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Curcumin-supplemented diets increase superoxide dismutase activity and mean lifespan in *Drosophila*

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Abstract Curcumin is a polyphenolic bioactive compound in turmeric. We examined if antioxidant effects of curcumin are associated with lifespan extension in *Drosophila.* In this experiment, females and males of *Drosophila* were fed diets either containing no curcumin (C0) or supplemented with curcumin at 0.5 (C1) and 1.0 (C2) mg/g of diet. The levels of malondialdehyde (MDA), enzyme activity of superoxide dismutase

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(SOD), and expression of seven age-related genes in females and males were analyzed. We found that C1 and C2 increased mean lifespan by 6.2 % and 25.8 % in females, and by 15.5 % and 12.6 % in males, respectively. Meanwhile, C1 and C2 significantly decreased MDA levels and increased SOD activity in both genders. Diets C1 in females and C2 in males are effective in extending mean lifespan and improving levels of two physiological and biochemical measures related to aging in *Drosophila*. Lifespan extension of curcumin in *Drosophila* was associated with the up-regulation of *Mn-SOD* and *CuZn-SOD* genes, and the down-regulation *of dInR*, *ATTD*, *Def*, *CecB*, and *DptB* genes. The present results suggest that curcumin increases mean lifespan of *Drosophila* via regulating gene expression of the key enzyme SOD and reducing accumulation of MDA and lipid peroxidation. This study provided new insights for understanding the anti-aging mechanism of curcumin in *Drosophila*.

Keywords Curcumin · Lifespan · MDA · SOD · Age-related genes

Introduction

Diet is an important factor in aging processes. Dietary phenolics are beneficial for health and longevity by reducing oxidative stress and regulating signal transduction and gene expression (Rossi et al. 2008). Curcumin is a main bioactive polyphenolic that is present in the rhizome of the plant *Curcumma longa* (turmeric), which has been widely used as a spice, food additive, and as a herbal medicine in Asia (Govindarajan 1980). A large number of studies have demonstrated that curcumin possesses potent biological activities, including antioxidant and anti-inflammatory activities (Sharma et al. 2005). A mouse model of Alzheimer disease (AD) suggested that curcumin prevented AD by lowing oxidized proteins, disaggregating amyloid-β, preventing fibril oligomer formation, and by reducing proinflammatory cytokines such as interleukin-1β, (Lim et al. 2001). Curcumin also has anti-cancer effect at various stages such as tumor initiation, promotion, and progression in different types of cancers (Surh and Chun 2007). Several clinical trials with curcumin have shown positive effect on cancer prevention (Schaffer et al. 2011). Curcumin also appeared to possess pharmacological activity to slow down the aging process and extend lifespan of *Drosophila* (Salvioli et al. 2007; Sikora et al. 2010; Suckow and Suckow 2006). Lifespan extension by curcumin in *Drosophila* was accompanied by its protection against oxidative stress, improvement in locomotion, and chemoprotective effects (Lee et al. 2010). However, the opposite effect was found in male Canton-S flies and female Ives flies (Lee et al. 2010). Thus, the molecular mechanism by which curcumin extends lifespan is not yet clear.

The free radical theory of aging considers that free radicals, a major determinant of lifespan, can produce oxidative damage to critical biological molecules (Harman 1992). A sustained or excessive increase in reactive oxygen species (ROS) can inflict random damage upon proteins, lipids, and nucleic acids (Valko et al. 2006). Superoxide dismutase (SOD) and malondialdehyde (MDA) are two important parameters to assess antioxidation and oxidation levels related to ROS. SOD is an essential enzyme in a network of biological antioxidants that is endogenously induced in order to eliminate superoxide radicals by conversion to hydrogen peroxide and oxygen (Barik et al. 2005). Among the isoforms of SOD, *CuZn-SOD* with copper (Cu) and zinc (Zn) in its catalytic center is localized to the intracellular cytoplasmic compartments, whereas *Mn-SOD* is primarily found as an important antioxidant enzyme in mitochondria (Miller 2012). MDA is the final product of lipid peroxidation mediated by free radicals (Nair et al. 2007).

To date, no study on the in vivo effects of curcumin on lipid peroxidation and SOD production has been reported for *Drosophila*. The present study aimed to examine the effect of curcumin on lifespan in *Drosophila* and to elucidate its association with curcumin-induced endogenous antioxidant SOD and lipid peroxidation.

Materials and methods

Reagents

Curcumin was purchased from Shanghai Winherb Medical Science Co. Ltd (Shanghai, China). Kits of MDA, SOD, and protein were purchased from Nanjing Jiancheng Bioengineering Company (Nanjing, China). Curcumin and kits were stored at 4°C.

Methanol and acetonitrile were purchased from Tjshield (Tianjing, China). Acetic acid and ethyl acetate were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). All chemicals used were either high-performance liquid chromatography (HPLC) grade or analytical reagents.

Diet

Based on the traditional corn–yeast medium (105-g corn, 75-g sucrose, 40-g yeast, 7.5-g agar, 10-mL propionic acid mixed with hot water to make 1,000-mL diet), the diet was prepared once a week. The base diet was cooked and then cooled to 50–60°C, curcumin extract dissolved in 2-mL ethanol was added to the base diet at 0.5 (C1) and 1.0 (C2) mg/g diet, and the same volume of ethanol, but no curcumin, was added to the base diet as the control (C0). The media were then mixed using a Hamilton Beach® hand mixer and distributed into vials or bottles. The experimental diets in the vials and bottles were packed in dark plastic bags, stored at 4°C, and used within 2–3 weeks. All tested diets were sampled and stored at -80°C for HPLC analysis.

Drosophila husbandry

Flies of the *Oregon-R* strain were used for this study. Flies were fed with either base or supplemented curcumin diets. Adult flies were removed after five days of mating, with the eggs developing into adult flies. Newly emerged flies of the same age were transferred into fresh bottles with base and test diets as described previously and allowed to mate for about 24 h. Three day-old mated female and male flies fed different diets were collected over light CO2 and systematically randomized into standard 50-mL vials with ~10 mL of corresponding base and test diets at a starting density of 30 male and 30 female flies. For each diet, a population of 300–320 flies was raised in 10 vials per sex at the density of ~30 flies per vial. As the flies aged, a similar density (~30 flies per vial) was maintained by combining flies from multiple vials within the same treatment. Flies were kept on a 12-h/12-h light: dark cycle at 25°C and 68 % humidity (Troen et al. 2007). Food was replaced every Tuesday and Friday. Survival was determined by recording dead flies during each transfer. Initial cohort sizes were calculated from the sum of dead flies observed during the whole lifespan. A second cohort of curcumin-feeding experiment of similar population sizes (about 300 flies per sex per diet) was conducted to collect flies at age of 1 day, 7 days, and 21 days for gene expression and SOD activity and MDA assays.

HPLC analysis

The content of curcumin in test diets was measured by HPLC as described (Schiborr et al. 2010). Briefly, curcumin was extracted by 95 % (v/v) ethyl acetate and 5 % (v/v) methanol. Samples (0.5 g) from each diet were weighed, and transferred into a tube containing 5-mL extraction solvent where the mixture was stirred using ultrasonic cleaning for 30 min. The samples were then centrifuged at 5,000 rpm for 10 min and filtrated through 0.45-μm filter membrane. The filtrate was concentrated under nitrogen. The dried residue was dissolved in 5-mL mobile phase and vortex-mixed for 30 s; then, 1.5 mL was transferred into an injection sample vial. A volume of 10 μL was injected into the HPLC system. About 4 mg of curcumin standard was weighed and dissolved directly in 20-mL mobile phase and vortex-mixed for 30 s; then, 10 mL was transferred into another tube containing 10-mL mobile phase. The chromatographic analysis was carried out by an Agilent HPLC system using Eclipse XDB-C_{18} column (4.6× 250 mm, 5 μm) with a mobile phase composed of 49 % (v/v) acetonitrile, 20 % (v/v) methanol, 30 % (v/v) deionized water, and 1 % (v/v) acetic acid. Flow rate was set to 0.4 mL/min. The column temperature was maintained at 25°C. The absorbance of detector was set at 430 nm. Each dietary treatment was prepared at least in three independent replicates. Curcumin standards at concentrations of 23.1, 46.2, 92.5, and 185 μg/mL were prepared, and 10 μL was injected into the HPLC system. The exact concentration of curcumin added in the diets of group C1, C2, and base was determined using HPLC.

Antioxidation assay

The samples (more than 300 flies per sample) of males and females of 7- and 21-day-old flies fed base and test diets were collected, respectively. The samples were frozen in liquid nitrogen and stored at -80°C for future analysis. For each parameter measured, at least three replicates were used from each of the control and treatments. Fifty micrograms of flies of each sample was homogenized in 450-μL physiological saline. Lipid peroxidation products were measured

using commercial MDA kit (Cat A003-1) using a spectrophotometer (PERSEE, Beijing Purkinje General Instrument, Co., Ltd, Beijing) at 532 nm according to the manufacturer's directions. The level of lipid peroxidation was expressed as mmol MDA per mg protein as described by Utley et al. (1967). The SOD activity was measured as reducing nitrite formation in 40 min at 37° C using a commercial SOD kit (Cat A001-1, Nanjing Jiancheng Bioengineering Company, China) according to the manufacturer's directions. This assay is based on the inhibition of nitrite formation from hydroxyl ammonium in the presence of O_2^- generators mediated by SOD. The spectrophotometric absorbance was set at 550 nm, and the results were expressed as units of SOD activity per mg protein as described by Elstner and Heupel (1976). The content of proteins was measured using commercial kit (Cat A045-2, Nanjing Jiancheng Bioengineering Company, China) and detected at 595 nm by a spectrophotometer according to the manufacturer's directions. SOD and MDA levels were measured in replicates of at least three independent samples.

Quantitative RT-PCR analysis

Each sample of flies (more than 100 flies per sample) was collected when the flies were at ages of 1 day and 21 days, separated by sex, and by diet. The samples were frozen in liquid nitrogen and stored at -80°C prior to all analyses. Total RNA was extracted from each sample using RNAiso Plus (TakaRa) and then was converted into cDNA using a PrimeScrit RT reagent Kit (TakaRa) according to the manufacturer's protocol. The seven genes related to aging, immune, and stress responses based on Lee et al. (2010): *Superoxide dismutase* (*SOD2, Mn-SOD*), *copper-zinc Superoxide dismutase* (*SOD1, CuZn-SOD*), *Insulin-like receptor* (dInR), *Attacin-D* (*ATTD*), *Defensin* (*Def*), *Cecropin B* (*CecB*), and *Diptericin B* (*DptB*), were selected for gene expression analysis using quantitative RT-PCR. The *Ribosomal protein 49* (*rp49*) gene was used as an internal control. Primers were synthesized by TakaRa. Mastercycler® ep *realplex* Real Time System (Eppendorf, Hamburg, Germany) and SYBR Premix Ex Taq (Tli RNaseH Plus) (TaKaRa, Japan) was used in the quantitative RT-PCR analysis. Comparative cycle threshold (Ct) was measured to quantify levels of gene expression. To measure gene expression changes in response to curcumin feeding, levels of gene expression in all groups were expressed as a ratio to the value of the control group (C0) at day 1. All experiments were repeated three times, and the data are presented as mean ± SD.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). Lifespan and mortality analyses were performed separately by sex on pooled data of the two cohorts. Survivorship between treatments were compared and tested for significance with log-rank tests. Survivorship is a cumulative function where differences between male and female flies are carried forward to subsequent age intervals. In some cases, this can inflate the effects of log-rank tests (Troen et al. 2007). In biochemical analyses, the levels of MDA and SOD were estimated at least in three independent replicates, and results are reported as mean ± standard deviation (SD). We conducted one-way ANOVA using Dunnett's test, with $p<0.05$ being statistically significant. Comparison in gene expression based on RT-PCR between the test diets and base diet was conducted using the comparative cycle threshold method (Lee et al. 2010). Correlation between SOD activity and MDA level was examined using a linear regression model.

Results

Stability of curcumin in diets

The HPLC retention time of curcumin was measured approximately at 9.44 min. The standard curve for curcumin was highly linear ($R^2>0.999$) within the range of 23.1–185 μg/mL. The tested diet C1, C2, and base diet contained 0.28±0.06, 0.66±0.05, and 0 mg/g, respectively, indicating that the curcumin in the test diets was chemically stable during the study period.

Effect of supplemented curcumin on lifespan in *Drosophila*

Survivorship (Fig. 1) differed significantly between diets by log-rank test ($p=0.0001$). The lifespan and change (%) of maximum mean and lifespan quartiles for each group are given in Tables 1 and 2, respectively. In females, diets C1 and C2 extended mean lifespan by 15.5 % from 24 to 28 days and by 12.7 % from 24 to 27 days compared to flies fed the base diet, respectively. In males, diets C1 and C2 increased mean

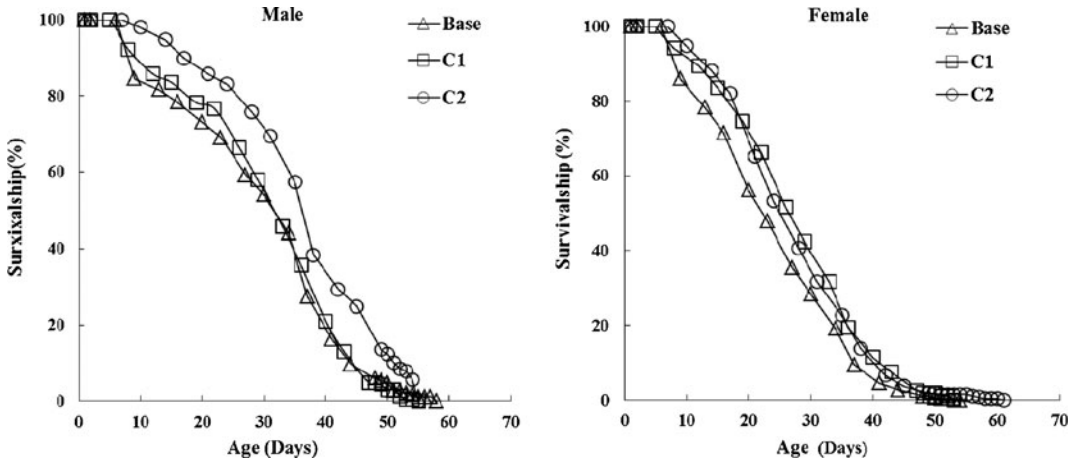

Fig. 1 Survivorship of *Drosophila* fed with supplemented curcumin and base diets. The survivorship curves show the proportion of living flies for each diet as a function of age. *Triangle* = the base diet (C0, 0 mg curcumin/g), *square* = C1 (0.5 mg curcumin/g, *circle* = C2 (1.0 mg curcumin/g). The median, top, and bottom quartile life spans are the ages at which the curves intersect with 50 %, 25 %, and 75 % survivorship (see Table 1), respectively

lifespan by 6.2 % from 29 to 31 days and by 25.8 % from 29 to 37 days compared to flies fed the base diet, respectively. This suggests that the mean lifespan extension in males was correlated with dietary curcumin. C2 (1.0 mg/g diet) was effective in extending mean lifespan in males, whereas C1 (0.5 mg/g diet) was more effective on lifespan extension in females.

Diet C2 extended lifespan for male flies in the top (25 %), median, and bottom (75 %) quartiles by 9.8 %, 26.7 %, and 75 %, respectively, but failed to extend the maximum lifespan (Table 2). In female flies, diet C2 increased lifespan in the top, median, bottom quartiles, and maximum by 2.9 %, 4.3 %, 31.3 %, and 13.0 %, respectively. On the other hand, diet C1 extended lifespan of male flies in median and bottom quartiles by 10.0 % and 37.5 %, respectively, but failed to extend the maximum and top quartile lifespan. In female flies, this diet extended lifespan in the top, median, and bottom quartiles by 5.9 %, 13.0 %, and 18.8 %, respectively, but failed to extend maximum lifespan.

The effect of curcumin supplementation on MDA level in *Drosophila*

The MDA levels of both male and female flies (7 and 21 days old) fed different diets are illustrated in Fig. 2. In 7-day-old flies, in relation to the base diet, diet C1 and C2 decreased the mean MDA levels by 26.6 % and

Table 1 Effect of curcumin at different concentrations on the lifespan in *Drosophila*

Gender	Diet*	Dead#	Maximum (d)	Mean (d)	Percentiles (±SE)		
					25th (d)	Median (d)	75th (d)
Male	C1	233	55	30.9±12.6[a]	40±0.82	33±1.01	22±1.83
	C2	177	55	36.6±11.9[b]	45±1.44	38±0.89	28±1.57
	Base	183	58	29.1±13.3[a]	41±0.79	30±1.52	16±3.10
Female	C1	281	53	27.6±11.1[b]	36±0.87	26±0.83	19±1.05
	C2	271	61	26.9±10.2[b]	35±0.99	24±0.80	21±0.85
	Base	286	54	23.9±10.8[a]	34±0.95	23±0.90	16±1.20

*The diet C1 and C2 were supplemented with 0.5- and 1.0-mg curcumin in 1-g base diet, respectively

Number of total dead flies.

Means shared the same letter (a or b) do not significantly differ (p>0.05) from each other, i.e., "a" vs "a" or "b" vs "b" do not differ significantly, whereas "a" vs "b" does (p=<0.05)

Table 2 Lifespan change (%)[a] of *Drosophila* fed to supplemented curcumin with different contents

Gender	Diet[b]	Maximum	Mean	25th	Median	75th
Male	C1	-5.1	6.2	-2.4	10.0	37.5
	C2	-5.1	25.8	9.8	26.7	75.0
Female	C1	-1.9	15.5	5.9	13.0	18.8
	C2	13.0	12.6	2.9	4.3	31.3

[a] Lifespan change (%) = (Treatment lifespan-reference lifespan)/reference lifespan×100, base diet is used as reference

[b] Diets are the same as in Table 1

27.8 % in females and by 25.6 % and 38.3 % in males ($p<0.05$), respectively (Fig. 2a, b). Similarly, in 21-day-old flies, compared to the base diet, diet C1 and C2 decreased mean MDA levels by 25.5 % and 70.2 % in females and by 34.6 % and 37.6 % in males ($p<0.01$), respectively (Fig. 2a, b). This suggests that the MDA level in *Drosophila* tends to correlate negatively with age and supplemented curcumin concentration.

The effect of dietary curcumin supplements on SOD activity in *Drosophila*

The SOD activity of both sexes at 21 days old on different diets is illustrated in Fig. 3. The level of SOD activity for both male and female flies on diets supplemented with curcumin was higher than that of the *Drosophila* fed the base diet. Compared to the base diet (the control), the C1 and C2 diets increased mean SOD activity in females by 13.8 % and 32.0 % ($p<0.01$), respectively, and increased the activity in males by 8.4 % and 16.7 %, respectively (Fig. 3). To examine the relationship between SOD activity and MDA levels, a linear regression analysis was used. The results showed that SOD activity was negatively correlated (r=-0.32 and p=0.304 in males, r=-0.21 and p=0.018 in females) with MDA levels in both sexes in 21-day-old *Drosophila* (Fig. 4).

The effect of curcumin on age-related gene expression

The effect of curcumin on age-related changes in gene expression was evaluated by quantitative RT-PCR. To measure gene expression changes in response to

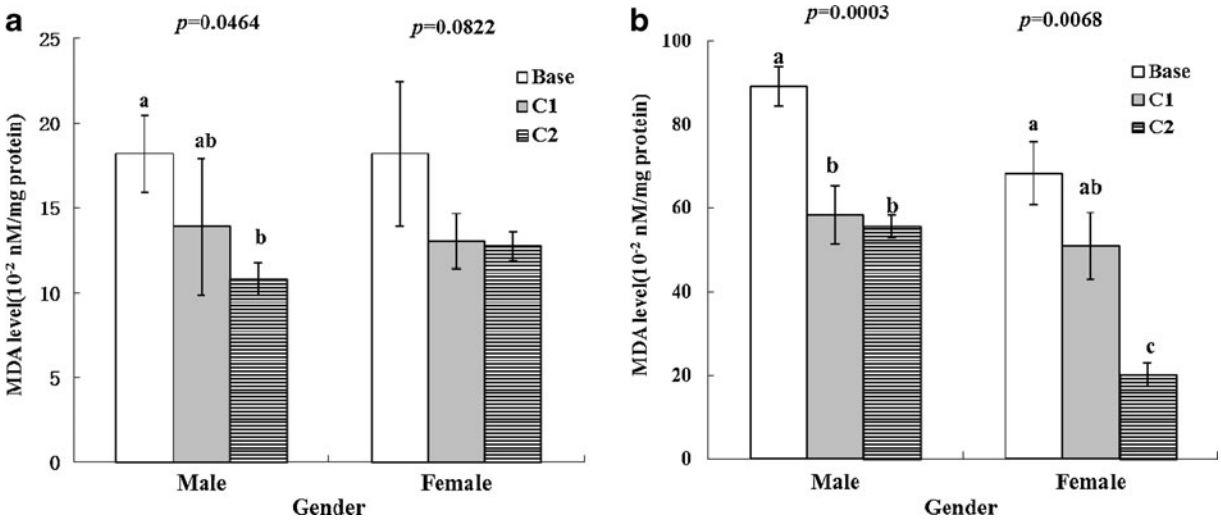

Fig. 2 The effect of supplemented curcumin at different concentrations on the MDA levels in *Drosophila.* The contents of MDA in vivo of 7- and 21-day-old male and female flies were detected with 10 % tissue homogenate of 50 mg flies, respectively. **a** *Drosophila* at 7 days old, **b** *Drosophila* at 21 days old. Data are mean ± SD, n=3 with each sample of about 100 flies. *Bars* shared the same letter (*a*, *b*, or *c*) do not significantly differ ($p>0.05$) from each other, i.e., "a" vs "a," "b" vs "b," or "a" vs "ab" do not differ significantly ($p>0.05$), whereas "a" vs "b" does ($p\leq0.05$)

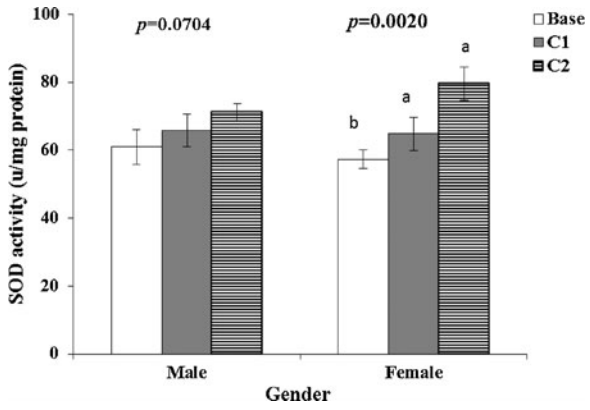

Fig. 3 The effect of supplemented curcumin at different concentrations on the SOD activity in *Drosophila.* The SOD activity in vivo of 21-day-old male and female flies was detected with 10 % tissue homogenate of 50 mg flies, respectively. Data are mean ± SD, n=3 with each sample of about 100 flies. *Bars* shared the same letter (*a* or *b*) do not significantly differ ($p>0.05$) from each other, i.e., "a" vs "a," "b" vs "b," or "a" vs "ab" do not differ significantly ($p>0.05$), whereas "a" vs "b" does ($p\leq0.05$)

curcumin feeding, levels of gene expression in all groups at day 21 were expressed as a ratio of the value of day 1 in the control group (Figs. 5 and 6). As shown in Fig. 5, compared to the control (no curcumin), expression of *MnSOD(Mn) and CuZn SOD* genes increased or did not change significantly in 21-day-old flies on curcumin diets, especially in females. This observation is consistent with the increased SOD activity and decreased MDA levels measured at day 21 as described previously, suggesting that curcumin acts as an antioxidant in both intracellular cytoplasmic

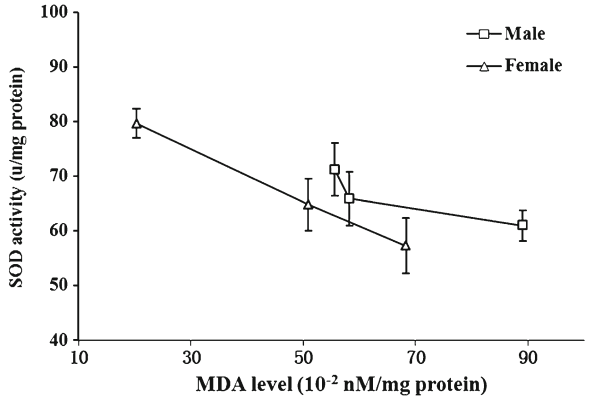

Fig. 4 The association of MDA level and SOD activity in *Drosophila* at 21 day old. Linear regressions were used to determine the relationship between SOD activity (*y*) and MDA level (*x*) separated by gender in *Drosophila*

compartments and mitochondria. Furthermore, in relation to the control, expression of *dInR*, *ATTD*, *Def*, *CecB*, and *DptB* was decreased or unchanged in response to dietary curcumin. Among the down-regulated genes, *DptB* showed the largest decrease, especially in females (Fig. 6). The degree to which the down-regulated genes show a change in expression is more dramatic in females than in males. These observations are consistent with the observations by Lee et al. (2010).

Discussion

The present study found that supplemented curcumin significantly prolonged mean lifespan of *Drosophila*. The beneficial effects of dietary curcumin are likely due to increased SOD activity and decreased accumulation of MDA. Diets C1 (0.5 mg/g) in females and C2 (1.0 mg/g) in males provided effective doses of curcumin for lifespan extension and improving biochemical measures related to aging. Lifespan extension was associated with the up-regulation of *Mn-SOD*, *CuZn-SOD*, and the down-regulation *of dInR*, *ATTD*, *Def*, *CecB*, *and DptB*. This suggests that curcumin could effectively inhibit oxidative stress associated with aging in *Drosophila*.

Damage to various cell components and activation of specific signaling pathways are two important biochemical events that are associated with aging and age-related diseases and modulated by oxidative stress. Oxidative stress by ROS contributes to aging through damage to lipids, proteins, and DNA in various tissues of many species (Martin and Grotewiel 2006). For example, hydroxyl radicals react with all components of DNA molecules by damaging both purine and pyrimidine bases. Besides DNA, ROS attacks polyunsaturated fatty acid residues of phospholipids (Valko et al. 2007). Protein conformational diseases such as Alzheimer, which can arise due to pathogenic accumulation of abnormal proteins, are associated with excessive production of reactive oxygen species (Calabrese 2007).

Curcumin is the main bioactive polyphenol in turmeric. In general, the antioxidant activity of polyphenols is related to the number of hydroxyl groups present on the aromatic ring structures (Rossi et al. 2008). In addition to the presence of two hydroxyl groups, a highly activated carbon atom exists between the two methoxyphenol rings. The C–H bonds on this carbon are very weak at pH below 7 and can serve as

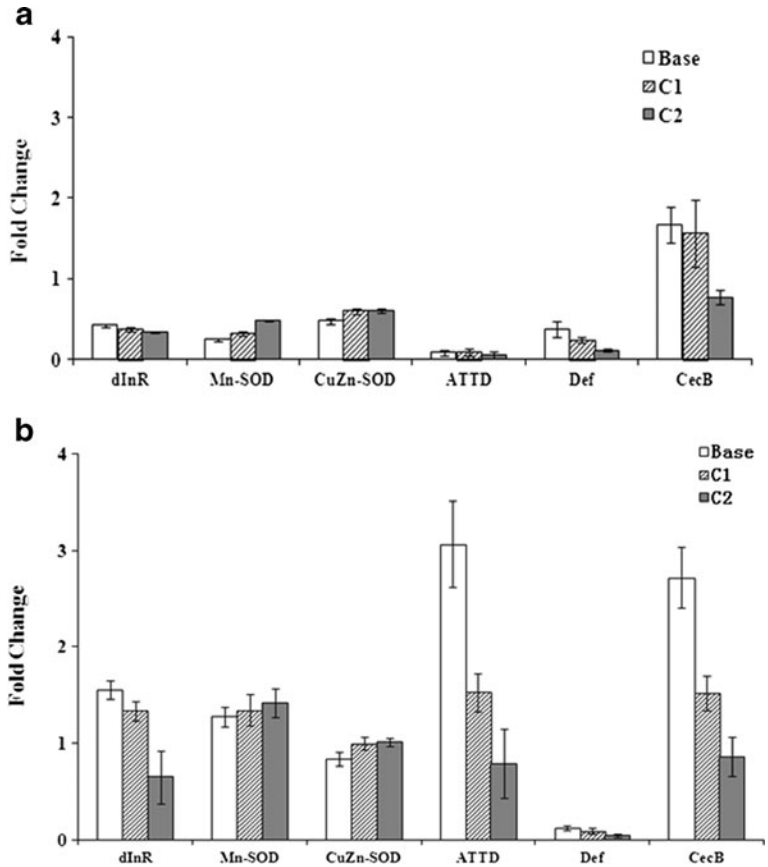

Fig. 5 Gene expression analysis of age-related genes using real-time PCR analysis in male (**a**) and female (**b**) of Oregon-R flies at 21 days old reared on control, C1, and C2 diets. Data are shown as fold changes of the base diet at day 1 based on three replicates using real-time PCR experiments. *SOD2* or *Mn-SOD* superoxide dismutase, *SOD1* or *CuZn-SOD* copper-zinc superoxide dismutase, *dInR* insulin-like receptor, *ATTD* Attacin-D, *Def* Defensin, *CecB* Cecropin B

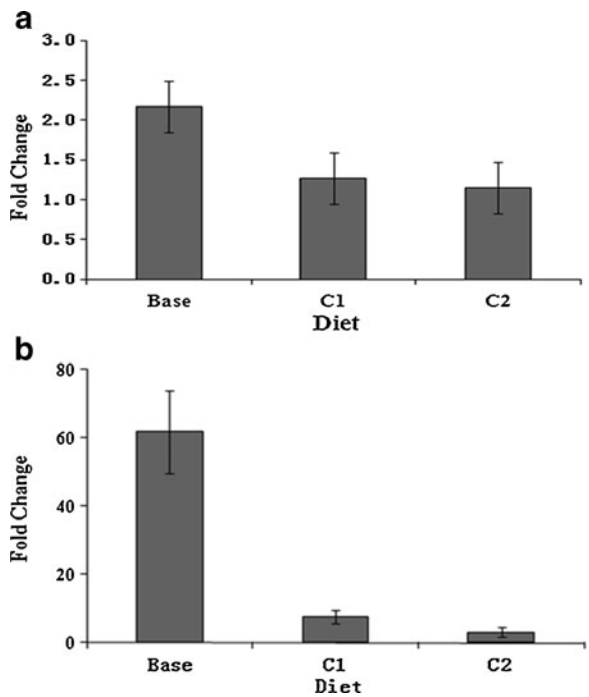

Fig. 6 Gene expression analysis of the *DptB* gene using real-time PCR analysis in male (**a**) and female (**b**) of *Oregon-R* flies at 21 days old reared on control diet, diet C1, and diet C2. *DptB* diptericin B

potent H-atom donors in scavenging free radicals (Jovanovic et al. 1999). In this regard, a number of studies have established the anti-oxidative activity of curcumin. For example, curcumin possesses potent activity inhibiting both initiation and propagation phases of low-density lipoprotein oxidation and preventing lipid peroxidation (Naidu and Thippeswamy 2002; Patro et al. 2002). In addition, curcumin effectively protects rat liver mitochondria from protein oxidation induced by free radicals (Patro et al. 2002; Wei et al. 2006).

MDA and SOD activity induced by curcumin has been estimated indirectly by supplementing with disulferam, an inhibitor of SOD activity using a small number of combined male and female flies (Suckow and Suckow 2006). Lee et al. (2010) found that curcumin at 100 μM extended lifespan of Canton-S female flies by 19 % and at 250 μM extended lifespan of Ives males by 16 %. The lifespan extension of curcumin in Canton-S was shown to be associated with changes in the expression of five age-associated genes: *dInR*, ToR, Hep, sun, and *mth*. The stress resistance of curcumin was measured with the percent survival of flies exposed to the free radial generator: hydrogen peroxide and paraquat within 36 h (Lee et al. (2010). However, the actual curcumin concentrations in all test diets were not determined, and the SOD activity and MDA levels in *Drosophila* fed curcumin were not measured in those experiments. The effects of curcumin on age-related genes were analyzed with a mixture of both sexes. In our curcumin feeding study, SOD activity, MDA levels, and age-related genes were measured directly in male and female flies separately fed higher doses of curcumin. We found that curcumin increased SOD activity and decreased the accumulation of MDA in *Drosophila* through its antioxidant activity in both the intracellular cytoplasmic compartments and mitochondria. In addition, we observed that two *SOD* genes (*Mn-SOD* and *CuZn-SOD*) showed increased expression and other age-related genes exhibited decreased expression in flies fed curcumin when compared to the control diet. Furthermore, gene expression changes were more apparent in females than in males. This could be due to the difference in biological ages between males and females. As shown in the survival curve (Fig. 1.), by day 21, almost half of the females were dead, whereas up to 70 % of the males were still alive. Our results further showed that the effective dose of curcumin for lifespan extension is different between male and female flies.

The present result is consistent with the previous study by Bala et al. (2006), in which chronic curcumin treatment on both 6- and 24-month-old rats resulted in significant increases in SOD activity in various brain regions compared to normal aging rats. Additionally, curcumin significantly decreased the level of superoxide anion which was induced by homocysteine in rat brain hippocampus, suggesting that curcumin might prevent oxidative damage in that tissue (Ataie et al. 2010).

Motterlini et al. (2000) reported that curcumin reduced oxidative stress by up-regulating the expression of *ho-1* to increase the activity of heme oxygenase in bovine aortic endothelial cells. In addition, curcumin inhibited NF-κB, which is the main mediator of inflammation, to activate the expression of many pro-inflammatory cytokines (Sikora et al. 2010). Furthermore, curcumin decreased or blocked the mammalian target of rapamycin (*mTOR*) whose function is to integrate the input from multiple upstream pathways and to act as a sensor of cellular nutrient and energy levels and of redox status in cells (Beever et al. 2009; Sikora et al. 2010). Lifespan extension by decreasing activity or expression of *mTOR*, *insulin*, *JNK*, and *Methuselah* signaling pathways in *Drosophila* has been demonstrated (Kapahi et al. 2004). Whether curcumin also can decrease flux through these pathways in *Drosophila* remains to be investigated.

In summary, this study has demonstrated that curcumin could increase mean lifespan of *Drosophila* via regulating gene expression of the key enzyme SOD and age-related genes, and by reducing accumulation of MDA. Male and female flies respond differentially to doses of curcumin in terms of lifespan extension, oxidative stress, and expression of age-related genes.

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