Aging is a multifaceted process associated with a gradual decline in physiological function and an increased incidence of various diseases, including cancer, neurodegenerative diseases, and diabetes [1,2]. Increasing evidence has demonstrated that many fruits and their extracts contain high levels of phytochemicals, and fruit consumption promotes health and prevents or delays age-related diseases [3–6]. However, few preclinical studies in animals have emerged demonstrating that specific fruits when supplemented in the diet promote longevity and health span.

Numerous genetic and environmental factors have been implicated in modulating aging processes [1,2]. Among many hypotheses of aging, the free radical hypothesis proposed more than 50 years ago states that cumulative oxidative damage to macromolecules in the cell is a causal factor of aging [7]. Since then, numerous lines of evidence have been discovered to support this hypothesis but also point out that the effect of oxidative stress on life span depends on the environmental context [8,9]. Genetic studies have identified hundreds of genes that are involved in modulating life span in yeast, worms, flies, and rodents. These genes can be categorized into a number of signaling pathways, including the insulin/insulin-like, target of rapamycin (TOR), Jun kinase (JNK), and sirtuin signaling pathways, which are functionally conserved across diverse species [1,2,10,11]. Single-nucleotide polymorphisms in some longevity-associated genes, such as insulin-like growth factor receptor, have been linked to human longevity because they are enriched in centenarians [12–15]. Some of the longevity-associated pathways, such as the JNK signaling pathway, mediate oxidative stress response in the cell. This is consistent with the free radical hypothesis of aging, although increasing resistance to oxidative stress does not necessarily result in longer life span.

Food consumption, an environmental factor, plays a pivotal role in modulating the life span of an organism. Dietary restriction (DR), a potent environmental intervention, can extend the life span and health span in various species, although DR does not always promote longevity [1,16–18]. DR is also effective in reducing the incidence of cancer, diabetes, and other diseases in mammals. Life span extension by DR may be mediated through several nonexclusive genetic pathways, such as the sirtuin, SKN-1, and TOR pathways [1,2,10].

Another prolongevity intervention is to supplement the regular diet with health-promoting nutraceutical compounds and extracts derived from plants and fruits. A prominent feature of nutraceutical extracts is that they have high levels of polyphenols, which possess...
high antioxidant activities and other health-promoting properties [4,19]. Polyphenols have been shown to reduce the incidence of various types of cancer, type 2 diabetes, and other diseases [4,19]. Several nutraceutical compounds and extracts have been shown to promote longevity in diverse species. Resveratrol, a polyphenol compound, can extend life span in yeast, worms, flies, and fishes on their respective regular diets, and mice on a high-fat diet [20–23], through activating several longevity-associated genes, including sirtuins [24]. Blueberry extract has been shown to extend the life span of Caenorhabditis elegans partly through an osmotic stress resistance-related Ca<sup>2+</sup>/calmodulin-dependent protein kinase pathway [25]. Depending on the composition of diets, a mixture of cranberry and oregano extracts can extend life span without compromising fecundity in Mexican fruit flies [26]. Pulp extract of açai, a berry indigenous to the Amazon River region, can improve survival of flies on a high-fat diet partly through activating the JNK pathway and reducing expression of genes in gluconeogenesis [27]. However, the number of longevity nutraceutical compounds and extracts identified so far is still small. In some cases, their effectiveness in promoting longevity has been challenged. Some other studies in yeast, worms, and flies have shown that no or marginal life span extension was induced by resveratrol [28,29]. Moreover, the mechanisms by which nutraceutical agents promote longevity remain elusive.

Nectarine (Prunus persica var. nectarine) is a subspecies of peach that is grown and consumed worldwide [30]. Nectarine contains a number of nutritionally important health-promoting ingredients, including dietary fiber, meaningful amounts of vitamin C and β-carotenoïds, and high contents of polyphenols [31–36]. A mouse study shows that both cytosolic and mitochondrial enzymatic activities in repairing 8-oxoguanine (8-oxoG) DNA lesions are significantly improved in aged mice fed a diet supplemented with a nectarine extract compared to age-matched control animals on a nonsupplemented diet [37]. Impairment of DNA repair capacities is linked to aging, cancer, and neurological diseases [38], and the mouse study supports the health benefits of nectarine consumption for its enhancement of DNA damage repair. However, there is no experimental evidence supporting any longevity-promoting property of nectarine at the organismic level.

Here we describe a series of studies designed to examine the effects of nectarine supplementation in the diet on life span in Drosophila melanogaster. D. melanogaster is an excellent model system to investigate the longevity-promoting properties of compounds and nutraceutical extracts because it has a short life span, can be cultured on simple diets, and has a rich genetic resource with a fully sequenced genome, and, more importantly, over half of the fly genes have mammalian homologs [39,40]. In this study, we demonstrate that nectarine can improve the survival of flies and that the extent of life span extension depends on gender, dietary conditions, and stress levels in flies.

**Materials and methods**

**Materials and media**

The cornmeal food for flies was made from cornmeal, sugar, yeast, and agar according to the published protocol [41]. The standard sugar–yeast extract (SY) diet contained 10% sugar, 10% yeast extract, and 1.5% agar. The high-fat diet was prepared by incorporating 2% palmitic acid (w/v) and 1% Tween 80 (v/v) into the standard SY diet as previously described [27]. The calorie-restricted diet contained 2.5% sugar, 2.5% yeast extract, and 1.5% agar. The freeze-dried nectarine extract was kindly provided by Paul Neipp at the USDA (Parlier, CA, USA) and stored at −80 °C until it was added to the standard, high-fat, and calorie-restricted diets at the final concentrations of 2, 4, and 8% (w/v).

Wild-type D. melanogaster strain Canton S was obtained from the Bloomington Drosophila Stock Center (Bloomington, IN, USA). Mutant flies with a sod1 reduction of function were generated using the Gal4-UAS system combined with the RNA interference (RNAi) technique [42]. Specifically, double-stranded RNA of sod1 was induced from a UAS-sod1 inverted-repeat (UAS-sod1IR) line by a ubiquitously expressed Gal4, daughterless-Gal4 (da-Gal4). The UAS-sod1IR stock (strain FI03) was originally generated by J. Phillips (University of Guelph, ON, Canada) [42]. Both UAS-sod1IR and da-Gal4 stocks were obtained from the Bloomington Drosophila Stock Center. All fly stocks were maintained on the cornmeal medium at 18 °C or room temperature before being used for various assays.

**Life span, food intake, locomotor activity, and fecundity assays**

To obtain adult flies for life span, food intake, locomotor activity, and fecundity assays, parental flies were allowed to mate and lay eggs on the cornmeal medium. After approximately 2 weeks, adult progeny flies of mixed sex within 24 h of eclosion were collected into new bottles with the standard SY medium. After mating for 24 h, males and females were sorted under light CO2 anesthesia and placed separately into vials with approximately 5 ml of the standard SY medium. Each vial contained approximately 20 male or female flies. After another 24 h, flies were transferred to their respective treatment diets for life span, food intake, locomotor activity, and fecundity assays.

For the life span assay, flies were transferred to fresh food once every 2–3 days, and the number of dead flies was counted at each transfer. Life span data were recorded using Microsoft Excel (Microsoft, Redmond, WA, USA). Around 100–120 flies in five or six vials were included for each life span experiment. Each life span assay was repeated at least twice.

The capillary feeder method was employed to measure food intake as previously described [43]. Female flies were first treated with the standard, calorie-restricted, or high-fat diet supplemented with 0 or 4% nectarine for 11 days. Subsequently, eight flies were selected from each treatment and individually housed in the capillary feeding chamber. Each capillary was filled with the same food that each fly was fed before the feeding assay, except that the food in the capillaries contained no agar so that it would remain a liquid. Two capillaries with the food were set up in two separate feeding chambers without flies to account for evaporation of liquid food. Food intake was measured within 24 h. Final values of food intake were calculated by subtracting the evaporation and averaging food intake of five to eight flies. Food intake was excluded for flies that died before the end of the experiment.

For the locomotor activity assay, spontaneous activity of 14-day-old females was measured after consumption of the standard, DR, or high-fat diet supplemented with or without 4% nectarine using the Drosophila Activity Monitor System (DAMS) from TriKinetics, Inc. (Waltham, MA, USA) according to the manufacturer’s suggested protocol. The DAMS measures the number of times that a fly breaks the infrared beams by walking back and forth in a horizontal glass vial. Specifically, a glass vial with approximately 10 flies and the food supplemented with or without nectarine was inserted into the DAMS. The number of times that flies broke the infrared beams was recorded once every 20 s during a 24-h recording window. At the end of recording, the total number of surviving flies was recorded for each vial. Because the flies were undisturbed during the recording period, we operationally defined this activity as spontaneous locomotor activity. The 24-h spontaneous activity level was calculated by dividing the total number of beam breaks in 24 h by the total number of surviving flies in the vial. Recording spontaneous activities of females on each treatment was replicated five or six times with five or six vials, each with 10 females.

For the fecundity assay, after female flies were mated with males for 24 h, they were separated from males, placed on their respective diets, and then transferred to vials with fresh food once every 2–3 days. The number of dead flies was counted at each transfer.
The vials with old food and eggs on them were kept for egg counting. The number of eggs laid was counted and recorded in Microsoft Excel until all the flies were dead in a vial. Each egg-laying assay was repeated five or six times in separate vials. The lifetime fecundity was calculated by dividing the lifetime egg production in each vial by the number of females (approximately 10 flies per vial) at the beginning of the experiment.

Quantitative polymerase chain reaction (qPCR)

Three-day-old female flies were fed the standard SY, DR, or high-fat diet supplemented with 0, 2, or 4% nectarine until 14 days of age. Fly heads and bodies were frozen with liquid nitrogen and separated using a sieve. Total RNA was prepared from heads using the Trizol reagent (Invitrogen; Carlsbad, CA, USA) according to the manufacturer’s suggested protocol. Quality and quantity of total RNA were assessed using the Nanodrop 1000 from Thermo Scientific (Wilmington, DE, USA). cDNA was synthesized from the total RNA by using SuperScript reverse transcriptase from Invitrogen. qPCR was performed using the StepOnePlus real-time PCR system from Applied Biosystems (Foster City, CA, USA) according to the manufacturer’s suggested protocol. The primer sequences for genes tested in this study were designed using the Primer3 program at http://primer3.sourceforge.net/ and are listed in Supplementary Table S2. Each qPCR measure was repeated three or four times with three or four biologically independent samples. Rp49 transcript was used as the reference for quantifying the relative transcript level of each selected gene.

Protein preparation

Mitochondrial and cytosolic proteins were prepared using the mitochondria isolation kit from MitoSciences (Cat. No. MS850). Briefly, approximately 40 14-day-old Canton S or sodRNAi female flies fed the standard or high-fat diet supplemented with or without 4% nectarine extract were homogenized in ice-cold isolation buffer containing protein inhibitor cocktail (Roche Cat. No. 04693159001) using a glass Dounce homogenizer. Each homogenate was centrifuged at 1000g at 4 °C for 10 min. The supernatant was then transferred to a new tube and centrifuged again at 12,000g at 4 °C for 15 min to separate cytosolic and mitochondria proteins. The supernatants were collected as the cytosolic protein sample, and the pellet was washed and resuspended in 30 μl isolation buffer as the mitochondria protein sample. Protein concentration was determined with the BCA protein assay kit (Thermo Fisher, Cat. No. 23225) according to the manufacturer’s suggested protocol.

Western blot analysis for 4-hydroxynonenal–protein adduct quantification

Protein samples (20 μg/lane) were separated by electrophoresis on a 7% NuPAGE Novex Tris–acetate SDS mini gel (Invitrogen, Cat. No. EAA0385BOX) under reducing conditions according to the manufacturer’s suggested protocol. After electrophoresis, proteins were transferred to a 0.2-μm polyvinyllidene difluoride membrane for 7.5 min with the iBlot Dry blotting system (Invitrogen, Cat. No. IB1001). Membrane-immobilized proteins were blocked with 5% nonfat dried milk in 1× Tris-buffered saline (pH 7.5) containing 0.1% Tween 20 (TBST) for 1 h at room temperature and then incubated overnight at 4 °C with anti-N-terminal acetyllysine-4-hydroxynonenal (4-HNE) fluorophore rabbit polyclonal primary antibody (Calbiochem, Cat. No. 393206) at a 1:4000 dilution in the blocking solution to detect 4-HNE–protein adducts [44]. The blots were washed three times (10 min each) with TBST and incubated in horseradish peroxidase-labeled goat anti-rabbit IgG diluted 1:3000 in the blocking solution for 90 min at room temperature. The immunoreactive proteins were detected with Amersham ECL Plus Western blotting detection reagents (GE Healthcare, Cat. No. RPN2132) and quantified using the ImageQuantTL software in the Typhoon Trio + imager system (GE Healthcare). The blots were then stripped and incubated with anti-β-actin antibody (Abcam, Cat. No. ab8224) and anti-complex 1 subunit NDUF533 antibody (Mitosciences, Cat. No. MS112), both at a 1:2000 dilution to detect β-actin and NDUF53 as the loading controls for cytosolic and mitochondrial proteins, respectively. For each protein sample, at least three biological replicates were used for immunostaining. For 4-HNE–protein adduct measurement, the protein region between molecular weights 460 and 268 kDa was quantified. Relative 4-HNE level was calculated by first normalizing the 4-HNE intensity value to that of β-actin and the ratios were adjusted again so that the ratio for the nonsupplemented control group was 1 for each comparison between supplemented and nonsupplemented groups.

Statistical analysis

All data were analyzed using StatView version 5.0 software (SAS, Cary, NC, USA). For the life span data, Mantel–Cox log rank tests were performed by comparing flies on the nectarine-supplemented diets to those on the nonsupplemented control diet. Maximum life span analysis was conducted on the longest-lived 10% of flies in each treatment. The two independent sets of life span data were analyzed either separately or by using data pooled from the two replicated experiments. For the spontaneous activity, food intake, fecundity, qPCR, and 4-HNE data, unpaired t tests were performed by comparing flies on the nectarine-supplemented diets to the nonsupplemented controls. The Bonferroni correction was conducted to adjust multiple comparisons. Statistical significance was set at p ≤ 0.05. All mean and maximum life span, food intake, locomotion, fecundity, qPCR, and 4-HNE values are presented as means ± standard error. In addition, for expression of a gene to be considered significantly altered by supplementation with nectarine, the percentage of changes at the transcript level had to meet a stringent and operationally set level of ≥ 20%.

Results

The effects of nectarine extract on life span of flies fed a standard diet

Nectarine is rich in polyphenols and other antioxidants [32–34]. We hypothesized that nectarine may counteract oxidative damage and therefore extend the life span in flies. To test this hypothesis, we supplemented the standard SY diet for wild-type Canton S flies with 2 or 4% nectarine extract. No significant life span increase in male flies under the tested conditions was observed (Fig. 1A, Table 1 for results from combining two replicates, and Supplementary Tables S1 and S2 for replicated experiments). Based on the pooled data, supplementation with 4% nectarine slightly decreased mean and maximum life span of males fed the standard diet, but only by < 8 and 2.5%, respectively (p < 0.01, Table 1). Supplementation with 4% but not 2% nectarine significantly increased the mean life span of female flies by approximately 14–22% (p < 0.01), but not consistently the maximum life span (Fig. 1B, Table 1, and Supplementary Tables S1 and S2). In one trial, supplementation with 2% nectarine decreased life span of females (Table S2). To determine whether food intake played a role in life span extension in female flies by nectarine, food intake within 24 h was measured. Supplementation with 4% nectarine did not significantly change the amount of food intake by females (Fig. 1C). These findings suggest that nectarine can promote longevity without affecting food intake, and this life span extension depends on gender and dosage of nectarine.

The effects of nectarine on life span of flies fed the DR diet

DR has been shown to extend life span in a wide variety of species [1]. To determine if nectarine and DR act on the same or different pathways, we measured the life span of Canton S flies fed the DR diet
supplemented with or without 2 or 4% nectarine. Supplementation with nectarine at both concentrations did not consistently affect the mean and maximum life span of males compared to the nonsupplemented controls (Fig. 2A, Table 1, and Supplementary Tables S1 and S2). However, supplementation with nectarine at 2 or 4% significantly extended the mean life span of females by 7–11% and the maximum life span by 10–14% (p<0.001, Fig. 2B, Table 1, and Supplementary Tables S1 and S2). No significant difference in food intake within 24 h was found between flies fed the DR diet supplemented with or without 4% nectarine (Fig. 2C). These results suggest that the prolongevity effect of nectarine is mediated at least partly through DR-independent pathways.

The effects of nectarine on life span of flies fed a high-fat diet

Consumption of a high-fat diet is often associated with an increase in oxidative damage and a decrease in life span. To evaluate whether nectarine could promote the survival of flies fed a high-fat diet, we compared the life span of flies fed a high-fat diet supplemented with or without nectarine. Supplementation with 2 or 4% nectarine significantly increased the mean and maximum life span of males, but only marginally, by up to 5% in most cases compared to the nonsupplemented controls (p<0.05, Fig. 3A, Table 1, and Supplementary Tables S1 and S2). However, supplementation with nectarine at 8% did not extend the mean and maximum life span of males (Table 1). On the other hand, supplementation with nectarine at 2, 4, or 8% significantly extended both mean and maximum life span of females fed the high-fat diet by 10–20% (p<0.01, Fig. 3B, Table 1, and Supplementary Tables S1 and S2). Food intake was measured for females fed the high-fat diet supplemented with 0, 2, or 4% nectarine. Although there is a trend of slight decrease in food intake by nectarine, no statistically significant differences were found among the three groups of females (p>0.1, Fig. 3C). These findings suggest that nectarine can limit the adverse effects of the high-fat diet and promote the survival of flies on the high-fat diet.

Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Gender</th>
<th>Diet±</th>
<th>Total No. of flies</th>
<th>Mean life span (days ± SE)b</th>
<th>p for all fliesc</th>
<th>No. of flies for max. life span</th>
<th>Max. life span (days ± SE)b</th>
<th>p for top 10% fliesd</th>
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<tbody>
<tr>
<td>Canton S</td>
<td>Female</td>
<td>Standard</td>
<td>219</td>
<td>33.6±0.9</td>
<td>0.002</td>
<td>22</td>
<td>54.6±0.9</td>
<td>0.0091</td>
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<tr>
<td>Canton S</td>
<td>Female</td>
<td>Standard + 2% nect</td>
<td>215</td>
<td>31.1±0.7</td>
<td>0.0003</td>
<td>22</td>
<td>48.1±1.5</td>
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<td>Standard + 4% nect</td>
<td>218</td>
<td>39.5±0.7</td>
<td>0.002</td>
<td>24</td>
<td>56.1±0.9</td>
<td>0.0001</td>
</tr>
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<td>260</td>
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<td>64.5±0.4</td>
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<td>Standard + 2% nect</td>
<td>222</td>
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<td>0.057</td>
<td>22</td>
<td>64.1±0.4</td>
<td>0.0002</td>
</tr>
<tr>
<td>Canton S</td>
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<td>Standard + 4% nect</td>
<td>245</td>
<td>47.1±0.9</td>
<td>0.0002</td>
<td>24</td>
<td>62.9±0.4</td>
<td>0.0002</td>
</tr>
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<td>82.5±1.2</td>
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<td>205</td>
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<td>91.3±0.9</td>
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<tr>
<td>Canton S</td>
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<td>DR</td>
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<td>46.7±0.7</td>
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<td>237</td>
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<td>12</td>
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<td>42.5±0.9</td>
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<td>0.0001</td>
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<tr>
<td>Canton S</td>
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<td>High fat + 8% nect</td>
<td>109</td>
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<td>0.0001</td>
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<td>26.8±0.3</td>
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<td>23</td>
<td>31.7±0.4</td>
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<td>0.3865</td>
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<td>25.3±0.8</td>
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</tbody>
</table>

a Standard, the standard diet containing 10% sugar and 10% yeast extract; nect, nectarine. The percentage value indicates the final concentration of nectarine in the food.
b Life span values are expressed as days ± standard error (SE).
c p values were calculated by comparing flies on the nectarine-supplemented diet to the corresponding nonsupplemented controls. Significant p values after the Bonferroni adjustment are in bold.
The effects of nectarine on spontaneous activity of flies

Locomotor activity generally declines with age [45]. To test whether the life span extension by nectarine was associated with any changes in locomotor activity, we measured spontaneous locomotor activity of 14-day-old females fed the standard, DR, or high-fat diet supplemented with or without 4% nectarine. No obvious changes of the spontaneous locomotor activity were found between flies fed the diets supplemented with or without nectarine (Fig. 4A). These findings suggest that nectarine is not detrimental to the mobility of flies.

The effects of nectarine on fecundity

Life span extension in animals in some cases is associated with a decrease in reproduction [46,47]. To determine if the extension of life span by nectarine was associated with a decrease in reproduction, we measured spontaneous locomotor activity of individually housed female flies fed the DR diet supplemented with 0, 2, or 4% nectarine. More than 90 females were used in each life span assay. Food intake was measured in 6–8 individually housed female flies fed the DR liquid diet supplemented with or without 4% nectarine. No agar was added to the food in the capillaries. Food intake in microliters was calculated by averaging food intake of 6–8 flies within 24 h after subtracting the evaporation of the food. Error bars indicate standard errors. The life span curves were derived from two independent experiments.

The effects of nectarine on expression changes of genes associated with metabolism, oxidative stress, and longevity

To investigate the molecular mechanisms by which nectarine promotes life span and health span, we measured expression changes of genes associated with metabolism, oxidative stress, and longevity, including those in the insulin-like, JNK, sirtuin, and TOR signaling pathways, in heads of female flies fed the standard, DR, or high-fat diet supplemented with 0, 2, or 4% nectarine. The relative transcript levels of the genes tested in this study are listed in Supplementary Table S3 and expression patterns of some genes with significant changes induced by nectarine are depicted in Fig. 5.

For metabolism, a significant decrease in the transcript level of phosphoenolpyruvate carboxykinase (PEPCK), a key enzyme in gluconeogenesis, was found in flies fed the standard, DR, or high-fat diet supplemented with nectarine compared to the respective nonsupplemented controls. Iron regulatory protein 1B (irp-1B), which is involved in iron metabolism, was down-regulated by nectarine in flies under all three dietary conditions.

For the oxidative stress response pathway, lethal (2) essential for life (l(2)ef) and heat shock protein 68 (Hsp68) were down-regulated, whereas metallothionein A (MtnA) was up-regulated by supplementation with 4% nectarine in flies fed the DR or high-fat diet but not the standard diet. These genes are downstream targets of the JNK pathway. However, the expression of other genes in the JNK pathway, including basket (bsk, the Drosophila NK gene), puckered (puc), and glutathione S-transferase D1 (GstD1), was not changed by supplementation with nectarine. Among other stress response genes examined, mitochondrial superoxide dismutase 2 (Sod2) was down-regulated by nectarine in flies fed the high-fat diet, and catalase (cat) was down-regulated by nectarine in flies fed the standard diet, whereas female-specific independent of transformer (fit), which is induced under various stress conditions, was down-regulated by nectarine in flies fed the DR and high-fat diets.

Peroxiredoxins (Prx’s) are a family of proteins with peroxidase activity and play an important role in oxidative stress response [48,49].
Transcript levels of four Prx genes, Prx2540, Prx5, Prx6005, and Prx5037, were down-regulated by supplementation with 4% nectarine in flies fed the high-fat diet. In addition, Prx6005 was down-regulated by nectarine in flies fed the standard but not the DR diet, whereas Prx2540 was down-regulated by nectarine in flies fed the standard or the DR diet.

For the TOR signaling pathway, the transcript level of S6K was significantly reduced by the supplementation with 4% nectarine but not the DR diet. No significant alterations in gene expression were observed for genes involved in the insulin-like signaling pathway, including three Drosophila insulin-like peptides (dlp2, dlp3, and dlp5), insulin-like receptor substrate (chico), and forkhead transcription factor (Foxo), and in mitochondrial biogenesis, including nitric oxide synthase (Nos), mitochondrial assembly regulatory factor (Mapf), and mitochondrial transcription factor A (TFAM) (Supplementary Table S3). Together, these findings indicate that supplementation with nectarine significantly influences the expression of genes involved in glucose metabolism, oxidative stress response, and detoxification.

**The effects of nectarine on life span of sod1 mutant flies**

To directly test whether the longevity effect of nectarine is related to antioxidative stress, we examined whether supplementation with nectarine could promote the survival of sod1 mutant flies. Sod1 is a major cytosolic enzyme that scavenges highly reactive superoxides within the cell [50]. Loss- or reduction-of-function sod1 mutants have higher amounts of oxidative damage to various macromolecules and shorter life span compared to wild-type flies [51]. We used the RNAi method to generate a reduction-of-function sod1 mutant (sod1RNAi) and fed these flies the standard diet supplemented with 0, 2, or 4% nectarine. Similar to the findings in wild-type Canton S flies, supplementation with up to 4% nectarine did not consistently extend the mean and maximum life span of sod1RNAi males (Fig. 6A, Table 1, and...
Supplementary Tables S1 and S2). However, supplementation with nectarine at 2 or 4% significantly increased the mean and maximum life span of sod1RNAi females by 14–28% (p < 0.0001, Fig. 6B, Table 1, and Supplementary Tables S1 and S2). Here the life span extension seems to be positively correlated with the concentration of nectarine. These results suggest that nectarine can alleviate the high level of oxidative damage and promote the survival in sod1RNAi female flies and also suggest that nectarine acts either downstream or independent of Sod1 to promote longevity.

**The effects of nectarine on oxidative damage**

To further examine the antioxidative stress property of nectarine, we determined the effect of nectarine on lipid oxidation as an indicator of oxidative damage by measuring the level of 4-HNE–protein adducts for wild-type Canton S or sod1RNAi female flies fed the standard SY or high-fat diet supplemented with or without 4% nectarine for 11 days. 4-HNE–protein adducts accumulate with age and under oxidative stress and are a commonly used biomarker of lipid oxidation [44, 52, 53]. We did not observe any significant change in the 4-HNE–protein adduct level in mitochondrial protein samples between any pair of diet-matched nectarine-supplemented and control female flies (data not shown). Supplementation with 4% nectarine did not induce any statistically significant change in the 4-HNE–protein adduct level in cytosolic protein samples for female flies on the standard SY diet compared to the nonsupplemented control, either (p = 0.20, Fig. 7A). However, supplementation with 4% nectarine significantly reduced the 4-HNE–protein adduct level in the cytosolic protein samples by more than twofold for flies on the high-fat diet compared to the diet-matched nonsupplemented control (p = 0.01, Fig. 7B). In addition, supplementation with 4% nectarine significantly reduced the 4-HNE–protein adduct level by approximately twofold for sod1RNAi female flies on the standard SY diet compared to the nonsupplemented control (p = 0.05, Fig. 7C). These findings further support the notion that nectarine has antioxidative properties in vivo, at least for flies on the high-fat diet or oxidatively stressed sod1RNAi flies.

**Discussion**

Free radicals play an important role in aging and age-associated diseases [8, 9]. Genetic mutations and environmental interventions that extend the life span of an organism are often associated with an increase in the resistance to oxidative stress in that organism. However, an alteration in oxidative stress response capacity alone does not necessarily lead to a change in life span, and the effects of oxidative stress response pathways on life span and aging depend on the environmental context and genetic background of an organism [9]. Nevertheless, reducing oxidative damage remains one of the promising interventions to delay the progression of aging and age-related diseases. Polyphenols are a family of phytochemicals possessing high antioxidant capacities and other health-promoting bioactivities, such as activators of enzymes and ligand mimetics of receptors [45, 19]. Many fruits contain significant amounts of polyphenols and, therefore, are ideal candidates for longevity interventions. However, evidence directly demonstrating the prolongevity properties of fruits is scarce. In this study, we have demonstrated that an extract from nectarine can promote longevity in female flies under various dietary conditions, including the standard, DR, and high-fat diets. Supplementation with nectarine can also promote the survival of flies with high levels of oxidative damage resulting from a reduction–of–function mutation in sod1, a major superoxide scavenger in the cell [50]. In addition, nectarine can increase fecundity and duration of egg production (data not shown), which suggests that nectarine can delay reproductive aging. Furthermore, qPCR and Western blot analyses suggest that nectarine promotes longevity at least partly through reducing oxidative damage. This study is the first demonstrating the prolongevity property of nectarine, a globally grown and consumed fruit.

Composition of dietary nutrients plays an important role in modulating life span [46, 54, 55]. DR has been shown to extend the life span in diverse species, although the genetic background of an organism also contributes to the DR life span extension effect [1, 17, 18]. Consumption of high-fat diets induces higher levels of oxidative stress and results in shorter life span compared to low-fat diets [20, 56]. In the studies reported here, we have investigated how supplementation with nectarine affects the life span in flies fed three different diets, standard, DR, and high fat. Supplementation with 4% nectarine is sufficient to extend life span of female flies under all the dietary conditions. Supplementation with 2% nectarine consistently extends lifespan only of females on the high-fat diet. However, supplementation with nectarine up to 4% only marginally extends life span in males fed the high-fat diet but not the standard or DR diet compared to the respective nonsupplemented controls. It is unclear why nectarine is much more effective in promoting longevity in females than in males. Further study is needed to determine whether the differences in physiology, such as the energy requirement for reproduction, contribute to the gender-specific response [57]. Nevertheless, our findings suggest that the extent of life span extension by nectarine in flies depends on gender, dosage of nectarine, and nutrient composition of the diet. This is consistent with previous observations using polyphenols and fruit extracts, e.g., the prolongevity effect of resveratrol in some organisms seems to depend on the dietary conditions [20, 22, 23, 58]. Resveratrol has been shown to promote the survival of mice on a high-fat diet but not on the standard diet.
One is that nectarine reduces the expression of PEPCK, a key enzyme in gluconeogenesis, in flies fed all three diets [62]. We have previously observed a decrease in PEPCK expression by supplementation with açai pulp extract in flies fed the high-fat diet. Inhibition of PEPCK activity by the drug fenofibrate is one of the therapeutic approaches to manage type 2 diabetes [63]. Although gluconeogenesis and the glucose level were not measured in our studies, our findings suggest that fruits rich in polyphenols have the potential to modulate glucose metabolism in a health-promoting manner.

The second finding revealed by gene expression analysis is that genes involved in the oxidative stress-response pathways tend to be down-regulated by nectarine. The extent of down-regulation depends on the dietary conditions. Nectarine decreases expression of two heat shock proteins, hsp68l(2)efl and Hsp68, but increases expression of Mtrn in flies fed the high-fat diet. These three genes are downstream targets of the JNK signaling pathway and are up-regulated in response to oxidative stress [64,65]. Interestingly, açai pulp extract has been shown to increase expression of these three genes in flies fed the high-fat diet. How nectarine differentially modulates expression of the JNK target genes is unclear. The JNK pathway has been implicated in modulating the life span in D. melanogaster [64,65]. We postulate that nectarine indirectly modulates the JNK signaling pathway by influencing the redox status and reducing oxidative damage in the cell, whereas açai may directly activate the JNK pathway.

Our hypothesis is further supported by expression patterns of three Prx's, Pxr2540, Pxr6005, and Pxr5037, a family of antioxidant enzymes involved in detoxifying peroxide in the cell [48,66]. Nectarine decreases expression of all three Prx's in flies fed the high-fat diet. Furthermore, nectarine decreases expression of Pxr2540 in flies fed the standard or DR diet and reduces the transcript level of Pxr6005 in flies fed the DR diet. Pxr's tend to be up-regulated by increased oxidative stress. Consistent with the expression patterns of the JNK downstream genes, decreased expression of Pxr's by supplementation with nectarine seems to be indirectly mediated by the effect of nectarine to reduce oxidative damage and/or improve the redox status. Furthermore, nectarine significantly reduces lipid oxidation as indicated by the decrease in the 4-HNE–protein adduct level in cytosolic proteins of wild-type female flies fed the high-fat diet and sod1RNAi female flies fed the standard DR diet compared to the respective nonsupplemented controls. These results are also consistent with observations made in mice fed nectarine. Mice fed nectarine have a higher 8-oxoG DNA lesion repair capacity than the nonsupplemented controls [37]. Several genes involved in detoxification, including Sod1, glutathione peroxidase 1, and glutathione S-transferase Alpha 3 (GSTA3), have been found to be down-regulated in 18- to 19-month-old mice fed a nectarine-supplemented diet for 14–16 weeks compared to nonsupplemented controls [37]. Although nectarine does not necessarily reduce...
expression of the fly genes homologous to these mouse genes, these observations suggest that down-regulation of genes involved in detoxification is a conserved property of nectarine. Furthermore, both nectarine and açai significantly extend the life span of sod RNAi female flies with high levels of oxidative damage despite the difference in modulating expression of stress-response genes. Taken together, these findings suggest that functional fruits such as nectarine and açai can promote survival by reducing oxidative damage and/or activating stress-response signaling pathways.

Consumption of fruits can provide numerous health benefits [5]. However, development of effective and relatively low-cost prolongevity interventions with functional fruits is still in the early stage. A survey conducted in five European countries indicates that Europeans consume two to four nectarines and peaches per week on average [30]. Nectarine detoxifies with high levels of oxidative damage despite the difference in lifespan extension by nectarine in an animal model and thus provides a possible European countries indicates that Europeans consume two to four nectarines and peaches per week on average [30]. Nectarine detoxifies with high levels of oxidative damage despite the difference in lifespan extension by nectarine in an animal model and thus provides a possible natural dietary bioactive compounds.

Supplementary materials related to this article can be found online at doi:10.1016/j.freeradbiomed.2011.03.011.

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References


