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Effects of resveratrol on lifespan in *Drosophila melanogaster* and *Caenorhabditis elegans*

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Abstract

It was recently reported that the plant polyphenol resveratrol, found, e.g., in grape berry skins, extended lifespan in the fruit fly *Drosophila melanogaster* and the nematode worm *Caenorhabditis elegans*. This lifespan extension was dependent on an NAD^+-dependent histone deacetylase, *Sir2* in *Drosophila* and SIR-2.1 in *C. elegans*. The extension of lifespan appeared to occur through a mechanism related to dietary restriction (DR), the reduction of available nutrients without causing malnutrition, an intervention that extends lifespan in diverse organisms from yeast to mammals. In *Drosophila*, lifespan extension by DR is associated with a reduction in fecundity. However, a slight increase in fecundity was reported upon treatment with resveratrol, suggesting a mode of action at least partially distinct from that of DR. To probe this mechanism further, we initiated a new study of the effects of resveratrol on *Drosophila*. We saw no significant effects on lifespan in seven independent trials. We analysed our resveratrol and found that its structure was normal, with no oxidative modifications. We therefore re-tested the effects of resveratrol in *C. elegans,* in both wild-type and *sir-2.1* mutant worms. The results were variable, with resveratrol treatment resulting in slight increases in lifespan in some trials but not others, in both wild type and *sir-2.1* mutant animals. We postulate that the effect of resveratrol upon lifespan in *C. elegans* could reflect induction of phase 2 drug detoxification or activation of AMP kinase.

Keywords: *Drosophila melanogaster*; *Caenorhabditis elegans*; Resveratrol; Lifespan

1. Introduction

Sir2 is an NAD^+-dependent protein deacetylase, important for a variety of biological processes related to regulation and maintenance of genes and DNA (Kaeberlein et al., 1999; Martin et al., 1999; Chen and Widom, 2004). It has also been reported to be a regulator of lifespan in yeast (Kaeberlein et al., 1999), nematodes (Tissenbaum and Guarente, 2001) and fruit flies (Rogina and Helfand, 2004). Sirtuin activating compounds (STACs) are small molecules that stimulate the deacetylation of proteins by members of the Sir2-like protein family (Howitz et al., 2003). Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a polyphenolic phytoallexin (anti-fungal defence compound) found in a range of plant products, including grape berry skins, and has been reported to act as a STAC (Howitz et al., 2003). This compound has a number of biological effects, including anticarcinogenic (Jang et al., 1997) and anticoagulant (Fauconneau et al., 1997) properties and is a suppressor of oxidative DNA damage (Mizutani et al., 2001). It was reported that its action to increase lifespan in the budding yeast *Saccharomyces cerevisiae* involves a similar mechanism to that of dietary restriction (DR) (Howitz et al., 2003). DR, the reduction of available nutrients without causing malnutrition, extends lifespan in a range of organisms, from yeast to mammals (McCay et al., 1935; Lin et al., 2002; Partridge et al., 2005b; Walker et al., 2005; Piper and Partridge, 2007). Activation of sirtuins by resveratrol in the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila* has also been suggested to extend lifespan, through a DR-like mechanism (Wood et al., 2004). The capacity of resveratrol to increase lifespan was verified in further studies using *Drosophila* (Bauer et al., 2004) and *C. elegans* (Viswanathan et al., 2005), and in the latter study the requirement of *sir2.1* for the lifespan extension by resveratrol was also reported.

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More recently, the role of Sir2 and resveratrol in aging and the relationship to the DR effect has become the subject of controversy (Kaeberlein et al., 2005; Baur and Sinclair, 2006). For example, it transpires that Sir2 is not required for the lifespan extension by DR in some strains of *S. cerevisiae* (Kaeberlein et al., 2004), deletion of all of the sirtuin family proteins does not prevent the response to DR in *S. cerevisiae* (Tsuchiya et al., 2006), *sir-2.1* is not required for the lifespan extension by DR in *C. elegans* (Kaeberlein et al., 2006; Lee et al., 2006; Hansen et al., 2007), resveratrol does not in fact activate Sir2 *in vivo* (Borra et al., 2005; Kaeberlein et al., 2005) and the extension of *S. cerevisiae* lifespan by treatment with resveratrol could not be reproduced (Kaeberlein et al., 2005). Resveratrol acts on a number of target proteins (Baur and Sinclair, 2006); for example it was recently shown to activate AMP kinase and have neuroprotective properties, but this effect is Sir2-independent (Dasgupta and Milbrandt, 2007). Thus resveratrol might exert some of its biological effects through pathways unrelated to Sir2.

These results raise a number of questions about the effects of resveratrol on *Drosophila* and *C. elegans*. How robust (i.e., reproducible) are the effects of resveratrol on lifespan? If resveratrol is not a STAC, then why should it show Sir2-dependent effects? A further question is: Does resveratrol really mimic DR? Extension of lifespan by resveratrol in *D. melanogaster* was associated with a slight increase in fecundity (Wood et al., 2004), whereas DR itself in *Drosophila* invariably reduces fertility (Chippindale et al., 1993; Chapman and Partridge, 1996; Partridge et al., 2005a). Our studies of resveratrol were initially motivated by a wish to examine the effect of resveratrol on risk and damage as determinants of mortality in *D. melanogaster* (Partridge et al., 2005c). The increased survival from DR in wild-type flies is due solely to a reduction in the short-term risk of death (Mair et al., 2003). We had hoped to establish the optimum dose of resveratrol for lifespan extension in *D. melanogaster* and to examine effects on risk and damage. Instead, we did not see increased survival in flies treated with resveratrol at any concentration. This led us both to examine the chemical integrity of our resveratrol and to extend our studies to *C. elegans*. In the latter, results were variable, with small, typically marginal increases in lifespan seen in resveratrol-treated groups in both wild-type and *sir-2.1* mutant worms in some trials but not others.

2. Materials and methods

2.1. Fly strains, culture conditions and lifespan experiments

To examine the effects of resveratrol on lifespan in two different types of wild type strains, Dahomey and Canton S wild-type flies were used in the lifespan studies. The former is a large, random-bred stock maintained in population cages while the latter is a standard, laboratory, inbred strain maintained in bottle and vial culture, and this strain was one of those previously shown to have increased survival when treated with resveratrol (Wood et al., 2004). All experimental flies were reared and the lifespan trials were conducted in a humidified, temperature-controlled incubator at 25 °C and 65% humidity on a 12-h light:12-h dark cycle. All larvae were reared in standard density culture (Clancy and Kennington, 2001) on standard laboratory fly medium (10% sugar/yeast: 2% agar, 10% sucrose, 10% autolysed yeast powder, 3% Nipagin, 0.3% propionic acid) and experimental flies used in the lifespan trials were maintained on either 15% sugar/yeast medium (1.5 SY) or standard laboratory maize/sugar/yeast medium (MSY) (15% sugar/yeast medium: 2% agar, 15% sucrose, 15% autolysed yeast powder, 3% Nipagin, 0.3% propionic acid, maize/sugar/yeast medium: 1% agar, 12% maize powder, 8.5% sucrose, 2% autolysed yeast powder, 2.5% Nipagin). Two sources of autolysed yeast powder were used in these experiments. Trial 4 used autolysed brewer's yeast powder (MP Biomedicals, Ohio, USA) and all other trials used autolysed baker's yeast powder (B.T.P. Drewitt, London, UK). Resveratrol (Biomol product # FR 104) was dissolved in ethanol and added to the molten media at 60 °C (10 ml ethanol/l medium), stirred vigorously and then agitated constantly while being poured. The final concentration of resveratrol in the food was 0, 1, 3.2, 10, 32, 100, 200, and 1000 μM. Flies were kept in 30 ml plastic vials containing 4 ml of food/drug media. For the no-drug control, just the carrier was added (ethanol 10 ml/l). Adult flies for the lifespan trials were transferred on the day of eclosion to fresh 10% sugar/yeast medium in bottles for 2 days to mate before they were collected under light CO_2 anaesthesia. Flies were transferred to experimental treatments at a density of 10 per vial, either 5 males and 5 females or 10 females alone, depending on the treatment. There were around 200 flies in each treatment. Flies were transferred to vials containing fresh medium every 2 days and deaths were scored daily.

2.2. Nematode strains, culture conditions and lifespan experiments

C. elegans were maintained on NGM agar and fed with *Escherichia coli* strain OP50 (Sulston and Hodgkin, 1988). Strains used were N2 (wild type) and XA2972 *sir-2.1(ok434) IV*, which has a deletion in the gene and is a putative null allele (Wood et al., 2004). XA2972 was outcrossed twice from VC199 (KO consortium) using N2. Effects of resveratrol on nematode lifespan were measured as previously (Wood et al., 2004). Animals were maintained at 24 °C. Resveratrol (to a final concentration of 100 μM) dissolved in DMSO, and DMSO alone (for the control) were added 1:1000 to molten agar NGM during preparation of plates. Plates also contained 40 μM fluoredeoxyuridine (FUdR) to prevent progeny-production. Animals were exposed to resveratrol and FUdR from late L4 onwards, and were transferred to fresh plates at approximately 2-day intervals for the first 8 days and then onwards once a week. All nematode lifespan studies were conducted blind. Two sets of trials were carried out independently by each of two researchers (D.W., UCL, and K.H., University of Ghent).

2.3. Analysis of survival data

Comparison of survivorship data was performed using the log rank and the Wilcoxon survival significance tests provided by JMP IN 5.1 statistical software package.

2.4. Mass spectroscopy analysis of resveratrol

Mass spectroscopy analysis of resveratrol was performed by Dr. Carolyn Hyde of the Scientific Support Services, Wolfson Institute for Biomedical Research, UCL. The sample of resveratrol was run on a Kratos Axima CFR without matrix due to the low molecular weight and aromatic nature of the compound.

2.5. Activity of resveratrol

The activity of resveratrol samples was measured using the SIRT1 Fluorimetric Drug Discovery Kit purchased from Biomol International Ltd. (product #AK555), and performed according to the kit's suggested protocol. Statistical analysis of the results was performed using the *t*-test implemented in JMP IN 5.1 statistical software package.

3. Results

3.1. Effect of resveratrol on lifespan in Drosophila

Flies were treated with resveratrol concentrations ranging from 1 to 1000 μM, in either single-sex or mixed-sex groups,

Table 1
Dahomey wild-type *Drosophila* resveratrol lifespan trials

Trial	1	2	3	3	4
Strain	Dahomey	Dahomey	Dahomey	Dahomey	Dahomey
Sex	Females	Females	Females	Males	Females
Status	Once-mated	Once-mated	Mating	Mating	Once-mated
Conditions	10 females/vial	10 females/vial	5m + 5f/vial	5m + 5f/vial	10 females/vial
Diet	1.5 SY (Baker's)	1.5 SY (Baker's)	1.5 SY (Baker's)	1.5 SY (Baker's)	1.5 SY (Brewer's)
[Resveratrol]	Median (*n*)				
0	36 (196)	29 (202)	27 (98)	33 (104)	71 (101)
1	34 (193)	31 (193)	26 (99)	38 (101)	72 (101)
3.2	35 (203)	28 (201)	25 (98)	32 (103)	73 (97)
10	34 (201)*	30 (199)	27 (98)	38 (96)	71 (105)
32	35 (199)	28 (193)	25 (95)	36 (94)	71 (99)
100	37 (201)	28 (202)	26 (102)	33 (99)	70 (106)*
200	35 (181)	29 (196)	25 (91)	37 (101)	70 (93)*
1000	34 (194)	31 (207)	25 (102)	34 (94)	69 (102)

Lifespan analysis was performed using JMP IN 5.1 statistical software package. Significant lifespan reductions are denoted by an asterisk (critical p value = 0.0071 after Bonferroni correction).

using two different wild-type strains and three different media recipes. We saw no significant increases in lifespan in resveratrol-treated *Drosophila*. Results of the survival assays using Dahomey wild-type flies are summarised in Table 1, and those using Canton S wild-type flies in Table 2. Resveratrol caused a significant reduction in lifespan (critical p value after Bonferroni adjustment for multiple comparisons = 0.05/7 = 0.0071) in some treatments (Trial 1 females, 10 μM resveratrol, log rank $X^2 = 11.547$, $p = 0.0007$, Wilcoxon $X^2 = 7.591$, $p = 0.0059$, Trial 4 females, 100 μM resveratrol, log rank $X^2 = 10.844$, $p = 0.001$, Wilcoxon $X^2 = 3.161$, $p = 0.075$, 200 μM resveratrol, log rank $X^2 = 8.381$, $p = 0.004$, Wilcoxon $X^2 = 7.591$, $p = 0.04$, Trial 5 females, 32 μM resveratrol, log rank $X^2 = 10.416$, $p = 0.001$, Wilcoxon $X^2 = 7.166$, $p = 0.0074$, Trial 6 females, 10 μM resveratrol, log rank $X^2 = 2.320$, $p = 0.128$, Wilcoxon $X^2 = 7.649$, $p = 0.006$, Trial 6 males, 10 μM resveratrol, log rank $X^2 = 7.294$, $p = 0.0069$, Wilcoxon $X^2 = 4.869$, $p = 0.027$, 32 μM resveratrol, log rank $X^2 = 11.424$, $p = 0.0007$, Wilcoxon $X^2 = 8.534$, $p = 0.0035$). Male Dahomey flies, kept in mixed-sex vials (Trial 3) and treated with 10 or 200 μM resveratrol showed slightly improved survival, but this was not significant with either the log rank or the Wilcoxon analysis after adjustment of the critical p value for multiple comparisons (Trial 3 males, 10 μM resveratrol, log rank $X^2 = 4.365$, $p = 0.037$, Wilcoxon $X^2 = 2.631$, $p = 0.105$, 200 μM resveratrol, log rank $X^2 = 4.016$, $p = 0.045$, Wilcoxon $X^2 = 4.208$, $p = 0.04$) and these were the only cases in which treatment with resveratrol caused an improvement in survival that gave a p value less than 0.05, the critical p value when multiple comparisons are not made.

3.2. Effect of resveratrol on lifespan in C. elegans

Treatment with 100 μM resveratrol resulted in variable effects on lifespan in *C. elegans* (Table 3, Fig. 1). In the

Table 2
Canton S wild-type *Drosophila* resveratrol lifespan trials

Trial	5	6	6	7	7
Strain	Canton S	Canton S	Canton S	Canton S	Canton S
Sex	Females	Females	Males	Females	Males
Status	Once-mated	Mating	Mating	Mating	Mating
Conditions	10 females/vial	5m + 5f/vial	5m + 5f/vial	5m + 5f/vial	5m + 5f/vial
Diet	1.5 SY (Baker's)	1.5 SY (Baker's)	1.5 SY (Baker's)	MSY	MSY
[Resveratrol]	Median (*n*)				
0	29 (193)	26 (97)	36 (91)	44 (96)	49 (88)
1	28 (198)	26 (85)	37 (99)	41 (101)	49 (90)
3.2	29 (185)	25 (90)	35 (95)	46 (101)	53 (92)
10	28 (195)	24 (97)*	33 (97)*	42 (99)	49 (97)
32	28 (196)*	22 (98)	31 (89)*	48 (101)	48 (90)
100	28 (197)	25 (96)	37 (86)	44 (112)	49 (81)
200	29 (199)	25 (95)	36 (91)	42 (99)	50 (92)
1000	30 (202)	25 (106)	33 (89)	46 (103)	48 (81)

Lifespan analysis was performed using JMP IN 5.1 statistical software package. Significant lifespan reductions are denoted by an asterisk (critical p value = 0.0071 after Bonferroni correction).

Table 3
Wild-type and *sir-2.1* mutant *C. elegans* resveratrol lifespan trials

Strain	Treatment	Trial	Median lifespan (days)	Mean lifespan (days)	*n*	Log rank X^2	*p*-Value	Wilcoxon X^2	*p*-Value
N2	DMSO	1	13	12.1	137				
N2	Resveratrol	1	13	13	134	1.71	0.191	4.67	0.031*
Sir2.1	DMSO	1	13	12	103				
Sir2.1	Resveratrol	1	13	13.2	125	3.11	0.078	6.85	0.009*
N2	DMSO	2	13	13.5	122				
N2	Resveratrol	2	15	14.6	123	3.76	0.053	3.64	0.056
Sir2.1	DMSO	2	13	14	110				
Sir2.1	Resveratrol	2	15	15.5	119	9.81	0.002*	6.47	0.011*
N2	DMSO	3	17	17.6	110				
N2	Resveratrol	3	19	18.2	112	3.04	0.081	3.56	0.059
Sir2.1	DMSO	3	16	16.6	106				
Sir2.1	Resveratrol	3	16	17.1	101	0.51	0.477	2.7	0.1
N2	DMSO	4	17	17.3	113				
N2	Resveratrol	4	17	18.4	112	7.31	0.007*	4.56	0.033*
Sir2.1	DMSO	4	12	13.7	93				
Sir2.1	Resveratrol	4	15	16.2	118	17.7	<0.0001*	19.06	<0.0001*

Lifespan analysis was performed using JMP IN 5.1 statistical software package. Significant lifespan extensions ($p < 0.05$) are denoted by an asterisk.

majority of trials, in both wild-type and *sir-2.1* mutant populations, resveratrol resulted in a slight shift in the lifespan curve to the right (Fig. 1). Sometimes these effects reached statistical significance ($p < 0.05$), in one trial (2) for wild type, and two trials (1 and 4) for *sir-2.1* mutants (Table 3). Thus, these small effects of resveratrol may be *sir-2.1*-independent. By contrast, in earlier studies the effects of resveratrol on *C. elegans* lifespan were found to be *sir-2.1*-dependent (Wood et al., 2004; Viswanathan et al., 2005).

3.3. Integrity of resveratrol

One possible explanation for the lack of lifespan-extending effect of resveratrol in the *Drosophila* trials is that the drug had

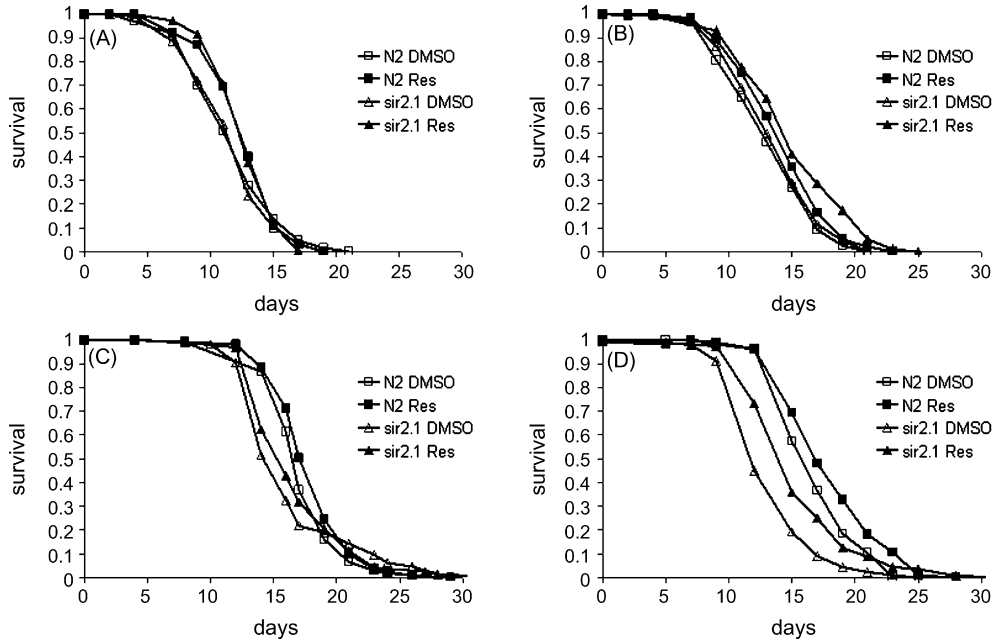

Fig. 1. One hundred micromolar resveratrol treatment causes a small, reproducible increase in lifespan in N2 wild-type and *sir-2.1* mutant *C. elegans* ((A) N2 Res compared with N2 DMSO, Wilcoxon $X^2 = 4.67$, $p = 0.031$, *sir-2.1* Res compared with *sir-2.1* DMSO, Wilcoxon $X^2 = 6.85$, $p = 0.009$, (B) *sir-2.1* Res compared with *sir-2.1* DMSO, log rank $X^2 = 9.81$, $p = 0.002$, Wilcoxon $X^2 = 6.47$, $p = 0.011$, (C) N2 Res compared with N2 DMSO, log rank $X^2 = 7.31$, $p = 0.007$, Wilcoxon $X^2 = 4.56$, $p = 0.033$, (D) *sir-2.1* Res compared with *sir-2.1* DMSO, log rank $X^2 = 16.82$, $p < 0.0001$, Wilcoxon $X^2 = 17.70$, $p < 0.0001$). (A and B) are trials 1 and 2, performed by operator K. Houthoofd and (C and D) are trials 3 and 4, performed by operator D. Weinkove.

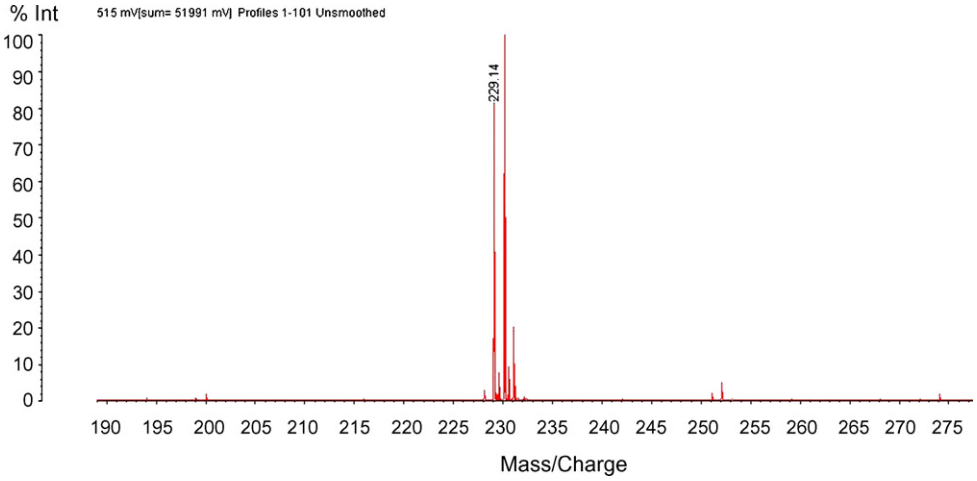

Fig. 2. Mass spectroscopy analysis of a resveratrol sample stored at −20 °C for several months after it was opened. The main peak with a mass to charge ratio of 229.14 corresponds to $(M+H)^+$. Other peaks around this reflect isotopic resolution of the analysis. The oxidised form of resveratrol would have a mass to charge ratio of 235. Mass spectroscopy analysis was performed by Dr. Carolyn Hyde of the Scientific Support Services, Wolfson Institute for Biomedical Research, UCL.

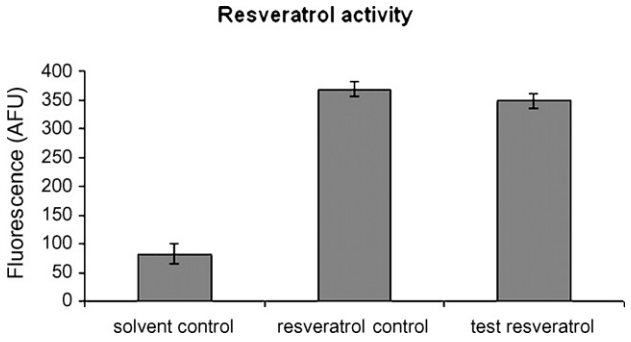

Fig. 3. There was no loss of activity in our sample of resveratrol (test resveratrol, mean activity = 348.4 Arbitrary Fluorescence Units (AFU), standard error = 12.7 AFU, $n = 16$) compared with the resveratrol provided as a positive control in the SIRT1 fluorimetric drug discovery kit (Biomol AK 555) ((resveratrol control, mean activity = 368.6 AFU, standard error = 12.0 AFU, $n = 8$), comparison of resveratrol sample with resveratrol control, (t-test, $p = 0.19$)). The negative control was a solvent only sample (solvent control, mean activity = 82.4 AFU, standard error = 16.5 AFU, $n = 8$).

reduced activity due to oxidation during storage. Storage of the drug was according to manufacturer's instructions and preparation of the nutrient media was according to the protocol outlined in the original study (Wood et al., 2004). We analysed the resveratrol using mass spectroscopy, with a sample of resveratrol taken from a container originally opened for use in the *Drosophila* lifespan trials, and the remainder stored at −20 °C for several months. Only the normal and no oxidised form of resveratrol was detected (Fig. 2). We then used the SIRT1 Fluorimetric Drug Discovery Kit, to measure the activity of our resveratrol sample in vitro, and compared it with the positive control provided with the kit. There was no detectable loss of activity (Fig. 3).

4. Discussion and conclusions

In this study, we tested the effects of resveratrol on lifespan in *Drosophila* and *C. elegans*. Resveratrol did not increase lifespan in *Drosophila*, but in some trials caused slight increases in lifespan in *C. elegans* in both wild-type and *sir-2.1* mutant populations.

The Bonferroni method for adjustment of critical p values in the statistical analysis of multiple comparisons may be overly strict and obscure a significant difference between control groups and treatments where one does exist (Bland and Altman, 1995). However, in *Drosophila* we found only two trials with a lifespan extension that gave rise to a p value less than the critical level before the Bonferroni adjustment, and four trials with a significant decrease in lifespan. Thus the results of these multiple trials with *Drosophila* suggest that the two lifespan extensions seen before Bonferroni correction were false positives, and that there is either no real effect or a slight negative effect of resveratrol on lifespan. We were unable to repeat the lifespan extension in *D. melanogaster* by resveratrol treatment, despite using the same strain of flies, the same nutrient media recipe and drug from the same source as that reported in the original study (Wood et al., 2004). Mass spectroscopy analysis of a sample of the drug that had been exposed to the air and then stored for several months according to the manufacturer's instructions showed no detectable oxidation of the drug, and the enzymic assay used in previous resveratrol studies (Howitz et al., 2003) showed that there had been no detectable loss in activity during storage. Possibly unknown environmental factors could have produced the different results between our own and the published studies. However, at the very least our results indicate that extension of lifespan in *Drosophila* by resveratrol is not a robust experimental finding.

Why might resveratrol not extend lifespan in *Drosophila*? In the wild, *Drosophila* are thought to co-consume fruit material and microbes from fermenting/rotting fruit (Spieth, 1974). Plant defence molecules (phytoallexins) such as resveratrol that are produced in response to pathogen attack (Dercks and Creasy, 1989) are therefore likely to be present in the normal diet of such animals. One possibility is that, because of the high levels of phytoallexins in their diet, fruit flies have evolved efficient mechanisms to detoxify such molecules (including

resveratrol), e.g., by means of drug metabolizing enzymes. This might explain why *Drosophila* are insensitive to resveratrol.

Given that it was initially thought that resveratrol activates Sir2 *in vivo* (Howitz et al., 2003), the observation of *sir-2.1*-dependence of the effects of resveratrol on *C. elegans* lifespan was easy to rationalize (Wood et al., 2004). However, it was recently demonstrated that resveratrol does not activate Sir2 *in vivo* (Borra et al., 2005; Kaeberlein et al., 2005). Thus, our observation, derived from trials conducted blind, suggesting that the small and intermittent lifespan extension in *C. elegans* by resveratrol does not depend on *sir-2.1*, is what one might expect. However, that there was a life extension at all is intriguing, and it seems likely that a different mechanism is at play from that originally proposed (Wood et al., 2004). One candidate for such a mechanism is induction of expression of genes involved in phase 2 drug detoxification, predicted to increase defences against pro-oxidants and other toxicants. Numerous electrophilic compounds can induce this gene battery by activation of the transcription factor Nrf-2 which stimulates transcriptions of genes with antioxidant response elements (AREs) (Talalay et al., 2003). Phase 2 enzymes protect against a range of oxidants and electrophiles, and include glutathione transferases, UDP-glucuronosyltransferases and NAD(P)H:quinone reductase (QR). A standard test for inducibility of this system by a compound is the Concentration required to Double (CD) QR specific activity (Talalay et al., 2003). Resveratrol is a potent inducer of QR in cultured murine cells, with a CD value of 21 μM (Jang et al., 1997). It is one of a number of phase 2 inducing electrophilic compounds which have the capacity to protect against carcinogenesis (so called chemoprotection) (Talalay, 2000). Possibly, resveratrol has a similar effect in *C. elegans*, and protects against the molecular damage underlying the aging process. A second candidate for the mechanism of lifespan extension by resveratrol in *C. elegans* is neuroprotection by stimulation of AMP kinase activity. AMP kinase, the central energy sensor in the cell (Bergeron et al., 2001), responds to alterations in the AMP:ATP ratio to maintain cellular energy homeostasis during energetic stress (Hardie et al., 1999). Resveratrol activates AMP kinase in neurons in a SIRT1-independent manner that could affect neuronal energy homeostasis and be neuroprotective (Dasgupta and Milbrandt, 2007). It is possible that resveratrol promotes neuroprotection by AMP kinase activation in *C. elegans* and extends lifespan.

In conclusion, our results fail to support the model that links DR, Sir2 and ageing in an evolutionarily conserved pathway, with resveratrol as an activator of the pathway. The slight and intermittent effect of resveratrol on lifespan in *C. elegans* is consistent with the possibility that the known chemoprotective property of resveratrol can also lead to geroprotection.

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