Tyrosol Bioavailability in Humans after Ingestion of Virgin Olive Oil, Elisabet Miró Casas,1 Magí Farré Albadalejo,1 María Isabel Covas Planells,2 Montserrat Fito Colomer,2 Rosa M. Lamuela Raventós,3 and Rafael de la Torre Fornell1*

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Results from epidemiological studies support the relationship between the consumption of phenolic-rich food and a low incidence of coronary heart disease (1, 2). The lower incidences of coronary heart disease and certain cancers in Mediterranean countries have been associated with diet, of which fruits, vegetables, legumes, and grains are the usual components and the major fat component is olive oil (3). The Mediterranean dietary pattern has been also shown to be effective in secondary prevention of coronary heart disease (4). Virgin olive oil is rich in phenolic compounds with strong antioxidant properties that protect olive oil from autoxidation (5). In addition, olive oil phenolic compounds have been shown to delay in vitro metal-induced and radical-dependent LDL oxidation (6, 7). Among the phenolic compounds in olive oil is tyrosol (4-hydroxyphenylethanol), which is also present in other dietary sources (8, 9) and has mild antioxidant properties (10–12). However, information on the bioavailability of dietary phenolic compounds in humans is scarce (13, 14). In bioavailability studies of phenolic compounds, one of the main problems is the estimation of the dose administered because these substances can be present in multiple forms in food. In the specific case of olive oil, phenolic compounds may be in the form of glycosides, polymers, and esters (12, 15). To our knowledge, the bioavailability of tyrosol from nonsupplemented dietary sources has not been described previously.

Eight healthy volunteers were recruited (five men and three women; age range, 25–52 years). The local ethics committee, CEIC-IMAS (register no. 98/798/I), approved the protocol, and participants signed an informed consent. All volunteers could be considered healthy on the basis of physical examination and standard biochemical and hematological tests. Subjects had a mean weight of 75 ± 13.47 kg (men, 83.2 ± 4.4 kg; women, 59.7 ± 6.11 kg) and a body mass index of 25.6 ± 3.1 kg/m² (men, 26.9 ± 3.29 kg/m²; women, 22.4 ± 2.4 kg/m²). Volunteers followed a phenolic-free diet for 4 days (wash-out period) before acute olive oil administration. A nutritionist designed dietary recommendations for 4 days (wash-out period) before acute olive oil administration. A nutritionist designed dietary recommendations and diet during the 24-h experimental period. Volunteers were instructed to exclude several foods from their diet (coffee, tea, fruits, vegetables, and wine) and olive oil). At 0800 on day 5, they were provided with 50 mL of extra virgin olive oil that was administered in a single dose ingested either directly (n = 2) or with some bread (n = 6). Olive oil was the sole phenolic dietary source in the next 24 h. Urine was collected before the start of the wash-out period (pre-washout period; first voided spot urine in the morning), during the wash-out period (first voided spot urine in the morning), and after the acute administration period (at 0–4, 4–8, 8–12, 12–16, and 16–24 h) and stored at −80 °C until analysis.

The tyrosol content in virgin olive oil usually is evaluated after extraction with methanolic water without other sample treatment (16). This method permits only the determination of free tyrosol in olive oil. In this bioavailability study, aliquots of methanolic water extracts of the olive oil administered were submitted to either an acidic or an alkaline treatment. This was done to release tyrosol from its conjugated form and to experimentally reproduce some of the gastrointestinal conditions occurring during digestion of oil in humans. Sample preparation was performed using a modification of a method previously described by Caruso et al. (12). A 15-mL aliquot of the olive oil administered in the study was extracted three times with methanol-water (80:20 by volume). Pooled extracts were treated with hydrochloric acid (1 mol/L HCl; sample pH adjusted to 1) or sodium hydroxide (0.1 mol/L NaOH; sample pH adjusted to 10) with further sonication (30 min at 45 kHz) at room temperature. After 60 min of incubation, the methanolic water extracts were evaporated under reduced pressure at 40 °C on a rotary evaporator (Rotavapor RE 121; Büchi). Aqueous
residues were extracted twice in ethyl acetate (final volume, 50 mL). After evaporation of organic phases to dryness, residues were reconstituted in 5 mL of methanol. Aliquots (50 μL) were evaporated under a nitrogen stream at 25 °C and analyzed by gas chromatography–mass spectrometry, after derivatization (60 °C for 30 min) with 100 μL of reaction mixture containing MSTFA (N-methyl-N-trimethylsilyl-trifluoroacetamide):NH₄I:2-mercaptoethanol (1000 mL:2 g:6 mL). For the quantification of tyrosol, calibrators were prepared by adding tyrosol to refined sunflower oil (which contains no tyrosol) and extracting as above; organic phases were dissolved in 1 mL of methanol.

Urine aliquots from the pre-wash-out period, days 3 and 4 of the wash-out period, and all samples collected after acute oil consumption were analyzed. Before analysis, 400 μg/L 3-(4-hydroxyphenyl)-propanol (10 μL of a 100 mg/L methanolic solution), the internal standard, was added to 2.5 mL of each urine aliquot. Samples were hydrolyzed by the addition of 500 μL of concentrated HCl and incubated for 20 min at 100 °C in a heating block. After samples cooled to room temperature, the pH was adjusted to 5.5 by the addition of 1 mL of 2 mol/L Tris, pH 8.2, and 450 μL of 10 mol/L KOH saturated with KHCO₃. A second aliquot of all urine samples was analyzed, omitting the hydrolysis step. After hydrolysis, samples were centrifuged at 300 g and applied to Waters Oasis™ HLB extraction cartridges (polydivinylbenzen-co-N-vinylpyrrolidone). Phenolic compounds were eluted with 1 mL of methanol and evaporated to dryness under a nitrogen stream at 25 °C. The residue was dissolved in 1 mL of 1.1 mol/L sodium acetate buffer, pH 5.2, and extracted once with 5 mL of ethyl acetate. After evaporation of the organic phase, samples were derivatized with 100 μL of MSTFA:NH₄I:2-mercaptoethanol (1000 mL:2 g:6 mL) for 30 min at 60 °C. Urine samples with high phenolic concentrations were diluted with synthetic urine (17) and treated as above.

Final extracts were analyzed by gas chromatography–mass spectrometry. The chromatographic separation was performed in a Ultra 2 cross-linked 5% phenyl-methyl siloxane capillary column connected to an HP 5973 MSD mass detector (Hewlett-Packard). The detector was operated in the single-ion monitoring mode, and compounds were ionized by electron impact. Ions at m/z 206 for bis-trimethylsilyl-3-(4-hydroxyphenyl)-propanol (retention time, 6.70 min) and m/z 179, 267, and 282 for bis-trimethylsilyl-tyrosol (retention time, 5.83 min) were recorded. Tyrosol calibration curves and control samples were prepared by adding tyrosol to synthetic urine and analyzed according to the urine sample preparation procedure. The calibration working range was 10–100 μg/L. Control concentrations were 15, 30, 60, and 90 μg/L. Within- and between-run imprecision (CVs) was 5.7–13% and 5.5–9.5%, respectively. Within- and between-run accuracy (relative error, as a percentage) was −5.8% to 5.8%, and −7.6 to 1.4%, respectively. The limit of detection was 1.9 μg/L (signal-to-noise ratio = 14). Tyrosol extraction recovery was >80% in all concentrations tested. A general linear model was used to test the best fitting of the repeated measurements: linear, quadratic, or cubic.

Tyrosol concentrations in human urine were 2–47.4 μg/L in the pre-wash-out period and 2.4–25.2 μg/L during the wash-out period. Subjects adhered to the phenolic-free diet as judged by diet records and the relatively low concentrations of tyrosol in their urine. The amount of tyrosol recovered in the urine during the 24 h after olive oil ingestion was 281–708 μg. Urine tyrosol concentrations increased in response to acute dietary olive oil ingestion. Maximal tyrosol values were obtained in the 0–4 h urine samples after olive oil intake and decreased thereafter to reach basal values in the 8–12 h samples. The best fitting of the distribution of values was cubic (P < 0.001). Urinary concentrations and the cumulative urinary excretion of tyrosol after olive oil intake are presented in Fig. 1. Tyrosol was excreted mainly in its conjugated form. Only 6–11% of the total tyrosol excreted in urine was in its free form, suggesting extensive hepatic metabolism.

The recovery of tyrosol in urine samples was estimated from the two different calculations of the dose applied. Differences in the calculations of the amount of tyrosol administered derived from the different treatments to which

Fig. 1. Bioavailability of tyrosol: tyrosol urinary concentrations during the study.

(A), concentration of tyrosol in urine samples. Samples identified as pre-wash-out (samples collected before the start of the tyrosol-free diet), −24h (4th day of tyrosol-free diet, but 1 day before the olive oil was administered), and pre-dose (urine collected just before oil administration) are spot urine samples. (B), recovery of tyrosol after olive oil intake. Columns indicate μg of tyrosol recovered during each time period. Bars, SD. Line indicates cumulative tyrosol recovery (%).
olive oil was submitted. Direct methanolic water extracts without any further treatment or extracts submitted to an acidic treatment yielded similar amounts of tyrosol present in olive oil: 216 μg (in 50 mL). From this value, urinary recoveries of tyrosol were unrealistic because they were 131.5–327.8% of the dose administered. When the tyrosol content was estimated after NaOH digestion and sonication of olive oil, the total tyrosol content was 1650 μg (in 50 mL).

From this value, recoveries of tyrosol in the 24-h urine were 17–43%, with a mean recovery of 24.7% ± 8.5% (mean ± SD). These values are likely to be a better estimate of the real bioavailability of this compound. The differences in recoveries between the extraction procedures probably reflect differences in the extent to which HCl and NaOH hydrolyze esterified forms of tyrosol present in olive oil (15).

The results from the present study clearly show for the first time that urinary tyrosol concentrations are responsive to dietary intake of virgin olive oil. The method developed for urinary tyrosol determination has the sensitivity and accuracy to detect tyrosol in amounts typically present in the urine of nonsupplemented individuals. These results also underline the relevance of adequate estimation of administered phenolic doses in studies linking them to biological effects. In a recent report, recoveries of ~20% in urine from tyrosol-enriched olive oil were described (18). These recoveries were obtained after urine samples were submitted to enzymatic hydrolysis by β-glucuronidase. Taking into account the similar recoveries of tyrosol when different hydrolytic procedures (enzymatic vs acidic) were used, it is very likely that tyrosol is excreted in the urine mainly as the glucuronon conjugate. From a theoretical perspective, studies after the administration of pure phenolic compounds or enriched foods have some interest. However, studies in which phenolic ingestion is closer to typical dietary patterns may be more appropriate for estimating bioavailability. Slight details in our study design may have some relevance in experimental results. As an example, subjects who ingested olive oil with bread had a tyrosol recovery (20% ± 3%; n = 6) lower than that observed in those who directly drank the olive oil (37.5% ± 5.5%; n = 2).

The present work is the first description of the bioavailability of a phenolic compound present in olive oil administered in its natural form. We found that tyrosol was absorbed from virgin olive oil administered and that one part of the dose was excreted over the next 24 h. This study also shows the feasibility of controlled bioavailability studies of phenolic compounds from dietary sources in humans.

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References

Novel Mutation and Polymorphisms of the HMBS Gene Detected by Denaturing HPLC, Ching-Wan Lam, T Priscilla Miu-Kuen Poon, T Sai-Fan Tong, T Anthony Wing-Lo Lai, T Chi-Kong Lau, T Kin-Lam Choi, T Sau-Cheung Tiu, T Yan-Wo Chan, T and Chi-Chung Shek (1 Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, China; 2 Department of Pathology, Princess Margaret Hospital, Hong Kong, China; Departments of 3 Medicine and 4 Pathology, Queen Elizabeth Hospital, Hong Kong, China; * author for correspondence: fax 852-2636-5090, e-mail chingwanlam@cuhk.edu.hk)

Acute intermittent porphyria (AIP) is an autosomal dominant, inborn error of the metabolism of heme biosynthesis caused by partial deficiency of hydroxymethylbilane synthase (HMBS). This enzyme catalyzes the condensa-