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Human bioavailability of olive oil secoiridoids: screening of metabolites in plasma and urine using UPLC coupled with high resolution mass spectrometry

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Olive oil (OO) is the primary source of fat in the Mediterranean diet and has been associated with a lower incidence of coronary heart disease(1). Minor components of OO include the phenolic compounds such as phenolic alcohols (hydroxytyrosol and tyrosol) and secoiridoids. The beneficial effects of OO consumption on cardiovascular risk factors were recognized by the Food and Drug Administration and European Food Safety Authority (EFSA) and attributed to the high levels of monounsaturated fatty acids and phenolic compounds respectively. The EFSA claim, in particular, identified that daily consumption of 5 mg of hydroxytyrosol and derivatives (per 20 g OO dose) could protect low density lipoproteins particles from oxidative damage if consumed daily. OO phenolic compounds *in vivo* effects are related with their bioavailability and it is known that OO phenolics undergo Phase I and II metabolism reactions(1). The majority of biovailability studies conducted in this area have focused on hydroxytyrosol and tyrosol metabolism. The aim of our study was to apply liquid chromatography (UPLC) coupled with high resolution mass spectrometry (HRMS) to identify metabolites of secoiridoids that could be used as biomarkers of OO intake. Secoiridoids account for more than 50 % of phenolic content of OO and have been associated with biological activity(1).

Self-reported healthy participants ($n = 9$) ingested 50 mL of virgin olive oil (VOO) in a single dose, with bread. Fasting venous blood was collected at baseline (before VOO ingestion) and at 0·5 h, 1 h, 2 h, 4 h and 6 h after VOO ingestion. Urine samples were collected prior to VOO ingestion and at four different time points (0–4 h, 4–8 h, 8–15 h and 15–24 h). Samples were analysed by UPLC-HRMS. Principal component analysis was applied as a statistical approach to discriminate between samples before and after VOO intake. By plotting correlation loadings, it was possible to understand which variables were responsible for sample discrimination. Five plasma metabolites were selected, three of them were Phase I metabolites (hydrogenated, hydrated and hydroxylated forms of secoiridoids) and two mixed forms of Phases I and II metabolism – glucuronides of hydrogenated secoiridoids. Seven urinary secoiridoid metabolites were detected, three were excreted as glucuronides and the other four as glucuronides of Phase I metabolites. Variability was observed among volunteers suggesting individual differences in absorption and metabolism of phenolic compounds, Table 1. From this exploratory approach we demonstrate that secoiriridoid metabolites can be used as biomarkers of OO intake.

Table 1 Pharmacokinetic parameters of the main metabolites detected in plasma after VOO intake

Metabolite	C_{max} (ppm)		T_{max} (h)	
	Mean	SD	Mean	SD
elenolic acid + H_2	183·8	184·4	1·3	0·6
p-HPEA-EDA + H_2O	34·9	13·6	1·1	1·2
3,4-DHPEA-EA + OH	7·1	7·0	2·0	1·9
p-HPEA-EDA + H_2 + glucuronide	27·7	21·2	0·7	0·3
p-HPEA-EA + H_2 + glucuronide	19·8	15·0	1·5	1·3

1. Silva S., Combet E., Figueira M.E., *et al.* (2015) *Proc Nutr Soc* **74**, 268–281.