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Diversity of Polyphenol Action in *Caenorhabditis elegans*: Between Toxicity and Longevity

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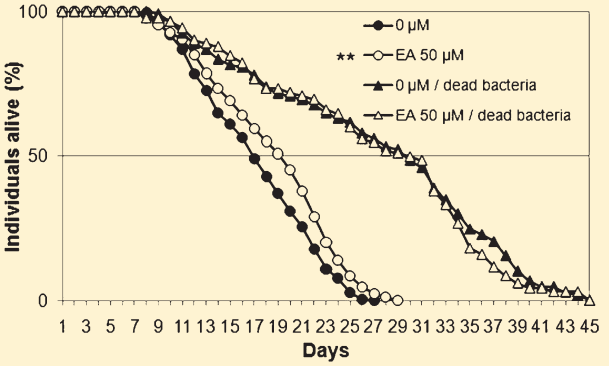
Supporting Information

ABSTRACT: The model organism *Caenorhabditis elegans* was utilized to determine, in vivo, the mode(s) of action of four plant polyphenols, namely, tannic acid (TA), gallic acid (GA), ellagic acid (EA), and catechin (CT). The determination of lifespan, stress resistance, growth, reproduction, eating-related behaviors, antioxidative capacities, and lifespan assays with the *mev-1* and the *eat-2* mutants as well as in the presence of dead bacteria provided new insights into their action. All four compounds prolonged lifespan, but only TA and CT mediated distinct stress protection. Longevity is unlikely the result of antioxidant capacities but rather due to calorie restriction imitating and hormetic properties in the case of TA and EA or antimicrobial capacities of GA and EA. Furthermore, the prominent "disposable soma theory" is only partly reflected by these polyphenols. In summary, this study underlines the diversity of polyphenolic phytochemicals and their mechanistic background.



Plants synthesize and secrete a diversity of polyphenolic secondary metabolites that, especially under stress conditions, act as a pathogenic defense mechanism with antifungal, antibacterial, and antiviral capacities.[1−3] Several health and antiaging benefits have been linked to these nutritional components, and antioxidant capacities are thought to be the main elicitor.[4] However, more recent reports suggest that other factors may contribute to the action of polyphenols. One has to question the presence of unequivocal benefits of polyphenols, since detrimental and mortality-increasing effects have been linked to some antioxidants, combined with the observation that prooxidant action of polyphenols can occur under certain conditions.[5−9] In addition to the prominent antioxidant mechanisms, several other health elicitors are thought to contribute to longevity, e.g., antimicrobial and hormetic effects, calorie restriction mimetic properties, and an energy redistribution effect in line with the "disposable soma theory".[10−16] How polyphenols act as health-promoting agents remains largely an unanswered question.

This study focuses on the commercial gallotannin tannic acid (TA), on the phenolic constituents of hydrolyzable tannins, namely, gallic acid (GA) and ellagic acid (EA), and on catechin (CT), a monomeric constituent unit of proanthocyanidins (condensed tannins). These compounds are of particular interest due to their health-supporting and antioxidant properties, as well as potential insalubrious effects, which include antinutritional and prooxidant capacities or even toxic effects resulting in hepatic lesions.[6,7,17−27]

CT and TA were recently found to prolong the lifespan and to promote stress resistance in the nematode model organism *Caenorhabditis elegans*, a finding that is in sync with many, but not all, "simple" polyphenols.[15,16,28−36] To date, no information is available that defines the life-prolonging and stress-resistant properties of GA and EA.

How, if at all, are these to some extent contradictory findings interconnected? If so, is a basic mode of action common to all/most polyphenols? A comparison of these compounds and their effects on different life parameters in organisms may aid our understanding and unravel their mode of action and activities. Moreover, a wide concentration series, which is typically absent in most studies, will help identify contrasting effects across different doses. The selected compounds were compared to investigate whether they share analogous lifespan and stress defense enhancing capacities in *C. elegans*. Furthermore, the following hypotheses were scrutinized:

I Antioxidant or antimicrobial effects are not responsible for the beneficial effects;

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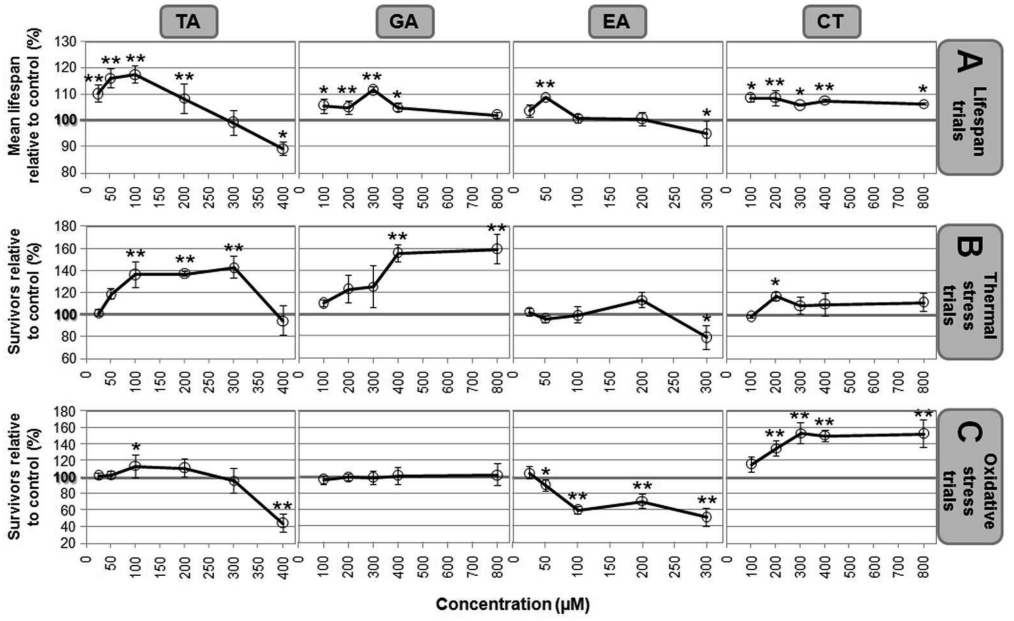

Figure 1. Concentration-dependent variation of lifespan and stress resistance. TA, GA, EA, and CT were tested in different concentrations for their ability to change the lifespan of *C. elegans* at 20 °C (A). The percentage variations of the mean lifespan compared to control are illustrated. In addition, the survival during thermal (B) and oxidative stress (C) of polyphenol-treated animals is shown. Wild-type nematodes were exposed to different polyphenol concentrations. At the sixth day of adulthood, dead and live animals were scored following an 8 h exposure to 35 °C (thermal stress) and 0.8 mM H_2O_2 (oxidative stress), respectively. Each data point of the stress trials (B and C) represents the average of 3−5 independent trials with about 100 animals per trial and concentration. Details about the lifespan assay (A) can be extracted from Table S2. The error bars represent the SEM, and differences were considered significant at $^*p < 0.05$ and $^{**}p < 0.005$, respectively.

II The polyphenols show a biphasic dose−response relationship on the basis of a hormetic effect;
III Fitness is inversely related to longevity, as defined by the "disposable soma theory";[37,38]
IV The selected polyphenols possess antinutritional capacities and elicit a life-extending "calorie restriction" effect.[39,40]

Answers to these hypotheses will further our understanding of polyphenolic action and will be instrumental in the identification of underlying mechanisms.

RESULTS AND DISCUSSION

Which biological effects are initiated by these polyphenols in the model organism *C. elegans*, and what are the main elicitors? Table S1 provides a short overview of the results. Some data concerning TA and CT were previously published, but for the purpose of comparison these data are included.[15,16]

EA and TA Are Hormetic Acting Polyphenols. How can polyphenols impose beneficial and detrimental effects? The hormesis effect may be able to provide an explanation for this seemingly contradictory notion. Hormesis is the reversal of response between low and high doses of diverse chemical, biological, or physical exposures, a background mechanism that may also be applicable to polyphenols.[12,41] The collation of concentration-dependent lifespan and stress resistance data allows a direct comparison of all polyphenols tested and an evaluation of a possible hormetic action (Figure 1). GA and CT are able to prolong the mean lifespan (relative to control) over a wide concentration range (Figure 1A) without imposing detrimental effects on thermal and oxidative stress resistance (Figure 1B and C). Both polyphenols are nontoxic even at concentrations of 800 μM, thus displaying no hormetic characteristics. In contrast, TA and EA act in a hormetic manner by displaying a relatively narrow beneficial concentration range, with toxic effects observed at higher concentrations. Nevertheless, TA was the most effective inducer of longevity. Further details specifying the mean and median lifespan, the quantity of animals, and the trial numbers are provided in Table S2.

The hormesis effect is also observed in the stress tests of TA and, by trend, of EA-exposed nematodes. Although EA was not able to exert stress protection, elevated levels induced adverse effects in both stress resistance trials. Another interesting finding is that TA was more effective in buffering thermal stress (Figure 1B), whereas CT was able to increase the resistance to oxidative stress (Figure 1C). Surprisingly, the GA concentration that was most effective in extending lifespan (300 μM GA) was not able to significantly enhance thermal or oxidative stress resistance (Figure 1B and C). However, higher GA concentrations improved thermal tolerance substantially.

The finding that TA and EA provoke hormetic patterns is in line with observations by Steinberg and colleagues, who proposed a hormetic mode of action for humic substances, which are mainly composed of polyphenolic tannins.[42,43] For all further tests, the most effective lifespan-increasing concentrations were chosen, namely, 100 μM TA, 300 μM GA, 50 μM EA, and 200 μM CT.

Antimicrobial Capacities Are Crucial for EA and GA Impact. Deleterious metabolites are produced during the proliferation process of *Escherichia coli*.[44] Any antimicrobial action can potentially reduce the synthesis of these metabolites, which in turn can influence longevity. In order to exclude simple antimicrobial effects as longevity elicitor, lifespan tests were also performed with heat-killed bacteria. If the antimicrobial

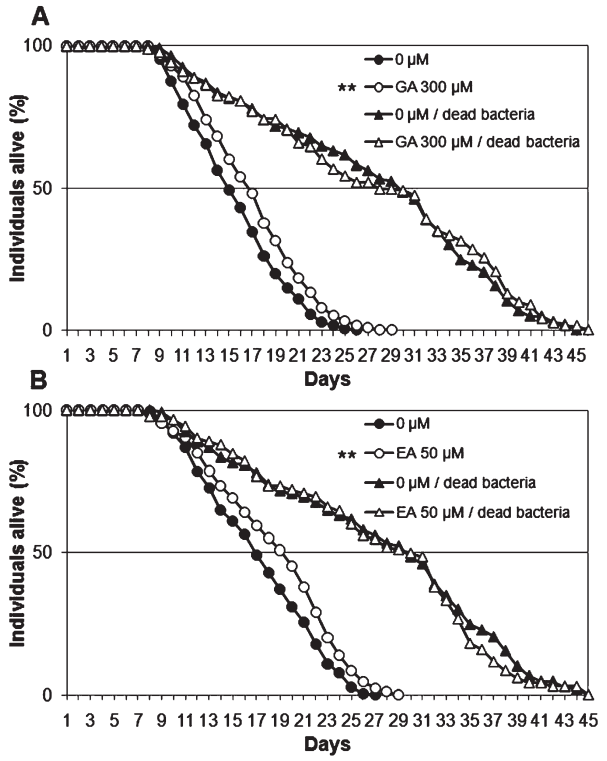

Figure 2. Survival curves with two different feeding regimes and polyphenol exposure. Nematodes were fed with live (circles) or dead (triangles) bacteria. The lifespan was monitored in the presence or absence of 300 μM GA (A) and 50 μM EA (B). Day 1 refers to the first day of adulthood, and the test was conducted at 20 °C. Differences were considered significant at $^{**}p < 0.005$. Details can be extracted from Tables S2 and S3.

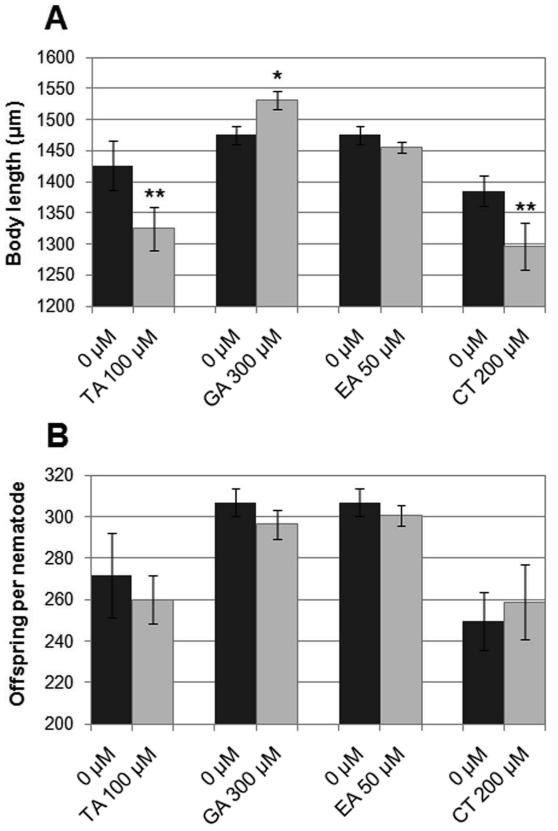

Figure 3. Impact of polyphenols on reproduction and growth. The lengths of untreated and polyphenol-treated nematodes were measured at the sixth day of adulthood (A). Shown is the average of 4 or 5 trials with 222−342 sized nematodes per concentration in total. Furthermore, the brood size of 30−40 animals in 3 or 4 independent trials was determined (B). The bars illustrate the average reproductive output. The error bars represent the SEM, and differences were considered significant at $^{*}p < 0.05$ and $^{**}p < 0.005$, respectively. Note: Since the compounds were tested separately in the reproduction and growth assays, no uniform control bar can be created, and each substance bar is displayed with its corresponding control bar.

properties of the polyphenols are an imperative factor of life extension, the compounds should be ineffective under these conditions. A previous study stated that longevity is increased by 200 μM CT and 100 μM TA in the presence of dead bacteria, therefore rejecting the antimicrobial hypothesis.[15,16] Here however, the addition of 300 μM GA and 50 μM EA to dead bacteria could not enhance the lifespan of *C. elegans* (Figure 2A and B); thus antibacterial capacities appear to be fundamental in GA- and EA-mediated lifespan extension. The full set of data, including TA and CT, is presented in Table S3.

"Disposable Soma" Does Not Apply to GA and EA, But to TA and CT. According to Kirkwood's "disposable soma theory", the energy for required life extension is subtracted from other sectors, such as reproduction and growth rate.[37,38] To verify this theory, the size of treated and untreated nematodes was determined at the sixth day of adulthood (Figure 3A). The exposure to 50 μM EA resulted in a slight, but statistically nonsignificant reduction of body length. A significant reduction of the body length was observed with 100 μM TA and 200 μM CT, but not with 300 μM GA, where the length of nematodes increased.

In addition, the reproductive output was determined for individual nematodes in the presence or absence of polyphenols; however, none of the compounds significantly affected brood size (Figure 3B). Two further reproductive behavior tests were conducted, i.e., the time to the first egg deposition and the reproductive output after 85 h. GA and EA delayed the beginning of egg deposition (Figure 4A). The difference appeared more distinct after 85 h: TA, GA, and EA elicited a significant reduction of initial reproduction rate (Figure 4B), whereas this effect was absent during CT exposure.

As discussed by Saul and co-workers, CT and TA might act in line with the "disposable soma theory", since both polyphenols exhibit significant growth-inhibiting effects.[15,16] However, GA and EA do not change the cumulative reproductive output or negatively affect size. The delay in the onset of reproduction is unlikely sufficient to support the theory (as the overall energy requirement might remain constant). Thus, GA and EA are not considered to act in line with Kirkwood's theory.

EA and TA Might Be CR Mimetics. Calorie restriction (CR) is a mechanism that prolongs the lifespan in various organisms, and the mode of action is still being discussed.[39,40,45−48] Owing to possible antinutritional capacities of certain polyphenols, it is conceivable that the test substances may elicit a CR effect and therefore act as "CR mimetics".

Nematodes were attracted to bacteria spiked with TA or GA, but not CT. In contrast, EA seemed to act as a chemorepellent and, therefore, possibly contributing to a reduced feeding behavior (Figure 5A). However, none of the tested compounds

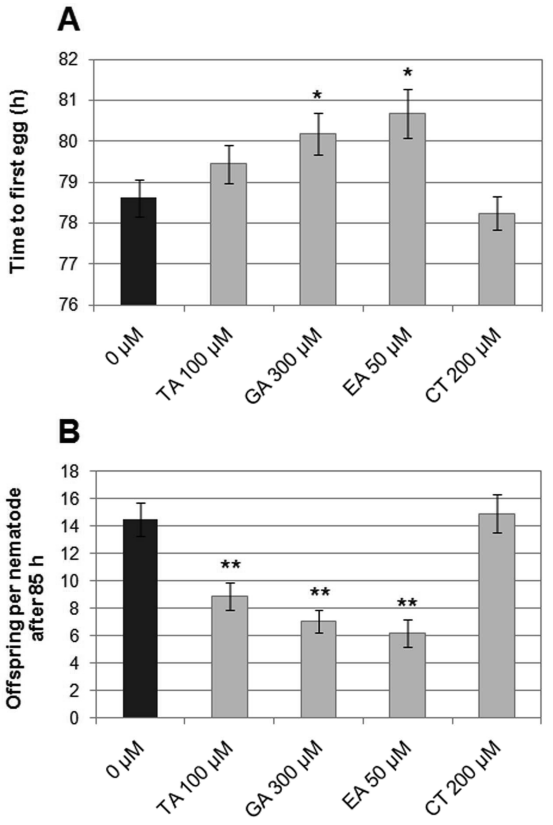

Figure 4. Impact of polyphenols on initial reproductive capacity. The time to the first laid egg was determined by hourly monitoring of 60 young adult animals per concentration in 2 independent trials (A). In addition the offspring of these 60 nematodes was counted 85 h after the egg stage (B). The error bars represent the SEM, and differences were considered significant at $^{*}p < 0.05$ and $^{**}p < 0.005$, respectively.

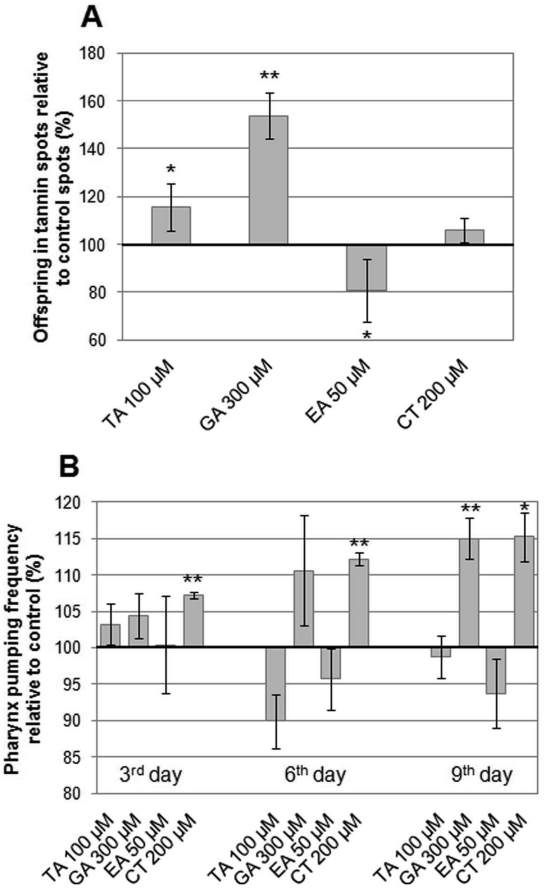

Figure 5. Eating-related behavior in polyphenol-treated nematodes. For the attraction assay (A) a 96 mm agar plate was prepared with 6 alternating bacterial spots (containing polyphenols or only DMSO). One untreated L1 larva was transferred to each spot, and the number of offspring per spot was counted after incubation for 96 h at 20 °C. The bars represent the percentage offspring variation compared to controls (100%) from 12 test plates per compound. Furthermore, the pharynx pumping rate (B) was determined in the absence and presence of polyphenol exposure. The pumping activity of 30 treated and untreated worms was monitored at the third, sixth, and ninth day of adulthood. Each nematode was quantified three times for 15 s. The bars represent the average percentage pumping variation of 3 independent trials compared to control (100%). The error bars represent the SEM, and differences were considered significant at $^{*}p < 0.05$ and $^{**}p < 0.005$, respectively.

significantly reduced the frequency of pharyngeal pumping (Figure 5B). GA increased the pumping frequency significantly at the ninth day of adulthood, while CT increased the pumping rate on all days.

Further evidence in support of CR was obtained via measurement of the fat content, which would be expected to decrease as a result of CR. Indeed, exposure to all polyphenols appeared to lead to a slight decrease in triglyceride content in young adults (Figure 6), although the effect was only significant at 100 μM TA.

In this context, lifespan changes of *eat-2(ad465)* mutants, which suffer from underfeeding due to decreased pharyngeal pumping, are of particular interest.[40] These animals did not benefit from the polyphenols (Figure 7). Although treatment with 300 μM GA increased median and mean survival by approximately 11% and 7%, respectively (Table S3), this lifespan modification is not statistically significant. Taken at face value, this result may suggest the presence of a CR-based mechanism; however when all results are taken into account, CR cannot be the explanation for GA action. GA acted as a chemo-attractant and enhanced the pharyngeal pumping frequency; thus there is no evidence for reduced ingestion, despite the delayed onset of reproduction. Moreover, GA-treated animals increased in size (Figure 3A), which is inconsistent with a CR effect.[49,50] The CT-mediated effects are less explicit. Although chemo-attraction was not modulated by CT, growth was distinctly reduced. However, as the pharyngeal pumping frequency was enhanced, there is not sufficient evidence in support of CR. In contrast, EA-containing bacteria seem to repulse *C. elegans*, which provides at least circumstantial evidence that worms exposed to EA eat less than their counterparts raised under control conditions. Furthermore, the reproduction rate was initially strongly reduced, an impact that in turn may be due to CR.[51−53] Results that argue against CR are the unchanged overall offspring number and the growth; thus EA may function as a weak CR mimetic.

The strong longevity effect of TA was negated in *eat-2(ad465)* mutants, the triglyceride content and the body length were reduced by TA treatment, and the initial reproduction period was delayed. Overall reproduction and the pharyngeal pumping frequency were unchanged; however attraction toward TA-containing bacteria was enhanced. Therefore TA might not reduce the food intake itself but act as molecular regulator within

the CR pathway or precipitate and bind nutritional proteins and digestive enzymes.

EA and TA Mediate Antioxidant Capacities *in Vivo*. Polyphenols can realize their antioxidant capacities in at least two possible ways: By direct quenching of free radicals and oxidants or by enhancing the synthesis or activity of antioxidant metabolites and enzymes in an organism. The ability of TA, EA, GA, and CT to quench reactive oxygen species (ROS) directly was described previously.[54−61] Here the antioxidant capacity was measured in vivo, which detects changes in the level of the antioxidant metabolite status and, to a lesser degree, the antioxidant enzyme activity. This capacity was determined for lipid-soluble metabolites (Trolox equivalents, Figure 8A) as well as for water-soluble metabolites (ascorbic acid equivalents, Figure 8B). Only TA was able to enhance the antioxidant status of lipid- and water-soluble metabolites. EA enhanced the antioxidant status of the water-soluble fraction, and GA and CT neither fraction.

At this point it should be noted that the water-soluble fraction is, due to the absence of an additional phase separation, prone to "contamination". Indeed, being of moderate molecular weight, TA and EA possess protein binding and precipitating capacities,[18] and therefore a contamination within the water-soluble fraction is a distinct possibility and calls for caution when interpreting these results.

The link between antioxidant activity and lifespan extension was further investigated using the *mev-1(kn1)* mutant. MEV-1 is part of the electron transport chain, and its absence results in ROS overproduction and subsequently in elevated stress sensitivity and premature aging.[62] Thus, if the antioxidant capacity of the polyphenols is elementary for longevity, *mev-1(kn1)* worms should clearly benefit from the treatment. However the results are striking (Figure 7): Although TA, GA, and EA treatment caused a significant extension of lifespan, the effect is modest compared to wild-type nematodes. Moreover, treatment with CT did not influence lifespan at all. Furthermore, the protection by TA and CT against oxidative and thermal stress (Figure 1) is evidence for antioxidant action.

Taken together, the CT-mediated enhancement of oxidative and thermal stress resistance are in line with an antioxidant action. However, *mev-1(kn1)* mutants do not benefit from CT treatment, and the antioxidant capacity tests yielded no antioxidative activity. GA and EA, both longevity elicitors in *mev-1(kn1)* mutants, did not enhance stress resistance or modulate antioxidant properties. It is therefore probable that the longevity effect in the *mev-1(kn1)* mutant is caused by an antimicrobial, rather than antioxidant activity. Thus, CT, GA, and EA are unlikely to be compounds that elicit major antioxidant capacities in vivo. Since TA treatment resulted in stress resistance, elevated antioxidant capacity, and longevity in *mev-1(kn1)* mutants, one might argue that TA acts as an antioxidant. However, given that TA extends the lifespan of *mev-1(kn1)* mutants by a mere

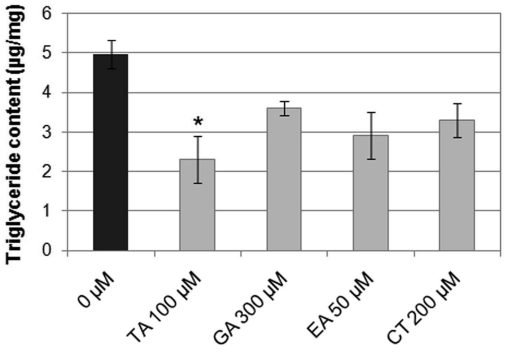

Figure 6. Triglyceride content of polyphenol-treated nematodes. Treated and untreated young-adult worms were homogenized. After a hydrolysis process the triglyceride content was determined photometrically. The data were normalized to the measured protein content. Each bar represents 2 independent trials. The error bars represent the SEM, and differences were considered significant at $^*p < 0.05$.

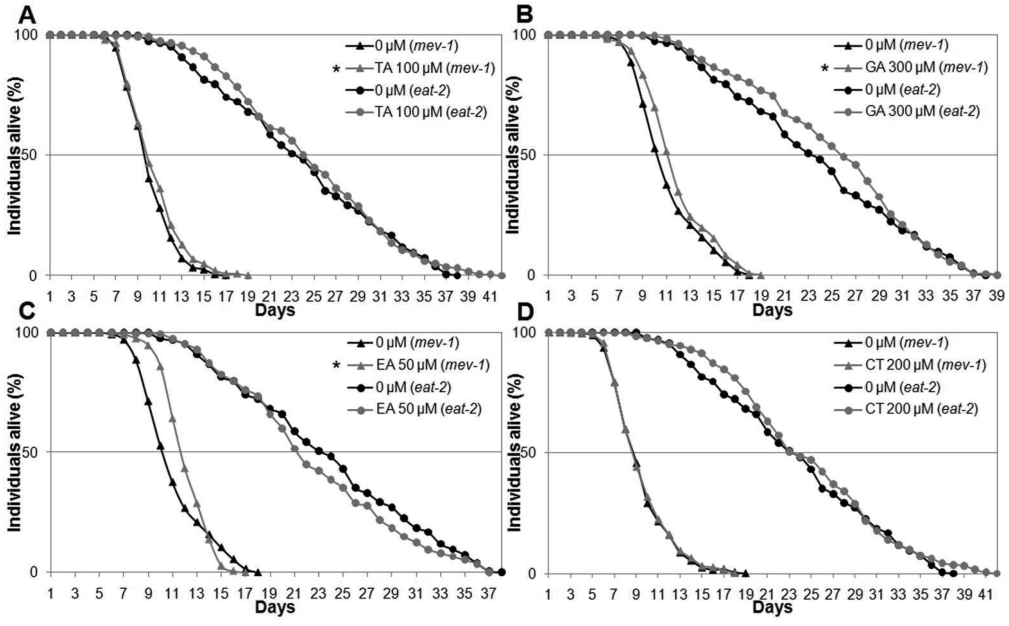

Figure 7. Lifespan of the eat-2 and mev-1 mutant strains. The mutants *eat-2(ad465)* and *mev-1(kn1)* were treated with 100 μM TA (A), 300 μM GA (B), 50 μM EA (C), and 200 μM CT (D). All tests were conducted at 20 °C, and day 1 refers to the first day of adulthood. Differences were considered significant at $^*p < 0.05$. Detailed experimental information can be extracted from Table S3.

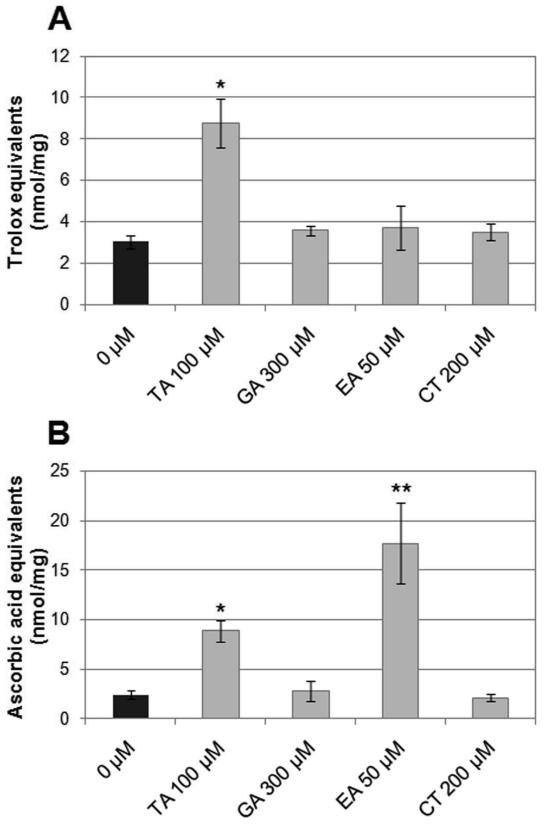

Figure 8. Antioxidant capacity of polyphenol-treated nematodes. Homogenized treated and untreated nematodes were assayed using the Photochem system. The antioxidant capacity of lipid-soluble (A) and water-soluble (B) metabolites was determined via photochemiluminescence and is expressed as Trolox and ascorbic acid equivalents per mg protein, respectively. The bars represent 3 independent trials, the error bars represent the SEM, and differences were considered significant at $^{*}p < 0.05$ and $^{**}p < 0.005$, respectively.

3.2%, a value that is well below the 17.6% observed in wild type, may indicate the presence of a prooxidant, rather than antioxidant action.

Overall, this report demonstrates the diversity of polyphenol action. Despite each tested polyphenol being unique and marked with an individual impact, they are universally able to extend the lifespan in *C. elegans*. Interestingly, their antioxidant capacity does not seem to correlate with lifespan extension. Instead, CR-imitating properties (TA and EA), antimicrobial effects (GA and EA), hormetic action (TA and EA), and an energy shift according to the "disposable soma theory" (CT and TA) are more likely to drive the action of these polyphenols.

Furthermore, elevated concentrations may not only shorten the lifespan but also increase stress sensitivity, inhibit growth, or decelerate reproductive output. In summary, although the health benefits of polyphenols are apparent, it should not be used as a universally applicable blanket statement.

EXPERIMENTAL SECTION

General Experimental Procedures. All *Caenorhabditis elegans* strains were maintained at 20 °C on nematode growth medium (NGM) seeded with *Escherichia coli* feeding strain OP50 according to Brenner.[63] The wild-type strain N2 (var. Bristol), the mutant strains DA465 (*eat-2(ad465)*) and TK22 (*mev-1(kn1)*), and the OP50 strain were obtained from the Caenorhabditis Genetics Centre, University of Minnesota. TA, GA, EA, and CT (Sigma-Aldrich, Taufkirchen, Germany) were added to the NGM and the OP50 bacteria with final concentrations ranging between 25 and 800 μM. Equal amounts of solvent (final concentration of 0.3% [v/v] DMSO; Applichem, Darmstadt, Germany) were used in all conditions.

Lifespan Assay. All compounds were initially tested at three concentrations [0 μM (solvent control), 100, 200, and 300 μM]. Thereafter, additional concentrations were chosen to expand the range to define, in more detail, life-prolonging or toxic conditions.

L4 larvae were transferred with a platinum wire to plastic Petri dishes (⌀ 96 mm) containing NGM dosed with TA, GA, EA, or CT (0 to 800 μM). About 15 L4 larvae of the following generation (generation F1) were transferred onto small plates (⌀ = 35 mm). Surviving and dead animals were counted daily (starting at the first day of adulthood) until all individuals had died. Nematodes that failed to respond to contact stimuli were considered to be dead. Nematodes suffering from internal hatch and those that escaped from the NGM agar were censored. Adult nematodes were regularly transferred to new treatment plates.

Ampicillin (final concentration 50 mg/mL) was added to the NGM agar in the lifespan assays with heat-killed OP50 bacteria (30 min at 65 °C; according to Gruber and co-workers).[30] Given that young adults frequently escape the plates and suffer from internal hatch when eating heat-killed bacteria, they were transferred to dead bacteria only once they had reached an age of six days.

Stress Resistance. Post L4 stage, all animals were transferred daily to fresh treatment plates. At the sixth day of adulthood, treated and untreated nematodes were either moved to 35 °C for 8 h (thermal stress trial) or transferred for the same duration to M9 buffer containing a final concentration of 0.8 mM H_2O_2 (oxidative stress trial). Thereafter, surviving and dead nematodes were counted.

Length Alterations. Nematode length was determined with the aid of a microscope and an integrated ocular micrometer. At the sixth day of adulthood, treated and untreated nematodes (F1 generation) were heat-killed (45 °C for 2.5 h) and subsequently sized.

Reproduction Assay. L4 larvae (generation F1) were transferred individually to treatment plates and moved to a fresh plate each day until reproduction was completed. The number of offspring per individual animal was determined. In addition, the time to first egg deposition was recorded. For that purpose, gravid animals of the parent generation were transferred to treatment plates for 30 min at the second day of adulthood (t_0). After 2 days the offspring were separated onto small plates and monitored hourly. The time to the first egg deposition (from t_0) was noted. In addition, the number of offspring after 85 h (from t_0) was counted.

Attraction Assay. Six alternating (absence and presence of polyphenol) spots of bacteria (OD_{595} = 5.0) were dropped on NGM plates (⌀ 96 mm) according to Menzel and co-workers.[64] A single L1 larva was transferred onto each bacteria spot. After incubation for 96 h at 20 °C, the number of offspring per spot was determined by manual counting. The sum of worms on the three control spots was taken as 100% and compared to the numbers observed on the polyphenol-containing spots.

Pharynx Pumping Rate. Nematodes of the F1 generation were randomly selected, and the pumping frequency was determined at the third, sixth, and ninth day of adulthood. The pharyngeal pumping was counted over three consecutive 15 s time frames using a microscope at 80-times magnification.

Antioxidant Capacity. Animals of the F1 generation were harvested with M9 buffer at the first day of adulthood. The nematodes were washed three times with M9 and finally centrifuged (200*g* at 4 °C). The pellets were transferred to lysis tubes (innuSPEED Lysis Tube C, Analytik Jena, Germany) and homogenized (Speedmill P12, Analytik Jena, Germany) six times for 30 s, with cooling steps on ice in between. The homogenates were centrifuged (3000*g* at 4 °C), and the

supernatant was transferred to a fresh tube. The protein content was calculated from 20 μL using the Bradford reagent (Sigma Aldrich, Taufkirchen, Germany) according to Bradford.[65] The remaining supernatant was directly used to determine the antioxidant capacity of water-soluble metabolites or was processed to extract the lipid-soluble fraction based on Bligh and Dyer.[66] The antioxidant capacity was quantified by photochemiluminescence using the PHOTOCHEM (Analytik Jena, Germany) and the provided antioxidant capacity of water-soluble compounds (ACW) and antioxidative capacity of lipid soluble compounds (ACL) protocols. Detailed background information can be found in Popov and Lewin, Popov et al., and Prior et al.[67−70]

Triglyceride Analysis. Young adult worms were washed and homogenized as described above. The triglycerides were enzymatically hydrolyzed and processed using the "serum triglyceride determination kit" (Sigma-Aldrich, Taufkirchen, Germany). The staining step was quantified by spectrophotometry at 540 nm and normalized to the protein content.

Data Interpretation and Statistical Analysis. Median and mean life span and percentage changes were determined and compared to controls. Statistical significance for alterations in the mean life span was calculated using a log-rank test (Bioinformatics at the Walter and Eliza Hall Institute of Medical Research; http://bioinf.wehi.edu.au/software/russell/logrank). Mean survival rates and percentage changes to the controls were calculated for the thermal and the oxidative stress resistance assays. Statistical significance was defined via the chi-square test (SigmaStat 3.5; SPSSInc., Chicago, IL) and one-way ANOVA (SigmaStat 3.5; SPSSInc., Chicago, IL) for the daily, initial, and total reproductive output, the body length, the attraction assay, the pharyngeal pumping rate, the antioxidant capacity, and the triglyceride content.

■ ASSOCIATED CONTENT

Supporting Information. The evaluation of five possible underlying longevity mechanisms is shown in Table S1. Table S2 presents the lifespan data at 20 °C of polyphenol-treated wild-type nematodes, and Table S3, those of polyphenol-treated wild-type nematodes with dead bacteria and of mutant strains. This material is available free of charge via the Internet at http://pubs.acs.org.

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