Nutrient Metabolism

Oleuropein, an Antioxidant Polyphenol from Olive Oil, Is Poorly Absorbed from Isolated Perfused Rat Intestine

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ABSTRACT  Epidemiological studies have shown that the incidence of heart disease and certain cancers is lower in the Mediterranean region. This has been attributed to the high consumption of olive oil in the Mediterranean diet, which contains polyphenolic compounds with antioxidant activity. Although many in vitro studies have been performed to elucidate mechanisms by which these compounds may act, there are virtually no data relating to their fate after ingestion. Therefore, we decided to investigate the intestinal absorption of one of the major olive oil polyphenolics, oleuropein. To do this, a novel in situ intestinal perfusion technique was developed, and the absorption of oleuropein was studied under both iso-osmotic and hypotonic luminal conditions. Oleuropein was absorbed, with an apparent permeability coefficient \( P_{app} \) of \( 1.47 \pm 0.13 \times 10^{-6} \) cm/s (±SE) observed under iso-osmotic conditions. The mechanism of absorption is unclear but may involve transcellular transport (SGLT1) or paracellular movement. Under hypotonic conditions, the permeability of oleuropein was significantly greater (\( 5.92 \pm 0.49 \times 10^{-6} \) cm/s, \( P < 0.001 \)). This increase is thought to be due to an increase in paracellular movement facilitated by the opening of paracellular junctions in response to hypotonicity. Overall, we determined that the olive oil polyphenolic oleuropein can be absorbed, albeit poorly, from isolated perfused rat intestine. Therefore, it is possible that it or its metabolites may confer a positive health benefit after the consumption of olive oil, most likely via an antioxidant mechanism. J. Nutr. 130: 2996–3002, 2000.

KEY WORDS: oleuropein • olive oil • absorption • Mediterranean diet • bioavailability • rats

Epidemiological studies have shown that the incidence of coronary heart disease (Keys 1970 and 1995) and certain cancers (especially prostate and colon; Martin-Moreno et al. 1994) is lower in the Mediterranean region. This has been attributed to the Mediterranean diet, which is largely vegetarian in nature and includes the consumption of large quantities of olive oil (Keys 1995). In addition to being the predominant source of fat in the Mediterranean diet (Petroni et al. 1995), olive oil is a source of at least 30 phenolic compounds, many of which have antioxidant properties (Kohyama et al. 1997, Manna et al. 1997, Saija et al. 1998, Visioli et al. 1995, Visioli and Galli 1994, Wiseman et al. 1996).

Many in vitro studies have been performed to elucidate mechanisms by which polyphenolic compounds may act to confer positive health effects. A number of these studies (Beecher et al. 1999, Manna et al. 1997, Visioli et al. 1998) have shown that polyphenolic compounds possess strong radical scavenging activity and appear to be at least, as if not more, effective than other important dietary antioxidants, such as vitamin C and \( \alpha \)-tocopherol. Because the uncontrolled production of free radicals has been hypothesized as contributing to the pathogenesis of diseases such as coronary heart disease and cancer (Visioli et al. 1998), the ability of polyphenols to scavenge free radicals could be important in explaining how polyphenols may play a role in preventing these diseases.

Studies have also demonstrated that polyphenolic compounds are potent inhibitors of LDL oxidation in vitro (De Whalley et al. 1990, Visioli 1995, Visioli and Galli 1994). Inhibition of LDL oxidation is important because the in vivo oxidation of LDL is strongly linked to the formation of atherosclerotic plaques, which in turn contribute to the development of coronary heart disease. Polyphenols have also been found to have activity in breaking peroxidative chain reactions and preventing metal ion chelation, processes that have also been linked to the pathogenesis of heart disease and cancer (Manna et al. 1997). In addition to their antioxidant properties, polyphenolic compounds have been shown to exhibit a range of indirect actions that may be beneficial to health, including the inhibition of enzymes involved in the inflammatory process (Kohyama et al. 1997, Laughton et al. 1991, Middleton and Kandoswami 1992), the inhibition of platelet aggregation (Petroni et al. 1995) and inhibition of the metabolic activation of procarcinogens (Stavric 1994). Surprisingly, despite the myriad of potential health benefits of the olive oil polyphenolics, there are virtually no data relating to their biological fate after oral ingestion. Bai et al. (1998) studied the oral fate of hydroxytyrosol (an olive oil polyphenolic) by measuring its concentration in plasma after oral dosing of rats and found that this compound was rapidly but poorly absorbed after a single oral dose. Another study (Visioli et al. 1999) investigated the absorption of two olive oil phenolic constituents, tyrosol and hydroxytyrosol, after
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MATERIALS AND METHODS

Chemicals and reagents. Oleuropein was obtained from the Indofine Chemical Company, Inc. (Belle Mead, NJ). -Coumaric acid was purchased from ICN BioMedicals Inc. (Aurora, OH). The radiolabeled (14C) marker polyethylene glycol (PEG)2 [average molecular weight (MW) 4000], which was used as an indicator of water flux across the intestine during in situ studies, was purchased from NEN Life Science Products (Boston, MA). Verapamil and mannitol were obtained from Sigma Chemical Co. (St. Louis, MO). All salts used in the preparation of perfusion media were of analytical grade and were purchased from BDH Chemicals (Poole, UK). Sulfuric acid was purchased from Ajax Chemicals (New South Wales, Australia). All water was freshly produced with a Milli-Q water purifier system from Millipore/Waters (New South Wales, Australia).

Blood-to-plasma concentration ratio of oleuropein. The blood-to-plasma concentration ratio of oleuropein had to be determined because an estimate of this variable was required for the mathematical calculation of the intestinal permeability of oleuropein. A method similar to that described by Milne et al. (1993), was used, in which preheated rat whole blood (37°C) was spiked to a concentration of 5 mmol/L using an aqueous oleuropein solution, and samples were mixed using a rotary mixer for 20 min and then centrifuged for 10 min at 3000 × g. The resulting plasma layer was removed, and three 500-µL aliquots were transferred to 10-mL tubes. Samples were then extracted into ethyl acetate (6 mL), centrifuged and evaporated under a nitrogen stream. After reconstitution in 200 µL of Milli-Q water, samples were analyzed with the HPLC method described later.

Oxotanol-water partition coefficient of oleuropein. This variable was determined using a modification of the method described by Hencii et al. (1995) and was important in predicting the extent to which oleuropein is likely to permeate the intestine. After saturation of the aqueous phase with n-octanol, oleuropein samples (25 µmol/L) were prepared by spiking the saturated aqueous phase (1 mL) with oleuropein solution. n-Octanol (1 mL) was added to each sample, and tubes were agitated for 20 min. After centrifugation, the aqueous layer was removed, and oleuropein concentrations were analyzed using HPLC.

Intestinal perfusion technique. The isolated perfused intestine method that we used was a minor adaptation of the validated method of Blanchard et al. (1990) from the model described by Windmueller and Spaeth (1981). Studies using this technique were approved by the Animal Ethics Committee of the Institute of Medical and Veterinary Science, Adelaide, South Australia, in February 1990. The perfusion medium used was an iso-osmotic medium called GoLYTELY (Davis et al., 1980), which contained 20 mmol NaHCO3/L, 25 mmol NaCl/L, 10 mmol KCl/L, 40 mmol NaSO4/L, 80 mmol mannitol/L and 1.25 mmol PEG 4000/L. This medium was chosen because it is associated with no net movement of fluid or electrolytes across the intestine, eliminating any effect of solvent flux on the absorption of oleuropein (Davis et al., 1980).

The surgical technique is illustrated in Figure 2. Before the start of the experiment, the GoLYTELY solution was adjusted to pH 2 with phosphoric acid. Then, 14C-PEG 4000 (250 µL of 0.37 GBq/L), a radiolabeled nonabsorbable marker used to assess net fluid movement during the experiment, was added to 100 mL of the GoLYTELY, which was placed in a water bath at 37°C. Collection of donor blood from two rats (~12-week-old, ~500-g male Sprague-Dawley) was then carried out, with 15–22 mL of blood typically being collected from each donor animal. Surgery was carried out in accordance with the methods described by Blanchard et al. (1990) to prepare the animal for the perfusion. In contrast to Blanchard et al. (1990), who recirculated their analyte-containing perfusate, flow of perfusate in this experiment was single pass (out-flowing perfusate discarded), and an aqueous solution of oleuropein was infused into the flow of blank perfusate just before it entered the intestinal segment. This technique served to prevent chemical degradation of the analyte in the perfusate.

After equilibration of the system with perfusate for 5 min, infusion of the aqueous oleuropein solution (1 mmol/L) was begun at a rate of 50 µL/min, and equilibration continued for an additional 3 min. The concentration and infusion rate of the oleuropein solution were determined based on the detection limit of the assay, with the

FIGURE 1 Structures of polyphenolic compounds from olive oil [hydroxytyrosol (A) and oleuropein glycoside (B)] and the apple tree [phlorizin (C)].

FIGURE 2 Experimental set-up used for the in situ intestinal perfusions (adapted from Blanchard et al. 1990).
prediction of ∼1% absorption from the small intestine. Samples were then taken for 40 min. The sampling regimen consisted of collecting the entire mesenteric outflow into preweighed 1.5-mL vials. Blood flow rate was determined by weighing the tubes and using a blood density of 1.054 kg/L (Blanchard et al. 1990), and the infusion rate of donor blood was adjusted accordingly. Blood samples were pooled into 5-min intervals, and at the completion of each interval, the samples were spun down in a microcentrifuge, and the resulting plasma was removed and frozen in solid CO₂. Both in-flowing and out-flowing perfusate samples (120 μL) were also taken every 5 min starting at 2.5 min, with 50 μL placed into a scintillation vial to allow assessment of the radiolabeled nonabsorbable marker, and an additional 50 μL placed into a 1.5-mL vial and frozen in solid CO₂ for subsequent evaluation of oleuropein content. After completion of the perfusion, the length of the perfused segment of intestine was measured using silk suture to allow its surface area and volume to be determined.

**Pharmacokinetic calculations.** The following calculations were made under the assumption that no net water movement occurred during the perfusion (Kim 1996).

The apparent permeability coefficient, $P_{app}$, was calculated using the model described by Lennerñas (1995):

$$P_{app} = \frac{dM_{int}}{dt} = \frac{A_r \cdot C_r}{A_T \cdot C_i}$$

where $dM_{int}/dt$ is the rate of absorption, $A_r$ is the total epithelial surface area of the perfused segment of intestine and $C_i$ is the concentration of compound entering the intestine. The rate of absorption was calculated as $Q \cdot C_m \cdot R$, where $Q_m$ is the blood flow rate over the collection period, $C_m$ is the concentration of compound in mesenteric blood during that period and $R$ is the blood-to-plasma concentration ratio of the compound. $A_T$ was calculated using the equation for the area of a cylinder ($A_T = 2\pi r L$), where $L$ is the length of the perfused segment of intestine and $r$ is the internal radius, 0.2 cm (Kim 1996).

Given a value for the $P_{app}$, a theoretical oral intestinal bioavailability may be predicted mathematically using the following equation (Lennerñas 1995):

$$F_{pred} = 1 - e^{-\left(\frac{P_{app} \times \text{intestinal surface area} \times \text{mean intestinal transit time}}{\text{intestinal volume}}\right)}$$

where $F_{pred}$ is the predicted intestinal bioavailability, intestinal surface area is calculated using the formula for a cylinder ($2 \cdot \pi \cdot r \cdot L$), mean intestinal transit time is estimated to be 2.5 h (Varga 1976) and intestinal volume is calculated as the volume of a cylinder ($\pi \cdot r^2 \cdot L$). The radius of the rat intestine is estimated at 0.2 cm (Kim 1996), and the length is estimated at 103 cm (Hebel and Stromberg 1976): therefore, intestinal surface area was calculated at 129.4 cm², and intestinal volume was calculated at 12.94 cm³.

**Study design.** The control series consisted of five male Sprague-Dawley rats, and it was designed to assess the intestinal absorption of oleuropein using the iso-osmotic GoLYTELY perfusion medium in single pass through the intestine. Another series ($n = 4$) examined the effect of fluid flux on the absorption of oleuropein. This series of experiments differed from the control series in that the perfusion medium used was pure Milli-Q water and the oleuropein was dissolved in the perfusate reservoir rather than being infused into the stream of perfusate. A subsequent series of pilot experiments consisting of two animals investigated the effect of a glucose-transporter inhibitor on the absorption of oleuropein by incorporating phlorizin (2 mmol/L) into the oleuropein infusion solution. Finally, the effect of verapamil (a commonly used inhibitor of P-glycoprotein) on the absorption of oleuropein was investigated in a series of three pilot experiments. Two perfusate reservoirs were used: one containing blank GoLYTELY medium and the other containing GoLYTELY medium with verapamil dissolved at a concentration of 200 μmol/L (a concentration previously found to be effective in inhibiting intestinal P-glycoprotein; Aungsr and Saitoh 1996). The first half of the experiment was performed using the blank GoLYTELY perfusate to establish a control level of oleuropein absorption; then, the intestine was perfused with the verapamil-containing perfusate (using a multidirectional tap to change the perfusate source). Absorption of oleuropein was compared over the two periods to determine the effect of verapamil.

**Analysis of oleuropein in plasma by HPLC.** The lack of in vivo work performed with the olive oil polyphenolics meant that there was no method established in the literature for their determination in biological matrices; therefore, a novel assay was developed. Before extraction into ethyl acetate, 80 μL of internal standard (1 mmol p-coumaric acid/L) was added to each 1-mL plasma sample. Ethyl acetate (6 mL) was added, and each sample was mixed using a rotary mixer. Next, samples were centrifuged, and the organic layer was separated and evaporated to dryness in a vortex evaporator, reconstituted in 200 μL of Milli-Q water and then injected onto the HPLC system. The HPLC system consisted of an LC-10AS single-piston pump, a SILD-10A autosampler and an SCL-10A system controller [Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Adelaide, Australia]. The detector was a Jasco 821-FP Spectrophorimeter (Japan Spectroscopic Co., Ltd., Hachijo City, Japan), and peaks were integrated using a C-R6A Chromatopac Integrator (Shimadzu). HPLC conditions consisted of a mobile phase of water/acetonitrile (80:20), adjusted to pH 2.9 with H₂SO₄, and an Alltima C₈ Rocket column (3 μm packing, 53 mm × 7 mm; Alltech Associates (Australia) Pty. Ltd., New South Wales, Australia). Then, 150 μL of each sample was injected onto the column, with a flow rate of 1.2 mL/min. Oleuropein and p-coumaric acid were detected by fluorescence, with excitation/emission wavelengths of 280/312 and 319/405 nm, respectively. The compounds eluted at ∼6.5 min (p-coumaric acid) and ∼14 min (oleuropein). A standard curve was also incorporated into each analytical run, with plasma standards having nominal oleuropein concentrations of 0.5, 1, 2.5, 5, 10, 50 and 100 μmol/L, whereas aqueous standards had nominal concentrations of 10, 20, 50, 100 and 200 μmol/L. Quality control samples of nominal concentrations of 5, 10, 50 and 60 μmol/L were also incorporated into the plasma standard curves. The assay was found to be accurate and reproducible over the concentration range, with maximum interday variability of 17% and 5.9% at the lower and higher ends of the curve and intraday variability of 2.7% and 6.5% at the lower and upper ends of the curve (using independent quality control samples of 2 and 80 μmol/L). The limit of quantification was 0.5 μmol/L.

**Statistical analysis.** Data were analyzed by one-way ANOVA and by unpaired t tests, assuming equal variance. Differences were considered significant at $P < 0.05$.

**RESULTS**

**Background studies.** The mean blood-to-plasma concentration ratio was 0.955 ± 0.119, whereas the octanol-water partition coefficient (D) was 1.57 ± 0.052, giving a logD value of 0.196 ± 0.014. Based on preliminary in vitro stability studies, oleuropein degraded significantly in GoLYTELY medium but was stable in Milli-Q water. Logarithmic transformation of the data indicated that the degradation followed apparent first-order kinetics, with a degradation rate constant of −0.023 min⁻¹ ($r^2 = 0.962$) (Fig. 3). Further investigation of the stability of oleuropein in various media under a range of pH conditions suggested that the degradation was pH dependent, with degradation occurring at pH 7 but not at pH 5.2 (Fig. 4). Assessment of the stability of oleuropein for 2 min followed by freezing in solid CO₂ found that there was no significant degradation over this time period in GoLYTELY medium, validating the use of snap freezing to preserve oleuropein concentrations.

**In vivo absorption studies.** Assessment of blood flow during each perfusion was important, because poor or widely fluctuating blood flow could be a source of error using our model, and could indicate problems with functionality of the
perfused intestinal segment. Both blood flow and absorption rate (assessed as the absorption rate constant) remained fairly consistent throughout the experiments (Fig. 5), indicating the maintained functionality of the perfusion system. The concentrations of 14C-PEG 4000 and oleuropein in both in- and out-flowing perfusate were also plotted together to assess any trends that may have indicated net water flux during the experiment. A typical plot is shown in Figure 6 and demonstrates no significant trends indicative of fluid movement during the perfusions. A summary of results from the in situ studies is shown in Table 1.

Absorption from an iso-osmotic lumen. Based on control experiments, the $P_{\text{app}}$ of oleuropein was $1.47 \pm 0.13 \times 10^{-6}$ cm/s ($\pm$SE). There was no significant difference ($P > 0.05$) in permeability between the time points, indicating that the absorption of oleuropein did not change with time. The rate of absorption remained fairly constant throughout the control series, with $0.19 \pm 0.2\%$ of the perfused dose being absorbed during each time interval.

Absorption from a hypotonic lumen. Absorption of oleuropein under hypotonic luminal conditions was $5.92 \pm 0.49 \times 10^{-6}$ cm/s ($\pm$SE) (Table 1), which was significantly higher ($P < 0.001$, unpaired t test assuming equal variance) than that found using the iso-osmotic perfusate in the control series. The absorption rate throughout the perfusions was again fairly constant, with $0.49 \pm 0.05\%$ of the perfused dose being absorbed during each time interval.

Effect of verapamil and phlorizin on oleuropein absorption. The addition of verapamil or phlorizin to the perfusion solution had no significant effect on the intestinal absorption of oleuropein (unpaired t test assuming equal variance) in the in situ model used in our pilot experiments. Mean $P_{\text{app}}$ values for oleuropein before and after the addition of verapamil to the perfusion solution were $2.22 \pm 1.71$ and $1.91 \pm 1.30 \times 10^{-6}$ cm/s ($\pm$SE), respectively, whereas the $P_{\text{app}}$ for oleuropein after perfusion with the phlorizin-containing perfusate was $1.11 \pm 0.38 \times 10^{-6}$ cm/s ($\pm$SE).

Mathematical estimation of intestinal bioavailability. Using the second equation, the intestinal bioavailability of oleuropein under conditions of no water flux across the intestine was calculated to be $0.124$ (12.4%). However, this does not take into account the effect of metabolism on the amount of compound that reaches the systemic circulation. When data from the hypotonic perfusate experiments were incorporated into this equation, the intestinal bioavailability was predicted to be $41.3\%$.

FIGURE 3 Concentration of oleuropein remaining versus time when dissolved in Milli-Q water and GoLYTELY medium. The compound was quite stable in the water and underwent first-order degradation in the GoLYTELY medium.

FIGURE 4 Concentration of oleuropein remaining versus time when dissolved in Milli-Q water and GoLYTELY medium. The compound was quite stable in the water and underwent first-order degradation in the GoLYTELY medium.

FIGURE 5 A typical representation of blood flow and the absorption rate constant over the course of a single intestinal perfusion (blood flow on left y-axis, absorption rate constant on right y-axis). The consistency of both parameters indicates that the functionality of the perfusion system was maintained throughout the experiment.

FIGURE 6 Concentration of oleuropein remaining versus time when dissolved in Milli-Q water and GoLYTELY medium. The compound was quite stable in the water and underwent first-order degradation in the GoLYTELY medium.

FIGURE 5 A typical representation of blood flow and the absorption rate constant over the course of a single intestinal perfusion (blood flow on left y-axis, absorption rate constant on right y-axis). The consistency of both parameters indicates that the functionality of the perfusion system was maintained throughout the experiment.

FIGURE 6 Trends in PEG 4000 and oleuropein concentrations in in- and out-flowing perfusate (PEG concentration on left y-axis, oleuropein concentration on right y-axis). Each point on the left represents a different time point during the experiment, and lines join these values to the corresponding values for out-flowing perfusate for that time point.
TABLE 1

Summary of apparent permeability coefficients ($P_{app}$) for oleuropein measured in isolated perfused rat intestine under various experimental conditions

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>$n$</th>
<th>$P_{app} \times 10^{-6}$ cm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>1.47 ± 0.13</td>
</tr>
<tr>
<td>Hypotonic lumen</td>
<td>4</td>
<td>5.92 ± 0.49*</td>
</tr>
<tr>
<td>Verapamil present</td>
<td>3</td>
<td>1.91 ± 1.30</td>
</tr>
<tr>
<td>Phlorizin present</td>
<td>2</td>
<td>1.15 ± 0.38</td>
</tr>
</tbody>
</table>

* Values are means ± se.
  * Different from control, $P < 0.01$ (unpaired t tests assuming equal variance).

DISCUSSION

Absorption from an iso-osmotic intestinal lumen. Results from this study showed that when the intestine is perfused in single pass with an iso-osmotic solution (Golytely medium) containing oleuropein, the compound is absorbed with a $P_{app}$ of $1.47 \pm 0.13 \times 10^{-6}$ cm/s (±se). This value is similar to values derived experimentally using this in situ model for clinically used drugs such as frusemide ($5.0 \pm 4.0 \times 10^{-6}$ cm/s; Wininwater et al. 1998), atenolol ($1.95 \pm 0.79 \times 10^{-6}$ cm/s) and hydrochlorothiazide ($1.71 \pm 0.61 \times 10^{-6}$ cm/s; Berry 1999) and classifies oleuropein as a poorly permeable compound. Because molecular modeling using a computer modeling package called Mopac (Fujitsu, Tokyo, Japan) found the minimum spherical radius of oleuropein in aqueous solution (0.828 nm) to be larger than the estimated radius of the paracellular junctions in the ileum (0.54 nm; Berry 1999), it appears that absorption of oleuropein under these conditions occurs predominantly via the transcellular route. Given that oleuropein is quite polar (logD 0.196), it seems unlikely that it would readily diffuse through the lipid bilayer of the epithelial cell membrane, making absorption via a transporter the most likely mechanism under these conditions. Being a glycoside, oleuropein could possibly access a glucose transporter, of which three have been identified in the epithelial cells of the small intestine. Two of these are facilitated-diffusion glucose transporters (Glut2 and Glut5), which act like channels to transport glucose passively, whereas the other is a sodium-dependent glucose transporter (SGLT1), which uses active transport to move glucose across a concentration gradient (Takata 1995). Both Glut 5 and SGLT1 are found on the apical side of intestinal epithelial cells, but Glut5 appears to be specific for the transport of fructose and therefore is unlikely to be involved in the movement of oleuropein into epithelial cells (Burant et al. 1992, Kane et al. 1997). The third intestinal glucose transporter, GluT2, has been localized to the basolateral side of epithelial cells and is likely to mediate the movement of glucose and like substrates from epithelial cells into the circulation (Kayano et al. 1990, Nomoto et al. 1998).

Given that oleuropein contains a conjugated glucose moiety, it would be feasible for it to access the SGLT1 transporter and be moved into the epithelial cell and then move into the blood stream via the Glut2 transporter on the basolateral side of the cell. This theory of glucose transporter–mediated absorption of oleuropein is supported in the literature, where Holman et al. (1995) observed the absorption of quercetin glycoside (a similar polyphenolic) after its oral administration to human ileostomy patients and postulated a mechanism involving active sugar transporters. Additional work with quercetin glycosides (Gee et al. 1998) has shown that these compounds are capable of interacting with the SGLT1 transporter, further supporting its role in the absorption of polyphenolic glycosides from the intestine, whereas work by Ohnishi et al. (1998) with another glycoside (p-nitrophenyl-β-D-glucopyranoside) has shown that this compound is absorbed from the rat small intestine via a carrier-mediated transport system.

However, this theory is not supported by preliminary work performed in the present study with an inhibitor of SGLT1, phlorizin, in the in situ perfusion model. At the concentrations used in the present study, phlorizin was observed to have no significant effect on the permeability of oleuropein under iso-osmotic luminal conditions, suggesting that SGLT1 is not involved in the absorption of oleuropein. This observation suggests that under iso-osmotic luminal conditions, oleuropein must move across the intestine either paracellularly (despite its large size) or via transcellular passive diffusion (despite its polarity). However, the involvement of SGLT1 cannot be discounted completely, because studies reported here represent only preliminary investigations. Considering the similarity in the structures of oleuropein and phlorizin, it is possible that oleuropein actually has a higher affinity for the SGLT1 transporter than does phlorizin; thus, the presence of phlorizin would have little effect on the absorption of oleuropein from the intestine in the isolated perfused intestine model. The involvement of the efflux pump P-glycoprotein in the absorption of oleuropein from the intestine was also not supported in pilot experiments performed as part of this study.

Although molecular modeling results from this study suggest that the paracellular movement of oleuropein under iso-osmotic luminal conditions is unlikely, it is not entirely unfeasible. Despite the fact that the minimum spherical radius of oleuropein has been estimated to be larger than the radius of the paracellular junctions in the ileum, it may be incorrect to consider the paracellular “pores” as cylindrical. It is probably more correct to consider this pathway as having only one truly restrictive dimension, the distance between adjacent epithelial cells (0.54 nm in the ileum), whereas the other two dimensions correspond more closely to the length and breadth of the epithelial cells. Therefore, it may be appropriate to consider the minimum cuboidal dimensions of oleuropein rather than its spherical dimensions. These have been estimated using computer modeling (0.861 × 1.25 × 1.16 nm) and reveal that there is one plane in which oleuropein is smaller than the distance between two epithelial cells (0.861 nm compared with a distance between cells of 1.08 nm). Therefore, it may be possible for oleuropein to fit between the cells if it is orientated correctly. However, because the number of spatial orientations in which oleuropein could pass through the paracellular junctions would be much smaller than the number in which oleuropein could not fit through, the contribution of the paracellular pathway to the absorption of oleuropein is likely to be minimal. Another possible explanation for the existence of paracellular absorption of oleuropein stems from recent work by He et al. (1998), who suggested that a small subpopulation of paracellular junctions exists that are larger than the normal junctions. It may be that these pores are large enough to accommodate the ready passage of oleuropein, facilitating the intestinal permeability observed in the control series of experiments via the paracellular route.

Absorption from a hypotonic intestinal lumen. The effect of fluid flux on the absorption of high-molecular-weight hydrophilic compounds (like oleuropein) from the intestine is a point of some conjecture. Most studies that used animal models show that the movement of water across the intestine results in increased absorption of these compounds (Fine et al.
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Although under normal iso-osmotic luminal conditions oleuropein is poorly absorbed, its absorption can be significantly increased by solvent flux through paracellular junctions, made possible by hypotonic conditions in the intestinal lumen. The data also suggest that hypotonic conditions are capable of opening the paracellular junctions to such an extent that even a molecule ~60% larger than the junction can pass through in significant amounts. The small population of larger intercellular junctions described by He et al. (1998) may also play a role in this increased absorption due to solvent drag. It must be remembered, however, that in the experiments described here, the hypotonic environment was sustained for the duration of the perfusion, whereas after the administration of a hypotonic solution under normal physiological conditions, the body most likely rapidly adjusts the luminal environment such that isotonicity is reestablished. Therefore, the observation of increased oleuropein absorption under hypotonic conditions may be largely irrelevant in vivo. However, it has been suggested by other authors (Pappenheimer 1987) that the presence of glucose or amino acids in the intestinal lumen after a meal stimulates water flux via the opening of paracellular junctions, and it may be that this mechanism has a similar effect on the absorption of oleuropein as does the use of a hypotonic solution.

In conclusion, it appears that oleuropein is capable of permeating the intestine, but the amount of oleuropein that reaches the systemic circulation unchanged is likely to be small. It may be that the active components contributing to a beneficial health outcome are those released via oleuropein metabolism, in either the intestine or the liver. Alternatively, oleuropein may be acting locally to protect other dietary antioxidants, such as vitamin C and α-tocopherol, from degradation in the intestine. This would contribute to a beneficial increase in the total antioxidant status of the body by enhancing the bioavailability of these other dietary antioxidants. Finally, it must be remembered that we examined the absorption of oleuropein from an aqueous solution rather than from the oily matrix in which it would be presented to the intestine after a meal. It is possible that absorption via the lymphatic system may occur when oleuropein is presented in an oily matrix, although being a hydrophilic compound it is more likely to move out of the oil into the aqueous medium of the intestine after oral ingestion. These experiments also excluded bile, which would normally be present in the intestine after a meal. Bile has been shown to be capable of opening the paracellular junctions in the intestine (Fricker et al. 1996, Swenson et al. 1994, Yamamoto et al. 1996), potentially increasing absorption of oleuropein via the paracellular route. Therefore, it may be that the absorption of oleuropein after oral ingestion is actually higher than that predicted in this study using the in situ intestinal perfusion model described.

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LITERATURE CITED


