Healthful Properties of Olive Oil
Minor Components

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Introduction

Adherence to a Mediterranean diet is likely to lower the risk for cardiovascular disease (Trichopoulou et al., 2003; Trichopoulou et al., 2005) and certain cancers (Trichopoulou et al., 2000; Trichopoulou et al., 2003). Even though cardiovascular risk and coronary heart disease (CHD) have always been associated with classic risk factors such as high serum cholesterol and blood pressure, evidence shows that the prevalence of such factors does not differ significantly between the populations of the Mediterranean area—where the incidence of CHD and certain cancers, e.g. breast and colon cancers, is lowest—and those of other North-European and Western countries (Parfitt et al., 1994). Moreover, there are several observations that do not completely link CHD incidence, fat intake, and absorption (Mancini and Rubba, 2000). Taken together, these data suggest that other, as yet unexplored, risk factors may be favorably affected by a healthful diet (Mancini and Rubba, 2000). Indeed, several studies demonstrate that oxidative processes in the endothelium play a role—the extent of which is yet to be fully understood—in the onset of atherosclerosis (Steinberg et al., 1989; Stocker and Keaney, 2004). These processes exacerbate inflammation and greatly increase the risk for atherosclerosis and CHD (Ross, 1999). Such experimental data led to the formulation of an oxidant/atherosclerosis hypothesis, which has been receiving increasing experimental support. The precise nature of the phenomena that trigger the development of atheroma and the extent of their contribution to CHD are yet to be fully elucidated. Based on this evidence, experimental and epidemiological studies are being carried out on the possible role of antioxidants in the relative protection from CHD observed in the Mediterranean area.

In the past, coupled with the low consumption of meat, major emphasis was put on the low saturated fat content (and the concomitant high proportion of monounsaturated fat) of the Mediterranean diet. More recently, research has underlined the importance of plant foods (including carbohydrates and non-digestible fiber) and of the regular use of olive oil. The latter has been traditionally endorsed with healthful
and even medicinal properties. As far as the cardiovascular system is concerned, the protective properties of olive oil have been, until recently, exclusively attributed to its high monounsaturated fatty acid (MFA) content, mostly in the form of oleic acid (18:1n-9). Indeed, monounsaturate supplementation leads to enhanced resistance of LDL to oxidation (Bonanome et al., 1992), hence lowering one of the risk factors for CHD (Witztum and Steinberg, 2001). Appropriately, the US Food and Drug Administration recently allowed a qualified health claim for monounsaturates from olive oil and reduced risk of CHD (FDA, P04-100, 2004). However, several observations argue against the hypothesis of oleic acid as the exclusive responsible factor for the lower rates of CHD of the Mediterranean area. For example, the effects of MFA on circulating lipids and lipoprotein have not been fully clarified. While the major effects of high monounsaturated fatty acid intakes on serum cholesterol are generally thought to be indirect and have been attributed to the associated replacement of saturated fatty acids (Belkner et al., 1993; Hegsted et al., 1993; Gardner and Kraemer, 1995), some studies (reviewed by Mensink et al., 2003), attributed a direct, although modest, cholesterol-lowering effect to MFA alone, when they equicalorically replace carbohydrates. Also, MFA increases the levels of the protective high-density lipoprotein (HDL) more than polyunsaturates (PUFAs) when these two classes of fatty acids replace carbohydrates in the diet (Mensink et al., 2003); however, there are reports of a neutral effect of MFA on plasma lipids or even a total- and LDL-cholesterol lowering activity. In turn, while oleic acid might exert some beneficial effects on the serum lipid profile, its actions are of moderate magnitude at best. Most important, oleic acid is one of the predominant fatty acids in largely-consumed animal foods such as poultry and pork. Thus, contrary to the common belief, the percentage of oleic acid in the Mediterranean diet as a whole is only slightly higher than that of other kinds of Western diets, e.g. the North American one (Dougherty et al., 1987; Katan, 1995). It is therefore unlikely that oleic acid is exclusively accountable for the healthful properties of olive oil. Finally, it is also noteworthy that several seed oils obtained through genetic selection, such as sunflower, soybean, and rapeseed oils are nowadays rich in monounsaturated, albeit devoid of phenolics (Owen et al., 2000), and are commercially available. Consumption of such oils, namely rapeseed, is widespread in several areas of the world (Gunstone, 2004): if oleic acid were endowed with strong cardioprotective effects, similar low incidence of CHD and high longevity would be observed outside the Mediterranean basin.

This chapter reviews the evidence that indicates how the phenolic components of extra virgin olive oil may play a role in the protection from CHD and cancer observed in the Mediterranean area.

**Olive Oil Minor Constituents—Role in Human Health?**

The epidemiological evidence of a lower incidence of CHD and certain cancers in the Mediterranean area (Keys, 1995) stimulated research on the potentially protective
activities of olive oil minor constituents, some of which have recently become commercially available.

It must be accentuated, as mentioned in other parts of this book, that only olive oil marketed as “extra virgin,” i.e. the one with a degree of acidity lower than 0.8% according to the current regulations, contains substantial amounts of phenolic compounds. Other kinds of olive oil, including the one simply marketed as “olive oil” are poor in or devoid of phenolics, due to the chemical procedures employed to reduce the acidity. This has important consequences in terms of agronomic and marketing policies. If olive oil phenols do have an impact on human health (see below), then the various procedures that influence olive oil quality are to be implemented and the general public should be informed to choose high quality olive oil. In particular, the phenolic constituents confer a bitter and pungent taste to the oil, as a result of complex interactions between such “minor constituents” and the taste buds, including inactivation of ptyalin. Slightly bitter and pungent tastes are positive attributes. This is known to members of panel tests, who evaluate the organoleptic quality of the oil and often appreciate high-phenol olive oils. Choice of an extra virgin olive oil, in addition to providing enjoyable meals, might thus positively influence human health, as described below.

Evaluation of the Role of Olive Oil Phenols in Human Health – Methodological Caveats

The major limitation of the qualitative/quantitative evaluation of olive oil phenolic compounds lies in the current lack of simple and easy to apply methodologies, as in the case of other foods (Hammerstone et al., 2000; Manach et al., 2004; Manach et al., 2005; Williamson and Manach, 2005). This negative aspect prevents the correct assessment of a relationship between olive oil phenol consumption and incidence of disease. Currently, the most widely employed methods for evaluating the total polyphenolic content of olive oil are the Folin-Ciocalteau colorimetric assay (Visioli et al., 1995b) and the HPLC (Montedoro et al., 1992). The former is simple to perform and does not require expensive equipment, but is limited by the low specificity of the reagent toward phenolic compounds; further, it does not provide qualitative information of the composition of the phenolic fraction. Conversely, HPLC is very sensitive and specific, but it is time-consuming (samples run for about one hour) and does not provide information on phenolic molecules for which reference standards are unavailable. An enzymatic assay for the quantitative determination of olive oil phenolics has been proposed by Mosca et al. (Mosca et al., 2000). This method is rapid and easy to perform and is more sensitive and specific for phenolic compounds than the Folin-Ciocalteau method. Alas, it also provides quantitative information only and does not detect other “minor constituents” such as cinnamic and vanillic acids. Finally, a rapid and sensitive method to evaluate the phenolic components of olive oil by Atmospher-
ic Pressure Chemical Ionization-Mass Spectrometry (APCI-MS) has been described by Caruso et al. (Caruso et al., 2000). This method allows for quick analyses of crude methanolic extracts of olive oil, does not need extensive analytical workup, and makes it possible to quantify oleuropein aglycone. The apparatus is, however, very expensive and requires trained personnel to operate.

**Olive Oil Phenolics and Human Health**

**In Vitro Studies**

**1. Antioxidant Activities**

The first experiments of our group on the biological activities of olive oil phenolics started over a decade ago thanks to the availability of oleuropein, obtained from Extrasynthese (France), and hydroxytyrosol, isolated in pure form from olive oil and provided to our lab by Professor Montedoro. The experimental model employed chemically-induced oxidation of LDL, which, back then, was considered prototypic to what was supposed to take place in vivo.

Both hydroxytyrosol (HT) and oleuropein (OE) potently and dose-dependently inhibit copper sulfate-induced and metal-independent oxidation of LDL, at concentrations of $10^{-6}$ to $10^{-4}$ M (Visioli and Galli, 1994; Visioli et al., 1995a). The protective effects of HT and OE were demonstrated through the assessment of various markers of LDL oxidation, such as a) a reduced formation of short-chain aldehydes (evaluated as thiobarbituric acid-reacting substances, TBARS) and of lipid peroxides, by b) a higher vitamin E content in the residual LDL (indicating sparing of endogenous antioxidants), by c) an extension of the lag phase to form conjugated dienes and by d) a reduced formation of malondialdehyde-lysine and 4-hydroxynonenal-lysine adducts, indicating protection of the apoprotein layer (Visioli et al., 1995a).

The antioxidant activities of hydroxytyrosol and oleuropein were further investigated and confirmed by the use of metal-independent oxidative systems, which indicated that these compounds are potent free radical scavengers (Visioli et al., 1998). In particular, both HT and OE effectively scavenge superoxide anion generated by either human polymorphonuclear cells or by the xanthine/xanthine oxidase system (Visioli et al., 1998); it is noteworthy that, in these experimental setups, both vitamin E and butylated hydroxytoluene were found to be inactive. The free radical-scavenging properties of olive oil phenolics have been confirmed over the past few years by several groups in various experimental models (see Table 8.1).

Relevant to the development of atherosclerosis, a scavenging effect of hydroxytyrosol and oleuropein was also demonstrated with respect to hypochlorous acid (Visioli et al., 1998), which is a potent oxidant species produced in vivo by activated neutrophils at the site of inflammation (Aruoma and Halliwell, 1987): evidence is rapidly
**TABLE 8.1**

**Biological activities of olive oil phenolics**

<table>
<thead>
<tr>
<th>Model</th>
<th>Activity</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td><strong>In vitro</strong></td>
<td>Antioxidant activity</td>
<td>(Grignaffini et al., 1994; Salami et al., 1995; Visioli et al., 1995a; Visioli et al., 1995b; Saenz et al., 1998; Saija et al., 1998; Speroni et al., 1998; Visioli et al., 1998; Delana et al., 1999; Manna et al., 1999; Fito et al., 2000; Pellegrini et al., 2001; Deiana et al., 2002; Lavelli, 2002; Leenen et al., 2002; Manna et al., 2002; Stupans et al., 2002; Carluccio et al., 2003; Gorinstein et al., 2003; Moreno, 2003; Pellegrini et al., 2003; Hashimoto et al., 2004; Masella et al., 2004; Valavanidis et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>Increase NO production</td>
<td>(Visioli et al., 2001)</td>
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<tr>
<td></td>
<td>Anti-inflammatory activity</td>
<td>(Petroni et al., 1995; Kohyama et al., 1997; de la Puerta et al., 1999; Miles et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>Antiatherogenic activity</td>
<td>(Carluccio et al., 2003; Turner et al., 2005)</td>
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<tr>
<td>Metabolism</td>
<td></td>
<td>(Edgecombe et al., 2000; Manna et al., 2000)</td>
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<td></td>
<td>Cytostatic activity</td>
<td>(Tranter et al., 1993; Saenz et al., 1998)</td>
</tr>
<tr>
<td></td>
<td>Antimicrobial activity</td>
<td>(Tranter et al., 1993; Bisignano et al., 1999)</td>
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<td>Animal</td>
<td>Chemoprevention</td>
<td>(Budiyanto et al., 2000 Perino et al., 1988)</td>
</tr>
<tr>
<td></td>
<td>Antioxidant activity</td>
<td>(Grignaffini et al., 1994; Wiseman et al., 1996; Coni et al., 2000; Alacon de la Lastra et al., 2002; Wiseman et al., 2002)</td>
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<tr>
<td></td>
<td>Anti inflammatory effect</td>
<td>(Martinez-Dominguez et al., 2001)</td>
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<td></td>
<td>Inhibition of progression of aortic lesions</td>
<td>(Agullera et al., 2002)</td>
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<td></td>
<td>Antithrombotic effect</td>
<td>(Brzosko et al., 2002)</td>
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<td></td>
<td>Absorption and metabolism</td>
<td>(Bai et al., 1998; Ruiz-Gutierrez et al., 2000; Tan et al., 2003; Visioli et al., 2003)</td>
</tr>
<tr>
<td>Human</td>
<td>Blood lipid modulation and eicosanoid production</td>
<td>(Visioli et al., 2000a; Oubina et al., 2001; Marrugat et al., 2004; Visioli et al., 2005)</td>
</tr>
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<td></td>
<td>Oxidative stress</td>
<td>(Ramirez-Tortosa et al., 1999a; Ramirez-Tortosa et al., 1999b; Masella et al., 2001; Vissers et al., 2001a; Vissers et al., 2001b; Nagyova et al., 2003; Haban et al., 2004)</td>
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<td></td>
<td>Postprandial lipemia</td>
<td>(Nicolaiew et al., 1998; Bonanome et al., 2000; Piers et al., 2002; Soares et al., 2004; Weinbrenner et al., 2004)</td>
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<tr>
<td></td>
<td>Absorption and metabolism</td>
<td>(Bonanome et al., 2000; Visioli et al., 2000a; D’Angelo et al., 2001; Miro-Casas et al., 2001; Visioli et al., 2001; Miro-Casas et al., 2003a; Visioli et al., 2003)</td>
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accumulating that the formation of chloramines via the myeloperoxidase-catalized formation of HOCl and subsequent chlorination of apoB-100 is responsible for LDL modification and peroxidation (Carr et al., 2000).

Most studies of olive oil phenolics focus on their cardioprotective potential. However, there is epidemiological evidence of lower incidence of certain cancers (breast, colon) in the Mediterranean area (Trichopoulou et al., 2000). As DNA mutation plays a key role in carcinogenesis, it is important to investigate the chemopreventive properties of olive oil minor constituents in ad hoc models (Owen et al., 2004). Deiana, Aruoma, and collaborators first investigated the activities of hydroxytyrosol toward chemically-induced DNA and aminoacid modification (Deiana et al., 1999). Low concentrations of hydroxytyrosol, i.e. 50 µM, are able to scavenge peroxynitrite and therefore to prevent ONOO−-dependent DNA damage and tyrosine nitration. The pro-oxidant activities of hydroxytyrosol (which are due to its copper-reducing properties and might potentially and paradoxically exacerbate DNA damage), were investigated in a model of copper-induced DNA damage. It was found that pro-oxidant actions were 40-fold weaker than those of ascorbate and occurred at very high, non-physiological concentrations (>500 µM) (Deiana et al., 1999). In synthesis, hydroxytyrosol is endowed with chemopreventive potential, which is being confirmed by human trials (see below). The hypothesis that both oleuropein and hydroxytyrosol, similarly to other phenolic compounds (Gehm et al., 1997) possess estrogenic or androgenic activities, was also tested. However, both compounds were found to be inactive (Visioli, Galli, and Poletti, unpublished data).

2. Activities on Enzymes

In addition to their antioxidant actions, the activities of olive oil phenolics on enzymes have been tested in a variety of cellular models, (i.e. platelets, leukocytes, and macrophages) relevant to human pathology. Most olive oil phenolics are amphiphilic and possess the ability to modulate enzymes such as cyclo- and lipoxygenases, NAD(P)H oxidase, and nitric oxide synthase, that are involved in key functions of those cells.

Hydroxytyrosol was found to inhibit a) chemically-induced in vitro platelet aggregation, b) the accumulation of the pro-aggregant agent thromboxane in human serum, c) the production of the pro-inflammatory molecules leukotrienes by activated human leukocytes, and d) to inhibit arachidonate lipoxygenase activity (Petroni et al., 1995; Kohyama et al., 1997; de la Puerta et al., 1999; Turner et al., 2005). IC_{50}s are in the 10^{-5} M range, similar to those of other NSAIDs and aspirin, indicating that the effects of olive oil phenolics on human health extend beyond mere antioxidant properties. Relevant to the onset and development of atherosclerosis as localized at the arterial wall level, a report by Carluccio et al. (Carluccio et al., 2003) demonstrated that olive oil phenolics inhibit endothelial activation and the related expression of adhesion molecules, hence lessening the consequences of inflammation, namely the recruitment of circulating cells (monocytes/macrophages). The effects of olive oil phe-
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Olive oil phenolics were comparable or even superior to those of red wine components such as resveratrol, providing yet another piece of evidence for the cardioprotective effects of the Mediterranean diet.

Modulation of macrophagic response to bacterial challenge is a multi-faceted phenomenon that is involved both in host immune response and in atherogenesis via intimal inflammation (Ross, 1999). In fact, during acute sepsis and inflammation, macrophages react to the endotoxin challenge by increasing the production of reactive species such as nitric oxide, which inhibits platelet aggregation and adherence, and maintains a proper end-organ perfusion rate through increased vasorelaxation. Accordingly, inhibition of nitric oxide synthesis during sepsis increases cellular damage and animal mortality. Moreover, macrophagic nitric oxide exerts a protective role in preventing oxidative LDL modification that may occur at the site of inflammation as a consequence of enhanced reactive oxygen species production (Jessup et al., 1999; Bloodsworth et al., 2000). Yet, macrophage-mediated production of inflammatory mediators in the arterial wall, namely in the intima, exacerbates the onset and development of atherosclerosis and is being recognized as a key contributor to atherogenesis and CHD. We reported that, when added to murine macrophages together with bacterial lipopolysaccharide (LPS), oleuropein increases the functional response of these immune-competent cells, as evaluated by a significant increase (+ 58.7 ± 4.6%, mean ± SD) in the production of nitric oxide (Visioli et al., 1998). This increase is due to a direct tonic effect of oleuropein on both the activity and the expression of the inducible form of the enzyme nitric oxide synthase (iNOS), as demonstrated by Western blot analyses of cell homogenates and by the coincubation of LPS-challenged cells with the iNOS inhibitor l-nitromethylarginine methylester (Visioli et al., 1998). These findings were not confirmed by a recent report (Turner et al., 2005) and await further investigation.

**In Vivo Studies**

The first step toward demonstrating *in vivo* effects of olive oil phenolics was to assess their bioavailability. In fact, experimental evidence that flavonoids and phenolic compounds are absorbed from the diet is accumulating (Williamson and Manach, 2005). Earlier suggestions of *in vivo* activities came from laboratory animals, *e.g.* rats or rabbits, which demonstrated a higher resistance to oxidation of LDL obtained from animals fed virgin olive oil, as compared to LDL separated from animals that were only administered an equivalent amount of oleic acid as either triolein (Scaccini et al., 1992) or olive oil devoid of phenols (Wiseman et al., 1996). In the year 2000, we demonstrated that olive oil phenolics are dose-dependently absorbed by humans and that they are excreted in the urine as glucuronide conjugates; another interesting finding of that study was that increasing amounts of phenolics administered with olive oil stimulated the rate of conjugation with glucuronide (Visioli et al., 2000b). Further studies elucidated the metabolic pathways of hydroxytyrosol and oleuropein,
which form elevated quantities of homovanillic alcohol and homovanillic acid (Caruso et al., 2001; Miro-Casas et al., 2003b). The most complete study in this area is from Miro-Casas and collaborators, who developed a method to quantify hydroxytyrosol and its metabolites in plasma (Miro-Casas et al., 2003a). In brief, absorption of hydroxytyrosol is nearly complete and its plasma half-life is 2.43 h.

It is noteworthy that hydroxytyrosol (previously also known as DOPET) is a derivative of dopamine metabolism, formed via monoamino oxidase-catalyzed deamination and subsequent reduction (Lamensdorf et al., 2000), and is found in the brain and other tissues. Accordingly, the formation of homovanillic alcohol in CACO-2 cells has been reported following incubation with hydroxytyrosol (Manna et al., 2000). Likely, hydroxytyrosol is recognized and metabolized by the catecholamine enzymatic systems, such as the catechol-\textit{O}-methyltransferase.

Mechanistically, studies carried out in CACO-2 intestinal cells demonstrated that hydroxytyrosol is absorbed from the gut by passive diffusion (Manna et al., 2000). Finally, Bonanome and coworkers (Bonanome et al., 2000) demonstrated the postprandial absorption of olive oil phenolics and their incorporation into human lipoproteins.

In addition to the elucidation of metabolic pathways that follow absorption, research is concentrating on the possible \textit{in vivo} activities of olive oil phenolics. While some evidence has been built by animal experiments, human studies are more scant. The first, albeit limited, evidence of \textit{in vivo} antioxidant activity was published in 2000 (Visioli et al., 2000a), when a moderate, but significant, decrease in \textit{F}_\text{\textsubscript{2}}-isoprostane excretion by healthy volunteers who ingested phenol-rich olive oils was reported. To date, approximately a dozen randomized, crossover, and controlled studies have been published yielding non-univocal results on the activities of olive oil phenolics. This is possibly due to limited sample sizes, different choices of biomarkers, confounding by other dietary components, etc. (Covas et al., in press).

To investigate the effects of olive oil phenols on postprandial events (Sies et al., 2005), we recently ran a study to evaluate the effects of moderate, real life doses of two olive oils, differing only in their phenolic content, on some \textit{in vivo} indexes of oxidative stress (plasma antioxidant capacity and urinary hydrogen peroxide levels) in a postprandial setting. Moreover, we assessed whether phenolic compounds influence a few arachidonic acid metabolites involved in the atherosclerotic processes, such as leukotriene \textit{B}_4 (LTB\textsubscript{4}) and thromboxane \textit{B}_2 (TXB\textsubscript{2}). Six subjects in each group received one of the two oils (30 mL/day of olive oil, OO, or extra virgin olive oil, EVOO, distributed among meals) over seven consecutive days (intervention period). On the morning of days 2 and 7 of the intervention and after an overnight fast, 50 mL of the same oil was administered with 25 g of bread to the volunteers to evaluate postprandial changes. On days 1 and 8, subjects received 75 mL of glucose in 150 mL of water to assess their glycemic response. Comparison of OO and EVOO treatments showed no significant differences between groups for all parameters (Figures 1 and
Fig. 8.1. Mean (± S.D.) plasma postprandial concentrations of LTB₄ (A) and TXB₂ (B). The volunteers received 30 mL of EVOO (n = 6) or OO (n = 6), distributed among meals, for one week. In the morning of days 2 and 7, 50 mL of the same oil was administered with 25 g of bread to evaluate postprandial changes. Blood aliquots at baseline (0 h), 2, and 4 h after meal were incubated for 1 h at 37° C, to stimulate TXB₂ production. Other aliquots were immediately added with the calcium-ionophore A37129 50 µM for 30 min at 37 °C, to stimulate LTB₄ production. The productions of LTB₄ and TXB₂ were evaluated by immunoassay (Cayman Chemical, Ann Arbor, MI). All data were subjected to repeated Measures Analysis of Variance (softwares: Stata version 8.0 and SAS version 8.2). EVOO: extra virgin olive oil; OO: olive oil.
It can be argued that one limitation of this study is the short-term period of sustained specific nutrient consumption. The short-term design, however, permitted volunteers to be restricted to a controlled low-antioxidant diet, thus avoiding consumption of other antioxidants as well as other possible confounding variables, such as fast changes in lifestyle factors which often mask and blur the results of this kind of study. In synthesis, even though human evidence is accumulating and many data are promising, the information available to date does not allow researchers to make any conclusive inference on the specific role played by olive oil phenolics in human health.

As far as toxicology is concerned, there are very few published data that address this issue. In all of the studies a toxic activity of hydroxytyrosol was excluded, even at high doses (D’Angelo et al., 2001; Babich and Visioli, 2003; Christian et al., 2004). However, in view of the potential future formulation of nutraceuticals, consolidation of these data is mandatory.

The recovery of olive phenolics from waste waters (for nutraceutical purposes) is another interesting and emerging field. It is noteworthy that the olive paste is continuously hosed with lukewarm water during the milling, a process that is called malaxation. The resulting “waste water” is produced in extremely large quantities (~800,000 tons/year in Italy) and, despite the fact that it contains a considerable
amount of phenols (more than 1% w/v), is currently disposed of. A decade ago, we demonstrated that waste water extracts have powerful (low ppm range) *in vitro* antioxidant activity (Visioli et al., 1995b; Visioli et al., 1999); thus, olive mill waste water could be recovered and employed as a cheap source of natural antioxidants. Indeed, animal experiments (Visioli et al., 2000c; Visioli et al., 2001) and a couple of human studies (Visioli et al., 2003; Leger et al., 2005) confirmed that waste waters are a source of bioactive phenols and dietary supplements derived from olive mill waste water are already available in the market.

**Conclusions**

The observation that in the Mediterranean area there is a lower incidence of CHD (Keys, 1995; Willett et al., 1995; Trichopoulou et al., 2003; Trichopoulou et al., 2005) and certain types of cancers (Trichopoulou, 1995; Lipworth et al., 1997) demonstrates that the Mediterranean diet, rich in grain, legumes, fresh fruits and vegetables, wine in moderate amounts, and olive oil has beneficial effects on human health, as further confirmed by a cross-cultural trial of Mediterranean diet (Singh et al., 2002). While the beneficial effects of the Mediterranean diet on the cardiovascular system have so far been mostly attributed to its lipid profile (i.e. high oleic acid and low saturates), evidence of the contribution of natural antioxidants and other components of the diet, such as fiber, to this effect is accumulating and should also be taken into account. The evidence reviewed in this chapter suggests that choosing a phenols-rich, extra virgin olive oil would contribute to the dietary intake of biologically-active compounds in quantities that have been correlated with a reduced risk of developing CHD (Hertog et al., 1993; Hertog et al., 1995). Moreover, a phenols-rich, tasty olive oil exerts “indirect” effects, such as the need to be used only in small amounts to dress foods. This reduces the overall calorie intake and is associated with the consumption of fresh vegetables.

In conclusion, even though solid human evidence is yet to be gathered, the biologically-relevant properties of olive oil phenolics described in this chapter and summarized in Table 1 provide substantial evidence to support the hypothesis that virgin olive oil consumption may contribute to lower CHD mortality.

**Acknowledgments**

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**References**


Oubina P., F. J. Sanchez-Muniz, S. Rodenas, et al., Eicosanoid Production, Thrombogenic Ratio,

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