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Olive Phenolics Increase Glutathione Levels in Healthy Volunteers

FRANCESCO VISIOLI,*,† ROSWITHA WOLFRAM,§ DORIANE RICHARD,† MUHAMMAD IMRAN CHONG B. ABDULLAH,§ AND ROBERTO CREA#

UPMC Univ Paris 06, Paris, France; Prince Court Medical Center, Kuala Lumpur, Malaysia; Division of Angiology, Department of Medicine II, Medical University, Vienna, Austria; and CreAgri Inc., Hayward, California 94545

Several in vitro and in vivo studies have shown that olive phenols exert potent biological activities including, but not limited to, antioxidant actions. These activities are shared by phenols found in olives, olive oil, and olive mill wastewater (OMWW). The aim of this study was to investigate whether a commercially available OMWW preparation could influence some parameters of oxidative status in healthy human volunteers. Ninety-eight healthy subjects with normal body weight were recruited, and 5 mL of blood was drawn from their antecubital vein after an overnight fast of at least 12 h. After this, subjects were asked to ingest 2 mL of a commercially available OMWW preparation. Another 5 mL of blood was drawn 1 h after ingestion of the preparation. Plasma antioxidant capacity and total and reduced glutathione were measured. No difference in plasma antioxidant capacity was observed between baseline and 1 h after the ingestion of the extract. Conversely, a significant increase in total plasma glutathione concentration was measured. This increase involved both the reduced and oxidized forms of glutathione; hence, their ratio was unaffected by the treatment. The observed effects of OMWW on glutathione levels might be governed by the antioxidant response element (ARE)-mediated increase in phase II enzyme expression, including that of γ-glutamylcysteine ligase and glutathione synthetase. Future studies on groups of individuals who may benefit from an increase in their glutathione levels, for example, the elderly, will further elucidate the biological activities of this formulation.

KEYWORDS: Polyphenols; hydroxytyrosol; nutraceuticals; olives; natural antioxidants; olive mill wastewater

INTRODUCTION

Olives contain high amounts of a variety of phenolic compounds, the most abundant being the secoiridoid oleuropein, which is a combination of elenolic acid and hydroxytyrosol (*1*). Several in vitro and in vivo studies have shown how both oleuropein and hydroxytyrosol exert potent biological activities including, but not limited to, antioxidant actions (*2, 3*). Indeed, interest in the healthful properties of olives and olive oil, mostly due to their phenolic components, has increased recently and has equaled that of other food items such as red wine and tea. During olive oil production, according to their partition coefficient, olive phenolics end up either in olive oil, in the olive mill wastewater (OMWW), or in the residual, solid phase (pomace) (*4, 5*).

In addition to the extensive research on extra virgin, that is, phenol-rich olive oil, data are also accumulating on the bioactive potential of OMWW preparations (*6, 7*). Results from both in vitro and in vivo (including rats and humans) studies suggest that OMWW, of which hydroxytyrosol is the most bioactive component, possesses anti-inflammatory and antithrombotic as well as antioxidant properties and as such is potentially capable of preventing passive smoke-induced oxidative stress, reducing thromboxane B_2 production by whole blood, and ameliorating symptoms of inflammatory disease such as osteoarthritis (*4, 5, 8–14*).

The aim of this study was to investigate whether a commercially available OMWW preparation could influence some parameters of oxidative status in healthy human volunteers.

MATERIALS AND METHODS

Design. The OMWW preparation we tested contained, as evaluated by HPLC coupled with UV detection, ≤6% of simple and complex (including secoiridoids) phenols, of which 45% was hydroxytyrosol and 7.5% was oleuropein (**Table 1**).

* Address correspondence to this author at the Laboratory of Micronutrients and Cardiovascular Disease, UR4, Université Pierre et Marie Curie, 7 quai St Bernard, Bât A 5 étage, 75005 Paris, France (e-mail francesco.visioli@upmc.fr).

† UPMC Univ Paris 06.

§ Prince Court Medical Center and Medical University Vienna.

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Table 1. Composition of the OMWW Preparation under Study

protein	5−10%
carbohydrates	45−68%
fat	17−30%
ash (% w/w)	8−15%
lead	<2 ppm
heavy metals	<10 ppm
simple and polyphenols	6% (minimum)
hydroxytyrosol	40−50% of total phenolics
gallic acid	2−5% of total phenolics
tyrosol	2−5% of total phenolics
oleuropein	5−10% of total phenolics
other secoiridoids	10−20% of total phenolics

Table 2. Plasma Antioxidant and Glutathione Status of Healthy Volunteers before and after the Administration of an OMWW Preparation[a]

	before	after
TAS (mmol/L)	2.067 ± 1.363	1.996 ± 1.291
GSH (μM)	1.794 ± 1.471	2.837 ± 1.617*
GSSG (μM)	0.403 ± 0.485	0.741 ± 0.620*
GSH/GSSG	13.55 ± 21.49	10.86 ± 18.47

[a] Abbreviations: TAS, total antioxidant status; GSH, reduced glutathione; GSSG, oxidized glutathione. Data are means ± SD. *, $p < 0.01$, Student's t test.

Ninety-eight apparently healthy subjects with normal body weight were recruited from within the Prince Court Medical Center (PCMC, a private hospital located in Kuala Lumpur, Malaysia, affiliated with the Medical University of Vienna) and in the city of Kuala Lumpur via word of mouth. The study protocol was approved by the local Ethic's committee and was fully explained to the participants, and written informed consent was obtained from all subjects prior to the start of the trial. Subjects were instructed not to eat or drink tea phenol-rich foods or beverages such as coffee, red wine, or chocolate the night prior to the experiment.

Experimental Procedure. For the assessment of the oxidative status of the study population, at baseline, 5 mL of blood was drawn from the antecubital vein after an overnight fast of at least 12 h. After this, subjects were asked to ingest 2 mL of a commercially available OMWW preparation (Olivenol Livin'), diluted in a glass of water. This amount provided ~50 mg of hydroxytyrosol and ~8 mg of oleuropein. This dose was chosen on the basis of previous studies of hydroxytyrosol's bioactivity (*9*, *12*) and is this commercial preparation's prescribed amount. Another 5 mL of blood was drawn exactly 1 h after the ingestion of the preparation. Plasma was separated by centrifugation at 2100*g* for 20 min at 4 °C, aliquoted, and stored at −80 °C.

Plasma antioxidant capacity was evaluated utilizing the TAS kit (Randox, Crumlin, U.K.) (*15*), according to the manufacturer's instructions. Total and reduced (GSH) glutathione levels were measured, after deproteinization, colorimetrically, following the manufacturer's (Cayman Chemical Co., Ann Arbor, MI) instructions (*16*).

RESULTS

We did not observe any difference in plasma antioxidant capacity between baseline and 1 h after the ingestion of the extract (**Table 2**). Conversely, a significant increase in total plasma glutathione concentration was measured. This increase involved both the reduced and oxidized forms of glutathione; hence, their ratio was unaffected by the treatment (**Table 2**).

DISCUSSION

This is the first study to report that a commercially available OMWW preparation is capable of increasing total plasma glutathione when administered to healthy volunteers of various Asian ethnic descent. This increase, however, did not translate into an increase in total antioxidant capacity in the studied population.

To counteract the potentially noxious effects of xenobiotics, higher vertebrates, including humans, have developed a battery of genes encoding phase II and antioxidant enzyme expression (*17*). These include the expression of various superoxide dismutase (SOD) isoforms, catalase, glutathione peroxidase, glutathione reductase, various glutathione-*S*-transferase (GST) isoforms, NAD(P)H:quinone oxidoreductase 1 (NQO1), and heme oxygenase (HO)-1, which can exert cytoprotective, antioxidant, and anti-inflammatory effects. One mechanism by which cells respond to oxidative injury is through the antioxidant response element (ARE), a cis-acting enhancer sequence that regulates the transcription of various cytoprotective genes (*18*). Upon toxic injury, the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) translocates to the nucleus and dimerizes with small Maf proteins to form a transactivation complex that, consequently, binds to the ARE (see ref *19* for details on Nrf2 regulation). Nrf2-induced ARE activation coordinates the expression of many genes involved in combating oxidative stress and toxicity in a broad range of tissues and cell types.

The synthesis of GSH from its constituent amino acids involves the actions of two ATP-dependent enzymes, γ-glutamyl-cysteine ligase (GCL) and GSH synthetase. GCL, the rate-controlling enzyme in the overall pathway, is a heterodimer composed of a catalytic (GCLC) and a modulatory (GCLM) subunit. The basal and inducible expressions of these GCL substituents are mediated by means of the ARE (*20*).

Pharmacological agents and natural substances shown to induce ARE-regulated gene expression (and, hence, endowed with chemopreventive activities) include oltipraz, anethole dithiolethione (ADT), sulforaphane, 6-ethylsulfinylhexyl isothiocyanate (6-HITC), curcumin, and caffeic acid phenethyl ester (CAPE), as well as 4′-bromoflavone (*18*). In synthesis, the observed effects of OMWW on glutathione levels might be governed by the ARE-mediated increase in phase II enzyme, namely, GCL and GSH synthetase, expression, in turn leading to enhanced glutathione synthesis. If confirmed, the potential applications of these preliminary findings are manifold and could extend to patients with reduced circulating glutathione levels, for example, patients on chronic hemodialysis (*21*, *22*) or patients suffering from Alzheimer's disease (*23*) or HIV infection (*24*). Furthermore, elderly people may potentially benefit from an increase of glutathione levels (*20*). It must be highlighted, though, that the orally ingested OMWW preparation investigated in the current trial acted solely on total glutathione levels and not on its reduced/oxidized ratio. Therefore, future investigations are crucial, in the context of a repeated administration of OMWW preparation as well as dose-finding studies, to evaluate the potential influence of OMWW on the reduced/oxidized ratio of glutathione.

The lack of effect on total antioxidant capacity observed in this study, which is in agreement with Kendall et al. (*25*) and Schaffer et al. (*13*), might be interpreted in terms of lower attainable concentrations of OMWW phenolics as compared to other endogenous antioxidants (*26*). Despite the paucity of data, it appears that OMWW phenolics, namely, hydroxytyrosol, attain plasma concentrations in the low micromolar range (*27*). In addition, OMWW phenols such as hydrotyrosol are extensively metabolized in humans (*28*), and data on the biological effects of metabolites are still scant. However, it is noteworthy that, as far as hydroxytyrosol is concerned, its 3-*O*-glucuronide conjugate exhibits strong antioxidant activities (stronger than those of the parent molecule) in vitro (*29*). Although these factors limit the capability of OMWW components to act as

antioxidants in plasma in vivo, especially compared to other antioxidants present in plasma at high steady-state concentrations, for example, 30−150 μM ascorbate (vitamin C), 160−450 μM urate, or 15−40 μM α-tocopherol (vitamin E) (*26*). Hence, it is possible that prolonged consumption of bioactive molecules such as olive phenols might exert long-term beneficial health effects, as emphasized by studies in which biological activities of OMWW preparations have been demonstrated in humans (*9*, *10*, *12*).

Concerns over OMWW toxicity have been extensively addressed by D'Angelo et al. (*30*), Christian et al. (*31*), and Soni et al. (*32*). These groups could not demonstrate toxic effects of hydroxytyrosol even at much higher doses as administered in this trial; hydroxytyrosol-rich OMWW extract has been granted GRAS (generally regarded as safe) status by the U.S. Food and Drug Association (FDA).

One limitation of our study was the lack of placebo. However, given the high number of subjects under study and the high statistical significance of our results, it is unlikely that the observed differences were due to chance. Another limitation was that we did not evaluate hydroxytyrosol's bioavailability. However, this issue has been already fully elucidated by us and other investigators (see, e.g., refs *27*, *28*, *33*, and *34*).

In conclusion, the ingestion of 2 mL of a commercially available OMWW preparation was shown to be able to significantly increase total glutathione levels in 98 healthy volunteers while not affecting plasma antioxidant capacity. Among the numerous groups of individuals who may benefit from increased glutathione levels, the elderly might be paid special attention, considering the fact that overall life expectancy is constantly increasing. Future studies, including the evaluation of various dosages and long-term administration of OMWW, are warranted to further investigate this formulation.

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