JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Distribution of Resveratrol Metabolites in Liver, Adipose Tissue, and Skeletal Muscle in Rats Fed Different Doses of This Polyphenol

Cristina Andres-Lacueva,[†,‡] M. Teresa Macarulla,[§] Maria Rotches-Ribalta,[†,‡] María Boto-Ordóñez,[†,∥] Mireia Urpi-Sarda,[∥] Víctor M. Rodríguez,[§] and María P. Portillo*[,§]

[†]Department of Nutrition and Food Science, XaRTA, INSA, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain

[‡]INGENIO−CONSOLIDER Program, Fun-C-food (CSD2007-063), Ministry of Science and Innovation, Barcelona, Spain

[§]Department of Nutrition and Food Science, Faculty of Pharmacy, RETIC RD06/0045/0003, Instituto de Salud Carlos III, University the Basque Country (UPV/EHU), Vitoria, Spain

[∥]CIBER: Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Barcelona, Spain

ABSTRACT: This study aimed to characterize resveratrol metabolite profiles in liver, skeletal muscle, and adipose tissue in rats treated for 6 weeks with 6, 30, or 60 mg of *trans*-resveratrol/kg body weight/d. Resveratrol metabolites were quantified by liquid chromatography−tandem mass spectrometry. The greatest number of metabolites was found in liver followed by adipose tissue. A great number of metabolites in muscle was below the limit of detection. The amounts of sulfate conjugates tended to increase when resveratrol dosage was enhanced, while the glucuronide ones increased only between 6 and 30 mg/kg/d. Microbiota metabolites were detected in higher amounts than resveratrol conjugates in liver, while the opposite occurred in adipose tissue and muscle. So, the largest amounts of resveratrol metabolites were found in liver, intermediate amounts in adipose tissue, and the lowest amounts in muscle. Sulfate conjugates, but not glucuronides, showed a dose−response pattern. Microbiota metabolites were predominant in liver.

KEYWORDS: *resveratrol, glucuronide metabolites, sulfate metabolites, dihydroresveratrol, liver, adipose tissue, skeletal muscle*

INTRODUCTION

Resveratrol (*trans*-3,5,4′-trihydroxystilbene) is a phytoalexin polyphenolic compound occurring naturally in various plants, including grapes, berries, and peanuts, in response to stress or as a defense mechanism against fungal, viral, bacterial infections and damage from exposure to ultraviolet radiation.[1]

Resveratrol has been reported to have several beneficial effects on health.[2] However, the efficacy of orally administered resveratrol depends on its absorption, metabolism, and tissue distribution, since it is quickly absorbed in the intestine, via simple intestinal transepithelial diffusion at a high rate (77−80%).[3,4] Active transport might occur as well, but this is only likely for resveratrol metabolites.[5] Most resveratrol undergoes rapid and extensive metabolism in enterocytes, before entering into the bloodstream, resulting in up to a 20-fold higher concentration of conjugates, and less than 1% resveratrol. Furthermore, it undergoes rapid first-pass metabolism in the liver.[6] Consequently, resveratrol bioavailability is very low and only a small proportion reaches plasma.[7] Besides this intestinal and hepatic metabolism, it has been proposed that bacterial metabolism should also be taken into account.[4] Several approaches that may increase the availability of resveratrol are under evaluation, such as dose escalation studies,[8] as well as repeated or long-term dosing, which might result in saturation of metabolism, leading to higher plasma and tissue levels of resveratrol.[4]

In vitro studies have suggested that concentrations in the range of 5−50 μM are needed to find significant changes in cells.[9−14] Taking into account the low bioavailability of orally administered resveratrol, the doses usually administered in in vivo studies are unlikely to furnish resveratrol levels sufficiently compatible with those that modulate biological actions in vitro.[15,16] A great number of biovailability studies have shown this.[6−8,17] Nevertheless, beneficial effects of resveratrol under in vivo conditions have been reported. This may be due to the fact that some resveratrol metabolites show biological activities[18] or due to the potential conversion of resveratrol metabolites back to resveratrol in target organs.[19]

Thus, the cause−effect relationship between resveratrol concentration in the systemic bloodstream and its reported biological effects is still a conundrum.[15] Consequently, it is essential (a) to establish whether resveratrol metabolites can conceivably contribute to, or account for, different effects of resveratrol in vivo and (b) to quantify resveratrol metabolites in vivo. Localization of tissue uptake, which determines target tissues, and the target/dose relationship are important to understand the positive health effects described for resveratrol.[20]

In this context, the present study aimed to characterize the resveratrol metabolite profile in liver, skeletal muscle, and adipose tissue in rats fed on obesogenic diets supplemented with resveratrol. Different doses of this polyphenol were used for supplementation in order to assess potential saturation of metabolic pathways responsible for transformation of resveratrol into metabolites. These tissues were chosen because they

Received: January 19, 2012
Revised: April 24, 2012
Accepted: April 25, 2012
Published: April 25, 2012

dx.doi.org/10.1021/jf3001108 | J. Agric. Food Chem. 2012, 60, 4833−4840

play a crucial role in the recently described body-fat-lowering effect of resveratrol. An interesting aspect of this study is that very few data have been published concerning metabolites found in two of these tissues, adipose tissue and skeletal muscle, as well as tissue distribution after a long-term treatment with resveratrol.

MATERIAL AND METHODS

Animals and Experimental Design. The experiment was conducted with 24 male Sprague−Dawley rats with an initial body weight of 180 ± 2 g purchased from Harlan Ibérica (Barcelona, Spain) and took place in accordance with the institution's guide for the care and use of laboratory animals (Reference protocol approval CUEID CEBA/30/2010). Animals from this cohort were previously used to study the potential body-fat-lowering effect of resveratrol.[21]

The animals were individually housed in polycarbonate metabolic cages (Techniplast Gazzada, Guguggiate, Italy) and placed in an air-conditioned room (22 ± 2 °C) with a 12-h day−night rhythm (light on at 21:00). After a 6-day adaptation period, the animals were fed on a commercial obesogenic diet supplied by Harlan Iberica (ref TD.06415) for 6 weeks. All animals had free access to food and water.

Before starting our animal studies with resveratrol, we analyzed the stability of this polyphenol when added into the diet in a reservoir protected from light. We found that resveratrol degraded almost completely over the feeding time.[21] Therefore, we concluded that mixing it in the diet, the most common method used to test the biological effects of a functional molecule intake, was not a suitable system. In a great number of studies performed by our group we have observed that the rats started eating the diet immediately when it was replaced every day at the beginning of the dark period. Taking advantage of this situation, we added resveratrol by using an ethanol solution (30 mg/mL) on the surface of the food reservoir. The amount of resveratrol solution added to the diet was adjusted on a daily basis according to the animal weight to reach the following doses: 6 mg resveratrol/kg body weight/d in RSV1 group, 30 mg resveratrol/kg body weight/d in RSV2 group, and 60 mg resveratrol/kg body weight/d in RSV3 group. These doses are equivalent to 68, 340, and 680 mg in a 70-kg person.[22] In order to avoid differences in the amount of ethanol received by each animal, ethanol was completed to reach 2 mL/kg body weight/d. Because a very small volume was added (0.36−0.69 mL for body weights ranging from 180 g, the initial body weight, to 345 g, the final body weight), the rats ate all the resveratrol provided in the first minutes of feeding period without any degradation. *trans*-Resveratrol (95% purity) was provided by Monteloeder (Elche, Spain) and added to the diet as explained above for 6 weeks.

Tissue Removal. At the end of the experimental period rats were fasted 12 h and sacrificed under anesthesia (chloral hydrate) by cardiac exsanguination. Subcutaneous white adipose tissue, gastrocnemious muscle, and liver were dissected, weighed, immediately frozen, and stored at −80 °C until analysis. Tissue removal was performed 24 h after resveratrol administration.

Standards and Reagents. All samples and standards were handled with no exposure to light. *trans*-Resveratrol (99% purity) and ethyl gallate, as internal standard (96% purity), were purchased from Sigma-Aldrich (St. Louis, MO). The external standard taxifolin (>90% purity) was purchased from Extrasynthese (Genay, France). Standards of *cis*-resveratrol (97% purity) and *trans*-resveratrol metabolites, i.e., *trans*-resveratrol-3-*O*-glucuronide (98% purity), *trans*-resveratrol-4′-*O*-glucuronide (98% purity) and *trans*-resveratrol-3-*O*-sulfate (98% purity), were acquired from Toronto Research Chemicals Inc. (North York, Canada). *trans*-Resveratrol-4′-*O*-sulfate and *trans*-resveratrol-3,4′-disulfate were obtained as reported previously for identification purposes.[23] Dihydroresveratrol aglycon was synthesized following the work by Thakkar et al.[24] Standards were prepared as stock solutions in 80% (v/v) methanol.

Liquid chromatography (LC) grade solvents methanol, ethyl acetate, acetonitrile, and ammonium acetate (>99%) were purchased from Scharlau Chemie, S.A. (Sentmenat, Spain). LC grade solvents glacial acetic acid, acetone, and ammonia (35%) were purchased from Panreac Quimica, SAU (Castellar del Vallès, Spain). Deuterated dimethyl sulfoxide (DMSO-d_6, 99.96% deuterated) was purchased from Euriso-top, SAS (Saint-Aubin Cedex, France). Ultrapure water (Milli-Q) was obtained from Millipore (Bedford, MA).

Structural Identification of Dihydroresveratrol. Dihydroresveratrol structure was confirmed by nuclear magnetic resonance (^{1}H NMR) measurements using a Varian 400-MHz instrument VNMR System (Varian, Palo Alto, CA). Synthesized dihydroresveratrol was dissolved in deuterated DMSO-d_6 using dinitrobenzene (1.6 mmol/L) as internal standard. ^{1}H NMR spectra were acquired with the following data: δ (ppm) 9.0 (br, 3H, −OH), 6.97 (d, 2H, J = 8.6 Hz, H-2′,6′), 6.63 (d, 2H, J = 8.6 Hz, H-3′,5′), 6.03 (d, 2H, J = 4.0 Hz, H-2,6), 6.00 (dd, 1H, J = 4.0 Hz, H-4), 2.68−2.56 (m, 4H, $-CH_2CH_2-$). Standard purity was calculated following the protocol validated by Malz and Jancke,[25] obtaining 80% purity. Only one peak was detected after a full scan MS experiment, and no peaks of the parent compound resveratrol (m/z 227) were detected.

Sample Preparation and Determination of Resveratrol Metabolites in Tissues. Analysis was carried out by LC−MS/MS after a solid-phase extraction (SPE), as described previously by Urpi-Sarda et al.[26] and Andres-Lacueva et al.[27] with some modifications. Briefly, ∼100 mg of frozen samples was extracted three times with 1 mL of a solution of 1.5 M formic acid with 5% of methanol using a mixer mill (Retsch MM 400, Qiagen, Hilden, Germany) at 30 Hz for 0.5 min and centrifuged each time at 14 000 rpm. After this homogenization, the tissue structure was broken and metabolites linked to protein were released. Pooled supernatant fractions with the internal standard were loaded onto a preconditioned Waters Oasis HLB 96-well plate (30 mg). Acetic acid 2 M in water (1 mL) and in water/methanol (85/15 v/v) (1 mL) were used to wash the plate. Elution was achieved with 0.5 mL of 1 M acetic acid in methanol, 1.5 mL of 1 M acetic acid in ethyl acetate, and 0.5 mL of methanol with 5% (v/v) ammonia. The eluate was evaporated to dryness. The residue was reconstituted with taxifolin dissolved in mobile phase as an additional external standard.

Liquid chromatography analyses were performed using an ACQUITY UPLC (Waters, Milford, MA) and a triple quadrupole mass spectrometer (API 3000) from Applied Biosystems (PE Sciex, Concord, Canada), equipped with a Turbo IonSpray source operated in the negative-ion mode. An ACQUITY UPLC BEH C_{18} column, 50 × 2.1 mm i.d., 1.7 μm, was used for chromatographic separation with mobile phase A (0.05% acetic acid in water, for glucuronide metabolites, or 10 mM ammonium acetate solution for sulfate metabolites) and mobile phase B (acetone:acetonitrile, 70:30). The linear gradient for the determination of resveratrol metabolites at a flow rate of 1 mL/min was [t (min), %B] (0, 10), (1, 30), (2, 100), (2.3, 100), (2.31, 10), (3, 10)]. In each case the sample volume injected was 5 μL. MS/MS parameters used were as follows: capillary voltage, −3500 V; focusing potential, −200 V; entrance potential, −10 V; nebulizer gas, 12 (arbitrary units), curtain gas, 12 (arbitrary units), collision gas, 6 (arbitrary units), auxiliary gas temperature, 400 °C; auxiliary gas flow rate, 7000 cm^3/min.

For quantification of resveratrol metabolites in tissue samples, the multiple reaction monitoring (MRM) mode was used with the following transitions: 227/185 for resveratrol; 403/227 and 307/227 for glucuronide and sulfate conjugates of resveratrol, respectively; 579/403 for resveratrol diglucuronides; 387/227 for resveratrol disulfates; 389/227 for resveratrol glucosides; 229/123 for dihydroresveratrol; 405/229 and 309/229 for glucuronide and sulfate conjugates of dihydroresveratrol and 197/169 and 303/285 for ethylgallate and taxifolin, respectively. Control tissues were used to prepare calibration curves since no resveratrol or its metabolites were detected. Calibration curves were prepared, in the range of expected concentrations, by supplementation with known concentrations of available standards. All of them were quantified using a six-point calibration curve between the limit of quantification (LOQ) and 10 μg/g determined by weighted ($1/x^2$) linear regression. Dihydroresveratrol metabolites and *cis*-resveratrol-3-*O*-sulfate were quantified using the calibration curve of dihydroresveratrol and *trans*-resveratrol-3-*O*-

Table 1. Limits of Detection (LOD) and Limits of Quantification (LOQ) for Each Tissue and Mean Recovery Efficiency from the Three Tissues[a]

analyte	LOD (nmol/g tissue)			LOQ (nmol/g tissue)			recovery (%)
	liver	adipose tissue	skeletal muscle	liver	adipose tissue	skeletal muscle	
trans-resveratrol	0.21 ± 0.01	0.07 ± 0.02	0.27 ± 0.02	0.70 ± 0.02	0.23 ± 0.05	0.90 ± 0.05	105.1 ± 11.5
cis-resveratrol	0.22 ± 0.02	0.14 ± 0.03	0.29 ± 0.02	0.73 ± 0.05	0.46 ± 0.08	1.31 ± 0.07	101.6 ± 8.4
trans- resveratrol-4′-*O*-glucuronide	0.11 ± 0.03	0.20 ± 0.04	0.29 ± 0.03	0.35 ± 0.09	0.66 ± 0.09	0.82 ± 0.94	101.2 ± 5.1
trans- resveratrol-3-*O*-glucuronide	0.09 ± 0.02	0.15 ± 0.04	0.21 ± 0.06	0.31 ± 0.06	0.51 ± 0.07	0.89 ± 0.07	98.7 ± 7.2
trans- resveratrol-3-*O*-sulfate	0.30 ± 0.00	0.06 ± 0.00	0.18 ± 0.04	1.00 ± 0.01	0.19 ± 0.01	0.61 ± 0.08	97.4 ± 10.0
dihydroresveratrol	0.06 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	0.21 ± 0.00	0.15 ± 0.01	0.15 ± 0.02	96.1 ± 5.4

[a]Values are means ± SD.

Table 2. Resveratrol Metabolite Profile (nmol/g tissue) in Liver and Adipose Tissue from Rats Receiving 30 mg of Resveratrol/kg Body Weight/d for 6 Weeks

analyte	liver			adipose tissue		
	mean ± SEM[c]	range	*n*	mean ± SEM[c]	range	*n*
cis-resveratrol	nq	nd−nq	−	nd	−	−
trans-resveratrol-3-*O*-glucuronide	8.66 ± 3.24	nd−28.79	7/8	1.31 ± 0.43	nd−3.24	6/8
trans-resveratrol-4′-*O*-sulfate[a]	1.07 ± 0.27	nq−2.64	7/8	nq	nd−nq	−
trans-resveratrol-3-*O*-sulfate	nq	nd−nq	−	0.24 ± 0.06	nd−0.41	6/8
cis-resveratrol-3-*O*-sulfate[a]	nq	nd−nq	−	nq	nd−0.41	4/8
trans-resveratrol-3,4′-disulfate[a]	nq	nd−nq	−	nq	nd−nq	−
dihydroresveratrol	nq	nd−0.42	2/8	nd	−	−
dihydroresveratrol glucuronide[b]	5.50 ± 1.45	1.77−14.86	8/8	nq	nd−0.18	2/8
dihydroresveratrol sulfate[b]	16.44 ± 4.27	2.90−41.35	8/8	0.32 ± 0.08	nd−0.65	6/8

[a]Quantified as *trans*-resveratrol-3-*O*-sulfate equivalents. [b]Quantified as dihydroresveratrol equivalents. [c]Values <LOQ considered as the LOD value. *n* is the number of rats in which the metabolite was quantified/total rats measured. nd and nq indicate not detected and not quantifiable, respectively (below the limit of detection or quantification, respectively; see Table 1).

Table 3. Resveratrol Metabolite Profile (nmol/g tissue) in Liver and Adipose Tissue from Rats Receiving 60 mg of Resveratrol/kg Body Weight/d for 6 Weeks

analyte	liver			adipose tissue		
	mean ± SEM[c]	range	*n*	mean ± SEM[c]	range	*n*
cis-resveratrol	nq	nd−nq	−	nq	nd−nq	−
trans-resveratrol-3-*O*-glucuronide	10.54 ± 3.06	2.26−25.36	8/8	1.08 ± 0.29	nq−2.33	6/8
trans-resveratrol-4′-*O*-sulfate[a]	3.01 ± 1.14	nq−8.55	6/8	0.26 ± 0.06	nq−0.65	6/8
trans-resveratrol-3-*O*-sulfate	nq	nd−nq	−	0.19 ± 0.06	nd−0.39	5/8
cis-resveratrol-3-*O*-sulfate[a]	nq	nd−nq	−	0.19 ± 0.06	nd−0.32	5/8
trans-resveratrol-3,4′-disulfate[a]	nq	nd−2.39	2/8	nq	nd−nq	−
dihydroresveratrol	0.75 ± 0.20	nd−1.42	7/8	nd	−	−
dihydroresveratrol glucuronide[b]	9.55 ± 2.45	2.65−18.96	8/8	nq	nd−0.30	4/8
dihydroresveratrol sulfate[b]	74.17 ± 23.39	13.00−186.68	8/8	1.02 ± 0.37	0.34−3.65	8/8

[a]Quantified as *trans*-resveratrol-3-*O*-sulfate equivalents. [b]Quantified as dihydroresveratrol equivalents. [c]Values <LOQ considered as the LOD value. *n* is the number of rats in which the metabolite was quantified/total rats measured. nd and nq indicate not detected and not quantifiable, respectively (below the limit of detection or quantification, respectively, see Table 1).

sulfate, respectively, and expressed as their equivalents, as was done previously,[8,26] since no standards are available.

The mean recovery efficiency of the available standards from the different samples was 99.2 ± 11.5%, ranging from 95.6 ± 9.9% (in the case of *trans*-resveratrol-4′-*O*-sulfate) to 105.1 ± 11.5% (in the case of *trans*-resveratrol) (Table 1). Calibration curves were linear over the concentration range with correlation coefficients for all the analytes >0.99. The detection limit (LOD) was defined as the concentration of analyte that produced a signal-to-noise ratio of at least 3, and the limit of quantification (LOQ) was the lowest standard with a signal-to-noise ratio of at least 10. The LOD and LOQ for each standard and evaluated tissue are shown in Table 1. The method had accuracy values (mean ± SD) of 97.25 ± 3.97%, 100.18 ± 3.05%, and 97.69 ± 5.49% for liver, adipose tissue, and skeletal muscle, respectively, and precision values ranged from 3.26 to 13.65% for liver, from 2.65 to 12.48% for adipose tissue, and from 3.22 to 12.85% for skeletal muscle, which met the acceptance criteria of the FDA.[28]

Statistical Analysis. Results are presented as mean ± SEM. Statistical analysis was performed using SPSS 17.0 (SPSS, Chicago, IL). Data were analyzed by one-way ANOVA followed by Newman Keuls post hoc test. Significance was assessed at the $P < 0.05$ level.

RESULTS

The profile of resveratrol metabolites in liver and adipose tissues is detailed in Tables 2 and 3. These metabolites included five phase II metabolites of resveratrol, *cis*-resveratrol, and dihydroresveratrol and its glucurono- and sulfo-conjugates (as microbial metabolites).

When the lowest dose of resveratrol was administered to rats (RSV1 group), two microbial metabolites of resveratrol (dihydroresveratrol conjugates) were found in quantifiable amounts in liver of all eight rats (mean ± SEM): dihydroresveratrol glucuronide (1.48 ± 0.32 nmol/g) and dihydroresveratrol sulfate (2.23 ± 0.32 nmol/g). Otherwise, two metabolites were found in adipose tissue of three rats (range): *trans*-resveratrol-3-*O*-sulfate [not detected (nd)−0.31 nmol/g] and *cis*-resveratrol-3-*O*-sulfate (nd−0.26 nmol/g). Any metabolite has been detected in skeletal muscle.

When higher doses were administered to rats (RSV2 and RSV3 groups) (Tables 2 and 3), the number of quantifiable compounds increased up to four in liver and three in adipose tissue in the RSV2 treatment and five metabolites in liver and adipose tissue in RSV3 treatment. The metabolite mainly detected in liver for all the treatment groups was dihydroresveratrol sulfate, a microbial metabolite, followed by *trans*-resveratrol-3-*O*-glucuronide and dihydroresveratrol glucuronide. Thus, it seems that microbial metabolites predominated in liver. *trans*-Resveratrol-3-*O*-glucuronide was quantified in only one rat in skeletal muscle after RSV2 and RSV3 treatments: 1.02 and 0.76 nmol/g, respectively. Dihydroresveratrol sulfate was quantified in skeletal muscle of two rats after RSV2 treatment (range: nd−0.31 nmol/g) and in six rats after RSV3 treatment [mean ± SEM (range): 0.30 ± 0.10 nmol/g (nd−0.86 nmol/g)]. *trans*-Resveratrol, *trans*-resveratrol-4′-*O*-glucuronide, and resveratrol diglucuronide were not detected in any tissue.

The highest concentrations of resveratrol metabolites were found in liver, followed by adipose tissue and skeletal muscle (Figure 1). A dose−response pattern was found in liver when total resveratrol metabolites were quantified. By contrast, in adipose tissue this pattern was only observed when RSV1 was compared with RSV2 and RSV3 groups (Figure 1). In skeletal muscle no resveratrol metabolites were detected in the RSV1 group, and no significant differences between groups RSV2 and RSV3 were observed (Figure 1).

Total glucuronide and sulfate metabolites in the three tissues are shown in Figure 1. When the distribution between these two kinds of metabolites was analyzed, different patterns of response were also found among tissues. In liver and adipose tissue, animals from the RSV2 group showed significantly higher amounts of glucuronide metabolites than animals from RSV1. Similar amounts of these metabolites were shown in the three tissues of animals from the RSV2 and RSV3 groups, since no statistical differences were obtained between them. A dose−response pattern in all the evaluated tissues was observed for sulfate conjugates. This pattern was also observed when metabolites derived from microbiota were considered (dihydroresveratrol and its conjugates), in the three tissues. Larger amounts of these metabolites than of resveratrol conjugates were detected in liver while the opposite was true in adipose tissue and skeletal muscle (Figure 2).

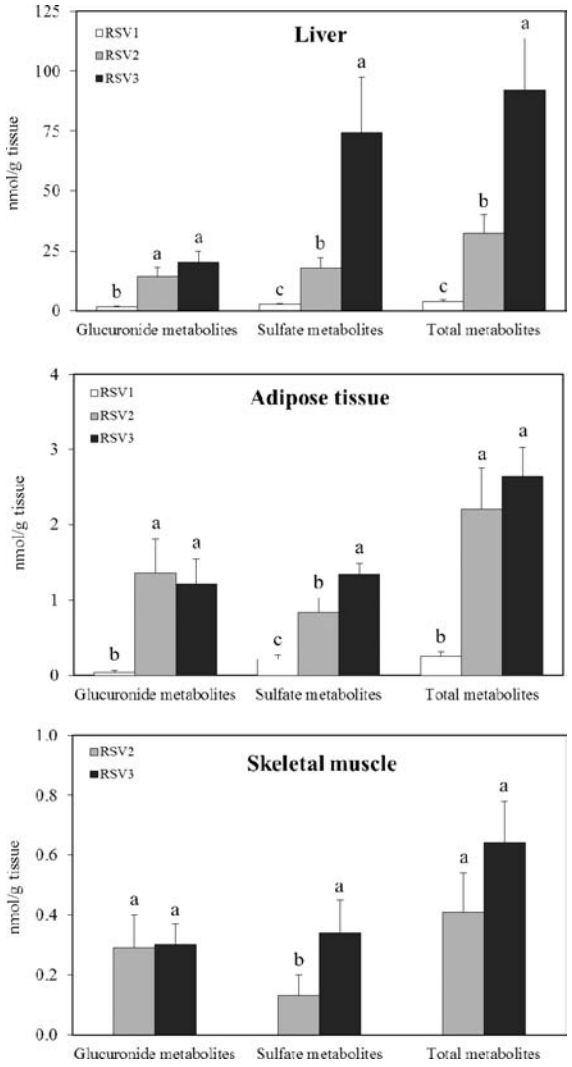

Figure 1. Glucuronide, sulfate, and total analyzed resveratrol metabolites in liver, adipose tissue, and skeletal muscle from rats receiving 6 mg/kg/d (RSV1), 30 mg/kg/d (RSV2), or 60 mg/kg/d (RSV3) of resveratrol for 6 weeks. Results are expressed as mean ± SEM. Statistics shows comparisons among the three dose groups for each metabolite type. Bars not sharing a common letter are significantly different ($P < 0.05$). No detectable amounts of resveratrol metabolites were found in the RSV1 group for the skeletal muscle. Data concerning total metabolites in adipose tissue from rats treated with 30 mg/kg/d have been previously presented.[43] (Values <LOQ considered as the LOD value.)

DISCUSSION

Resveratrol is a polyphenol with a very low availability. Due to its extensive metabolism, free resveratrol reaches very low concentrations in plasma and tissues.[6] Despite that, a great number of published studies have reported beneficial effects on health in animal experimental models. It can be hypothesized that resveratrol metabolites may be active per se, or that they provide a pool for local or systemic regeneration of resveratrol in vivo.[29] Due to these facts, it is essential to quantify resveratrol metabolites in tissues in in vivo studies. Nevertheless, tissue distribution of resveratrol and its metabolites has rarely been assessed compared with the number of papers that refer to the plasmatic concentrations of this polyphenol so far.

It is important to point out that our experimental design was not specifically planned to carry out a pharmacokinetic study. In fact, our first interest was to analyze the effects of resveratrol on body fat accumulation and serum parameters, and thus, rats were fed on an obesogenic diet.[21] We observed that the lowest dose (6 mg/kg/d) was without effect, the intermediate dose (30 mg/kg/d) induced a significant reduction in body fat, and the highest dose (60 mg/kg/d) did not improve the effect of 30 mg/kg/d. In the light of these results, we considered it very interesting to determine the amount of resveratrol and resveratrol metabolites found in target tissues involved in the

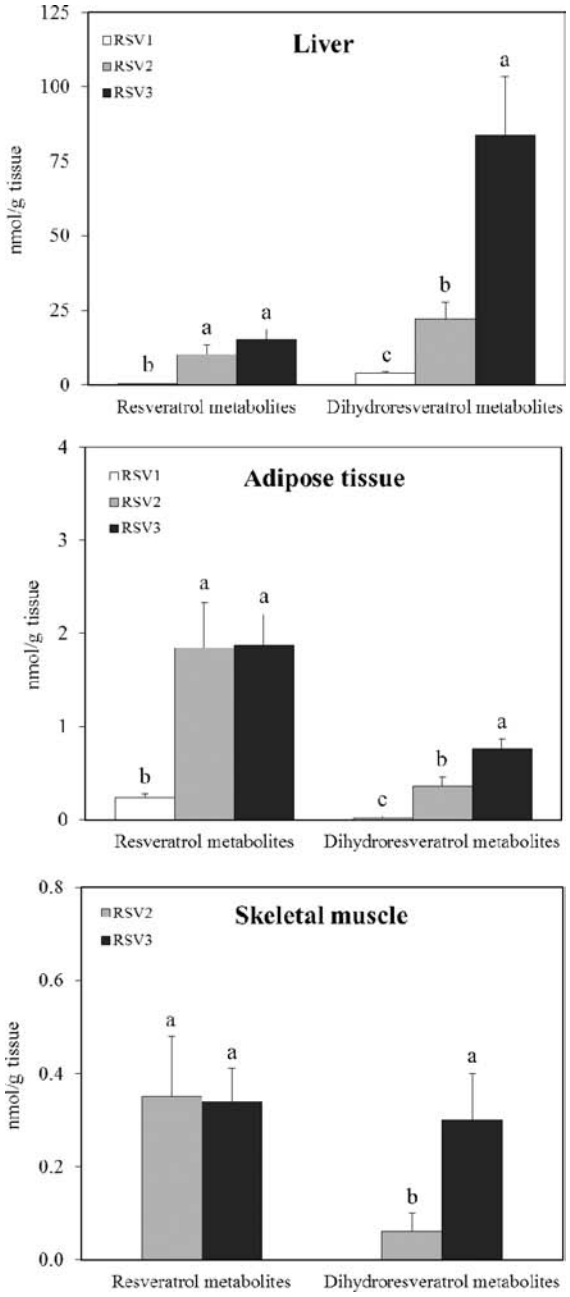

Figure 2. Resveratrol (glucuronide and sulfate conjugates) and dihydroresveratrol (free and its glucurono- and sulfoconjugates; microbial metabolism) metabolites in liver, adipose tissue, and skeletal muscle from rats receiving 6 mg/kg/d (RSV1), 30 mg/kg/d (RSV2), or 60 mg/kg/d (RSV3) of resveratrol for 6 weeks. Results are expressed as mean ± SEM. Statistics shows comparisons among the three dose groups for each metabolite type. Bars not sharing a common letter are significantly different ($P < 0.05$). No detectable amounts resveratrol metabolites were found in the RSV1 group for the skeletal muscle. (Values <LOQ considered as the LOD value.)

body-fat-lowering effect of this polyphenol, such as adipose tissue, liver, and skeletal muscle,[30−32] and we carried out the present study in the same cohort of animals, which were fed an obesogenic diet and fasted overnight before sacrifice.

These experimental conditions were not ideal for this type of study, and obviously this is a limitation of this work. Nevertheless, an interesting aspect should also be emphasized. Usually, resveratrol metabolite distribution has been analyzed after a single dose of resveratrol, or repeated doses for a short time.[6,15,33,34] By contrast, in the present study resveratrol metabolite distribution was analyzed after a long-term treatment (6 weeks) and this can provide additional data on this issue.

With regard to diet composition, feeding rats an obesogenic diet (high-fat diet) can modify resveratrol absorption. Vaz-da-Silva et al.[35] showed that a high-fat breakfast delayed the absorption of *trans*-resveratrol compared with fasted intake, but it did not influence the extent of absorption in humans after a single dose of 400 mg. La Porte et al.[36] reported decreased and delayed absorption of *trans*-resveratrol in combination with a high-fat breakfast as compared with a standard breakfast in humans treated with 2000 mg of resveratrol twice daily. Despite this fact, we believe that results concerning resveratrol metabolite distribution obtained under the experimental conditions which allow resveratrol to show body fat-lowering properties, can be helpful to explain this biological effect. The distribution of resveratrol under other experimental conditions should also be analyzed in order to gain more insight into this issue.

In the present study, resveratrol metabolites, but not their parent compound, were detected in the three tissues analyzed by the method described. Taking into account that rats were fasted overnight, the results are to be expected because other authors have shown that after its administration, free resveratrol is either absent or present only as trace amounts in plasma and tissues and that resveratrol metabolite concentrations are much higher than the concentrations of the parent compound.[4,5] It has been also reported that resveratrol is detected in plasma as soon as 15 min after administration, reaching peak concentrations at 60 min and then sharply decreasing. In contrast, some resveratrol metabolites are detectable in plasma even 3 h after administration; at this time only trace amounts of free resveratrol are found in plasma.[3,37]

Our results are in line with those published by Wenzel et al.,[6] who found resveratrol metabolites, but not free resveratrol, in kidneys after an overnight fast when rats were administered 300 mg/kg/d of this polyphenol. In liver they found a small amount of free resveratrol and higher amounts of its metabolites but they used a dose far higher than that used in the present study. Nevertheless, the fact that resveratrol was present but below the LOD cannot be discarded.

Most of the studies devoted to analyzing resveratrol metabolic profile in tissues focus on liver, kidney, lungs, brain, and testis.[6,34,38,39] However, as far as we know, only one study has evaluated resveratrol metabolites in adipose tissue and skeletal muscle.[40] In recent years the effectiveness of resveratrol as a body-fat-lowering agent has been demonstrated in rodents.[21,30−32,41] Adipose tissue and skeletal muscle, as well as liver, are targets for resveratrol; in fact different actions of this polyphenol, such as decreasing lipogenesis, increasing lipolysis, and increasing fatty acid oxidation, take place in these tissues and organs and they are mechanisms of action that explain the effect of resveratrol on the reduction of body fat accumulation.[21,30−32,41−43] Thus, describing the resveratrol metabolic profile in these tissues is an important issue.

As expected, in general terms, resveratrol metabolite amounts increased with increasing concentrations of the polyphenol in the diet. A clear dose−response pattern was found in liver when the three experimental groups were analyzed. By contrast, this pattern was observed in adipose tissue only when RSV1 and RSV2 groups were compared. In skeletal muscle, resveratrol

metabolites were not detected in the RSV1 group, and very similar amounts were detected in the RSV2 and RSV3 groups. This could suggest that these two tissues have a maximal capacity for resveratrol metabolite incorporation, which cannot be exceeded by increasing resveratrol administration. In the present study, the highest amounts of total resveratrol metabolites for the three analyzed doses were detected in liver. Indeed, other authors have reported that this organ has been found to be subjected to an important accumulation of these compounds in rodents.[6,34,38,39] Resveratrol metabolite amounts in adipose tissue were much higher than those in skeletal muscle. It has been reported that resveratrol is mainly distributed in abundant blood-supplied tissues, such as liver, lung, and kidney.[34,40] This is logical, but other factors also may influence this process because although skeletal muscle has a more active blood flow than adipose tissue, lower amounts of resveratrol metabolites were detected in this tissue. Thus, the potential contribution of differences among tissues in terms of transporters that excreted xenobiotics from different organs (members of the family of ATP-binding cassette) cannot be discarded.[34]

In this work, the resveratrol metabolic profile evaluated in the three tissues under fasting conditions and during long-term treatment includes glucuronide and sulfate conjugates of resveratrol together with those derived from the microbiota. Although glucuronide and sulfate conjugates of resveratrol have blood and tissue peaks about 3−6 h postadministration, it could be hypothesized that resveratrol is accumulated in tissues and released slowly over a longer period of time, as previously published for resveratrol[44] or other polyphenols.[45]

Only a few studies have considered the determination of microbial metabolites after resveratrol administration: Azorín-Ortuño et al. in a great number of tissues[40] and Wang et al.[46] in urine samples. Dihydroresveratrol conjugates appear later in the organism after the colonic microbial degradation of resveratrol and further absorption and phase II metabolism. Thus, in our study, dihydroresveratrol as free or as glucuronide and sulfate conjugates was assessed. These were the main metabolites in liver, but not in adipose tissue and skeletal muscle.

When the distribution between glucuronide and sulfate metabolites was analyzed, it was noteworthy that while sulfates increased when resveratrol dosage was enhanced, glucuronides increased between 6 and 30 mg/kg/d, but remained unchanged when resveratrol dosage reached 60 mg/kg/d. These results may suggest that glucuronidation, but not sulfation, is a saturable metabolic pathway, at least in the range of doses used in the present study. Nevertheless, the potential degradation of glucuronide metabolites, as well as a more rapid elimination, cannot be discarded. Consequently, further studies are needed to better analyze this issue. In the literature there are other studies that provide data that lend support to this theory. Thus, Kapetanovic et al.[47] observed a shift from glucuronidation to sulfation of resveratrol possibly due to the saturation of the glucuronide pathway. Maier-Salamon et al.[48] have already described a possible saturation of the enzymes responsible for resveratrol glucuronidation (UDP-glucuronosyltransferases UGT1A7 and UGT1A10), when increasing administered resveratrol concentrations, while noncompetitive substrate inhibition was shown for resveratrol sulfation.

These data can provide a clue to explain the results that we obtained, by using this cohort of animals, when the effects of resveratrol on body fat accumulation were assessed.[21] As we have explained before in this section, while resveratrol reduced body fat at a dose of 30 mg/kg/d, it did not at 6 mg/kg/d. Surprisingly, rats treated with 60 mg/kg/d resveratrol did not show further reduction. This means that a plateau was reached when the dose of resveratrol increased. Moreover, in a previous experiment that we performed in 3T-L1 adipocytes, glucuronide, but not sulfate metabolites, were shown to be active in reducing triacylglycerol accumulation (data submitted). Thus, it can be hypothesized that the lack of increase in glucuronides, metabolites that could be responsible in part for the body-fat-lowering effect of resveratrol, when the dose of this polyphenol increases from 30 to 60 mg/kg/d could help to explain the above-mentioned plateau effect. Nevertheless, the levels of these metabolites detected in adipose tissue and skeletal muscle are very low and thus it is difficult to understand how they are contributing to the effects observed. Consequently, more studies are needed to explain the in vivo effects induced by resveratrol after a long-term treatment.

In conclusion, this study describes that the largest amounts of resveratrol metabolites were found in liver, intermediate amounts were observed in adipose tissue, and the lowest amounts were seen in skeletal muscle. In general terms, a dose−response pattern was found in sulfate conjugates but not glucuronides. Metabolites derived from microbiota were detected in greater amounts in liver than resveratrol conjugates were, whereas the opposite was the case in adipose tissue and skeletal muscle.

AUTHOR INFORMATION

Corresponding Author

*Tel: +34-945-013067. Fax: +34-945-013014. E-mail: mariapuy.portillo@ehu.es.

Funding

This work was partially supported by the INGENIO−CONSOLIDER Program, Fun-C-Food CSD2007-063, Instituto de Salud Carlos III (RETIC PREDIMED), AGL2008-1005-ALI and AGL2009-13906-C02-01 from the Spanish Ministerio de Ciencia e Innovación, AGL2011-27406-ALI from de Spanish Ministerio de Economía y Competitividad, IT-386-10 from the Government of the Basque Country and (UFI11/32) from the University of the Basque Country (UPV/EHU). M.R.-R. would like to thank the FI-DGR 2010 (AGAUR) fellowship from the Generalitat de Catalunya, M.B-O. the FPU AP2008-01922 fellowship from Education Ministry, and M.U.-S. the postdoctoral program Sara Borrell CD09/00134, from the Ministry of Science and Innovation.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Resveratrol was a generous gift from Monteloeder (Elche, Alicante, Spain).

REFERENCES

(1) Signorelli, P.; Ghidoni, R. Resveratrol as an anticancer nutrient: Molecular basis, open questions and promises. *J. Nutr. Biochem.* **2005**, *16*, 449−466.

(2) Frémont, L. Biological effects of resveratrol. *Life Sci.* **2000**, *66*, 663−673.

(3) Soleas, G. J.; Angelini, M.; Grass, L.; Diamendis, E. P.; Goldberg, D. M. Absorption of *trans*-resveratrol in rats. *Methods Enzymol.* **2001**, *335*, 145−154.

(4) Walle, T. Biovailability of resveratrol. *Ann. N.Y. Acad. Sci.* **2011**, *1215*, 9−15.

(5) Maier-Salamon, A.; Hagenauer, B.; Reznicek, G.; Szekeres, T.; Thalhammer, T.; Jäger, W. Metabolism and disposition of resveratrol in the isolated perfused rat liver: Role of Mrp2 in the biliary excretion of glucuronides. *J. Pharm. Sci.* **2008**, *97*, 1615−1628.
(6) Wenzel, E.; Somoza, V. Metabolism and bioavailability of *trans*-resveratrol. *Mol. Nutr. Food Res.* **2005**, *49*, 472−481.
(7) Andrés-Lacueva, C.; Urpí-Sardá, M.; Zamora-Ros, R.; Lamuela-Raventós, R. M. Bioavailability and metabolism of resveratrol. In *Plant Phenolics and Human Health: Biochemistry, Nutrition and Pharmacology*; Fraga, C. G., Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, 2009; pp 265−299.
(8) Boocock, D. J.; Faust, G. E.; Patel, K. R.; Schinas, A. M.; Brown, V. A.; Ducharme, M. P.; Booth, T. D.; Crowell, J. A.; Perloff, M.; Gescher, A. J.; Steward, W. P.; Brenner, D. E. Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. *Cancer Epidemiol. Biomarkers Prev.* **2007**, *16*, 1246−1252.
(9) Jang, M.; Cai, L.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W.; Fong, H. H.; Farnsworth, N. R.; Kinghorn, A. D.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* **1997**, *275*, 218−220.
(10) Weng, C. J.; Wu, C. F.; Huang, H. W.; Wu, C. H.; Ho, C. T.; Yen, G. C. Evaluation of anti-invasion effect of resveratrol and related methoxy analogues on human hepatocarcinoma cells. *J. Agric. Food Chem.* **2010**, *58*, 2886−2894.
(11) Picard, F.; Kurlev, M.; Chung, N.; Topark-Ngarm, A.; Senawong, T.; Machado de Oliveira, R.; Leid, M.; McBurney, M. W.; Guarante, L. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. *Nature* **2004**, *429*, 771−776.
(12) Floyd, Z. E.; Wang, Z. Q.; Kilroy, G.; Cefalu, W. T. Modulation of peroxisome proliferator-activated receptor gamma stability and transcriptional activity in adipocytes by resveratrol. *Metabolism* **2008**, *57* (Suppl. 1), S32−S38.
(13) Bai, L.; Pang, W. J.; Yang, Y. J.; Yang, G. S. Modulation of Sirt1 by resveratrol and nicotinamide alters proliferation and differentiation for pig adipocytes. *Mol. Cell. Biochem.* **2008**, *307*, 129−140.
(14) Rayalam, S.; Yang, J. Y.; Ambati, S.; Della-Ferra, M. A.; Baile, C. A. Resveratrol induces apoptosis and inhibits adipogenesis in 3T3-L1 adipocytes. *Phytother. Res.* **2008**, *22*, 1367−1371.
(15) Azorín-Ortuño, M.; Yañéz-Gascón, M. J.; Pallarés, F. J.; Vallejo, F.; Larrosa, M.; García-Conesa, M. T.; Tomás-Barberán, F.; Espín, J. C. Pharmacokinetic study of *trans*-resveratrol in adult pigs. *J. Agric. Food Chem.* **2010**, *58*, 11165−11171.
(16) Crowell, J. A.; Korytko, P. J.; Morrissey, R. L.; Booth, T. D.; Levine, B. S. Resveratrol-associated renal toxicity. *Toxicol. Sci.* **2004**, *82*, 614−619.
(17) Brown, V. A.; Patel, K. R.; Viskaduraki, M.; Crowell, J. A.; Perloff, M.; Booth, T. D.; Vasilinin, G.; Sen, A.; Schinas, A. M.; Piccirilli, G.; Brown, K.; Steward, W. P.; Gescher, A. J.; Brenner, D. E. Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: Safety, pharmacokinetics, and effect on the insulin-like growth factor axis. *Cancer Res.* **2010**, *70*, 9003−9011.
(18) Hoshino, J.; Park, E. J.; Kondratyuk, T. P.; Marler, L.; Pezzuto, J. M.; van Breemen, R. B.; Mo, S.; Li, Y.; Cushman, M. Selective synthesis and biological evaluation of sulfate-conjugated resveratrol metabolites. *J. Med. Chem.* **2010**, *53*, 5033−5043.
(19) Delmas, D.; Aires, V.; Limagne, E.; Dutartre, P.; Mazué, F.; Ghiringhelli, F.; Latruffe, N. Transport, stability, and biological activity of resveratrol. *Ann. N.Y. Acad. Sci.* **2011**, *1215*, 48−59.
(20) Chachay, V. S.; Kirkpatrick, C. M.; Hickman, I. J.; Ferguson, M.; Prins, J. B.; Martin, J. H. Resveratrol—Pills to replace a healthy diet? *Br. J. Clin. Pharmacol.* **2011**, *72*, 27−38.
(21) Macarulla, M. T.; Alberdi, G.; Gómez, S.; Tueros, I.; Bald, C.; Rodríguez, V. M.; Martínez, J. A.; Portillo, M. P. Effects of different doses of resveratrol on body fat and serum parameters in rats fed a hypecaloric diet. *J. Physiol. Biochem.* **2009**, *65*, 369−376.
(22) Reagan-Shaw, S.; Nihal, M.; Ahmad, N. Dose translation from animal to human studies revisited. *FASEB J.* **2007**, *22*, 659−661.
(23) Miksits, M.; Maier-Salamon, A.; Aust, S.; Thalhammer, T.; Reznicek, G.; Kunert, O.; Haslinger, E.; Szekeres, T.; Jaeger, W. Sulfation of resveratrol in human liver: Evidence of a major role for the sulfotransferases SULT1A1 and SULT1E1. *Xenobiotica* **2005**, *35*, 1101−1119.
(24) Thakkar, K.; Geahlen, R. L.; Cushman, M. Synthesis and protein-tyrosine kinase inhibitory activity of polyhydroxylated stilbene analogues of piceatannol. *J. Med. Chem.* **1993**, *36*, 2950−2955.
(25) Malz, F.; Jancke, H. Validation of quantitative NMR. *J. Pharm. Biomed. Anal.* **2005**, *38*, 813−823.
(26) Urpí-Sardà, M.; Zamora-Ros, R.; Lamuela-Raventós, R.; Cherubini, A.; Jáuregui, O.; de la Torre, R.; Covas, M. I.; Estruch, R.; Jaeger, W.; Andrés-Lacueva, C. HPLC−tandem mass spectrometric method to characterize resveratrol metabolism in humans. *Clin. Chem.* **2007**, *53*, 292−299.
(27) Andrés-Lacueva, C.; Shukitt-Hale, B.; Galli, R. L.; Jauregui, O.; Lamuela-Raventos, R.; Joseph, J. A. Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. *Nutr. Neurosci.* **2005**, *8*, 111−120.
(28) US Department of Health and Human Services, Food and Drug Administration. *Guidance for Industry Bioanalytical Method Validation.* 2001; http://www.fda.gov/cder/guidance/4252fnl.htm
(29) Wang, L. X.; Heredia, A.; Song, H.; Zhang, Z.; Yu, B.; Davis, C.; Redfield, R. Resveratrol glucuronides as the metabolites of resveratrol in humans: Characterization, synthesis, and anti-HIV activity. *J. Pharm. Sci.* **2004**, *93*, 2448−2457.
(30) Szkudelska, K.; Szkudelski, T. Resveratrol, obesity and diabetes. *Eur. J. Pharmacol.* **2010**, *635*, 1−8.
(31) Lagouge, M.; Argmann, C.; Gerhart-Hines, Z.; Meziane, H.; Lerin, C.; Daussin, F.; Messadeq, N.; Milne, J.; Lambert, P.; Elliott, P.; Geny, B.; Laakso, M.; Puigserver, P.; Auwerx, J. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* **2006**, *127*, 1109−1122.
(32) Baur, J. A.; Pearson, K. J.; Price, N. L.; Jamieson, H. A.; Lerin, C.; Kalra, A.; Prabhu, V. V.; Allard, J. S.; Lopez-Lluch, G.; Lewis, K.; Pistell, P. J.; Poosala, S.; Becker, K. G.; Boss, O.; Gwinn, D.; Wang, M.; Ramaswamy, S.; Fishbein, K. W.; Spencer, R. G.; Lakatta., E. G.; Le Couteur, D.; Shaw, R. J.; Navas, P.; Puigserver, P.; Ingram, D. K.; de Cabo, R.; Sinclair, D. A. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **2006**, *444*, 337−342.
(33) Patel, K. R.; Scott, E.; Brown, V. A.; Gescher, A. J.; Steward, W. P.; Brown, K. Clinical trials of resveratrol. *Ann. N.Y. Acad. Sci.* **2011**, *1215*, 161−169.
(34) Juan, M. E.; Maijó, M.; Planas, J. M. Quantification of *trans*-resveratrol and its metabolites in rat plasma and tissues by HPLC. *J. Pharm. Biomed. Anal.* **2010**, *51*, 391−398.
(35) Vaz-da-Silva, M.; Loureiro, A. I.; Falcao, A.; Nunes, T.; Rocha, J. F.; Fernandes-Lopes, C.; Soares, E.; Wright, L.; Almeida, L.; Soares-da-Silva, P. Effect of food on pharmakokinetics profile of *trans*-resveratrol. *Int. J. Clin. Pharmacol. Ther.* **2008**, *46*, 564−570.
(36) La Porte, C.; Voduc, N.; Zhang, G.; Seguin, I.; Tardiff, D.; Singhal, N.; Cameron, D. W. Steady-state pharmacokinetics and tolerability of *trans*-resveratrol 2000 mg twice daily with food, quercetin and alcohol (ethanol) in healthy human subjects. *Clin. Pharmacokinet.* **2010**, *49*, 449−454.
(37) Yu, C.; Shin, Y. G.; Chow, A.; Li, Y.; Kosmeder, J. W.; Lee, Y. S.; Hirschelman, W. H.; Pezzuto, J. M.; Mehta, R. G.; van Breemen, R. B. Human, rat and mouse metabolism of resveratrol. *Pharm. Res.* **2002**, *19*, 1907−1914.
(38) Vitrac, X.; Desmoulière, A.; Brouillaud, B.; Krisa, S.; Deffieux, G.; Barthe, N.; Rosenbaum, J.; Mérillon, J. M. Distribution of [^{14}C]-*trans*-resveratrol, a cancer chemopreventive polyphenol, in mouse tissues after oral administration. *Life Sci.* **2003**, *72*, 2219−2233.
(39) Sale, S.; Verschoyle, R. D.; Boocock, D.; Jones, D. J.; Wilsher, N.; Ruparelia, K. C.; Potter, G. A.; Farmer, P. B.; Steward, W. P.; Gescher, A. J. 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* **2004**, *90*, 736−744.

(40) Azorín-Ortuño, M.; Yáñez-Gascón, M. J.; Vallejo, F.; Pallarés, F. J.; Larrosa, M.; Lucas, R.; Morales, J. C.; Tomás-Barberán, F. A.; García-Conesa, M. T.; Espín, J. C. Metabolites and tissue distribution of resveratrol in the pig. *Mol. Nutr. Food Res.* **2011**, *55*, 1154−1168.
(41) Rivera, L.; Morón, R.; Zarzuelo, A.; Galisteo, M. Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats. *Biochem. Pharmacol.* **2009**, *77*, 1053−1063.
(42) Baile, C. A.; Yang, J. Y.; Rayalam, S.; Hartzell, D. L.; Lai, C. Y.; Andersen, C.; Della-Fera, M. A. Effect of resveratrol on fat mobilization. *Ann. N.Y. Acad. Sci.* **2011**, *1215*, 40−47.
(43) Alberdi, G.; Rodríguez, V. M.; Miranda, J.; Macarulla, M. T.; Arias, N.; Andrés-Lacueva, C.; Portillo, M. P. Changes in white adipose tissue metabolism induced by resveratrol in rats. *Nutr. Metab.* **2011**, *8*, 29.
(44) Zamora-Ros, R.; Urpí-Sardà, M.; Lamuela-Raventós, R. M.; Estruch, R.; Martínez-González, M. A.; Bulló, M.; Arós, F.; Cherubini, A.; Andres-Lacueva, C. Resveratrol metabolites in urine as a biomarker of wine intake in free-living subjects: The PREDIMED Study. *Free Radical Biol. Med.* **2009**, *46*, 1562−1566.
(45) Weinbrenner, T.; Fitó, M.; de la Torre, R.; Saez, G. T.; Rijken, P.; Tormos, C.; Coolen, S.; Albaladejo, M. F.; Abanades, S.; Schroder, H.; Marrugat, J.; Covas, M. I. Olive oils high in phenolic compounds modulate oxidative/antioxidative status in men. *J. Nutr.* **2004**, *134*, 2314−2321.
(46) Wang, D.; Hang, T.; Wu, C.; Liu, W. Identification of the major metabolites of resveratrol in rat urine by HPLC-MS/MS. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2005**, *829*, 97−106.
(47) Kapetanovic, I. M.; Muzzio, M.; Huang, Z.; Thompson, T. N.; McCormick, D. L. Pharmacokinetics, oral bioavailability, and metabolic profile of resveratrol and its dimethylether analog, pterostilbene, in rats. *Cancer Chemother. Pharmacol.* **2011**, *68*, 593−601.
(48) Maier-Salamon, A.; Hagenauer, B.; Wirth, M.; Gabor, F.; Szekeres, T.; Jäger, W. Increased transport of resveratrol across monolayers of the human intestinal Caco-2 cells is mediated by inhibition and saturation of metabolites. *Pharm. Res.* **2006**, *23*, 2107−2115.