israel as early as 6,500 years ago (5).

The pioneer Seven Countries Study, a multicenter intercohort study carried out in Finland, Greece, Italy, Japan, The Netherlands, the former Yugoslavia and the United States conducted by Keys et al. (6), found a relatively low incidence of cardiovascular diseases and cancer in the Mediterranean cohorts.

Olive oil is the major source of fat in the Mediterranean diet. Olive oil, especially virgin oil, provides antioxidants in abundance and contributes in this way to cancer prevention (7). Olive oil has a fatty acid fraction of 98-99% and a minor component fraction of 1-2%. Oleic acid (18:1n-9) represents 70-80% of the fatty acids found in olive oil (8). The single double bond in oleic acid makes it less susceptible to oxidation than the seed oils, which have multiple double bonds. Its monounsaturated character protects the olive fruit from oxidation in the Mediterranean climate and contributes to the oil's high stability

and relatively long shelf-life. Minor components of olive oil are non-nutrient molecules and constitute 1-2% of the total content of an olive oil. Among these, the most well known are squalene and the phenolic compounds. Squalene is a precursor in the biosynthesis of cholesterol and all of the steroid hormones. Compared with other vegetable oils, squalene appears in elevated proportions in olive oil (around 400 mg/kg). Phenolic compounds are present in olive oil, with a higher content in the virgin olive oils (around 230 mg/kg; common range 130-350 mg/kg) (9). Other vegetable oils do not have phenolic compounds due to the fact that they are submitted to refining processes in which the soluble compounds, the phenolics, are lost. Olive oil-rich diets reduce the levels of low-density lipoprotein (LDL) cholesterol compared to saturated oil-rich diets and increase the level of high-density lipoprotein (HDL) cholesterol compared to saturated and polyunsaturated oil-rich diets, thus diminishing the risk of atherosclerosis (10, 11). Moreover, circulating lipoproteins rich in oleic acid are more resistant to oxidation than those rich in linoleic acid (12, 13).

In addition to cardiovascular health benefits, it has been reported that virgin olive oil has anti-inflammatory and antimicrobial activity (8). Also, there is growing evidence that olive oil has antitumor activity. This review will discuss the anticancer properties of olive oil and its constituents, focusing on experimental and epidemiological data. The mechanism by which oleic acid, phenolics and squalene could exert this antitumor effect will also be reviewed. The human data on primary cancer endpoints that will be presented in this review will concentrate on breast cancer and colorectal cancer, as the majority of experimental and human studies have dealt with these common cancers.

MECHANISTIC ASPECTS OF OLIVE OIL COMPONENTS

The mechanisms suggested to be involved in the anticarcinogenic effect of olive oil primarily entail anti-inflammatory and antioxidant properties (8). Inflammation is part of the host defense system induced by cellular injury and is basically a self-limiting process. However, inadequate resolution of this inflammatory response may lead to chronic disease, including cancer. Sustained inflammation causes immune cells to produce excessive reactive oxygen species (ROS) and reactive nitrogen species (RNS), causing DNA damage and modification, as well as activation of oncogenes and inactivation of repair genes and tumor suppressor genes. These detrimental actions also comprise alterations in signaling processes. For example, ROS and RNS can activate the transcription factor AP-1 (activator protein 1) and nuclear factor NF-kappa-B signaling pathways involved in cell transformation and proliferation (14). Interestingly, both ROS and RNS can play a beneficial role at relatively low to moderate concentrations by causing apoptosis. Thus, active species-mediated actions could protect the cell against induced damage, a process called "redox homeostasis" (15).

The intake of dietary lipids gives rise to changes in the lipid profile of the cell membrane and can affect its structure and function. The most widely studied effects are altered membrane architecture and biochemical make-up after the intake of polyunsaturated fatty acids, notably omega-3 fats (16). These molecules alter the basic characteristics of the cell membrane due to steric incompatibility with the cholesterol and sphingolipids present in the lipid raft (17). Also, highly unsaturated fatty acids present in the membrane phospholipids are more susceptible to oxidation relative to monounsaturated fats like olive oil oleic acid (7, 18).

Olive oil phenolic antioxidants present in the cell may interact with the inflammatory cascade (19). The inhibition of the enzymatic activity of phospholipase A_2 (PLA_2), cyclooxygenase and lipoxygenase reduces the production of the proinflammatory arachidonic acid and its metabolites, the prostanoids and leukotrienes. These molecules have been linked experimentally to carcinogenesis. For example, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been associated with growth-associated proteins, resistance to apoptosis, tumor angiogenesis and promotion of tumor cell adhesion (20). The antioxidant activity of phenolic compounds is related to their ability to scavenge free radicals, preventing cellular injury (21).

Taken together, the generation of ROS and RNS in inflamed tissue and the subsequent detrimental effect on DNA, as well as the activation of oncogenes and the deactivation of repair and tumor suppressor genes, cause cancer to grow. Olive oil components may alleviate inflammatory reactions and oxidative responses following cell injury. Relatively high concentrations of oleic acid in the cell membrane reduce the formation of proinflammatory molecules. In addition, olive oil antioxidants reduce the harmful effect of reactive species in the inflammatory environment of the injured cell.

OLEIC ACID

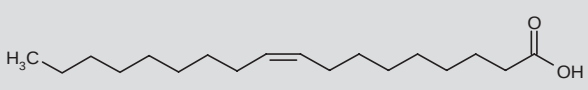
Experimental studies

The chemoprotective properties of **oleic acid** have been studied using breast cancer cell lines. Data from Menendez et al. (22, 23) showed that oleic acid suppresses overexpressed *HER2/NEU* oncogenes. A synergistic effect between oleic acid and trastuzumab in downregulating this oncogene resulted in increased apoptosis (22). In contrast with these findings, results obtained by Soto-Guzman et al. (24) showed that oleic acid stimulates breast cancer cells in those cell lines expressing GPR40, a G-protein coupled receptor. This process appeared to be mediated by EGFR transactivation. Thus, controversial data exist on the in vitro antitumor activity of oleic acid on breast cancer cells.

As far as colorectal cancer cells are concerned, Llor et al. (25) demonstrated that olive oil oleic acid is capable of inducing apoptosis and cell differentiation. Their cell line experiments also showed that oleic acid induced downregulation of COX-2, which was followed by a decrease in Bcl-2 expression, but not by decreased cell proliferation.

Epidemiological studies

A review of studies performed before 2005 shows that the association between monounsaturated fatty acid (MUFA) intake and breast



Oleic acid

cancer is neutral or beneficial for vegetable oils (26). A study in 1,703 breast cancer patients in the San Francisco Bay area showed that cooking with olive oil rich in oleic acid reduced the risk of breast cancer when compared with hydrogenated fats (odds ratio [OR]: 1.58; 95% confidence interval [CI]: 1.20-2.10) or with vegetable/corn oil rich in linoleic acid (OR: 1.30; 95% CI: 1.06-1.58) (27). The so-called Potsdam (Germany) study investigated the effect of high-fat food choices on the incidence of breast cancer in a prospective manner (28). This study comprised 15,351 female subjects who were followed over an average period of 6.0 years. The investigators identified a food pattern characterized by high consumption of processed meat, fish, butter and other animal fats and low consumption of bread and fruit juices. The intake of all fatty acid fractions, including MUFAs, appeared to be positively related with the incidence of breast cancer, with a twofold increase when comparing extreme tertiles. The authors concluded that total dietary fat, rather than specific fatty acids, is associated with the risk of breast cancer. However, the French component of the European Prospective Investigation into Cancer and Nutrition (EPIC), published in 2008, documented the monounsaturated fat intake in 19,934 women, of whom 363 cases developed breast cancer during an average follow-up of 7 years. An increased incidence of breast cancer was associated with increasing levels of serum *trans*-MUFA as opposed to *cis*-MUFA (a group to which oleic acid belongs), which appeared unrelated (29).

A large epidemiological study on oleic acid consumption and colon cancer risk was performed by Theodoratou et al. (30) and published in 2007. This case-control study included 1,455 cases and 1,455 matched controls, and lasted 7 years. The results of this study showed no clear association between oleic acid intake and colorectal cancer risk, as opposed to the inverse relationship found for omega-3 fatty acids.

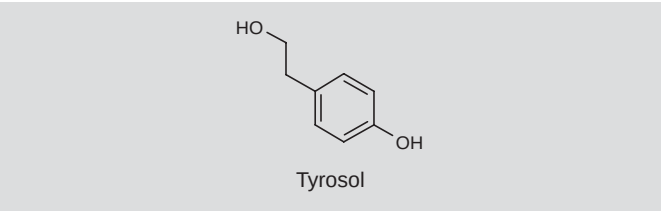
Taken together, experimental studies revealing an anticancer effect for oleic acid in breast cancer have recently been confirmed by a number of epidemiological studies, but controversial data preclude a definitive conclusion. With regard to colorectal cancer, human studies have not shown a positive effect on colorectal cancer risk, in contrast to the available experimental results.

PHENOLIC CONSTITUENTS

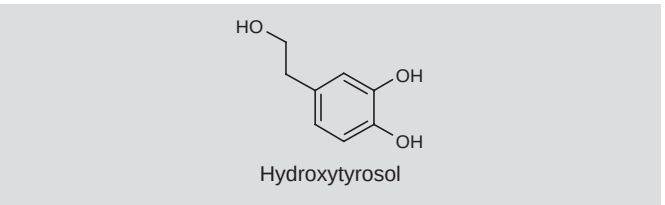
The phenolic content of olives depends on their (climatological) growth site and maturation conditions (31). Olives cultivated in warmer climates have a higher phenolic content. The concentration of phenolics in olive oil also depends on the variety of olives and the extraction and storage processes (32). The range of phenolic compounds in virgin olive oil consists of simple phenols such as **tyrosol** and **hydroxytyrosol**, polyphenols such as **oleuropein**, secoiridoids such as **ligstroside** lacking a methyl group and the aglycone of oleuropein, and lignans such as **pinoresinol** and **acetoxypinoresinol** (9, 33). Oleuropein, tyrosol and hydroxytyrosol are present in the highest concentrations (34).

Experimental studies

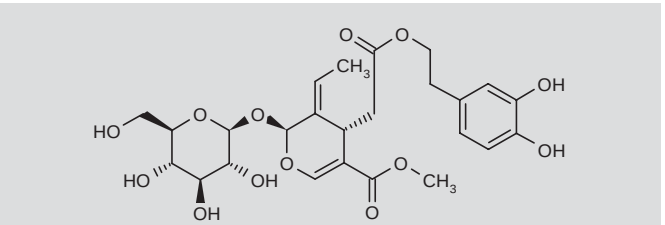
The antioxidant action of olive oil phenolic compounds in relation to their possible anticancer effect has been the subject of numerous experimental studies, which were summarized in 2005 by Tripoli et



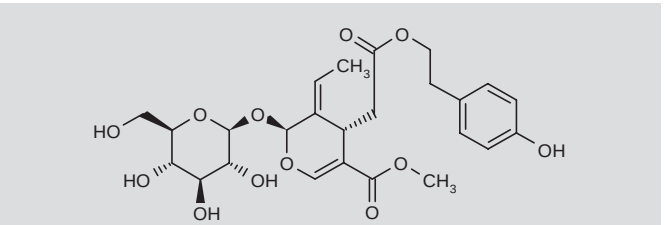
Tyrosol



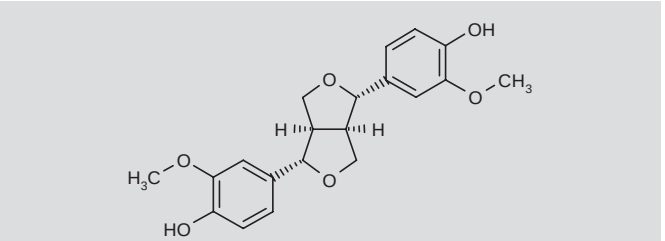
Hydroxytyrosol



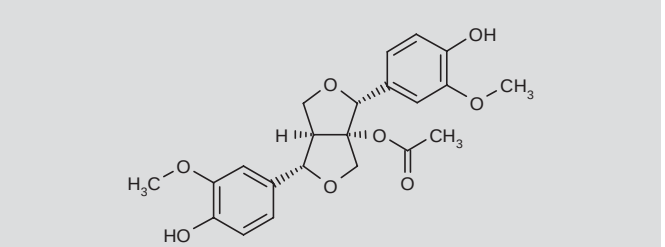
Oleuropein



Ligstroside



Pinoresinol



Acetoxypinoresinol

al. (8 and references therein). The results indicate that free radical scavenging is accompanied by inhibition of platelet aggregation, reduction of proinflammatory molecule formation, upregulation of the immune system, prevention of oxidative damage to erythrocytes and intestinal cells, as well as inhibition of carcinogenesis-promoting enzymes such as xanthine oxidase. Recent experimental data show an anticancer effect for olive oil phenolics in, e.g., colon cancer cells (34), breast cancer cells (35), leukemia cells (36) and hepatoma cells (37). An overall effect of olive oil phenolics on the initial step of carcinogenesis has recently been reported by Fabiani et al. (38). Their in vitro studies showed prevention of oxidative DNA damage in human leukemia cells by a complex mixture of phenols extracted from olive oil and olive mill waste water.

Epidemiological studies

In humans the bioactive effects of olive oil phenolics have been mostly investigated by assessing the resistance of LDL to oxidation. A protective effect on lipid oxidation by virgin olive oil high in phenolic compounds has been reported by numerous authors and has been reviewed by Fito et al. (39), Carlucci et al. (40) and Covas (41). Literature on the anticancer effects of olive oil phenolics in controlled human intervention is more scarce.

An international team of researchers (42) performed a multicenter, double-blind, randomized, crossover, controlled intervention trial in southern, central and northern European countries to investigate the impact of olive oil consumption and the effects of the phenolic content of olive oils from picual olives (Andalucia, Spain) on oxidative damage. The oils used were virgin (phenolic content [PC] = 366 mg/kg), common (PC = 164 mg/kg) and refined (PC = 2.7 mg/kg) from the same cultivar, soil and harvest. The various oils were similar with regard to nutrient content. This study comprised 200 healthy nonsmoking male volunteers who fulfilled the inclusion criteria from Denmark, Finland, Germany, Italy and Spain. The effect of olive oil from baseline to post-treatment showed a reduction in DNA oxidation of 13% (P = 0.008) in all types of olive oil consumed, although it did not appear possible to relate this to the concentration of the phenolics. The authors suggest that the beneficial effect of olive oil on oxidative stress to DNA was related to the monounsaturated fatty acid content.

Their results are in accordance with previous studies on the impact of olive oil phenolic concentration. These investigations demonstrated a decrease in the oxidative lipid damage dependent on the phenolic content of the olive oil administered, whereas no association betwen the degree of oxidative DNA damage and the phenolic content of the olive oil could be found (43, 44). In contrast, in a randomized, crossover intervention trial of high-phenol versus low-phenol olive oil in 10 postmenopausal Italian women from the Florence area, a 30% reduction in DNA oxidative damage was found when comparing two oils "from different parts of the Mediterranean basin" (45). Interestingly, for this study the participants consumed at least 50 g/day of the assigned study oil in raw form, whereas the participants of the above-mentioned international study consumed 25 mL/day (a supposed density of 800-920 kg/m^3 makes 50 g equal to about 55 mL; reference www.simetric.co.uk). This is in line with the findings by Weinbrenner et al. (46), who used 25 mL/day of Cretan olive oil. In this short-term study lasting 4 days and comprising 12 healthy nonsmoking males, the researchers found decreased DNA damage as a function of the olive oil phenolic content, evidenced by reduced 8-oxodeoxyguanosine, an oxidized base formed by free radical attack on DNA.

In summary, although some results point to a concerted action of olive oil constituents, there is no full evidence that the positive experimental anticarcinogenic effects of olive oil phenolics translate into reduced oxidative DNA stress in humans. On the contrary, consumption of oleic acid promotes a reduction in DNA damage. This is an encouraging observation, as DNA oxidation is generally considered an initial step in the carcinogenic process. These findings need further epidemiological study to determine to what extent this results in a decreased cancer incidence.

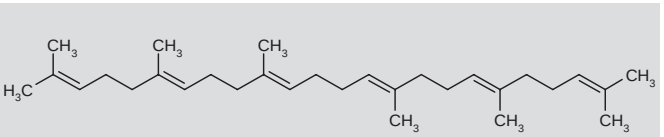
SQUALENE

Squalene is a triterpene found abundantly in nature, an important biochemical precursor of cholesterol and other steroids in flora and fauna. It is present in olive oil at concentrations between 0.2% and 0.7% (47). One of the early experiments that showed its chemoprotective effect was reported by Murakoshi et al. (48), who applied squalene topically and found suppression of experimental skin tumors in mice. In other animal experiments it was found that dietary squalene suppressed the formation of aberrant crypt foci (a biomarker for preneoplastic colon lesions) (49, 50). Skopinska-Rozewska et al. (51) have provided evidence that shark liver oil rich in squalene is able to inhibit cutaneous angiogenesis induced by transplanted sarcoma in mice[1].

However, none of these studies has been carried out with squalene from olive oil and, according to our knowledge, no such studies, if ever feasible in vitro or in vivo, have been reported and no epidemiological data are available. Nevertheless, from a theoretical standpoint its chemical structure allows for the scavenging of reactive species, which makes it a potential antioxidant (7, 56).

DISCUSSION AND COMMENTS

Since the Mediterranean diet as a whole is associated with a reduction in cancer incidence (2, 57, 58), it seems reasonable to assume that olive oil – with its central dietary position – could play an important role in the chemoprotective properties of this diet. This may be due to the effect of increased amounts of both antioxidants and oleic



Squalene

[1]The results of these experiments have led to the commercialization of "pure" squalene from shark liver tissue and cartilage without evidence of a clear-cut beneficial effect in humans. It should be noted that a well-performed trial in breast and colon cancer patients was unable to demonstrate "any suggestion of efficacy" (52). Various authors have relegated the use of more or less pure squalene to the realm of "pseudoscience" (53) or called it "ignorance-based treatment" (54). Moreover, sharks do get cancer (55).

acid relative to a non-Mediterranean diet. Phenolic compounds are abundantly present in plasma in conjugated form (mainly glucurono conjugates) after olive oil consumption (60). Oleuropein and other phenols are known to be absorbed by about 60 mol% in the intestine and are metabolized and recovered in urine as hydroxytyrosol (61, 62). It is also known that phenols are dose-dependently absorbed in humans (63) and that both a relatively high single-dose ingestion of about 55 mL and a sustained real-life dose of 25 mL/day for a week results in an increase in the urinary recovery of tyrosol and hydroxytyrosol (64).

Oleuropein and its hydrolysis product hydroxytyrosol are among the most potent antioxidants and retain their antioxidant activity in vivo (21, 32, 65, 66). This characteristic was confirmed in the Eurolive study, which showed that the administration of olive oil to volunteers resulted in a dose-dependent decrease in lipid oxidative damage (67). This is a remarkable result, as this prospective, randomized, multicenter study in 200 healthy subjects from 5 different European countries was carried out with three types of "similar" olive oil. This study also revealed that the relative amount of oleic acid in LDL increased, whereas the concentration of linoleic acid and arachidonic acid decreased. These results support the hypothesis that a sustained daily intake of a feasible dose of 25 mL of virgin oil promotes protective LDL changes prior to oxidation (68).

There is no reason why the modulation of the oxidative/antioxidant status associated with sustained olive oil consumption, fitting well in the culinary traditions of the Mediterranean diet, would not have a beneficial effect on carcinogenesis. Interestingly, cooking with olive oil may affect the bioavailability of antioxidants other than those present in olive oil. Fielding et al. (69) found a marked increase in the plasma concentration of lycopene (an antioxidant hypothesized to have an anticancer effect, particularly in colorectal and prostate cancer) from tomatoes after being cooked in olive oil as compared to those cooked without olive oil. In this context, ongoing experiments are studying the trend of antioxidant capacity and total phenolic concentration in heated olive oil (70).

An open issue is the amount of virgin olive oil required to achieve health benefits. Obviously, the concentration and total content of antioxidant compounds is an important factor and comparative studies need to be done to clarify this. One of the few comparative investigations concerned phenolic molecules in some French and Spanish olive oils (71). It was found that the quantity of individual phenolics was similar, except for pinoresinol, which was lower in French oil samples. More studies in this field can be expected soon, as analytical methods have improved considerably over the past years (72, 73).

With regard to the beneficial effect of the Mediterranean diet as a whole, Sofi et al. (58) recently performed a meta-analysis among 8 cohorts comprising 514,816 subjects and 33,576 deaths and showed a reduced incidence of or mortality from cancer (hazard ratio: 0.94; 95% CI: 0.92-0.96). It is interesting to note that six of these eight cohort studies concentrated on Greek populations. What is so special about the Greek Mediterranean diet? Simopoulos (74) answered this question in 2001, emphasizing the Cretan diet: "high intake of fruits and fruit juice, vegetables, herbs and spices, nuts (notably walnuts), garlic, onions, cereals (whole wheat and sourdough rather than pasta), olive oil and olives, less milk, more (goat) cheese, less meat, more fish, yoghurt, water and moderate amounts of red wine with meals". This diet is significantly differently from the diet that has been positively associated with cancer in Europe (see, e.g., 75, 76 and references therein).

In conclusion, several experimental studies provide support for the hypothesis that olive oil components such as oleic acid and phenolics play an important role in the anticancer properties of olive oil. However, the anticancer effect of these molecules still needs to be confirmed in human intervention trials or large long-lasting cohort studies with primary cancer as an endpoint. On the other hand, there is also solid epidemiological evidence that adherence to the Mediterranean diet as a whole is positively correlated with a low incidence of cancer (77). This fits well with the growing understanding that olive oil components act in an integrated manner within the network of molecules present in the Mediterranean diet (78, 79). Thus, it seems that, with regard to the beneficial anticancer effects of olive oil, the sum of the whole is greater than the sum of the individual parts.

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