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REVIEW ARTICLE

The estrogenic activity of resveratrol: a comprehensive review of *in vitro* and *in vivo* evidence and the potential for endocrine disruption

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ABSTRACT

trans-Resveratrol, a polyphenolic stilbene of plant origin is structurally similar to natural and synthetic estrogens and has been classified a phytoestrogen. Direct binding of resveratrol to the nuclear estrogen receptor (ER) and modulation of its genomic activity was among the first of its reported pharmacological actions. Additionally, resveratrol in some investigations interacted with membrane bound ER and modulated non-genomic estrogenic activities. The compound was also reported to interfere in steroidogenesis and estrogen biosynthesis at multiple steps along the pathway. Resveratrol also inhibited hepatic and intestinal metabolism of estrogens and increased circulating levels of sex hormone binding globulin (SHBG). Recent investigations report estrogenic activities for resveratrol metabolites, especially for the predominant sulfate conjugate. The majority of these estrogenic effects have been observed *in vitro* using micro-molar concentrations. However, the daily consumption of 0.5–1 *g* of resveratrol supplements is sufficient to furnish plasma levels sufficient to initiate most of these actions. The diverse modes of estrogenic and hormonal activities of resveratrol can produce a progressive shift in the homeostatic balance of estrogens and other steroidal hormones to a new operational set point. While this could represent an opportunity for therapeutic benefit in a variety of endocrine related diseases, it may also pose risk of endocrine disruption following chronic exposure that warrants caution. Herein, a review of the current knowledge of resveratrol's estrogenic activity at the molecular, cellular and whole organism since it was reported two decades ago is provided followed by an assessment of endocrine disruption via an estrogenic mode of action.

KEY MESSAGE

Resveratrol interacts with ER and modulates its genomic and non-genomic activities. It also inhibits several enzymes in steroidogenesis and competes in estrogen metabolism. Commercial supplements reach dosages of 1000 mg per serving and the consumption of 0.5–1 *g* per day furnishes low micro-molar plasma levels sufficient to start these activities. The pleiotropic hormonal actions of resveratrol open an opportunity for clinical benefit, but also risk endocrine disruption if exposure is chronic or during critical windows of development.

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1. Introduction

Resveratrol (3,5,4′-trihydroxystilbene, MW 228.24 g/mol, Figure 1) is a natural polyphenolic stilbene and common constituent of several edible plants. Two geometric isomers have been purified for resveratrol, the most abundant sterically stable trans-isomer and the cis-isomer. Naturally, both isomers undergo glucosylation to form the glucoside derivatives *trans*-piceid and *cis*-piceid, respectively (Romero-Pérez et al. 1999). They also undergo polymerization at low concentrations to form natural oligostilbenes known as viniferins (Korhammer et al. 1995). The biosynthesis of resveratrol and its derivatives is particularly increased following specific physiological and pathological stresses. In addition, regional and cultivar variations influence the levels of resveratrol and its glucosylated and oligomeric products. The level of resveratrol in a variety of food and beverage products has been reported by several studies and ranges from a fraction of a microgram to less than 2 milligrams per product gram or liter (Langcake and Pryce 1976; Siemann and Creasy 1992; Burns et al. 2002; Weiskirchen and Weiskirchen 2016). Generally, the biological actions and therapeutic properties of the *trans*-isomer dominated most of the scientific literature and are the present focus of this review. However, it is noteworthy that there has been a simultaneous interest in the actions of the *cis*-isomer- albeit to a lower extent, and a rising interest in the actions of the glucoside and oligomeric derivatives and most recently, the biological metabolites of the *trans*-isomer.

Scientific and public interest in the pharmacological properties and possible health benefits of resveratrol initiated following its detection in red wine. Regular consumption of red wine has been associated with a lower incidence of cardiovascular disease in France despite of dietary patterns and demographics that are much in common with other western nations. The speculative role of resveratrol in this paradox has been suggested by preliminary *in vitro* results and findings in animals where it favorably modulated lipoprotein metabolism, decreased the biosynthesis of thromboxane B_2 and related eicosanoids and inhibited platelet aggregation (Goldberg et al. 1995; Pace-Asciak et al. 1995). Increased interest in resveratrol sparked following a report describing its beneficial effects in cancer (Jang et al. 1997). This report demonstrated that resveratrol inhibited tumor initiation and interfered with tumor propagation and progression. In yet another set of interesting studies, resveratrol was shown to prolong the lifespan of *Saccharomyces cerevisiae* and middle aged C57BL/6 mice fed a high calorie diet by programing a metabolic state that mimicked caloric restriction (Howitz et al. 2003; Baur et al. 2006). On these bases, several clinical studies were designed to address the preventative and therapeutic efficacy of resveratrol on a range of human ailments that include cancer, metabolic, cardiovascular and neurodegenerative diseases and excellent reviews describing the outcomes of these trials have been published (Baur and Sinclair 2006; Vang 2013; Park and Pezzuto 2015; Singh et al. 2015; Berman et al. 2017).

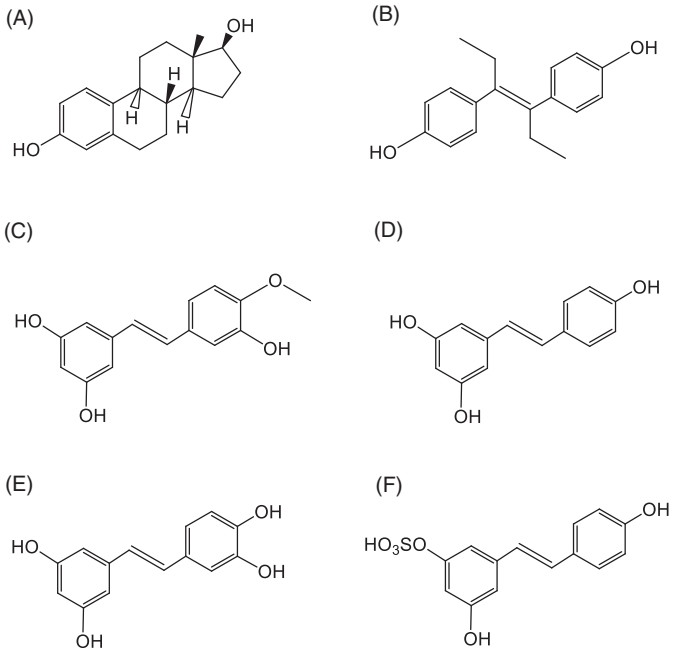

Figure 1. The chemical structures of (A) 17β-estradiol (E2), (B) diethylstilbestrol (DES), (C) rhapontigenin, (D) *trans*-resveratrol, (E) piceatannol, (F) *trans*-resveratrol-3-*O*-sulfate.

Extensive efforts to identify a molecular target for resveratrol over the past two decades revealed a wide range of cellular effectors that potentially mediate its health benefits. For example, studies showed that resveratrol inhibited the activity of cyclooxygenase 1 and 2 (COX1 and 2), ribonucleotide reductase (RNR), nuclear factor kappa B (NF-κB), signal transducer and activator of transcription 3 (STAT3) and aryl hydrocarbon receptor (AhR). On the other hand, resveratrol was shown to activate AMP activated protein kinase (AMPK), mitogen-activated protein kinase (MAPK), p53 and NAD-dependent deacetylase sirtuin-1 (Sirtuin 1), although the latter was riddled with controversy (Pacholec et al. 2010; Pezzuto 2011). Extensive reviews on the many targets of resveratrol have been published in recent years (Saiko et al. 2008; Athar et al. 2009; Kulkarni and Canto 2015). One early identified biological target of resveratrol has been the estrogen receptor (ER) and the compound has been classified a phytoestrogen by the late 1990s, early 2000s. The primary drive that initiated the investigation of resveratrol's

estrogenic effects was its structural similarity to primary estrogenic compounds such as the natural 17β-estradiol (E2), synthetic diethylstilbestrol (DES; 4-[(E)-4-(4-hydroxyphenyl)-hex-3-en-3-yl]phenol) and rhapontigenin, a plant glycoside that has been used in the treatment of estrogen deficiency disorders (Figure 1) (Gehm et al. 1997; Freyberger et al. 2001). Resveratrol shares a stilbene structure with DES and rhapontigenin and the phenolic A ring characteristic of natural estrogens. In addition to these structural similarities, resveratrol also shared in preclinical studies some of the effects of estrogens. For example, preclinical investigation revealed resveratrol produced cardioprotective effects, a long-known biological effect of estrogens (Mendelsohn and Karas 1999). The aim of this review was to collate all experimental and clinical evidence of direct and indirect estrogenic activities of resveratrol. This is followed by a discussion of the potential for endocrine disruption via an estrogenic mode of action.

2. Methods

To evaluate the diverse estrogenic activities of resveratrol, a brief overview of the molecular mechanisms of action of mammalian estrogens and the experimental approach used in thier detection is provided here followed by the approach undertaken in the development of this review. ER belongs to the steroid subfamily of nuclear receptors that also includes the androgen, progesterone, glucocorticoid and mineralocorticoid receptors (Evans 1988; Mangelsdorf et al. 1995). The natural ligands for ER are the most potent E2, estrone (E1) and estriol (E3). Activation of ER by these ligands produces mainly genomic effects that lead to transcriptional gene activation or repression, depending on the specific gene and cell-type. Estrogens are also known to bind a membrane associated version of ER or a G-protein coupled receptor designated GPER. This interaction produces quick non-genomic cellular responses such as alterations in the activity of important cytoplasmic and membrane associated regulatory proteins. In mammals, two ER isoforms have been identified and are designated ERα and ERβ. The two isoforms have a low 60% homology in the hormone binding domain offering them distinctive affinities to various ligands. The relative expression of the two isoforms varies between different tissues and their activation produces opposite transcriptional effects on some genes. For instance, the activation of ERα increases cellular proliferation while the activation of ERβ reverses the effects of ERα and suppresses proliferation (Duffy 2006; Heldring et al. 2007; Hewitt and Korach 2018).

A battery of *in vitro* and *in vivo* assays are recommended by the United States Environmental and Protection Agency (US EPA) and the European Organization for Economic Cooperation and Development (OECD) in screening natural and synthetic compounds for putative estrogenic activity. The *in vitro* assays determine if a compound modulates the genomic activity of ER in cell culture and are designed to address various aspects of receptor biology such as ligand-receptor binding, receptor transactivation, transcription of estrogen responsive genes and proliferation of estrogen responsive cells. The *in vivo* assays are performed in rodents and explore the estrogenic efficacy of a compound on estrogen-responsive tissues. Their primary advantage over *in vitro* assays is that they account for metabolic and pharmacokinetic attributes of the compound that can be crucial for estrogenic activity. They also give a global perspective of the hormonal effects and whether the compound disrupts the homoeostatic balance of endogenous estrogens. The US EPA and OECD consider demonstration of a positive test result in more than one assay evidence of estrogenic activity (Diel et al. 1999; Ashby 2000; Clode 2006; Shanle and Xu 2011).

In the planning phase of this review, literature search was steered to retrieve all experimental and clinical studies that address the effects of resveratrol on ER biology and the pathways involved in estrogen and steroid hormone homeostasis such as steroidogenesis and steroid hormone disposition. The titles, abstracts and methodology were reviewed for assays required by the US EPA and OECD in the identification of estrogenic compounds. If a controversial result was identified, care was taken to include all relevant studies covering the controversy. Pharmacokinetic studies essential to the scope of this review were also retrieved. Additional studies were identified by scrutinizing the reference list of individual studies. Database search was performed in PubMed and covered the period from 1997, the year when the first report on the estrogenic activity of resveratrol was published, to the date of this review. Keywords used in the search included: Resveratrol + Estrogen Receptor; Resveratrol + Steroidogenesis; Resveratrol + Estrogen metabolism; Resveratrol + Safety studies; Resveratrol + Toxicity; Resveratrol + Pharmacokinetics; Resveratrol + Aromatase; Resveratrol + Clinical trial + Estrogen.

Generally, all of the *in vitro* and *in vivo* assays approved by the US EPA and OECD have been reported for resveratrol by several research departments. The genomic estrogenic effects were the primary focus of early studies. However, the results reported in subsequent studies suggested resveratrol produced estrogenic effects via multiple mechanisms that included genomic and non-genomic ER activation, changes in ER expression, proteasomal degradation of ER and alterations in estrogen biosynthesis and metabolism. Of noteworthy, some studies were controversial due to the weak nature of the reported effects, technical drawbacks of individual assays and lack of reproducibility by other research groups. The next section addresses the results of the literature search beginning with the experimental *in vitro* and *in vivo* evidence of resveratrol's direct modulation of ER activity.

3. Effects of resveratrol on ER biology

3.1. Effects on genomic ER signaling

3.1.1. Evidence from in vitro assays

3.1.1.1. Competitive receptor binding and ligand displacement. Competitive binding studies were first described by Gehm et al. using ER rich cytosolic extracts prepared from human MCF-7 breast cancer cells. Micro-molar concentrations

of resveratrol were required to displace competing nano-molar concentrations of ^{125}I-E2 (the IC_{50} for resveratrol was ≈10 and 50 μM for 0.1 and 0.3 nM of ^{125}I-E2, respectively). These results suggested resveratrol had significantly weaker affinity to ER than natural estrogens. The inhibition of receptor binding plateaued at high resveratrol concentrations approaching 100 μM and complete displacement of ^{125}I-E2 was never achieved suggesting a large proportion of the dose was dissipated in interaction with other cellular proteins. Because MCF-7 cells predominantly express ERα, the affinity of resveratrol to ERβ was not inferred from the results of this study (Gehm et al. 1997). Another study followed by Ashby et al. who performed competitive binding assays using 5 nM of ^{3}H-E2 in rat uterine cytosolic extracts that express both ER subtypes. The estimated IC_{50} for resveratrol was 100 μM and this was approximately 5 orders of magnitude greater than the IC_{50} values of unlabeled E2 (3 nM) and DES (1 nM) (Ashby et al. 1999). Other groups performed competitive binding assays in other cellular models and reported similar results. For example, micro-molar concentrations of resveratrol were required to displace nano-molar levels of ^{3}H-E2 in whole cellular extracts prepared from immortalized PR1 pituitary cells that express ERα. In this study, resveratrol had significantly weaker affinity to ER than other phytoestrogens with a calculated IC_{50} that was 6.21 μM (Stahl et al. 1998). Bowers et al. investigated the binding affinity of resveratrol to either ER isoform. Competitive binding studies were performed using ^{3}H-E2 and nuclear extracts isolated from ovarian Sf-21 cells that express ERα or ERβ. The investigation confirmed the weaker affinity of resveratrol compared to E2 on either ER isoform. The estimated IC_{50} was 58.5 μM and 130 μM for ERα and ERβ, respectively (Bowers et al. 2000). Bhat et al. performed binding studies using recombinant human ERα and ERβ. Resveratrol was not reported to bind either isoform at concentrations below 100 μM but exhibited relative preference to ERβ at higher concentrations with an IC_{50} of 125 μM. Complete inhibition of ^{3}H-E2 binding couldn't be achieved, even at a concentration of 1000 μM (Bhat and Pezzuto 2001). Taken together, these studies indicated that resveratrol has an affinity that is comparable to either ER isoform, or of slight preference to the ERβ isoform, but that is significantly inferior by several orders of magnitude to natural estrogens. As discussed in later sections of this review, the IC_{50} values reported in the majority of these studies are much higher than the plasma levels established following the consumption of supplemental doses of resveratrol.

3.1.1.2. Functional transactivation of a reporter gene construct. The transactivation of ER following resveratrol treatment has been studied in genetically engineered yeast and mammalian cells that express β-galactosidase and luciferase reporter genes, respectively. Both of these cellular models have been extensively used in screening estrogenic compounds and identification of endocrine disruptors. The effect of resveratrol on ER transactivation in these models has been inconsistent between different research departments. The first transactivation assay was performed in MCF-7 cells transfected with a reporter construct carrying one or two copies of the *Xenopus* vitellogenin A_2 estrogen response element (ERE) upstream of a minimal thymidine kinase promoter and a luciferase reporter gene. Resveratrol stimulated luciferase expression with an EC_{50} in the range of 5–10 μM and the effect was abrogated by ERE deletion or treatment with an ER antagonist. These results indicated resveratrol directly activated ER. Surprisingly, resveratrol was substantially less potent to E2 in this investigation but produced 2-3-times greater reporter gene activity. The investigators suggested resveratrol could function as a superagonist. However, in the same study, expression of the reporter gene in transfected human BG-1 ovarian carcinoma cells following resveratrol treatment was comparatively lower to E2. The discrepancy in the results between MCF-7 and BG-1 cells suggested the agonistic activity of resveratrol is dictated by the cellular milieu that includes the relative expression of the ER subtypes and balance of coactivators and corepressors (Gehm et al. 1997). Of noteworthy, the superagonistic activity of resveratrol in MCF-7 cells was subsequently reported by two other groups of investigators (Basly et al. 2000; Ruotolo et al. 2013) and in a later study by the same group of investigators in MDA-MB-231 cells stably expressing wild type ERα (Gehm et al. 2004). However, many of the investigations discussed below attempted to reproduce these results and failed to detect the superagonistic activity for resveratrol in MCF-7 cells.

Ashby et al. used the yeast transactivation assay to study the estrogenic activity of resveratrol on human ERα. Resveratrol produced submaximal response at doses 6 orders of magnitude greater than E2 and DES. The investigation was repeated in monkey COS-1 kidney fibroblast cells transfected with ERα or ERβ and one of two different EREs. The potency of resveratrol was substantially inferior to E2 and DES on either receptor subtype and produced weaker responses. It was concluded that resveratrol was significantly less portent to E2 and acted as an ER partial agonist at best. The investigation also concluded that the effect of resveratrol was preferentially mediated via ERβ and the consensus vitellogenin A_2 ERE (Ashby et al. 1999). The findings of this study were replicated by two independent studies in hamster CHO-K1 ovarian cells transiently transfected with either ERα or ERβ. Resveratrol acted as an agonist that preferentially activated ERβ (Bowers et al. 2000; Klinge et al. 2003). Taken together, these results implied that tissues where ERβ is preferentially expressed are more sensitive to the estrogenic activity of resveratrol.

The agonistic activity of resveratrol on ER has been questioned by several other studies. For example, in a study by Bhat et al., resveratrol in the absence of E2 exhibited mixed agonistic and antagonistic activities on several ER positive mammary cancer cell lines transfected with the vitellogenin A_2 ERE coupled to a luciferase reporter gene construct. However, in the presence of E2, resveratrol functioned as an antiestrogen (Bhat et al. 2001). In another study by the same group, the treatment of endometrial human adenocarcinoma (Ishikawa) cells with resveratrol did not increase luciferase gene activity. Instead, the compound inhibited E2 stimulation of luciferase activity in a dose dependent manner suggesting it functioned as an ER antagonist. Notably, the degree of

inhibition approached 100% at 15 μM resveratrol (Bhat and Pezzuto 2001). One group of investigators suggested resveratrol's antagonism of receptor function depends on the nucleotide sequence of ERE. For example, in CHO-K1 cells transfected with ERα or ERβ, resveratrol produced some degree of antagonism to E2 that was dependent on the specific nucleotide sequence of the ERE in the transfection construct (Bowers et al. 2000).

The inconsistent results between these studies are believed to reflect technical shortcomings in the reporter gene assays. The assays are sensitive to a number of variables that produce discrepant results. These include the cellular stability of the construct and the levels of growth factors and traces of steroids in the culture medium. In addition, variations in the nucleotide sequence of ERE and differences between the cellular milieu of mammalian and yeast cells impact receptor activity and reporter gene expression. The studies reviewed above also varied in the concentration of resveratrol used in the experiments and in some cases, the concentrations were criticized for being too high that androgens at similar concentration produce a positive test result. The solubility of high resveratrol concentrations was also questioned by some investigators. Perhaps investigating receptor transactivation and expression of endogenous estrogen responsive genes in mammalian cells may overcome some of these deficiencies and these studies are discussed next.

3.1.1.3. Functional transactivation of endogenous estrogen responsive genes. The effects of resveratrol on the expression of natural estrogen responsive genes were first described by Gehm et al. in MCF7 cells. Resveratrol was substantially less potent but as effective as E2 in activating the transcription of the progesterone receptor (PR) gene, a classic estrogen regulated gene. This indicated resveratrol acted as a full agonist of ER (Gehm et al. 1997). In two later studies by the same group, ER negative MDA-MB-231 cells were stably transfected with wild type ER and treated with increasing resveratrol concentrations. The cells exhibited a dose dependent increase in mRNA expression of transforming growth factor alpha (TGFα), another estrogen responsive gene. The maximal response was observed at 50 μM whereas E2 produced a maximal response at 1 nM. These results are congruent with the weak potency of resveratrol deduced from pervious experiments. The combination of E2 and resveratrol at their optimum concentrations did not produce synergistic effects on TGFα expression. Unlike in the reporter gene assays, there was no evidence of superagonistic activity on endogenous estrogenic genes in these experiments. In addition, resveratrol acted as a full agonist when combined with low 0.01 nM E2 concentration and there was no evidence of antagonism at high 1 nM E2 concentration (Levenson et al. 2003; Gehm et al. 2004).

Lu and Serrero evaluated the effects of resveratrol on the expression of estrogen responsive genes in MCF-7 cells in the presence and absence of E2. Resveratrol in the absence of E2 stimulated PR expression, but the stimulation was only half that of E2. When cells were cultured with both compounds, resveratrol decreased E2 stimulated transcription of the PR gene. This effect was accompanied by a decrease in mRNA expression of TGF-α and insulin-like growth factor 1 receptor (IGF-1R), two positive regulators of estrogen mediated cellular growth and proliferation, and a reciprocal increase in TGF-β2 mRNA expression, a known negative regulator of cellular growth and marker of antiestrogenic activity. The investigators concluded resveratrol acted as a partial agonist that antagonizes the effects of E2 (Lu and Serrero 1999).

Bhat et al. investigated the effects of resveratrol on the expression of two estrogen responsive genes in four mammary cancer cell lines. Resveratrol antagonized E2 and suppressed PR and presenilin 2 (pS2) gene expression. The decrease in expression was dose dependent with a maximal effect observed in the range of 10–15 μM (Bhat et al. 2001). In another study by the same group, resveratrol suppressed in a dose dependent manner E2 induced alkaline phosphatase protein expression (IC_{50} 2.3 μM) and PR mRNA levels (range 5 - 15 μM) in human endometrial adenocarcinoma (Ishikawa) cells. The decrease in PR expression was associated with a decrease in its cellular function as evidenced by the decrease in expression of α1 integrin, a collagen-laminin receptor protein that is hormonally regulated in the endometrium (Bhat and Pezzuto 2001). These studies by Bhat and et al. concluded resveratrol acted as an antiestrogen on endogenous estrogen responsive genes.

The combined results of these studies created a scientific dilemma and there has not been a consensus on resveratrol whether it functioned as a partial or full agonist of ER or as an antagonist. However, most investigators were certain of a direct resveratrol interaction with ER. Some suggested resveratrol exerts both estrogenic and anti-estrogenic activities reminiscent of the clinically approved selective estrogen receptor modulators (SERMs). Molecular dynamic simulation of the interaction of resveratrol with the ligand binding domain of ERα was undertaken to examine the conformational changes in the receptor following interaction with resveratrol and maybe resolve the controversy. The binding energy for the interaction of resveratrol with the receptor was significantly weaker than DES, a finding that is in agreement with the results of the binding assays. However, the pattern of hydrogen bond interaction with the receptor was different between the two molecules. Unlike DES, the receptor assumed an antagonistic confirmation with resveratrol that obscured the co-activator binding groove. This conformation was thermodynamically preferable to any agonistic conformations. These molecular dynamics studies concluded resveratrol is likely to act as an ER antagonist (el-Mowafy et al. 2002; Chakraborty et al. 2013).

3.1.1.4. Cellular proliferation assay (E-screen assay). Typically, ER positive MCF-7 cells have been used in this assay. The culture of these cells in the presence of E2 or plant-derived and synthetic estrogenic compounds increases their proliferation. Because the cells predominantly express ERα, the assay does not predict the proliferative response of cells and tissues where ERβ is predominantly expressed. Therefore, the assay may overlook the detection of selective

estrogen receptor modulators (SERMs). Additionally, different stocks of MCF-7 cells respond differently to E2 and environmental estrogens in proliferation assays. To overcome these deficiencies, most investigators use a variety of estrogen responsive cell lines that express ERα or ERβ when screening environmental chemicals for proliferative estrogenic activity (Villalobos et al. 1995; Klotz et al. 1996; Diel et al. 1999; Borgert et al. 2003; Shanle and Xu 2011).

The first report of an anti-proliferative effect for resveratrol was demonstrated in human breast carcinoma cells by Mgbonyebi et al. Resveratrol inhibited cellular growth and reduced viability of ER positive MCF-7 cells and ER negative non-tumorigenic MCF-10F and invasive MDA-MB-231 cells. The tested concentrations were exceedingly higher than subsequent studies and ranged from 22 to 175 μM. The study concluded resveratrol produced ER independent anti-proliferative effects that were likely mediated via activation of cellular apoptosis (Mgbonyebi et al. 1998). A subsequent study confirmed through a series of *in vitro* experiments that MDA-MB-231 cells treated with a resveratrol concentration of 100 μM died via activation of apoptosis, a result that was also replicated for tumor growth *in vivo* (Garvin et al. 2006).

Gehm et al. studied the effects of resveratrol on the proliferation of estrogen responsive T47D breast cancer cells. Surprisingly, resveratrol at low 10 μM concentration stimulated cellular proliferation to an extent similar to 0.1 nM E2. The effect was blocked by the ER antagonist ICI 182,780 indicating it was mediated via ER activation. This was the first demonstration of mitogenic estrogenic properties of low micro-molar doses of resveratrol (Gehm et al. 1997). In another study, Gehm et al. investigated the effect of high resveratrol concentrations on the proliferation of MDA-MB-231 human breast cancer cells transfected with either wild type or mutant ER (Levenson et al. 2003). Resveratrol inhibited cellular growth in the wild type and mutant ER transfected cells with an IC_{50} of 6.7 and 1.5 μM, respectively. The inhibition of cellular proliferation was also observed in ER negative MDA-MB-23. The investigators concluded that resveratrol exerts weak inhibitory effects on cellular proliferation with increasing concentrations approaching 100 μM and that it was mediated via ER independent pathways. Nakagawa et al examined the effects of low and high resveratrol concentrations on the proliferation of ER positive KPL-1 and MCF-7 cells and ER negative MKL-F cells (Nakagawa et al. 2001). Low resveratrol concentrations promoted cellular proliferation of ER positive cells while higher concentrations suppressed growth and proliferation of all types of cells and induced apoptosis evidenced by the accumulation of cells in the sub G1 fraction, upregulation of Bax and Bak, downregulation of $Bcl\text{-}_{xL}$ and activation of caspase-3. This biphasic effect of resveratrol on cellular proliferation has been reportedly observed with other phytoestrogens such as coumestrol and genistein which also exert stimulatory effects at low concentrations and inhibitory effects at high concentrations (Whitten and Patisaul 2001; Borgert et al. 2003).

Lu and Serrero attributed the conflicting effects of resveratrol on cellular proliferation to differences in the estrogenic composition of the culture medium. The investigators studied the effect of resveratrol on the proliferation of MCF-7 cells in the presence and absence of E2. Resveratrol at concentrations in the range of 1 to 10 μM inhibited cellular proliferation in a regular DME-F12 medium plus 5% FBS and was toxic at 100 μM. However, cellular proliferation increased in response to 0.1 μM in an E2-stripped medium and reached a plateau at 1 μM before it declined at higher concentrations. The maximal effect of resveratrol on cellular proliferation was half the effect produced by E2 suggesting the compound functioned as an ER partial agonist. Higher concentrations of resveratrol approaching 10 μM resulted in complete inhibition of MCF-7 proliferation that was not due to cellular toxicity. The authors also report that resveratrol interfered with E2 stimulated proliferation with an IC_{50} of 5 μM and a maximal inhibitory effect at 10 μM. These *in vitro* experiments indicated that resveratrol was a weak stimulator of cellular growth in an estrogen depleted medium and an inhibitor when combined with E2 (Lu and Serrero 1999). Similar results were reported by Basly et al.. The investigators determined whether the geometric isomers of resveratrol had differential effects on the proliferation of MCF-7 cells in the presence and absence of E2. Either isomer was found to encourage cellular proliferation in the absence of E2 with an effective concentration in the range of 10–25 μM. The *(E)*- trans-isomer was more effective compared to the (Z)- cis-isomer. At 50 μM, both isomers decreased cellular proliferation suggesting it was the cut off point for cytotoxicity. Interestingly, when E2 was added to the culture medium at a concentration of 0.1 nM, MCF-7 proliferation decreased with both isomers, mainly the *(E)*- trans-isomer beginning at a concentration of 25 μM (Basly et al. 2000).

The anti-proliferative effect of resveratrol in culture has been observed in other cell lines including BG-1 ovarian cancer cells, endometrial adenocarcinoma (Ishikawa) cells and prostate cancer cell lines (Hsieh and Wu 1999; Bhat and Pezzuto 2001; Kang et al. 2012). Interestingly, resveratrol at a concentration of 10 μM inhibited E2 driven cellular proliferation of Ishikawa cells without decreasing the number of cellular colonies suggesting its antiproliferative effect was cytostatic instead of cytotoxic at this concentration. Collectively, results of the E-screen assay suggested resveratrol has anti-proliferative activity on mammary and other carcinoma cell lines in culture that was ER independent and mediated via activation of apoptosis. However, studies also indicate that lower micro-molar concentrations of the compound encourage cellular proliferation that is mediated, in part, by ER activation.

3.1.2. Evidence from in vivo assays

3.1.2.1. Uterotrophic assay in rodents. The uterotrophic assay is considered the "gold standard" in screening natural and synthetic compounds for *in vivo* estrogenic activity (Kleinstreuer et al. 2016). The assay is performed in juvenile immature rats or ovariectomized adult rats and accounts for metabolic transformation and pharmacokinetic attributes that can influence *in vivo* potency and efficacy of estrogenic compounds. Ashby and colleagues reported the assay for resveratrol in immature rats. The drug was administered orally (PO) and subcutaneously (SC) and the dose was matched to that

where the antiplatelet effects were observed for resveratrol in humans (0.03 mg/kg/day) (Pace-Asciak et al. 1996). Unlike DES, resveratrol produced a weak uterotrophic response that was not reproducible, even at a dose of 120 mg/kg/day, a dose 5000 times greater than the amount consumed by a modest red wine drinker. The lack of reproducibility in the assay implied resveratrol does not exert a uterotrophic response (Ashby et al. 1999). However, these results did not preclude the possibility of an anti-estrogenic activity *in vivo*.

In another study, resveratrol was administered orally for 6 consecutive days to weanling juvenile rats in doses of 1, 4, 10, 40 and 100 μg/day (range of 0.02–2 mg/kg/day) and the effect on estrogen target tissues was compared to E2. Resveratrol in contrast to E2 did not decrease animal growth, body weight, serum cholesterol and radial bone growth and did not affect uterine indices of growth and differentiation. However, resveratrol did produce small increases in uterine weight at the highest dose and the investigators suggested it possibly exerts a greater effect if higher doses were given. These results indicated resveratrol does not possess sufficient *in vivo* agonistic properties on ER-expressing tissues. A second set of experiments were designed to test if resveratrol antagonized the estrogenic activity of E2 *in vivo*. Resveratrol at a dose of 1000 μg/day (equivalent to 20 mg/kg/day) for 6 consecutive days antagonized E2 mediated decreases in plasma cholesterol, but not any of the other physiological effects of E2. This result is perhaps the most direct *in vivo* evidence of a competition between resveratrol and E2 on ER. The investigators performed a similar study in ovariectomized sexually mature rats. Resveratrol pretreatment decreased by 50% the nuclear levels of the tracer ^{3}H-E2 in uterine cells. It was concluded based on these results that resveratrol produced *in vivo* estrogenic antagonism (Turner et al. 1999). Bhat and Pezzuto performed a similar study in ovariectomized rats to explore possible estrogenic effects of resveratrol on uterine tissues. However, the rats were treated for 30 days with resveratrol added in the diet at a concentration of 3000 mg/kg and a comparison was made with E2 injected SC. Unlike E2, resveratrol did not affect uterine weight or type of cells in different stages of the estrous cycle. Resveratrol also did not antagonize the effects of E2 on both parameters. The investigation concluded resveratrol had no estrogenic activity on its own, and no anti-estrogenic activity in combination with E2 (Bhat and Pezzuto 2002; Pezzuto 2011).

The uterotrophic assay was also reported by another group of investigators (Freyberger et al. 2001). Resveratrol was dosed SC instead of PO to immature juvenile rats in an attempt to maximize detection of weak estrogenic activity. The dosing rates were 19, 58 and 575 mg/kg/day for three successive days. Comparison was made with E2 for a host of histological features and protein markers in addition to uterine gravimetric measurements. The chosen doses of resveratrol produced plasma concentrations of 1, 1.6 and 1.8 μM, respectively. These concentrations corresponded to those where some of the estrogenic effects were observed *in vitro*. However, they were inadequate to observe *in vivo* estrogenic effects on uterine tissues. Resveratrol did not produce the classic histological changes observed with E2, but it did reduce uterine weight and the effect was dose independent. Immunohistochemistry showed a dose dependent reduction in ERα immunostaining in epithelial, stromal and myometrial nuclei. Overall, these results are in line with previous uterotrophic assays. The authors suggested that the mild reduction in uterine weight and ERα immunostaining implies some degree of estrogenic antagonism as they resemble the effects of the ER antagonist ICI 182,780.

In conclusion, these series of *in vivo* experiments suggested resveratrol in microgram up to milligram doses is likely to act as an ER antagonist in the short term. The lack of a positive estrogenic effect on uterine growth and differentiation and on other estrogen responsive tissues substantiates this conclusion. However, it is important that these results are approached with caution because the assay reportedly gives discordant results and many chemicals were classified as both ER active and inactive in the literature. An elaborate review on the uterotrophic assay that addresses the results of 2,615 tests and discusses various sources of variability has been published recently (Kleinstreuer et al. 2016).

3.2. Effects on non-genomic ER signaling

The genomic effects of resveratrol mediated via nuclear localized ER has been the focus of most studies. Little has been mentioned on the non-genomic estrogenic effects of resveratrol mediated via membrane associated ER. The activation of this subpopulation of the receptor produces rapid responses via a network of intracellular signaling cascades. Prominent examples include estrogen mediated phosphorylation and activation of MAPK and endothelial nitric oxide synthase (eNOS) and their downstream targets. Experimental findings indicated that resveratrol similar to E2 increased in a concentration dependent manner the activity of MAPK and eNOS in cultured endothelial cells via activation of membrane associated ER (Klinge et al. 2005). The cellular responses were quick and reached its maximum within 5–20 min of treatment and were mediated via a complex cascade of signaling events that involved mitogen-activated protein kinase kinase 1 (MAP2K1, MEK1), Src kinase, caveolin-1, matrix metalloproteinase and epidermal growth factor receptor (EGF-R). Pharmacological inhibition or siRNA transfection experiments targeting any of these proteins abolished phosphorylation and activation of MAPK and eNOS and subsequent production of nitric oxide (NO). These non-genomic estrogenic effects have been demonstrated with low nano-molar concentrations of resveratrol. Interestingly, because the consumption of resveratrol rich diets such as red wine furnishes nano-molar concentrations of resveratrol in human serum within 30 min, the results of these studies have been in support of a cardio-protective role for resveratrol in the French paradox (Klinge et al. 2005, 2008).

3.3. Effects on ER expression

The effectiveness of resveratrol in the treatment of cancer has been founded on experimental evidence that it

enhanced the activity of the p53 tumor suppressor protein. In MCF-7 cells, resveratrol activated MAPK and induced phosphorylation of p53, an essential step for subsequent p53 acetylation and activation of its transcriptional and apoptotic programs (Zhang et al. 2004). The connection between p53 activation and ER expression has been demonstrated in one interesting study by De Amicis and colleagues (De Amicis et al. 2011). In this study, treatment of parental and tamoxifen resistant MCF-7 cells with resveratrol concentrations in the range of 20 to 100 μM decreased the promoter activity of ERα gene and mRNA and protein expression of ER and induced cell cycle arrest in the G1/S phase within 24 h. These effects were mediated through induction of p53 expression and activity and decreased activity of the proximal promoter region of the ERα gene. The promoter region contains two binding sites for Sp1 protein and a regulatory CCAAT box that are responsible for maintaining basal ERα gene expression. The experiments showed that active p53 interacted with the transcriptional co-repressor Sin3A and phosphorylated histone deacetylase 1 (HDAC1) to create a ternary complex. This complex blocked Sp1 from binding to the promoter region and released RNA polymerase II resulting in transcriptional repression of the ERα gene. Site directed mutagenesis, EMSA and Chip experiments suggested that active p53 also encouraged the binding of the transcriptional repressor NF-Y to the CCAAT box, further suppressing ERα expression. The investigators also revealed that the induction of p53 by resveratrol was mediated via $p38^{MAPK}$ phosphorylation. The activation of $p38^{MAPK}$ by resveratrol possibly occurred via a cell surface receptor and the integrin protein αVβ3 was named candidate because it harbors sites for resveratrol binding (Lin et al. 2006; De Amicis et al. 2011). Because of the critical role of ERα in initiating and supporting the growth of mammary carcinoma in coordination with other growth factors, the downregulation of ERα in this study was viewed favorably in the context of breast cancer treatment (De Amicis et al. 2011).

Experimental evidence of a reduction in the expression of ER following resveratrol treatment is also seen in a study addressing the effects of resveratrol on the progression of HER2 and ERα-positive breast cancer. The study was performed in transgenic female mice expressing Δ16HER2, a common splice variant of the human HER2 gene that is co-expressed with the wild-type receptor. The expression of HER2 is inversely associated with ERα expression and breast cancer cells with increased expression of HER2 have been resistant to endocrine therapy. The study showed that mice treated with low 4 μg oral daily doses of resveratrol in tap water for 15 weeks showed increased Δ16HER2 protein levels and decreased ER expression in mammary tissue, an effect that mimicked resistance to endocrine therapy. These changes in protein expression resulted in accelerated growth and multiplication of mammary tumors (Andreani et al. 2017).

The effect of resveratrol on ER expression was also reported in some of the early investigations of resveratrol's estrogenic activity. Levenson and colleagues showed in breast cancer cell lines stably expressing wild type ER and mutant D351Y ER that resveratrol decreased the level of expression of wild type ER, but unexpectedly increased expression of the mutant ER. These findings were difficult to explain in the context of receptor agonist/antagonism. The study suggested the decrease in ER levels may involve ubiquitination and proteasomal degradation of the receptor (Levenson et al. 2003). The possibility that resveratrol promoted proteasomal degradation of ER was confirmed in a later study in MCF-7 cells at a concentration of 50 μM and the proteasome inhibitor MG132 blocked the effect (Pozo-Guisado et al. 2004). In the group of studies carried by Bhat et al., the antiestrogenic activity of resveratrol occurred at concentrations that were much lower than those required for interaction with ER. The investigators proposed resveratrol may produce its anti-estrogenic effects through receptor downregulation. They showed in Ishikawa cells that resveratrol at a concentration of 15 μM decreased basal ERα expression and antagonized E2 induced expression of ERα at the mRNA and protein levels. Interestingly, resveratrol did not suppress basal and E2 induced expression of ERβ in these cells (Bhat and Pezzuto 2001). Taken together, the reviewed literature supports the notion that resveratrol treatment decreases ER expression, the clinical consequences of this effect depends on the cellular context and role of ER in the pathogenic process.

3.4. Effects on steroidogenesis and estrogen biosynthesis

The biosynthetic pathways for the production of steroid hormones are illustrated in Figure 2. The rate of steroidogenesis is determined by the steroidogenic acute regulatory protein (StAR), a carrier protein that controls mitochondrial cholesterol import. Expression of StAR is especially abundant in the gonadal and adrenal tissues where steroidogenesis is active and is regulated by cellular cAMP levels. The effects of resveratrol on StAR expression and steroid hormone synthesis were investigated in MA-10 mouse Leydig cells. Progesterone is the main product in these cells because they do not express cytochrome P450c17, the key enzyme that catalyzes the flow of metabolic substrates into pathways that produce corticosteroids and androgens. Resveratrol in these cells reversed cAMP mediated synthesis of progesterone in a dose dependent fashion and effective concentration in the range of 10-50 μM. These effects were associated with decreased cAMP mediated promoter activity and expression of the StAR gene. These results suggested resveratrol potentially slows steroid hormone biosynthesis via downregulation of StAR and decreased mitochondrial influx of cholesterol (Chen et al. 2007).

Another group of investigators reported similar results. Resveratrol and several synthetic acetoxy and methoxy derivatives decreased StAR expression in addition to cytochrome P450c17 (Cyp17a1) in rat Leydig cells (Svechnikov et al. 2009). These genetic effects suppressed human chorionic gonadotropin (hCG) induced steroidogenesis in the cells resulting in the accumulation of progesterone and decreased production of androstenedione. The IC_{50} value for resveratrol was 7.5 μM while the synthetic derivatives had IC_{50} values in

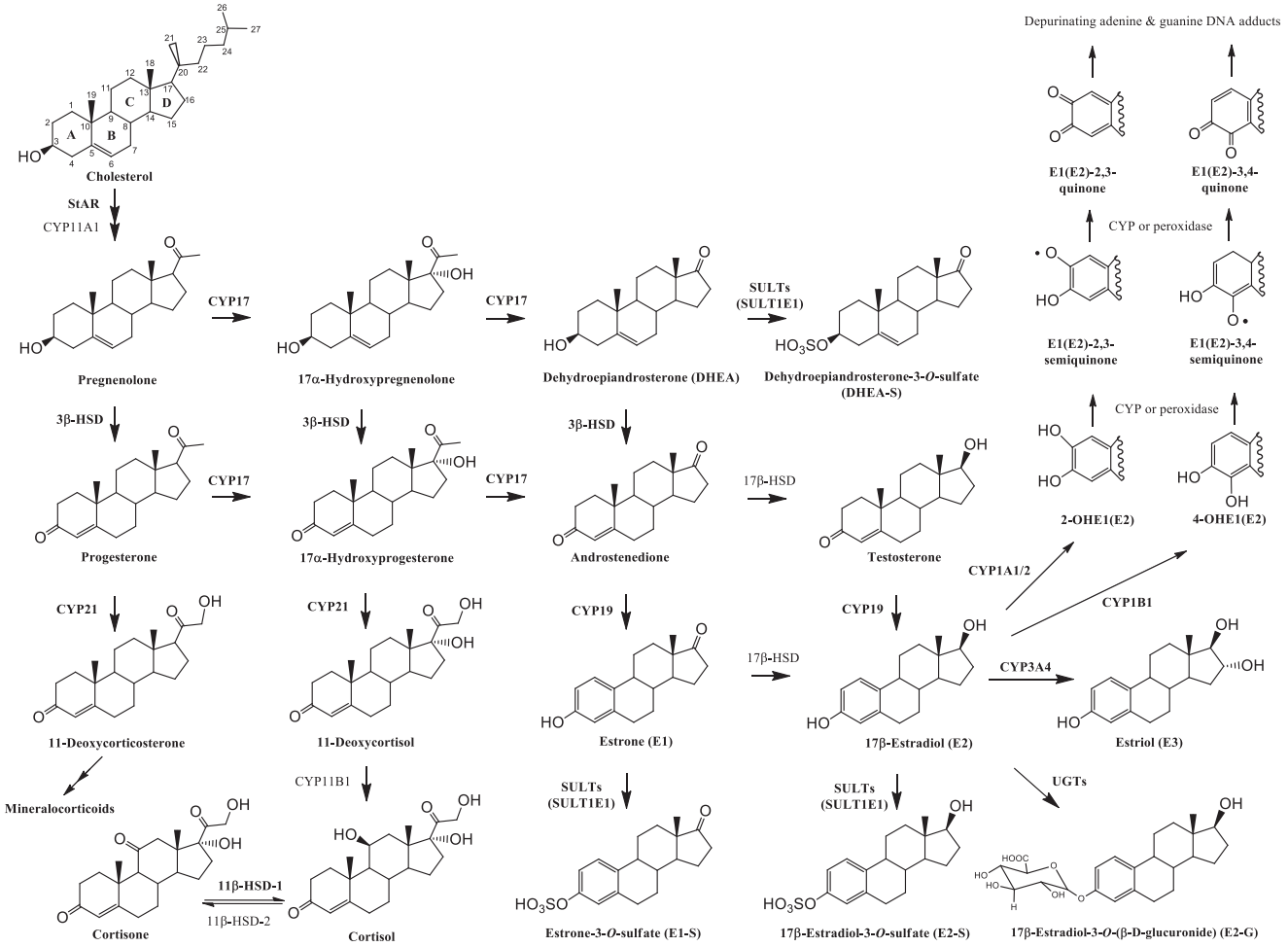

Figure 2. Enzymatic targets of resveratrol in the pathways of steroid hormone biosynthesis and metabolism. The targets are outlined in bold and have been described in various literature reports. Details of the effects of resveratrol on these enzymes and inhibitory concentrations are mentioned in the text. Not shown but described in the text is the enzymatic reduction of estrogenic quinones to E1 and E2 catechols via quinone reductase (NQO1) and their subsequent elimination via phase II conjugation reactions. Resveratrol induces the expression of NQO1, therefore, protecting against the formation of reactive quinones. The effect of resveratrol on the catalytic activity of CYP1A2 has been inconsistent with reports of enhanced and suppressed phenotypic activities. The hydroxylation of estrogens into catechol derivatives (2-OHE1(E2) and 4-OHE1(E2)) is presumed for E1 and E2 and the full chemical structures of the catechol estrogens have been abbreviated to display enzymatic modifications on the A ring. StAR: steroidogenic acute regulatory protein that carries mitochondrial import of cholesterol.

the range of 5.9–35.3 μM. The study indicated that inhibition of StAR and CYPc17 gene expression could be mediated via activation of ERα. This suggestion was based on substantiated evidence that E2 decreased StAR expression and steroidogenesis in cultured cells, such as MA-10 mouse tumor Leydig cells and primary pig Leydig cells, and that mutant mice deficient in ERα gene exhibited elevations in testosterone levels (Li 1991; Akingbemi et al. 2003; Houk et al. 2004). It is noteworthy that human Leydig cells lack ERα expression but the suppressive effects of resveratrol on androgen biosynthesis in this study can be extrapolated to tissues that express ERα and are active in steroid hormone synthesis. A subsequent study reported that resveratrol and several of its acetylated analogs inhibited the catalytic activity of CYP17A1 in human H295R adrenocortical carcinoma cells. Interestingly, resveratrol preferentially inhibited the 17-20 lyase activity of the enzyme (Figure 2). The selective inhibition of the 17–20 lyase activity of the enzyme over its 17α-hydroxylase activity decreased the production of dehydroepiandrosterone (DHEA) and testosterone and caused a significant increase in the production of progesterone and aldosterone (Oskarsson et al. 2014).

In further pursue of the effects of resveratrol on steroidogenesis, it was reported that the culture of primary rat adrenocortical cells with 50 μM resveratrol decreased expression of cytochrome P450 c21-hydroxylase (Cyp21, Cyp21a1, human ortholog CYP21A2) (Figure 2). This caused a significant 47% decline in the production of corticosterone and disproportionate 400% increase in the production of progesterone. These findings were replicated in primary adrenocortical cells prepared from rats fed for 12 weeks approximately 20 mg per day resveratrol mixed in the diet. The cells exhibited 20% reduction in corticosterone production and an associated decrease in P450 c21-hydroxylase expression. In addition, the study reported an increase in the weight of the adrenal glands implying a disruption to the hypothalamic pituitary adrenal (HPA) axis. The study compared the reduction in P450 c21 hydroxylase activity to autosomal-recessive congenital adrenal hyperplasia (c21 type) and cautioned from resveratrol exposure during embryonic development. It also advised to monitor adrenal function following prolonged administration of supplemental doses for chemoprevention and antiaging effects (Supornsilchai et al. 2005). In another

relevant study, resveratrol inhibited corticosteroid 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in microsomal preparations from rodent adipose tissue and mouse 3T3-L1 cells. The enzyme activates glucocorticoids in rodents by reducing 11-dehydrocorticosterone to corticosterone and in humans by converting cortisone into cortisol (Figure 2). Resveratrol produced noncompetitive inhibition of the enzyme with Ki and IC_{50} values of 39.6 and 35.2 μM, respectively. The investigation suggested that inhibition of the enzyme and decreased production of active corticosteroids might underlie the beneficial effects of resveratrol on central adiposity (Tagawa et al. 2013). In yet another investigation of the impact of resveratrol on steroidogenesis, the compound at a concentration of 100 μM inhibited the activity of 3β-hydroxysteroid dehydrogenase (3β-HSD) in cultured immature rat Leydig cells (Figure 2). The study also reported inhibition of the enzyme in rat and human testicular microsomal preparations with an IC_{50} of 3.87 and 8.49 μM, respectively. The inhibition was competitive and the study indicated that the consumption of resveratrol supplements in excess of 2 *g* per day may furnish plasma levels sufficient to inhibit the enzyme. The study also demonstrated that resveratrol gavaged to rats at a dose of 50 and 100 mg/kg for 2 days lowered serum testosterone levels to approximately half the levels in the placebo group (Li et al. 2014).

Aromatase (cytochrome P450 19, CYP 19) catalyzes the final step in the biosynthesis of estrogens (Figure 2). The enzyme aromatizes the A ring in androgens to produce estrogens. Inhibition of the enzyme and consequent depletion of estrogens is a common clinical approach for chemoprevention and treatment of estrogen dependent cancers. The effects of resveratrol on aromatase were investigated in an aromatase expressing MCF-7 cell line (MCF-7aro cells) (Wang et al. 2006). The investigation was initiated based on experimental evidence of aromatase inhibition and abrogation of mammary hyperplasia in CYP19 overexpressing transgenic mice after treatment with a red wine extract (Eng et al. 2001; 2002). Resveratrol inhibited aromatase in MCF-7aro cells with an IC_{50} value of 25 μM and decreased testosterone driven cellular proliferation. Analysis of the enzyme-kinetic data showed that resveratrol produced a mixed-type inhibition with a Ki value of 25.4 μM. Additional experiments were performed in SK-BR-3 human breast cancer cells to determine if resveratrol affects endogenous mRNA expression of aromatase. Quantitative RT-PCR analysis showed that resveratrol at a concentration of 50 and 100 μM decreased aromatase mRNA expression by 55 and 75%, respectively, and this resulted from decreased transcriptional activity of promoters I.3 and II in the aromatase gene. The decrease in aromatase mRNA expression resulted in decreased protein levels and activity in the cells. The study suggested that aromatase transcriptional repression could be mediated via the inhibitory effects of resveratrol on cyclooxygenases (COX) because inhibition of COX transcription and activity has been linked to decreased expression of the enzyme (Subbaramaiah et al. 1998; Wang et al. 2006). These results are supported by findings of another study that used co-cultures of ERα positive T47D cells and aromatase expressing human primary breast fibroblasts (BAFs). The co-culture model is believed to be a better representation of the tumor microenvironment and role of paracrine androgen signals in breast cancer. In this system, aromatase catalyzed conversion of testosterone into E2 in BAF cells is required to drive T47D cellular proliferation. Resveratrol at 20 μM decreased testosterone driven T47D cellular proliferation suggesting inhibition of aromatase and decreased E2 production in the BAF cells. The decreased proliferation of the T47D cells was associated with decreased expression of the prognostic marker *k1-67* gene and estrogen responsive *pS2* gene (Chottanapund et al. 2014). Collectively, the results of these studies constitute evidence of resveratrol interference in steroidogenesis. Additional evidence is found in a number of clinical trials which are discussed in a later section in this review.

3.5. Effects on estrogen metabolism

There has been mounting experimental and clinical evidence to indicate that resveratrol interferes in the metabolic disposition of estrogens. For example, the metabolism of resveratrol by sulfotransferases (SULT) and possible competition in the sulfation of steroids was investigated by Furimsky and his colleagues. E2 and resveratrol sulfation were studied in human liver and jejunum S9 fractions and using recombinant human SULTs. Resveratrol was incubated at 0.5, 1 and 2 μM, concentrations that are relevant to plasma levels achieved following oral doses of resveratrol supplements. Resveratrol inhibited E2 sulfation in the S9 fractions. It was also shown to inhibit sulfation with recombinant SULT1E1, the main isoform that carries hepatic and intestinal sulfation of estrogens. The estimated Ki values were 1.1 μM (liver), 0.6 μM (jejunum) and 2.3 μM (SULT1E1). The study suggested that the inhibition of sulfation was mediated by resveratrol or any of its metabolites including its sulfated conjugates. Additional investigation showed that piceatannol (Figure 1), the only cytochrome P450 metabolite of resveratrol also inhibited E2 sulfation with Ki values of 1.6, 0.4 and 1.2 μM in human liver and jejunum S9 fractions and recombinant SULT1E1, respectively. These results indicated that resveratrol at pharmacological doses alters the homeostasis of estrogens by inhibiting their metabolism. The results of this study also suggested a potential interaction between resveratrol and estrogenic or steroidal drugs metabolized via sulfation (Furimsky et al. 2008).

In another study, the effect of resveratrol on the metabolism of estrogens and other steroids to sulfate and glucuronide conjugates was investigated in MCF-7 and MDA-MB-231 cells. The study used dehydroepiandrosterone (DHEA) as a hormone precursor and monitored the proliferation of the cells and formation of a range of conjugate metabolites. Resveratrol concentrations less than 10 μM inhibited the metabolism of DHEA to the corresponding sulfate conjugate in MCF-7 cells, but not in MDA-MB-231. This resulted in the accumulation of DHEA and increased production of androstenedione and testosterone but not estrogens because MCF-7 cells express low levels of aromatase. To overcome the deficiency in aromatase, DHEA was replaced with E1 as a hormone precursor. The hormone is converted to E2 in MCF-

7 cells by 17-β-hydroxysteroid dehydrogenase (17β-HSD) and both E1 and E2 are converted to their corresponding sulfate and glucuronide conjugates (Figure 2). Resveratrol inhibited formation of the conjugated metabolites and inhibited CYP3A4 hydroxylation of E2 to E3. This inhibition of estrogen metabolism resulted in a 2-fold increase in the levels of E2, and this was accompanied by increased MCF-7 proliferation, especially at low resveratrol concentrations (<10 μM). The inhibition of steroid sulfation in the study occurred over a concentration range from 5 to 100 μM and was via direct noncompetitive interaction with the metabolizing enzymes. These results were placed in the context of cancer promotion if high supplemental doses of resveratrol are consumed over prolonged periods. The study also indicated that resveratrol metabolites may accumulate in tissues and produce similar effects on estrogen metabolism (Poschner et al. 2018). The effects of resveratrol on the metabolism of estrogens and steroidal hormones is also seen in a study on isolated human H295R adrenocortical carcinoma cells. Resveratrol increased E2 levels and reduced E1 sulfate formation via inhibition of SULT1E1 sulfation (Oskarsson et al. 2014).

The predisposition of estrogens to cancer developemnt is well established in epidemiological studies and has been effectively reviewed by many investigators (Cavalieri and Rogan 2011; Cavalieri et al. 2012). There are several mechanims that underlie the carcinogenic potential of E1 and E2, one substantiated mechanism is via chemical carcinogenesis. A group of activating cytochrome P450 enzymes, mainly CYP1A1/2 and CYP1B1 catalyze hydroxylation of estrogens to their corresponding catechol derivatives 2-OHE1(E2) and 4-OHE1(E2), respectively. These catechols are typically conjugated and eliminated, but inhibition of the conjugating enzymes or deficiency in conjugating precursors increases their conversion via peroxidases and CYPs to semiquinones and subsequently to quinones. The quinone products of estrogens react with the DNA to form N3-adenine and N7-guanine adducts. The cell depurinates these adducts at variable rates via error prone mechanims that can produce mutations and initiate cancer, especially if this happens in critical genes (Figure 2). The damaging potential of estrogenic quinones is offset by a group of protective processes such as conjugation with glutathione (GSH) or reduction via quinone reductase (NQO1 and NQO2). Disruption of estrogen metabolism due to a homeostatic imbalance between activating and protecting enzymes increases the formation of the catechol metabolites and their corresponding estrogenic quinones, which predisposes to the development of cancer (Cavalieri and Rogan 2011; Cavalieri et al. 2012; Yager 2015). Resveratrol was shown in several studies to inhibit formation of the catechol derivatives. In immortal non-transformed ERα negative MCF-10F cells cultured with E2, 4-OHE2 and estradiol-3,4-quinone (E2-3,4-Q), the compound in a concentration range of 12.5–50 μM decreased the rate of adduct formation in a dose and time dependant manner and protected the cells from undergoing malignant transformation. This protection occured via induction of NQO1 and competitive inhibition of CYP1B1 activity with Ki values of 9 and 28 μM according to the model system used in the assay (Guengerich et al. 2003; Zahid et al. 2007; Lu et al. 2008; Zahid et al. 2008). The protective effect also involves inhibition of CYP1A1 and 1A2 activities and therefore formation of 2-OHE1(E2) with IC_{50} values – depending on the assay system used – that were 11 and 23 μM for the 1A1 isozyme and 0.58 and 1 mM for the 1A2 isozyme (Chun et al. 1999). These effects have been viewed positively in the context of protecting against the genotoxic effects of estrogens, but are indeed evidnce of the compound's potential to manipulate estrogen metabolism.

Findings in animal studies also support resveratrol's interefece in the metabolic disposition of estrogens. One study examined postnatal ovairian toxicity in offspring of rats exposed during lactation to chromium in dirinking water. Chromium exposure of the mothers decreased sysnthesis and enhanced metabolic clearnce of E2 in the offspring on postnatal day 25. Resvertrol adminstration to the mothers by oral gavage at a rate of 10 mg/kg/day during lactation restored E2 levels in the offspring. This occurred via inhibition of many isozymes responsible for the hydroxylation, glucuronidation and sulfation of E2 (Banu et al. 2016). Evidence of resveratrol interference in estrogen metabolism is also found in some clinical trials discussed in a subsequent section of this review.

3.6. Effects on plasma levels of sex hormone binding globulin (SHBG)

The effect of resveratrol on hepatic production of SHBG has been demonstrated in several *in vitro* and *in vivo* studies and were reported by a few clinical trials. The treatment of HepG2 cells with red wine or pure resveratrol stimulated the production of SHBG. This occurred through activation of the human constitutive androstane receptor (CAR). CAR is a nuclear receptor that binds a consensus direct repeat-1 element in the proximal promoter of the human SHBG gene. Interestingly, red wine that contains nano-molar levels of resveratrol produced a greater effect than micro-molar levels of pure resveratrol. It was suggested that several components in red wine synergize with resveratrol or possibly stabilize resveratrol against metabolism to facilitate expression of SHBG. These results agree with *in vivo* studies in a human SHBG and CAR double transgenic mouse model. Mice were treated with 60 μg/mL resveratrol in drinking water for 20 days. Hepatic mRNA expression of SHBG and plasma SHBG levels increased progressively between days 10 and 20 (Saez-Lopez et al. 2017). The results of these studies support the clinical scenario that low plasma levels of SHBG increase the bioavailability of steroid hormones to various tissues and predispose to metabolic diseases, whereas high levels of SHBG are protective. In clinical trials, the evidence of an increase in SHBG production following resveratrol treatment has been inconsistent. For example, a statistically significant 10% average increase in circulating SHBG was noticed in postmenopausal obese women treated daily with 1 *g* of resveratrol for 12 weeks (Chow et al. 2014). In a study involving a group of women with polycystic ovary syndrome (PCOS), the increase in SHBG with a daily resveratrol dose of 1500 mg for 3 months was double the placebo group, although the

difference was not statistically significant (Banaszewska et al. 2016). In contrast, a study that involved middle-aged men with metabolic syndrome and treated with two daily doses of 150 or 1000 mg resveratrol for 4 months reported no effects on plasma levels of SHBG (Kjaer et al. 2015). A clinical study that investigated the differential effects of drinking red vs white wine in premenopausal women also showed no effects on SHBG levels (Shufelt et al. 2012).

4. Experimental evidence in animals that resveratrol induces endocrine disruption via an estrogenic mode of action

A number of safety studies were reported for resveratrol in several animal species, a few of them were specifically designed to address the effects of resveratrol exposure during various stages of development or during adult-life on reproductive organ development, function and sexual behavior. A review of these studies is provided below in addition to safety studies where the estrogenic and hormonal properties of resveratrol were determined within a broad investigation of the compounds acute and subchronic toxicities.

4.1. Prenatal exposure

The effects of maternal exposure to resveratrol on mammary and reproductive tract development in the female offspring were studied in CD-1 mice. On gestation day 15, resveratrol was injected SC for four days at a rate of 0.5 mg/kg/day to mimic human dietary exposure and at 10 mg/kg/day. Comparison was made with untreated controls and mice injected SC with DES at a rate of 0.5 μg/kg/day and 10 μg/kg/day. Like DES, maternal exposure to resveratrol accelerated the growth of the offspring. The absolute body weight was greater than the control offspring at 16 weeks of age. Resveratrol treatment, unlike DES, did not shorten the time for vaginal opening. However, both drugs prolonged the estrous cycle and extended the diestrus phase of the cycle between weeks 9 and 11 of age. At 4 weeks of age, histological examination of the ovaries revealed missing corpora lutea in 17% of the animals exposed to the high resveratrol dose while the rate was 85 and 100% in animals exposed to the low and high DES doses, respectively. Because the absence of corpora lutea is a frequent cause of infertility in humans, these findings represented important evidence that resveratrol consumption during critical windows of development might be associated with deleterious endocrine effects in the offspring that warrant investigation. The study did not report noticeable changes in uterine weight or in the development and differentiation of the mammary glands in the resveratrol exposed groups up until 16 weeks of age (Nikaido et al. 2004).

4.2. Neonatal exposure

Henry and Witt reported in rats the effects of maternal exposure to resveratrol during lactation on the integrity and function of the neuroendocrine system in the offspring during adult life. Following parturition, dams were exposed throughout lactation to resveratrol in drinking water at levels of 5, 50 and 100 μM. The levels covered the range of resveratrol concentrations found in red wine (range of 1.2–12.1 mg/L). Maternal resveratrol exposure had a greater impact on the adult male offspring. There was a dose dependent reduction in plasma testosterone levels and increase in testicular weight. These effects were accompanied with decreased sociosexual behavior. Histological examination of hypothalamic brain sections revealed significant morphological changes in the sexually dimorphic preoptic nucleus (SDN-POA) and the anteroventral periventricular (AVPV) nucleus. The offspring exhibited significant shrinkage in the SDN-POA nucleus that is typically larger in males compared to females and this was associated with a reciprocal enlargement in the AVPV volume that is relatively smaller in males than females. These morphological changes in the hypothalamic nuclei are the opposite of those produced by E2 suggesting resveratrol produced estrogenic antagonism in the CNS. Importantly, these results indicated that resveratrol disrupted the hypothalamic pituitary gonadal (HPG) axis in the male offspring. In the female offspring, maternal resveratrol exposure during lactation did not affect the size of the hypothalamic SDN-POA and AVPV nuclei, but produced peripheral effects that were typical of an estrogen agonist. These included ovarian hypertrophy and a reduction in body weight throughout the period of investigation until post-natal day 132. Taken together, it seems that the effects of maternal exposure to resveratrol during lactation on reproductive physiology and sociosexual behavior of the offspring during adult life are gender-specific. In addition, the study concluded that resveratrol resembled SERMs in that it produced opposite estrogenic effects in different organ systems, i.e., peripheral estrogenic versus central anti-estrogenic effects (Henry and Witt 2006). To this end, it highly unlikely that resveratrol or any natural dietary supplement is recommended for consumption during pregnancy and lactation. However, the forgoing preclinical studies on the exposure to resveratrol during the perinatal and neonatal periods are insightful because they shed light on the endocrine activity of the compound and its potential to act as an endocrine disruptor if exposure occurs during other physiological stages of the human life.

4.3. Prepubertal exposure

The levels of estrogens are low during the prepubertal period and exposure to xenoestrogens in this period can produce morphological and functional abnormalities in estrogen target organs. In one study that examined in female CD-1 mice the effects of prepubertal exposure to resveratrol on the development and function of the mammary and reproductive system, resveratrol was dosed SC at a rate of 10 mg/kg/day for 4 days starting from day 15 of age and the mice were monitored until 24 weeks old. The dose was chosen to exceed the amount of resveratrol consumed in a single glass of wine that was estimated at an average rate of 0.02 mg/kg/day. The effects of resveratrol were compared to DES injected

SC at a rate of 10 μg/kg/day. Resveratrol did not change the morphology of the mammary glands and reproductive organs. Additionally, resveratrol unlike DES did not shorten the time for vaginal opening or extend the estrus phase of the estrous cycle. However, the *corpora lutea* were absent from the ovaries of 50% of the mice at 4 weeks of age compared to 100% in the DES treated group, but this effect was not seen at 24 weeks of age suggesting it was temporary in nature (Nikaido et al. 2005). In another study by the same group of investigators, prepubertal exposure of female rats to the same dose of resveratrol did not accelerate the development of *N*-methyl-*N*-nitrosourea (MNU) induced mammary carcinoma, but exposure to a dose of 100 mg/kg/day increased their susceptibility. Resveratrol increased the percentage of ERα and PR positive mammary glandular cells and the relative uterine and ovarian wet weights and slightly accelerated vaginal opening compared to controls. The offspring also exhibited increased irregularity in the estrous cycle with more time spent in the estrus phase (Sato et al. 2003). These results differ from those reported by Bhat et al. in their investigation of the potential role of resveratrol in promoting or protecting against chemical induced MNU-carcinogenesis in rats. In their study, resveratrol administered orally at a rate of 100 mg/kg/day initially delayed tumorigenesis and suppressed tumor multiplication before it eventually caught up with the control group at the end of the experiment. The investigators attributed the delay in tumorigenesis to the anti-estrogenic effects of resveratrol on ER positive cells that dominate early premalignant and hyperplastic mammary lesions. This effect progressively weakens as the tumor grows and populates nonresponsive ER negative cells which explains why the tumor caught up with the control group in later stages of the experiment (Bhat et al. 2001). Taken together, the results of these studies indicate that pharmacological doses of resveratrol prior to puberty disrupt endocrine homeostasis, in part, via an estrogenic mode of action that could be agonistic, antagonistic, or a mixture of both actions.

4.4. Adult exposure

Henry and Witt studied in gonadally intact and ovariectomized adult female rats the effects of resveratrol on reproductive physiology and behavior. Resveratrol was added in drinking water to a concentration of 100 μM. The gonadally intact females consumed a total amount of 15 mg over the course of 7 days. Resveratrol caused significant weight loss in these animals and because estrogens induce weight loss in rats (Ramirez 1981), the investigators suggested it could be mediated via ERβ activation. The consumption of resveratrol also induced ovarian hypertrophy and disrupted the estrous cycle with 59% of the rats exhibiting either constant estrus or irregular cycles. The molecular mechanism behind the ovarian hypertrophy was unknown, but sustained estrogenic activity is believed to underlie the disruption in the estrous cycle. The investigators cautioned these effects are commonly associated with infertility. In a second set of experiments, the investigators demonstrated in ovariectomized rats that SC injection of 10 μg of E2 on two successive days followed by an injection of progesterone facilitated sexual receptivity for several days post-treatment. In quite contrast, SC injections of resveratrol at doses of 10, 100 and 1000 μg over two successive days followed by an injection of progesterone did not stimulate lordosis or facilitate sociosexual behavior. The injection of resveratrol with E2 did not reverse or augment the behavioral effects of E2. The animals exhibited the full complement of sociosexual behavior similar to the E2 alone treated group. These results suggested resveratrol did not antagonize E2 action *in vivo*. Interestingly, the injection of 1000 μg of resveratrol with E2 resulted in a significant increase in plasma E2 levels compared to animals dosed with either drug alone. The investigators did not provide an explanation for the increased plasma E2 levels, but it could represent inhibition of E2 metabolism. Taken together, the effects of resveratrol in this study were consistent with the actions of a weak ER agonist or an ER antagonist of diminished potency. Alternatively, the effects could represent the actions of a mixed agonist/antagonist that are dictated by tissue specific expression of ERα and ERβ (Henry and Witt 2002).

4.5. Short term and sub-chronic repeated dosing toxicity studies

Several safety studies were performed to determine the toxic effects of resveratrol and the estrogenic and hormonal effects have been one toxic end point of interest. The studies have been performed in several species and used different pharmacological doses. In one study, a single oral dose of 2000 mg/kg of resveratrol or a daily dose of 20 mg/kg for 28 days in male Sprague Dawley rats was not associated with alternations in feeding and drinking behaviors or in the body weight and growth of the animals. There was no evidence of hematological, biochemical and histological abnormalities suggesting the compound has a large margin of safety. However, the study reported a relative increase in brain and testicular weights. The study did not report any endocrine related anomalies. The oral dose used in this study was estimated at 1000 times the average daily consumption of 0.02 mg/kg of resveratrol in red wine (Juan et al. 2002). A similar study design was repeated by Crowell et al. with resveratrol doses of 300, 1000 and 3000 mg/kg/day administered by oral gavage for 4 weeks. The majority of the toxic symptoms were associated with the highest dose and were related to nephropathy and renal toxicity. Body weight and food intake were reduced in both sexes and there was an elevation in liver enzymes without histopathological changes. The female rats were more susceptible to toxicity than the male counterparts, but the study did not report any endocrine related adverse effects (Crowell et al. 2004).

The toxic effects of resveratrol following subchronic exposure have been investigated in rats. The drug was mixed with standard laboratory diet and dosed at a rate of 50, 150 and 500 mg/kg/day for 28 days and at a rate of 120, 300 and 750 mg/kg/day for 90 days. The toxicities of resveratrol in this study were not significant, were observed at the highest

dose in one of the genders and were dose independent (Williams et al. 2009). In another subchronic study, resveratrol was dosed to CD rats by oral gavage at a rate of 200, 400 and 1000 mg/kg/day and to beagle dogs in capsules at a rate 200, 600 and 1200 mg/kg/day. Animals of both genders were monitored for signs of toxicity for a period of 90 days. In rats, resveratrol induced a dose dependent reduction in mean body weight only in the females and it was not associated with changes in food consumption. Resveratrol did not alter the weight of reproductive organs or induce histopathological changes in both sexes. However, in the female group exposed to 1000 mg/kg/day, there was an increase in relative ovarian weight that was attributed to the reduction in body weight. Microscopic examination of reproductive organs did not reveal any signs of hormonal activity. In dogs, resveratrol only reduced the body weight of both genders and this was attributed to a decrease in food consumption. The primary toxicities described in this study were hepatomegaly and elevation in bilirubin levels. These effects were gender nonspecific and observed only in rats suggesting resveratrol toxicity is species-specific. Based on the lowest dose that reduced body weight in both species, the study concluded that the No Observed Adverse Effect Level (NOAEL) for resveratrol was 200 mg/kg/day in rats and 600 mg/kg/day in dogs. Although the reduction in body weight could represent increased estrogenic activity as previously described, the study did not favor this conclusion. Most importantly, this study raised the safety margin of resveratrol to doses that potentially establish plasma concentrations necessary for the compounds putative chemopreventive effects (Johnson et al. 2011). Collectively, these stduies did not report evidence of hormonal activity for resveratrol. However, it needs emphasized that the investigations were not long enough to detect hormonal effects for resveratrol that are presumably weak according to *in vitro* results. In addition, acute or progressive organ toxicity in these studies could have masked detection of subtle hormonal activities of the compound. The hormonal end-points of toxicity were also limited and plasma levels of steroid hormones and their precursors were lacking in many of these studies. Therefore, there is a need for long-term studies to monitor subtle hormonal changes that gradually evolve following prolonged periods of exposure to pharmacological doses of resveratrol.

5. Evidence of estrogenic and hormonal activities of resveratrol in therapeutic applications

The effectiveness of resveratrol in the treatment of a range of diseases has been evaluated in many animal models of disease and in clinical trials. In some studies, the *in vitro* estrogenic activity of the compound was viewed as an opportunity for clinical benefit in the treatment of specific endocrine related pathologies. Generally, the results of these studies provide evidence that supports the compound's hormonal activity. A sample of these studies is discussed below to help researchers and endocrine specialists deduce the dosage of resveratrol and time needed to detect the hormonal effects of the compound in future preclinical and clinical studies.

5.1. Preclinical studies

The estrogenic activity of resveratrol was demonstrated to alleviate insulin resistance and normalize plasma glucose levels in animal models of diet induced obesity and diabetes. In one study, the estrogenic activity of resveratrol was responsible, in part, for alleviating peripheral insulin resistance in rats fed a high cholesterol fructose diet for 15 weeks. Resveratrol administered by oral gavage at a rate of 1 mg/kg/day enhanced insulin-mediated whole body glucose uptake, mainly in the soleus and C2Cl2 muscles and liver and this was attributed to increased membrane translocation and expression of GLUT4 transporter. These effects were partially mediated via ERα activation and reversed by the ER antagonist ICI 182,780 (Deng et al. 2008). Of noteworthy, resveratrol in a randomized placebo-controlled clinical trial on patients with the metabolic syndrome was dosed at a daily rate of 150 and 1000 mg for 16 weeks, but had no effects on glucose and lipid metabolism as well as biological markers of other components of the metabolic syndrome (Kjaer et al. 2017).

The literature also describes a promising role for the estrogenic properties of resveratrol in suppressing vascular smooth muscle proliferation and promoting re-endothelialization when using balloon angioplasty and a metallic stent to open narrowed arteries. Increased proliferation of the arterial smooth muscle cells and delayed re-endothelialization are the primary drawbacks of bare metal stents that lead to several complications that include re-stenosis, narrowing of the blood vessel and increased possibility of re-thrombosis. Stents loaded with anti-mitogenic drugs have been used instead to prevent smooth muscle proliferation, but these stents prevented re-endothelialization and encouraged in-stent thrombosis. Studies *in vitro* and in animals showed that resveratrol slowed the proliferation of vascular smooth muscle cells and luminal stenosis by inhibiting NF-κB activation of inflammatory processes and increasing eNOS activity and NO production. These effects were mediated via non-genomic activation of ERα (Kolodgie et al. 1996; Zou et al. 2000; Ekshyyan et al. 2007). On the other hand, resveratrol added to the diet and consumed at a rate of 50 mg/kg/day encouraged re-endothelialization under physiological conditions of laminar shear stress in a mouse model of arterial injury, also via non-genomic ERα activation of extracellular signal regulated kinase (ERK/MAPK) (Yurdagul et al. 2014). Similar results were obtained in a rat model of injured aorta. Resveratrol oral gavage for two weeks at a rate of 10 mg/kg/day prior to surgery increased circulating progenitor endothelial cells, accelerated reendothelialization and inhibited neointimal formation following inflated balloon catheter injury of the aorta (Gu et al. 2006). These non-genomic estrogenic activities were observed in various types of cultured endothelial cells at nanomolar concentrations of resveratrol, three orders of magnitude less than the levels needed for genomic activities. The opposite mitogenic effects of resveratrol on cellular proliferation of the endothelial and smooth muscle cells in these studies exemplified the differential effects of ER activation on different cells (Klinge et al. 2005, 2008).

There has also been a role for the estrogenic effects of resveratrol in attenuating hepatic and intestinal injury following trauma hemorrhage. The release of pro-inflammatory cytokines during a hemorrhagic shock contributes to the malfunction and eventual failure of many organs. The liver is a critical organ that is affected by several cytokines during hemorrhagic shock. These include cytokine induced neutrophil chemoattractant 1 and 3 (CINC-1 and CINC-3). These cytokines attract large numbers of neutrophils that infiltrate the organ and accumulate on endothelial cells due to increased expression of adhesion molecules such as the intracellular adhesion molecule 1 (ICAM-1). Resveratrol has been known for its anti-inflammatory properties and a group of investigators examined in a rat model of trauma hemorrhage whether resveratrol imparts anti-inflammatory activities that attenuate liver damage following hemorrhagic shock. Resveratrol was administered intravenously (IV) at a rate of 3, 10, 30, 90 and 150 mg/kg and was shown to decrease markers of liver damage at 24 h following injury and at 2 and 24 h after resuscitation with Ringer's lactate solution. The biological markers included plasma levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), hepatic activity of myeloperoxidase (MPO) and hepatic levels of interleukin-6 (IL-6), ICAM-1, CINC-1 and CINC-3. The effects of resveratrol were abolished when co-administered with ICI 182,780 suggesting they were mediated via ER activation. Another study by the same group reported similar results on intestinal injury following trauma hemorrhage. Resveratrol reduced inflammatory injury, intestinal water accumulation, MPO activity and TNF-α, IL-6, ICAM-1, CINC-1 and CINC-3 levels. Again, these effects were mediated via ER activation of p38 MAPK which in turn activated heme-oxidase-1 (HO-1) to protect the intestine from inflammatory injury and the co-administration of ICI 182,780 attenuated them (Yu et al. 2008, 2011).

The estrogenic activity of resveratrol is also reported to mitigate inflammatory and oxidative injuries after cerebral ischemia/reperfusion. In a rat model of cerebral ischemia/reperfusion, resveratrol was injected IV in 200 nL volumes at a rate of 2×10^{-3}, 2×10^{-4}, 1×10^{-4} mg/kg prior to occluding the middle cerebral artery. Resveratrol significantly reduced the infarct area following reperfusion, an effect that was reversed with the nonselective ER antagonist ICI 182,780. These results suggested resveratrol mediated its neuroprotective effects via ER activation (Saleh et al. 2010). In a subsequent study, intra-cortical injection of resveratrol in a volume of 200 nL and concentration range from 0.0001 to 1 μM prior to arterial occlusion produced dose dependent neuroprotection and reduction in infarct size. On the other hand, the concomitant injection of a selective ERα or ERβ antagonist with resveratrol reversed the neuroprotective effect suggesting it was mediated via direct activation of either ER subtype in the ischemic cortex (Saleh et al. 2013). Collectively, these preclinical studies indicate that resveratrol mediates some of its beneficial health effects via activation of ER.

5.2. Clinical studies

A list of clinical studies that report hormonal effects of resveratrol is provided in Table 1. The interference of resveratrol in steroidogenesis has been utilized in treating a number of endocrinopathies. In one trial, the compound was used in treating polycystic ovary syndrome (PCOS). The disease is the most common endocrinopathy in women of reproductive age. It produces reproductive dysfunction and symptoms of metabolic derangements such as hyperlipidemia and insulin resistance. The condition is also associated with hypertrophy of the theca-interstitial ovarian cells and the adrenal glands which results in excessive steroidogenesis and production of androgens. Clinical management involves treatment with metformin or combination contraceptives. Studies *in vitro* indicated that resveratrol decreased androgen production and proliferation of rat theca cells and induced apoptosis via activation of key caspases. The decrease in androgen production was attributed to inhibition of 17α-hydroxylase/C17-20-lyase (CYP17; Cyp17a1) and decreased expression of HMG-Co reductase and activity of the mevalonate pathway (Wong et al. 2010; Ortega and Duleba 2015). In a randomized double blinded placebo controlled trial on 30 patients with PCOS, resveratrol was given at a daily rate of 1500 mg PO for 3 months and a number of endocrine and metabolic indices were investigated. Resveratrol treatment caused a significant 22–23% reduction in total serum testosterone and dehydroepiandrosterone and 66% increase in insulin sensitivity. Interestingly, resveratrol did not affect serum lipids or inflammatory parameters which otherwise would have been elevated if contraceptive pills were used instead (Banaszewska et al. 2016).

In another clinical trial, the utility of resveratrol in the treatment of benign prostatic hyperplasia and prostate cancer were investigated in middle aged men with the metabolic syndrome. In this study, patients were given an oral dose of 1000 mg daily for 4 months and plasma androgen levels and prostate size were examined. Resveratrol decreased androstenedione, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEAS), but testosterone and dihydrotestosterone levels were not affected, probably because resveratrol does not effectively penetrate into testicular tissues, the primary site for testosterone production in men. Additionally, resveratrol did not affect prostate size or plasma levels of the prostate specific antigen. The reduction in androgenic precursors was believed to occur through the selective inhibition of the 17-20 lyase activity of CYP17A1 (P450C17) without drastically affecting the 17 hydroxylase activity of the enzyme. This selective inhibition decreased biosynthesis of androgens but did not affect glucocorticoid and mineralocorticoid production. The study was not supportive of a clinical role for resveratrol in the treatment of prostate hypertrophy or cancer, but the changes in plasma steroid levels are evidence of the compound's impact on steroidogenesis (Kjaer et al. 2015). Yet another example of the impact of resveratrol on steroidogenesis is in relieving endometriosis-related pain that doesn't resolve with a combination of drospirenone and ethinylestradiol. In an open office based clinical study, the addition of 30 mg of daily resveratrol to the treatment decreased dysmenorrheal and pelvic pain in 85% of the patients after 2 months. In a follow up study by the same group, the expression of aromatase was determined in the endometrial tissue of patients

Table 1. A sample of clinical trials reporting outcomes reflective of estrogenic and hormonal activity of resveratrol.

Study	Study design	Participants condition	n^*	Average age (years) of resveratrol treated group	Oral dose	Duration	Outcomes
Banaszewska et al. 2016	Randomized double blind placebo controlled	Women with PCOS	30	26.8 ± 1.1	Daily 1500 mg	3 months	23.1% Decrease in testosterone (p=.01), 22.2% decrease in dehydroepiandrosterone (p=.01), there was a statistically non-significant reduction in SHBG and increase in volume of both ovaries.
Chow et al. 2010	Not available	Healthy volunteers (men and women)	42	40 (19-64)	Daily 1000 mg	4 weeks	Decreased phenotypic activity of CYP3A4 (p=.01) increased phenotypic activity of CYP1A2 (p=.0005), increased lymphocyte GST-π (p<.005) and UGT1A1 activity (p<.01) in the lowest tertile only, report of menstrual irregularities and mood changes in 4.8% and hot flashes in 2.4% of the participants.
Chow et al. 2010	Pilot open label, single arm intervention	Postmenopausal women with high body mass index ≥25 kg/m^2	34	58 ± 8	Daily 1000 mg	12 weeks	10% Increase in SHBG (p<.01), 22.4% increase in E2 (p=.47), 73.2% increase in 2-OHE1 ng/mg creatinine (p<.01), 5.9% increase in 16α-OHE1 ng/mg creatinine (p=.08), 84.5% increase in 2-OHE1/16α-OHE1 ratio (p<.01).
Kjaer et al. 2015	Randomized double blind placebo controlled	Men with metabolic syndrome	66	Low dose 48.9 ± 6.5 High dose: 50.9 ± 5.9	Daily 150 mg and 1000 mg	4 months	Highest dose effects only: 25% reduction in serum androstenedione (p=.052), 41% reduction in serum dehydroepiandrosterone (p<.01), 50% reduction in serum dehydroepiandrosterone sulfate (p<.01).
Ornstrup et al. 2014	Randomized double blind placebo controlled	Men with metabolic syndrome	66	Low dose 48.9 ± 6.5 High dose: 50.9 ± 5.9	Daily 150 mg and 1000 mg	4 months	Dose dependant increase in bone alkaline phosphatase (BAP), (p<.01)[#], dose dependant increase in lumbar spine trabecular volumetric bone mineral density (LS $vBMD_{trab}$), (significant increase only in the high dose group, p<.036)[#].
Maia et al., 2012	1st Study: open office based study	1st Study: women with endometriosis treated with an oral combination contraceptive containing drospirenone and ethinylestradiol for 6 months without pain relief	12	30 ± 5 (22–37)	Daily 30 mg added to the contraceptive pill	2 months	Decrease in pain score, and dysmenorrheal and pelvic pain.
	2nd Study immunohistochemistry of placental tissue	2nd Study: women with endometriosis subsmitted to laparoscopy and hysteroscopy	42	31 ± 4 (24–40)	Daily 30 mg added to the contraceptive pill	2 months	Inhibition of endometrial aromatase and COX-2 expression.

*Number of participants who completed the trial.
[#]The results of *in vitro* studies suggest these outcomes could reflect estrogenic activity of resveratrol, but the study did not discuss this possibility.

submitted to laparoscopy and hysteroscopy to treat endometriosis. Patients were given drospirenone and ethinylestradiol alone or in combination with resveratrol. Patients who received resveratrol had significant suppression in the expression of aromatase in the eutopic endometrium (Maia et al., 2012).

The anti-estrogenic properties of resveratrol were investigated in the treatment of breast cancer in obese postmenopausal women. High abdominal adiposity in postmenopausal women is associated with decreased hepatic synthesis of SHBG and increased synthesis of estrogens. The decrease in circulating SHBG increases the biologically active free fraction of steroid hormones. Indeed, this predisposes postmenopausal obese women to develop breast cancer (Key et al. 2002). The increase in tissue exposure to endogenous estrogens is believed to induce neoplastic transformation through two primary pathways. The first is via activation of genomic and non-genomic ER signaling to promote cellular proliferation. The second is via metabolic conversion of E1 and E2 to catechol derivatives and then to reactive quninones that produce DNA oxidative damage and depurinating adenine and guanine adducts. As discussed previously, resveratrol in cell culture stimulated the production of SHBG and inhibited oxidative metabolism of E1 and E2 to catechols and DNA reactive quinone metabolites (Cavalieri and Rogan 2011; Cavalieri et al. 2012; Yager 2015). In a pilot study that enrolled obese postmenopausal women with a BMI $>$ 25 kg/m^3, the daily administration of 1 *g* of resveratrol for 12 weeks produced a significant 10% average increase in the concentration of SHBG. This was not associated with significant changes to the levels of E1, E2 and testosterone, however there was a noticeable 22.4% increase in E2 levels. Resveratrol produced a favorable increase in the concentration of urinary 2-hydroxyestrone (2-OHE1) and the ratio of 2-OHE1 to 16α-hydroxyestrone (16α-OHE1), the former is an ER antagonist and the latter is a known mitogen (Chow et al. 2014). These results were favorably viewed in the context of preventing the development of breast cancer in this subpopulation of women. They also support a role for resveratrol in modulating steroid hormone metabolism for a beneficial outcome. This conclusion is also supported by results of a trial on forty-two healthy female participants. The daily intake of 1000 mg for one month inhibited the phenotypic index of CYP3A4 and induced CYP1A2, enzymes that are important for estrogen metabolism. These changes in enzyme activities were associated with menstrual irregularities and mood changes in 4.8% and hot flashes in 2.4% of the participants suggesting alterations in the homeostatic balance of estrogens and steroidal hormones (Chow et al. 2010).

The potential role of resveratrol in the treatment of many diseases has been promising and in many cases could be mediated via its direct activation of ER. Perhaps the role of resveratrol in the treatment of osteoporosis is a prominent example. In early *in vitro* investigations, resveratrol treatment of MC3T3-E1 osteoblastic cells increased prolyl hydroxylase activity and production of alkaline phosphatase suggesting it can be used to protect bone mass in osteoporotic patients (Mizutani et al. 1998). Both activities were inhibited by tamoxifen suggesting they were mediated via ER activation. In a clinical trial examining the effects of resveratrol on bone formation and mineralization, middle aged men with the metabolic syndrome were treated with a daily dose of 150 or 1000 mg for 4 months. Resveratrol produced a dose dependent increase in bone alkaline phosphatase (BAP), a bone formation marker, and in the mineral density of the lumbar spine trabecular volumetric bone (vBMD) that was attributed to calcium deposition. The increase in BAP was 16% at all time points (4, 8 and 16 weeks of treatment) and the increase in mineralization of the trabecular vBMD was by 2.6% at the end of the study in the high dose group. Resveratrol did not affect bone resorption markers in the study, but the investigators suggested the positive impact of the compound on bone formation and mineralization could be associated with decreased osteoclast activity. The potential roles of vitamin D and PTH in mediating the reported effects were discussed, but interpretation of their levels and possible roles were difficult because of the variable baseline levels in the participants. The study did not address the role of the estrogenic properties of resveratrol in the reported outcomes although estrogen replacement therapy is commonly used to manage osteoporosis in menopausal women (Ornstrup et al. 2014, 2015).

6. Discussion

The clinical benefit of resveratrol on a wide range of human diseases was investigated in several trials. Unfortunately, the therapeutic efficacy in the majority of these studies was below expectations considering the hype associated with its *in vitro* effects. In early trials, it was indicated that the extensive intestinal and hepatic metabolism of resveratrol into glucuronide and sulfate conjugates reduced its oral bioavailability and that systemic concentrations were below the therapeutic threshold. Therefore, efforts were focused on enhancing the oral bioavailability of resveratrol in an attempt to advance its therapeutic utility and the literature has been continuously reporting a variety of approaches. These included the synthesis of metabolically stable derivatives and formulation of innovative dosage forms (Hoshino et al. 2010; Kapetanovic et al. 2011; Szekeres et al. 2011; Amiot et al. 2013; Yeo et al. 2013; Horgan et al. 2019). However, dose-escalation coupled to repeated dosing has been the most effective approach to this date in surmounting metabolic deactivation and enhancing the bioavailability of the compound. In this approach, the doses of resveratrol exceeded natural dietary levels by several orders of magnitude and in some cases reached up to 5 *g* per day. The clinical studies, doses and number of studies that used each dose have been summarized in recent reviews (Park and Pezzuto 2015; Pezzuto 2019). The plasma levels of unchanged resveratrol increased progressively with escalating doses to the lower micro-molar range. For example, single oral doses of 0.5, 1, 2.5 and 5 *g* furnished maximal plasma concentrations (C_{max}) of 0.3, 0.5, 1.2 and 2.3 μM, respectively (Boocock et al. 2007). The repeated administration of the same doses for 21 to 28 days produced 0.19, 0.62, 1.45 and 4.24 μM, respectively (Brown et al. 2010). These levels were deemed sufficient for

biological activity, especially for cancer chemoprevention. The side effects reported with the high dosing rates were generally mild suggesting resveratrol had a large margin of safety. However, the therapeutic benefits in most clinical studies remained limited and not supportive of a clinical role. It is important to note that dietary doses of resveratrol similar to those in red wine produced plasma levels of unchanged resveratrol in preclinical and clinical studies that were non-measureable or brief traces in the nano-molar range, three orders of magnitude less than what is needed for biological activity in most *in vitro* studies (Marier et al. 2002; Goldberg et al. 2003; Walle et al. 2004; Vitaglione et al. 2005). Because of resveratrol's relative safety in clinical trials, it was decided to demote the compound to the ranks of a dietary supplement. The daily dietary allowance was implied in light of the doses used in clinical trials to range up to 2 *g* per day (Boocock et al. 2007; Almeida et al. 2009; Brown et al. 2010). Commercial supplements of resveratrol were initially marketed by the health food industry in dosages as little as 50 μg, however the dose was progressively increased in later varieties to reach the level of 600 mg per serving, and was ramped up in recent years to 1000 mg per serving to allow convenient once-a-day administration and super antioxidant activity (Boocock et al. 2007; Williams et al. 2009; Edwards et al. 2011; EFSA Scientific Panel NDA 2016).

The overwhelming interest in the health benefits of resveratrol and need to improve its oral bioavailability have been approached with relative disregard to its endocrine effects. The main basis for this has been the lack of a reproducible positive result for resveratrol in the uterotrophic assay. In addition, estrogenic and hormonal effects were not reported in short and subchronic toxicity studies. However, as reviewed herein, resveratrol is unique in its pleiotropic hormonal actions mediated via a multitude of biological effectors. These include mixed agonistic and antagonistic activities on ER in different tissues and organs, inhibition of enzymes in steroidogenesis and modulation of enzymes and proteins important in the disposition of estrogens and steroids (Figure 3). In many cases, the actions of resveratrol on individual targets and its impact on estrogen and steroid hormone homeostasis are found in sporadic studies throughout the vast resveratrol literature, but little attention has been given to these studies and attempts to replicate their findings have been limited. In experimental *in vitro* tests, almost all of the estrogenic and hormonal effects of resveratrol required single and double digit micromolar concentrations. The administration of supplemental doses in the range of 0.5–1 *g* per day establishes low micro-molar levels that are just enough to initiate biological effects on all of its targets simultaneously, albeit to different extents. Following chronic exposure, the combined activities of different strengths on multiple targets can lead to a progressive drift in the homeostatic balance of estrogens and other steroids to a new physiological set point. This pharmacological outcome may represent an opportunity for the management of many endocrine related anomalies such as PCOS, endometriosis and endocrine related cancers. However, it may as well predispose to endocrine disruption, the manifestations of it can include but are not limited to abnormalities in reproductive organ development and physiology, aberrations in sexual behavior and flare of endocrine related diseases such as hormone dependent cancers. The clinical and experimental evidence reviewed herein is sufficient to raise reasonable concern that resveratrol can produce endocrine disruption. For example, resveratrol consumed at a rate of 1 *g* or more per day in clinical trials altered the plasma levels of steroidal hormones and their precursors within 3–4 months (Kjaer et al. 2015; Banaszewska et al. 2016). The long term health consequences of these effects are currently unknown from an endocrine perspective.

It is difficult to identify the most susceptible target to resveratrol within the network of enzymes and proteins that regulate estrogen and steroid hormone homeostasis. However, a comparison of the EC_{50} and IC_{50} values presented in this review suggest that hepatic metabolism of steroids is most vulnerable. The interference of resveratrol in steroid hormone metabolism can produce elevations in the concentration of estrogens and other steroids and profound potentiation of their central and peripheral effects. Therefore, if the direct estrogenic effects of resveratrol mediated via ER interaction have been marginalized due to their weak and non-reproducible nature in the uterotrophic assay, the indirect estrogenic effects via inhibition of estrogen metabolism and increase in plasma E2 levels warrant careful consideration, especially when approving high strength resveratrol supplements. Subtle elevations in E2 are likely to happen following chronic exposure and this has been reported in some of the studies reviewed herein (Henry and Witt 2002; Chow et al. 2014). In addition, the ability of resveratrol to potentiate the actions of oral contraceptives in treating endometriosis-related pain lends support to this aspect (Maia et al. 2012). Contraceptives share many of their metabolic pathways with endogenous estrogens and steroids and it is conceivable that resveratrol maximizes their therapeutic efficacy by inhibiting their metabolism. Another important consideration in resveratrol's interference in common pathways of steroid hormone metabolism is that high dosing rates may overwhelmingly deplete these metabolic pathways of essential cofactors and precursors required by their respective enzymes. This can forge estrogens and steroids into alternative less common pathways of metabolism that yield metabolites of mutagenic potential. It also alters the metabolism of drugs taken concomitantly, increasing their systemic levels and potential for toxicity (Chow et al. 2010, 2014).

Recent investigations are beginning to uncover the role of resveratrol's metabolic conjugates in mediating some of the compounds beneficial health effects. In clinical trials, the plasma levels of the glucuronide and sulfate conjugates reached micro-molar concentrations that were significantly greater than the parent compound (Boocock et al. 2007; Brown et al. 2010; Patel et al. 2010). Studies report these conjugates possess biological properties that are similar to resveratrol in many perspectives. For example, the sulfate conjugates induced quinone reductase 1 and acted as a radical scavenger to prevent oxidative stress just like resveratrol. They also activated SIRT1 and inhibited COX1 and 2 activities and NFκB expression to suppress inflammation (Calamini et al. 2010; Hoshino et al. 2010). On the other hand, the glucuronide conjugate possessed antioxidant and radical

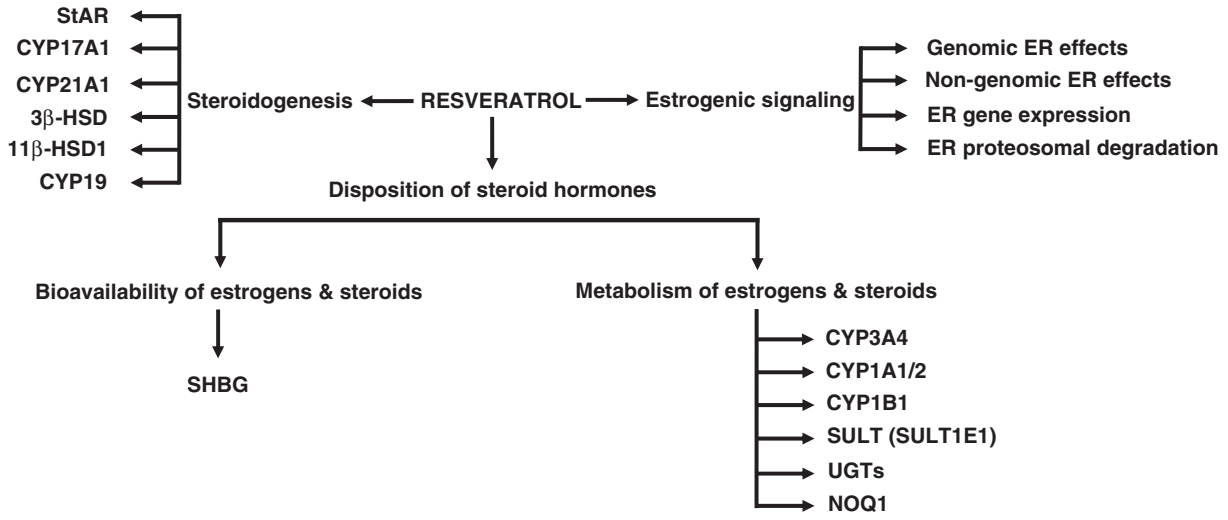

Figure 3. The pleiotropic influence of resveratrol on estrogen and steroid hormone homeostasis. Resveratrol exerts direct effects on estrogenic tissues that has been a mixture of agonistic and antagonistic actions. It also acts indirectly to alter systemic levels of estrogens and steroids by interfering in steroidogenesis and estrogen and steroid hormone disposition. The overall impact can shift the homeostatic balance of estrogens to a new physiological set point.

scavenging activities that were more powerful than resveratrol (Mikulski and Molski 2010). However, these metabolic conjugates are not free of side effects and could be responsible for some of the toxic effects of resveratrol, especially following prolonged intake of high-strength supplements. From an endocrine perspective, they may mediate some of resveratrol's hormonal effects and potentially disrupt steroid hormone homeostasis. As a matter of fact, they are in a better position to impart hormonal actions of their own because of their higher plasma concentrations relative to the parent compound. For example, the systemic levels of resveratrol-3-*O*-sulfate (Figure 1), the primary sulfate conjugate following the administration of high doses of resveratrol, exceeded the parent compound by 18 and 23 fold following oral administration of 0.5 and 5 grams of resveratrol, respectively (Boocock et al. 2007). Notably, this was the only sulfate conjugate detected in human breast cancer with a rate of production that was 33.5 times greater than in non-tumoral adjacent tissues (Miksits et al. 2010). In a recent study, this sulfate conjugate exhibited strong anti-estrogenic activity on both ER isoforms in a yeast two hybrid system. In addition, 50 μM of the conjugate decreased E2 driven luciferase expression by 40% in MCF-7 cells stably transfected with a luciferase reporter gene. Surprisingly however, it stimulated the proliferation of ERα positive MCF-7 cells with an EC_{50} of only 4.9 μM, a concentration that is easily achieved in plasma following the consumption of high-strength resveratrol supplements (Ruotolo et al. 2013). For example, the maximal plasma concentration (C_{max}) of resveratrol-3-*O*-sulfate following the oral administration of 0.5 and 1 *g* of resveratrol in humans was 3.7 and 6.8 μM, respectively (Boocock et al, 2007). Therefore, this metabolite may flare ERα positive breast cancer and other estrogen dependent cancers if supplemental doses in excess of 0.5 *g* are taken on chronic basis. However, in another group of studies, resveratrol-3-*O*-sulfate at clinically relevant concentrations inhibited the proliferation of three human colorectal cancer cell lines via a combination of autophagy and induction of senescence (Patel et al. 2013; Andreadi et al. 2014). It also exhibited synergistic antiproliferative effects on colon cancer cells when combined with other resveratrol conjugates, namely resveratrol-3-*O*-glucuronide and resveratrol-4′-*O*-glucuronide (Aires et al. 2013). Because normal colorectal tissues express ERβ and the receptor is progressively lost during the progression phase of colon cancer (Jassam et al. 2005; Castiglione et al. 2008), the different proliferative responses of colon and breast cancer cells to resveratrol-3-*O*-sulfate could be a reflection of the differential expression of either ER isoform in the two cell types and the respective biological outcomes of their activation. To this end, it is worth mentioning that there has been experimental data attributing some of the beneficial cellular effects of resveratrol, such as inhibition of replicative growth and improvement in cellular defense to oxidative stress, to its activation of ERβ (Robb and Stuart 2011). Collectively, these findings emphasize the importance of investigating the biological properties of resveratrol metabolites, especially their effects on the endocrine system as the results may uncover previously unknown but important implications to the homeostasis of steroid hormones and management of hormone dependent cancers and endocrine related diseases.

The oral doses of resveratrol and other phytoestrogens correlate well with plasma levels, but there has not been a clear correlation between plasma levels of phytoestrogens and the amounts that accumulate in tissues (Whitten and Patisaul 2001; van Duursen 2017). Therefore, plasma levels are not reliable enough to predict tissue exposure levels. This potentially complicates assessment of the long term hormonal risks of resveratrol exposure. There is enough evidence from animal studies that resveratrol and its metabolic conjugates accumulate in tissues to levels that induce biological activity (Vitrac et al. 2003; Bresciani et al. 2014; Wang et al. 2017). For example, resveratrol was reported to accumulate in intestinal cells to levels that inhibited colon cancer (Patel et al. 2010). Studies in rodents indicated that a large proportion of resveratrol accumulation occurred in the heart, liver and kidneys (Bertelli et al. 1998). In a study in rats, resveratrol conjugates accumulated in the heart in a dose and time dependant manner following daily treatment with 1 and

5 mg/kg body weight for 3-6 weeks (Bresciani et al. 2014). Because resveratrol can be regenerated *in vivo* from its metabolic conjugates via a group of sulfatases and β-glucuronidases found in blood and tissues, their bioaccumulation helps sustain systemic levels and tissue exposure to resveratrol and prolongs its biological effects including its hormonal activities (Walle et al. 2004; Boocock et al. 2007; Patel et al. 2013). The *in vivo* regeneration of resveratrol has been confirmed in mice treated via oral gavage with resveratrol-3-*O*-sulfate in doses that established clinically relevant concentrations of the conjugate. In addition, the intracellular generation of resveratrol via de-conjugation was 10 fold greater than the levels achieved from resveratrol penetrating through the plasma membrane when clinically relevant concentrations of the conjugate were used in culture (Patel et al. 2013; Andreadi et al. 2014). However, the literature remains deficient in tissue exposure levels to resveratrol and its conjugates, especially in endocrine and reproductive organs, and more data is needed to allow a detailed risk assessment in these tissues. It is noteworthy that the intracellular accumulation of resveratrol via interaction with cellular proteins was implied in very early *in vitro* studies and was attributed to its low water solubility (Gehm et al. 1997).

The estrogenic activities of resveratrol have been confirmed in many *in vitro* investigations, yet *in vivo* ER activity is still vague, has not been reproducible and the statistical risk of such activity is unpredictable at this point. It is generally accepted that human exposure to the strongest environmental estrogen, whether *in utero* or during the period from adolescence to adulthood should be at least 1000 fold greater than any natural level in order to trigger clinically significant adverse effects via an endocrine disruption mode of action (Sharpe 2003; Golden et al. 2005). In the case of resveratrol supplements, the consumption of 1 *g* per day fulfills the exposure criterion because the dose surpasses natural dietary levels by several orders of magnitude. For example, the consumption of 1 *g* of resveratrol is equivalent to many years of daily consumption of white and red wines, grapes, peanuts and other resveratrol rich diets (Weiskirchen and Weiskirchen 2016). Resveratrol is unique compared to common endocrine disruptors in its pleiotropic effects on the systems involved in estrogen and steroid hormone homeostasis. High dosing rates of resveratrol combined with its pleiotropic mechanisms of hormonal action qualify it from a hypothetical standpoint to the category of endocrine disrupters, especially if taken over prolonged periods or during critical windows of development. Therefore, there is great need to monitor the endocrine effects of high strength oral resveratrol supplements in long term clinical trials. In the meantime, it is prudent that specific consumer subpopulations avoid large supplemental doses (van Duursen 2017). These include women of reproductive age in addition to patients treated with estrogen replacement therapies or who use contraceptive pills and drugs that require hepatic metabolism, or those who have hepatic and renal disease or endocrine related diseases such as hormone dependent cancers or are vulnerable to develop such diseases. Consumers may choose to consume doses that match natural dietary levels of the compound, estimated in Europe to range from 0.01 to 0.45 mg per day (Edwards et al. 2011). The likely fact that the health benefits of red wine, grapes and other diets are mediated via a complex mixture of polyphenols rather than resveratrol alone (resveratrol being one molecule of 1600 molecules in grape (Pezzuto 2011)), and because the therapeutic benefits of pharmacological doses of resveratrol have been limited in clinical trials substantiate this recommendation (Vitaglione et al. 2005). Interestingly, a case control study conducted between the years 1993 and 2003 and evaluated dietary resveratrol intake and the risk of breast cancer in the Swiss Canton of Vaud reported an inverse association only for resveratrol from grapes suggesting a nonspecific favorable effect from fruit (Levi et al. 2005). On the other hand, if supplements in the dosage range of 0.5–1 *g* per day are used on long term basis for antioxidant, anti-aging and metabolic benefits, it is important consumers are kept aware of the potential estrogenic and hormonal hazards of resveratrol.

7. Summary

Literature evidence suggests that the hormonal effects of resveratrol are subtle, require time to manifest and are likely to go unnoticed or masked by an acute or progressive toxicity to specific organ systems in acute and sub-chronic toxicity studies. Resveratrol modulates estrogenic activity via multiple mechanisms. The compound directly interacts with ER and functions as a mixed agonist/antagonist. It also interferes in intestinal and hepatic metabolism of estrogens, which increases their levels and potentially intensifies their central and peripheral actions. Resveratrol also inhibits critical enzymes in steroidogenesis which alters the plasma levels of steroids and their precursors. The short term consumption of dietary supplements may not produce endocrine related abnormalities, but chronic exposure to pharmacological doses of the compound potentially shifts the homeostatic balance of estrogens and other steroid hormones to a new operational set point. This outcome opens a window for therapeutic benefit in some endocrine diseases, but also predisposes to detrimental effects on reproductive organ development and function, socio-sexual behavior and endocrine related pathologies such as hormonal dependent cancers. Therefore, there is a need to determine the long term effects of resveratrol on steroid hormone homeostasis in future preclinical and clinical trials, especially with pharmacological doses in excess of 0.5 *g*. Until sufficient data is available, the consumption of high-strength resveratrol supplements for general health benefits on long-term basis or during critical windows of development or by specific consumer subpopulations warrants caution.

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