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Nutritional Benefit of Olive Oil: The Biological Effects of Hydroxytyrosol and Its Arylating Quinone Adducts

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Olive oil is the essential component of the Mediterranean diet, a nutritional regimen gaining ever-increasing renown for its beneficial effects on inflammation, cardiovascular disease, and cancer. A unique characteristic of olive oil is its enrichment in oleuropein, a member of the secoiridoid family, which hydrolyzes to the catechol hydroxytyrosol and functions as a hydrophilic phenolic antioxidant that is oxidized to its catechol quinone during redox cycling. Little effort has been spent on exploring the biological properties of the catechol hydroxytyrosol quinone, a strong arylating electrophile that forms Michael adducts with thiol nucleophiles in glutathione and proteins. This study compares the chemical and biological characteristics of hydroxytyrosol with those of the tocopherol family in which Michael adducts of arylating desmethyltocopherol quinones have been identified and correlated with biologic properties including cytotoxicity and induction of endoplasmic reticulum stress. It is noted that hydroxytyrosol and desmethyltocopherols share many similarities, suggesting that Michael adduct formation by an arylating quinone electrophile may contribute to the biological properties of both families, including the unique nutritional benefit of olive oil.

KEYWORDS: Olive oil; secoiridoids; oleuropein; ligstroside; hydroxytyrosol; tyrosol; phenolic antioxidant; Michael reaction; arylating catechol quinone; NF-κB

INTRODUCTION

The Mediterranean diet is associated with the emergence of agriculture within the Natufian culture in the Levant around 13000 years before the present (BP) and was refined by the continued development of agriculture throughout the Neolithic period, 5500−10000 BP (*1, 2*). Olives and olive oil are described in documents from Mycenaean Greece, and olive tree cultivation was extended throughout the later Roman Empire, ultimately centering in the Guadalquivir River valley of Spain (*2*). The present-day Mediterranean diet originates from this period and is most clearly defined by its olive oil and cereal grain content (*2*−*4*).

Within the general area of nutrition, beneficial effects are ascribed to the Mediterranean diet, particularly in the areas of cancer, cardiovascular disease, and inflammation, and these effects have been subjected to extensive review (*1, 5*−*22*). High contents of oleic acid and minor components including squalene, plant sterols, tocopherols, and polyphenols all contribute to the unique composition of olive oil; several of these components are considered to be candidates, either individually or in mixtures, responsible for its beneficial effects. Mixtures, which may express synergy, require an almost exponential increase in independent variables and unconditional logical regression (*23*), are seldom studied in the original literature, and are not analyzed in this review.

CHARACTERISTIC COMPONENTS OF OLIVE OIL

Oleic Acid. The striking difference in fatty acid content between olive oil with its high oleic acid (18:1n-9) and other seed oils with their high linoleic acid (18:2n-6) content (**Table 1**) was noted early and continues to be a subject of great interest (*1, 5, 15, 20, 24*−*30*). Investigators in the 1990s found little real difference when saturated fats were replaced by either 18:1n-9 or 18:2n-6 in the diet (*31*). Furthermore, meat (**Table 1**) contributes significantly to the 18:1n-9 levels in human tissues (*32*), and because olive oil is not the only major source of dietary 18:1n-9, a unique role for this acid is an unlikely explanation for the beneficial effects of the Mediterranean diet (*20*). In fact, different olive oil preparations with similar oleic acid contents have shown different biological effects in living animals (*33*).

Table 1. Fatty Acid Composition of Seed Oils and Pork Lard (Percent of Total Lipids)[a]

	palmitic acid (16:0)	oleic acid (18:1n-9)	linoleic acid (18:2n-6)	linolenic acid (18:3n-3)
olive oil	14	69	12	<1
corn oil	13	31	53	1
soybean oil	11	23	53	9
pork lard	26	48	9	<1

[a] Data summarized from ref *28*.

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Table 2. Isoprenoid Content of Seed Oils (Milligrams per Kilogram)[a]

	olive oil	corn oil	soybean oil
squalene	3300	280	110
β-sitosterol	77	9130	1530
squalene/β-sitosterol	45.9	0.03	0.07
α-tocopherol	99	11	180
γ-tocopherol	11	1370	1240
δ-tocopherol	0	49	541

[a] Data summarized from refs *28, 34, and 35*.

Squalene and Phytosterols. A high squalene content and a low phytosterol content have been known as defining characteristics of olive oil (*8, 10, 29, 30, 34, 35*). Squalene and the major phytosterol, β-sitosterol (*28, 29, 36*), show dramatic differences between olive and other oils (**Table 2**). Squalene is synthesized in the isoprenoid biosynthetic pathway (*36*−*38*), whereas little is known about the regulation of phytosterol biosynthesis from squalene in higher plants including olive (*39*). Isoprenoids and phytosterols are being discussed increasingly for their roles in transcription and signaling transduction pathways (*38, 39*), and the unusual squalene/phytosterol profile in olive oil has drawn much attention. A number of studies promulgate a squalene hypothesis as an explanation for the beneficial effects of olive oil (*7, 8, 20, 40*−*43*); however, the evidence is not compelling (*44, 45*).

Tocopherols. The literature is replete with numerous studies on the nutritional effects of tocopherols in vegetable oils, and two important observations on the tocopherol content of olive oil are found in these studies. Olive oil is low in total tocopherol (T) compared to other vegetable oils, and it contains mostly α-T, being almost devoid of partially methylated β-, γ-, and δ-T (**Table 2**). Thus, olive oil resembles animal fat in composition (*1*) and if, as was recently suggested (*46*), the primary role of α-T is as a phenolic antioxidant, the tocopherol content of olive oil is unremarkable, suggesting at the most the possibility of a mild antioxidant deficiency, which could be compensated in part by its relatively high secoiridoid content.

Secoiridoids. Many studies link specific secoiridoids as key minor components in an explanation of the extraordinary nutritional effects of olive oil (*6, 8*−*10, 13*−*20*). Secoiridoids are found in Oleaceae, a family consisting of 600 species grouped in 25 genera and containing 258 different secoiridoid compounds. Olive oil contains both iridoids derived from iridane and secoiridoids derived from iridoids (*10, 19, 20, 36*−*38, 47*−*49*). The biosynthesis of iridoids begins with mevalonic acid, as does the biosynthesis of many olive oil minor components including squalene. Iridane is formed, and this is followed by a ring opening and conversion to elenolic acid, which is the core structure of secoiridoid compounds (**Figure 1**). Secoiridoids are glycoside derivatives at the hydroxyl group (the aglycon is also found) of elenolic acid and either hydroxytyrosol (HTy) or tyrosol (Ty) esters of elenolic acid, which are known as oleuropein and ligstroside, respectively.

Secoiridoids are found not only in olive fruit but also in small branches and leaves of the olive tree (*50*−*53*). Their levels in olive oil differ widely depending on variety, ripening, extraction, and storage conditions (*54*−*63*). Secoiridoid elenolic acid esters are readily hydrolyzed in mild acid (**Figure 1**), yielding the hydrophilic catechol HTy (*64*−*67*), a process likely occurring during gastric digestion. Ligstrosides are also hydrolyzed with mild acid (**Figure 1**), yielding Ty, which, as will be discussed later, has very different biological properties from HTy. The free HTy content of virgin olive oil increases with the maturation of the fruit (*63*), but many studies summarized above and elsewhere (see HTy Concentration in Olive Oil) show that the mole fractions of free HTy and Ty are only a few percent of the mole fractions of total (free and bound) phenolics in olive oil.

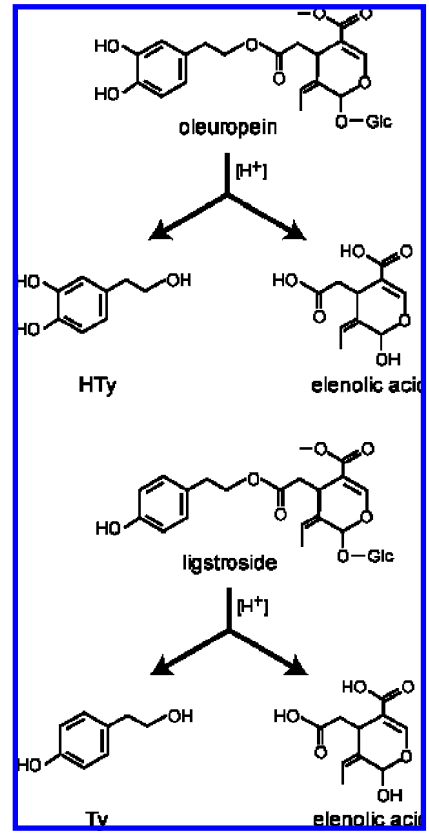

Figure 1. Generation of hydroxytyrosol (HTy) and tyrosol (Ty).

HTY AND THE ARYLATING QUINONE HYPOTHESIS

After considering the composition of olive tissue, we propose as a working hypothesis that the beneficial effects of olive oil reside in polyphenol secoiridoids and their hydrolysis product, HTy. These are hydrophilic catechol phenolic antioxidants that will convert to a catechol quinone electrophile, HTyQ, during redox cycling and, therefore, possess many properties similar to those of lipophilic arylating tocopherol quinone electrophiles, including formation of Michael adducts with cellular thiol nucleophiles, particularly thiols on cysteinyl proteins. The hydrophilicity of HTyQ distinguishes it from the lipophilic tocopherol quinones in that it more readily undergoes rapid detoxification through arylation with hydrophilic thiol nucleophiles such as glutathione (GSH). In addition, hydrophilicity allows HTyQ to react with available thiol nucleophilic groups in enzymes and signaling molecules within the hydrophilic environment of the cell. The ability of HTyQ to react with more hydrophilic cysteinyl proteins may result in different biological properties for this arylating quinone compared to hydrophobic arylating tocopherol quinones, which are concentrated in lipid membranes. Supporting this notion, differences between hydrophilic and lipophilic arylating quinones are found when lipophilic γ-TQ is compared with a much more reactive hydrophilic hydroxyethyl hydroxychroman derivative (γ-CEHC) in which the phytyl side chain of the tocopherol is oxidized to a short chain with a terminal carboxylic acid group (*1, 68, 69*). Enhanced hydrophilicity may be an important contributor to the unique beneficial effects associated with secoiridoids and HTy in olive oil.

Table 3. Hydroxytyrosol (HTy) and Tyrosol (Ty) Contents of Olive Oils Produced in Different Areas and Different Years (Milligrams per Kilogram)[a]

area/year	HTy	Ty
Coratina		
2000	103	162
2001	169	290
2002	85	138
average	119	197
Peranzana		
2000	124	83
2001	154	172
2002	94	66
average	124	107

[a] Data summarized from ref *67*.

Despite extensive literature on olive oil, there is little description of the arylation chemistry of secoiridoid quinone electrophiles, and no suggestion attributing the nutritional biochemistry of secoiridoids and HTy to the formation of arylating ortho quinone electrophiles and their subsequent interactions with cellular thiols. Here, we summarize evidence in the literature that supports this hypothesis and propose future directions for investigation.

CHEMICAL PROPERTIES OF HTY

HTy Content in Olive Oil. A number of studies use liquid chromatography or capillary electrophoresis and mass spectroscopy, ^{1}H and ^{31}P NMR, and amperometric techniques for the quantitative analysis of bioactive phenolic substances in olive oil. Phenolic compounds from olive oil are also derivatized with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide and then analyzed by various procedures described above. A representative selection of experimental studies is listed here (*67, 70*−*76*).

As many as 23 phenolic compounds have been identified and quantified as members of the secoiridoid oleuropein and ligstroside families (*70*). Because phenolic conjugates are readily hydrolyzed, in vitro and in vivo, to free HTy and Ty (**Figure 1**), we find great merit in a procedure (*67*) that involves acid hydrolysis followed by the estimation of total free HTy and Ty as a practical alternative for the estimation of polyphenols in olive oils as their secoiridoid precursors. Data from a study originally reported as milligrams per liter for different oils are recalculated as milligrams per kilogram (**Table 3**) and compared with α-T (**Table 2**), which has a lower concentration than HTy in olive oil. Oils from two regions in Italy were collected in three different years and pressed from whole and stoned olives, but only data for pressed whole olives are summarized (*67*). HTy and Ty contents depended on growing conditions (region and year) and the method of isolation (whole and stoned). The analytical procedures cited here are important in the isolation, purification, and quantitative estimation of secoiridoids, but they do not address an important question, namely, the occurrence and metabolism of the hydrophilic HTyQ electrophile derived from HTy.

Role of HTy as an Antioxidant. The antioxidant properties of tocopherols are generally considered as the basis for their role in nutrition (*46*), and this role, although not stated explicitly, has been assigned by many scientists to olive oil secoiridoids (*13, 19, 20, 27, 77, 78*). The phenolic antioxidant activity of olive oil and its purified secoiridoid catechols has been studied exhaustively using many different techniques in abiotic model systems where reactive oxygen species (ROS) and other radicals are generated by a variety of agents. These studies correlate antioxidant activity with total phenolics, being principally linked to the secoiridoids oleuropein and its hydrolysis product HTy (*8, 47, 50, 51, 53, 54, 62, 63, 78*−*90*).

Interestingly, low concentrations of polyphenols appear to function as pro- instead of antioxidants (*91*). This paradoxical effect might be related to ROS generation through hydroquinone/quinone redox cycling, a process that has been studied extensively with aromatic xenobiotic quinones and their Michael adducts (*92*−*94*). It is important to point out that both arylating quinone electrophiles and their nonarylating Michael adducts undergo redox cycling and function as phenolic antioxidants and, therefore, the dramatic differences in the biologic activities of arylating quinones and their adducts cannot be explained solely by their common role as phenolic antioxidants.

The high catechol (*o*-dihydroxyl) antioxidant functionality of HTy, which is probably related to O−H bond dissociation enthalpy (*95, 96*), is most impressive, showing a stronger correlation with oxidative stability than that of *p*-dihydroxy α-T and leading to longer induction times for rancidity with oleuropein, homovanillic acid, and HTy compared to α-T, ascorbic acid, and the synthetic antioxidant butylated hydroxytoluene (BHT) (*90, 97*−*99*). The 3-*O*-monoglucuronide conjugate of HTy is a stronger antioxidant than HTy itself, whereas the 4-*O*-monosulfate conjugate is not, suggesting that both the hydrophilicity and the 4-*O*-hydroxy group are probably important for its antioxidant activity (*99*). Interestingly, HTy with its catechol structure is a much more effective antioxidant than Ty (*100, 101*), which has the 4-*O*-hydroxy functionality and might presumably oxidize to an arylating quinone methide (*1, 102*). As has been noted, *p*-coumaric acid and 2-(4-hydroxyphenyl)ethanol, compounds with 4-*O*-hydroxy groups, function as weak antioxidants in a lipoprotein diene formation test but do not function as radical scavengers in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test (*103*). Evidently, antioxidant activity does not always correlate in different assay methods, and the catechol structure is not always required for antioxidant activity. However, as a general rule, the catechols such as HTy and oleuropein have the highest antioxidant activity followed by 4-*O*-monohydroxy compounds, ligstrosides, Ty, and 3-*O*-hydroxy-substituted catechols, and all of these compounds are stronger antioxidants than either ascorbic acid or α-T (*90, 99*).

In many of the studies cited above, short-chain acyl (hydrophilic) HTy derivatives had a higher protective effect against oxidative damage than long-chain acyl (hydrophobic) HTy derivatives and homovanillic alcohol long-chain acyl derivatives (*104*). However, long-chain acyl HTy derivatives had a higher protective effect against oxidative damage in an ex vivo brain homogenate model (*105*). This is suggestive to us of a homogeneous lipid phase emulsion in the brain homogenate leading to the so-called "polar paradox", where hydrophobic antioxidants function more effectively than hydrophilic antioxidants when emulsions and bulk lipid phases are compared (*90, 106, 107*).

Thermal oxidation of vegetable oils causes the conversion of fully methylated α-T to nonarylating α-TQ, whereas in oils such as corn oil and soybean oil that are rich in partially methylated γ-T and δ-T, thermal oxidation causes tocopherol loss, seemingly without the formation of oxidation products such as arylating γ-TQ and δ-TQ (*1, 108*). We attribute this phenomenon to the formation of thiol adducts of the arylating tocopherol quinones, which we have identified in model systems by tetramethylammonium hydroxide (TMAH) thermochemolysis (*68, 69, 109*).

Similarly, thermal oxidation of virgin olive oil causes a significant exponential decrease in the catechol HTy, seemingly without the formation of its catechol quinone, whereas α-TQ, the oxidation products of α-T, was detected (*110*−*116*). As discussed above, HTy is a more effective antioxidant than α-T and, consequently, more HTy should enter redox cycling and yield more HTyQ electrophiles. Again, this paradoxical observation can be explained by the formation of adducts between HTyQ electrophiles and nucleophiles in the oil, which will prevent the detection of HTyQ by the methods used in those studies. Therefore, detection of Michael adducts in these systems would support the formation of HTyQ during thermo-oxidation, which could be achieved through TMAH thermochemolysis.

Role of HTyQ as an Arylating Quinine. Isoprenoids and their metabolites, particularly phenols, have many purported roles in cell signaling (*117*), and there are compelling reasons to focus on their roles in nutrition as naturally occurring phenolic antioxidants (*46*). Two aspects to the biochemistry of phenolic antioxidants, protection against ROS and the formation of a variety of highly interesting arylating oxidation products, are particularly important.

As was discussed previously, our HTy arylation hypothesis is formulated on the seldomly recognized fact that phenols, as they function as antioxidants, are ultimately transformed into quinones, many of which are arylating quinone electrophiles (*1, 92*−*94*). We find within the tocopherol family of *p*-diphenolic antioxidants, a striking step-function difference between nonarylating α-TQ and arylating γ- and δ-TQ in their biological properties (*1*). Catechols, which include xenobiotics and naturally occurring *o*-diphenolic antioxidants, readily oxidize to their quinones, and many studies, particularly with xenobiotic catechols, show striking similarities between catechol *o*-quinones and *p*-quinones in their roles as arylating electrophiles (*1, 92*−*94*). Both catechols and tocopherols disappear during oxidation, which likely results from the formation of thiol adduct by arylating quinone electrophiles (*1*). Yet, no effort has been made to identify *o*-quinone thiol adducts in olive oil processing and metabolism, and this should be explored as a possible explanation for the absence of arylating quinones formed through oxidation.

As mentioned above, the Michael addition reaction between an arylating quinone electrophile and thiol nucleophile is readily demonstrated by the formation of a thiol adduct with *N*-acetylcysteine (NAC), GSH, or protein with accessible cysteinyl groups (*1*). Early investigators reasoned that a Michael adduct had been formed when they isolated phthiocol (2-methyl-3-hydroxyl-1,4-naphthoquinone) by saponifying the ether extract from human tubercle bacilli in 1 N NaOH with heating. Hydrolysis occurred through a nucleophilic attack by the hydroxyl group at the relatively electron deficient ring carbon of a C−S bond (*118*). This reaction, which was later explored by the saponification/hydrolysis of thiodione (2-methyl-3-*S*-glutathionyl-1,4-naphthoquinone) (*119*), has been adapted using the strong base TMAH in a thermochemolysis reaction that was introduced to cleave Michael adducts in conjugated macromolecules with humic substances and plant biopolymers (*120*−*122*). With TMAH thermochemolysis, tocopherols are methylated, tocopherol quinones reduced and methylated, and thiol adducts of their arylating quinones cleaved, reduced, and methylated (*1, 68, 69, 109*). Fully methylated derivatives are ideally suited for separation and identification by capillary GC-MS without prior trimethylsilylation (*76*). A scheme based on published studies with γ-T, γ-TQ and its thiol adducts (*1*) is outlined for HTy in **Figure 2**.

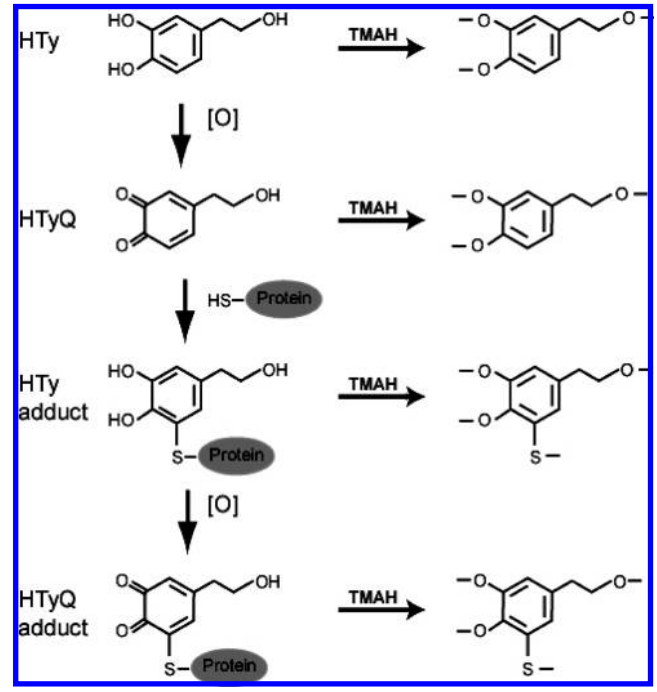

Figure 2. TMAH fragmentation/reductive methylation of hydroxytyrosol (HTy), hydroxytyrosol quinone (HTyQ), and thiol nucleophile adducts.

Information in the pharmacologic literature suggests that olive oil catechols and other polyphenols are multipotent agents (*123*) with biological effects that may have little to do with their antioxidant activity (*124, 125*). For example, Ty, a much less effective antioxidant than HTy, is, like HTy, able to restore intracellular antioxidant defenses with little impact on other biological effects (*126*). Indeed, a large body of evidence is accumulating that within another polyphenol family, the flavonoids, antioxidant and cell signaling functions, particularly those functions where a catechol group in the B-ring of the flavonoid is involved, are separated from each other (*127*−*129*). Metabolites of these flavonoid antioxidants, particularly their arylating quinone electrophiles, readily form thiol nucleophile adducts that may be involved in cell signaling functions independent of redox cycling (*1, 127*−*130*).

Synthesis of Catechol Quinones from Secoiridoids. The remarkable commonality between the biological properties of arylating γ-TQ and those of arylating HTyQ (discussed below) may indeed be explained by the oxidation of HTy to its arylating quinone HTyQ. Arylating *o*-quinones derived from catechols have been studied in xenobiotic systems (*92*−*94*), and recently several studies have begun to explore the role of naturally occurring HTyQ derived from the oxidation of HTy as an arylating quinone electrophile. HTy oxidation with H_2O_2 yields a hydroxyquinone intermediate, which is then subjected to a nucleophilic attack with HTy in a quinone−quinone coupling mechanism (*131*). Tyrosinase oxidation of HTy to HTyQ may be followed by a nucleophilic attack by *o*-hydroxyl groups yielding methanooxocinobenzodioxinones (*132*). HTy is also subject to phase I/II biotransformation and the formation of glutathionyl conjugates when it is oxidized by tyrosinase in the presence of GSH, but specific conjugates are not always characterized (*133, 134*). Recent studies have identified electrophilic quinones from botanicals activated by hepatic microsomes and then treated with NAC or GSH to form conjugates

identified by LC-MS/MS (*135, 136*). In these studies, an HTyQ conjugate was identified among many conjugates from black cohosh, but this methodology, which does not allow for the identification of total protein thiol conjugates, has not yet been extended to secoiridoids and HTy in olive oil.

BIOLOGICAL PROPERTIES OF HTY

HTy Metabolism. A review of the literature suggests that the normal Mediterranean diet does not provide sufficient amounts of HTy equivalents to affect antioxidant status, in vivo (*137*). Polyphenols do function as phenolic antioxidants in isolated cells, tissues, and animal models and, as a consequence of this function, polyphenols generate arylating catechol quinones with specificities far exceeding those of ROS. Studies with individual polyphenols in model systems only hint at the complexities found in olive oil polyphenol metabolism. The high polyphenol levels supplied to cells in tissue culture and the very high polyphenol levels provided in studies involving animal nutrition result in many biological effects, but their interpretation has to be very careful because these observations do not establish with certainty that effects will be attained in free-living human populations.

In Caco-2 cells, HTy is transported across the membrane by bidirectional passive diffusion and 3-hydroxyl-4-methoxyphenylethanol, a product of catechol-*O*-methyltrasferase, is found in the culture medium (*138*). In whole animals, oleuropein is hydrolyzed to HTy in the intestine and transported across rat jejunum and ileum (*134*). When animals are fed large amounts of olive oil, the oleuropein hydrolysis product HTy, its catechol-*O*-methyl transferase metabolite 3-hydroxyl-4-methoxyphenylethanol, and the ligstroside hydrolysis product Ty are increased in plasma and rapidly excreted in urine, largely as the glucuronide but also as sulfo and other conjugates (*9, 99, 139−146*). The methyltransferase enzyme reaction will actually function as a detoxification mechanism because the methoxy derivative does not oxidize to the biologically active arylating catechol quinone. Interestingly, urinary Ty is a better marker than HTy for secoiridoid uptake (*70, 143*), an observation that could be attributed both to HTy methylation and to HTy oxidation to HTyQ followed by its sequestration as an adduct, similar to that of arylating tocopherol quinones (*1*). Studies with HepG2 cells, a human hepatoma cell line, suggest that HTy can be metabolized by the liver (*147*). Other studies report that the antioxidant activity of virgin olive oil is caused by the dialdehydic form of elenolic acid linked to HTy, so both secoiridoids and HTy should be compared in studies on antioxidant activity in cell cultures. However, secoiridoid hydrolysis in the intestine (*134*) suggests that HTy precursors will not be involved in vivo as phenolic antioxidants. and indeed there are few, if any, reports that measurable oleuropein levels are found, in vivo, to any significant extent, although oleuropein itself diminishes oxidative myocardial injury induced by tissue ischemia and reperfusion (*148*).

Antioxidant Functions of HTy in Cells and Whole Animals. The catechol HTy reacts with H_2O_2, forming the quinone, but its scavenging action on superoxide released during the respiratory burst is controversial (*149, 150*). HTy counteracts the cytotoxicity induced by ROS in Caco-2 (*80*) and HepG2 cells (*151*), and when added to the diet, HTy improves antioxidant status in rabbits (*152, 153*) and diminishes the urinary excretion of 8-iso-PGF2α, an index of LDL oxidation, in both rats and humans (*154−156*). A study comparing urinary 8-oxodeoxyguanosine (DNA oxidation) in northern and southern Europeans suggests an antioxidant effect of olive oil (Mediterranean diet) in southern Europeans, but this effect was small in short-term diet studies with olive oil (*157*). When Jurkat cells were exposed to continuous H_2O_2 generation with glucose oxidase, the comet assay (single-cell gel electrophoresis methodology) showed diminished DNA damage at a low level of oleuropein, but at high concentrations, oleuropein alone had a cytotoxic effect (*158*). Another study, in which oxidative DNA damage was measured in postmenopausal women by the comet assay in peripheral blood lymphocytes, showed reduced damage with dietary olive oil and particularly with HTy (*159*).

High-phenolic olive oil diets alter not only antioxidant function in cells and tissues but also the properties of metabolites, particularly LDL isolated from peripheral blood of humans and animals pretreated with diets rich in olive oil. This is a good indirect measure of phenolic antioxidant uptake followed by enhanced in vivo antioxidant status. Thus, an increase in plasma and urinary HTy and Ty levels (intestinal hydrolysis) is found with high olive oil diets and, as a consequence, the susceptibility of LDL, isolated from the plasma of both healthy volunteers and hyderlipidemic patients, to various oxidants including the cupric ion and AAPH [2,2′-azobis(2-amidinopropanol)hydrochloride] is diminished (*152, 160−165*). In fact, HTy monosulfate and other soluble derivatives have been found in isolated LDL after olive oil ingestion (*163−166*).

Consequences of HTyQ Arylation in Cells and Whole Animals. Although the overwhelming preponderance of data strongly supports the role of olive oil polyphenols as antioxidants, this role for polyphenols in animal models has been questioned (*167*), and the possibility of confounders in nutritional studies must always be considered (*23*). Two examples of polyphenol derivatives with unique biological properties will suffice here. HTy reacts with aldehydes and ketones to form hydroxyisochromans, some of which are found in very small amounts in olive oil (*168*) and inhibit TXB_2 formation from arachidonic acid (*169*). Olive oil contains oleocanthal (a derivative of ligstroside aglicone) that inhibits COX-1 and -2 even more strongly than ibuprofen (*170, 171*). Many biological properties of olive oil cannot be attributed solely to its antioxidant activity, and we cannot exclude the possibility that these effects may result from some yet to be identified minor components of olive oil. However, a much simpler explanation involving arylating HTyQ formation should be explored.

The high antioxidant activity of dietary olive oil catechols strongly suggests that hydrophilic catechols are much more readily oxidized in vivo than lipophilic tocopherols (*90, 95−101*) and, consequently, some of the biological effects of olive oil may actually be attributed to the rapid generation of the arylating catechol quinone as an oxidation product. Because of their susceptibility to rapid oxidation, almost all studies reported in the literature utilize secoiridoid mixtures. Refined olive oil mixtures are now available (*172*), and it will be important to isolate pure oleuropein and ligstroside and use these compounds to generate HTy and Ty, specifically, by acid hydrolysis (**Figure 1**) and to generate HTyQ from TyQ by tyrosinase oxidation (*173*).

Commonalities between Arylating HTyQ and γ-TQ. Many studies posit a fundamental role for plant phenolics in regulating ROS generation through redox-initiated metabolism, a position forcefully expressed in a recent review (*46*). We propose that phenolic antioxidants, which undoubtedly regulate ROS, are also converted to quinones, which alter the biological properties of cells and tissues through arylation (*1*). Therefore, the oxidative conversion of HTy to arylating HTyQ may contribute to the unique beneficial effects of olive oil in nutrition. The generation

of HTyQ and its subsequent arylation reactions have not received much attention, but we have investigated the formation of quinones and the biological properties associated with arylation from an analogous family of phenolic antioxidants, the tocopherols. Comparing the properties associated these two families of antioxidants, secoiridoids and tocopherols, provides strong evidence supporting the arylating quinone hypothesis.

Tocopherols are ideal molecules for distinguishing biological properties resulting from antioxidant activity and arylation. All tocopherols are phenolic antioxidants, but only partially methylated tocopherols and, under certain conditions, tocopherol quinone methides function as arylating quinone electrophiles, which are associated with unique biological properties (*1*). We noted above that the *o*-quinone derivative HTyQ, although not identical to arylating tocopherol *p*-quinone derivatives γ-TQ and δ-TQ, shares many of the same biologicalroperties. The data that support this commonality and are summarized below allow us to promulgate the hypothesis that HTy and partially methylated tocopherols are both oxidized to bioactive arylating quinones.

When polyphenol antioxidants serve as precursors of arylating quinone electrophiles in vitro, for example, the thermal oxidation of γ-T to the arylating *p*-quinone γ-TQ and the conversion of HTy to the arylating *o*-quinone HTyQ, the apparent yield of the arylating quinone oxidation product is dramatically lower than the initial concentration of the precursor polyphenols (*110*−*116*), a situation not found with nonarylating quinones such as α-TQ (*1*). This phenomenon has been studied extensively with tocopherols and could be explained, as described above, by Michael adduct formation of arylating tocopherol quinones (*1*). A similar explanation could explain the disappearance of HTy during thermal oxidation (*110*−*116*) and should be investigated.

The environment inside a mammalian cell is much more complicated than that of in vitro thermo-oxidation. Many studies revealed that the phenolic antioxidant activity promotes cell integrity and proliferation. Yet, high concentrations of HTy have been found to cause toxicity in cultured tumor cells. HTy diminishes the proliferation and survival of HL60, HT-29, and Caco2 cells, and HTy stimulates apoptosis in these cells as demonstrated by PARP cleavage, annexin labeling, cytochrome *c* release, and caspase activation (*174, 175*). The HTy-induced apoptosis in these tumor cells is thought to contribute to the beneficial anticancer activity of olive oil.

The cytotoxic effect of HTy cannot be explained by its antioxidant activity, but rather the formation of arylating HTyQ during redox cycling offers a plausible explanation. Indeed, observations in cells treated with high levels of HTy are very similar to the data we reported for proliferation, survival, and apoptosis in cells treated with arylating γ-TQ (*1, 68, 69, 109, 176*−*180*). Notably, a comparable cytotoxic effect can be demonstrated with the HTy itself, whereas, with γ-T, the phenolic antioxidant must be first oxidized to the quinone. We attribute this difference to the fact, summarized extensively above, that hydrophilic HTy is a much stronger antioxidant and more readily oxidized to its arylating catechol quinone than lipophilic γ-T (*90, 97*−*99, 181*). In accordance with this explanation, less cytotoxicity is found with high levels of γ-T (*68, 69, 109, 180*) when it is compared to HTy (*174, 175, 182*).

Many studies describe multifaceted effects of arylating tocopherol quinone electrophiles, particularly γ-TQ, in cell biology (*1*). Similarities with catechol precursors of arylating catechol quinones are yet to be explored extensively. However, there are remarkable studies where the effects of the arylating quinone electrophile γ-TQ in endoplasmic reticulum (ER) stress in our studies (*69*) map onto the effects of HTy in other published work on ER stress (*182*). HTy induces ER stress, leading to the activation of two main branches of the unfolded protein response (UPR), Ire1/XBP-1/ Bip and PERK/eIF2, and the induction of pro-apoptotic transcription factor CHOP. We reported that γ-TQ induces ER stress by activating the PERK signaling pathway including elF2α, ATF4, and CHOP (*69*). ER stress is closely associated with arylating γ-TQ toxicity through Michael adduction as demonstrated by time-of-flight mass spectroscopy (*69*). More importantly, ROS and Michael adduction mechanisms are clearly distinguished in these studies by the observation that thiol antioxidants, including NAC and dithiothreitol (DTT), detoxify through adduct formation, whereas other antioxidants including BHA and ascorbic acid deplete ROS without affecting the cytotoxicity. Again, more study should be focused on the formation of an arylating quinone as a common initiating event leading to common and/or distinct biological mechanisms in which hydrophilic HTy and hydrophobic tocopherol phenolic antioxidants diminish cell proliferation and promote cell death through apoptosis and other means.

PURPORTED ROLE OF HTY IN INFLAMMATION AND CANCER

Arachidonic Acid Cascades. The apparent effects of inflammation on cancer are a subject frequently included in reviews that summarize the role of olive oil in nutrition (*1, 5*−*22*). HTy evidently influences two families of inflammatory lipid mediators, leukotrienes (LTs) and prostaglandin (PGs). LTs are generated from arachidonic acid (AA) through a metabolic process initiated by 5-lipoxygenase (5-LOX), whereas the actions of constitutive cyclooxygenase (COX-1) and inducible cyclooxygenase (COX-2) convert arachidonic acid to PGs (*183*−*186*). Notably, elevated cellular levels of key intermediates and products of the AA/LTs cascade, 5-HETE, 5-oxo-ETE, LTB_4, and cys-LTs, have strong positive correlations with various types of cancers, whereas inhibitors of 5-LOX have a strong negative correlation with carcinogenesis (*183*−*188*). Converging pathways (*187*) involve both 5-LOX and COX-2 in carcinogenesis, with both enzymes stimulating inflammation, cancer cell proliferation, and neo-angiogenesis and both enzymes inhibiting apoptosis.

An extraordinary number of natural compounds of plant origin regulate the AA/LT cascade (*183, 186*). The olive oil component of the Mediterranean diet is a classic example of a plant source for an inhibitory 5-LOX catechol, namely, HTy derived from secoiridoids. Several investigators in the late 1990s described the inhibition of 5-LOX with HTy and other related phenolic antioxidants, yielding the inhibitory sequence HTy > caffeic acid > oleuropein > Ty for the generation of LTB4 in leukocytes stimulated with the calcium ionophore A23187, yet without having a substantial effect on thromboxane generation (*189*−*191*) through the constitutive COX-1 pathway (*192*). In contrast, HTy does inhibit the generation of PGE2 through a pathway involving the inducible COX-2 enzyme (*193, 194*), placing HTy at the nexus of what has been described as the converging functions of COX-2 and 5-LOX. Despite a general interest in inflammation and cancer, little effort has been made to propound a mechanism explaining the sequential inhibitory roles for the catechol HTy on 5-LOX and PGE2 production in inflammation and carcinogenesis.

We explain the fact that arylating HTyQ was not shown in any of HTy studies by the formation of Michael thiol adducts, which cannot be identified by classic analytical techniques (see Role of HTy as an Antioxidant). Similar results were obtained with arylating γ-TQ (*195*), and its Michael adduct was identified by only TMAH thermochemolysis instead of classic quinone detection methods (*68, 69, 109*). Yet, a comparison between α-T and γ-T does suggest an important role for quinone arylation in inflammatory responses. Whereas fully methylated α-T has little effect on inflammation, partially methylated γ-T functions as an anti-inflammatory agent, inhibiting both COX-2 and 5-LOX and diminishing the concentrations of PGE2 and LTB4 in LPS (lipopolysaccharide)-treated and IL-1β-treated cells and in carrageenan-induced inflammation in rats (*1, 196*). This effect cannot be attributed simply to the antioxidant activity of tocopherols because both α-T and γ-T are antioxidants. On the contrary, the specific arylation capability associated with γ-TQ, an oxidative product of γ-T, may offer a plausible explanation.

NF-κB at the Nexus. It is well established that the receptors for both LPS and IL-1β belong to the Toll-like receptor/IL-1 receptor superfamily, which plays an important role in regulating inflammatory responses (*197, 198*). A key intracellular response to activated IL-1β receptor is the activation of transcription factor NF-κB (*199*), which regulates the expression of both COX-2 and 5-LOX (*200−202*). Obviously, arylating quinones may react with different intracellular cysteinyl proteins, leading to different biological effects, but NF-κB is surely a plausible and interesting target, which has been shown to be inhibited by the arylating agent PGJ2 (*203*). Similarly, the inhibition by γ-T, but not α-T, of COX-2 and 5-LOX in LPS- or IL-1β-treated cells (*1, 196*) could be via the formation of arylating/alkylating adducts with NF-κB that contains a critical cysteine residue for its DNA binding capability (*204, 205*). Inhibition of NF-κB, a key signaling molecule in the inflammatory response (*198, 206−208*), will reduce both COX-2 and 5-LOX activity, resulting in lower PGs and LTs and diminishing the inflammatory reactions.

Although not proved, a similar Michael reaction mechanism could be attributed to arylating HTyQ, which would lead to NF-κB inhibition and, consequently, reductions in COX-2 and 5-LOX activity and the production of PGs and LTs. Notably, olive oil, polar components isolated from olive oil, and HTy have been shown to inhibit NF-κB activation, respectively (*193, 209, 210*). More importantly, the inhibitory effect is observed when HTy concentration is comparable to that in human blood. In addition to the regulation of 5-LOX expression, the inhibition of NF-κB can also affect the activation of 5-LOX in a living cell, which requires the translocation of cytosolic 5-LOX to the nuclear membrane-associated protein complex (*211−214*). On the membrane, 5-LOX forms a complex with 5-LOX activating protein (FLAP), which enhances its enzyme activity by transferring substrate AA to 5-LOX for LT production (*215*). The expression of FLAP is under regulation by NF-κB (*216*), and it has been reported that NF-κB is involved in the redistribution of cytosolic 5-LOX to nuclear membrane (*217*). These observations are in accordance with the hypothesis that arylating HTyQ inhibits NF-κB activation and, as a result, mitigates inflammatory reactions.

The connection between chronic inflammation and tumor growth has received much attention, and it is estimated that inflammation contributes to 15−20% of all cancers (*218, 219*). Activation of NF-κB in inflammatory cells promotes tumor growth, whereas activation of NF-κB in cancer cells inhibits apoptosis and increases metastatic capability (*220−222*). Another aspect of the chronic inflammation and cancer connection is highlighted by the myeloid-derived suppressor cell (MDSC) mediated suppression of immune surveillance against cancer cells (*223*). PGE2 and IL-1β produced by tumor cells stimulate MDSC proliferation, which allows tumor cells to evade immune surveillance (*224−226*). Because NF-κB plays a key role in IL-1β signaling and in the production of PGs, HTyQ could potentially inhibit NF-κB activity in the inflammatory cells or in cancer cells, decreasing tumor growth and increasing apoptosis. It may also act on MDSC, suppressing IL-1β-stimulated proliferation and allowing immune surveillance against cancer cells. Thus, the oxidative conversion of HTy to arylating HTyQ and the subsequent inhibition of NF-κB through Michael adduct formation may play a central role in the beneficial effects of olive oil by diminishing the inflammatory reaction, inhibiting tumor growth, and enhancing immune surveillance against cancer cells.

CONCLUDING REMARKS

The beneficial effects of Mediterranean diet, particularly its olive oil component, against inflammation, cancer, and cardiovascular disease are well documented (*1, 5−22*), but the mechanism that explains these effects remains obscure. The arylating HTyQ hypothesis, which we present here, is supported by many experimental observations that allow us to map the properties of the catechol HTy and its arylating quinone oxidation product HTyQ onto the properties of tocopherols and their arylating quinones. We foresee a fruitful research field, centered on HTy and HTyQ, which will enhance our understanding of the nutritional benefit of olive oil, an area that was first recognized in antiquity and continues to amaze nutritionists, biochemists, and molecular biologists even to the present time.

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