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Lifespan Extension by Cranberry Supplementation Partially Requires SOD2 and is Life Stage Independent

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Abstract

Many nutraceuticals and pharmaceuticals have been shown to promote healthspan and lifespan. However, the mechanisms underlying the beneficial effects of longevity interventions and the time points at which interventions should be implemented to achieve beneficial effects are not well characterized. We have previously shown that a cranberry-containing nutraceutical can promote lifespan in worms and flies and delay age-related functional decline of pancreatic cells in rats. Here we investigated the mechanism underlying lifespan extension induced by cranberry and the effects of short-term or life stage-specific interventions with cranberry on lifespan in *Drosophila*. We found that lifespan extension induced by cranberry was associated with reduced phosphorylation of ERK, a component of oxidative stress response MAPK signaling, and slightly increased phosphorylation of AKT, a component of insulin-like signaling. Lifespan extension was also associated with a reduced level of 4-Hydroxynonenal protein adducts, a biomarker of lipid oxidation. Moreover, lifespan extension induced by cranberry was partially suppressed by knockdown of SOD2, a major mitochondrial superoxide scavenger. Furthermore, cranberry supplementation was administered in three life stages of adult flies, health span (3–30 days), transition span (31–60 days) and senescence span (61 days to the end when all flies died). Cranberry supplementation during any of these life stages extended the remaining lifespan relative to the non-supplemented and life stage-matched controls. These findings suggest that cranberry supplementation is sufficient to promote longevity when implemented during any life stage, likely through reducing oxidative damage.

Keywords

aging; dietary intervention; nutraceutical; botanical; oxidative stress; life stage

Introduction

Aging is modulated by both genetic and environmental factors (Fontana et al., 2010). A number of interventions using pharmaceuticals and nutraceuticals have been identified to promote healthspan and lifespan in model organisms (Dong et al., 2012; Frankel et al., 2011; Hada et al., 2013; Lee et al., 2010; Lucanic et al., 2013). However, aging is a complex biological process and many roadblocks remain to be overcome before any of these

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interventions can be applied to humans. On one hand, lifespan is modulated by multiple genetic pathways, including insulin-like, target-of-rapamycin (TOR) and sirtuin signaling pathways (Fontana et al., 2010; Hall et al., 2013). On the other hand, an adult life can be divided into three separate life stages, health span, transition span and senescence span from young to old, based on the age-specific mortality rate of the population of an organism (Arking, 2006; McDonald et al., 2013). Age-specific mortality rate is negligible in the health span, gradually rises in the transition span and exponentially increases in the senescence span. At the molecular level, the three life stages are characterized by different gene expression patterns and increasing levels of oxidative damage to macromolecules with age. At the physiological level, the three life stages are associated with gradual disruption of organelle function, declining metabolic capacity and sensitivity to stress with age. At the behavioral and cognitive level, the three life stages are associated with gradual declines in locomotor activity and learning and memory capacity with age.

The complex nature of life stages poses a major challenge to develop effective interventions for promoting healthspan and lifespan. Many interventions tested so far have been initiated in very young adults and continued throughout adult life in model organisms (Lucanic et al., 2013; Roth et al., 2005). Any intervention starting in young adults can be costly and impractical to implement in humans. Moreover, it may miss interventions effective in certain life stages. A few studies have started to address the effects of life stage-specific interventions on healthspan and lifespan. For example, some investigators have tested the longevity effect of drugs by initiating the intervention in middle-aged mice. The median lifespan of mice under standard lab conditions is approximately two years. The effects of rapamycin and resveratrol on lifespan and healthspan have been revealed by feeding these two drugs to one-year old mice, respectively (Baur et al., 2006; Harrison et al., 2009). More systematic life stage-specific interventions have been tested for curcumin and sodium butyrate in *Drosophila*, which has mean lifespan of a couple of months, depending on culture conditions. Curcumin supplementation at a modest level throughout adult life can extend lifespan in *Drosophila* (Lee et al., 2010; Shen et al., 2013). However, supplementation with a high concentration of curcumin extends lifespan when implemented during the health span, but decreases lifespan when administered chronically throughout adult life in *Drosophila* (Soh et al., 2013). Supplementation of sodium butyrate can extend the lifespan when implemented during the transition or senescent span, but decrease the lifespan when applied during the health span in flies (McDonald et al., 2013). These studies point out the importance to test the effect of any intervention during different life stage on healthspan and lifespan. Such studies are still very limited in the number of cases.

Cranberry is a popularly consumed berry rich in numerous kinds of bioactive phytochemicals (He and Liu, 2006; Howell, 2007; Neto, 2007). Cranberry derived nutraceuticals have been shown to extend lifespan in worms and flies (Guha et al., 2013; Wang et al., 2013). Cranberry feeding also delays age-related functional decline of pancreatic β -cells in rats (Zhu et al., 2011). Here we investigated the mechanisms underlying the longevity effect of cranberry and determined the effect of short-term cranberry supplementation in three life stages on lifespan in *Drosophila*. We found that cranberry reduces accumulated oxidative damage and does not substantially increase lifespan of *sod2* knockdown flies. We also found that implementation of cranberry supplementation during any of the health, transition or senescence span is sufficient to extend the remaining lifespan of flies. Our findings suggest that cranberry supplementation promotes healthy aging at least partially through reducing oxidative damage and is effective even when implemented for a short-term.

Materials and Methods

Fly stocks and culture

Fly stocks were maintained on the standard cornmeal-yeast-agar medium at 25 ± 1 °C, $60 \pm 5\%$ humidity and a 12:12 h light/dark cycle (Ashburner et al., 2005). The wild-type strain *Canton S*, Gal4 driver da-Gal4 (w^{1118} ; P{w[+mW.hs]=GAL4-da.G32}, 3) and UAS line UAS-*sod2 inverted repeat (IR)* (w^1 ; P{UAS-Sod2.dsRNA.K}15/SM5) were obtained from the Bloomington Drosophila Stock Center (Bloomington, IN). *sod2* knockdown flies were generated from crossing UAS-*sod2IR* with da-Gal4 flies. Two isocaloric sugar-yeast (SY)-agar based diets, SY9:1 and SY1:1, described previously were used for assays (Lee et al., 2008; Skorupa et al., 2008; Sun et al., 2012). The SY9:1 diet contained 18% sugar and 2% autolyzed yeast (MP Biochemicals, Solon, Ohio), which has SY at 9:1 ratio, and the SY1:1 diet had 10% sugar and 10% yeast. The cranberry supplemented diets were prepared as previously described (Wang et al., 2013). Briefly, proanthocyanidin-enriched cranberry extract, kindly provided by Decas Botanical Synergies (Carver, MA), was added to the cooled SY diet to the final concentrations of 2% by weight/volume. The main ingredients of the cranberry extract include 4.0% proanthocyanidins, 37.9% sugars, 4.1% dietary fiber, 0.5% protein, 5.0 moisture, 7.2% ash and 4.0% total vitamin and minerals as described by Decas Botanical Synergies (<http://www.decasbotanical.com>).

Lifespan assay

Lifespan assay was performed as previously described (Sun et al., 2012). For life stage-specific interventions, adult flies were transferred to the SY1:1 diet in bottles within 24 h after eclosion. After mating for 24 h, female flies were sorted out and placed in SY1:1 vials each with approximately 20 flies. After another 24 h on SY1:1, cranberry supplementation was initiated in three life stages. For supplementation during the health span, flies were fed SY9:1 with and without 2% cranberry from the age of day 3 to 30; for supplementation during the transition span, cranberry supplementation was implemented from the age of day 31 to 60; for intervention during the senescence stage, cranberry supplementation was administered from the age of day 61 to the end when all flies died. For measuring the lifespan of *sod2* knockdown flies, sorted females of 2-day old were transferred to SY9:1 or SY1:1 with and without 2% cranberry. All flies were transferred to fresh food once every 2–3 days. The number of dead flies was recorded at each transfer until all flies were dead. Each lifespan assay used approximately 100–200 flies in 5–8 vials.

Western blot analysis

The heads or whole bodies of 14-d old flies were collected for protein preparation with tissue extraction reagent 1 (Cat # FNN0071, Life Technologies Inc.). The flies were fed the SY9:1 diet with and without 2% cranberry starting at the age of day 3 for 11 days before sample collection. Protein samples were separated by electrophoresis in a 10% Bis-Tris NuPAGE gel (Cat. # NP0303BOX, Life Technologies Inc.) and transferred to a polyvinylidene difluoride membrane. Proteins of interest were probed with primary antibodies against ERK and pERK (Cat # 4695 and 9101, Cell signaling, Danvers, MA), 4-HNE-protein adducts (Cat # 393206, CalBiochem), AKT and pAKT (Cat # 9272 and 4691, Cell signaling), and β -Actin (Cat #8224, Abcam) at a dilution from 1000–5000. Secondary antibodies were HRP goat anti-rabbit IgG (Abcam Cat # ab6721), HRP goat anti-mouse IgG (Abcam Cat # ab6789) or HRP donkey anti-goat IgG (Santa cruz Cat # sc-2020) at a dilution of 5000–20,000. The signals from Western blot analysis were quantified by using Image Quant TL software in the Typhoon TRIO+ Variable Mode Imager (GE Healthcare life sciences). Each assay was repeated with 5–6 biologically independent replicates.

Statistical analysis

Statistical analyses were performed using StatView version 5.0 software (SAS, Cary, NC), SPSS version 18 software (IBM) and R program. Lifespan data were analyzed by Mantel-Cox logrank tests and a likelihood ratio test. All other measurements were analyzed by Student's *t*-test. Maximum lifespan was calculated as mean lifespan of the 10% longest surviving flies of a population. $p < 0.05$ was considered statistically significant.

Results

Cranberry supplementation affects insulin-like and MAPK signaling

We previously found that 2% cranberry can extend lifespan of female flies fed a high sugar-low protein SY9:1 diet (Wang et al., 2013). To understand the underlying mechanisms, we measured changes in the phosphorylation of proteins in three key metabolism and stress response pathways using the head protein samples of flies fed the SY9:1 diet supplemented with and without 2% cranberry for 11 days. The proteins tested are S6 kinase (S6K) in TOR signaling (Kim and Guan, 2011; Zoncu et al., 2011), AKT in insulin-like signaling (Broughton and Partridge, 2009) and ERK1/2 in stress response MAP kinase (MAPK) signaling (Rubinfeld and Seger, 2005). We chose head samples to reduce the complexity of protein samples since it is known that gene expression patterns are different among tissues. Cranberry supplementation did not significantly change the ratio of phosphorylated S6K (pS6K) to the total S6K in fly heads (Fig. 1A). However, cranberry supplementation slightly but significantly increased the ratio of pAKT/AKT ($p < 0.05$, Fig. 1B), while it significantly reduced the ratio of phosphorylated ERK1/2 to the total ERK1/2 ($p < 0.05$, Fig. 1C), when compared to the non-supplemented controls, respectively. These findings suggest that cranberry supplementation improves insulin-like signaling and reduces oxidative stress response signaling.

Cranberry supplementation reduced oxidative damage

To determine the effect of cranberry supplementation on oxidative damage, we measured the level of 4-hydroxynonenal protein adducts (4-HNE), which is a biomarker of lipid oxidation (Tsai et al., 1998). By Western blot analysis, we found that flies fed the SY9:1 diet supplemented with 2% cranberry for 11 days had significantly lower levels of 4-HNE in the whole body relative to the non-supplemented age-matched controls by approximately 25% ($p < 0.05$, Fig. 2). This suggests that cranberry supplementation reduces the accumulation of oxidative damage.

Lifespan extension induced by cranberry supplementation partially requires SOD2

We previously showed that 2% cranberry could extend lifespan of *sod1* knockdown flies under SY9:1 by >36% (Wang et al., 2013). To further determine the effect of cranberry supplementation on oxidative damage, we measured the lifespan of *sod2* knockdown female flies on SY9:1. SOD2 is a key superoxide scavenger in mitochondria, while SOD1 is a superoxide scavenger mostly in cytosol and partly in mitochondria (Fridovich, 1995). Supplementation of 2% cranberry only slightly increased mean lifespan of *sod2* knockdown flies fed the SY9:1 diet relative to the non-supplemented controls by approximately 12% ($p < 0.02$, Fig. 3A and Table 1). However, cranberry supplementation did not appear to affect the median lifespan of *sod2* knockdown flies (Table 1). Our previous findings indicate that 2% cranberry is not sufficient to extend the lifespan of wild type *Canton S* flies, but significantly increases the lifespan of *sod1* knockdown flies by >27% under the SY1:1 diet, which is a commonly used standard SY diet with modest amounts of sugar and yeast (Wang et al., 2013). Here we further tested the effect of cranberry supplementation on the lifespan of *sod2* knockdown flies on SY1:1. Consistent with the findings under the SY9:1 diet, 2%

cranberry did not change the lifespan of *sod2* knockdown female flies fed SY1:1, relative to the non-supplemented controls (Fig. 3B and Table 1). These findings suggest that SOD2 is at least partially required for lifespan extension induced by cranberry supplementation.

The effect of short-term life stage-specific supplementation of cranberry on lifespan and age-specific mortality rate

We next tested the impact of cranberry supplementation during three different life stages, health span from 3 to 30 days, transition span from 31 to 60 days and senescence span from 60 days to the end when all flies died. We measured the remaining lifespan after the initiation of cranberry supplementation in each of the three life stages in *Canton S* female flies. Supplementation of 2% cranberry during the health span increased mean lifespan by approximately 13 days or 25% relative to the life stage-matched non-supplemented controls ($p < 0.001$, Fig 4A and Table 1). Cranberry supplementation during the transition span increased the remaining mean lifespan of the population by approximately 8 days or 30% relative to the life stage-matched controls ($p < 0.001$, Fig 4C and Table 1). Cranberry supplementation during the senescence span also increased the remaining mean lifespan of the population by approximately 3 days or 30% relative to the controls ($p < 0.001$, Fig 4E and Table 1). The later the intervention was administered, the smaller the difference observed in mean lifespan in days was between life stage-matched cranberry supplemented and non-supplemented groups (Table 1). The remaining maximum lifespan of flies was also extended by cranberry supplementation during any of three life stages relative to the stage-matched controls (Table 1).

We then calculated the age-specific mortality rate for flies under the stage-specific supplementation of cranberry. Flies under cranberry supplementation during any of the three life stages showed a significant overall decrease in age-specific mortality rate relative to the life stage-matched controls after the initiation of cranberry supplementation ($p < 0.05$, Fig. 4B, D and F). The extent of the decrease in age-specific mortality rate was most prominent for flies under cranberry supplement during the health span when compared to those during the transition and senescence spans. The decrease of age-specific mortality was maintained throughout most of the rest of the life even when cranberry supplementation was stopped for flies under cranberry supplementation during health and transition spans. These findings suggest that cranberry supplementation can induce long-lasting prolongevity effects and reduce age-specific mortality rate when administered in different life stages.

Discussion

An adult life can be divided into three life stages, health, transition and senescence span, based on the age-specific mortality rate of the population (Arking, 2006). Each life stage has distinct characteristics and duration of each stage is plastic and modulated by both genetic and environmental factors. The complex nature of life stages poses challenges but also opportunities for developing cost effective but efficient interventions for promoting health and lifespan. Since it is almost impractical to implement any prolongevity intervention during the entire adult life span or even half of it in humans, it is critical to evaluate how an intervention during different life stages impact health and lifespan. In addition, life stage-specific studies may increase the chance to uncover many effective interventions. One type of intervention may be beneficial when implemented in early life but detrimental in late life, such as curcumin. A high dose of curcumin can extend lifespan when implemented during the health span, but decrease lifespan when administered during the transition or senescence span (Soh et al., 2013). A second type of intervention may be effective to promote healthy aging only when administered during late life, but detrimental or not effective when implemented during or initiated in early life. Sodium butyrate is such a drug, which does not

extend and even shortens lifespan when administered throughout adult life, but increases the lifespan when implemented during the transition or senesce span in *Drosophila* (McDonald et al., 2013). Another type of intervention may be potent regardless of when implemented if the duration of the intervention is long enough. The cranberry study described here is important not just because the effectiveness of cranberry supplementation has been investigated during three adult life stages. To our knowledge, cranberry is the first case showing that a pharmaceutical or nutraceutical can promote longevity when administered during any of the three distinct life stages. Future studies are warranted to determine how cranberry extends lifespan during different life stages. Such studies are important because different mechanisms may be involved during different life stages.

The free radical hypothesis of aging suggests that aging is caused by accumulated oxidative damage on macromolecules (Muller et al., 2007). We have found that lifespan extension induced by cranberry is associated with reduced oxidative damage at least in the health span. Two possible mechanisms may explain the effect of cranberry supplementation on oxidative damage. One is that cranberry acts as an antioxidant to promote longevity. Like many fruits and vegetables, cranberry is rich in many phytochemicals, such as proanthocyanins, which have many bioactivities, such as anti-oxidant, anti-cancer, anti-inflammation and anti-microbial infection capacities (He and Liu, 2006; Howell, 2007; Neto, 2007). Moreover, cranberry is among the fruits and vegetables with highest antioxidant activities as determined by the oxygen radical absorbance capacity (ORAC) assay (White et al., 2010; Wu et al., 2004). It is therefore possible that cranberry acts directly as an antioxidant to remove ROS and promote longevity. Consistent with this hypothesis, cranberry reduces accumulated 4-HNE, a biomarker for lipid oxidation (Tsai et al., 1998), and reduces the phosphorylation level of ERK, a component in oxidative stress response pathways (Rubinfeld and Seger, 2005). Lower pERK may indicate the higher reducing environment induced by cranberry supplementation. Moreover, cranberry can extend the lifespan of *sod1* knockdown flies, which have high oxidative damage due to the reduction of the major ROS scavenging enzyme SOD1 (Phillips et al., 1989; Wang et al., 2013).

Another possible mechanism by which cranberry may promote healthy aging is that cranberry may function as a molecular switch to epigenetically alter signaling pathways important for aging processes. We think that the second hypothesis may better explain the effect of cranberry in promoting longevity than the first one for at least two reasons. First, although cranberry can extend lifespan of oxidatively stressed *sod1* knockdown flies, it is not effective in extending lifespan of *sod2* knockdown flies. Like *sod1* knockdown flies, *sod2* knockdown flies have high oxidative damage (Kirby et al., 2002). The difference is that SOD1 predominantly resides in cytosol and SOD2 is primarily located in mitochondria to scavenge ROS (Fridovich, 1995). It is unlikely that *sod2* knockdown flies are generally too sick to be refractory to any lifespan extension intervention, since the SY9:1 diet significantly extends their lifespan when compared to the SY1:1 diet (Table 1). This suggests that cranberry stimulates signaling pathways that require SOD2 to promote longevity. However, we cannot exclude the possibility that cranberry has a different antioxidant capacity for ROS in different cellular compartment or cranberry and SOD2 act in a parallel manner to scavenge some overlapped sources of ROS. Second, the signaling hypothesis is supported by the life stage-specific experiments showing that life stage-specific interventions are sufficient to promote longevity and reduce age-specific mortality even after cranberry supplementation stops. The long lasting effect would unlikely sustain if cranberry acts merely as an antioxidant. One plausible scenario would be that phytochemicals in cranberry at least partially alter signaling profiles in the cell through epigenetic modulation.

A number of signaling pathways have been shown to modulate lifespan. These include nutrient sensing TOR and Insulin-like signaling and oxidative stress response MAPK

signaling. TOR signaling mostly senses amino acid concentration and insulin-like signaling mainly responds to glucose and energy levels (Fontana et al., 2010; Rubinfeld and Seger, 2005; Zoncu et al., 2011). Lower TOR or insulin-like signaling typically leads to a longer lifespan (Zoncu et al., 2011). We have observed little or no change in the phosphorylation of S6K, a component of TOR signaling, suggesting that cranberry may not have much impact on TOR signaling. Paradoxically, we observed a slightly elevated phosphorylation level of AKT, a key component of insulin-like signaling (Broughton and Partridge, 2009). The increase of pAKT may indicate improved glucose metabolism induced by cranberry. Higher pAKT may render flies better able to cope with any detrimental effect of a high sugar level in the diet, although it remains to be determined what impact of the slight increase of pAKT/AKT ratio has on lifespan. The conclusions drawn from studies of pS6K and pAKT levels are further supported by our previous study of a diet composition-dependent effect of cranberry on lifespan. Flies on the high sugar-low protein diet generally live longer than those on the low sugar-high protein diet at least partly due to lower TOR signaling under the high sugar-low protein diet (Kapahi et al., 2004; Lee et al., 2008; Sun et al., 2012; Zid et al., 2009). We have previously found that cranberry can further extend the long lifespan of flies fed the high sugar-low protein diet but not the relatively short lifespan of flies on the low sugar-high protein diet when compared to the diet-matched controls (Wang et al., 2013). Therefore, it is unlikely cranberry extends lifespan by reducing TOR signaling. Otherwise, cranberry supplementation should increase lifespan of flies on the high protein diet. Furthermore, cranberry supplementation has been shown to increase insulin-releasing β -cells in the pancreas of aged rats (Zhu et al., 2011). These findings support the role of cranberry supplement in improving glucose metabolism. Another signaling pathway affected by cranberry supplementation is the oxidative stress response MAPK signaling, suggested by the fact that cranberry reduces the phosphorylation level of ERK. Cranberry may promote a cellular environment with less oxidative damage, which in turn results in less active MAPK signaling. Consistent with our findings, curcumin treatment reduced the ERK1/2 protein level in leiomyoma cells from patients with symptomatic leiomyomata (Malik et al., 2009). More mechanistic studies are needed to pinpoint signaling pathways and genome-wide epigenetic status, which are directly affected by cranberry supplementation. Given the fact that lifespan extension induced by cranberry at least partly requires SOD2, signaling pathways involving SOD2 are likely candidates to evaluate.

In conclusion, we have demonstrated that cranberry supplementation reduces accumulated oxidative damage and oxidative stress response signaling in *Drosophila*. We have also shown that cranberry supplementation during any of the three life stages, health, transition and senescence span, is sufficient to lower the age-specific mortality rate and extends lifespan. The long-lasting effect of cranberry supplementation is probably due to its ability to change signaling pathways and epigenetic status. Our findings suggest that cranberry as an abundant and popular fruit is a potentially viable option for aging interventions in humans of different ages. This study also supports the plastic nature of health and longevity at different life stages and the need to develop a combination of interventions each optimized at different life stages to promote overall healthy aging. Life stage-specific studies should provide valuable guidance to reduce intervention duration and minimize potentially detrimental effects of any drug.

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Abbreviations

4-HNE	4-Hydroxynonenal
cran	cranberry
IR	inverted repeat
MAPK	MAP kinase
RNAi	RNA interference
S6K	S6 kinase
SOD1	superoxide dismutase 1
SOD2	superoxide dismutase 2
SY	sugar-yeast diet
TOR	target of rapamycin

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Highlights

- Cranberry supplementation reduces accumulated oxidative damage.
- Lifespan extension induced by cranberry partially requires SOD2.
- Short-term Cranberry feeding in three different life stages can extend lifespan.

Fig. 1.

The effect of cranberry supplementation on metabolic and stress response pathways. (A) Western blot images of S6 kinase (S6K) and phosphorylated S6K (pS6K) for flies fed the SY9:1 diet with and without 2% cranberry (cran). (B) Quantification of pS6K/S6K ratios in flies fed SY9:1 with and without 2% cranberry. (C) Western blot images of AKT and phosphorylated AKT (pAKT) for flies fed SY9:1 with and without 2% cranberry. (D) Quantification of pAKT/AKT ratios in flies fed SY9:1 with and without 2% cranberry. (E) Western blot images of ERK and phosphorylated ERK (pERK) for flies fed SY9:1 with and without 2% cranberry. (F) Quantification of pERK/ERK ratios in flies fed SY9:1 with and without 2% cranberry. The flies were collected at 14 d old. Each treatment was repeated with five biologically independent samples. Three replicates are shown for each treatment. n.s., not significant; * $p < 0.05$ by Student's t -test.

Fig. 2.

The effect of cranberry supplementation on accumulated oxidative damage. (A) Western blot images of 4-HNE protein adducts (4-HNE) and loading control β -Actin for flies fed the SY9:1 diet with and without 2% cranberry (cran). (B) Quantification of the 4-HNE levels normalized with β -Actin in flies fed SY9:1 with and without 2% cranberry. The flies were collected at 14 d old. Each treatment was repeated with five biologically independent samples. Three replicates are shown for each treatment. * $p < 0.05$ by Student's t -test.

Fig. 3.

The effect of cranberry supplementation on lifespan of *sod2* knockdown female flies. (A) Lifespan of *sod2* knockdown flies fed the SY9:1 diet supplemented with and without 2% cranberry (cran). *sod2* knockdown was induced by RNA interference (RNAi). (B) Lifespan of *sod2* knockdown flies fed the SY1:1 diet supplemented with and without 2% cranberry (cran). p values from logrank test between “no cran” and “2% cran” groups are shown inside the lifespan curves.

Fig. 4.

The effect of cranberry supplementation during different life stage on lifespan. (A) Lifespan of wild-type *Canton S* flies under supplementation of 2% cranberry during the health span (3–30 days) and non-supplemented controls. (B) Age-specific mortality rate of flies under supplementation of 2% cranberry during the health span and non-supplemented controls. (C) The remaining lifespan of wild-type *Canton S* flies under supplementation of 2% cranberry during the transition span (31–60 days) and non-supplemented controls. (D) The remaining age-specific mortality rate of flies under supplementation of 2% cranberry during the transition span and non-supplemented controls. (E) The remaining lifespan of wild-type *Canton S* flies under supplementation of 2% cranberry during the senescence span (60–90 days) and non-supplemented controls. (F) The remaining age-specific mortality rate of flies under supplementation of 2% cranberry during the senescence span and non-supplemented controls. The duration of cranberry treatment in each life stage is marked with a dash line bordered with arrows. Thick dash trend lines are for mortality rates of control flies and thick solid lines are for mortality rates of flies under supplementation of 2% cranberry.

solid trend lines are for mortality rates of flies treated with 2% cranberry. The estimated regression equation for the non-supplemented control is $y=0.0556x-6.070$ ($R^2=0.795$, $p<0.0001$); for the health span intervention group, $y=0.0552x-6.729$ ($R^2=0.674$, $p<0.0001$); for the transition span intervention group, $y=0.0516x-6.238$ ($R^2=0.718$, $p<0.0001$); for the senescence intervention group, $y=0.045x-5.760$ ($R^2=0.622$, $p<0.0001$), in which y represents age and x represents $\text{Ln}(\text{age-specific mortality rate})$. The p values inside the lifespan curves were from logrank test between life stage- matched “no cran” and “2% cran” groups. The p values inside the age-specific mortality rates were from likelihood ratio test between life stage- matched “no cran” and “2% cran” groups.

Table 1

Lifespan of flies with and without 2% cranberry supplementation.

Strain	SY Diet ^a	Intervention period	Cran Conc.	# of flies	Mean lifespan (days±SE) ^b	Change of mean lifespan	P value by LogRank	Median lifespan ^b	Maximum lifespan (days±SE) ^b	Change of maximum lifespan
<i>Canton S</i>	SY9:1	3–30 d	0%	327	51.4±1.0			51	79.7±0.5	
			2%	474	64.4±0.9	25.2%	<0001	70	89.3±0.3	12.0%
		31–60 d	0%	290	25.3±0.9			23	50.2±0.5	
			2%	280	32.9±1.0	30.1%	<0001	35	57.8±0.5	13.1%
		61 d–	0%	108	11.3±0.6			11	23.1±0.7	
<i>sod2RNAi</i>	SY9:1	Adult life	2%	154	14.7±0.8	29.8%	<0001	13	30.4±0.4	31.6%
			0%	99	14.8±0.4			15	n. d.	
		Adult life	2%	87	16.5±0.6	11.8%	0.0222	15	n. d.	n. d.
			0%	83	9.7±0.4			8	n. d.	
		Adult life	2%	71	9.4±0.3	–3.1%	0.37	10	n. d.	n. d.

^aSY diet refers to the diet with sugar-yeast at the ratio of 9:1 or 1:1.

^bLifespan refers to the remaining mean, median or maximum lifespan after the initiation of intervention.
n. d.: not determined.