THE ROLE OF FUNCTIONAL FOOD SECURITY IN GLOBAL HEALTH

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CHAPTER

BIOACTIVE OLIVE OIL POLYPHENOLS IN THE PROMOTION OF HEALTH

36

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36.1 INTRODUCTION

Olive polyphenols are part of the minor components that comprise approximately 2% of the total weight of olive oil. They are found in the unsaponifiable fraction that includes the phenolic alcohols, hydroxytyrosol and tyrosol, and the secoiridoids, oleuropein and oleocanthal [1,2], with the latter two responsible for the pungent and throat-irritating qualities of olive oil, respectively [1,3,4]. This chapter review will focus on these polyphenols due to the substantial quantity of evidence that exists concerning these compounds in relation to human health as discussed in recent reviews [5,6].

The concentration of polyphenols in olive oil is dependent upon the olive tree variety, the stage of ripening and extraction, as well as storage conditions and other variables [1,7]. Olive oil is a main component of the Mediterranean diet that has been shown to have various positive health effects [8], and olive polyphenols have been associated with many of the beneficial effects of olive oil on health including antioxidant, antiinflammatory, enhanced wound healing, and digestive health, as well as effects against diabetes, osteoporosis, heart disease, neurological disease, and cancer [7–12]. In fact, it has been proposed that extra-virgin olive oil (EVOO) fits the definition of a functional food due to its "clinically proven and documented health benefits for the prevention, management or treatment of chronic disease" [13] (Fig. 36.1).

The polyphenols found in olive oil, like other polyphenols are produced by the olive tree during stress to protect the olive fruit and other parts of the tree from pests and microbial infections [1] Interestingly, the polyphenols found in olive oil including hydroxytyrosol, oleuropein, tyrosol, and oleocanthal are also antioxidants that help prevent autooxidation of the oil [14,15]. Hydroxytyrosol, which is produced from oleuropein by hydrolysis, has the strongest antioxidant effect of the polyphenols found in EVOO [16], and tyrosol has the weakest antioxidant effect [17].

36.2 ORAL BIOAVAILABILITY AND METABOLISM OF OLIVE POLYPHENOLS

Most important polyphenols from olive oil include the phenolic alcohols hydroxytyrosol and tyrosol as well as the secoiridoids oleuropein and ligstroside as glycosides and aglycones [18]. Olives also

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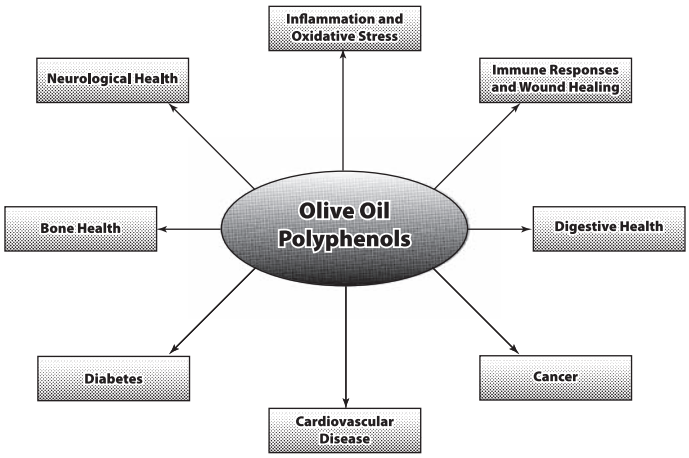

FIGURE 36.1

Accumulating evidence suggests that olive oil polyphenols have various beneficial effects, represented by the arrows directed at eight highlighted areas of health.

contain phenolic acids (benzoic and cinnamic acids), lignans (pinoresinols), and flavonoids (apigenin and luteolin); however, this section will focus on the phenolic alcohols and secoiridoids as they have more research behind them.

The absorption and bioavailability of olive polyphenols has predominately been conducted on hydroxytyrosol, tyrosol, and oleuropein and their derivatives, but the findings have been somewhat inconsistent, conflicting, and sometimes controversial [18]. The metabolic pathways of hydroxytyrosol, tyrosol, and oleuropein are further complicated by the fact that there are endogenous sources of these compounds and/or their metabolites as part of natural dopamine metabolism. Hydroxytyrosol, also referred to as 3,4-dihydroxyphenyethanol (DOPET), is a known metabolite of dopamine, as are many of the hydroxytyrosol metabolites including homovanillic acid (HVA), HVA1c, 3,4-dihydroxyphenylacetic acid (DOPAC), and 3,4-dihydroxyphenyl acetaldehyde (DOPAL) [19,20]. Hydroxytyrosol and other metabolites can freely pass through the blood−brain-barrier making it somewhat difficult to separate endogenous production from exogenous sources in pharmacokinetic studies [18,20,21].

The majority of in vivo data on olive polyphenols has been completed in rat models and some research has indicated as much as a 25-fold increase in excretion of metabolites when comparing rats to humans [18]. It is also notable that many of the studies in both rats and humans have utilized preparations that are enriched with polyphenols, and may not represent the typical exposure from regular consumption of standard olive oil preparations [18].

Some forms of olive polyphenols are susceptible to hydrolysis in low pH environment of the stomach, however it appears that significant portions of these compounds can survive into the small intestines where most absorption occurs [18]. Hydroxytyrosol and tyrosol are predominantly absorbed in the small intestine; however, oleuropein is absorbed mostly in the colon following bioconversion by intestinal bacteria [22].

Olive polyphenols are absorbed into the bloodstream relatively well following oral administration; however, most of the compounds are subject to significant first-pass hepatic metabolism that potentially reduces their distribution and bioavailability throughout the body. However, researchers have identified dose-dependent effects throughout the body leading many investigators to believe that several of the metabolites have biological activity. Some hypothesize that certain metabolites such as glucuronide and sulfate conjugates may even function as depot forms of the polyphenols, slowly releasing the free polyphenols into the bloodstream [20]. As mentioned before, the research is clouded by the fact that many of the polyphenols and metabolites are endogenously produced as a part of normal dopamine metabolism, so there is still significant research needed to better understand the complexities.

Typically, olive polyphenols are quickly absorbed in 5−10 minutes after administration, with more efficient absorption observed with oil-based formulations versus water-based [21]. While some of the polyphenol derivatives and conjugates may persist longer by certain mechanisms including binding to circulating lipoproteins, free-circulating hydroxytyrosol has a short 1−2 minutes half-life before being excreted by the kidneys [21].

There is significant absorption of most olive polyphenols and their derivatives into the bloodstream, however it is hard to pinpoint the exact bioavailability and activity without further research in human models. However, dose-dependent increases in plasma levels, urinary excretion, and biochemical effects have been identified, indicating that oral administration can deliver olive polyphenols into the bloodstream with subsequent biochemical activity throughout the body by the polyphenols and/or their derivatives. The average consumption of olive oil per capita per day in the three Mediterranean countries where it is consumed the most (Greece, Italy, and Spain) is approximately 42 mL/day with Greece having the highest consumption per capita of approximately 55 mL/day [23] The full extent of oral bioavailability is still in need of further research.

36.3 ANTIOXIDANT AND ANTIINFLAMMATORY PROPERTIES

Many health issues including type 2 diabetes mellitus, vascular disease, and neurodegenerative diseases are associated with oxidative stress [24,25]. Oxidative stress occurs when cells are unable to eliminate free radicals known as reactive oxygen species (ROS) using the natural antioxidant defense system that includes glutathione and defense enzymes such as superoxide dismutase (SOD) and hydrogen peroxidase (HO)-1 [26,27]. When cells undergo oxidative stress, they express genes encoding detoxifying enzymes in the cytoplasm, under the control of the nuclear factor erythroid 2-related factor 2 (Nrf2), such as glutathione S-transferase (GST) and HO-1 [27].

Mitochondria, the powerhouses of cells, produce free radicals including ROS during metabolism and can contribute substantially to oxidative stress when damaged or dysfunctional [28]. The mitochondrial antioxidant system is regulated by the forkhead box “O” (FOXO) transcription factor, FOX03a that activates manganese (Mn) SOD [27]. As mentioned, hydroxytyrosol, oleuropein, tyrosol, and oleocanthal possess antioxidant activity. Hydroxytyrosol has been found to activate Nrf2, FOXO3a, and MnSOD, as well as decrease mitochondrial dysfunction [27,29]. In addition, hydroxytyrosol and oleuropein have been shown to upregulate Nfr2 and activate HO-1 [30,31].

Oxidative stress is associated with inflammation and ROS can activate signaling pathways and transcription factors involved in inflammatory cascades, including NFkB, a key transcription factor involved in inflammation regulation [32]. All of the antioxidant olive polyphenols previously mentioned also possess antiinflammatory activities [4,9,33,34]. In fact, hydroxytyrosol has been shown to inhibit activation of NFkB [21], which upregulates many inflammatory cytokines during the acute phase of inflammation, including IL-1, IL-6, and TNF-alpha. Inflammation is the body's response to infection and injury, and accumulating evidence indicates that it plays an important role in many diseases including diabetes, arthritis, cancer, and cardiovascular disease [35,36].

Markers of inflammation include matrix metalloproteinases (MMPs), C-reactive protein (CRP), and cyclooxygenase (COX)-2, an enzyme that produces prostaglandins, which help mediate the inflammatory response [35–37]. Interestingly, oleocanthal was observed to have irritating qualities similar to the NSAID, ibuprofen, and was found to inhibit COX-1 and COX-2 enzymes, mimicking the antiinflammatory activity of ibuprofen [38]. Hydroxytyrosol has also been shown to decrease the level of COX-2 messages [39], and oleuropein has been found to inhibit the production of inflammatory cytokines and lipoxygenase (involved in prostaglandin metabolism) activity [33,40]. Recently, tyrosol has also been shown to have antiinflammatory activity, partially induced by preventing the phosphorylation of NFkB signaling proteins [34].

36.4 IMMUNE CELL RESPONSES AND WOUND HEALING

During inflammation induced by invading pathogens or injury, sensor cells including mast cells, dendritic cells, and macrophages detect infection or tissue damage and express inflammatory mediators including inflammatory cytokines. Neutrophils are recruited from the circulation and certain inflammatory cytokines induce the liver to produce acute phase proteins such as CRP. Normally, a transition from neutrophil to monocyte/macrophage recruitment results in the removal of debris, resolution of inflammation, and initiation of tissue repair. If the inflammatory trigger is not removed, chronic inflammation and chronic wounds may occur. In addition, some health conditions have less distinct inflammatory triggers including diabetes, obesity, neurodegenerative diseases, and cancer that are associated with chronic inflammation [41].

Oral olive oil administration has resulted in reduced inflammation and enhanced wound healing [42,43]. Hydroxytyrosol and oleuropein have been shown to increase wound healing by increasing cell endothelial cell migration (even with high glucose simulating diabetic wounds) and have been found to increase angiogenesis (unpublished results from our laboratory). Endothelial cell repair and wound healing has also been correlated with Nrf2 activation or nuclear accumulation and increased HO-1 expression [30,31]. Evidence for hydroxytyrosol and oleuropein reversing endothelial dysfunction, which occurs with diabetes, has also been found [14,31]. In addition, evidence indicates that mast cells regulate wound healing in diabetes [44] and that therapies that inhibit mast cell degranulation (including histamine release) could improve wound healing in diabetes. Interestingly, hydroxytyrosol and oleuropein have both been shown to inhibit mast cell degranulation [45]. Antimicrobial properties of olive polyphenols are likely to benefit wound healing. Hydroxytyrosol and/or oleuropein have been shown to inhibit the growth of wound pathogens including *Staphylococcus aureus* and *Candida albicans* [46,47].

36.5 DIGESTIVE HEALTH

The potential impacts of olive polyphenols in digestive health are particularly interesting because the polyphenols have direct contact with the gastric and intestinal mucosa, allowing for direct cellular exposure while avoiding the first-pass hepatic metabolism previously discussed. Studies have demonstrated that several of the olive polyphenols and/or derivatives can passively diffuse into enterocytes quite readily [18,48]. The mucosa, therefore, does not rely on increasing plasma levels to observe some of the researched benefits, though there is likely added benefit from circulating polyphenols and derivatives. Olive oil polyphenols can also directly impact digestive health by promoting an intestinal microbiome that supports intestinal immune homeostasis, which expands the potential benefits to a broad spectrum of disease processes known to be tied into this system [49,50].

The findings that topical application of olive polyphenols can improve wound healing processes and is also relevant in digestive health as oral administration of the polyphenols can serve as a topical application to the gastric and intestinal mucosa [51,52]. The implications are that topical would healing properties have utility in restoring damaged and inflamed mucosa. The combination of systemic effects paired with direct topical effects on mucosa, modulation of microbiome, and immunemodulation all contribute to the observed benefits of olive polyphenols in a wide variety of digestive issues including IBS, Crohn's disease, ulcerative colitis, and even cancer [53–55].

36.6 CANCER

Cancer is a complex disease that involves the transformation of normal cells into malignant cells that can grow beyond normal boundaries, forming tumors and frequently invading other tissues or spreading. The increased proliferation of cancer cells compared to normal cells is due to the lack of growth regulation that is found in normal cells. In addition, normal cells monitor DNA damage that may occur and arrest their growth until the damage is repaired, or if unable to repair damage, activate apoptosis. Cancer cells, however, are fairly resistant to apoptosis [56,57].

VOO consumption and the incidence of many forms of cancer including colon, breast, and skin cancer have been inversely correlated [7]. In fact, a systematic review and meta-analysis that included 13,800 patients came to the conclusion that olive oil consumption is inversely related to cancer prevalence [58]. The anticancer properties of olive oil polyphenols have recently been reviewed [7,56]. Some anticancer mechanisms and targets of olive polyphenols are summarized in Table 36.1 including cytoskeleton disruption [59], lysosomal membrane permeabilization [60], C-Met signaling inhibition [61,62], and epidermal growth factor receptor (EGFR) downregulation and degradation [63].

Many studies have demonstrated antiproliferative and proapoptotic effects of oleuropein with different cancer types including studies involving breast, colorectal, lung, leukemia, and prostate cancer cell lines (unpublished results from our laboratory) [3]. Other studies have also shown that hydroxytyrosol decreases proliferation and induces apoptosis in various types of cancer cells including breast, prostate, as well as in various leukemia cell lines (unpublished results from our laboratory) [9,11,64]. In fact, oleuropein and hydroxytyrosol have invariably been found in

Table 36.1 Some Anticancer Mechanisms and Targets of Olive Oil Phenols

Mechanism	Effect	Cancer Cells	Target	Polyphenol	References
Estrogen competition	Proliferation inhibition	Breast	Estrogen receptor	Oleuropein, hydroxytyrosol	[73]
Cytoskeleton disruption	Proliferation, migration, inhibition	Leukemia, renal, breast, colorectal melanoma	Actin filaments	Oleuropein	[62]
Lysosomal membrane permeabilization	Apoptosis, necrosis	Prostate, breast, pancreatic	Lysosome	Oleocanthal	[63]
C-Met signaling inhibition	Proliferation, migration, invasion inhibition	Breast, prostate	C-Met kinase	Oleocanthal	[64,65]
EGFR down regulation, degradation	Proliferation inhibition	Colon	EGFR	Hydroxytyrosol	[66]

numerous studies to have these effects (inhibition of proliferation and induction of apoptosis) in cancer cells without affecting normal cells [36]. In addition, oleocanthal has been shown to induce death or inhibit proliferation and/or migration and invasion of breast, colon, prostate, or pancreatic cancer cell lines, as well as multiple myeloma cells [60,61,65].

In animal model studies, orally delivered oleuropein induced complete soft tissue sarcoma tumor regression [59] and in another study, decreased the incidence and volume of skin tumors [66]. Hydroxytyrosol delivered orally, was shown to decrease breast tumor volume [21]. In addition, oleocanthal treatment was found to suppress tumor cell growth in a model of breast cancer [61].

As mentioned, various olive polyphenol anticancer activities have been demonstrated, including those involving apoptosis. Cancer cells have greater ROS production due to their higher metabolism [67], and olive polyphenols including hydroxytyrosol and oleuropein can induce prooxidative effects in cancer cells that lead to apoptosis. Interestingly, one hypothesis (xenohormesis) proposes that polyphenols produced by plants under stress might also activate stress responses in humans, including oxidative stress responses that protect against diseases such as cancer [36].

Cancer is an inflammatory disease and NFkB plays an important role in many inflammatory pathways [35]. Evidence indicates that the olive polyphenols, oleuropein, and hydroxytyrosol can inhibit NFkB and modulate these pathways that are related to inflammatory cytokine, COX-2, and iNOS production in various cancer types. Interestingly, therapies that inhibit COX-2 have decreased breast cancer risk and have had proapoptotic effects in a breast cancer cell line [36]. The tumor suppressor gene, p53, mediates apoptosis following damage to cellular DNA. In an important study, oleuropein was shown to induce apoptosis in breast cancer cells via a p53-dependent pathway [68]. High levels of estrogen are linked with breast cancer development. An interesting mechanism proposed to explain certain anticancer activities of hydroxytyrosol and oleuropein on breast cancer cells, highlights the fact that these polyphenols have an aromatic ring analogous to estradiol that could compete with estrogen [69].

Inhibition of a heat shock protein is involved in an additional olive polyphenol anticancer activity. Interestingly, oleocanthal was found in a study to inhibit Hsp90, a key target in cancer therapy [70]. Cells exposed to stress, including wounding, produce heat-shock proteins (HSPs) whose primary role is in aiding protein folding and maintaining the structures and functions of the proteins that they chaperone. Molecular chaperones protect proteins under stress that results in their unfolding, and their expression and activities are substantially increased in a variety of cancer types, resulting in the suppression of apoptosis [71]. Oleocanthal had a proapoptotic effect on leukemia cells in the aforementioned study with only a slight decrease in the viability of normal cells, which is typical of other Hsp90 inhibitors [4].

36.7 CARDIOVASCULAR DISEASE

There are many etiologies behind the various forms of cardiovascular disease, however there are some common motifs that all tend to include components of inflammation, endothelial dysfunction, and metabolic impairments associated with metabolic syndrome, obesity, and/or dyslipidemia [72,73]. Accumulating evidence suggests that olive oil polyphenols can influence these processes, indicating that polyphenols have tremendous potential in addressing the causative and contributing factors in a wide variety of cardiovascular disorders [8,11]. In fact, olive polyphenols are thought to play a significant role in the improved cardiovascular health observed in adherents to Mediterranean diets that incorporate significant amounts of olives and olive oil [74]. Some examples of the effects of olive oil polyphenols against cardiovascular disease are summarized in this section including the observation that hydroxytyrosol was able to reverse chronic inflammation and oxidative stress that can lead to the development of cardiovascular, hepatic, and metabolic syndrome from a high-carbohydrate and high-fat diet [75]. Polyphenols have also been shown to improve vascular function and reduce inflammation and fibrosis in heart tissues, reduce left ventricle stiffness, and improve aortic reactivity, while simultaneously improving abdominal fat deposition, plasma triglycerides, total cholesterol, glucose tolerance, and insulin sensitivity [75,76]. Moreover, the antiinflammatory and antioxidant properties of oleuropein appear to contribute to the antithrombotic and antiatherogenic properties of oleuropein, which has also been shown to have beneficial effects in dyslipidemia and preventing LDL oxidation [33]. In addition, studies in hypertensive patients have shown therapeutic benefits from high-phenolic olive oil that was not observed with low-phenolic olive oil, leading researchers to attribute the observed effects to the natural polyphenols [77]. Moreover, oleuropein has been shown to demonstrate cardioprotective effects following ischemia and doxorubicin-induced cardiomyopathy [8]. Finally, there have been some clinical trials that have examined the effects of olive oil on cardiovascular disease and related health problems, including diabetes, that have indicated that the consumption of olive oil can provide protection against various aspects of cardiovascular disease [1,7].

36.8 DIABETES

People with type 2 diabetes mellitus have an increased risk of cardiovascular disease, neurodegenerative disorders, retinopathy, impaired wound healing, and cancer. Throughout this chapter, we

have or will discuss the impact olive polyphenols can have in each of those conditions, and the benefits are observed in the diabetic patient as well. Many of the studies that attribute benefits of olive polyphenols in people with diabetes have done so due to the aforementioned reasoning, however there is also evidence suggesting the polyphenols have more direct effect on sugar metabolism, fasting glucose levels, glycosylated hemoglobin (HbA1C), and BMI [8,17,78]. In addition, oleuropein has recently been shown to protect beta cells from cytotoxicity induced by amylin amyloids that are characteristic of type 2 diabetes [79].

36.9 NEURODEGENERATIVE DISEASE

There is a continuously increasing body of evidence implicating oxidative and inflammatory processes, as well as mitochondrial dysfunction in a variety of neurological and neurodegenerative disorders [80,81]. Naturally, due to their well-documented antiinflammatory and antioxidant properties, olive polyphenols are increasingly being investigated for their potential neuroprotective effects [82]. Hydroxytyrosol has been shown to increase genetic expression of key cellular antioxidants that protect brain cells from free radical damage that contributes to neurodegenerative processes [29,83]. Hydroxytyrosol has also been shown to protect against mitochondrial dysfunction [28] including in rat brains [84].

Oleuropein has demonstrated protective effects on dopaminergic neurons located in the substantia nigra, implying possible roles in preventing or treating Parkinson's disease [33]. Oleuropein also interacts with some of the biochemical processes behind the formation and deposition of beta-amyloid plaques and Tau proteins associated with Alzheimer's Disease, with promising studies showing possible protective effects [33]. In addition, oleocanthal has been found to enhance amyloid-beta clearance from the brains of mice [85,86]. Moreover, oleocanthal was found to interfere with Tau aggregation [87].

36.10 OSTEOPOROSIS AND BONE LOSS

Bone formation and maintenance is regulated by bone-producing osteoblasts and bone-reabsorbing osteoclasts, and imbalance of these processes can result in diseases including osteoporosis [88], which has the lowest incidence in the Mediterranean region where the Mediterranean diet is common [89]. Interestingly, EVOO phenolic extracts have been shown to increase osteoblastic cell proliferation [90]. In addition, olive oil oral supplementation has been found to increase bone thickness and density in ovariectomized mice [91,92], and hydroxytyrosol has been found to suppress bone loss in ovariectomized mice [93]. Importantly, female patients 30−50 years of age who had hysterectomies were provided with 50 mL of olive oil per day or nothing (control) in a study where it was found that bone density decreased in the control group, but not in the treatment group [92].

Estrogen deficiency is a major factor contributing to osteoporosis, however oxidative stress and inflammation are thought to play critical roles as well, and notably, patients with inflammatory diseases including inflammatory bowel disease, rheumatoid arthritis, and chronic obstructive pulmonary disease experience decreased bone mass and increased fractures. In fact, inflammatory

mediators have been found to affect bone remodeling, and antiinflammatory therapies have positive effects on bone fragility markers [12].

36.11 RESPIRATORY HEALTH

Olive polyphenols have been shown to play a role in reducing oxidative stress and modulating inflammatory responses in the lungs [94,95]. Studies have shown a variety of positive effects including reductions in inflammatory markers, improved lung function, and fewer exacerbations of conditions like COPD, emphysema, and asthma [94].

36.12 CONCLUSION

The positive health effects of the Mediterranean diet have indicated that olive oil, a main component, may also have various positive health effects, which in recent years have been investigated and substantiated. Here, we have reviewed evidence for many of these health effects including against diabetes, cancer, and heart disease. There are numerous other favorable effects of olive oil polyphenols on health that were not reviewed such as against arthritis, obesity, and liver disease. Although evidence is rapidly accumulating to support the beneficial health effects of olive oil polyphenols, more human trials may be needed before the general public is convinced and willing to incorporate olive oil into their diet, suggesting that olive polyphenol supplementation is important.

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