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Green tea catechins and broccoli reduce fat-induced mortality in *Drosophila melanogaster*☆

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Abstract

Dietary fat accelerates the ageing process and causes a greater mortality by accumulating lipid hydroperoxide (LPO) in *Drosophila melanogaster*. The present study found that the life span of *D. melanogaster* was shortened from 54 to 6 days in a dose-dependent manner when fat in diet increased from 0% to 25%. The results showed that supplementation of both green tea catechins (GTC) and broccoli extract (BE) reversed partially the fat-induced mortality. The maximum life span was 44 days for the control group fed with a 5% fat, whereas it increased to 50 and 59 days in the GTC- and BE-supplemented groups, respectively. The 50% survival time for the control flies fed with a 5% fat diet was 30 days. In contrast, it increased to 32 and 48 days when GTC and BE were supplemented in the diet. This was consistent with a significant reduction in total body LPO level in *D. melanogaster* maintained on the GTC- and BE-supplemented diet. Accordingly, catalase and superoxide dismutase (SOD) activities increased significantly in the flies fed with a GTC or a BE diet compared with those fed with a control 5% fat diet. Reverse transcriptase–polymerase chain reaction analysis indicated that the increase in enzymatic activities of catalase and SOD was accompanied by up-regulation of genes for catalase, copper–zinc containing SOD and manganese-containing SOD. It was concluded that GTC and BE reversed the fat-induced mortality in *D. melanogaster*, most likely but necessarily solely, by up-regulation of endogenous antioxidant enzymes.

Keywords: Broccoli; Catalase; Green tea catechins; Life span; Lipid peroxidation; Superoxide dismutase

1. Introduction

The link of a high fat intake with ageing in humans remains unknown. It also remains controversial if dietary fat increases the risk of chronic diseases including coronary heart diseases (CHD), stroke and cancer. It has been reported that countries with a greater consumption of fat had much greater morbidity and mortality of CHD and cancer than those countries with a lesser fat intake [1,2]. Systematic reviews support the view that reduction of dietary fat consumption is associated with reduced risk of cardiovascular disease [3–5]. However, the Women's Health Initiative Randomized Controlled Dietary Modification Trial failed to demonstrate that a dietary intervention that had reduced total fat intake with increased intakes of vegetables, fruits and grains decreased the risk of CHD, stroke and colorectal cancer [6,7].

Free radicals have been implicated in ageing [8]. Aerobic organisms develop a cellular metabolism that adapts oxygen as an electron acceptor while it continuously generates reactive oxygen species (ROS), namely, hydroxyl radical, superoxide anion and hydrogen peroxide. Aerobic organisms also possess antioxidant defense systems that can effectively remove these ROS. One of antioxidant defense systems consists of a series of enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase [9]. In addition to ROS, other free radicals also exist as products of nonenzymatic reactions of oxygen and oxidants. In this regard, a wide array of nonenzymatic antioxidants are also

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present in diets, including ascorbic acid, vitamin A, vitamin C, α-tocopherol and plant flavonoids [10]. These dietary antioxidants and antioxidant enzymes work synergistically or independently to scavenge the free radicals [11]. Theoretically, these antioxidant systems shall terminate the propagation of the free radicals reactions, limit the formation of new free radicals and slow down the ageing process.

Fruit fly, *Drosophila melanogaster*, is one of the most commonly used models to investigate the genetic determinants of ageing [12]. It had been shown that a high-fat diet accelerated the ageing process and shortened the life span of *D. melanogaster* due to accumulation of lipid hydroperoxide (LPO) products [13]. Both green tea and broccoli are rich in antioxidants [14,15]. We hypothesized that supplementation of green tea catechins (GTC) and broccoli extract (BE) could reverse the fat-induced mortality and prolong the life span of *D. melanogaster* fed with a high-fat diet. It had been reported that the long-lived strain of *D. melanogaster* had a significantly increased expression and activity of SOD and catalase compared with the normal-lived strain [16]. We therefore focused on the interaction of dietary GTC and BE with gene expression of endogenous antioxidant enzymes, namely, SOD and catalase in fruit flies reared on a high-fat diet.

2. Materials and methods

2.1. Fly line and diet

D. melanogaster (Oregon-R-C) was obtained from Bloomington Drosophila Stock Center, Department of Biology, Indiana University, Bloomington, IN, USA. Basal fly diet was prepared according to the standard formulation described by Roberts and Standen [17]. In brief, 1000 ml of diet contained 105 g cornmeal, 21 g yeast, 105 g dextrose and 13 g agar. Ethyl-4-hydroxybenzoate (0.4%) was added to inhibit the growth of mold. To the basal diet, various amounts of fatty acids derived from lard, GTC and BE were added. The mixture was cooked. For rearing the stocks, 15 ml of the cooked mixture was poured into each vial. For the experimental flies, 5 ml of the cooked mixture was prepared per vial.

2.2. Preparation of GTC and BE

GTC from Chinese Longjing green tea was extracted as previously described [18]. Dry green tea (700 g) was soaked in 4 L of boiling water for 30 min; the infusion was filtered and condensed in a rotary evaporator. Equal volume of chloroform was used to remove the caffeine. After the chloroform was removed, the aqueous phase was extracted using an equal volume of ethyl acetate. GTC (30 g) was obtained after the ethyl acetate was evaporated in a rotary evaporator. The content of GTC was analyzed and quantified using a Shimadzu LC-10AD HPLC (Tokyo, Japan) equipped with a ternary pump delivery system. The high-performance liquid chromatography (HPLC) analysis found that it contained 62% epigallocatechin gallate, 19% epigallocatechin, 7% epicatechin ad 9% epicatechin gallate.

BE was obtained from the washed fresh broccoli using a juice extruder. The juice was filtered and subjected to freeze drying. The resultant extract powders were stored at −20°C. In general, 90–100 g of fresh vegetable tissue produced 150 ml fresh vegetable juice and subsequently 10 g of dry vegetable extract powders.

2.3. Preparation of fatty acids from lard

Lard was regarded as antioxidant-free compared with other dietary fat [19]. Free fatty acids were prepared according to Gerhardt et al. [20] with some modification. In brief, 35 g potassium hydroxide was ground into power and dissolved into 2 L methanol solution containing 130 g of lard. The mixture was then heated for 2 h in a water bath under a gentle stream of nitrogen gas to prevent oxidation. The methanol was removed in a rotary evaporator. The resultant saponified mixture was then acidified using 10% sulfuric acid to precipitate the free fatty acids followed by washing five times with distilled water. The free fatty acids were then stored at −20°C.

2.4. Dose effect of fatty acids on life span

Male flies were divided into seven groups with 200 flies in each group rearing in 10 vials (n=20 flies per vial). The first group was maintained on the control diet, while the remaining six groups were fed with one of the six diets containing 1%, 5%, 10%, 15%, 20% and 25% lard fatty acids. The dead flies were counted every 3 days, and the remaining alive flies were then transferred to a new vial containing the same diet. The feeding experiment lasted for about 60 days.

2.5. Effect of GTC and BE on life span

Male flies were maintained on diets containing 5% and 10% lard fatty acids with or without supplementation of 10 mg GTC or 50 mg BE per milliliter. For each set of fat diet, 1800 flies (600 for the control and 1200 for the GTC and BE groups) were used with 20 flies per vial containing 5 ml of diet. The flies were incubated at 25°C. Every 2–3 days, flies were transferred to a new vial containing the same diet. At day 10, half of the flies in each group were first starved for 2 h and then killed by storing at −80°C for 10 min. At day 10, the remaining flies were similarly killed.

2.6. Measurement of LPO

LPO level was measured using an LPO assay kit (Cayman Chemical, Ann Arbor, MI, USA), which measured the amount of hydroperoxides reacted with ferrous ions and resulted in the formation of ferric ions. The ferric ions formed were detected using thiocyanate ion as the chromogen. The flies were maintained on either the control diet or the experimental diet containing 10 mg GTC or BE per milliliter for a period of 10 or 20 days. In each measurement,

every 100 fruit flies (n=3×100) were weighted and homogenized in 2 ml of HPLC-grade water. Debris was spun down at the speed of 1500×g for 5 min at 4°C. Supernatant (500 μl) was aliquoted into a tube in triplicate. The sample was deproteinized and extracted using methanol/chloroform (vov/vol, 1:2) saturated with nitrogen gas and followed by centrifugation at 1500×g for 5 min at 0°C. Fifty microliters FTS Reagent 1 containing 4.5 mM ferrous sulfate in 0.2 M hydrochloric acid and 50 μl ferric thiocyanide solution (FTS) Reagent 2 containing 3% methanolic solution of ammonium thiocyanate were added. After being incubated for 5 min at room temperature, absorbance of each sample was measured in a spectrometer at 500 nm with 1 ml quartz cuvette.

2.7. SOD activity

SOD activity was quantified using an SOD assay kit (Cayman Chemical). In brief, SOD present in the sample competes with the superoxide radicals with tetrazolium salt. As SOD concentration increased, the rate of formazan dye formed from tetrazolium salt decreased. Flies (100 per homogenization, n=3×100) were homogenized in 1 ml of cold 20 mM HEPES buffer (pH 7.2, with 1 mM EGTA, 210 mM mannitol and 70 mM sucrose). Debris was spun down at a speed of 1500 g for 5 min at 4°C. The supernatant was transferred to a tube on ice and then subjected to centrifugation at 10,000×g for 15 min at 4°C. The supernatant contained the cytosolic copper–zinc-containing SOD (CuZnSOD), and the pellet contained mitochondrial manganese-containing SOD (MnSOD). The supernatant was removed to a new eppendorf, and the mitochondrial pellet was suspended in 0.5 ml cold HEPES buffer. The sample (10 μl) in triplicate was used for each test. The radical detector (200 μl) containing tetrazolium salt was added onto 96-well plates together with 10 μl sample. The reaction was initiated by adding 20 μl of diluted xanthine oxidase (147 mU/ml) followed by shaking the plate for 20 min at room temperature. After incubation, the absorbance was recorded at 450 nm with a microplate reader.

2.8. Catalase activity

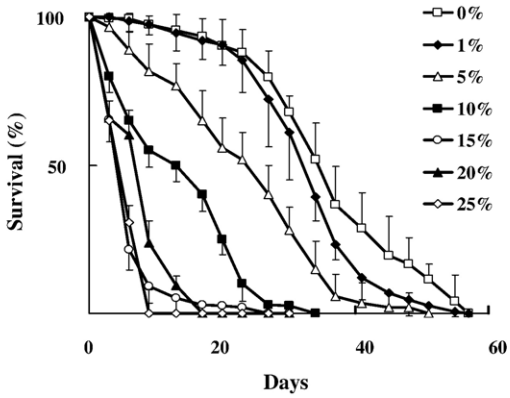
A catalase assay kit (Sigma, St. Louis, MO, USA) was used to measure the catalase activity. In brief, this method measured the remaining hydrogen peroxide substrate after it reacted with the catalase present in the sample. The remaining hydrogen peroxide was measured with 3,5-dichloro-2-hydroxybenzene-sulfonic acid, which couples oxidatively to 4-aminoantipyrine in the presence of hydrogen peroxide and horseradish peroxidase to give a red quinoneimine dye. Flies (n=100 per homogenization, n=3×100) were homogenized in 1 ml enzyme dilution buffer (5 mM potassium phosphate buffer, pH 7.0, containing 0.1% Triton ×100). Debris was centrifuged at the speed of 1500×g for 5 min at 4°C. The supernatant was transferred to a new tube on ice and diluted by mixing 3 μl of the supernatant with 42 μl 1× assay buffer (5 mM potassium phosphate buffer, pH 7.0) in triplicate. The resultant sample (10 μl) was again diluted with 65 μl of 1× assay buffer. Then, 25 μl of 200 mM hydrogen peroxide solution was added as the substrate solution to initiate the reaction. At exactly 1 min, 900 μl of stop solution (15 mM sodium azide) was added. The reaction mixture (10 μl) was mixed with 1 ml of color reagent containing 0.25 mM 4-aminoantipyrine, 2 mM 3,5-dichloro-2-hydroxybenzenesulfonic acid and freshly added peroxidase (0.8–1.2 U/mg). After incubation for 15 min at room temperature, absorbance of each sample was measured in a spectrometer at 520 nm.

2.9. RNA isolation

Total RNA was isolated from 12 flies (n=3×12) in triplicate by *TRI*zol Reagent (Invitrogen Corporation, Carlsbad, CA, USA). *TRI*zol Reagent (800 μl) was added, and the flies were homogenized in an eppendorf. It was then centrifuged at 12,000×g at 4°C for 10 min. Debris was removed, and the homogenized samples were incubated at room temperature for 5 min followed by the addition of 160 μl chloroform. After 3 min, the samples were subjected to centrifugation at 12,000×g at 4°C for 15 min. The upper layer was transferred to a new Eppendorf with 400 μl of isopropanol and then stored at −20°C overnight. After overnight incubation, samples were subjected to centrifugation at 12,000×g at 4°C for 10 min. The supernatant was discarded, and the RNA pellets were mixed with 1 ml of 75% ethanol for washing. The RNA was repelleted by centrifugation and subjected to be air dried. Finally, 25 μl of DEPC-treated water was used to resuspend the RNA pellet. For DNase digestion, 3 μl 10× DNase buffer, 0.5 μl DEPC-treated water and 1.5 μl DNase (Promega Corporation, Madison, WI, USA) were added and incubated at room temperature for 15 min followed by the addition of 3 μl EDTA and then incubation at 65°C for 10 min. Sodium acetate (4 μl, 3 M) and 3 μl DEPC-treated water were mixed. Eighty microliters of 100% ethanol was introduced, and then the samples were stored at −80°C overnight. After incubation, the samples were centrifuged at 12,000×g at 4°C for 15 min, and 1 ml 70% ethanol was added afterwards for washing. Followed by being repelleted and air dried, the RNA pellet was dissolved in 30 μl DEPC-treated water and stored at −80°C. The quantity and purity of RNA were determined by absorbance reading at 260 and 280 nm. The quantity of RNA (mg ml^{-1}) was calculated by the formula OD_{260}×40 mg ml^{-1}, and the ratio of OD_{260}/OD_{280} should be higher than 1.8 to show a high purity of RNA.

2.10. Primer sequences

The forward and reverse primer sequences for CuZnSOD were 5′-taaattgattaattcattcg-3′ and 5′-acatcggaatagattatcgc-3′; for MnSOD, they were 5′-gcagatatgttcgtggcccgta-3′ and 5′-agttgcagtttgcccgacttct-3′; for catalase, they were 5′-



	Maximum Lifespan of Last Fly (Days)	50 % Survival (Days)	Mean Lifespan (Days)
0 %Fat	55	38	32±1.9[a]
1 %Fat	55	36	31±2.2[ab]
5 %Fat	48	25	25±1.3[b]
10 %Fat	30	13	17±2.6[bc]
15 %Fat	23	6	7±3.9[c]
20 %Fat	13	7	10±1.3[c]
25 %Fat	6	4	4±1.7[c]

Fig. 1. Life span curve of fruit flies fed with diets containing 0% to 25% lard fatty acids. Data are expressed as the maximum life span of the last fly, 50% survival time and mean life span (n=200 flies per group, n=20 flies per vial). The flies were incubated at 25°C. [a,b,c]Means at the same column with different superscripts differ significantly at $P<.05$.

ttcctggatgagatgtcgcact-3′ and 5′-ttctgggtgtgaatgaagctgg-3′, respectively.

2.11. Reverse transcriptase polymerase chain reaction

A reverse transcriptase–polymerase chain reaction assay was carried out to quantify the m RNA level. RNA (5 μg) was responsible for cDNA synthesis together with the addition of oligo dT, 5×1st strand buffer (with DTT), 10 mM dNTP, RNA inhibitor and M-MLV reverse transcriptase (Promega Corporation). The final volume was diluted to 20 μl. cDNA was amplified by polymerase chain reaction (PCR). In each reaction, 0.4 μl 10 mM dNTP, 1 μl cDNA template, 5 mM of each primer, 10× PCR buffer, 1 U Taq polymerase (Promega Corporation) and double-distilled water were added to obtain a final volume of 20 μl. The PCR products were separated on a 1.5% agarose gel, stained with 0.01 μg/ml ethidium bromide, and photographed.

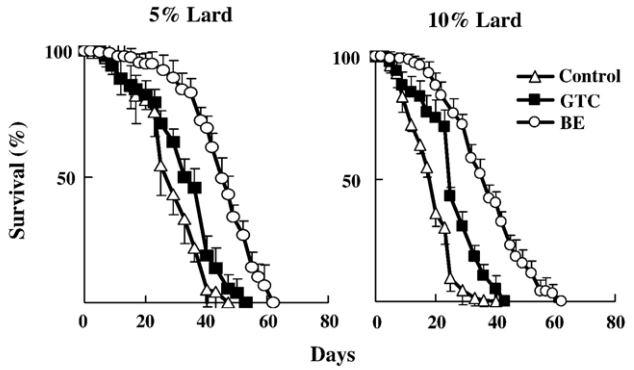
2.12. Statistics

Data were expressed as mean±S.D. Each treatment against the control was compared using Dunnett's method, while the significance across all treatments and treatment trend were assessed using Bonferroni's method. Significance was defined as a P value of less than .05 (SPSS version 11.0, Statistical Package for the Social Sciences software, SPSS Inc, Chicago, IL, USA). $P<.05$ was considered statistically significant.

3. Results

The present study demonstrated clearly that fat accelerated the ageing process of *D. melanogaster* in a dose-dependent manner. As shown in Fig. 1, both 50% survival time and mean survival time of the flies were inversely related to the amount of lard fatty acids in the diet. When the life span was calculated by the survival time of the last dead fly, the control group (0% fat) reached a maximum life span of 55 days, but the 25% fat group had only 6 days; the life span was shortened by 91%. In addition, 50% survival time decreased as the amount of fat increased. It was found that the 0% fat group had a 50% survival time of 38 days, whereas the 25% fat group had only 4 days. When the fat was increased from 0 to 25%, the mean life span of the flies was decreased from 32 to 4 days.

Supplementation of both GTC and BE reversed the fat-induced ageing. As shown in Fig. 2, the maximum life span was 44 days for the control group fed with a 5% fat diet, whereas it increased to 50 and 59 days in the GTC- and BE-supplemented groups, respectively. The 50% survival time for the control flies fed with a 5% fat diet was 30 days. In contrast, it increased to 32 and 48 days when GTC and BE were supplemented in the diet. The control had a mean life span of 25 days, whereas that of the GTC and BE groups increased to 29 and 40 days. A similar trend was observed in the flies fed with a 10% fat diet. The maximum life span was



	Maximum Lifespan of Last Fly (Days)	50 % Survival (Days)	Mean Lifespan (Days)
5 % Lard Diet			
Control	44	30	25±1.9[c]
GTC	50	32	29±2.3[b]
BE	59	48	40±2.7[a]
10 % Lard Diet			
Control	33	20	17±1.9[c]
GTC	40	25	24±1.8[b]
BE	59	28	33±1.6[a]

Fig. 2. Effect of GTC and BE on life span of fruit flies fed with either a 5% lard diet or a 10% lard diet at 25°C. Data are expressed as the maximum life span of the last fly, 50% survival time and mean life span (n=200 flies per group, n=20 flies per vial) for each group. [a,b,c]Means at the same column with different superscripts differ significantly at $P<.05$.

shortened to 33 days in the control group fed with a 10% fat diet, while it was recovered to 40 and 59 days in the GTC and BE groups, respectively. The control flies maintained on a 10% fat diet had a 50% survival time of 20 days, while the GTC and BE groups had 25 and 28 days, respectively. The flies on GTC and BE diets had 24 and 33 days of mean life span compared with 17 days for the control fed with a 10% lard diet.

Incorporation of fat into the diet induced production of LPO in *D. melanogaster*. Supplementation of GTC and BE reduced the amount of LPO compared with the control flies (Fig. 3). Supplementation of GTC and BE in a 5% fat diet for 10 days decreased LPO production by 11% and 16%, respectively. Similarly, GTC and BE supplementation reduced production of LPO by 34% and 40%, respectively, in the flies maintained on a 10% fat diet for 10 days, while they further reduced LPO by 51% and 49% in the flies maintained on the same diet for 20 days (Fig. 3).

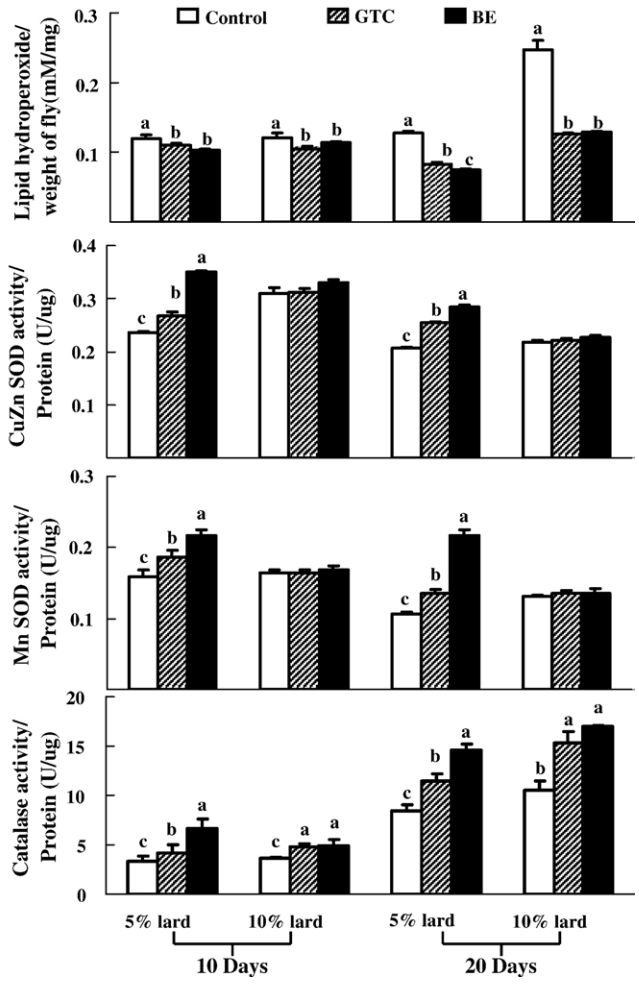


Fig. 3. Effect of GTC and BE on the whole-body LPO level, and enzymatic activity of CuZnSOD, MnSOD and catalase, in fruit flies reared on a 5% lard diet or a 10% lard diet for a period of 10 or 20 days at 25°C. Data are expressed as mean±S.D., n=3 homogenizations (100 flies per homogenization). [a,b,c]Means with different superscripts differ significantly at $P<.05$.

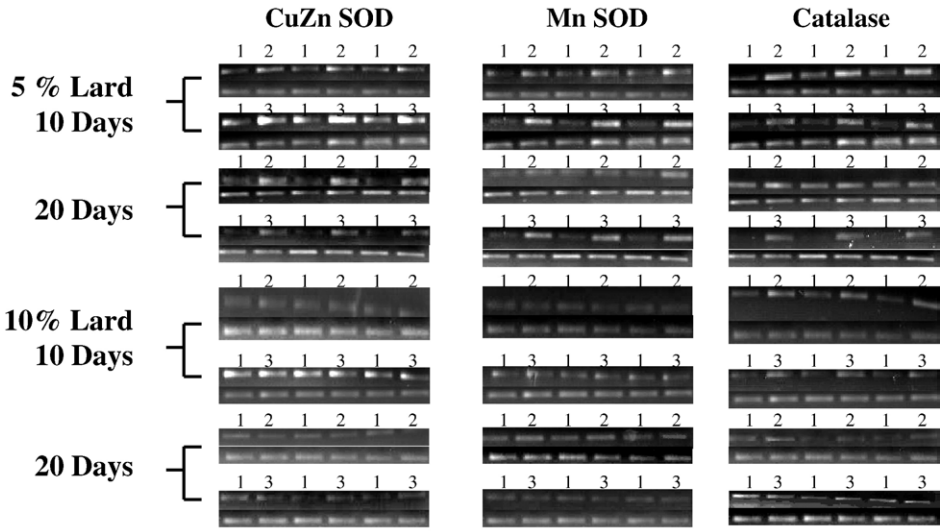
GTC and BE supplementation increased catalase activity in the flies reared on either a 5% lard diet or a 10% lard diet (Fig. 3). When the flies were maintained on a 5% lard diet for 10 days, the catalase activity for the control was 3.3 U/μg protein, while the values for the GTC and BE groups were 4.2 and 6.7 U/μg protein, respectively. When the flies were maintained on a 10% lard diet for 20 days, the control had a catalase activity of 10.5 U/μg protein. In contrast, the GTC and BE groups had a catalase activity increased to up to 15.3 and 17.0 U/μg protein, respectively. The increase in catalase activity associated with GTC and BE supplementation was mediated by gene up-regulation of catalase. As shown in Fig. 4, the ratio of catalase to β-actin was significantly greater in the GTC and BE groups compared with that in the control flies.

GTC and BE supplementation increased both CuZnSOD and MnSOD activity only in the flies reared on a 5% lard diet (Fig. 3). This was consistent with gene up-regulation of CuZnSOD and MnSOD in the flies fed with a 5% lard diet (Fig. 4). When the flies were fed with a 10% lard diet for a period of either 10 or 20 days, no difference in CuZnSOD and MnSOD activity was seen among the control, GTC and BE groups. Similarly, the gene expression of CuZnSOD and MnSOD was similar among the control, GTC and BE flies reared on a 10% lard diet.

4. Discussion

The present study demonstrated clearly that fat shortened the life span of *D. melanogaster* in a dose-dependent manner. This observation was in agreement with the study by Driver and Cosopodiotis [13], who found that dietary incorporation of palmitic acid, a saturated fatty acid, could shorten the life span of flies by 20%. The present study used a mixture of fatty acids derived from lard rather than a single fatty acid, suggesting that fat as a whole shortened the life span and caused a greater mortality in *D. melanogaster*. Interestingly, the decrease in life span of *D. melanogaster* induced by a high-fat diet was partially reversible if the diet contained antioxidants, GTC and BE.

Both GTC and BE are an excellent source of antioxidants [14,18]. The present study is the first report to demonstrate that GTC and BE can reverse the fat-induced mortality in *D. melanogaster*. Cui et al. [21] investigated a Chinese medicine capsule containing 50% green tea extract, 30% spine date and 20% Chinese wolfberry, finding *Drosophila*'s life span increased by 33% when the flies were maintained on a fat-free diet. In male mice orally given a water solution containing 80 mg/L of GTC at the age of 13 months until death, they had a statistical increase in an average life span by 6.4% [22]. In a human cohort study, it was reported that daily consumption of green tea in sufficient amounts could prolong life by avoiding premature death, particularly death caused by cancer [23]. Zhou and Lu [24] examined antioxidant activity and phenolic contents of 38 commonly



	CuZn SOD/β-actin	Mn SOD/β-actin	Catalase/β-actin
5% lard for 10 days			
Control	1.319 ± 0.06	0.84 ± 0.03	0.74 ± 0.03
GTC	1.65 ± 0.11*	0.98 ± 0.02*	0.96 ± 0.01*
BE	1.81 ± 0.03*	1.08 ± 0.03*	0.99 ± 0.06*
5% lard for 20 days			
Control	1.44 ± 0.16	0.81 ± 0.04	0.73 ± 0.03
GTC	1.59 ± 0.04*	0.93 ± 0.02*	0.79 ± 0.01*
BE	1.61 ± 0.25*	1.16 ± 0.01*	0.95 ± 0.04*
10% lard for 10 days			
Control	1.51 ± 0.04	0.97 ± 0.02	0.80 ± 0.02
GTC	1.53 ± 0.02	0.91 ± 0.04	0.90 ± 0.03*
BE	1.53 ± 0.04	0.99 ± 0.03	0.92 ± 0.02*
10% lard for 20 days			
Control	1.52 ± 0.08	0.81 ± 0.04	0.82 ± 0.02
GTC	1.50 ± 0.01	0.88 ± 0.06	0.81 ± 0.03*
BE	1.53 ± 0.23	0.77 ± 0.04	0.93 ± 0.04*

Fig. 4. Effect of GTC and BE supplementation on gene expression of CuZnSOD, MnSOD and catalase, compared with the control diet. The flies were incubated at 25°C and reared on either a 5% lard diet or a 10% lard diet for a period of 10 or 20 days. The values were normalized to the corresponding amount of β-actin (top band=target gene, bottom band=β-actin). Lane 1=control; Lane 2=GTC group; Lane 3=BE group. Data are expressed as mean±S.D., n=3 homogenizations (12 flies per homogenization). *Differs significantly from that of the control value at $P<.05$.

consumed vegetables, finding that broccoli was rich in phenolics and its antioxidant activity ranked top 3 among these vegetables. Culcin et al. [25] studied the antioxidant activity of water and ethanol extracts of broccoli, finding that both extracts displayed effective reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activity. In this regard, broccoli contains various antioxidants including carotenoids, tocopherol, ascorbic acid, glucosinolates, isothiocyanates, dithiothiones, inodoles, sulfonates and flavonoids [26,27].

Supplementation of GTC and BE in diet was associated with a lower LPO production in *D. melanogaster* fed with a high-fat diet. One of indications for ageing is accumulation of LPO products. In fact, the present study clearly demonstrated that GTC and BE groups led to a decrease in total body LPO level compared with the control group, suggesting that dietary GTE and BE had antioxidant activity in vivo in *D. melanogaster*. Arking et al. [16] showed that genetic-manipulated, long-lived strain of *D. melanogaster* had a lower LPO level with greater SOD and catalase activities in every time point throughout the whole life compared with the short-lived strain. In rats, GTC feeding decreased plasma LPO level [9]. Another in vitro study also showed that GTC inhibited generation of superoxide, hydroxyl radicals and LPO [28]. Various models have also demonstrated the antioxidant activity of broccoli. In Hepa 1c1c7 cells, broccoli extracts reduced oxidative stress and inhibited hydrogen

peroxide-induced DNA single strand break [29]. In HepG2 cells, broccoli extracts scavenged the ROS induced by AAPH radical [27]. When broccoli was supplemented in the diet of chicken, it stabilized erythrocytes and reduced oxidation of insoluble muscle proteins and lipids in the liver [30]. Murashima et al. [31] investigated the effect of broccoli sprouts on the induction of various biochemical oxidative stress markers in 12 healthy subjects, finding fresh broccoli sprouts for 1 week reduced the oxidative stress markers including phosphatidylcholine hydroperoxide, urinary 8-isoprostane and 8-hydroxydeoxyguanosine.

In general, GTC and BE supplementation was associated with an increase in endogenous antioxidant enzymatic activity of CuZnSOD, MnSOD and catalase in *D. melanogaster* fed with a 5% fat diet. The observed increase in these antioxidant enzymes was accompanied by up-regulation of expression of both SOD and catalase in flies maintained on a 5% fat diet supplemented with GTC and BE. The present result was in agreement with the report of Sohal et al. [32], who found that flies had increased SOD activity by 26% and catalase activity by 73% in response to 34% increase in life span. Chan et al. [33] found that both activity and m RNA of SOD were markedly up-regulated when the cultured rat brain astrocytes were incubated with tea catechin. A similar increase in activity and gene expression of SOD was observed when pheochromocytoma cells were incubated with tea catechin [34]. In mice, dietary catechin supplementation could protect epidermal cells against ultraviolet irradiation–induced damage by modulating SOD and catalase activity [35]. However, Orr and Sohal [36] showed that overexpression of CuZnSOD alone did not increase the life span of the flies, and the increase in the oxidative resistance was insignificant. Similarly, overexpression of catalase gene alone did not increase the longevity of the flies [37]. It appears that longer life span is only associated with up-regulation of both SOD and catalase. We had no explanation for the observation that supplementation of GTC and BE in a 10% fat diet did not alter SOD activity. Perhaps, expression of SOD is a function of LPO level. In this regard, LPO level in the 10% fat diet supplemented with GTC and BE was too low to activate expression of CuZnSOD and MnSOD compared with that in the control flies.

The present study investigated neither the mechanism by which GTC and BE induced the up-regulation of genes for catalase and SOD nor the interaction of GTC and BE with other antioxidants. It is known that GTC can regenerate α-tocopherol in cells and human low-density lipoprotein [38]. It is also evident that GTC are able to modulate the expression of cytochrome P450 enzymes in various cell lines [39]. There has been no similar study to date carried out in fruit flies. It was possible that GTC and BE prolonged the life span of the files fed with a high-fat diet by not only up-regulation of catalase and SOD but also modulation of other enzymes and interactions with other antioxidants to protect against oxidants.

It was concluded that the life span-prolonging effect of GTC and BE in the fruit flies maintained on a high-fat diet was accompanied by up-regulation of catalase and SOD in both transcriptional and translational levels. The present study mainly focused on interaction of GTC and BE with endogenous catalase and SOD but not with GPx as glutathione pathway in *D. melanogaster* is quantitatively minor in contribution to overall antioxidant capacity [40]. Dietary fat accelerated the ageing process and caused a greater mortality in *D. melanogaster*, but GTC and BE could reverse it partially, most likely but not necessarily causative, by up-regulation of the expression of endogenous SOD and catalase.

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