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Olive oil phenolics are dose-dependently absorbed in humans

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Abstract Olive oil phenolic constituents have been shown, in vitro, to be endowed with potent biological activities including, but not limited to, an antioxidant action. To date, there is no information on the absorption and disposition of such compounds in humans. We report that olive oil phenolics, namely tyrosol and hydroxytyrosol, are dose-dependently absorbed in humans after ingestion and that they are excreted in the urine as glucuronide conjugates. Furthermore, an increase in the dose of phenolics administered increased the proportion of conjugation with glucuronide.

Key words: Olive oil; Phenol; Atherosclerosis; Mediterranean diet; Antioxidant; Hydroxytyrosol

1. Introduction

Extra virgin olive oil, as opposed to seed oils, contains a series of phenolic 'minor components' that grant its particular aroma and taste [1]. Olive oil is the principal fat component of the Mediterranean diet, that has been associated with a lower incidence of coronary heart disease (CHD) and certain cancers [2]. In light of the growing evidence of the role played by low-density lipoprotein (LDL) oxidation in the onset of CHD, several studies have been carried out to elucidate the antioxidant activities of olive oil phenolics. In particular, hydroxytyrosol (HT) and oleuropein inhibit copper- and peroxyl radical-induced LDL oxidation, scavenge free radicals including superoxide, and exert other biological activities including inhibition of platelet aggregation and potentiation of the nitric oxide-mediated macrophagic immune response [3].

To date, there is little quantitative information on the bioavailability of phenolic compounds, e.g. flavonoids, in humans [4–6]. As the absorption of olive oil phenolics has not been reported as yet, the current investigation was carried out to quantitatively assess their uptake from oils containing different amounts of phenols. Some simple olive oil phenols, e.g. tyrosol (T) and HT, are rather polar and, after absorption, are likely to be excreted through the kidneys either as such or as their metabolites. Thus, our study focused on the urinary excretion of simple phenols as free compounds or in conjugated form.

2. Materials and methods

The local ethics committee approved the study and written informed consent was obtained. Four olive oil samples (hereinafter referred to as A, B, C, and D) were prepared by adding different amounts of an olive oil phenolic extract to a phenol-poor oil. The final phenolic contents of the samples, as evaluated by the colorimetric method [7], are shown in Table 1. The extracts were characterized by gas chromatography-mass spectrometry (GC-MS, see below) in order to identify and quantify T and HT, the two most representative simple phenols of olive oil (Table 1); the latter is also the most potent antioxidant in olive oil.

Six male human healthy volunteers (age 30 (27–33), body mass index 23.35 (22.42–24.16)) were instructed not to consume olive oil for 48 h before and 24 h after the beginning of the experiment. Fasting subjects were given 50 ml of oil samples, accompanied by 40 g of bread, between 8:00 a.m. and 8:30 a.m. This treatment was repeated four times, after a 1-month period of washout, so that, overall, each subject received the four different oil samples. Urine was collected for 24 h, the volume was recorded and an aliquot was extracted as follows: for the quantitation of free, unconjugated phenols, 1 ml of urine was added with 1 μg/ml of α-naphthol, as internal standard, it was acidified with HCl (0.3 M):acetonitrile (1:1, by volume) and it was extracted twice with three volumes of ethyl acetate; the organic phase was evaporated to dryness under nitrogen. The residue was dissolved in a mixture of bis-trimethylsilyl-trifluoro-acetamide:pyridine (4:1, by volume). For the quantitation of *total* simple phenols in urine, 360 U of β-glucuronidase (Sigma, St. Louis, MO, USA) were added to 1 ml of urine and, after an overnight incubation at 37°C and pH 5, extraction was carried out as described above. Calibration curves for the quantitative determinations were prepared using 1-ml samples of urine that did not contain the compounds under investigation, as shown by preliminary GC-MS analysis. These samples were spiked with α-naphthol (1 μg/ml) and increasing amounts, i.e. from 10 to 2000 ng/ml, of authentic T and HT; each sample was then extracted and analyzed. GC-MS analyses were performed on a BP1 fused silica capillary column (SGE s.r.l., Italy), connected with an HP 5970 MSD mass spectrometer (Hewlett-Packard, USA). For EI-selected ion monitoring, ions at *m/z* 216 for α-naphthol, at *m/z* 197, 267, and 282 for T, and at *m/z* 197, 267, and 370 for HT were recorded. These ions were selected after mass spectra obtained from authentic standards.

3. Results and discussion

The urinary levels of free, i.e. unconjugated, T and HT are reported in Fig. 1A. The urinary excretion of both free T and HT correlated with their intake, with the exception of oil D, i.e. the oil with the highest phenol concentration, that resulted in a T and HT excretion lower than that recorded following the ingestion of oil C. However, when urine extracts were treated with glucuronidase, significant correlations ($R^2 = 0.76$ and 0.72, respectively) between the amounts of simple phenols ingested and excreted were observed (Fig. 1B). Thus, the data indicate that the simple olive oil phenols, namely T and HT, are absorbed after ingestion and are excreted as glucuronide conjugates. The proportions of total T and HT, with respect

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Table 1
Content in total phenols, HT, and T of the olive oil samples employed in the study

Oil sample	A	B	C	D
Total phenols (mg/l)	487.5	975	1462.5	1950
HT (μg/ml)	20	44	66	84
T (μg/ml)	36	72	110	140

Table 2
Excretion of T and HT as percentage of the total administered amount

Oil sample	A	B	C	D
T (% of administered amount)	21 ± 11	28 ± 21	21 ± 6	24 ± 14
HT (% of administered amount)	29 ± 27	64 ± 36	35 ± 19	40 ± 21

Data are the means ± S.D. $n = 6$ for each oil sample.

to the ingested dose, were in the range of 20–22% for T and 30–60% for HT. The T/HT ratios found in urines were, however, similar to that present in the oil (~1.7).

An important finding of this investigation is that higher doses of phenolics increase their rate of conjugation with glucuronide, which is generally considered to be the most common final metabolic step of intact phenolic compounds [5].

Not the entire quantity of T or HT that was given was subsequently found in the urines (Table 2); whether the remaining amount was not absorbed, excreted with the feces, destroyed in the gut, accumulated in organs or circulating cells such as red blood cells, or was excreted after 24 h remains to be elucidated. Future development of appropriate techniques or availability of labeled compounds will eventually clarify this issue.

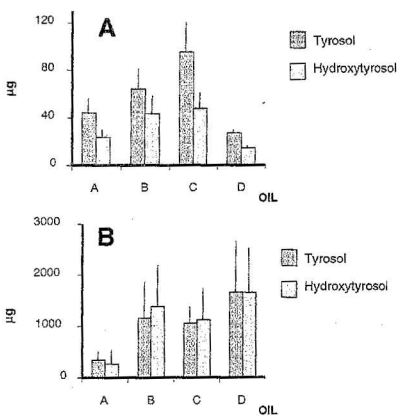

Fig. 1. A: Levels of unconjugated T and HT in urines of human volunteers who ingested olive oil samples with increasing concentrations of T and HT. B: Levels of total T and HT in urines of human volunteers who ingested olive oil samples with increasing concentrations of T and HT.

Caution should be taken in extrapolating these results to a typical Mediterranean diet, in which the daily intake of olive oil is, on the average, lower than the 50 ml we administered in this study as a single load. In addition, the phenolic content of the oils we employed was several fold higher than that of a typical virgin olive oil. It cannot be excluded, however, that continual exposure to olive oil phenols results in a long-term accumulation; our data clearly demonstrate a dose-dependent and not saturable (at least at the doses we employed) absorption of simple phenols. Unanswered questions concern the fate of the remaining amount of phenolics and their hypothetical distribution in tissues or circulating cells. Moreover, whether the potent biological activities of olive oil phenols thus far demonstrated in vitro are also exerted in vivo remains to be elucidated.

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