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RESVERATROL AND HORMESIS

RESVERATROL: AN ASSESSMENT OF ITS DOSE-RESPONSE *An Introduction*

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Resveratrol has become a major focus of biomedical research over the past two decades. This interest was initially based on reproducible epidemiological investigations showing an inverse relationship between the consumption of red wine and various types of cardiovascular disease (Mochly-Rosen and Zakhari, 2010; Ramprasath and Jones, 2010; Tosun and Ikaya, 2010). According to the Web of Science Database the first citation of resveratrol was in 1977. Scientific interest in resveratrol grew slowly with approximately 200 cumulative citations over the next 15 years. However, from the mid 1990s to the present this field has expanded considerably. For example, in the year 2001 alone there were nearly 3100 citations concerning resveratrol. In 2009 this number had jumped to nearly 18,000 (Figure 1) with 2010 projected to easily exceed 20,000 citations. These citations are based on the publication of now over 5000 articles on resveratrol in the biomedical literature (Figure 2).

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While the initial biomedical interest in resveratrol focused on its implications for cardiovascular health, research interest has broadly expanded, now involving a plethora of biological endpoints and disease conditions which may be affected by consumption of resveratrol. These areas are very broad and significant, affecting neurodegenerative diseases (Sun et al., 2010), immunological responsiveness (Yusuf et al., 2009), a broad spectrum of cancers (Alfaras et al., 2010), and the aging process (Imai, 2010), amongst others. These findings have lead to numerous efforts to explore how resveratrol can be used to enhance the public health.

Despite this rather striking proliferation of research on the biomedical and public health implications of resveratrol there has been a surprising lack on focus on the nature of its dose response and how this type of information could guide both research activities as well as recommendations concerning the consumption of resveratrol. Clarification of the nature of the dose response for resveratrol is therefore an important consideration.

The nature of the dose response in toxicology and pharmacology has become a topic of considerable interest. During the past century the fields of toxicology and pharmacology came to accept the assumption that the most fundamental nature of the dose response was that of a threshold. Despite never validating this assumption via scientific evaluation (Calabrese, 2010), regulatory agencies in the middle decades of the 20th century adopted the use of the threshold model and built major regulatory programs based upon it (Calabrese, 2009a). The only exception to this general dose response perspective was the introduction of linearity at low dose modeling for genotoxic carcinogens, a perspective based principally on a public health protectionist philosophy in the late 1950's for ionizing radiation and the late 1970's for chemical carcinogens (Calabrese, 2009b). However, over the past two decades acceptance of these two dose response theories (i.e., the threshold model and the linear at low dose model) has been challenged by the hormetic dose response model (Calabrese and Baldwin, 2003a; 2003b; 2001a; Calabrese and Blain, 2005). These findings indicate that the biphasic hormetic dose response was very common and highly generalizable, being independent of biological model, endpoint measured and chemical class. Furthermore, in head to head competition the hormetic dose response model has repeatedly far outperformed the threshold and linear dose response models in making accurate predictions of biological responses in the low dose area (Calabrese and Baldwin, 2003c, 2001b; Calabrese et al., 2010, 2008, 2006). These findings lead to the conclusion that the hormetic dose response is more fundamental, common and generalizable than the threshold and linear dose response models. It also strongly suggested that the hormetic dose response model might have considerable relevance to the design of studies and in the interpretation of biomedical research on chemicals, pharmaceuticals and natural products like resveratrol. Consequently, a comprehensive effort was made to assess the dose response features of resveratrol. The results of this assessment indicated that the hormetic dose response is a highly common feature of the resveratrol literature, having important and broadly based implications. Consequently, this issue of the BELLE Newsletter provides a detailed assessment of hormetic dose response relationships for resveratrol in the biomedical and toxicological literature. This comprehensive assessment is followed by multiple unedited expert commentaries on this paper by leading researchers in this area. Finally, this issue closes with a final response by the authors of the comprehensive review to the expert commentaries.

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Figure 1. Number of articles published on resveratrol and listed in Web of Science database

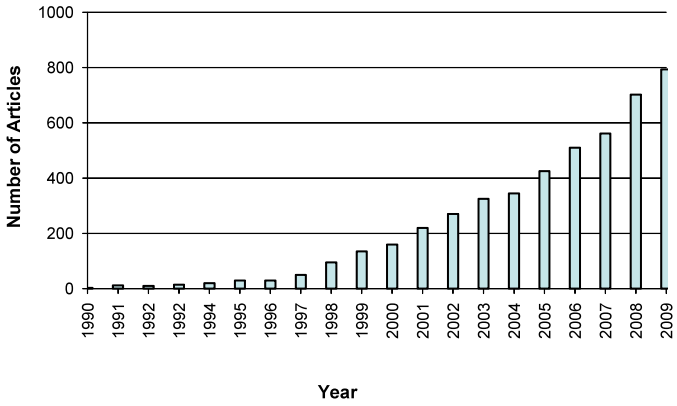
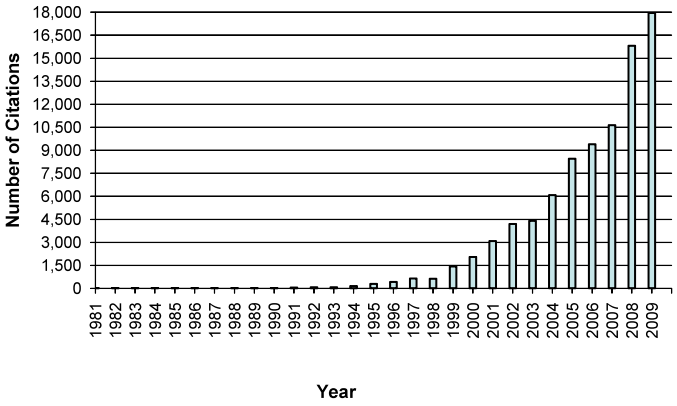


Figure 2. Citations of the term resveratrol in Web of Science database



RESVERATROL COMMONLY DISPLAYS HORMESIS: OCCURRENCE AND BIOMEDICAL SIGNIFICANCE

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ABSTRACT

Resveratrol induces hormetic dose responses in a wide range of biological models, affecting numerous endpoints of biomedical and therapeutic significance. These responses were reported for numerous human tumor cell lines affecting breast, prostate, colon, lung, uterine and leukemia. In such cases low concentrations of resveratrol enhanced tumor cell proliferation whereas higher concentrations were inhibitory. Similar resveratrol induced biphasic dose responses were seen with several parasitic diseases, including leishmaniasis and trichinella. Hormetic effects were also reported in animal models for cardiovascular induced injury, gastric lesions, ischemic stroke, Alzheimer's disease and osteoporosis. In these cases there was often a protective effect at low doses but an adverse effect at higher doses, exacerbating the disease process/incidence. This analysis indicates that many effects induced by resveratrol are dependent on dose and that opposite effects occur at low and high doses, being indicative of a hormetic dose response. Despite consistent occurrence of hormetic dose responses of resveratrol in a wide range of biomedical models, epidemiologic and clinical trials are needed to assess the nature of its dose-response in humans.

Key Words: resveratrol, hormesis, hormetic, biphasic, U-shaped

INTRODUCTION

Resveratrol (3,4',5-trihydroxystilbene) is a phenolic compound found naturally in numerous plant species as the *cis* and *trans* isomers. Resveratrol has been characterized as a phytoalexin, an anti-infectious compound produced by plants in particularly high amounts as a response to injury, pathogenic-induced damage, nutrient deficiency, temperature fluctuations as well as other environmental factors such as exposure to ozone, ultraviolet radiation and other stressors.[1,2] Interest in resveratrol, particularly the *trans* isomer, markedly increased in the early 1990s when investigators reported that mortality associated with coronary artery disease was lower in Southern France than in other industrialized countries in spite of consumption of a diet relatively high in saturated fat. This phenomenon soon became known as the "French Paradox".[3] This term became "coined" in November 1991 during the CBS program "60 Minutes" by Dr. Serge Renaud with the lower risk being attributed to the prolonged daily consumption of moderate amounts of red wine by the southern France population.[4] Later, resveratrol was proposed to be one of the active agents in red wines responsible for the French Paradox. These comments and perspectives led to a major outpouring of research on the biological (e.g., pharmacokinetic/pharmacodynamic aspects)[2,5-7] and biomedical effects of resveratrol, demonstrating its capacity to protect against coronary heart disease, retard tumor cell proliferation and to enhance longevity of lower organisms.

The present assessment was undertaken to evaluate whether and to what extent resveratrol displays hormetic dose responses and how this might affect its public health and therapeutic implications. Hormesis is a biphasic dose response phenomenon that is characterized by a low dose stimulation and a high dose inhibition[8-14] with specific quantitative features with respect to the magnitude and width of the low dose stimulation

(Figure 1).[15-17] Hormetic dose responses, which have long been reported in the biomedical literature,[18-22] have been found to be highly generalizable, being independent of biology model, endpoint measured, and chemical class. Hormesis has also been able to account for numerous types of adaptive responses such as preconditioning and post-conditioning in a broad spectrum of biomedical models, as well as for adaptive responses to radiation exposure.[23,24] Resveratrol causes a plethora of biological responses in cells and organisms that involve a spectrum of adaptive mechanisms, suggesting that such responses may be mediated by hormetic mechanisms and display its characteristic quantitative features including a modest stimulatory response (30-60% increase over controls) in the low dose zone. What has emerged from the assessment is substantial evidence that resveratrol often displays a biphasic dose response with quantitative features consistent with the hormetic dose response. Tables 1 and 2 provide a summary overview to the range of hormetic dose responses addressed in this paper, with more detailed considerations being provided in the body of the text. These observations extend across a very broad range of biological models and endpoints and may profoundly impact the public health and clinical applications of resveratrol and structurally/functionally related agents.

TUMOR CELL LINES

Human Breast Cancer Cell Lines

Estrogen Receptor-Positive

Cell Proliferation

Considerable research efforts have explored the effects of resveratrol on human breast cancer cell lines. This research includes both estrogen receptor (ERα) positive (i.e., MCF-7, TD-47 and KPL-1) and ER(α) negative (i.e., MKL-F, MDA-MB-231, Bcap37 and SK-BR-3) cell lines. MCF-7, the most frequently studied human breast tumor cell line with respect to resveratrol, has commonly displayed a resveratrol induced biphasic dose response (Figures 2-5). A similar type of biphasic dose response was also reported for the other two ER positive cell lines (Figure 6).[25-28]

In the above cited studies resveratrol was consistently demonstrated to act as an estrogen agonist at low concentrations and an antagonist at higher concentrations. Such concentration-dependent agonist and antagonist behavior was employed to account for mechanisms underlying the biphasic concentration response. These observations were generalizable as they occurred for either the *trans* or *cis* form of resveratrol, regardless of the source of the MCF-7 cells as well as independent of the use of a wide variety of experimental methods (Figures 7A and 7B).[25-30] Not only was the biphasic dose response consistently demonstrated but so to were its quantitative features (i.e., maximum stimulation and width of the stimulatory response). That is, in most cases the maximum stimulation was about 30-60% greater than the control, although the paper by Liu and Serrero[28] displayed an increase of about 300%. The width of the stimulatory zone was reasonably well defined in several of these studies and suggested a 50-100-fold concentration range. While the optimal concentration for cell proliferation was study-dependent, the basic hormetic/biphasic concentration response was nonetheless consistently seen. The reason for the variation in optimal cell proliferation concentration is most likely related to the use of differing experimental conditions during the various assays. Despite these observations of a consistent resveratrol-induced low dose stimulation of ERα positive human tumor cell lines, the overriding interest, based upon the focus of essentially all relevant published papers, concerned the capacity of resveratrol to inhibit the growth/proliferation of the various human breast tumor cell lines for application in the treatment of cancer. Consequently, efforts of these investigators were typically directed toward clarifying mechanisms by which resveratrol induced cell death, typically exploring various apoptotic pathways.

Mechanism of Hormetic Cell Proliferation Response

Despite the lower priority placed on clarifying the nature of the low concentration-induced stimulation, a possible mechanism to account for the resveratrol induced hormetic dose response was published by Pozo-Guisado et al.[30] They proposed that resveratrol

modulates the ERα dependent phosphatidylinositol-3 kinase (PI3K) pathway which in turn mediates cell proliferation and apoptotic pathways. PI3K activity was enhanced at low concentrations of resveratrol (10 μM) while being inhibited at concentrations > 50 μM. This occurred in complete media with E2 at 10^{-12}M or in E2-depleted media. The effect of resveratrol on PI3K was blocked by the pure anti-estrogen 182,780, suggesting that resveratrol had an agonist effect on the ERα dependent PI3K activity. The hormetic profile of PI3K activity therefore is consistent with the overall concentration response of resveratrol on cell proliferation in the MCF-7 cell line and is most likely a determining factor of this low concentration stimulatory process.

Significance of Hormetic Cell Proliferation Response

-Key for optimizing chemotherapeutic strategy

The low concentration induced stimulation of human breast tumor cell lines was consistently reported but typically placed into an area of secondary importance, essentially never generating detailed and insightful discussion. In fact, it was only the report of Li et al.[31] that highlighted this biphasic concentration response in their assessment, pointing out that this phenomenon was an example of hormesis and that further research was needed before resveratrol should be considered as a chemotherapeutic drug for breast cancer. The concern raised by Li et al.[31] related to the capacity of resveratrol to enhance the proliferation of human breast tumor cell lines at low concentrations. The maximum proliferation increase has generally been in the 20-60% range, typical of most hormetic dose responses. While one may hypothesize on the biological significance of the "modest" increase in cell proliferation response, it should be noted that the magnitude of linoleic acid induced increases in cell proliferation of various human breast cancer cell lines is quantitatively similar to that induced by resveratrol.[25] Many authors tend to highlight the "major" stimulative effect of linoleic acid, yet this response is in the same range as observed for resveratrol. This suggests that the low concentration stimulatory effects of resveratrol could be considered within the same quantitative context as the "highly" stimulatory linoleic acid, raising similar clinical concerns for both agents.

-Molecular targets and clinical applications

While hormetic responses have been commonly reported, it has been unclear how this biphasic concentration response may be exploited in the clinical management of human breast cancer treatment as well as in a chemopreventive manner. Important insights into this question have been obtained by de Leon and her colleagues[32] over the past several years. Their general research strategy has centered on the identification of molecular targets for resveratrol and how clinical approaches might to be guided by the biphasic nature of the dose response relationship.

Molecular Target # 1

IGF-II

This group identified two potentially key molecular targets for chemo-preventive action, insulin growth factor (IGF)-II and cathepsin D, indicating how resveratrol may affect the occurrence of breast cancer via interactions with these two targets.[32] Starting with IGF-II, this protein is synthesized in breast cells, becoming secreted under certain conditions, inducing a strong mitogenic response which facilitates the expansion of a breast tumor cell mass. This protein also inhibits the occurrence of apoptosis in breast cancer cells. Once secreted, IGF-II may thereby contribute to an increased risk of breast cancer. In a developmental sense, IGF-II plays an important role in early life stages when more active cell division is required but is much less active during reduced growth periods. IGF-II, with its mitogenic mediating functions, is kept in check by cell cycle watchdog genes such as p53 and WT-1. When these genes become mutated or deleted the IGF-II loses "supervision" thereby facilitating tumor promotion.

IGF-II is comprised of several forms, but typically is referred to as either proIGF-II or mature IGF-II. The proIGF-II form of IGF-II is the predominant isoform that is secreted by most tumors. It is biologically active with its effects being mediated via multiple receptors. These include the M6P receptor which affects a pathway that leads to the degradation of IGF-II and the slowing down of other biological functions of IGF-II. The other receptors, IGF-1 and insulin receptor isoform A, when activated, facilitate mitogenic activities. IGF-II itself is

regulated by estrogen and it is via the action of estrogen that IGF-II facilitates breast cancer cell proliferation and tumor development. Since estrogen regulates IGF-II, and since resveratrol can serve as an estrogen agonist and antagonist, de Leon and her colleagues proposed that resveratrol may have inhibitory and stimulatory effects on human breast cancer cell lines that are mediated by IGF-II.

This group systematically assessed the capacity of resveratrol to affect secretion of the IGF-II and IGF-1 in two human breast cancer cell lines, MCF-7 and T47D, both ERα positive. They then assessed two types of chemical interactions, that is, a joint exposure to resveratrol and estrogen and between resveratrol and the estrogen antagonist tamoxifen. These experiments were followed by assessing the effects of resveratrol on IGF-II gene expression and then on cell growth and survival. The resveratrol was demonstrated to biphasically affect each of the endpoints studied with a low concentration (10^{-6}M) affecting an approximate 2-fold increase over control values (Figure 8). However, at the higher concentration of 10^{-4}M there was a decreased responsiveness by about 30-40% from the control group. While estrogen was also stimulatory at low concentrations (10^{-9}M), it prevented the stimulatory effects of resveratrol when they were administered jointly at the low concentrations that are normally stimulatory when administered separately. Furthermore, the administration of tamoxifen, an estrogen antagonist, was also able to prevent the low dose stimulatory effects of resveratrol. The stimulatory effects of resveratrol were blocked by an IGF-I antibody, thereby identifying activation of this receptor as critical to the stimulatory response. Complementary experiments using the higher inhibitory concentrations of resveratrol revealed that these effects were independent of the estrogen receptor, in contrast to the responses at low concentrations. This observation was particularly important, suggesting that high doses of resveratrol could have therapeutic utility for women with breast tumors, independent of their ER status. Furthermore, the low dose stimulation would more likely be a health concern for those women with ER positive status.

Molecular Target #2

Cathepsin D

While the above assessment of resveratrol principally concerned its capacity to stimulate human breast tumor cell proliferation via the modulation of IGF-II, de Leon and colleagues[32,33] extended their inquiry to cathepsin D (CD), a protease that is also regulated by estrogen and over-expressed in many human tumor cells, including those positive for the ER. CD secretion, like that of IGF-II, is strongly associated with a risk of metastasis and mitogenic activity. There also are a number of ways in which IGF-II and CD interact and it is the nature of these inter-relationships that led de Leon to assess whether resveratrol could modulate CD activity/effects. For example, it was known that proIGF-II administration can stimulate an increase in CD secretion from MCF cells. In fact, IGF-II binding to IGF-M6P is needed for CD secretion. Since IGF-II can interfere with CD routing, these researchers decided to explore whether resveratrol would modulate CD secretion and several of its other related effects. Following the experimental procedural plan of the earlier study with IGF-II, they tested whether resveratrol might affect CD secretion in MCF-7 and T47D cells and its effects on CD mRNA levels. Furthermore, they assessed the effects of resveratrol and IGF-II on CD secretion in ER negative human breast cancer cell lines and epithelial cells. In the case of CD secretion and mRNA levels the resveratrol displayed a consistent hormetic-like biphasic concentration response in both cell lines (Figure 9A and 9B). In contrast, resveratrol did not enhance the secretion of either IGF-II (mature or precursor forms) or CD in the ER negative cell lines. However, it again displayed an inhibitory response. The authors suggested that resveratrol has the capacity to be a useful therapeutic agent for ER positive and negative breast tumors based on its effects at high doses. They also indicated that it was not likely that the low dose stimulation would occur since it would likely be antagonized by naturally occurring estrogen or by the administration of tamoxifen.

Molecular Target #3

Vitamin D Receptor

Resveratrol may also affect the risk of breast cancer via its capacity to affect the vitamin D receptor (VDR). In breast cancer cells, $1,25(OH)_2D_3$ causes a decrease in growth and an increase apoptosis *in vitro* and *in vivo*, thereby implying a therapeutic application for vitamin D in breast cancer treatment. However, since the levels of $1,25(OH)_2D_3$ required to affect such growth regulation can also induce hypercalcemia, structural analogs have been developed which display increased regulatory responses with less side effects. Additional studies have also demonstrated that VDR promoters are responsive to estrogen and may be up-regulated by estrogen in breast cancer cells. These findings led Wietzke and Welsch[34] to explore the capacity of several phytoestrogens, including resveratrol, to affect VDR signaling in two ER positive human breast cancer cell lines, MCF-7 and T47D. Similar to natural estrogen, resveratrol can interact with both ERα and ERβ subtypes, although with lower affinity than estrogen. It can also activate estrogen responsive genes containing the classical estrogen response elements (ERE) and can activate the SP1 transcription factor. Since activation of the VDR promoter activity was expected to reduce cell proliferation, it was hoped that the resveratrol could display a capacity to inhibit cell proliferation in the two cell lines. The study revealed that resveratrol affected a biphasic dose response for both the VDR promoter activity and for cell proliferation in both cell lines (Figures 10A, 10B). The concentration responses were such as to be non-overlapping. That is, the resveratrol effects on the VDR promoter activity occurred at a concentration lower than its effects on cell proliferation. Furthermore, as expected, when the VDR site was activated there was a decrease by about 15% in cell counts. At a 10-fold higher concentration the resveratrol increased cell proliferation. This study, therefore, yielded a triphasic dose response with a low concentration decreasing cell proliferation and higher concentrations stimulating cell proliferation. Further increases in the resveratrol concentration resulted in decreased cell numbers. It is interesting to note that at the highest concentration of resveratrol that caused an increase in the VDR promoter activity (40 nM) there was also a modest increase in T47D cell number (Figure 10B). This suggests that there is a competition between pathways for cell proliferation and inhibition, which in this case favored proliferation.

Estrogen Receptor Negative Tumor Cell Lines and Low Concentration Stimulation

At high concentrations resveratrol inhibits the growth of ER negative tumor cell lines. Of further interest is that a low concentration of resveratrol stimulated cell proliferation in ER negative cells[31,35] (Figure 11). With the observations of the biphasic concentration response in the ER negative cell lines it suggested that resveratrol could induce cell proliferation and inhibition via a mechanism(s) that are independent of the ER. Regardless of the mechanism by which the biphasic concentration response was occurring in the ER positive or negative cell lines (Figure 12), the quantitative features of these concentration responses were similar. While one may have predicted that the magnitude of the stimulation would be significantly less than that reported in ER positive cells, this was not the case.

These curious observations have received little follow up attention since resveratrol effects on cell lines with an ER positive status have been so convincingly prevented by the use of estrogen antagonists. Yet if resveratrol also affects a stimulatory response for cell proliferation in ER negative cells this may have considerable biomedical and therapeutic implications.

Metastatic Breast Cancer

Considerable research has focused on resveratrol as a chemo-preventive agent, reducing the risks of multiple cancers, including breast cancer. This suggested that resveratrol may act, at least in part, via the ERα receptor. However, in the case of metastatic breast cancer the ERα is typically lost, yet these cells retain the ERβ receptor isoform. This led to the use of the cell line MDA-MB-231 which is ERα(-) and ER(β)(+). Since resveratrol can interact with ERβ, Azios and colleagues[36,37] assessed its capacity to affect processes that are related to tumor metastasis, including focal adhesion activity, cell migration, cell invasion, and cytoskeleton characteristics predictive of metastasis. While it was the intention

of these investigators to explore rather high concentrations of resveratrol, they broadened the concentration range in order to assess multiple endpoints more effectively. To their surprise, they noted the occurrence of a biphasic concentration response for focal adhesion kinase (FAK) activity (Figure 13)[36] as well as for cell migration, cell invasion, and lamellipodia formation (a cytoskeleton change indicative of metastasis). Whereas a high concentration (50 μM) of resveratrol consistently reduced these multiple indices of metastasis, a 5 μM concentration of resveratrol consistently induced the opposite response, that is, an enhancement of metastatic parameters by about 60% over control values (Figure 14). According to the authors, the biphasic concentration-dependent effects of resveratrol have been widely reported and may be explained by its mixed ER agonist/antagonist properties. Thus, it is commonly seen that lower concentrations (0.01-10 μM) of resveratrol behave as an estrogen, activating pathways such as MAPK or P13K/AKT while higher concentrations (50-100 μM) are inhibitory.

Morphological investigations using the breast cancer metastatic model indicated that resveratrol displayed hormetic-like biphasic concentration effects on actin cytoskeletal morphology which were associated with differential effects on migration of the tumor cells.[36,37] While resveratrol at 5 μM induced a rapid, continuous and leading edge lamellipodia response, increasing the concentration by ten-fold (i.e. to 50 μM) quickly induced the formation of filopodia, leading to a non-migrating response of the tumor cells. The authors noted that while resveratrol can act as a preventive agent at high doses with respect to breast cancer metastasis, the opposite (i.e., hormetic) effect at low dose suggested the need for a cautionary note. The biphasic dose response characteristics of resveratrol should guide strategic considerations with respect to its use in breast cancer prevention and therapy. This perspective needs to be experimentally assessed in a manner analogous to that of de Leon and colleagues[32,33] which also revealed biphasic responses with respect to CD. In the case of CD, as noted above, the low dose stimulation could be inhibited by estrogen and/or estrogen antagonists thereby providing therapeutic options in clinical settings. Finally, the capacity of resveratrol to enhance cellular migration of MDA-MB231 cells was not only confirmed by Castillo-Pichardo et al.[38] but also extended to quercetin and catehin, which collectively comprise up to 65-70% of the polyphenols in red wines.

Human Prostate Cancer Cell Lines

Resveratrol has been assessed in human prostate tumor cell lines, displaying anti-proliferative effects.[39] These observations indicate that resveratrol may have chemopreventive effects on prostate cancer which are mediated by altered cell cycle transition, concentration effects, and multiple receptor interactions, much like it has shown with other types of tumor cells (e.g., breast). Of particular interest in this regard is that resveratrol displays a biphasic concentration response in human androgen receptor positive LNCaP cells.[40] This biphasic concentration effect was also time dependent, occurring at 24 hours while at 1 hour there was a concentration dependent inhibition. In contrast to the stimulatory response at 24 hours with respect to DNA synthesis, there was no evidence of enhanced protein synthesis. The increase in DNA synthesis, which was in the 2-3 fold range, was associated with an enhanced recruitment of cells from G1 into the S phase of the cell cycle.

The maximum stimulatory response in the LNCaP cells was about 270-300% (Figure 15A). With respect to the other two cell lines tested, the authors concluded that there was no treatment effect. In contrast to the LNCap cells the maximum response in DU145 cells was 130-140% as displayed in two adjacent concentrations (i.e. the two lowest concentrations were in this response range) (Figure 15B). In fact, the rather modest 30-40% increase is much more in line with an expected magnitude of a hormetic stimulatory response. Support for the observations of resveratrol inducing biphasic concentration responses was recently provided by Benitez et al.[41] and Wang et al.[42] using the LNCaP and PC-3 prostate cell line models.

Kuwajerwala et al.[40] speculated on the therapeutic potential of these biphasic concentration response observations. They indicated that since prostate cancer often is a slowly progressing disease, only a small per-

centage of the cells are in the proliferative phase at any given time. This creates a problem in the treatment strategy since most chemotherapeutics and ionizing radiation are optimally successful in killing tumor cells that are in the proliferative phase. Thus, it was suggested that resveratrol might have treatment utility in the recruitment of cells into a cell proliferative phase at which point they would become more sensitive to treatment. On the other hand, there may be a risk in speeding up the entry into the proliferative phase unless the cell killing effect of the chemotherapeutic agents/ionizing radiation were extremely effective.

Human Leukemia Cell Lines

Other chemo-preventive research with resveratrol has been directed toward human leukemia cell lines. Of particular interest with respect to hormetic dose responses was the report of Lee et al.[43] concerning the effects of resveratrol on HL-60 cells, a malignant, promyelocytic leukemia cell line. Using standard procedures the authors exposed these cells to five different concentrations of resveratrol (10-500 μM) over a single 24 hour period and evaluated their responses in the MTT assay for cell viability. Figure 16 demonstrates that resveratrol induced a hormetic-biphasic concentration response relationship. This concentration response was acknowledged by the authors, who also noted the earlier research of Nakagawa et al.[25] in which resveratrol induced a low concentration stimulation in human breast cancer cells. In the case of Lee et al.[43] the quantitative features of the biphasic concentration responses were consistent with the hormetic dose response.

Resveratrol was also tested in the MTT assay with CEM-C7H2 leukemia cells. While this assay has been thought to estimate cell viability this interpretation has been challenged based on findings of several experimental systems in which MTT activity increased while cell counts were actually decreased. The MTT assay involves the cleaving of tetrazolium salt in mitochondria of living cells to formazan by the succinate-tetrazolium reductase system of the respiratory chain. The amount of formazan formed is assumed to be directly related to the number of metabolically active cells. However, this interpretation has been challenged by findings indicating that MTT reduction activity can occur outside the mitochondria. With respect to the effects of resveratrol, it induced a hormetic response in CEM-C7H2 leukemia cells in the MTT assay. The low concentration stimulatory response was associated with a decrease in cell number (Figure 17), just the opposite of what would normally be expected.[44] It is therefore not clear what the low concentration increase in MTT represented; it could have resulted from mitochondrial proliferation and/or an increase in mitochondrial metabolism. A similar type of response was reported by Holian and Walter[45] in studies with human keratinocytes in which an increase in MTT reduction was not associated with an increase in cell viability (Figure 18).

Human Uterine Tumor Cell Lines

Endometrial carcinoma, the fourth most frequent of all female cancers in Western countries, displays a broad array of molecular alterations, including the up-regulation of COX-1 and COX-2, rate limiting enzymes involved in the biosynthesis of prostaglandins (PGs). These observations lead Sexton et al.[46] to suggest that the malignant uterus may be a potential target of resveratrol, since it inhibits both COX-1[47,48] and COX-2[49] activities. In fact, COX-2 is commonly over-expressed in endometrial tumor cell lines[50] and in most human endometrial carcinoma specimens.[51,52] Since the presence of COX-2 promotes the survival of endometrial tumor cells,[50] Sexton et al.[46] theorized that resveratrol may display tumoricidal activity to uterine cancer cells as a result of its inhibition of COX-2 expression and/or activity. The chemo-preventive potential of resveratrol on uterine tumor cells was suggested as also acting via the inhibition of COX-1, which is present in many endometrial tumor tissues.[51] Consequently, Sexton et al.[46] assessed how resveratrol may affect survival and COX expression in various human uterine cancer cell lines. Using six human uterine tumor cell lines, they assessed six different resveratrol concentrations over three time periods. With respect to cell proliferation, these authors reported that resveratrol generally induced a low con-

centration stimulation (3.125 – 12 μM) and a high concentration (50 -100 μM) inhibition (see Figure 19 for the response of HeLa cells).

Follow up studies revealed that low concentrations of resveratrol also up-regulated COX activity in the multiple human uterine cancer cell lines.[46] Since increased COX-2 activity has been reported in uterine cancer cells, it was speculated that this enhanced COX enzymatic activity could underlie the proliferative activity and be causally associated with the low concentration stimulatory effect of resveratrol on cellular proliferation. However, indomethacin, which inhibits both COX-1 and COX-2 enzymatic activities, did not block the effects of resveratrol on cell proliferation. Similarly, NS-398, a selective inhibitor of COX-2 activity, did not alter the effects of low concentrations of resveratrol on cellular proliferation. These findings indicated that the increase in cellular proliferation following the low concentration of resveratrol did not result from an increase in COX activity. Furthermore, the high concentration inhibitory effects of resveratrol on cell proliferation were also not affected by COX activity. PGE2 was also thought to affect the resveratrol-induced proliferation effect. However, exogenous PGE2 was unable to affect the capacity of resveratrol to induce either stimulatory or inhibitory effects in the uterine cell line En-1078D. Other experiments by Sexton et al.[46] revealed that levels of Akt were not increased in uterine cancer cells following exposure to low concentrations of resveratrol, suggesting that resveratrol does not enhance the proliferation of uterine cells via Akt regulation.

Resveratrol may act as an estrogen agonist or antagonist. Whether resveratrol acts as an agonist or antagonist is contingent on several factors on the response element sequence, if it binds to ERα or ERβ, as well as the concentration administered. Of the six uterine cancer cell lines tested by Sexton et al.,[46] Hela cells displayed the highest ERβ to ERα ratio while En-1078D cells (the only cell-type that expressed ERα in the Sexton et al.,[46] study) has the lowest ratio. Proliferation of uterine cancer cell lines was increased in response to estrogen.[53] If the resveratrol acts as an estrogenic agonist via the ERβ receptor on uterine cancer cell lines, it should stimulate proliferation in Hela cells, but probably not with En1078D cells. However, Sexton et al.[46] reported just the opposite, suggesting that the low concentration stimulation of cell proliferation by resveratrol is not mediated via the ER pathway.

Human Colonic Tumor Cell Lines (HT-29 and SW-620)

In an effort to extend research that had characterized the effects of resveratrol on human tumor cell lines, Szende et al.[54] assessed the effects of resveratrol on two human colonic tumor cell lines (HT-29 and SW-620). Both cell types displayed a hormetic biphasic concentration response relationship at 24 and 48 hours (Figures 20A and 20B). These findings are generally consistent with the effects of resveratrol on other types of tumor cell lines such as the human breast tumor cell line MCF-7[25] and numerous human uterine cell tumor lines.[46] A later study by Hope et al.[55] using the human colon cancer cell line (RKO) also reported an hormetic-like biphasic concentration response for resveratrol on cell proliferation with a maximum stimulation of about 20-25%. Follow up studies concerning the effects of resveratrol on Wnt signaling in the HT29 and Rko cancer cell models and a non-tumor colon cell line (i.e., NCM460) revealed a diverse set of non-linear dose responses for various protein products that are associated with early events in the process of carcinogenesis. In general the inhibition of these signals is believed to represent a possible chemopreventive action in some instances.

Lewis Lung Carcinoma Cells

A number of papers indicated that resveratrol inhibits tumor growth via apoptosis.[56-63] Such studies have addressed the effects of resveratrol at relatively high concentrations. However, a study by Kimura and Okuda,[64] which assessed the effects of resveratrol on Lewis lung carcinoma cells over a broad concentration range, revealed a J-shaped dose response for apoptosis (Figure 21). These findings suggest that at the highest concentration (100 μmol/L) tested the resveratrol would have chemopreventive effects while at the two lowest concentrations survival of the lung tumor cells would be enhanced.

NON-TUMOR CELL LINES

Endothelial Cells

Progenitor Cells and Re-Endothelialization

Injuries to vascular tissue often result in a repair process that can lead to the development of new pathological conditions such as neointimal hyperplasia. It is well known that endothelial cell loss is a principal factor leading to the pathological repair of damaged vascular tissue. Damage to the vascular integrity can also affect a diminished production of vascular protective mediators and increase levels of vasoconstrictor and growth-promoting agents 3 and 4. This can lead to an elevated vascular tone, platelet adhesion, inflammatory responses, and medial smooth muscle cell proliferation.[65] This disease affecting process can lead to the occurrence of restenosis (i.e., narrowing of a blood vessel) following revascularization procedures such as angioplasty with or without stenting and bypass grafting.

Since endothelial cell damage/loss can have a significant role in the pathogenesis of intimal hyperplasia following vascular injury, it is widely believed that a therapeutic strategy that enhances early re-endothelialization of the damaged tissue would inhibit intimal lesion development, enhance vascular repair and enhance long term patency. Based on the strong association of moderate consumption of red wine with a lower incidence of coronary artery disease in humans and laboratory studies demonstrating that resveratrol can inhibit platelet aggregation and adhesion, lower oxidative stress in platelets, protect against low density lipoprotein oxidation, suppress proliferation and hypertrophy of smooth muscle and increase high density lipoprotein (HDL) cholesterol, as well as decrease neointimal thickening of injured arteries in animal models, Gu et al.[65] evaluated the effects of resveratrol on the angiogenic activities and eNOS expression of isolated human endothelial cells *in vitro* and the effects of resveratrol on the mobilization of endothelial cells from bone marrow, as well as the re-endothelialization, neointimal hyperplasia and eNOS expression in injured arteries. Using a four concentration protocol (1, 5, 15 and 60 μM), Gu et al.[65] demonstrated a consistent hormetic-like biphasic concentration response for endothelial cell proliferation (Figure 22A), cellular migration (Figure 22B), cell adherence (Figure 22C), and eNOS expression/concentration (Figures 22D and 22E). There was a remarkable qualitative and quantitative consistency of the findings with a stimulatory response consistently observed for the 1 μM concentration with the magnitude of the stimulatory response being about 40-60% greater than the control, with all such responses achieving statistical significance. In each of the concentration responses the 60 μM concentration was inhibitory with the response decreased to only 30-60% of the control group.

These *in vitro* findings with isolated human endothelial cells were supported in subsequent *in vivo* studies with a rat model. Such studies demonstrated that a low dose of resveratrol enhanced the mobilization of endothelial cells, facilitated re-endothelialization, reduced the occurrence of neointimal formation and up-regulated the expression of eNOS following an induced balloon injury. These findings were not only supported in subsequent research of Xia et al.,[66] which corroborated the hormetic-like response of resveratrol on endothelial progenitor cell proliferation (Figure 23A) and cell migration (Figure 23B), but further extended other observations by linking these responses to telomerase activity via AKT-dependent mechanisms. As in the case of the endothelial cell parameters measured, the resveratrol induced alterations in telomerase activity were also indicative of hormetic responses.

Proliferation/Apoptosis

In 2000 Szende et al.[54] assessed the effects of resveratrol on proliferation and apoptosis in human endothelial cells. The justification of this study was to extend research with resveratrol to other cell types that have generally showed an anti-proliferative effect at high doses of resveratrol. In contrast to studies that they cited showing resveratrol to be an inhibitor of cell proliferation, resveratrol biphasically affected proliferation with a low concentration stimulation and a high concentration inhibition. Using a low concentration protocol, only the lowest concentration of resveratrol increased proliferation as was the case at both 24 and 48 hours (Figure 24). Of further interest is that the occurrence of apoptosis offers suggestive evidence of a J-shaped dose response.

Endothelial Cell Migration: Anti-Angiogenesis Potential

It is well known that tumor growth is an angiogenesis-dependent process. This perspective is supported by observations that a tumor cannot become greater than 2-3 mm^3 without new blood capillary formation. Since resveratrol has displayed chemo-preventive effects in several cancer bioassays,[60,67] In et al.[68] speculated that such effects may be related to possible anti-angiogenesis effects. In order to test this hypothesis, In et al.[68] assessed the effects of resveratrol on endothelial cell migration since it is an essential activity for several vascular functions which may affect tumor growth, vascular remodeling as well as wound healing.[69] Using bovine aortic endothelial cells (BAECs) the resveratrol biphasically affected endothelial cell migration[68] with a low concentration being stimulatory while high concentrations were inhibitory (Figure 25). While the authors acknowledged the capacity of resveratrol to induce a biphasic concentration response, they emphasized that their data revealed that high concentrations of resveratrol might have utility as a potent anti-angiogenesis drug. However, at lower concentrations the response could switch to angiogenic, leading to a tumor promotion effect.

Immune Cell Responses

T-Cells

Numerous studies have revealed that resveratrol can induce a broad range of biomedical effects, having potentially important therapeutic implications. These effects are seen in multiple biological systems for a diverse set of endpoints, including those related to immune function. Within this context resveratrol has anti-inflammatory properties such as shown with the inhibition of COX-1[60] and COX-2,[62] down regulation of prostaglandin biosynthesis and suppression of carrageenan-induced paw edema.[60,70] Since these collective findings suggested a possible effect on immune response, Falchetti et al.[71] explored, in detail, the effects of resveratrol on multiple immune functions of human T-cells *in vitro*. These included the development of cytokine-producing CD4+ and CD8+ T cells by stimulating peripheral blood mononuclear cells (PBMC) with anti-CD3/antiCD28, specific antigen-induced generation of cytotoxic T lymphocytes and natural killer (NK) activity of peripheral blood mononuclear cells (PBMC) (Figures F26A – F26F). These authors reported that there was a hormetic-like biphasic dose response for each endpoint assessed. According to Falchetti et al.[71] these findings suggested a regulatory effect of resveratrol on the immune response.

Spleen Cells

Studies of the effects of resveratrol on the proliferation of spleen lymphocytes using thymidine incorporation were reported by Gao et al.[72,73] Using Con A to induce proliferation, the authors then explored whether resveratrol would modulate that response. The findings indicated that resveratrol inhibited the Con A-induced proliferation of the spleen cells but only at high concentrations (25 and 50 μM). At lower concentrations (6.25 and 2.5 μM) the proliferative response was significantly increased (Figure 27). These findings generally supported the conclusion that resveratrol has the capacity to suppress or upregulate immune response depending on the concentration.

Cytotoxicity of H22 Cells by Peritoneal Macrophages

While the antitumor activity of resveratrol has generated considerable interest, the mechanisms by which this chemo-preventive effect is initiated remains to be clarified. To this end, Liu et al.[74] assessed the antitumor capacity of resveratrol by evaluating the capacity of peritoneal macrophages to kill H22 cells in Balb/c mice. In their experiments five concentrations of resveratrol (1.25-20.0 mg/L) were assessed. At higher concentrations of resveratrol there was a progressive increase in the cytotoxicity of peritoneal macrophages against the H22 cells. However, at the lowest concentration tested there was a non-significant decrease of cytotoxicity with two experimental conditions (Figure 28), suggesting a possible hormetic concentration response.

Interferon-γ

Patients with vascular disease commonly display enhanced production and secretion of pro-inflammatory cytokines. According to Wirleitner et al.[75] the Th1-type

cytokine interferon-γ (INF-γ) has a central role in this inflammatory process, facilitating the production of reactive oxygen species (ROS) by monocytes. In addition, INF-γ enhances the synthesis of neopterin by activating the enzyme GTP-cyclohydrolase I. It enhances the activity of indoleamine 2,3-dioxygenase (IDO) which metabolizes tryptophan. Based on these metabolic interrelationships Wirleitner et al.[75] assessed the effects of resveratrol on tryptophan degradation in human PBMC. This was accomplished by estimating the activity of IDO by determining the ratio of kynurenine to tryptophan. These data revealed that low concentrations of resveratrol enhance kynurenine/tryptophan ratios whereas high concentrations decreased this ratio below that of the controls (Figure 29). While the high concentration of resveratrol response suggested that it could suppress INF-γ-mediated pathways that might lead to vascular disease, including atherosclerosis, the low concentration results suggest an opposite effect.

Astrocytes

Astrocytes have an important role in preventing oxidative stress-related neuronal cell death. For example, during cell culture experiments astrocytes protect neurons from cell death caused by hydrogen peroxide and nitric oxide.[76] Further, astrocytes facilitated the protection of adjacent neurons by providing glutathione precursors.[77] Even though resveratrol has been suggested as a pharmacological/nutrient to enhance neuroprotection in several experimental systems, there are only limited studies concerning the effects of resveratrol on astrocytes.[78,79] Consequently, dos Santos et al.[80] assessed the effects of resveratrol on several specific parameters using the C6 glioma cell line, since it displays a variety of astrocytic properties (Figure 30). While high concentrations of resveratrol increased the release of LDH, low concentrations (10-100 μM) had the opposite effect, resulting in enhanced membrane integrity.

Aortic Smooth Muscle

It is not very common that the mechanisms underlying dose/concentration dependent transitions are explored, especially when the dose-response is complex such as with hormetic-like biphasic dose response relationships. However, a 2005 study by Juan et al.[81] provided such a mechanistic insight into how resveratrol affects a hormetic response in human aortic smooth muscle cells (HASMCs). Resveratrol induced HO-1 expression in a manner that conformed to the hormetic dose response, markedly enhancing HO-1 expression at low concentrations (< 10 μM) whereas at higher concentrations (> 20 μM) this response was diminished. The magnitude of the stimulatory response was slightly greater than three fold with the width of the stimulatory response being at least 200-fold and likely considerably broader (Figure 31).

Employing various pharmacological inhibitors, these authors established that there was no involvement of ERK, JNK and p38MAP kinase in the HO-1 induction process. Other experiments using transcriptional and translational inhibitors prevented resveratrol mediated HO-1-mRNA and protein expression, suggesting that HO-1 is affected by transcriptional and translational control in HASMCs administered resveratrol. Resveratrol induction of the HO-1 gene was mediated via the NF-κB pathway. In fact, two separate NF-κB inhibitors abolished the capacity of resveratrol to induce HO-1 expression and the activity of the HO-1 promoter. Low concentrations of resveratrol enhanced NF-κB binding activity based upon experiments assessing electrophoretic mobility shifts. At these low concentrations the resveratrol trans-activated the NF-κB by enhancing IκBα phosphorylation and degradation. This activation process enhanced the transmigration of NF-κB into the nucleus which then led to the modulation of HO-1 gene expression.

Chinese Hamster Fibroblast Cell Line (V79)

The antioxidative properties of resveratrol were tested by Muller et al.,[82] using V79 cells, a Chinese hamster fibroblast cell line. This was accomplished using a dual-luciferase reporter gene assay. In their system the V79 cells were transfected with plasmid pGL3-SOD along with the human SOD1 promoter or pGL3-GPx with the GPx1 promoter. Using three concentrations of resveratrol (1.1-4.6 μg/ml), a biphasic concentration response was observed for both SOD and GPx with a maximum

stimulatory response of 130-140% at the lowest concentration tested (Figure 32). Similar findings were also observed for the commercial product Vineatrol® 30 which contains resveratrol and considerable quantities of resveratrol oligomers. These findings indicated that resveratrol and Vineatrol® likely act as free radical scavengers at low concentrations. However, at higher concentrations the antioxidant properties are diminished, switching to a pro-oxidant activity.

Leydig Cells/Testosterone Production

Resveratrol has estrogenic and anti-estrogenic effects depending on the specific cell type when bound to the ER. Since Leydig cells have estrogen receptors, the question was posed as to whether resveratrol would act as an agonist or antagonist on ER mediated responses by these cells. Since Leydig cells are necessary for the production of testosterone by the mammalian testis, it was of interest to assess whether resveratrol may effect testosterone production. Follow up experiments revealed that resveratrol induced a hormetic biphasic concentration response on hCG stimulated testosterone production, basic testosterone levels as well as on Leydig cell viability (Figure 33).[83] These findings were consistent with the findings of Juan et al.[84] that resveratrol increased sperm output in healthy rats.

Liver – Paraoxonase Activity: Human Liver Cell Line (HCO4)

Paraoxonase-1 (PON-1) is a high-density lipoprotein (HDLP)-associated with serum enzymes. It is secreted principally from the liver.[85] PON-1 can affect the occurrence of CVD due to inactivation of oxidized phospholipids carried by HDL and low-density lipoprotein.[86] Further supporting this perspective were findings by Shih et al.[87] that PON-1 deficient mice are more susceptible to lipoprotein oxidation, atherosclerosis and organophosphate toxicity. In addition, PON-1 genetic polymorphisms have also been associated with individual susceptibility to CVD.[88]

Such findings lead to the hypothesis that some of the CVD benefits induced by resveratrol may be due to its capacity to affect PON-1. In 2004 Gouedard et al.[89,90] reported that resveratrol increased the PON-1 gene expression in human hepatocyte primary cultures and in the HUH7 hepatoma cell line with the transcription mechanism mediated via the aryl hydrocarbon receptor (AhR).

Since polyphenolic compounds such as resveratrol increase PON-1 mRNA and activity, Curtin et al.[91] hypothesized that resveratrol may protect against low dose exposure to chemical warfare nerve agents (CWNAs) or reduce the prophylactic therapeutic dose of PON-1 needed for protection against lethal doses of neurotoxins. Using the human liver cell line HCO4 this hypothesis was tested by Curtin et al.,[91] who demonstrated that resveratrol biphasically affected PON-1 activity (Figure 34). The induced PON-1 nearly completely protected against toxicity induced by the CWNAs somin and sarin. The authors did not assess the effects of resveratrol on susceptibility at doses/concentrations of resveratrol that cause a decrease in PON-1 activity.

HUMAN DISEASE/INJURY

Gastric Lesions

Resveratrol has displayed a broad range of chemo-preventive effects, including protecting against a number of chemically induced tumors in experimental studies. The mechanism by which resveratrol may affect such protection is thought to involve the inhibition of cyclooxygenase (COX) expression.[60] Resveratrol has also been shown to delay the healing of acetic acid induced gastric lesions in rats,[92] a process that depended on its inhibition of COX-1 activity. Thus, resveratrol appears to protect against cancer by a process similar to which it may affect the healing of gastric ulcers. Pro-angiogenic processes may enhance the healing of gastric lesions while providing a promotional stimulus during the process of carcinogenesis while the reverse may also be the case as well.

Despite the demonstrated capacity of resveratrol to diminish the gastric ulcer healing process in rats, Dey et al.[93] hypothesized that this adverse response may be the result of administering an excessive dose of resveratrol.

Furthermore, the hypothesis that resveratrol may affect the acceleration of healing via a lower dose was based on the assumption that it may display a biphasic rather than a linear dose response. They claimed that an important characteristic of resveratrol was that it often acted via a biphasic dose response. However, the only support that Dey et al.[93] provided for this statement was the report of Kuwajerwala et al.[40] which indicated a biphasic concentration response for DNA synthesis in prostate cells (LNCaP). However, even though the authors only cited one paper to support this assertion, a broad search of the literature concerning resveratrol would confirm their conclusion on this point as has been documented in the present article.

In their study male Swiss Albino mice (6-8 weeks) were administered a single large dose of indomethacin which induced ulcerogenic gastric lesions over the next several days. Graded doses of resveratrol were then administered six hours after the exposure to indomethacin. Mice were sacrificed at 1, 2, 3, 4, 7, 10 and 15 days after indomethacin treatment and assessed for gastric lesions as well as for MPO activity, a marker of neutrophil aggregation at the site of inflammation. Figures 35A-35E reveal a striking hormetic-like biphasic dose response for the occurrence of gastric lesions and the MPO activity on Days 2, 3, 4, 7 and 10. These dose responses were highly consistent with each other, both qualitatively and quantitatively. Resveratrol treatment at 2 mg/kg was associated with the most pronounced decrease in gastric lesions and MPO activity. Such protection became apparent at two days after indomethacin treatment and continued throughout the 15 day study period. The only other dose of resveratrol that showed protection was 1 mg/kg.

The authors indicated that the biphasic dose responses were due to the effects of resveratrol on COX-1 and eNOS, a constitutive form of NOS. The capacity to affect COX-1 and eNOS controlled the synthesis of PGE2 and angiogenic processes which then affected critical gastric healing processes in the mice. More specifically, a high dose of resveratrol (10 mg/kg) suppressed COX-1 expression and led to a decreased production of mucosal PGE2, promoting gastric ulcer formation. In contrast, low doses of resveratrol enhanced the expression of eNOS, and lowered iNOS (inducible) activity leading to a higher eNOS/iNOS ratio, and lower gastric damage. This interpretation was supported by observations that resveratrol doses (e.g. 0.05 mg/kg) which did not affect COX-1, eNOS and PGE2 levels did not have an effect on the occurrence of gastric damage.

These findings are interesting in several respects, including the occurrence of the hormetic dose response, but also with respect to the very narrow therapeutic zone with protection being seen at only 1 and 2 mg/kg. The stimulatory range in several other cases of resveratrol induced hormetic responses has been broader than that reported for the gastric lesion endpoint (e.g. 4-fold for various immune parameters[71]; between 4-30 fold for various endpoints in mouse primary Leydig cells,[83] about 10 fold for PON-1 activity in human liver cell lines,[91] about 5-fold for cell proliferation in endothelial progenitor cells[66] and over a 1000-fold for thymidine incorporation in mesenchymal stem cells.[94] However, there were several cases reported where the width of the low dose/concentration stimulation was in the two fold range,[40] a width similar to that reported for the gastric lesion/MPO response. The factors underlying the width of the low dose/concentration stimulatory response were not addressed experimentally nor discussed in any of these papers.

While it was clear that the resveratrol treatment at low doses resulted in less damage than the control group it was not possible to assess if this treatment was also affecting the healing process. This experiment was not designed to differentiate the capacity of resveratrol to reduce damage induction from the acceleration of healing. In order to have done this other experimental groups would have been necessary. In such cases resveratrol treatment would need to be administered at near maximum damage rather than soon after the administration of the indomethacin. This is an important issue to resolve because it has important therapeutic implications.

Osteoporosis

Osteoporosis is a significant public health concern associated with estrogen deficiency following menopause.[95]

While hormone replacement therapy (HRT) is widely employed to prevent postmenopausal osteoporosis, HRT also enhances the risk of breast and endometrial cancers.[96] As a result of these limitations/concerns, phytoestrogens have been considered as a potentially important alternative therapy.

In order to explore the potential of resveratrol to affect bone cell function, Dai et al.[94] assessed its *in vitro* effects on the proliferation and osteoblast maturation of cultured human bone marrow stem cells (HBMSCs) (Figures 36A and 36B). Resveratrol stimulated the proliferation and osteogenic differentiation of HBMSCs in a hormetic-like biphasic manner. The magnitude of the stimulatory response was considerably different depending on the endpoint with cell proliferation approaching a 3-fold increase whereas the osteogenic differentiation increased by only 15%, although still statistically significant. These effects of resveratrol were mediated via the ER signaling pathway as they were abolished by the administration of ICI82,780, a complete antagonist of ER. The ER pathway in HBMSCs is linked to the MAPK pathway principally via ERK1/2 affecting cell proliferation and osteoblastic differentiation endpoints. The data of Dai et al.[94] represent the first evidence that resveratrol directly enhances HBMSCs proliferation, osteoblast differentiation and osteogenic gene expression via mechanisms involving the ER dependent MAPK pathway. The findings suggest that resveratrol or its mimetics may be able to activate this pathway in bone cells and facilitate therapeutic options in the treatment of osteoporosis. Zhou et al.[97] has extended the research of Dai et al.[94] by showing that resveratrol promotes the osteoblastic differentiation of multi-potent mesenchymal cells (i.e., cell line ST2). As in the case of Dai et al.,[94] these authors also reported that resveratrol affected osteoblastic differentiation in a manner indicative of the hormetic dose response.

Ischemic-Reperfusion and Heart Damage

Resveratrol has been widely reported to provide cardioprotection from a multiplicity of routes, including mechanisms mediated by antioxidant activity,[98,99] inhibition of low-density lipoprotein,[100] activating NO production,[98,101] preventing platelet aggregation[102] and promoting anti-inflammatory effects.[103] While relatively low doses of resveratrol have been chemo-protective, high doses induce a broad spectrum of toxic responses in multiple systems, including cell death via apoptosis mechanisms,[104-108] retarding wound healing,[109] cell proliferation,[110] enhancing genotoxicity,[111] nephrotoxicity,[109] and neurotoxicity to primary cortical astrocytes of the cerebral cortex of neonatal rats.[112] According to Das and colleagues[113, 115] such dose related effects with high and low doses of resveratrol were consistent with a report of Wilson et al.[107] suggesting that high doses of trans-resveratrol induced atherosclerotic lesions in hypercholesterolemic rabbits while lower doses of resveratrol may be protective.

These observations led Dudley et al.[113] to hypothesize that at low doses resveratrol may act as an antioxidant whereas at higher doses it may function as a pro-oxidant (see Miura et al.,[114]). Using this theoretical framework Dudley et al.[113] assessed the effects of resveratrol on a wide range of cardio-protective indices/endpoints across a broad range of resveratrol doses (2.5, 5.0, 25 and 50 mg/kg) in male Sprague-Dawley rats. Rats were administered resveratrol orally for 14 consecutive days prior to receiving an ischemic (30 minutes)/reperfusion (2 hours) event. These findings revealed that the resveratrol induced hormetic-biphasic dose responses for aortic flow, coronary flow, left ventricular developed pressure (LVDP), maximum first derivative of LVDP as well as myocardial infarct size (Figure 37A) and cardiomyocyte apoptosis (Figure 37B). The dose response patterns were strikingly consistent with 2.5 and 5.0 mg/kg displaying a cardio-protective response while 25 and 50 mg/kg dosages were progressively cardio-toxic. Additional experiments also revealed similar hormetic dose responses for protein and RNA transcripts of redox proteins including thioredoxin (Trx)-1, Trx-2, glutaredoxin (Grx-)-1, Grx-2, redox factor (Ref-1), redox –sensitive transcript factor NFkB and several survival factors (i.e., phosphorylated Akt (pAkt) and Bcl-2). According to Das and colleagues[113, 115] at low doses resveratrol induces survival signaling by up-regulating anti-apoptosis and redox proteins, Akt and Bcl-2, while at higher doses it induces a death signal by decreasing redox proteins and up-regulating pro-apoptotic proteins. The study of Dudley et al.[113] was extraordinary based on the

nature of the study design and endpoint selection, both of which took advantage of considerable preliminary research that had identified the likely hormetic dosage zone. According to the authors the amount of resveratrol in the hormetic (i.e., protective) zone on a mg/kg basis would be similar that consumed in one or two glasses of red wine per day. The elevated doses of 25 and 50 mg/kg would require the consumption of at least 1.75 L to 3.5 L of red wine per day.

β-Amyloid Induced Neurotoxicity (Alzheimer's Disease Model)

While the β-amyloid precursor protein (β-APP) is neurotropic,[116,117] it can undergo proteolysis by various secretases to produce 1-40 and 1-42 peptides. The amyloid β-peptide (Aβ) (Aβ40-42 peptide) is the principal component of amyloid deposits in the brain parenchima in patients with Alzheimer's disease (AD).[118] Experimental studies with neural tumor cell lines as well as primary rodent and human neurons in culture have shown that aggregating forms of Aβ are cytotoxic,[119-121] acting via a complex array of mechanisms including disruption of cellular redox systems, formation of ROS/NOS, perturbation of Ca^{2+} homeostasis and hyperphosphorylation of Tau and Ser protein residues.[122] In one study, the treatment of cultured rat hippocampal neurons with resveratrol significantly attenuated Aβ-induced cell death in a concentration-dependent manner, with a concentration of 25 μM being maximally effective.[123] In an effort to assess whether dietary factors may alleviate the toxicity of Aβ, Conte et al.[124,125] assessed the affects of resveratrol on Aβ treated PC12 cells. The rationale for testing resveratrol was based on evidence of its cardioprotective properties. In this investigation the effects of Aβ (1-41) on PC 12 cells were studied with seven concentrations ranging from 10-120 μM of resveratrol using the MTT assay. Resveratrol displayed a concentration-dependent biphasic effect at each of four concentrations of Aβ (1-41) used to initiate damage. Figure 38 provides a graphic representation of these four dose responses. The basic approach involved the induction of toxicity by the different concentrations of Aβ and then the assessment of whether the resveratrol treatment could reverse the damage. In all cases the resveratrol was successful in reducing the toxicity of Aβ at lower concentrations. However, at higher concentrations the toxicity response re-appeared with responses at 120 μM approaching the damage induced by Aβ alone. These findings indicate that resveratrol affects a consistent biphasic concentration response regardless of the initial level of damage induced by the Aβ. While the underlying mechanism(s) by which resveratrol acts to prevent Aβ toxicity is uncertain it involves, at least in part, reducing the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2), thus preventing the uncontrolled release of NO and prostaglandin E2 (PGE2).[126]

Ischemic Stroke

Transient global forebrain ischemia in rats (a model of brain damage caused by cardiac arrest) causes selective degeneration of CA1 hippocampal neurons. However, when rats were pretreated (intraperitoneal injection) with resveratrol (10, 50 and 100 mg/kg dosages) neuronal degeneration was prevented. This chemo-protective affect was associated with SIRT1 activation and upregulation of uncoupling protein 2.[127] In another study, resveratrol at concentrations from 5 to 25 μM also protected cultured hippocampal neurons against nitric oxide-induced cell death.[128] Resveratrol might also suppress inflammatory processes in ischemic stroke because relatively low doses of resveratrol (0.1 – 5 μM) have been shown to suppress inflammation and oxidative stress in cultured neural tumor cells by a mechanism involving the inhibition of COX2.[129] However, recent findings have shown that when neurons are under conditions of reduced energy (oxygen and glucose) availability, as occurs in a stroke, resveratrol can enhance their death.[130] The latter study showed that resveratrol may deplete cellular NAD^+ levels as a result of the activation of the NAD^+-dependent histone deacetylase SIRT1. In the case of neurons under energetic stress, as occurs most notably in ischemic stroke, resveratrol may exert both beneficial and detrimental effects depending on its dose and time of administration in relation to the ischemic event (Figure 39).

Experimental Models of Parkinson's, Huntington's and Motor Neuron Diseases

Daily oral administration of resveratrol (10, 20 and 40 mg/kg) to "Parkinson's disease" rats improved their motor function within two weeks of administration.[131] In experimental models of Huntington's disease resveratrol protected neurons against the toxicity of polyglutamine proteins.[132] However, resveratrol reduced the resistance of Wld(S) neurons to axonal degeneration induced by colchicine, a microtubule depolymerizing drug.[133] This adverse effect of resveratrol was dependent upon sirtuin activation.

The Aging Process

Resveratrol has been touted as an "anti-aging" dietary supplement.[134] Studies of mice have shown that resveratrol can induce gene expression patterns in several tissues that are similar to the changes in gene expression induced by dietary energy restriction,[135] consistent with a hormetic mechanism of action. Old mice that had been treated with resveratrol beginning at 12 months of age exhibited reduced signs of aging including decreased inflammation, increased aortic elasticity, greater motor coordination and reduced cataract formation. Nevertheless, the mice treated with resveratrol did not live longer than the control mice. In another study, mice fed a high calorie diet were treated with resveratrol.[136] Although resveratrol did not prevent the mice from becoming obese, it did exert multiple health benefits including increased insulin sensitivity, increased levels of mitochondria, improved motor function and an extension of survival. An analysis of gene expression showed that most changes in gene expression caused by the high energy diet were counteracted by resveratrol treatment. Dose response studies were not performed, but the biological end-point data are consistent with the dose given being in the hormetic (low dose) zone.

PARASITIC DISEASES

Leishmaniasis

Leishmaniasis is a protozoan disease with serious risks of fatality. It is widely distributed, affecting people in nearly 90 countries. The treatment of choice for this disease has been pentavalent antimony. However, the use of this agent has become problematic because of the emergence of drug resistance and the accompanying loss of efficacy. According to Davis and Kedzierski,[137] there are few drug options besides the use of pentavalent antimony due to issues of cost, side effects and toxicity. As a result of these concerns investigators have explored other options with one being resveratrol based on its cost, safety profile and activity against skin pathogens. Furthermore, resveratrol has also been shown to have activity against dermatophytes,[138] parasitic fungi[139] and viruses.[140] In their investigations Kedzierski et al.,[141] assessed the effects of resveratrol and its hydroxylated analogues on *L. major* promastigotes [i.e. the flagellate stage of a trypanosomatid protozoan in which the flagellum originates from a kinetoplast in front of the nucleus and extends from the front of the organism; usually an extracellular phase, as in the insect intermediate host (or in culture) of leishmania parasites and intracellular amastigotes (i.e. the nonflagellate, intracellular, morphologic stage in the development of certain hemoflagellates, resembling the typical adult form of *Leishmania*] *in vitro* and assessed the toxicity of these agents on several cell types. The evaluation revealed that the resveratrol and most of its hydroxylated metabolites had the potential to markedly reduce the viability of *L. major* promastigotes. However, of particular interest to the present evaluation was that five of the hydroxylated analogues of resveratrol displayed a biphasic concentration response, enhancing the viability of the promatigotes *in vitro* at low concentrations, including the analogue that had displayed the greatest killing potential (Figure 40). It is interesting to note that the low concentration stimulation of viability was not discussed in the paper but is evident in the published figures. The implication of a low concentration stimulation of the *L. major* promatigotes represents a potentially important finding that would need to be further considered prior to the use of these agents within a therapeutic context.

Trichinella spiralis

Resveratrol has been reported to display antibacterial, antifungal and antiviral activity.[138,140,142] However, assessment of its effects on human parasites has been quite limited. In such investigations resveratrol has had an inhibitory effect on the growth of *Leishmania major*, *Plasmodium falciparum* and *Encepholitozoon cuniculi*.[139,141,143] Since there were no papers published on the effects of resveratrol on human trichinellosis, Ozkoc et al.[144] decided to assess the effects of resveratrol on the viability of *Trichinella sprialis* life stages *in vitro*. Of particular relevance to the present assessment were observations that resveratrol induced a hormetic-like biphasic concentration response in the *T. spiralis* muscle larvae. As seen in Figure 41, the 220 μM concentration increased the viability of *T. spiralis* muscle larvae by nearly 2-fold, becoming markedly inhibitory at twice that concentration (440 μM). A suggestive hormetic response trend was also reported at the 72 hour period for the adult worm stage at the lowest concentration (55 μM). Interestingly, the resveratrol was highly toxic even at the lowest concentration (55 μM) tested for the newborn larvae. Thus, the resveratrol displayed stage specific toxicity and hormesis, a finding conceptually similar to that reported by Nascarella et al.[145] concerning the effects of cadmium on blow flies.

Grape Parasite-*Plasmopara viticola*

Resveratrol and related stilbenes are synthesized in grapes, becoming concentrated in the skin of red grapes during various stressful conditions such as infections. Detailed concentration response studies by Pezet et al.[146] have shown that at high concentrations resveratrol and related agents act to prevent the capacity of parasites to spread across the plant surfaces. However, at low concentrations these agents consistently demonstrated a capacity to enhance the mobility of the spores, in a manner consistent with the hormetic dose response (Figure 42). The magnitude of the increase in mobility was modest and varied somewhat across the chemicals studied. The range of maximum stimulation was approximately 8-20%.

OTHERS

Sirtuins (SIRT1) and Pro-inflammatory/Anti-inflammatory Effects

Histone-modifying proteins play a fundamental role in gene regulation, with linkages to various human disease processes. Of particular interest in this regard is histone deacetylation and its mediation via sirtuins or SIR-proteins (silent information regulator). SIRT1, a highly conserved nictotinamide adenine dinucleotide (NAD)-dependent protein deacetylase, affects a wide range of cellular processes including metabolism, stress and DNA damage response, differentiation as well as multi-drug resistance in cancer. In addition to targeting nuclear histones for deacetylation, SIRT1 also effects the removal of acetyl-groups from transcription factors such as p53, the FOXO family, and NF-κB. As a result of its role in modulation of acetylation status, SIRT1 has displayed a long list of pleiotropic effects which are implicated in a variety of human diseases. SIRT1, via its modulation of various transcription factors, exerts protective effects on various biological systems, especially in studies dealing with neuroprotection. As a result of its broadly based chemo-preventive effects, it has been of considerable interest to identify SIRT1 activators as well as inhibitors. In a survey of 147,000 compounds Nayagam et al.[147] reported that 0.48% of compounds (705) tested satisfied their criteria of an activator (≥ 150% of control deacetylation activity in 3T3L1 mouse fibroblast and/or THP-1-leukemia cells). One such compound acting as a SIRT1 activator was resveratrol. It was subsequently evaluated for its anti-inflammatory properties in an assay designed to monitor TNF-α modulation in the human leukemia cell line THP-1, which produces and releases TNF-α and other cytokines following stimulation with LPS. In this bioassay, the resveratrol treatment diminished the release of TNF-α at elevated concentrations. However, the opposite affect occurred at lower concentrations (Figure 43). This hormetic-like biphasic dose response was not addressed by the authors. However, it suggests that resveratrol has the capacity to induce pro-inflammatory and anti-inflammatory responses depending on the concentration.

Co-Hormesis Concept

Elevated doses of H_20_2 can cause a concentration dependent decrease in retinal pigment epithelial (RPE) cell survival. However, when resveratrol (100 μmol/L) was administered l hour prior to the addition of H_20_2 a biphasic concentration response was reported (Figure 44).[148] The prior dose of resveratrol reduced the capacity of the H_20_2 to cause toxicity across the broad range of concentrations tested. Of particular interest to the present assessment is that at 150 μmol H_20_2/L there was a nearly 20% increase in cell proliferation as compared to the nearly 25% decrease in survival with H_20_2 alone. Despite such a striking disparity (i.e., 45% difference) it was not mentioned in the results or discussion. These findings are of particular interest since they suggest a role for resveratrol as an example of co-hormesis, analogous to a co-carcinogen. That is, the co-hormesis agent did not induce hormesis within the experiment by itself but was necessary for the H_20_2 to do so.

UV and Resveratrol

A major mechanism of the chemo-preventive effects induced by resveratrol is mediated via the inhibition of quinine reductase 2 activity, which induces the up-regulation of cellular antioxidants and detoxification enzymes (e.g., catalase, gluthathione S-transferase). Other protective mechanisms involve the activation of SIRT which enhances cellular resistance to stress and increases longevity. Despite these beneficial effects of resveratrol it is limited by its low bioavailability, low water solubility and stability. Using liposomes to enhance the biological activity of resveratrol, Kristl et al.[149] assessed whether resveratrol would affect the capacity of UV to alter cell metabolic activity using the MTS assay. They demonstrated that low concentrations of resveratrol (10 μM) enhanced metabolic activity whereas the activity was lost at higher concentrations (100 μM).

DISCUSSION

While numerous papers have assessed the hormesis concept and its toxicological, biomedical and clinical implications[150-155] this is the first paper that has comprehensively assembled and analyzed whether resveratrol displays hormesis and its potential clinical significance. This paper builds upon the suggestion of Howitz and Sinclair[156] that resveratrol acts as an xenohormetic agent, reflecting a highly conserved and integrated adaptive set of responses selected during evolution for dealing with a broad spectrum of environmental stressor agents.

The present analysis has shown that resveratrol acts in a hormetic-like biphasic dose/concentration response manner in numerous biological models, affecting a plethora of endpoints of biomedical importance. For example, resveratrol acts hormetically in numerous human cancer cell lines, affecting six major types of tumor cells, including breast (Figures 2-8), prostate (Figures 15A and 15B), leukemia (Figure 17), colon (Figures 20A and 20B) uterus (Figure 19) and lung (Figure 21) in a manner consistent with numerous other xenobiotics.[157] Resveratrol acts hormetically on human breast tumor cells with and without an estrogen receptor (Figure 12), affecting both cell proliferation and endpoints predictive of metastasis. These findings are particularly striking because the overriding emphasis of resveratrol research on cancer has been its potential chemo-preventive effects. Yet at low concentrations it has been consistently shown to stimulate cell proliferation of many human tumor cell types. This perspective has only recently begun to be discussed with respect to its implications for humans[31] who ingest foods or commercial products with resveratrol. This emerging perspective suggests that resveratrol may prevent or enhance tumor development, with the outcome depending to a large extent on the dose. These findings indicate that the hormesis concept presents unique challenges to the biomedical and clinical communities to better clarify the therapeutic implications of the hormetic dose response as treatment success or failure is likely to be highly dependent on "getting the dose right".

Resveratrol has also been shown to have chemo-preventive effects with respect to several human disease conditions such as cardiovascular disease (Figure 37A and 37B),[113] Alzheimer's disease (Figure 30),[124] osteoporosis (Figure 36A and 36B),[94] and gastric ulcers (Figure 35A-

35E).[93] In each of these conditions, a low dose of resveratrol was shown to confer protection in experimental models whereas at higher doses there is a transition to adverse health effects. These findings have been particularly convincing with respect to heart damage due to ischemia reperfusion (IR) in studies with rodents. Of particular interest is that the optimized daily dose could be extrapolated to the equivalent of a single glass of red wine per day.[113] Consistent with these studies on IR induced heart injury have been reports that low doses of resveratrol enhance the re-endothelialization of damaged vascular tissue, thereby facilitating tissue repair processes that would contribute to the cardioprotection.

Hormetic responses for other biomedical endpoints with considerable clinical implications have been reported.[158] This has been especially emphasized for neurological diseases[159,160] such as Alzheimer's disease[161] and osteoporosis[158], medical conditions for which resveratrol has a modulatory effect which closely conforms to the hormetic dose response.

While resveratrol affects the stimulation of cell proliferation of prostate tumor cells *in vitro*, it has also been shown to affect the production of testosterone in a biphasic manner. These observations were associated with the capacity of resveratrol to enhance sperm production in healthy animals.[84] Similar hormetic biphasic dose responses have also been reported for androgens induced by alcohol and other agents.[162,163]

Resveratrol also has the potential to affect to occurrence and progression of various parasitic diseases based on animal model studies. However, despite the capacity of resveratrol to protect against Leishmaniasis and Trichinella at elevated doses, it consistently enhances their proliferation at lower doses, thereby suggesting an increasing risk of infection and disease progression. This capacity of resveratrol to enhance the proliferation of these human parasites closely parallels its capacity to enhance tumor growth at low concentrations as emphasized in this paper.

Resveratrol has emerged as an agent with very complex biological activity. Due to its remarkably broad range of effects, especially with respect to cardiovascular protection and longevity, resveratrol has attracted numerous researchers and widespread consumer interest. In fact, various consumer products are now available with resveratrol and advertised for their health benefits. The emerging body of work indicates that resveratrol commonly acts in a hormetic fashion, displaying biphasic dose responses across numerous biological systems, affecting a wide range of endpoints. These hormetic dose responses of resveratrol have been reported principally in in vitro studies in the case of tumor cell lines[157] and in vivo with animal models with endpoints such as heart attacks[113] and gastric ulcers.[93] While it would be expected that the hormetic dose response findings within these experimental systems could be reliably extrapolated to humans this has yet to be demonstrated. However, it does appear that the documented cardiovascular protective effects of resveratrol research as reported in experimental animal model studies may be able to account, at least in part, for the French Paradox which itself provided the initial stimulation to better understand the clinical implications of resveratrol exposure.

The current assessment indicates that low concentrations of resveratrol can be potentially beneficial or harmful, depending on the endpoint of interest. The data suggest that low doses of resveratrol would have the capacity to increase the risk of tumor development of a number of organs based on its capacity to enhance cell proliferation in multiple human tumor cell lines. In contrast, a strong case can be made that low doses of resveratrol can be significantly cardio-protective. It is likely that these general conditions could occur simultaneously within individuals. While the data are insufficient to answer this question, this type of conflict is not uncommon and we are likely to encounter it more often as investigators become more knowledgeable of the general nature of the hormetic dose response and its capacity to enhance potentially beneficial or harmful effects depending on the biological system, tissue and chemical agent. Resveratrol is important because it is part of the normal diet of vast numbers of people and also because of its substantial commercial applications. However, resveratrol is simply the tip of the chemical iceberg in a toxicological and pharmacological world that is likely to be markedly affected by the hormetic dose response.

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Table 1. The hormetic/biphasic effects of resveratrol on specific cell types

Low Concentration	Cell Type	High Concentration
Human Tumor Cell Lines		
Enhances cell proliferation by 30-60% Implications - hypothesized to increase tumor cell proliferation	Estrogen receptor – Positive/ Negative - Prostate Leukemia Uterine Colonic Lung	Suppresses cell proliferation relative to control group
Non-Tumor Cell Lines		
Enhances re-endothelialization -> implications – enhances repair of vascular damage	Endothelial	Suppresses tissue repair of damaged vascular tissue
Endothelial cell migration is enhanced -> implications – hypothesized to increase the risk of tumor development	Endothelial	Suppressed cell migration may retard/slow tumor development
Enhances response from multiple immune cells –> implications – such responses are believed to mediate a range of protective effects associated with resveratrol consumption	Immune	Suppresses multiple immune cell responses -> implications – may contribute to exacerbation of multiple disease conditions
Low concentrations of resveratrol act as an antioxidant -> implications – protection against chemically induced damage is associated with the antioxidant properties	Lung fibroblasts (V79)	Switches at higher concentration to pro-oxidant -> implications – at high doses resveratrol may become directly toxic
Enhances testosterone production -> implications – uncertain at present	Leydig	Suppresses Testosterone production - > implications – uncertain at present

Table 2. The hormetic/biphasic effects of resveratrol on the occurrence of selected human diseases

Low Concentration	Cell Type	High Concentration
Human Disease		
Enhances healing	Gastric lesions	Delays healing
Facilitates bone formation -> implications - has potential therapeutic utility	Osteoporosis	Uncertain effects at high doses
Low doses prevent damage to cardiac tissue due to ischemia/reperfusion -> implications – relatively low doses of resveratrol are chemopreventive	Heart damage	Ischemic reperfusion induced cardiac damage is enhanced
Enhanced viability of L. major -> implications – low doses may enhance disease progression	Leishmaniasis	Suppresses viability of L. major -> implications – high doses may be chemopreventive

Figure 1. Dose-response curve depicting the quantitative features of hormesis

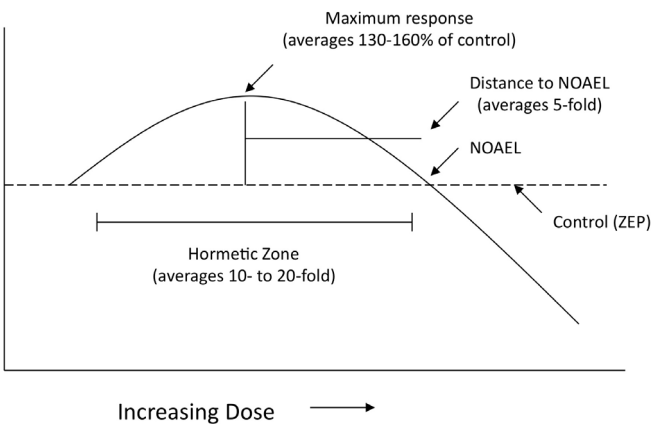


Figure 2. Effects of resveratrol on cell proliferation in the human estrogen-sensitive breast cancer cell line MCF-7[27]

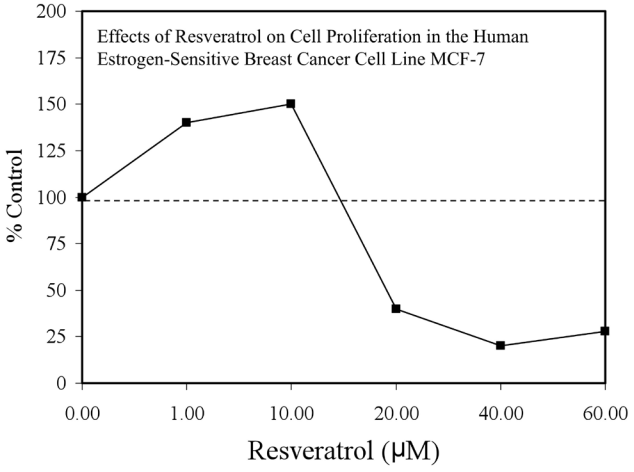


Figure 3. Effects of resveratrol on growth stimulation/ inhibition of ER-positive human breast cancer cell line MCF-7[25]

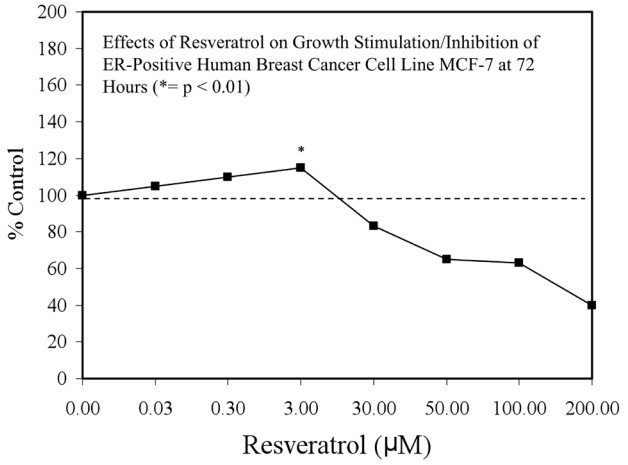


Figure 4. Effects of resveratrol on MCF-7 cell proliferation (6 days)[26]

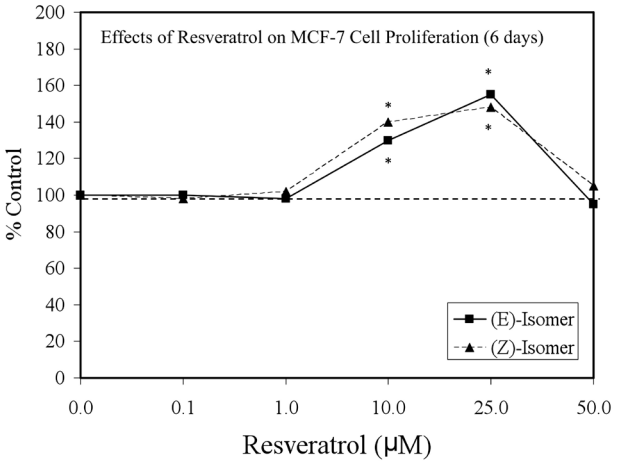


Figure 5. Effects of resveratrol on growth of MCF-7 cells[28]

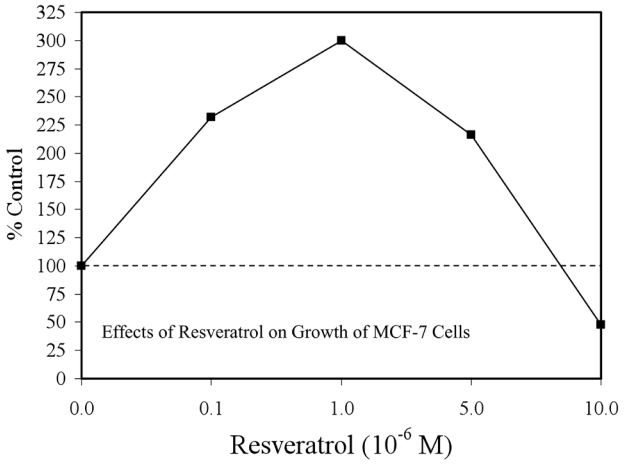


Figure 6. Effects of resveratrol on growth stimulation/ inhibition of ER-positive human breast cancer cell line KPL-1[25]

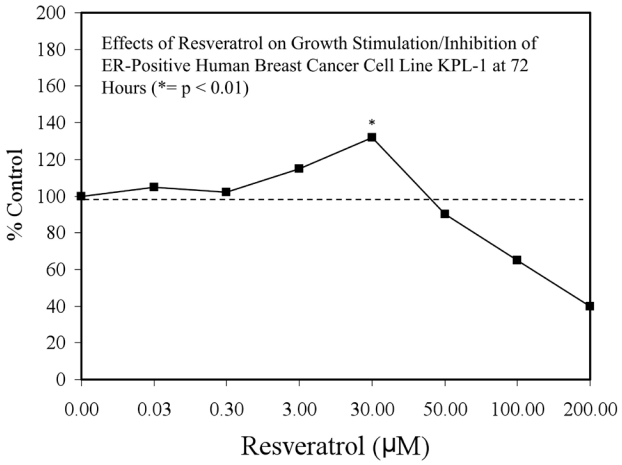


Figure 7A. Effects of resveratrol on MCF-7 cell proliferation (complete medium)[30]

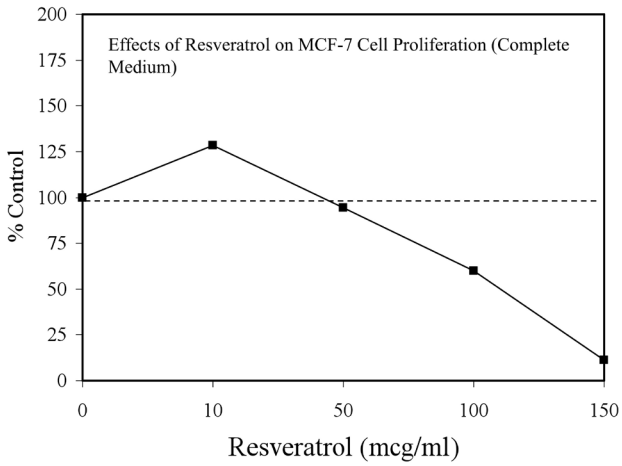


Figure 7B. Effects of resveratrol on MCF-7 cell proliferation (estrogen-depleted medium)[30]

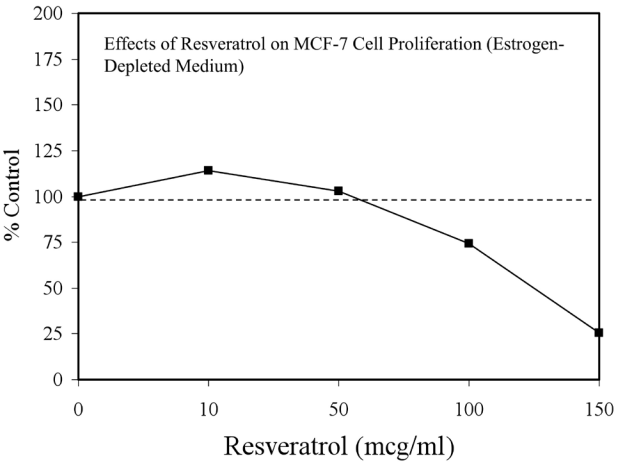


Figure 8. Effects of resveratrol on IGF-I secreted from MCF-7 cells[33]

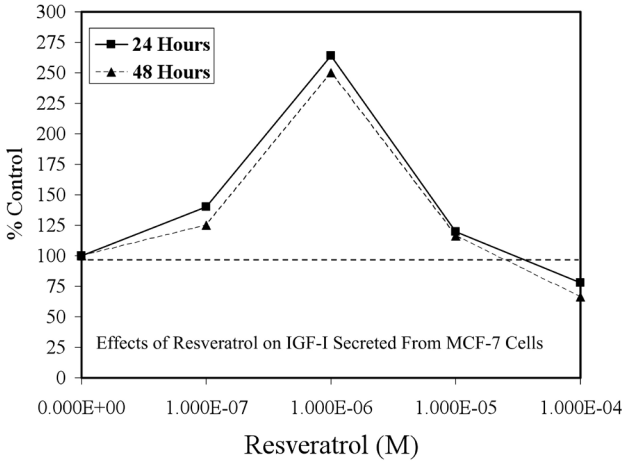


Figure 9A. Effects of resveratrol on cathepsin D secreted from MCF-7 cells[33]

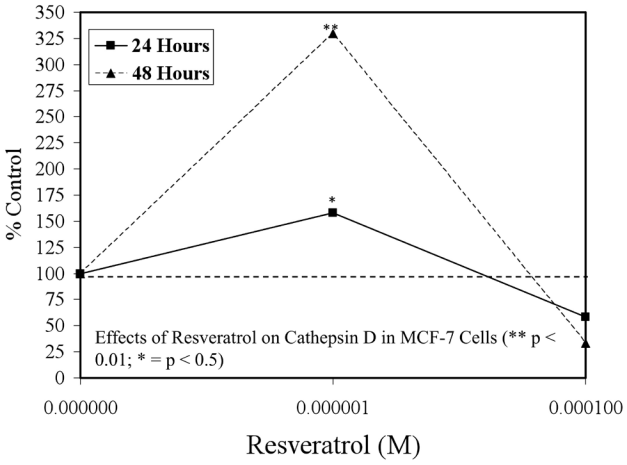


Figure 9B. Effects of resveratrol on cathepsin D secreted from T47D cells[33]

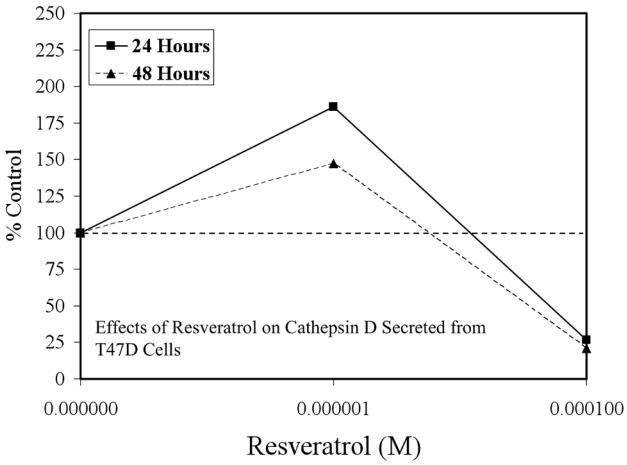


Figure 10A. Effects of resveratrol on human vitamin D receptor (VDR) promoter activity (relative luciferase units) in MCF-7 breast cancer cells[34]

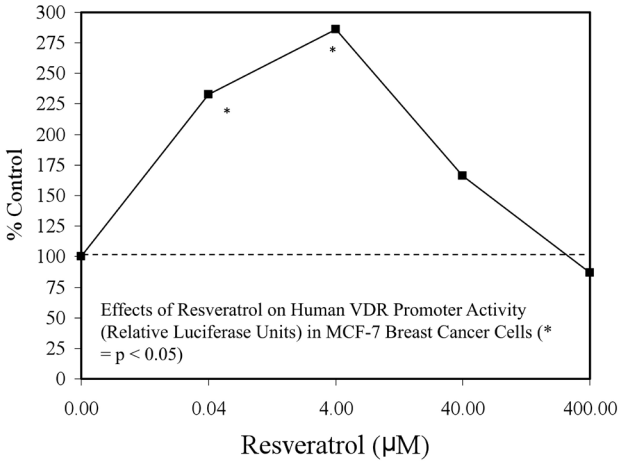


Figure 10B. Effects of resveratrol on human VDR promoter activity (relative luciferase units) in T47D breast cancer cells[34]

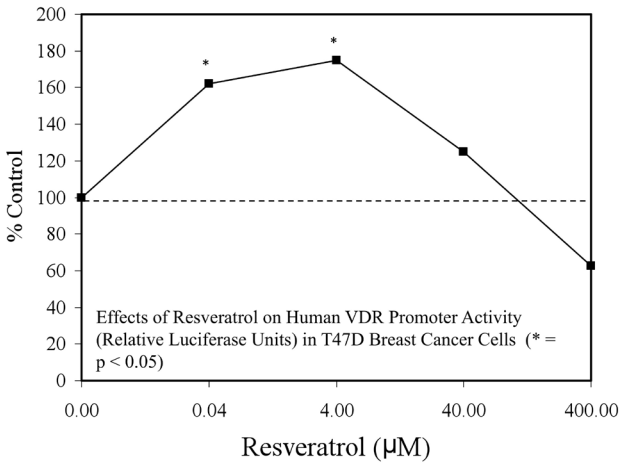


Figure 11. Effects of resveratrol on growth of ER-negative human breast cancer cell line MDA-MB-231[35]

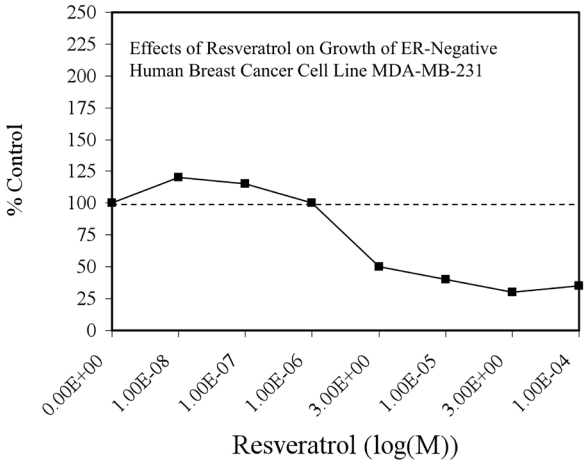


Figure 12. Effects of resveratrol on multiple human breast cancer cell lines[31]

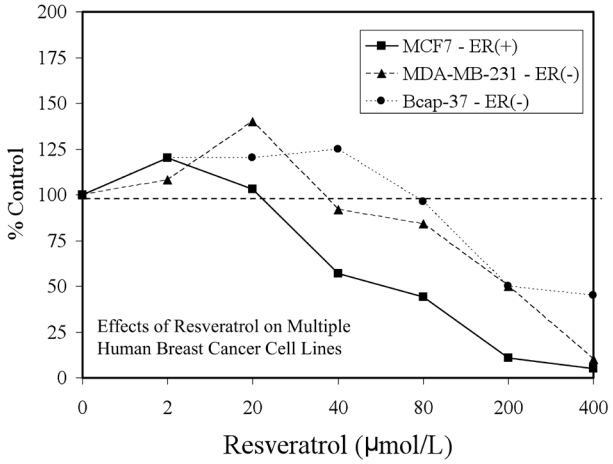


Figure 13. Effects of resveratrol on focal adhesion kinase (FAK) activity in MDA-MB-231 cells[36]

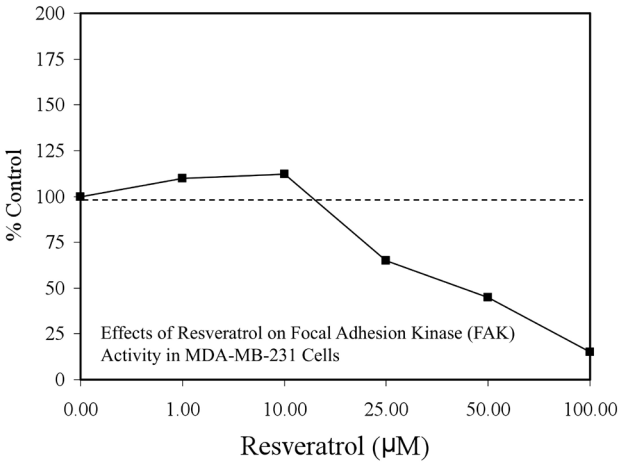


Figure 14. Effects of resveratrol on MDA-MB-231 cells[37]

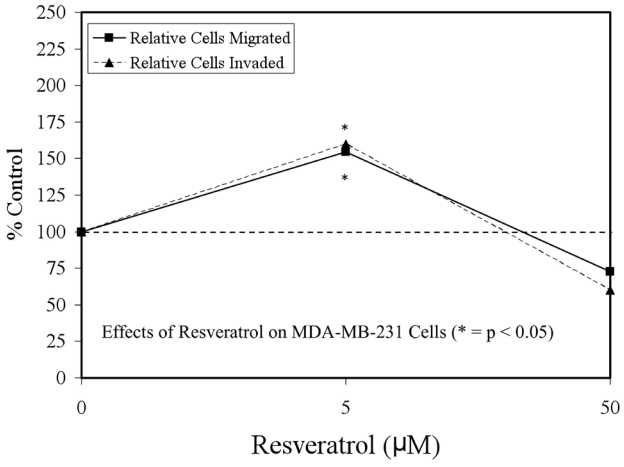


Figure 15A. Effects of resveratrol on [^{3}H]thymidine and [5-^{3}H]uridine incorporation into LNCaP cells[40]

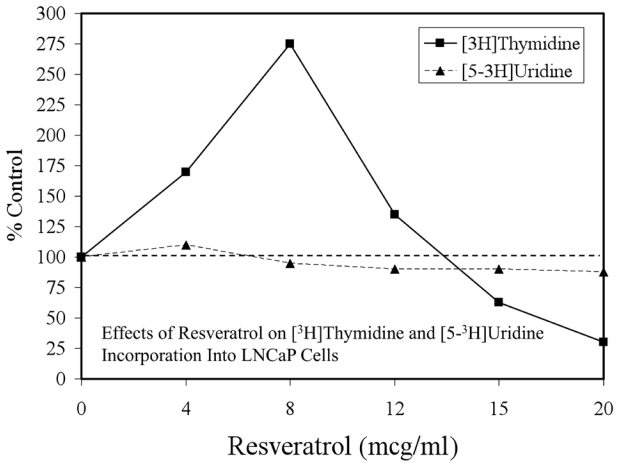


Figure 15B. Effects of resveratrol on prostate tumor cell lines[40]

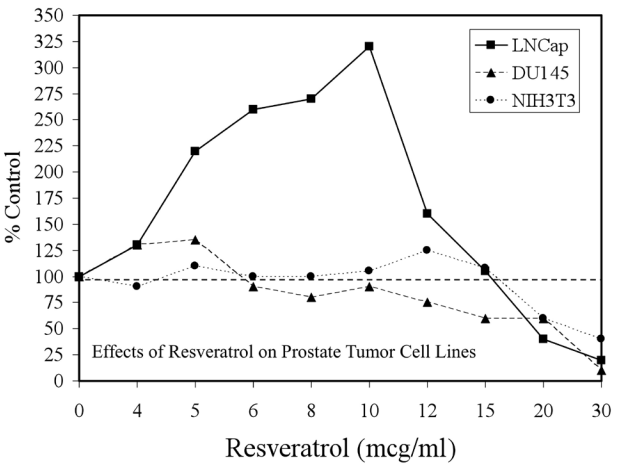


Figure 16. Effects of resveratrol on relative survival in WIL2-NS and HL-60 cells[43]

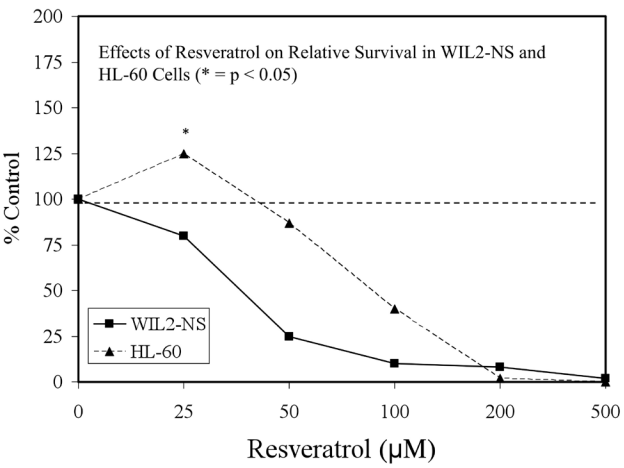


Figure 17. Effects of resveratrol on CEM-C7H2 leukemia cell proliferation[44]

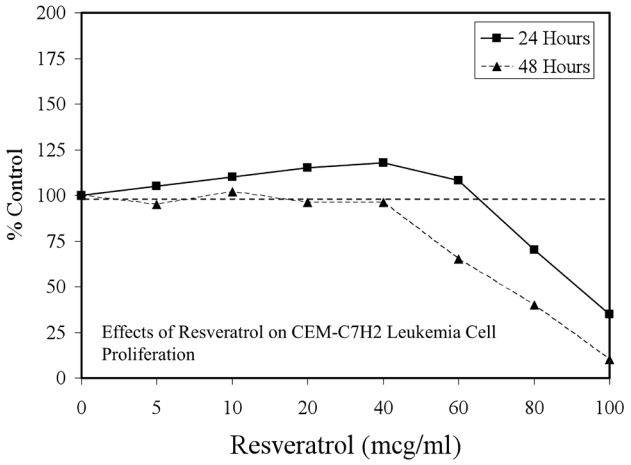


Figure 18. Effects of resveratrol on MTS reduction to formazan in human keratinocytes (72 Hours)[45]

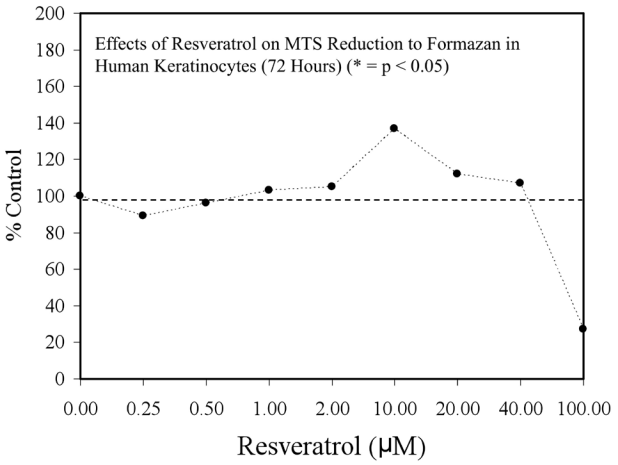


Figure 19. Effects of resveratrol on cellular proliferation in HeLa cells (24 hours)[46]

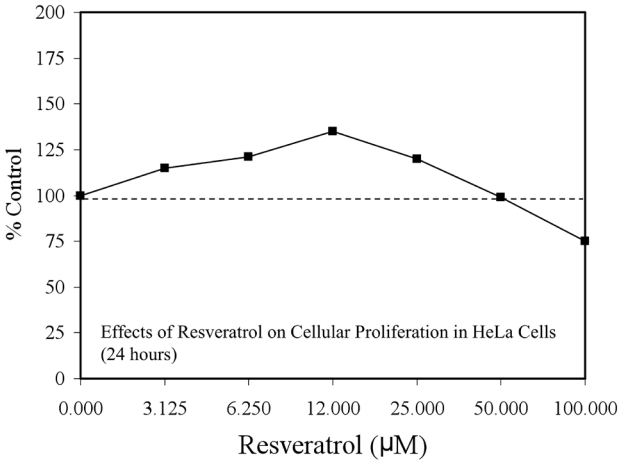


Figure 20A. Effects of resveratrol on proliferation of HT-29 colon carcinoma cells[54]

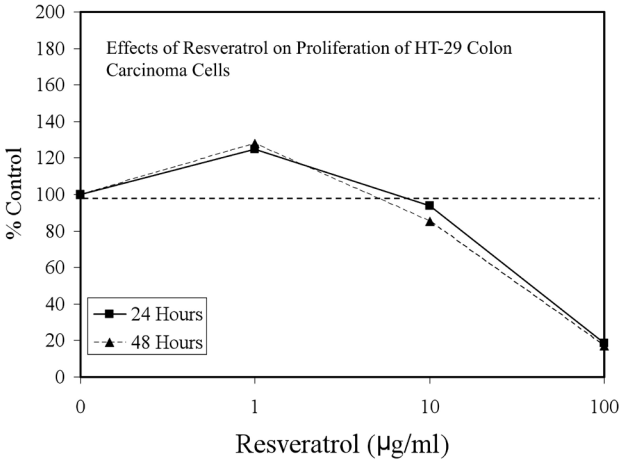


Figure 20B. Effects of resveratrol on proliferation of SW-620 colon carcinoma cells[54]

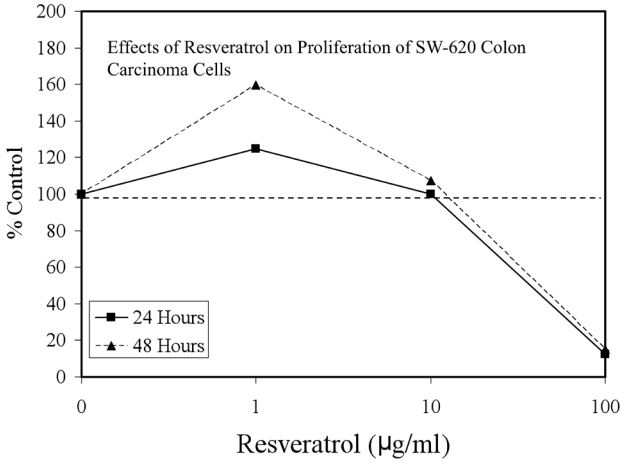


Figure 21. Effects of resveratrol on apoptosis in Lewis lung carcinoma cells[64]

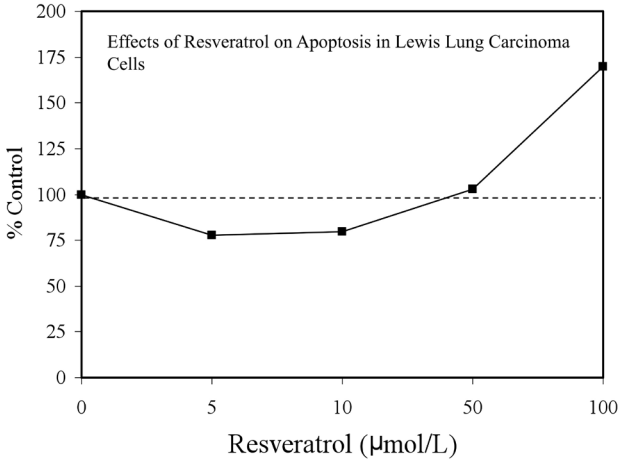


Figure 22A. Effects of resveratrol on endothelial progenitor cell proliferation[65]

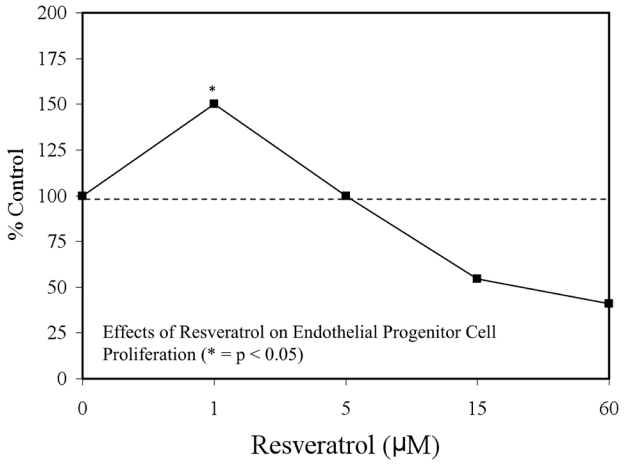


Figure 22B. Effects of resveratrol on endothelial progenitor cell migration[65]

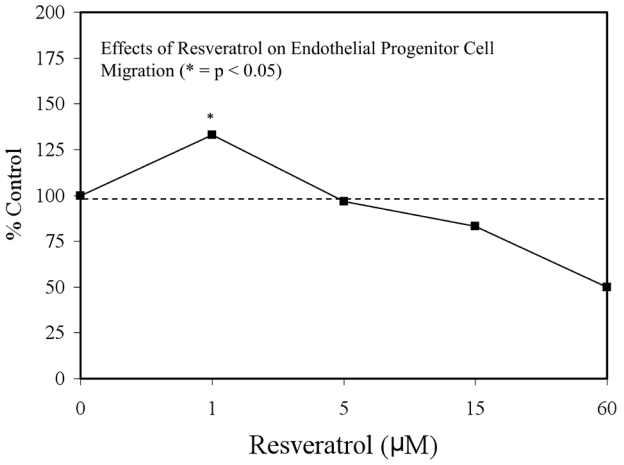


Figure 22C. Effects of resveratrol on endothelial progenitor adherence cells[65]

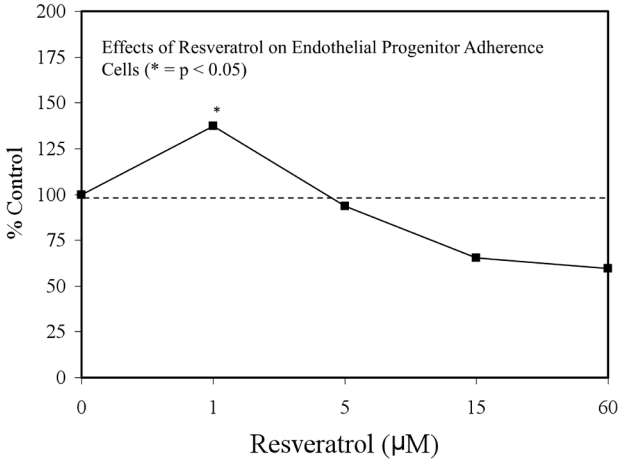


Figure 22D. Effects of resveratrol on eNOS protein expression in endothelial progenitor cells[65]

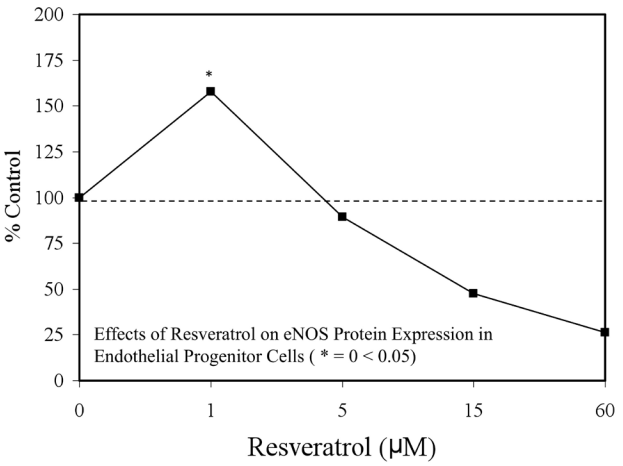


Figure 22E. Effects of resveratrol on eNOS/beta-actin in endothelial progenitor cells[65]

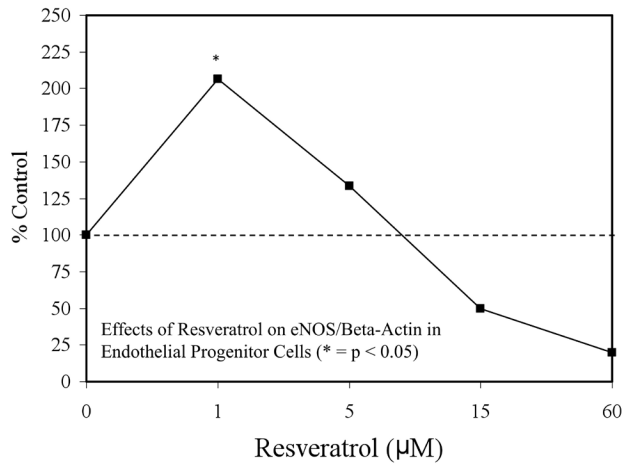


Figure 23A. Effects of resveratrol on cell proliferation of endothelial progenitor cells[66]

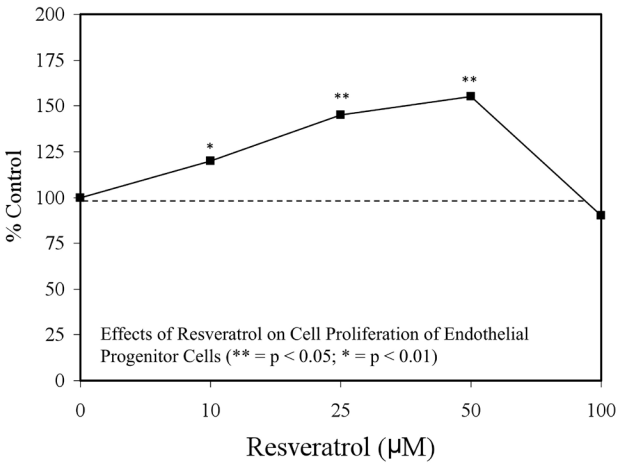


Figure 23B. Effects of resveratrol on migration of endothelial progenitor cells[66]

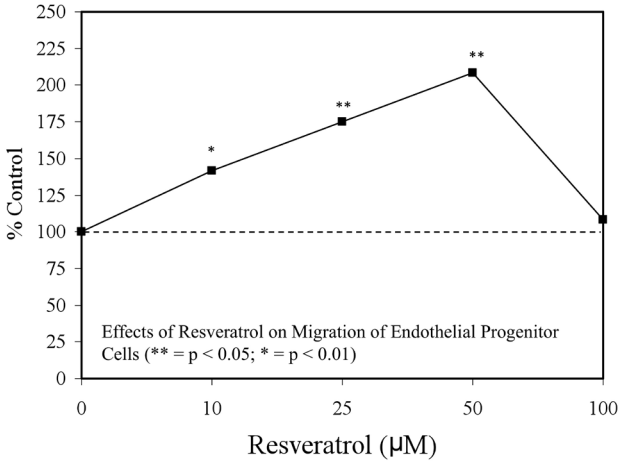


Figure 24. Effects of resveratrol on proliferation of human endothelial (HUV-EC-C) cells[54]

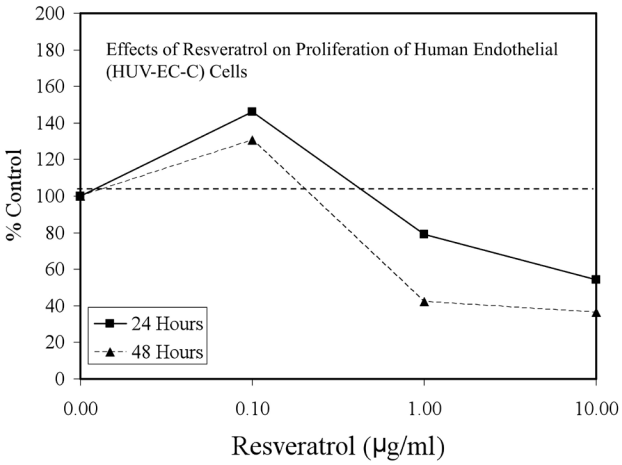


Figure 25. Effects of resveratrol on endothelial cell migration[68]

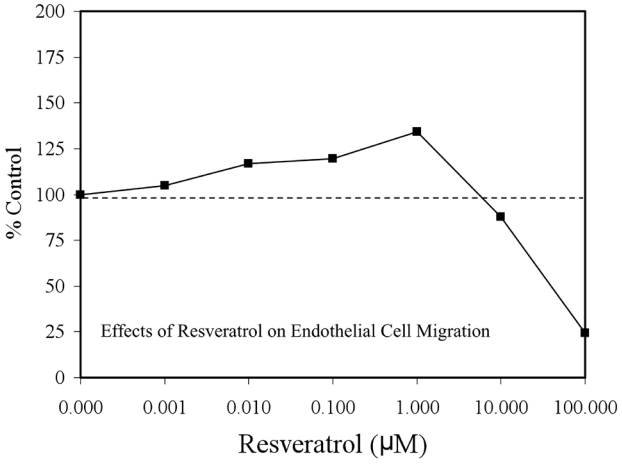


Figure 26A. Effects of resveratrol on frequency of CD8 + T cells expressing IFN-γ[71]

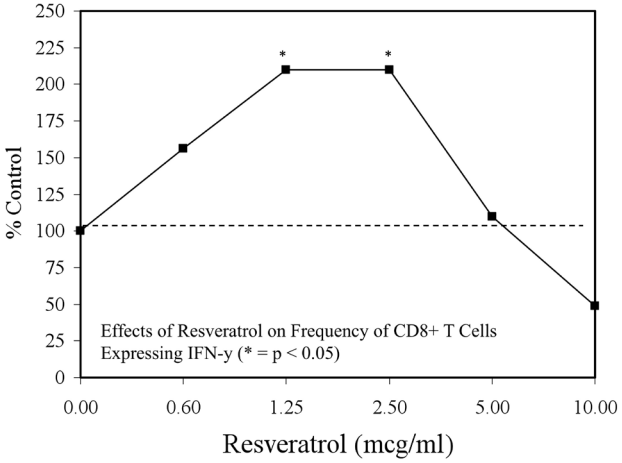


Figure 26B. Effects of resveratrol on frequency of CD8+ T cells expressing IL2[71]

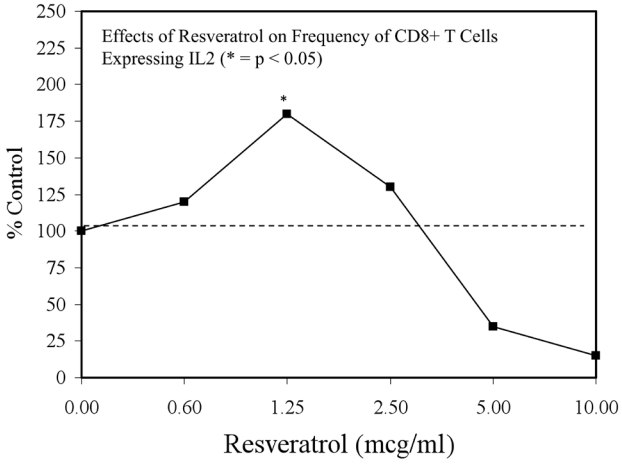


Figure 26C. Effects of resveratrol on frequency of CD8+ T cells expressing IL4[71]

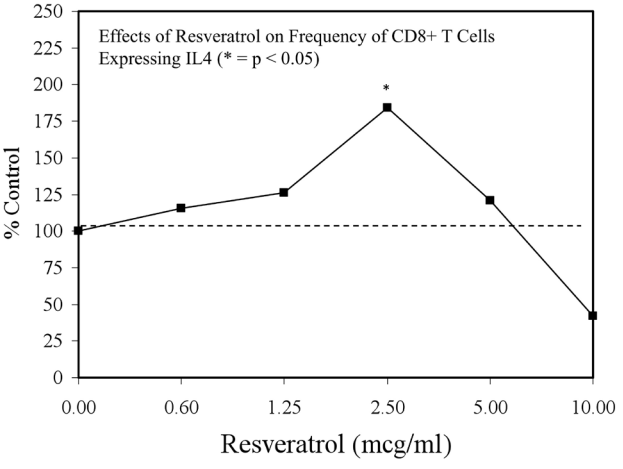


Figure 26D. Effects of resveratrol on frequency of CD4+ T cells expressing IFN-γ[71]

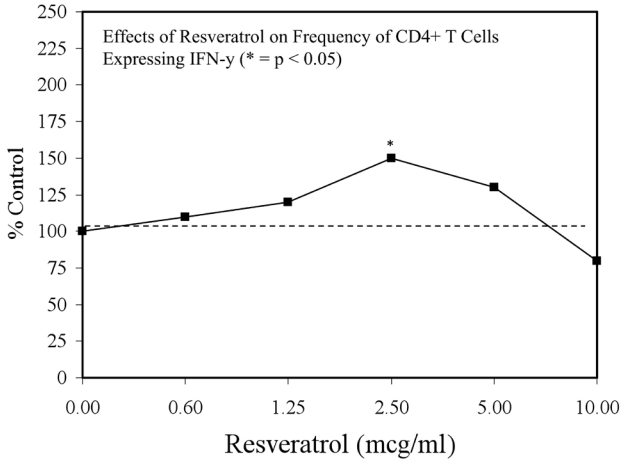


Figure 26E. Effects of resveratrol on frequency of CD4+ T cells expressing IL2[71]

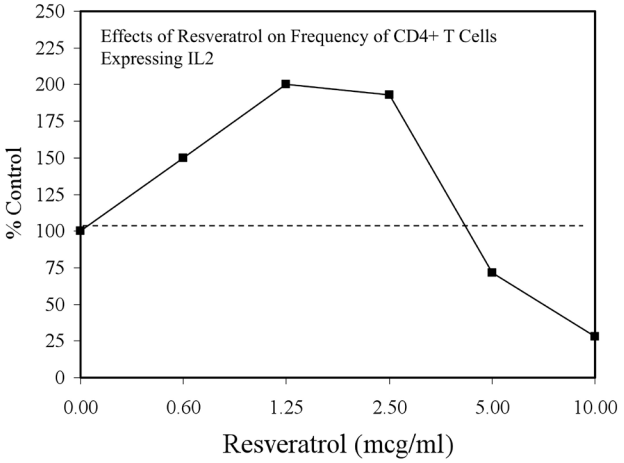


Figure 26F. Effects of resveratrol on frequency of CD4+ T cells expressing IL4[71]

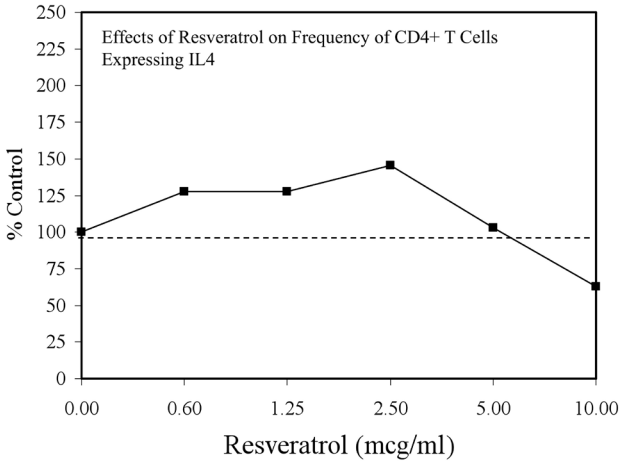


Figure 27. Effects of resveratrol on the proliferation of spleen cells[73]

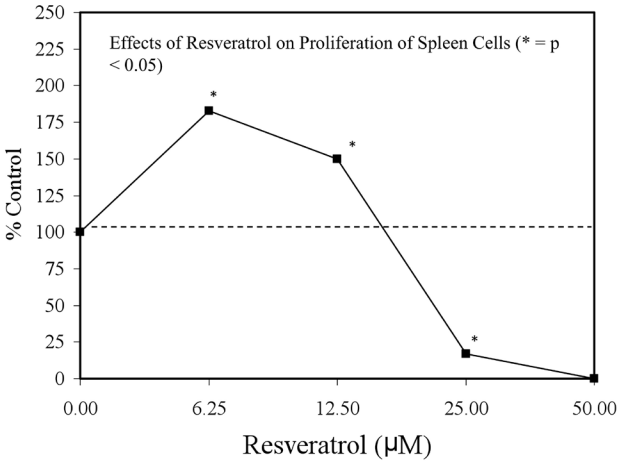


Figure 28. Effect of resveratrol on H22 tumor cell killing by peritoneal macrophages[74]

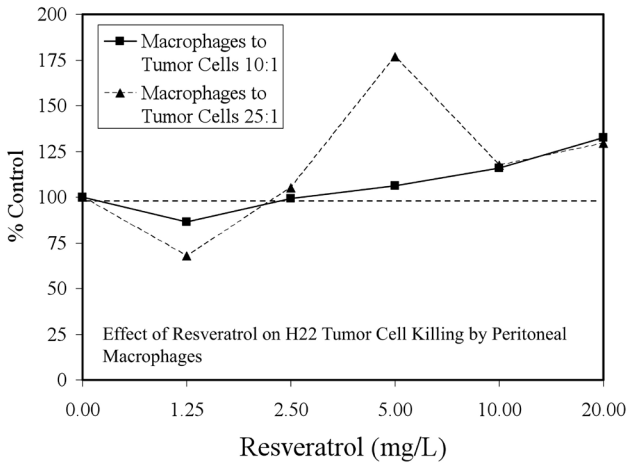


Figure 29. Effects of resveratrol on the ratio of kynurenine to tryptophan[75]

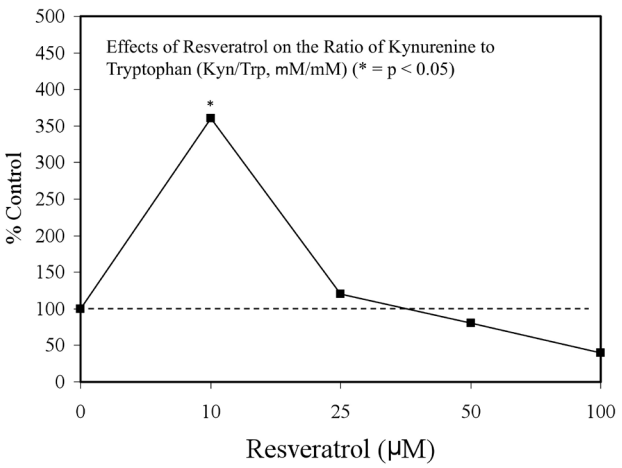


Figure 30. Effects of resveratrol on C6 glioma (rat brain tumor) cell damage measured by LDH[80]

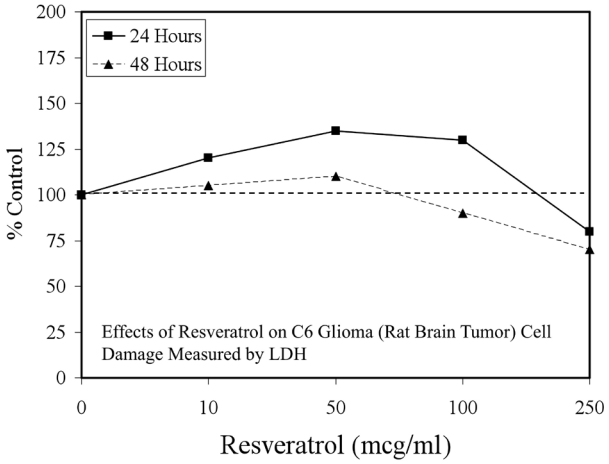


Figure 31. Effects of resveratrol on HO-1 promoter activity in human aortic smooth muscle cells[81]

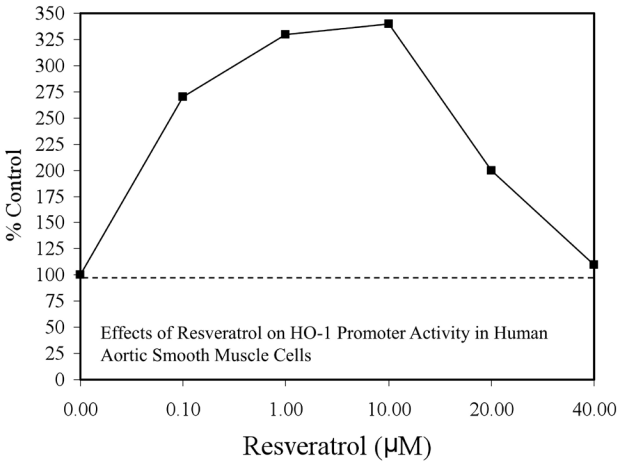


Figure 32. Effects of resveratrol on SOD and GPx in V79 cells[82]

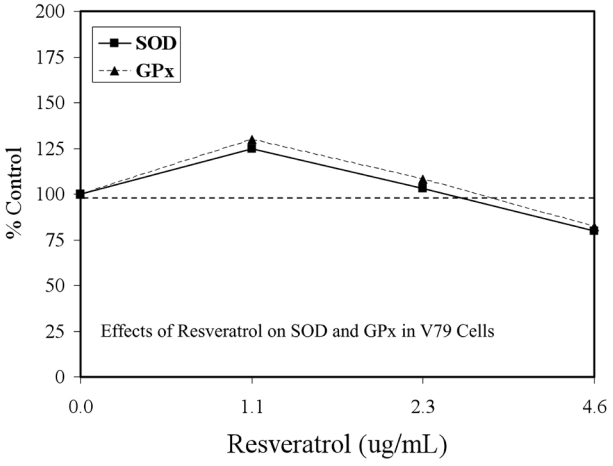


Figure 33. Effects of resveratrol on testosterone production in mouse primary Leydig cells[83]

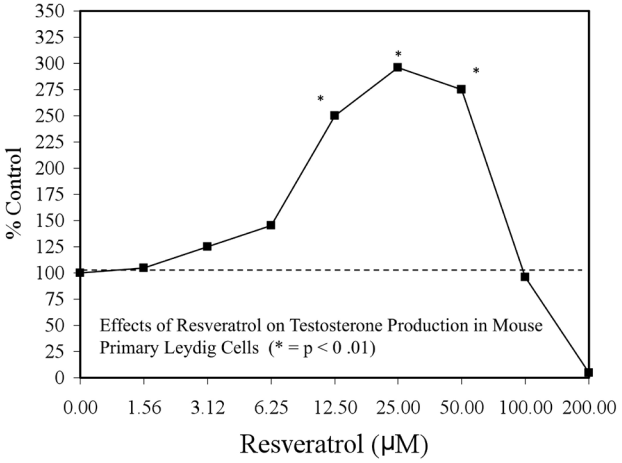


Figure 34. Effects of resveratrol on paraoxonase-1 (PON-1) activity in the human liver cell line (HC04)[91]

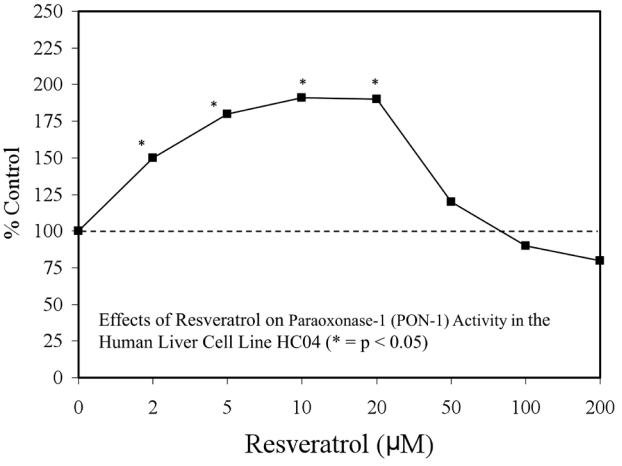


Figure 35A. Effects of resveratrol on ulcer index and myeloperoxidase (MPO) activity in mice – Day 2[93]

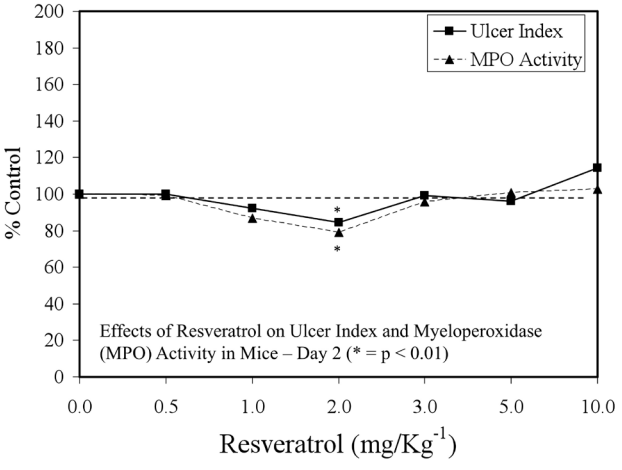


Figure 35B. Effects of resveratrol on ulcer index and myeloperoxidase (MPO) activity in mice – Day 3[93]

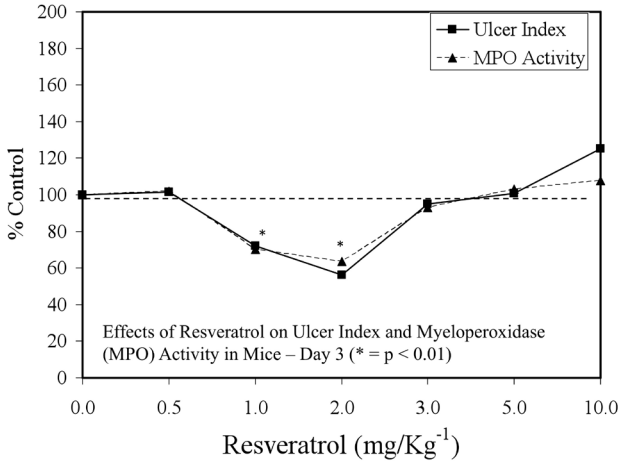


Figure 35C. Effects of resveratrol on ulcer index and myeloperoxidase (MPO) activity in mice – Day 4[93]

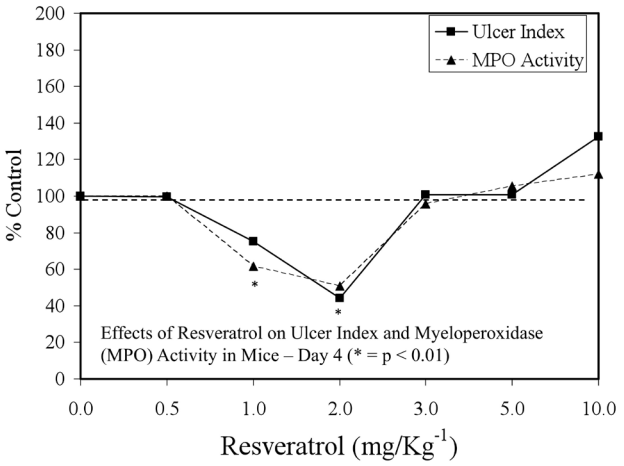


Figure 35D. Effects of resveratrol on ulcer index and myeloperoxidase (MPO) activity in mice – Day 7[93]

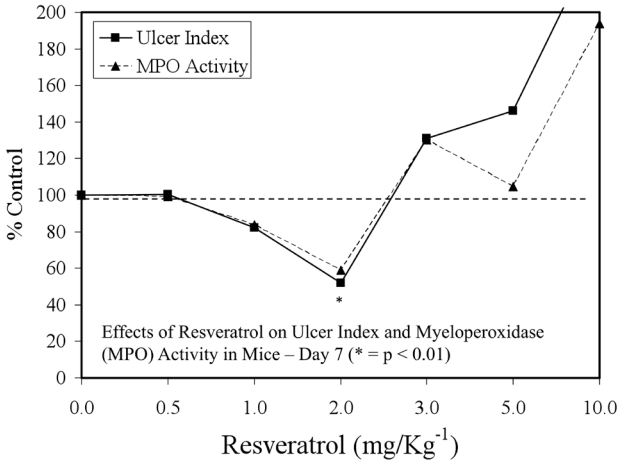


Figure 35E. Effects of resveratrol on ulcer index and myeloperoxidase (MPO) activity in mice – Day 10[93]

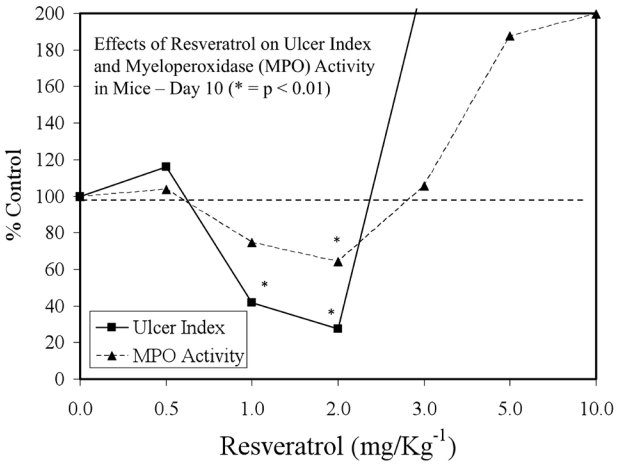


Figure 36A. Effects of resveratrol on cell proliferation in human bone marrow-derived mesenchymal stem cells (HBMSCs)[94]

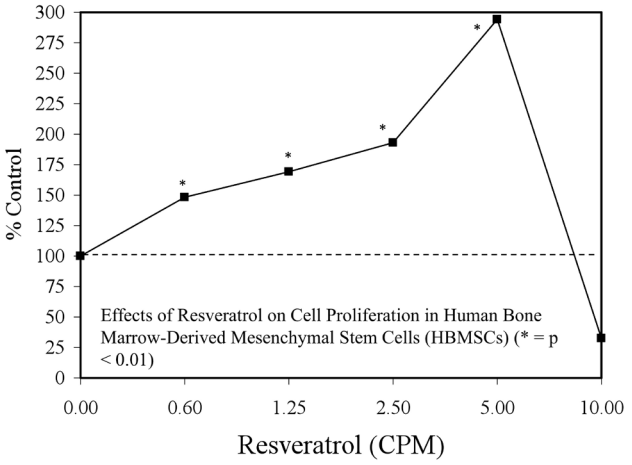


Figure 36B. Effects of resveratrol on cell proliferation in human bone marrow-derived mesenchymal stem cells (HBMSCs)[94]

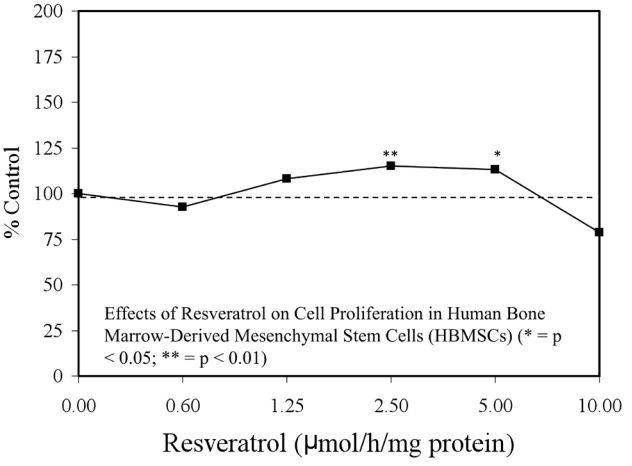


Figure 37A. Effects of resveratrol on myocardial infarct damage[113]

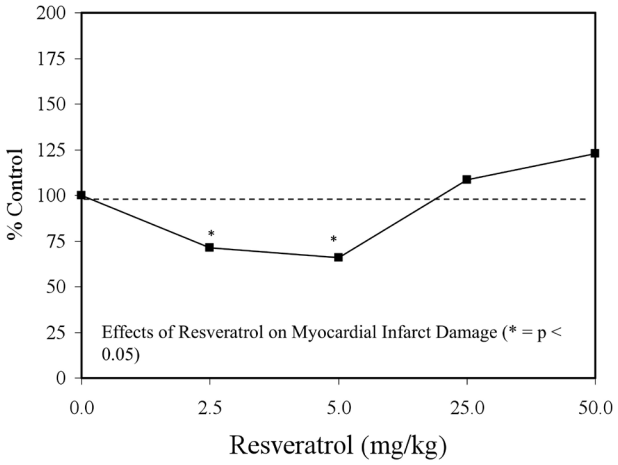


Figure 37B. Effects of resveratrol on cardiomyocyte apoptosis[113]

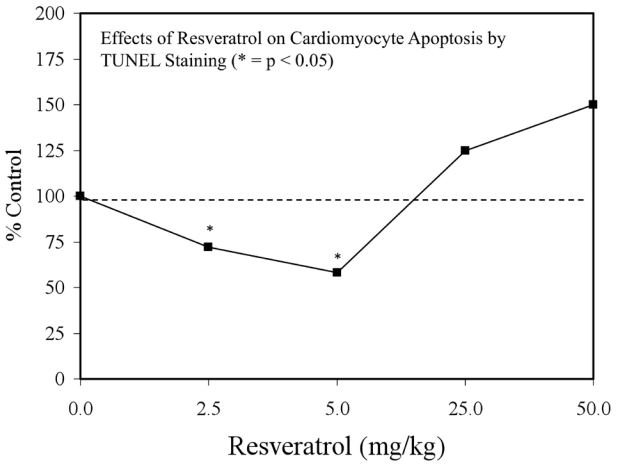


Figure 38. Effects of resveratrol on beta-amyloid (BA) peptide induced cytotoxicity (reversal of damage) in PC12 cells using the MTT assay. Beta-amyloid dose decreases from Tox 1 (highest) to Tox 4 (lowest)[123,124]

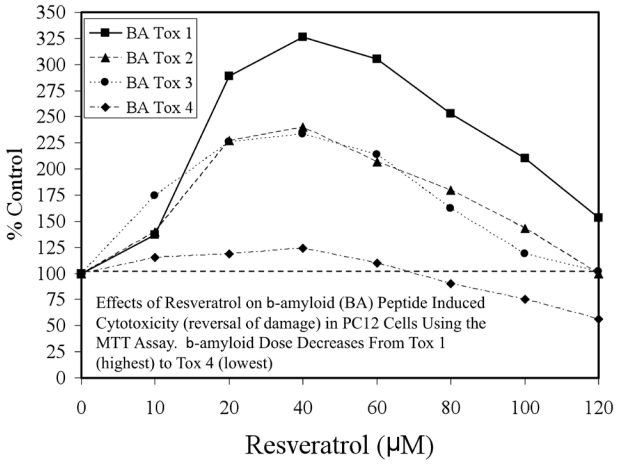


Figure 39. Resveratrol can protect neurons against ischemic and excitotoxic injury by suppressing oxidative stress, but may also endanger neurons by depleting NAD+ levels via the activation of SIRT1.

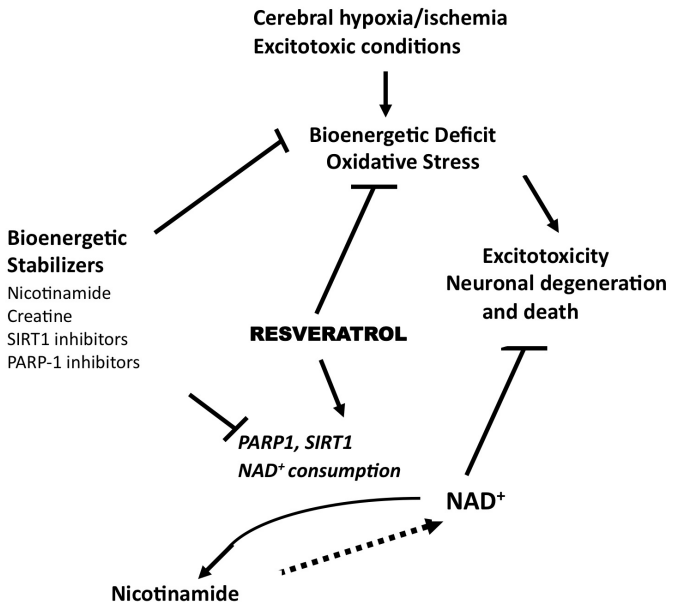


Figure 40. Effects of 3,4,4',5'-tetrahydroxy-trans-stilbene on promastigotes in vitro[141]

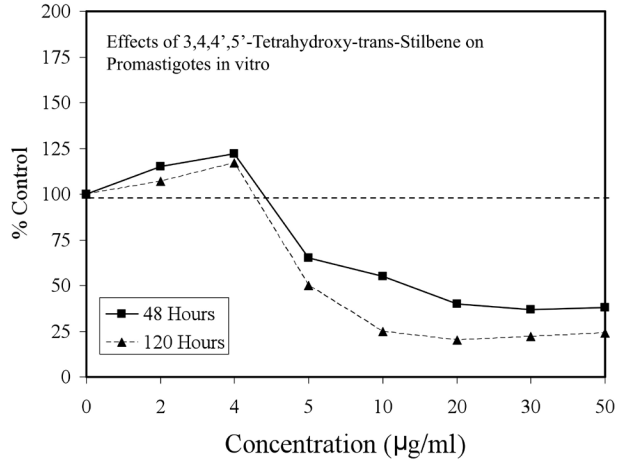


Figure 41. Effects of resveratrol on viability rates of T. spiralis muscle larvae[144]

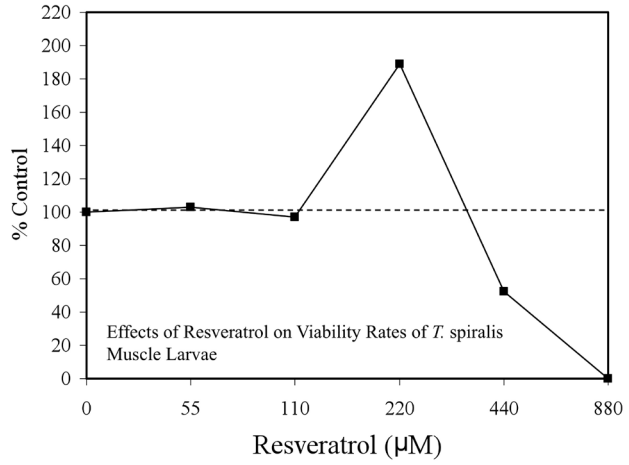


Figure 42. Effects of resveratrol of grape leaf infectivity[146]

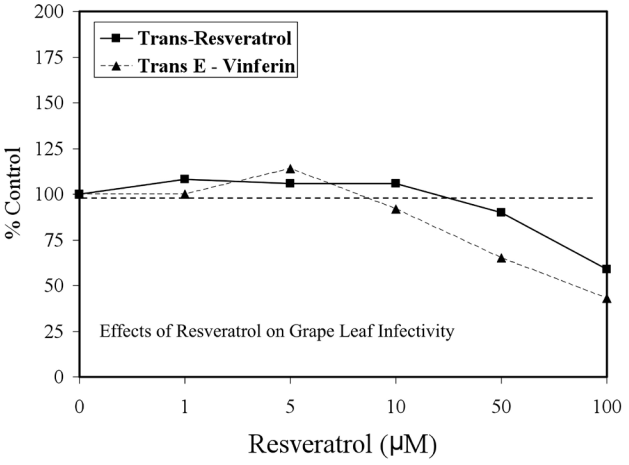


Figure 43. Down-regulation of tumor necrosis factor-alpha by resveratrol, a SIRT1 activator, using THP-1 cells[147]

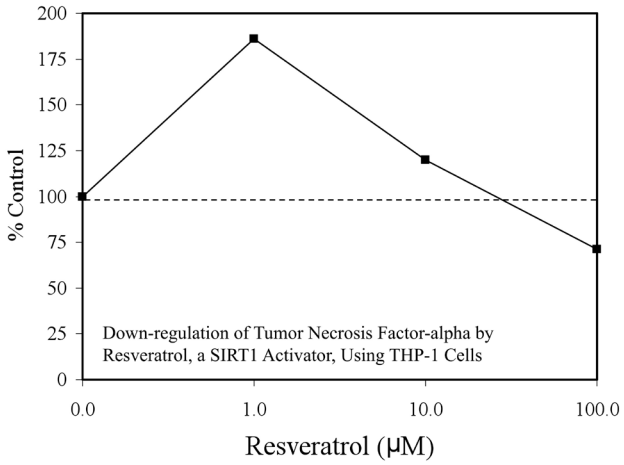
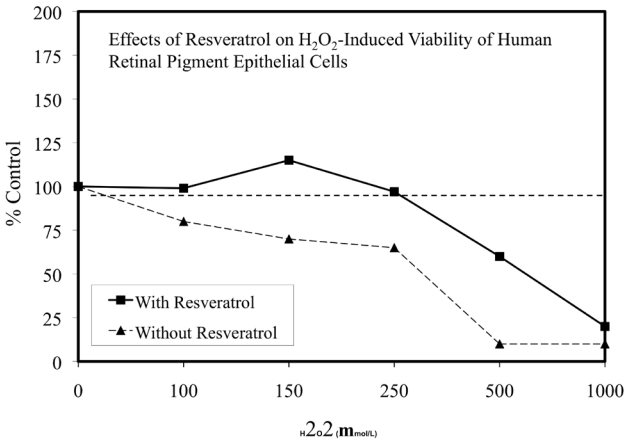


Figure 44. Effects of resveratrol on H_2O_2-induced viability of human retinal pigment epithelial cells[148]



COMMENTARY ON RESVERATROL AND HORMESIS

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ABSTRACT

Resveratrol, a grape skin and red wine-derived polyphenolic phytoalexin exhibits hormetic action delivering numerous health benefits at lower doses while being detrimental at higher doses. Epidemiologic and clinical trials need to be based on the clear understanding of hormetic health benefits of resveratrol

Hormesis is defined as a dose response relationship that is stimulatory at low doses, but detrimental at higher doses resulting in a J-shaped or an inverted U-shaped dose response curve. It has been known for quite some time that cardioprotective effects of alcohol or wine intake follow a J-shaped curve (1). Extensive literature search implicates that resveratrol present in red wine also demonstrates a simlar health benefits, being highly effective at lower doses and detrimental at higher doses. Such hormesis has been known for more than hunred years, and frequently observed among the toxins. Resveratrol is a phytoalexin, whose growth is stimulated by environmental stress such as fungal infection, UV radiation and water deprivation (2). Cardioprotective effects of resveratrol is exerted through its ability to precondition a heart, which causes the development of intracellular stress leading to the upregulation of intracellular defense system such as antioxidants and heat shock protein (3). Preconditioning is another example of hormesis, which is potentiated by subjecting an organ like heart to cyclic episodes of short durations of ischemia, each followed by another short durations of reperfusion (4). Such small but therapeutic amount of stress renders the heart resistant to subsequent lethal ischemic injury. Such an adaptive response is commonly oberved with aging. Consistent with this idea, resveratrol has been found to stimulate longevity genes, and at least in prokaryotic species extent the life span (5, 6). In this respect, resveratrol may fulfil the defination of a hormetins (7). There is no doubt that alcohol, wine, and wine-derived resveratrol -all display hormesis.

The present review by Calabrese and his co-authors from an extensive literature search describes how resveratrol displays hormesis. The review should be very important for the basic scientists, clinicians as well as for the common people to understand the importance of using resveratrol only at lower doses as completely opposite effects can occur at higher doses resulting in adverse effects on health. Epidemiologic and clinical trials need to be based on the clear understanding of hormetic beneficial effects of resveratrol

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COMMENTARY ON RESVERATROL AND HORMESIS

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ABSTRACT

The plan of attack for this Commentary is threefold. First: to consider, place in perspective and judge some of the claims that have been advanced for resveratrol's utility. Second: to evaluate and judge the scientific basis and validity of the paper being commented upon. The judgment is very positive. Third: to place in perspective the importance of hormesis in being a major factor determining resveratrol's ultimate efficacy.

PROLOGUE

Before offering specific comments on "Resveratrol Commonly Displays Hormesis: Occurrence and Biomedical Significance", henceforth referred to as the "report", it is worthwhile to make two general comments. First, the report was stated to be built upon the suggestion of Howitz and Sinclair1 that resveratrol acts as a zenohormetic agent, with zenohormesis referring to inter-species hormesis whereby an animal or fungal species uses chemical cues from other species about the status of its environment or food supply to mount a preemptive defense response that increases its chances of survival. They explicitly distinguished zenohormetic from "straightforward" hormetic effects. While admitting that the two mechanisms may not be mutually exclusive and conceding that they may even function synergistically, Howitz and Sinclair considered it unlikely that straightforward hormesis is the primary mode of action. Substantial evidence for straightforward hormesis as established in the report calls into question the relative importance of zenohormesis.

Second, in the report's penultimate paragraph it was perceptively and correctly stated that animal studies show that resveratrol may be able to account "at least in part" for the French Paradox's cardioprotective effects. Actually alcohol (ethanol) provides a broader and more encompassing explanation for the French Paradox with strong psychological, behavioral and biochemical evidence for alcohol affecting the experimental behavior of animals in a hormetic biphasic dose-dependent manner.2 Both case-control and cohort epidemiological studies strongly indicate that light-to-moderate alcoholic drinkers are at lower risk of cardiovascular disease and death than non-drinkers, although heavier alcohol consumption can negatively affect the neurological, gastrointestinal, hematologic, immune, psychiatric and musculoskeletal organ systems. It has been stated that the totality of evidence suggests that the major beneficial component of alcoholic beverages on cardiovascular mortality is in fact ethanol per se rather than some other component,3 and that it is likely that any apparent additional beneficial effect of wine on health in addition to the effect of ethanol itself is a consequence of confounding.4 These facts demonstrate that at least in this instance resveratrol may not be the all-encompassing elixir it has oftentimes been hyped to be. A proper understanding and appreciation of hormetic effects in resveratrol should place resveratrol's efficacy in proper perspective. The report significantly contributes to that understanding and appreciation.

My commentary will be based on a deconstruction of the report centered on a particular word and phrase in its title: "occurrence" and "biomedical significance".

OCCURRENCE

The report's Abstract states that resveratrol induces opposite effects at low vis-à-vis high doses, indicative of hormetic dose response. A plethora of specific confirmatory dose-response examples has been provided in the body of the report. Perusal of the report's host of figures reveals qualitative features of hormesis in tandem with characteristic quantitative features of hormetic dose-response with respect to maximum stimulation and width of stimulation. These examples cover the gamut of biomedical models: tumor cell lines, non-tumor cell

lines, and human and parasitic disease conditions, with both in vitro and in vivo animal model studies being reported. These figures are remarkable and demonstrate the occurrence of hormesis, but some questions arise about them. They suffer from a dearth of error bars. If given, error bars would have provided even more convincing evidence. The report would have been further enhanced by a discussion of the data's a prior entry and evaluative criteria. Rigorous and convincing criteria have been employed in previous surveys of this type by one of the report's authors.5,6 Convincing demonstrations that rigorous criteria had been employed would serve to allay possible concerns that the data had been cherry-picked to demonstrate hormesis.

The report may have been unintentionally misleading in quoting out of context a statement by Azios et al7 that "the biphasic concentration-dependent effects of resveratrol have been widely reported". While "widely reported" is certainly open to debate, "acceptance" of biphasic concentration-dependent effects is another matter entirely and certainly even less widely accepted. In reality, most hormesis proponents would ruefully admit that they are still awaiting Kuhn's hormesis paradigm shift to kick in. A less important point: since nontoxicologists may not be acquainted with the acronym NOEL, the term should either be defined or deleted from Figure 1.

BIOMEDICAL SIGNIFICANCE

Most of the report's efforts were devoted to presenting experimental laboratory evidence establishing resveratrol's hormetic dose-responses. The evidence demonstrates that resveratrol-hormesis functions as a double-edged sword with low resveratrol concentrations being either potentially beneficial or harmful, depending on the endpoint of interest. While the biomedical significance of resveratrol-hormesis has been noted in the report, further discussions of the significance of hormetic resveratrol to public health and clinical applications are warranted and will now be presented.

The report noted that the hormesis concept presents unique challenges to the biomedical and clinical communities to better clarify therapeutic implications of hormetic dose-responses, as treatment success or failure is likely to be highly dependent on "getting the dose right". The correctness of this observation is consonant with the fact that both the Selenium and Vitamin E Cancer Prevention Trial (SELECT) and the Nutritional Prevention of Cancer (NPC) Trial have been critiqued for administering the same dose to all participants under the operational dictum that one dose fits all without considering hormetic responses.8,9 The lesson to be learned from this has important ramifications in that any future or ongoing human-use trials of resveratrol should take hormetic dose-responses into account and tailor dose to the individual.

The report's discussion of resveratrol affecting triphasic dose-responses in vitamin D receptor (VDR) promoter activity has important biomedical implications in general and to resveratrol trials in particular. Only over the past few decades has it become appreciated that vitamin D generates important biological responses in the immune, heart-cardiovascular, muscle, and brain systems as well as involvement in control of the cell cycle and thus of the disease process of cancer. For most individuals, casual sunlight exposure accounts for more than 90% of vitamin D in the body.10 The best clinical indicator of vitamin D status in humans is measurement of serum concentrations of 25-hydroxyvitamin D [25(OH)D], the major circulating metabolite of vitamin D.11 While the report's subsection "Vitamin D Receptor" was restricted to breast cancer cell lines, other parts of the report discussed laboratory evidence for resveratrol inducing hormetic responses in the following processes: ageing, angiogenesis, immune function, inflammation, neurological, oxidation: anti- and pro-, programmed cell death: apoptosis and autophagy, as well as in cardiovascular disease and prostate cancer. The plot begins to thicken as other laboratory studies have independently established that vitamin D also exerts control in each of those processes.12 There are also laboratory studies attesting to vitamin D-induced biphasic dose-responses.13 In addition , biphasic vitamin D dose-responses in humans have been uncovered in epidemiological studies of cardiovascular disease14-16 and prostate cancer.17,18

These facts have important ramifications in any human-use trials of resveratrol. If the 25(OH)D vitamin D levels of trial participants are not monitored before and during such trials, the trial results would prove futile, or at least ambiguous, since they could be influenced, if not determined, by vitamin D status. Without vitamin D monitoring, the end-result would be a confounding culde-sac. Of course other possible confounding factors should also be considered in formulating and carrying out resveratrol trials.

EPILOGUE

A recent search of the PubMed data base uncovered some thirty-two hundred publication citations for "resveratrol". In the interim that number has most likely appreciably increased. It is my considered judgment that the authors of most of these publications would profit from reading, digesting and being cognizant of the report and the ideas and concepts that would most likely in due course spring forth from doing so.

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COMMENTARY ON RESVERATROL AND HORMESIS

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SUMMARY

During the last years, resveratrol has been the focus of numerous in vitro and in vivo studies investigating its biological attributes, which include mostly antioxidant and anti-inflammatory activities, immunomodulation and chemoprevention. Nevertheless, depending on the concentration of the phytoalexin and the cell type, resveratrol commonly may act in a hormetic fashion, displaying biphasic dose responses mainly in tumor cell lines and in animal models of heart attacks and gastric ulcers. In relation to digestive disorders, there are contradictory results. Several studies including ours, have identified resveratrol as a beneficial agent at different doses (5 and 10 mg/Kg), without detecting any biphasic dose responses. Anyway, it is possible that the opposing results of the mentioned studies could be due to variations in conditions of administration, protocols and methods of assessment. In addition, concerning toxicity, resveratrol seems to be well tolerated although a lot of questions remain unanswered. For example, the disparity between bioavailability and efficacy is a topic that merits investigation. Besides, more long- term toxicity studies and extensive randomized, placebo-controlled double- blind clinical trials will be needed to fully evaluate the benefit-risk profile of resveratrol, its potential in terms of optimal dose, route of administration, disease targets and possible interactions with other drugs.

EXPERT COMMENTARY

During the last years, resveratrol (3,4_,5-trihydroxystilbene has been the focus of numerous in vitro and in vivo studies investigating its biological attributes, which include mainly antioxidant and anti-inflammatory activities, immunomodulation and chemoprevention. In fact, recently, it has been demonstrated that the stilbene blocks the multistep process of carcinogenesis at various stages: tumour initiation, promotion and progression. Nevertheless, depending on the concentration of the phytoalexin and the cell type, it has also been shown that resveratrol can exhibit pro-oxidant properties, leading to oxidative breakage of cellular DNA in the presence of transition metal ions such as copper (De la Lastra et al., 2005, 2007). Additionally, in western populations, where obesity is difficult to control, resveratrol is commercially available as a dietary supplement with aggressive marketing and represents a potential life-long medicine.

This interesting review indicates that resveratrol commonly acts in a hormetic fashion, displaying biphasic dose responses across numerous biological systems, affecting a wide range of endpoints. These hormetic dose responses of resveratrol have been reported mainly in in vitro studies in the case of tumor cell lines (Calabrese 2005) and in vivo with animal models with endpoints such as heart attacks (Dudley et al., 2009) and gastric ulcers (Dey et al.,.2009). For example, in relation to digestive disorders, Dey et al. observed that resveratrol administered at 5-10 mg /kg severely delayed healing of pre-existing gastric ulcers, in contrast results from (Solmaz et al., 2009) demonstrated that resveratrol at the same dose of 10 mg /kg had both protective and therapeutic effects on oxidative gastric damage by suppressing pro-inflammatory cascades, including the activation of pro-inflammatory cytokines, accumulation of neutrophils and release of oxygen-derived free radicals.

With regard with other digestive disorders such as ulcerative colitis several studies, including ours, have identified resveratrol as a beneficial agent at different doses (5 and 10 mg/Kg body weight) in an acute and chronic-induced colitis (Martin et al., 2004, 2006). Similarly in a recent study, (Sanchez-Fidalgo et al., 2010), we have demonstrated the protective/preventive effects of dietary resveratrol intake in a chronic colitis model. Resveratrol group consumed an average of 3 g/day of diet resulting in a dose of 3 mg/kg body weight of resveratrol ingested.

These effects were observed despite extremely low bioavailability and rapid clearance from the circulation. Potential mechanisms implicated comprised inhibition of synthesis and release of pro-inflammatory mediators, modification of eicosanoid synthesis, inhibition of activate immune cells and inflammatory enzymes such as iNOS [inducible NOS (nitric oxide synthase)] and COX-2 (cyclo-oxygenase-2) through its inhibitory effects on NF-κB (nuclear factor κB) or the AP-1 (activator protein-1) signalling pathways. Concerning toxicity, we have not observed any adverse effects at this range of dose. Anyway, it is possible that the contradictory results of the mentioned studies could be due to variations in conditions of administration, protocols and methods of assessment.

In addition, in a recent review by Cottart et al., 2010 who summarise data from toxicological studies performed with resveratrol indicated that the phytoalexin administered to rats and rabbits at 0.3, 1 and 3 g/kg/day for 4 wk (corresponding to 21, 70, and 210 g/day, respectively, in a human weighing 70 kg) and up to 750 mg/kg/day for 3 months was well tolerated and non-toxic (Crowell et al.,2004; Juan et al., 2002; Williams et al., 2009).

The adverse effects in humans have been investigated in several studies with healthy patients after high-dose resveratrol intake (Boocock et al., 2007; Almeida et al., 2009; representing a total of 104 patients (including placebo). The highest doses were 5 g/70 kg for a single intake and 0.9 g/day for iterative administration, no serious adverse event was detected in any of these studies. After a single administration of 400mg of resveratrol, Vaz-da-Silva et al. 2008 exhibited one or more minor biological adverse event, consisting of a small increase in blood bilirubin or alanine amino transferase level. In the multiple-dose study, 40 volunteers received one dose of resveratrol (25, 50 100, 150 mg, or placebo) every 4 h for 48 h. The most frequent adverse event was frontal headache (three cases) (Almedia et al., 2009).

We conclude first, resveratrol seems to be well tolerated although a lot of questions remain unanswered; the answers could depend on the experimental model and conditions of administration. Second, several clinical projects have been yet recorded on clinical trial. gov concerning resveratrol effects for instance in cancer, metabolic syndrome and Alzheimer's disease, however, no information is available on long-term administration. Third, the disparity between bioavailability and efficacy is a topic that merits investigation. There is great inter-individual variability in metabolism and no all resveratrol metabolites have been identified yet. Among them, piceatannol, a monohydroxylated derivative of resveratrol, has physiological effects which may contribute to the action of resveratrol.

Thus given the enthusiam for resveratrol it is necessary to be careful until the data from ongoing clinical trials have been fully assessed. More long- term toxicity studies and extensive randomized, placebo-controlled double- blind clinical trials will be needed to fully evaluate the benefit-risk profile of resveratrol , its potential in terms of optimal dose, route of administration, disease targets and possible interactions with other drugs.

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COMMENTARY ON RESVERATROL AND HORMESIS

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ABSTRACT

Many phenolics found naturally in food have the capacity to show beneficial effects at low doses and toxicity at high doses in in vitro systems. Resvertol is no exception . Nonetheless in the nutritional context the evidence that resveratrol shows hormetic effects is very limited and is of questionable relevance given its rapid metabolism in the stomach. Hormesis can only be confirmed if evidence for a J- or U-shaped dose-response relationship is found in in-vivo doses that are relevant to human intakes.

RESVERATROL

According to this review the biological characteristics of resveratrol are varied and frequently show a biphasic dose-response relationship. These conclusions may have validity in in vitro systems but have to be put into a realistic biological framework to determine whether or not these effects are relevant to human health. In particular there must be a clear distinction drawn between the potential benefits, in the likely dose range to which humans are likely to be exposed through the diet and those that are only relevant to the use of this plant secondary metabolite as a food supplement or pharmaceutical product. It is difficult to answer this critical question from the information provided in this review.

Human bioavailability of resveratrol is relatively low due to its rapid metabolism and elimination. The available studies suggest that plasma concentrations of resveratrol and metabolites peak rapidly after ingestion at concentrations around 0.5 milligrams/litre with an oral dose of 25mg present in various food substrates. Grape juice which contains mostly glucosides of resveratrol (piceid) indicates an even lower bioavailability. Is this dose achievable through normal dietary patterns?

Much of the basic research on resveratrol has been conducted in cultured cells exposed to unmetabolized resveratrol at unrealistic concentrations relevant to likely human nutritional effects. Although cells that line the digestive tract are exposed to unmetabolized resveratrol, other tissues are exposed primarily to resveratrol metabolites. The plasma metabolites resveratrol-3-sulfate and resveratrol monoglucuronides are up to 23 times greater than those of resveratrol in plasma. Little is known about the biological activity of these metabolites at the concentrations present in plasma or other tissues, nor what tissues are capable of converting resveratrol metabolites back to resveratrol. This review has not focussed on the likelihood that the major metabolites show a hormetic effect.

Many of the effects attributable to resveratrol are similar to those found in other secondary plant metabolites and this raises the issue of what particular benefit there is in emphasizing the effects of resveratrol as opposed to secondary metabolites in general. The possibility of synergistic effects needs to be seriously considered in the dietary context.

The scientific literature is replete with claims made for the protective effects of phytochemicals that are present in the diet but in very few cases, if any, has a properly conducted pharmacokinetic study been undertaken with the rigour of that undertaken by de Leon and her colleagues. Their work clearly delineates the dose range for protective as opposed to toxic effects. To demonstrate a hormetic effect it is necessary that there are doses that maximise a benefit rather than that simply show a benefit. In other words a clear demonstration of a J- or U-shaped dose-response has been shown within the dose range that could be relevant in vivo. Finally consideration needs to be given to whether the effects observed are general or are only likely to apply to a specific sub-group of the population.

The review has provided insufficient data to evaluate whether the hormetic effects observed by the experimental data are results of the experimental design of much of the work or are real and applicable to human nutrition.

COMMENTARY ON RESVERATROL AND HORMESIS: RESVERATROL – A HORMETIC MARVEL IN WAITING?

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ABSTRACT

Hormesis is a phenomenon in which adaptive responses to low doses of otherwise harmful factors (also called mild stressors) make cells and organisms more robust. In their review, Calabrese et al. provide evidence for resveratrol acting hormetically in different types of human cell lines. The effects of resveratrol represent a "two-edged sword" in that it has contrasting effects at low and high doses in healthy and cancerogenous cells. What demarkates a low and a high dose needs to be clarified. Concentrations tested in cell cultures, moreover, may not be relevant to whole organisms. And data from animal models need not apply to humans. Co-morbidities should also be considered. More research is needed to understand the action of resveratrol on all cell types and conditions, and the optimum therapeutic concentration that applies to each of these. Future research needs to determine the dynamics of the effects of resveratrol in different subcellular compartments and the interactions of these. In addition, the interactions between resveratrol, environmental factors, other compounds and medications, diseases and the genetic background of the individual will need to be appreciated in order to gain a complete understanding of the hormetic response of resveratrol.

Keywords: hormesis, resveratrol, doses, co-morbidities, interaction.

COMMENTARY

Hormesis refers to the phenomenon by which benefits are seen for low doses of external stressors that at higher concentrations are noxious.[1,2] Resveratrol, a natural polyphenolic flavonoid found in grapes, red wine, berries, knotweed, peanuts and diverse other plants, mediates the beneficial effects of mild environmental stressors on lifespan and health. Its potent antioxidant effects might explain some of its diverse health benefits.[3-5] One well-established effect of resveratrol is its potential to inhibit the initiation and growth of tumours in different cancer models in mice and rats.[6,7] But an immense number of other health benefits have also been reported.[3-5]

In the present issue of Human & Experimental Toxicology, Calabrese et al.[8] have reviewed the evidence for resveratrol acting hormetically and find in favour of this possibility. The effects of resveratrol therefore represent a "two-edged sword" in that it has contrasting effects at low and high doses. At low doses, it appears to stimulate the proliferation of healthy and cancer cells, and has therapeutic effects on healthy cells. These effects include enhancement of metabolism and cardiovascular function, and improvement of osteoporosis. High doses, were, however, found to be able to inhibit proliferation of cells, healthy or not, so highlighting the potential of resveratrol as a new treatment for cancer. At these same doses, nevertheless, resveratrol had adverse clinical effects on other conditions. Its adverse effects included increased toxicity, suppression of immune response, and delayed healing. The extensive review by Calabrese et al.[8] gives numerous examples of such a biphasic response of resveratrol, a property not evident when assessing only individual studies. As highlighted by the authors, most of the mechanisms underlying the effects of resveratrol remain to be elucidated completely. The idea that res-

veratrol is a direct activator of sirtuins arose from a technical problem with the assay, and no longer holds.[9] Sirtuin activation is in fact probably indirect. Interestingly the most studied sirtuin, SIRT1, also has a dual role in cell death and survival.[10] More research is, however, needed to understand the action of resveratrol on all cell types and other conditions, and the optimum therapeutic concentration that applies to each of these.

Although there are well-known cardiovascular benefits of resveratrol, we consider that the authors' contention that the resveratrol present in red wine could be responsible for the "French Paradox" might be a little too simplistic. An optimistic estimate of the amount of resveratrol consumed in one glass (185 ml) of red wine (5 mg/l of resveratrol) is 13.5 μg/kg in a 70 kg person.[6] This is much lower than the low doses used in the cell culture experiments referred to in the review. Thus low and high doses should be better defined, because the low doses used in the cell experiments reported in their review[8] might be high doses if extrapolated to whole organisms. Resveratrol is available in natural foods at a low concentration,[3-5] and increased intake of fruits and vegetables may offer a small but protective effect against certain cancers.11 Its effects on vascular health and thus cardiovascular disease could be mediated at still lower concentrations, such as in red wine,[12] and might support the "French paradox". More evidence is, however, needed before this claim can be accepted.

The fact that low doses can stimulate cancer proliferation, however, should raise concerns. And how low? … especially given the fact that dietary intake is highly unlikely to provide enough to be dangerous – if anything, the reverse. The benefits on different diseases may involve different concentrations. Concentrations tested in cell cultures may not be relevant to whole organisms. And in whole organisms different doses can yield different effects. For example, in mice, low doses of resveratrol increase body weight, intermediate doses have no effect, whereas high doses decrease body weight.[13] Of course, data from animal models may or may not apply to humans. One disease might benefit, but the dose used could fail to affect another condition, or even make it worse. Thus co-morbidities should be considered.

There are considerable potential risks of resveratrol supplements available over the counter in pharmacies or on the internet. Origin, purity, and age of the product are often not known. On the top of this, resveratrol has low water solubility and is sensitive to light and heat, so customers may be purchasing an inactive product or a product that will not be absorbed. In this way, novel formulations of resveratrol, with improved oral bioavailability and pharmacokinetic properties, such as SRT501,[14,15] and new chemicals which also activate, directly or indirectly, sirtuins, such as SRT1720,[15] should be studied, and the risk of cancer should be re-evaluated. The potential of resveratrol, and perhaps the new compounds, to increase the risk of cancer in naïve consumers, however, should be of concern.

Considering the low solubility of resveratrol in water, it would be interesting to know how it was diluted in the experiments cited in the review, and if proper controls containing the same vehicle were used. Our own experience, based on the recommendations of the supplier, is to dilute resveratrol in absolute ethanol or dimethylsulfoxide (DMSO). But these chemicals could have adverse effects on cells during culture, and this would likely include cancer cells. DMSO, for example, increases proliferation of ovarian carcinoma cell lines.[16]

Future research needs to determine the dynamics of the effects of resveratrol in different subcellular compartments and the interactions of these. In addition, the interactions between resveratrol, environmental factors, other compounds and medications, diseases and the genetic background of the individual will need to be appreciated in order to gain a complete understanding of the hormetic response of resveratrol. In a practical sense, it is hoped that gaining such an understanding will assist in improvements in health at the cellular and organismal level, so reducing cancer rates, and preventing or treating metabolic, neurological, cardiovascular and other diseases. It would be wonderful if resveratrol and compounds with similar beneficial effects become novel therapeutic agents and thereby enhance lifespan and/or healthspan. As a general principle, however, the potential adverse consequences of introduction of new treatments based on benefits that any compound may have at

low-doses, but that is noxious at higher doses, means we must end with a note of caution pending the outcome of much more research in this fascinating area.

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COMMENTARY ON RESVERATROL AND HORMESIS

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ABSTRACT

The review by Calabrese et al. describes the hormetic dose responses induced by phytoalexin resveratrol in a wide range of biological models. We agree and support the authors' strategy to present an impressive number of experiments furnished with an exhaustive bibliography to emphasize that "many effects induced by resveratrol are dependent on dose and that opposite effects occur at low and high doses, being indicative of a hormetic dose response." We also highly appreciate the holistic view of the hormetic behavior of resveratrol provided by the authors spanning from tumor and non-tumor cell lines to human and parasitic diseases. In our comments, we touched minor points whose discussion would have strengthened the work of Calabrese, such as contradictions on the role of resveratrol in the "French Paradox", its effect on aromatase activity, glutamate cysteine ligase expression and glutathione levels. Overall, we encourage colleagues working in this field to read the present review and consider its relevant biological implications. The vision of Calabrese et al. is far too important to be ignored.

COMMENTS

In the present article, Calabrese et al. address the question of the existence of a hormetic dose response induced by resveratrol, a naturally occurring compound, in a wide range of biological models and its significance in biomedical and therapeutic researches. Reaching the end of the article the readers may lean asking why the Authors had to resort to so many different examples, spanning from tumor cell lines of different origin to several parasitic diseases, in order to sustain their unique message which can be summarized as follows: "many effects induced by resveratrol are dependent on dose and that opposite effects occur at low and high doses, being indicative of a hormetic dose response." In fact, from a preliminary analysis, the article appears redundant in listing a large number of examples of cell lines all showing a similar hormetic behavior with a biphasic dose response phenomenon. For those working on resveratrol, this may not represent a novelty. In our daily laboratory experience on cancer cell lines, we often observed a minor but constant and significant increase in cell number at low concentrations of resveratrol (1-5 μM), which was reverted by higher concentrations applied to the same cell lines (> 10 μM) resulting in cell death and generally associated to apoptosis or necrosis (depending on the concentration applied and time of treatment). However, we must admit, and, in this respect, we totally agree with Calabrese et al., that many research groups tend to ignore, hide or underestimate the existence of a hormetic response associated to resveratrol. In our view, this could be due to a limited knowledge (or total ignorance) of the phenomenon which can induce colleagues to fall into the temptation to shift the dose-response curve in their experiments to concentrations which "bypass" the hormetic range, moving towards higher values usually associated to cytotoxicity. It is easier for many to consider that weird and apparently inexplicable "proliferative peak" appearing at low concentrations of resveratrol as an artifact which is better to avoid than to accept the explanation that the molecule can improve proliferation of cancer cells, facilitating, perhaps, their oncogenic capacity! This embarrassment can also be registered in public scientific contexts when the term "hormesis" is pronounced. Based on these considerations, we now understand and justify the strategy of Calabrese et al. in this article to present an abundant number of data showing almost the same biphasic pattern of biological activities triggered by resveratrol. Also from an iconographic point of view, the fact that graphs reproduced in figures 1-30 are

largely super-imposable helps the readers to conclude that resveratrol can be considered a classical molecule possessing hormetic behavior.

In addition to the considerations reported above, we are reporting below minor suggestions and criticisms whose discussion could have been further strengthened the work of Calabrese et al..

The impressive number of studies reported in this review will significantly contribute to our knowledge on the hormetic role of resveratrol in the fields of experimental biology and human health. Resveratrol has become synonymous of the beneficial effects of red wine in the Western diet, because of its relatively abundance in this beverage (1). The review of Calabrese raises additional and interesting questions. Firstly, the Authors cite the work of Dudley et al. (2) which generates, probably, a misunderstanding. In fact, they reported that a dose of 2.5 mg/kg of resveratrol, administered to rats, corresponds approximately to a glass of red wine (170 ml). However, to reach the same dosage in humans, the amount of wine to be ingested should be of about 12 liters! The assumption reported by Calabrese et al. (pag. 34) is far more optimistic. Fortunately, this calculation is overestimated since it implies a direct equivalence between the quantity of compound administered to humans and rats. The Food and Drug Administration (FDA) suggests that the dose administered to animals, extrapolated to the human body should take into account of body surface area (BSA), often represented as mg/m^2 (3). Making this correction, a subject who would assume a dose of resveratrol corresponding to 2.5 mg/kg should consume approximately 1 liter of red wine instead of 12 liters. In any case, such a large daily consumption of red wine is highly discouraged by the presence of alcohol (4), making impracticable the assumption of resveratrol through red wine. A valid alternative could is represented by a direct consumption of resveratrol. The pure molecule can be administered to humans at relatively high concentrations without apparent side effects (5). A recent study found that a single dose up to 5 gr in humans resulted in no particular side effects (6). Similarly, in rats, oral administration of resveratrol at doses up to 300 mg/kg of body weight for several weeks resulted in absence of toxicity (7). Finally, in a proprietary formulation of Sirtris Pharmaceuticals (SRT-501), the circulating concentration of resveratrol can be increased from 5 to 8-fold. These levels do approach the concentration necessary to exert the effects shown in animal models and in vitro experiments (8). Secondly, according to Lee et al. (9) resveratrol increases HL-60 survival (Fig. 16 in Calabrese et al.) in a dose-dependent concentration up to 20-25 μM. The same range of concentrations, however, resulted cytotoxic in other works performed on the same cell line (10,11), without any evidence of hormetic response. Interestingly, resveratrol induces HL-60 cellular proliferation at sub-micromolar concentration in part due to its pro-oxidant capacity (12). Thirdly, in the context of possible biological targets of resveratrol, we suggest its effect on aromatase activity. It is well known that aromatase, also known as cytochrome P450 (CYP19), is an enzyme responsible for a key step in the biosynthesis of estrogens. Estrogens are involved in breast cancer risk because of its role in stimulating cell division in mammary gland (13). Wang et al. demonstrated that a hormetic concentration of resveratrol inhibits breast cancer cells proliferation when stimulated in the presence of testosterone (10 nM). This effect can be attributed to the specific inhibition of aromatase. Kinetic analysis indicated that both competitive and non-competitive inhibition might be involved (14). Fourthly, in the chapter regarding the hormetic effect of resveratrol on non-tumor cell lines, we would like to underline its important role in increasing glutamate cysteine ligase expression and glutathione in human bronchial epithelial cells (15). Glutathione is a crucial component of the long-term adaptive system against oxidative stress, which can be implicated in chronic diseases, such as neurological and cardiovascular pathologies, and cancer (16). The result of Zhang et al. (15) is of considerable importance since the low concentrations of resveratrol employed (0.5-2 μM) to induce its beneficial, antioxidant effects. These values are not far from those detectable into the blood through reasonable dietary consumption.

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COMMENTARY ON RESVERATROL AND HORMESIS

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It is evident from this very thorough review by E.J. Calabrese et al. that the dose response of resveratrol is predominantly hormetic when tested in various assay systems reflecting aspects of relevance for human diseases/disease prevention. However, it is also evident that the effect in relation to the individual disease is not always favorable. Also the large divergence in effective dosage when tested in model systems of relevance for diseases is noticeable.

The overall purpose for investigating effects of various biologically active compounds like resveratol is to reveal possible recommendations in relation to 1) prevention of disease development by means of consumption or 2) as therapeutic biomedical in relation to specific diseases.

For possible medical use the concentration of resveratrol has to be strictly controlled, in order to ensure a specific effect in relation to a given disease. Hence to administer a very specific dosage within a narrow effective concentration range the compound has to be administered intravenously.

However, the greatest potential advantage from the huge range of possible beneficial effects of resveratrol outlined in the review would be if this compound could be recommended to the broad population as a protective agent. The consumed concentration would still have to be controlled/restricted especially regarding toxic effects of high concentrations. One of the very effective ways of ensuring a natural restriction on the consumed concentration is by only consuming the compound as part of its natural environment e.g. as an integral part of grapes or wine. Nonetheless, the issue of differences in effective doses as well as negative effects is not eliminated by consuming the bioactive compound as it occurs naturally. An issue worth investigating is if resveratrol consumed as grapes or wine displays a hormetic response in respect to the various disease models described in the review. This may not be the case, since the consumption of the compound together with the inherent food matrix is very likely co-administered with other bioactive compounds as is the case in e.g. tea where a large amount of different catechins are present (1) and indeed the co-occurrence of catechin, quercetin and resveratol in wine (2). Various bioactive components within a food item may act through very different mechanisms as e.g. β-carotene and falcarinol in carrots (3) while others like the two polyacetylens; falcarinol and falcarindiol act in similar ways but just with different potency (4), and may thus act additionally or even synergistically when consumed together in the food-item. On the other hand the food matrix may contain compounds with antagonistic effect or compounds preventing or potentiating the uptake as discussed by Manach et al. (5) in relation to polyphenols.

In this light it may be worth paying attention to studies of more complex systems. Studies of individual compounds in various cell assays are invaluable in the investigation of toxic and beneficial effects of biologically active compounds which may have a potential therapeutic use. However, it is equally important to investigate how co-occurring bioactive compounds from the matrix of its natural environment affects the hormetic response of e.g. resveratrol in relation to disease models. Studies on single compounds are also valuable for generating hypothesis and understanding reaction pathways, but combined effects of two or more compounds could pave the way for understanding the biology behind epidemiological observations like the “French paradox”

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DOSE RESPONSE BIOLOGY: THE CASE OF RESVERATROL

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ABSTRACT

Resveratrol often displays hormesis-like biphasic dose responses. This occurs in a broad range of biological models and for numerous endpoints of biomedical interest and public health concern. Recognition of the widespread occurrence of the hormetic nature of many of the responses of resveratrol is important on multiple levels. It can help optimize study design protocols by investigators, create a dose response framework for better addressing dose-related biological complexities and assist in the development of public health and medical guidance with respect to considerations for what is an optimal dose not just for an agent such as resveratrol, but also for the plethora of agents that also act via hormetic mechanisms.

Key Words: resveratrol, hormesis, hormetic, dose response, biphasic, U-shaped, adaptive response

Resveratrol is a molecule that has reached the equivalent of "Main St." It has been the object of numerous articles in the popular press, the subject of widespread national television programs and a topic commonly discussed on syndicated radio health programs. Resveratrol has now become quite widely known and placed within a general public health context by a large number of the general population, even though most of its purported health benefits have not been established (Espin et al., 2007). The successful marketing of resveratrol has been due, at least in part, to the fact that a substantial proportion of adults would like to keep healthy while doing as little as possible and by doing what they enjoy, in this case, consuming moderate amounts of red wine on a regular basis. The media has widely reported that regular consumption of low to moderate amounts of red wine can lower the risks of various cardiovascular diseases and that the most likely so-called "magic" ingredient in the red wine was something called resveratrol. As discussed by Calabrese et al. (2010) numerous credible researchers have reported on the topic in leading scientific and medical journals. This was not a topic like cold fusion that was going to be disproven once closer scrutiny was given to the issue. In fact, the red wine story and resveratrol had numerous and detailed scientific evaluations, and some of the original health claims were not only verified but expanded beyond the cardiovascular areas to the CNS, the gastro-intestinal tract, the immune system, and to aging itself. So popular was this topic that resveratrol tablets became a marketable commodity and in nationwide food and health related stores (Seward, 2008).

It is indeed rare for the public to embrace, at least at the concept level, what is principally a scientific topic and incorporate it into their daily lives. It was this fact that lead to the creation of the current BELLE Newsletter (Calabrese et al., 2010; Das, 21010; De La Lastra, 2010; Hayes, 2010; Lindsay, 2010; Marques and Morris, 2010; Tedesco et al, 2010; Young and Bhattacharya, 2010). The BELLE Newsletter is devoted to enhancing an understanding of the nature of the dose response, especially in the low dose zone, and its underlying mechanisms and implications. While the BELLE Newsletter has principally focused on toxicological and pharmacological concepts rather than an individual chemical it was thought that resveratrol would make an appropriate exception. While it was known that resveratrol commonly displayed hormetic-like biphasic dose responses (Mukherjee et al., 2010), no prior broad and detailed assessment of its hormetic potential had been published.

Once it became clear that hormetic dose responses were a dominant aspect of the biological effects of resveratrol, it was thought that this might present the opportunity of gaining more widespread interest in the hormesis concept, using resveratrol as the lure. Thus, it was hoped to achieve two goals, that is, a detailed assessment of the dose response features of resveratrol, their mechanistic underpinnings, and public health implications, as well as providing a means to introduce the concept of hormesis to a broader range of biomedical scientists.

What emerged from this detailing of resveratrol dose responses was the consistent observation that many dose responses were clearly biphasic, having quantitative features of hormetic dose responses. This was the case with respect to the magnitude of the stimulatory response, its width and its relationship to the pharmacological or toxicological threshold. The resveratrol induced hormetic effects were observed in multiple biological systems, affecting numerous normal cell types including endothelial cells, immune cells, male and female reproductive cells, various types of bone cells, heart cells, and lung cells amongst others. In the assessment of these findings the authors typically speculated on the potential biomedical implications of the low dose stimulatory effects of resveratrol. In most cases the findings were clear and reproducible but the capacity to extrapolate from either an animal model or cell line to the human populations can be highly uncertain both qualitatively as well as quantitatively. Even in the case of the detailed in vivo animal studies of Mukherjee et al. (2010) which demonstrated that resveratrol reduced the magnitude and severity of indomethecin-induced gastric ulcers, there is still uncertainty over the precise application to human populations, especially when widespread inter-individual variability of human responses has to be taken into account at multiple levels of assessment from the regulatory agency to the physician to the affected individual. Thus, the comments of Lindsay (2010) are quite relevant as he noted that the capacity to apply the predominantly in vitro experimental findings with respect to reservatrol induced hormetic effects in humans, awaits further and more detailed testing and evaluation. However, the transition from the striking laboratory findings to the use within society is rarely straight forward, often presenting significant challenges. For example, epidemiological validation of laboratory studies is often susceptible to false negative findings for pharmaceutical agents. This is due to the modest nature of the drug-induced enhancements of biological performance which are usually only in the percentage range over controls and the occurrence of widespread variability within the highly heterogeneous human population. This has been a major issue in failure of candidate drugs that have looked so promising in preclinical clinical trials yet to fail so frequently when evaluated via the clinical trial. Thus, while the comment of Lindsay (2010) is appropriate and valid, there is no easy and pragmatic solution to this scientific validation conundrum.

There is also further uncertainty in the extrapolation of resveratrol from animal models to humans with respect to dose normalization. For example, Tedesco et al., (2010) has indicated that some authors, such as Mukherjee et al (2010), have assumed dose normalization via body weight for interspecies extrapolation, whereas the FDA has recommended a surface area normalization approach. Such dose normalization procedures for the application of in vivo animal study findings have long been a source of considerable debate within regulatory agencies and it is likely to present a challenge in the application of in vivo animal model data to humans for agents such as resveratrol. Despite its potentially contentious nature, this concern seems minor when compared to the extrapolation of in vitro data concerning resveratrol to in vivo studies, including those of a clinical and epidemiological nature. Yet both challenges are important to address with respect to many of the intriguing chemoprotective findings reported for resveratrol.

Of particular interest are the numerous observations that resveratrol can enhance the proliferation of multiple types of human tumor cell lines at low concentrations in a means fully consistent with the quantitative features of the hormetic dose response (Calabrese et al., 2010). Reservatrol was shown to induce such proliferation of numerous types of tumor cells as well as tumor cells in different stages of tumorigenesis including non-metastasis and metastasis stages as in the case of human breast tumor cell models. Furthermore, even though

resveratrol acts as a weak estrogen agonist, it is effective inducing cell proliferation via hormetic processes in human tumor cell lines independently of whether the tumor cells are estrogen dependent or not. Whether such findings are directly relevant to humans is not yet known. Furthermore, the effects of resveratrol on tumor cells must also be seen within the context of its capacity to enhance immune function, possibly affecting susceptibility to various types of tumors. For example, it is reasonable to speculate that resveratrol may enhance susceptibility to certain types of diseases at one or more stages of development (e.g., cell proliferation of tumor cells) while decreasing risks to the same disease at other stages (e.g., tumor suppressive effects due to immune stimulation). How such potentially complex biological interactions may affect the incidence of disease can be assessed in future animal experimental studies as well as epidemiological investigations. While this situation presents important challenges to developing an integrative assessment of resveratrol, this type of challenge is similar to that faced by numerous other agents consumed by humans that also display hormetic dose responses.

These observations clearly indicate the complexity of the resveratrol biomedical literature and the challenges and opportunities that the hormetic dose response model presents to biomedical researchers, clinicians, risk assessment specialists and regulatory agencies. Society is still generally guided by the assumption that the nature of the dose response follows a threshold model with respect to non-cancerous outcomes or a linear model for carcinogens (Calabrese, 2005; Calabrese 2009a,b). In these instances the guidance that can be offered is rather straight forward. In the case of threshold acting agents the goal has been to keep the exposure below the toxic threshold with an adequate margin of safety. In the case of carcinogens, in which risk has been assumed to be proportionate to dose, the lower the exposure the better. Voluminous findings over the past decade have revealed that these standard dose response models fail to make accurate predictions in the low dose zone, the zone where people tend to live. However, the hormetic dose response has been shown to commonly occur (Calabrese and Baldwin, 2001, 2003a,b; Calabrese and Blain, 2005; Calabrese et al., 2006, 2008, 2010) and to make far more accurate predictions of biological responses in the low zone than either the threshold or linear models (Calabrese, 2010). The key challenge for the biomedical and regulatory communities is take advantage of these developments in the area of dose response biology in terms of how to improve study designs so as not to miss important low dose effects of a beneficial or undesirable nature, and how to use the information provided by the hormetic framework to improve the preclinical and clinical testing of chemotherapeutic and chemopreventive agents and to enhance the likelihood of "getting the dose right".

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