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File: ■ Olive (*Olea europaea*) Leaf
■ Menopause
■ Antioxidants

HC 021466-499

Date: June 30, 2014

RE: Pharmacokinetics of Olive Leaf Bioactives

García-Villalba R, Larrosa M, Possemiers S, Tomás-Barberán FA, Espín JC. Bioavailability of phenolics from an oleuropein-rich olive (*Olea europaea*) leaf extract and its acute effect on plasma antioxidant status: comparison between pre- and postmenopausal women. *Eur J Nutr*. June 2014;53(4):1015-1027.

Olive (*Olea europaea*) leaves contain the bioactive phenolic compounds oleuropein and hydroxytyrosol, shown to be bioactive in treating chronic diseases in clinical, in vivo, and in vitro studies. Oleuropein and hydroxytyrosol are reported to alleviate bone loss in in vivo and in vitro models of osteoporosis. As excess reactive oxygen species has been correlated with bone loss in postmenopausal women, this clinical, observational study addressed whether an oleuropein-rich olive leaf extract would impact antioxidant activity in pre- and postmenopausal women. The pharmacokinetics and urine excretion of phenolics in these subjects was also investigated.

Subjects were recruited through the Drug Research Unit of Maastricht, Maastricht, Netherlands, and were 18-75 years old. Women were subject to a physical exam including vital signs, height, weight, and blood collection for laboratory parameters. If the women were premenopausal, they underwent a pregnancy test, and those with prior hormone problems and permanent hormone modifications were excluded. Included premenopausal women were required to use oral contraception, and the study could not occur on the first 3 days of its usage.

Included postmenopausal women were required to have been in menopause for at least 2 years. Women were not permitted to consume hormones, "medicinal products," food supplements, anti-osteoporosis medication, or vitamins. Subjects were excluded if they smoked, had liver function problems, "abnormal" serum creatinine, body mass index (BMI) either < 18 or > 30 kg/m^2, were using medications, supplements, or had donated blood in the 4 weeks prior to the study. In total, 8 each of pre- and postmenopausal women participated.

The premenopausal women were 19-25 years old with an average BMI of 22.7 kg/m^2, and postmenopausal women, 51-66 years old, had an average BMI of 23.8 kg/m^2.

Baseline physical parameters were normal for all women. After fasting (except for water) from midnight, subjects arrived at the clinic the morning of the study day, and blood was collected within 2 hours of ingesting olive leaf extract (250 mg capsules taken with water in 1 minute). The source of the plant material was not provided. Blood was taken at 30 minutes, 1, 2, 3, 4, 6, 8, 12, and 24 hours. Urine was also taken and combined into 0-4, 4-8, 8-12, 12-16, and 16-24 hour samples. Subjects were fed at 2, 4, 9, and 13 hours, and had vital signs measured and any adverse side effects recorded at the end of the day.

High-performance liquid chromatography (HPLC), both alone and coupled to mass spectrometry, was used to detect and quantify phenolics in both the olive leaf extract and in plasma and urine samples. To assess antioxidant activity, the ferric reducing antioxidant power (FRAP) assay was used and malondialdehyde (MDA, an indicator of lipid peroxidation) concentrations were determined.

No adverse side effects were noted during the study, and all subjects completed the protocol. In plant material, oleuropein was the most abundant compound detected, with hydroxytyrosol found at smaller concentrations. Metabolites found in plasma and urine consisted of methylated, sulfated, or glucuronidated compounds of olive leaf phenolics and other compounds. None of the parent or metabolite compounds were found in plasma or urine at baseline. Compounds showed comparable absorption patterns in both pre- and postmenopausal women, with hydroxytyrosol glucuronide arriving at maximum peak concentration (C_{max}) first and hydroxytyrosol sulfate last, and all metabolites reaching C_{max} within the first 35-75 minutes. All metabolites found in the serum were also detected in the urine.

The C_{max} of hydroxytyrosol glucuronide, hydroxytyrosol sulfate, oleuropein aglycon glucuronide, and an oleuropein aglycon derivative were significantly more elevated in postmenopausal women than premenopausal women ($P<0.05$). Additionally, the excretion of hydroxytyrosol sulfate and hydroxytyrosol sulfoglucuronide was significantly higher in premenopausal women than postmenopausal women ($P<0.05$). In plasma, there was a significant positive correlation with the area under the curve from beginning timepoint to ending timepoint (AUCall) of hydroxytyrosol glucuronide and oleuropein aglycon glucuronide ($P<0.05$), and hydroxytyrosol sulfate and oleuropein aglycon glucuronide ($P<0.05$). In urine, there was a significant positive correlation with hydroxytyrosol glucuronide AUCall and that of hydroxytyrosol sulfoglucuronide ($P=0.03$), homovanillic alcohol glucuronide ($P<0.05$), oleuropein aglycon glucuronide ($P=0.04$), and an oleuropein aglycon derivative ($P=0.01$).

In urine, the total amount recovered, AUCall, and AUC∞ (final measured concentration divided by elimination rate constant) of hydroxytyrosol sulfoglucuronide were significantly positively correlated with this compound's plasma AUCall, AUC∞, and C_{max} ($P<0.05$). These statistical relationships were observed when analyses were done of all women and pre- and postmenopausal women separately. Plasma FRAP activity and MDA concentrations were not different between pre- and postmenopausal women at baseline. At 30 minutes after ingestion of the olive leaf extract, postmenopausal women had significantly less MDA than premenopausal women ($P=0.016$).

In summary, this study reports the detection of hydroxytyrosol glucuronide and hydroxytyrosol sulfoglucuronide in plasma, which is somewhat contradictory with other studies. It is mentioned that the correlations among compounds in plasma may reflect

compound modifications in the gastrointestinal tract. Additionally, hormonal changes may be one factor among others in the significantly different amounts of compounds seen in premenopausal compared to postmenopausal women. The lesser amount of MDA in postmenopausal women 30 minutes after consumption of oleuropein-rich olive leaf extract may suggest its use in alleviating oxidative damage; however, the bioactivity of these compounds and metabolites in chronic disease needs further investigation.

—Amy C. Keller, PhD