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Review

Metabolism and bioavailability of *trans*-resveratrol

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Resveratrol (3,4′,5-trihydroxy-*trans*-stilbene) is a polyphenolic compound accounting to the stilbene class. Most stilbenes in plants act as antifungal phytoalexins, compounds that are usually synthesized only in response to infection or injury. Resveratrol has been detected in trees, in a few flowering plants, in peanuts, and in grapevines. The major dietary sources of resveratrol include grapes, wine, peanuts, and peanut products. Numerous *in vitro* studies describe different biological effects of resveratrol. The major impacts are the antioxidative, anti-inflammatory, and estrogenic effects as well as anticancer and chemopreventive activities. In order to reveal information on absorption, metabolism, and the consequent bioavailability of resveratrol, different research approaches were performed, including *in vitro*, *ex vivo*, and *in vivo* models, all of which are considered in this review. Summarizing the data, resveratrol is absorbed and metabolized. Around 75% of this polyphenol are excreted *via* feces and urine. The oral bioavailability of resveratrol is almost zero due to rapid and extensive metabolism and the consequent formation of various metabolites as resveratrol glucuronides and resveratrol sulfates. The potential biologic activity of resveratrol conjugates should be considered in future investigations.

Keywords: Bioavailability / Metabolism / Resveratrol / Review

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1 Introduction

High amounts of resveratrol (Fig. 1) in wine have been speculated to be responsible for the decreased risk of coronary heart disease, called the "French paradox". This phenomenon describes the situation in France where the high intake of saturated fat correlates with a low mortality from coronary heart disease [1 – 3]. At present, analysis of red wine originating from diverse countries show some variations in ranges of resveratrol concentrations (up to 6.8 mg/L). Considerable amounts of piceid (Fig. 2), one of the glycosidic forms of resveratrol, also occur in red wine in varying amounts up to 50.8 mg/L [4 – 6]. But the resveratrol saga does not begin with its discovery in wine. It started in the early 1980s among Japanese scientists. In 1982, Arichi *et al.* [7] noted that the dried roots of *Polygonum cuspidatum* have been used in traditional Japanese and Chinese medicine in a product called 'Kojo-kon'. Kojo-kon, also named Itadori, was used to treat a wide range of afflictions, including fungal diseases, various skin inflammations, and diseases of the heart, liver, and blood vessels. Resveratrol has been shown to be the primary active ingredient of *Polygonum cuspidatum*, which is one of the richest sources of resveratrol [7]. Indeed, numerous *in vitro* studies describe different biological effects of resveratrol. The major

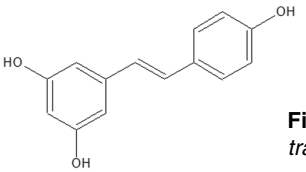

Figure 1. 3,4′,5-Trihydroxy-*trans*-stilbene (resveratrol).

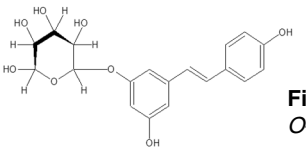

Figure 2. *trans*-Resveratrol-3-*O*-D-glycoside (piceid).

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impacts are the antioxidative, anti-inflammatory, and estrogenic effects as well as anticancer and chemopreventive activities [8, 9]. In order to reveal informations on absorption, metabolism, and the consequent bioavailability of resveratrol different research approaches were performed, including *in vitro*, *ex vivo*, and *in vivo* models, all of which are discussed in this review.

2 Metabolism of resveratrol in *in vitro* and *ex vivo* models

Since 2000, several *in vitro* and *ex vivo* studies have contributed to the clarification of the resveratrol metabolism. Initially, absorption of resveratrol was evaluated by the use of isolated rat small intestine perfusion models. Apart from investigations of glucuronidation and sulfation of resveratrol in liver and duodenum, transport and uptake models were established using intestinal as well as hepatic cells [10 – 19].

The use of an isolated rat small intestine perfusion model facilitates the direct investigation of luminal disappearance and the venous appearance of administered resveratrol, thus allowing the assessment of intestinal absorption of this compound. In such *ex vivo* studies, Andlauer *et al.* [10] applied an amount of resveratrol (837 – 1704 nmol) corresponding to its usual nutritional intake. In a single pass perfusion, 46% of the luminally administered resveratrol was extracted by the small intestine whereas 21% of administered resveratrol appeared at the vascular side and 2% was located in the intestinal tissue. In the luminal effluent, most of the resveratrol was free (40%), while 11% was glucuronidated and 3% was sulfated. The conjugates were both absorbed to the vascular side and secreted to the luminal side, with over 80% of the vascularly absorbed resveratrol being conjugated. Glucuronide, the major conjugate, was preferentially released to the vascular side (17% of administered resveratrol) whereas most of the sulfate conjugate was secreted to the luminal side [10]. Nearly at the same time, Kuhnle and co-workers [11] established a similar model. The absorption of resveratrol across the jejunum and ileum was investigated as a function of time up to 90 min. Resveratrol was detected at the serosal side of the enterocytes but the amount which was transferred unmetabolized was very small (0.03 nmol/cm jejunum). In contrast, significant amounts (1.19 nmol/cm jejunum) of a more polar product with similar spectral characteristics were detected by HPLC analysis of the serosal fluid samples. The presence of a glucuronide conjugate was investigated by treatment of the samples with β-glucuronidase, suggesting the glucuronidation of resveratrol as it crosses the jejunum. Levels of resveratrol glucuronide in the serosal fluid after perfusion of the ileum, with the same concentration of resveratrol, were only 38% of that transferred across the jejunum. Approximately 99% of the resveratrol equivalents recovered on the serosal side of the gut were identified as resveratrol glucuronide [11].

Quite different from the *ex vivo* studies, De Santi *et al.* [12 – 14] performed very basic experiments on resveratrol sulfation and glucuronidation in *in vitro* human liver and duodenal models. Initially, an assay was described to measure the rate of resveratrol sulfation. The sulfation of resveratrol was 91 ± 21 and 74 ± 60 pmol/min/mg cytosolic protein (mean ± SD) for liver and duodenum samples, respectively. The Michaelis constants (K_M) of resveratrol sulfotransferase were 0.60 μM (liver) and 0.50 μM (duodenum), which indicates that resveratrol is a good substrate for the human hepatic and duodenal sulfotransferase [12]. For resveratrol glucuronidation in human liver, a second radiometric assay was developed. The rate of resveratrol glucuronidation was measured in ten liver samples and the glucuronidation rate was 0.69 ± 0.34 nmol/min/mg. Resveratrol glucuronosyl transferase followed Michaelis-Menten kinetics (K_M 0.15 ± 0.09 mM). The presented results show that resveratrol is glucuronidated in the human liver [13]. In the following, experiments on the inhibition of resveratrol sulfation and glucuronidation by flavonoids were performed [13, 14]. In the liver, for example, IC_{50} for the inhibition of resveratrol sulfation was 12 ± 2 pM for quercetin. Similarly, in the duodenum, IC_{50} was 15.2 ± 2 pM for quercetin. Also resveratrol glucuronidation was inhibited with an IC_{50} of 10 ± 1 μM for quercetin in liver samples. A competitive metabolization of quercetin by the same xenobiotic enzyme systems *via* the formation of glucuronide and sulfate conjugates is the most probable explanation for the inhibition of resveratrol conjugation by quercetin [14]. The glucuronidation rate of *trans*- and *cis*-resveratrol by human liver microsomes was also evaluated by Aumont *et al.* [15]. In doing so, the authors went a step further, as different glucuronide metabolites were identified. The results indicate that the *in vitro* glucuronidation of *trans*- and *cis*-resveratrol by human liver microsomes led to the formation of two monoglucuronides, 3- and 4′-glucuronide. *Trans*- and *cis*-resveratrol were efficiently glucuronidated by human liver microsomes. Glucuronidation was stereoselective, the *cis*-isomer being glucuronidated much faster (5 – 10 ×) than the *trans*-isomer at both positions. Moreover, the two *trans*- and *cis*-resveratrol glucuronides were formed at very different rates, suggesting a preference for the 3-position. *trans*-Resveratrol was mainly glucuronidated by UGT1A1, UGT1A9, and UGT1A10. Since resveratrol presents three hydroxyl groups, the formation of diglucuronides cannot be ruled out. Indeed, such a very polar metabolite was detected, but in a very low amount under the experimental conditions used [15].

Alongside with these *in vitro* investigations on glucuronidation and sulfation of resveratrol, more recent studies on intestinal and hepatic cells describe particularly the absorption and transport mechanisms of resveratrol and its meta-

bolites by different cell lines. The transcellular absorption was investigated at different concentrations in Caco-2 cells, a human intestinal cell line, and appeared to be concentration-dependent. The limited linearity in transport with time suggests extensive metabolism of resveratrol by the Caco-2 cells [16]. In a similar approach, the transport mechanisms of resveratrol, the apical-to-basolateral and the basolateral-to-apical permeabilities of resveratrol, were measured with resveratrol concentrations ranging from 5–250 μM on the initial side [17]. There was no significant difference between the two permeabilities, which suggests that the transport of resveratrol across Caco-2 monolayers is probably through a passive and concentration-independent diffusion mechanism. P-glycoprotein and multiple resistance-associated proteins (MRPs) are significant determinants of drug absorption in the human intestine by acting as efflux pumps. Further experiments confirmed that P-glycoprotein and MRPs were not involved in resveratrol transport. These efflux inhibition results, along with those of permeability experiments, indicate that the flux of resveratrol across Caco-2 cell monolayers is rapid and that resveratrol diffuses in a direction-independent manner [17]. The metabolism of resveratrol by Caco-2 cells was also investigated. HPLC-UV (λ = 305 nm) detection permitted the detection of two metabolites, resveratrol-3-sulfate and resveratrol-3-glucuronide, as phase II biotransformation products. There was no evidence of phase I metabolites [16, 17]. In further experiments, the major sulfate conjugate was examined under transport conditions. The conjugate appeared on both the apical and the basolateral sides after loading on the apical side. At low concentrations, most of the resveratrol sulfate is effluxed to the apical side. However, increasing resveratrol concentrations resulted in a shift of the cellular export in favor of the basolateral side. The amount of the glucuronic acid conjugate formed was very low in comparison (*i.e.*, less than 5% of total conjugates) and all of it was exported to the apical side [16]. The concentration of the metabolites increased over time. After incubation for 4 h, 53% of the apically administered resveratrol appeared on the basolateral side, 32% remained on the apical side, and 4% was located in cells. Approximately 9% of the inital dose was transformed into resveratrol-3-sulfate and 4% was metabolized to resveratrol-3-glucuronide [17]. Similar analysis of metabolites was done after loading resveratrol on the basolateral side. The results were virtually identical to those obtained after apical loading of resveratrol. An accumulation of resveratrol in Caco-2 cells was also observed, suggesting that the enterocyte could be a major biological target site [16]. The precondition for an accumulation of resveratrol in different organic tissues is the uptake of this stilbene by the respective cells.

Apart from the studies on the intestinal cell line Caco-2, the uptake of resveratrol was only investigated for hepatic cells to date [18]. Two different cellular models were used: a human hepatoblastoma cell line (HepG2), which is responsive to antiproliferative effects of resveratrol, and human hepatocytes in order to compare resveratrol transport in normal and in tumor cells. The fluorescent properties of resveratrol were used to observe its cellular uptake. Cells treated with resveratrol showed an apparent increase in fluorescence intensity, pointing to the uptake of this stilbene. The time-course of resveratrol uptake was similar in hepatocytes and in HepG2 cells. In further experiments, the possibility of a carrier-mediated transport of [^{3}H]resveratrol was investigated. The results indicate that the uptake of resveratrol probably results from the contribution of two processes, a passive one and a carrier-mediated one. Under physiological conditions, this process would allow an efficient hepatic uptake of resveratrol present in circulating blood [18]. Regrettably, this study does not provide any data on the metabolism and the possible formation of resveratrol metabolites. But interestingly, additional experiments on the interference of serum proteins on the bioavailability of resveratrol were established. Apparently, resveratrol is trapped by serum constituents. This binding seems to occur particularly with albumin. Tritiated resveratrol uptake decreased with increasing concentrations of bovine serum albumin. At the initial concentration of 5 μM, all incubated resveratrol was bound to proteins after 24 h. Spectrophotometric analysis demonstrated the formation of resveratrol-albumin complexes by an increase of absorbance. The results confirmed the interaction of resveratrol with albumin [19].

Considering the experiments with the Caco-2 cell line *in vitro* [16, 17] and the studies of the isolated rat small intestine perfusion models *ex vivo* [10, 11], the accordance of the results is quite striking. Concluding, resveratrol is fairly well absorbed and metabolized by the small intestine. The mechanism of resveratrol movement across the intestinal epithelium appears to be a rapid passive diffusion [17]. In contrast, the uptake of resveratrol in hepatic cells probably results from the contribution of two processes, a passive one and a carrier-mediated one. Under physiological conditions, this active process would allow an efficient hepatic uptake of resveratrol present in circulating blood [18]. However, the uptake of resveratrol might be reduced due to interferences with serum proteins [19]. It was also shown that resveratrol is glucuronidated and sulfated in liver and duodenum samples [12–15] as well as by Caco-2 cells [16, 17]. This is of particular interest, because extensive presystemic metabolism might significantly restrict the bioavailability of resveratrol.

3 Metabolism of resveratrol *in vivo* in rodent models

The bioavailability of a nutrient is defined by its degree to which it becomes available to the target tissue after admin-

istration [20]. Subsequently, the knowledge of absorption, distribution, and metabolism of a compound *in vivo* is the precondition to determine its bioavailability. The absorption of resveratrol in rats was first described in 1996 [21]. Until then, little was known about the metabolic fate of resveratrol. In the beginning of the millenium a boost of absorption studies in different species followed, leading to a better understanding of the resveratrol metabolism.

In the first experiments, the group of Bertelli [21–23] measured the absorption of resveratrol in rats. Red wine with a known resveratrol content (6.5 mg/L) was administered. Each experiment was carried out on 84 male Wistar rats with an average weight of 300 g. The first group consisted of 42 animals, 36 rats were each administered 4 mL of red wine by gastric intubation, corresponding to a dose of 86 μg/kg resveratrol. The remaining 6 rats were used as a control. All the rats (in groups of 6) were sacrificed at different time intervals: immediately before wine administration (control), after 30 min, and after 1, 2, 4, 8, and 12 h. In a second experiment, 42 rats were devided as follows: 36 were given 2 mL per day of the same red wine, corresponding to the dose of 43 μg/kg resveratrol per day, for 15 days, and six rats were kept as control. The results of these studies indicate that resveratrol contained in wine was quickly absorbed, reaching its peak concentration approximately 60 min after wine ingestion, with initial resveratrol concentrations observed after 30 min. After a short period of time, resveratrol was detectable in the liver and in the kidneys. In these two organs, the highest concentrations were already reached after 1 h. Resveratrol concentrations in the kidneys seem to decrease with time and the kidneys appear to be the preferential organ of excretion. Studies of the kinetics of the absorption of resveratrol administered for a prolonged period of time also showed that after a sufficiently high concentration of resveratrol has been attained, an equilibrium is reached between the absorbed and the eliminated resveratrol.

After these initial experiments a break in the resveratrol research of nearly five years occurred, which was ended with the publication of the investigations of Soleas and coworkers in 2001 [24]. Intragastric administration to male Wistar rats was performed in experiments with tritiated resveratrol. The polyphenol was added to the following matrices: 10% (v/v) ethanol, V-8 homogenized vegetable cocktail, and white grape juice. Around 75% of the dose administered with each matrix is accounted for the measured stool and urine radioactivity. Taking as the measure of absorption the difference beween the amount of radioactivity given and the amount recovered in the stool, it appears that 77–80% resveratrol may be absorbed in the rat intestine, with no differences among the three liquid matrices. All of the tritiated label present in the urine must have been absorbed and subsequently excreted, with values ranging from 49 to 61%. It therefore appears that, by any criteria, at least 50% resveratrol is absorbed by the rat and that alcohol up to 10% by volume does not enhance absorption. The 24-h urinary excretion of radioactivity was also measured in rats in which unlabeled resveratrol, catechin, and quercetin were coadministered in a matrix of 10% (v/v) ethanol in concentrations ranging from 10 nM to 1 mM. No significant inhibition by these compounds was observed. Significant radioactivity was present at 30 min after administration of the different dosages (10 nM, 100 nM, 1 mM), but remained around the same concentrations over the next 90 min. The mean percentage of the dose administered measurable in the 24-h urine ranged from 2.5% (10 nM) to 7.4% (100 nM) to 14.7% (1 mM). These results suggest that whereas the absorption of resveratrol is not affected by the dose administered, the metabolic conversion of the parent compound is saturable so that as the dose is increased, a higher percentage is excreted unchanged in the urine. Only trace amounts of radioactivity were detectable in liver, kidneys, heart, or spleen, with the aggregate value for these tissues being <2%. An additional set of experiments was conducted, in the first of which rats were given 0.5, 1.5, and 2.5 mg resveratrol in 1 mL of 20% (v/v) ethanol and blood was withdrawn by cardiac puncture 60 min later. The mean concentrations in serum were 2.5 μg/L after 0.5 mg, 3.6 μg/L after 1.5 mg, and 5.7 μg/L after 2.5 mg. In the second set, the amount given was 5 mg and for two rats, blood was withdrawn at 15, 30, and 60 min. High concentrations of resveratrol were already evident in serum by 15 min after gavage, peaking at 30 min, and decreasing over the next 30 min. A similar time course was seen in whole blood, although the concentrations were consistently lower than those of serum. Summarizing the results, peak concentrations of resveratrol occur in blood and serum very rapidly but in a low range [24].

The experiments of Vitrac *et al.* [25] showed similar results after oral administration of [^{14}C]resveratrol to mice. The radioactivity in the blood 1.5 h after dose was low and did not increase significantly during the experimental period (6 h). This low concentration is probably due to the timepoint of 1.5 h as Soleas *et al.* [24] desribed peak concentrations as soon as 30 min after administration with the concentration of parent compound falling sharply after this peak in plasma. In contrast, in urine and bile, a very high concentration of radioactivity was shown 1.5 h after administration. In bile, radioactivity was maintained at the same level until the end of the experimental period (6 h), whereas it increased slowly with time in urine. Three hours after administration, radioactivity was found in various organs with highest concentration in the duodenum. Kidney was the next most labeled organ followed by lung and liver. To confirm whether administered ^{14}C-label was incorporated into tissues, sections of liver and kidney obtained from mice sacrificed 3 h after oral administration of [^{14}C]resvera-

trol were subjected to microautoradiography. In the kidney, radioactivity was detected generally in the cortex. In the liver, radioactivity was observed in the parenchyma, suggesting that hepatocytes in particular are able to incorporate [^{14}C]resveratrol-derived radioactivity. In kidney extracts, HPLC analysis demonstrated the presence of the parent drug as the major radioactive product. HPLC analysis of liver extracts revealed the presence of the parent drug (25 μM) and, surprisingly, the absence of the 3-glucuronide conjugate at the time examined. [^{14}C]resveratrol constituted 27% of the total radioactivity whereas the majority of the radioactivity was present in peaks of retention times between 0 – 5 min (60%) and 5 – 10 min (13%). The presence of glucuronide and/or sulfated conjugates in these radioactive peaks was investigated by treatment of the extract with a β-glucuronidase. At least one compound was present as a glucuronide conjugate, distinct from the 3-glucuronide of resveratrol [25].

The formation of distinct resveratrol metabolites through enzymes of phase II of the biotransformation system as the UDP-glucuronosyltransferases or sulfotransferases is a main focus of further investigations on the metabolism of resveratrol. But prior to a detailed description of the hitherto existing results a short excursus might help to comprehend the metabolic pathway of resveratrol as aglycone and glucuronide. In 2002, Marier *et al.* [26] established a linked-rat model to explain the metabolic fate of resveratrol. Thereby, resveratrol aglycone was administered to bile-donor rats, and their bile flowed directly into the duodenum of bile-recipient rats *via* surgically implanted catheters so that the contribution of enterohepatic recirculation to the overall disposition could be determined. The plasma concentration of aglycone and glucuronide in bile-donor rats declined with no sudden increase in plasma concentration after the initial absorption phase. This is most likely due to the interruption of the recirculatory pathway in bile-donor rats. Enterohepatic recirculation was confirmed by the presence of significant plasma concentrations of aglycone and glucuronide in bile-recipient rats over the 4- to 8-h time period. These observations suggest that glucuronide is most likely excreted in the bile of bile-donor rats and reabsorbed in the intestine of bile-recipient rats in its aglycone and/or glucuronide forms. Plasma concentrations of aglycone and glucuronide in bile-recipient rats coincided with the sudden peaks in plasma concentrations observed in intact rats receiving i.v. (15 mg/kg) or p.o. (50 mg/kg) doses. In addition, the fraction of the drug excreted in urine reached a maximum value during 4- to 8-h time interval in bile-recipient rats, coinciding with their respective time to maximum plasma concentrations. Glucuronide exposures were approximately 7- and 46-fold higher than those of aglycone after intravenous and oral administration, respectively. Concluding, enterohepatic recirculation contributes to the overall systemic exposures of aglycone and glucuronide in rats [26].

The identification of resveratrol metabolites was in the focus of further investigations. Resveratrol (0.1 mM) incubated with human hepatocytes for 4 h showed several new peaks. One resveratrol metabolite was detected, which was synthesized and confirmed to be resveratrol-3-sulfate [27]. Additionaly, LC-UV-MS/MS showed three resveratrol glucuronide peaks, resveratrol-4′-glucuronide, resveratrol-3-glucuronide and *cis*-resveratrol-4′-glucuronide. No peaks corresponding to a diglucuronide were detected. Resveratrol sulfate seems to be a minor human hepatic metabolite compared with the formation of the glucuronide in the incubations with human hepatocytes. After administration of 20 mg/kg (i.p.) resveratrol to rats, analysis of rat hepatocytes showed two major peaks which were assigned as resveratrol-3-glucuronide and resveratrol-3-sulfate. In contrast to human hepatocytes, resveratrol sulfate was the more abundant metabolite in rat hepatocytes. Resveratrol-3-sulfate and resveratrol-3-glucuronide were as well detected in urine of the animals [27]. Similar results were obtained from Asensi *et al.* [28]. HPLC analysis revealed two peaks in the 24-h urine of rats treated with 20 mg/kg resveratrol (p.o.). Free resveratrol was not detected in the urine. Mass spectrometry identified one peak as a glucuronide conjugate. However, the second peak remained undefined [28]. In another set of experiments, Yu and co-workers [27] administered resveratrol to mice at two different doses (20 and 60 mg/kg) using i.p. or i.g. injection. Resveratrol glucuronide and resveratrol sulfate were detected as the only resveratrol metabolites in the mouse serum samples, and both of these metabolites were detected after i.p. or i.g. administration. After i.p. injection of 20 mg/kg to mice, the maximum concentrations of both metabolites were observed in the serum samples obtained at the first time point of 15 min. In these samples, the concentration of resveratrol sulfate (13 μM) was almost 3-fold higher than that of resveratrol glucuronide (5 μM). Only traces of unconjugated resveratrol were detected. Furthermore, no resveratrol or its metabolites were detected after 1 h. In the second set of experiments, 60 mg/kg resveratrol was given i.g. In these serum samples, also the same resveratrol sulfate and resveratrol glucuronide metabolites were detected. The resveratrol sulfate concentration reached a maximum value in mouse serum after 30 min instead of 15 min as described after administration of 20 mg/kg resveratrol. Serum resveratrol disappeared after 30 min but resveratrol sulfate and resveratrol glucuronide were still detectable 3 h after the higher dosage. The effect of different administration forms of resveratrol on its metabolism was also investigated in rabbits, comparing i.v. and p.o. administration of 20 mg/kg resveratrol [28]. After i.v. application, its highest concentration in plasma (42.8 ± 4.4 μM) decreased rapidly to 0.9 ± 0.2 μM within 60 min. If the same amount of resver-

atrol is administered orally, the highest concentration in plasma (approx. 1 μM) was found within the first 5 min after administration followed by a decrease to less than 0.1 μM at 60 min. Unfortunately, in these samples, no distinction was made between resveratrol and resveratrol metabolites. In further experiments, extravascular tissue bioavailability of resveratrol was evaluated by the authors. After its oral administration to rabbits, rats, or mice, resveratrol content in brain, lung, liver, and kidney was always below 1 nmol per g of fresh tissue. The highest levels were found within the first 10 min after administration [28].

The distribution of resveratrol and the formation of its metabolites in different organic tissues was also described in a recent publication by Sale and co-workers [29]. Beyond the metabolism of resveratrol, the question if a replacement of the phenol functionalities in resveratrol by methoxy moieties and an addition of a further methoxy group to 3,4,5,4′-tetramethoxystilbene would affect the pharmacokinetic properties of the parent molecule was considered in this article. Mice received intragastric resveratrol or 3,4,5,4′-tetramethoxystilbene (1 or 9.8 mmol/kg), and drug levels were measured in plasma and tissue, such as liver, kidney, lung, brain, small intestinal mucosa, and colonic mucosa. Both agents were rapidly cleared from blood and tisssues within 1 h of administration. Concentrations of resveratrol were consistently higher than those of 3,4,5,4′-tetramethoxystilbene in the plasma, liver, and heart. The most dramatic discrepancy in levels occurred in the liver, in which the resveratrol concentration was five times higher than 3,4,5,4′-tetramethoxystilbene, and in the small intestinal and colonic mucosae, where 3,4,5,4′-tetramethoxystilbene exceeded the resveratrol concentration by factors of 10 and 7, respectively. HPLC analysis of liver and kidney samples from animals on resveratrol displayed two peaks in addition to the parent compound, which were identified as a resveratrol glucuronide and resveratrol-3-sulfate. These metabolites were not observed in plasma. The systemic bioavailability could not be increased by 3,4,5,4′-tetramethoxystilbene. The lower availability of resveratrol in the small intestine and colon compared to 3,4,5,4′-tetramethoxystilbene is possibly the consequence of the high capacity of resveratrol to undergo conjugation reactions.

Summarizing the results, resveratrol is absorbed, distributed to various organs, and metabolized to glucuronide and sulfate conjugates by different rodent species. The first results of Bertelli *et al.* [21 – 23] already indicated the rapid absorption of resveratrol. In plasma, resveratrol is detectable as soon as 15 min after administration and reaches peak concentrations after 30 min [24, 27]. With resveratrol-3-glucuronide and resveratrol-3-sulfate also conjugated forms of resveratrol were detected in plasma of rodents. These resveratrol metabolites were even detectable 3 h after administration. At this time, only trace amounts of free resveratrol were found in plasma samples [27, 29]. This prolonged detection of low levels of resveratrol metabolites in plasma suggests that resveratrol is partly metabolized in the small intestine and distributed to various tissues also in its conjugated forms [24, 25, 27, 29]. The liver seems subjected to an important accumulation of resveratrol and its metabolites. Thereby it is not yet known, if accumulation of resveratrol metabolites in the liver, namely resveratrol-3-sulfate and resveratrol-3-glucuronide, takes place due to either a metabolism of resveratrol in the small intestine and its subsequent absorption, or a metabolism *in situ* [25, 27, 29]. The kinetic studies of Bertelli *et al.* [22, 23] showed yet decreasing concentrations of resveratrol in kidneys. This result was confirmed by decreasing concentrations of resveratrol derived radioactivity in the kidney over the time indicating that renal excretion might be one of the major ways of elimination of the ^{14}C-label [25]. This hypothesis was also supported by the high concentrations of resveratrol and its metabolites, resveratrol-3-sulfate and resveratrol-3-glucuronide, found in urine [24, 25, 27, 28]. The results of these studies provide basic information for the evaluation of the biological effects of resveratrol described in numerous *in vitro* studies. However, as the settings of the described studies were very different for each experiment, species specific differences cannot be ruled out. For a reliable evaluation of the bioavailability and benefical effects of resveratrol in humans, absorption and metabolism of this polyphenol should also be investigated in humans.

4 Metabolism and bioavailability of resveratrol in humans

A beginning of investigating the metabolism of resveratrol in humans was made quite recently. During the last two years, some papers were published in which resveratrol was administered to human subjects in different servings like wine or juice. Lately, even the application of ^{14}C-labeled resveratrol was described in one of the studies.

In 2003, the group of Goldberg [24] was the first to administer resveratrol to humans. The experiments were conducted in a similar manner as in previous studies, in which the absorption of resveratrol in rats was examined. Apart from resveratrol, the absorption and bioavailability of catechin and quercetin were evaluated in 12 healthy male human subjects (age 25 – 45 years) after oral ingestion. Because of the lipophilic nature of these compounds and their limited solubility, the absorption from three different matrices, one aqueous (grape juice), one containing ethanol (white wine), and one simulating a vegetable homogenate (V-8 juice), were compared additionally. Resveratrol and catechin were given at a dose of 25 mg/70 kg body weight, quercetin at a dose of 10 mg/70 kg body weight. The poly-

phenols were dissolved in 100 mL of the respective beverage. For 24 h before and during the 24 h of the test, the subjects were asked to abstain from foods and beverages rich in polyphenols. Baseline urine and blood samples were taken before drinking the respective beverage. Further blood samples were taken at 30 min, 60 min, 120 min, and 4 h. Urine was collected for 24 h after drinking the beverage. For total resveratrol (aglycone and metabolites), the highest recorded serum level occurred at 30 min, the mean value for the three matrices ranging from 416 to 471 μg/L. Thereafter, the concentration declined most rapidly when V8 was the matrix, and least rapidly in the case of grape juice. The total absorption was similar for the three matrices. For resveratrol aglycone, the highest recorded concentrations in serum also occurred 30 min after consumption in all three matrices, with little differences in mean concentrations at any time interval. Values returned to baseline within 4 h. The serum concentration of free resveratrol aglycone was a small fraction of the total resveratrol concentration, highest observed values being only 1.7 to 1.9%. Urinary 24 h resveratrol excretion did not show any matrix effect and approximated 17% of the administered dose [30]. In a similar approach, the fate of resveratrol given as pure aglycone or as constituent of grape juice was determined. Three adult male volunteers between 30 and 50 years of age (45–85 kg) took part in the study. After they had fasted overnight, two subjects each received a single oral dose of resveratrol (0.5 or 1 mg/kg dissolved in 5 mL whisky and mixed with 50 mL water). After a 3-day washout period, one subject received another dose of resveratrol (0.03 mg/kg). The grape preparations used contained 0.16 mg resveratrol per serving of 100 mL. In experiments with these preparations, one subject initially received 200 mL grape juice, and the experiment was repeated with 400, 600, and 1200 mL grape juice preparation after at least a 2-week washout period. Urine samples collected after administration of resveratrol aglycone as well as after ingesting high doses of grape juice preparations (600 and 1200 mL) showed detectable peaks of resveratrol. The identity of resveratrol and resveratrol glucuronide in the urine samples was confirmed by LC-MS/MS. With a dose of 0.03 mg/kg, most of the resveratrol was excreted in the first 2 or 3 h. In the same subject with a higher dose (1 mg/kg), it took more than 7–10 h to excrete most of the resveratrol in the urine. The recovery of resveratrol in the subject receiving 0.03, 0.5, and 1 mg/kg was 52, 34, and 26% of the respective dose. Resveratrol was principally present as a glucuronide. The enzyme-hydrolyzed plasma samples obtained from the human subject receiving resveratrol (1 mg/kg) showed peaks of resveratrol. After consumption of standard grape juice, resveratrol was not detectable in plasma samples [31].

In a very recent publication, ^{14}C-labeled resveratrol was administered both orally and intravenously to six healthy volunteers. Oral and intravenous resveratrol doses were administered in the morning after an overnight fast. As a representative oral dose, 25 mg were selected. To be able to determine the absolute absorption and bioavailability, a small intravenous dose of 0.2 mg were also administered. After the oral dose of 25 mg [^{14}C]resveratrol, an early peak plasma resveratrol equivalent concentration, *i.e.*, total radioactivity, of 491 ± 90 ng/mL, was reached at about 1 h after the dose. At 6 h after the dose, there was a second peak in all subjects with a mean concentration of 290 ± 68 ng/mL. The plasma concentrations then declined exponentially. After the i.v. dose of 0.2 mg [^{14}C]resveratrol there was a rapid fall of the plasma concentrations of total radioactivity over the first 1 h after the bolus injection, indicating the distribution phase. The plasma concentrations then fell in parallel with those after the oral dose. Most of the radioactivity after the oral doses was recovered in urine (53–85%). The recovery in feces was highly variable (0.3–38%). After the i.v. dose, the recoveries in urine were 42–83% of the dose with 0.6–23% found in the feces. Thus, the overall recoveries in urine and feces were 71–98% after the oral and 54–91% after the intravenous dose. For structure identification of resveratrol metabolites by LC-MS, a larger unlabeled dose (100 mg) was given orally to one of the subjects. LC-MS-UV of the 0–12 h urine detected five major metabolites, two resveratrol monoglucuronides, a dihydroresveratrol monoglucuronide, a resveratrol monosulfate, and a dihydroresveratrol sulfate. The dihydroresveratrol conjugates had no UV absorption peaks at λ = 305 nm. After the oral dose, the sulfate conjugates excreted in the urine accounted for 24 ± 3% of the dose and the glucuronic acid conjugates for 13 ± 1%. The recovery of these metabolites was similar after the smaller intravenous dose. Also, fecal samples contained both resveratrol and the major resveratrol sulfate conjugate. All attempts to find measurable levels of resveratrol in plasma after the oral dose in the six volunteers failed at any time-point. Only trace amounts of less than 5 ng/mL could be seen. However, evidence of both sulfate and glucuronic acid conjugates could be found. To test how fast resveratrol may be metabolized in the body plasma, samples at early time-points after the i.v. dose were examined. At the end of the 10-min i.v. resveratrol infusion all three subjects showed unchanged resveratrol. Two subjects also demonstrated a major metabolite peak, which is suggested to be a sulfate conjugate. When examining the 30-min samples from the same subjects, plasma from subjects 1 and 2 had no resveratrol, whereas in subject 3 there was a small amount of resveratrol left. In samples obtained beyond 30 min, there was no unchanged resveratrol detected in any of the subjects [32].

Concluding, in humans resveratrol, whether as aglycone or in its glycosidic form, is also absorbed quite rapidly after oral consumption. Resveratrol levels were readily detectable in both plasma and urine with highest plasma concentrations being reached around 30–60 min after ingestion.

After oral administration the amount of free resveratrol in plasma and serum accounted for less than 2% of total resveratrol or even was not detectable. However, sulfate and glucuronide conjugates occured [30 – 32]. Furthermore, the appearance of a new resveratrol plasma peak 6 h after consumption suggests enteric recirculation of conjugated metabolites by reabsorption after intestinal hydrolysis. Although the presystemic metabolism of resveratrol may be important for the oral dose, the observations for i.v. doses also demonstrate a highly efficient systemic metabolism [32]. Total urine and feces recovery of resveratrol varied between 54 and 98%. In urine, two isomeric glucuronic acid conjugates and one sulfate conjugate were identified. Interestingly, strong evidence for hydrogenation of the aliphatic double bound was obtained, with this reduced metabolite excreted both in a glucuronic acid and a sulfate conjugate form [32]. Finally, absorption does not require the presence of alcohol; aqueous media and vegetable suspensions are, with a few exceptions, as effective as wine [30]. However, the cumulative excretion of resveratrol after drinking of grape juice was only about 5% of the dose administered. This is one-tenth of that obtained with oral administration of the same dosage of pure resveratrol [31]. Unfortunately, in all these studies the number of volunteers was small, ranging from one to six persons per test group. This is particularly critical as interindividual variability in xenobiotic metabolism occurs, what might reduce the significance of the presented results.

5 Conclusions

In different rodent species as well as in humans, resveratrol is well absorbed, distributed to various organs, and metabolized to glucuronide and sulfate conjugates. In plasma, resveratrol reaches peak concentrations after 30 min [24, 27, 30, 31]. With resveratrol glucuronide and resveratrol sulfate the conjugated resveratrol predominantly circulates in plasma. The prolonged detection of low plasma levels of these resveratrol metabolites suggests that resveratrol is partly metabolized in the small intestine and distributed to various tissues mainly in its conjugated forms [24, 25, 27, 29 – 32]. This hypothesis of a presystemic metabolism of resveratrol is supported by different *ex vivo* and *in vitro* models, describing phase II conjugation of resveratrol by the intestine, which might significantly restrict the bioavailability of resveratrol *in vivo* [10 – 17].

In humans, enteric recirculation of conjugated metabolites by reabsorption after intestinal hydrolysis was suggested, a hypothesis supported by experiments with rats, in which enterohepatic recirculation contributes to the overall systemic exposure of aglycone and glucuronide [32, 26]. *In vitro* experiments showed an efficient active uptake of resveratrol in hepatic cells. Indeed, the liver seems to be subjected to an important accumulation of resveratrol and its metabolites in rodents [18, 25, 27, 29]. This accumulation of resveratrol metabolites in the liver probably results from the contribution of two processes: a metabolism of resveratrol in the small intestine and its subsequent absorption, and a metabolism *in situ* [24]. Decreasing concentrations of resveratrol in the kidneys of rats over the time indicates that renal excretion is one of the major ways of elimination of this stilbene, resulting in high levels of resveratrol, resveratrol glucuronides, and resveratrol sulfates in urine of rats as well as in urine of human subjects [24, 25, 27, 28, 30 – 32].

It is important to note that no phase I reactions of resveratrol, such as oxidations, reductions, or hydrolyzes, were observed in any of these systems. Furthermore, conjugated resveratrol and not its free form was found to predominate in the circulation. The voluminous literature reporting powerful anticancer, anti-inflammatory, and anti-atherogenic activities of resveratrol based on *in vitro* experiments utilizing the aglycone is weakened in face of the virtual absence of the aglycone from the circulation or the urine following absorption [27, 30]. However, when resveratrol was administered to rats it was much more potent in blocking the function of the aryl hydrocarbon receptor than when incubated with cell cultures *in vitro*, suggesting that hepatic biotransformation generated one or more metabolites with much greater activity than the parent compound [33]. These data suggest that in future investigations resveratrol metabolites should be considered to evaluate the potential biological activity of resveratrol *in vivo*.

But, in evaluating the bioavailability of resveratrol, it should also be accounted for that in red wine, the main dietary source of resveratrol and piceid in the western diet, numerous polyphenols, like stilbenes, flavonoids, or anthocyanins occur. It cannot be ruled out, that resveratrol metabolism *in vivo* is inhibited by other polyphenols due to competitive metabolization *via* the same phase II enzyme systems. Although by now, after coadministration of resveratrol, catechin, and quercetin to rats no inhibition of resveratrol metabolism could be observed, *in vitro* experiments showed the inhibition of resveratrol glucuronidation and sulfation in liver and duodenum samples by quercetin [14, 24]. Furthermore, it is known that epicatechin gallate and epigallocatechin gallate are potent inhibitors of human liver sulfotransferases [34]. Apparently, it seems as if the reduced bioavailability of resveratrol *in vivo* could be improved by application of competitive effective substances.

Although interindividual variability in xenobiotic metabolism occurs, the presented results give no explicit hints on significant variations in resveratrol metabolism between the different species considered in the cited publications.

But the results also show that it is questionable to extrapolate the insights of *in vitro* experiments to the conditions *in vivo*, especially if only one food ingredient is considered. Therefore, in future experiments, the focus should be laid on the evaluation of the potential biological impacts of resveratrol metabolites as well as on synergistic or antisynergistic effects of different food compounds.

6 References

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