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**Resveratrol requires red wine polyphenols for optimum antioxidant activity**  
 --Manuscript Draft--

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<b>Corresponding Author:</b>	Sara Straniero, PH.D. Karolinska Institutet Stockholm, SWEDEN
<b>Corresponding Author Secondary Information:</b>	
<b>Corresponding Author's Institution:</b>	Karolinska Institutet
<b>Corresponding Author's Secondary Institution:</b>	
<b>First Author:</b>	Sara Straniero, PH.D.
<b>First Author Secondary Information:</b>	
<b>Order of Authors:</b>	Sara Straniero, PH.D. Gabriella Cavallini, PhD Alessio Donati, PhD Ettore Bergamini, MD, PhD
<b>Order of Authors Secondary Information:</b>	
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<b>Abstract:</b>	<p>Objective: There is substantial evidence that a diet rich in fruit and vegetables may reduce the risk of aging and stress oxidative associated diseases. It has been suggested that benefits associated with fruit and red wine consumption could be due to pooled antioxidant microcomponents in diet. The aim of this study was to investigate the antioxidant activities of pure resveratrol (a well known phytoalexin, RSV) and red wine polyphenols (RWP), using UV-B radiated isolated rat hepatocytes as a model of oxidative stress.</p> <p>Methods: Rat hepatocytes were isolated by the collagenase method. The cells were loaded with resveratrol and/or polyphenols at different concentrations. The production of thiobarbituric acid reactive substances (TBARS) released by UV-B radiated cells and the levels of lipid-soluble antioxidants (Dolichol, Vitamin E, Coenzyme Q9 and Q10) were measured.</p> <p>Results: Resveratrol had pro-oxidant or antioxidant effects depending on (lower or higher) dosage. RWP protection from photolipoperoxidation was dose-dependent and increased with dosage. Combination of the two compounds exhibited synergistic antioxidant effect, and made resveratrol effective both at lower and higher dosages.</p> <p>Conclusions: These results suggest that resveratrol requires red wine polyphenols for optimum antioxidant activity.</p>
<b>Response to Reviewers:</b>	See attachment

We warmly thank reviewers for helpful comments and criticisms.

**Note for reviewing purposes - *Our comments/answers in bold italic***

**Revisions and corrections in the text all in red bold**

**Reviewer #1:** I've read the manuscript entitled, "Resveratrol requires red wine polyphenols for optimum antioxidant activity" written by Cavallini G. et al. and sent for publication in the Journal of Nutrition Health and Aging. In this work, the authors studied the effects of resveratrol and polyphenols on the oxidative stress in rat hepatocytes. Globally, the manuscript is interesting, clear and well written and could be published in JNHA.

***We thank the reviewer.***

In the next paragraphs I will include some suggestions that could help authors to improve the quality of this manuscript.

1. Figures 3 and 4 are not clear, mainly in the y-axis. Why "percent changes " are used in Fig.3 and other units in Fig. 4?

***In Fig. 4, results are given as concentration of lipid-soluble antioxidants, because figure shows many treatment conditions and in our opinion, this data presentation can be more demonstrative and understandable for readers, as stated by reviewer's #3 comment.***

2. It would be interesting to evaluate the effect of resveratrol and polyphenols on human hepatocytes (from primary cultures) or using human hepatocytes cell lines.

3. What about the effect of resveratrol and polyphenols in vivo using animals of different age (young vs old).

***We thank the reviewer for these helpful suggestions that can be a starting point for further studies both in vitro and in vivo.***

4. Do you expect any clinical application in humans?

***Natural products are widely used as complementary and alternative medications for the prevention and treatment of various human diseases. Our results highlight that the use of antioxidants contained in food matrices and their potential synergistic combinations, should be carefully evaluated before being recommended to individuals, especially to the sick or elderly subjects. Preliminary data showed that the oral administration of 250 mg of a mixture resveratrol:polyphenol (1:20) increases the resistance of red blood cell membranes to photooxidative stress significantly both in dogs and humans.***

**Reviewer #2:** The authors report that when resveratrol and red wine polyphenols are combined, they exert a synergistic antioxidant effect on UV-B-irradiated hepatocytes. The results are interesting but the manuscript needs another round of proofreading to get rid of some minor issues. A few examples:

Introduction, Page 3

Line 7: Change to ...oxidative stress (OS) that results from an imbalance between formation and neutralization of antioxidants. It is initiated by free radicals like...

***The change was made.***

Line 12 Change to ...and DNA in cells causing protein and DNA damage along..

***The change was made.***

Line 49 Correct 'reservatrol'

***The mistake was amended.***

Line 56 Change to ...a powerful antioxidant effect...

***The change was made.***

Intro, Page 4

Line 37: Perhaps it should be changed to: ...UV light and infection by fungi and pathogenic microorganisms, providing... Authors may leave it without change

***The change was made.***

Materials and Methods, Page 5

Animals: Start by saying "All of the experiments were conducted following the Official Italian Regulation n. 116/92 for the care and use of laboratory animals and were approved by the Independent Ethics Committee of the University of Pisa (Approval number: 2A/42155)"

***The beginning of the paragraph has been changed.***

Lines 17-23: The liver samples were obtained from 3-month-old Sprague-Dawley rats raised at the Pisa University Interdepartmental research Centre on Biology and Pathology of Aging vivarium. The animals were kept... It should then be stated how the study groups were formed.

***The sentence was reformulated as suggested. The study groups were stated.***

Line 56: Remove 'substantially'

***'Substantially' was removed.***

M&M, Page 6

Line 1 ...designed and built by the...

***The mistake was amended.***

Line 7 (and throughout the manuscript, if it is to be published in an American journal) Use decimal point instead of comma

***The mistake was amended.***

Line 12 What was the 'given time'? Then "After centrifugation of the cell suspension the supernatant was used to assay the release of..."

***The sentence was reformulated as suggested.***

**Reviewer #3:** The paper significantly contributes to the understanding of effects and role of antioxidants present in red wine. There are many misunderstandings, overvaluations or even as underestimations regarding this issue. Of course, the in vitro experiments may provide highly valuable conclusions although their transposition to living systems, mainly to the human organism requires well-founded consideration. The paper contains important findings on pro- and antioxidant properties of resveratrol, on the synergism of different antioxidant items of red wine.

***We thank the reviewer.***

Some remarks:

Page 3, line 4: 'today's diseases are due to the oxidant stress...' The so called civilisation diseases have numerous causes; the oxidative stress more or less contributes to their development.

***The sentence was reformulated as suggested.***

Page 3, lines 28-29: '...natural antioxidant defense mechanisms may not be fully effective...' Maybe, because of their (environmental etc.) overload..

***The sentence was reformulated as suggested.***

Page 5, UV-B radiation: It is advised to include the duration of the UV radiation.

***The duration of the UV radiation is included (p. 6, line 6).***

Page 6, lines 22-34 and page 9, line 41: The TBARS method does not seem to be the best choice; however, in this case it is acceptable.

***We thank the reviewer, we agree with his criticism.***

Page 10, line 1: The human biological consequences of a high-fat diet depend partly on amount of fat and partly on fatty acid pattern. The latter issue should to be emphasized. More favourable fatty acid content allows higher dietary total fat level without physiological disadvantages.

***Criticism has been accepted. Sentence now reads: "the apparent compatibility of a high fat diet rich in cholesterol and saturated (and low in unsaturated) fatty acids with a low incidence of coronary atherosclerosis" (p. 10, line 1,2).***

The figures are well constructed, understandable and demonstrative.

***We thank the reviewer.***

**Reviewer #5:** Major comments

1, This paper focused on antioxidative effects of resveratrol, however, major beneficial effects of resveratrol do not depend on the antioxidative properties.

***We agree with reviewer's comment, but the aim of this study was to investigate one of the many properties resveratrol: the antioxidant activity.***

2, Phenolic compounds usually exist as conjugated forms in the blood. Authors must consider resveratrol do not reach hepatocytes as aglycone form.

***We accepted this very useful comment. Now the paper reads: "... in this research, the antioxidant action of resveratrol was studied in vitro in the absence or presence of the polyphenols normally present in red wine, using UV-B radiated isolated rat hepatocytes as a model of oxidative stress. Perhaps it may be worthwhile to mention here that phenolic compounds usually exist as conjugated forms in the blood and that in vivo resveratrol may not reach hepatocytes as aglycone form". (p. 4, lines 18-23).***

3, Authors should identify effective compounds in RWP on suppression of TBARS production, and also examine molecular mechanisms for synergistic effects between resveratrol and the identified compound in RWP.

***We thank the reviewer for these helpful comments that can be a starting point for further studies.***

Minor comments

1, Authors do not explain the increase in TBARS production by low level of resveratorol in Fig. 1.

***An explanation of this result was given in Discussion: "An interesting hypothesis is that loading the membranes of mammalian cells with a small number of resveratrol molecules (and no polyphenols) may produced a structural perturbation on the membrane bilayer and change the metabolism of free radicals in lipophilic compartment of cytomembranes (27). More recently, data demonstrate that resveratrol produce a moderate but significant structural perturbation of the lipid bilayer and induce echinocytosis in human erythrocytes: the extent of these changes is dependent on resveratrol concentration (39). Cell membrane is a diffusion barrier which protects the interior of the cell. Therefore, its structure and functions are susceptible to alterations as a consequence of interactions with chemical species". (p. 10, lines 8-15).***

2, Authors should show TBARS levels in non-UV irradiated cells.

***Results are given as changes with respect to not-radiated cells, however TBARS levels in non-irradiated cells were:  $17.4 \pm 2.27$  nmol MDA equiv/g/min (n =18).***

3, Show hepatocyte viability in the conditions observed.

***Hepatocyte viability was given in Materials e Methods (p.6, lines 7,8).***

4. Fig. 4: The results may suggest no relevant effects on cell oxidative conditions by RWP+RSV20 because the reduction of antioxidative compounds do not recover clearly.

***We agree, this is an interesting finding, which is under investigation. The working hypothesis is that phenomenon might be the consequence of a perturbation in the proposed highly matched free-radical-transfer chain endowed in membranes (see ref. n. 27 and 40).***

5, P. 5, Line 51: Show vehicle (solvent) to add resveratrol.

***Vehicle was given (p.6, line 2).***

**Reviewer #6:** Please note that this recent paper (please see below) is not cited in your paper and will increase your paper update if you feel that it is appropriate. Can you send us the final version of your paper and it will be printed soon.

***The suggested reference was cited in the manuscript (ref. n. 1).***

## Resveratrol requires red wine polyphenols for optimum antioxidant activity

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**Authors:** Gabriella Cavallini<sup>a</sup>, Sara Straniero<sup>a\*</sup>, Alessio Donati, Ettore Bergamini

**Affiliations:** Interdepartmental Research Centre on Biology and Pathology of Aging, University of Pisa; Pisa, Italy

<sup>a</sup>Gabriella Cavallini and Sara Straniero contributed equally to this manuscript

\* address correspondence to this author at:

Department of Medicine, Center for Diabetes, Metabolism and Endocrinology, Karolinska Institute,

Karolinska University Hospital, Huddinge, 141 86 Stockholm, Sweden

Tel +46 8 52481097

e-mail: sara.straniero@ki.se



## Abstract

1  
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5 *Objective:* There is substantial evidence that a diet rich in fruit and vegetables may reduce the risk  
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7 of aging and stress oxidative associated diseases. It has been suggested that benefits associated with  
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9 fruit and red wine consumption could be due to pooled antioxidant microcomponents in diet. The  
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11 aim of this study was to investigate the antioxidant activities of pure resveratrol (a well known  
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13 phytoalexin, RSV) and red wine polyphenols (RWP), using UV-B radiated isolated rat hepatocytes  
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15 as a model of oxidative stress.  
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19 *Methods:* Rat hepatocytes were isolated by the collagenase method. The cells were loaded with  
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21 resveratrol and/or polyphenols at different concentrations. The production of thiobarbituric acid  
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23 reactive substances (TBARS) released by UV-B radiated cells and the levels of lipid-soluble  
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25 antioxidants (Dolichol, Vitamin E, Coenzyme Q9 and Q10) were measured.  
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29 *Results:* Resveratrol had pro-oxidant or antioxidant effects depending on (lower or higher) dosage.  
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31 RWP protection from photolipoperoxidation was dose-dependent and increased with dosage.  
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33 Combination of the two compounds exhibited synergistic antioxidant effect, and made resveratrol  
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35 effective both at lower and higher dosages.  
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39 *Conclusions:* These results suggest that resveratrol requires red wine polyphenols for optimum  
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41 antioxidant activity.  
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48 **Keywords:** Resveratrol, red wine polyphenols, oxidative stress, lipid-soluble antioxidants.  
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## Introduction

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4 The so called civilisation diseases have numerous causes (1); the oxidative stress (OS) more or less  
5 contributes to their development. OS results from an imbalance between formation and  
6  
7 neutralization of antioxidants. It is initiated by free radicals like hydroxyl, peroxy and superoxide  
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9 radicals, which become stable through electron pairing with biological macromolecules such as  
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11 proteins, lipids and DNA in cells causing protein and DNA damage along with lipid peroxidation.  
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16 The damage caused by OS has been implicated as a potential contributor to the pathogenesis of  
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18 cancer, diabetes, atherosclerosis, cardiovascular diseases, inflammatory diseases and aging (2,3).  
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21 The damage can become more widespread due to weakened cellular antioxidant defense systems.  
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24 All biological systems have antioxidant defense mechanisms that protects against oxidative  
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26 damages and repairs enzymes to remove damaged molecules. However, these natural antioxidant  
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28 defense mechanisms may not be fully effective, maybe because of their overload, and dietary intake  
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30 of antioxidant may be required. Fruits and vegetables contain different antioxidant compounds, such  
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32 as Vitamin C, Vitamin E, carotenoids or polyphenol molecules which interact with various  
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34 biological systems (4-6).  
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38 Red wine contains a rich source of a large number of antioxidants, namely the phenolic acids and  
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40 polyphenols, which provide it with its protective redox potential (7,8). Studies on squamous  
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42 carcinoma cells showed an inhibitory effect of red wine polyphenols on cell growth (9) and studies  
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44 on the antimutagenic activity of grape extracts suggested that the protective effect of many  
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46 compounds in the extract was synergistic (11). An important role in the antioxidant activity of red  
47  
48 wine, has been attributed to the presence of resveratrol, a triphenolic stilbene present in the black  
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50 skin of grapes and proanthocyanidins (11). Mounting evidence suggests that resveratrol could act as  
51  
52 a powerful anti-cancer (12), anti-inflammatory (13), anti-diabetes (14) and anti-oxidation agent  
53  
54 (15). Most strikingly, resveratrol can exert a powerful antioxidant effect in organisms: resveratrol  
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56 scavenges cellular reactive oxygen species (ROS) and corrects radical-induced responses such as  
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1 DNA damage (16), imbalance of mitochondria redox state (17) and interfering cellular signal  
2 transductions (18). Furthermore, resveratrol increases yeast cell survival (19), and subsequent  
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4 studies on worms, fruit flies, fish and mice have also linked resveratrol effects on longevity (20,21).  
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6 The beneficial effects of RSV on aging have been suggested to resemble and potentially mimic  
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8 caloric restriction (CR) in *Caenorhabditis elegans* (22), *Drosophila melanogaster* (20) and mice  
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10 (21).  
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14 However, both the anti-aging benefits and the counteraction of all aging-related diseases were  
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16 observed only at much higher doses of resveratrol (tens or hundreds mg per kg/body weight) than  
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18 those that may be involved in the so-called "French paradox": the strikingly low incidences of  
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20 coronary heart diseases in France, despite intake of a high-fat diet, have been attributed to the  
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22 consumption of red wine (23). Furthermore, unlike the case with functional foods, resveratrol were  
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24 said to have both favorable and harmful dose-dependent effects (24).  
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28 These observations, the incomplete knowledge of the mechanisms of action, the growing public  
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30 interest and the vast (not always well-controlled) consumption of resveratrol, prompted us to  
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32 further investigate its action as antioxidant compound. Recalling that resveratrol is a naturally  
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34 occurring phytoalexin ("defender of the plant") that is produced in response to injury, such as  
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36 mechanical trauma, UV light and infection by fungi and pathogenic microorganisms, providing  
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38 means for defense together with others constitutive or inducible compounds (25), in this research  
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40 the antioxidant action of resveratrol was studied in vitro in the absence or presence of the  
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42 polyphenols normally present in red wine, using UV-B radiated isolated rat hepatocytes as a model  
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44 of oxidative stress (26). Perhaps it may be worthwhile to mention here that phenolic compounds  
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46 usually exist as conjugated forms in the blood and that in vivo resveratrol may not reach  
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48 hepatocytes as aglycone form. In order to evaluate effects on lipid peroxidation, the production of  
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50 thiobarbituric acid reactive substances (TBARS) released by UV-B radiated cells was assayed.  
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52 Furthermore, the levels of natural fat-soluble antioxidants (Dolichol, Vitamin E, Coenzyme Q9 and  
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54 Q10), which protect membrane lipids from oxidative damage (27,28), were measured.  
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## Materials and Methods

### *Materials*

All reagents were of analytical and HPLC grade. Solvents were purchased from Panreac Química S.L.U. (Barcelona, Spain). Standard molecules and chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Milli-Q (Millipore-Lab, Bedford, MA) purified water was used for all analyses.

### *Animals*

All of the experiments were conducted following the Official Italian Regulation n. 116/92 for the care and use of laboratory animals and were approved by the Independent Ethics Committee of the University of Pisa (Approval number: 2A/42155).

The liver samples were obtained from 3-month-old Sprague-Dawley rats raised at the Pisa University Interdepartmental research Centre on Biology and Pathology of Aging vivarium. The animals were kept in a controlled environment (22 °C; 12/12 h light/dark cycle) and were maintained on a standard laboratory diet (Teklad, Harlan, Italy). All rats had free access to water.

Animals were randomly assigned to experimental groups: resveratrol ( $n = 6$ ), red wine polyphenols ( $n = 6$ ), resveratrol and red wine polyphenols ( $n = 6$ ).

Rats were anaesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg body weight) and the liver was removed.

### *Preparation of isolated liver cells*

Isolated hepatocytes were prepared by the method of collagenase perfusion (29). Cell viability was tested with the trypan-blue exclusion and was always higher than 90%. The obtained cells were divided in different aliquots and antioxidants loaded by the incubation with 20 volumes of washing buffer (29) containing resveratrol and/or polyphenols for 10 minutes, centrifuged, washed once with washing buffer and diluted with suspension buffer fortified with pyruvate (15 mM) (29). An aliquot without incubation with resveratrol and/or polyphenols was used as control.

### *Antioxidant solution*

Resveratrol and polyphenols was dissolved in pure ethanol and then diluted with washing buffer.

### *Ultraviolet-B (UV-B) radiation*

UV-B radiation of hepatocytes was performed as described by Parentini et al. (26). A cell monolayer was obtained by pouring 50 mL of a 6 mg/mL cell suspension in a Petri dish with a surface of 150 cm<sup>2</sup> (no cover top). The dish was placed under the radiation source for 40 minutes.

At the end of radiation, hepatocytes viability was 70% and incubation with resveratrol and/or polyphenols had no additional effect on cell viability.

The radiation source was a flat-bed chamber for cell irradiation, designed and built by the Laboratory of Biophysics Institute of the National Research Council (IFB-CNR), Pisa. UV-B sources were two UV-B-313 fluorescent tubes, mounted about 7.0 cm above the sample holder. UV-B irradiance was about 1.7 W/m<sup>2</sup>, slightly lower than the UV-B present in a natural environment. Light irradiance was measured by means of a United Detector Technology radiometer (UDT instruments, San Diego, CA, USA). After centrifugation of the cell suspension, the supernatant was used to assay the release of thiobarbituric acid reactive substances (TBARS) in the medium and cells were used to study the effects of radiation on Dolichol, Vitamin E, Coenzyme Q9 and Q10 levels.

### *Thiobarbituric acid reactive substances (TBARS) assay*

TBARS were assayed by taking 1 mL aliquot of the medium at the end of radiation. Assay was performed substantially according to Cavallini et al. (30). The quantification of TBARS production was analyzed by high performance liquid chromatography-VIS detection (532 nm) using a reverse-phase column and eluted as described by Grotto et al. (31). The calibration curve was prepared with malondialdehyde (MDA) and the results were reported as MDA equivalents.

### *Extraction of Dolichol, Vitamin E and Coenzyme Q*

Dolichol (a long-chain polyisoprenoid broadly distributed in all tissues and membranes), Vitamin E and Coenzyme Q (CoQ) were extracted simultaneously into hexane from a sodium dodecyl

1 sulphate-treated homogenate (32). Aliquots of the extract were taken for Dolichol, Vitamin E and  
2 CoQ assay, dried under nitrogen and redissolved in isopropanol, methanol and methanol/reagent  
3 alcohol solution, respectively.  
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### 6 ***High-pressure liquid chromatography (HPLC) assay***

7 Dolichol was assayed by an HPLC-UV detection (210 nm) using a reverse-phase column and  
8 eluted as described by Maltese et al. (33). Coenzyme Q9 and Q10 was assayed by an HPLC-UV  
9 detection (275 nm) using a reverse-phase column and eluted as described by Lang et al. (32).  
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11 Vitamin E was assayed by an HPLC-fluorometric detection (excitation at 295 nm, emission at 350  
12 nm) using a reverse-phase column and eluted as described by Ruperez et al. (34).  
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### 21 ***Statistical analyses***

22 The analysis of variance (ANOVA) test was used to evaluate differences among multiple  
23 conditions. If positive, the Tukey test was used to test for their statistical significance. Student's *t*  
24 test was used to evaluate differences between two conditions. Values of  $p < 0.05$  were considered to  
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31 be statistically significant.  
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## Results

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5 The production of TBARS, released by UV-B radiated cells without resveratrol or polyphenols,  
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7 increased in a linear manner for at least 40 min (20 min:  $214 \pm 24$  nmol MDA equivalent/g/min; 40  
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9 min:  $394 \pm 25$  nmol MDA equivalent/g/min;  $p < 0.01$ ). The release of TBARS from the radiated  
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11 liver cells loading with solutions containing resveratrol alone was dose-dependent: resveratrol had a  
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13 pro-oxidant effect at a concentration similar to that found in vivo after a moderate consumption of  
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15 red wine ( $0.7 \mu\text{mol/L}$ ), and an antioxidant effect at a higher concentration ( $20 \mu\text{mol/L}$ ), (Figure  
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17 1A).  
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21 Loading cells with polyphenols reduced lipid peroxidation in a dose-dependent manner after the 40  
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23 min exposure to UV-B radiation: maximum protection was attained by the use of a polyphenol  
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25 concentration equal to or higher than  $0.25 \text{ g/100 mL}$  (Figure 1B).  
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29 Figure 2 shows that loading cells with both resveratrol and polyphenols had synergistic effects (i.e.  
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31 protection was much higher than that given by resveratrol or polyphenols alone): protection by  
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33 resveratrol was seen at any tested concentrations (including  $0.7 \mu\text{mol/L}$ ), and increased with the  
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35 resveratrol concentration in the loading solution up to  $20 \mu\text{mol/L}$ .  
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39 Figure 3 shows the effects of UV-B radiation and resveratrol solutions on lipid-soluble antioxidants  
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41 isolated rat hepatocytes. UV-B radiation caused a 65% decrease in alpha-tocopherol and CoQ9,  
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43 40% in CoQ10 and 25% in dolichol levels, in keeping with previous observations (26). Loading  
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45 cells with the lower resveratrol solution (but not with a  $20 \mu\text{M}$  solution), caused a bigger loss of  
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47 vitamin E, CoQ9 and CoQ10 in UV-B radiated cells, but had no significant effect on dolichol.  
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51 Figure 4 shows that pre-loading with red wine polyphenols had no effect on the UV-B induced  
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53 depletion in fat-soluble antioxidants, but prevented the decrease observed with lower resveratrol  
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55 solution. Polyphenols together with increasing concentrations of resveratrol counteracted in part  
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57 (but significantly:  $p < 0.05$ ) the effects of radiation on CoQ9 and Vitamin E levels.  
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## Discussion

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4 UV-induced peroxidation of fatty acids containing more than two methylene-linked double bonds  
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6 and lipid peroxidation is a fundamental parameter of oxidative stress. Furthermore, UV-B radiation  
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8 is a method to increase oxidative stress from extracellular environment and may offer several  
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10 advantages over classical chemical tests (e.g. oxidation with  $\text{FeSO}_4$  or  $\text{H}_2\text{O}_2$  might interfere with  
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12 antioxidant activity of phytochemicals). A recent study show that the effects UV-B radiation can be  
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14 used to evaluate the antioxidant activity of plant extract and their interactions on red blood cell  
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16 ghosts (30).

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19 Natural products are widely used as complementary and alternative medications for the prevention  
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21 and treatment of various human diseases (35). Resveratrol, a naturally occurring phytoalexin found  
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23 abundance in grapes, red wines and other edible plants, has been reported to exert a variety of  
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25 pharmacological effects. In recent years, resveratrol has received considerable attention for its  
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27 antioxidant and free radical scavenging properties.  
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34 In this research, attention was focused on the antioxidant action of resveratrol in the absence or  
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36 presence of the polyphenols normally present in red wine, using UV-B radiated isolated rat  
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38 hepatocytes as a model of oxidative stress.  
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41 The routine use of TBARS determination in a wide array of sample types was criticized (31,36),  
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43 but assay proved to be a satisfactory index of the occurrence/extent of lipid peroxidation with UV-  
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45 B radiated isolated rat hepatocytes (27).  
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48 A surprising finding was that loading cells with a solution containing resveratrol alone, at a  
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50 concentration similar to that found in vivo after a moderate consumption of red wine, increased the  
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52 release of TBARS and led to a decrease in Vitamin E, Coenzyme Q9 and Q10 levels in the radiated  
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54 liver cells. This pro-oxidant effect was not seen at a higher resveratrol concentration. The presence  
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56 of resveratrol and polyphenols had synergistic antioxidant effect, and made resveratrol effective  
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58 both at lower and higher dosages. These results might provide answer to the “French paradox”, i.e.,  
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1 the apparent compatibility of a high-fat diet rich in cholesterol and saturated (and low in  
2 unsaturated) fatty acids with a low incidence of coronary atherosclerosis (37): the antioxidant and  
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4 anti-inflammatory activities of red wine might derive not only from the mere chemical composition  
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6 but also from the interaction occurring within the bulk of different molecules (38). Even though the  
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8 antioxidant activities of the wines vary over a factor of 2, the ratios of the activities to the total  
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10 phenol content are approximately the same (about a factor of 10), indicating the direct relationship  
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12 between the two (38).  
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16 An interesting hypothesis is that loading the membranes of mammalian cells with a small number  
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18 of resveratrol molecules (and no polyphenols) may produced a structural perturbation on the  
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20 membrane bilayer and change the metabolism of free radicals in lipophilic compartment of  
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22 cytomembranes (27). More recently, data demonstrate that resveratrol produce a moderate but  
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24 significant structural perturbation of the lipid bilayer and induce echinocytosis in human  
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26 erythrocytes: the extent of these changes is dependent on resveratrol concentration (39). Cell  
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28 membrane is a diffusion barrier which protects the interior of the cell. Therefore, its structure and  
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30 functions are susceptible to alterations as a consequence of interactions with chemical species.  
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32 Preceding studies show an earlier loss of Vitamin E than the others membrane lipid-soluble  
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34 antioxidants in isolated rat hepatocytes treated with NADPH-ADP-Fe system generating free  
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36 radicals from hydrophilic, extracellular environment (40). Also UV-B radiation is a method to  
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38 increase oxidative stress from extracellular environment and the effect of polyphenols together  
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40 with increasing concentrations of resveratrol may be protective because preserve an innate  
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42 antioxidant membrane machinery.  
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51 Overall, our results demonstrate that resveratrol and red wine polyphenols together can protect  
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53 mammalian cells from oxidative stress, whereas resveratrol alone may have pro-oxidant or anti-  
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55 oxidant effects depending on concentration. This conclusion is in line with the hypothesis that the  
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57 benefits of diets rich in fruits and vegetables are attributable to the uptake of phytocomplexes rather  
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59 than to individual micro-compounds. Several authors have hypothesized that some health effects  
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1 of plant polyphenols may not require their efficient absorption through the gut and may be due to  
2 the direct, protective effects on the intestinal mucosa against oxidative stress or the action of  
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4 carcinogens (41). Studies on human cells show that polyphenols can protect skin fibroblasts and  
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6 keratinocytes from photo-radiation damage (42); that resveratrol protects cells from an oxidative  
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8 stress by tert-butylhydroperoxide only at high concentrations (in the micromolar range (43)); and  
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10 that the protection is enhanced if resveratrol is used in combination with polyphenols such as  
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12 quercetina and pterostilbene (44). Furthermore, recent data demonstrate that low dose resveratrol  
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14 treatment causes premature senescence in lung cancer cells via ROS-mediated DNA damage (45).  
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17 Finally, our results may contribute to move forward in the further studies on the antioxidants  
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19 contained in food matrices and their potential synergistic combinations, since non-physiological,  
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21 excessive exogenous supply of dietary antioxidants could therefore even interfere with the delicate  
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23 redox balance of the cell, especially in elderly individuals.  
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31 *Conflict of interest* : The authors Dr. Straniero, Dr. Cavallini and Prof. Bergamini has a patent  
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33 10798587.1 pending.  
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## Figure Captions

**Fig. 1** Effect of the pre-incubation with solutions containing resveratrol (RSV) (A) or red wine polyphenols (RWP) (B) on photolipoperoxidation of UV-B radiated rat hepatocytes. Results are given as changes with respect to not-radiated cells. No changes in the levels of the TBARS production were observed in absence of radiation regardless of the concentration of RSV or RWP in the loading-solution. Results represent the means  $\pm$  SEM of six cases.

RSV: ANOVA statistical analysis showed that effect of resveratrol solutions was highly significant ( $p < 0.01$ ). Post-ANOVA Tukey test ( $p < 0.05$ ): # versus No RSV; § versus 0.7  $\mu\text{mol/L}$ .

RWP: ANOVA statistical analysis showed that effect of red wine polyphenols solutions was highly significant ( $p < 0.01$ ). Post-ANOVA Tukey test ( $p < 0.05$ ): # versus No RWP

**Fig. 2** Protection of rat hepatocytes from photolipoperoxidation after UV-B radiation (UV-B) by pre-incubation with red wine polyphenols (RWP) 0.25 g/100mL and increasing amounts of resveratrol (RSV: 0.7, 2, 6 or 20  $\mu\text{mol/L}$ ). Results are given as changes with respect to not-radiated cells. No changes in the levels of the TBARS production were observed in absence of radiation regardless of the concentration of RWP and RSV in the loading-solution. Results represent the means  $\pm$  SEM of six cases. ANOVA statistical analysis showed that the protective effects of red wine polyphenols and resveratrol were highly significant ( $p < 0.01$ ). Post-ANOVA Tukey test ( $p < 0.05$ ): \* versus UV-B; # versus RWP+RSV 20  $\mu\text{mol/L}$ ; § versus RWP

**Fig. 3** Effect of UV-B radiation on the level of lipid-soluble antioxidants (Coenzyme Q9 and Q10, Vitamin E and Dolichol) in isolated liver cells pre-loaded or not with resveratrol (RSV: 0.7 or 20  $\mu\text{mol/L}$ ). Results are given as percent changes with respect to not-radiated cells. No changes in the levels of the lipophilic antioxidants were observed in absence of radiation regardless of RSV concentration in the loading-solution. Results represent the means  $\pm$  SEM of six cases. ANOVA



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statistical analysis showed that effects of UV-B radiation on lipophilic antioxidant levels with or without resveratrol in the loading-solution were highly significant ( $p < 0.01$ ). Post-ANOVA Tukey test ( $p < 0.05$ ): \* versus UV-B

**Fig. 4** Effect of UV-B radiation on the level of lipid-soluble antioxidants (Coenzyme Q9 and Q10, Vitamin E and Dolichol) in isolated liver cells pre-loaded with red wine polyphenols (RWP) 0.25 g/100mL and increasing amounts of resveratrol (RSV: 0.7, 2, 6 or 20  $\mu\text{mol/L}$ ). Results are given as  $\mu\text{g}$  Coenzyme Q9/g cell,  $\mu\text{g}$  Coenzyme Q10/g cell, nmol Vitamin E/g cell and  $\mu\text{g}$  Dolichol/g cell. No changes in the levels of the TBARS production were observed in absence of radiation regardless of the concentration of RWP and RSV in the loading-solution. Results represent the means  $\pm$  SEM of six cases. ANOVA statistical analysis showed that effects of UV-B radiation on lipophilic antioxidant levels with or without increasing amounts of RSV in the loading-solution were highly significant ( $p < 0.01$ ). Post-ANOVA Tukey test ( $p < 0.05$ ): \* versus UV-B+RWP

Fig. 1

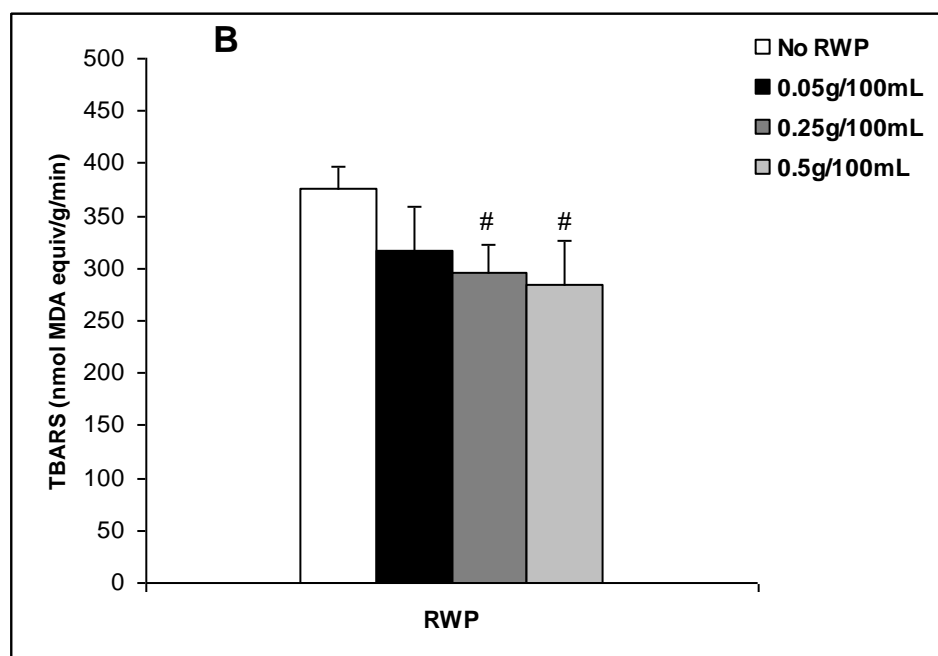
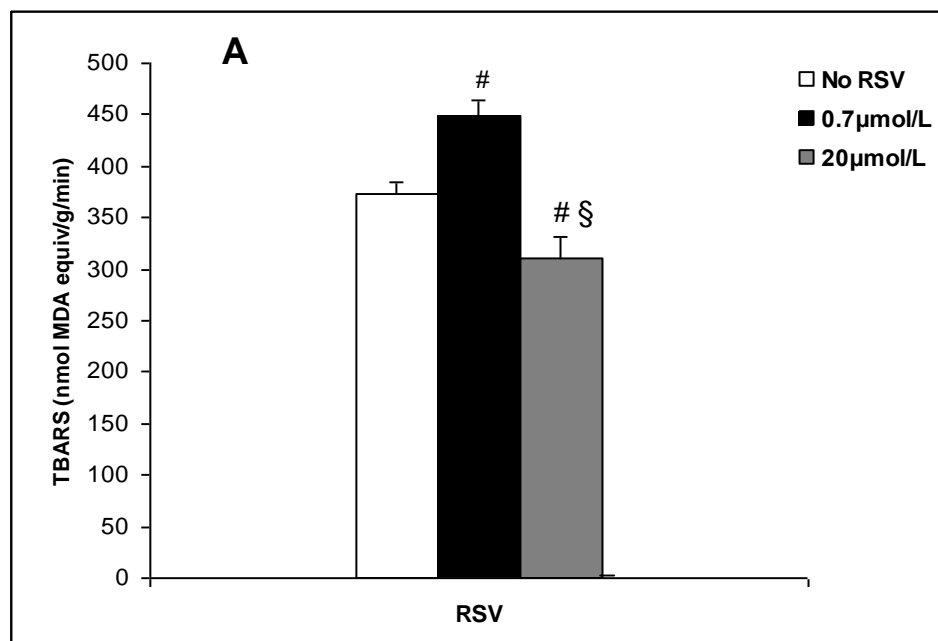


Fig. 2

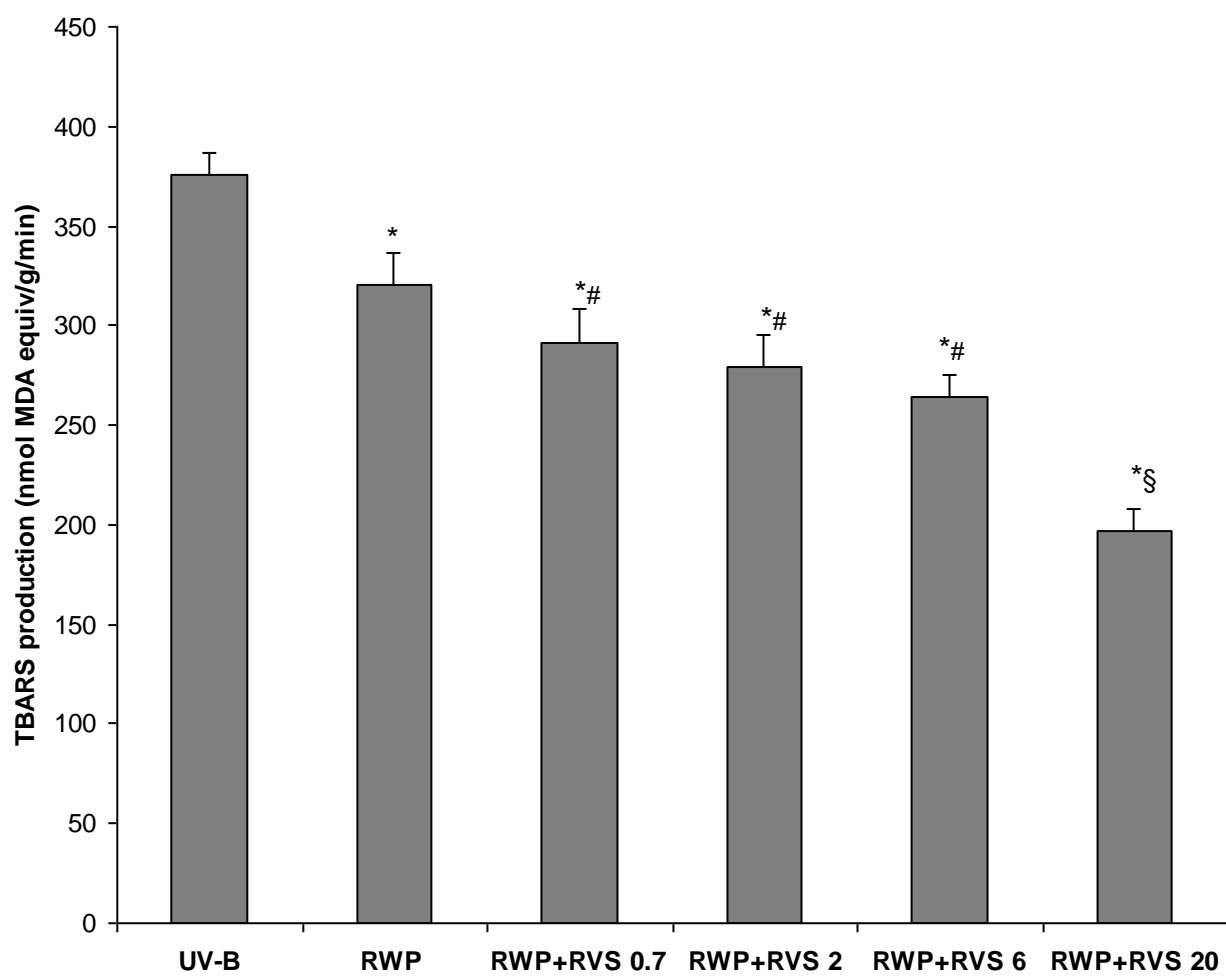


Fig. 3

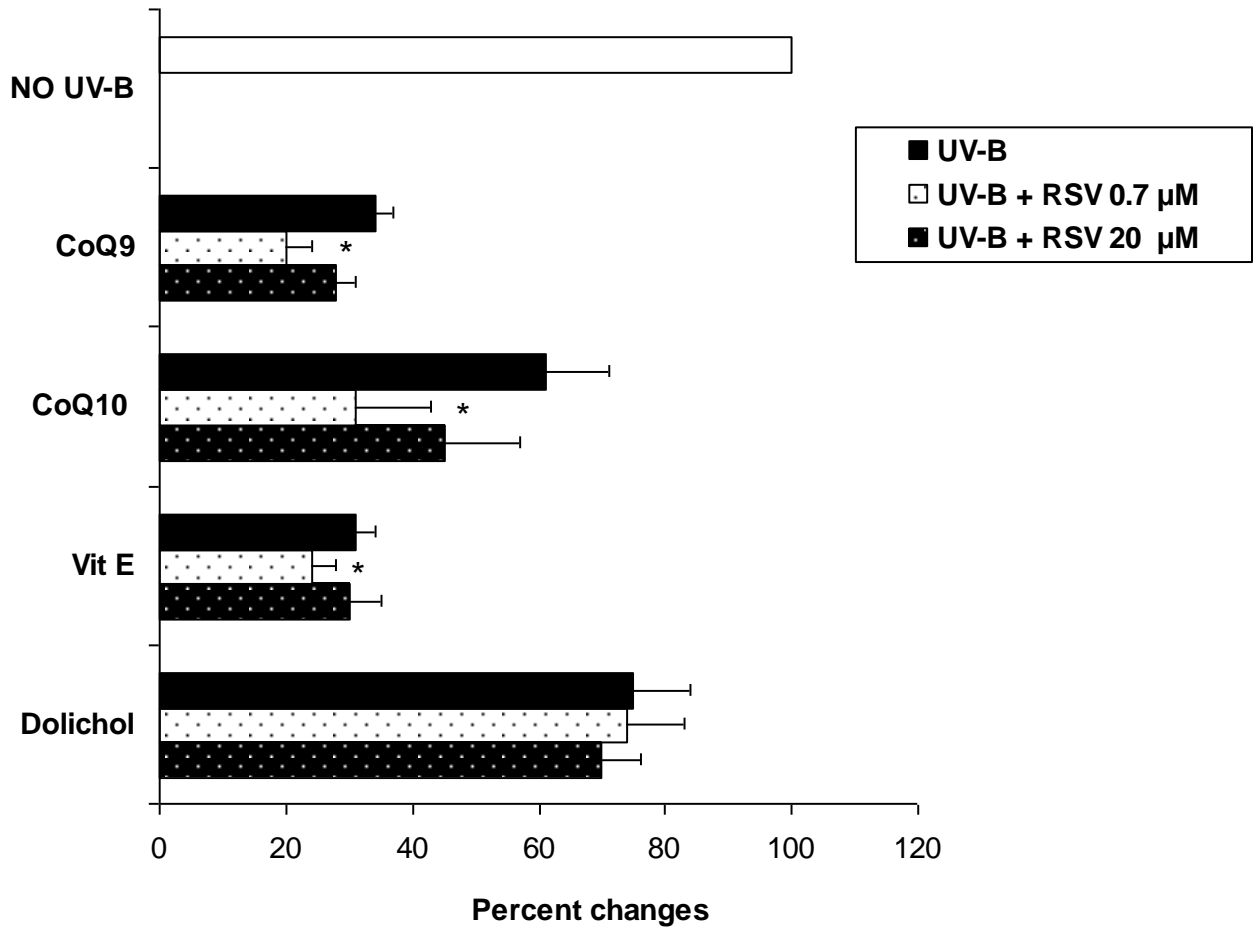
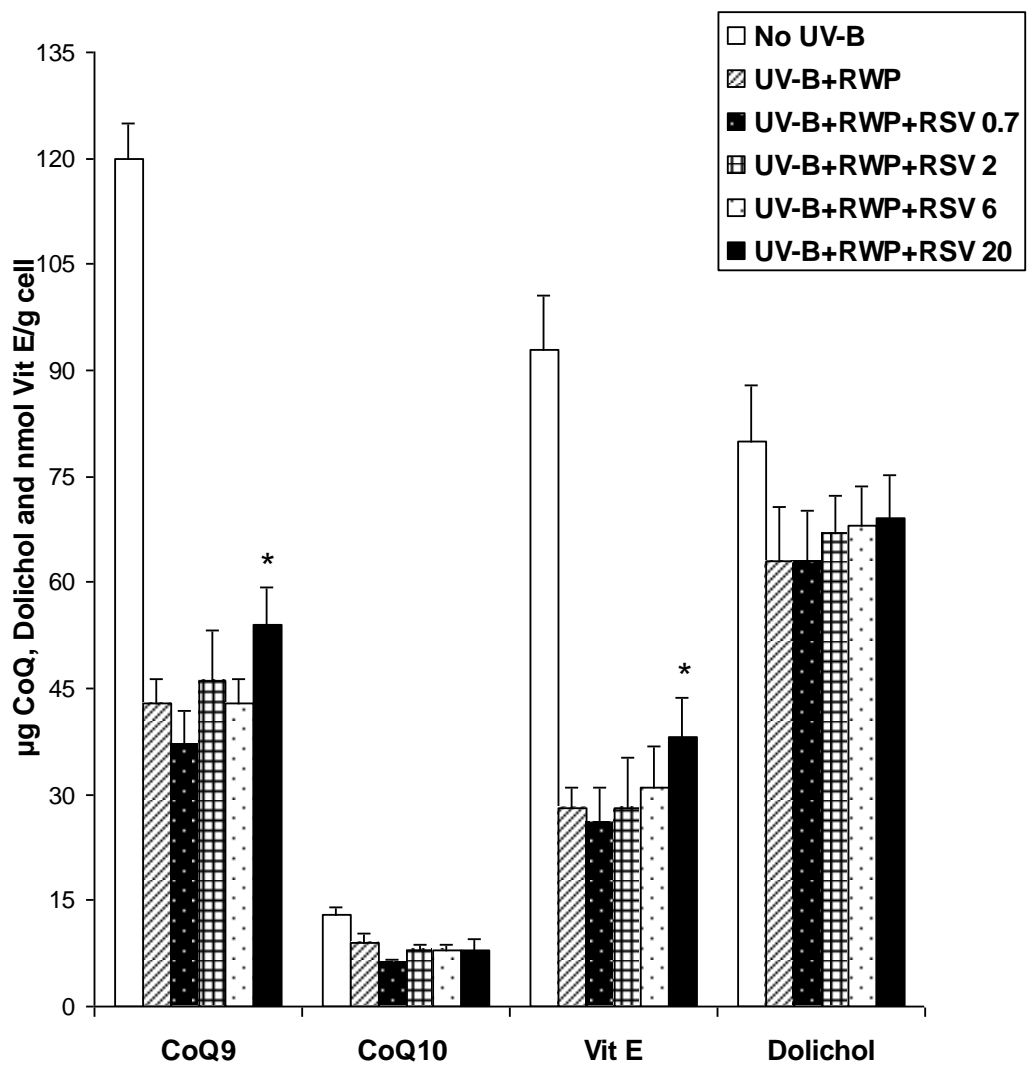
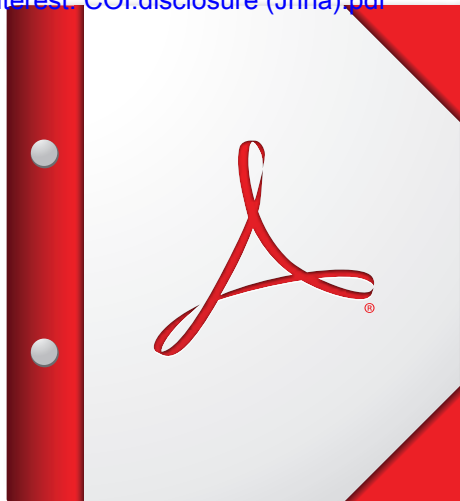


Fig. 4





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