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Review Article

Active Components and Clinical Applications of Olive Oil

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Abstract

The olive tree, *Olea europaea*, is native to the Mediterranean basin and parts of Asia Minor. The fruit and compression-extracted oil have a wide range of therapeutic and culinary applications. Olive oil also constitutes a major component of the "Mediterranean diet." The chief active components of olive oil include oleic acid, phenolic constituents, and squalene. The main phenolics include hydroxytyrosol, tyrosol, and oleuropein, which occur in highest levels in virgin olive oil and have demonstrated antioxidant activity. Antioxidants are believed to be responsible for a number of olive oil's biological activities. Oleic acid, a monounsaturated fatty acid, has shown activity in cancer prevention, while squalene has also been identified as having anticancer effects. Olive oil consumption has benefit for colon and breast cancer prevention. The oil has been widely studied for its effects on coronary heart disease (CHD), specifically for its ability to reduce blood pressure and low-density lipoprotein (LDL) cholesterol. Antimicrobial activity of hydroxytyrosol, tyrosol, and oleuropein has been demonstrated against several strains of bacteria implicated in intestinal and respiratory infections. Although the majority of research has been conducted on the oil, consumption of whole olives might also confer health benefits. *(Altern Med Rev* 2007;12(4):331-342)

Introduction

The olive tree, *Olea europaea*, produces the olive fruit. Olives are grown widely in the Mediterranean basin and parts of Asia Minor. References to the olive tree date back to Biblical and Roman times and to Greek mythology. Historically, the products of *Olea europaea* have been used as aphrodisiacs, emollients, laxatives, nutritives, sedatives, and tonics. Specific conditions traditionally treated include colic, alopecia, paralysis, rheumatic pain, sciatica, and hypertension.[1] The olive can be consumed whole as either the fully ripe black fruit or as the unripe green fruit. Olive oil, the major source of dietary fat in the countries where olives are grown,[2,3] constitutes part the commonly referred to "Mediterranean diet" of countries that surround the Mediterranean Sea and tend to have a low incidence of chronic degenerative disease.[4] Although there are dietary variations among Mediterranean countries, a common feature is the high consumption of olive oil, either uncooked or as the primary cooking fat.[4] Half the total fat consumed in the Mediterranean diet comes from cooking with olive oil, with deep fat frying being the most common method used.[4]

In the latter part of the 20th century, Keys et al conducted the Seven Countries Study, which revealed the Mediterranean diet is linked to a reduced incidence of degenerative diseases, particularly coronary heart disease (CHD) and cancers of the breast, skin, and colon.[5,6] This study inspired much research into the Mediterranean diet. In addition to olive oil, the Mediterranean diet is rich in healthful fiber, fish, fruits, and vegetables.[6] Since olive oil is the major energy source in the Mediterranean diet, recent research has focused on the contribution it makes to reported health benefits of the diet. Compared to diets of other countries, the

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Mediterranean diet has a relatively high fat content; however, as the diet is associated with a low incidence of cancer and CHD, despite the high fat intake, it has been suggested the type of fat is more important than the total amount consumed.[6]

To manufacture olive oil, olives are crushed to create a pomace, which is then homogenized before being pressed to produce oil. The first oil extracted is the high quality extra virgin olive oil – produced using centrifugation and water only. The pomace can then be processed again to yield the lower quality refined virgin olive oil. Further extraction with organic solvents can be undertaken to produce low quality refined husk oil.[7]

Olive oil is believed to exert its biological benefits mainly via constituent antioxidants. Although the composition of olive oil is complex, the major groups of compounds thought to contribute to its observed health benefits include oleic acid, phenolics, and squalene,[7] all of which have been found to inhibit oxidative stress. Antioxidants in olives protect them from oxidation by the high temperatures and ultraviolet radiation of the Mediterranean climate.[8] The physical methods used to produce olive oil preserve many of its antioxidant compounds. This is not seen with other vegetable and seed oils, which tend to be more refined. Factors affecting the environmental conditions of growing olives alter the constituents of the oil, including its antioxidant properties.[8]

Oleic Acid

Olive oil is approximately 72-percent oleic acid, a monounsaturated fatty acid.[9] Olive oil is unique with respect to the high oleic acid content because the majority of seed oils are composed primarily of polyunsaturated fatty acids, including the essential omega-6 fatty acid, linoleic acid. Compared to polyunsaturated fatty acids, oleic acid is monounsaturated, meaning it has one double bond, making it much less susceptible to oxidation and contributing to the antioxidant action, high stability, and long shelf life of olive oil.[10]

Data concerning the health benefits of oleic acid are conflicting. It has been reported that oleic acid plays a role in cancer prevention. Whether this is a secondary effect of the fatty acid on oil stability (preventing oxidative stress) or a direct anticancer effect remains debatable.[8] Preference for the latter theory is based on the fact that, although oleic acid is found in high concentration in olive oil,[11] it is also found in relatively high levels in foodstuffs that form a major part of Western diets in non-Mediterranean countries.[12] For example, beef and poultry contain 30- to 45-percent oleic acid, while oils such as palm, peanut, soybean, and sunflower contain 25- to 49-percent oleic acid.[9] These countries do not have the low incidence of CHD and cancer typical of the Mediterranean countries. This fact could be due to the comparatively low levels of oleic acid and concomitant high levels of other fatty acids.

Several *in vitro* and *in vivo* studies have examined the effect of oleic acid on cancer. Llor and Pons conducted *in vitro* experiments on the effect of olive oil or isolated oleic acid on colorectal neoplasia. They concluded olive oil induces apoptosis and cell differentiation and down-regulates the expression of cyclooxygenase-2 (COX-2) and Bcl-2. COX-2 is believed to play an important role in colorectal cancer development, while Bcl-2 is an intracellular protein that inhibits apoptosis. Oleic acid alone exhibited cell-line specific apoptotic induction, since HT-29 cells were affected but not Caco-2 cells. Oleic acid had no effect on the down-regulation of COX-2 and Bcl-2. Olive oil was found to have no effect on cell proliferation. The researchers concluded oleic acid plays a minor role, if any, in colorectal chemoprotection and that other components of olive oil are involved in this protective process.[13]

In vitro studies conducted by Menendez et al examined the effect of oleic acid on breast cancer cell lines.[11] The study results are encouraging and support the theory that oleic acid is important in chemoprotection. The researchers reported oleic acid down-regulates the over-expression of Her-2/neu, an oncogene over-expressed in approximately 20-percent of breast carcinomas. The gene, also know as erb-B2, encodes for the $p185^{Her\text{-}2/neu}$ oncoprotein, a transmembrane tyrosine kinase orphan receptor that, under normal cellular conditions, is highly regulated because it controls many cell functions, such as differentiation, proliferation, and apoptosis. Deregulation of $p185^{Her\text{-}2/neu}$ greatly increases the risk of cancer development. In addition to oleic acid alone, the authors also looked at the effect of oleic acid when compared to, and given simultaneously with, the anticancer drug trastuzumab (Herceptin®). Trastuzumab is a human monoclonal antibody that targets

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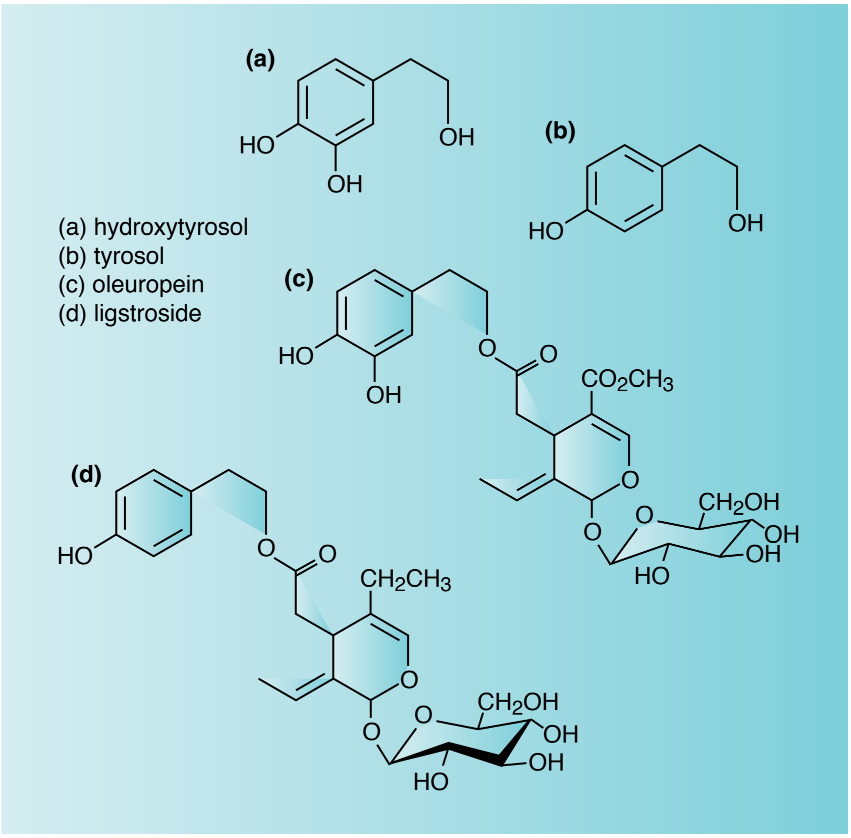
p185[Her-2/neu]. Menendez et al found oleic acid acts synergistically with trastuzumab to enhance its action when used against cell cultures that over-express the Her-2/neu oncogene.[11]

Following these results, Menendez et al sought to identify the mechanism of action for the down-regulation of the Her-2/neu oncogene by oleic acid.[14] The research focused on polyomavirus enhancer activator 3 (PEA3), a protein that represses the expression of Her-2/neu. They found oleic acid up-regulates PEA3. Low levels of PEA3 are found in cells over-expressing Her-2/neu; whereas, high levels of PEA3 are associated with low p185[Her-2/neu] expression.[14] Since these data are from *in vitro* cell lines, the authors warn the results cannot be extrapolated to prove exogenous consumption of oleic acid down-regulates Her-2/neu expression via up-regulation of PEA3 *in vivo*.

Figure 1. Olive Oil Phenols



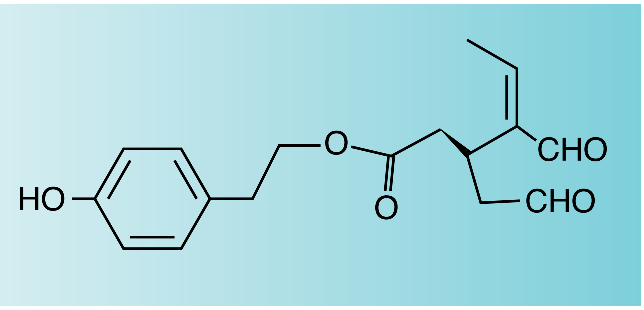
Phenolic Constituents

A range of phenols in olive oil provides some of its health benefits. The total phenolic content has been reported to be in the range of 196-500 mg/kg.[7] Although the reported levels of phenolic compounds in olive oil vary widely, one consistent conclusion is that extra virgin olive oil has a higher phenolic content than refined virgin olive oil.[7,15] Owen et al showed this difference was reflected in the levels of individual phenols as well as the total quantity of phenols in the oil.[10] The concentration of phenols depends on a number of factors, including environmental growth conditions, method of oil production, and storage conditions.[3]

Olive oil phenols can be divided into three categories: simple phenols, secoiridoids, and lignans, all of which inhibit auto-oxidation. Major phenols include hydroxytyrosol, tyrosol, oleuropein,[16] and ligstroside.[7] Hydroxytyrosol and tyrosol are simple phenols and oleuropein is a secoiridoid (Figure 1). The simple phenols hydroxytyrosol and tyrosol are formed from the hydrolysis of the secoiridoid aglycones of oleuropein and ligstroside. Hydrolysis of oleuropein, which occurs during olive oil storage,[17] results in the formation of hydroxytyrosol, tyrosol, and ethanol.[18] As well as being present in olive oil, hydroxytyrosol is endogenous to the brain as a catabolite of neurotransmitter breakdown.[8]

The phenolic content of the olive fruit changes as it grows and develops. After six months of growth, the major phenols are the glucosides of ligstroside and oleuropein.[7] As the olive matures these compounds are deglycosylated by glucosidase enzymes to free secoiridoids.[7] Unlike the glucosides, free secoiridoids can be detected in olive oil. Because the free secoiridoids are able to cross the oil/water barrier, these compounds partition into the oil.[7] Black olive pericarp extract (from the outer layer of the black olive) has a higher concentration of phenolic compounds and a higher antioxidant capacity than green olive pericarp extract.[6]

Figure 2. The Structure of Oleocanthal



It has been known for many years that compounds with a catechol group exhibit antioxidant activity.[3] The catechol group is able to stabilize free radicals through the formation of intramolecular hydrogen bonds. Of the three main phenols in olive oil, hydroxytyrosol and oleuropein are catechols and tyrosol is a mono-phenol. It has been suggested that, of all the phenols present in olive oil, only the catechols are important.[3]

Hydroxytyrosol and oleuropein scavenge free radicals and inhibit low density lipoprotein (LDL) oxidation.[3,7] These two phenols show dose-dependent activity and are considered potent antioxidants, demonstrating activity in the micro-molar range. Both are more potent at scavenging free radicals than the endogenous antioxidant vitamin E and the exogenous antioxidants dimethyl sulfoxide (DMSO) and butylated hydroxytoluene (BHT).[3,7] These two catechols have been shown to scavenge a variety of endogenous and exogenous free radicals and oxidants, including those generated by hydrogen peroxide,[7] hypochlorous acid, and xanthine/xanthine oxidase.[3] Higher concentrations of tyrosol are needed to exert an antioxidant effect.

Using hydroxyl radical scavenging as a measure of antioxidant capacity, Owen et al concluded olive oil has a higher antioxidant capacity than seed oils and extra virgin olive oil is more potent than refined virgin olive oil[10] due to its higher concentration of antioxidants. Similar results were obtained when xanthine oxidase[10] and hypochlorous acid[3] were used. Olive oil phenols are capable of scavenging free radicals produced in the fecal matrix, which is thought to explain the epidemiological data suggesting a colonic chemoprotective effect of olive oil.[7]

One mechanism associated with the anticancer effects of hydroxytyrosol and oleuropein is prevention of DNA damage, which can prevent mutagenesis and carcinogenesis.[3] Hydroxytyrosol, however, has biological activity beyond its antioxidant capacity, as it can affect a range of enzymes, including cyclooxygenase and NAD(P)H oxidase,[3] and reduce platelet aggregation.[3,19]

Recently a secoiridoid derivative, oleocanthal – the dialdehydic form of deacetoxy-ligstroside aglycone – was identified (Figure 2). This compound, having an extreme irritant effect on the throat, has demonstrated inhibition of cyclooxygenase enzymes and anti-inflammatory activity.[20]

Squalene

Squalene, ubiquitous in nature, is a triterpene hydrocarbon (Figure 3) and a major intermediate in the biosynthesis of cholesterol. Although found in both plants and animals, it is found in vastly different amounts.[9] While olive oil is composed of approximately 0.7-percent squalene,[9] other foods and oils typically have squalene levels in the range of 0.002-0.03 percent. Only a slight difference is observed between the level of squalene in extra virgin and refined virgin olive oils (extra virgin having higher levels).[7]

Although squalene is widely distributed throughout the body, the majority is transported to the skin.[9] Sebum has high levels (12%); whereas, adipose tissue has much lower levels (0.001-0.04%).[9]

Due to squalene's structure, it is more likely to scavenge singlet oxygen species than hydroxyl radicals.[9] Exposure to high levels of ultraviolet radiation causes the formation of carcinogenic singlet oxygen species within the skin, where a high concentration of squalene may provide a chemoprotective effect.[9] Squalene, found in high amounts in the Mediterranean diet, is believed to be responsible for the lower incidence of skin cancer seen in epidemiological studies of populations consuming this diet.

Animal studies have shown topical squalene has an inhibitory action on chemically-induced skin carcinomas.[9] Squalene added to the diet of rats resulted in an 80-percent increase in serum squalene levels and inhibition of the hepatic enzyme HMG-CoA reductase.[9]

Figure 3. The Structure of Squalene

The enzyme inhibition may be due to squalene or its metabolites. HMG-CoA reductase, the rate-limiting enzyme in the biosynthesis of cholesterol, results in decreased production of cholesterol and the intermediates formed during its biosynthesis. These intermediates are commonly needed to activate oncogenes.[9] One important intermediate is the compound farnesyl pyrophosphate (FPP), which is involved in the prenylation of several oncoproteins. Because other dietary substances that cause a reduction in FPP levels cause a reduction in tumor growth, squalene is hypothesized to work in the same manner.[9]

Following acute administration of squalene, the rate of cholesterol synthesis increased 9-24 hours post-administration.[21] This apparent conflicting data may be a result of the single acute dose of squalene used in this study; whereas, chronic long-term administration results in reduced HMG CoA reductase activity and increased fecal elimination of cholesterol.[21]

Longer-term studies of the effect of chronic squalene intake on serum cholesterol levels have reported increased, decreased, or unchanged levels. These observed differences may be due to the dose of squalene. Short-term studies have shown increased dietary squalene, while increasing serum squalene levels, does not cause an increase in serum cholesterol or atherosclerosis.[22]

Cooking with Olive Oil

In the Mediterranean diet, olive oil is consumed cold as a dressing for salads and pasta and used for sauteing and deep frying. Therefore, it is important to determine the stability of the identified active components when subjected to heat. The major process contributing to the instability of olive oil when stored or heated is fat oxidation.[4] Sufficient exposure and degradation can lead to significant changes in the composition of olive oil, and these changes affect its biological properties. Due to the reduction in polyphenol content during heating, cooking with olive oil produces a number of degradation products,[23] with lipid peroxidation occurring to a limited extent.

The heating method also affects degradation. In traditional Mediterranean cooking, olive oil is boiled or heated conventionally. In recent times, the introduction of the microwave oven has added another method to heat olive oil. Research has shown the composition of heated olive oil to be different for all three methods.[23,24] For conventional heating, a time-dependent effect is observed, with the phenolic content being reduced as heating time increases.[23] Individual phenols react differently to conventional heating; for example, hydroxytyrosol levels decrease rapidly, as do lignans, but at a slower rate.[23] With respect to microwave heating, Brenes et al report only minor changes in polyphenol content; whereas, Caponi et al report microwave heating produces a greater amount of degradation products than conventional heating.[23,24] The reported differences are possibly due to different experimental methods used. For example, the length of time the oil is heated in a microwave appears to have a dramatic effect on degradation of the oil.[23,24] Brenes et al also looked at the effects of boiling olive oil in a pressure cooker and concluded that water of pH 4-5 (acidic) was a major contributing factor to the level of degradation. They reported a reduction in the levels of polyphenols occurred, but the olive oil was not oxidized following boiling.[23]

Lipid peroxidation products have been linked to cancer and cardiovascular disease.[4] Compared with other oils used for cooking, olive oil has a high concentration of monounsaturated fatty acid and a low concentration of polyunsaturated fatty acids. This means fewer targets for reactive oxygen species (ROS), making olive oil more stable and less likely to undergo peroxidation. In addition, olive oil contains many antioxidants that reduce lipid peroxidation.[4] Although antioxidants protect olive oil from thermal degradation,[25] frying reduces the oil's antioxidative capacity,[26] a particularly important fact when the same oil is used repeatedly.

Deep fat frying has both advantages and disadvantages related to olive oil degradation. The low oxygen exposure of the oil and a short cooking time reduce the potential for lipid peroxidation.[4] However, because the oil is more likely to be re-used, accumulation of polymeric compounds occurs as the antioxidant capacity is being reduced.[4,27] Compared to other oils, olive oil has a relatively long deep-fat frying "shelf-life" and is comparatively more stable than other oils for repeated frying.[4,27,28] Because exchange between lipids in the food and the oil occurs during cooking, the type of food fried also plays a role. For example, frying fish increases the oil's instability because the oil becomes enriched with polyunsaturated fatty acids, which are more susceptible to oxidative degradation than monounsaturated fatty acids.[4] Although frying foods with a high protein content such as meat, fish, and eggs can potentially produce carcinogenic heterocyclic amines (HCAs), the antioxidants present in olive oil limit the formation of HCAs.[28,29]

The Effect of Olive Oil on Specific Conditions

Coronary Heart Disease

Epidemiological studies demonstrate the Mediterranean diet reduces the incidence of CHD.[30,31] The antioxidant effects of olive oil may contribute to these protective effects.

To understand how olive oil might help prevent atherosclerosis, a review of atherosclerotic plaque formation is in order. Oxidation of LDL cholesterol has been identified as one of the first steps in the development of atherosclerotic lesions by promoting injury to the arterial wall through several mechanisms, including growth factor and chemotactic protein expression, inflammation, and increased local macrophages. Macrophages bind to and engulf oxidized LDL – an innate immune response to tissue damage. This engulfment produces a fatty foam cell, which, when combined with other cells, produces a fatty streak in the blood vessel.[32] Oxidized LDL can also be taken up directly by endothelial and smooth muscles cells, leading to formation of fatty streaks, which is the first sign of atherosclerosis. The lesions forming atherosclerotic plaques are made up of lipids, endothelial and smooth muscle cells, and extracellular matrix. The plaque environment is proinflammatory.[4] Inflammation occurring prior to the formation of fatty streaks and atherosclerotic lesions causes alterations to the endothelial cell wall, which increases the adhesion of leukocytes, LDL cholesterol, and platelets. This contributes to the development of atherosclerosis and cardiovascular disease.[32]

In vitro studies have demonstrated hydroxytyrosol and oleuropein are capable of inhibiting production of isoprostanes, a marker of LDL oxidation.[33] It has been suggested that phenols present in olive oil may act synergistically with these constituents to prevent LDL oxidation.

Hypertension

As with other aspects of cardiovascular diseases, there is a reduced incidence of hypertension in populations that consume the Mediterranean diet,[16,34] and adherence to the Mediterranean diet is inversely related to systolic and diastolic blood pressure.[35] Several studies have demonstrated the antihypertensive properties of olive oil.[1,5,36-38] Giliani et al found intravenous administration of olive oil extract reduced systolic, diastolic, and mean arterial blood pressures in normotensive rats.[1] Epidemiological data from studies in three Mediterranean countries – Italy, Greece, and Spain – as well as non-Mediterranean countries, suggest a protective effect for monounsaturated fatty acids or olive oil, while non-Mediterranean countries show little or no positive effects.[37]

Ferrara et al compared a diet rich in polyunsaturated fatty acids (from sunflower oil) with a diet high in monounsaturated fatty acids (from olive oil) in patients taking antihypertensive medications[38] and found individuals who consumed an olive oil-rich diet were able to reduce the dosage of antihypertensive medication.

Olive oil's precise mechanism of action for blood pressure reduction is unknown, although several theories have been proposed. Giliani et al concluded that olive oil is a calcium channel antagonist, closely mimicking the effects of the calcium channel blocker drug verapamil.[1] Another suggested mechanism is via improved endothelial function.[16,37,39] Phenols and oleic acid may contribute to improved endothelial function by reducing ROS.[16] Other potential mechanisms have been suggested, including decreasing vascular tone and changes to the fatty acid and phospholipid composition of the aorta.[38]

Cancer

Because strong epidemiological evidence suggests people who consume the Mediterranean diet have a lower incidence of certain cancers, including breast, skin, and colon,[4,7] research has focused on possible mechanisms to explain this phenomenon. Oxidation of proteins, DNA, and lipids has been shown to contribute to cancer development, and consumption of antioxidants is believed to reduce the risk of mutagenesis and carcinogenesis.[12] Antioxidants are present in olive oil, fruits, and vegetables that constitute a large part of the Mediterranean diet. The exact contribution olive oil makes to the apparent dietary chemoprotection is debatable; *in vitro* studies have found olive oil phenols are potent antioxidants, which may provide potential chemoprotective properties, although *in vivo* data are lacking.

Research examining individual phenolic compounds has found hydroxytyrosol is capable of protecting cells from hydrogen peroxide damage and DNA from peroxynitrite-induced damage, blocking cell cycle progression at the G_1 phase, and inducing apoptosis.[19] *In vivo* and *in vitro* studies on the activity of oleuropein have found, in addition to antioxidant properties, it has antiangiogenic action and inhibits cell growth, motility, and invasiveness.[40] Oleuropein has also been found to cause cell rounding, which disrupts the cell actin cytoskeleton. Oleuropein also affects and disrupts purified actin filaments, providing direct antitumor effects due to cell disruption.[40] In *in vivo* animal studies, rapid tumor regression was observed when mice were given one-percent oleuropein in drinking water.[40]

Saturated animal fats and polyunsaturated plant fats in the diet have been implicated in colon, breast, prostate, and ovarian cancers.[41] The substitution of olive oil in the Mediterranean diet may explain its apparent cancer-protective effect and accentuate the importance of the type, rather than the amount, of fat consumed.

Colon Cancer

The HCAs produced when protein-containing food is fried have been found to induce breast, colon, and pancreatic cancer in rats.[28] Based on this evidence, Galeone et al used data from a multinational, case control study to examine the relationship between fried foods and colorectal cancer.[28] When olive oil was compared to other oils, it was found that fried olive oil has a protective effect against colon cancer. This agrees with data that unheated olive oil is beneficial in protecting against colon cancer.[28] As described previously, when olive oil is used for frying, fewer HCAs are produced than when oils high in polyunsaturated fatty acids are used.

Later *in vitro* research by Gill et al looked at the effect of virgin olive oil phenols on colorectal carcinogenesis.[42] Using specific cell lines, they investigated processes involved in cancer initiation, promotion, and metastasis – the three main stages in cancer development – and concluded olive oil phenols exert beneficial effects in all three stages. The oil extract was shown to reduce DNA damage (initiation), increase barrier function (promotion), and reduce cell invasion of surrounding tissue (metastasis).[42]

Breast Cancer

Most of the active compounds in olive oil are lipid soluble; however, even though the phenolic glycosides are less so, they are likely to be stored in fat tissue. This may explain the chemoprotective effect against breast cancer and the low incidence of breast cancer in Mediterranean countries. In addition, oleic acid is incorporated into the phospholipid membrane of breast tissue cells, resulting in a reduction in lipid peroxidation.[42]

Although in developed countries breast cancer is the most common cancer seen in women, there is vast geographical variation in its incidence.[42,43] Epidemiological data show women in the Mediterranean basin have a lower incidence of breast cancer than women in other "Western" countries.[5,7] Case control studies that looked at women in several Mediterranean countries have shown an inverse correlation between olive oil consumption and the incidence of breast cancer.[43,44]

High mammographic breast density (H-MBD) is associated with greater breast cancer risk.[44] Using volunteers from the European Prospective Investigation into Cancer and Nutrition (EPIC) study, Masala et al examined the effect of diet and lifestyle on MBD and concluded consumption of olive oil is inversely related to the risk of H-MBD. Women who reported olive oil intake of ≥30.5 g/day were 30-percent less likely to be classified into the H-MBD group.

Using data from the European Community Multicenter Study on Antioxidants, Myocardial Infarction and Breast Cancer (EURAMIC), Simonsen et al looked at the relationship between monounsaturated fat intake, the storage of monounsaturated fatty acids in breast tissue, and postmenopausal breast cancer.[41] They found a strong inverse relationship between oleic acid consumption and breast cancer only in the Spanish group,[42] possibly due to the reported high consumption of olive oil in Spain.

Animal studies using dimethylbenz(α)anthracene-induced cancer have shown a diet rich in olive oil has a non-promoting effect on carcinogenesis. This effect is backed up by histopathological and morphological features.[45,46]

Antimicrobial Activity

In vitro studies have demonstrated the antimicrobial activity of hydroxytyrosol, tyrosol, and oleuropein against several strains of bacteria implicated in intestinal and respiratory infections. Hydroxytyrosol and oleuropein have antimicrobial action against both American Type Culture Collection (ATCC) and patient-derived clinical bacterial strains, with slightly greater activity against ATCC strains. It has been proposed that this action is due to the two ortho-positioned phenolic groups in their structure (Figure 1).[15]

A recent study found virgin olive oil has bactericidal action against *Helicobacter pylori*,[17] the primary cause of gastric ulcers and linked to gastric cancers. In recent years some strains have shown resistance to the typical antibiotics used to eradicate the infection and aid ulcer healing, spurring research on other compounds to treat the infection. Because phenolic compounds have been identified as having antibacterial properties, olive oil, with its high phenolic content, has been studied for *H. pylori*. Romero et al concluded phenols inhibited bacterial growth at low concentration and were stable for several hours in the highly acidic environment of the stomach. They found the secoiridoid aglycones, particularly the dialdehydic form of decarboxymethyl ligstroside, have the greatest anti-*H. pylori* activity and are not hydrolyzed in the stomach;[17] hydrolysis, if it occurs, produces the less active hydroxytyrosol and tyrosol. As the concentration of phenolics needed to kill *H. pylori* cells is higher than that for antibiotics, the researchers suggest virgin olive oil should be considered as preventive rather than a treatment agent. Because the research was conducted *in vitro*, the researchers also suggest *in vivo* testing is needed to confirm or reject the conclusions, an especially important note since other foods that demonstrate good activity against *H. pylori in vitro* do not appear to have any action *in vivo*. The mechanism by which phenolic compounds affect *H. pylori* is unknown at present.[17]

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic joint inflammation and damage. The initial autoimmune stimulus is unknown; however, joint and tissue damage occurs by a variety of mechanisms, many of which involve reactive oxygen species. ROS can cause destruction of hyaluronic acid and disruption to collagen, proteoglycans, protease inhibitors, and membrane function, the latter via oxidation of membrane fatty acids.[47] The initiation of RA is believed to result in an increase in the concentration of macrophages and neutrophils in the synovial fluid and free-radical-producing enzymes. This leads to high levels of ROS in the joints, which increases and prolongs inflammation and damage.[47] The antioxidant effect of olive oil has been found to reduce inflammation. In addition, dietary omega-9 monounsaturated fatty acids, such as oleic acid, have been found to replace omega-6 polyunsaturated fatty acids (PUFAs) in several aspects of cell metabolism. Reducing the competition between omega-6 and omega-3-PUFAs can lead to an increased use and incorporation of omega-3-PUFAs.[47]

A number of studies that examined the benefits of fish oils in RA used an olive oil placebo for the control groups.[48-50] Although results highlighted the benefits of fish oils, unexpected significant improvements were also seen in the control groups. Benefits including pain reduction, reduced morning stiffness, and improved patient evaluation of global disease were reported by patients receiving olive oil only. No explanation of the improvements shown by the olive oil groups were proposed, although changes in immune function may be responsible.[50]

As a result of these data, Berbert et al conducted research to establish whether olive oil improved RA symptoms in patients already receiving fish oil.[51] Olive oil appears to act synergistically with omega-3 fish oils to improve the symptoms of RA; the benefits are thought to be exerted through the oleic acid component. Oleic acid is converted to eicosatrienoic acid (ETA) and then leukotriene A_3 (LTA_3). LTA_3 is a potent inhibitor of proinflammatory leukotriene B_4 synthesis.[51] It has also been shown that olive oil consumption decreases the risk of developing RA.[52]

Conclusion

Considerable evidence indicates the Mediterranean diet is linked to a decreased incidence of cardiovascular disease and certain cancer types, despite the fact that this diet is higher in fat than other Western diets. An important component of this diet is the main source of fat – olive oil. Since previous research concludes fat intake has a positive correlation with the risk of CHD and cancer, and the Mediterranean diet is high in fat, a conclusion can be drawn that the type of fat is more important than the total amount consumed. Saturated fatty acids have been linked to unfavorable health outcomes; whereas, monounsaturated fatty acids have been found to be beneficial, even though olive oil contains no essential omega-3 or omega-6 PUFAs. The high level of the monounsaturated fatty acid oleic acid in olive oil is therefore believed to contribute to the low incidence of chronic diseases in populations that consume the Mediterranean diet.

Although initial studies focused on the overall Mediterranean diet, more recent studies compare a diet rich in olive oil to one low in olive oil. These studies provide good evidence olive oil may be beneficial for reducing high blood pressure and preventing breast and colon cancer, although the latter is *in vitro* evidence. Based on the positive results seen with epidemiological, case control, and cohort studies, *in vitro* studies have been designed to identify how olive oil exerts its effects at the cellular level.

The antioxidant capacity of olive oil contributes to many of its health benefits. Oleuropein and its hydrolysis product hydroxytyrosol are the most potent antioxidants. The antioxidant action of olive oil *in vitro* has been highly documented and linked to such benefits as chemoprotection, anti-inflammatory action, and prevention of atherosclerotic plaque formation. Care is needed when extrapolating *in vitro* data to *in vivo* models because it cannot be assumed that the effects seen when cells are exposed directly to olive oil extracts will be seen when olive oil is consumed in the diet. There is, however, evidence that the active compounds in olive oil are capable of distribution throughout the body. It has been estimated that 55-66 percent of olive oil phenols are absorbed after ingestion, the majority in the small intestine.[53] Phenols are believed to act in the blood vessels to prevent LDL oxidation and in tissues to protect against DNA damage. In addition, it has been demonstrated that olive oil phenols retain antioxidant activity *in vivo* when given orally.[3] Squalene absorption has also been demonstrated by the increase in squalene blood levels following long-term dosing.

Research has focused on the effects of olive oil rather than whole olives because it is easier to analyze, extract, and administer individual compounds or fractions from the oil. However, it seems safe to assume the benefits of olive oil also apply to consumption of whole olives because the therapeutic components of the oil are also found in the whole olive. Research has repeatedly shown extra virgin olive oil contains significantly more antioxidants than refined virgin olive oil and husk oil.

Because *in vitro* studies have shown the antioxidant activity of hydroxytyrosol and oleuropein is dose dependent, the amount of olive oil consumed is likely to affect its chemoprotective and cardioprotective effects. Although it is not unreasonable to assume greater protection is provided when olive oil comprises a greater proportion of the diet, minimal data exists to indicate the amount of olive oil or length of consumption needed to achieve health benefits. Research indicates annual olive oil consumption in Mediterranean countries can be as high as 15 kg per person.[7] In the studies comparing an olive oil-rich diet to an olive oil-poor diet, the level of olive oil consumed was not always recorded and reported. In some cases this was impossible due to the design of the study; however, some data are available. Regarding breast cancer, the highest protective effect of olive oil was seen in women who consumed ≥30.5 g/day.[44] With respect to blood pressure reduction, an effect was seen with dietary supplementation of 40 g/day for men and 30 g/day for women,[38] which equates to approximately 15 kg/year and 11 kg/year, respectively. These values are at the higher end of the reported olive oil consumption for Mediterranean countries.

Although the diet consumed in Mediterranean countries is thought to be a significant, if not the primary, contributor to the reduced incidence of CHD and certain cancers seen in these populations, it may not be the only factor. Genetic, cultural, and lifestyle influences likely play a factor. Olive oil is also not the only component of the Mediterranean diet that has been found to have biological benefits. Other foods consumed in the diet have been shown to provide important health-promoting nutrients, including garlic, fruits and vegetables rich in antioxidants, and fish high in omega-3 fatty acids. The evidence indicates, however, that olive oil and its components contribute significantly to the health benefits of the Mediterranean diet, with more of an effect on prevention than treatment.

References

1. Gilani AH, Khan AU, Shah AJ, et al. Blood pressure lowering effect of olive is mediated through calcium channel blockade. *Int J Food Sci Nutr* 2005;56:613-620.
2. Wahrburg U, Kratz M, Cullen P. Mediterranean diet, olive oil and health. *Eur J Lipid Sci Technol* 2002;104:698-705.
3. Visioli F, Poli A, Gall C. Antioxidant and other biological activities of phenols from olives and olive oil. *Med Res Rev* 2002;22:65-75.
4. Harwood JL, Yaqoob P. Nutritional and health aspects of olive oil. *Eur J Lipid Sci Technol* 2002;104:685-697.
5. Keys A, Menotti A, Karvonen MJ, et al. The diet and 15-year death rate in the Seven Countries Study. *Am J Epidemiol* 1986;124:903-915.
6. Owen RW, Haubner R, Wurtele G, et al. Olives and olive oil in cancer prevention. *Eur J Cancer Prev* 2004;13:319-326.
7. Owen RW, Giacosa A, Hull WE, et al. Olive-oil consumption and health: the possible role of antioxidants. *Lancet Oncol* 2000;1:107-112.
8. Visioli F, Galli C, Galli G, Caruso D. Biological activities and metabolic fate of olive oil phenols. *Eur J Lipid Sci Technol* 2002;104:677-684.
9. Newmark HL. Squalene, olive oil, and cancer risk: a review and hypothesis. *Cancer Epidemiol Biomarkers Prev* 1997;6:1101-1103.
10. Owen RW, Mier W, Giacosa A, et al. Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignans and squalene. *Food Chem Toxicol* 2000;38:647-659.
11. Menendez JA, Vellon L, Colomer R, Lupu R. Oleic acid, the main monounsaturated fatty acid of olive oil, suppresses Her-2/*neu* (erbB-2) expression and synergistically enhances the growth inhibitory effects of trastuzumab (Herceptin) in breast cancer cells with Her-2/*neu* oncogene amplification. *Ann Oncol* 2005;16:359-371.
12. Visioli F, Grande S, Bogani P, Galli C. The role of antioxidants in the Mediterranean diets: focus on cancer. *Eur J Cancer Prev* 2004;13:337-343.
13. Llor X, Pons E, Roca A, et al. The effects of fish oil, olive oil, oleic acid and linoleic acid on colorectal neoplastic processes. *Clin Nutr* 2003;22:71-79.
14. Menendez JA, Papadimitropoulou A, Vellon L, Lupu R. A genomic explanation connecting "Mediterranean diet," olive oil and cancer: oleic acid, the main monounsaturated fatty acid of olive oil, induces formation of inhibitory "PEA3 transcription factor-PEA3 DNA binding site" complexes at the Her-2/*neu* (erbB-2) oncogene promoter in breast, ovarian and stomach cancer cells. *Eur J Cancer* 2006;42:2425-2432.
15. Tuck KL, Hayball PJ. Major phenolic compounds in olive oil: metabolism and health effects. *J Nutr Biochem* 2002;13:636-644.
16. Perona JS, Cabello-Moruno R, Ruiz-Gutierrez V. The role of virgin olive oil components in the modulation of endothelial function. *J Nutr Biochem* 2006;17:429-445.
17. Romero C, Medina E, Vargas J, et al. *In vitro* activity of olive oil polyphenols against *Helicobacter pylori*. *J Agric Food Chem* 2007;55:680-686.
18. Martinez-Dominguez E, De la Puerta R, Ruiz-Gutierrez V. Protective effects upon experimental inflammation models of a polyphenol-supplemented virgin olive oil diet. *Inflamm Res* 2001;50:102-106.

19. Fabiani R, De Bartolomeo A, Rosignoli P, et al. Cancer chemoprevention by hydroxytyrosol isolated from virgin olive oil through G1 cell cycle arrest and apoptosis. *Eur J Cancer Prev* 2002;11:351-358.
20. Beauchamp GK, Keast RS, Morel D, et al. Phytochemistry: Ibuprofen-like activity in extra-virgin olive oil. *Nature* 2005;437:45-46.
21. Relas H, Gylling H, Miettinen TA. Dietary squalene increases cholesterol synthesis measured with serum non-cholesterol sterols after a single oral dose in humans. *Atherosclerosis* 2000;152:377-383.
22. Strandberg TE, Tilvis RS, Miettinen TA. Metabolic variables of cholesterol during squalene feeding in humans: comparison with cholestyramine treatment. *J Lipid Res* 1990;31:1637-1643.
23. Brenes M, Garcia A, Dobarganes MC, et al. Influence of thermal treatments simulating cooking processes on the polyphenol content in virgin olive oil. *J Agric Food Chem* 2002;50:5962-5967.
24. Caponio F, Pasqualone A, Gomes T. Effects of conventional and microwave heating on the degradation of olive oil. *Eur Food Res Technol* 2002;215:114-117.
25. Gennaro L, Bocca AP, Modesti D, et al. Effect of biophenols on olive oil stability evaluated by thermogravimetric analysis. *J Agric Food Chem* 1998;6:4465-4469.
26. Quiles JL, Ramirez-Tortosa MC, Gomez JA, et al. Role of vitamin E and phenolic compounds in the antioxidant capacity, measured by ESR, of virgin olive, olive and sunflower oils after frying. *Food Chem* 2002;76:461-468.
27. Galeone C, Talamini R, Levi F, et al. Fried foods, olive oil and colorectal cancer. *Ann Oncol* 2007;18:36-39.
28. Bastida S, Sanchez-Muniz FJ. Thermal oxidation of olive oil, sunflower oil and a mix of both oils during forty discontinuous domestic fryings of different foods. *Food Sci Technol Int* 2001;7:15-21.
29. Monti SM, Ritieni A, Sacchi R, et al. Characterization of phenolic compounds in virgin olive oil and their effect on the formation of carcinogenic/mutagenic heterocyclic amines in a model system. *J Agric Food Chem* 2001;49:3969-3975.
30. de Lorgeril M, Salen P. The Mediterranean diet in secondary prevention of coronary heart disease. *Clin Invest Med* 2006;29:154-158.
31. de Lorgeril M, Salen P. The Mediterranean-style diet for the prevention of cardiovascular diseases. *Public Health Nutr* 2006;9:118-123.
32. Patrick L, Uzick M. Cardiovascular disease: C-reactive protein and the inflammatory disease paradigm: HMG-CoA reductase inhibitors, alpha-tocopherol, red yeast rice, and olive oil polyphenols. A review of the literature. *Altern Med Rev* 2001;6:248-271.
33. Salami M, Galli C, De Angelis L, Visioli F. Formation of F2-isoprostanes in oxidized low density lipoprotein: inhibitory effect of hydroxytyrosol. *Pharmacol Res* 1995;31:275-279.
34. Carollo C, Presti RL, Caimi G. Wine, diet, and arterial hypertension. *Angiology* 2007;58:92-96.
35. Psaltopoulou T, Naska A, Orfanos P, et al. Olive oil, the Mediterranean diet, and arterial blood pressure: the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Am J Clin Nutr* 2004;80:1012-1018.
36. Ruiz-Gutierrez V, Muriana FJ, Guerrero A, et al. Role of dietary oleic acid from different sources on fatty acid composition of erythrocyte membrane and blood pressure in healthy subjects. *J Nutr Biochem* 1997;8:689-695.
37. Alonso A, Ruiz-Gutierrez V, Martinez-Gonzalez MA. Monounsaturated fatty acids, olive oil and blood pressure: epidemiological, clinical and experimental evidence. *Public Health Nutr* 2006;9:251-257.
38. Ferrara LA, Raimondi AS, d'Episcopo L, et al. Olive oil and reduced need for antihypertensive medications. *Arch Intern Med* 2000;160:837-842.
39. Herrera MD, Perez-Guerrero C, Marhuenda E, Ruiz-Gutierrez V. Effects of dietary oleic-rich oils (virgin olive and high-oleic-acid sunflower) on vascular reactivity in Wistar-Kyoto and spontaneously hypertensive rats. *Br J Nutr* 2001;86:349-357.
40. Hamdi HK, Castellon R. Oleuropein, a non-toxic olive iridoid, is an anti-tumor agent and cytoskeleton disruptor. *Biochem Biophys Res Commun* 2005;334:769-778.
41. Simonsen NR, Fernandez-Crehuet Navajas J, Martin-Moreno JM, et al. Tissue stores of individual monounsaturated fatty acids and breast cancer: the EURAMIC study. European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Breast Cancer. *Am J Clin Nutr* 1998;68:134-141.
42. Gill CI, Boyd A, McDermott E, et al. Potential anti-cancer effects of virgin olive oil phenols on colorectal carcinogenesis models *in vitro*. *Int J Cancer* 2005;117:1-7.
43. Sieri S, Krogh V, Pala V, et al. Dietary patterns and risk of breast cancer in the ORDET cohort. *Cancer Epidemiol Biomarkers Prev* 2004;13:567-572.
44. Masala G, Ambrogetti D, Assedi M, et al. Dietary and lifestyle determinants of mammographic breast density. A longitudinal study in a Mediterranean population. *Int J Cancer* 2006;118:1782-1789.
45. Solanas M, Hurtado A, Costa I, et al. Effects of a high olive oil diet on the clinical behavior and histopathological features of rat DMBA-induced mammary tumors compared with a high corn oil diet. *Int J Oncol* 2002;21:745-753.

46. Costa I, Moral R, Solanas M, Escrich E. High-fat corn oil diet promotes the development of high histologic grade rat DMBA-induced mammary adenocarcinomas, while high olive oil diet does not. *Breast Cancer Res Treat* 2004;86:225-235.
47. Darlington LG, Stone TW. Antioxidants and fatty acids in the amelioration of rheumatoid arthritis and related disorders. *Br J Nutr* 2001;85:251-269.
48. Soeken KL, Miller SA, Ernst E. Herbal medicines for the treatment of rheumatoid arthritis: a systematic review. *Rheumatology (Oxford)* 2003;42:652-659.
49. Jantti J, Seppala E, Vapaatalo H, Isomaki H. Evening primrose oil and olive oil in treatment of rheumatoid arthritis. *Clin Rheumatol* 1989;8:238-244.
50. Kremer JM, Lawrence DA, Jubiz W, et al. Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. Clinical and immunologic effects. *Arthritis Rheum* 1990;33:810-820.
51. Berbert AA, Kondo CR, Almendra CL, et al. Supplementation of fish oil and olive oil in patients with rheumatoid arthritis. *Nutrition* 2005;21:131-136.
52. Linos A, Kaklamani VG, Kaklamani E, et al. Dietary factors in relation to rheumatoid arthritis: a role for olive oil and cooked vegetables? *Am J Clin Nutr* 1999;70:1077-1082.
53. Vissers MN, Zock PL, Roodenburg AJ, et al. Olive oil phenols are absorbed in humans. *J Nutr* 2002;132:409-417.

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