Eur J Nutr (2006) 45:307–310
DOI 10.1007/s00394-006-0596-9

SHORT CONTRIBUTION

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Is dopamine behind the health benefits of red wine?

Received: 8 October 2005
Accepted: 14 February 2006
Published online: 3 April 2006

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Abstract *Background* The contribution of biologically active non-nutrient chemicals to the health benefits of the Mediterranean diet is controversial because of their low dietary concentrations. Hydroxytyrosol is a dopamine metabolite, and also a very active naturally occurring phenol compound in olive oil. *Aim of the study* To examine the disposition of hydroxytyrosol in humans, given that we discovered its presence in red wine in the frame of the study. *Methods* The pharmacokinetics of hydroxytyrosol from two clinical trials, designed to assess the short-term and postprandial effects of moderate doses of wine and olive oil in healthy volunteers, were compared. *Results* Despite a five-fold difference in the doses of hydroxytyrosol administered (0.35 mg for red wine and 1.7 mg for olive oil), urinary recoveries of hydroxytyrosol were higher after red wine administration. An interaction between ethanol and dopamine after red wine ingestion leading to the formation of hydroxytyrosol was observed. *Conclusions* Biological effects after red wine ingestion should be re-examined on the basis of combined hydroxytyrosol concentrations from red wine and dopamine turnover.

The Mediterranean diet has a significant protective effect against cancer and cardiovascular diseases [1]. The principal components of this diet are olive oil, fruits, vegetables, wine, and fish. Beneficial health effects have been partially attributed to non-nutrient chemicals, including phenolic compounds, such as hydroxytyrosol [2] and oleuropein found in olive oil, or resveratrol in wine [3]. It has been recognized that consumption of low doses of alcohol may have some beneficial properties. It is also acknowledged that the amounts of phenolic compounds (i.e. resveratrol) associated with moderate wine consumption are unlikely to contribute further to its biological effects [4]. In the present report, biochemical data from clinical studies give insights into how alcohol and antioxidants might interact synergistically to provide beneficial effects for health.

During the early stages of a clinical trial designed to assess the short-term and postprandial effects of moderate doses of grape products, it was found that hydroxytyrosol—the antioxidant prototype of olive oil phenolic compounds—occurs also in wine in significant amounts. This antioxidant compound has not been previously described in red wine. Subsequently, therefore, hydroxytyrosol concentrations in different biological fluids of the volunteers involved in the clinical trial were determined.

A total of 12 healthy male volunteers aged 18–27 years participated in this controlled, randomized

EJN 596

cross-over study with three grape products. All volunteers were in good health as assessed by medical history, physical examination, and routine blood and urine laboratory tests. Wine (250 ml/day), grape juice (1 l/day), and tablets of grape extracts enriched with trans-resveratrol (equivalent to 1 mg/day) were administered daily over three intervention periods of 4 days each one preceded by a 10-day washout period. During the first 7 days of the washout period participants were asked to avoid excessive antioxidant intake. During the last 3 days before the day of the intervention (days 8–10 of the washout period) they followed a strict diet low in phenolic compounds. A nutritionist instructed them on excluding several foods, rich in phenolic compounds, from their diet (vegetables, legumes, fruit, juice, wine, coffee, tea, caffeine-containing soft drinks, beer, cocoa, marmalade, and olives). Daily dietary records were obtained from each volunteer. After 4 days of wine intake a decrease in the LDL/HDL cholesterol ratio (-0.17 ± 0.22, mean ± SD, $P=0.026$) and oxidized LDL levels (-28.3 ± 36.1 U/l, $P=0.048$), together with an increase in the antioxidant enzyme glutathione peroxidase activity (64 ± 71 U/l, $P=0.025$) was observed. The content of resveratrol and hydroxytyrosol for the dose of red wine administered was 6.9 μmol and 2.3 μmol, respectively. Hydroxytyrosol (HT) and its metabolite homovanillic alcohol (3-*O*-methyl-hydroxytyrosol, HVALC) were measured by gas chromatography-mass spectrometry (GC/MS). In short, HT and HVALC analyses were carried out on a Hewlett-Packard (Palo Alto, CA) gas chromatograph coupled to a mass spectrometer detector system consisting of an HP5980 gas chromatograph, a HP5973 mass-selective detector, and a HP7683 series injector. Separation of hydroxytyrosol and tyrosol was carried out using a HP Ultra 2 (12.5 m × 0.2-mm i.d. and 0.33-μm film thickness) cross-linked 5% phenylmethyl silicone capillary column (Hewlett-Packard). Instrumental, hydrolytic and extraction conditions of samples were previously described [5, 6]. All chemicals and organic solvents used were of analytical grade. The application of mass spectrometry for the analysis of these analytes confers a high specificity and selectivity to the assay. It has not only been applied for the evaluation of hydroxytyrosol disposition in humans [5] but also has been validated as biomarker of olive oil consumption in nutritional intervention studies and clinical trials [7, 8]. Plasma and urinary (0–24 h) concentrations of both compounds were higher after wine ingestion compared with baseline values (values after the washout period). Experimental pharmacokinetic results for the first 24 h (Table 1) were compared with those observed in a group of 12 male healthy volunteers given 25 ml of extra virgin olive oil (11 μmol of hydroxytyrosol per dose) who participated in another study of a similar design with three types of olive oils differing in their phenolic content [9]. The maximum plasma concentrations observed in subjects given red wine were lower than those observed amongst subjects in the olive oil group. This would be in agreement with the doses of hydroxytyrosol administered with each dietary product, although the areas under the curve (0–24 h) were quite similar despite dose differences. Both treatments differed in the time to reach peak plasma concentrations (Table). Urinary recoveries of hydroxytyrosol in the red wine group were 40% higher than those observed in the virgin olive oil treatment (Fig. 1a). The recovery of hydroxytyrosol in urine could not be explained by the dose administered of red wine as it was 200% times higher than expected. This observation, combined with differences in plasma pharmacokinetic parameters, suggested another source of hydroxytyrosol besides red wine.

Hydroxytyrosol, in addition to being a natural antioxidant, is also a metabolite of dopamine (DOPET). Two hypotheses were tested in relation to the role of dopamine metabolism. The first one evaluated the possible contribution of naturally present tyramine in red wine (2.2 μmol per dose of red wine administered), as it has been proposed that dopamine can be synthesized from tyramine in a biosynthetic reaction catalysed by the isoenzyme of cytochrome P450, CYP2D6 [10]. Therefore, urinary concentrations of tyramine were measured by GC/MS [11]. Recoveries of tyramine in the red wine group were higher than those observed in the virgin olive oil group (Fig. 1b), but not sufficient to explain the observed hydroxytyrosol urinary recoveries. Alternatively, a second hypothesis regarding the possible direct effects of alcohol on dopamine release was examined. A recent report provided neuroimaging data supporting the release of dopamine induced by ethanol in humans [12]. Urinary concentrations of homovanillic acid (HVA) in urine, a typical biomarker of dopamine turnover in

Table 1 Pharmacokinetics results for virgin olive oil and red wine phenol compounds in human plasma

Phenol compound	Parameter	Olive oil	Red wine	
Hydroxytyrosol	T_{max} (h)*	1.3 (1–2)	2.2 (1–4)	$P=0.015$**
	C_{max} (ng/ml)	11.7±2.3	7.9±2.1	$P=0.05$
	AUC 0–8 (ng/ml*h^{-1})	42.4±15.0	36.1±7.2	NS
Homovanillic alcohol	T_{max} (h)*	1.3 (1–2)	1.2 (1–2)	NS
	C_{max} (ng/ml)	3.0±0.8	3.4±0.4	NS
	AUC 0–8 (ng/ml*h^{-1})	9.8±2.2	8.8±3.3	NS

* T_{max}: mean values and range
** All statistical comparisons performed with the Mann–Whitney U test

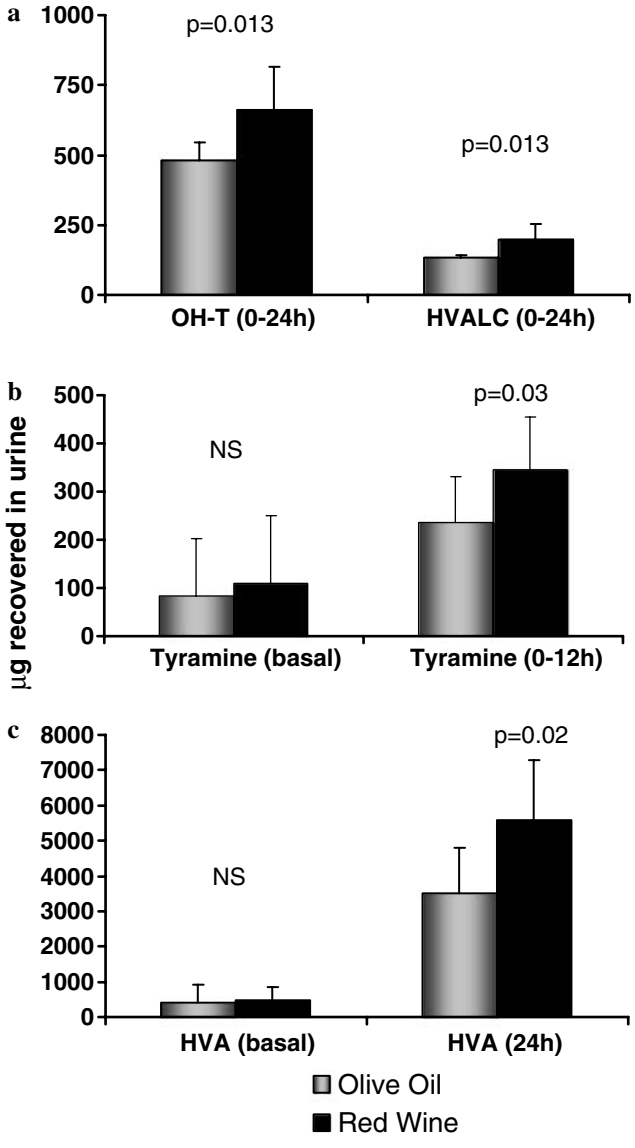

Fig. 1 Red wine administration (250 ml) vs. virgin olive oil administration (25 ml). urinary recoveries (Mann–Whitney U test, used for statistical comparisons): (a) Hydroxytyrosol (OH-T) concentrations (μg) in 0–24 h were 4.3±1.0 vs. 3.1±0.4 for wine and virgin olive oil, respectively. Homovanillic alcohol (HVALC) values were 1.2±0.3 and 0.8±0.1 for wine and virgin olive oil, respectively. (b) Tyramine concentrations (μg) in 0–12 h were 0.8±1.0 vs 0.6±0.9 in basal conditions, and 2.5±0.8 vs 1.7±0.7 after treatment administration, for wine and virgin olive oil, respectively. (c) Homovanillic acid (HVA) concentrations (μg) in basal conditions were 2.6±2.2 vs. 2.3±2.7 and 0–24 h were 30.7±9.4 vs. 19.2±7.2 for wine and virgin olive oil, respectively

humans, were determined by high-performance liquid chromatography and electrochemical detection [13]. Higher recoveries of HVA in subjects from the red wine group compared with the virgin olive oil group were found (Fig. 1c). This could be related to the unexpectedly high recoveries of hydroxytyrosol in urine on the basis of a high ethanol-induced peripheral turnover of dopamine. This hypothesis is further supported by results in the grape juice treatment, ethanol free. In the grape juice, concentrations of hydroxytyrosol per ml (in the drink beverage) were similar to those present in red wine (1.65 μg/ml vs. 1.39 μg/ml). Nevertheless there was a four fold difference in the volume administered to volunteers: 1000 ml for grape juice vs. 250 ml for red wine; therefore the doses administered of hydroxytyrosol were 1.65 mg vs. 0.35 mg respectively. Despite these differences in dosage, urinary recoveries of hydroxytyrosol (0–12 h) were twice higher in the red wine treatment (0.26 mg for grape juice vs. 0.54 mg for red wine).

In summary, we report for the first time that red wine is a natural source of hydroxytyrosol, and also that ingestion of red wine can promote endogenous hydroxytyrosol generation. The physiological pathway for endogenous hydroxytyrosol production would be via dopamine metabolism. Mechanisms for an enhancement of dopamine metabolism after red wine ingestion could be both the interaction of ethanol with the dopaminergic neurotransmission system or, to a lesser extent, the conversion of natural tyramine ingested with red wine to dopamine.

In recent clinical studies, we have shown that secondary biomarkers of lipids and DNA oxidation improve with dietary doses (25 ml/day) of olive oil. Such changes were related in a dose-dependent manner with the content in phenols of the olive oil, and among these phenols, because its biological activities, hydroxytyrosol [2]. If we accept that hydroxytyrosol plays a role in the beneficial effects of olive oil on health, this report suggests that in the case of wine, a single glass is at least equivalent to 25 ml (22 g) of virgin olive oil in its capacity to increase hydroxytyrosol concentrations in the body.

Acknowledgement Financial support from Instituto Nacional de Investigaciones Agrarias (INIA-MAPA VIN00-027-C3-01) and the PREDIMED network (ISCIII G03/140) is acknowledged.

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