13 Bioavailability and Metabolism of Resveratrol

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CONTENTS

INTRODUCTION

The essential chemical structure of stilbenes is the *trans*-1,2-diphenylethylene. The parent molecule of this group is the resveratrol (3,4′,5- trihydroxystilbene) that exists as two geometric isomers: *cis*- (*Z*) and *trans*- (*E*). The *trans*-form can undergo isomerization to the *cis*-form when exposed to ultraviolet irradiation. Piceid (resveratrol glucoside) is the major resveratrol derivative in plants. Other stilbenes in the vegetal kingdom are pterostilbene, piceatannol, astringin, and viniferins (Fig. 13.1).

Resveratrol and piceid are mainly present in grape and grape products, and its composition is affected by grape variety, maturity degree at harvest, fungal

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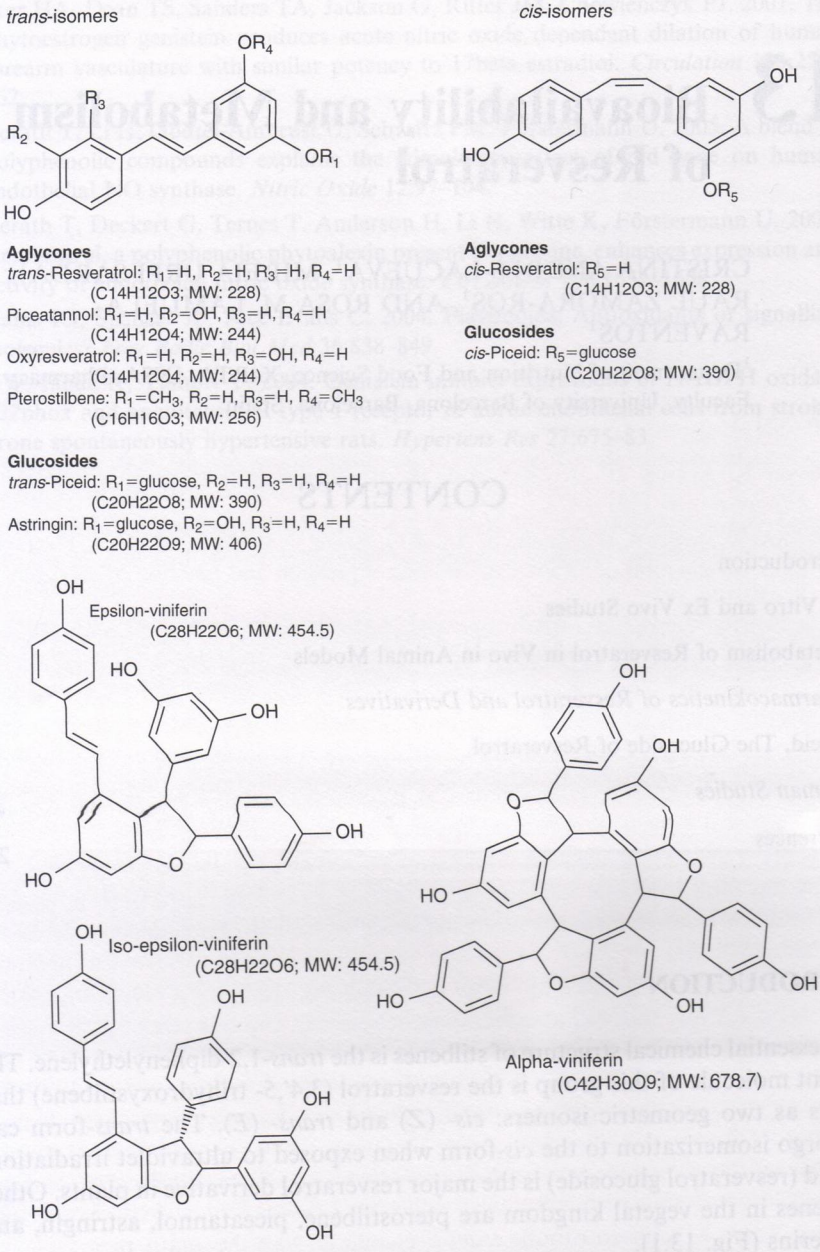


Figure 13.1 Chemical structure of stilbenes present in foods.

stress, climate, soil characteristics (*terroir*), and wine-making process as well as technology [de Andrés-de Prado et al., 2007; González-Barrio et al., 2006; Romero-Perez et al., 2001]. Other slight food sources of stilbenes are peanuts, pistachios, and berries such as bilberry, blueberry, and cranberry [Burns et al.,

2002; Rimando et al., 2004; Sobolev and Cole, 1999; Tokusoglu et al., 2005]. In an adult Spanish cohort resveratrol and piceid, *trans* and *cis*-forms, were evaluated. Estimated median and mean of resveratrol and piceid were 100 and 933 μg/day, respectively, of which 98.4, 1.6, and less than 0.1% come from wines, grape and grape juice, and peanuts, pistachios, and berries, respectively [Zamora-Ros et al., 2008].

IN VITRO AND EX VIVO STUDIES

Several studies have investigated the absorption, transport, and metabolism of resveratrol in vitro and ex vivo. They are summarized in Table 13.1.

Caco-2 cells and isolated small intestine are models of basic nutrition that contributed to the understanding of resveratrol absorption and bioavailability. While the Caco-2 absorption model is a well-defined cellular in vitro system based on a human colonic adenocarcinoma cell line, the isolated small intestine model is nearer to in vivo conditions and is also simpler to handle. It also avoids the methodological problems of in vivo perfusion models [Barthe et al., 1998, 1999].

The Caco-2 cells incubated in vitro with *trans*-resveratrol (5–40 μM) showed a concentration-dependent transcellular absorption up to 3 h, with a linear rate for the first hour [Kaldas et al., 2003]. At 3 h of incubation, the concentration of resveratrol reached a plateau [Kaldas et al., 2003; Maier-Salamon et al., 2006]. However, incubations with higher amounts of this polyphenol (40 μM) increased their concentration in the Caco-2 cells, so there was no saturation of the transport systems [Kaldas et al., 2003]. The cellular uptake of *trans*-piceid was also investigated in Caco-2 cells and its transport was slower and about 20% of the resveratrol aglycone [Henry et al., 2005]. *trans*-Resveratrol crosses the apical membrane of the Caco-2 cells using a passive transport, whereas *trans*-piceid seems to use both the active transporter sodium-dependent glucose transporter 1 (SGLT1) and the multidrug resistance protein 2 (MRP2) [Henry et al., 2005]. After piceid absorption, it can be hydrolized to resveratrol by the cytosolic-β-glucosidase [Henry-Vitrac et al., 2006]. Another possible pathway to absorb piceid is through its deglycosilation by membrane-bound lactase phlorizin hydrolase, since it then goes across the apical membrane as resveratrol [Henry-Vitrac et al., 2006]. The basolateral to apical efflux also occurs at similar concentrations to apical and basolateral efflux [Henry-Vitrac et al., 2006; Kaldas et al., 2003]. After resveratrol absorption, this is conjugated rapidly in intestinal cells. At 10 μM concentration, *trans*-3-*O*-sulfate was the main metabolite of resveratrol, however, its formation drastically decreased at higher resveratrol concentrations (200 μM), possibly due to saturation or inhibition of metabolism at higher stilbene concentrations [Maier-Salamon et al., 2006]. Glucuronidate forms such as *trans*-resveratrol-4′-*O*-glucuronide and *trans*-resveratrol-3-*O*-glucuronide were also released at less levels than sulfate forms (Fig.13.2) [Maier-Salamon et al., 2006].

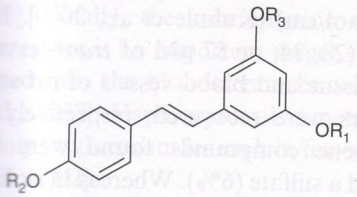
Table 13.1 Metabolism of Resveratrol in Vitro and Ex Vivo Models[a]

Tissue	Dose, Source (time)	Metabolites (C found)	Reference
Human erythrocytes (1.6×10^9)	21.9 nmol *t*-Resv (0.25 h)	*t-Resv 10.0 ± 1.7 nmol/10^9	Blache et al., 1997
Rat erythrocytes (1.6×10^9)		*t-Resv 10.8 ± 2.2 nmol/10^9	
Rat platelets (10^9)		*t-Resv 2.2 ± 1.2 nmol/10^9	
Human LDL (0.5 mg/mL)	17.5 μM *t*-Resv (0.5 h)	*t-Resv 3.8 ± 0.9 nmol/mg protein	
Jejunum and ileum of Sprague–Dawley male rats	200 μM Resv (1.5 h)	Resv (0.03 nmol/cm jejunum) Gluc (1.19 nmol/cm jejunum)	Kuhnle et al., 2000
Small intestine of male Sprague–Dawley rats	28, 34, 57 μM Resv (1 h)	Vascular effluent: Gluc (16.8%), Resv (3.4%), Sulf (0.3%) Luminal effluent: Gluc (11.2%), Resv (39.7%), Sulf (3.0%) Intestinal tissue: Gluc (0.1%), Resv (1.5%), Sulf (0.3%)	Andlauer et al., 2000
Human liver microsomes	1 mM *cis* and *t*-Resv	*c*-3-Gluc (+), *t*-3-Gluc (+), *c*-4′-Gluc (-), *t*-4′-Gluc (-)	Aumont et al., 2001
Rat hepatocytes	20 μM *t*- Resv (1 h)		Asensi et al., 2002
Human liver microsomes	5 mM Resv (1 h)	Free Resv	Yu et al., 2002
Human hepatocytes	0.1 mL of 0.1 mM *t*-resveratrol (4 h)	*t*-3-Gluc, *t*-4′-Gluc, *c*-3-Gluc, *t*-3-Sulf	
Caco-2 cells	5–40 μM Resv (6 h)	Resv (200–4000 pmol)	Kaldas et al., 2003
Caco-2 cells	150–300 μM *t*-Resv and *t*-piceid (0.03–0.5 h)	*t*-Resv > *t*-piceid	Henry et al., 2005
Caco-2 cells	10–200 μM Resv	*t*-4′-Gluc, *t*-3-Gluc, *t*-3-Sulf	Maier-Salamon et al., 2006
Human liver microsomes Human intestional microsomes	500 μM *t*-Resv (5 h)	3-Gluc > 4′-Gluc 3-Gluc < 4′-Gluc	Brill et al., 2006

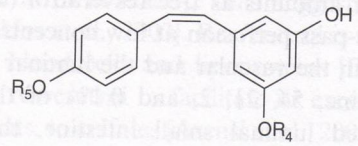
[a] Resv: resveratrol; Gluc: glucuronide; Sulf: sulfate; *t*-3-Gluc: *trans*-resveratrol-3-*O*-glucuronide; *t*-4′-Gluc: *trans*-resveratrol-4′-*O*-glucuronide; *c*-4′-Gluc: *cis*-resveratrol-4′-*O*-glucuronide; *c*-3-Gluc: *cis*-resveratrol-3-*O*-glucuronide, *t*-3-Sulf: *trans*-resveratrol-3-sulfate.
*Normalized values.

INTESTINAL METABOLISM

trans-Isomers



cis-Isomers



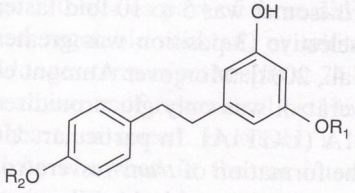
trans-Isomers

trans-Resveratrol-3-O-glucuronide: R_1=glucuronic acid, R_2=H, R_3=H ($C_{20}H_{20}O_9$; MW: 404)
trans-Resveratrol-4′-O-glucuronide: R_1=H, R_2=glucuronic acid, R_3=H, ($C_{20}H_{20}O_9$; MW: 404)
trans-Resveratrol-3,4′-diglucuronide: R_1=glucuronic acid, R_2=glucuronic acid, R_3=H ($C_{26}H_{28}O_{15}$; MW: 580)
trans-Resveratrol-3-sulfate: R_1=SO_3H, R_2=H ($C_{14}H_{12}O_6S$; MW: 308)
trans-Resveratrol-4′-sulfate: R_1=H, R_2=SO_3H, R_3=H ($C_{14}H_{12}O_6S$; MW: 308)
trans-Resveratrol-3,4′-sulfate: R_1=SO_3H, R_2=SO_3H, R_3=H ($C_{14}H_{12}O_9S_2$; MW: 388)
trans-Resveratrol-3,5′-disulfate: R_1=SO_3H, R_2=H, R_3=SO_3H ($C_{14}H_{12}O_9S_2$; MW: 388)
trans-Resveratrol-3,5,4′-trisulfate: R_1=SO_3H, R_2=SO_3H, R_3=SO_3H ($C_{14}H_{12}O_{12}S_3$; MW: 468)

cis-Isomers

cis-Resveratrol-3-O-glucuronide: R_4=glucuronic acid, R_5=H ($C_{20}H_{20}O_9$; MW: 404)
cis-Resveratrol-4′-O-glucuronide: R_4=H, R_5=glucuronic acid ($C_{20}H_{20}O_9$; MW: 404)
cis-Resveratrol-3-4′-diglucuronide: R_4=glucuronic acid, R_5=glucuronic acid ($C_{26}H_{28}O_{15}$; MW: 580)
cis-Resveratrol-3-sulfate: R_4=SO_3H, R_5=H ($C_{14}H_{12}O_6S$; MW: 308)
cis-Resveratrol-4′-sulfate: R_4=H, R_5=SO_3H ($C_{14}H_{12}O_6S$; MW: 308)
cis-Resveratrol-3,4′-disulfate: R_4=SO_3H, R_5=SO_3H ($C_{14}H_{12}O_6S_2$; MW: 388)

MICROBIAL METABOLISM



Dihydroresveratrol: R_1=H, R_2=H, ($C_{14}H_{14}O_3$; MW: 230)
Dihydroresveratrol-glucuronide: R_1=H or glucuronic acid, R_2=glucuronic acid or H ($C_{20}H_{22}O_9$; MW: 406)
Dihydroresveratrol-sulfate: R_1=H or SO_3H, R_2=SO_3H or H ($C_{14}H_{14}O_6S$; MW: 310)

Figure 13.2 Chemical structures of resveratrol metabolites.

Maier-Salomon et al. [2006] showed the high influence of metabolized resveratrol on the transepithelial transport of resveratrol and its intracellular accumulation. At 10 μM concentration in the Caco-2 cells, resveratrol was 84, 8, and 12% conjugated, transported, and accumulated, respectively. Whereas at higher doses (200 μM) resveratrol was 8, 26, and 61% conjugated, transported, and accumulated, respectively. Moreover, Kaldas et al. [2003] also found a resveratrol accumulation higher than 35-fold when transported through the Caco-2 cells, which suggests enterocytes as a major target site for this polyphenol.

Perfusion of 200 μM *trans*-resveratrol in isolated jejunum and ileum only transferred 6 and 2% of the total amount of resveratrol available, respectively. Resveratrol passed across jejunum as a glucuronide form (1.1 nmol/cm) and in minor amounts as free resveratrol (0.03 nmol/cm) [Kuhnle et al., 2000]. In a single-pass perfusion at low concentrations (28, 34, or 57 μM of *trans*-resveratrol) in the vascular and the luminar side, tissue and blood vessels of rat small intestine, 54, 21, 2, and 0.1% of the doses were recovered, respectively. In isolated luminal small intestine, the stilbene compounds found were free resveratrol (74%), 2-glucuronides (21%), and a sulfate (6%). Whereas in isolated vascular small intestine, the main metabolites were the glucuronidate forms (82%), free resveratrol (17%), and the sulfate form (1.5%). No differences were found in the recoveries using several different doses of resveratrol [Andlauer et al., 2000]. *trans*-Resveratrol was able to bind to human and rat erythrocytes, rat platelets, and low-density lipoprotein (LDL) as free resveratrol after in vitro incubations, confirming that resveratrol can diffuse throughout the body by means of its incorporation into blood cells and lipoproteins [Blache et al., 1997].

Conjugation forms of resveratrol can occur in both enterocytes and hepatocytes. After intestinal microsome incubation with 500 μM *trans*-resveratrol, only glucuronides were formed, in which *trans*-resveratrol-4′-*O*-glucuronide was more abundant than *trans*-resveratrol-3-*O*-glucuronide. In comparison with liver ability to metabolize, glucuronides were formed at higher levels (up to 10-fold) by the intestinal microsomes than the liver microsomes [Brill et al., 2006]. Likewise in liver microsomes, only glucuronidate forms and aglycones were observed after incubation with *trans*- and *cis*-resveratrol. Glucuronidation in liver was stereoselective (*cis*-isomer was 5 to 10-fold faster than *trans*-isomer) at both positions and regioselective (3-position was greater than 4′-position) for both isomers [Aumont et al., 2001]. Moreover Aumont et al. [2001] and Brill et al. [2006] found that resveratrol was only glucuronidized by the UDP-glucuronosyltransferase family 1A (UGT1A). In particular, UGT1A1 and UGT1A9 were mainly involved in the formation of *trans*-resveratrol-4′-*O*-glucuronide and *trans*-resveratrol-3-*O*-glucuronide, respectively. Likewise, in biopsies of human liver it was found that resveratrol is a better substrate for glucuronosyl transferase (K_m 0.15 mM) [de Santi et al., 2000a] than sulfotransferases (K_m 0.60 microM)) [de Santi et al., 2000b].

trans-Resveratrol was rapidly metabolized in a dose-dependent manner in rat hepatocytes, approximately 80 and 100% of the resveratrol (20 μM) incubated were conjugated at 20 min and at 1 h, respectively [Asensi et al., 2002]. In rat hepatocytes the main metabolite formed was *trans*-resveratrol-3-*O*-sulfate followed by *trans*-resveratrol-3-*O*-glucuronide. In contrast, in human hepatocytes glucuronides, that is, *trans*-resveratrol-4′-*O*-glucuronide, *trans*-resveratrol-3-*O*-glucuronide, and *cis*-resveratrol-3-*O*-glucuronide, were more abundant than sulfates (*trans*-resveratrol-3-*O*-sulfate) [Yu et al., 2002]. Based on these results, sulfate forms seem to be a minor human hepatic metabolite.

In summary, resveratrol absorption is higher as aglycon than piceid, although piceid can be hydrolyzed by β-glucosidases in the enterocytes [Henry-Vitrac

et al., 2006]. Enterocytes metabolize resveratrol up to a certain extent. At low concentrations it seems that there is mainly a sulfate pathway, but at higher amounts glucuronidate forms become more abundant [Maier-Salamon et al., 2006]. Finally, the accumulation of resveratrol in Caco-2 cells suggests that enterocytes is an important target site [Kaldas et al., 2003]. Resveratrol is transported into the bloodstream bounded to blood cells and lipoproteins [Blache et al., 1997]. Hepatocytes also metabolize resveratrol to facilitate its excretion, mainly to glucuronides and, in fewer amounts, to sulfates [Asensi et al., 2002; Yu et al., 2002].

METABOLISM OF RESVERATROL IN VIVO IN ANIMAL MODELS

There is an extensive amount of literature on the in vitro activities of resveratrol. Bertelli et al. [1996] were the first to study the bioavailability of resveratrol in rats. They assessed analysis of plasma and tissues from rats that were administered red wine in a single dose or a regular dose for 15 day. Results indicated that resveratrol is quickly absorbed with a maximum peak at 1 h in plasma as well as in liver and kidney, although in the heart it peak at 2 h. Values obtained after regular consumption of red wine were higher than without the regular consumption in the different organs studies, in particular in the liver. The kidney seemed to be the main route of excretion (Table 13.2) [Bertelli et al., 1996].

Soleas et al. [2001a] performed experiments using *trans*-resveratrol radiolabeled with [^{3}H] in a fixed position in the first benzene ring. Male Wistar rats were administered by gavage 120 nCi as part of three different matrices: 10% (v/v) ethanol, a homogenized vegetable cocktail, or white grape juice. Short-term experiments at 2 h and longer-term experiments at 24 h were carried out. Over a 24-h period only trace amounts of resveratrol (<1%) were detected in tissues such as liver, kidney, heart, or spleen. However, it appeared that 77–80% of *trans*-resveratrol could be absorbed in the rat intestine, with no differences among the three foo matrices when absorption was measured as the difference between the dose of adioactivity given and the recovered radioactivity in the stool (24-h feces plus colonic contents and colon). Nevertheless, values ranging from 49 to 61% were present in the urine, regardless the matrix. This 25% difference could be accounted for by excretion via sweat and the respiratory system, metabolism to CO_2, and accumulation in fat-rich tissues such as the brain and nervous system [Soleas et al., 2001a]. Short-term experiments (2 h) showed that over the first 30 min after administration, a significant amount of radioactivity accumulated blood and serum. The radioactivity remained at similar levels during the following 90 min. These observations included the parent compound as well as the metabolites (Table 13.3).

Experiments with unlabeled resveratrol were also conducted in rats after administration of 5 mg of resveratrol. *trans*-Resveratrol concentrations appeared in serum at 15 min, peaked at 30 min, and declined abruptly over the next 30 min. Soleas et al. [2001a] also measured the competition of several polyphenols

Table 13.2 Tissue Distribution in Animal Models[a]

Species	Source and Dose	Administration	Mean (SD) nmol/g												Plasma (μmol/l)	Feces (%)	Urine (%)	Time	Reference
			Brain	Lung	Liver	Kidney	Heart	Spleen	Gastrointestinal tract	Small intestine	Stomach	Testis	Colonic Mucosae	Bile					
6 Wistar rats	4 ml red wine (0.09 mg/kg)	Intragastric	–	–	0.09	–	–	–	–	–	–	–	–	–	–	–	–	0.5 h	Bertelli et al. 1996
6 Wistar rats			–	–	–	0.09	–	–	–	–	–	–	–	–	0.09	–	–	1 h	
6 Wistar rats			–	–	–	–	0.009	–	–	–	–	–	–	–	–	–	–	2 h	
36 Wistar rats	2 ml red wine/day (0.04 mg/kg/day) during 15 days	Intragastric			0.23 (0.006)	0.19 (0.005)	0.01 (0.001)	–	–	–	–	–	–	–	0.03 (0.002)	–	0.29 (0.007)	24 h	
5 C57BL/6J male mice	20 mg/kg *t*-Resv	Intragastric	0.50 (0.35)	0.40 (0.23)	1.03 (0.80)	0.17 (0.06)		–	–	–	–	–	–	–	1.7 (0.9)	–	–	5 min	Asensi et al. 2002
								–	–	–	–	–	–	–		–	2% Gluc	24 h	
5 Wistar male rats		Intragastric	0.11 (0.05)	0.10 (0.05)	0.45 (0.11)	0.06 (0.03)		–	–	–	–	–	–	–	1.2 (0.4)	–	–	5 min	
								–	–	–	–	–	–	–		–	Gluc	24 h	
5 ESD NZW male rabbits		Intragastric	0.08 (0.03)	0.62 (0.17)	0.35 (0.14)	0.05 (0.02)		–	–	–	–	–	–	–	0.7 (0.5)	–	–	5 min	

Table 13.2 *(Continued)*

Species	Source and Dose	Administration	Mean (SD) nmol/g											Plasma (μmol/l)	Feces (%)	Urine (%)	Time	Reference
			Brain	Lung	Liver	Kidney	Heart	Spleen	Gastrointestinal tract	Small intestine	Stomach	Testis	Colonic Mucosae	Bile				
3 Mice per time point	240 mg/kg *t*-Resv	Intragastric	1.2 (0.1)	50 (25)	51 (15)	16 (10)	75 (27)	–	–	960 (100)	–	–	30 (5)	–	32 (14)	–	–	10 min	Sale et al. 2004
	240 mg/kg *t*-3,4,5,4′-tetramethoxystilbene	Intragastric	5 (1)	11 (0)	8 (3)	11 (4)	10 (5)	–	–	7600 (6500)	–	–	330 (300) 30 min	–	5 (2)	–	–	10 min	
10 Male Wistar rats	300 mg/kg/day *t*-Resv during 8 weeks	Oral	–	–	triSulf: n.d. diSulf: n.d. 3-Sulf: 1.92 (0.78) 4′-Sulf: 5.75 (2.08) 3-Gluc: 1.58 (0.59) Resv: 3.20 (1.01)	triSulf: n.d. diSulf: 5.00 (1.31) 3-Sulf: 1.10 (0.36) 4′-Sulf: n.d. 3-Gluc: 6.71 (1.49) Resv: n.d.		–	–	–	–	–	–	–	TriSulf: 7.0 (2.6) 3,4′-diSulf: 19.23 (6.0) 3,5-diSulf: 3.30 (0.8) 3-Sulf: 1.20 (0.3) 3-Gluc: 7.75 (2.2) Resv: n.d.	TriSulf: n.d. 3,4′-diSulf: 3.42% 3,5-diSulf: 0.80% 3-Sulf: 5.68% 4′-Sulf: 3.83% 3-Gluc: n.d. Resv: 17.2%	TriSulf: 1.42% 3,4′-diSulf: 3.5% 3,5-diSulf: 10% 3-Sulf: 5.93% 4′-Sulf: n.d. 3-Gluc: 28.6% Resv: 3.99%	24h	Wenzel et al. 2005
10 Male Wistar rats	50 mg/kg/day *t*-Resv during 8 weeks	Oral	–	–	n.d.	n.d.		–	–	–	–	–	–	–	n.d.	TriSulf: n.d. 3,4′-diSulf: n.d. 3,5-diSulf: n.d. 3-Sulf: 1.73% 4′-Sulf: n.d. 3-Gluc: n.d. Resv: 13.1%	TriSulf: n.d. 3,4′-diSulf: 0.56% 3,5-diSulf: 0.26% 3-Sulf: 0.60% 4′-Sulf: n.d. 3-Gluc: 8.90% Resv: 5.03%		
18 Male Wistar rats	50 mg/kg Piceid	Oral	15.56 (7.3)	26.72 (9.9)	11.46 (6.4)	6.62 (3.1)	1.28 (0.7)	71.87 (35.4)	-	278.60 (76.4)	432.78 (198.6)	13.60 (6.2)	–	–	–	–	–	10 min	Lv et al. 2006

Table 13.2 *(Continued)*

Species	Source and Dose	Administration	Mean (SD) nmol/g												Plasma (μmol/l)	Feces (%)	Urine (%)	Time	Reference
			Brain	Lung	Liver	Kidney	Heart	Spleen	Gastrointestinal tract	Small intestine	Stomach	Testis	Colonic Mucosae	Bile					
6 Male Sprague-Dawley rats	50 mg/kg resveratrol + 1.85 MBq [3H]resveratrol	Gavage	< 0.1% dpm	< 0.1% dpm	0.98% dpm	0.59% dpm	< 0.1% dpm	< 0.1% dpm	76.2% dpm	–	–	–	–	–	1.7% dpm	–	–	2 h	El-Mohsen et al. 2006
			Gluc: 0.2 (0.0) Resv: 0.1 (0.0)	Gluc: 0.5 (0.2) Rev: 0.2 (0.2)	Gluc: 1.2 (0.3)	Gluc: 4.0 (0.6)	Gluc: 0.4 (0.0)	n.d.	–	–	–	–	–	–	Gluc: 7.0 (1.0) Resv	2 Gluc Resv	Resv 2 Gluc		
6 Male Sprague-Dawley rats	50 mg/kg resveratrol + 1.85MBq [3H]resveratrol	Gavage	0.35% dpm						5.1% dpm	–	–	–	1.55% dpm	–	0.48% dpm	–	3.3% dpm	18 h	El-Mohsen et al. 2006
			Gluc: 0.13 (0.05) Resv: 0.07(0.05)	Gluc: 0.1 (0.03) Resv: 0.2 (0.03)	Gluc: 0.1 (0.03) Resv: 0.15 (0.03)	n.d.	Resv: 0.09 (0.02)	n.d.	–	–	–	–	–	–	n.d.	–	–		

Table 13.2 *(Continued)*

Species	Source and Dose	Administration	Mean (SD) nmol/g											Plasma (µmol/l)	Feces (%)	Urine (%)	Time	Reference
			Brain	Lung	Liver	Kidney	Heart	Spleen	Gastrointestinal tract	Small intestine	Stomach	Testis	Colonic Mucosae	Bile					
3 Balb/c male mice	7.4 kBq ^{14}C- *t*-Resv (5 mg/kg)	Intragastric	n.c.	n.c.	441 (90) dpm/100 mg	342 (165)	n.c.	n.c.	–	2208 (1436) dpm/100 mg	–	n.c.	106 (80) dpm/100 mg	460 (70) ·10^{3}dpm/ml	0.5 (0.2) ·10^{3}dpm/ml	–	100 (26.7) ·10^{3}dpm/ml	1.5h	Vitrac et al. 2003
3 Balb/c male mice			196 (47) dpm/100 mg	380 (148) dpm/100 mg	374 (48) dpm/100 mg	552 (51) dpm/100 mg	210 (132) dpm/100 mg	312 (62) dpm/100 mg	–	1841 (183) dpm/100 mg	–	**193 (9)**	300 (83) dpm/100 mg	1170 (500) ·10^{3}dpm/ml	0.6 (0.8) ·10^{3}dpm/ml	–	286 (16.7) ·10^{3}dpm/ml	3h	
3 Balb/c male mice			n.c.	n.c.	189 (28) dpm/100 mg	263 (123) dpm/100 mg	n.c.	n.c.	–	933 (201) dpm/100 mg	–	n.c.	88 (26) dpm/100 mg	340 (330) ·10^{3}dpm/ml	1.25 (0.8) ·10^{3}dpm/ml	–	235 (23.3) ·10^{3}dpm/ml	6h	
3 Balb/c male mice	74 kBq ^{14}C- *t*-Resv (50 mg/kg)	Intragastric	-	-	0.09 (0.03) cpm/mm^2	0.05 (0.02) cpm/mm^2	–	–	–	**n.d.**	1.09 (0.72) cpm/mm^2	–	–	–	–	–	–	1.5h	
3 Balb/c male mice			-	-	0.1 (0.01) cpm/mm^2	0.07 (0.02) cpm/mm^2	–	–	–	1.7 (0.16) cpm/mm^2	0.93 (0.55) cpm/mm^2	–	–	–	–	–	–	3h	
3 Balb/c male mice			-	-	0.05 (0.01) cpm/mm^2	0.03 (0.01) cpm/mm^2	–	–	–	0.2 (0.04) cpm/mm^2	0.4 (0.04) cpm/mm^2	–	–	–	–	–	–	6h	
3 Balb/c male mice	92.5 kBq ^{14}C- *t*-Resv (66 mg/kg)	Intragastric			^{14}C- *t*-Resv: 25 µmol/l	^{14}C- *t*-Resv: 30 µmol/l	–	–	–	-	-	–	–	–	–	–	–	3h	

[a] TriSulf: resveratrol-3,4′,5-trisulfate; diSulf: resveratrol-disulfate; Sulf: resveratrol-sulfate; Gluc: resveratrol-glucuronide; *t*-Resv: *trans*-resveratrol; Radioactivity was measured by dpm or cpm: dpm: desintegrations per minute and cpm: counts per minute; MBq: megabecquerel; kBq: kilobecquerel; n.d.: not detected; n.c.: not collected.

Table 13.3 Metabolism of Resveratrol in Vivo in Animal Models[a]

Animals	Source	Administration	Dose mg/kg	Blood or Serum or Plasma	Urinary excretion		Time (h)	Reference
				Metabolites (Concentration)	%	Metabolites		
8 male Wistar rats	*trans*-Resveratrol	Intragastric	2 μL of	—	49–61%	—	24	Soleas et al.,
2 male Wistar rats	tritiated in 4-position with 10%EtOH or V-8 homogenized vegetable cocktail or white grape juice		120 nCi/mL	Serum: 690 dpm/mL Blood: 600 dpm/ml	—	—	0.5	2001
Male Wistar rats	*t*-Resv	Intragastric	1.43 mg/kg	*t*-Resv: Serum: 0.01 μM Blood: 0.01 μM	—	—	1	
			4.29 mg/kg	*t*-Resv: Serum: 0.02 μM Blood: 0.01 μM	—	—	1	
			7.14 mg/kg	*t*-Resv: Serum: 0.03 μM Blood: 0.02 μM	—	—	1	
2 male Wistar rats	*t*-Resv	Intragastric	14.28 mg/kg	*t*-Resv Serum: 1.44 μM Blood: 1.00 μM	—	—	0.5	
3 Sprague–Dawley female rats	Resv	Intraperitoneal	20 mg/kg	—	—	*t*-3-Gluc	2 h	Yu et al., 2002
12 Balb/c female mice	Resv	Intraperitoneal	20 mg/kg	*t*-3-Gluc (3 μM), *t*-3-Sulf (13 μM)	—	—	0.25	
12 Balb/c female mice		Gavage	20 mg/kg	*t*-3-Gluc (1 μM), *t*-3-Sulf (5 μM)	—	—	0.25	
12 Balb/c female mice		Gavage	60 mg/kg	*t*-3-Gluc (175 μM), *t*-3-Sulf (300 μM)	—	—	0.25, 0.5	

(Continued)

Table 13.3 *(Continued)*

Animals	Source	Administration	Dose mg/kg	Blood or Serum or Plasma	Urinary excretion		Time (h)	Reference
				Metabolites (Concentration)	%	Metabolites		
5 male Sprague–Dawley rats	*t*-Resv	Oral	20 mg/kg	—	—	Gluc, Sulf, DHR, DHR-Sulf	—	Wang et al., 2005
Female CF-1 mice	Grape juice	Oral	~0.7 mg/kg Resv	n.d.	n.d.	—	—	Meng et al., 2004
			~1.4 mg/kg Resv	n.d.	1–2%	*t*-Resv *	24	
			~2.7 mg/kg Resv	n.d.	0.9–2.3%	*t*-Resv *	24	
2 female Wistar rats	*t*-Resv	Intragastric	2 mg/kg	Resv: 0.09 μM	—	—	4	
				Total: 1.2 μM	—	—	4	
			5 mg/kg	Resv: 0.11 μM	—	—	1.5	
				Total:1.5μM	—	—	4	
5 Sprague–Dawley rats	*t*-Resv *c*-Resv	Intravenous	5.13 mg/kg	*t*-Resv (~21.9 μM)	—	—	0.08	Chen et al., 2007
			4.87 mg/kg	*c*-Resv (~17.5 μM)	—	—	0.08	
Male Sprague–Dawley rats		Oral	2 mg/kg	*t*-Resv (0.77 μM)	—	—	0.25	Juan et al., 1999

[a] t-Resv: *trans*-resveratrol; *t*-3-Gluc: *trans*-resveratrol-3-*O*-glucuronide; *t*-3-Sulf: *trans*-resveratrol-3-sulfate; Gluc: resveratrol glucuronide; Sulf: resveratrol-sulfate; DHR: 7,8-dihydroresveratrol; DHR-Sulf: 7,8-dihydroresveratrol sulfate; radioactivity was measured by dpm: desintegrations per minute.
* Quantified after hydrolysis.

when catechin, quercetin, and resveratrol (1–10 nM) were co-administered in rats. They found no competition between the three polyphenols and the absorption of *trans*-resveratrol was not saturable in the used concentrations [Soleas et al., 2001a].

In 2006, El-Mohsen et al. investigated the time-dependent appearance and disappearance in various organs of metabolic products of [^{3}H] *trans*-resveratrol (2 and 18 h following gastric administration of 50 mg/kg + 1.85 MBq) to Sprague–Dawley rats. At 2 h postgavage, most of the recovered radioactivity was still present in the gastrointestinal tract. The total dose administered reached only 1.7% in plasma. The only tissues with high concentrations were the liver and the kidney while the amount detected in other tissues was $<0.1\%$. In contrast, at 2 and 18 h postadministration, approximately 11% of total dose was accounted for in all of the studied tissues (Table 13.2). They found that around 90% of the administered dose was absorbed, however, only 3.3% of absorbed resveratrol was detected in urine. The undetected radioactivity is expected to be lost via respiration and/or accumulation in other tissues, such as skeletal muscle and adipose tissue [El-Mohsen et al., 2006; Soleas et al., 2001a].

The metabolites of [^{3}H] *trans*-resveratrol detected in tissues and plasma were also investigated. In kidney, liver, heart, lungs, brain, and plasma (2-h samples), the only metabolite found was resveratrol–glucuronide. Glucuronides in plasma and kidney disappeared completely at 18 h. In lungs, liver, heart, and brain, the main detected metabolite at 18 h was the *trans*-resveratrol (Table 13.2). This study provided data on the metabolic fate of resveratrol. While glucuronides were predominant in plasma and tissues at the earlier times, the aglycone represented the main form at later times [El-Mohsen et al., 2006].

The kinetics of absorption, tissue distribution, and excretion was assessed after a single oral dose of ^{14}C-*trans*-resveratrol to male Balb/c mice. Blood and tissue samples were collected at 1.5, 3, and 6 h postadministration [Vitrac et al., 2003]. The autoradiographic examination of mice tissue sections and the radioactivity quantification revealed a higher fixation of ^{14}C-*trans*-resveratrol in the stomach, liver, kidney, intestine, bile, and urine and other organs of absorption and elimination. The concentration of radioactivity in blood was low and regular during the experimental period. During the entire experimental period, nearly complete absorption occurred in the small intestine as suggested by the higher concentration found in the proximal section compared to the distal section. After 6 h of oral administration the high concentration in stomach and duodenum was perhaps located in the mucosa, although this was also probably due to residual stomach content. The low concentrations found in the colon suggests that it was a minor way of elimination. The major concentrations found in urine and the decreasing concentrations in kidney showed that renal excretion was a major way of elimination. The kidney and the liver were the organs with highest deposition of ^{14}C-*trans*-resveratrol. In contrast to the kidney in which the parent drug was the major radioactive product, liver extracts 3 h after administration showed the presence of ^{14}C-*trans*-resveratrol with a high concentration of radioactive glucuronide or sulfated conjugated [Vitrac et al., 2003].

Contrasting with other studies that showed the accumulation of labeled resveratrol in tissues, the following studies addressed the search for the formed metabolites in biological fluids and tissues. Yu et al. [2002] synthesized and identified for the first time, the monosulfate isomers of resveratrol, resveratrol-3 and 4′-sulfate. These studies were performed in female Sprage–Dawley rats after intraperitoneal administration of 20 mg/kg of resveratrol with urine collection at 2 h and in female Balb/c mice after intraperitoneal (20 mg/kg) and oral administration (20 and 60 mg/kg) with collection of serum samples up to 4 h. The mass spectrometry analysis of rat urine samples only detected *trans*-resveratrol-3-glucuronide. In the mouse serum samples, after administration of 20 mg/kg via intraperitoneal or oral, maximum concentrations of resveratrol-3-glucuronide and resveratrol-3-sulfate were observed at 15 min. The sulfate was almost three-fold greater than the glucuronide (Table 13.3). Only traces of free resveratrol were observed. Furthermore, no resveratrol or metabolites were detected after 1 h. When higher doses were orally administered to mouse (60 mg/kg), the same metabolites as before were observed, but the maximum value for sulfate metabolite were reached after 30 min instead of 15 min, probably because more time was required to absorb the large volume that was administered. This was not the case for the minor dosage both glucuronide and sulfate metabolite were still detected after 3 h, suggesting that resveratrol was distributed to tissues and cleared slowly.

Wenzel et al. [2005] synthesized the same resveratrol-3 and 4′-sulfates as Yu et al [2002] but also the 3 and 4′-glucuronide, 3,4′-disulfate, 3,5-disulfate, and 3,4′,5-trisulfate metabolites of resveratrol. To search for these compounds in in vivo conditions, two experiments were carried out. Resveratrol aglycone was administered to male Wistar rats in a dosage of 50 or 300 mg/kg/day during 8 weeks, and urine, feces, and tissue samples were collected. As shown in Table 13.2, the administration of 50 mg/kg of resveratrol resulted in the formation of *trans*-resveratrol-3-sulfate, *trans*-resveratrol-disulfate, *trans*-resveratrol-3-glucuronide, and resveratrol in urine. Furthermore, an increase of the dosage (sixfold) showed the additional formation of *trans*-resveratrol-trisulfate. In both experiments, the 3-glucuronide was the main metabolite, and sulfate (3-sulfate and 3,5-disulfate) derivatives were 100- and 50-fold less, in relation to the dosage, respectively. The total recovery in urine of rats on 50 and 300 mg/kg was 15 and 54%, respectively. In feces samples of rats of the 50-mg/kg group, only 3-sulfate and resveratrol were determined, and 300 mg/kg administration resulted in the formation of all possible sulfates except trisulfate metabolite. The total recovery in feces samples was 15 and 31%, respectively, for both dosages [Wenzel et al., 2005]. They also studied the different distribution of these metabolites in plasma, kidney, and liver tissues and only after feeding 300 mg/kg were metabolites observed in 50% of the animals. The main metabolite in plasma samples was 3,4′-disulfate followed by 3,4′,5-trisulfate and 3-glucuronide metabolites (Fig. 13.2). In liver samples only 3 and 4′-monosulfates and 3-monoglucuronide were identified. The main metabolite in kidneys was the 3-glucuronide metabolite, followed by disulfates and minor 3-sulfate. In contrast to plasma and kidney, free resveratrol was only observed in liver samples.

Similarly in 2005, Wang et al. identified the microbial metabolites of resveratrol in rats (Table 13.3). Urine samples were obtained after oral administration of 20 mg/kg to Sprague–Dawley rats. They identified resveratrol–glucuronide, resveratrol–sulfate, 7,8-dihydroresveratrol, and 7,8-dihydroresveratrol–sulfate as the main 12-h urinary metabolite in rats by mass spectrometry after are SPE treatment [Wang et al., 2005].

In 2004, Meng et al. investigated the urinary and plasma levels of resveratrol and quercetin after their administration as constituents of grape juice or as pure aglycones. The first study was carried out during 4 days with female CF-1 mice receiving solutions containing 18.4 and 36.8% of grape preparation. The urinary excretion of resveratrol increased gradually during the study period, excreting a cumulative amount of approximately 1–2% of the ingested dose when receiving 18.4% grape juice and 0.9–2.3% when the dose was 36.8%. The second study was done after the oral dose of 2 and 5 mg/kg resveratrol to female Wistar rats. In plasma for both doses, resveratrol was mainly present as conjugates and the resveratrol aglycone constituted around 10–11% of total resveratrol at the beginning and declined to 5–7% at 4 h (Table 13.3) [Meng et al., 2004].

PHARMACOKINETICS OF RESVERATROL AND DERIVATIVES

The evaluation of pharmacokinetic analysis of resveratrol was carried out in different animal models such as rats, mice, and rabbits [Asensi et al., 2002; Chen et al., 2007; Juan et al., 2002; Marier et al., 2002; Sale et al., 2004]; furthermore, pharmacokinetic analysis was also evaluated with other resveratrol derivatives such as piceid [Lv et al., 2006; Zhou et al., 2007], a *Smilax china* root extract [Huang et al., 2008], 3,4,5,4′-tetramethoxystilbene [Sale et al., 2004], piceatannol, pinosylvin, and rhapontigenin [Roupe et al., 2006] (Table 13.4).

In the first kinetic study, Juan et al. [2002] focused on the determination of the time course of *trans*-resveratrol level in plasma after the 2-mg/kg orally administrated dose to rats. Resveratrol had already reached the bloodstream at 5 min, presented its maximum level at around 10 min, and was still detected after 60 min [Juan et al., 2002].

Another pharmacokinetic study was carried out after intravenous administration of 4.87 mg/kg of *cis*-resveratrol and 5.13 mg/kg of *trans*-resveratrol to Sprague–Dawley rats. The study showed that both isomers showed a rapid eliminate disposition in 90 min [Chen et al., 2007].

There is a further pharmacokinetic study in which *trans*-resveratrol in the aglycone form and the glucuronide forms were examined following intravenous (15 mg/kg) and oral (50 mg/kg) administration of *trans*-resveratrol to rats [Marier et al., 2002]. After intravenous administration, the plasmatic resveratrol concentrations declined rapidly over the first 2 h. Then, concentration profiles of resveratrol and resveratrol–glucuronide from intravenous or oral administration increased abruptly due to enterohepatic recirculation over the 4- to 8-h time period that resulted in a significant maintenance in the terminal elimination

Table 13.4 Pharmacokinetic Studies of Resveratrol in Animal Models and Humans[a]

Species	Source (dose)	Administration	C_{max} (μmol/L)	T_{max} (h)	$T_{1/2}$ (h)	AUC $_{0-\infty}$ (μmol · h/L)	Urinary Excretion (%)	References
6 male Wistar rats	Piceid (50 mg/kg)	Oral	0.93 (0.39)	0.35 (0.14)	1.68 (0.3)	2.23 (0.69)	—	Lv et al., 2006
C57BL/6J male mice	*t*-Resv (20 mg/kg)	Intragastric	2.6 (1.0)	2.5	—	—	—	Asensi et al., 2002
Wistar male rats		Intragastric	1.2 (0.4)	5	—	—	2	
6 ESD NZW male rabbits		Intragastric	1.1 (0.8)	2.5	—	—	—	
		IV	—	—	0.24	—	—	
3 mice	*t*-Resv (240 mg/kg)	Intragastric	32	0.2	—	—	—	Sale et al., 2004
6 male Sprague–Dawley rats	*t*-Resv (15 mg/kg)	Intravenous	—	—	Resv: 0.13 (0.02) Gluc: n.a.	Resv: 5.64 (0.5) Gluc: 38.7 (5.5)	—	Marier et al., 2002
6 male Sprague–Dawley rats	*t*-Resv (50 mg/kg)	Oral	Resv: 6.57 (1.55) Gluc:105.2 (32.4)	Resv: 0.29 (0.1) Gluc: 0.42 (0.3)	Resv: 1.48 (0.4) Gluc:1.55 (0.4)	Resv: 7.1 (2.0) Gluc: 324.7 (57.6)	—	
5 female Sprague–Dawley rats	1 g/kg *Smilax china* root extract equivalent to 180 mg/kg *O*-Resv and 80 mg/kg Resv	Oral	*O*-Resv: 21.93 (3.1) Resv: 9.61 (1.5)	0.25 0.25	— —	91.78 (13.7) 17.81 (0.6)	— —	Huang et al., 2008
Male Sprague–Dawley rats	*t*-Resv (2 mg/kg)	Intragastric	Resv: 2.57	*t*-Resv 0.16	0.25	—	—	Juan et al., 2002
8 Wistar rats	*t*-Piceid (150 mg/kg)	Oral	*t*-Gluc: 64.85 (18.5) *t*-Resv: 3.55 (0.7) *t*-Piceid: 4.35 (1.3)	2 1 0.5	4 2 1	— — —	— — —	Zhou et al., 2007
5 male Sprague–Dawley rats	Piceatannol: (10 mg/kg)	Intravenous	—	—	4.23 (1.25)	34.75 (10.2)	32.8	Roupe et al., 2006
	Pinosylvin: (10 mg/kg)		—	—	0.82 (0.05)	24.67 (5.7)	9.46	

(*Continued*)

Table 13.4 *(Continued)*

Species	Source (dose)	Administration	C_{max} (μmol/L)	T_{max} (h)	$T_{1/2}$ (h)	AUC $_{0-\infty}$ (μmol · h/L)	Urinary Excretion (%)	References
5 male Sprague–Dawley rats								
5 male Sprague–Dawley rats	Rhapontigenin: (10 mg/kg)		—	—	3.0 (1.35)	32.52 (0.4)	1.25	
10 humans (45% males)	*t*-Resv (7.7 mg/kg)	Oral	*t*-Resv: 0.32 (0.16) Gluc 1: 1.00 (0.35) Gluc 2: 0.91 (0.36) 3-Sulf: 3.69 (0.95)	*t*-Resv: 0.83 (0.5-1.5) Gluc 1: 2.00 (1.0-6.0) Gluc 2: 1.50 (1.0-5.0) 3-Sulf: 1.50 (1.0-5.0)	*t*-Resv: 2.85 Gluc 1: 2.85 Gluc 2: 3.09 3-Sulf: 3.21	*t*-Resv: 0.98 Gluc 1: 8.4 (0.1) Gluc 2: 5.6 (0.1) 3-Sulf: 17.8(0.1)	*t*-Resv: 0.04 (0.05) Gluc 1: 2.0 (0.4) Gluc 2: 8.9 (2.6) 3-Sulf: 11.4 (2.3)	Boocock et al 2007
10 humans (45% males)	*t*-Resv (15.4 mg/kg)		*t*-Resv: 0.51 (0.38) Gluc 1: 1.17 (0.90) Gluc 2: 1.66 (1.35) 3-Sulf: 6.82(21.39)	*t*-Resv: 0.759 (0.5-4.0) Gluc 1: 2.25 (1.0-6.0) Gluc 2: 1.75 (1.0-5.1) 3-Sulf: 2.00 (1.0-5.0)	*t*-Resv: 8.87 Gluc: 1 7.27 Gluc: 2 6.64 3-Sulf: 4.51	*t*-Resv: 2.4 (0.3) Gluc 1: 13.4(0.3) Gluc 2: 11.4(0.3) 3-Sulf: 44.1(0.3)	*t*-Resv: 0.1 (0.1) Gluc 1: 2.1 (1.1) Gluc 2: 3.2 (1.7) 3-Sulf: 7.3 (3.1)	
10 humans (45% males)	*t*-Resv (38.5 mg/kg)		*t*-Resv: 1.18 (0.65) Gluc 1: 2.16 (0.81) Gluc 2: 4.02 (2.88) 3-Sulf: 9.05 (2.46)	*t*-Resv: 1.38 (0.5-4.0) Gluc 1: 2.375 (1.0-8.0) Gluc 2: 2.00	*t*-Resv: 4.22 Gluc 1: 10.6 Gluc 2: 8.42 3-Sulf: 11.5	*t*-Resv: 3.4 (0.2) Gluc 1: 24.8(1.2) Gluc 2: 18.9(0.1) 3-Sulf: 74.5(0.2)	*t*-Resv: 0.1 (0.1) Gluc 1: 1.7 (1.7) Gluc 2: 3.1 (1.4)	

(Continued)

Table 13.4 *(Continued)*

Species	Source (dose)	Administration	C_{max} (μmol/L)	T_{max} (h)	$T_{1/2}$ (h)	AUC $_{0-\infty}$ (μmol · h/L)	Urinary Excretion (%)	References
				(1.0-6.0) 3-Sulf: 2.00 (1.0-5.2)			3-Sulf: 5.2 (2.6)	
10 Humans (45% males)	*t*-Resv (76.9 mg/kg)		*t*-Resv: 2.36 (1.71) Gluc 1: 3.18 (1.47) Gluc 2: 4.29 (2.85) 3-Sulf: 13.94(6.69)	*t*-Resv: 0.83 (0.5-1.5) Gluc 1: 2.00 (1.0-6.0) Gluc 2: 1.50 (1.0-5.0) 3-Sulf: 1.50 (1.0-5.0)	*t*-Resv: 8.52 Gluc 1: 7.90 Gluc 2: 5.83 3-Sulf: 7.71	*t*-Resv: 5.8(0.3) Gluc 1: 43.5(0.2) Gluc 2: 37.5(0.3) 3-Sulf: 135.5(0.2)	*t*-Resv: 0.1 (0.1) Gluc 1: 0.5 (0.3) Gluc 2: 3.0 (1.4) 3-Sulf: 5.0 (1.6)	

[a] *t*-Resv: *trans*-resveratrol; *O*-Resv: oxyresveratrol; Gluc: resveratrol-glucuronide; 3-Sulf: resveratrol-3-sulfate.

half-life of resveratrol aglycone. The clearance of resveratrol after oral or intravenous administration was higher than that of resvertarol–glucuronide, which resulted in a systemic exposure of approximately 46- or 7-fold, respectively, lower than that of glucuronide.

Asensi et al. [2002] studied tissue levels and pharmacokinetics of resveratrol after intravenous (20 mg/kg) and oral (20 mg/kg) administration to rabbits, rats, and mice. The highest concentration levels in plasma of *trans*-resveratrol were reached within the first 5 min in all animals studied but showed a short half-life and a rapid clearance. They found extravascular levels of resveratrol after its oral administration to rabbits, rats, and mice with the highest levels occurring within the first 10 min in liver, lung, brain, and kidney; therefore, it appears that resveratrol does not accumulate extravascularly and its presence in the tissues is parallel in time to its bioavailability in blood [Asensi et al., 2002]. They suggested that most or all circulating resveratrol may be removed by liver metabolism and if high doses are administered, high rates of hepatic metabolism could be occurring [Asensi et al., 2002].

Sale et al. [2004] studied the pharmacokinetic properties of 3,4,5,4′-tetramethoxystilbene compared with those of resveratrol in the plasma and mice tissues. This analog compound was a novel congener of pharmacological interest, and it was under preclinical evaluation as a potential antitumor prodrug. This tetramethoxystilbene was capable of preferentially interfering with the proliferation and survival of transformed human lung-derived cells, with much lower growth inhibitory and apoptotic properties in its untransformed counterparts than resveratrol, which does not possess this discriminatory potential. Therefore, a kinetic evaluation and a tissue distribution for both were applied after a single dose (240 mg/kg) oral administration to mice. The results suggested that the introduction of four methoxy groups into the stilbene structure, three of which replaced the hydroxyl moieties in resveratrol, did not increase the systemic availability of the molecule in comparison to resveratrol [Sale et al., 2004].

They also evaluated the pharmacokinetic properties of resveratrol compared to the synthetic analog in different mice tissues in which resveratrol might prevent malignancy or delay its onset. The availability of the synthetic analog was inferior in plasma, liver, kidney, lung, and heart than resveratrol; meanwhile, it was more available in intestinal and colonic mucosa and in brain [Sale et al., 2004]. These results provided a good argument to assess 3,4,5,4′-tetramethoxystilbene as a colorectal cancer chemo preventive agent. Furthermore, in the search for the main conjugated forms, resveratrol showed its glucuronided and sulfated conjugates, while, the 3,4,5,4′-tetramethoxystilbene underwent metabolic hydroxylation or single and double *O*-demethylation [Sale et al., 2004].

The pharmacokinetic dispositions of other stilbenes that are structurally similar to resveratrol and have pharmacological activity across many anticancer, anti-inflammatory, and antioxidant assays have been studied. The pharmacokinetics was characterized in male Sprague–Dawley rats after single intravenous

doses of 10 mg/kg of piceatannol, pinosylvin, or rhanpontigenin. The detectable plasma half-lives of these compounds appear to be relatively short. The estimates of oral bioavailability characterize these stilbenes as poorly bioavailable compounds. All three stilbenes undergo extensive glucuronidation upon intravenous administration, as was determined by plasma and urine concentrations. The total amount excreted shows that the three stilbenes excreted in urine—32.8, 9.5, and 1.3%, respectively—are very small compared with the overall dose given of each one (3.5 mg). This indicates that the three stilbenes in contrast to resveratrol are eliminated predominantly via nonrenal excretion [Roupe et al., 2006].

Recently, the pharmacokinetics of resveratrol from *Smilax china,* a rhizome extensively used in traditional Chinese medicine was evaluated [Huang et al., 2008]. Forty-five female rats were orally administered with 1 g/kg *S. china* extract equivalent to 180 mg/kg of oxyresveratrol and 80 mg/kg of resveratrol. The pharmacokinetic parameters showed that the two stilbenes were rapidly absorbed into the body fluid from the gastrointestinal tract and could still be detected in the plasma at least 6 h after the administration [Huang et al., 2008], probably due to the high dose administered.

PICEID, THE GLUCOSIDE OF RESVERATROL

Various studies have focused on the metabolism of piceid, the glucoside of resveratrol. These studies are of great interest due to the higher amount of piceid compared to resveratrol in food. Therefore, bioavailability studies of this compound are required. In vitro studies have already observed the absorption of piceid in the enterocytes, but in vivo studies in animal models are still scarce.

The bioavailability and tissue distribution of *trans*-piceid was studied in Wistar rats after its oral administration [Lv et al., 2006; Zhou et al., 2007]. At present, the studies of the pharmacokinetics and distribution of piceid have been poorly documented. The first study with piceid administration to rats, measured its pharmacokinetics and its tissue distribution after a single oral administration of 50 mg/kg to 6 male Wistar rats. This was the first in vivo study that demonstrated the absorption of piceid with maximum plasma concentrations of 0.93 (0.4) μM at 21 min (Table 13.2) [Lv et al., 2006]. Another relevant result of this study was the diffusion of piceid to tissues. The highest concentrations were found in the stomach, then, in the small intestine, followed by spleen, lung, brain, testis, liver, kidney, and heart at 10 min. At 30 min, high concentrations were still detected in stomach and relatively high concentrations were present at 120 min. The absorption, distribution, and elimination of piceid were quick after the oral administration. The major distribution organs in rats were stomach, small intestine, and spleen; furthermore, it was able to cross the blood–brain and blood–testis barriers. Nevertheless, no long-term accumulation of piceid in tissues took place [Lv et al., 2006].

A recent study showed the bioavailability of piceid after oral administration of 150 mg/kg of piceid to rats. *trans*-Piceid was absorbed, with maximum plasma levels at 30 min, and metabolized to *trans*-resveratrol, with a maximum plasma concentration at 60 min, and this to *trans*-resveratrol–glucuronide, with maximum concentration at 120 min [Zhou et al., 2007]. The resveratrol–glucoside was absorbed by transepithelial transport across the intestine with maximum concentration occurring at 30 min after administration. This glycosytated derivative is deglycosylated in *trans*-resveratrol in the intestine with a cleavage by the CBG after passing the brush–border membrane by SGLT1 or by the membrane-bound enzyme LPH followed by passive diffusion of the released *trans*-resveratrol, which is further metabolized inside the cells into glucuronoconjugates [Henry-Vitrac et al., 2006]. The constant absorption of piceid from the first minutes of ingestion is reflected in the *trans*-resveratrol–glucuronide with the highest concentrations (30-fold higher) taking place in plasma and having a relatively long elimination half-life. Furthermore, the parent drug and the metabolites, *trans*-resveratrol and *trans*-resveratrol–glucuronide were detected at 8, 12, and 24 h after the oral dose [Zhou et al., 2007].

In conclusion, resveratrol is absorbed and already shows plasmatic and serum levels between 5 min and 4 h, depending on the dose and the animal species. Furthermore, plasmatic levels increase between 4 and 8 h due to the enterohepatic recirculation [Marier et al., 2002]. When absorbed, resveratrol is metabolized and the major conjugated forms in plasma consisted of sulfate conjugates, in which minor concentrations of the 3-glucuronide were observed. Further studies are required to obtain more standards of metabolites and more data about the major sulfate metabolite since Wenzel et al. [2005] and Yu et al. [2002] found major amounts for 3,4′-disulfate and 3-sulfate conjugates, respectively. Resveratrol is also distributed to different tissues such as liver and kidney, the major organs of deposition, and also in the lung, spleen, and heart. It crosses the blood–brain and blood–testis barriers showing major concentration levels in the intestinal tract. Maximum concentrations were found at early hours and trace amounts at later hours, showing no accumulation extravascularly [Asensi et al., 2002]. The main metabolite found in tissues at early hours was the glucuronide form, and the free form of resveratrol predominated at later hours [El-Mohsen et al., 2006].

Renal excretion was the major way of elimination compared to the colonic one. Urinary excretion varied between 3 and 61%, depending on the study, animal species, and dose. Some studies had also shown a possible excretion via sweat and respiratory system and metabolism to CO_2 [El-Mohsen et al., 2006; Soleas et al., 2001a]. The major conjugated form present in urine is the 3-glucuronide metabolite [Wenzel et al., 2005; Yu et al., 2002] although mono-, di-, and tri-sulfated metabolites and free resveratrol were also determined [Wang et al., 2005; Wenzel et al., 2005]. Furthermore, microbial metabolites such as dihydroresveratrol and its sulfate conjugate were also identified in 12 h rat urine [Wang et al., 2005].

HUMAN STUDIES

Studies that investigate the bioavailability of resveratrol in humans are scarce. Moreover, the research in this area is quite recent. It has been summarized in Table 13.5. The experimental approaches have been improved with the use of new analytical techniques such as mass spectrometry to identify and quantify metabolites present in very low concentrations. Resveratrol and its metabolites have been measured in several biofluids: plasma or serum, urine, LDL, and feces.

In 2001, the group of Soleas, Yang, and Goldberg [Soleas et al., 2001b] was the first to investigate the oral administration of resveratrol: 25 mg of *trans*-resveratrol standard dissolved in 120 mL of white wine were consumed by 10 healthy volunteers. The analyses were performed by gas chromatography–mass spectrometry (GC-MS) after treatment with β-glucuronidases and sulfatases. After stilbene intake, free resveratrol and its conjugates were found in all subjects and at all times, even after an abstinence of at least 24 h from food sources of this polyphenol. In plasma samples, they found that the highest resveratrol concentration (345.1 μg/L) occurred at 30 min. Moreover, resveratrol conjugates were 20- to 50-fold more abundant than free resveratrol. After resveratrol intake, the recovery in urine of 24 h was 24.6% as free and conjugated forms. Likewise, the urine concentrations of resveratrol conjugates were 30- to 50-fold higher than aglycone. At 2 h the highest concentration of resveratrol was observed, it was nearly 8 mg/L.

Three years later the same group [Goldberg et al., 2003] tested the absorptive efficiency of *trans*-resveratrol standard (25 mg) dissolved in three different matrices: white wine, grape juice, and vegetable juice. The conditions of study were the same as in the previous work. The results were also similar and serum showed the highest level at 30 min. Furthermore, the total absorption curves were similar regardless the matrices. In this study slight amounts of resveratrol at basal time were detected too. Urinary 24-h resveratrol excretions were 17.0, 16.8, and 16.0% after oral administration of vegetable juice, wine, and grape juice, respectively. The results in plasma and urine supported that there were no differences in resveratrol absorption by using the different matrices.

In 2004, Meng et al. published the first study that investigated the bioavailability of grape juice (ranged from 200 to 1200 mL) after oral ingestion, whose composition was 1.6 mg/L of stilbene, mainly as piceid [Meng et al., 2004]. Oral consumption of *trans*-resveratrol standard at several concentrations (0.03, 0.5, and 1 mg/kg) were also studied. In this case, the analyses were performed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) after enzymatic hydrolysis. Resveratrol was only found in plasma when high doses (1 mg/kg) were ingested. At lower concentrations of standard or grape juice were ingested, no peaks were reached. However, urinary recoveries were 52, 34, and 26% after 0.03, 0.5, and 1 mg/kg, respectively. These results could suggest an inversely dose-dependent manner. Furthermore with a dose of 0.03 mg/kg, resveratrol was mainly excreted in the first 2–3 h; however, with 1 mg/kg, 7–10 h were necessary to excrete most of the resveratrol. Resveratrol was mainly found in the conjugated form as

Table 13.5 Human Bioavailability Studies[a]

Participants	Gender (%)	Age (y[c])	Resveratrol Source	Dose (mg/kg body weight)	Concentration (μmol/L)	Time (h)	Urinary Excretion (%) (Time, h)	Reference
10	Male (45%)	19–61	RESV Standard in 120 mL of white wine	0.357 mg/kg (25 mg total)	Resv 0.031 Conjugated[b] 1.48	0.5–1	Total 24.6% (24 h)	Soleas et al., 2001
4	Males (100%)	25–45	RESV Standard dissolved in 100 mL vegetable juice	0.357 mg/kg (25 mg total)	Resv 0.037 Conjugated[b] 2.03	0.5	17.0 (24 h)	Goldberg et al., 2003
4			RESV Standard dissolved in 100 mL white wine	0.357 mg/kg (25 mg total)	Resv 0.031 Conjugated[b] 1.80	0.5	16.8 (24 h)	
4			RESV Standard dissolved in 100 mL grape juice	0.357 mg/kg (25 mg total)	Resv 0.035 Conjugated[b] 1.82	0.5	16.0 (24 h)	
1	Male	30–50	RESV Standard	0.03 mg/kg	n.d.		Gluc: 52 (24 h)	Meng et al., 2004
1				0.5 mg/kg	n.d.		Gluc: 34 (24 h)	
1				1 mg/kg	Gluc 1.86	1.5	Gluc: 26 (24 h)	
1			200 mL grape juice	0.005 mg/kg[c] (0.32 mg total)	n.d.		n.d. (24 h)	
1			400 mL grape juice	0.009 mg/kg[c] (0.64 mg total)	n.d.		n.d. (24 h)	
1			600 mL grape juice	0.014 mg/kg[c] (0.96 mg total)	n.d.		Gluc: (24 h)	
1			1200 mL grape juice	0.027 mg/kg[c] (1.92 mg total)	n.d.		Gluc: 5.0 (24 h)	
6	3 Males (50%)	23–34	^{14}C- RESV standard Oral	0.385 mg/kg[cc] (25 mg total)			53.4–84.9% (72 h)	Walle et al., 2004
5			^{14}C- RESV standard Intravenous	0.023 mg/kg[c] (1.5 mg total)			42.3–83.2% (72 h)	
1			RESV Standard ORAL	1.538 mg/kg[c] (100 mg total)	Tr.		Gluc: 13 (1) Sulf: 24 (3) Total 37 (12 h)	
10	Males (100%)	30 (25–40)	300 mL red wine Lambrusco + meal	0.0034 g/kg	Gluc 0.096	1		Vitaglione et al., 2005
5	1 Male (20%)	29 (24–38)	600 mL red wine Cabernet Franc	0.0329 μg/kg	Gluc 0.687	0.5–2		
10	3 Males (30%)	31 (24–54)	600 mL red wine Aglianico + meal	0.0075 μg/kg	Resv 0.004 Gluc 0.150	0.5 1–2		
10	Male (100%)	28.2 (25–41)	300 mL/d sparkling wine (28 d)	0.005 mg/kg/d (0.357 mg/d)[c]	n.d.		*t*-3-Gluc: 4.8 (2.5) *c*-3-Gluc: 2.4 (1.3) Total 7.2 (2.7) (morning urine)	Zamora-Ros et al., 2006

(*Continued*)

Table 13.5 *(Continued)*

Participants	Gender (%)	Age (y[c])	Resveratrol Source	Dose (mg/kg body weight)	Concentration (μmol/L)	Time (h)	Urinary Excretion (%) (Time, h)	Reference
10	Women (100%)	38.1 (25–50)	200 mL/d white wine (28 d)	0.007 mg/kg/d (0.398 mg/d)[c]	n.d.		*t*-3-Gluc: 11.7 (2.8) *c*-3-Gluc: 3.3 (3.6) Total 15.0 (4.3) (morning urine)	
10	Women (100%)	38.1 (25–50)	200 mL/d red wine (28 d)	0.043 mg/kg/d (2.56 mg/d)[c]	n.d.		*t*-3-Gluc: 4.2 (3.2) *c*-3-Gluc: 1.2 (1.2) Total 5.4 (3.9) (morning urine)	
5	Male (100%)	25–28	250 mL red wine	0.077 mg/kg[c] (5.4 mg total)			*t*-4' Gluc: 0.13 (0.19) *t*-3 Gluc: 0.38 (0.59) *c*-4' Gluc: 0.75 (1.2) *c*-3 Gluc: 1.9 (1.9) *t*-4' Sulf: 0.01 (0.03) *t*-3 Sulf: 0.16 (0.67) *c*-4' Sulf: 19.6 (17.4) *c*-3 Sulf: 0.47 (2.2) Total 23.4 (4 h)	Urpi-Sarda et al., 2007
9	Males (100%)	23–41	PICEID Standard dissolved in 100 mL ethanol (15%) + 400 mL milk (1.5% fat)	1.22 mg/kg (85.5 mg/70 kg)	*t*-3-Sulf: 0.95 (0.16) *t*-3,4′-Disulf: 0.33 (0.07) *t*-3,5-Disulf: 0.94 (0.17) 3-Gluc: 0.16 (0.04) 4′-Gluc: 0.19 (0.05) (2) *t*-Digluc: 0.35 (0.09)	*t*-3-Sulf: 1 *t*-3,4′-Disulf: 6- *t*-3,5-Disulf: 8 3-Gluc: 6-8 4′-Gluc: 6 (2) *t*-Digluc: 6	*t*-3-Sulf: 4.53 *t*-3,4′-Disulf: 1.71 *t*-3,5-Disulf: 7.18 3-Gluc: 2.99 4′-Gluc: 0.69 (2) *t*-Digluc: 2.65 Total 13.6-35.7 (48 h)	Burkon and Somoza 2008

[a] Resv: resveratrol; Gluc: glucuronide; Sulf: sulfate; *t*-4 Gluc: *trans*-resveratrol-4′-*O*-glucuronide; *t*-3 Gluc: *trans*-resveratrol-3-*O*-glucuronide; *c*-4 Gluc: *cis*-resveratrol-4′-*O*-glucuronide; *c*-3 Gluc: *cis*-resveratrol-3-*O*-glucuronide; *t*-4 Sulf: *trans*-resveratrol-4′-sulfate; *t*-3 Sulf: *trans*-resveratrol-3-sulfate; *c*-4 Sulf: *cis*-resveratrol-4′-sulfate; *c*-3 Sulf: *cis*-resveratrol-3-sulfate; *t*-3,4′-Disulf: *trans*-resveratrol-3,4′-disulfates; *t*-3,5-Disulf: *trans*-resveratrol-3,5-disulfates; *t*-digluc:*trans*-resveratrol-*C*/*O*-diglucuronides.

[b] Quantified after hydrolysis of resveratrol conjugates.

[c] Calculated as weight estimation 70 and 60 kg for males and females, respectively. n.d. non detected.

glucuronide. After grape juice consumption, at low doses (200 and 400 mL) peaks of resveratrol in urine were not detected. Although at high doses (600 and 1200 mL) only conjugated forms were found. Moreover, after 1200 mL of grape juice, the recovery was only about 5% of the dose administered. This study showed that the glycoside forms are absorbed less than aglycones.

In the same year, Walle et al. [2004] were the first to administer intravenous and oral labeled resveratrol in humans. After 25 mg of an oral ^{14}C-resveratrol dose (6 healthy subjects), total radioactivity in plasma was maximum (491 ng/mL) at approximately 1 h after the intake, and then it kept falling during the following 72 h over the study. After 1.5 mg intravenous ^{14}C-resveratrol (5 healthy subjects) total radioactivity fell rapidly, but plasma radioactivity remained for the following 72 h. Moreover, both half-lives ranged from 7 to 14 h after any dose. This data is important because a single high dose of resveratrol can be active in plasma at least half a day. After oral dosage, 53–85 and 0.3–38 radioactivity were recovered in urine and feces, respectively. Similar results were observed after intravenous doses: 42–83 and 0.6–23 of total radioactivity were found in urine and feces, respectively. High variability was observed in the urinary and fecal recoveries. Elimination half-lives in urine ranged from 6.5 to 18.8 h after oral or intravenous doses. The authors also tested the metabolites formed after a large unlabeled oral dose of 100 mg of resveratrol. The analyses were performed by LC-MS-UV. This was the first study of human urine that analyzed resveratrol metabolite profile, showing the presence of two monoglucuronides, a monosulfate, a dihydroresveratrol monoglucuronide, and a dihydroresveratrol monosulfate. Dihydroresveratrol metabolites could be formed by the intestinal microbiota as occurs with other polyphenols [Gonthier et al., 2003]. The sulfate and glucuronide conjugates excreted in the urine accounted for 24 and 13% of the dose, respectively. However, in plasma resveratrol or its metabolites were not detected at any time. Only trace amounts (less than 5 ng/mL) could be found in plasma after an oral dose of the 100 mg.

Vitaglione et al. [2005] evaluated the bioavailability of red wine resveratrol consumed with several meals: standard, fat, or lean meal. Identification and quantification of resveratrol and its metabolites in serums were done by LC-MS/MS. In the first experiment, 10 healthy males were involved in the assessment of the bioavailability of Lambrusco red wine (0.82 mg *trans*-resveratrol/L) consumed with a standard meal (Milanese beef cutlet and chips). Only in 4 of the volunteers at 1 h were some amount of resveratrol glucuronides that ranged from 15 to 168 ng/mL found. In the second experiment, 5 healthy volunteers were recruited to intake 600 mL of Cabernet Franc red wine (3.2 mg *trans*-resveratrol/L) over night while fasting. Only 3 of the 5 subjects showed resveratrol free or metabolites in serum. In 2 volunteers resveratrol aglycone was detected but not in quantifiable amounts. Resveratrol glucuronides (isomers 3 and 4′) were reached at different times (0.5–2 h) and different concentrations (77–900 ng/mL). In the third experiment, 10 healthy subjects consumed 600 mL of aglianico red wine (0.8 mg *trans*-resveratrol/L) with either a lean meal or with a fat meal. Free resveratrol was detected in 2 of the subjects

at concentrations ranging from 1 to 6 ng/mL at 30 min after wine consumption. Resveratrol–glucuronides were only detected in one subject of each intervention at 1–2 h after intake. This study clearly showed a high interindividual variation in the absorption and bioavailability of resveratrol.

Zamora-Ros et al [2006] carried out the first work that assessed the bioavailability of resveratrol (provided by different wines) in a regular intervention during 28 day. The analyses were performed by LC-MS/MS. In the first study, 10 healthy males were recruited to consume 300 mL/day of sparkling wine (1.19 mg resveratrol/l). After 28 day of supplementation, urinary *trans*- and *cis*-resveratrol-3-*O*-glucuronides were 75 and 38 nmol/g creatinine, respectively. In the second study, 10 healthy females were selected to consume 200 mL of white wine (1.99 mg resveratrol/L) or 200 mL of red wine (12.8 mg resveratrol/L) in a crossover clinical trial. Likewise after 28 days only resveratrol metabolites were detected in morning urine. *trans*- (205 and 473 nmol/g creatinine) and *cis*-resveratrol-3-*O*-glucuronides (58 and 140 nmol/g creatinine) were found after white and red wine intake, respectively. Those studies showed that urinary excretion was dose dependent. Furthermore, slight amounts of resveratrol metabolites were also detected at baseline periods. No free resveratrol or piceid were detected in any of the studies.

Urpi-Sarda et al. [2005, 2007] published the first works that investigated the presence of resveratrol in LDL. The analyses were performed by LC-MS/MS. Eleven healthy males were recruited to consume 250 mL of merlot wine (10.2 mg resveratrol/L). Free resveratrol, glycoside, glucuronidate, and sulfate forms were found in 24-h LDL. The detected metabolites were *cis*- and *trans*-, 3- and 4′-position, glucuronides, and sulfates. The more abundant metabolites were 88% glucuronides (*trans*-resveratrol-3-*O*-glucuronide, 112 pmol/mg LDL protein), 10.4% sulfates, and 2.0% *trans*-aglycone. Piceid was also found in LDL 24 h in lower concentrations (1.1–28.5 pmol/mg LDL protein). In the second experiment the metabolic profile was assessed at low resveratrol doses. Five healthy males were recruited to consume 250 mL of merlot wine (10.2 mg/L). Only conjugated forms were detected in urine 4 h after wine consumption. The more abundant metabolites were *cis*-resveratrol-4′-*O*-sulfate (9.3 μmol/g creatinine) and *cis*-resveratrol-3-*O*-glucuronide (0.9 μmol/g creatinine). Sulfation and glucuronidation represented 86.6 and 13.4% of total urinary resveratrol excretion, respectively.

In 2007, Boocock et al. [2007] were the first to publish a complete phase I dose pharmacokinetic study in humans. Ten healthy volunteers were recruited to consume single doses of oral resveratrol (0.5, 1, 2.5, or 5 g). Consumption of resveratrol did not cause serious adverse events. Analyses of resveratrol and its metabolites were performed by LC-MS/MS. In plasma in all intake doses resveratrol-3-sulfate (56%) was the highest metabolite, the second and third metabolites were monoglucuronides (17 and 23%, respectively), and, finally, the lowest was free resveratrol (5%). Resveratrol was rapidly absorbed, the T_{max} for all metabolites ranged between 0.8 and 2.4 h, although the half-lives of free resveratrol and the conjugated forms remained for a long time in plasma, between 2.9 and 11.5 h.

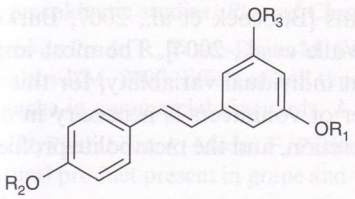
Urinary excretion mainly took place in the first 4 h after consumption (77% of total excretion), although resveratrol metabolites remained in urine between 12and24 h after intake. Free resveratrol, 2 glucuronides, and the 3-sulfate excreted in the urine 24 h after intake were below 0.04, 2, 9, and 11% of the 0.5 mg provided, respectively. At higher dose (5 mg) resveratrol, glucuronides and sulfate recoveries in the urine at 24 h were 0.1, 0.5, 3, and 5% of the dose, respectively. In urinary excretion, the sulfate forms were also higher than the glucuronide and free forms.

The piceid absorption was recently investigated for the first time. Nine healthy males participated in this controlled trial, which consisted of the administration of a single oral dose of 85.5 mg piceid standard per 70 kg. Resveratrol metabolites in plasma and urine were identified by LC-MS/MS, although these were quantified by HPLC-DAD. The same number of resveratrol metabolites were detected in both urine and plasma: *trans*-resveratrol-3-sulfate, *trans*-resveratrol-3,4′-disulfate, *trans*-resveratrol-3,5-disulfate, *trans*-3-*O*-glucuronide, *trans*-4′-*O*-glucuronide, and two resveratrol diglucuronides (*trans*-resveratrol-2-C-β-/4′-β-*O*-diglucuronide and *trans*-resveratrol-2-C-β-/5-β-*O*-diglucuronide). The two disulfates, previously identified in animals, and the two diglucuronides have been found in humans for the first time, thereby increasing the classical metabolic profile (monosulfates and monoglucoronides). The authors did not detect piceid nor resveratrol aglycone in any sample. Piceid was absorbed rapidly (1 h) in the form of *trans*-resveratrol-3-sulfate, whereas the other resveratrol metabolites reached their maximum concentration between 6 and 8 h after piceid administration. Sulfation pathway was more efficient than glucuronide. These authors also observed that 34, 44, and 46% of sulfates, disulfates, and diglucuronides, respectively, were noncovalently bound to plasma proteins; the rest of the percentage of conjugates were transported freely in plasma. After 24 h of piceid intake, no resveratrol metabolites were detected. The total urinary recovery ranged between 14 and 36%. The metabolic profile was approximately 15 and 8% as sulfate and glucuronide conjugates, respectively. Urinary excretion was completed within 48 h of oral piceid administration (Fig. 13.3).

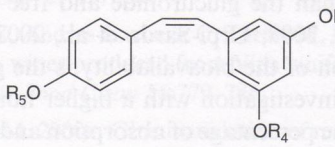
In conclusion, resveratrol seems to have a greater absorption than piceid at nutritional doses [Meng et al., 2004], although pharmacological doses of piceid standard is also recovered at similar percentages than resveratrol standard [Burkon and Somoza, 2008]. Moreover, resveratrol and piceid are absorbed and metabolized quickly. In blood samples, the highest resveratrol peak is detected at around 30–60 min [Boocock et al., 2007; Goldberg et al., 2003; Soleas et al., 2001b; Vitaglione et al., 2005] and 6 h [Burkon and Somoza, 2008] after consumption of resveratrol or piceid, respectively. Nevertheless traces of resveratrol could remain in plasma for at least 72 h after ingestion [Walle et al., 2004]. Resveratrol conjugates are more abundant than the free form. It seems that sulfation is a more efficient metabolic pathway than glucuronidation (56 vs. 39%) [Boocock et al., 2007; Burkon and Somoza, 2008]. Part of resveratrol is transported through the body bound to LDL mainly as glucuronides (88%)

INTESTINAL METABOLISM

trans-isomers



cis-isomers



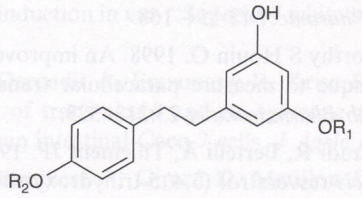
trans-Isomers

trans-Resveratrol-3-O-glucuronide: R_1=glucuronid acid, R_2=H, R_3=H (C20H20O9; MW: 404)
trans-Resveratrol-4′-O-glucuronide: R_1=H, R_2=glucuronid acid, R_3=H, (C20H20O9; MW: 404)
trans-Resveratrol-3,4′-diglucuronide: R_1=glucuronid acid, R_2=glucuronid acid, R_3=H (C26H28O15; MW: 580)
trans-Resveratrol-3-sulfate: R_1=SO_3H, R_2=H (C14H12O6S; MW: 308)
trans-Resveratrol-4′-sulfate: R_1=H, R_2=SO_3H, R_3=H (C14H12O6S; MW: 308)
trans-Resveratrol-3,4′-sulfate: R_1=SO_3H, R_2=SO_3H, R_3=H (C14H12O9S2; MW: 388)
trans-Resveratrol-3,5′-disulfate: R_1=SO_3H, R_2=H, R_3=SO_3H (C14H12O9S2; MW: 388)
trans-Resveratrol-3,5,4′-trisulfate: R_1=SO_3H, R_2=SO_3H, R_3=SO_3H (C14H12O12S3; MW: 468)

cis-Isomers

cis-Resveratrol-3-O-glucuronide: R_4=glucuronid acid, R_5=H (C20H20O9; MW: 404)
cis-Resveratrol-4′-O-glucuronide: R_4=H, R_5=glucuronid acid (C20H20O9; MW: 404)
cis-Resveratrol-3-4′-diglucuronide: R_4=glucuronid acid, R_5=glucuronid acid (C26H28O15; MW: 580)
cis-Resveratrol-3-sulfate: R_4=SO_3H, R_5=H (C14H21O6S; MW: 308)
cis-Resveratrol-4′-sulfate: R_4=H, R_5=SO_3H (C14H12O6S; MW: 308)
cis-Resveratrol-3,4′-disulfate: R_4=SO_3H, R_5=SO_3H (C14H12O9S2; MW: 388)

MICROBIAL METABOLISM



Dihydroresveratrol: R_1=H, R_2=H, (C14H14O3; MW: 230)
Dihydroresveratrol-glucuronide: R_1=H or glucuronide acid, R_2=glucuronide acid or H (C20H22O9; MW: 406)
Dihydroresveratrol-sulfate: R_1=H or SO_3H, R_2=SO_3H or H (C14H14O6S; MW: 310)

Figure 13.3 Pathways of resveratrol absorption, distribution, metabolism, and excretion.

and sulfates (11%) [Urpi-Sarda et al., 2005, 2007]. In a recent study, it was shown that more than 50% of resveratrol conjugates (sulfates, disulfates, and C/*O*-diglucuronides) were bound to proteins in plasma [Burkon and Somoza, 2008]. Urine (53–85%) and fecal (0.3–38%) were the most important ways of excretion of resveratrol measured by total radioactivity [Walle et al., 2004]. However, urinary recoveries by mass spectrometry ranged from 5 to 37%, depending on dose and the kind of resveratrol source [Boocock et al., 2007; Burkon and Somoza, 2008; Goldberg et al., 2003; Meng et al., 2004; Soleas et al., 2001b; Urpi-Sarda et al., 2007; Walle et al., 2004; Zamora-Ros et al., 2006]. Metabolites identified in urine are four monoglucuronides, four monosulfates, two

disulfates, two C/*O*-diglucuronides, free aglycone, dihydroresveratrol monoglucuronide, and monosulfate. Likewise in urinary excretion the sulfate forms seem to be higher than the glucuronide and free forms [Boocock et al., 2007; Burkon and Somoza, 2008; Urpi-Sarda et al., 2007; Walle et al., 2004]. The most important limitation of the bioavailability is the great individual variability; for this reason, further investigation with a higher number of volunteers is necessary in order to assess the percentage of absorption and excretion, and the metabolite profile of this polyphenol.

REFERENCES

Andlauer W, Kolb J, Siebert K, Furst P. 2000. Assessment of resveratrol bioavailability in the perfused small intestine of the rat. *Drugs Exp Clin Res* 26:47–55.

Asensi M, Medina I, Ortega A, Carretero J, Bano MC, Obrador E, Estrela J. 2002. Inhibition of cancer growth by resveratrol is related to its low bioavailability. *Free Radic Biol Med* 33:387–398.

Aumont V, Krisa S, Battaglia E, Netter P, Richard T, Merillon JM, Magdalou J, Sabolovic N. 2001. Regioselective and stereospecific glucuronidation of *trans*- and *cis*-resveratrol in human. *Arch Biochem Biophys* 393:281–289.

Barthe L, Woodley J, Houin G. 1999. Gastrointestinal absorption of drugs: Methods and studies. *Fundam Clin Pharmacol* 13:154–168.

Barthe L, Woodley JF, Kenworthy S Houin G. 1998. An improved everted gut sac as a simple and accurate technique to measure paracellular transport across the small intestine. *Eur J Drug Metab Pharmacokinet* 23:313–323.

Bertelli AA, Giovannini L, Stradi R, Bertelli A, Tillement JP. 1996. Plasma, urine and tissue levels of *trans*- and *cis*-resveratrol (3,4′,5-trihydroxystilbene) after short-term or prolonged administration of red wine to rats. *Int J Tissue React* 18:67–71.

Blache D, Rustan I, Durand P, Lesgards G, Loreau N. 1997. Gas chromatographic analysis of resveratrol in plasma, lipoproteins and cells after in vitro incubations. *J Chromatogr B Biomed Sci Appl* 702:103–110.

Boocock DJ, Faust GE, Patel KR, Schinas AM, Brown VA, Ducharme MP, Booth TD, Crowell JA, Perloff M, Gescher AJ, Steward WP, Brenner DE. 2007. Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. *Cancer Epidemiol Biomarkers Prev* 16:1246–1252.

Brill SS, Furimsky AM, Ho MN, Furniss MJ, Li Y, Green AG, Bradford WW, Green CE, Kapetanovic IM, Iyer LV. 2006. Glucuronidation of *trans*-resveratrol by human liver and intestinal microsomes and UGT isoforms. *J Pharm Pharmacol* 58:469–479.

Burkon A, Somoza V. 2008. Quantification of free and protein-bound *trans*-resveratrol metabolites and identification of *trans*-resveratrol-C/O-conjugated diglucuronides—Two novel resveratrol metabolites in human plasma. *Mol Nutr Food Res* 52:549–557.

Burns J, Yokota T, Ashihara H, Lean ME, Crozier A. 2002. Plant foods and herbal sources of resveratrol. *J Agric Food Chem* 50:3337–3340.

Chen X, He H, Wang G, Yang B, Ren W, Ma L, Yu Q. 2007. Stereospecific determination of *cis*- and *trans*-resveratrol in rat plasma by HPLC: Application to pharmacokinetic studies. *Biomed Chromatogr* 21:257–265.

de Andrés-de Prado R, Yuste-Rojas M, Sort X, Andres-Lacueva C, Torres M, Lamuela-Raventos RM. 2007. Effect of soil type on wines produced from Vitis vinifera L. cv. Grenache in commercial vineyards. *J Agric Food Chem* 55:779–786.

de Santi C, Pietrabissa A, Mosca F, Pacifici GM. 2000a. Glucuronidation of resveratrol, a natural product present in grape and wine, in the human liver. *Xenobiotica* 30:1047–1054.

de Santi C, Pietrabissa A, Spisni R, Mosca F, Pacifici GM. 2000b. Sulphation of resveratrol, a natural product present in grapes and wine, in the human liver and duodenum. *Xenobiotica* 30:609–617.

El-Mohsen M, Bayele H, Kuhnle G, Gibson G, Debnam E, Kaila SS, Rice-Evans C, Spencer JP. 2006. Distribution of [3H]*trans*-resveratrol in rat tissues following oral administration. *Br J Nutr* 96:62–70.

Goldberg DM, Yan J, Soleas GJ. 2003. Absorption of three wine-related polyphenols in three different matrices by healthy subjects. *Clin Biochem* 36:79–87.

Gonthier MP, Cheynier V, Donovan JL, Manach C, Morand C, Mila I, Lapierre C, Remesy C, Scalbert A. 2003. Microbial aromatic acid metabolites formed in the gut account for a major fraction of the polyphenols excreted in urine of rats fed red wine polyphenols. *J Nutr* 133:461–467.

González-Barrio R, Beltran D, Cantos E, Gil MI, Espín JC, Tomás-Barberán FA. 2006. Comparison of ozone and UV-C treatments on the postharvest stilbenoid monomer, dimer, and trimer induction in var. "Superior" white table grapes. *J Agric Food Chem* 54:4222–4228.

Henry C, Vitrac X, Decendit A, Ennamany R, Krisa S, Merillon JM. 2005. Cellular uptake and efflux of trans-piceid and its aglycone *trans*-resveratrol on the apical membrane of human intestinal Caco-2 cells. *J Agric Food Chem* 53:798–803.

Henry-Vitrac C, Desmouliere A, Girard D, Merillon JM, Krisa S. 2006. Transport, deglycosylation, and metabolism of trans-piceid by small intestinal epithelial cells. *Eur J Nutr* 45:376–382.

Huang H, Zhang J, Chen G, Lu Z, Wang X, Sha N, Shao B, Li P, Guo DA. 2008. High performance liquid chromatographic method for the determination and pharmacokinetic studies of oxyresveratrol and resveratrol in rat plasma after oral administration of Smilax china extract. *Biomed Chromatogr* 22:421–427.

Juan ME, Buenafuente J, Casals I, Planas JM. 2002. Plasmatic levels of trans-resveratrol in rats. *Food Res Int* 35:195–199.

Kaldas MI, Walle UK, Walle T. 2003. Resveratrol transport and metabolism by human intestinal Caco-2 cells. *J Pharm Pharmacol* 55:307–312.

Kuhnle G, Spencer JPE, Chowrimootoo G, Schroeter H, Debnam ES, Srai SKS, Rice-Evans C, Hahn U. 2000. Resveratrol is absorbed in the small intestine as resveratrol glucuronide. *Biochem Biophys Res Commun* 272:212–217.

Lv C, Zhang L, Wang Q, Liu W, Wang C, Jing X, Liu Y. 2006. Determination of piceid in rat plasma and tissues by high-performance liquid chromatographic method with UV detection. *Biomed Chromatogr* 20:1260–1266.

Maier-Salamon A, Hagenauer B, Wirth M, Gabor F, Szekeres T, Jager W. 2006. Increased transport of resveratrol across monolayers of the human intestinal Caco-2 cells is mediated by inhibition and saturation of metabolites. *Pharm Res* 23:2107–2115.

Marier JF, Vachon P, Gritsas A, Zhang J, Moreau JP, Ducharme MP. 2002. Metabolism and disposition of resveratrol in rats: Extent of absorption, glucuronidation, and enterohepatic recirculation evidenced by a linked-rat model. *J Pharmacol Exp Ther* 302:369–373.

Meng X, Maliakal P, Lu H, Lee MJ, Yang CS. 2004. Urinary and plasma levels of resveratrol and quercetin in humans, mice, and rats after ingestion of pure compounds and grape juice. *J Agric Food Chem* 52:35–942.

Rimando AM, Kalt W, Magee JB, Dewey J, Ballington JR. 2004. Resveratrol, pterostilbene, and piceatannol in vaccinium berries. *J Agric Food Chem* 52:4713–4719.

Romero-Perez AI, Lamuela-Raventos RM, Andres-Lacueva C, Torre-Boronat MC. 2001. Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. *J Agric Food Chem* 49:210–215.

Roupe KA, Yáñez JA, Teng XW, Davies NM. 2006. Pharmacokinetics of selected stilbenes: rhapontigenin, piceatannol and pinosylvin in rats. *J Pharm Pharmacol* 58:1443–1450.

Sale S, Verschoyle RD, Boocock D, Jones DJ, Wilsher N, Ruparelia KC, Potter GA, Farmer PB, Steward WP, Gescher AJ. 2004. Pharmacokinetics in mice and growth-inhibitory properties of the putative cancer chemopreventive agent resveratrol and the synthetic analogue trans 3,4,5,4′-tetramethoxystilbene. *Br J Cancer* 90:736–744.

Sobolev VS, Cole RJ. 1999. *trans*-Resveratrol content in commercial peanuts and peanut products. *J Agric Food Chem* 47:1435–1439.

Soleas GJ, Angelini M, Grass L, Diamandis EP, Goldberg DM. 2001a. Absorption of *trans*-resveratrol in rats. *Methods Enzymol* 335:145–154.

Soleas GJ, Yan J, Goldberg DM. 2001b. Ultrasensitive assay for three polyphenols (catechin, quercetin and resveratrol) and their conjugates in biological fluids utilizing gas chromatography with mass selective detection. *J Chromatogr B Biomed Sci Appl* 757:161–172.

Tokusoglu O, Unal MK, Yemis F. 2005. Determination of the phytoalexin resveratrol (3,5,4′-trihydroxystilbene) in peanuts and pistachios by high-performance liquid chromatographic diode array (HPLC-DAD) and gas chromatography-mass spectrometry (GC-MS). *J Agric Food Chem* 53:5003–5009.

Urpi-Sarda M, Zamora-Ros R, Lamuela-Raventós RM, Cherubini A, Jauregui O, de la Torre R, Covas M, Estruch R, Jaeger W, Andres-Lacueva C. 2007. HPLC-tandem mass spectrometric method to characterize resveratrol metabolism in humans. *Clin Chem* 53:292–299.

Urpi-Sarda M, Jauregui O, Lamuela-Raventós RM, Jaeger W, Miksits M, Covas M, Andres-Lacueva C. 2005. Uptake of diet resveratrol into the human low density lipoprotein. Identification and quantification of resveratrol metabolites by liquid chromatography coupled with tandem mass spectrometry. *Anal Chem* 77:3149–3155.

Vitaglione P, Sforza S, Galaverna G, Ghidini C, Caporaso N, Vescovi PP, Fogliano V, Marchelli R. 2005. Bioavailability of *trans*-resveratrol from red wine in humans. *Mol Nutr Food Res* 49:495–504.

Vitrac X, Desmouliere A, Brouillaud B, Krisa S, Deffieux G, Barthe N, Rosenbaum J, Merillon JM. 2003. Distribution of [^{14}C]-trans-resveratrol, a cancer chemopreventive polyphenol, in mouse tissues after oral administration. *Life Sci* 72:2219–2233.

Walle T, Hsieh F, DeLegge MH, Oatis JE, Walle UK. 2004. High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab Dispos* 32:1377–1382.

Wang D, Hang T, Wu C, Liu W. 2005. Identification of the major metabolites of resveratrol in rat urine by HPLC-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 829:97–106.

Wenzel E, Soldo T, Erbersdobler H, Somoza V. 2005. Bioactivity and metabolism of trans-resveratrol orally administered to Wistar rats1847. *Mol Nutr Food Res* 49:482–494.

Yu C, Shin YG, Chow A, Li Y, Kosmeder, JW, Lee YS, Hirschelman WH, Pezzuto JM, Mehta RG, van Breemen RB. 2002. Human, rat, and mouse metabolism of resveratrol. *Pharm Res* 19:1907–1914.

Zamora-Ros R, Andres-Lacueva C, Lamuela-Raventós RM, Berenguer T, Jakszyn P, Martínez C, Sánchez MJ, Navarro C, Chirlaque MD, Tormo MJ, Quirós JR, Amiano P, Dorronsoro M, Larrañaga N, Barricarte A, Ardanaz E, González CA. 2008. Concentrations of resveratrol and derivatives in foods and estimation of dietary intake in a Spanish population: European Prospective Investigation into Cancer and Nutrition (EPIC)—Spain cohort. *Br J Nutr* 100:188–196.

Zamora-Ros R, Urpi-Sarda M, Lamuela-Raventós RM, Estruch R, Vázquez-Agell M, Serrano-Martínez M, Jaeger W, Andres-Lacueva C. 2006. Diagnostic performance of urinary resveratrol metabolites as a biomarker of moderate wine consumption. *Clin Chem* 52:1373–1380.

Zhou M, Chen X, Zhong D. 2007. Simultaneous determination of *trans*-resveratrol-3-*O*-glucoside and its two metabolites in rat plasma using liquid chromatography with ultraviolet detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 854:219–223.