*Proceedings of the Nutrition Society* (2017), **76**, 145–162 doi:10.1017/S0029665116000720
 First published online 9 September 2016

*Nutrition Society Scottish Section Meeting held at The Royal College of Physicians, Edinburgh on 21–22 March 2016*

# Conference on 'Phytochemicals and health: new perspectives on plant-based nutrition' Symposium 3: Phytochemicals for healthier foods

# A literature review of flavonoids and lifespan in model organisms

Kathrin Pallauf, Nils Duckstein and Gerald Rimbach*
*Institute of Human Nutrition and Food Science, University of Kiel, Germany*

Epidemiological data on consumption of flavonoid-containing food points to the notion that some of these secondary plant metabolites may favour healthy ageing. The aim of the present paper was to review the literature on lifespan extension by flavonoids in worms, flies and mice. In most studies, worms and flies experienced lifespan extension when supplemented with flavonoids either as extracts or single compounds. Studies with mutant worms and flies give hints as to which gene products may be regulated by flavonoids and consequently enhance longevity. We discuss the data considering putative mechanisms that may underlie flavonoid action such as energy-restriction-like effects, inhibition of insulin-like-growth-factor signalling, induction of antioxidant defence mechanisms, hormesis as well as antimicrobial properties. However, it remains uncertain whether human lifespan could be prolonged by increased flavonoid intake.

**Polyphenols: Longevity: Catechin: Quercetin**

Flavonoids are the biggest group of polyphenolic secondary plant metabolites found in human diets. It has been estimated that for human subjects the daily intake ranges from 20 to 70 mg/d and might even be as high as 500 mg/d with tea and fruit being the main source[1–4]. Flavonoid consumption may correlate inversely with ageing-related pathologies such as CHD. In 1993, Hertog *et al*. reported that flavonoid intake was inversely associated with mortality from CHD[5]. Since then, further studies on ageing-related diseases have shown that flavonoids may reduce or delay onset of CVD[6–8] or diabetes type II[9]. Moreover, flavonoids may promote longevity by somewhat imitating energy restriction (ER). Such a reduction of energy intake by 10–40 % without malnutrition has been repeatedly shown to increase lifespan in model organisms and may improve healthspan in human subjects[10]. In ageing organisms, increasing oxidative damage and inflammation can be observed and ER may decrease these ageing-related symptoms[11,12]. Lowering energy intake down-regulates the phosphatidylinositide 3-kinase/Akt/mechanistic target of rapamycin pathway. Activation of this pathway leads to proliferation and cell growth and its inhibition prolongs lifespan in various model organisms[13]. For a review on ER and compounds that may mimic ER, see[12,14,15].

The common phenolic and pyrane three-ring $C_6$–$C_3$–$C_6$ structure (the rings are referred to as A, C and B, respectively) of flavonoids is called flavan. According to their hydroxylation pattern and oxidation of the pyrane ring, flavonoids can be divided into the compound classes flavones, flavonols, flavanones, flavanols and anthocyanidins[16]. The flavan structure and the flavonoids used in the studies reviewed are depicted in Fig. 1.

*In vitro* studies give mechanistic insights into how flavonoids could influence cellular pathways that are known to affect ageing. In particular, flavonoids such as quercetin and epigallocatechin gallate (EGCG) were shown to reduce inflammation and induce endogenous antioxidative defence mechanisms in the cell[17–19] and the flavonol fisetin inhibited the target of rapamycin pathway in various cell models[20].

While epidemiological studies point to the notion that consuming flavonoid-rich food or beverages may prolong human lifespan by lowering cardiovascular mortality[21] or improving insulin sensitivity[22], some of these studies also show that flavonoid subclasses (flavanols, catechins)

**Abbreviations:** BE, blueberry extract; BTE, black tea extract; EGCG, epigallocatechin gallate; ER, energy restriction; FOXO, forkhead box O; GTE, green tea extract; IGF, insulin-like growth factor; PG, pomegranate powder; SOD, superoxide dismutases.

***Corresponding author**: Professor G. Rimbach, fax +49(0)431-8802628, email rimbach@foodsci.uni-kiel.de

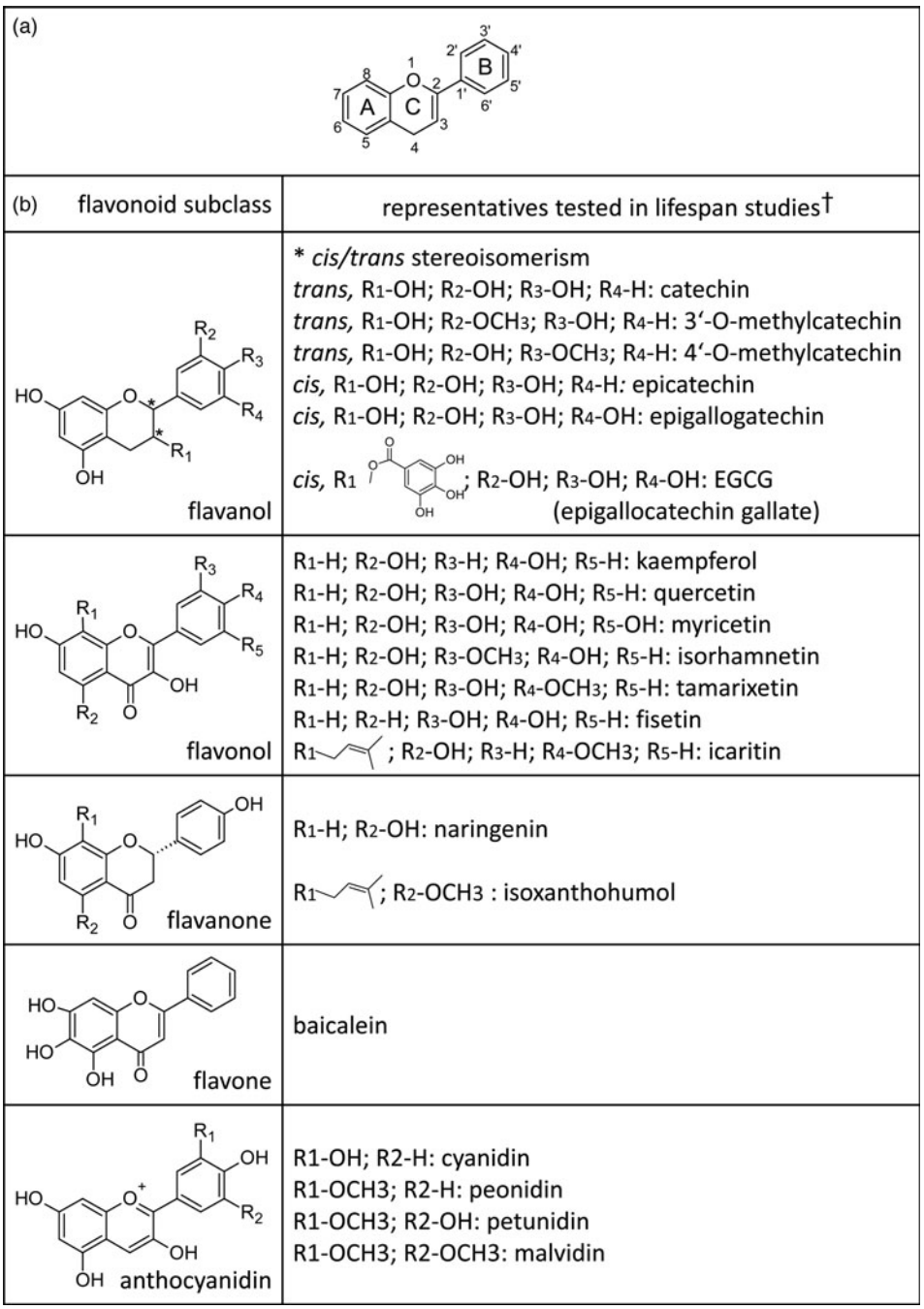


**Fig. 1.** (a) Common phenolic structure of flavonoids, flavan, the numbering of its C-atoms and names of its rings. (b) Structures of flavonoid subclasses and flavonoids tested in lifespan studies in worms, flies or mice † tested as single compounds, as part of an extract and/or glycosylated.

or sources (cocoa, tea) may differ in their potential to prevent disease[9,23]. Additionally, findings from the observational studies on mortality could result from other factors than flavonoid intake. However, lifespan studies in human subjects are difficult to pursue and the data are limited to findings from model organisms.

In order to review the available data on longevity in wild-type and mutant mice, flies and worms supplemented with flavonoids, we searched PubMed and Web of Science for articles reporting lifespan studies in these models. We included forty-four original research articles written in English on feed supplementation with flavonoids in wild-type or mutant animals compared with wild-type species that reported lifespan studies under non-extreme conditions. Extreme experimental conditions such as high temperature, known infection or exogenous oxidative stress were not included. In the following, we review and discuss the studies we found on worms, flies and mice examining how single flavonoid compounds or plant extracts known to contain

https://doi.org/10.1017/S0029665116000720 Published online by Cambridge University Press

considerable amounts of flavonoids affected the lifespan of these model organisms. Isoflavones were not included; however, it has been shown that isoflavones may improve health and prolong lifespan in flies[24,25].

## Flavonoids and the lifespan of the worm *Caenorhabditis elegans*

This nematode is widely used as a model organism since it is easy to culture under laboratory conditions and very well characterised. *Caenorhabditis elegans* was the first multicellular organism to have its genome fully sequenced[26] and many of its gene functions have been characterised by generating mutant strains. Approximately 60–80 % of human genes have homologues in *C. elegans*, which, similar to human subjects, is affected by ageing. In *C. elegans*, hundreds of genes that modulate longevity have been identified[27]. Information on the worm genome and gene functions can be found on the website www.wormbase.org. *C. elegans* is a self-fertilising hermaphrodite, which develops rapidly passing through four larval stages with a generation time of 3 d (www.wormbook.org). Because of its multicellularity and its different tissues and cell types such as neurons, gut and muscle cells, the nematode is a suitable model organism to study metabolic functions and other processes related to ageing such as neurodegeneration. *C. elegans* can be maintained from about 12 to 25°C on agar with *Escherichia coli* as its diet (www.wormbook.org). At 25°C the worm has a life expectancy of approximately 15 d, with a maximum lifespan of approximately 22 d[28]. Such a short lifespan makes it a useful organism for longevity studies.

In our search, we found a total of twenty-seven reports on flavonoids or flavonoid-containing plant extracts and their impact on *C. elegans* lifespan. Three of these studies dealt with catechin, two studies were on epicatechin, one study on methylated epicatechins, three studies on EGCG, nine studies on quercetin, three studies on glycosylated quercetins, two on methoxylated quercetins, two on kaempferol, one on kaempferol derivatives, two on myrecitin, one on naringenin, one on isoxanthohumol, one on baicalein, one on flavolignan and seven on plant extracts (Table 1).

The flavanol catechin increases *C. elegans* lifespan in some settings. Saul *et al.* found that catechin extended lifespan of the wild-type N2 strain at concentrations from 100 to 800 μM and temperatures ranging from 15 to 23°C[29,30]. However, Surco-Laos *et al.*[31] could not reproduce this finding when they tested the effect of 200 μM quercetin at 20°C in the same worm strain. In contrast to Saul *et al.* who used the $F_1$ generation of parents that had been exposed to catechin, Surco-Laos *et al.* started the exposition of the worm to the flavanol later in life (larval stage 1).

The findings by Saul *et al.* were followed by studies in mutant worms. Interestingly, catechin did not extend lifespan in the mutant strains *daf-2* (the orthologue of the human insulin/insulin-like growth factor (IGF) receptor), *nhr-8* (a nuclear hormone receptor), *akt-2* (homologue of the serine/threonine kinase Akt/protein kinase B), *mev-1* (a subunit of the mitochondrial respiratory chain complex II, the worms have increased levels of oxidative stress) and *eat-2* (worms carrying this mutation show decreased pharyngeal feeding and therefore are energy restricted).

It remains unclear whether epicatechin benefits longevity of worms. While Bartholome *et al.*[32] reported lifespan extension at 100 μM epicatechin in the feed, Surco-Laos *et al.* did not observe any changes using the concentration of 200 μM. However, they found that the two derivatives of epicatechin, which are methylated at one of the hydroxyl groups of the B ring (3′-O-methylepicatechin and 4′-O-methylepicatechin) increased *C. elegans* lifespan.

EGCG, the ester of gallocatechin (a flavanol with three hydroxyl groups in the B ring) with gallic acid also appears to prolong lifespan if supplemented at concentrations from 100 to 220 μM[32,33]. At concentrations of 22 μM and lower there were no effects observed under normal growth conditions[34]. Interestingly, EGCG could also prolong lifespan in *mev-1* mutants.

Of the nine studies on quercetin, seven reported quercetin as prolonging lifespan at concentrations ranging from approximately 70 to 200 μM[35–41]. One study supplementing quercetin at 166 μM could not detect lifespan extension[42], another study applying quercetin at 50 and 250 μM reported no change and a decrease in lifespan, respectively[43]. Furthermore, quercetin supplementation was tested in various mutant strains. These studies concluded that mutations in *sek-1* (a mitogen-activated protein kinase kinase), *daf-2, age-1* (the orthologue of the human phosphoinositide-3-kinase p110 catalytic subunit) and *unc-43* (the orthologue of the human type II calcium/calmodulin-dependent protein kinase) abrogated lifespan extension[43]. Pietsch *et al.* also found that quercetin could prolong the lifespan of *mev-1* mutants, which experience high levels of oxidant stress. Contrarily, Grunz *et al.*[40] found no lifespan increase in quercetin supplemented *mev-1* mutants.

Beside its concentration, the glycosylation of quercetin also seems to influence lifespan extension. At position 3 or 3′ glycosylated quercetin prolonged lifespan when used at concentrations of 10 and 25 μM (quercetin-3-O-glycoside)[41] or 66 μM (quercetin-3′-O-glycoside)[39]. In contrast, higher concentrations of quercetin-3-O-lycoside (50 and 100 μM) had no effect and 200 μM even shortened lifespan[41]. The authors who also observed that quercetin at 100 μM increased lifespan explain this finding with a higher uptake and bioavailability of the glycosylated form. In the worm, the glycoside is metabolised into the active quercetin by deglycosylation. The *klo-1* and *klo-2* mutant worms with defects in β-glucosidase activity benefited from quercetin-3-O-glucoside concentrations as high as 200 μM possibly the lack of β-glucosidase prevented the formation of quercetin from quercetin-3-O-glycoside at detrimental levels. Furthermore, the type of sugar that is conjugated to quercetin also seems to be important for lifespan increase. While quercetin that was glycosylated at position 3 with a disaccharide consisting of two glucose monomers (Q3M) or two rhamnose monomers

https://doi.org/10.1017/S0029665116000720 Published online by Cambridge University Press

**Table 1.** Lifespan and flavonoids in *Caenorhabditis elegans*

| Flavonoid | Dose in diet | Worm mutant/strain | *T* (°C) | Start of treatment | Mean lifespan | Median lifespan | Maximum lifespan | Number of animals* | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *Catechins* (*Flavanols*) | | | | | | | | | |
| Catechin | 200 µM | N2 (wild-type) | 20 | L1 stage, lifelong | NS | | | 199 | Surco-Laos *et al.*[31] |
| | 100 µM | N2 (wild-type) | | $F_1$ generation of exposed parents, lifelong | +9 % | +11 % | | 349 | Saul *et al.*[29]; Saul *et al.*[30] |
| | 200 µM | | | | +9 % | +13 % | | 499 | |
| | 300 µM | | | | +6 % | +10 % | | 272 | |
| | 400 µM | | | | +8 % | +11 % | | 307 | |
| | 800 µM | | | | +6 % | +10 % | | 269 | |
| | 200 µM | N2 (wild-type) | 15 | | +15 % | +14 % | | 163 | Saul *et al.*[29] |
| | | | 23 | | +11 % | +9 % | | 162 | Saul *et al.*[29] |
| | | TJ 1052 (*age-1* (*hx546*)) | 20 | | +11 % | +6 % | | 306 | |
| | | VC 204 (*akt-2* (*ok393*)) | | | NS | NS | | 309 | |
| | | DR 1567 (*daf-2* (*m577*)) | | | NS | NS | | 321 | |
| | | DR 20 (*daf-12* (*m20*)) | | | +9 % | +9 % | | 171 | |
| | | GR1307 (*daf-16* (*mgDf50*)) | | | +8 % | +8 % | | 709 | |
| | | VC8 (*jnk-1* (*gk7*)) | | | +6 % | +6 % | | 398 | |
| | | TK22 (*mev-1* (*kn1*)) | | | NS | NS | | 310 | |
| | | AE501 (*nhr-8* (*ok186*)) | | | NS | NS | | 508 | |
| | | AM1 (*osr-1* (*rm1*)) | | | +13 % | +19 % | | 228 | |
| | | AU1 (*sek-1* (*ag1*)) | | | +10 % | +20 % | | 104 | |
| | | VC199 (*sir-2.1* (*ok434*)) | | | +6 % | +8 % | | 302 | |
| | | EU1 (*skn-1* (*zu67*)) | | | +13 % | +3 % | | 149 | |
| | | MT2605 (*unc-43* (*n498n1186*)) | | | +4 % | +9 % | | 485 | |
| | | DA465 (*eat-2* (*ad465*)) | | | NS | NS | | 178 | Saul *et al.*[30] |
| Epicatechin | 100 µM | N2 (wild-type) var. Bristol | 20 | 3 d post-hatching, lifelong | +15 % | | +11 % | 60 | Bartholome *et al.*[32] |
| | 200 µM | N2 (wild-type) | 20 | L1 stage, lifelong | NS | | | 199 | Surco-Laos *et al.*[31] |
| 3′-O-methylepicatechin | 200 µM | N2 (wild-type) | 20 | L1 stage, lifelong | +6 % | +11 % | +4 % | 199 | Surco-Laos *et al.*[31] |
| 4′-O-methylepicatechin | 200 µM | N2 (wild-type) | 20 | L1 stage, lifelong | +13 % | +11 % | +10 % | 199 | Surco-Laos *et al.*[31] |
| Epigallocatechin gallate | 100 µM | N2 (wild-type) var. Bristol | 20 | 3 d post-hatching, lifelong | +20 % | | +13 % | 60 | Bartholome *et al.*[32] |
| | 0·1 mg/l (0·22 µM) | N2 (wild-type) | 20 | beginning of egg-laying, lifelong | NS | | | 210 | Zhang *et al.*[34] |
| | 1·0 mg/l (2·2 µM) | | | | NS | | | 208 | |
| | 10 mg/l (22 µM) | | | | NS | | | 194 | |
| | 220 µM | N2 (wild-type) | 20 | 3 d post-hatching, lifelong | +10 % | | NS | ≥71 | Abbas & Wink[33] |
| | | BA17 (*fem-1* (*hc17*)) (infertile when grown over 25°C) | 25 | | +14 % | | NS | ≥93 | |
| | | TK22 ((*mev-1* (*kn1*)) | 20 | | +16 % | | NS | ≥49 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Flavonols* | | | | | | | | | |
| Quercetin | 100 μM | N2 (wild-type) var. Bristol | 20 | 3 d post-hatching, lifelong | +15 % | +19 % | +15 % | 120 | Kampkotter *et al.*[35] |
| | 50 mg/l (166 μM) | N2 (wild-type) | 22 | 4 d old (young adults) | NS | | | 100 | Wu *et al.*[42] |
| | 200 μM | | 20 | L1 stage, lifelong | +11 % | +29 % | +5 % | 304 | Surco-Laos *et al.*[38] |
| | 50 μM | | 20 | After hatching, lifelong | NS | NS | | 180 | Pietsch *et al.*[43] |
| | 250 μM | | | | −7 % | −6 % | | 171 | |
| | 100 μM | | 20 | L1 stage, lifelong | +16 % | +3 % | +1 % | 300 | Duenas *et al.*[41] |
| | 100 μM | | 20 | L4 stage, lifelong | +6 % | +1 % | +18 % | 171 | Grunz *et al.*[40] |
| | 100 μM | TK22 (*mev-1* (*kn1*)*III*) | 20 | L4 stage, lifelong | NS | | | 256 | Grunz *et al.* [40] |
| | 100 μM | N2 (wild-type) | 20 | $F_1$ generation of | +11 % | | | 260 | Pietsch *et al.*[37] |
| | 200 μM | | | exposed parents, | +18 % | | | 260 | Pietsch *et al.*[37] |
| | | | 15 | lifelong | +9 % | | | 122 | |
| | | GR1307 (*daf-16* (*mgDf50*)) | 20 | | +15 % | | | 475 | |
| | | VC199 (*sir-2.1.* (*ok 434*)) | | | +8 % | | | 262 | |
| | | AM1 (*osr-1* (*rm1*)) | | | +12 % | | | 156 | |
| | | AU1 (*sek-1* (*ag1*)) | | | NS | | | 101 | |
| | | MT2605 (*unc-43* (*n498n1186*)) | | | NS | | | 283 | |
| | | TK22 (*mev-1* (*kn1*)) | | | +10 % | | | 173 | |
| | | DR 157 (*daf-2* (*e1168*)) | | | NS | | | 352 | |
| | | VC8 (*jnk-1* (*gk7*)) | | | +11 % | | | 305 | |
| | | TJ 1052 (*age-1* (*hx546*)) | | | NS | | | 599 | |
| | | DR20 (*daf-12* (*m20*)) | | | +16 % | | | 207 | |
| | | EU1 (*skn-1* (*zu67*)) | | | +18 % | | | 262 | |
| | | VC204 (*akt-2* (*ok 393*)) | | | +10 % | | | 241 | |
| | | AE501 (*nhr-8* (*ok186*)) | | | +12 % | | | 324 | |
| | 100 μM | N2 (wild-type) | | | +10 % | | | 344 | Saul *et al.*[36] |
| | 200 μM | | | | +10 % | | | 806 | |
| | 100 μM | *daf 16* (*mgDf50*) | | | +4 % | | | 258 | |
| | 200 μM | | | | +15 % | | | 475 | |
| Flavonoids from onion | 20 mg/l | N2 (wild-type) | 20 | 2 d post-hatching | +14 % | | | 82 | Xue *et al.*[39] |
| super kito momiji | (approximately | | | | +20 % | | | 83 | |
| Quercetin | 66 μM) | | | | +12 % | | | 85 | |
| Quercetinglucoside Q3M | | | | | NS | | | NS | |
| Quercetinglucoside Q3′G | | | | | | | | | |
| Rutin | | | | | | | | | |
| Quercetin-3-O-α-L-rhamnopyranosyl(1→2) O –α-L-rhamnopyranoside | 100 μM | N2 (wild-type) | 20 | Eggs after embryo | +10 % | | | ≥71 | Ahn *et al.*[44] |
| | 200 μM | var. Bristol | | isolation | +22 % | | | ≥71 | |
| Quercetin-3-O-glucoside | 10 μM | N2 (wild-type) | 20 | L1 stage, lifelong | +12 % | +29 % | +1 % | 300 | Duenas *et al.*[41] |
| | 25 μM | | | | +23 % | +29 % | +7 % | 300 | Duenas *et al.*[41] |
| | 50 μM | | | | NS | NS | NS | 300 | |
| | 100 μM | | | | NS | NS | NS | 300 | |
| | 200 μM | | | | −17 % | −29 % | −10 % | 300 | |
| | | *klo-1* (*ok2925*)IV | 20 | | +24 % | +33 % | +3 % | 300 | |
| | | *klo-2* (*ok1862*)III | | | +39 % | +125 % | +17 % | 300 | |
| | | *klo-2* (*ok1830*)III | | | +24 % | +22 % | +24 % | 300 | |
| Isorhamnetin (quercetin-3′-O-methylether) | 200 μM | N2 (wild-type) | 20 | L1 stage, lifelong | +16 % | +29 % | +16 % | 304 | Surco-Laos *et al.*[38] |

Table 1. (*Cont.*)

| Flavonoid | Dose in diet | Worm mutant/strain | *T* (°C) | Start of treatment | Mean lifespan | Median lifespan | Maximum lifespan | Number of animals* | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Tamarixetin (quercetin-4′-O-methylether) | 200 μM | N2 (wild-type) | 20 | L1 stage, lifelong | +12 % | +29 % | NS | 304 | Surco-Laos *et al.*[38] |
| | 50 mg/l (158 μM) | | 22 | 4 d old (young adults) | | +25 % | | 100 | Wu *et al.*[42] |
| Kaempferol | 50 mg/l (175 μM) | N2 (wild-type) | 22 | 4 d old (young adults) | | NS | | 100 | Wu *et al.* [42] |
| | 100 μM | | 20 | L4 stage, lifelong | +6 % | +4 % | +7 % | 173 | Grunz *et al.*[40] |
| | | TK22 (*mev-1* (*kn1*)*III*) | | | NS | | | 256 | |
| Icariin | 15 μM | N2 (wild-type) | 25 | Day 1 of adult life, lifelong | NS | | | 301 | Cai *et al.*[45] |
| | 45 μM | | | | +21 % | | | 503 | |
| | 75 μM | | | | NS | | | 306 | |
| | 45 μM | CB 1370 (*daf-2* (*e1370*)) | | | NS | | | 144 | |
| | | CF 1038 (*daf-16* (*mu86*)) | | | NS | | | 119 | |
| Icaritin | 20 μM | N2 (wild-type) | 25 | Day 1 of adult life, lifelong | NS | | | 185 | Cai *et al.*[45] |
| Icariside I | 20 μM | N2 (wild-type) | 25 | Day 1 of adult life, lifelong | NS | | | 176 | Cai *et al.*[45] |
| Icariside II | 10 μM | N2 (wild-type) | 25 | Day 1 of adult life, lifelong | NS | | | 119 | Cai *et al.*[45] |
| | 20 μM | | | | +23 % | | | 458 | |
| | 40 μM | | | | NS | | | 124 | |
| | 20 μM | CB 1370 (*daf-2* (*e1370*)) | | | NS | | | 264 | |
| | | CF 1038 (*daf-16* (*mu86*)) | | | NS | | | 235 | |
| | | SY3551 (*hsf-1* (*sy441*)) | | | NS | | | 216 | |
| | | DA1116 (*eat-2* (*ad1113*)) | | | +14–15 % | | | 257 | |
| | | XA8223 (*rsks-1* (*ok1255*)) | | | +17–21 % | | | 228 | |
| Myricetin | 100 μM | N2 (wild-type) | 25 | L4 stage/adult, lifelong | +33 % | | | 90 | Buchter *et al.*[46] |
| | | CF1038 (*daf-16*(*mu86*)*I*) | 25 | | NS | | | ≥153 | |
| | | N2 (wild-type) | 20 | L4 stage, lifelong | +18 % | +16 % | +22 % | 169 | Grunz *et al.*[40] |
| | | TK22 (*mev-1* (*kn1*)*III*) | | | +16 % | +16 % | +30 % | 256 | |
| *Flavanones* | | | | | | | | | |
| Naringenin | 100 μM | N2 (wild-type) | 20 | L4 stage, lifelong | NS | | | 181 | Grunz *et al.*[40] |
| | | TK22 (*mev-1* (*kn1*)*III*) | | | NS | | | 256 | |
| Isoxanthohumol | 100 μM | N2 (wild-type) | 20 | L4 stage/adult, lifelong | NS | | | 154 | Buchter *et al.*[47] |
| | 200 μM | | | | NS. | | | 154 | |
| | 100 μM | CF1038 (*daf-16*(*mu86*)I) | | | NS | | | 158 | |
| | 200 μM | | | | −5 % | | | 155 | |
| *Flavone* | | | | | | | | | |
| Baicalein | 100 μM | N2 (wild-type) | | Day 3 after egg-laying, lifelong | +45 % | +57 % | +24 % | 360 | Havermann *et al.*[48] |
| Flavolignans | | | | | | | | | |
| Silymarin (flavolignan isomers) | 25 μM | N2 (wild-type) | 20 | L4 stage | +10 % | | | Approximately 400 | Kumar *et al.*[49] |
| | 50 μM | | | | +25 % | | | Approximately 400 | |
| | 100 μM | | | | −9 % | | | Approximately 400 | |
| *Extracts* | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Polyphenol-enriched cocoa powder (containing approx. 120 g/kg procyanidins) | 4 g/l | Wild-type N2<br>GR1307(*daf-16* (*mgDF50*))<br>VC199 (*sir-2.1.*(*ok434*)) (*daf-2* (*e1370*)) | 20 | Young adult stage, lifelong | | +17 %<br>NS<br>NS<br>NS | | 100 | Martorell *et al.*[50] |
| Hot water extract from Taiwan hinoki (containing catechin, quercetin, quercetin-3-O-rhamnoside and myricetin-3-O-rhamnoside) | 2 mg/l<br>20 mg/l | N2 (wild-type) | 20 | | +8 %<br>+20 % | | +9 %<br>+17 % | n.g. | Cheng *et al.*[51] |
| Tea seed pomace extract (methanolic fraction containing approx. 44 % kaempferol glycosides) | 1 mg/l<br>10 mg/l | N2 (wild-type) | 20 | L1 stage, lifelong | | +25 %<br>+25 % | +13 %<br>+25 % | 180<br>180 | Wei *et al.*[52] |
| Total flavones from nymphea hybrid | 100 mg/l | N2 (wild-type) | 20 | L4 stage | Increased | | | 3 trials | Zhuang *et al.*[53] |
| Ginkgo biloba extract EGb761 (containing 24 % flavone glycosides) | 100 mg/l | N2 (wild-type) | 22 | 4 d old (young adults) | | +8 % | | 100 | Wu *et al.*[42] |
| Proanthocyanidin-enriched fraction from blueberry extract | 67 mg/l<br>200 mg/l | Fem-1 (hc17) | 25 | Day 1 of adult life, lifelong | | +20 %<br>+14 % | | 88<br>92 | Wilson *et al.*[54] |
| Anthocyanin-rich extract from purple wheat (containing cyanidin-3-O-glycoside, peonidin-3-O-glucoside and malvidin-3-O-galactoside) | 100 mg/l | N2 (wild-type)<br>TK22 (*mev-1* (*kn1*))<br>GR1307 (*daf16* (*mgDf50*)) | 20 | 3 d after hatching, lifelong | +11 %<br>+9 %<br>NS | | | 100<br>90<br>50 | Chen *et al.*[55] |

* If the numbers in the control and treatment groups are different, the lower number is given. If various trials were carried out the sum of the animals used is given; L-larval.

(quercetin-3-O-α-L-rhamnopyranosyl(1→2) O-α-L-rhamnopyranoside) increased lifespan in N2 worms, rutin, which is glycosylated at the same position with the disaccharide rutinose, could not prolong longevity[39,44]. Similarly to the aglycon, quercetin with a single methylation of either hydroxyl group in the B ring (isorhamnetin: 3′methylation, tamarixetin: 4′ methylation) extends lifespan in worms at 200 μM[38] and tamarixetin also at 158 μM[42].

For kaempferol we found two studies that reported contradicting data. While the study that used the higher concentration of 175 μM did not observe lifespan extension in supplemented N2 worms[42], the second study using 100 μM kaempferol observed a slight lifespan increase, which was absent in the *mev-1* mutant[40]. The kaempferol derivative icariin prolonged lifespan at 45 μM but not at 15 or 75 μM and not in *daf-2* or *daf-16* (forkhead box O (FOXO) homologue) mutants. While deglycosylation of icariin, which is glycosylated at positions 3 and 7, at both (icaritin) or at the 3 position (icariside I) seems to cancel out its lifespan-expanding properties, the derivative that is deglycosylated at position seven (icariside II) extended lifespan at 20 μM but not at 10 or 40 μM. In studies with *daf-2, daf-16, hsf-1, rsks-1* and *eat-2 mutants*, the impact of icariside II depended on the insulin/IGF receptor orthologue (*daf-2*), the FOXO homologue (*daf-16*), the heat-shock transcription factor orthologue (*hsf-1*) but not on a putative ribosomal protein S6 kinase (*rsks-1*). This was also observed in energy restricted worms (*eat-2*)[45].

Myricetin was used in two studies and both the studies concluded that it prolonged lifespan at 100 μM. This seems to depend on the *C. elegans* FOXO orthologue[46], whereas *mev-1* mutants also benefitted from myricetin supplementation[40].

The flavanones naringenin and isoxanthohumol did not promote longevity in the worm at 100 or 200 μM and in FOXO mutants, 200 μM isoxanthohumol even shortened lifespan[40,47].

In contrast, the flavone baicalein prolonged lifespan at 100 μM in wild-type worms[48]. In the case of the flavolignan isomers silymarin, the effect appeared to be dose dependent, since 25 and 50 μM increased the worm's lifespan, while 100 μM shortened it[49].

All seven studies on plant extracts reported positive outcomes on wild-type worm lifespan. The extracts studied were a polyphenol-enriched cocoa powder containing approximately 120 g/kg procyanidins[50], a hot water extract from Taiwan hinoki containing catechin, quercetin, quercetin-3-O-rhamnoside and myricetin-3-O-rhamnoside[51], a tea seed pomace extract containing approximately 44 % kaempferol glycosides[52], the total flavones from nymphea hybrid[53], the ginkgo biloba extract EGb761 (24 % flavone glycosides)[42], a proanthocyanidin-enriched fraction from a blueberry extract (BE)[54] and an anthocyanin-rich extract from purple wheat-containing cyanidin-3-O-glycoside, peonidin3-O-glucoside and malvidin-3-O-galactoside[55]. The cocoa powder and the purple wheat extract were also tested in mutants where the effect of the procyanidin-rich cocoa powder depended on *daf-16, sir2.1* and *daf-2*[50]. Similarly, the anthocyanin-rich wheat extract did not prolong lifespan in *daf-16* mutants. However, this extract extended lifespan in the more sensitive towards oxidative stress than wild-type worms, the *mev-1* mutants[55], which were not tested with the cocoa powder. Interestingly, some of the extracts such as the tea seed pomace[52] or the Taiwan hinoki extract[51] extended lifespan at concentrations that were as low as 1–2 mg/l.

Most studies in *C. elegans* report positive effects on worm lifespan when used at μM concentrations, however, with the concentration depending on the flavonoid supplemented, high doses may shorten lifespan. Interestingly, not all flavonoids seem to affect the same molecular targets. While the action of kaempferol, its derivatives and myricetin depended on a functional FOXO homologue, quercetin and catechin increased lifespan irrespectively of *daf-16*. However, IGF receptor mutants (*daf-2*) did not live longer when supplemented with any of the flavonoids tested.

## Flavonoids and the lifespan of *Drosophila melanogaster*

Similar to *C. elegans*, the fruit fly *Drosophila melanogaster* is a multicellular organism that is easy to house and extensively used as a model organism. Its organs, development and behaviour show similarities to human subjects. The fly has a relatively short lifespan of 2–3 months and shows symptoms of ageing[56]. Its genome has been fully sequenced[57] and information on genes and mutants has been deposited on www.flybase.org.

We found ten studies on flavonoids and lifespan studies in *D. melanogaster*. Three of them used single compounds such as the flavanol epicatechin and the flavonol fisetin[58–60], the other seven studies were on cocoa powder, green tea extract, *Ludwigia octovalvis* extract, apple polyphenols, black tea extract (BTE) and BE[61–67] (Table 2).

Epicatechin at 100–8000 μM increased fly lifespan, while 10 μM had no effect[59]. The other single compound tested in flies, the flavonol fisetin, was tested as part of a standard diet containing 3 % yeast and an energy restricted diet with 2 % yeast. Interestingly, 10 μM fisetin did not change the lifespan of male or female flies on a 3 % yeast diet, but 100 μM fisitin in the 3 % yeast diet increased lifespan in both sexes. However, on a 2 % yeast diet female flies did not benefit from fisetin at 10 or 100 μM and male flies lived for a shorter period when supplemented with fisetin as compared with the 2 % yeast diet control. The authors hypothesise that fisitin may mimic the ER effect on lifespan and thus not be effective when used as part of an energy restricted diet[58]. EGCG increased the lifespan of male flies at 10 g/l diet. However, the authors also state that EGCG did not affect lifespan in female flies[60].

Cocoa powder improved fly longevity at 50 g/l but showed no effect at 100 g/l[62]. Although the flavonoid content of this powder had not been analysed as part of the study, we included it because cocoa powder is known to have high flavonoid content[68]. Bahdorani

https://doi.org/10.1017/S0029665116000720 Published online by Cambridge University Press

**Table 2.** Lifespan and flavonoids in *Drosophila melanogaster*

| Flavonoid | Dose in diet | Fly genotype | Sex | Diet | Start of treatment | Mean lifespan | Median lifespan | Maximum lifespan | Number of animals* | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| *Flavanols* | | | | | | | | | | |
| Epicatechin | 10 μM | Canton-S (wild-type) | ♂ | 2 % agar | Newly | NS | | | 120 | Si *et al.*[59] |
| | 100 μM | | | 10 % yeast | eclosed | Increase | | | 120 | |
| | 1000 μM | | | 10 % sucrose | | Increase | | | 120 | |
| | 8000 μM | | | | | Increase | | | 120 | |
| EGCG | 10 g/l | $w^{1118}$ (wild-type) | ♂ | 5·5 % dextrose<br>3·0 % sucrose<br>6·0 % corn meal, 2·5 % yeast<br>1·0 % agar<br>0·3 % nipagin<br>0·3 % propionic acid | 2 d after eclosure | Increase | | | 120 | Wagner *et al.*[60] |
| *Flavonol* | | | | | | | | | | |
| Fisetin | 10 μM | yw-marked (wild-type) | ♀ | 3 % CSY:<br>5 % cornmeal, 10·5 % sucrose,<br>3 % yeast, 0·7 % agar | Newly eclosed adults | NS | NS | | 237 | Wood *et al.*[58] |
| | | | | 2 % CSY: as earlier with 2 % yeast (energy restricted diet) | | NS | NS | | 307 | |
| | 100 μM | | | 3 % CSY | | +7 % | +24 % | | 237 | |
| | | | | 2 % CSY | | NS | NS | | 300 | |
| | 10 μM | | ♂ | 3 % CSY | | NS | NS | | 210 | |
| | | | | 2 % CSY | | −6 % | −7 % | | 274 | |
| | 100 μM | | | 3 % CSY | | +13 % | +12 % | | 210 | |
| | | | | 2 % CSY | | −11 % | −10 % | | 281 | |
| *Extracts* | | | | | | | | | | |
| Cocoa powder | 50 g/l | Rosy $^{+5}$ (wild-type) | ♂ | 100 g sugar<br>50 g yeast<br>18 g agar<br>8 g sodium potassium tartrate | Newly eclosed | Increase | | | 200 | Bahadorani & Hilliker[62] |
| | 100 g/l | | | | | NS | | | 200 | |
| | 50 g/l | F1 UAS-SOD1-IR × $da^{G32}$Gal4 driver stock (SOD1-deficient) | | | | Increase | | | 200 | |
| | 100 g/l | | | | | Increase | | | 200 | |
| | 50 g/l | F1 UAS-SOD2-IR x $da^{G32}$Gal4 driver stock (SOD2-deficient) | | | | Decrease | | | 200 | |
| | 100 g/l | | | | | Decrease | | | 200 | |
| Green tea extract (containing 62 % EGCG, 19 % epigallocatechin, 9 % epicatechin gallate 7 % epicatechin) | 1 g/l | Oregon-R-C (wild-type) | ♂ | Standard diet: 105 g cornmeal<br>21 g yeast<br>105 g dextrose<br>13 g agar/l (0·4 % ethyl-4-hydroxybenzoate<br>0·5 % acetic acid) | 2 d old | +6 % (NS) | +14 % | +5 % | 200 | Li *et al.*[61] |
| | 5 g/l | | | | | +21 % | +5 % | | 200 | |
| | 10 g/l | | | | | +8 % | +36 % | +5 % | 200 | |
| | | | | | | +16 % | | | | |
| | | | | Standard diet | | +16 % | +7 % | +14 % | 200 | Li *et al.*[69] |
| | | | | +5 % lard | | +71 % | +25 % | +33 % | 200 | |
| | | | | Standard diet<br>+10 % lard | | | | | | |

Table 2. (*Cont.*)

| Flavonoid | Dose in diet | Fly genotype | Sex | Diet | Start of treatment | Mean lifespan | Median lifespan | Maximum lifespan | Number of animals* | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Black tea extract (containing 50 % theaflavins, 12 % epicatechin, 12 % epigallocatechin, 10 % EGCG and 6 % epicatechin gallate) | 5 g/l<br>10 g/l | Oregon R-C (wild-type) | ♂ | Standard diet: 105 g cornmeal 21 g yeast 105 g glucose 13 g agar/l (0·4 % ethyl-4-hydroxybenzoate)<br>Standard diet +10 % lard | 2 d old | +8 %<br>+10 % | +10 %<br>+12 % | +4 %<br>+4 %<br>+87 % | 200<br>200<br>200 | Peng *et al.*[63] |
| Ludwigia octovalvis extract (containing 90 mg flavonoids/g measured as rutin equivalents) | 0·05 g/l<br>0·1 g/l<br>1 g/l<br>0·05 g/l<br>0·1 g/l<br>1 g/l<br>0·1 g/l<br>0·1 g/l<br>0·1 g/l | Canton S (wild-type)<br>Canton S (wild-type)<br>w$^{1118}$ (wild-type)<br>w$^{1118}$ (wild-type) | ♂<br>♀<br>♂<br>♀<br>♂<br>♀ | High-energy diet (containing 15 % dextrose and 15 % yeast)<br>Low-energy diet (containing 5 % dextrose and 5 % yeast) | 48 h after eclosure | +9 %<br>+16 %<br>+10 %<br>+13 %<br>+24 %<br>+12 %<br>+8 %<br>+11 %<br>NS<br>NS | | | | Lin *et al.*[67] |
| Apple polyphenols (containing 4 % proanthocyanidins, 3 % epicatechin, 1 % catechin and other proanthocyanidins and phenolics) | 2 g/l<br>10 g/l | Oregon R-C (wild-type) | ♂ | Standard diet: 105 g cornmeal 21 g yeast 105 g glucose 13 g agar/ L (0·4 % ethyl-4-hydroxybenzoate) | Newly eclosed | NS<br>+10 % | +10 % | +3 % | 200<br>200 | Peng *et al.*[64] |
| Blueberry extract (containing 49 % cyanidin-3-O-glucoside and 20 % petunidin-3-O-glucoside) | 2 g/l<br>5 g/l | Oregon R-C (wild-type) | ♂ | Standard diet: 105 g cornmeal 21 g yeast 105 g glucose 13 g agar/l (0·4 % ethyl-4-hydroxybenzoate) | Newly eclosed | NS<br>+12 % | +13 % | +9 % | 400<br>400 | Peng *et al.*[65] |
| Black rice extract (containing 24 % cyanidine-3-O-glucoside and 1·5 % petunidin-3-O-glucoside) | 10 g/l<br>30 g/l | Oregon R-C (wild-type) | ♂ | standard diet: 105 g maize 21 g yeast 105 g dextrose 13 g agar/l (0·4 % ethyl-4-hydroxybenzoate) | Newly eclosed (1–3 d old) | NS<br>+14 % | +15 % | +23 % | | Zuo *et al.*[66] |

EGCG, epigallocatechin gallate; CSY, cornmeal–sugar–yeast.
* If the numbers in the control and treatment groups are different, the lower number is given. If various trials were carried out the sum of the animals used is given.

and Hilliker also used mutant strains for the superoxide dismutases (SOD) 1 and 2 and observed that in animals deficient in SOD1, which is also referred to as Cu/ZnSOD and found in the cytosol of the cell, both concentrations of cocoa powder (50 and 100 g/l) increased lifespan, while in animals deficient in SOD2, also referred to as MgSOD and found in the mitochondrial matrix, lifespan was shortened.

Green tea extract (GTE) containing 62 % EGCG, 19 % epigallocatechin, 9 % epicatechin gallate, 7 % epicatechin extended lifespan in male Oregon-R-C on a diet with 10·5 % cornmeal, 2·1 % yeast and 10·5 % dextrose (m/V). The extract was tested at 1; 5 and 10 g/l with 5 and 10 g/l extending lifespan significantly[61]. Adding fat to fly diets shortens lifespan dose-dependently[69]. However, adding the 10 g/l GTE to a 5 or 10 % lard-containing diet improved longevity compared with the non-supplemented high-fat diet[69].

When using a BTE containing 50 % theaflavins, 12 % epicatechin, 12 % epigallocatechin, 10 % EGCG and 6 % epicatechin gallate with a similar diet (glucose instead of dextrose) and the same type of flies at 5 and 10 g/l, lifespan was also increased. Furthermore, BTE at 10 g/l could counteract, in part, the detrimental effect of 10 % lard addition to the diet on fly lifespan[63].

*Ludwigia octovalvis* is used as herbal medicine and consumed in parts of Asia as a drink[67]. Its extract containing 90 mg flavonoids/g (measured as rutin equivalents) prolonged the lifespan of male and female Canton-S or $w^{1118}$ flies at concentrations from 0·05 – 1 g/l when applied with a high-energy diet (15 % dextrose and 15 % yeast). Supplementation of the low-energy diet (5 % dextrose and 5 % yeast) at 0·1 g/l did not affect lifespan in $w^{1118}$ flies[67].

Apple polyphenols containing 4 % proanthocyanidins, 3 % epicatechin, 1 % catechin and other unidentified proanthocyanidins and phenolics increased the lifespan of male Oregon-R-C flies fed on 10·5 % cornmeal, 2·1 % yeast and 10·5 % glucose (m/V) when supplemented at 10 g/l, whereas 2 g/l did not improve longevity[64].

BE containing 49 % cyanidin-3-O-glucoside and 20 % petunidin-3-O-glucoside and used with the same type of flies and diet as the apple polyphenols increased the lifespan supplemented at a concentration of 5 g/l, while 2 g/l did not change lifespan[65].

Black rice extract containing lower levels of cyanidin and petunidin glycosides than the BE (24 % cyanidine-3-O-glucoside and 1·5 % petunidin-3-O-glucoside) extended lifespan in the same type of flies as the BE and apple polyphenols at a sixfold higher concentration (30 g/l) than BE, which is also high in anthocyanins. The twofold higher concentration (10 g/l) did not affect lifespan[66]. This finding is consistent with the BE having no effect at 2 g/l since the black rice extract has less than half of the cyanidin content and an approximately sevenfold lower petunidin content compared with the BE.

Overall, experiments in flies showed a beneficial effect of flavonoids or flavonoid-containing extracts on the lifespan of *D. melanogaster* even with concentrations in the feed that were sixtyfold higher than in the worm. Of interest, the diet composition strongly influenced the outcome of the studies, since energy restricted diets abrogated or reversed the flavonoid effect on longevity while high-energy diets increased it.

## Flavonoids and the lifespan of *Mus musculus*

Being mammals and vertebrates, mice share even more similarities with human subjects than worms and flies. Human homologues have been found for approximately 98 % of mouse genes[70] and many genetic functions have been studied in knockout mice. Compared with other mammals, mice are easy to house and have a short lifespan of 2–3 years. This makes them a useful model organism for ageing research[71].

Of six mouse trials that studied the influence of flavonoid-containing extracts on lifespan, two studies also tested the flavonols quercetin and icariin as single compounds (Table 3).

While icariin extended mean lifespan at 0·02 % diet when fed to C57BL/6 from age 12 months but did not extend maximum lifespan[72], feeding of quercetin at a fivefold higher dose than icariin (0·1 % diet) and beginning at age 5 weeks decreased the lifespan of LACA strain mice[73]. Two out of six studies testing flavonoid-containing extracts observed a lifespan increase in supplemented mice. These extracts were a triple combination of BE, GTE and pomegranate powder (PG) in the study by Aires *et al.*[74] and green tea polyphenols containing approximately 70 % epigallocatechins, epicatechins and gallocatechins in a study by Kitani *et al.*[75]. Interestingly, the combination of BE, EGCG and PG was compared with a non-supplemented high-fat diet in C57BL/6 mice that had access to feed for 24 h followed by 24 h fasting (intermittent fasting). While intermittent fasting compared with *ad libitum* feeding increased lifespan when started at age 20 weeks, a 2 % BE, 0·015 % GTE and 0·3 % PG diet further prolonged lifespan in intermittent fasting-fed mice[74]. The green tea polyphenols were tested in 13-month-old C57BL/6 on an *ad libitum* moderate fat diet. By supplementing their drinking water at 80 mg/l the mice lived longer than the unsupplemented controls[75]. However, four studies testing blackcurrant juice (containing anthocyanins, quercetin and quercetin glycosides)[73], epimedium flavonoids (containing 20 % icariin)[72], GTE[76], BE, GTE, a triple combination of GTE, BTE and morin, PG or a triple combination of quercetin, taxifolin and pynogenol®[77] did not detect lifespan increase in the mice.

In mice, it remains unclear whether flavonoid supplementation could prolong lifespan. While in C57BL/6 mice there are indications that these mice could benefit from elevated flavonoid intake, genetically heterogenous mice do not live longer when supplemented. Moreover, a very high flavonoid dose (1 g/kg diet) shortened the lifespan of LACA mice.

https://doi.org/10.1017/S0029665116000720 Published online by Cambridge University Press

**Table 3.** Lifespan and flavonoids in *Mus musculus*

| Flavonoid | Dose in diet | Mouse genotype | Sex | Diet | Start of treatment | Mean lifespan | Median lifespan | Maximum lifespan | Number of animals* | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| *Flavonols* | | | | | | | | | | |
| Icariin | 0·02 % | C57BL/6 | ♂ | GB14924.3–2001 AL | 12 months | +8 % | | NS | 49 | Zhang *et al.*[72] |
| Quercetin | 0·1 % | LACA strain | ♂ & ♀ | semi-synthetic scorbutogenic diet MG1 + vitamin C | 5 weeks | −10 % | | | 50/sex | Jones & Hughes [73] |
| *Extracts and combinations* | | | | | | | | | | |
| Blackcurrant juice | 220 ml juice/kg (approximately 0·038 % flavonoids/kg, mainly anthocyanins, 11 % quercetin and its glycosides) | LACA strain | ♂ & ♀ | MG1 | 5 weeks | NS | | | 50/sex | Jones & Hughes [73] |
| Epimedium flavonoids (containing 20 % icariin) | 0·06 % | C57BL/6 | ♂ | GB14924.3–2001 AL | 12 months | NS | | NS | 49 | Zhang *et al.*[72] |
| Triple combination of BE, GTE, PG | 2 % BE, 0·015 % GTE, 0·3 % PG | C57BL/6 | ♂ | High-fat chow TD94045 AIN-93G, IF (24 h access to feed followed by 24 h fasting) | 20 weeks | | +6 % | | 68 | Aires *et al.*[74] |
| Green tea polyphenols (containing approximately 70 % epigallocatechins, epicatechins and gallocatechins) | 80 mg/l in drinking water | C57BL/6 | ♂ | Moderate fat diet, 23·8 % protein, Oriental Yeast Ltd, Japan | 13 months | +6 % | | NS | 50 | Kitani *et al.*[75] |
| GTE | 0·2 % | Genetically heterogeneous mice (progeny CB6F1*C3D2F1) | ♂<br>♀ | Purina5LG6 control diet | 4 months | NS<br>NS | | | ≥153<br>≥129 | Strong *et al.*[76] |
| BE | 684 mg/kg | $F_1$ mice from | ♂ | AIN-93M (Purina chow feed up to 12 months) | 12 months | NS | | | 36 | Spindler *et al.*[77] |
| GTE | 931 mg/kg | C57BL/6NHsd x | | | | NS | | | 36 | |
| Triple combination of GTE, black tea extract, morin | 931, 440, 500 mg/kg | C3H/HeNHsd cross | | | | NS | | | 36 | |
| Pomegranate extract | 818 mg/kg | | | | | NS | | | 36 | |
| Triple combination of quercetin, taxifolin, pynogenol® (pine bark extract: condensed tannins) | 584, 630, 94 mg/kg | | | | | NS | | | 36 | |

BE, blueberry extract; GTE, green tea extract; PG, pomegranate powder.
* If the numbers in the control and treatment groups are different, the lower number is given. If various trials were carried out the sum of the animals used is given.

## Factors putatively affecting lifespan modulation by flavonoids

Although flavonoids that extend worm lifespan do not necessarily expand mammalian lifespan, experiments in worms and flies can help to understand which proteins or signalling pathways are affected by the flavonoids. Thus, invertebrate models could be a useful tool to investigate structure–activity relationships. As can be deduced from studies in mutant worms and flies (Tables 1 and 2), flavonoids affect various molecular targets and may influence lifespan by numerous mechanisms (Fig. 2). Flavonoid pleiotropy and human subjects consuming many different flavonoids with their diet and not just single compounds complicate the evaluation of flavonoids in the context of lifespan extension.

### Extracts or single compounds

It remains unclear whether different flavonoids that are consumed at the same time have additive or even synergistic effects on lifespan. It is also possible that other compounds in the diet attenuate the effect of life-prolonging flavonoids. To test this, studies experimenting with single compounds and combinations of flavonoids and/or other secondary plant metabolites are needed. Although there are studies that tested a flavonoid-containing extract and single flavonoids from this extract, the concentrations in the extracts are different from the tested dose of the single compounds. This makes it difficult to deduce whether varying results are due to changes in concentration or the combination with other polyphenols. For a ginkgo biloba extract, the authors found that one of its flavonoids, kaempferol, did not affect lifespan on its own while isolated tamarixetin prolonged lifespan to a greater extent than the extract. This could have occurred because the tamarixetin dose the worms were exposed to was higher in the single compound experiment than in the ginkgo extract experiment. Alternatively, components of the extract could have attenuated the lifespan-prolonging action of tamarixetin or may be slightly toxic[42].

Interestingly, some of the extracts used in worms such as the tea seed pomace or the Taiwan hinoki extract increased lifespan at lower concentrations than single compounds[51,52]. Assuming these extracts consisted entirely of a flavonoid with the molecular mass of catechin, 1–2 mg/l would correspond to 3·5–7 μM flavonoid/l. If extracts worked at lower doses than single compounds this might be due to synergisms between different flavonoids and/or other polyphenols. In flies, the concentrations used to test the extracts tended to be higher than the single compounds and manifold higher than in the worms. In wild-type flies approximately 10 g flavonoids/l seemed non-toxic[61,63]. However in flies, only epicatechin and fisetin were tested on their own, making it difficult to hypothesise whether flavonoids in extracts show additive, synergistic or antagonistic actions.

In mice, blackcurrant juice was tested and did not alter lifespan, but when testing one of the flavonols in blackcurrant juice, quercetin, a manifold higher concentration shortened the lifespan of the mice[73]. When testing epimedium flavonoids of which 20 % are icariin at 0·06 %, these did not prolong mouse lifespan whereas, when supplementing icariin at 0·02 %, the mice lived longer[72]. Comparing icariin intake of a 30 g mouse consuming 5 g feed daily to an estimated human intake of 70 mg flavonoids daily[1], the intake/kg bodyweight is thirty times higher in the mouse. Thus, the lifespan extending concentrations of flavonoids are not consumed in a non-supplemented human diet. Moreover, the toxic dose (0·1 %) of quercetin in mice[73] is over 100 times higher than the estimated human intake in mg/kg bodyweight.

### Antimicrobial properties

Antimicrobial, antioxidative and ER-like mechanisms have been proposed to prolong lifespan in flavonoid-supplemented animals. It has been shown that flavonoids and other polyphenols possess antimicrobial properties and that feeding worms dead bacteria as opposed to live bacteria prolongs lifespan. When supplementing gallic acid or ellagic acid to heat-killed bacteria no further lifespan enhancing effect is observed because of the polyphenols although both extend lifespan when fed as part of a live bacteria diet[30,78]. Interestingly, catechin and quercetin extended lifespan also when the worms were fed dead bacteria[29,37], thereby making it unlikely that these flavonoids promote longevity because of their antimicrobial properties. Furthermore, it was shown in mice and rats that flavonoids influenced the gut microbiota, which could benefit the host and as a consequence promote longevity[79,80].

### Antioxidant properties

In worms, it has been shown that flavonoids decrease oxidative stress[52,81]. Although flavonoids can work as radical scavengers because of their chemical structure, their *in vivo* antioxidative action is more likely caused by them stabilising and up-regulating the antioxidant transcription factor nuclear factor (erythroid-derived 2)-like 2. Nuclear factor (erythroid-derived 2)-like 2 activation leads to the expression of its target genes that remove peroxides and eliminate xenobiotics[17]. Some studies found that EGCG could increase the lifespan of oxidatively stressed worms[34]. Additionally, experiments in *mev-1* mutants that experience high levels of oxidative damage and have a shorter lifespan than wild-type worms, showed that myricetin, quercetin and purple wheat extract could prolong their lifespan[36,46,55]. However, quercetin does not always benefit *mev-1* worms and kaempferol did not increase lifespan in the mutants[40]. In flies, cocoa powder was also tested in SOD-deficient mutants. Interestingly, it prolonged lifespan in flies that did not have the cytosolic SOD1, but was detrimental for flies without the mitochondrial SOD2. These findings point to the notion that when

https://doi.org/10.1017/S0029665116000720 Published online by Cambridge University Press

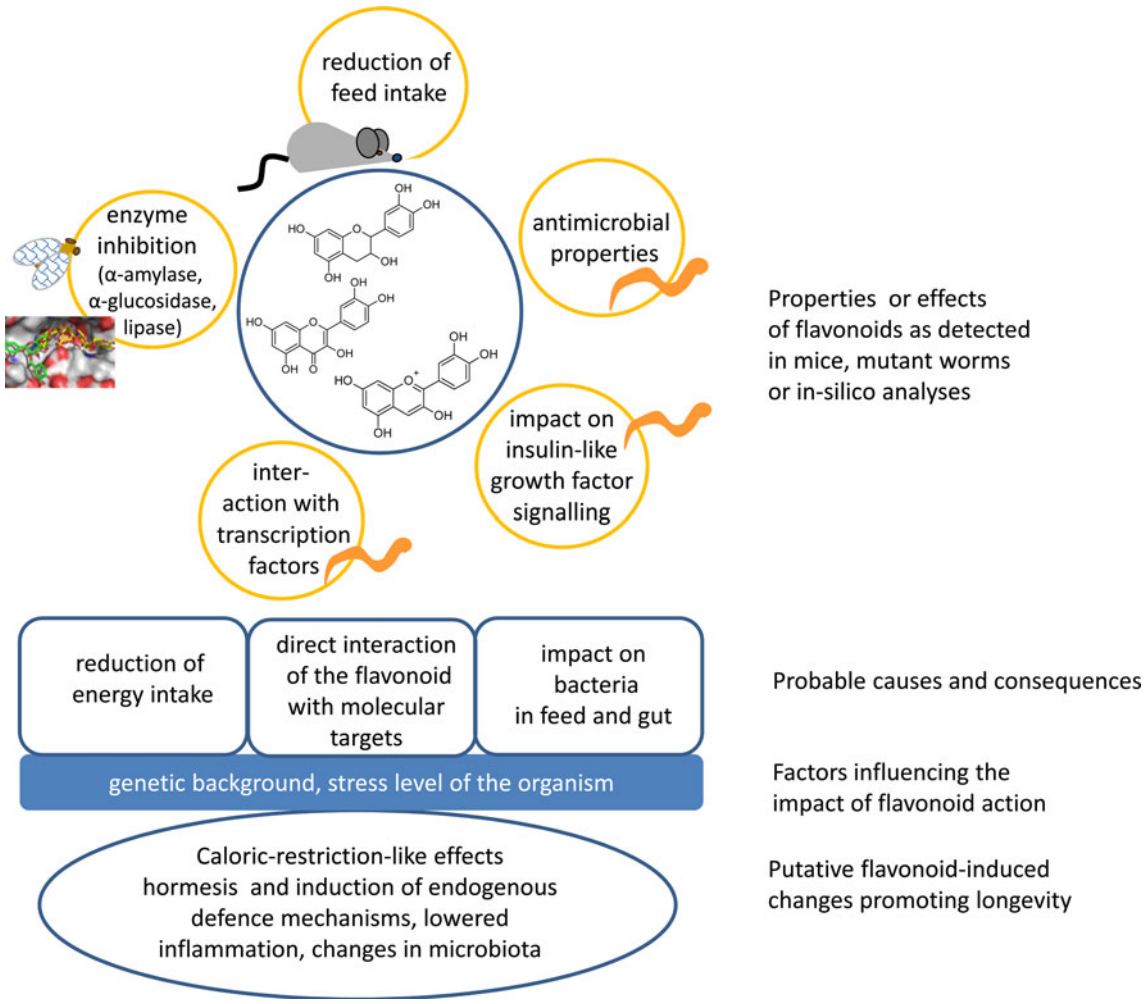


**Fig. 2.** (Colour online) Putative pleiotropic effects by which flavonoids may affect lifespan in model organisms.

oxidative stress levels are very high, flavonoids could further increase oxidative damage[62]. Moreover, it has been hypothesised that low levels of oxidative stress may increase the lifespan of model organisms by activating their antioxidant defence[82]. Thus, flavonoids may not only activate nuclear factor (erythroid-derived 2)-like 2 by reacting with its inhibitor Kelch-like ECH-associated protein 1 and thereby allowing the transcription factor to locate to the nucleus[83], but also by producing low levels of oxidative stress, which in turn induce the transcription of antioxidative enzymes. The observation that a low dose of a stimulus has a beneficial and a high dose of a stimulus has the opposite (negative) effect has been described as hormesis[84]. In experiments with worms, it was shown that lifespan extension by quercetin was dose dependent with low doses prolonging lifespan and higher doses shortening lifespan[41,43]. Interestingly, this dose–response curve may not apply to all flavonoids since catechin prolonged worm lifespan to a very similar extent over a concentration range of 100–800 μM[30]. The finding that some flavonoids are toxic at high doses and others do not appear to harm the organism even at higher concentrations points to the notion that different flavonoids affect lifespan via different mechanisms[85]. In the studies reviewed on quercetin and worms (Table 1), lifespan extension depended on methyl groups or sugars conjugated to the flavonoid[38,39,42]. Apart from changing the interaction with molecular targets inside the organism these modifications also affect the bioavailability of the flavonoid[41].

## Studies in mutants

Studies in other mutant worms also showed that the mechanisms underlying the lifespan increase might differ depending on the flavonoid. The longevity-promoting impact of icariin, icariside II, quercetin, catechin and cocoa powder was not triggered in *daf-2* mutant worms (Table 2). None of the flavonoids or extracts tested prolonged the lifespan of this mutant lacking the human IGF homologue. However, in an *age-1* mutant (phosphoinositide-3-kinase subunit p110 homologue) catechin increased lifespan, while quercetin did not. In turn, catechin failed to increase lifespan in an *akt-2* (AKT homologue) mutant, while quercetin could

https://doi.org/10.1017/S0029665116000720 Published online by Cambridge University Press

prolong lifespan in these worms[30,37]. In contrast to quercetin and catechin, the life-expanding impact of icariside II, myricetin, cocoa powder and purple wheat extract was abolished by mutating *daf-16*, the worm FOXO homologue[45,46]. Furthermore, the effect of cocoa powder but not quercetin or catechin depended on *sir-2.1*, a histone deacetylase which is similar to mammalian SIRT1[50].

## Energy-restriction-like effects

Oxidative stress and IGF signalling are reduced in animals undergoing ER. It could be hypothesised that flavonoids mimic, in part, the effects or ER, which is known to prolong lifespan in worms, flies and mice[14]. However, while catechin failed to prolong lifespan in a worm that was GM to reduce its feed intake, quercetin and icariside II prolonged lifespan in an ER worm model[29,37,45]. Interestingly, in mice, intermittent fasting and supplementing a combination of GTE, BE and PG had additive effects on lifespan increase. In contrast in flies, fisetin did not extend the lifespan of flies fed an ER diet, but as part of a diet with higher yeast content it could prolong lifespan. Flavonoids could protect an organism from the negative impact of overeating since BTE and GTE partly attenuated the lifespan decrease caused by feeding flies fat-containing diets. In worms, GTE was also shown to prevent glucose-induced survival reduction[86]. In a mouse study by Spindler *et al.*[77], although ER extended lifespan, the tested flavonoid-containing extracts did not improve longevity. Of interest, the control group was fed isoenergically compared with the group receiving the flavonoid extracts because the researchers observed a lower feed intake in the supplemented group. Therefore, in future studies feed intake should be monitored carefully in order to detect whether the flavonoid or a flavonoid-caused decrease in food intake extends lifespan of the model organism. However, since antinutritional qualities of flavonoids have been described, it is possible that flavonoids mimic ER by binding to proteins and inhibiting enzymes[87,88], e.g. lipases[89], α-amylase, α-glucosidase[60,90,91], proteases such as trypsin[92] or glucose transporters[93,94]. Moreover, flavonoids may affect the microbiota composition[79] and thus exert ER-like effects via interaction with the microbiota[95].

## Mice do not always benefit from flavonoid supplementation

The negative outcomes of most mouse studies show that the lifespan-extending effects of flavonoids in worms and flies need to be verified in mammals since mice seem less likely to respond to flavonoid supplements. Moreover, flavonoid metabolism may differ between species.

In contrast to the findings in worms, quercetin supplemented as a single compound decreased mouse lifespan and diets with approximately 40 or 580 mg quercetin/kg diet did not change lifespan. However, in the case of icariin, studies in mice and worms showed positive effects. The data on GTE and mouse longevity are contradicting. In one out of three studies GTE extended lifespan, whereas in the other two no differences were observed. In addition to differences in the dose, diet and onset of supplementation, the mouse strains were different. While in heterogeneous mice GTE did not expand lifespan when supplemented from month 4[76] or 12[77], in male C57BL/6 mice, supplementing GTE from age 13 months promoted longevity[75]. In the case of the stilbene resveratrol lifespan extension also seems to depend on the genotype of the mice. While in C57BL/6 mice[96] resveratrol extended lifespan, in heterogeneous mice it did not[97]. Furthermore, the impact of flavonoids on health may depend on the sex of the model organism. In male flies, EGCG extended lifespan although female flies did not benefit from EGCG supplementation[60]. Therefore, the species and the genotype appear to be important for the responsiveness of an organism to polyphenol treatment and it seems that the more complex the organism, the more complex the effect of flavonoids on it is.

## Challenges of deducing whether human subjects could benefit from flavonoid supplementation

Human flavonoid intake varies considerably. Vogiatzoglou *et al.*[4] have reported that although the mean intake in Europe is 430 mg/d, median intake is much lower, 160 mg/d, meaning that some human subjects consume high amounts of flavonoids with their diets, while others consume very little. Thus, based on murine intake in the reviewed articles with a positive outcome for lifespan extension, human intake would be four to ten times lower compared with the amount mice are usually supplemented with (calculated as mg flavonoid/kg bodyweight) and the amounts supplemented in worm and fly studies are even higher. Interestingly, in human subjects, much lower doses than in animal trials benefitted health since in intervention trials with doses from 100 to 800 mg/d[98,99], positive effects on CVD-related parameters were reported. Furthermore, it would be interesting to study whether flavonoid consumption may abrogate the negative impact of high-fat and high-sugar diets on human health as it seems the case in flies and possibly mice. However, in some human trials, flavonoid consumption could not decrease CVD risk[100] and cancer does not seem to be affected[101]. Unfortunately, randomised controlled or prospective trials studying human morbidity or mortality as an endpoint for flavonoid supplementation are scarce.

## Conclusion

On one hand, epidemiological studies have concluded that a high flavonoid intake benefits health[23,99] and certain lifespan studies in worms, flies and even mice show that flavonoids could promote longevity. On the other hand, the findings in mammalian model organisms are contradictory and health-related data on flavonoids in human subjects have been obtained from epidemiological

https://doi.org/10.1017/S0029665116000720 Published online by Cambridge University Press

and not prospective studies. Thus, it remains uncertain whether human individuals would benefit from increased flavonoid intake as far as lifespan extension is concerned.


## Financial Support

None.


## Conflict of Interest

None.

## Authorship

G. R. and K. P. wrote the manuscript. N. D. conducted a literature search and provided the tables.

## References


1. Beecher GR (2003) Overview of dietary flavonoids: nomenclature, occurrence and intake. *J Nutr* **133**, 3248S–3254S.
2. Chun OK, Chung SJ & Song WO (2007) Estimated dietary flavonoid intake and major food sources of U.S. adults. *J Nutr* **137**, 1244–1252.
3. Perez-Jimenez J, Hubert J, Hooper L *et al.* (2010) Urinary metabolites as biomarkers of polyphenol intake in humans: a systematic review. *Am J Clin Nutr* **92**, 801–809.
4. Vogiatzoglou A, Mulligan AA, Lentjes MA *et al.* (2015) Flavonoid intake in European adults (18 to 64 years). *PLoS ONE* **10**, e0128132.
5. Hertog MG, Feskens EJ, Hollman PC *et al.* (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* **342**, 1007–1011.
6. Hertog MG, Kromhout D, Aravanis C *et al.* (1995) Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch Intern Med* **155**, 381–386.
7. Hertog MG, Feskens EJ & Kromhout D (1997) Antioxidant flavonols and coronary heart disease risk. *Lancet* **349**, 699.
8. Pallauf K, Giller K, Huebbe P *et al.* (2013) Nutrition and healthy ageing: calorie restriction or polyphenol-rich 'MediterrAsian' diet? *Oxid Med Cell Longev* **2013**, 707421.
9. Zamora-Ros R, Forouhi NG, Sharp SJ *et al.* (2014) Dietary intakes of individual flavanols and flavonols are inversely associated with incident type 2 diabetes in European populations. *J Nutr* **144**, 335–343.
10. Speakman JR & Mitchell SE (2011) Caloric restriction. *Mol Aspects Med* **32**, 159–221.
11. El Assar M, Angulo J & Rodriguez-Manas L (2013) Oxidative stress and vascular inflammation in aging. *Free Radic Biol Med* **65**, 380–401.
12. Testa G, Biasi F, Poli G *et al.* (2014) Calorie restriction and dietary restriction mimetics: a strategy for improving healthy aging and longevity. *Curr Pharm Des* **20**, 2950–2977.
13. Blagosklonny MV (2010) Calorie restriction: decelerating mTOR-driven aging from cells to organisms (including humans). *Cell Cycle* **9**, 683–688.
14. Nikolai S, Pallauf K, Huebbe P *et al.* (2015) Energy restriction and potential energy restriction mimetics. *Nutr Res Rev* **28**, 100–120.
15. Roth GS & Ingram DK (2016) Manipulation of health span and function by dietary caloric restriction mimetics. *Ann N Y Acad Sci* **1363**, 5–10.
16. Heim KE, Tagliaferro AR & Bobilya DJ (2002) Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J Nutr Biochem* **13**, 572–584.
17. Tanigawa S, Fujii M & Hou DX (2007) Action of Nrf2 and Keap1 in ARE-mediated NQO1 expression by quercetin. *Free Radic Biol Med* **42**, 1690–1703.
18. Jiang J, Mo ZC, Yin K *et al.* (2012) Epigallocatechin-3-gallate prevents TNF-alpha-induced NF-kappaB activation thereby upregulating ABCA1 via the Nrf2/Keap1 pathway in macrophage foam cells. *Int J Mol Med* **29**, 946–956.
19. Indra MR, Karyono S, Ratnawati R *et al.* (2013) Quercetin suppresses inflammation by reducing ERK1/2 phosphorylation and NF kappa B activation in Leptin-induced Human Umbilical Vein Endothelial Cells (HUVECs). *BMC Res Notes* **6**, 275.
20. Syed DN, Adhami VM, Khan MI *et al.* (2013) Inhibition of Akt/mTOR signaling by the dietary flavonoid fisetin. *Anticancer Agents Med Chem* **13**, 995–1001.
21. Kuriyama S, Shimazu T, Ohmori K *et al.* (2006) Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. *JAMA* **296**, 1255–1265.
22. Grassi D, Desideri G, Necozione S *et al.* (2008) Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. *J Nutr* **138**, 1671–1676.
23. Wang X, Ouyang YY, Liu J *et al.* (2014) Flavonoid intake and risk of CVD: a systematic review and meta-analysis of prospective cohort studies. *Br J Nutr* **111**, 1–11.
24. Piegholdt S, Rimbach G & Wagner AE (2016) The phytoestrogen prunetin affects body composition and improves fitness and lifespan in male *Drosophila melanogaster*. *FASEB J* **30**, 948–958.
25. Piegholdt S, Rimbach G & Wagner AE (2016) Effects of the isoflavone prunetin on gut health and stress response in male *Drosophila melanogaster*. *Redox Biol* **8**, 119–126.
26. Consortium TCeS (1998) Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* **282**, 2012–2018.
27. Tissenbaum HA (2012) Genetics, life span, health span, and the aging process in *Caenorhabditis elegans*. *J Gerontol A Biol Sci Med Sci* **67**, 503–510.
28. Friedman DB & Johnson TE (1988) A mutation in the age-1 gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. *Genetics* **118**, 75–86.
29. Saul N, Pietsch K, Menzel R *et al.* (2009) Catechin induced longevity in *C. elegans*: from key regulator genes to disposable soma. *Mech Ageing Dev* **130**, 477–486.
30. Saul N, Pietsch K, Sturzenbaum SR *et al.* (2011) Diversity of polyphenol action in *Caenorhabditis elegans*: between toxicity and longevity. *J Nat Prod* **74**, 1713–1720.
31. Surco-Laos F, Duenas M, Gonzalez-Manzano S *et al.* (2012) Influence of catechins and their methylated metabolites on lifespan and resistance to oxidative and thermal stress of *Caenorhabditis elegans* and epicatechin uptake. *Food Res Int* **46**, 514–521.
32. Bartholome A, Kampkotter A, Tanner S *et al.* (2010) Epigallocatechin gallate-induced modulation of FoxO signaling in mammalian cells and *C. elegans*: FoxO

stimulation is masked via PI3K/Akt activation by hydrogen peroxide formed in cell culture. *Arch Biochem Biophys* **501**, 58–64.
33. Abbas S & Wink M (2009) Epigallocatechin gallate from green tea (*Camellia sinensis*) increases lifespan and stress resistance in *Caenorhabditis elegans*. *Planta Med* **75**, 216–221.
34. Zhang L, Jie G, Zhang J *et al*. (2009) Significant longevity-extending effects of EGCG on *Caenorhabditis elegans* under stress. *Free Radic Biol Med* **46**, 414–421.
35. Kampkotter A, Timpel C, Zurawski RF *et al*. (2008) Increase of stress resistance and lifespan of *Caenorhabditis elegans* by quercetin. *Comp Biochem Physiol B Biochem Mol Biol* **149**, 314–323.
36. Saul N, Pietsch K, Menzel R *et al*. (2008) Quercetin-mediated longevity in *Caenorhabditis elegans*: is DAF-16 involved? *Mech Ageing Dev* **129**, 611–613.
37. Pietsch K, Saul N, Menzel R *et al*. (2009) Quercetin mediated lifespan extension in *Caenorhabditis elegans* is modulated by age-1, daf-2, sek-1 and unc-43. *Biogerontology* **10**, 565–578.
38. Surco-Laos F, Cabello J, Gomez-Orte E *et al*. (2011) Effects of O-methylated metabolites of quercetin on oxidative stress, thermotolerance, lifespan and bioavailability on *Caenorhabditis elegans*. *Food Funct* **2**, 445–456.
39. Xue YL, Ahiko T, Miyakawa T *et al*. (2011) Isolation and *Caenorhabditis elegans* lifespan assay of flavonoids from onion. *J Agric Food Chem* **59**, 5927–5934.
40. Grunz G, Haas K, Soukup S *et al*. (2012) Structural features and bioavailability of four flavonoids and their implications for lifespan-extending and antioxidant actions in *C. elegans*. *Mech Ageing Dev* **133**, 1–10.
41. Duenas M, Surco-Laos F, Gonzalez-Manzano S *et al*. (2013) Deglycosylation is a key step in biotransformation and lifespan effects of quercetin-3-O-glucoside in *Caenorhabditis elegans*. *Pharmacol Res* **76**, 41–48.
42. Wu Z, Smith JV, Paramasivam V *et al*. (2002) *Ginkgo biloba* extract EGb 761 increases stress resistance and extends life span of *Caenorhabditis elegans*. Cell Mol Biol *(Noisy-le-grand)* **48**, 725–731.
43. Pietsch K, Saul N, Chakrabarti S *et al*. (2011) Hormetins, antioxidants and prooxidants: defining quercetin-, caffeic acid- and rosmarinic acid-mediated life extension in *C. elegans*. *Biogerontology* **12**, 329–347.
44. Ahn D, Lee EB, Kim BJ *et al*. (2014) Antioxidant and lifespan extending property of quercetin-3-O-dirhamnoside from *Curcuma longa* L. In *Caenorhabditis elegans*. *J Korean Soc Appl Biol Chem* **57**, 709–714.
45. Cai WJ, Huang JH, Zhang SQ *et al*. (2011) Icariin and its derivative icariside II extend healthspan via insulin/IGF-1 pathway in *C. elegans*. *PLoS ONE* **6**, e28835.
46. Buchter C, Ackermann D, Havermann S *et al*. (2013) Myricetin-mediated lifespan extension in *Caenorhabditis elegans* is modulated by DAF-16. *Int J Mol Sci* **14**, 11895–11914.
47. Buchter C, Havermann S, Koch K *et al*. (2016) Isoxanthohumol, a constituent of hop (*Humulus lupulus* L.), increases stress resistance in *Caenorhabditis elegans* dependent on the transcription factor DAF-16. *Eur J Nutr* **55**, 257–265.
48. Havermann S, Rohrig R, Chovolou Y *et al*. (2013) Molecular effects of baicalein in Hct116 cells and *Caenorhabditis elegans*: activation of the Nrf2 signaling pathway and prolongation of lifespan. *J Agric Food Chem* **61**, 2158–2164.
49. Kumar J, Park K-C, Awasthi A *et al*. (2015) Silymarin extends lifespan and reduces proteotoxicity in *C. elegans* Alzheimer's model. *CNS Neurol Disord – Drug Targets* **14**, 295–302.
50. Martorell P, Forment JV, de Llanos R *et al*. (2011) Use of *Saccharomyces cerevisiae* and *Caenorhabditis elegans* as model organisms to study the effect of cocoa polyphenols in the resistance to oxidative stress. *J Agric Food Chem* **59**, 2077–2085.
51. Cheng SC, Li WH, Shi YC *et al*. (2014) Antioxidant activity and delayed aging effects of hot water extract from *Chamaecyparis obtusa* var. formosana leaves. *J Agric Food Chem* **62**, 4159–4165.
52. Wei CC, Yu CW, Yen PL *et al*. (2014) Antioxidant activity, delayed aging, and reduced amyloid-beta toxicity of methanol extracts of tea seed pomace from *Camellia tenuifolia*. *J Agric Food Chem* **62**, 10701–10707.
53. Zhuang Z, Lv T, Li M *et al*. (2014) The lifespan-extending effects of Nymphaea hybrid root extract in the nematode *Caenorhabditis elegans*. *Plant Foods Hum Nutr* **69**, 304–309.
54. Wilson MA, Shukitt-Hale B, Kalt W *et al*. (2006) Blueberry polyphenols increase lifespan and thermotolerance in *Caenorhabditis elegans*. *Aging Cell* **5**, 59–68.
55. Chen W, Muller D, Richling E *et al*. (2013) Anthocyanin-rich purple wheat prolongs the life span of *Caenorhabditis elegans* probably by activating the DAF-16/FOXO transcription factor. *J Agric Food Chem* **61**, 3047–3053.
56. He Y & Jasper H (2014) Studying aging in *Drosophila*. *Methods* **68**, 129–133.
57. Adams MD, Celniker SE, Holt RA *et al*. (2000) The genome sequence of *Drosophila melanogaster*. *Science* **287**, 2185–2195.
58. Wood JG, Rogina B, Lavu S *et al*. (2004) Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* **430**, 686–689.
59. Si H, Fu Z, Babu PV *et al*. (2011) Dietary epicatechin promotes survival of obese diabetic mice and *Drosophila melanogaster*. *J Nutr* **141**, 1095–1100.
60. Wagner AE, Piegholdt S, Rabe D *et al*. (2015) Epigallocatechin gallate affects glucose metabolism and increases fitness and lifespan in *Drosophila melanogaster*. *Oncotarget* **6**, 30568–30578.
61. Li YM, Chan HY, Huang Y *et al*. (2007) Green tea catechins upregulate superoxide dismutase and catalase in fruit flies. *Mol Nutr Food Res* **51**, 546–554.
62. Bahadorani S & Hilliker AJ (2008) Cocoa confers life span extension in *Drosophila melanogaster*. *Nutr Res* **28**, 377–382.
63. Peng C, Chan HYE, Li YM *et al*. (2009) Black tea theaflavins extend the lifespan of fruit flies. *Exp Gerontol* **44**, 773–783.
64. Peng C, Chan HY, Huang Y *et al*. (2011) Apple polyphenols extend the mean lifespan of *Drosophila melanogaster*. *J Agric Food Chem* **59**, 2097–2106.
65. Peng C, Zuo YY, Kwan KM *et al*. (2012) Blueberry extract prolongs lifespan of *Drosophila melanogaster*. *Exp Gerontol* **47**, 170–178.
66. Zuo YY, Peng C, Liang YT *et al*. (2012) Black rice extract extends the lifespan of fruit flies. *Food Funct* **3**, 1271–1279.
67. Lin WS, Chen JY, Wang JC *et al*. (2014) The anti-aging effects of *Ludwigia octovalvis* on *Drosophila melanogaster* and SAMP8 mice. Age *(Dordr)* **36**, 689–703.
68. Lee KW, Kim YJ, Lee HJ *et al*. (2003) Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. *J Agric Food Chem* **51**, 7292–7295.
69. Li YM, Chan HY, Yao XQ *et al*. (2008) Green tea catechins and broccoli reduce fat-induced mortality in *Drosophila melanogaster*. *J Nutr Biochem* **19**, 376–383.

https://doi.org/10.1017/S0029665116000720 Published online by Cambridge University Press

70. Mural RJ, Adams MD, Myers EW *et al.* (2002) A comparison of whole-genome shotgun-derived mouse chromosome 16 and the human genome. *Science* **296**, 1661–1671.
71. Selman C & Withers DJ (2011) Mammalian models of extended healthy lifespan. *Philos Trans R Soc Lond B Biol Sci* **366**, 99–107.
72. Zhang SQ, Cai WJ, Huang JH *et al.* (2015) Icariin, a natural flavonol glycoside, extends healthspan in mice. *Exp Gerontol* **69**, 226–235.
73. Jones E & Hughes RE (1982) Quercetin, flavonoids and the life-span of mice. *Exp Gerontol* **17**, 213–217.
74. Aires DJ, Rockwell G, Wang T *et al.* (2012) Potentiation of dietary restriction-induced lifespan extension by polyphenols. *Biochim Biophys Acta* **1822**, 522–526.
75. Kitani K, Osawa T & Yokozawa T (2007) The effects of tetrahydrocurcumin and green tea polyphenol on the survival of male C57BL/6 mice. *Biogerontology* **8**, 567–573.
76. Strong R, Miller RA, Astle CM *et al.* (2013) Evaluation of resveratrol, green tea extract, curcumin, oxaloacetic acid, and medium-chain triglyceride oil on life span of genetically heterogeneous mice. *J Gerontol Ser a - Biol Sci Med Sci* **68**, 6–16.
77. Spindler SR, Mote PL, Flegal JM *et al.* (2013) 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. *Rejuven Res* **16**, 143–151.
78. Kim HI, Kim JA, Choi EJ *et al.* (2015) *In vitro* and *in vivo* antimicrobial efficacy of natural plant-derived compounds against *Vibrio cholerae* of O1 El Tor Inaba serotype. *Biosci Biotechnol Biochem* **79**, 475–483.
79. Massot-Cladera M, Abril-Gil M, Torres S *et al.* (2014) Impact of cocoa polyphenol extracts on the immune system and microbiota in two strains of young rats. *Br J Nutr* **112**, 1944–1954.
80. Anhe FF, Roy D, Pilon G *et al.* (2015) A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased *Akkermansia* spp. population in the gut microbiota of mice. *Gut* **64**, 872–883.
81. Fitzenberger E, Deusing DJ, Marx C *et al.* (2014) The polyphenol quercetin protects the mev-1 mutant of *Caenorhabditis elegans* from glucose-induced reduction of survival under heat-stress depending on SIR-2.1, DAF-12, and proteasomal activity. *Mol Nutr Food Res* **58**, 984–994.
82. Schulz TJ, Zarse K, Voigt A *et al.* (2007) Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab* **6**, 280–293.
83. Dinkova-Kostova AT, Holtzclaw WD, Cole RN *et al.* (2002) Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc Natl Acad Sci USA* **99**, 11908–11913.
84. Mattson MP (2008) Hormesis defined. *Ageing Res Rev* **7**, 1–7.
85. Pallauf K & Rimbach G (2013) Autophagy, polyphenols and healthy ageing. *Ageing Res Rev* **12**, 237–252.
86. Deusing DJ, Winter S, Kler A *et al.* (2015) A catechin-enriched green tea extract prevents glucose-induced survival reduction in *Caenorhabditis elegans* through sir-2.1 and uba-1 dependent hormesis. *Fitoterapia* **102**, 163–170.
87. Rawel HA, Meidtner K & Kroll J (2005) Binding of selected phenolic compounds to proteins. *J Agric Food Chem* **53**, 4228–4235.
88. Wiese S, Gartner S, Rawel HM *et al.* (2009) Protein interactions with cyanidin-3-glucoside and its influence on alpha-amylase activity. *J Sci Food Agric* **89**, 33–40.
89. Buchholz T & Melzig MF (2015) Polyphenolic compounds as pancreatic lipase inhibitors. *Planta Med* **81**, 771–783.
90. Ong KC & Khoo HE (1997) Biological effects of myricetin. *Gen Pharmacol* **29**, 121–126.
91. Wang H, Du YJ & Song HC (2010) Alpha-glucosidase and alpha-amylase inhibitory activities of guava leaves. *Food Chemistry* **123**, 6–13.
92. Shahwar D, Raza MA & Atta Ur R (2013) Identification of flavonoids with trypsin inhibitory activity extracted from orange peel and green tea leaves. *J Sci Food Agric* **93**, 1420–1426.
93. Noteborn HP, Jansen E, Benito S *et al.* (1997) Oral absorption and metabolism of quercetin and sugar-conjugated derivatives in specific transport systems. *Cancer Lett* **114**, 175–177.
94. Ader P, Block M, Pietzsch S *et al.* (2001) Interaction of quercetin glucosides with the intestinal sodium/glucose co-transporter (SGLT-1). *Cancer Lett* **162**, 175–180.
95. Esposito D, Damsud T, Wilson M *et al.* (2015) Black currant anthocyanins attenuate weight gain and improve glucose metabolism in diet-induced obese mice with intact, but not disrupted, gut microbiome. *J Agric Food Chem* **63**, 6172–6180.
96. Baur JA, Pearson KJ, Price NL *et al.* (2006) Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **444**, 337–342.
97. Miller RA, Harrison DE, Astle CM *et al.* (2011) Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterogeneous mice. *J Gerontol A Biol Sci Med Sci* **66**, 191–201.
98. Hooper L, Kroon PA, Rimm EB *et al.* (2008) Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* **88**, 38–50.
99. Egert S, Bosy-Westphal A, Seiberl J *et al.* (2009) Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: a double-blinded, placebo-controlled cross-over study. *Br J Nutr* **102**, 1065–1074.
100. Vogiatzoglou A, Mulligan AA, Bhaniani A *et al.* (2015) Associations between flavan-3-ol intake and CVD risk in the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk). *Free Radic Biol Med* **84**, 1–10.
101. Hui C, Qi X, Qianyong Z *et al.* (2013) Flavonoids, flavonoid subclasses and breast cancer risk: a meta-analysis of epidemiologic studies. *PLoS ONE* **8**, e54318.



https://doi.org/10.1017/S0029665116000720 Published online by Cambridge University Press