EFFECTS OF RESVERATROL ON DRUG- AND CARCINOGEN-
METABOLIZING ENZYMES, IMPLICATIONS FOR CHEMOPREVENTION

By

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ABSTRACT

Resveratrol is a polyphenol found in grape skins and peanuts that has demonstrated many health benefits including protection against aging, cardiovascular and metabolic disease, neurological decline and cancer. The anticancer properties of resveratrol have been attributed to a variety of mechanisms, including its general inhibition of phase I metabolism and induction of phase II metabolism. The effects of resveratrol on these enzymes, however, are still unclear, as in vitro evidence often contrasts with animal studies and clinical trials. Reasons for these variances could include the low bioavailability of resveratrol and activity of resveratrol metabolites. Due to resveratrol’s interactions with drug-metabolizing enzymes and drug transporters, individuals concurrently taking pharmacological doses of resveratrol with other supplements or medications could potentially experience nutrient-drug interactions. The first part of this thesis reviews the known effects of resveratrol and its main metabolites on drug metabolism. The second portion introduces preliminary data from the metabolomic analysis of stored urine samples collected from a previous resveratrol clinical study. The overarching aim of this work is to characterize which populations might benefit from resveratrol for the prevention of cancer, as well as identify those that may need to avoid supplementation due to potential drug interactions.
BACKGROUND

Resveratrol is a polyphenol found in red wine and peanuts that has been attributed to a variety of health benefits, including cancer prevention (Jang et al., 1997; Bhat and Pezzuto, 2002; Dong, 2003; Whitlock et al., 2012; Carter et al., 2014). While resveratrol has shown promising results in preclinical trials, its interactions with drug- and carcinogen-metabolizing enzymes create the potential for nutrient-drug interactions (Chow et al., 2010; Detampel et al., 2012). Supplements like resveratrol are often overlooked when reviewing patient medications, even though they could create significant interactions. This is particularly relevant for oncology patients, as 69% reported supplement use after their diagnosis (Ferrucci et al., 2009). Such interactions could be harmful for other patient populations as well, such as those receiving medications with a narrow therapeutic ranges, e.g. warfarin (Sanderson et al., 2005).

My Honors Thesis will review the literature regarding the effects of resveratrol and its major metabolites on drug- and carcinogen-metabolizing enzymes in order to determine its potential as a chemoprevention agent as well as predict which patient populations may benefit most from supplementation. This review (which has been submitted for publication and is currently under revision) will serve as the background for my thesis, emphasizing the necessity for further studies that analyze the effects of resveratrol on human metabolism.

The second portion of my thesis will present preliminary data from metabolomics analyses that we conducted on stored urine samples from a study in which postmenopausal women with a high body mass index (BMI) were given 1 gram daily resveratrol for 12 weeks (Chow et al., 2014). Metabolomics is the systematic analysis of the small-molecule end products, or “fingerprints,” of specific cellular processes (Hunter, 2009). Since the metabolome, or sum of these end products, changes in response to diet, drugs, supplements, or environmental conditions, metabolic profiling
can provide a snapshot of the systemic effect of a therapeutic intervention (Want et al., 2010). In fact, metabolomics is quickly becoming a promising tool for personalized medicine (Schnackenberg and Beger, 2006; Gonzalez de Castro et al., 2013). For this Honors Thesis, metabolomics was utilized to measure the systemic effect of resveratrol. Global metabolic profiling was conducted using ultra-performance liquid chromatography tandem quadrupole time of flight mass spectrometry (UPLC-QTOF). In addition to characterizing overall metabolomic changes with resveratrol, metabolites that changed as a result of resveratrol supplementation are putatively identified. These metabolites could potentially serve as biomarkers, allowing for faster determination of whether resveratrol supplementation is effectively modulating intermediate biomarkers of cancer risk in a patient.
Introduction

Resveratrol (3, 4’,5-trihydroxy-trans-stilbene; see Figure 1) is a polyphenol found in grapes and peanuts. In preclinical models, there is convincing evidence for anticancer, anti-aging, and anti-inflammatory effects of trans-resveratrol as well as protection against metabolic and cardiovascular disease (Zordoky et al., 2015). Evidence supporting the clinical benefit of resveratrol supplementation is mixed, but recent reviews examine evidence for resveratrol as an agent to prevent and/or treat obesity (de Ligt et al., 2015), cardiovascular disease (Zordoky et al., 2015), diabetes (Szkudelski and Szkudelska, 2015), neurological disease (Bastianetto et al., 2015; Pasinetti et al., 2015), aging (Ramis et al., 2015) and cancer (Baur and Sinclair, 2006; Gescher et al., 2013; Carter et al., 2014). Many questions remain regarding the dose and duration of resveratrol supplementation for either prevention in the general population or as a therapeutic in the clinical setting and for which conditions (Novelle et al., 2015). In order to determine which populations or individuals might benefit from resveratrol for cancer prevention, it is imperative to understand the ideal dose, the potential activity of resveratrol metabolites, and pharmacological targets.

Resveratrol Dosing

Because of the success in preclinical models as well as media attention, resveratrol is
available over the counter with single capsules containing between 20-500 mg pure resveratrol (Chachay et al., 2011). With pharmaceutical doses being not only achievable but also easily accessible, questions of toxicity and safety have emerged. Detailed reviews of the bioavailability, metabolism, and toxicity of resveratrol are available (Wenzel and Somoza, 2005; Cottart et al., 2014). Based on human equivalent dose (HED) calculations from animal models (Crowell et al., 2004) and on clinical trials (Brown et al., 2010; Chow et al., 2010; Anton et al., 2014), the evidence supports an upper limit of 1 g daily for humans, although a recent clinical study showed that doses as high as 2 g daily were safe and well-tolerated in older adults (Turner et al., 2015).

The ideal dose for resveratrol supplementation, however, may be less. In recent clinical trials, 5-250 mg doses of resveratrol show a positive effect on metabolic parameters in diabetics (Brasnyó et al., 2011; Bhatt et al., 2012) as well as healthy obese adults (Timmers et al., 2011; Witte et al., 2014). A higher dose of 1 g daily resveratrol had significant improvements in several metabolic parameters in diabetic men (Movahed et al., 2013); however, doses ranging from 1-2 g daily resveratrol had little or no effect in older adults with glucose intolerance (Crandall et al., 2012) or healthy obese males (Dash et al., 2013; Poulsen et al., 2013). Diabetics who consumed
150 mL of red wine daily for two years, the equivalent of only 2 mg/day resveratrol, had improved cholesterol profiles over diabetics that consumed white wine or mineral water (Gepner et al., 2015). These studies suggest that lower doses of resveratrol are better able to reverse metabolic disturbances, which is favorable as these disturbances have been linked to cancer (Fay et al., 2009; Seyfried and Shelton, 2010; Sinicrope and Dannenberg, 2011). In mice fed a high fat diet, 5 mg resveratrol (HED of 28 mg for a 70 kg human) reduced intestinal adenoma number and volume more potently than a 1 g dose (Cai et al., 2015). One proposed explanation for this nonlinear dose response is that lower doses of antioxidants like resveratrol allow for low levels of reactive oxygen species (ROS) to activate natural cellular defense mechanisms (Cai et al., 2015). It is also possible that higher doses of resveratrol compete with other polyphenols for transporters, reducing their uptake and potential synergistic effects.

**Resveratrol metabolites**

The benefits of oral resveratrol in humans may also be derived from its metabolites, which have demonstrated more potent activity than the parent compound against multiple cancer types in some, but not all, preclinical models (Aires et al., 2013; Ruotolo et al., 2013). Resveratrol has high oral absorption (up to 70%) but is rapidly metabolized (Walle et al., 2004). It undergoes extensive pre-systemic circulation by phase II enzymes, particularly sulfotransferases (SULTs) (Miksits et al., 2005) and UDP-glucuronosyltransferases (UGTs) (de Santi et al., 2000; Brill et al., 2006), however emerging evidence also indicates a role for the gut microbiome in resveratrol metabolism (Qiao et al., 2014; Cai et al., 2015). Evidence also suggests that sulfation is the main limiting factor for bioavailability (Walle et al., 2004). Sulfated metabolites include resveratrol-3-\(O\)-sulfate (R3S), resveratrol-4’-\(O\)-sulfate (R4S) and resveratrol-3-\(O\)-4’-\(O\)-disulfate (R34S)
Glucuronidated metabolites include resveratrol-3-\textit{O}-glucuronide (R3G) and resveratrol-4’-\textit{O}-glucuronide (R4G). A glucuronide-sulfate metabolite has also been detected in humans after resveratrol administration (Chow et al., 2010) and piceatannol (3,4,3’,5’-tetrahydroxy-\textit{trans}-stilbene) is a minor metabolite which results from metabolism of resveratrol by cytochrome P450 enzymes (Chang et al., 2007). In addition, 3,4’-dihydroxy-\textit{trans}-stilbene, 3,4’-dihydroxybibenzyl (lunularin), and dihydroresveratrol have been identified as human gut microbial metabolites, although it is still unclear whether these metabolites confer health benefits (Bode et al., 2013).

The primary metabolite in humans is R3S, with plasma levels reaching a C\textit{max} of 6.4 µM after treatment with 1.0 g resveratrol (Boocock et al., 2007). The median C\textit{max} for R3S following a 250 mg dose, however, was 7.1 µM (Wightman et al., 2014), suggesting a nonlinear dose response. Following a 52-week intervention with doses of resveratrol reaching 2.0 g daily, C\textit{max} values for R3S, R4G, and R3G were 28.2 µM, 19.4 µM, and 15.2 µM, respectively (Turner et al., 2015). Figure 2 illustrates the structures of these main metabolites of resveratrol, as well as R4S and piceatannol.

**Resveratrol and chemoprevention**

For prevention of various cancers, it has been hypothesized that resveratrol’s activity may be a result of action on multiple pathways and various molecular targets within those pathways. Specifically, resveratrol has demonstrated that it may act as a calorie-restriction mimetic, antioxidant, and anti-inflammatory agent, to provide an anticancer environment (Bhat and Pezzuto, 2002; Kulkarni and Cantó, 2014; Bitterman and Chung, 2015). Less attention has been given to another mechanism by which resveratrol may prevent carcinogenesis, namely, through
Figure 2. Structures of resveratrol’s main metabolites. (A) *trans*-resveratrol-4’-O-sulfate (main metabolite found in mice), (B) *trans*-resveratrol-3-O-sulfate (main metabolite found in humans), (C) *trans*-resveratrol-3-O-4’-O-disulfate (D) *trans*-resveratrol-3-O-glucuronide (major glucuronide product), (E) *trans*-resveratrol-4’-O-glucuronide, (F) piceatannol (a minor resveratrol metabolite that is quickly metabolized by phase II enzymes).
the inhibition of procarcinogen-activating enzymes (phases I metabolism, or cytochrome P450 enzymes) and induction of elimination enzymes (phase II metabolism). While some of resveratrol’s interactions with cytochrome P450 enzymes have been summarized in an existing review (Detampel et al., 2012), this review will include more recent evidence and focus on implications of these interactions for cancer prevention. Furthermore, we will include a discussion of resveratrol’s effect on phase II metabolism. We will also discuss the anticancer activities of resveratrol’s main glucuronide and sulfate metabolites when evidence is available.

Resveratrol and phase I metabolism

Phase I metabolism of pharmaceutical drugs, phytochemicals, environmental pollutants and various other endogenous and exogenous compounds is accomplished mainly by the cytochrome P450 (CYP) enzymes. CYPs catalyze oxidation, reduction, aromatic hydroxylation, hydrolysis, deamination, and other reactions that add or expose polar substituents to a compound (Omiecinski et al., 2011). This increased polarity may facilitate the elimination of these compounds or, in some cases, may create a bioactive metabolite. The effects of resveratrol on isolated isozymes and in microsomes are summarized in Table 1, and Table 2 summarizes the effects of resveratrol on drug- and carcinogen-metabolizing enzymes in clinical and animal models.
Table 1. Inhibitory effect of resveratrol in liver microsomes and heterologously expressed isozymes (only studies that measured enzyme activity are included).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Model</th>
<th>IC$_{50}$ (µM)</th>
<th>K$_i$ (µM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>EROD</td>
<td>Human HepG2 microsome</td>
<td>1.0</td>
<td>0.42</td>
<td>Ciolo and Yeh, 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human HepG2 cell</td>
<td>1.0</td>
<td></td>
<td>Ciolo and Yeh, 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human MCF-7 cell</td>
<td>0.5</td>
<td></td>
<td>Ciolo and Yeh, 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recombinant isozyme</td>
<td></td>
<td>1.2</td>
<td>Chang et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recombinant isozyme</td>
<td></td>
<td>40</td>
<td>Piver et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recombinant isozyme</td>
<td></td>
<td>30</td>
<td>Piver et al., 2003</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>EROD</td>
<td>Recombinant isozyme</td>
<td></td>
<td>15.5</td>
<td>Chang et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recombinant isozyme</td>
<td></td>
<td>30</td>
<td>Piver et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human liver microsome</td>
<td></td>
<td>25</td>
<td>Piver et al., 2003</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>EROD</td>
<td>Recombinant isozyme</td>
<td></td>
<td>0.8</td>
<td>Chang et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recombinant isozyme</td>
<td></td>
<td></td>
<td>Piver et al., 2003</td>
</tr>
<tr>
<td>CYP2B1</td>
<td>EFC</td>
<td>Rat liver microsome*</td>
<td></td>
<td>40</td>
<td>Huynh and Teel, 2002</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>BROD</td>
<td>Recombinant isozyme</td>
<td>&gt; 50</td>
<td></td>
<td>Piver et al., 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recombinant isozyme</td>
<td>100</td>
<td></td>
<td>Piver et al., 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human liver microsome</td>
<td>&gt; 50</td>
<td>100</td>
<td>Piver et al., 2003</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Diclofenac</td>
<td>Human liver microsome</td>
<td>&gt; 50</td>
<td></td>
<td>Yu et al., 2003</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Bufuralol</td>
<td>Human liver microsome</td>
<td>&gt; 50</td>
<td></td>
<td>Yu et al., 2003</td>
</tr>
<tr>
<td></td>
<td>Aripiprazole</td>
<td>Recombinant isozyme</td>
<td>87.9</td>
<td></td>
<td>Zhan et al., 2015</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Chlorzoxazone</td>
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<td>Recombinant isozyme</td>
<td>50</td>
<td></td>
<td>Piver et al., 2003</td>
</tr>
<tr>
<td></td>
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<td>Human liver microsome</td>
<td>50</td>
<td></td>
<td>Piver et al., 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rat liver microsome</td>
<td>18.5</td>
<td></td>
<td>Mikstacka et al., 2002</td>
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<tr>
<td>CYP3A4</td>
<td>Testosterone</td>
<td>Human liver microsome</td>
<td>4.0</td>
<td></td>
<td>Piver et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rat liver microsome</td>
<td>20</td>
<td></td>
<td>Piver et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recombinant isozyme</td>
<td>10</td>
<td></td>
<td>Piver et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recombinant isozyme</td>
<td>15</td>
<td></td>
<td>Piver et al., 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human liver microsome</td>
<td>25</td>
<td></td>
<td>Piver et al., 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recombinant isozyme</td>
<td>1.1</td>
<td></td>
<td>Yu et al., 2003</td>
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<tr>
<td></td>
<td></td>
<td>Recombinant isozyme</td>
<td>6.8</td>
<td></td>
<td>Zhan et al., 2015</td>
</tr>
</tbody>
</table>

* $p < 0.05$ for percent inhibition

BROD: 7-benzoxy resorufin; EFC: 7-ethoxy-4-trifluormethyl courmarin; EROD: 7-ethoxyresorufin; PNPH: $p$-nitrophenol hydroxylase
Table 2. Effect of resveratrol on drug- and carcinogen-metabolizing enzymes in clinical trials and animal studies (only studies which measured enzymatic activity are included).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Species</th>
<th>Dose per day (HED)</th>
<th>Administration</th>
<th>Effect</th>
<th>Reference</th>
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<tbody>
<tr>
<td>CYP1A2</td>
<td>Caffeine</td>
<td>Human</td>
<td>1.0 g&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Oral</td>
<td>Induction&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Chow et al., 2010</td>
</tr>
<tr>
<td>CYP2B1/2</td>
<td>Testosterone</td>
<td>Mice</td>
<td>50 mg/kg (284 mg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Intraperitoneal</td>
<td>Inhibition&lt;sup&gt;**&lt;/sup&gt;</td>
<td>Canistro et al., 2009</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Losartan</td>
<td>Human</td>
<td>1.0 g&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Oral</td>
<td>Inhibition&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Chow et al., 2010</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Dextromethorphan</td>
<td>Human</td>
<td>1.0 g&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Oral</td>
<td>Inhibition&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Chow et al., 2010</td>
</tr>
<tr>
<td>CYP3A1/2</td>
<td>Testosterone</td>
<td>Mice</td>
<td>50 mg/kg (284 mg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Intraperitoneal</td>
<td>Inhibition&lt;sup&gt;**&lt;/sup&gt;</td>
<td>Canistro et al., 2009</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Diltiazem</td>
<td>Rat</td>
<td>2.5 mg/kg (28 mg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral</td>
<td>Inhibition&lt;sup&gt;W&lt;/sup&gt;</td>
<td>Hong et al., 2008</td>
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<td></td>
<td>Nicardipine</td>
<td>Rat</td>
<td>0.5 mg/kg (6 mg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral</td>
<td>Inhibition&lt;sup&gt;W&lt;/sup&gt;</td>
<td>Choi et al., 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.5 mg/kg (28 mg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral</td>
<td>Inhibition&lt;sup&gt;W&lt;sup&gt;**&lt;/sup&gt;&lt;/sup&gt;</td>
<td>Choi et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Buspirone</td>
<td>Human</td>
<td>1.0 g&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Oral</td>
<td>Inhibition&lt;sup&gt;W&lt;sup&gt;**&lt;/sup&gt;&lt;/sup&gt;</td>
<td>Chow et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Carbamazepine</td>
<td>Human</td>
<td>0.5 g&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral</td>
<td>Inhibition&lt;sup&gt;W&lt;/sup&gt;</td>
<td>Bedada and Nearati, 2015</td>
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<tr>
<td>GST</td>
<td>CDNB</td>
<td>Mice</td>
<td>25 mg/kg (142 mg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Intraperitoneal</td>
<td>Inhibition&lt;sup&gt;**&lt;/sup&gt;</td>
<td>Canistro et al., 2009</td>
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<tr>
<td></td>
<td></td>
<td>Human</td>
<td>1.0 g&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Oral</td>
<td>Induction&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>Chow et al., 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mice</td>
<td>Low dose&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Oral</td>
<td>Induction&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Liu et al., 2015</td>
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<td></td>
<td></td>
<td>Mice</td>
<td>16.7 mg/kg (95 mg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral</td>
<td>Induction&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Tung et al., 2015</td>
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<td></td>
<td>Measured directly</td>
<td>Rat</td>
<td>10 mg/kg (114 mg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral</td>
<td>Induction&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Ali et al., 2015</td>
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<td></td>
<td>Rat</td>
<td>20 mg/kg (227 mg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral</td>
<td>Induction&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Sadi et al., 2015</td>
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<tr>
<td></td>
<td></td>
<td>Rat</td>
<td>~2.5 mg (28 mg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral</td>
<td>Induction&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Javkhedkar et al., 2015</td>
</tr>
<tr>
<td>UGT</td>
<td>1-naphthol</td>
<td>Mice</td>
<td>25 mg/kg (142 mg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Intraperitoneal</td>
<td>Induction&lt;sup&gt;**&lt;/sup&gt;</td>
<td>Canistro et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Bilirubin as indirect</td>
<td>Human</td>
<td>1.0 g&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral</td>
<td>Induction&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>Chow et al., 2010</td>
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<td>NQO1</td>
<td>2.6-dichlorophenol</td>
<td>Mice</td>
<td>16.7 mg/kg (95 mg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral</td>
<td>Induction&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Tung et al., 2013</td>
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<td></td>
<td>indophenol</td>
<td>Mice</td>
<td>16.7 mg/kg (95 mg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral</td>
<td>Induction&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Tung et al., 2015</td>
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<tr>
<td>P-gp (transporter)</td>
<td>Nicardipine</td>
<td>Rat</td>
<td>0.5 mg/kg (6 mg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral</td>
<td>Inhibition&lt;sup&gt;W&lt;/sup&gt;</td>
<td>Choi et al., 2009</td>
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<td>2.5 mg/kg (28 mg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral</td>
<td>Inhibition&lt;sup&gt;W&lt;sup&gt;**&lt;/sup&gt;&lt;/sup&gt;</td>
<td>Choi et al., 2009</td>
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<td>Fexofenadine</td>
<td>Human</td>
<td>500 mg</td>
<td>Oral</td>
<td>Inhibition&lt;sup&gt;W&lt;sup&gt;**&lt;/sup&gt;&lt;/sup&gt;</td>
<td>Bedada et al., 2014</td>
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**EFFECTS OF RESVERATROL ON DRUG AND CARCINOGEN METABOLISM**

*b* Blood concentrations averaged 72.7 ng/mL (0.32 µM); *b* Blood concentration not given; *c* 5.7 µg/mL resveratrol mixed with drinking water three times weekly; *p* < 0.05; ***p* < 0.001; NS Not significant; W Weak (1.25- to 2-fold change in AUC); M Moderate (2- to 5-fold change in AUC).

CDNB: 1-chloro-2,4-dinitrobenzene; HED: human equivalent dose (based on 70 kg human); PROD: pentoxyresorufin dealkylase

**CYP1A1 and CYP1A2**

CYP1A1 (mainly extrahepatic) and 1A2 (exclusively hepatic) help metabolize a range of medications including antipsychotics (clozapine), CNS stimulants (caffeine), heart medications (propranolol and verapamil), and endogenous compounds like estradiol (Zhou *et al.*, 2010). Additionally, they metabolize many anticancer drugs such as tegafur (solid tumors), flutamide (prostate), dacarbazine (skin), and aminoflavone (solid tumors). CYP1A enzymes also activate procarcinogens such as benzo[a]pyrene (B[a]P), a polycyclic aromatic hydrocarbon found in cigarette smoke and grilled/smoked/barbequed food products (Ding *et al.*, 2014; Rose *et al.*, 2015) and 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD), a contaminant in chlorophenoxy herbicides (Saberi Hosnijeh *et al.*, 2012). Resveratrol has been shown to inhibit both the expression and activity of CYP1A enzymes (Detampel *et al.*, 2012). While supplementation with resveratrol may be a viable cancer prevention strategy for individuals with exposure to B[a]P or TCDD (Go *et al.*, 2015), it could potentially create nutrient-drug interactions for individuals taking any of the anticancer medication listed above.

In preclinical models, resveratrol inhibits CYP1 gene expression by blocking transcription through the aryl hydrocarbon receptor (AHR) pathway. CYP1 gene expression is induced by the binding of a ligand (e.g. B[a]P or TCDD) to the AHR, its translocation to the nucleus, and binding of the complex to the xenobiotic response element (XRE) (Zanger and Schwab, 2013). The exact target of resveratrol’s inhibition along this AHR pathway is still an active area of research. Figure 3 illustrates AHR induced expression of CYP1 genes and proposed mechanisms for resveratrol
EFFECTS OF RESVERATROL ON DRUG AND CARCINOGEN METABOLISM

inhibition. Early work by Ciolino et al demonstrated that in human HepG2 hematoma cells, 10 µM resveratrol prevented the TCDD-induced transformation of AHR to its nuclear form and also inhibited AHR function in the absence of TCDD.

![Proposed mechanisms for resveratrol’s suppression of CYP1 induction](image)

Figure 3. Proposed mechanisms for resveratrol’s suppression of CYP1 induction. An early model showed that resveratrol inhibited the transformation of the aryl hydrocarbon receptor (AHR) to its nuclear form by forming a complex with the aryl hydrocarbon nuclear translocator (ARNT). Resveratrol has also been shown to act later in the pathway, inhibiting the binding of the nuclear AHR complex to the xenobiotic response element (XRE) or inhibiting the recruitment of RNA polymerase II to the DNA. These mechanisms block the transcription of *CYP1A1* and *CYP1B1*. Still, others have proposed that CYP1 inhibition occurs post-transcriptionally. AHR: aryl hydrocarbon receptor; ARNT: aryl hydrocarbon receptor nuclear translocator; CYP: cytochrome P450; XRE: xenobiotic response element.
Based on this evidence, the authors concluded resveratrol does not act through competitive inhibition for the TCDD binding site on AHR but may act on a separate binding region (Ciolino et al., 1998). Subsequent studies have suggested that resveratrol acts later in the AHR pathway, affecting binding to the XRE and recruitment of RNA polymerase II. This was demonstrated at exposures as low as 1 µM in B[a]P-induced HepG2 cells and 7,12-dimethylbenz[a]anthracene (DMBA)-induced MCF-7 cells (Ciolino and Yeh, 1999) and at 10 µM in TCDD-induced HepG2 and MCF-7 cells (Beedanagari et al., 2009). Other researchers, however, proposed a post-transcriptional mechanism of inhibition after discovering that 10 µM resveratrol increased the rate of CYP1A1 mRNA degradation in T47D breast cancer cells (Lee and Safe, 2001). In contrast to inhibitory evidence for CYP1A1, high levels of resveratrol (50 µM) weakly induced CYP1A2 mRNA expression in HepG2 cells (Koe et al., 2014). It is possible that resveratrol’s effect on the AHR pathway and subsequent expression of CYP1A enzymes may depend on the carcinogen and model used.

While in vitro evidence has demonstrated direct inhibition of CYP1A1 and CYP1A2 by resveratrol, as well as mechanism-based inactivation of CYP1A2 by the polyphenol (Chang et al., 2001), resveratrol induced CYP1A2 activity in an early phase clinical study. When participants were given 100 mg caffeine after treatment with 1 g daily resveratrol for four weeks, the metabolic ratio of caffeine/paraxanthine decreased significantly compared to baseline measurements (indicative of CYP1A2 induction) (Chow et al., 2010). The observed difference between this clinical observation and previous in vitro studies could be attributed to the indirect assessment of CYP1A2 activity, or to resveratrol metabolism. In the microsomal assay by Chang et al, the requirement of NADPH for inhibition of CYP1A2 indicated that a resveratrol metabolite was responsible for the observed inhibitory activity (Chang et al., 2001). Although a subsequent study
found that RS3 did not significantly inhibit CYP1A2 in cell cultures containing the recombinant human isozyme (Yu et al., 2003), piceatannol has been shown to inhibit CYP1 activity to an extent similar to that of resveratrol (Chang et al., 2007) and could affect its interactions with CYP1A enzymes. While it is difficult to compare across models, inhibition of CYP1A enzymes would support a role for resveratrol as a chemoprevention agent and necessitates additional human studies.

**CYP1B1**

CYP1B1 is an extrahepatic enzyme that is over-expressed in breast, prostate, endometrial, and ovarian cancers (Gajjar et al., 2012). It has been shown to biotransform anticancer agents *in vitro* and may contribute to drug therapy resistance (Rochat et al., 2001). Furthermore, CYP1B1 is involved in the metabolism of 17β-estradiol and the formation of a toxicologically active metabolite, 4-hydroxyestradiol (Tsuchiya et al., 2004). Therefore, inhibition of CYP1B1 is an attractive target for hormonally-driven cancers such as breast (Gajjar et al., 2012). While there are approved agents for breast cancer prevention, uptake of these agents by eligible women is less than 5%. One of these agents, the selective estrogen receptor modulator, tamoxifen, has been associated with a small increased risk of endometrial cancer (Hu et al., 2015). This effect may be attributed to the upregulation of the *CYP1B1* gene by tamoxifen metabolites (Gajjar et al., 2012) or to the formation of DNA adducts by its α-hydroxytamoxifen metabolite (Sharma et al., 2003). An agent like resveratrol that already has high uptake by the general population could potentially serve as an alternate chemoprevention option. An understanding of resveratrol’s clinical interactions is critical, however, and resveratrol probably should not be used for combination treatment (see continued discussion of tamoxifen under “CYP2D6”).
Preclinical studies have demonstrated that resveratrol may act as a CYP1B1 inhibitor. In baculovirus-infected insect cell microsomes, the apparent $K_i$ of heterologously expressed human CYP1B1 was found to be 0.8 µM, similar to the apparent $K_i$ of 1.2 µM for CYP1A1 (Chang et al., 2001). Considering that transcription of CYP1B1 is also mediated by the AHR pathway (Go et al., 2015), it can be expected that inhibition by resveratrol also occurs at the transcription level (see Figure 3). Indeed, treatment of human MCF-7 breast cancer cells with 10 µM resveratrol inhibited TCDD-induced expression of CYP1B1 (Beedanagari et al., 2009). Others have shown that 200 µM resveratrol itself was metabolized by CYP1B1 in human lymphoblast microsomes and that the product is piceatannol, a known anticancer compound (Potter et al., 2002). While these findings are promising for the chemopreventive use of resveratrol, further studies are needed in order to understand effect of resveratrol on CYP1B1 in the clinical setting, especially since its low bioavailability may hinder resveratrol from reaching breast and other endocrine tissues.

**CYP2B6**

CYP2B6 is a major enzyme involved in the activation of cyclophosphamide, an anticancer prodrug (Zanger and Schwab, 2013), as well as bupropion, a drug used for smoking cessation and depression (Pekthong et al., 2012). Expression of CYP2B6 is regulated by xenosensing nuclear receptors, constitutive androstane receptor (CAR) and pregnane X receptor (PXR) (Zanger and Schwab, 2013). While resveratrol has been shown to inhibit PXR-induced expression of CYP3A4 at levels as low as 25 µM in transfected cell cell lines (Deng et al., 2014), it has also been suggested that resveratrol may inhibit the PXR-induced expression of other CYP enzymes, such as CYP2B6 (Smutny and Pavek, 2014). There is also evidence, however, to suggest that the polyphenol activates CAR; in transfected HepG2 cells, 5 µM resveratrol activated CAR (Yao et
al., 2011). Consistent with this CAR study, multiple injections of with 50 mg/kg (HED of 284 mg for a 70 kg human) resveratrol in CD1 mice induced testosterone metabolism by hepatic Cyp2b1/2, which shares a high sequence identity with human CYP2B6 (Canistro et al., 2009). Conversely, liver microsomes from 6- and 12-month old male Fisher F344 rats incubated with high micromolar concentrations (100-250 µM) resveratrol had significantly inhibited CYP2B1 activity (Huynh and Teel, 2002). Resveratrol likewise demonstrated inhibition of CYP2B6 in human liver microsomes, but also at supraphysiological concentrations (IC$_{50}$ of 100 µM) (Piver et al., 2003). Differences in resveratrol’s effect on CYP2B enzymes may be related to the fact that microsomal experiments tend to only observe inhibitory effects. It is also possible that CYP2B6 induction is caused by resveratrol metabolites.

**CYP2C9**

CYP2C9 metabolizes several notable drugs, including non-steroidal anti-inflammatory drugs (NSAIDs), which target COX-2 and may protect against cancer development (Ghosh et al., 2010). It also metabolizes drugs with narrow therapeutic indices like (S)-warfarin, phenytoin, and tolbutamide (Miners and Birkett, 1998). CYP2C9 expression is regulated by the CAR and PXR (Zanger and Schwab, 2013). Resveratrol, through activation of the CAR (Yao et al., 2011) and attenuation of PXR-induced enzyme expression (Deng et al., 2014; Smutny and Pavek, 2014), may alter the expression of CYP2C9, although there are currently no studies to support this hypothesis.

Few studies have investigated the effects of resveratrol on CYP2C9 activity. In humans, a dose of 1 g daily resveratrol for four weeks was shown to inhibit CYP2C9 by 2.71-fold using losartan as a probe drug. Blood concentrations of resveratrol reached an average of 72.7 ng/mL (0.32 µM) in this study (Chow et al., 2010). While low concentrations of resveratrol (0.5 µM) have
been shown to reduce oxidative stress \textit{in vitro}, 10 µM and 25 µM resveratrol induced pro-oxidative stress in human endothelial cells (Posadino et al., 2015). Researchers have proposed that the opposite effects seen in the vasculature could be attributed to dose, as CYP2C9 may recognize resveratrol as a xenobiotic at higher doses and metabolize the compound to produce ROS (Posadino et al., 2015). Evidence for interactions of resveratrol’s metabolites with CYP2C9 is limited, but one study found that inhibition of the recombinant isozyme by R3S was not significant (Yu et al., 2003). Pharmacogenetic variation may also alter resveratrol’s effect on CYP2C9, as variant alleles have been shown to affect the metabolism of xenobiotics like warfarin, which are largely metabolized by CYP2C9 (Sanderson et al., 2005). Though it is unknown whether variant alleles can alter the effects of resveratrol on CYP2C9, it could be expected that larger changes in drug clearance could be seen in wild-types or ultra-metabolizers compared to poor metabolizers. High doses of resveratrol, therefore, could become problematic for individuals on medications with narrow therapeutic ranges who may be more sensitive to changes in CYP2C9.

\textit{CYP2C19}

CYP2C19 plays a major role in the metabolism of proton pump inhibitors such as omeprazole and pantoprazole, antidepressants like citalopram, and endogenous compounds including progesterone and, to a lesser extent, melatonin (Brosen, 2004; Zanger and Schwab, 2013). The enzyme is mainly found in hepatic tissue and its expression is regulated by ligands of the PXR and CAR, glucocorticoid, and vitamin D nuclear receptors (Zanger and Schwab, 2013). Resveratrol moderately inhibited CYP2C19 in microsomes containing the heterologously expressed isoform (IC\textsubscript{50} value of 11.6 µM) (Yu et al., 2003). Additionally, a naturally occurring trimer of resveratrol, \(\alpha\)-viniferin, potently inhibited CYP2C19 activity in human liver microsomes
at an IC\textsubscript{50} of 0.93 µM (Sim \textit{et al.}, 2014). Further research is needed to determine whether these \textit{in vitro} observations translate into clinically relevant nutrient-drug interactions, especially since drugs metabolized by CYP2C19 are widely prescribed.

\textbf{CYP2D6}

CYP2D6 is found mainly in the liver, but has also been expressed in the gastrointestinal tract and brain (Zanger and Schwab, 2013). It has been characterized as one of the most polymorphic phase I enzymes, which is of high clinical importance as it metabolizes approximately 25\% of prescribed medications (Ingelman-Sundberg, 2005). This inter-individual variability also has implications for chemoprevention. CYP2D6 is the enzyme responsible for converting tamoxifen, a selective estrogen receptor modulator used for both treatment and prevention of breast cancer, to endoxifen, its active metabolite (Jin \textit{et al.}, 2005). Phenotypic variations of CYP2D6 which result in loss of function or increased susceptibility to inhibition may impede tamoxifen's effectiveness (Sim \textit{et al.}, 2012).

In cell culture, inhibition of CYP2D6 by resveratrol and its main metabolite, R3S, was rather insignificant, with IC\textsubscript{50} values of 87.9 µM for resveratrol (Zhan \textit{et al.}, 2015) and > 50 µM for R3S (Yu \textit{et al.}, 2003). Despite these high IC\textsubscript{50} values, resveratrol was found to inhibit CYP2D6 activity by 1.7-fold in humans administered 1 g/day for four weeks (Chow \textit{et al.}, 2010). In this study, resveratrol intervention significantly increased the dextromethorphan/dextrorphan molar ratio, indicating inhibition of CYP2D6. Given these clinical results, supplementation with resveratrol may not be beneficial for individuals who are already using tamoxifen for breast cancer prevention and treatment, as it may significantly inhibit its activation.
CYP2E1

CYP2E1 is involved in the metabolism of various carcinogens, including volatile organic solvents like toluene and nitrosamines (Piver et al., 2001). Common carcinogenic compounds like alcohol, nicotine and tobacco smoke increase the expression of this enzyme (Jiménez-Garza et al., 2015), and inhibition of CYP2E1 could serve as a way to prevent carcinogenesis among those highly exposed to such xenobiotics. While expression of CYP2E1 is highest in the liver, low expression has also been detected in extrahepatic tissues including the brain, adrenal cortex, ovaries, testes, gastrointestinal tract, and cardiac tissue (Zanger and Schwab, 2013).

Resveratrol has been found to inhibit CYP2E1, however the mechanism is still unclear. In human and rat microsomes, 100 μM resveratrol acted as a reversible, non-competitive inhibitor of CYP2E1, as determined by kinetic studies (Piver et al., 2001). Pre-incubation of murine liver microsomes with NADPH, however, resulted in irreversible inhibition of CYP2E1 by lower doses of resveratrol (IC₅₀ value of 18.5 µM) (Mikstacka et al., 2002). The requirement of NADPH in this study suggests that resveratrol itself is metabolized by CYP2E1 and a reactive intermediate is responsible for inactivation of the enzyme. Mechanistic differences regarding resveratrol’s inhibition of CYP2E1 could be due to differences in doses and substrates, or could be a result of the enzyme’s multiple active sites (Liu et al., 2013).

CYP2E1 has also been found to activate compounds which induce hepatocarcinogenesis; in rats with chemically induced hepatic cancer, 60 mg/kg resveratrol (HED of 681 mg for a 70 kg human) reduced Cyp2e1 expression and suppressed diethylnitrosamine-induced carcinogenesis (Wu et al., 2013). Although resveratrol displayed weak inhibition (IC₅₀ of 150 µM) of CYP2E1 in human liver microsomes (Piver et al., 2001), the role of CYP2E1 in the activation of carcinogens, as well as in the metabolism of common xenobiotics like acetaminophen and ethanol (Joshi and
Tyndale, 2006) warrants the need for further research regarding resveratrol’s effect on CYP2E1 in vivo.

**CYP3A4**

Among the most important and investigated components of xenobiotic metabolism, CYP3A enzymes, particularly CYP3A4, are involved in the metabolism at least 40% of human prescription drugs (Thummel and Wilkinson, 1998), including many anticancer drugs (Kacevska et al., 2008). Therefore, when considering resveratrol as a cancer prevention agent, it is necessary to predict potential interactions with CYP3A4 to ensure the safety of patients receiving chemotherapeutics (Goey et al., 2013).

Resveratrol has been shown to attenuate PXR-induced expression of Cyp3a11, an ortholog of CYP3A4, in mouse hepatocytes (Deng et al., 2014). It also irreversibly inactivates CYP3A4 by undergoing metabolism by the enzyme, then forming an irreversible complex by means of a reactive intermediate (Chan and Delucchi, 2000; Regev-Shoshani et al., 2004; Zhou et al., 2005). The stilbene has been shown to inhibit CYP3A4 at IC50 values of 4.0 µM in human liver microsomes (Piver et al., 2001) and in heterologously expressed isozymes at IC50 values ranging from 1.1-10 µM (Piver et al., 2001; Yu et al., 2003; Zhan et al., 2015). Injection of CD1 mice with 50 mg/kg resveratrol (HED of 284 mg for a 70 kg human) resulted in an approximately 61% loss of hepatic Cyp3a activity (Canistro et al., 2009).

CYP3A4 is one of the few CYP enzymes with data that directly shows resveratrol-drug interactions. Rats treated with 2.5 and 10 mg/kg resveratrol (HED of 28 mg and 114 mg, respectively, for a 70 kg human), experienced increased plasma levels of calcium channel blockers, nicardipine (Choi et al., 2009) and diltiazem (Hong et al., 2008), which are both substrates of
CYP3A4. In a clinical trial with healthy volunteers, 500 mg/day resveratrol for 10 days significantly decreased the metabolite to parent ratio of carbamazepine and increased the AUC of carbamazepine by 1.49-fold, indicating that metabolism of the anticonvulsant by CYP3A4 was inhibited (Bedada and Nearati, 2015). In another clinical study, 1 g daily resveratrol significantly inhibited CYP3A4 activity among participants with a high baseline activity, as measured from changes in the AUC of buspirone, a CYP3A4 probe drug (Chow et al., 2010). These studies strongly suggest that individuals using drugs that are metabolized by CYP3A4 should carefully review the effect of nutrient-drug interactions on their treatment plan before supplementation with resveratrol.

**Resveratrol and phase II metabolism**

In phase II metabolism, xenobiotics and endogenous compounds are primed for elimination via bile or urine. The conjugating enzymes involved in this process typically act as transferases, adding large polar groups to their substrates (Jancova et al., 2010). Such enzymes include glutathione S-transferase, UDP-glucuronosyl transferase, NAD(P)H hydrogenase, quinone 1, and catechol-O-methyl transferase. By inducing phase II enzymes, resveratrol may facilitate the removal of carcinogens from the body. The effects of resveratrol *in vivo* and *in vitro* are summarized in Tables 1 and 2, respectively.

**Glutathione S-transferase (GST)**

Glutathione S-transferase (GST) is a common phase II enzyme that facilitates the transfer reaction between the thiol moiety of glutathione and the substrate intended for elimination (Van Bladeren, 2000). This enzyme is found in most organisms and protects against exposure to
carcinogens and oxidative stress. Substrates of GST include N-nitrosamines and polycyclic aromatic hydrocarbons like B[a]P (Pool-Zobel et al., 2005). GST also inactivates estrogen quinones, protecting against estrogen-induced carcinogenesis (Yager, 2015). Thus, induction of GST is considered a potential strategy for prevention of multiple conditions, including various cancers.

A growing body of evidence from animal models strongly supports an induction effect of resveratrol on GST. In rats with nonalcoholic fatty liver disease, 10 mg/kg resveratrol (HED of 114 mg for a 70 kg human) induced total Gst activity by 84% (Ali et al., 2015). In aged mice, administration of 16.67 mg/kg resveratrol (95 mg for a 70 kg human) for 6 months increased heart and liver Gst levels (Tung et al., 2015); this is particularly interesting considering that decreased GST levels in aging humans has been associated with neurological decline (Salminen and Paul, 2014). Resveratrol treatment also restored overall Gst activity in spontaneously hypertensive rats (Javkhedkar et al., 2015), streptozotocin-induced diabetic rats (Sadi et al., 2015), and in mice with B[a]P-induced lung carcinogenesis (Liu et al., 2015). In humans, 1 g daily dose of resveratrol for four weeks induced GST-π activity among individuals with low baseline expression, although the overall effect was found to be insignificant (Chow et al., 2010). Given the evidence that supports and association between GST induction and reduced cancer risk (Pool-Zobel et al., 2005; McIlwain et al., 2006), induction of this enzyme could be a significant mechanism by which resveratrol provides protection against cancer.

**Uridine diphosphate- glucuronosyl transferase (UGT)**

UDP-glucuronosyl transferases (UGTs) are detoxifying enzymes that facilitate the elimination of xenobiotics, such as carcinogens and pollutants, and endogenous substances, such
as bile acids and hormones, through glucuronidation (Jancova et al., 2010). Increased expression of UGTs, therefore, has been associated with reduced risk of various cancer types (Guillemette et al., 2014). In Caco-2 intestinal cancer cells, 20 µM resveratrol significantly induced UGT1A1 expression (Iwuchukwu et al., 2011). In a clinical trial, 1 g daily resveratrol for four weeks significantly increased bilirubin clearance among subjects with low baseline UGT1A1 activity; however, the resveratrol intervention had a minimal effect on overall bilirubin clearance (Chow et al., 2010). The observed lack of UGT1A1 induction in this clinical study could be due to resveratrol’s low bioavailability, as resveratrol may not reach adequate concentrations in the liver to significantly induce bilirubin-UGT; however, hepatic UGT activity was increased 83% in mice injected with 25 mg/kg (HED of 142 mg for a 70 kg human) (Canistro et al., 2009). While induction of UGT could be an effective means by which resveratrol provides protection against cancer, evidence is lacking for this particular mechanism.

**NAD(P)H dehydrogenase, quinone 1 and 2 (NQO1 and NQO2)**

NAD(P)H dehydrogenase, quinone 1 (NQO1) plays an important role in chemoprevention due to its ability to catalyze the reduction of quinones to catechols (Lu et al., 2008). This reduction is thought to decrease quinone-induced oxidative stress and therefore protect the cell (Zhang et al., 2012). Conversely, its homolog, NAD(P)H dehydrogenase, quinone 2 (NQO2) does not appear to share the protective effects of NQO1, as NQO2 knockout mice demonstrated increased antioxidant and detoxifying enzyme expression (Buryanovskyy et al., 2004). Resveratrol has been shown to induce NQO1 (Mayhoub et al., 2012) and inhibit NQO2 (Buryanovskyy et al., 2004), which is doubly favorable in terms of chemoprevention. Furthermore, R3S has been found to induce NQO1 more potently than resveratrol in murine hepatoma cells, with only 2.6 µM needed to double the
concentration of NQO1 (Hoshino et al., 2010).

The induction of NQO1 by resveratrol has significant implications for breast cancer prevention. Estrogens can be metabolized into catechols and further oxidized into *ortho*-quinones, which react with DNA to form adducts (Cavalieri and Rogan, 2014). By inducing NQO1, resveratrol may facilitate the reduction of semiquinones to catechols and subsequent inactivation by catechol-*O*-methyl transferase (Cavalieri and Rogan, 2014; Yager, 2015). Rats co-treated with 3 mg estradiol daily and 50 mg resveratrol every other month for eight months had increased tumor latency and reduced estradiol-induced tumor development, as well as upregulation of the *Nqo1* gene in mammary tissue due to increased expression of nuclear factor erythroid 2-related factor 2 (Singh et al., 2014).

The protective benefits of resveratrol, however, may be age- or tissue-dependent. When mice of varying ages were given 16.67 mg/kg resveratrol daily (HED of 95 mg for a 70 kg human) for six months, Nqo1 activity increased in young (2-month-old) and aged (18-month-old) mice but decreased in mature (12-month-old) mice (Tung et al., 2013). Aged mice given the same resveratrol treatment as described above had increased Nqo1 activity in the brain and liver, but decreased activity in the heart (Tung et al., 2015).

Clinical evidence has suggested that the effects of resveratrol may differ among patient populations. In patients with nonalcoholic fatty liver disease, for example, 3 g daily resveratrol for eight weeks decreased NQO1 expression in peripheral blood mononuclear cells (Chachay et al., 2014), suggesting a disease-dependent response. These results may also reflect dose-dependence, as 3 g daily can be considered a very high dose of resveratrol. Dose-dependent effects were also reported in a clinical trial during which patients receiving 5 mg resveratrol had higher levels of NQO1 in colorectal mucosa than those receiving 1 g resveratrol (Cai et al., 2015). Resveratrol’s
varied effects on NQO1 further underscore the need to determine which populations may benefit from supplementation.

**Transport proteins**

Transport proteins play a major role in drug elimination. These transporters also work in tandem with phase I and II metabolism, as various drugs can be oxidized or conjugated to form substrates for these transporters (Chan et al., 2004). Transporters include ATP-binding cassette (ABC) transport proteins, breast cancer resistance proteins (BCRP), multidrug resistance proteins 2 and 3 (MDR2 and MDR3), and organic anion transporting polypeptides (OATPs).

ABC transport proteins are present in the apical membrane of the small intestine, liver, and kidney, facilitating the elimination of xenobiotics by active efflux (Chan et al., 2004). Interactions with these transporters, therefore, could alter the bioavailability of various xenobiotics and lead to significant drug interactions. Supplemental doses of resveratrol (500 mg/day) have been shown to inhibit P-glycoprotein (P-gp), an ABC transporter (Bedada et al., 2014), as well as increase the bioavailability of P-gp substrates, including doxorubicin, a first-line breast cancer drug (Kim et al., 2014). Resveratrol’s inhibitory effect on ABC transport proteins like P-gp demand further research. While its enhancement of P-gp substrate bioavailability may be beneficial, identifying the extent of resveratrol’s activity is necessary to prevent toxicity.

Resveratrol and its metabolites are substrates for BCRPs, which are responsible for their active efflux at the enterocyte; the stilbene’s glucuronidated metabolites, however, act as substrates for MDR2 and MDR3, which allow these metabolites to enter the bloodstream (Maier-Salamon et al., 2013). Additionally, resveratrol and its major sulfate metabolite, R3S, have been shown to interact with OATPs in Chinese hamster ovary and breast cancer cells, allowing for their uptake
into enterocytes and hepatocytes (Riha et al., 2014). Competition for these transporters could result in significant interactions with drugs, including anticancer drugs like docetaxel and imatinib (Kalliokoski and Niemi, 2009), or with flavonoids and other polyphenols that could potentially confer protection against cancer.

Conclusions

Resveratrol has received a great amount of attention over the past two decades due to its ability to inhibit cancer initiation, promotion, and progression in vitro and in multiple animal models (Singh et al., 2015). While these chemoprevention properties have been attributed many different mechanisms, we have focused on resveratrol’s effects on xenobiotic metabolism and drug transporters. The stilbene has been shown to inhibit several important cytochrome P450 enzymes, many of which are responsible for the bioactivation of carcinogens. Furthermore, resveratrol induces conjugating enzymes, facilitating the elimination of toxic substances.

While in vitro studies provide a controlled environment for precise quantification of resveratrol’s effect on phase I and II metabolism, such studies fail to capture its clinical activity. With the evidence available, we have attempted to consider the activities of resveratrol’s sulfated and glucuronidated metabolites along with the parent compound in order to evaluate the translatability of certain animal models to humans; however, direct evaluation of the activity of resveratrol’s metabolites, which may each have different targets, is lacking.

In summary, caution should be taken when using supplemental doses of resveratrol for health benefits such as chemoprevention. This polyphenol has demonstrated significant interactions with phase I and II enzymes both in vitro and in vivo. While these interactions may be beneficial for some individuals in terms of reduced activation and higher clearance of carcinogens,
they could also result in nutrient-drug interactions. This is particularly concerning for those individuals taking anticancer medications like tamoxifen, or those on medications with narrow therapeutic ranges such as warfarin (Sanderson et al., 2005). In addition to these concerns, emerging evidence suggests that low-dose resveratrol may be more beneficial for disease prevention (Cai et al., 2015; Posadino et al., 2015). Additional research is needed before recommendations can be made for resveratrol supplementation above dietary levels for either prevention or for therapeutic purposes.
PART 2 - EFFECT OF RESVERATROL ON THE URINE METABOLOME

Introduction

Resveratrol, a naturally occurring trans-stilbene found in grape skins and peanuts, has been attributed to many health benefits, including cancer prevention (Bhat and Pezzuto, 2002; Dong, 2003). Multiple mechanisms have been proposed to supporting resveratrol’s putative chemopreventive activity including modulation of drug- and carcinogen-metabolizing enzymes, (Chow et al., 2010, 2014; Detampel et al., 2012), ability to act as an antioxidant, and anti-inflammatory capability (Zykova et al., 2008). Resveratrol is also suspected to correct metabolic dysregulation, thereby decreasing the risk for cancer, particularly obesity-related cancers such as breast (Fay et al., 2009; Seyfried and Shelton, 2010). It is logical, therefore, to evaluate the systemic effects of resveratrol among postmenopausal women with a high BMI, whose circulating estrogen levels correlate with adiposity (Cleary and Grossmann, 2009; Maccio and Madeddu, 2011). It is necessary to begin looking at the systemic effect of resveratrol in order to begin elucidating its metabolic targets in vivo. One method of detecting these interactions is through metabolomic analysis.

The metabolome is the sum of all small molecules (lipids, carbohydrates, proteins, vitamins) present in a given biological sample (Fiehn, 2002). Many of these low molecular weight molecules are byproducts of both the host’s metabolism and the metabolism of its microbiome (Matsumoto et al., 2012). Metabolomics, or the study of the metabolome, aims to identify these small molecule byproducts in order to elucidate the metabolic processes that have occurred (Gomase et al., 2008). By providing a “snapshot” of the metabolome, metabolomics can be used as a tool for biomarker identification (Vinayavekhin and Saghatelian, 2010; Vinayavekhin et al.,
2010), early disease detection (Nordström and Lewensohn, 2010), and for evaluating the systemic effects of drugs, environmental pollutants, and other xenobiotics (Johnson et al., 2012).

Previous authors have used targeted metabolomics to characterize the biomarker imprinting of resveratrol-containing foods (Ye et al., 2007; Brito et al., 2014; Urpi-Sarda et al., 2015), while others have investigated the systemic effects of supplemental resveratrol in murine models (Zhou et al., 2012; Etxeberria et al., 2015; Wang et al., 2015). The method employed in this study allows for the discovery of novel mechanisms and metabolic pathways by which resveratrol may protect against cancer. It also allows for the detection and characterization of resveratrol metabolites, for which there is currently limited data.

For this study, metabolomics was used to identify the systemic effects of resveratrol at a high pharmacological dose. An untargeted metabolomic analysis was performed on stored urine samples from a clinical study in which postmenopausal women with a high BMI were given 1 g daily resveratrol for 12 weeks (Chow et al., 2014). As opposed to targeted analyses, which aim to quantify known metabolites, this untargeted metabolomic profiling experiment captured all metabolites within a particular mass range in order to provide both known and structurally novel metabolites (Vinayavekhin and Saghatelian, 2010; Want et al., 2010). The goal of this study is to identify and characterize metabolic perturbations that occurred in response to resveratrol supplementation in order to determine its effects on multiple metabolic pathways. In elucidating these metabolic changes, targeted analyses may be employed in the future to capture biomarkers that indicate individuals who may benefit from resveratrol supplementation. To the best of our knowledge, this is the first study to use untargeted metabolomics to characterize the systemic effect of supplemental resveratrol in humans.
Methods

Sample collection. Urine was collected from postmenopausal women with a high BMI (≥25 kg/m²) participating in pilot study in which they were given resveratrol (1 g QD) for 12 weeks (Chow et al., 2014). Samples were stored at -80 °C prior to analysis.

Reagents. Methanol, acetonitrile, formic acid, isopropanol, leucine enkephalin acetate salt hydrate, and sodium azide were obtained from Sigma Aldrich Corporation (St. Louis, MO).

Urine sample preparation. Urine samples were centrifuged at 4°C in volumes of 60 µL at 10,000 x g for 10 minutes to remove particulates, after which 50 µL was mixed with 100 µL water and placed in glass LC vials. The samples were stored at -20 °C.

UPLC-MS data acquisition and preprocessing. Prepared vials were centrifuged at 10,000 x g for 5 minutes at 4 °C, then loaded into an autosampler also maintained at 4 °C. The electrospray ionization mode was selected (positive or negative). UPLC-QTOF instrument was set up using leucine enkephalin (200 pg/µL in water:acetonitrile 50:50 solution) as the lockmass solution and sodium formate (0.1 mg/mL in water, diluted to 0.01 mg/mL solution in 90% isopropanol and 10% water) as the calibration solution.

UPLC-MS of urine samples. Urine samples (5 µL) were injected onto a 2.1 x 100 mm (1.7 µm) Acquity UPLC HSS T3 column (Waters Corporation, Milford, MA) and eluted using optimized gradients (A = water, 0.1% formic acid; B = acetonitrile, 0.1% formic acid). Samples were analyzed using a UPLC system (UPLC Acquity, Waters MS Technologies Corporation, Manchester, UK) coupled online to a Xevo G2S-QTOF (Waters MS Technologies Corporation, Manchester, UK). Samples were analyzed in positive and negative electrospray modes, with a scan range of 50–1000 m/z. Fragmentation information was generated using MS² experiments collected at both low (6V) and high (40V) collision energies. Intra-day and inter-day quality control (QC) samples (pooled urine samples from sample set) were injected at the start of the analyses for
column conditioning and at multiple points during each batch. Metabolomic profile alignment, peak detection and integration data were achieved using Progenesis QI software (Nonlinear Dynamics, Durham, North Carolina, USA).

**Data analysis.** Aligned data sets were exported into SIMCA-P (Umetrics) for further multivariate analysis. Principal components analysis (PCA) and orthogonal partial least squares-discriminate analysis (OPLS-DA) were used to determine which metabolites were most important in separating the treatment groups (baseline versus post-intervention). Subsequent construction of an S-plot provided a visual model to identify metabolites that were affected most by resveratrol supplementation. Pre-to post-intervention changes in metabolite levels were compared using two-sample t-tests.

**Results**

Multivariate analysis revealed significant differences between participants’ metabolomes prior to resveratrol supplementation and post-intervention. Figures 4 and 5 depict the multivariate analysis plots for positive and negative ionization mode, respectively. Principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) plots depict the significance of these changes. There is a clear distinction between profiles collected at baseline (green) and profiles collected at the end of the trial (blue). Figures 4c and 5c show that the OPLS-DA plots had an excellent summary of fit, and show good predictability of this model. S-plots (4d and 5d) show those metabolites that were present in higher concentrations at baseline compared to post-intervention, as well as metabolites that were higher post-intervention compared to baseline. Data from participants who ended the trial early was excluded from analysis.
Figure 4. Multivariate analysis of urine metabolites detected in positive ionization mode.

(a) Principal component analysis (PCA) illustrates a summary of baseline and post-intervention groups (shown in green and blue, respectively). (b) Orthogonal partial least squares- discriminant analysis (OPLS-DA) plot illustrates distinct differences in the metabolic profiles. (c) The predictability of this model is shown by an excellent summary of fit for the OPLS-DA plot. (d) An S-plot of the data shows those metabolites that had the greatest changes between baseline and post-intervention groups. These plots were generated after excluding data from patients who ended the trial early.
Figure 5. Multivariate analysis of urine metabolites detected in negative ionization mode.

(a) Principal component analysis (PCA) illustrates a summary of baseline and post-intervention groups (shown in green and blue, respectively). (b) Orthogonal partial least squares- discriminant analysis (OPLS-DA) plot illustrates distinct differences in the metabolic profiles. (c) The predictability of this model is shown by an excellent summary of fit for the OPLS-DA plot. (d) An S-plot of the data shows those metabolites that had the greatest changes between baseline and post-intervention groups. These plots do not include data points for patients who ended the trial early.
The high baseline and high post-intervention metabolites were putatively identified by comparing their mass and MS\textsuperscript{E} fragmentation patterns to compounds in online databases. Putative identifications and fold-changes are listed in Table 3.

Many of the high post-intervention compounds are suspected to be resveratrol or its metabolites. This assumption is supported by multi-fold changes in compound concentrations, ranging from 98-fold changes to infinitely higher concentrations. Other identified compounds include dietary metabolites and several drug metabolites, particularly naproxen-O-glucuronide.
Table 3. Putative urine metabolite identification, sorted by fold change (descending order).

<table>
<thead>
<tr>
<th>Retention time_m/z</th>
<th>Identification</th>
<th>Source</th>
<th>Ion Mode</th>
<th>Pre-Mean (standard deviation)</th>
<th>Post-Mean (standard deviation)</th>
<th>Fold Change</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher Pre-Intervention</td>
<td>3.93_319.1269m/z</td>
<td>Parakmerin A</td>
<td>Nutmeg</td>
<td>(+) 185.4 (146.1)</td>
<td>13.6 (21.5)</td>
<td>13.6 &lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.42_239.0900m/z</td>
<td>3,4,5-Trimethoxycinnaminic acid</td>
<td>Organic acid found in urine</td>
<td>(+) 158.976 (107.1)</td>
<td>21.1 (31.1)</td>
<td>7.5 &lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.87_484.2532n--507.2425m/z</td>
<td>17-hydroxyandrostan-3-glucuronide</td>
<td>Steroid metabolite</td>
<td>(+) 261.9 (238.8)</td>
<td>37.7 (92.5)</td>
<td>6.9 &lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.87_442.1587m/z</td>
<td>Simulansamide</td>
<td>Szechuan pepper</td>
<td>(-) 105.27 (65.4)</td>
<td>19.32 (37.5)</td>
<td>5.4 &lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.93_230.0122m/z</td>
<td>Parakmerin A</td>
<td>Nutmeg</td>
<td>(-) 1,187.9 (1,393.2)</td>
<td>241.54 (1,100.4)</td>
<td>4.9 0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.58_274.2018m/z</td>
<td>Heptanoylcarnitine</td>
<td>Amino acid derivative</td>
<td>(+) 270.6 (265.0)</td>
<td>56.9 (108.2)</td>
<td>4.8 &lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.56_276.0903m/z</td>
<td>Unidentified</td>
<td>Product of Rosaceae family</td>
<td>(-) 128.35 (71.6)</td>
<td>26.72 (51.7)</td>
<td>4.8 &lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.58_454.1828n--4.58_453.1755m/z</td>
<td>Koenoline</td>
<td>Curryleaf tree</td>
<td>(-) 997.5 (1,199.7)</td>
<td>210.6 (615.2)</td>
<td>4.7 0.001</td>
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</tr>
<tr>
<td></td>
<td>3.99_332.2073m/z</td>
<td>Unidentified</td>
<td>Curryleaf tree</td>
<td>(+) 632.7 (480.1)</td>
<td>146.5 (172.9)</td>
<td>4.3 &lt;0.0001</td>
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</tr>
<tr>
<td></td>
<td>4.45_397.1454m/z</td>
<td>Unidentified</td>
<td>---</td>
<td>(-) 66.85 (19.5)</td>
<td>16.02 (24.7)</td>
<td>4.2 &lt;0.0001</td>
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<tr>
<td></td>
<td>4.45_185.0812m/z</td>
<td>Unidentified</td>
<td>Milk and milk products</td>
<td>(-) 855.46 (404.4)</td>
<td>212.68 (205.8)</td>
<td>4.0 &lt;0.0001</td>
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</tr>
<tr>
<td></td>
<td>4.43_439.1599m/z</td>
<td>3-Hydroxychavicol 1-[rhamnosyl-(1-&gt;6)-glucoside]</td>
<td>Herbs and spices</td>
<td>(-) 632.44 (231.2)</td>
<td>189.03 (176.8)</td>
<td>3.3 &lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>m/z</td>
<td>Description</td>
<td>Change</td>
<td><em>P</em></td>
<td>Change</td>
<td><em>P</em></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4.42_216.0991n</td>
<td>Small peptide</td>
<td>(-)</td>
<td>172.32</td>
<td>56.95</td>
<td>3.0</td>
<td></td>
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<tr>
<td>4.42_215.0917m/z</td>
<td></td>
<td>(+)</td>
<td>1,412.3</td>
<td>529.1</td>
<td>2.7</td>
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<td>4.45_284.1863m/z</td>
<td>Unidentified</td>
<td>(+)</td>
<td>0.3</td>
<td>0.0</td>
<td>&lt;0.0001</td>
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<tr>
<td>4.29_809.2307m/z</td>
<td>Resveratrol monoglucuronide (2M)</td>
<td>(+)</td>
<td>0.0</td>
<td>3,484.2</td>
<td>Infinity</td>
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<tr>
<td>3.97_808.2235n</td>
<td>Resveratrol monoglucuronide (2M)</td>
<td>(+)</td>
<td>0.0</td>
<td>1,397.7</td>
<td>Infinity</td>
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<tr>
<td>4.44_812.2548n</td>
<td>(E)-Oxyresveratrol 3'-O-b-D-glucoside</td>
<td>(+)</td>
<td>0.0</td>
<td>1,178.7</td>
<td>Infinity</td>
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<tr>
<td>4.30_808.2237n</td>
<td>Unidentified</td>
<td>(+)</td>
<td>0.0</td>
<td>1,097.5</td>
<td>Infinity</td>
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<tr>
<td>3.39_580.1438n</td>
<td>Graveobioside A</td>
<td>(+)</td>
<td>0.3</td>
<td>750.8</td>
<td>Infinity</td>
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<tr>
<td>3.84_483.0600m/z</td>
<td>Unidentified</td>
<td>(-)</td>
<td>0.8</td>
<td>16,277.4</td>
<td>20,346.8</td>
<td></td>
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<tr>
<td>4.57_227.0707m/z</td>
<td>Resveratrol</td>
<td>(-)</td>
<td>0.1</td>
<td>11,189.9</td>
<td>111,899.0</td>
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<tr>
<td>4.46_309.0433m/z</td>
<td>Unidentified</td>
<td>(-)</td>
<td>0.8</td>
<td>16,277.4</td>
<td>20,346.8</td>
<td></td>
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<tr>
<td>4.57_307.0295m/z</td>
<td>Resveratrol monosulfate</td>
<td>(-)</td>
<td>10.0</td>
<td>186,222.4</td>
<td>18,622.2</td>
<td></td>
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<tr>
<td>4.34_307.0281m/z</td>
<td>Resveratrol monosulfate</td>
<td>(-)</td>
<td>2.0</td>
<td>23,477.2</td>
<td>11,738.6</td>
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<tr>
<td>4.60_309.1789m/z</td>
<td>Dihydroresveratrol sulfate</td>
<td>(-)</td>
<td>0.7</td>
<td>6,897.4</td>
<td>9,853.4</td>
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<tr>
<td>4.29_404.1105n</td>
<td>Resveratrol monoglucuronide</td>
<td>(-)</td>
<td>9.5</td>
<td>75,004.2</td>
<td>7,895.2</td>
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<tr>
<td>4.29_405.1186m/z</td>
<td>Resveratrol monoglucuronide</td>
<td>(+)</td>
<td>1.0</td>
<td>7,332.6</td>
<td>7,332.6</td>
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<td></td>
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</table>

Higher Post-Intervention
<table>
<thead>
<tr>
<th>m/z</th>
<th>Compounds</th>
<th>Species</th>
<th>Status</th>
<th>Ratio</th>
<th>MConf</th>
<th>RConf</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.65_483.064</td>
<td>Resveratrol sulfate glucuronide</td>
<td>(-)</td>
<td>10.6</td>
<td>71,281.3</td>
<td>6,724.7</td>
<td>&lt;0.0001</td>
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<tr>
<td>3.94_485.075</td>
<td>Resveratrol sulfate glucuronide</td>
<td>(-)</td>
<td>18.7</td>
<td>103,685.8</td>
<td>5,544.7</td>
<td>&lt;0.0001</td>
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<tr>
<td>3.94_486.084</td>
<td>Resveratrol metabolite</td>
<td>(+)</td>
<td>0.2</td>
<td>926.6</td>
<td>4,633.0</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>3.69_309.044</td>
<td>Resveratrol monosulfate</td>
<td>(+)</td>
<td>0.4</td>
<td>899.2</td>
<td>2,248.0</td>
<td>&lt;0.0001</td>
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<tr>
<td>4.44_389.123</td>
<td>Dihydroresveratrol sulfate glucuronide</td>
<td>(+)</td>
<td>0.4</td>
<td>896.0</td>
<td>2,240.0</td>
<td>&lt;0.0001</td>
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<tr>
<td>4.44_406.126</td>
<td>Naproxen O-glucuronide</td>
<td>(-)</td>
<td>71.9</td>
<td>120,077.9</td>
<td>1,670.1</td>
<td>&lt;0.0001</td>
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<tr>
<td>4.27_406.126</td>
<td>Edulisin VI</td>
<td>(-)</td>
<td>18.3</td>
<td>29,703.8</td>
<td>1,623.2</td>
<td>&lt;0.0001</td>
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<tr>
<td>3.97_615.063</td>
<td>Dihydroresveratrol sulfate</td>
<td>(-)</td>
<td>11.7</td>
<td>16,081.4</td>
<td>1,374.5</td>
<td>&lt;0.0001</td>
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<tr>
<td>3.83_469.080</td>
<td>Resveratrol sulfoglucoside</td>
<td>(-)</td>
<td>20.9</td>
<td>15,752.3</td>
<td>753.7</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>3.4_229.086</td>
<td>Resveratrol (cis?)</td>
<td>(+)</td>
<td>12.4</td>
<td>8,158.4</td>
<td>657.9</td>
<td>&lt;0.0001</td>
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<tr>
<td>4.27_230.082</td>
<td>3,4-Dihydro-6-methoxy-2,2-dimethyl-2H-1-benzopyran-4-ol</td>
<td>(+)</td>
<td>5.3</td>
<td>2,918.0</td>
<td>550.6</td>
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<td>4.44_371.113</td>
<td>Glycyrrhizaflavonol A</td>
<td>(+)</td>
<td>8.9</td>
<td>3,730.1</td>
<td>419.1</td>
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<td>4.44_407.134</td>
<td>(E)-Oxyresveratrol 3'-O-b-D-glucoside</td>
<td>(+)</td>
<td>19.1</td>
<td>7,753.2</td>
<td>405.9</td>
<td>&lt;0.0001</td>
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</tr>
<tr>
<td>Retention Time</td>
<td>Substance</td>
<td>Metabolite</td>
<td>Change</td>
<td>Mass (amu)</td>
<td>Mass Error (ppm)</td>
<td>p-Value</td>
<td></td>
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<tr>
<td>-----------------</td>
<td>-----------------------------------------------</td>
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<td></td>
</tr>
<tr>
<td>3.64_405.1187m/z</td>
<td>Resveratrol monoglucuronide</td>
<td>Resveratrol</td>
<td>(+)</td>
<td>3.9 (9.4)</td>
<td>975.7 (588.3)</td>
<td>&lt;0.0001</td>
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<td>4.57_386.9842m/z</td>
<td>Resveratrol disulfate</td>
<td>Resveratrol</td>
<td>(-)</td>
<td>45.8 (107.7)</td>
<td>11,019.4 (5,461.8)</td>
<td>&lt;0.0001</td>
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<td>3.64_229.0869m/z</td>
<td>Resveratrol (trans?)</td>
<td>Grape skins</td>
<td>(+)</td>
<td>4.8 (9.0)</td>
<td>935.2 (486.6)</td>
<td>&lt;0.0001</td>
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<tr>
<td>3.97_405.1187m/z</td>
<td>Resveratrol monoglucuronide</td>
<td>Resveratrol</td>
<td>(+)</td>
<td>60.6 (171.8)</td>
<td>5,957.1 (3,262.1)</td>
<td>&lt;0.0001</td>
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<tr>
<td>4.27_406.1263n</td>
<td>Naproxen O-glucuronide</td>
<td>Naproxen</td>
<td>(+)</td>
<td>51.1 (264.1)</td>
<td>4,316.9 (2,836.2)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

This study demonstrated that supplementation with resveratrol (1 g QD) for 12 weeks significantly altered the metabolomic profiles of post-menopausal women with a high BMI. UPLC-QTOF was used to detect metabolites in urine samples from a previous resveratrol study (Chow et al., 2014) and multivariate analysis was performed on the aligned data in order to determine metabolites whose concentrations were highest at baseline and post-intervention. Identifying these compounds could further elucidate resveratrol’s systemic effect and provide biomarkers for future resveratrol interventions.

Multivariate analysis showed significant differences between the metabolic profiles of samples collected pre- and post-intervention. Interestingly, data from participants who ended the trial early had metabolic profiles comparable to baseline measures (data not shown), suggesting that longer durations of resveratrol supplementation are necessary in order to observe these metabolic alterations. This finding is not surprising, as it may take longer for adequate concentrations of resveratrol and its metabolites to be present systemically due to limited bioavailability (Wenzel and Somoza, 2005; Neves et al., 2012; Pangeni et al., 2014). Indeed, the majority of metabolites putatively identified were resveratrol metabolites as well as the parent compound. Confidence in these identifications is supported by large fold-changes, some infinitely higher relative to baseline measurements. These compounds also contained characteristic fragmentation patterns for resveratrol and its metabolites, further increasing confidence in these putative identifications.

In addition to resveratrol-related compounds, other dietary metabolites were identified from sources such as root vegetables, green vegetables, herbs and spices, and flavoring agents. Although fasting samples were obtained during the study, significant changes in dietary behavior during the trial may have affected participants’ metabolomes. Concurrent supplement use could
have also created these observed effects. In the United States, over 40 million adults use herbs or supplements, with higher rates seen among women and older adults (Wu et al., 2014). Participants in this study seemed to follow this trend, as 76% of these women were taking at least one supplement during the resveratrol trial. It is unknown whether these compounds derived from the diet alone, or were present as additives in supplements. Identifying these food compounds, however, highlights the fact that, at concentrated doses, resveratrol and other dietary supplements become pharmacological agents, with the potential to interact with other pharmacological agents, including medications.

One drug metabolite, naproxen-\textit{O}-glucuronide (a non-steroidal anti-inflammatory drug metabolite), was also putatively identified. There were two participants taking naproxen continuously throughout the duration of the study, and an additional two that took it at least once. There was an 84.5-fold increase in naproxen positive ion mode and 1,670.1-fold increase in negative ion mode. This increase could reflect induction of UDP-glucuronosyl transferase (UGT) by resveratrol. This induction has been reported previously as a mechanism by which resveratrol may prevent carcinogenesis (Iwuchukwu et al., 2011). Participants taking naproxen did not report an increase in dosage towards the end of the trial, which increases our confidence in this proposed mechanism, however, a direct study of the interaction between naproxen and resveratrol is necessary in order to confirm this result.

This study had several limitations. First, the urine samples used for this study had been stored for approximately four years. Although the samples were stored at -80°C and care was taken to avoid premature thawing, composition of the urine samples may have changed slightly from initial acquisition. Second, six participants of the clinical study from which urine samples were obtained discontinued the trial early due to adverse events (Chow et al., 2014). While these women
were excluded from our analysis, resveratrol could have exerted significant effects among these women; if true, such effects were not captured by our analysis. Future exploratory analyses will also determine whether these participants had different metabolomes at baseline. If consistent patterns are observed, this could ultimately allow us to predict individuals that might have an adverse reaction to resveratrol.

Strengths of this study include the well-controlled nature of the initial trial and the accuracy of the instrumentation used. Chow et al. maintained a detailed record of participant demographics and concurrent medications, and attempted to account for dietary variability by obtaining fasting pre- and post-intervention samples and employing a 2-week “washout” period prior to the trial, during which resveratrol-containing items were limited in the diet. The study also contained a well-characterized sample (postmenopausal women with a BMI ≥25 kg/m²); while we consider this to be a strength, it should be noted that such a limited sample population could be a weakness, as resveratrol may have different effects on those without these characteristics. Caution should be taken, therefore, when applying results from our analyses to different patient populations. Finally, our advanced instrumentation is a strength of this study, as the Xevo G2S Q-TOF has a high mass accuracy and is among the newest of models.

This study demonstrated that supplementation with a pharmacological dose of resveratrol for 12 weeks resulted in significant metabolic changes among postmenopausal women with a high BMI. Such metabolic changes could confer protection against carcinogenesis, although the preliminary data presented cannot confirm specific pathways. It is suspected that resveratrol induces UGT enzymes, thereby facilitating the elimination of potentially harmful xenobiotics. This mechanism was supported by elevated levels of the glucuronidated metabolite of naproxen. The next steps for our team is to further confirm the putatively identified compounds by comparing
their fragmentation patterns with those of pure standards. Future studies should aim to continue evaluating the systemic effects of resveratrol in additional patient populations to determine those who may benefit most from supplementation. Furthermore, identifying biomarkers of resveratrol supplementation could allow for earlier evaluation of treatment efficacy, aligning with the goals of individualized medicine.
SUMMARY

Resveratrol has demonstrated numerous health benefits in preclinical studies, including chemopreventive activity. These benefits are not always replicated *in vivo*, likely due to resveratrol’s low bioavailability. In fact, resveratrol is rapidly metabolized to its main sulfated and glucuronidated metabolites upon consumption, which necessitates the evaluation of these metabolites and their potential as chemoprevention agents. We found that resveratrol supplementation (1 g QD) altered the urine metabolomes of postmenopausal women with a high BMI. Metabolites that were altered the most by the intervention included resveratrol and its metabolites, dietary compounds, and naproxen-\(\text{-O-glucuronide}\), an NSAID metabolite. Although these are putative identifications, they suggest that resveratrol, despite low bioavailability, can significantly alter the metabolome and may elicit nutrient-drug interactions.
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EFFECTS OF RESVERATROL ON DRUG AND CARCINOGEN METABOLISM


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ABBREVIATIONS

ABC: ATP-binding cassette; AHR: aryl hydrocarbon receptor; B[a]P: benzo-a-pyrene; BCRP: breast cancer resistance proteins; BMI: Body Mass Index; BROD: 7-benzoxy resorufin O-deethylolation; CDNB: 1-chloro-2,4-dinitrobenzene; CNS: central nervous system; COX-1: cyclooxygenase-1; COX-2: cyclooxygenase-2; CYP: cytochrome P450; DMBA: dimethylbenz[a]anthracene; ECF: 7-ethoxy-4-trifluormethyl courmarin; EROD: ethoxyresorufin-O-deethylase; GST: glutathione-S-transferase; HED: human equivalent dose; MDR2/3: multidrug resistance proteins 2 and 3; NMS: N-methyl-N-nitrosourea; NQO1 : NAD(P)H dehydrogenase, quinone 1; NQO2: NAD(P)H dehydrogenase, quinone 2; NSAID: nonsteroidal anti-inflammatory drug; OATP: organic anion transporting polypeptide; P-gp: P-glycoprotein; PNPH: p-nitrophenol hydroxylase; R3G: resveratrol-3-O-glucuronide; R3S: resveratrol-3-O-sulfate; R34S: resveratrol-3-O-4'-O-disulfate; R4S: resveratrol-4'-O-sulfate; ROS: reactive oxygen species; TCDD: 2,3,7,8-tetrachloro-dibenzo-p-dioxin; TMS: 2,3,3'5'-tetramethoxystilbene; UPLC-QTOF: ultra-performance liquid chromatography quadrupole time of flight mass spectrometry; UGT: UDP-glucuronosyl transferase; XRE: xenobiotic response element