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Hydroxytyrosol
An examination of its potential role in cardiovascular disease, inflammation, and longevity

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ABSTRACT: Multiple lines of converging evidence link polyphenols, particularly hydroxytyrosol, to improved clinical outcomes. In human and animal studies, hydroxytyrosol has been shown to induce beneficial effects on the cardiovascular system and exert anti-inflammatory effects. Hydroxytyrosol has also been shown to be a critical regulator of cell signalling pathways that have been implicated in longevity; in a series of experiments, hydroxytyrosol was shown to be superior to resveratrol in preventing cell death and increasing the expression of longevity proteins. In this review article we describe the experimental and clinical research that has been performed on hydroxytyrosol, and a novel hydroxytyrosol-based product, to highlight the therapeutic potential, across a broad range of disease states, of this polyphenol.

INTRODUCTION

The Mediterranean diet has been associated with a lower incidence of morbidity, mortality, and improved clinical outcomes. A prospective study of over 22,000 people determined that strong adherence to the Mediterranean diet, which is rich in fruit, vegetables, and fish, was associated with a reduced incidence of cardiovascular disease, cancer, and death (1). The purported clinical benefits of the Mediterranean diet have been attributed to the consumption of foods containing high levels of free-radical scavenging polyphenols, particularly olives and olive oil. Of the olive polyphenols that have been characterized, hydroxytyrosol has emerged as the polyphenol with one of the greatest potential therapeutic effects; it has the highest level of free radical protection activity ever reported for any natural antioxidant compound and a variety of laboratory and clinical studies have demonstrated its beneficial effects in a broad range of diseases. The effects of isolated polyphenols, such as hydroxytyrosol, have been extensively documented, by the Visioli laboratory (32, 34, 37, 38), in olive mill waste water (OMWW), which is one of the major by-products of olive oil production (35). Polyphenols have been shown to be present at considerably higher concentrations in OMWW, which could act as an important source of bioactive polyphenols for laboratory studies and clinical research (32). For example, an OMWW extract, containing hydroxytyrosol was shown to increase the plasma antioxidant activity of animals (34). In the following sections we discuss studies of hydroxytyrosol's effects on cardiovascular disease, inflammatory disorders, and its potential role in regulating longevity.

THE POTENTIAL BENEFITS OF HYDROXYTYROSOL IN CARDIOVASCULAR DISEASE

Sudden cardiac death (SCD) is the leading cause of mortality in the western world; in the United States, it has been estimated to account 400,000 deaths per year (2-3) while in the United Kingdom up to 70,000 deaths per years are attributed to SCD (4). SCD therefore represents a major public health burden. Numerous human and animal studies have shown that olive polyphenols, particularly hydroxytyrosol, can improve blood cholesterol profiles and reduce the risk of potentially lethal thrombosis. These effects of hydroxytyrosol, which are described in the following sections, could have an important impact on the prevention of cardiovascular disease and SCD.

The Eurolive trial was one of the first major, multicentre clinical trials to provide strong evidence in support of the beneficial effects of olive oil polyphenols on blood cholesterol levels. In this study 200 healthy male volunteers received 25 ml of olive oil containing either low, medium, or high concentrations of polyphenols once daily for three weeks. High polyphenol olive oil resulted in significantly raised levels of HDL cholesterol (5). HDL levels in study participants increased by 0.045 mmol/L (P=0.046) (5); previously in a separate study, a 0.026 mmol/L increase in HDL was associated with a 2-3 percent decrease in the risk of cardiovascular disease (30). A number of emerging pharmaceutical therapies, such as the cholesterylester transfer protein inhibitors anacetrapib and dalcetrapib, are in mid- to late-stage development for the treatment of dyslipidemia and the prevention of cardiovascular disease; the beneficial effects of these agents is thought to be partly due to their ability to raise HDL levels. Therefore by similarly raising HDL, polyphenol-rich olive oil has the potential to exert beneficial effects on the cardiovascular system.

The Eurolive clinical trial also demonstrated that high polyphenol olive oil reduced the levels of oxidized LDL cholesterol in healthy volunteers (5). Numerous studies have also associated oxidized LDL with the development of obstructive, atherosclerotic plaques within arteries (5-6). Oxidation of LDL results in a conformational change which permits its entrance into the arterial wall where it may promote atherosclerotic processes and the subsequent formation of plaques which impair the flow of blood to vital organs, including the heart. Such is the correlation between oxidized LDL and atherosclerosis, measurements of LDL oxidation have been shown to be powerful predictors of acute cardiovascular events in both coronary heart disease patients and in larger patient populations (7-8).

Importantly virgin olive oil also contains a wide array of other minor components, in addition to polyphenols, such as α- and γ-tocopherols, phytosterols, and flavonoids (32), all of which may have, in part, contributed to the effects observed in the Eurolive clinical study.

Hydroxytyrosol has been shown to play a role in atherogenesis; however, conflicting findings have been reported in different studies. For example, in one report administration of hydroxytyrosol to rabbits for one month resulted in a prominent reduction of the size of plaques formed in the aorta (9). Additionally, hydroxytyrosol resulted in

a 50 percent reduction in total cholesterol levels (9). However, a different study found that administration of pure hydroxytyrosol for 10 ten weeks to apo E deficient mice resulted in increased total cholesterol and atherosclerotic lesions (31). The authors of this latter study speculated that phenolic products, when taken out of their original matrix and natural environment could induce adverse effects (13); such a theory may support the use of hydroxytyrosol products containing the full complement of naturally occurring, additional phenolic compounds as studied in previous reports (11, 13-14). Additionally, evidence suggests that isolated, purified polyphenols may suffer from a reduced bioavailability compared to polyphenols that are contained within their natural matrix; hydroxytyrosol's bioavailability was shown to be higher when given as a natural component of olive oil versus when it was added to olive oil or yoghurt (33).

Clinical studies have demonstrated that hydroxytyrosol associates with LDL following oral administration of the polyphenol. In one report, 10 healthy volunteers were administered an oral formulation of hydroxytyrosol and blood sampling was performed at regular intervals to elucidate the polyphenol's pharmacokinetic profile and investigate whether hydroxytyrosol could be detected within LDL (10). In this study hydroxytyrosol was detected in volunteers' LDL fractions at 10 minutes and 20 minutes following oral administration (10). Similar findings have also been seen in a study of 12 healthy volunteers administered virgin olive oil with varying polyphenol concentrations (6). Following oral administration of 40 ml of polyphenol-enriched olive oil, total polyphenol compounds present in the subjects' LDL fractions increased by 57 percent after 1.5 hours; these changes directly correlated with plasma hydroxytyrosol and tyrosol concentrations (6). This same study also demonstrated that polyphenol-enriched olive oil reduced LDL oxidation in healthy volunteers (6). Collectively these clinical findings support the potential beneficial effects of hydroxytyrosol in atherogenesis through the reduction of LDL oxidation. In addition to hydroxytyrosol, oleuropein has also been shown to effectively inhibit LDL oxidation induced by copper sulphate (39).

An important clinical trial assessed the therapeutic benefits of a novel, hydroxytyrosol-based product generated from OMWW, HIDROX®, in patients with hyperlipidemia (11). In this clinical study, 35 patients were assigned to twice daily oral administration of the hydroxytyrosol-based supplement for six months. After six months of treatment, a statistically significant reduction in the levels of V-LDL cholesterol was documented in the patient population (P=0.001) (11). V-LDL cholesterol contains the highest amount of triglyceride fat of all cholesterol subtypes and high levels of V-LDL cholesterol can significantly raise the risk of coronary artery disease and myocardial infarction. By reducing V-LDL cholesterol, hydroxytyrosol could play an important role in reducing the risk of cardiovascular disease.

Hydroxytyrosol, in addition to its aforementioned effects on blood lipid profiles, has also been shown to play an important role in the prevention of thrombosis in a clinical study; thrombosis is an important cause of myocardial infarction and SCD. A small clinical trial, conducted in a high risk patient population, demonstrated that hydroxytyrosol could reduce a thrombosis biomarker, thus highlighting its potentially therapeutic antithrombotic effects (12). In this study, type I diabetic patients were administered 12.5-25 mg of a hydroxytyrosol solution once daily for 4 days. Hydroxytyrosol produced a notable decrease in plasma levels of thromboxane B_2 (a metabolite of thromboxane A_2, which is responsible for platelet aggregation and the formation of thromboses). Importantly, these beneficial effects occurred within a very short time frame (4 days) (12).

THE ANTI-INFLAMMATORY EFFECTS OF HYDROXYTYROSOL

Laboratory and clinical studies have shown that hydroxytyrosol exerts anti-inflammatory effects; experiments performed using a hydroxytyrosol-based supplement have reported a reduction in biomarkers of inflammation and improved quality of life in osteoarthritis patients (13-14). In an animal model of joint inflammation, using lipopolysaccharide (LPS) mice, the effects of pure hydroxytyrosol and a hydroxytyrosol-based supplement generated from OMWW on tumour necrosis factor-a (TNF-a) (a pivotal cytokine involved in inflammation) were investigated (13). Whereas administration of the hydroxytyrosol-based supplement obtained from OMWW evoked a statistically significant reduction in TNF-a levels in an LPS-treated human cell line (P<0.01), pure hydroxytyrosol failed to exert any significant effects on the levels of this inflammatory cytokine (13). Pure hydroxytyrosol was also shown to be ineffective in other cell models of inflammation (13); such findings imply that other components that are present within the hydroxytyrosol-based OMWW product, but are absent in pure hydroxytyrosol, could be responsible for these differential effects. Indeed cellular studies measuring the antioxidant profiles of a hydroxytyrosol-based supplement from OMWW and pure hydroxytyrosol demonstrated that the former had a greater antioxidant capability (13), thus further indicating that additional components present within the OMWW product may provide supplementary benefits over pure hydroxytyrosol alone.

To explore if anti-inflammatory effects could also be seen in the *in vivo* setting with the OMWW hydroxytyrosol-based supplement, LPS-treated animals were administered the supplement; significant reductions in TNF-a levels were recorded (P<0.05) (13). Further experiments also determined that the OMWW hydroxytyrosol-based supplement acted synergistically with glucosamine to induce notable anti-inflammatory effects. In LPS-treated mice administered 35 mg of the hydroxytyrosol-based supplement or 12.5 mg of glucosamine TNF-a levels were significantly reduced;

however, if both compounds were administered to animals an even greater reduction in TNF-a was seen (13). These important findings demonstrated, for the first time, that an OMWW hydroxytyrosol-based supplement acted synergistically with glucosamine, and that a combination of both products could evoke prominent anti-inflammatory effects in animals.

Hydroxytyrosol has been shown to reduce inflammation in animal paws and to also reduce levels of pain associated with inflammation. Oral administration of hydroxytyrosol to rats with experimentally-induced paw inflammation induced reductions in the degree of paw swelling and pain induced by paw inflammation (15). An additional study assessed the anti-inflammatory effects of olive polyphenols in animals with acute and chronic inflammation (16). Rats were fed on diets of either sunflower oil, palm oil, fish oil, or an olive polyphenol diet for 8 weeks. After the study period, inflammatory biomarkers were the lowest in animals given olive polyphenols (16).

In keeping with the above animal studies of hydroxytyrosol, human studies have also demonstrated its powerful anti-inflammatory effects. A placebo-controlled clinical study of the OMWW hydroxytyrosol-based product in patients suffering from rheumatoid arthritis revealed that the supplement reduced levels of C-reactive protein after eight weeks (14). C-reactive protein is an important biomarker of inflammation and it has been previously associated with rheumatoid arthritis, cardiovascular disease, and mortality. The same clinical study also demonstrated that the supplement significantly reduced levels of homocysteine after eight weeks (P<0.01) (14). Homocysteine is also an important biomarker of inflammation and a number of large clinical studies have established homocysteine as an independent risk factor for venous thromboembolism, stroke, coronary heart disease, and death (17). Additionally, an eight-week placebo-controlled clinical study of the OMWW hydroxytyrosol-based supplement in patients with osteoarthritis demonstrated that it induced significant improvements in patient's quality of life as measured by a health assessment questionnaire disability index (HAQ-DI) (14). The HAQ-DI evaluated nine categories of functional activity; 69 percent of osteoarthritis patients receiving the supplement reported a greater than 20 percent improvement in their HAQ-DI scores (14).

Biological effects	References
Association with LDL cholesterol and the prevention of its oxidation (clinical study).	6., 10.
Reduction of V-LDL cholesterol (clinical study).	11.
Reduction of thromboxane B_2, a thrombosis biomarker (clinical study).	12.
Reduction of tumour necrosis factor-α, an inflammation biomarker (laboratory study).	13.
Reduction of limb swelling and pain induced by inflammation (laboratory study).	15.
Reduction of C reactive protein and homocysteine, biomarkers of inflammation linked to cardiovascular disease (clinical study).	14.
Improvement in the quality of life associated with osteoarthritis (clinical study).	14.
Increased expression of SirT-1, SirT-3, and SirT-4, key proteins implicated in the regulation of longevity (laboratory study).	23.
Reduced size of cardiac infarct and cell death following myocardial infarction (laboratory study).	23.

Table. The biological effects of hydroxytyrosol reported in laboratory and clinical studies.

THE EFFECTS OF HYDROXYTYROSOL ON CELLULAR PATHWATS IMPLICATED IN LONGEVITY

The sirtuin (SirT) family of proteins, of which seven human forms have been described, have been implicated in variety of biological processes including fatty acid metabolism and the extension of life span (18-19). To date the most widely researched SirT protein is SirT-1; among other functions, SirT-1 is associated with muscle development, gene silencing, and longevity (20-21). Resveratrol has previously been shown to activate SirT-1 and its effects on longevity have been attributed to this mechanism of action (22-23). In a compelling series of experiments using rat hearts, hydroxytyrosol was shown to increase the expression of several longevity proteins, prevent cell death, and reduce the size of infarcted tissue following ischemia; in the majority of these experiments hydroxytyrosol exerted greater effects than resveratrol (23).

Following administration of experimental compounds, including hydroxytyrosol and resveratrol, for 14 days, the expression levels of various SirT proteins (SirT-1, SirT-3, and SirT-4) were evaluated in excised hearts (23). Hydroxytyrosol led to a statistically significantly increase in SirT-1 in cardiac tissue (P<0.05); of all the test compounds studied, including resveratrol, hydroxytyrosol was the most effective at increasing the levels of this longevity protein (23). Hydroxytyrosol also evoked significant increases in the SirT-3 and SirT- 4 longevity proteins (P<0.05); SirT-3 and SirT-4 are localized in the mitochondria and they have been implicated in the regulation of aging and energy metabolism (23). In contrast, resveratrol failed to evoke an increase in the expression of SirT-3 (23).

In subsequent experiments the potential cardioprotective effects of hydroxytyrosol were evaluated in isolated rat hearts that had been subjected to 30 minutes of ischemia followed by reperfusion. In control hearts the size of the infarct, following the ischemic episode, was approximately 40 percent of the total heart tissue (23). Hydroxytyrosol treatment resulted in a significant reduction in the size of the infarct to approximately 21 percent (P<0.05) (23). Hydroxytyrosol also evoked the lowest levels of cell death following the ischemic episode compared to other 5 test compounds, including resveratrol (23). These important findings demonstrate that hydroxytyrosol can increase the expression of multiple SirT longevity proteins and potentially reduce the deleterious pathophysiological effects of myocardial infarction through reducing the size of the infarct and preventing cell death. Additionally, hydroxytyrosol was shown to be more effective than resveratrol in these studies (23).

CONCLUSION

Hydroxytyrosol, a polyphenol found at high concentrations in OMWW, is a potent natural antioxidant. Multiple experimental and clinical studies have identified a number of potential therapeutic effects of hydroxytyrosol (see Table). Hydroxytyrosol has been shown to reduce V-LDL in a clinical study (11) and have notable antithrombotic effects. Hydroxytyrosol's anti-inflammatory effects have been demonstrated in a number of laboratory studies; a hydroxytyrosol-based supplement reduced levels of TNF-a in an animal model of joint inflammation (13), and improved the quality of life in osteoarthritis patients (14). Finally, animal studies have indicated that hydroxytyrosol may play a role in the extension of life span through its effects on the

upregulation of longevity proteins and its ability to exert protective effects on the heart following myocardial infarction.

In addition to these effects, several studies have demonstrated hydroxytyrosol's notable antibacterial and antimicrobial properties (24-26). Recently a number of studies have also reported that hydroxytyrosol induces mitochondrial biogenesis and the stimulation of mitochondrial function, which could have important implications in the management of diseases that are linked to mitochondrial dysfunction including diabetes and obesity (27-28).

To the best of our knowledge, only one hydroxytyrosol-based product has been subjected to a broad range of dedicated preclinical toxicology studies (29). Administration of this hydroxytyrosol-based product, in single or multiple dosages, up to 2000 mg/kg/day resulted in no adverse clinical, haematological, biochemical, or reproductive adverse effects (29). These preclinical data correspond with a lack of major adverse events in patients studied in clinical trials with the same product. The potential beneficial effects of hydroxytyrosol, coupled with its strong safety profile, merit investigations in larger clinical studies to further determine its role in the prevention of diseases that represent substantial causes of morbidity and mortality.

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