

RESEARCH ARTICLE

Metformin modulates the unfolded protein responses, altering lifespan and health-promoting effects in UPR-activated worms

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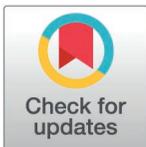
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Abstract

Metformin has been demonstrated to extend lifespan in various model organisms, and its molecular effects are observed in the cytoplasm and multiple organelles, including mitochondria. However, its association with the unfolded protein response (UPR) and its impact on stress resistance and locomotion remain uncertain. In this study, metformin was found to exert differential influences on both UPR^{mt} and UPR^{er}. The correlation between metformin's lifespan-mediating effect and its interaction with UPRs was also inconsistent. We identified a metformin-mediated lifespan extension in wild-type *C. elegans* and in UPR^{mt}-activated *tomm-22* and *cco-1* RNAi worms. Metformin suppressed the UPR^{mt} without compromising the lifespan extension observed in *tomm-22* worms. Conversely, metformin did not affect the UPR^{mt} but extended the lifespan of long-lived *cco-1* RNAi worms. Furthermore, we investigated the effects of metformin on UPR^{er}-activated nematodes. We observed that metformin exhibited a slight increase in the UPR^{er} in *mdt-15* RNAi worms and failed to induce lifespan extension. Surprisingly, metformin appeared to mediate lifespan extension in *tmem-131* RNAi worms while suppressing the UPR^{er}. Notably, the correlation between thermotolerance, oxidative stress resistance, and the lifespan effects of metformin in UPR-activated worms was inconsistent. Activation of UPRs, but not metformin treatment, enhanced the locomotor phenotype of these worms.

Introduction

Metformin is a biguanide compound commonly used to manage type 2 diabetes mellitus (T2DM). Notably, it exhibits lower toxicity compared to its predecessors,

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Abbreviations: UPR^{mt}, mitochondrial unfolded protein response; UPR^{er}, endoplasmic reticulum unfolded protein response; GFP, green fluorescent protein; T2DM, type 2 diabetes mellitus.

phenformin and buformin [1]. Beyond its primary role in treating T2DM, studies suggests that metformin may reduce the risk of T2DM-related diseases, including dementia [2], cardiovascular disease [3] and cancer [4]. Furthermore, longevity studies have demonstrated an extension of lifespan with a reduction of age-related symptoms in animal models administered metformin at various doses [5–9], implying that metformin not only possesses pro-longevity properties but also exhibits health-promoting effects. Several pathways have been identified as mediating metformin-induced longevity, including activation of the lysosomal pathway [7], inhibition of mitochondrial complex I [8,10,11], inhibition of adenylate cyclase [12], alteration of folate metabolism [13], direct regulation of AMP-kinase [14–16], and enhancement of autophagy [17].

Several of these pathways are associated with the mitochondria, where positively charged molecules such as metformin may accumulate [18–21]. These associations led us to consider the possibility that the mitochondrial unfolded protein response (UPR^{mt}) may have a role to play in metformin-mediated lifespan extension, stress resistance and health promotion. In *C. elegans*, the UPR^{mt} is a mitochondrial stress response that can extend lifespan by preserving mitochondrial transport [22,23] in a cell-non-autonomous manner [24]. Upon mitochondrial proteomic stress, the UPR^{mt} signaling pathway is activated, which initiates the translocation of ATFS-1, a transcription factor, into the nucleus. ATFS-1, in conjunction with other proteins such as UBL-5 and DVE-1 regulates the expression of genes essential for mitigating the effects of proteomic stress [25–27]. Another similar stress response is the unfolded protein response for the endoplasmic reticulum (UPR^{er}), which can be activated by proteomic stress events within the endoplasmic reticulum. In addition to stress response to proteomic toxicity in the endoplasmic reticulum, UPR^{er} is essential for normal development of *C. elegans* [28–30]. However, UPR^{mt} may not be required for several types of lifespan extension in the nematode, and UPR^{mt} *per se* does not predict longevity in the nematodes [31,32]. Interestingly, the effect of metformin on UPR^{mt} has only been briefly discussed previously, stating only that metformin itself does not activate UPR^{mt} [8]. Furthermore, little is known about the interaction of metformin in worms exhibiting a short-lived phenotype, whether it can display a beneficial effect on their lifespan, stress resistance, and health.

In this study, we investigated the effect of metformin on lifespan, its interaction with UPR^{mt}, UPR^{er}, and its influence on health promotion (stress resistance and locomotion) in UPR-activated *C. elegans*. We hypothesized that metformin may mediate its beneficial effect in the nematode through UPR^{mt}, but not UPR^{er}. To investigate UPR^{mt}, we employed *tomm-22* and *cco-1* RNAi knockdowns, both constitutively activating UPR^{mt} but with opposing effects on worm lifespan [32]. TOMM-22 is involved in protein transport across the outer mitochondrial membrane, while CCO-1 serves as a subunit of the respiratory complex IV. For UPR^{er}, we employed *mdt-15* and *tmem-131* RNAi knockdowns, both resulting in activated UPR^{er} [33–35] and shortened lifespan. MDT-15 is a mediator subunit required for transcription of genes involved in fatty acid metabolism [36,37], while TMEM-131 is a chaperone in the ER and is essential for collagen recruitment and secretion [34].

Here, we report that metformin induces lifespan extension and mildly increases thermotolerance and oxidative stress resistance in middle-aged wild-type worms. In the UPR-activated worms, metformin extended lifespan in *tomm-22*, *cco-1* and *tmem-131* worms while suppressing UPR^{mt} in *tomm-22* but not *cco-1*. Metformin reduced the lifespan of *mdt-15* worms and slightly increased their UPR^{er}. Notably, metformin suppressed the UPR^{er} in *tmem-131* while simultaneously extending the worms' lifespan. Stress resistance in metformin-treated UPR-activated worms deviated from the wild-type patterns and was not directly correlated with lifespan. Activation of UPRs resulted in enhanced locomotion, particularly in *tomm-22*. However, metformin did not exhibit any beneficial effect on locomotion in the nematodes. These findings indicate that metformin exhibits a multifaceted interaction with lifespan, UPR activation, and stress resistance, but not locomotory function in *C. elegans*.

Materials and methods

Nematode strains and bacteria cultures

Nematode strains were obtained from the Caenorhabditis Genetics Centre (CGC, University of Minnesota, USA), including: Wild-type N2 Bristol, SJ4100 *zcls13[hsp-6::GFP]*, SJ4005 *zcls4[hsp-4::GFP]*. *E. coli* strains from Ahringer's Library used include OP50, HT115 harboring the empty vector L4440 (EV), *tomm-22* (W10D9.5), *cco-1* (F26E4.9), *nuo-6* (W01A8.4), *mdt-15* (R12B2.5), *tmem-131* (C27F2.8), *tag-335*(C42C1.5), *atfs-1*(ZC376.7), and *ubl-5*(F46F11.4)

Nematode culture and RNA interference

Nematode Growth Media (NGM) was prepared as described [38], with the volume adjusted to accommodate the addition of metformin to molten agar. The final concentrations of metformin used were 25 mM and 50 mM. The cooled plates were subsequently seeded with *E. coli* and incubated overnight at 37 °C in an incubator. RNA interference by feeding was performed as described [39].

Lifespan analysis

Wild-type N2 worms were synchronized by transferring egg-laying day-1 adult worms to freshly seeded EV or OP50 for 4–6 hours. Subsequently, they were removed from the plates and the plates were incubated at 20 °C for 3 days before late L4 worms were picked to individual plates (20–30 per plate) for maintenance and data collection. Worms were synchronized at a well-fed state for at least two generations prior to experimentation. RNAi bacteria were utilized to knock down the genes *tomm-22*, *cco-1*, *nuo-6*, *mdt-15*, *tmem-131*, *tag-335*, *atfs-1*, and *ubl-5*. Worms were transferred to fresh plates daily for the initial 10 days, followed by every other day thereafter. The presence of life was determined by touch provocation and pharyngeal pumping. Worms exhibiting vulvar deformities, desiccation, or bagging were censored and excluded from the analysis.

Green fluorescent protein image capturing and signal quantitation

An inverted fluorescence microscope equipped with a camera (Ti-S Intensilight Ri1 NIS-D) (Nikon, Tokyo, Japan) was used to visualize GFP fluorescence under the FITC Filter (Excitation 465–495 nm, Emission at 515–555 nm). The NIS-D Elements software was used to capture the images in.tif format.

For quantification of worm fluorescence, the mean pixel density of individual worms was calculated using Fiji (Version 2.1.0). Regions of interests (ROIs) were drawn using the polygonal selection tool around each individual worm (n=5) in the brightfield image and saved in the ROI manager for superimposition in the fluorescence image. Then, using the same ROI in the brightfield image, the fluorescence intensity of each individual worm was quantified. The background normalization signal was calculated by manual thresholding of the blue channel, binarization, then filling holes and erosion--dilation to select the worms before using inverse selection for the area outside of the worms.

The final fluorescence was then calculated by deducting the individual worm fluorescence with the background signal, and the mean and standard deviation was calculated and plotted. Student *t*-test was used for statistical analysis.

Thermotolerance and oxidative stress assays

Wild-type N2 worms were synchronized in a manner similar to the lifespan experiments conducted prior to the commencement of the stress experiments, with the addition of 30 μ M FUdR. Subsequently, worms were transferred to 96-well plates containing 100 μ l S-basal every 3 days for analysis ($n = 10$ per well, 3 wells per strain per condition). SYTOX Green was added to a final concentration of 5 μ M prior to the commencement of the experiment. For thermotolerance, worms were exposed to 42°C for 1 hour before manual scoring for death (using a fluorescence microscope equipped with a FITC Filter) every subsequent hour until all the worms were dead. For oxidative stress, worms were exposed to 1.4% tert-Butyl peroxide for 2 hours at 20°C before manual scoring every hour until all the worms were dead.

Locomotion assays

Wild-type N2 worms were cultured synchronously on EV and different RNAi bacteria including *tomm-22* and *mdt-15*, and tested on the 3rd, 6th, 9th and 12th day of growth at 20°C. Locomotion assays included body trashing and crawling velocity measurements. In a trashing assay, 10–20 worms were placed into 200 μ l of M9 solution. The worms were allowed to equilibrate in the solution for 1 minute before the trashing movie of the worms was recorded for 30 seconds using a digital camera mounted onto a stereomicroscope. In a crawling velocity assay, 10–20 worms were transferred onto an unseeded NGM plate and allowed to crawl freely for 1 minute before the movement movie of the worms was recorded for 30 seconds. Each video ensured worms moved independently, without overlap, and under uniform illumination. The videos were exported and analyzed using ImageJ (Fiji) (Version 2.1.0). The data were collected from 3 independent cultures of each worm strain.

Statistical analysis

The lifespan results were analyzed using the log-rank test implemented in OASIS [40]. Thermotolerance, oxidative stress, trashing and velocity assays were analyzed by either Student *t*-test or the log-rank test.

Results

The lifespan-extending effects of metformin are not consistently observed in all UPR-activated worm strains

We observed a significant lifespan extension ($p < 0.01$) in wild-type worms treated with 50 mM metformin (Fig 1A). The nematodes did not exhibit consistent lifespan responses to RNAi-activated UPR. UPR^{er} activation by *mdt-15* and *tmem-131* (RNAi) reduced the nematode's lifespan, while UPR^{mt} activation led to lifespan extension in *cco-1* (RNAi) and a reduction in *tomm-22* (RNAi) (Fig 1B). Interestingly, exposing metformin to the UPR-activated worms also yielded varying results. For UPR^{mt}-activated worms, metformin treatment at 50 mM in short-lived *tomm-22* and long-lived *cco-1* worms resulted in a significant lifespan extension when compared to the corresponding strain that was not treated with metformin ($p < 0.01$) (Fig 1C and 1D). Conversely, the UPR^{er}-activated short-lived *mdt-15* worms exhibited a significant lifespan reduction upon metformin treatment at 50 mM when compared to *mdt-15* worms that were not treated with metformin ($p < 0.01$) (Fig 1E). Metformin treatment of UPR^{er}-activated short-lived *tmem-131* worms significantly extended the lifespan of the animals compared to *tmem-131* worms that were not treated with metformin ($p < 0.05$) (Fig 1F). In summary, metformin extends lifespan of UPR^{mt}-activated *tomm-22* and *cco-1*, and UPR^{er}-activated *tmem-131* but not UPR^{er}-activated *mdt-15*. Statistical analysis details are presented in Table 1.

A previous study by Espada *et al.* [41] reported that metformin-mediated longevity *via* UPR^{mt} required *ubl-5*. To further distinguish whether the lifespan-extending effect of metformin was UPR^{mt}-dependent in the *tomm-22* background, we investigated the effect of *ubl-5* in the *tomm-22* background. Logically, if *ubl-5* was required for metformin-mediated longevity, then the effect of metformin would be lost in the presence of the *tomm-22* background. Surprisingly, we found that the double knockdown of *tomm-22* and *ubl-5* in the presence of metformin did not abrogate the lifespan-extending effects of

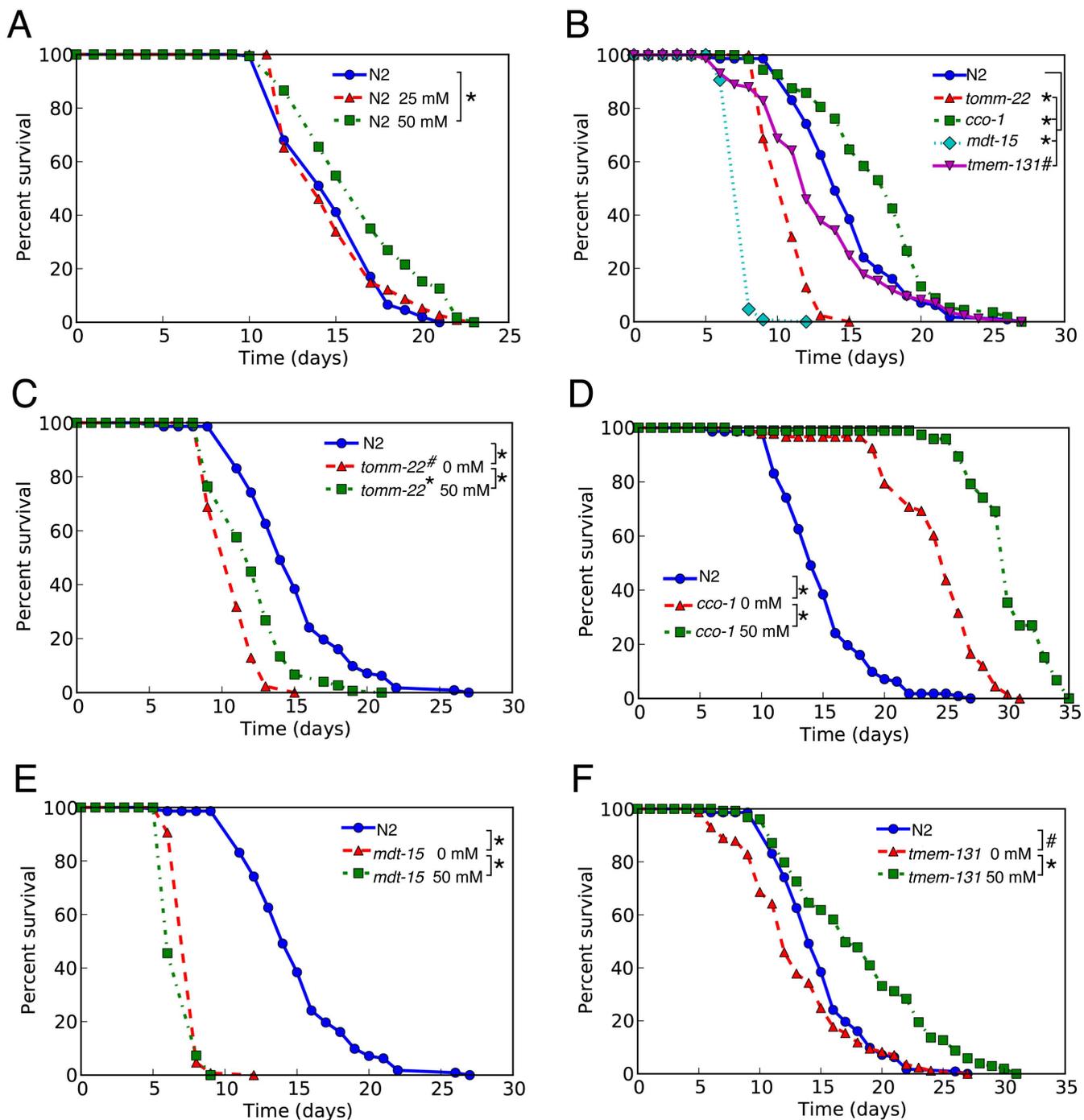


Fig 1. Effect of metformin on lifespan of wild-type and UPR-activated *C. elegans*. Lifespan assays were conducted in N2 wild-type nematodes under different conditions. The nematodes were grown on OP50 in the presence of 25 mM or 50 mM metformin or without metformin (A). Nematodes were grown on either EV or RNAi bacteria for UPR^{mt} activation (*tomm-22* and *cco-1*) or UPR^{er} activation (*mdt-15* and *tmem-131*) without metformin treatment (B). The lifespan of UPR-activated nematodes in the presence of 50 mM metformin was assayed in comparison to the control, which is N2 grown on EV, as shown in (C-F). Each assay consisted of 100-200 age-synchronized worms. Statistical analysis was performed using the log-rank test. * and # denote statistical significance where Bonferroni's p-value < 0.01 and < 0.05, respectively. Statistical analysis details are presented in Table 1.

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Table 1. Statistical output of Log-Rank test for lifespan of wild-type and UPR-activated worms.

Strain	Metformin	n	Lifespan			p-value	Bonferroni's p-value	p-value condition
			Mean	Median	Max			
N2 fed OP50	0 mM	153	14.98±0.21	15	21			
	25 mM	115	14.89±0.27	14	23	0.8702	1.0000	vs N2 fed OP50 0 mM
	50 mM	156	16.60±0.29	17	23	5.3E-07	1.1E-06*	
N2 fed EV	0 mM	150	14.92±0.32	14	27			
<i>tomm-22</i> (UPR ^{mt})	0 mM	173	10.87±0.12	11	15	0.0E+00	0.0E+00*	vs N2 fed EV 0 mM
	50 mM	165	12.17±0.20	12	21	1.1E-08	2.3E-08*	vs <i>tomm-22</i> 0 mM
<i>cco-1</i> (UPR ^{mt})	0 mM	100	24.32±0.46	25	31	0.0E+00	0.0E+00*	vs N2 fed EV 0 mM
	50 mM	100	29.97±0.42	30	35	0.0E+00	0.0E+00*	vs <i>cco-1</i> 0 mM
<i>mdt-15</i> (UPR ^{er})	0 mM	128	7.88±0.06	9	12	0.0E+00	0.0E+00*	vs N2 fed EV 0 mM
	50 mM	123	6.98±0.10	8	9	0.0E+00	0.0E+00*	vs <i>mdt-15</i> 0 mM
<i>tmem-131</i> (UPR ^{er})	0 mM	150	13.06±0.45	12	27	0.0075	0.0151#	vs N2 fed EV 0 mM
	50 mM	150	18.16±0.53	17	31	0.0E+00	0.0E+00*	vs <i>tmem-131</i> 0 mM

Statistical analysis was conducted using the log-rank test.

*and

#denote statistical significance where Bonferroni's p-value < 0.01 and < 0.05, respectively.

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metformin but further synergized to extend lifespan (S1 Fig). These findings suggest that both *tomm-22* and *ubl-5* do not overlap in their pathways, supporting our hypothesis that metformin's effect in longevity is independent of UPR^{mt}.

Metformin exposure affected both UPR^{mt} and UPR^{er}

We next investigated whether metformin exhibits differential interactions with both UPR systems. To this end, we employed the GFP-tagged reporter proteins *hsp-6::GFP* and *hsp-4::GFP* to observe the effect of metformin on the activation of the UPR^{mt} and UPR^{er}, respectively.

Our findings revealed that *cco-1*(RNAi);*hsp-6::GFP* worms exhibited no discernible differences in fluorescence profiles between metformin-treated and non-treated groups (Fig 2A–B). When *tomm-22*(RNAi);*hsp-6::GFP* and *tmem-131*(RNAi);*hsp-4::GFP* worms were subjected to 50 mM metformin, a decrease in the UPR^{mt} was observed. In addition, metformin appeared to enhance UPR^{er}, slightly but significantly, in *mdt-15*(RNAi);*hsp-4::GFP* (Fig 2A–B). To enhance the comprehensiveness of our analysis regarding the interaction between metformin and the UPRs, we also tested additional UPR-activated worms. These included *nuo-6* RNAi (UPR^{mt}) and *tag-335* RNAi (UPR^{er}) worms, whose lifespan and stress resistance phenotypes, however, were not carried on as in others. We found that metformin suppressed UPR^{mt} in *nuo-6*(RNAi);*hsp-6::GFP* while increased UPR^{er} in *tag-335*(RNAi);*hsp-4::GFP*. (Fig 2A–B).

These findings indicate that metformin's action is not mitochondria-specific, as it can influence both the UPR^{mt} and UPR^{er} pathways. Furthermore, they imply that UPR^{mt} is dispensable for lifespan extension, as the metformin-induced reduction of UPR^{mt} (Fig 2) did not diminish its lifespan-extending effect in *tomm-22* (Fig 1C). It is then plausible that UPR^{mt} can be inhibited by metformin. Similarly, these results indicate that UPR^{er} is not necessary for the lifespan-extending effect of metformin, as *tmem-131* exhibited extended lifespan upon metformin treatment despite decreasing UPR^{er} (Figs 1F and 2).

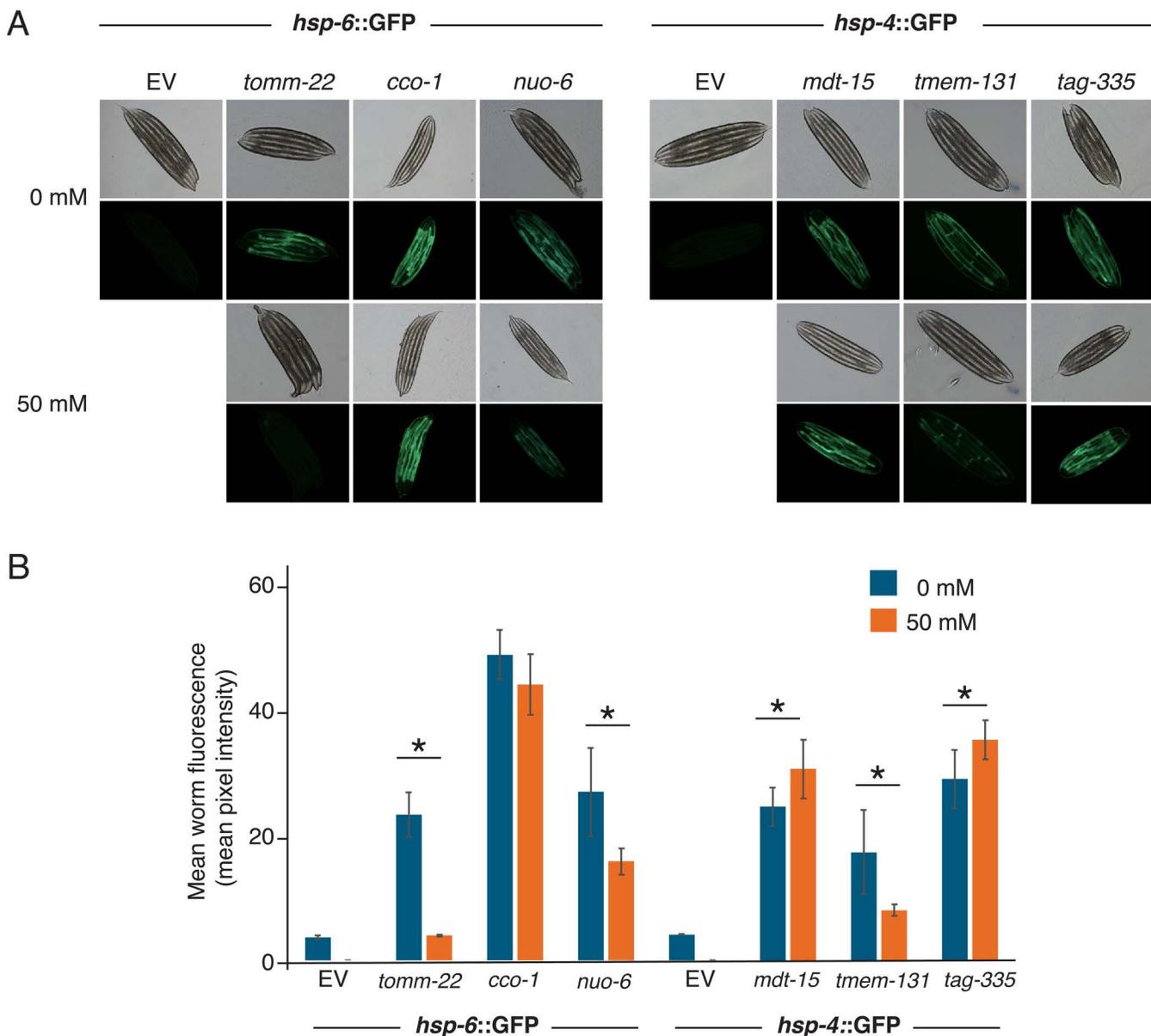


Fig 2. Effect of metformin on UPRs activation. Expression profiles of UPR^{mt} and UPR^{er} were conducted in SJ4100 *zcls13[hsp-6::GFP]* and SJ4005 *zcls4[hsp-4::GFP]* worms, accordingly. The expression of *hsp-6::GFP* in worms fed with EV, *tomm-22*, *cco-1* and *nuo-6* RNAi, and of *hsp-4::GFP* in worms fed with EV, *mdt-15*, *tmem-131* and *tag-335* RNAi is presented as shown (A). The treated nematodes were administered metformin at a concentration of 50 mM. Quantitative analysis of GFP signals is presented in (B). The assay was conducted on adult day 1 worms. (n=5 for each experiment). * denotes p-value < 0.05.

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Metformin enhances thermotolerance in young wild-type adults, but not in UPR-activated nematodes

We showed that metformin extended the lifespan in *tomm-22* worms while simultaneously suppressing the activated UPR^{mt}, a mechanism that mitigates mitochondrial stress. We next investigated the effects of metformin on the resistance to two stressors: heat and oxidative stress.

Young metformin-treated wild-type worms showed moderately but significantly enhanced thermotolerance, a characteristic that diminished from Day 9 of life. Metformin did not alter thermotolerance throughout the entire adult life in both *tomm-22* and *mdt-15* (Fig 3B,C, Table 2). It is noteworthy that the mean thermotolerance of untreated *mdt-15* surpassed 10 hours, comparable to that of 50 mM metformin-treated wild-type worms but lower than that of the untreated ones (Table 2). Therefore, UPR^{er} activation may enhance thermotolerance in worms, but metformin treatment does not further augment this effect.

In summary, metformin treatment resulted in increased thermotolerance only in young wild-type adults. Activation of UPR^{mt} and UPR^{er} by knocking down *tomm-22* and *mdt-15* caused young metformin-treated worms to lose their enhanced thermotolerance. This outcome is somewhat surprising, as the induced UPR would be anticipated to protect the worms against heat stress. Notably, while mean heat stress survival is not statistically different, metformin occasionally induces a slightly stronger shoulder but steeper decline.

Metformin promotes late-life oxidative stress resistance in UPR^{mt}-activated *tomm-22* nematodes, but not in UPR^{er}-activated *mdt-15* nematodes

Wild-type worms given metformin exhibited enhanced oxidative stress resistance in Day-6 worms (Fig 4A, Table 3). In contrast, the UPR^{mt}-activated worms displayed a more intricate response. Although oxidative stress resistance in

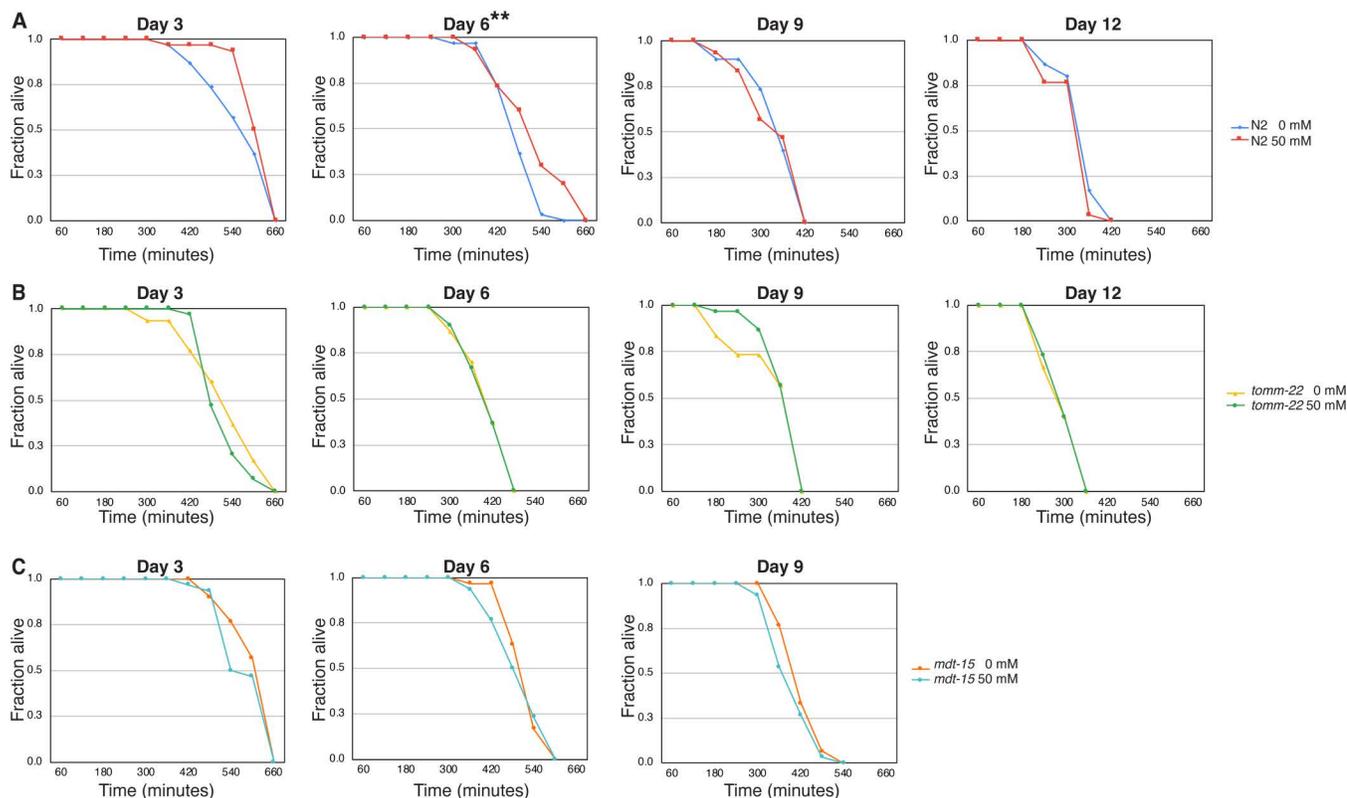


Fig 3. Effect of metformin on thermotolerance in wild-type and UPR-activated *C. elegans*. Thermotolerance assays were performed on N2 wild-type worms fed with EV (A), *tomm-22* RNAi (B), and *mdt-15* RNAi (C). Worms were exposed to metformin for their entire life (0 mM and 50 mM, as indicated). The assay was conducted every 3 days. (n = 30 for each experiment). * denotes p-value < 0.05. Statistical analysis details are presented in Table 2.

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Table 2. Statistical output of overall and mean thermotolerance survival of wild-type and UPR-activated worms.

Worm	Age	Overall Thermotolerance Survival		Mean Thermotolerance Survival		
		p-value	Bonferroni's p-value	0 mM Metformin (min)	50 mM Metformin (min)	p-value (t-test)
N2 fed EV	Day 3	0.0503	0.0503	570 ± 16.43	620 ± 10.71	0.0135*
	Day 6	0.0141	0.0141*	484 ± 10.93	526 ± 16.90	0.0413*
	Day 9	0.9825	0.9825	356 ± 13.25	348 ± 14.25	0.6825
	Day 12	0.1945	0.1945	350 ± 9.42	334 ± 9.67	0.2408
<i>tomm-22</i> (UPR ^{mt})	Day 3	0.3480	0.3480	526 ± 18.04	522 ± 10.06	0.8492
	Day 6	1.0000	1.0000	416 ± 11.29	416 ± 10.93	1.0000
	Day 9	0.0961	0.0961	352 ± 17.14	382 ± 9.99	0.1359
	Day 12	0.8591	0.8591	304 ± 9.35	308 ± 8.82	0.7568
<i>mdt-15</i> (UPR ^{er})	Day 3	0.3707	0.3707	614 ± 11.20	592 ± 12.56	0.1910
	Day 6	0.5981	0.5981	524 ± 9.35	506 ± 13.17	0.2697
	Day 9	0.0961	0.0961	430 ± 9.42	406 ± 10.84	0.1001

Log Rank analysis was used to calculate statistical significance of overall thermotolerance survival, comparing survival rates between 0 mM and 50 mM metformin.

*denotes statistical significance where Bonferroni's p value or Student *t*-test p-value < 0.05.

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metformin-treated *tomm-22* worms was decreased in young Day-3 worms, it was significantly increased during late life at Days 9 and 12 (Fig 4B, and Table 3).

It is noteworthy that untreated Day-3 *tomm-22* worms survived the oxidative stress for 610 minutes while the corresponding wildtypes survived only for 522 mins (Table 3). This increased oxidative stress resistance in *tomm-22* worms declined with age (Table 3). Furthermore, the enhanced oxidative stress resistance observed at early age was obliterated by metformin. The mean oxidative stress resistance of Day-3 *tomm-22* worms decreased from 610 minutes (untreated) to 536 minutes (50 mM metformin), which is close to the untreated Day 3 wildtypes (522 minutes) (Table 3). The potential reversal of this effect in older worms may be attributed to the extended healthspan effect of metformin in *tomm-22* worms.

The activation of UPR^{er} by *mdt-15* RNAi resulted in a slight increase in oxidative stress resistance in young to middle-aged worms compared to the wildtype (Fig 4C, and Table 3). Conversely, metformin caused a slight but significant reduction in mean survival time in oxidative stress resistance in Day-6 worms (Table 3).

UPR activation, but not metformin, has a beneficial effect on locomotor phenotypes in the nematodes

Next, we investigated whether metformin could exhibit a beneficial effect on locomotion of the worms by measuring body trashing and crawling velocity in N2 wild-type and UPR-activated nematodes. Without metformin, UPR^{mt}-activated nematode *tomm-22* exhibited significantly higher locomotory rates (both trashing and crawling velocity) compared to N2 wild-type worms (Fig 5A and 5E) from Day 6 to late life. Nematodes with UPR^{er} activated by *mdt-15* exhibited significantly lower trashing rates in D3 but higher trashing rates in D6, compared to N2. When metformin was supplemented, N2 wild-type worms showed significantly slower trashing rates in older worms (Fig 5B) while crawling velocity remained unchanged (Fig 5F). In metformin-treated UPR-activated worms, neither *tomm-22* nor *mdt-15* exhibited any alteration in trashing or crawling velocity for the most extensively tested lifetime, with the exception of *mdt-15* at D9, which displayed a significantly elevated trashing rate (Fig 5C–D and 5G–H).

Discussion

For several decades, mitochondria have been considered the primary driver of the aging process [42] and the UPR^{mt} has been associated with increased longevity in several model organisms [43–47]. This prompted us to

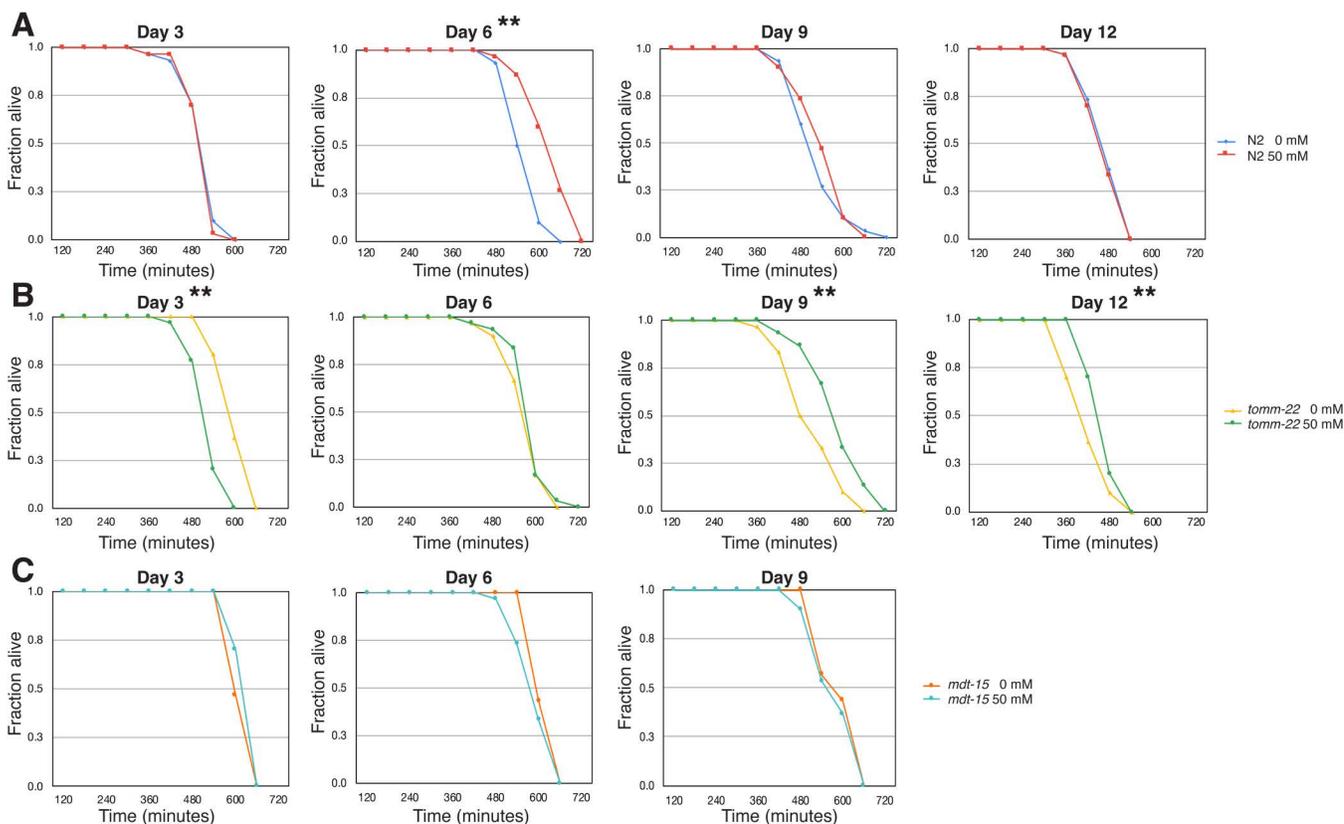


Fig 4. Effect of metformin on oxidative stress resistance in wild-type and UPR-activated *C. elegans*. Oxidative stress assays were performed on N2 wild-type worms fed with EV (A), *tomm-22* RNAi (B), and *mdt-15* RNAi (C). Worms were treated with metformin for their entire life (0 mM and 50 mM, as indicated). The assay was conducted every 3 days. (n = 30 for each experiment). ** denotes p-value < 0.01. Statistical analysis details are presented in Table 3.

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investigate whether UPR^{mt}, a biological response that specifically designed to counteract mitochondrial stress, can be modulated by metformin, a compound that is known to interact with mitochondrial pathways and exhibits anti-aging properties.

In this study, we demonstrated that metformin extends the lifespan of wild-type worms, aligning with previous studies findings [6–8,13]. The use of 50 mM metformin for nematodes appears to be sufficient to induce the longevity phenotype. However, a higher dosage may be necessary for polar drugs, as the protective cuticle of the nematode can restrict their uptake. Nevertheless, metformin has also been shown to reduce lifespan in some circumstances, primarily due to overstimulation of the phenotype or drug toxicity [48,49]. We demonstrated that the pro-longevity effect of metformin is maintained in both *tomm-22* (membrane transport associated-UPR^{mt}) and *cco-1* (electron transport associated-UPR^{mt}). Furthermore, by investigating *ubl-5;tomm-22* double RNAi knockdown, we found that the lifespan extending effect by metformin was not lost but further synergized to extend lifespan. Interestingly, we also demonstrated that metformin extends the lifespan of *tmem-131* (protein sorting associated-UPR^{er}) while decreasing UPR^{er}. Conversely, metformin decreased the lifespan of *mdt-15* (modulator associated-UPR^{er}) while increasing UPR^{er}. These findings suggest that metformin's beneficial effect on worm lifespan is independent of UPR^{mt} or UPR^{er} activation. We propose that metformin may have some underlying interplay between various components in the unfolded stress response systems and other pathway, secondary to the target gene knockdown.

Table 3. Statistical output of overall and mean oxidative stress resistance of wild-type and UPR-activated worms.

Worm	Age	Overall Oxidative Stress Resistance		Mean Oxidative Stress Resistance		
		p-value	Bonferroni's p-value	0 mM Metformin (min)	50 mM Metformin (min)	p-value (t-test)
N2 fed EV	Day 3	0.6816	0.6816	522±9.01	520±7.66	0.8663
	Day 6	9.8E-06	9.8E-06**	512±8.36	582±11.71	0.0001**
	Day 9	0.3801	0.3801	536±12.63	552±12.46	0.3709
	Day 12	0.7590	0.7590	484±9.35	480±9.38	0.7637
<i>tomm-22</i> (UPR ^{mt})	Day 3	2.0E-07	2.0E-07**	610±8.04	536±7.97	0.0001**
	Day 6	0.2802	0.2802	582±10.26	596±9.77	0.3272
	Day 9	0.0014	0.0014**	524±14.40	596±14.68	0.0009**
	Day 12	0.0031	0.0031**	428±10.09	474±7.67	0.0006**
<i>mdt-15</i> (UPR ^{er})	Day 3	0.0691	0.0691	568±5.47	582±5.02	0.0643
	Day 6	0.0904	0.0904	626±5.43	602±9.16	0.0280*
	Day 9	0.4681	0.4681	540±10.20	528±11.45	0.4371

Log Rank analysis was used to calculate statistical significance of overall oxidative stress resistance comparing 0 mM and 50 mM metformin. Statistical significance was denoted by asterisks

(*) and double asterisks

(**) when Bonferroni's p-value or Student t-test p-value < 0.05 and < 0.01, respectively.

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We had initially expected that metformin may be beneficial for short-lived phenotypes by exerting its lifespan-extending effect through UPR^{mt}. Nonetheless, we demonstrated that metformin did not activate UPR^{mt}, consistent with previous studies (8, 51). In addition, we presented evidence suggesting that metformin was able to significantly reduce UPR^{mt} activation in *tomm-22* and *nuo-6* worms, but not in *cco-1*. This might be attributed to the negative effect of *tomm-22* RNAi on mitochondrial import as recently demonstrated by Xin, *et al.* [22]. Upon activation of UPR^{mt} in *tomm-22*, mitochondrial import was also affected, potentially leading to degraded stress-responsive signals emanating from the nucleus. Conversely, mitochondrial import was found to be enhanced in *cco-1*, which can attenuate stress response. Therefore, we hypothesize that UPR^{mt} suppression by metformin can outcompete the stress-responsive signals from the nucleus to restore proteostasis in *tomm-22* but not in *cco-1*. Interactions between metformin and other UPR^{mt} effectors such as HSP-6 or ATFS-1 maybe unlikely, as metformin has no inhibitory effect on the *cco-1*-induced UPR^{mt}. In addition, the primary role of *tomm-22* is a mitochondrial outer membrane transporter rather than a mitochondrial UPR stress regulator. The activation of *hsp-6* response in *tomm-22* RNAi worms in our study was likely a response to changes in the mitochondrial import machinery instead of a direct regulatory response to mitochondrial stress.

In a previous study conducted by Espada *et al.* [41] the role of *ubl-5*, *atfs-1*, and *isp-1* in metformin-mediated longevity was investigated, with a primary focus on mitochondrial stress-signalling and the UPR^{mt}. The findings indicated that knocking down *atfs-1* and *isp-1* resulted in a shortened lifespan of the nematodes. Notably, when metformin was administered, the lifespan was further shortened and exacerbated metformin toxicity. However, *ubl-5* did not have a significant effect and thus was reported to be required for lifespan extension. Furthermore, the study by Benedetti *et al.* (25) showed that *ubl-5* knockdown did not induce *hsp-6* and *hsp-60* in response to heat stress, suggesting that *ubl-5* is required for the initiation of UPR^{mt} and subsequent activation of chaperones.

Notably, our finding demonstrated that UPR^{er} can also be influenced by metformin through both inhibition and enhancement mechanisms. These findings indicate that the impact of metformin on the UPR extends beyond its effects on mitochondria. Furthermore, the effects of metformin on UPR systems may be the result of the interplay between the knockdown of target genes and their subsequent responses.

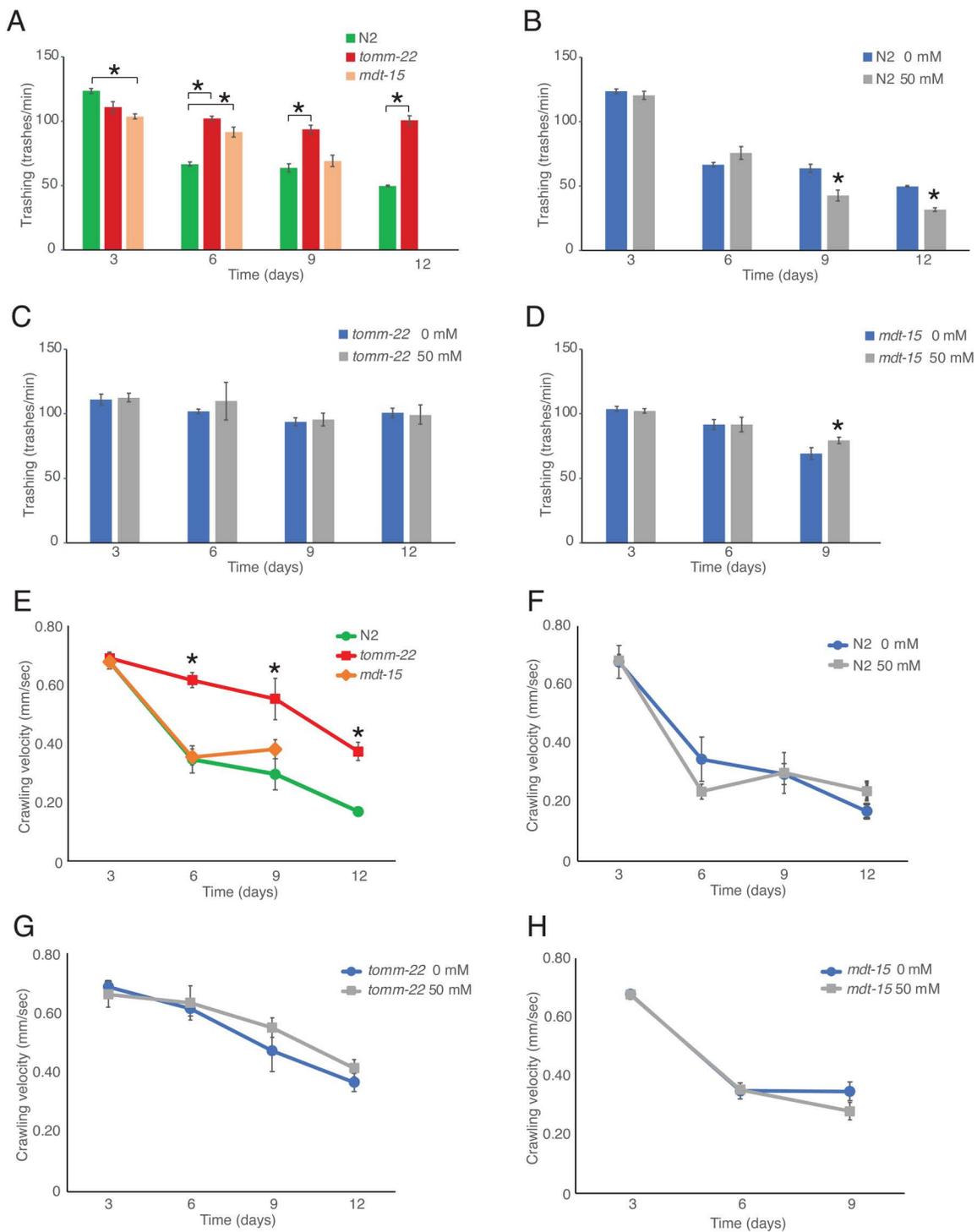


Fig 5. Effect of metformin on locomotion in wild-type and UPR-activated *C. elegans*. Body bending (A-D) and movement velocity (E-H) assays were performed on N2 wild-type worms fed with EV, *tomm-22* RNAi, and *mdt-15* RNAi. Worms were treated with metformin for their entire life (0 mM and 50 mM, as indicated). The assay was conducted every 3 days. Each data point represents 3 independent cultures (n = 10-20). * denotes $p < 0.05$.

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UPRs regulate the cellular protection against various stresses, including heat and oxidative stress [50–53]. Given that many UPR proteins are heat shock proteins, the association between UPR activation and heat stress resistance is readily apparent [54]. Oxidative stress, mediated by free radicals, can disrupt protein folding, which may be counteracted by UPR responses [55]. Contrary to our expectations, the UPR^{mt}-activated *tomm-22* worms that gained lifespan extension by metformin did not necessarily acquire thermotolerance, while the beneficial effect of UPR^{mt} activation against oxidative stress was neutralized upon metformin treatment in young adults. This effect was clear especially in wild-type worms. While young to middle-aged wild-type worms exhibited a modest increase in stress resistance upon metformin treatment, this effect rapidly diminished as they aged. In contrast, activation of the UPR^{er} in *mdt-15* knockdown worms appeared to enhance thermotolerance. However, unlike wild-type worms, metformin did not further alleviate this phenotype. The oxidative stress resistance of *mdt-15* knockdown worms remained unaffected by metformin treatment, although UPR^{er} was enhanced.

Mechanistically, metformin may affect cellular oxidative stress in various ways. On the one hand, metformin induces endogenous hydrogen peroxide in the worm, rendering peroxiredoxin mutants highly susceptible to metformin [8]. Conversely, metformin stimulates the oxidative stress response by upregulating nuclear accumulation of SKN-1 [6]. These multifaceted effects of metformin on cellular oxidative stress systems could complicate the longevity and stress resistance phenotypes, particularly in UPR-activated backgrounds. In the UPR^{er}-activated *mdt-15* worms, metformin treatment appears to induce a slight reduction in lifespan, which is concurrent with a corresponding reduction in oxidative stress resistance. However, the effect size is very small and these phenotypes may not be causally related. This is further supported by the absence of a consistent correlation between stress resistance and lifespan phenotypes in wild-type and *tomm-22* worms. Metformin extends lifespan in both wild-type and certain UPR-activated worms (*tomm-22* and *tmem-131*), but increases thermotolerance only in young wild types. Furthermore, its impact on oxidative stress resistance exhibits considerable variability and complexity over time.

We also investigated locomotory parameters including trashing and crawling velocity, as locomotion can reflect health conditions related to neuromuscular activity [56]. Activation of both UPR systems exerts a beneficial effect on locomotion. This effect was particularly striking in *tomm-22* knockdowns, as evidenced by significantly increased trashing and crawling throughout the entire tested lifespan. We hypothesized that this might result from an increase in chaperonic activity of heat shock proteins by activation of UPR^{mt}, which mitigates proteotoxicity that generally increases over life span. However, other mechanisms secondarily to UPR^{mt} activation may also play a role in this effect. Given the short lifespan of *tomm-22*, this suggests that lifespan extension does not necessarily translate into improved health conditions, as evidenced by the healthspan metrics. Our study contributes to this observation, complementing the findings of Bansal *et al.* [57]. Notably, metformin did not appear to improve locomotion in old-aged worms. This contradicts our expectation, as it has been demonstrated that metformin can prevent sarcopenia and enhance physical performance in human subjects [58–60].

Conclusion

Metformin extends lifespan of wild-type *C. elegans*, although its effects vary among UPR^{mt}-activated and UPR^{er}-activated worms. While metformin exerts diverse effects on the UPR systems, it can reduce UPR^{mt} and UPR^{er} in certain UPR-activated strains and temporarily decreases oxidative stress resistance in young worms. In some UPR, locomotor phenotypes exhibit improvement, whereas metformin treatment does not induce such changes. Therefore, metformin exhibits diverse effects on UPR, lifespan and stress resistance, contingent upon the mitochondrial and ER status of the worms.

Supporting information

S1 Fig. Inactivation of UPR^{mt} by *atfs-1* and *ubl-1* contributes to longevity observed in metformin-treated *tomm-22*. (PDF)

S1 File. Raw data for each experiment.

(ZIP)

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References

1. Bailey CJ. Metformin: historical overview. *Diabetologia*. 2017;60(9):1566–76. <https://doi.org/10.1007/s00125-017-4318-z> PMID: [28776081](https://pubmed.ncbi.nlm.nih.gov/28776081/)
2. Campbell JM, Stephenson MD, de Courten B, Chapman I, Bellman SM, Aromataris E. Metformin use associated with reduced risk of dementia in patients with diabetes: a systematic review and meta-analysis. *J Alzheimers Dis*. 2018;65(4):1225–36. <https://doi.org/10.3233/JAD-180263> PMID: [30149446](https://pubmed.ncbi.nlm.nih.gov/30149446/)
3. Griffin SJ, Leaver JK, Irving GJ. Impact of metformin on cardiovascular disease: a meta-analysis of randomised trials among people with type 2 diabetes. *Diabetologia*. 2017;60(9):1620–9. <https://doi.org/10.1007/s00125-017-4337-9> PMID: [28770324](https://pubmed.ncbi.nlm.nih.gov/28770324/)
4. Kim HJ, Lee S, Chun KH, Jeon JY, Han SJ, Kim DJ, et al. Metformin reduces the risk of cancer in patients with type 2 diabetes: an analysis based on the Korean National Diabetes Program Cohort. *Medicine (Baltimore)*. 2018;97(8):e0036. <https://doi.org/10.1097/MD.000000000010036> PMID: [29465545](https://pubmed.ncbi.nlm.nih.gov/29465545/)
5. Martin-Montalvo A, Mercken EM, Mitchell SJ, Palacios HH, Mote PL, Scheibye-Knudsen M, et al. Metformin improves healthspan and lifespan in mice. *Nat Commun*. 2013;4:2192. <https://doi.org/10.1038/ncomms3192> PMID: [23900241](https://pubmed.ncbi.nlm.nih.gov/23900241/)
6. Onken B, Driscoll M. Metformin induces a dietary restriction-like state and the oxidative stress response to extend *C. elegans* Healthspan via AMPK, LKB1, and SKN-1. *PLoS One*. 2010;5(1):e8758. <https://doi.org/10.1371/journal.pone.0008758> PMID: [20090912](https://pubmed.ncbi.nlm.nih.gov/20090912/)
7. Chen J, Ou Y, Li Y, Hu S, Shao L-W, Liu Y. Metformin extends *C. elegans* lifespan through lysosomal pathway. *Elife*. 2017;6:e31268. <https://doi.org/10.7554/eLife.31268> PMID: [29027899](https://pubmed.ncbi.nlm.nih.gov/29027899/)
8. De Haes W, Froninckx L, Van Assche R, Smolders A, Depuydt G, Billen J, et al. Metformin promotes lifespan through mitohormesis via the peroxiredoxin PRDX-2. *Proc Natl Acad Sci U S A*. 2014;111(24):E2501–9. <https://doi.org/10.1073/pnas.1321776111> PMID: [24889636](https://pubmed.ncbi.nlm.nih.gov/24889636/)
9. Song J, Jiang G, Zhang J, Guo J, Li Z, Hao K, et al. Metformin prolongs lifespan through remodeling the energy distribution strategy in silkworm, *Bombyx mori*. *Aging (Albany NY)*. 2019;11(1):240–8. <https://doi.org/10.18632/aging.101746> PMID: [30636724](https://pubmed.ncbi.nlm.nih.gov/30636724/)
10. Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochem J*. 2000;348 Pt 3(Pt 3):607–14. <https://doi.org/10.1042/bj3480607> PMID: [10839993](https://pubmed.ncbi.nlm.nih.gov/10839993/)
11. Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. *Clin Sci (Lond)*. 2012;122(6):253–70. <https://doi.org/10.1042/CS20110386> PMID: [22117616](https://pubmed.ncbi.nlm.nih.gov/22117616/)
12. Miller RA, Chu Q, Xie J, Foretz M, Viollet B, Birnbaum MJ. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. *Nature*. 2013;494(7436):256–60. <https://doi.org/10.1038/nature11808> PMID: [23292513](https://pubmed.ncbi.nlm.nih.gov/23292513/)
13. Cabreiro F, Au C, Leung K-Y, Vergara-Irigaray N, Cochemé HM, Noori T, et al. Metformin retards aging in *C. elegans* by altering microbial folate and methionine metabolism. *Cell*. 2013;153(1):228–39. <https://doi.org/10.1016/j.cell.2013.02.035> PMID: [23540700](https://pubmed.ncbi.nlm.nih.gov/23540700/)
14. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest*. 2001;108(8):1167–74. <https://doi.org/10.1172/JCI13505> PMID: [11602624](https://pubmed.ncbi.nlm.nih.gov/11602624/)

15. Ouyang J, Parakhia RA, Ochs RS. Metformin activates AMP kinase through inhibition of AMP deaminase. *J Biol Chem*. 2011;286(1):1–11. <https://doi.org/10.1074/jbc.M110.121806> PMID: [21059655](https://pubmed.ncbi.nlm.nih.gov/21059655/)
16. Stephenne X, Foretz M, Taleux N, van der Zon GC, Sokal E, Hue L, et al. Metformin activates AMP-activated protein kinase in primary human hepatocytes by decreasing cellular energy status. *Diabetologia*. 2011;54(12):3101–10. <https://doi.org/10.1007/s00125-011-2311-5> PMID: [21947382](https://pubmed.ncbi.nlm.nih.gov/21947382/)
17. Bharath LP, Agrawal M, McCambridge G, Nicholas DA, Hasturk H, Liu J, et al. Metformin enhances autophagy and normalizes mitochondrial function to alleviate aging-associated inflammation. *Cell Metab*. 2020;32(1):44–55.e6. <https://doi.org/10.1016/j.cmet.2020.04.015> PMID: [32402267](https://pubmed.ncbi.nlm.nih.gov/32402267/)
18. Bridges HR, Jones AJY, Pollak MN, Hirst J. Effects of metformin and other biguanides on oxidative phosphorylation in mitochondria. *Biochem J*. 2014;462(3):475–87. <https://doi.org/10.1042/BJ20140620> PMID: [25017630](https://pubmed.ncbi.nlm.nih.gov/25017630/)
19. Wheaton WW, Weinberg SE, Hamanaka RB, Soberanes S, Sullivan LB, Anso E, et al. Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis. *Elife*. 2014;3:e02242. <https://doi.org/10.7554/eLife.02242> PMID: [24843020](https://pubmed.ncbi.nlm.nih.gov/24843020/)
20. Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong JK, et al. Clinical pharmacokinetics of metformin. *Clin Pharmacokinet*. 2011;50(2):81–98. <https://doi.org/10.2165/11534750-000000000-00000> PMID: [21241070](https://pubmed.ncbi.nlm.nih.gov/21241070/)
21. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. *Diabetologia*. 2017;60(9):1577–85. <https://doi.org/10.1007/s00125-017-4342-z> PMID: [28776086](https://pubmed.ncbi.nlm.nih.gov/28776086/)
22. Xin N, Durieux J, Yang C, Wolff S, Kim H-E, Dillin A. The UPRmt preserves mitochondrial import to extend lifespan. *J Cell Biol*. 2022;221(7):e202201071. <https://doi.org/10.1083/jcb.202201071> PMID: [35608535](https://pubmed.ncbi.nlm.nih.gov/35608535/)
23. Lionaki E, Gkikas I, Daskalaki I, Ioannidi M-K, Klapa MI, Tavernarakis N. Mitochondrial protein import determines lifespan through metabolic reprogramming and de novo serine biosynthesis. *Nat Commun*. 2022;13(1):651. <https://doi.org/10.1038/s41467-022-28272-1> PMID: [35115503](https://pubmed.ncbi.nlm.nih.gov/35115503/)
24. Durieux J, Wolff S, Dillin A. The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell*. 2011;144(1):79–91. <https://doi.org/10.1016/j.cell.2010.12.016> PMID: [21215371](https://pubmed.ncbi.nlm.nih.gov/21215371/)
25. Benedetti C, Haynes CM, Yang Y, Harding HP, Ron D. Ubiquitin-like protein 5 positively regulates chaperone gene expression in the mitochondrial unfolded protein response. *Genetics*. 2006;174(1):229–39. <https://doi.org/10.1534/genetics.106.061580> PMID: [16816413](https://pubmed.ncbi.nlm.nih.gov/16816413/)
26. Shpilka T, Du Y, Yang Q, Melber A, Uma Naresh N, Lavelle J, et al. UPRmt scales mitochondrial network expansion with protein synthesis via mitochondrial import in *Caenorhabditis elegans*. *Nat Commun*. 2021;12(1):479. <https://doi.org/10.1038/s41467-020-20784-y> PMID: [33473112](https://pubmed.ncbi.nlm.nih.gov/33473112/)
27. Melber A, Haynes CM. UPRmt regulation and output: a stress response mediated by mitochondrial-nuclear communication. *Cell Res*. 2018;28(3):281–95. <https://doi.org/10.1038/cr.2018.16> PMID: [29424373](https://pubmed.ncbi.nlm.nih.gov/29424373/)
28. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol*. 2007;8(7):519–29. <https://doi.org/10.1038/nrm2199> PMID: [17565364](https://pubmed.ncbi.nlm.nih.gov/17565364/)
29. Shen X, Ellis RE, Lee K, Liu CY, Yang K, Solomon A, et al. Complementary signaling pathways regulate the unfolded protein response and are required for *C. elegans* development. *Cell*. 2001;107(7):893–903. [https://doi.org/10.1016/s0092-8674\(01\)00612-2](https://doi.org/10.1016/s0092-8674(01)00612-2) PMID: [11779465](https://pubmed.ncbi.nlm.nih.gov/11779465/)
30. Richardson CE, Kinkel S, Kim DH. Physiological IRE-1-XBP-1 and PEK-1 signaling in *Caenorhabditis elegans* larval development and immunity. *PLoS Genet*. 2011;7(11):e1002391. <https://doi.org/10.1371/journal.pgen.1002391> PMID: [22125500](https://pubmed.ncbi.nlm.nih.gov/22125500/)
31. Houtkooper RH, Mouchiroud L, Ryu D, Moullan N, Katsyuba E, Knott G, et al. Mitonuclear protein imbalance as a conserved longevity mechanism. *Nature*. 2013;497(7450):451–7. <https://doi.org/10.1038/nature12188> PMID: [23698443](https://pubmed.ncbi.nlm.nih.gov/23698443/)
32. Bennett CF, Vander Wende H, Simko M, Klum S, Barfield S, Choi H, et al. Activation of the mitochondrial unfolded protein response does not predict longevity in *Caenorhabditis elegans*. *Nat Commun*. 2014;5:3483. <https://doi.org/10.1038/ncomms4483> PMID: [24662282](https://pubmed.ncbi.nlm.nih.gov/24662282/)
33. Hou NS, Gutschmidt A, Choi DY, Pather K, Shi X, Watts JL, et al. Activation of the endoplasmic reticulum unfolded protein response by lipid disequilibrium without disturbed proteostasis in vivo. *Proc Natl Acad Sci U S A*. 2014;111(22):E2271–80. <https://doi.org/10.1073/pnas.1318262111> PMID: [24843123](https://pubmed.ncbi.nlm.nih.gov/24843123/)
34. Zhang Z, Bai M, Barbosa GO, Chen A, Wei Y, Luo S, et al. Broadly conserved roles of TMEM131 family proteins in intracellular collagen assembly and secretory cargo trafficking. *Sci Adv*. 2020;6(7):eaay7667. <https://doi.org/10.1126/sciadv.aay7667> PMID: [32095531](https://pubmed.ncbi.nlm.nih.gov/32095531/)
35. Goh GYS, Martelli KL, Parhar KS, Kwong AWL, Wong MA, Mah A, et al. The conserved mediator subunit MDT-15 is required for oxidative stress responses in *Caenorhabditis elegans*. *Aging Cell*. 2014;13(1):70–9. <https://doi.org/10.1111/acer.12154> PMID: [23957350](https://pubmed.ncbi.nlm.nih.gov/23957350/)
36. Taubert S, Van Gilst MR, Hansen M, Yamamoto KR. A mediator subunit, MDT-15, integrates regulation of fatty acid metabolism by NHR-49-dependent and -independent pathways in *C. elegans*. *Genes Dev*. 2006;20(9):1137–49. <https://doi.org/10.1101/gad.1395406> PMID: [16651656](https://pubmed.ncbi.nlm.nih.gov/16651656/)
37. Taubert S, Hansen M, Van Gilst MR, Cooper SB, Yamamoto KR. The mediator subunit MDT-15 confers metabolic adaptation to ingested material. *PLoS Genet*. 2008;4(2):e1000021. <https://doi.org/10.1371/journal.pgen.1000021> PMID: [18454197](https://pubmed.ncbi.nlm.nih.gov/18454197/)
38. Brenner S. The genetics of *Caenorhabditis elegans*. *Genetics*. 1974;77(1):71–94. <https://doi.org/10.1093/genetics/77.1.71> PMID: [4366476](https://pubmed.ncbi.nlm.nih.gov/4366476/)
39. Kamath RS, Martinez-Campos M, Zipperlen P, Fraser AG, Ahringer J. Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegans*. *Genome Biol*. 2001;2(1). <https://doi.org/10.1186/gb-2000-2-1-research0002> PMID: [11178279](https://pubmed.ncbi.nlm.nih.gov/11178279/)
40. Yang J-S, Nam H-J, Seo M, Han SK, Choi Y, Nam HG, et al. OASIS: online application for the survival analysis of lifespan assays performed in aging research. *PLoS One*. 2011;6(8):e23525. <https://doi.org/10.1371/journal.pone.0023525> PMID: [21858155](https://pubmed.ncbi.nlm.nih.gov/21858155/)
41. Espada L, Dakhovnik A, Chaudhari P, Martirosyan A, Miek L, Poliezhaieva T, et al. Loss of metabolic plasticity underlies metformin toxicity in aged *Caenorhabditis elegans*. *Nat Metab*. 2020;2(11):1316–31. <https://doi.org/10.1038/s42255-020-00307-1> PMID: [33139960](https://pubmed.ncbi.nlm.nih.gov/33139960/)

42. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol.* 1956;11(3):298–300. <https://doi.org/10.1093/geronj/11.3.298> PMID: [13332224](https://pubmed.ncbi.nlm.nih.gov/13332224/)
43. Chadwick SR, Fazio EN, Etedali-Zadeh P, Genereaux J, Duennwald ML, Lajoie P. A functional unfolded protein response is required for chronological aging in *Saccharomyces cerevisiae*. *Curr Genet.* 2020;66(1):263–77. <https://doi.org/10.1007/s00294-019-01019-0> PMID: [31346745](https://pubmed.ncbi.nlm.nih.gov/31346745/)
44. Matai L, Sarkar GC, Chamoli M, Malik Y, Kumar SS, Rautela U, et al. Dietary restriction improves proteostasis and increases life span through endoplasmic reticulum hormesis. *Proc Natl Acad Sci U S A.* 2019;116(35):17383–92. <https://doi.org/10.1073/pnas.1900055116> PMID: [31413197](https://pubmed.ncbi.nlm.nih.gov/31413197/)
45. Wu Z, Senchuk MM, Dues DJ, Johnson BK, Cooper JF, Lew L, et al. Mitochondrial unfolded protein response transcription factor ATFS-1 promotes longevity in a long-lived mitochondrial mutant through activation of stress response pathways. *BMC Biol.* 2018;16(1):147. <https://doi.org/10.1186/s12915-018-0615-3> PMID: [30563508](https://pubmed.ncbi.nlm.nih.gov/30563508/)
46. Copeland JM, Cho J, Lo T Jr, Hur JH, Bahadorani S, Arabyan T, et al. Extension of *Drosophila* life span by RNAi of the mitochondrial respiratory chain. *Curr Biol.* 2009;19(19):1591–8. <https://doi.org/10.1016/j.cub.2009.08.016> PMID: [19747824](https://pubmed.ncbi.nlm.nih.gov/19747824/)
47. Lapointe J, Stepanyan Z, Bigras E, Hekimi S. Reversal of the mitochondrial phenotype and slow development of oxidative biomarkers of aging in long-lived *Mclk1*^{-/-} mice. *J Biol Chem.* 2009;284(30):20364–74. <https://doi.org/10.1074/jbc.M109.006569> PMID: [19478076](https://pubmed.ncbi.nlm.nih.gov/19478076/)
48. Slack C, Foley A, Partridge L. Activation of AMPK by the putative dietary restriction mimetic metformin is insufficient to extend lifespan in *Drosophila*. *PLoS One.* 2012;7(10):e47699. <https://doi.org/10.1371/journal.pone.0047699> PMID: [23077661](https://pubmed.ncbi.nlm.nih.gov/23077661/)
49. Kim J, Lee H-Y, Ahn J, Hyun M, Lee I, Min K-J, et al. NHX-5, an endosomal Na⁺/H⁺ exchanger, is associated with metformin action. *J Biol Chem.* 2016;291(35):18591–9. <https://doi.org/10.1074/jbc.C116.744037> PMID: [27435670](https://pubmed.ncbi.nlm.nih.gov/27435670/)
50. Wang S, Kaufman RJ. The impact of the unfolded protein response on human disease. *J Cell Biol.* 2012;197(7):857–67. <https://doi.org/10.1083/jcb.201110131> PMID: [22733998](https://pubmed.ncbi.nlm.nih.gov/22733998/)
51. Hetz C, Zhang K, Kaufman RJ. Mechanisms, regulation and functions of the unfolded protein response. *Nat Rev Mol Cell Biol.* 2020;21(8):421–38. <https://doi.org/10.1038/s41580-020-0250-z> PMID: [32457508](https://pubmed.ncbi.nlm.nih.gov/32457508/)
52. Hetz C, Chevet E, Oakes SA. Proteostasis control by the unfolded protein response. *Nat Cell Biol.* 2015;17(7):829–38. <https://doi.org/10.1038/ncb3184> PMID: [26123108](https://pubmed.ncbi.nlm.nih.gov/26123108/)
53. Lionaki E, Tavernarakis N. Oxidative stress and mitochondrial protein quality control in aging. *J Proteomics.* 2013;92:181–94. <https://doi.org/10.1016/j.jprot.2013.03.022> PMID: [23563202](https://pubmed.ncbi.nlm.nih.gov/23563202/)
54. Pallepati P, Averill-Bates DA. Activation of ER stress and apoptosis by hydrogen peroxide in HeLa cells: protective role of mild heat preconditioning at 40°C. *Biochim Biophys Acta.* 2011;1813(12):1987–99. <https://doi.org/10.1016/j.bbamcr.2011.07.021> PMID: [21875624](https://pubmed.ncbi.nlm.nih.gov/21875624/)
55. Hotamisligil GS, Davis RJ. Cell signaling and stress responses. *Cold Spring Harb Perspect Biol.* 2016;8(10):a006072. <https://doi.org/10.1101/csh-perspect.a006072> PMID: [27698029](https://pubmed.ncbi.nlm.nih.gov/27698029/)
56. Pierce-Shimomura JT, Chen BL, Mun JJ, Ho R, Sarkis R, McIntire SL. Genetic analysis of crawling and swimming locomotory patterns in *C. elegans*. *Proc Natl Acad Sci U S A.* 2008;105(52):20982–7. <https://doi.org/10.1073/pnas.0810359105> PMID: [19074276](https://pubmed.ncbi.nlm.nih.gov/19074276/)
57. Bansal A, Zhu LJ, Yen K, Tissenbaum HA. Uncoupling lifespan and healthspan in *Caenorhabditis elegans* longevity mutants. *Proc Natl Acad Sci U S A.* 2015;112(3):E277–86. <https://doi.org/10.1073/pnas.1412192112> PMID: [25561524](https://pubmed.ncbi.nlm.nih.gov/25561524/)
58. Rennie KJ, Witham M, Bradley P, Clegg A, Connolly S, Hancock HC, et al. MET-PREVENT: metformin to improve physical performance in older people with sarcopenia and physical prefrailty/frailty - protocol for a double-blind, randomised controlled proof-of-concept trial. *BMJ Open.* 2022;12(7):e061823. <https://doi.org/10.1136/bmjopen-2022-061823> PMID: [35851031](https://pubmed.ncbi.nlm.nih.gov/35851031/)
59. Qaisar R, Karim A, Muhammad T, Iqbal MS, Ahmad F. Metformin improves sarcopenia-related quality of life in geriatric adults: a randomized controlled trial. *Arch Med Res.* 2024;55(4):102998. <https://doi.org/10.1016/j.arcmed.2024.102998> PMID: [38615625](https://pubmed.ncbi.nlm.nih.gov/38615625/)
60. Petrocelli JJ, McKenzie AI, de Hart NMMP, Reidy PT, Mahmassani ZS, Keeble AR, et al. Disuse-induced muscle fibrosis, cellular senescence, and senescence-associated secretory phenotype in older adults are alleviated during re-ambulation with metformin pre-treatment. *Aging Cell.* 2023;22(11):e13936. <https://doi.org/10.1111/accel.13936> PMID: [37486024](https://pubmed.ncbi.nlm.nih.gov/37486024/)