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Influence on Longevity of Blueberry, Cinnamon, Green and Black Tea, Pomegranate, Sesame, Curcumin, Morin, Pycnogenol, Quercetin, and Taxifolin Fed Iso-Calorically to Long-Lived, F1 Hybrid Mice

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Abstract

Phytonutrients reportedly extend the life span of *Caenorhabditis elegans*, *Drosophila*, and mice. We tested extracts of blueberry, pomegranate, green and black tea, cinnamon, sesame, and French maritime pine bark (Pycnogenol and taxifolin), as well as curcumin, morin, and quercetin for their effects on the life span of mice. While many of these phytonutrients reportedly extend the life span of model organisms, we found no significant effect on the life span of male F1 hybrid mice, even though the dosages used reportedly produce defined therapeutic end points in mice. The compounds were fed beginning at 12 months of age. The control and treatment groups were iso-caloric with respect to one another. A 40% calorically restricted and other groups not reported here did experience life span extension. Body weights were unchanged relative to controls for all but two supplemented groups, indicating most supplements did not change energy absorption or utilization. Tea extracts with morin decreased weight, whereas quercetin, taxifolin, and Pycnogenol together increased weight. These changes may be due to altered locomotion or fatty acid biosynthesis. Published reports of murine life span extension using curcumin or tea components may have resulted from induced caloric restriction. Together, our results do not support the idea that isolated phytonutrient anti-oxidants and anti-inflammatories are potential longevity therapeutics, even though consumption of whole fruits and vegetables is associated with enhanced health span and life span.

Introduction

Dietary vegetables and fruits are a major source of phytochemicals, nutrients, and fiber.[1] Consumption of plant-based foods is associated with improved cardiovascular and neurological health, reduced cancer incidence, and increased longevity in humans.[2,3] Extracts and isolated components of fruits, spices, leaves, and seeds are consumed by about half of the adults in the United States with the expectation that they will be as, or more, efficacious than their botanical sources.[4] Expenditures for dietary supplements are estimated to be in excess of $30 billion annually.[5]

The studies described here were designed to test the hypothesis that popular fruit, leaf, spice, and seed extracts, and individual phytochemicals isolated from these botanicals can increase mammalian life span. Many of the extracts and compounds tested have been reported to increase the health and longevity of model organisms and, in a few cases, of mice.[6] To increase the likelihood that our studies would provide a reliable measure of the effects of the compounds *per se*, long-lived, F1 hybrid mice were used, and their body weights were determined throughout the treatment regimen, as discussed previously.[6] The control and supplement-treated mice were fed the same number of calories, *e.g.*, they were fed iso-calorically.

Materials and Methods

Mouse life span and food consumption

The studies used male B6C3F1 mice (Harlan Breeders, Indianapolis, IN) randomly assigned to treatment groups at 12 months of age. The notation indicates that the mice are a cross between C57BL/6NHsd inbred females and C3H/HeNHsd inbred males. The mice were un-mated. No mice had to be removed from the study due to aggression. A total of 297 mice (the control mice) were shifted from *ad libitum*

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chow feeding (Diet # 5001, Purina Mills, Richmond, IN) to daily feeding with 13.3 kcal/day per mouse of control diet (AIN-93M, Diet No. F05312; Bio-Serv, Frenchtown, NJ). Groups of 36 mice were shifted to daily feeding with an identical quantity of control diet supplemented with the compounds indicated in Table 1. The 40% calorie restriction (CR) group (36 mice) was shifted to 11 kcal/day per mouse of AIN-93M 20% Restricted Diet for 2 weeks, and thereafter to 7.46 kcal/day of AIN-93M 40% Restricted Diet (Diet No. F05314, Bio-Serv). The 20% and 40% CR diets were fortified so that the mice received fewer calories in the form of carbohydrates than the other groups but approximately equal amounts of fat, protein, vitamins, and minerals. Carbohydrate restriction was used because good experimental design specifies that to the degree possible only one variable should be changed at a time to simplify the interpretation of results. All mice were fed daily between 9 a.m. and 2 p.m. As the study progressed, feeding concluded sooner as the number of mice declined. Mice were moved as numbers declined to maintain densities of two to four per cage. Food consumption and mouse health were monitored at the time of feeding, and any un-eaten food was noted. With few exceptions, all food was eaten each day. The drugs were mixed with powdered diet and cold-pressed into 1-gram pellets by Bio-Serv. The food was stored moisture free at 4°C until used. The mice drank acidified (pH 4.0) tap water *ad libitum*. Acidification greatly reduces *Pseudomonas* colonization of water bottles and sippers, as well as the nasopharynx and intestines of mice.[7,8] The mice were weighed bi-monthly. The mice were maintained in shoebox cages with 0.22-micron covers, under barrier conditions, four per cage, on a 12-hr light/dark cycle at 22°C and 56% humidity. Bedding was sterilized with radiation. The health of the mice was examined twice daily by laboratory staff and weekly by a veterinarian. Dead mice were stored at −20°C until necropsy. Two sentinel Swiss Webster female mice were housed in each vivarium room with mice for this study. They were exposed to dirty bedding from the cages in that room, and this treatment was renewed weekly. Quarterly, the sentinels from each room were tested serologically by Idexx Radil for *Mycoplasma pulmonis*, ectromelia virus, epizootic diarrhea of infant mice virus, lymphocytic choriomeningitis virus, mouse hepatitis virus, murine norovirus, mouse parvovirus (MPV), mouse minute virus, pneumonia virus of mice, respiratory enteric orphan virus-3 (REO3), Sendai virus, and Theiler's encephalomyelitis virus. Sentinels also were tested for *Aspiculuris tetraptera* by fecal flotation and for *Syphacia obvelata*, *S. muris*, *Myobia musculi*, *Mycoptes musculinus*, and *Radfordia affinis* using tape tests. On one occasion (1/30/2009), when the mice were approximately 450 days old, sentinels from one of five rooms housing the mice returned a positive immunological test for MPV. Feces from all cages in that room and from cages in the other rooms were immediately tested for MPV using a polymerase chain reaction assay (Charles River Laboratories), which returned uniformly negative results. Positive controls were used. Thereafter, all serological tests for all the sentinels from all rooms tested uniformly negative for all pathogens. This study was approved by the Institutional Animal Care and Use Committee at the University of California, Riverside.

Table 1. Dosages of the Supplements

Treatment	*Concentration in food (mg supplement/kg diet)*[a]	*Approximate daily dose (mg supplement/kg body weight/day)*
Blueberry extract	684	73
Cinnamon extract	438	47
Curcumin (0.1%)	1000	106
Curcumin (0.2%)	2000	212
Green tea extract	931	99
Green tea extract, Black tea extract, morin	931, 440, 500	99, 47, 53
Pomegranate extract	818	67
Quercetin,[b] taxifolin, Pycnogenol	584, 630, 94	62, 67, 10
Sesame extract (sesamin)[c]	4.94	525

[a]The concentrations are for the components listed less excipient.
[b]Quercetin was dissolved in the corn oil component of the AIN-93M diet.
[c]An equivalent volume of corn oil was removed from the diet.

Statistical analysis

For the survival data, Kaplan–Meier survival curves were compared using the Gehan-Breslow–Wilcoxon test implemented in GraphPad Prism 5.01. The tests were not adjusted for multiple testing. For body weight, the significance of the differences between groups were judged using a linear mixed effects model.[9,10] Specifically, we modeled the mean response by a set of fixed effects assumed to be shared by mice and a set of random effects that are unique to a particular mouse. Additionally, our model imposes a common intercept because all mice were on the same diet at the time of the first measurement. We denote Y_{ijt} as the weight of mouse $i \in \{1,\ldots 657\}$ for diet $j \in \{1, 4, 11, 25, 29, 32, 33, 36, 49, 52, 62\}$; where diet 1 is the control diet, 4 is 40% CR diet, 11 the blueberry diet, 25 the cinnamon diet, 29 the Tea/morin diet, 32 the 0.1% curcumin diet, 33 the 0.2% curcumin diet, 36 the green tea diet, 49 the pomegranate diet, 52 the sesame diet, and 62 the quercetin/taxifolin/Pycnogenol-containing diet; taken at time $t \in \{0, 30, 91, 152, 213, 274, 335, 395, 456, 517, 578, 639, 700, 760, 821, 882, 943, \text{and } 1004\}$, where the numerals correspond to numbers days on the diets. Our analysis uses the linear mixed model, $Y_{ijt} = \beta_0 + \beta_1 t + \beta_2 t^2 + \beta_{1j} t + \beta_{2j} t^2 + b_{0i} + b_{1i} t + b_{2i} t^2 + e_{ijt}$; where β_0 is the global intercept, β_1 and β_2 are the control diet quadratic coefficients, β_{1j} and β_{2j} are diet-specific coefficient adjustments for diet j, and e_{ijt} is the measurement error. Note that β_{11} and β_{21} will be zero for mice within the control group, *i.e.*, when $j=1$. Further, we denote the subject specific random effects as b_{0i}, b_{1i}, and b_{2i} for $i \in \{1,\ldots 670\}$, and hence we allow each mouse to have a subject specific quadratic trajectory. Use of these random effects induces a suitable covariance model for the data in this study. Application of a Bayesian information criterion (BIC) model selection criteria suggests that the 40% CR and tea/morin diets show significant weight loss, whereas the mice consuming the quercetin/taxifolin/Pycnogenol diet maintained a significantly higher weight than the control group and the groups consuming the other diets.

Sources of dietary supplements

Blueberry and pomegranate (*Punica granatum*) extracts were formulated by the Life Extension Foundation; black tea extract was formulated by Solaray; cinnamon extract was formulated by Pure Encapsulations; microencapsulated curcumin was a gift from Verdure Sciences; green tea extract was formulated by Now; Pycnogenol was formulated by Source Naturals; quercetin was formulated by Jarrow; and sesamin was formulated by SciVation. All of the previously listed compounds were purchased from the least expensive vendors. Morin was from Sigma and taxifolin from APAC Pharmaceuticals.

Results

Life span results

The natural products tested in these studies were extracts of blueberry, pomegranate, green and black tea, cinnamon, sesame, French maritime pine bark (Pycnogenol), curcumin, quercetin, and taxifolin. We used a microencapsulated form of curcumin designed to increase its bioavailability.[11,12] Most of the extracts and supplements tested have been described as anti-oxidants or anti-inflammatories (see the Discussion section). The doses for each of the compounds are shown in Table 1.The agents were cold packed into the food, which was the chemically defined AIN-93M diet. The supplemented diets were fed iso-calorically to male B6C3F1 mice beginning at 12 months of age. Because the food was administered daily in measured amounts, un-eaten food could be noted and the next day's allotment adjusted to maintain the daily quota of calories. None of the agents significantly altered the life span of the mice, as judged by comparing Kaplan–Meier survival curves using either the Gehan–Breslow-Wilcoxon or the Mantel–Cox statistical tests (Figs. 1–5). In contrast, 40% CR, begun at 12 months of age, resulted in a highly significant 23% extension of median life span and also extended maximum life span ($p < 0.001$; Fig. 5). Statistically significant life span extension also was found in seven other treatment groups from this study (*e.g.*, ref. 13). These positive results demonstrate that, despite their long life spans, the mice were responsive to longevity-extending treatments begun at 1 year of age under these husbandry conditions.

This study was part of a larger study using a total of 2400 mice in which groups of 36 treated mice and 297 control mice were used to maximize the number of treatment groups.[14]

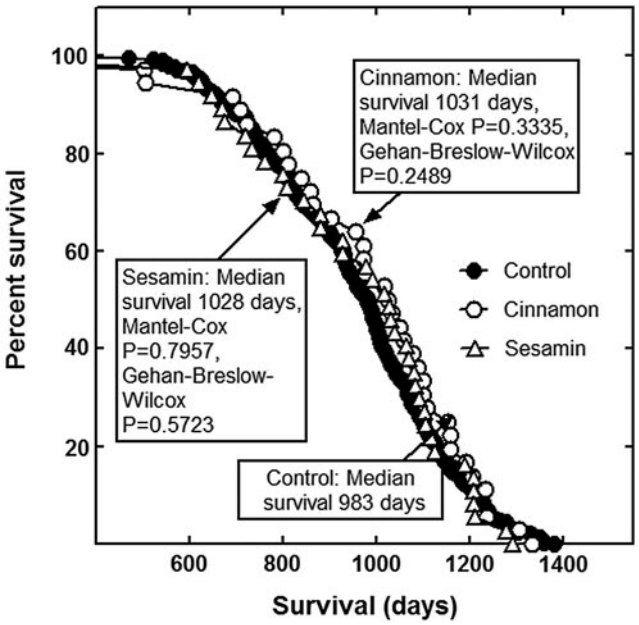

FIG. 2. Life span of mice fed a control diet or a diet containing cinnamon extract or sesamin extract.

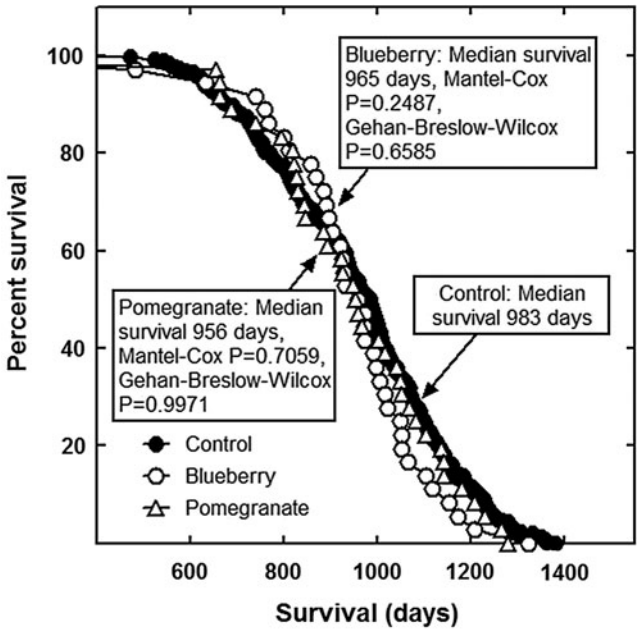

FIG. 1. Life span of mice fed a control diet or a diet supplemented with blueberry extract or pomegranate extract.

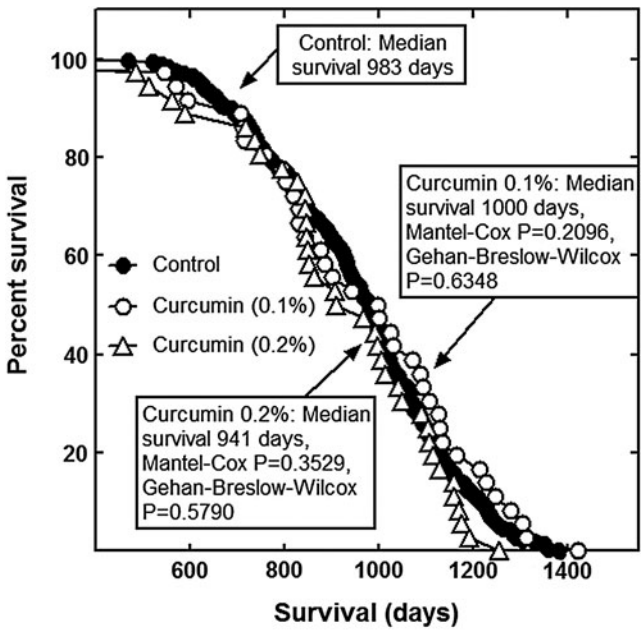

FIG. 3. Life span of mice fed a control diet or a diet containing one of two concentrations of curcumin.

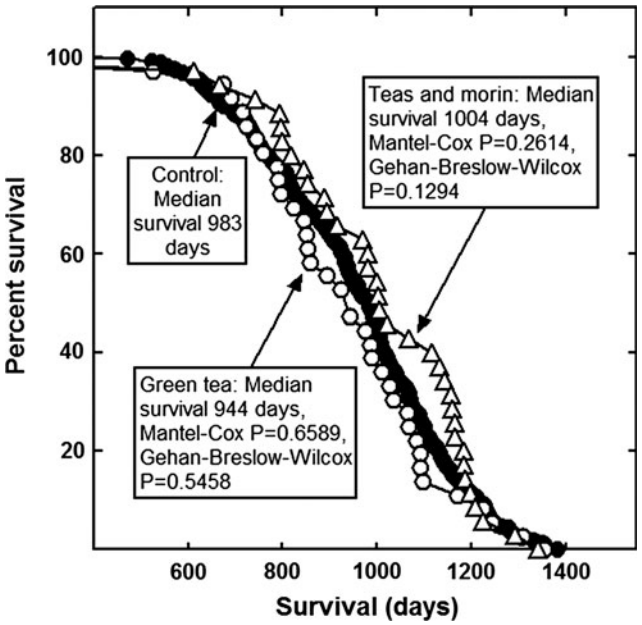

FIG. 4. Life span of mice fed a control diet or a diet supplemented with green tea extract alone or with green and black tea extract and morin combined.

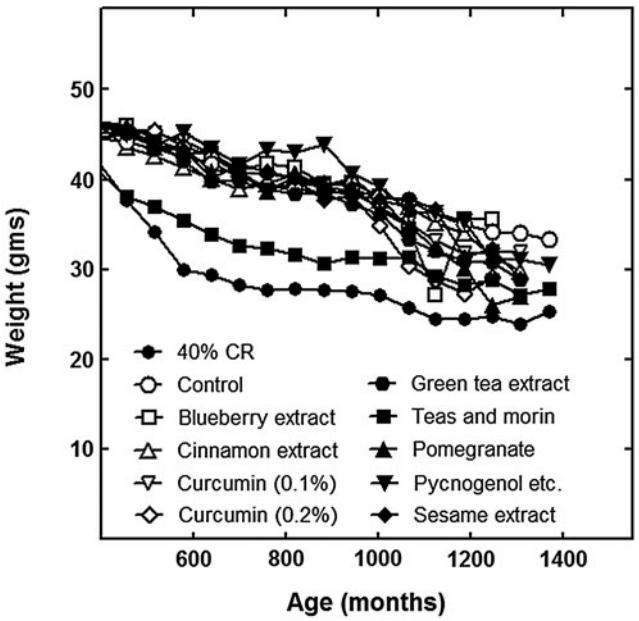

FIG. 6. Body weight of the mice in each group. For clarity, error bars were omitted. The groups fed the 40% calorie restricted (CR) and tea/morin diets were significantly underweight relative to the controls, whereas the group fed the quercetin/taxifolin/Pycnogenol-containing diet was significantly heavier than the controls (see Materials and Methods for details regarding the statistical analysis). The body weights of the CR mice have been published previously and are used by permission.[9]

Food consumption and body weight

The body weights of the mice are shown in Fig. 6. The mice were randomly assigned to groups at 1 year of age. After their shift to the defined diets at that time, the weights of the supplemented groups were not different than those of the controls, with two exceptions. The median weight of the mice fed the diet supplemented with green tea, black tea, and morin (tea/morin diet) was significantly less than that of the controls at every age (Fig. 6). Because the mice ate all of their food (Table 2), they either absorbed fewer calories or metabolized the calories more quickly. These effects may be due to increased spontaneous locomotion or reduced fatty acid synthesis (see Discussion). In contrast, the quercetin-, taxifolin-, and Pycnogenol-supplemented group was heavier than the controls, perhaps due to either increased absorption or decreased use of calories. With these exceptions, there were only sporadic differences between the weights of the other groups and the controls (Fig. 6). Because the ratio of food consumption to body weight was not different for these groups, most of the supplements had no apparent effect on the rates of energy adsorption or use and therefore on the metabolic rate.

All of the mice gradually lost weight after they were shifted from chow to the AIN diets, even though all of their food was eaten each time (Table 2). Despite this weight loss, they appear to have been close to *ad libitum* caloric intakes. The control and most treated groups weighed ~41 grams at 665 days of age, 300 days after the switch to the AIN diets (Fig. 6). This is approximately the same weight as chow fed to B6C3F1 male mice of the same age.[15] The weight loss that occurred after 2 years of age was probably due to the characteristic loss of body weight by B6C3F1 mice during aging.[15]

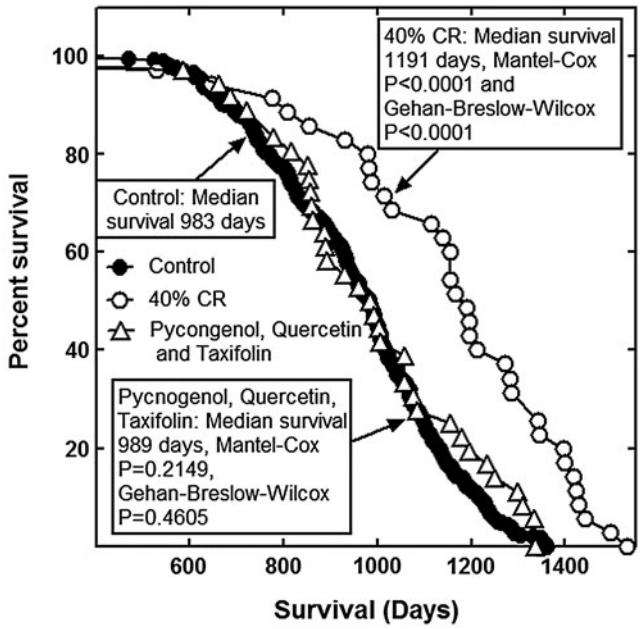

FIG. 5. Life span of mice fed a control diet, a 40% calorie restricted (CR) diet, or a diet containing quercetin, Pycnogenol, and taxifolin. The life span data for the 40% CR diet fed mice were published previously and are used by permission.[9]

Discussion

The work presented here does not support the notion that fruit, leaf, spice, and seed phytonutrient extracts and isolated phytonutrients extend murine life span when they are fed

Table 2. Food Consumption of the Treated Mice As a Percent of Control Food Consumption

	Age (days)[a]													
Diet	*456*	*487*	*517*	*548*	*578*	*608*	*639*	*669*	*700*	*730*	*760*	*791*	*821*	*852*
Blueberry extract	99.0	99.9	100.1	100.0	100.0	100.0	100.1	100.1	99.8	99.8	98.6	98.6	95.5	95.5
Cinnamon extract	99.7	99.8	100.0	100.0	100.0	100.0	100.1	100.1	100.1	100.1	100.0	100.0	100.0	99.8
Curcumin (0.1%)	99.3	100.0	100.0	100.0	100.0	100.0	100.1	100.1	100.0	100.1	100.0	100.0	99.1	98.4
Curcumin (0.2%)	99.3	99.5	100.0	100.0	100.0	99.7	100.1	100.1	99.7	99.0	99.9	100.0	99.8	100.0
Green tea extract	99.1	99.6	99.1	99.4	100.0	10.0	99.9	100.1	100.0	100.0	99.7	99.7	99.2	100.0
Green and black tea extract, morin	94.5	95.7	98.3	99.8	100.0	100.0	99.8	99.8	99.9	100.1	99.9	99.8	100.0	100.0
Pomegranate extract	99.3	99.8	100.1	100.0	100.0	100.0	99.9	100.1	100.0	99.7	99.9	100.0	100.0	100.0
Quercetin, taxifolin, Pycnogenol	99.6	99.7	99.9	100.0	100.0	100.0	100.1	100.1	100.1	100.1	100.0	100.0	99.8	100.1
Sesame extract (Sesamin)	97.7	99.5	99.9	100.0	100.0	99.5	99.3	99.0	99.4	98.0	99.6	99.7	99.4	99.2

	Age (days)[a]												
Diet	*882*	*913*	*943*	*973*	*1004*	*1034*	*1065*	*1095*	*1125*	*1156*	*1186*	*1217*	*1247*
Blueberry extract	97.3	98.1	100.2	100.5	100.5	100.6	102.1	102.6	100.8	105.8	102.6	102.2	102.7
Cinnamon extract	99.9	100.0	100.2	100.7	100.5	100.6	101.1	102.6	107.2	106.0	102.6	101.9	101.9
Curcumin (0.1%)	100.0	99.6	100.2	100.7	100.5	100.6	101.8	102.5	107.1	106.0	102.6	102.2	102.5
Curcumin (0.2%)	99.5	99.7	100.1	100.6	100.4	99.7	101.9	102.3	107.2	105.8	102.2	101.7	102.7
Green tea extract	99.9	99.6	100.1	100.7	100.5	100.6	102.1	102.6	107.2	106.0	102.6	102.2	102.7
Green and black tea extract, morin	99.7	100.0	100.2	100.7	100.5	100.5	100.4	102.4	106.3	102.5	100.3	100.2	97.8
Pomegranate extract	100.0	100.0	100.2	100.5	100.5	100.6	102.1	102.3	107.2	106.0	102.6	102.2	102.7
Quercetin, taxifolin, Pycnogenol	99.3	99.1	100.0	100.7	100.5	100.4	102.0	102.6	107.2	106.0	102.6	102.2	102.4
Sesame extract (Sesamin)	100.0	100.0	100.2	100.7	100.4	100.4	97.7	96.5	104.4	102.1	97.2	98.3	102.7

[a]Values shown are the food consumption as a percent of control for all mice alive in each group at the age indicated. Mean food consumption was not statistically different at any age compared to control as judged using two sample t-tests.

iso-calorically to long-lived F1 mice. These results are in contrast to previous reports indicating that many of the tested compounds extend the life spans of nematodes, flies, and/or mice. Our results are not due to a general lack of responsiveness of these mice to treatments, since they responded to β-adrenergic receptor blockers,[13] CR (Fig. 5), and other compounds (data not shown). One interpretation of our results and those in previous reports is that longevity studies in lower eukaryotes and enfeebled rodents are prone to false positives. However, it is clear that at least some compounds that increase the life span of lower eukaryotes do increase the life span of iso-calorically fed F1 mice (*e.g.* ref. 13). Because many of the compounds we tested are thought to be biologically relevant anti-oxidants, our studies are consistent with the growing body of literature suggesting that oxidative stress may play a more limited role than once thought in determining the life span of healthy mammals.[16]

We used F1 hybrid mice for these studies because they are heterozygous for all alleles that differ between the parental strains. They tend to be more robust and longer lived than their inbred parents.[6] The mice also were fed iso-calorically, rather than being fed *ad libitum*, as is the practice in most supplement-feeding studies. In *ad libitum* feeding studies, supplement-related food aversion could lead to reduced caloric consumption, and hence to CR-related life span extension. We found that approximately half of the supplements fed to mice reduce *ad libitum* food consumption.[6] We also monitored body weight regularly.

The possible reasons the mice supplemented with the tea/morin diet were underweight despite consuming the same number of calories as the control group are not known. However, this may be related to either the caffeine or tea polyphenols in the diet. Caffeine and several of its metabolites are non-selective antagonists of the adenosine receptors, which promote increased locomotor activity and decreased weight and adiposity in mice.[17–19]Tea also contains a large number of polyphenolic flavonoids, including morin (a gallated catechin).[20] Many of these flavonoids are potent inhibitors of fatty acid synthase, a rate-limiting enzyme in fatty acid biosynthesis.[21] These inhibitors may also have reduced the weight of the treated mice. Because the tea and morin group did not experience an increase in longevity, we did not investigate its effect on body weight further.

A majority of the compounds tested in our study were previously shown to extend the life span of nematodes, flies, and/or mice. For example, blueberry extract, which is a rich source of phytonutrients, including flavonoids, was shown to extend the life span the nematode *Caenorhabditis elegans*[22] and the fly *Drosophila melanogaster*.[23] Blueberries also were reported to have beneficial neurological and cardiovascular effects in rats and humans.[24–27] Pomegranate consumption is associated with decreased basal cell and squamous cell carcinoma risk in European populations,[28] and with amelioration of the adverse effects of metabolic syndrome.[29] Pomegranate juice and its specific components also may inhibit cellular and molecular processes required for the metastasis of prostate and breast cancer in mice.[30–33] Curcumin, the major curcuminoid of the spice turmeric, or its metabolite tetrahydrocurcumin, reportedly extend the life spans of *Drosophila*,[34–36] *C. elegans*,[37] and mice.[38] However, the

published mouse study may be confounded by reduced caloric intake, since the treated mice weighed significantly less than the controls.[38] We found here that when caloric intake is unchanged, weight is not affected by curcumin and there is no effect on life span.

Tea leaf extracts contain principally epicatechins and theaflavins, and their gallated derivatives, in addition to many other compounds.[39] Positive health effects have been reported for tea leaf extracts and specific subcomponents. For example, daily consumption of green tea catechins delays memory regression in aged senescence-accelerated mice.[40] Morin, a plant flavone widely distributed in human foods such as tea, vegetables, fruits, and red wine, has anti-oxidant and anti-inflammatory activity.[41,42] Morin has been reported to attenuate *N*-nitrosodiethylamine–induced hepatocellular carcinogenesis in rats.[42] Its anti-carcinogenic activities may stem from its inhibitory effects on fatty acid synthase.[21] Black tea extracts extend the life span of *Drosophila*,[43] whereas green tea polyphenols have been reported to both extend and to not extend mouse life span.[12,38] The flavonoid epigallocatechin gallate (EGCG), isolated from green tea, reportedly extends,[44] or does not extend[45,46] the life span of *C. elegans*. We found no effect of tea extracts or morin on the life span of our mice, in agreement with a similar study by others.[12]

Pycnogenol, a standardized, flavonoid, catechin, proanthocyanidin, and phenolic acid–rich extract of *Pinus pinaster* (French maritime pine) bark,[47] is currently being evaluated in a randomized, placebo-controlled, double-blind, three-arm clinical trial for its effects on human cardiovascular and cognitive brain health.[48] Taxifolin, a flavonoid component of Pycnogenol,[47] extends the life span of *C. elegans*.[49] Quercetin, which is structurally related to taxifolin, increases the life span of *C. elegans*[50,51]and *Drosophila*.[52] Despite these longevity effects in worms and flies, and the possible effects of Pycnogenol on human brain health, we found no effects of a cocktail composed of all three compounds on the longevity of mice. The literature regarding Pycnogenol and its components and metabolites is limited, and there is presently insufficient information for informed speculation as to why it increased the weight of our iso-calorically fed mice.

Drug dosages

The dosages used in these studies were chosen based on published studies associating specific dosages with well-defined health-related end points in mice. The dosages used are shown in Table 1. The oral administration of blueberry extract at 10–1000 mg/kg body weight to mice decreased hemangioendothelioma tumor growth *in vivo* and prolonged survival.[53] Black tea extract administered orally at 50 mg/kg body weight suppressed blood triglyceride levels after the oral administration of corn oil to male mice.[54] Cinnamon extract administered in drinking water at 100 mg/L led to a marked decrease in 56-kD amyloidβ (Aβ) oligomers and plaque number and an improvement in the cognitive behavior of a transgenic mouse model of Alzheimer disease.[55] Curcumin fed at 500 mg/kg chow (0.05%) reduced brain amyloid plaque burden, insoluble Aβ peptide, and carbonyls in Tg2576 APPsw transgenic mice.[11] Green tea extract at 500 and 1000 mg/kg in food attenuated hepatic steatosis by decreasing adipose lipogenesis and enhancing hepatic anti-oxidant defenses in *ob/ob* mice.[56] Morin administered at 500 mg/kg food protected against *N*-nitosodiethylamine–induced hepatocellular carcinogenesis in mice.[42] Pomegranate extract administered to mice at 1000 mg/L in drinking water inhibited the development of prostate cancer in TRAMP mice and the growth of tumor xenografts in nude mice.[32,33] This dose is approximately equivalent to the dosage used in the present study, adjusted for the differences in food versus water consumption. Pycnogenol at 10 mg/kg body weight/day significantly reduced encephalomyocarditis virus–induced myocarditis by decreasing viral replication.[57] Quercetin at 0.04% (400 mg/kg food) and 0.08% (800 mg/kg food) decreased plasma glucose levels dose responsively, increased plasma adiponectin, improved plasma lipid and lipoprotein profiles, reduced thiobarbituric acid reactive substances, and elevated anti-oxidant enzyme level in the liver of C57BL/KsJ-db/db mice.[58] Sesamin at 5 grams/kg food attenuated intimal thickening and intercellular adhesion molecule-1 (ICAM-1) expression in the aortas of apolipoprotein E–deficient mice.[59] Taxifolin administered to male mice at 669 mg/kg in food reduced formalin/acetic acid, and carrageenan/bradykinin/dextran induced pain and edema more potently than did acetaminophen.[60]

Study design

We began compound treatment at 12 months of age. There were several rationales for this choice. First, at least some therapeutics have negative as well as positive effects on health and longevity.[13] Administering a compound beginning later in life confines the treatment to the time in life when the effects of aging are most pronounced. Second, the most useful and approvable human therapeutics may be those that are effective when treatment starts later in life, after growth, development and reproduction have ceased.[61] Third, at least some therapeutics are equally effective whether begun early or later in life. For example, rapamycin, a model longevity therapeutic, is approximately as effective whether treatment is begun at 9 or at 20 months of age.[62]

An un-balanced statistical design was employed, wherein a large group of control mice and smaller groups of treated mice were used to maximize the total number of study groups in the study (see, for example, ref. 14 for a discussion of this in the context of Weibull analyses). The sample sizes in this study are similar to those required for a Weibull survival analyses set up to have a 75% probability of detecting an 10% increase in mean life span with a 1% ($\alpha \leq 0.01$) probability of a false positive across 58 test groups.[14] There were 58 test groups used for these studies, and the statistics reported are not corrected for multiple testing.

Summary

The results of the studies reported do not support the idea that extracts of blueberry, cinnamon, curcumin, green tea, black tea, morin, pomegranate, quercetin, Pycnogenol, taxifolin, and sesamin increase the life span of healthy male mice. The mice were fed daily, and both food consumption and body weight were measured. The supplements, with only two exceptions, did not affect energy absorption or utilization (metabolic rate). The results reported here do not support the idea that the consumption of isolated and concentrated botanical components enhances life span.

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Author Disclosure statement

The authors declare they have no conflict of interest.

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