Cellular and Molecular Effects of Bioactive Phenolic Compounds in Olives and Olive Oil

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Introduction

Olea europaea belongs to the family Oleaceae of the genus Olea, and it is known commonly as the olive tree, bearing the widely consumed and studied olive fruit. The cultivated olive tree can be found distributed across the globe, from Greece and the Levant, southward to Africa and Australia, west into Italy and Spain, and east reaching across to China. The Oleaceae family encompasses 600 different species, and the genus Olea includes approximately 40 taxa of shrubs and trees divided into the subgenera Olea, Paniculatae, and Tetrapilus. The subgenre Olea includes the subspecies Olea europaea and included also within the subgenre is the section ligustroides.

The olive tree is a hardy plant that can survive in a wide range of environments, but it is found prominently distributed in tropical and subtropical areas; the forests of the Mediterranean, the Sahara, and lowland tropical forests are some indications of its habitat (Besnard et al., 2009). The olive tree has adapted its growth to a semi-arid environment including calcareous and sandy, sunny slopes. It is tolerant of hot summers and can be sustained despite limited water supplies (Wahbi et al., 2005).

In this chapter we explore the traditional medicinal properties and early uses of olives and olive oil. Further, we discuss the evolution and health benefits of the Mediterranean diet. In particular, we focus on the cellular and molecular effects of bioactive phenolic compounds in olives and olive oil. Wound healing, cardiovascular effects, and anticancer properties of the major phenolics, hydroxytyrosol and oleuropein, are also considered.

Traditional Medicinal Applications of Olives and Olive Oil

Historical Applications of Olives and Olive Oil

The scientific denomination of the cultivated olive is Olea europaea L.sativa, which distinguishes it from the wild olive, Olea oleaster. Used most commonly now in its
table oil form, mainly for cooking, the olive and its oil are preceded by a long and revered history. Unlike its debated geographical origins, it is known that *O. europaea* and its fruit find their historical homes in the Mediterranean: from the west in Italy, Greece, and Crete, to the east in the Levant, and particularly in the countries of Syria, Lebanon, and Palestine. Although it is speculated that the use of the wild olive extends as far back in history as the Neolithic Period, 6000 years before the birth of Christ, the recorded cultivation of olives can be traced to it beginning in the Chalcolithic period, early Bronze Age. From that point on, olives, derived from the Greek name *Elaia* (also termed *Oleum* in Latin and *Zayit* in Hebrew) became entwined in the mythology, history, religion, and lives of the Mediterranean people.

In the west, archaeological evidence indicates that the olive tree was central to Greek mythology, religion, and the livelihoods and sustenance of its people. Beyond the mythological, olive oil can often be traced to many facets of ancient Mediterranean life; it was used in cooking, as a cosmetic, and as a fuel source. The importance of olive oil has reached much further than local domestic use and has formed a significant part of Mediterranean commerce. The eastern Mediterranean presents a similar central role for the olive. Here it is also intertwined with religion, cited many times in the texts of the Babylonian Talmud, the Bible, and the Quran.

However, in describing a brief history of the olive and its uses, the final application for olive oil that has survived mythology and that is garnering increasing interest is its use in medicine. This use finds its roots in the traditional remedies of antiquity. For example, the leaves of *O. europaea* formed the basis of the olive-leaf tea, which provided health benefits that now are recognized as anti-inflammatory, antioxidant, and anti-atherogenic (Caramia et al., 2012). In turn, the ubiquitous use of olive oil by the ancient cultures has naturally alluded to its potential medicinal properties. Given its reference as liquid gold in Homeric texts, oil derived from the olive fruit was highly prized as a remedy for burned skin wounds and stomach and liver pains in both Western and Eastern cultures.

In alleviating sores and chills and in strengthening the skin and muscles, Pliny the Elder, in his encyclopedic works the Historia Naturalis, extensively documented the medicinal uses of olives during the rule of Vespasian, during which olives were also applied to ward off headaches, and the juice produced by olives was found efficacious in the treatment of ulcers. In progression to the Middle Ages, olives continued to be used in medicinal ointments and in applications against infections. This trend continued into the Renaissance, during which the health properties of the olive were recognized and used in a broader range against pathologies including heart conditions and diabetes.

Based on largely anecdotal evidence, numerous traditional cultures continually used olive oil and crude olive extracts for their medicinal properties. In more recent times, accumulating epidemiological and clinical evidence has provided the scientific basis for the health benefits of olives and olive oil.
The original stimulus for the current interest in olive-derived health benefits stems from the differential mortality rates observed among populations from different regions of the world, elucidated by the pioneering epidemiological study by Ancel Keys and his collaborators (Keys, 1966). The study addressed factors associated with the incidence and mortality of coronary heart disease (CHD), and the efforts of these investigators and participating countries produced what is now the enduring title of the investigation: the Seven Countries Study. Bringing together participant data from complying nations—Finland, Italy, Greece, Japan, the United States, Yugoslavia, and Serbia—Ancel Keys’s research aimed to satisfy the queries that surround disparities of CHD incidence between populations under a common and unifying set of protocols and methods (Keys et al., 1984).

The results accumulated in the Seven Countries Study indicated the highest coronary death rate belonged to the northern Europeans, with the U.S. closely trailing behind (Mariotti et al., 1982). In contrast, southern Europeans and the Japanese yielded a much lower coronary death rate. Further analysis of the study indicated several critical risk factors associated with the higher incidence of coronary death rate; age, serum cholesterol, systolic blood pressure, and cigarette smoking.

This difference in coronary death rates prompted the analysis of dietary intakes in light of lacking significance between cohorts for the factors of age, body weight, fat composition, and habitual physical activity (Keys et al., 1986). From dietary analyses, Keys draws a direct comparison between the rural European cohorts, highlighting the variation of dietary fat intakes in relation to CHD incidences. Though having very similar lifestyles and physical activity levels, the dietary composition of the cohorts varied greatly with respect to their fat consumption. Southern Europeans favored a Mediterranean diet rich in fruits and vegetables, and fat consumption was primarily through olive oil, making up 15–30% of total dietary energy, whereas the contrasting European cohorts derived their dietary fats from milk and milk products containing saturated fats.

From these observations, subsequent interest in the Mediterranean diet was stirred and much emphasis has been placed on olive oil, one of the defining differences in diet in Keys’s study and believed to be a prime contributor to the lower mortality rates shared by the populace of the Mediterranean. The Mediterranean diet existed far before its recent prominence as a model for good dietary habit. Though seemingly vague, because the term Mediterranean can refer to any or all of the countries that border the Mediterranean, the title of the Mediterranean diet often refers to the diet consumed by the populace of the island of Crete and the people of Naples and southern Italy. This view came about primarily from the earlier observations of Keys in his investigations of how diet contributes to coronary disease (Keys, 1966). The original
postulations by Keys linked a low-fat diet, the opposite of the prevailing eating habits, to a decreased risk of heart disease. The subsequent publishing of his Seven Countries Study seemingly confirmed his theories of diet and disease correlation. The positive health effects derived from the diet were by themselves worthy of elucidation. The diet prominently featured plant produce, moderate consumption of seafood, limited amounts of red meat, and the critical ingredient of the Mediterranean diet, olive oil (Hu, 2003). Despite being a culinary staple, the benefits of olive oil extend beyond gustatory satisfaction into the medicinal and scientific sphere.

Previously, the parallels drawn between olive oil and subsequent health benefits against coronary disease and cancer were attributed to the high concentration of monounsaturated fatty acid (MUFA) in olive oil composition. The persisting interest in MUFAs has drawn the focus on olive studies toward oleic acid (18:1n-9). As the predominant MUFA in olive oil, investigations into its properties have unveiled its potential to reduce the LDL/HDL ratio, which is associated with cardiovascular risk (Martínez-González and Sanchez-Villegas, 2004). In addition, olive oil has hypertension-preventative properties, whereby its use is associated with a decrease in systolic and diastolic blood pressure, along with an inverse association with developing hypertension in males (Álvaro et al., 2006). To expound upon the benefits of olive oil, oleic acid has also been implicated in anti-oncogenic activity as well as lower incidences in and decreased risk of breast cancer (Menendez and Lupu, 2006).

Although not exclusive to olives, the extensive health benefits conferred by MUFA consumption cannot be understated. The MUFA content within olives strengthens the evidence that advocates for the health and medicinal properties that have been long observed throughout its use since prebiblical times. Yet despite its age old prevalence, human understanding of the olive fruit is still incomplete. An indication of this is the recent paradigm shift concerning the focus on the compounds that define the olive’s inherent properties. Although MUFAs and oleic acid were at the forefront of these studies in the past, more recent research has revealed phenolic compounds as the prime contributors to the protective and medicinal characteristics of olives.

**Phenolic Compounds in Olives and Olive Oil**

**Overview**

Accumulating evidence suggests the emerging value of phenolic compounds as potent anti-inflammatory, antimicrobial, and antioxidant factors that contribute substantially to the health advantages of an olive oil–dominant diet (Cicerale et al., 2012). The abundance of these naturally occurring phenols and the phenolic profile of the olive and its oil are produced as a result of natural external factors and the developmental stages of the fruit (Charoenprasert and Mitchell, 2012) (see also Chapters 4 and...
5). The translation of these phenolic compounds to the human taste of olive fruit is interpreted as sensations of bitterness/pungency and governs the overall palatability of the olive and its oil. These traits, however, are ancillary to the broadening appreciation toward the medicinal value of phenolic compounds found in the olive, which have become the primary interest of scientific study.

The phenol constituents in olives, and by extension olive oil, are many, numbering 36 distinct phenols that show considerable variation in both presence and concentration as determined by the olive variant, ripeness, and location of cultivation.

The assessment of structural components of phenolic molecules has enabled the determination of relationships between individual phenolic constituents as well as the identification of the potential origins of the biological effects stimulated by phenolic compounds. This is exemplified by the compound and structural component, elenolic acid. A defining structural component of the secoiridoid group of olive phenolic compounds, elenolic acid finds itself integrated within oleuropein, dimethyleuropoiein, ligstroside, and elenolic acid–linked hydroxytyrosol and tyrosol. Its presence within certain phenolic compounds allows differentiation of biological activity within otherwise identical or similar compounds that are devoid of its presence. This is true in the instance of hydroxytyrosol (3, 4-DHPEA) and elenolic acid–linked hydroxytyrosol (3, 4-DHPEA-EDA), for which a comparison study indicated a greater than 50% favor of elenolic acid–linked hydroxytyrosol in cell apoptotic percentage, comparable to catalase-induced apoptosis (Fabiani et al., 2009b).

Elenolic acid participation in the biological activity of olives is noted as a significant component that confers antimicrobial activity (Brenes et al., 2007). Its location within olive phenol structures may provide an explanation of the bactericidal and bacterial inhibitory activity of olive phenols against food-borne pathogens (Listeria monocytogenes, Staphylococcus aureus, Salmonella enterica), respiratory tract infection agents (Haemophilus influenza, Moraxella catarrhalis, Salmonella typhi), as well as probiotic bacterium (Lactobacillus acidophilus and Bifidobacterium bifidum) (Bisignano et al., 1999; Medina et al., 2006). A specific study targeting Lactobacillus pentosus, Escherichia coli, and Helicobacter pylori using free elenolic acid and hydroxytyrosol placed significant weight on elenolic acid as the responsible agent for olive bactericidal activity (Medina et al., 2009).

A similar example of biological activity conferred by a structural component can be shown with homovanillic alcohol (Figure 3.1). However, in contrast to elenolic acid, homovanillic alcohol is studied and observed primarily as a metabolite of hydroxytyrosol within the renal system. Nonetheless, as a metabolite of hydroxytyrosol, homovanillic alcohol is known as a potent antioxidant similar to its parent molecule (Tuck et al., 2002). As a hydroxytyrosol metabolite, homovanillic alcohol directly affects the renal system, where it is thought to have protective effects against cell injury. Within the renal context, hydrogen peroxide (H$_2$O$_2$) oxidative damage of tubular...
epithelial cells results in acute tubular cell injury via lipid peroxidation (Sheridan et al., 1996). Pretreatment with hydroxytyrosol and the subsequent generation of homovanillic alcohol, led to reductions in lipid peroxidation, as measured by malondialdehyde (MDA), a lipid peroxidation marker in renal tubular epithelial cells. Although not as potent as its hydroxytyrosol parent molecule, homovanillic alcohol reduced MDA concentrations significantly and established its position as an important renal cell protective metabolite (Deiana et al., 2008). Using a comparable concentration of homovanillic alcohol (10 µM) to the previously described study, Incani et al. (2010) found protection of renal cells via several intracellular signaling pathways. Along with the reported protection against H$_2$O$_2$-induced cytotoxicity, homovanillic alcohol pretreatment also inhibited H$_2$O$_2$-mediated ERK (extracellular signal-regulated kinase) phosphorylation (Incani et al., 2010). Such modulation of the MAPK (mitogen-activated protein kinase) family has significant pathogenic implications due to its influence on cell survival regulation and cellular responses to toxic substances (Sinha et al., 2004; Wang et al., 2000).

In relation to the minor phenolic constituents that contribute to the antioxidative activity of olives and olive oil, oleocanthal plays a major role in the anti-inflammatory effects of olive-derived phenols. A contributor to pharyngeal pungency stimulated by high-quality olive oils, oleocanthal prompts a sensation of irritation by engaging ion channel activation in sensory neurons within the upper airways (Peyrot des Gauchons et al., 2011). This sensation is likened to ibuprofen oral irritation, with which oleocanthal shares similar anti-inflammatory activity mediated via the inhibition of cyclooxygenase enzymes COX-1 and COX-2 (Beauchamp et al., 2005). Other oleocanthal signaling pathways include inducible nitric oxide synthase (iNOS) suppression in chondrocytes in arthritis pathogenesis and tau protein interaction inhibition in neurodegenerative disorders (Lucas et al., 2011). Despite the wealth of therapeutic benefits derived from phenolic compounds, olive phenol composition is dominated by the presence of oleuropein and hydroxytyrosol (see Figure 3.1).

Identification of Hydroxytyrosol and Oleuropein as Key Antioxidants in Olives and Olive Oil

Although the various olive compositions each include distinct phenolic profiles spanning a number of compounds, oleuropein and hydroxytyrosol are among the most prevalent compounds in high-phenolic olive cultivars, having 2- to 10-fold higher concentrations than other phenolic compounds (Alagna et al., 2012; Charoenprasert and Mitchell, 2012). Originally an undesirable compound due to its bitterness/pungency, oleuropein is a primary antioxidant that is noted for its benefits to human health (Esti et al., 1998). It is a major compound in olive fruit and a secoiridoid ester,
derived from secologanin and secoxyglanin precursors, which include hydroxytyrosol and elenolic acid (Omar, 2010). Its hydrolysis metabolite, hydroxytyrosol, incorporates a similar chemical structure and is synthesized from esterase or acidic catalysis reactions (Bernini et al., 2008).

Both oleuropein and hydroxytyrosol confer antioxidant properties from an underlying orthodiphenolic structure that contributes to their protective role against
oxidation damage–induced disease, including cardiovascular disease and cancers. The antioxidant action of oleuropein and hydroxytyrosol is demonstrated by their capacity to prevent hydroxylation reactions on salicylic acid, through competition as a substrate or classical proton donation. Although similar in structure, hydroxytyrosol is the stronger of the two compounds (Carrasco-Pancorbo et al., 2005).

Focusing on cardiovascular pathology, oleuropein and hydroxytyrosol’s influence is illustrated. The extension of their oxidation prevention on low-density lipoproteins, via inhibition of copper sulfate–induced oxidation and hypochlorous acid scavenging, represents a mechanism for preventing atherosclerotic lesion development (Visioli and Galli, 1994). Yet, their utility against cardiovascular disease is not solely dependent on their antioxidant interference but is complemented by their activity in anti-inflammatory processes through the attenuation of nitric oxide (NO) production resulting from decreased iNOS activity in macrophages (Visioli et al., 1998b).

The significance of olive phenolic efficacy in the prevention of human disease is not exclusively related to cardiovascular pathology, but is shared by its involvement in anti-carcinogenic activities in both in vitro and in vivo contexts (Carrera-González et al., 2013). The study of olive phenols has revealed positive effects in inducing apoptosis and inhibiting cell proliferation primarily in leukemia, breast cancer, and colorectal cancers.

Though very briefly reviewed at this point, the current literature and mechanisms concerning oleuropein and hydroxytyrosol contribution to human pathology prevention will now be given greater focus, and the importance of olive phenols in the areas of wound healing, cardiovascular pathology, and carcinogenesis will be discussed.

Olive and Olive Oil Products in Cosmetics

Since ancient times, plant oils have traditionally been used to treat a range of cutaneous conditions from rashes to tumors (Brussell, 2004), and recently, their derivatives have elicited a revived popularity in modern cosmetic and skincare formulations. The ethnobotanical applications of plant-derived compounds in relation to skin care have formed the basis of their use in cosmetics products. Specifically, the olive and its obtained oils and extracts have long been used in the ancient Mediterranean cultures in skin protectants, soaps, bath oils, and cosmetics (Boardman et al., 1976). Olive oil was also used by the ancient Egyptians to make creams and perfumes (Brun, 2000), and the Romans kept their skin elastic by applying olive oil during bathing.

There are numerous attributes that have contributed to the timeless appeal and suitability of olive oil for its usage in a range of skin-care products. The beneficial properties of olive oil as a topical agent include its function as a solvent, surfactant, emollient, antimicrobial, fragrance enhancer, and skin conditioner. Considered an
“occlusive hydrophobic ingredient” (Weber et al., 2010), olive oil is instrumental in protecting the skin from external elements. It is efficient at removing the ashen appearance of dry skin, since the squalene and triglycerol content of the oil resembles human sebum (Nikkari, 1974; Picardo et al., 2009). Its ability to retard the growth of a range of microorganisms, as well as to inhibit the oxidation of fats, allows olive oil to have a long storage life, and hence enables its capacity to function as an epidermal antimicrobial and preservative for perishable goods.

The fatty acids that comprise the saponifiable fraction of extra virgin olive oil constitute almost 99% of the oil by weight, and of this fraction, 70–80% is oleic acid (Dugo et al., 2004; Gomez-Alonso et al., 2002; Ollivier et al., 2006). These fatty acids form an occlusive layer on the skin that maintains the integrity of the epidermal barrier in a process involving epidermal keratinocyte metabolism of lipids derived from topically applied emollients, due to a fatty acid transporter on their surface, permitting the cells to form a functional epidermal barrier through the imported lipids (Schurer et al., 1995). It has also been shown that ingestion of olive oil fatty acid constituents, including oleic acid and linoleic acid, accelerates the wound healing process (Rodrigues et al., 2012).

Oleic acid in particular has also been found to function as a penetration enhancer (Walker and Hadgraft, 1991), wherein it facilitates transdermal drug delivery through the stratum corneum (Naik et al., 1995; Wang et al., 2004), thereby raising epidermal penetrability. Through the temporary and reversible disordering of the stratum corneum, oleic acid increases the fluidization of the intercellular lipid medium, making the epidermis more permissible to a range of compounds (Larrucea et al., 2001). However, because of the increased permeability of the skin barrier, there is the concern of excessive transepidermal water loss, which may result in the impairment of skin barrier function.

The polar phenolic fraction of the olive provides considerable delivery of antioxidant and anti-inflammatory properties to the skin, primarily derived from oleuropein and hydroxytyrosol. These properties have proven to be beneficial in the mitigation of photo damage and skin aging (Ichihashi et al., 2000; Perugini et al., 2008) as well as in improving the course of cutaneous wound healing (Mehraein et al., 2014). The antioxidant activity mitigates the oxidizing effects of external aggressors, including UVA-B light damage and ambient particulate matter (D’Angelo et al., 2005; Guo et al., 2010; Mehraein et al., 2014) through the activation of enzyme p53, which prevents and repairs skin damage following UV exposure (Budiyanto et al., 2000).

The repairing capacity of its high fatty acid and antioxidant content endows olive oil with a unique ability to afford protection and hydration when applied topically to the skin. Its enduring use in cosmetic concoctions and its subsequent reemergence as a key ingredient in cleansers, moisturizers, and body oils solidify its popularity and ubiquitous use as a cosmetic product throughout the world.
There are many applications for the use of the olive and its oil in the dermatological arena. Olive oil is used extensively to improve a range of skin conditions in neonates and infants (Kiechl-Kohlendorfer et al., 2008), as well as in the treatment of wounds and burns (Sakazaki et al., 2007), through both oral administration and topical application. The treatment objective of many cutaneous ailments is the restoration of integrity, tensile strength, and barrier function of the skin (Singer et al., 2000), a process that invariably draws on the wound healing cascade. It has been shown that the leaf extract and the extra virgin oil fraction of the olive can improve dermatitis and accelerate the course of wound healing (Koca et al., 2011). The wound healing process encompasses a multifaceted series of interactions between existing keratinocytes, the extracellular matrix (ECM), immune cells, cytokine mediators, and growth factors (Singer and Clark, 1999). The phases consist of: inflammation, proliferation, and re-epithelialization, leading to scar formation (Werner and Grose, 2003). The spatial and temporal levels of a range of cytokines, growth factors, and reactive oxygen species (ROS) within the wound remain in flux, and this dynamic balance between stimulators and inhibitors can lose its equilibrium and lead to a pathologic state.

Normal wound healing processes are reliant on the categorical transition of the aforementioned phases to achieve partial reconstruction of the wound area. Initially, platelets release eicosanoids, including COX-1 and COX-2, that convert arachidonic acid into proinflammatory prostaglandins such as PGE$_2$, producing dilated and porous blood vessels and thereby facilitating inflammatory migration. The pool of cytokines and growth factors released upon platelet degranulation are among other mediators involved, including PDGF, bFGF, TGF$\beta$, EGF, and VEGF, which attract neutrophils and monocytes (Bahou and Gnatenko, 2004; Singer and Clark, 1999). Subsequently, resident immune cells such as mast cells, T-cells, and Langerhans cells (Noli and Miolo, 2001) are activated, releasing a range of cytokines including tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and interleukin-1 (IL-1) (Szpaderska et al., 2003).

Early on in the wound healing process, the onset of acute inflammation ensues, characterized by the dense level of neutrophils that constitute around 50% of cells at the wound site (Engelhardt et al., 1998). Accordingly, neutrophils perform their function of cleansing the wound bed of foreign pathogens (Gillitzer and Goebeler, 2001), mainly through bursts of ROS, which serve as signaling molecules and are involved in the destruction of foreign microbes and regulating the activation of transcription factors (Roy et al., 2006). However, the perpetuation of oxidant activity is detrimental to the wound healing process because it contributes to fibrotic scarring, damages tissue architecture (van der Vliet and Janssen-Heininger, 2014), and induces oxidative stress in adjacent normal tissue, which enhance pathologic processes. The
destructive capacity of the inflammatory phase may convey impaired healing ability upon prolonged tissue inflammation (Ashcroft et al., 1999) through the exaggerated activity of macrophages and neutrophils (Chen et al., 2014; Cooper et al., 2005). It is seen that excessive platelet activation, generation of arachidonic acid derivatives, and the subsequent secretion of proinflammatory cytokines have been correlated with an augmented inflammatory response (Diegelmann, 2003; Muscara et al., 2000). Previous studies have found improved wound healing in the setting of decreased inflammation (Ponrasu et al., 2014). Particularly, neutrophil infiltration has been attributed to the damaging effects of inflammation within the skin (Henson and Johnston, 1987).

It has been demonstrated that hydroxytyrosol and oleuropein can offset the deleterious effects of extreme inflammatory responses through their capacity to inhibit the respiratory burst of neutrophils (Visioli et al., 1998a), which reduces the oxidative load to surrounding keratinocytes and promotes wound closure and connective tissue formation (Loo et al., 2012). Oleuropein and hydroxytyrosol have also exhibited protection against genotoxic damage induced by ROS in the DNA of several cell lines (Fabiani et al., 2008a; Grasso et al., 2007; Nousis et al., 2005), limiting damage-associated molecular pattern molecules (DAMPs) (Bianchi, 2007) and further curbing the inflammatory response in light of their potent antioxidant activity (Figure 3.2). Furthermore, hydroxytyrosol has been shown to abrogate the secretion of several proinflammatory cytokines within macrophages including TNF-α, NO, and COX-2 (Maiuri et al., 2005), mediated through the attenuation of deleterious signalling cascades, such as NF-κβ (Scoditti et al., 2012). Moreover, it has been elucidated that olive phenols possess antithrombotic and antiplatelet properties (see also Chapter 2). This can be attributed to the reduction in expression levels of adhesion molecules involved in leukocyte diapedis within endothelial cells, including intercellular and vascular cell-adhesion molecules (ICAM-1 and VCAM-1) and E-selectin (Manna et al., 2009), leading to the mitigation of the coagulation response and platelet-released proinflammatory cytokines and growth factors (Singer and Clark, 1999).

The proliferative and re-epithelialization phases of wound repair are dependent upon the activity of fibroblasts that produce granulation tissue (Fernandes et al., 2004; Rajkumar et al., 2006) and the subsequent synthesis, bundling, and alignment of collagen fibers, which eventually allow for wound closure. The dense ECM that facilitates cellular migration must undergo restoration to normal histoarchitecture, which is achieved through the action of MMPs that degrade ECM components and are secreted by a range of dermal cells (Singer and Clark, 1999).

A distinguishing trait of granulation tissue is the formation of blood vessels, which are necessary to deliver nutrients and oxygen to the new tissue. This neo-vascularization is prompted by several growth factors including VEGF, TGFβ, and bFGF (Folkman and D’Amore, 1996) and is facilitated by MMP activity (Haas et al., 2000) and hypoxic conditions (Detmar et al., 1997). However, in nonhealing
wounds, reduced levels of these proangiogenic factors combined with endothelial cell dysfunction (Sluimer and Daemen, 2009) contribute to impaired angiogenesis and healing. Thus, therapeutic strategies are centered on mechanisms related to the cytoprotection and proliferation of the vasculature within the wound.

Olive phenols including oleuropein and hydroxytyrosol have been discovered to impart protective effects on vascular endothelial cells through limiting oxidative injury (Palmieri et al., 2012; Zrelli et al., 2011b) and inflammatory damage mediated by TNF-α (Zrelli et al., 2013), thereby supporting vascular cell proliferation. In contrast, these phenols have also been shown to abrogate angiogenesis through the inhibition of MMP-2 (Fortes et al., 2012) and MMP-9 activity (Scoditti et al., 2012), as well as the down regulation of VEGF expression (Lamy et al., 2014). However, these inhibitory effects on neovascularization have generally been found in vitro using high concentrations of hydroxytyrosol (≥100 μm) (Fortes et al., 2012) as well as in neoplastic settings (Zhao et al., 2014). It has also been shown that abnormal vessel growth, which promotes tumorigenesis, is attributed to aberrant VEGF expression (Kerbel

**Figure 3.2** Schematic representation of wound repair mechanisms. Growth factors necessary for cell motility and proliferation are shown, as are the effects of hydroxytyrosol (HT) and oleuropein (OL) on key cytokines and DAMPs involved in the inflammatory response of wound healing.
and Folkman, 2002) and numerous signaling pathways unique to cancer cells, which differ from the processes within normal wounds, where the dynamic reciprocity between cellular components, matrix proteins, and bioactive molecules propel conserved angiogenic mechanisms that allow for adequate tissue repair (Greaves et al., 2013). However, within impaired wound healing, decreased VEGF expression is observed, which is credited to DNA damage and lipid peroxidation (Warletta et al., 2011) induced by the wound environment. Interestingly, oleuropein has been shown to increase VEGF expression during in vivo wound healing studies, which was correlated with the observed acceleration of re-epithelialization (Mehraein et al., 2014). Thus, olive phenols may serve as suitable proangiogenic compounds in the context of inflammatory environments that are associated with wound healing.

Re-epithelialization of wounds involves both the migration and proliferation of keratinocytes within the epidermal parameters of the wound. Ultimately, the outcome of mammalian wound healing is the formation of a relatively acellular scar with refined vasculature (Adams and Alitalo, 2007). The inflammatory response completely resolves with the apoptosis of neutrophils (Haslett, 1992), and some leukocytes return to the vasculature (Mathias et al., 2006) or emigrate through the lymph vessels (Schwab et al., 2007). The mitigation of inflammation is achieved through the deactivation of macrophages by anti-inflammatory cytokines (Ma et al., 2003), sequestration of proinflammatory chemokines (D’Amico et al., 2000), and production of endogenous anti-inflammatory molecules (Schwab et al., 2007). Failure to resolve inflammation results in nonhealing wounds, increased scar formation, and reduced tensile strength.

Oleuropein and hydroxytyrosol assert their anti-inflammatory capabilities via several mechanisms. Concurrently, oleuropein and hydroxytyrosol have been shown to influence cytosolic Ca\(^{2+}\) levels, augmenting the activation of T and B lymphocytes (Palmerini et al., 2005; Zbidi et al., 2009). In vivo, oleuropein and hydroxytyrosol have been found to reduce the level of acute inflammation via the reduction of cytokines IL-\(\beta\) and TNF-\(\alpha\) (Gong et al., 2009), as well as by decreasing the number of infiltrating neutrophils (de la Puerta et al., 2000).

These anti-inflammatory effects are demonstrated with topical application to the skin such that the olive phenols reduce edematous tissue by almost 50% (de la Puerta et al., 2000), effectively accelerating the contraction of the wound area in vivo (Mehraein et al., 2014). In conjunction, the antifibrotic value and scar minimization of hydroxytyrosol may be mediated through its effects in up-regulating keratinocyte SMAD7 (Rafehi et al., 2012), which is known to be highly expressed in the wound stroma (Han et al., 2011). SMAD7 acts as a TGF-\(\beta\) antagonist and thereby limits fibrosis through blocking its chief facilitator (Flanders et al., 2003). The healing value of olive oil is diverse in its cellular effects on the epidermis and underlying dermis. As antioxidants, the scavenging abilities of the olive phenolic compounds oleuropein and hydroxytyrosol contribute to its therapeutic capabilities.
(Manna et al., 2002; Owen et al., 2000). Because excessive ROS production during the proliferative and re-epithelialization phases is implicated in perturbed wound healing, olive oil is a suitable restorative product. Moreover, the therapeutic qualities conveyed by the olive phenols include their preventative capacities to ameliorate excessive inflammatory responses and tissue damage during impaired wound healing.

**Molecular Mechanisms Accounting for the Wound Healing Properties of Phenolic Compounds from Olives and Olive Oil**

The typical epidermal wound involves multiple cell types of several distinct lineages, each responding to and generating different signals at different times. The underlying basis for these marked responses is the activation of conserved signal transduction pathways leading to the transcription of wound-response genes expressed at the wound site that orchestrate the repair process (Cole et al., 2001). However, as previously mentioned, an excessive and prolonged inflammatory response can impair wound healing due to the exacerbation of cytokine production (Martin and Leibovich, 2005). Specifically, olive phenolic compounds have the capacity to suppress cytokine signaling through the regulation of gene expression, in which perturbations of gene transcription have been implicated in nonhealing chronic wounds (Trengove et al., 1999) as well as hypertrophic scarring (Russell et al., 1989).

Receptor tyrosine kinases such as EGFR, PDGF (Campos et al., 2010; Wang et al., 2009), VEGF, and FGF (Komi-Kuramochi et al., 2005) bind ligands that initiate from damaged cells within the wound. The subsequent signaling via cytosolic phosphorylation in several pathways potentiates the nuclear activation of transcription factors, which modulate gene expression in cells near the wound site. Hydroxytyrosol has been found to promote such signaling activity, which selectively modulates the phosphorylation of kinases implicated in wound repair, including PI3K/AKT (Zou et al., 2012) and ERK1/2 pathways (Zrelli et al., 2011b). It has been discovered that these pathways are partly responsible for the activation of Nrf2, which is essential for the transcriptional activation of antioxidant and phase II detoxifying genes (Mann et al., 2007; Motohashi and Yamamoto, 2004; Nguyen et al., 2009), including glutathione peroxidise, superoxide dismutase-1, catalase, and heme-oxygenase (HO-1) (Surh et al., 2008). Within keratinocytes, hydroxytyrosol has been shown to up-regulate several of these antioxidant defense enzymes, especially HO-1 (Rafehi et al., 2012), which also promotes proliferation and inhibits apoptosis (Clark et al., 1997; Petrache et al., 2000). The transcriptional activation of Nrf2 by hydroxytyrosol has been demonstrated to stimulate and protect vascular endothelial cells (Zrelli et al., 2011b) from oxidative stress through the phosphorylation of PI3K/AKT and
ERK1/2 and the downstream induction of HO-1 (Zrelli et al., 2011b), which has been implicated in promoting neovascularization (Florczyk et al., 2014) and tissue repair (Bussolati and Mason, 2006). Upon phosphorylation, these kinases induce COX-2 and subsequent secretion of MMP-9 (Zhang et al., 2009a), for which elevated levels of MMP-9 have been associated with detrimental protease activity leading to growth factor degradation and the obstruction of cellular proliferation (Jang et al., 2011). Thus, hydroxytyrosol may prove to be a valuable therapeutic against destructive eicosanoids and proteases.

Notably, olive phenols have been shown to prevent the stimulation of redox-sensitive transcription factors such as NF-κB (Figure 3.3). Bound to its inhibitory subunit IκB within the cytosol, NF-κB may be activated by several initiators, including ROS, TNF-α, IL-1β, and ionizing radiation (Basu et al., 1998; Chandel et al., 2000; Renard et al., 1997), via the phosphorylation and subsequent degradation

![Figure 3.3](image)

**Figure 3.3** Molecular mechanisms of damage-signaling pathways in wound healing. The transcriptional modulating effects of hydroxytyrosol on NF-κB and Nrf-2 are depicted, mitigating inflammation and promoting cellular proliferation during wound repair.
of IkxB, which allows NF-κB to translocate into the nucleus. Hydroxytyrosol has been shown to interfere with this nuclear translocation (Scoditti et al., 2012), reducing the expression of NF-κB-governed pro-inflammatory genes that mediate TNF-α, IL-1β, ICAM-1, and MCP-1 secretion (Basu et al., 1998; Chandel et al., 2000; Renard et al., 1997). This supports the hypothesis that hydroxytyrosol induces NF-κB inhibition activity and the subsequent production of key inflammatory cytokines, which have been implicated in aberrant wound healing. Furthermore, the enzyme p38 MAPK, found upstream in the NF-κB signaling pathway, has been shown to attenuate neutrophil and monocyte release of TNF-α and IL-1β when inhibited (Song et al., 2001). In line with this, hydroxytyrosol has been shown to reduce p38 MAPK activity (Sánchez-Fidalgo et al., 2012) and to exert chemopreventative effects through the same mechanism.

In parallel, the transcription of wound response genes may also be modulated through various epigenetic mechanisms, which appear to be uniquely related to the stage of repair. Although limited research has been conducted on the specific effects of olive phenols on epigenetic factors, it is known that phenolic compounds regulate the epigenome through their antioxidant activity.

DNA methylation, histone modifications, and posttranscriptional regulation by microRNAs are involved in the regulation of many biological processes through gene expression modulation (Jaenisch and Bird, 2003; Lin and Hannon, 2004), and numerous natural compounds have been identified as histone deacetylase (HDAC) inhibitors, histone acetyltransferase (HAT) activators, and HAT inhibitors. The generation of ROS and hypoxic conditions have been found to increase DNA methylation, in which the reduced expression of PKCε gene was observed; however, treatment with ROS scavengers blocked hypoxia-induced CpG methylation. Because PKCε expression is pertinent to cutaneous wound closure and limiting fibrosis (Leask et al., 2008; Mesquita et al., 2014), the introduction of antioxidants such as olive phenols to the wound environment may convey therapeutic effects. Similarly, in the case of histone acetylation, several thiol antioxidants were discovered to inhibit acetylation induced by hydrogen peroxide (Gilmour et al., 2003), thereby limiting the secretion of the proinflammatory cytokine IL-8 (Tomita et al., 2003).

Furthermore, it is seen that under proinflammatory conditions, human monocytes are susceptible to acetylation of NF-κB and subsequent cytokine gene expression; however, the inhibition of NF-κB target genes, including IL-6 and TNF-α, in THP-1 monocytes cells was achieved through the up-regulation of HDAC activity and inhibition of HAT activity, preventing NF-κB-mediated chromatin acetylation and subsequent transcription of cytokines (Hye Joo et al., 2012). Although olive phenols demonstrate significant anti-inflammatory effects, their structurally related cousins elicit HDAC activity in a proinflammatory setting, which suggests oleuropein and hydroxytyrosol may operate in a similar manner.
Antioxidant and Anti-Inflammatory Effects of Phenolic Compounds in Olives and Olive Oil

Conventional Antioxidant Mechanisms of Phenolic Compounds in Olives and Olive Oil

Since its discovery in 1960, when the phenolic compound oleuropein was isolated from olive leaves (Panizzi, 1960), it has been elucidated that oleuropein and its derivative, hydroxytyrosol, possess powerful scavenging abilities through reducing a range of ROS, including peroxyl radicals, HClO, superoxide radical, $\text{H}_2\text{O}_2$, and synthetic radicals that contribute to the olive’s health promoting effects.

In particular, oleuropein’s antioxidant activity is characterized by its ability to scavenge aqueous and chain-propagating lipid peroxyl radicals. This ability is substantiated and complemented by its concurrent indirect inhibitory effect on xanthine oxidase, an ROS-generating enzyme; following its inhibition, there is a compounding loss of super-oxide generation (Visioli et al., 1998a). As previously mentioned, the potent antioxidant activity of oleuropein and hydroxytyrosol may be attributed to the orthodiphenolic group found in their molecular structures and the high electron donating effect of the second hydroxyl group. Together these olive phenols convey protection through regulating antioxidant/pro-oxidant enzymes (Martinez-Gonzalez and Sanchez-Villegas, 2004). The activation of phase II detoxifying enzyme systems, including glutathione (GSH), SOD, HO-1, and NAD (P)H quinone oxidoreductase-1 (Copple et al., 2008), may be induced through activation of the transcription factors Nrf2 and AMPK-FOXO3a. Thus, activating alternate antioxidant expression at the gene level contributes to their antioxidant activities. In addition, the activation of peroxisome proliferator-activated receptor coactivator 1α (PPARGC1α) in retinal epithelial cells has resulted in increased protein expression of mitochondrial transcription factors, leading to enhanced mitochondrial biogenesis. This further contributes to its protective effects against damage resulting from oxidative stress.

In congruence with the antioxidant effects of olive phenols are their anti-inflammatory activities. It has been demonstrated that hydroxytyrosol attenuates oxidative stress-associated inflammation. These effects are facilitated by the abrogation of intracellular redox-sensitive gene expression, including expression of the gene encoding COX-2 (Babieri et al., 2003), and is exemplified with human monocytes in which hydroxytyrosol diminished ROS-mediated COX-2 expression (Zhang et al., 2009b). It is also through the inhibition of proinflammatory transcription factors, such as PKCa/PKCβ1, NF-κB (Scoditti et al., 2012), STAT-1α, and IRF-1 (Maiuri et al., 2005), that olive phenol compounds convey their anti-inflammatory action. PKCa and PKCβ 1 induce COX-2 secretion, whereas hydroxytyrosol has been shown to obstruct their phosphorylation (Scoditti et al., 2012; Zhang et al., 2009a). Through NF-κB inhibition, hydroxytyrosol causes a reduction in the expression of proinflammatory genes that facilitate TNF-α,
IL-1β, ICAM-1, and MCP-1 secretion (Basu et al., 1998; Chandel et al., 2000; Renard et al., 1997), thereby mitigating the ROS-induced inflammation.

The conventional antioxidant activities together with their transcriptional anti-inflammatory effects support the therapeutic utility of olive phenol compounds.

Emerging Insights from Genome-Wide Transcription Studies Relating to the Antioxidant and Anti-Inflammatory Effects of Phenolic Compounds in Olives and Olive Oil

Insights into the molecular mechanisms of olive phenolic antioxidant and anti-inflammatory activities have been revealed through study of their modulation of gene expression. Recent identification of specific antioxidant and anti-inflammatory pathways of olive phenols have reinforced previous epidemiological studies that highlighted the chemopreventive and cardioprotective properties of olive oil.

Modulation of inflammation-associated gene expression has been documented following treatment with hydroxytyrosol. For example, mRNA sequencing analysis demonstrated the up-regulation of the anti-inflammatory receptor IFN-γ and down-regulation of the cytokine IL-11 (Rafehi et al., 2012). The same study also identified genes associated with the antioxidant activity of hydroxytyrosol, including the gene-encoding HO-1, which is up-regulated in response to oxidative stress via the Nrf-2/ARE pathway (Chen et al., 2011; Kang et al., 2005; Park et al., 2011), and which was expressed in treated keratinocytes, along with the antioxidant enzyme glutathione peroxidase 3 (Rafehi et al., 2012).

It was discovered that the phenol fraction of the olive repressed the expression of several genes related to inflammation pathways in vivo. Consumption of phenol-rich virgin olive oil in humans was associated with decreased expression in PBMCs of both postprandial IL1B and PTGS2, which are up-regulated in chronic inflammatory conditions (FitzGerald, 2003). This finding is analogous to previous in vitro studies that demonstrated olive oil–induced attenuation of LPS-induced PTGS2 transcription (Zhang et al., 2009b). In vivo reduction of NF-κB activation following olive oil consumption was found (Bellido et al., 2004; Perez-Martinez et al., 2007) and is supported by the reported under-expression of NFKBIA, which encodes IκBα, a member of an inhibitory IκB family that retains NFκB in the cytoplasm (Aggarwal, 2004). Furthermore, in vitro studies showing reduced NF-κB activation by resveratrol support the hypothesis that phenols use similar pathway mechanisms involving the mitigation of genes encoding inflammatory cytokines and adhesion molecules (Zern and Luz-Fernandez, 2005).

The antineoplastic activity of olive phenol compounds has also been attributed to their gene-modulating abilities. Olive oil consumption with high phenol content has resulted in decreased PBMC expression of DUSP1 and DUSP2, which are known
to regulate members of the MAPK pathway, including p38, MAPK/ERK, and SAPK/JNK (Lang et al., 2006). In vitro, it has been demonstrated that the STAT family of transcription factors, which are associated with the survival of a variety of different tumors (Das et al., 2007; Pedranzini et al., 2004; Zhang et al., 2006), were down-regulated in human chronic myelogenous leukemia K562 cells following treatment with hydroxytyrosol (Rafehi et al., 2012). Moreover, it has been revealed that hydroxytyrosol and oleuropein induce antiproliferative and proapoptotic effects in certain human colorectal cancer cell types, mediated by the down-regulation of FAS gene expression. Thus, the modulation of the aforementioned genes may prove to be a molecular target for the antiproliferative activity of olive oil phenols in a subset of cancers. Other possible mechanisms through which olive phenols convey chemopreventive activity include their anti-inflammatory action against processes previously described, involving the ability of proinflammatory cytokines to promote the tumor microenvironment (Colotta et al., 2009).

Although olive phenols may act solely as conventional antioxidants, they also promote cellular defense against oxidative stress, conveying anti-inflammatory and antitumorigenic properties.

Cardiovascular Effects of Hydroxytyrosol and Oleuropein

Anti-Atherogenic Effects of Hydroxytyrosol and Oleuropein

Of the phenolic compounds found in olives, hydroxytyrosol and oleuropein are some of the most potent antioxidants and radical scavengers that protect against cardiovascular disease. These phenols protect the heart by decreasing oxidative stress, atherosclerosis risk factors, and cardiotoxicity and by promoting high-density lipoprotein (HDL) cholesterol and angiogenesis (see Chapter 2).

Given that atherosclerosis and cardiovascular disease are dependent on accumulative oxidative damage to biomolecules, it is reasonable to link phenolic compounds to their prevention. Hydroxytyrosol was shown to prevent atherosclerotic plaque formation by inhibiting the oxidation of LDL cholesterol in macrophage-like J774 A.1 cells (Di Benedetto et al., 2007). In rats, a cholesterol-rich diet induced hypercholesterolemia associated with elevated total cholesterol, triglycerols, and LDL cholesterol (Jemai et al., 2008). Rats fed a cholesterol-rich diet supplemented with hydroxytyrosol in drinking water had decreased total cholesterol, triacylglycerol, and LDL cholesterol and increased HDL cholesterol in their serum, in contrast to rats that consumed only cholesterol-rich diets (Jemai et al., 2008). Furthermore, following human consumption, hydroxytyrosol was detected in the plasma gradient fractions containing LDL, suggesting that the phenol has a transient association with LDL, revealing a physiological relevance to atherosclerosis development (Gonzalez-Santiago et al., 2010). In
vitro, hydroxytyrosol has also been implicated in the inhibition of platelet aggregation and prevention of eicosanoid formation (Petroni et al., 1995). Hydroxytyrosol diet supplementation in hyperlipemic rabbits improved blood lipids and antioxidant status and decreased atherosclerotic lesion size (Gonzalez-Santiago et al., 2006). In addition, hydroxytyrosol protected the aorta against oxidative stress in rats (Rietjens et al., 2007).

Monocyte adhesion to vascular endothelium is a known contributor to atherosclerosis development. ICAM-1, VCAM-1, and E-selectin have been found to play a role in endothelial activation. Extra virgin olive oil was shown to reduce ICAM-1 and VCAM-1 expression in human umbilical vascular endothelial cells, which was primarily mediated by oleuropein and hydroxytyrosol (Dell’Agli et al., 2006). Specifically, hydroxytyrosol and oleuropein treatment has also decreased VCAM-1 in endothelial cells (Carluccio et al., 2003; Manna et al., 2009). In addition, hydroxytyrosol has been shown to have anti-atherogenic and antioxidant effects on porcine pulmonary artery endothelial cells mediated by an increase in catalase and activation of the AMP-FOXO3a signaling pathway (Zrelli et al., 2011c).

Excessive vascular smooth muscle cell (VSMC) proliferation and migration also contribute to atherosclerosis development, and hydroxytyrosol was found to induce apoptosis of rat VSMCs mediated by NO production and inactivation of Akt (Zrelli et al., 2011a). Furthermore, oleuropein was shown to inhibit bovine VSMCs by blocking the G1 to S cell transition, possibly mediated by ERK1/2 (Abe et al., 2011). Together these studies suggest hydroxytyrosol and oleuropein may decrease the onset of atherosclerosis by preventing excessive VSMCs.

**Hydroxytyrosol and Oleuropein Protect the Heart**

The anti-atherogenic effects of olive phenols rest on their ability to attenuate the damage associated with oxidative stress. Because LDL oxidation and its downstream affects, including ischaemia, may be inhibited by olive phenols, these compounds serve as both a prophylactic and treatment for cardiac pathologies.

For example, pretreatment with oleuropein in isolated rat hearts with sustained ischemia-reperfusion induced ROS (oxidized glutathione and membrane lipid peroxidation) afforded protection from oxidative stress (Manna et al., 2004). In a separate study, rabbits fed a normal or hypercholesterolemic diet were subjected to ischemia, and it was seen that ischemia-treated rabbits having diets supplemented with oleuropein had a reduction in infarct size, total cholesterol, and triacylglycerols (Andreadou et al., 2006).

In ischemic tissues, endothelial progenitor cells (EPCs) play an important role in neovascularisation and cardiac repair. Arterial hypertension affects cardiovascular disease, during which angiotensin II damages vascular endothelium (through oxidative stress) and inhibits the regenerative functions of EPCs. An interesting study by
Parzonko (2013) and colleagues investigated the effects of oleuropein on EPCs. Pretreatment of EPCs with oleuropein partially prevented angiotensin II-mediated decreased proliferation and telomerase activity. Angiotensin II impaired EPC migration and adhesion; however, pretreatment with oleuropein abrogated this effect. Oleuropein also induced EPCs to undergo increased tube formation. Collectively, oleuropein promoted angiogenesis in a process possibly mediated by Nrf2 activation and increased HO-1 expression. In addition, dietary supplementation of hydroxytyrosol and oleuropein was able to reverse high-fat diet induced cardiac stiffness and reduce high-fat diet induced cardiac collagen deposition in rats (Poudyal et al., 2010).

As a cancer chemotherapeutic drug with damaging cardiac side effects, doxorubicin-associated cardiotoxicity is defined by the increased oxidative stress and mitochondrial dysfunction it induces. Hydroxytyrosol has been shown to reduce oxidative damage and the percentage of altered mitochondria in the heart following doxorubicin administration (Granados-Principal et al., 2014). Oleuropein has also been demonstrated to reduce doxorubicin-induced cardiotoxicity. Doxorubicin-treated rats that had cardiotoxicity as characterized by increased lipid peroxidation and iNOS in cardiomyocytes (which lead to higher NO production and subsequent oxidative apoptosis/necrosis) were protected by oleuropein, which was able to prevent these increases (Andreadou et al., 2007). In the same study, doxorubicin changed the metabolic profile of rat hearts by increasing myocardial acetate and succinate; however, pre- and post-treatment with oleuropein abrogated these doxorubicin-induced changes.

Although there are no reports on the effects of hydroxytyrosol on cardiomyocytes, recent work revealed a decrease in mitochondrial-dependent oxidative stress, which favors cardiomyocyte proliferation (Puente et al., 2014). Therefore, it is possible that the antioxidant effects of hydroxytyrosol and oleuropein could potentially promote cardiomyocyte proliferation by decreasing oxidative stress and DNA damage. In light of recent literature supporting adult mammalian cardiomyocyte renewal (Bergmann et al., 2009; Mollova et al., 2013; Senyo et al., 2013), and given the favorable effect of olive oil on the heart, future studies should be performed to determine whether hydroxytyrosol and oleuropein might possibly promote cardiomyocyte proliferation to favor cardiac regeneration or increase the low homeostatic levels of cardiomyocyte renewal.

Anticancer Effects of Phenolic Compounds in Olives and Olive Oil

In Vitro and in Vivo Evidence

The proposal of olive antagonism against cancer is founded on epidemiological studies correlating a lower incidence of certain cancers within the Mediterranean region. In these studies, in addition to the observed reduced death rates, the prevalence of
cancer also saw a marked decrease of 61% in the case of the Lyon Heart Study, due to the adherence to a Mediterranean diet (de Lorgeril et al., 1998). These lower relative mortality rates were seen particularly in breast and colorectal cancers, as well as leukemia (Casaburi et al., 2013). The association between olive consumption and its anti-inflammatory and antioxidant activities forms the basis for the proposed effects against tumorigenesis. The phenolic compounds of the olive demonstrated a number of actions that counter the classical hallmarks of cancer: apoptosis evasion, angiogenesis, growth signal self-sufficiency, invasion of tissue, and insensitivity antigrowth signals (Hanahan and Weinberg, 2000). Here, we discuss some insights into phenolic compound interactions with cancer and oncogenesis.

When discussing classical cancer characteristics, tumor formation is typified by uninhibited cellular proliferation. This proliferation is derived from an accumulation of cellular abnormalities that enable cancerous cells to grow uncontrollably and is achieved via the dysregulation of growth promoting signals, evasion of apoptosis, and a proangiogenic environment.

The antitumoral potential of oleuropein has been investigated in vitro on the cancer cell lines TF-1 glioblastoma, 786-O renal cell adenocarcinoma, RPMI7951 skin lymph node melanoma, and LoVo colorectal adenocarcinoma (Hamdi and Castellon, 2005). The findings indicate growth inhibition induced by oleuropein treatment in a dose-dependent manner in all cell lines. However, this effect was considerably diminished in LoVo colorectal adenocarcinoma (Hamdi and Castellon, 2005). The varying efficacy of oleuropein treatment on these cancer cell lines is partially explained by glucose transporter (GLUT)–mediated oleuropein diffusion. Variable expression of GLUT in cancer cell lines leads to varying concentrations of oleuropein delivered to different cell lines. This manifests as differential sensitivity toward oleuropein, whereby cervical, thyroid, breast, and colon cancers up-regulate GLUT transporters and are therefore more susceptible to treatment. In a subsequent in vivo investigation, partial to complete tumor regression was observed in Swiss albino mice that consumed 1% oleuropein in their drinking water (Hamdi and Castellon, 2005).

Comparable observations in which oleuropein induced inhibition of tumor proliferation were made in a Mediterranean study that linked colorectal and breast cancers. In the instance of human colorectal cancer, the down-regulation of HIF-1α, a regulator of angiogenic cytokine and cell motility adhesion genes, was identified as the mechanism of olive phenolic action in the inhibition of HT-29 grade II adenocarcinoma cell line proliferation (Cardeno et al., 2013). The same study revealed that the induction of apoptotic events in HT-29 cells was time dependent, where hydroxytyrosol induced significant dose-dependent early and late apoptotic populations. This was correspondingly linked to the proapoptotic tumor suppressor p53 protein, which was significantly up-regulated 48 hours post–hydroxytyrosol treatment, such that a greater than threefold increase of p53 expression comparative to
controls was found. This olive phenol stimulation of apoptosis via the p53 pathway was corroborated in MCF-7 breast cancer cells that were treated with oleuropein, in which 3.5-fold up-regulation of p53 expression was found (Hassan et al., 2013). This result was correlated with an increase in observed Bax gene expression that promoted the disruption of mitochondrial integrity and initiated the apoptotic pathway. A related breast cancer study investigated the variant SKBR3, which has a type-1 receptor tyrosine kinase HER-2 overexpressing phenotype, where HER-2 regulates cellular proliferation, differentiation, and apoptosis (Yarden, 2001). Olive oil phenolic fractions, including oleuropein aglycone, played a multifaceted role in diminishing SKBR3 cell viability. The first observation included reduced cell viability of SKBR3 cancer cells through the induction of cytotoxic activity (Menendez et al., 2008). In addition, overall down-regulation of the HER-2 oncoprotein was found. In the same study, a synergistic activity of oleuropein aglycone and ligstroside aglycone, caused a 68–85% reduction of HER-2 expression. In single phenol treatments, the compound hydroxytyrosol alone reduced HER-2 expression by 35% (Menendez et al., 2008).

The most interesting aspect of oleuropein’s cellular effects is its interaction with drug therapy. A complementary study of HER-2 breast cancer presents additional data that suggest that a group of 30 olive phenolic compounds may reverse autoresistance to Trastuzumab, a HER-2 breast cancer monoclonal antibody therapy (Menendez et al., 2007). While supporting oleuropein aglycone dose-dependent inhibition of SKBR3, the results suggested a correlation between the levels of HER-2 expression and the efficacy of oleuropein aglycone treatment. Co-exposure of oleuropein aglycone with Trastuzumab in HER-2 overexpressing breast cancer cells indicated a synergistic enhancement of Trastuzumab activity, with a reported 50-fold increase in Trastuzumab efficacy in reducing cell viability, hinting at the reversal of Trastuzumab autoresistance (Menendez et al., 2007).

In an alternative model utilizing breast cancer cell lines derived from estrogen receptor alpha (ER-α) negative SKBR-3 cells, which have 25–30% prevalence among breast cancers, proliferation inhibition by oleuropein and hydroxytyrosol involved GPER30 dependent pathways. ER-α negative breast cancer cell proliferation is activated upon binding to the G-protein coupled receptor, GPER30, and cell proliferation is initiated via triggering ERK1/2 signalling (Patani et al., 2013).

Illumination of oleuropein, hydroxytyrosol, and GPER binding pocket biochemistry indicated a degree of binding affinity of phenols to the GPER binding site. In contrast to tamoxifen, an antagonist to ER-α positive breast cancer proliferation and an agonist of GPER that stimulates growth in ER-α negative breast cancer, oleuropein and hydroxytyrosol are growth inhibitors that reduce cyclin D1 protein expression and enhance expression of the p53 tumor suppressor. Cyclin D1 is a component of cell cycle progression that is up-regulated in cases of human neoplasia (Weinstein-Saslow et al., 1995). Apoptosis stimulation resulted from GPER binding to oleuropein
and hydroxytyrosol and subsequent sustained ERK phosphorylation and nuclear accumulation (Chimento et al., 2014).

As mentioned previously, besides the more commonly studied breast and colorectal cancers that are less prevalent in the Mediterranean region, there has been some interest in phenolic interactions with human leukemia. In experiments using HL-60 cells as part of a broader investigation, concentrated efforts were limited to human leukemia as the focal point of study in relation to olive phenol fractions (Hamdi and Castellon, 2005). K562 cells stand as an important target for therapeutics due to their stem cell–like properties, such as pluripotency, as well as their resistance against chemical inducers. Application of olive leaf extract, rich in oleuropein, inhibited the proliferation of K562 in a dose-dependent manner. Effects were particularly evident at 72 hours post treatment, in which olive extract treatment reduced cell proliferation levels to below 20% relative to controls.

Although cell viability and proliferation assays feature prominently in assessing treatment efficacy, genotypic expression alterations are also correlated with olive leaf extract treatment (Samet et al., 2014). Oleuropein and hydroxytyrosol have also been shown to up-regulate CHEK2 gene expression (Frazer and Young, 2012), which is involved in cell cycle arrest. In addition, observed apoptotic effects can be linked to the up-regulation of CASP6, CASP8, DFFA, and BID proapoptotic genes by olive leaf extract (Samet et al., 2014).

As knowledge of the biological activity and influences of phenolic compounds, such as oleuropein and hydroxytyrosol, become increasingly clear and extensive, it should be appreciated that their therapeutic benefits extend beyond classical anti-inflammatory and antioxidant activities. It has been demonstrated that phenolic compounds possess the potential to modulate both protein expression and interfere with cell signaling pathways to exert their effects. With increasing research, however, it appears that among the results garnered, anomalies have surfaced that present an almost paradoxical model of phenolic compound influence on the instigation of cell death in tumors.

The Radical-Mediated Hypothesis: Controversies Surrounding in Vitro Evidence for the Anticancer Effects of Phenolic Compounds in Olives and Olive Oil

The broad and extensive cellular modulatory effects of phenolic compounds from olives and olive oil on cancer cells have produced conflicting evidence of their mechanism of action. This controversy stems from the varying results of in vitro studies on different cell lines using similar phenolic treatment doses. A 100 μM dose of hydroxytyrosol was sufficient to inhibit growth and induce apoptosis in HL-60 leukaemia cells; however, in MCF-7 and SKBR3, cell resistance was found with the same treatment (Fabiani et al., 2008b; Han et al., 2009). Yet, in an opposing study, a 12.5 μM treatment dose of
hydroxytyrosol was sufficient to attenuate MCF-7 cell growth (Goulas et al., 2009). This variation has been considered to be an anomaly resulting from the accumulation of H$_2$O$_2$ within the culture medium, wherein H$_2$O$_2$ encourages antiproliferative and pro-apoptotic reactions as a pro-oxidant (Fabiani et al., 2009a). Hydroxytyrosol generation of H$_2$O$_2$ was identified as a partial explanation of the proapoptotic conditions instigated by the phenol. Contrary to its known antioxidant activity, it was observed that oxidative stress from H$_2$O$_2$ generated by hydroxytyrosol resulted in the apoptosis of HL-60 cells. In support of this, detoxification of H$_2$O$_2$ prevented cell apoptosis, which was further supported by the finding that the structurally similar compounds tyrosol and caffeic acid, which are known to generate H$_2$O$_2$, were ineffective at increasing the percentage of apoptotic cells (Fabiani et al., 2009b).

A further study of olive phenolic compounds clarified the significance of the contribution of H$_2$O$_2$ generation to their cellular effects (Fabiani et al., 2009b). The results confirmed that H$_2$O$_2$-generating phenolic compounds were particularly capable in promoting apoptosis, an effect that was reduced following catalase treatment. Despite this, it was shown concurrently that oleuropein and ligrostride aglycone were able to induce apoptosis with minimal production of H$_2$O$_2$ (Fabiani et al., 2009b).

In an additional study that sought to further explain the effect of hydroxytyrosol on a number of tumor cell lines, it was found that, H$_2$O$_2$-induced tumor cell apoptosis was not a complete explanation (Fabiani et al., 2012). An aspect of the radical-mediated hypothesis proposes culture media influence on H$_2$O$_2$ accumulation and apoptosis instigation. Although H$_2$O$_2$ release is observed with hydroxytyrosol, simple phosphate buffered saline solution also produced this effect, indicating a dependence on experimental conditions. More importantly, apoptosis of tumor cells following hydroxytyrosol application is still greater than the direct application of H$_2$O$_2$ (Fabiani et al., 2012).

In addressing the controversial aspects of phenol anticancer properties, the current literature cannot adequately elaborate and amalgamate the mechanisms of phenolic activity against cancers. Although the radical-mediated hypothesis presents a viable explanation of the experimental results of apoptosis induced by phenolic compounds, it is not uniformly observed with a single compound, let alone all olive phenols, as shown with oleuropein and ligrostride aglycones. This controversy stems from an in vitro setting in which the cell culture medium may produce adverse effects that are unlikely to occur in vivo. With more rigorous and extensive research, the biological outcomes of olive phenols may be elucidated.

**Acknowledgments**

Tom C. Karagiannis, a future fellow, and the Epigenomic Medicine Laboratory are supported by the Australian Research Council, the Victorian Government’s Operational Infrastructure Support Program and McCord Research. Tom C. Karagiannis
and Nancy B. Ray are directly supported by McCord Research, and the authors would like to acknowledge the intellectual contribution of Dr. Darlene McCord.

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