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Urinary and Plasma Levels of Resveratrol and Quercetin in Humans, Mice, and Rats after Ingestion of Pure Compounds and Grape Juice

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The present study investigates the bioavailability of resveratrol and quercetin in humans, mice, and rats after oral ingestion of grape juice preparations or pure aglycones. Oral administration of resveratrol and quercetin to humans yielded detectable levels of resveratrol, quercetin, and their derivatives in the plasma and urine. Urinary levels of resveratrol, quercetin, and their metabolites were observed in human subjects receiving 600 and 1200 mL of grape juice, whereas quercetin metabolites were identified in urine samples even after receiving 200 mL of grape juice. The cumulative amounts of resveratrol and quercetin excreted in the urine of mice receiving concentrated grape juice for 4 days were 2.3 and 0.7% of the ingested doses, respectively. After i.g. administration of resveratrol to rats (2 mg/kg), up to 1.2 μM resveratrol was observed in the plasma. The study demonstrates that the glycoside forms of resveratrol and quercetin in grape juice are absorbed to a lesser extent than the aglycones.

KEYWORDS: Resveratrol; quercetin; grape juice; human; rodents; plasma; urine

INTRODUCTION

Several studies have suggested that consumption of red wine in moderation is associated with the reduction in the risk of coronary heart disease (*1*, *2*) and cancer (*3*). Grape juice, which is available to a broader range of populations, may also have beneficial health effects similar to that of wine. Grape juice consumption has been reported to decrease platelet aggregation, which in turn might prevent coronary artery disease (*4*, *5*).

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene; **Figure 1**) and piceid (resveratrol 3-*O*-β-glucoside) present in grapes have been studied for their potential cardioprotective effects (*6*). Four resveratrol derivatives have been characterized in wine: the aglycone and piceid both existing in cis and trans forms (*7*, *8*). Resveratrol is formed in the skin of grape berries but not in the flesh, and fresh grape skin contains 50−100 mg/g resveratrol (*9*). Consequently, the amount of these compounds varies considerably in different types of grape juice and wine depending on the grape variety, environmental factors in the vineyard, juice extraction, and wine processing techniques. Two studies have reported highly different resveratrol content in grape juices with ranges varying from 3 to 15 μg/L (*10*) and 690 to 14 500 μg/L (*1*). Recent studies show that total resveratrol and piceid levels in wines could vary from 0 to 25 000 μg/L (*11*).

Resveratrol has been reported to inhibit the oxidation of human low density lipoproteins (*12*) and the aggregation of platelets (*13*). Moreover, *trans*-resveratrol also possesses antiproliferative and anticancer activities (*14*, *15*). Resveratrol inhibits the growth of several human cancer cell lines, including that of oral squamous carcinoma (SCC-25) (*16*), promyelocytic leukemia (*17*), and breast cancer (*18*). Resveratrol can bind to estrogen receptors and activate estrogen responsive genes in vitro. It has been suggested that the estrogenic actions of resveratrol may contribute to the reported cardiovascular benefits of drinking wine or grape juice (*19*).

Quercetin, a major flavonol constituent found in many fruits, vegetables, and beverages including grape juice, exhibits a multitude of biological activities in vitro including antioxidative (*20*) and anticancer (*21*, *22*) effects. Recent studies have shown that quercetin inhibited the growth of B16 melanoma cells in vitro and reduced the number of lung tumor colonies in vivo in a dose-dependent manner when given intraperitoneally to tumor-bearing mice (*23*). Several epidemiological studies have shown a significant inverse association between consumption of flavonoids and cardiovascular disease (*24*, *25*).

Although many studies have implicated the roles of resveratrol and quercetin in disease prevention, their biological effects in vivo and modes of action are unclear. The activities of resveratrol and quercetin in vivo depend on their bioavailabilities. The information relating to the bioavailability of these compounds is inconclusive. One study on the plasma kinetics and tissue bioavailability of resveratrol after oral administration of red wine to rats suggested significant cardiac bioavailability and affinity of resveratrol to the liver and kidneys (*26*). A recent

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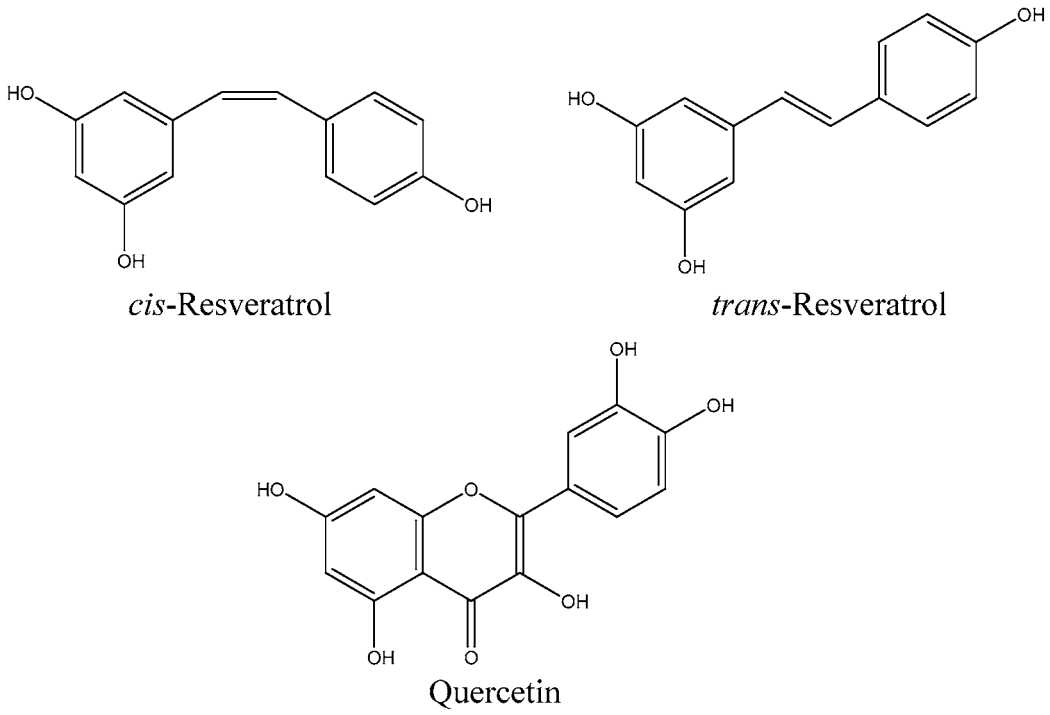

Figure 1. Chemical structures of resveratrol and quercetin.

study reported that vascular uptake of luminally administered resveratrol in rats was 20.5%. The majority of the absorbed resveratrol was converted to resveratrol glucuronide (16.8%), which was also the main luminal metabolite (*27*). Glucuronidation and sulfation of resveratrol were shown to decrease the bioavailability of resveratrol in humans (*28*). Several studies on the bioavailability of quercetin have been reported (*29*−*31*). A comparative bioavailability study of flavonols, especially quercetin, from red wine, onions, and black tea showed that the human plasma quercetin concentration after the consumption of wine was lower than that after onion and similar to that after tea consumption (*32*).

There is a dearth of information on the bioavailability of resveratrol and quercetin in human and animal models from grape juice and other food products. The purpose of this study was to investigate the fate of resveratrol and quercetin in terms of their urinary and plasma levels when they were given as pure aglycones or as constituents of grape juice.

MATERIALS AND METHODS

Chemicals and Reagents. Resveratrol, quercetin, 3′-O-methyl quercetin, trifluoroacetic acid, β-D-glucuronidase (EC 3.2.1.31), and sulfatase (EC 3.1.6.1) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Other reagents and solvents [high-performance liquid chromatography (HPLC) grade] were obtained from EM Sciences (Gibbstown, NJ). The standard stock solutions of resveratrol and quercetin (10 μg/mL each) were made in 50% methanol containing 0.2% ascorbic acid and stored at −80 °C until use. The grape preparation was obtained from the California Grape Commission (Fresno, CA). This pinkish-brown, freeze-dried powder contains (per kg) approximately 38 μmol of total resveratrol and 69 μmol of total quercetin. The preparation was stored in sealed opaque sachets at −80 °C. An 18.4% aqueous solution of this preparation is expected to approximate natural grape juice composition. This will be referred to as the grape juice preparation.

Human Studies. The protocol (No. 92-034) was approved by the Institutional Review Board at Rutgers University (Piscataway, NJ). Three adult volunteers (males) between 30 and 50 years of age, weighing between 45 and 85 kg, who did not smoke or drink alcoholic beverages prior to the experiment and during the sample collection period, took part in the study. After they fasted overnight, two subjects each received a single oral dose of resveratrol (0.5 or 1 mg/kg dissolved in 5 mL of whisky and mixed with 50 mL of water). After a 3 day washout period, one subject received another dose of resveratrol (0.03 mg/kg). After a 1 week washout period, each of these two subjects received a dose of pure quercetin (0.5 or 1 mg/kg in 5 mL of whisky and mixed with 50 mL of water). In experiments with grape preparations, one subject initially received 200 mL of grape juice preparation, and the experiment was repeated with 400, 600, and 1200 mL of grape juice preparation after at least a 2 week washout period. In all of the experiments, urine samples were collected before the dose and at different time points following the dose. The total volume of each urine sample was recorded. Blood was collected at 0 and 1.5 h after the ingestion of 1 mg/kg of resveratrol or quercetin and at 0, 2, 3, and 5 h after consumption of 1200 mL of grape juice. Urine and plasma samples were stored at −80 °C after mixing with 100 μL of 20% ascorbic acid per mL of plasma/urine.

Animal Experiments. The protocol (No. 91-024) was approved by the Animal Care and Facilities Committee at Rutgers University. Female CF-1 mice (from Charles River, NJ), 8 weeks old (weighing 18−21 g), were housed five per cage in metabolism cages. A week after acclimation, the mice were divided into four groups. The control group ($n = 5$) received a 12% sugar solution. Group 1 ($n = 10$), group 2 ($n = 15$), and group 3 ($n = 20$) received solutions containing 9.2, 18.4, and 36.8% of the grape preparation, respectively, as their sole source of drinking fluid. All mice were fed the AIN-76 diet during the experimental period. The volume of fluid consumed was recorded daily. Urine samples were collected during the treatment. Four days after commencing the experiment, the mice were sacrificed and the blood samples were collected at the time of sacrifice. The plasma and urine samples were preserved and stored at −80 °C as described earlier. Two female Wistar rats (250 g in body weight) (Jackson Lab, ME) were given a dose of 2 or 5 mg/kg resveratrol (in 0.5 mL of 10% ethanol) i.g. after overnight fasting. Urine and blood samples were collected at 0, 0.5, 1.5, and 4 h, which were preserved and stored at −80 °C until use.

HPLC Analysis of Resveratrol and Quercetin in Biological Fluids. The plasma and urinary levels of resveratrol, quercetin, and their metabolites were analyzed by HPLC. The samples were prepared using our previous method for catechin analysis (*33*). The sample was

incubated with β-D-glucuronidase and sulfatase at 37 °C. The reaction mixture was then extracted with ethyl acetate, and the extract was evaporated to dryness under vacuum. The residues were reconstituted in 200 μL of 20% methanol (for resveratrol) or 30% methanol (for quercetin). After the resultant solution was centrifuged, 50 μL of the supernatant was injected onto the HPLC. For the identification of conjugates of resveratrol and quercetin, plasma and urine samples without the enzyme digestion were used. Typically, 200 μL of urine was extracted with 200 μL of methylene chloride, the aqueous layer was filtered through a 0.22 μm filter, and 50 μL of the filtrate was injected onto the HPLC column.

A 150 mm × 4.6 mm i.d., 5 μm, Supelcosil C_{18} reversed phase column (Supelco, Bellefonte, PA) was used for the separation of the analytes. The column was eluted at 30 °C initially with 100% solvent A (5% methanol containing 0.1% TFA) at a flow rate of 1 mL/min. The gradient started at 6 min with the introduction of 5% solvent B (90% methanol containing 0.1% TFA), and then, solvent B was gradually increased to 15, 25, 50, 70, and 100% at 15, 25, 30, 35, and 40 min, respectively. At 44 min, the column was reequilibrated to 100% solvent A. The eluent was monitored by the ESA model 5500 coulochem electrode array system (ESA, Inc., Bedford, MA) with potential settings at −100, 100, 300, and 500 mV with simultaneous recordings of the four channel response. The peak height was used for the quantitation of plasma and urine levels of resveratrol and quercetin after calibration with the standard solutions.

Detection and Characterization of Resveratrol, Quercetin, and Their Metabolites by LC/MS. LC/MS analysis was carried out with a Finnigan Spectra System, which consisted of a Finnigan model P4000 pump, model AS3000 refrigerated autosampler, model UV6000LP photodiode array UV detector, and a Thermo Finnigan LCQ Deca mass detector (San Jose, CA) incorporated with an electrospray ionization (ESI) interface. The separation of the compounds was achieved on a 75 mm × 2.1 mm i.d., 3 μm, Supelco Discovery HS C_{18} column. The mobile phase consisted of 10% aqueous methanol (solvent A) and 70% aqueous methanol (solvent B) delivered at a flow rate of 0.2 mL/min. A binary linear gradient started with 90% solvent A and 10% solvent B, which was then increased to 31% B at 3 min, 33% B at 17 min, and subsequently to 90% B at 30 min. Then, the column was equilibrated with 10% B from 30.1 to 40 min before the next sample injection. The negative ion polarity mode was set for ESI ion source with the voltage on the ESI interface maintained at approximately −4 kV. With tandem mass spectrometry (MS/MS), the negative molecular ions *m*/*z* 227, 301, 315, 403, and 477 were used for identification of resveratrol, quercetin, monomethylated quercetin, resveratrol mono-glucuronide, and quercetin mono-glucuronide, respectively.

RESULTS

Detection of Resveratrol, Quercetin, and Their Metabolites in Human Urine by HPLC and LC/MS. With the present HPLC electrochemical detection method, standard resveratrol and quercetin were eluted at 26.5 and 29.5 min, respectively (**Figure 2A**,**D**). The calibration curves generated from standard solutions of resveratrol and quercetin showed a linear relationship between peak height and concentration in the range of 12.5 ng/mL to 10 μg/mL. The detection limits for resveratrol and quercetin were 3 and 6 ng/mL, respectively. In human urine samples after ingestion of pure resveratrol and quercetin, two peaks with the same retention time as standard resveratrol and quercetin were detected after hydrolysis with β-glucuronidase and sulfatase (**Figure 2C**,**E**). Comparison of the retention times and electrochemical detector responses of the authentic samples facilitated the preliminary identification of these peaks in urine samples as resveratrol and quercetin. Without enzymatic hydrolysis, urine samples collected from human subjects receiving resveratrol (0.03, 0.5, or 1 mg/kg) showed a major peak eluting at 23.9 min instead of 26.5 min for resveratrol (**Figure 2B**), suggesting that this peak is a conjugation derivative of resveratrol. Human urine samples collected from subjects ingesting high

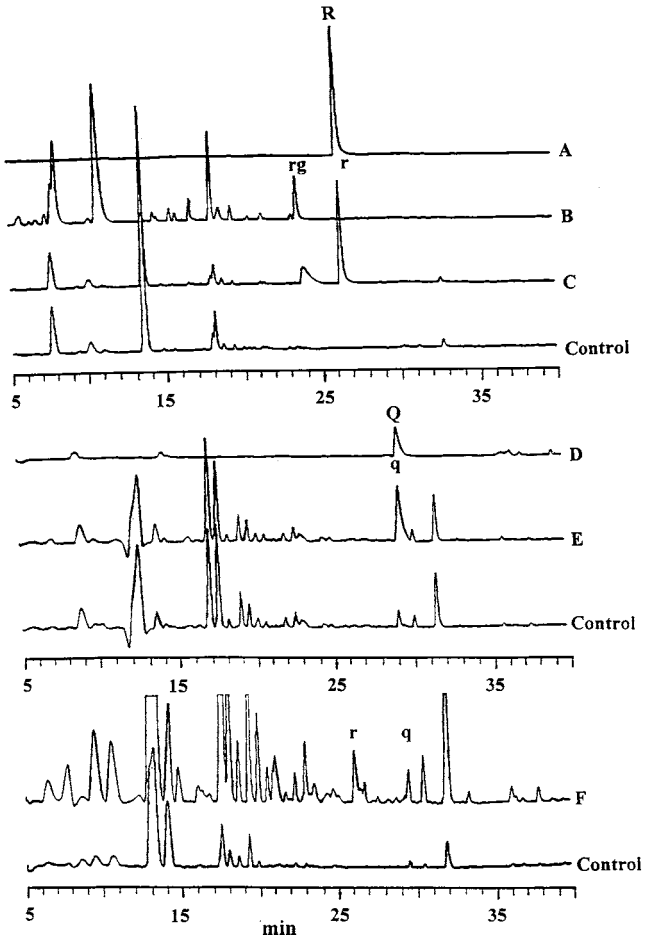

Figure 2. HPLC chromatograms of resveratrol and quercetin in human urine after oral ingestion of pure compounds or grape juice. (**A**) Resveratrol standard (2.5 μg/mL); (**B**) human urine collected 1 h after ingestion of *trans*-resveratrol (1 mg/kg) without enzyme hydrolysis; (**C**) human urine collected 1 h after ingestion of pure resveratrol (1 mg/kg); (**D**) quercetin standard (2.5 μg/mL); (**E**) human urine sample collected 3 h after ingestion of pure quercetin (0.5 mg/kg); and (**F**) human urine collected 4 h after ingestion of 1200 mL of 18.4% grape juice. R and r, resveratrol; rg, resveratrol glucuronide; and Q and q, quercetin.

doses of grape juice preparation (600 and 1200 mL) after enzyme digestion also showed detectable peaks of resveratrol and quercetin (**Figure 2F**).

The identities of resveratrol and quercetin in the urine samples were confirmed by LC/MS/MS. The mass spectrum of standard resveratrol gave fragments with *m*/*z* values of 159, 185, and 227, which were also seen with the plasma and urine samples (**Figure 3A**). The base peak *m*/*z* 227 corresponds to the pseudomolecular ion $[M - H]^-$ for resveratrol. Other fragment ions correspond to the losses of hydroxyl groups from the phenolic nucleus. The same fragmentation pattern was observed from the major peak detected in human urine after administration of resveratrol (**Figure 3B**). In the case of quercetin, both the standard and the biological samples yielded peaks (30 min) with fragments *m*/*z* 151, 179, 273, and 301 (**Figure 3C**,**D**) in their MS/MS spectra. The fragment with *m*/*z* 151 is considered to originate from a retro-Diels−Alder fission, resulting in the cleavage of the heterocyclic ring of quercetin (*34*). The formation of the fragment with *m*/*z* 179 probably involves a reopening and reclosing of the heterocyclic ring of quercetin along with a loss of a carbon in this ring.

Resveratrol and quercetin can be extensively metabolized by glucuronidation and methylation reactions. Standards of res-

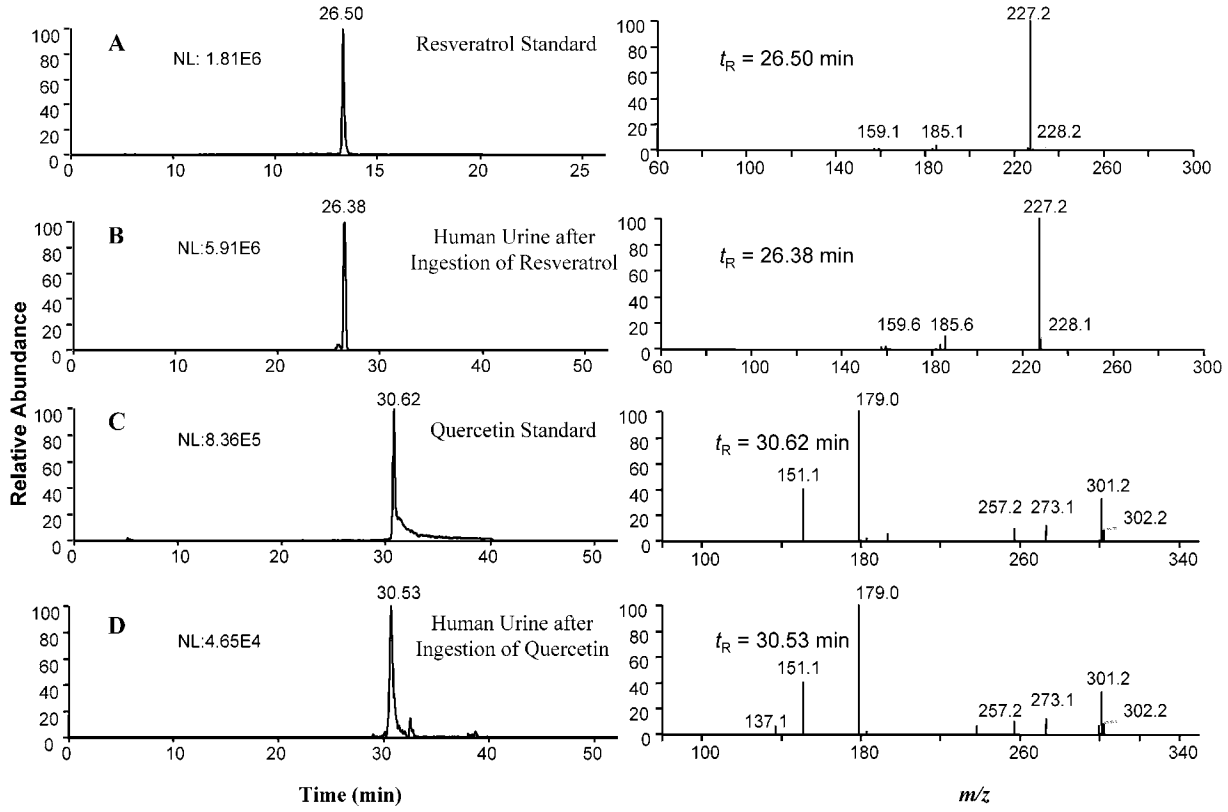

Figure 3. LC/MS/MS identification of resveratrol and quercetin in human urine samples. (**A**) Resveratrol standard (2.5 μg/mL); (**B**) human urine collected 1 h after ingestion of pure resveratrol (1 mg/kg); (**C**) quercetin standard (2.5 μg/mL); and (**D**) human urine sample collected 3 h after ingestion of pure quercetin (0.5 mg/kg).

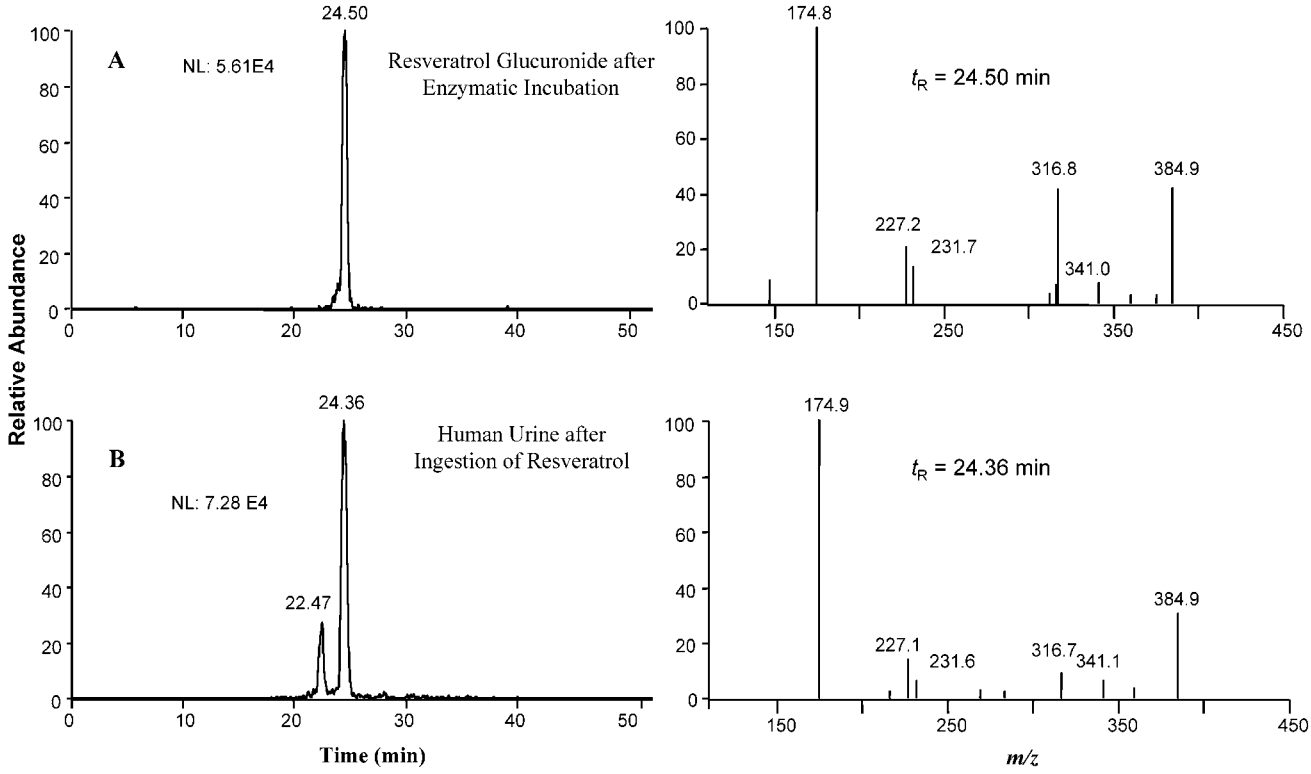

Figure 4. LC/MS/MS identification of resveratrol glucuronides. (**A**) Resveratrol glucuronide obtained after incubation of resveratrol with rat liver microsomes and (**B**) human urine sample collected 4 h after ingestion of 1200 mL of grape juice.

veratrol and quercetin glucuronides were prepared by incubating the aglycones with mouse liver microsomal protein and UDP-glucuronic acid. Negative molecular ion *m/z* 403 was applied for the detection of resveratrol mono-glucuronide. As shown in **Figure 4A**, a single peak was detected with a retention time 24.50 min after incubation of resveratrol with mouse liver microsomes. The presence of the fragments of resveratrol aglycone (*m/z* 227) and glucuronic acid (*m/z* 175) indicated that

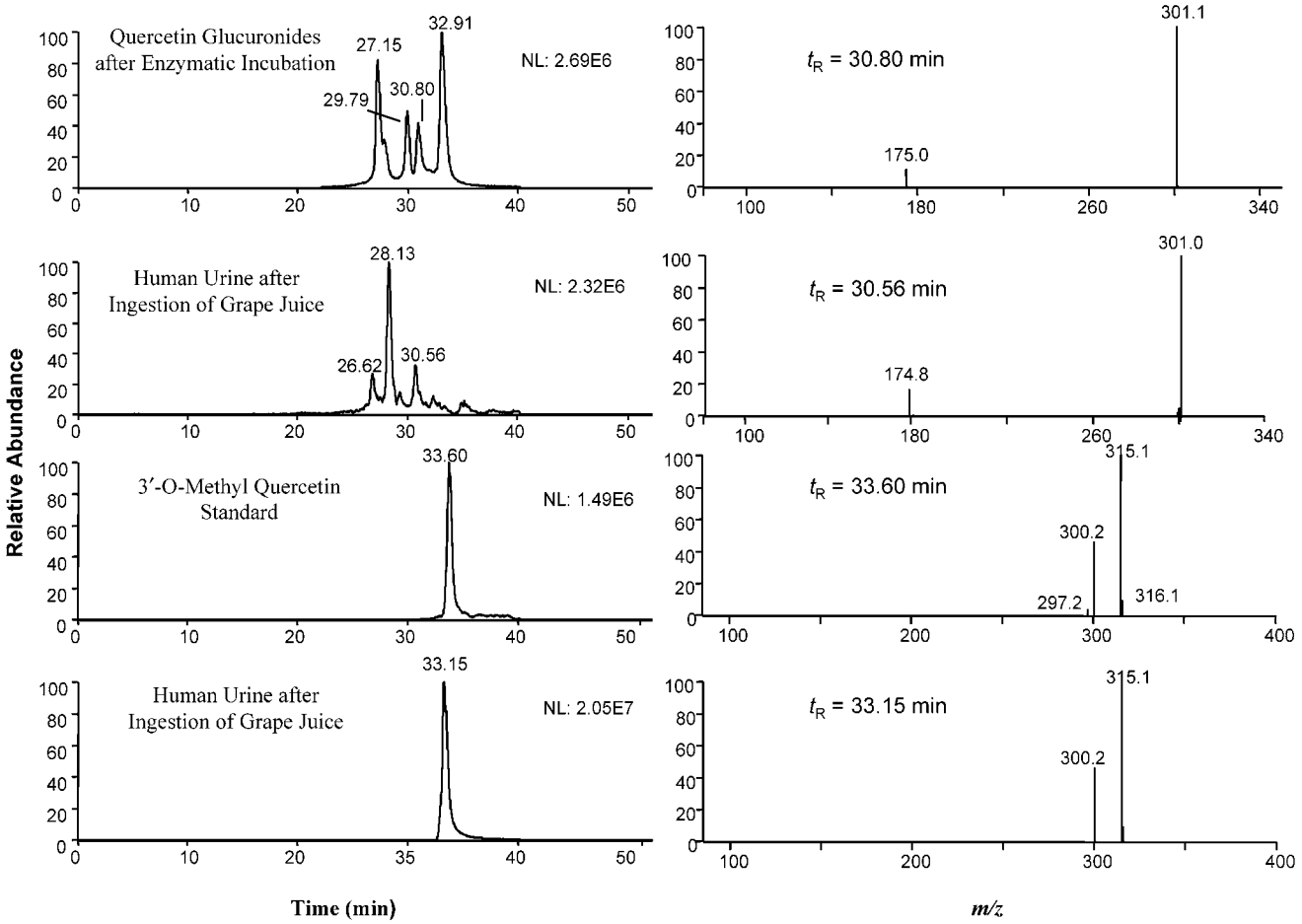

Figure 5. LC/MS/MS identification of 3′-*O*-methyl quercetin and quercetin glucuronides in human urine samples. (**A**) Quercetin glucuronides obtained after incubation of quercetin with rat liver microsomes; (**B**) human urine sample collected 4 h after ingestion of 200 mL of grape juice; (**C**) 3′-*O*-methyl quercetin standard solution (25 μg/mL); and (**D**) Human urine collected 4 h after ingestion of 200 mL of grape juice.

this peak was a resveratrol glucuronide. The same peak along with another peak (22.47 min) was detected in the human urine samples from subjects receiving resveratrol or high doses of grape juice preparation (600 and 1200 mL) (**Figure 4B**).

In the case of quercetin, four distinct peaks were detected with negative molecular ion *m*/*z* 447 after enzymatic reaction (27.15, 29.79, 30.80, and 32.91 min) (**Figure 5A**). These peaks were confirmed to be quercetin mono-glucuronides by LC/MS/MS with typical fragments of quercetin aglycone (*m*/*z* 301) and glucuronic acid (*m*/*z* 175). In human urine samples after ingestion of quercetin or high doses of grape juice preparation, three peaks were detected with slightly different retention times (26.62, 28.13, and 30.56 min) and the same fragment pattern (**Figure 5B**). The methylated derivative of quercetin in human urine sample after ingestion of grape juice preparation showed a major peak with fragments of *m*/*z* 300 and 315 in its mass spectrum (**Figure 5D**). This peak showed identical characteristics in comparison with authentic 3′-*O*-methyl quercetin (**Figure 5C**).

Urinary and Plasma Levels of Resveratrol and Quercetin in Human Subjects after Ingestion of Resveratrol and Quercetin or Grape Juice. After ingestion of resveratrol, the typical human urinary excretion profile of total resveratrol is depicted in **Figure 6A**. 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 cumulative excretion of resveratrol in the subject receiving 0.03 and 1 mg/kg amounted to 0.79 and 15.4 mg, respectively. These values correspond to 52 and 26% of the administered dose, respectively. In another subject receiving 0.5 mg/kg resveratrol, the cumulative amount of resveratrol excreted was 13.6 mg corresponding to 34% of the administered dose (data not shown). Resveratrol was principally present in the conjugated form as a glucuronide. The enzyme-hydrolyzed plasma samples obtained from the human subject receiving resveratrol (1 mg/kg) showed peaks of resveratrol. Considering the plasma volume as 6% of the body weight (3.6 L), the circulating plasma level of total resveratrol was calculated to be approximately 2.7 mg at 1.5 h. Confirmation of the identity of the peaks seen in the plasma samples was achieved by both HPLC-ECD and LC/MS/MS analyses.

After ingestion of quercetin (0.5 mg/kg), most of the urinary excretion of quercetin occurred in the first 12 h and peaked after about 10 h (**Figure 6B**). The urinary quercetin was still detected 24 h following the dosing. This residual level of quercetin may be of a dietary origin because it was also seen in the control urine samples. Most of the quercetin present in the urine appeared to be in conjugated form, as only the hydrolyzed samples had detectable levels of quercetin. Surprisingly, only trace amounts of quercetin were observed in the plasma samples after the enzymatic hydrolysis.

After consumption of standard grape juice (18.4%) in doses of 200 and 400 mL, quercetin, 3′-*O*-methyl quercetin, and quercetin glucuronides were detectable in the urine by LC/MS/MS, but resveratrol was not detectable. The 3′-*O*-methyl

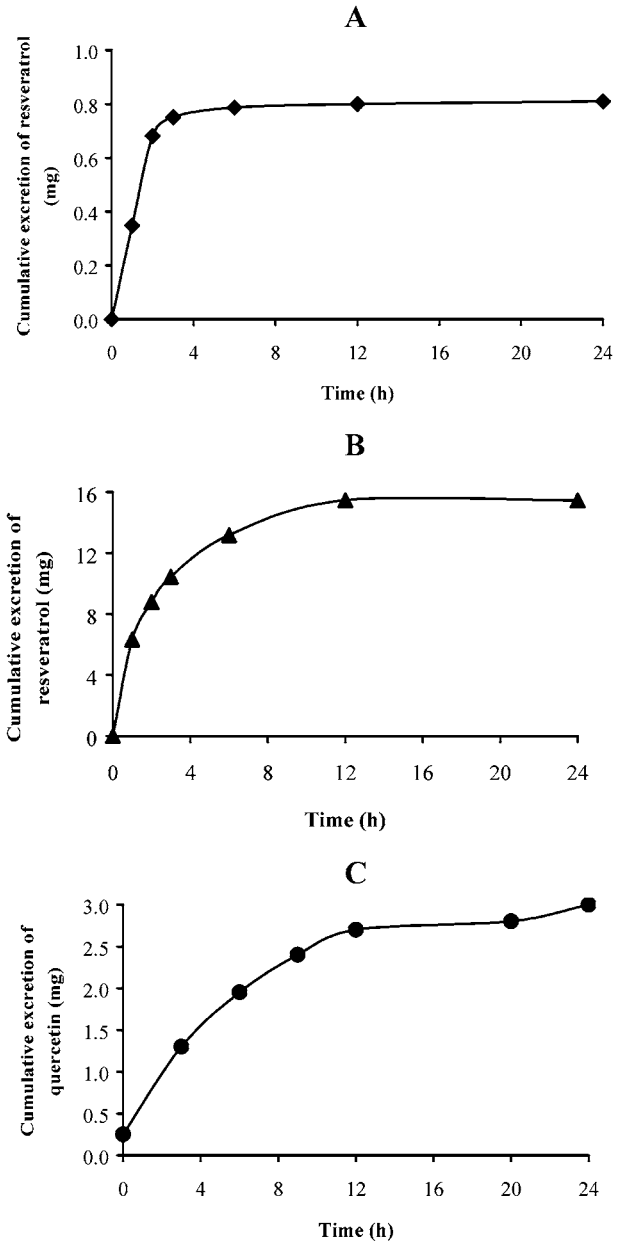

Figure 6. Urinary excretion profile of resveratrol and quercetin in human. Cumulative urinary excretion profile of resveratrol during 24 h in a subject receiving an oral dose of pure resveratrol: (**A**) 1 or (**B**) 0.03 mg/kg. (**C**) Cumulative excretion profile of quercetin in human urine after ingestion of pure quercetin aglycone (0.5 mg/kg).

quercetin peak, eluting 3 min after quercetin, was observed in the urine samples 3−4 h after grape juice administration. The fragmentation pattern of this peak in the LC/MS/MS analysis showed characteristic fragmentation peaks with *m*/*z* 300 and 315 of the authentic 3′-*O*-methyl quercetin. At high doses (600 and 1200 mL), the levels of resveratrol and quercetin were evident in the human urine samples after enzyme hydrolysis, which only accounted for less than 1% of the ingested dose. However, resveratrol or quercetin was not detectable in the plasma samples.

Urinary Levels of Resveratrol and Quercetin in the Mouse after Oral Administration of Grape Juice. Groups of mice receiving high concentrations of grape juice (18.4 and 36.8%) as drinking fluid had resveratrol and quercetin in the urine after enzyme hydrolysis. The identification of the resveratrol and quercetin in these samples was confirmed by LC/MS/MS.

Table 1. Total and Free Plasma Resveratrol Levels in Rats Receiving Oral Doses of Pure Resveratrol (2 or 5 mg/kg in 0.5 mL of 10% Ethanol)[a]

	plasma resveratrol concentration (μM)					
	0.5 h		1.5 h		4 h	
dose	free	total	free	total	free	total
2 mg/kg	0.04	0.35	0.09	0.87	0.09	1.2
5 mg/kg	0.06	0.52	0.11	1.3	0.08	1.5

[a] The values are means of duplicated determinations.

Because grape juice was available to the mice during the entire study period, the urinary excretion of resveratrol increased gradually during the study period. The cumulative amount of resveratrol excreted in urine by mice receiving 18.4% grape juice ranged from 0.28 to 0.55 μg corresponding to approximately 1−2% of the ingested dose whereas those receiving 36.8% grape juice ranged from 0.41 to 0.96 μg, corresponding to 0.9−2.3% of the dose. The excretion of quercetin also was found to increase gradually in the mouse urine and the cumulative amount of quercetin excreted by mice receiving 18.4 and 36.8% grape juice amounted to 0.4 and 1 μg or 1.1 and 0.7% of the dose, respectively. Plasma levels of resveratrol and quercetin, however, were not detected.

Plasma Levels of Resveratrol in Rats after Oral Administration of Resveratrol. The plasma samples obtained from rats were analyzed for the presence of both free and conjugated resveratrol and its metabolites. In rats receiving pure resveratrol (2 mg/kg), plasma resveratrol was detectable as early as 0.5 h. The plasma level increased more than 2-fold at 1.5 h and further rose moderately at 4 h. The plasma concentrations of both free and total resveratrol at 0.5, 2, and 4 h in rats receiving two different doses of resveratrol are given in **Table 1**. The majority of resveratrol was present as conjugates at both doses with the free form constituting only about 10−11% of the total resveratrol level at the initial time points (0.5 and 1.5 h) and declining to 5−7% at 4 h.

DISCUSSION

A variety of methods have been developed for the quantification of resveratrol and quercetin. The presently reported simultaneous analysis of resveratrol and quercetin in biological fluids by HPLC coupled with electrochemical or MS detections is convenient and precise.

After oral administration of pure resveratrol to humans, resveratrol levels were readily detectable in both the plasma and the urine. The low dose of resveratrol given to human subjects (0.03 mg/kg) is comparable to 2−3 glasses of wine. The recovery of resveratrol in the circulating plasma suggested a rapid absorption of resveratrol in the gastrointestinal tract. At a low dose (0.03 mg/kg), more than half of the ingested resveratrol was recovered in the urine in 24 h, whereas at a higher dose (1 mg/kg), only a quarter of the administered dose could be recovered during the same period.

The grape juice preparation (18.4% solution of the freeze-dried powder) that we used contained 0.16 mg of resveratrol per serving of 100 mL. Most of this resveratrol exists as glucosides. In the present experiment with 200 and 400 mL of grape juice, the level of this compound in the urine and plasma was below the level of detection. When 600 and 1200 mL of grape juice, which contained approximately 1 and 2 mg of total resveratrol, respectively, were administered, resveratrol was detectable in the urine samples. However, the cumulative

excretion of resveratrol after drinking 1200 mL of grape juice (containing 1.96 mg of resveratrol) was only about 5% of the dose administered. This is one-tenth that obtained with oral administration of pure resveratrol (1.95 mg for a 65 kg person). In grape juice, the level of free resveratrol is rather low. *cis*- and *trans*-Piceid are the major resveratrol derivatives in grape juice. This result suggests the lower bioavailability of resveratrol glycosides in grape juice in comparison to its pure aglycone. The glycosidase in grape juice and in the intestinal microflora can hydrolyze piceid to the aglycone and thus may affect systemic absorption. It is also possible that the high sugar content in the grape preparation used has adverse effects on the bioavailability of resveratrol.

In our experiments with pure quercetin, quercetin was readily detectable in human urine samples. In experiments where human subjects consumed 600 and 1200 mL of grape juice (containing approximately 2.3 and 4.6 mg of quercetin, respectively), quantitative estimation of the urinary quercetin excretion was feasible. There is only limited knowledge on the bioavailability of quercetin in humans. Recent studies indicated that quercetin is absorbed from the intestine after hydrolysis of its glycosides by the intestinal glucosidases (*35*), and the type and position of sugar moiety in the quercetin glycoside can influence the absorption of quercetin (*36*). There are also suggestions that quercetin glycosides could be absorbed intact from the small intestine (*38*, *39*). Our observations showed that the human subjects receiving pure quercetin excreted approximately 7.6% of the dose ingested in 24 h, while this value was only 0.5% with grape juice as the source for quercetin. This would suggest that absorption of quercetin glycosides in the grape juice is much less than from the pure aglycones. Poor bioavailability of quercetin (mostly in the glycoside form) from red wine was also reported recently (*32*). In contrast to resveratrol, little or no quercetin was observed in the plasma after the ingestion of either pure aglycone or grape juice.

After administration, resveratrol will undergo glucuronidation. There is evidence that the major form of resveratrol transferred across the rat intestinal epithelium into the blood stream is resveratrol glucuronides (*40*). Glucuronidation and sulfation of resveratrol by human liver microsomes have been reported (*28*). Another study (*41*) has shown the formation of 3-*O*-glucuronide and 4′-*O*-glucuronides by human liver microsomes. Consistent with this study, two major peaks of resveratrol glucuronides were detected in human urine samples after the ingestion of resveratrol. However, only one peak was observed following the incubation of resveratrol with mouse liver microsomes, which might be due to the different glucuronosyltransferase isoforms in the mouse. The plasma level of resveratrol in rats was also investigated after administration of pure resveratrol. In rats, more than 90% of total resveratrol circulating in the plasma was in the conjugated form at almost every time point studied.

The glucuronidation of quercetin could take place at the 3-, 7-, 3′-, and 4′-positions when quercetin was incubated with human liver cell-free extract (*42*). In our study, four peaks were obtained when quercetin was incubated with mouse liver microsomes, whereas only one major peak along with several other minor peaks was detected in human urine after ingestion of grape juice. The species difference might contribute to this result. Quercetin is readily O-methylated by catechol-*O*-methyltransferase because of its catechol structure. Methylation of quercetin from food products such as onion have been reported earlier (*43*, *44*). The present study reports for the first time the presence of O-methylated quercetin in human urine after consuming grape juice. Even in subjects receiving the lowest dose of grape juice (200 mL), 3′-*O*-methyl quercetin was detectable, which may suggest the high activity of *O*-methylation toward quercetin. Although the methylation of quercetin could occur at both 3′- and 4′-positions (*45*), only 3′-*O*-methyl quercetin was observed in human urine after ingestion of quercetin or grape juice.

In conclusion, the present study provides information on the comparative bioavailability of resveratrol and quercetin as pure compounds and as constituents of grape juice. The information obtained from this study will facilitate the design and interpretation of future large-scale human studies in this area. The results show the presence of resveratrol and quercetin metabolites in the body. Future studies are warranted to further explore the biological effects of methylated or glucuronidated metabolites of these polyphenols.

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