The oral bioavailability of *trans*-resveratrol from a grapevine-shoot extract in healthy humans is significantly increased by micellar solubilization

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Abbreviations: AUC, area under the plasma concentration–time curve; **AP**, apical; **BL**, basolateral; $\mathbf{C_{max}}$, maximum plasma concentration; $\mathbf{T_{max}}$, time to reach the maximum plasma concentration.

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Abstract

Scope. Grapevine-shoot extract Vineatrol®30 contains abundant resveratrol monomers and oligomers with health-promoting potential. However, the oral bioavailability of these compounds in humans is low (<1 – 2%). The aim of this study was to improve the oral bioavailability of resveratrol from vineatrol by micellar solubilization.

Methods and results. Twelve healthy volunteers (6 women, 6 men) randomly ingested a single dose of 500 mg vineatrol (30 mg *trans*-resveratrol, 75 mg *trans*-ε-viniferin) as native powder or liquid micelles. Plasma and urine were collected at baseline and over 24 hours after intake. Resveratrol and viniferin were analyzed by HPLC. The area under the plasma concentration–time curve (AUC) and mean maximum plasma *trans*-resveratrol concentrations were 5.0-fold and 10.6-fold higher, respectively, after micellar supplementation relative to the native powder. However, no detectable amounts of *trans*-ε-viniferin were found in either plasma or urine. The transepithelial permeability of *trans*-resveratrol and *trans*-ε-viniferin across differentiated Caco-2 monolayers was consistent to the absorbed fractions *in vivo*.

Conclusion. The oral bioavailability of *trans*-resveratrol from the grapevine-shoot extract Vineatrol®30 was significantly increased using a liquid micellar formulation, without any treatment-related adverse effects, making it a suitable system for improved supplementation of *trans*-resveratrol.

1. Introduction

Vineatrol®30 (subsequently referred to as vineatrol) is a standardized ethanolic extract of grapevine shoots that contains at least 30% stilbenes, mostly *trans*-resveratrol and the resveratrol dimer *trans*-ε-viniferin, as well as a mixture of higher oligomeric stilbenes of up to four resveratrol subunits [1]. The complex polyphenol mixture in vineatrol displays stronger apoptotic and anti-proliferative properties against cancer cells than resveratrol alone [2–4]. Resveratrol (3,5,4'-*trans*-trihydroxy stilbene) is a low molecular weight phenolic phytoalexin [5], which has been described as the main bioactive compound responsible for the cardioprotective effects of red wine [6]. Resveratrol might also have other biological activities, including anti-oxidant, anti-inflammatory, anti-cancer, neuroprotective, anti-diabetic and anti-microbial activities [7,8].

In spite of high intestinal absorption (>70% of the administered dose), the oral bioavailability of resveratrol is low (less than 1-2% of the dose) [9,10]. This has been mainly attributed to extensive enteric and hepatic phase II metabolism, which results in almost complete conversion of the ingested compound to conjugated metabolites that are rapidly excreted, and explains the low concentrations of free *trans*-resveratrol in blood and tissues [10–14].

Several approaches to increase the bioavailability of resveratrol have been described, most of which rely on enhancing its low aqueous solubility [8,11–14]. It has also been suggested that competitive inhibition of phase II metabolism by simultaneous administration of other polyphenols could improve resveratrol bioavailability [10]. However, clinical reports using novel formulations of oligomeric resveratrol are still scarce.

Thus, in order to improve the oral bioavailability of oligomeric resveratrol, the native vineatrol powder was incorporated into micelles. The absorption and excretion kinetics of *trans*-resveratrol from native and micellar vineatrol were investigated in a single dose trial in healthy women and men. In addition, the transepithelial permeability of *trans*-resveratrol and *trans*-ε-viniferin across differentiated Caco-2 monolayers was determined for comparison with the absorbed fractions *in vivo*.

2. Materials and methods

2.1. Vineatrol formulations

Native Vineatrol®30 powder was extracted from grape vine shoots (*Vitis vinifera*) and was kindly provided by Breko GmbH (Bremen, Germany). The native extract contained 33.3% resveratrol monomers and oligomers, including 5.8% *trans*-resveratrol and 14.5% *trans*-ε-viniferin and minor amounts of other resveratrol oligomers, including ampelopsin (2.4%), hopeaphenol (2.2%), piceatannol (0.5%), R2-viniferin (1.2%), R-viniferin (1.8%), miyabenol C (2.3%), and E-omega-viniferin (2.6%). The solubilized Vineatrol®30 liquid formulation NovaSOL® Vineatrol®30 (AQUANOVA AG, Darmstadt, Germany) had a micelle structure (consisting of polysorbate 80, polysorbate 20, and medium chain triacylglycerols) and contained 9% native Vineatrol®30. Both, the

native powder and the liquid micellar formulation were filled into hydroxypropyl methyl cellulose capsules (Licaps; Capsugel France SAS, Colmar Cedex, France).

2.2. Subjects

The study protocol (F-2014-108) was in conformance with the Declaration of Helsinki and was approved by the ethics committee of the State Medical Society of Baden-Württemberg (Germany). The trial was registered at clinicaltrials.gov as NCT02944097. The study was advertised at the University of Hohenheim (Germany) and six healthy women (22 – 29 years) and six healthy men (24 – 32 years) with routine blood chemistry values within the normal ranges (**Table 1**) were selected after a screening appointment. The exclusion criteria were: obesity (BMI >30 kg/m²); medication (except contraceptives); pregnancy or lactation; previous cardiac infarction; drug or alcohol abuse; chronic diseases; smoking; use of dietary supplements; >5 h exercise per week; restrictive dietary regimes; participation in a clinical trial up to 3 months prior to recruitment; and a known intolerance or hypersensitivity against grapes, wine or resveratrol. All participants provided written informed consent before inclusion in the trial.

2.3. Study design

The participants were given a list of resveratrol-containing foods (grapes, red-blue berries, peanuts, grape juice, wine, brandy and more) and were asked to abstain from their consumption from one week before and throughout the entire study. The study followed a single-blinded (participants), two-armed crossover design with at least one week washout periods between interventions. Standardized (resveratrol-free) meals and water (ad libitum) were provided 10 h before vineatrol intake and during the entire intervention days. Blood samples were drawn from an indwelling venous cannula and collected into potassium-EDTA-containing tubes at baseline (0 h) and 0.5, 1, 2, 4, 6, 8 and 24 h after the ingestion of 500 mg Vineatrol®30 (30 mg *trans*-resveratrol, 72.5 mg *trans*-ε-viniferin) as native powder or liquid micelles, in random order. Allocation to the treatments was done using a table of random numbers. Centrifuged plasma (3,005 × g for 10 min at 4 °C) was collected and stored at -20 °C until further analysis. Serum was obtained from blood collected at 0, 4 and 24 h after intake into

tubes with clotting activator (Z-Gel, Sarstedt AG & Co, Nümbrecht, Germany) for analysis of liver and kidney function markers, as well as blood glucose and lipids. Serum analyses were performed by the clinical laboratory Laborärzte Sindelfingen (Sindelfingen, Germany).

Urine was collected at baseline (0 h) and in intervals after intake (1-6, 6-12, 12-24 h) in opaque containers (3 L) with 30 mL of 10 % phosphoric acid. The collection started with the second morning urine before intake and ended with the first urination 24 h later. Urine volume was recorded by weighing and aliquots of each time period were stored at -20 °C for analysis. Urinary creatinine was quantified in a clinical laboratory (Laborärzte Sindelfingen).

2.4. Transepithelial permeability in differentiated Caco-2 monolayers

Caco-2 cells (passages 8-20) were cultivated in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich Chemie GmbH; Steinheim, Germany) containing 10% fetal calf serum (GIBCO; Darmstadt, Germany), 1% non-essential amino acids (Life Technologies GmbH; Darmstadt, Germany), 1% sodium pyruvate (Life Technologies GmbH) and 1% penicillin/streptomycin (Biochrome AG; Berlin, Germany) at 37°C, 95% relative humidity and 5% CO_2. Caco-2 monolayers were grown for 25 days on 12-well transwell inserts (1.2 cm^2 polycarbonate membrane, 0.4 µm pore size; Corning; NY, USA) until enterocytic differentiation as previously described [15]. The integrity of the differentiated epithelia was confirmed by measuring the transepithelial electrical resistance (TEER) and lucifer yellow permeability. TEER was measured with an EVOM-G Ohm Meter (World Precision Instruments; Florida, USA) and inserts with values under 250 $\Omega \cdot cm^2$ were discarded [16]. Lucifer yellow (100 µmol/L) was added to the apical (AP) chamber and its concentration was measured in the basolateral (BL) fluid after 1 h incubation by comparing the fluorescence signal (485 nm excitation, 530 nm emission) against a linearity curve (0.5-50 µmol/L). Inserts with lucifer yellow permeability above 3% were excluded.

The transepithelial transport of *trans*-resveratrol and *trans*-ε-viniferin was determined in the apical-to-basolateral direction at 37 °C. Differentiated monolayers (n = 3) were incubated for 1 h with 25 µmol/L *trans*-resveratrol, 25 µmol/L *trans*-ε-viniferin or 25 µmol/L *trans*-resveratrol + 25 µmol/L *trans*-ε-viniferin added in 400 µL of HBSS (pH 6.5, Sigma-Aldrich Chemie GmbH) on the AP

chamber. The BL chamber was filled with 1 mL HBSS (pH 7.4). The concentrations of the compounds in the BL chamber after 1 h were determined by HPLC as described below. The apparent permeability coefficient (Papp, cm/s) of total (after enzymatic deconjugation) *trans*-resveratrol and *trans*-ε-viniferin were calculated as (C_f x V) x (1/(*t* x C_i x A), where C_f is the final concentration in the BL chamber, V is the volume of the BL chamber (mL), *t* is the duration of the experiment, C_i is the initial concentration in the AP chamber, and A is the surface area of the cell monolayer (1.12 cm^2) [17].

2.5. HPLC analysis of *trans*-resveratrol and *trans*-ε-viniferin

Plasma and urine samples (1 mL, in duplicates) were adjusted to pH 6.0 – 6.2 and then incubated at 37 °C for 1 h (~90 rpm) with 100 µL β-glucuronidase/sulfatase (100 µL) type H-1 from *Helix pomatia* (3 mg/100 µL in 0.1 sodium acetate buffer, pH 4.5; Sigma-Aldrich, Schnelldorf, Germany) for deconjugation of metabolites. The pH of the samples was adjusted to the pH optimum for the sulfatase activity of the enzyme (pH ~6.2) based on previous reports [18,19]. After cooling down on ice, 500 µL 0.5 N sodium hydroxide was added for inactivation of the enzyme and protein precipitation. The resveratrol fraction was extracted with a total of 6 mL of ethyl acetate in three 2 mL extractions on a vertical rotator (10 min at 90 rpm, each), with centrifugation steps (1,690 × g for 15 min at 4 °C) in-between extractions for better separation of the aqueous and organic layers. The organic layer was collected and a total of 5.5 mL pooled supernatant was evaporated to dryness on a centrifugal evaporator. Urine samples were extracted the same way, but the pooled supernatants were filtered using nylon DMSO-safe Acrodisc®-syringe filters (Pall GmbH, Dreieich, Germany) before evaporation. All the dried residues were dissolved in 100 µL of mobile phase B and 20 µL were injected into the HPLC system.

Resveratrol and viniferin were analyzed on a JASCO HPLC system (LC Net II ADC, AS-2059-SF Plus, PU-2080 Plus, CO-2060 Plus, DG-2080-53, LG-2080-02S, FP-2020-Plus; Groß-Umstadt, Germany) with a Luna C8 (2) column (150 x 4.6 mm, 3 µm; Phenomenex, Aschaffenburg, Germany) maintained at 35 °C. Chromatographic separation was achieved using a multistep gradient method with mobile phase A (5 mM ammonium acetate in deionized water with 0.5 % acetic acid)

and mobile phase B (5 mM ammonium acetate in methanol with 0.5 % acetic acid) at a flow rate of 1.0 mL/min. The gradient profile was 0 min 0 % B, 1 min 2 % B, 18.0 min 85 % B, 20.0 min 2 % B. The eluent was monitored by fluorescence detection (excitation, 313 nm; emission, 400 nm) and *trans*-resveratrol and *trans*-ε-viniferin were quantified by comparing the baseline-normalized peak areas of the analytes to authentic external standards. The lower limits of quantification (LLOQ) of *trans*-resveratrol and *trans*-ε-viniferin were 0.02 ± 0.01 ng/mL and 0.01 ± 0.01 ng/mL, respectively (*n* = 6). *Trans*-resveratrol (≥98%) and *trans*-ε-viniferin (98%) standards were from Cayman Chemical (Ann Arbor, MI, USA) and AppliChem GmbH (Darmstadt, Germany), respectively. JASCO ChromNAV software (version 1.19.1) was used for equipment control and data evaluation.

Resveratrol and viniferin were extracted from the AP and BL fluids from the transepithelial permeability experiments following the same protocol, but a shorter isocratic HPLC method was used for simplicity. A JASCO HPLC system (LC Net II ADC, X-LC 3185 PU, X-LC 3180 MX, X-LC 3080 DG, X-LC 3159 AS, X-LC 3067 CO; Groβ-Umstadt, Germany) was fitted with a Reprosil-Pur 120 C18-AQ (150 x 4 mm, 3 μm) column (Dr. Maisch GmbH, Amerbuch-Entringen, Germany) maintained at 45°C. Mobile phase (58% water pH 3, 30% acetonitrile, 12% methanol) was delivered at 0.6 mL/min. Fluorescent detection (JASCO FP-2020 Plus) was performed at excitation (Ex) and emission (Em) wavelengths of 300 and 380 nm, respectively, from 0 to 5.6 min, and 313 (Ex) and 400 (Em) from 5.6 to 10 min. The dried residues after extraction were reconstituted in 100 μL water-acetonitrile (50:50, v/v) and 20 μL were injected in the HPLC. Data acquisition and evaluation were done with ChromPass II (version 1.8.6.1; JASCO) and quantification was performed by comparison against authentic external standards.

2.6. Pharmacokinetic and statistical analyses

The primary outcomes measured were the pharmacokinetic parameters of total resveratrol (after enzymatic de-conjugation of metabolites): the area under the plasma concentration–time curve (AUC), the maximum plasma concentration (C_{max}), and the time to reach C_{max} (T_{max}). Data is shown as the mean with SD or SEM. Normality was tested with the Kolmogorov-Smirnov test or with the

D'Agostino-Pearson test. An unpaired Student's *t*-test or a Mann-Whitney U test was used to compare the baseline characteristics between women and men (**Table 1**). Differences in safety parameters before and after vineatrol intake were determined by repeated measures two-way ANOVA (**Supporting Information Table S1**). A two-way ANOVA was used to analyze the effect of the different vineatrol formulations and sex in resveratrol absorption (**Table 2**) and excretion kinetics (**Table 3**), followed by Bonferroni post-hoc tests, Student's *t*-tests or Mann-Whitney U tests, to assess differences within groups. AUC and all statistical analyses were calculated with the software package GraphPad Prism 5 for Windows (version 5.02; GraphPad Software, Inc., La Jolla, CA, USA). Differences were considered significant at $p < 0.05$.

3. Results

3.1. Subjects and tolerability

Twelve healthy volunteers (6 women, 6 men) participated in and completed the study. BMI and routine blood chemistry values were within the reference ranges (**Table 1**) for all subjects at the time of screening, with expected differences between women and men. Mean blood pressure at screening was slightly elevated, but still within the normal range.

No adverse effects were reported after the intake of either of the formulations. The serum safety parameters (mean ± SD) did not differ between the vineatrol formulations and remained within the reference ranges at all the time points (see **Supporting Information Table S1**).

3.2. Resveratrol pharmacokinetics

The pharmacokinetic parameters (AUC, C_{max}, T_{max}) were calculated from plasma samples collected from the 12 healthy volunteers at baseline and over 24 h following the oral administration of 500 mg vineatrol as native powder or liquid micelles (**Figure 1**). No *trans*-ε-viniferin was detected in plasma and urine.

Maximum total (after enzymatic de-conjugation with β-glucuronidase/sulfatase) *trans*-resveratrol concentrations (C_{max}) in plasma ranged between 14.9 – 93.6 nmol/L (native) and 167.7 – 475.9 nmol/L (micelles) and were 9.6-, 12.5-, and 10.6-fold higher in women, men, and all subjects,

respectively, when ingesting micellar compared to native vineatrol (**Table 2**). Significantly higher C_{max} were observed in women relative to men, but only after consumption of the vineatrol micelles. Very low amounts of *trans*-resveratrol were detected in fasting plasma in 5 subjects before the intake of the native formulation (11.3 ± 3.5 nmol/L, mean ± SD) and in 4 subjects before ingestion of the micelles (9.6 ± 3.0 nmol/L, mean ± SD).

The micellar formulation significantly reduced the time to reach the maximum plasma *trans*-resveratrol concentrations (T_{max}), with maximum peaks occurring 0.5 to 1 h after intake (**Table 2**), and a possible secondary peak around 8 h post administration. Maximum peak times after native vineatrol intake were irregularly dispersed over the 0.5 to 8 h monitoring period due to wide interindividual variation.

Based on the AUC, the relative systemic bioavailability of total *trans*-resveratrol was 4.2, 6.4, and 5.0 times higher in women, men, and all subjects, respectively, after ingestion of the micellar vineatrol compared to the native form (**Table 2**), with no significant differences between men and women.

3.3. Urinary excretion of resveratrol

The 24 h cumulative urinary excretion of total *trans*-resveratrol following the intake of vineatrol micelles was 5.2-, 3.9-, and 4.5-fold higher in women, men and all subjects, respectively, than after intake of the native formulation (**Table 3**). No significant differences in the urinary excretion of *trans*-resveratrol between women and men were observed. Maximum excretion of *trans*-resveratrol occurred 1-6 hours post-administration (**Figure 2, Supporting Information Tables S2 and S3**). Only 2.0 ± 0.6% (mean ± SD) of the ingested dose (30 mg *trans*-resveratrol) was recovered in the 24-hour urine samples of subjects treated with the native vineatrol and 8.7 ± 3.5% in subjects taking the micellar formulation. Low amounts of *trans*-resveratrol were detected in baseline urine (0 h) in 11 subjects before the intake of the native powder (19.4 ± 14.8 μg, mean ± SD) and in 11 subjects before ingestion of the micelles (18.3 ± 9.8 μg, mean ± SD).

3.4. Transepithelial permeability of resveratrol and viniferin in Caco-2 monolayers

The apical-to-basolateral permeability of total *trans*-resveratrol in differentiated Caco-2 monolayers, calculated as the apparent permeability coefficient, was slightly lower when incubated together with an equimolar concentration of *trans*-ε-viniferin (4.4×10^{-6} cm/s) than when incubated alone (4.8×10^{-6} cm/s). The transepithelial permeability of *trans*-ε-viniferin (6.4×10^{-7} cm/s) was not changed when incubated alone or simultaneously with *trans*-resveratrol.

4. Discussion

Resveratrol has a large number of potentially beneficial health effects [7,8], but its oral bioavailability in humans is limited [10–14]. Even though resveratrol is a lipophilic molecule and as such may easily permeate membranes by passive diffusion, its low solubility in water limits its ability to cross the unstirred water layer lining the intestinal surface and thus its reaching the membranes of enterocytes for resorption [12]. The oral bioavailability of resveratrol is further limited by its extensive metabolism to sulfate and glucuronide conjugates in the intestine and liver and their subsequent rapid elimination [14,19].

In this study, we aimed towards enhancing the absorption of resveratrol by increasing its hydrophilicity by micellar solubilization. The relative systemic bioavailability (based on AUC) of total *trans*-resveratrol was 5-fold higher after ingestion of the soluble vineatrol micelles compared to the native powder (**Table 2**) without any indication of adverse effects (**Supporting Information Table S1**). The mean maximum total plasma *trans*-resveratrol concentrations (C_{max}) were 28 and 300 nmol/L after the intake of native and micellar vineatrol (30 mg *trans*-resveratrol), respectively. After the intake of the micellar formulation, a significantly higher C_{max} was reached in women (196 – 357 nmol/L) than in men (167 – 243 nmol/L; **Table 2**), which can be explained by the higher dose per kg bodyweight and the lower volumes of distribution in females. A second *trans*-resveratrol peak, suggestive of enterohepatic recirculation, was observed around 8 h post administration and is in agreement with previous observations in rats [20].

Several studies have explored the oral bioavailability in humans of *trans*-resveratrol from different sources, including purified formulations and complex food matrices (reviewed in [7,11,14]).

Maximum plasma concentrations (C_{max}) of total *trans*-resveratrol (including metabolites) after oral administration reached ~2 μmol/L (~0.4 μg/mL), as measured by total radioactivity after the intake of a relatively low oral dose of 25 mg ^{14}C-resveratrol [19]. In contrast, the same dose (25 mg *trans*-resveratrol) resulted in only 6.5 nmol/L (C_{max}) free (intact) *trans*-resveratrol, as measured by HPLC-MS in another trial [21]. Plasma concentrations of free *trans*-resveratrol in the low micromolar range, comparable to the total *trans*-resveratrol concentrations in the above mentioned study [19] were only achieved after the intake of a much larger oral dose (5 g; C_{max}, 2.4 μmol/L) [18]. On the other hand, repeated administration of 5 g *trans*-resveratrol for 21-28 days doubled the maximum blood concentrations of free resveratrol (C_{max}, 4.2 μmol/L) relative to single dosing; however, the differences were not significant when comparing the AUC values [22], which take into consideration the overall exposure to the substance over time and not only at a single time point. Moreover, in the same study, resveratrol metabolites reached up to ~10 times higher C_{max} and AUC values than the unconjugated *trans*-resveratrol [22]. Thus, only a small fraction of orally administered resveratrol escapes biotransformation *in vivo*, even under high-dose supplementation, which has been proposed as a major hurdle for reaching effective systemic concentrations [11]. Of note, in a proof-of-concept study with two healthy men, relatively high plasma concentrations of free resveratrol (1.4 μmol/L) were achieved by delivering *trans*-resveratrol (140 mg) in lozenges through absorption via the oral mucosa, which circumvents first-pass metabolism in the intestine and liver [23].

Several novel formulations have attempted to enhance the bioavailability of resveratrol, mostly by means of increasing its hydrophilicity, stability and bioaccesibility, including: micronization, microencapsulation with polymeric nanoparticles and solid lipid nanoparticles, encapsulation into colloidal lipid- and/or biopolymer-based delivery systems (liposomes, micelles, nanoemulsions and microemulsions), complexation with cyclodextrins, and encapsulation into pectinate beads, to name a few [8,11–14,24]. Food-grade surfactant-based micelles may constitute a relatively easy and cost-effective way for the solubilization of hydrophobic molecules due to their spontaneous self-assembly intro structures with a hydrophobic core and a hydrophilic outer shell [25]. Solubilization of resveratrol into emulsion-based colloidal formulations has already been investigated [24] but human trials using micellar resveratrol formulations for oral consumption are scarce.

We propose that, in addition to enhanced passage through the unstirred water layer, the increased uptake of total *trans*-resveratrol from the vineatrol micellar formulation, compared to the vineatrol native powder, was aided by i) improved stability in the gastrointestinal tract due to incorporation into a micellar shell and/or ii) decreased biotransformation during transit through the enterocytes due to competitive inhibition of phase II metabolism by increased substrate concentrations, and/or presence of other resveratrol oligomers from the grape shoot extract [1].

Our findings are in agreement with published data reporting a ca. 9-fold increase in the AUC in 15 healthy subjects (4 women, 11 men) orally dosed with 40 mg of a comparable emulsified formulation of *trans*-resveratrol (mixed with polysorbate 20 and polyglyceryl-3-dioleate) relative to the native compound [26]. The C_{max} in these 15 volunteers, who only ingested 10 mg more *trans*-resveratrol than in our trial, were 470 nmol/L for the native and 5707 nmol/L for the micellar formulation, and thus roughly one order of magnitude higher than in our volunteers (**Table 2**). The discrepancy might be explained by one of the following reasons or a combination thereof: 1) matrix effects may have limited the liberation –and thus the fraction available for resorption– of *trans*-resveratrol from the grapevine shoot extract; 2) the presence of oligomeric resveratrol may have interfered with absorption; 3) the higher dose, which was ca. 133% of the dose administered in the present study, may result in higher intracellular concentrations, which may reach a level sufficient to (partly) saturate metabolic enzymes and thus proportionally reduce elimination; and 4) oligomeric resveratrol, similar to resveratrol itself [27] may induce xenobiotic enzymes involved in the metabolism and elimination of *trans*-resveratrol. It has indeed been reported that resveratrol, as well as a root extract rich in resveratrol oligomers, induced Nrf2 [28,29], a transcription factor regulating the transcription of xenobiotic enzymes and efflux transporters [28]. Nrf2 induction by 10 µmol/L viniferin (dimeric resveratrol) was comparable to that of a threefold higher concentration of 30 µmol/L resveratrol in vascular smooth muscle cells [30], and thus more potent.

As mentioned before, C_{max}, based on total radioactivity, reached ~2 µmol/L in six healthy subjects (3 women, 3 men) given 25 mg ^{14}C-resveratrol [19]. Since this method quantified total radioactivity, this value may also account for resveratrol metabolites that are not detectable by our analytical method. Likewise, also the previously mentioned study of Amiot and co-workers [26]

found up to ~93% of the total resveratrol in plasma in the form of sulfate conjugates. This is in agreement with other publications reporting resveratrol sulfates as the predominant human metabolites [18,19,22,31]. Therefore, the significantly lower plasma concentrations in our study might also be attributed to the different analytical methods employed. We hydrolyzed resveratrol conjugates with a β-glucuronidase/sulfatase and adjusted the pH of the samples to facilitate optimum cleavage of sulfates. The recovery of sulfated metabolites of our method may nevertheless be lower than that of radioactivity- and MS-based methods. Because sulfated metabolites are not commercially available, we cannot at present quantify the efficacy of sulfate hydrolysis of our method. However, our main objective with this study was to compare the relative bioavailability of native versus micellar resveratrol, which was still possible, even though the total plasma concentrations are lower than expected. An efficient and precise HPLC-DAD method for the quantification of free and protein-bound resveratrol metabolites has been published and offers an alternative when radioactivity- and MS-based methods are not available [32,33].

Even though renal excretion has been described as the main route of elimination in humans [12], the mean urinary excretion of total *trans*-resveratrol over 24 h accounted for less than 2.0% (native) and 8.7% (micelles) of the ingested dose (**Table 3, Figure 2**). Walle et al. [19] recovered 53-85% of the resveratrol dose in urine and 0.3-38 % in feces after the intake of 25 mg ^{14}C-resveratrol based on radioactively [19]. Likewise, Bode et al. [34] recovered 36-83% of resveratrol and its metabolites in 24 h urine after the intake of ~38 mg *trans*-resveratrol from soluble vineatrol. However, in the latter study, 8-63% of the dose was recovered as transformation products of resveratrol by the human gut microbiota [34]. As proposed above, these metabolites may not be detectable with our method, which would explain the lower recovery of resveratrol, in the form of its metabolites, in the urine of our subjects compared to these studies [19,34].

In our volunteers, viniferin could not be detected in any of the plasma or urine samples, suggesting that it has none or very low oral bioavailability in humans. Preliminary results from our laboratory revealed accumulation of viniferin in differentiated Caco-2 monolayers (data not shown), but marginal transepithelial transport, showing efflux of less than 0.3% of the dose into the basolateral compartment and an apparent permeability coefficient (6.4×10^{-7} cm/s) within the reported range of

compounds with poor transepithelial absorption (<10 x 10^{-6} cm/s) [17]. Both findings are in agreement with an earlier publication also reporting that viniferin can be found in the apical chamber and in Caco-2 cell pellets, but not in the basolateral compartment [17]. Together, these data suggest that the resveratrol dimer viniferin is not absorbed in humans, as proposed by Willenberg and colleagues [17], but may still enter small intestinal cells, where it could modify the expression of xenobiotic enzymes and thereby alter resveratrol metabolism. The transepithelial permeability of *trans*-resveratrol was only slightly improved by co-incubation with *trans*-ε-viniferin at equimolar concentrations in Caco-2 differentiated monolayers (see Results); however, the amount of viniferin and the duration of exposure in this experiment could have been insufficient to observe more relevant effects.

In conclusion, a single oral dose of 500 mg vineatrol (30 mg *trans*-resveratrol, 75 mg *trans*-ε-viniferin) as liquid micelles significantly increased the oral bioavailability of *trans*-resveratrol in women and men relative to the native powder without any adverse effects, showing that a micellar microemulsion may provide better possibilities for *trans*-resveratrol to be safely delivered in relevant bioactive concentrations to systemic circulation.

Author contributions

JF designed and LACC, CS, FD, HE and ABW conducted the human trial. NS developed the HPLC method. JV performed the cell culture experiments. LACC and JF wrote the first draft of the manuscript and all authors read, edited, and approved the final document. JF is responsible for the final content.

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Conflict of interest statement

Dariush Behnam is the founder and CEO of AQUANOVA AG, the company producing and selling the micellar formulations for profit. Jan Frank has received honoraria for scientific consultation from AQUANOVA AG. None of the other authors has a no known conflict of interest to declare.

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Figure 1. Mean (± SEM) total plasma *trans*-resveratrol concentrations (nmol/L) following the ingestion of a single dose of 500 mg Vineatrol®30 (30 mg *trans*-resveratrol, 75 mg *trans*-ε-viniferin) as native powder (black line) or liquid micelles (dotted line), in six healthy women (A) and six healthy men (B). Statistical differences between formulations were evaluated by an unpaired Student's *t*-test or a Mann-Whitney U test, as appropriate (GraphPad Prism 5, v. 5.02); $p < 0.05$ (*).

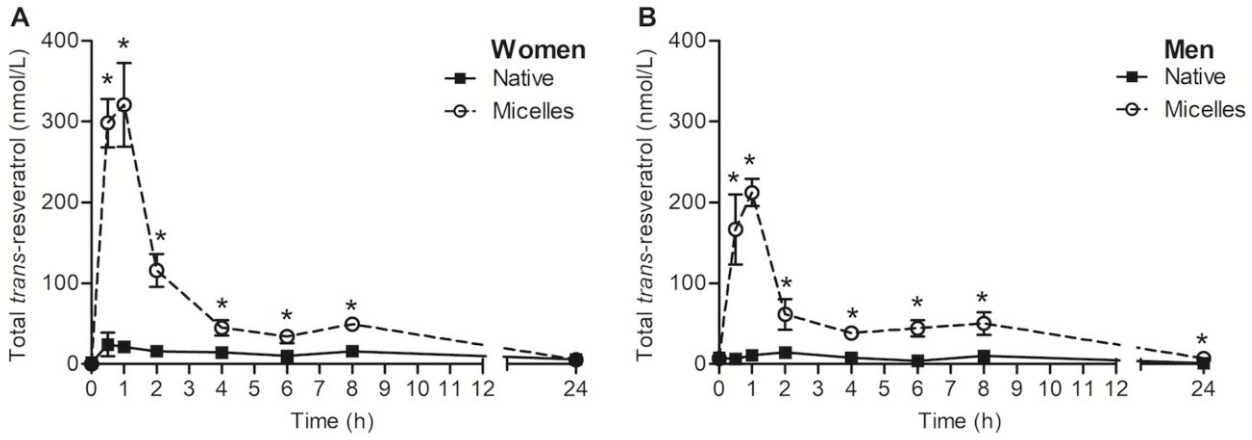
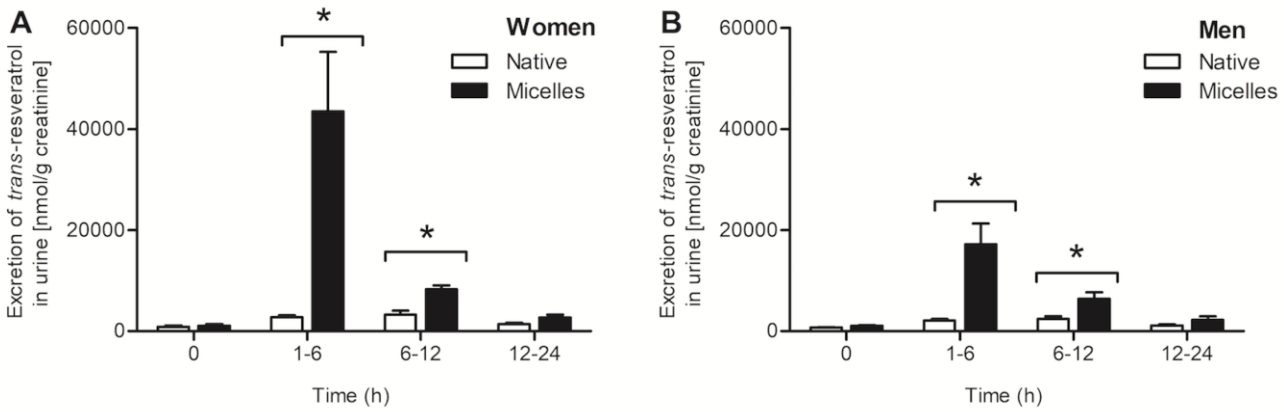


Figure 2. *Trans*-resveratrol urinary excretion profile (nmol/g creatinine; mean ± SEM) over 24 h in six women (A) and six men (B) after a single oral dose of 500 mg Vineatrol®30 (30 mg *trans*-resveratrol, 75 mg *trans*-ε-viniferin) as native powder (open columns) or liquid micelles (closed columns). Statistical differences between formulations were evaluated by an unpaired Student's *t*-test (GraphPad Prism 5, v. 5.02); $p < 0.05$ (*).



Graphical Abstract

The oral bioavailability in humans of *trans*-resveratrol was significantly higher after ingesting a grapevine-shoot extract (vineatrol, 500 mg) encapsulated in liquid micelles relative to the native vineatrol powder, without any adverse effects. Resveratrol's dimer, *trans*-ε-viniferin, was not detected in human plasma or urine, and its low intestinal permeability was confirmed *in vitro*. The vineatrol micelles may improve the delivery of resveratrol for reaching relevant systemic concentrations.

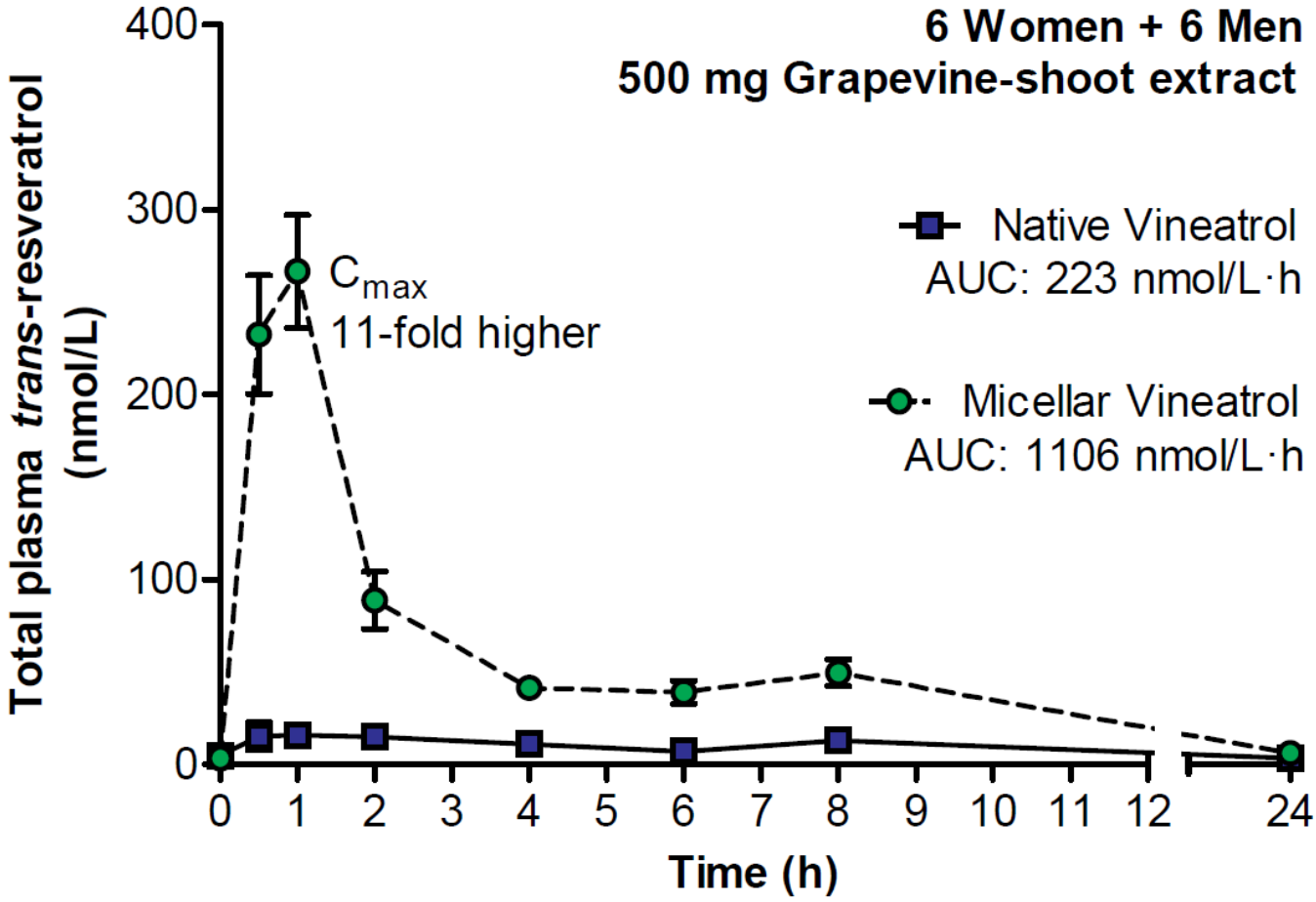


Table 1. Baseline characteristics (mean ± SD) of the participants at screening[1].

Parameter	Reference range	Women (*n*=6)	Men (*n*=6)
Anthropometric measurements			
Age (years)	18 – 35	25 ± 3	26 ± 3
Body height (m)	–	1.68 ± 0.05*	1.82 ± 0.07*
Body mass (kg)	–	64.1 ± 8.05*	77.6 ± 6.7*
BMI (kg/m²)	19 – 25	22.6 ± 1.8	23.3 ± 1.2
Systolic blood pressure (mmHg)	90 – 120	130.3 ± 11.9	135.8 ± 13.9
Diastolic blood pressure (mmHg)	60 – 80	80.8 ± 8.9	77.0 ± 5.2
Glucose and lipids			
Fasting plasma glucose (mg/dL)	75 – 126	88.2 ± 7.4	90.0 ± 6.4
Total cholesterol (mg/dL)	< 200	188.8 ± 40.5	173.7 ± 24.8
LDL cholesterol (mg/dL)	< 155	110.7 ± 22.3	116.8 ± 15.4
HDL cholesterol (mg/dL)	35 – 100	71.8 ± 20.8*	47.8 ± 9.6*
LDL/HDL cholesterol ratio	0.5 – 3.5	1.6 ± 0.4*	2.5 ± 0.4*
Triacylglycerols (mg/dL)	< 200	94.2 ± 35.9	79.2 ± 15.2
Kidney and liver function markers			
γ-GT (U/L)	♀< 40, ♂< 60	15.8 ± 10.0	23.7 ± 13.3
AST (U/L)	♀< 35, ♂< 50	26.0 ± 7.6	27.7 ± 7.8
ALT (U/L)	♀< 35, ♂< 50	19.3 ± 7.3	29.2 ± 10.0
Alkaline phosphatase (U/L)	♀35 – 105, ♂40 – 130	75.7 ± 17.2	77.8 ± 18.2
Creatinine i.S. (mg/dL)	♀0.50 – 1.00, ♂0.70 – 1.20	0.7 ± 0.04*	0.9 ± 0.08*
Bilirrubin (mg/dL)	< 1.10	0.5 ± 0.1	0.6 ± 0.2
Uric acid (mg/dL)	♀< 6, ♂< 7	4.3 ± 0.4*	5.4 ± 0.9*
Blood count			
Hematocrit (%)	♀34.7 – 44.7, ♂36.0 – 48.2	39.4 ± 1.3*	43.8 ± 2.5*
Hemoglobin (g/dL)	♀12.3 – 15.3, ♂14.0 – 17.5	13.6 ± 0.8*	15.3 ± 0.9*
Erythrocytes (/pl)	♀4.10 – 5.10, ♂4.50 – 5.90	4.6 ± 0.15*	5.05 ± 0.3*
Thrombocytes (/nl)	150 – 400	239 ± 18.8	241 ± 41.7
Leukocytes (/nl)	4 – 11	6.9 ± 0.8	6.4 ± 0.7
MCHC (g/dL)	30 – 37	34.2 ± 1.0	35.0 ± 0.6
MCH (HBE) (pg)	28 – 33	29.4 ± 1.7	30.3 ± 1.1
MCV (fl)	80 – 96	85.3 ± 2.8	86.8 ± 3.4

[1]Statistical differences ($p < 0.05$, indicated with asterisks) between sexes were calculated by an unpaired Student's *t*-test or a Mann Whitney U test (GraphPad Prism 5, v. 5.02).

Table 2. Pharmacokinetic variables (mean ± SD) calculated from plasma total *trans*-resveratrol concentrations in 12 healthy human subjects (6 women, 6 men) after a single oral dose of 500 mg Vineatrol®30 (30 mg *trans*-resveratrol, 75 mg *trans*-ε-viniferin) as native powder or liquid micelles [1].

	trans-resveratrol	
	Native vineatrol	Micellar vineatrol
AUC [2,3] [nmol/L · h] (ng/ml · h)		
Women	288.7 ± 115.9 (65.9 ± 26.5)***	1203.8 ± 265.3 (274.8 ± 60.5)***
Men	156.7 ± 89.0 (35.8 ± 20.3)***	1007.8 ± 367.3 (230.0 ± 83.8)***
All	222.7 ± 120.2 (50.8 ± 27.4)***	1105.8 ± 322.1 (252.4 ± 73.5)***
p for formulation	< 0.0001	
p for sex	ns	
p for formulation × sex	ns	
C_{max} [nmol/L] (ng/ml)		
Women	37.2 ± 28.1 (8.5 ± 6.4)***	357.3 ± 90.1 (81.5 ± 20.6)***
Men	19.4 ± 4.6 (4.4 ± 1.0)***	242.8 ± 55.8 (55.4 ± 12.7)***
All	28.3 ± 21.3 (6.5 ± 4.9)***	300.0 ± 93.2 (68.8 ± 21.3)***
p for formulation	< 0.0001	
p for sex	0.0079	
p for formulation × sex	0.0436	
T_{max} [h]		
Women	2.0 ± 2.9	0.8 ± 0.3
Men	4.0 ± 3.3*	0.8 ± 0.3*
All	3.0 ± 3.2*	0.8 ± 0.2*
p for formulation	0.0265	
p for sex	ns	
p for formulation × sex	ns	

[1] AUC, area under the plasma concentration time curve; C_{max}, maximum plasma concentration; T_{max}, time to reach C_{max}. Concentrations in ng/ml are shown between parentheses.
[2] AUC was calculated with GraphPad Prism 5 for Windows (v. 5.02).
[3] The effects of formulation and sex on the availability of *trans*-resveratrol were calculated in a two-way ANOVA, followed by Bonferroni post-hoc tests for within-formulation differences. Differences between formulations comparing all participants (All) were calculated by an unpaired Student's *t*-test or a Mann Whitney U test as appropriate. Statistical differences between formulations are marked with asterisks (* $p < 0.05$; *** $p < 0.001$) (GraphPad Prism 5, v.5.02).

Table 3. Cumulative 24 h urinary excretion of *trans*-resveratrol (mean ± SD) in 12 healthy human subjects (6 women, 6 men) after a single oral dose of 500 mg Vineatrol®30 (30 mg *trans*-resveratrol, 75 mg *trans*-ε-viniferin) as native powder or liquid micelles[1].

	trans-resveratrol urinary excretion [mg]	
	Native vineatrol	Micellar vineatrol
Women	0.5 ± 0.2***	2.8 ± 1.2***
Men	0.6 ± 0.2**	2.5 ± 1.1**
All	0.6 ± 0.2***	2.7 ± 1.1***
p for formulation	< 0.0001	
p for sex	ns	
p for formulation × sex	ns	

[1]Two-way ANOVA was calculated to determine the effects of formulation and sex on *trans*-resveratrol excretion, followed by Bonferroni post-hoc tests for within-formulation differences. Differences between formulations comparing all participants (All) were calculated by an unpaired Student's *t*-test. Statistical differences between formulations are marked with asterisks (** $p < 0.01$; *** $p < 0.001$) (GraphPad Prism 5, v.5.02).