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Pharmacokinetic Study of *trans*-Resveratrol in Adult Pigs

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A number of pharmacokinetic studies have shown marked differences in the plasma metabolic profile of resveratrol (RES) between humans and animals and between individuals of the same species, which complicates the identification of the putative bioactive metabolites responsible for the beneficial effects of RES. On the basis of the physiological similarity between pigs and humans, the aim of this work was to characterize the metabolic profile and pharmacokinetics of RES in the plasma of pigs and to compare this to values reported in humans. RES (5.9 mg/kg of body weight) was orally administered to pigs. The following metabolites were identified in plasma using HPLC-MS/MS: RES-diglucuronide (**1**), two isomers of RES-sulfoglucuronide (**2**, **3**), two isomers of RES-glucuronide (**4**, **5**), RES-sulfate (**6**), and RES. The most abundant metabolites were **2**, **5** (identified as resveratrol 3-*O*-glucuronide), and **6**. The t_{max} ranged from 0.9 h for compounds **2** and **5** to 2 h for compound **3**. The highest C_{max} value was 2223 ng/mL (5.5 μM) for metabolite **5**, which was 2.6-, 3.3-, and 12-fold higher than that for metabolites **6**, **2**, and **3**, respectively. Peak plasma levels of RES (53 ng/mL; 0.23 μM) were detected 0.5 h after RES ingestion. Apart from the low levels of RES aglycone, the RES metabolic profile in pigs differs from that found in humans. The identification of the actual active RES metabolites is a challenge that requires more complex studies which should take into account many possible influencing factors such as age, gender, and methodological approaches.

KEYWORDS: Swine; bioavailability; polyphenol; metabolism; plasma; metabolites

INTRODUCTION

The polyphenol resveratrol (3,5,4′-trihydroxy-*trans*-stilbene; RES) has been reported to slow the progression of a number of pathologies such as cancer, cardiovascular diseases, and inflammation as well as to increase the lifespan of several organisms (*1*). RES naturally occurs in red wine, grapes, peanuts, and some berries; however, the consumption of this compound as part of the Western diet is very low (*2*). For example, the estimated daily intake of RES in the Spanish population is 0.2 mg/day, which matches the pattern of red wine intake (*3*). This low dietary intake may be increased by the regular intake of RES-enriched nutraceuticals (*2*). Nutrapharmacology is a recently coined term that refers to the study of therapeutic effects derived from the consumption of nutraceuticals. The cardiovascular nutrapharmacology of RES has been recently reviewed (*4*) and suggests that this compound is not only a health-beneficial dietary compound but a drug with promising therapeutic effects.

Despite its well-established beneficial effects, RES has a very limited bioavailability, and the levels of this molecule detected in plasma are very low (*5*, *6*). In this context, the cause−effect relationship between RES concentration in the systemic bloodstream and the biological effects reported is still a conundrum (*7*), and the mechanisms by which RES may exert its many effects are not yet fully understood (*1*). It is essential to establish the metabolism of this compound and to determine the metabolic species formed in vivo that may contribute to or be responsible for the observed effects. The bioavailability and pharmacokinetics of RES have been already approached both in humans and in animal models (*6*,*8*−*10*) and have been the subject of some reviews (*5*, *11*, *12*). These studies show that, in general, the metabolism of rodents is far from that of humans and that there are important differences between species. To identify the putative bioactive metabolites of RES and to contribute to the clarification of the mechanisms by which RES may exert its effects, it is recommended to carry out pharmacokinetic studies in animal models more similar to humans. From the anatomy and physiology points of view, the pig gastrointestinal system is more similar to the human digestive system than that of rodents (*13*). Although the size and habits of pigs require special animal facilities as well as the help of experts in the handling and care of these animals, the use of pigs has been reported to be a valuable tool when looking at the metabolism of nutrients and, in particular, to that of polyphenols. Several research groups have used the pig to describe the pharmacokinetics,

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metabolism, and tissue distribution of polyphenols such as anthocyanins (*14*−*16*), quercetin (*17*−*19*), and ellagitannins (*20*).

The aim of the present study was to characterize the metabolic profile of RES in the plasma of pigs and to determine the pharmacokinetic parameters of the main individual resveratrol metabolites after oral administration of RES in adult pigs.

MATERIALS AND METHODS

Materials. *trans*-Resveratrol (resveratrol, RES, 3,5,4′-trihydroxy-*trans*-stilbene, >99% purity) was obtained from Sigma-Aldrich (St. Louis, MO). Resveratrol 3-*O*-glucuronide was obtained according to the method of Lucas et al. (*21*). Tiletamine-zolazepam (Zoletil 50) was purchased from Virbac España S.A. (Esplugues de Llobregat, Spain) and isoflurane (Isoba Vet) from Schering-Plough (Brussels, Belgium). Acetonitrile, methanol, and formic acid (HPLC grade) were purchased from Scharlau Chemie S.A. (Sentmenat, Spain).

Animal Study Design. Experiments followed a protocol approved by the local animal ethics committee and the local government. All experiments were in accordance with the recommendations of the European Union regarding animal experimentation (Directive of the European Council 86/609/EC). Housing and animal interventions were carried out in the Veterinary Teaching Farm of the University of Murcia (Murcia, Spain). Four healthy female pigs (cross-breed 25% Landrace × 25% Large White × 50% Duroc) were provided by the same Teaching Farm. Pigs were anesthetized with isoflurane and surgically equipped with a permanent catheter (Cook España, Barcelona, Spain) placed in the jugular vein. The catheter was filled with heparinized saline solution (1000 U/L) and flushed with saline every other day and filled with heparinized saline. Each pig was allowed 1 week to recover after the catheter had been implanted.

The pigs (mean weight of 80 ± 8.2 kg) were fasted overnight with free access to water before RES administration. All animal interventions were carried out at the same hour in the morning. Animals were tranquilized with intramuscular administration of tiletamine-zolazepam (3.5 mg/kg of body weight) to allow animal handling during RES administration and blood sampling. The dose of RES was adjusted to 6.25 mg/kg of body weight (500 mg for an 80 kg animal) and prepared as a suspension in tap water (50 mL). The RES aqueous suspension was prepared freshly and shaken vigorously before oral administration using an intragastric probe. The probe was washed twice with 50 mL of tap water, which was also administered to the animal to ensure maximal delivery of the initial dose. Animals were immobilized using a muzzle loop for each blood withdrawal. Blood samples (2 mL) were taken at 15, 30, 45, 60, 120, 180, 240, and 300 min after RES administration. Blood samples were collected in BD Vacutainer lithium heparin tubes (BD, Franklin Lakes, NJ). The collected blood was immediately separated in plasma by centrifugation at 3000*g* for 10 min at 4 °C in a Sigma 1-13 microcentrifuge (Braun Biotech. Int., Melsungen, Germany). The plasma was immediately frozen at −80 °C for further analyses.

To quantify the amount of RES that might have been left in the gastric probe after oral administration, the probe was washed three times with methanol and the collected methanolic solution was analyzed by HPLC-DAD-MS/MS.

Sampling Procedure and Recovery Efficiency. Three different extraction protocols were compared. The first method was based on the use of acetonitrile (*22*). Plasma samples (300 μL) were mixed with 750 μL of acetonitrile, vigorously stirred in a vortex for 1 min, and centrifuged at 3000*g* for 15 min at 4 °C in the microcentrifuge. The supernatant was evaporated under vacuum in a SpeedVac Concentrator Savant SPD121P (Thermo Scientific, Alcobendas, Spain) equipped with a refrigerated vapor trap RVT4104 (Thermo Scientific) and a vacuum pump v-710 (BÜCHI Labortechnik AG, Postfach, Switzerland). The pellet was redissolved in 150 μL of methanol. Methanolic samples were diluted 1:1 (v/v) with ultrapure Milli-Q water (Millipore Corp., Bedford, MA), filtered through a 0.45 μm membrane filter Millex-HV13 (Millipore Corp.), and a 50 μL aliquot was analyzed by HPLC-DAD-MS/MS. The second protocol used acidified methanol (2 HCl:98 methanol, v/v) instead of acetonitrile to extract the RES metabolites from plasma (*23*). The rest of the steps were similar to those described above for the method based on acetonitrile. The third method consisted of a solid-phase plasma extraction with a C_{18} cartridge (*24*). In this case, the same volume of plasma was filtered through a Sep-Pak solid-phase extraction cartridge (a reverse phase C-18 cartridge; Waters, Milford, MA). The cartridges were previously activated with 10 mL of methanol and 10 mL of water and subsequently emptied with air to remove the remaining water. The plasma was acidified to pH 4 with acetic acid. After stirring in the vortex for 2 min, the sample was loaded onto the cartridge and washed with 10 mL of water. The remaining volume in the cartridge was eluted with 2 mL of methanol. The rest of the steps, that is, evaporation, dilution, etc., were as described above for the method based on acetonitrile. In all cases, sample manipulation was performed in the dark to minimize the possible photochemical isomerization of *trans*-resveratrol to the *cis*-form.

The recovery efficiency of RES from plasma was determined by spiking a plasma sample from a control animal with known concentrations of RES. The plasma samples were processed according to the protocols described above and the recoveries calculated by comparing the peak area ratios from spiked samples to those of the corresponding concentrations of RES standard in methanol directly injected into the HPLC system. Linearity ($r^2 = 0.999$) was confirmed for both RES-spiked plasma samples as well as RES in methanol in the range from 0.05 to 125 μM.

HPLC-DAD-MS/MS Analyses. Identification of plasma metabolites was carried out in an 1100 series HPLC-DAD system (Agilent Technologies, Waldbronn, Germany) equipped with a mass spectrometer (Agilent). The mass detector was an ion-trap mass spectrometer equipped with an electrospray ionization system (ESI). The heated capillary and voltage were maintained at 350 °C and 4 kV, respectively. Mass scan (MS) and MS/MS daughter spectra were measured from *m*/*z* 100 to *m*/*z* 800. Collision-induced fragmentation experiments were performed in the ion trap using helium as collision gas, and the collision energy was set at 50%. Mass spectrometry data were acquired in the negative ionization modes. Chromatographic separations were achieved on a 250 × 4 mm i.d., 5 μm, C_{18} Mediterranea Sea column (Teknokroma, Barcelona, Spain) using water/formic acid (99:1, v/v) (A) and acetonitrile (B) as the mobile phases at a flow rate of 1 mL/min. The gradient started with 5% B in A to reach 55% B at 30 min and 90% B at 31 min for 5 min and returned to the initial conditions (5% B).

Quantification of RES-derived metabolites in pig plasma was carried out in an Elite LaChrom-Hitachi HPLC system with a pump model 2130, a diode array detector 2455, and an autosampler model 2200 (VWR-Hitachi, Barcelona, Spain). Chromatographic separations were achieved on the same Mediterranea Sea column described above using water/formic acid (95:5, v/v) (A) and acetonitrile (B) as the mobile phases at a flow rate of 1 mL/min. The gradient started with 5% B in A to reach 32% B at 30 min and 95% B at 31 min for 5 min and returned to the initial conditions (5% B). The different plasma metabolites were identified by their UV spectrum, molecular mass, daughter ions, and fragmentation pattern in the 1100 Agilent equipment. Quantification was performed in the Merck equipment using UV detection at 320 nm and commercial RES as external standard. The limit of quantification was 110 ± 8 nM.

Pharmacokinetic Analysis. Data of RES metabolites concentrations in plasma versus postingestion time of RES were analyzed by noncompartmental pharmacokinetic analysis. Pharmacokinetic parameters were estimated using the WinNonlin software package (WinNonlin Professional version 5.2.1., Pharsight Corp., Sunnyvale, CA). WinNonlin model 200 was used for the analysis. The area under the plasma concentration−time curve (AUC_{last}) from time 0 to the last point (t_{last}) with measurable concentration (C_{last}) was estimated using a linear/log trapezoidal approximation. The time to reach peak concentration (t_{max}) and peak concentration (C_{max}) were obtained directly from the plasma concentration−time curves. Other pharmacokinetic parameters were the lag time (t_{lag}), defined as the time prior to the first measurable (nonzero) concentration; the elimination half-life ($t_{1/2}$), which was calculated using $\ln 2/k_{el}$ (where k_{el} is the rate constant of elimination); the mean residence time (MRT_{last}), which is defined as the average time for all drug molecules to reside in the body calculated from the time of dosing to the time of the last quantifiable concentration; and the area under the curve extrapolated to infinity (AUC_{∞}).

RESULTS

Resveratrol Administration and Extraction Protocols. The actual RES dose administered to the animals was calculated by subtracting the RES quantity left in the probe after intragastric

administration (mean value = 27.9 ± 10.6 mg of RES, $n = 4$ animal interventions) from the initial weighed dose (6.25 mg/kg of body weight, 500 mg for an 80 kg animal). The effective intragastric RES dose administered was therefore 5.9 mg/kg of body weight or 472 mg of RES for an 80 kg animal (which is equivalent to the same human dose) (*25*).

Three different plasma extraction procedures were compared by calculating the efficiency of recovery of RES in samples of plasma obtained after 60 min of the RES administration. The recovery of RES in spiked-plasma samples was 65% using methanol as a solvent, whereas the best values were obtained using acetonitrile or the C_{18} cartridge, which yielded 99 and 95% of RES, respectively. The profile of metabolites extracted and detected in plasma was qualitatively and quantitatively very similar using both the acetonitrile and the C_{18} cartridge methods. Because the method based on the use of acetonitrile was much faster, we chose to process the rest of the samples using this method.

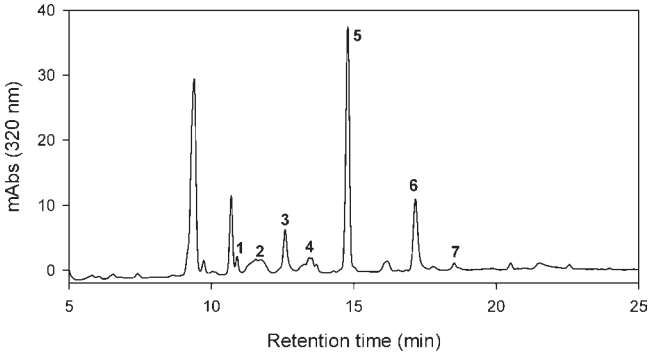

Figure 1. Representative HPLC chromatogram (320 nm) of a pig plasma sample after 60 min of oral administration of RES. Peaks: (1) resveratrol diglucuronide; (2, 3) resveratrol sulfoglucuronide isomers; (4) resveratrol glucuronide isomer; (5) resveratrol 3-*O*-glucuronide; (6) resveratrol sulfate; (7) RES.

Identification of Resveratrol Metabolites in Pig Plasma. Several metabolites of RES were detected and analyzed in the plasma samples obtained from the pigs at different times after intragastric administration of pure RES resuspended in water. A representative chromatogram is shown in **Figure 1**. Resveratrol diglucuronide (peak 1), two isomers of resveratrol sulfoglucuronide (peaks 2 and 3), two isomers of resveratrol glucuronide (peaks 4 and 5), resveratrol sulfate (peak 6), and resveratrol (peak 7) were all identified according to their UV spectral properties, molecular mass, daughter ions, and fragmentation pattern (**Figures 1** and **2**). In addition, the identity of the most abundant resveratrol metabolite (**Figure 1**, peak 5) was confirmed to be resveratrol 3-*O*-glucuronide by coelution with the authentic standard. The microbiota-derived resveratrol metabolite dihydroresveratrol as well as its conjugates were not detected in the pig plasma under the described assay conditions.

Pharmacokinetic Analysis of Resveratrol Metabolites. The main metabolites detected in the plasma of pigs after RES administration

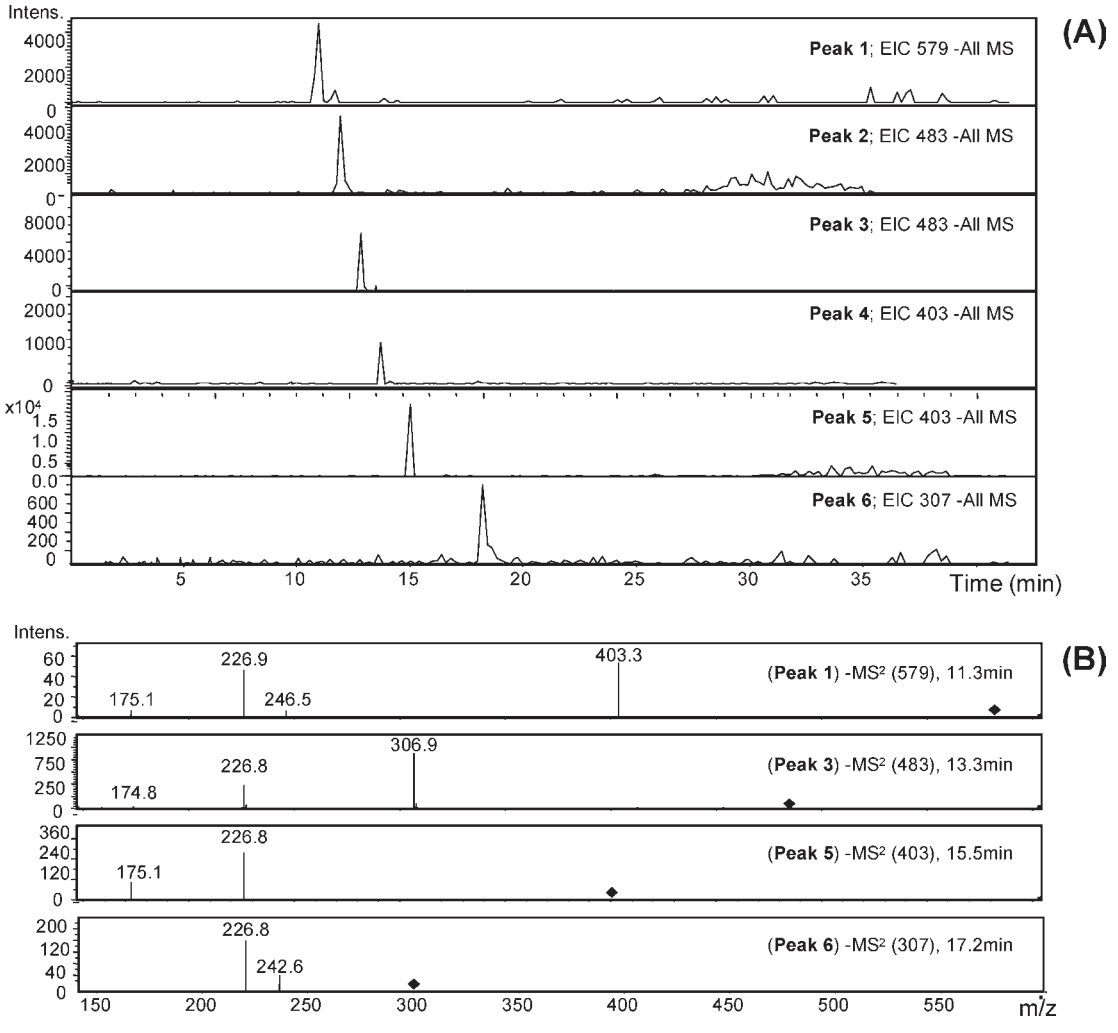

Figure 2. (**A**) Extracted ion chromatograms (EIC) and (**B**) ion fragmentation patterns of the most representative plasma RES metabolites.

Table 1. Pharmacokinetic Parameters of Total and Individual RES Metabolites[a]

parameter[b]	total metabolites[c]	RES sulfoglucuronide (peak 2)[d]	RES sulfoglucuronide (peak 3)[d]	RES 3-*O*-glucuronide (peak 5)[d]	RES sulfate (peak 6)[d]
t_{max} (h)	1 (0)	0.9 (13.3)	2	0.9 (28.6)	1.3 (43.3)
t_{last} (h)	4.3 (13.3)	3.7 (33.5)	4	5 (0)	5 (0)
t_{lag} (h)	0	0	0	0	0
$t_{1/2}$ (h)	1.2 (17.4)	0.8 (16.7)	1.2	1.3 (12.2)	0.7 (1.1)
C_{max} (ng/mL)	3643.7 (17.5)	666.8 (22.5)	185.4	2223.8 (23.1)	847.5 (33.6)
C_{last} (ng/mL)	351.3 (39.5)	110.0 (50.8)	57.7	351.3 (39.6)	135.5 (26.7)
AUC_{∞} (ng h/mL)	8162.2 (9.1)	1213.1 (32.1)	597.2	4617.1 (28.2)	1948.7 (17.1)
AUC_{last} (ng h/mL)	6993.9 (6.2)	1089.4 (35.7)	498.3	3942.3 (25.6)	1801.5 (20.4)
MRT_{last} (h)	1.6 (10.9)	1.39 (24.5)	1.9	1.8 (8.4)	1.8 (7.6)

[a] Results are presented as the mean value (n = 4) followed by the coefficient of variation (%). [b] Pharmacokinetic parameters are defined under Materials and Methods. [c] Total metabolites did not include peak 3, which was quantified in only one pig. [d] Peak numbers refer to **Figure 1**.

were a sulfoglucuronide isomer (peak 2), resveratrol 3-*O*-glucuronide (peak 5), and a sulfate conjugate (peak 6). The time course dispositions of these metabolites were quite similar (**Figure 3**). RES was rapidly absorbed after oral administration as no lag time (t_{lag}) was obtained for any metabolite (**Figure 3**; **Table 1**). Peak plasma levels of RES aglycone (53 ± 20 ng/mL or 0.23 ± 0.08 μM, mean value ± standard error) occurred at 0.5 h postdose (**Table 2**). The kinetics of total RES metabolites (**2** + **3** + **5** + **6**) showed a maximum concentration at 1 h with t_{max} values of 0.9 h for compounds **2** and **5**, 1.3 h for compound **6**, and 2 h for compound **3** (**Table 1**). The C_{max} value for the total metabolites was 3643 ng/mL ± 369.4), the highest value (2223 ng/mL ± 256.3 or 5.5 ± 0.63 μM) being for resveratrol 3-*O*-glucuronide (**5**). The C_{max} for this metabolite was 2.6-, 3.3-, and 12-fold higher than that for the sulfate derivative (**6**), the sulfoglucuronide isomer-1 (**2**), and the sulfoglucuronide isomer-2 (**3**), respectively (**Table 1**). The MRT_{last} of total metabolites was 1.6 h, ranging from 1.4 h (compound **2**) to 1.9 h (compound **3**) for the individual metabolites. The t_{last} value was 5 h for compounds **5** and **6**, whereas this value was 3.7 and 4 h for compounds **2** and **3**, respectively (**Table 1**).

The rest of the RES metabolites (peaks 1, 4, and 7 from **Figure 1**) were not included in the pharmacokinetic analysis because not enough measurable time points were obtained for these compounds (values below the limit of quantification) (**Table 2**).

DISCUSSION

The pig has been previously acknowledged as a valuable model in studies looking at the metabolism of polyphenols (*14*−*20*). For example, the overall metabolic profile of isoflavones in female pigs has been shown to be closer to that of women than the metabolic profile of female rats or female monkeys (*26*). Along these lines, the metabolism of ellagitannins and ellagic acid in the pig (*20*) is also more similar to that in humans (*27*−*29*) than the metabolism of these polyphenols in the rat (*30*, *31*), and the metabolism and tissue distribution of quercetin in the pig have also been reported to be different from that in rats (*18*). In the present study, we describe, for the first time, the metabolic profile of RES and the pharmacokinetics of the individual RES metabolites in the plasma of pigs after the oral administration of RES. We show that these metabolites differ from those reported in humans.

The absorption, metabolism, and plasma detection of RES and RES conjugates in humans have been studied in a number of interventions as recently reviewed by Cottart et al. (*12*). Comparison between the different studies clearly shows some differences between the metabolic profiles of RES detected in humans. In fact, the pharmacokinetics of the individual RES metabolites (the most abundant RES derived compounds detected in plasma) has not been studied in depth. In many studies, the formation of

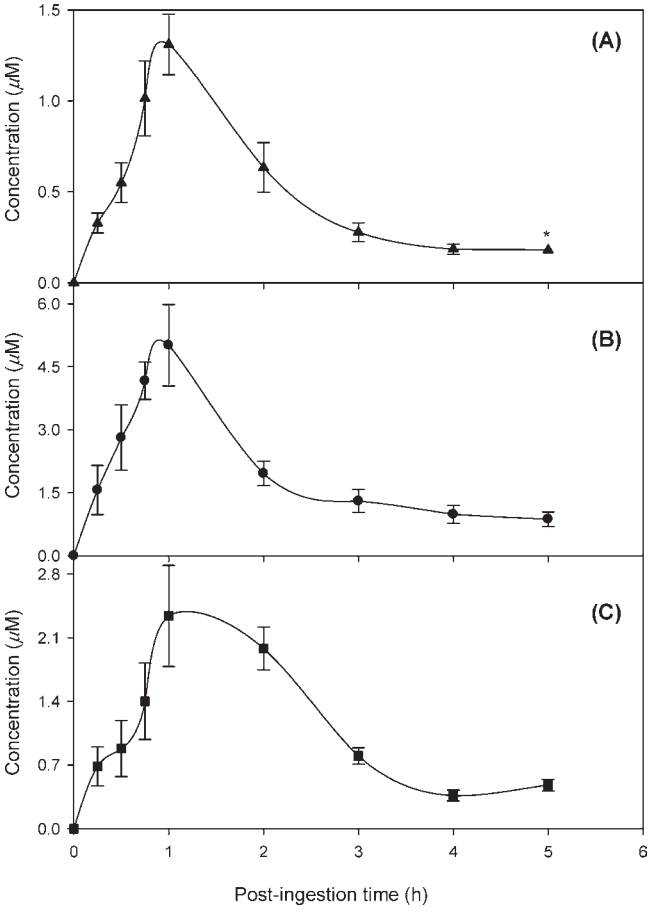

Figure 3. Plasma concentration−time curve of the main metabolites after oral administration of RES (5.9 mg/kg of body weight): (**A**) resveratrol sulfoglucuronide (peak 2 from **Figure 1**) (at 300 min after the oral dose, the concentration of this metabolite was quantified only in one animal, whereas in the rest of the pigs (n = 3) this metabolite was detected but the values were below the limit of quantification); (**B**) resveratrol 3-*O*-glucuronide (peak 5 from **Figure 1**); (**C**) resveratrol sulfate (peak 6 from **Figure 1**). Error bars are standard errors (n = 4).

RES conjugates was indirectly determined by enzymatic treatment of the plasma samples with the enzymes glucuronidase and sulfatase, which indicates the presence or absence of these conjugates but does not identify the actual compounds (*6*, *10*, *32*, *33*). A more detailed description of the RES metabolites was provided by Urpí-Sardá et al. (*34*), who reported the presence of different RES metabolites in human LDL particles after the intake of red wine, with RES glucuronides being the most abundant metabolites. These results did not agree, however, with the results reported by

Table 2. RES and Derived Metabolites Detected in Plasma but Not Included in the Pharmacokinetic Analysis[a]

postingestion time (h)	RES diglucuronide (peak 1)[b,c]	RES glucuronide isomer (peak 4)[b,c]	RES (peak 7)[b,c]
0.25	77.4 (n = 1); Det. (n = 2)	119.1 ± 43.6 (n = 3); Det. (n = 1)	27.3 (n = 1); Det. (n = 2)
0.50	95.3 (n = 1); Det. (n = 2)	121.4 ± 41.4 (n = 3); Det. (n = 1)	52.9 ± 20.5 (n = 3)
0.75	95.6 ± 15.7 (n = 3)	128.7 ± 29.9 (n = 3); Det. (n = 1)	30.1 ± 0.6 (n = 3); Det. (n = 1)
1	82.4 ± 9.2 (n = 2); Det. (n = 1)	124.1 ± 11.5 (n = 4)	36.3 ± 1.1 (n = 2); Det. (n = 1)
2	95.7 ± 21.9 (n = 2); Det. (n = 2)	32.1 (n = 1); Det. (n = 1)	Det. (n = 3)
3	94.6 ± 28.6 (n = 2); Det. (n = 2)	Det. (n = 3)	Det. (n = 3)
4	69.9 ± 3.1 (n = 2); Det. (n = 2)	Det. (n = 4)	Det. (n = 3)
5	Det. (n = 4)	Det. (n = 3)	Det. (n = 1)

[a] Results are presented as mean values ± SE ng/mL. [b] Peak numbers refer to **Figure 1**. [c] Sample size is shown in parentheses. Values without standard error indicate that the metabolite was quantified in only one animal. Det., value below the limit of quantification (the number of pigs in which the compound was detected is indicated).

others (*35*), who detected RES disulfates as the main RES metabolites in human plasma after the administration of a single dose of piceid (resveratrol 3-*O*-glucoside; 85.5 mg/70 kg of body weight). One of the most detailed studies was carried out by Boocock et al. (*36*), who reported the pharmacokinetics of RES and three individual metabolites in the plasma of volunteers after a single oral dose of 0.5, 1, 2.5, or 5 g of RES. Two monosulfates, one disulfate, two monoglucuronides, and one sulfoglucuronide were detected, although the main metabolites were resveratrol 3-*O*-sulfate and two monoglucuronides (*36*). In the present study, the most abundant metabolite of RES detected in the plasma of pigs was resveratrol 3-*O*-glucuronide (**Figure 1**, peak 5) followed by a resveratrol sulfate (**Figure 1**, peak 6) (**Table 1**). These results reflect clear differences against the human profile described by Boocock et al. (*36*). Our results in pigs are more in agreement with the plasma profile of RES metabolites observed in rats after intravenous administration of RES (15 mg/kg) (*24*). Therefore, despite the well-accepted physiology similarity between pigs and humans, our results suggest that the metabolism of RES in pigs may be different from that in humans.

Besides the inherent differences of species, there are many other factors that may influence the different metabolic profiles of RES reported in the different studies. One first interfering factor could be the plasma sample preparation procedure. In our study we tested three different extraction protocols and found no significant differences in the plasma metabolic profile of RES. Wenzel et al. (*22*) found some sulfate conjugates as the main RES metabolites in the plasma of rat using the method with acetonitrile approached in our pig study. In addition, in a preliminary human study carried out in our laboratory and also using the extraction protocol with acetonitrile used in the pig study, we have identified two resveratrol monoglucuronides and a resveratrol sulfate as the main human plasma metabolites after the oral administration of RES in aqueous solution (9 mg/kg of body weight) (unpublished results). Our results and others suggest that plasma sample preparation and solvent extraction may not be critical factors affecting the plasma metabolic profile of RES metabolites.

Different oral administration vehicles and doses may also have an effect on the plasma metabolic profile and pharmacokinetics of RES. Due to the low solubility of RES, some biovailability studies have used the cyclic oligosaccharides β-cyclodextrins to improve aqueous solubility of RES (*9*). β-Cyclodextrins have been proposed as carriers to deliver drugs into the colon as they may prevent the molecules from absorption and metabolism in the small intestine (*37*) and, consequently, they may have an effect on the metabolic profile and kinetic parameters. Das et al. (*38*) reported in the rat different t_{max} and C_{max} plasma values for RES after oral administration of RES with different β-cyclodextrins-based formulations. However, AUC values remained unchanged, and a qualitative effect on the metabolic profile was not assessed (*38*). In the present pig study, the half-life ($t_{1/2}$) of resveratrol 3-*O*-glucuronide was 1.3 h, which is within the range of values reported in other animal studies (*5*). However, in humans, and using a similar dose of RES, the $t_{1/2}$ of the main metabolites of RES calculated by Boocock et al. (*36*) was about 3 h after the intake of RES in caplets. The difference in the $t_{1/2}$ values may be due to the different vehicles used to administer RES, water versus caplets. The AUC_{∞} values as well as the C_{max} of total RES metabolites obtained in our present study in pigs (**Table 1**) were on the same order of those obtained by Boocock et al. (*36*) at a similar RES dosage (0.5 g). These results suggest that different oral RES administration vehicles can affect pharmacokinetic parameters without changing the metabolic profile. In general, resveratrol sulfates have been reported to be the most abundant conjugates in humans, whereas in animals, resveratrol glucuronides are the main metabolites detected (*35*). The high doses usually administered to animals (50−300 mg/kg of body weight) have been suggested to explain these differences. These high doses could cause a shift in the profile of metabolites from the sulfate to the glucuronide conjugates (*35*) based on the dose-dependent substrate affinity of glucuronyl and sulfate transferases. However, and in disagreement with this hypothesis, in the human study by Boocock et al. (*36*) the dose administered of RES ranged from 7.1 to 71 mg/kg and the most abundant metabolite detected was always resveratrol 3-*O*-sulfate.

Gender is another critical factor with an effect on metabolism and pharmacokinetics. Different pharmacokinetic results have been reported for isoflavones in men and women (*39*). The possible gender-specific differences in the pharmacokinetics of RES have not been investigated so far. The study of Boocock et al. (*36*) does not indicate any possible differences between males and females. Multridrug-resistant-associated proteins such as MRP2 and BCRP, involved in the intestinal efflux of RES metabolites (*40*), have been suggested to exhibit gender-specific differences (*41*). Our study in pigs was carried out in female animals, and possible differences from males cannot be ruled out.

Phase I and II enzyme polymorphisms and different enzymatic properties of drug-metabolizing enzymes may also contribute to the differences between animals and human metabolic profiles (*42*). In addition, in a multiple-dose study in humans with increasing doses of RES, the highest C_{max} values for RES were obtained in the morning, suggesting an effect of the circadian variation of enterohepatic circulation on the kinetics parameters (*43*).

Another important factor, often disregarded in animal studies, is the possible effect of the anesthetic procedure on RES absorption and metabolism. This is especially relevant when animal and human studies are compared because animal handling commonly requires anesthesia, whereas in humans anesthesia is not required. Drugs used in anesthesia could affect important aspects of the metabolism such as gastric emptying, induction and/or inhibition of phase I and II enzymes, and intestinal motility. Virtually all of the anesthetic drugs are metabolized by either phase I or phase II

enzymes (*44*). In addition, another possible effect of the drugs used in anesthesia may be related to the inhibition of intestinal transporters such as the RES ATP-binding cassette transporters BCRP and MRP. The specific effect of anesthetic drugs commonly used in pharmacokinetic assays with animal models on intestinal transporters such as BCRP and MRP has not been studied in depth. To the best of our knowledge, no specific studies that evaluate the effect of different anesthetic drugs on the absorption and metabolism of polyphenols, including RES, have been reported.

In this work we have shown that the metabolic profile of RES in the plasma of pigs differs from that reported previously in humans. These results add to the increasing number of metabolic studies that evidence the important differences which exist in the metabolism between species, even between species considered to be physiologically very similar. We have highlighted several other factors that can have a critical effect on the metabolism: gender, age, the type of drugs and protocols used in anesthesia, polymorphisms (phase I and II enzymes, transporters, etc.), different oral administration procedures, sample extraction, and analytical protocols. The involvement of these factors on the pharmacokinetics of RES cannot be ruled out and should be critically approached in future studies as very few papers take into account all of these variables in the metabolism of polyphenols (*45*). More robust pharmacokinetic studies are needed and should be designed to carefully assess as many of the interfering factors as possible to understand the role of metabolism in the bioactivity of beneficial dietary compounds such as RES.

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