Impacts of metformin and aspirin on life history features and longevity of crickets: trade-offs versus cost-free life extension?

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Abstract We examined the impacts of aspirin and metformin on the life history of the cricket *Acheta domesticus* (growth rate, maturation time, mature body size, survivorship, and maximal longevity). Both drugs significantly increased survivorship and maximal life span. Maximal longevity was 136 days for controls, 188 days (138 % of controls) for metformin, and 194 days (143 % of controls) for aspirin. Metformin and aspirin in combination extended longevity to a lesser degree (163 days, 120 % of controls). Increases in general survivorship were even more pronounced, with low-dose aspirin yielding mean longevity 234 % of controls (i.e., health span). Metformin strongly reduced growth rates of both genders (<60 % of controls), whereas aspirin only slightly reduced the growth rate of females and slightly increased that of males. Both drugs delayed maturation age relative to controls, but metformin had a much greater impact (>140 % of controls) than aspirin (~118 % of controls). Crickets maturing on low aspirin showed no evidence of a trade-off between maturation mass and life extension. Remarkably, by 100 days of age, aspirin-treated females were significantly larger than controls (largely reflecting egg complement). Unlike the reigning dietary restriction paradigm, low aspirin conformed to a paradigm of “eat

more, live longer.” In contrast, metformin-treated females were only ~67 % of the mass of controls. Our results suggest that hormetic agents like metformin may derive significant trade-offs with life extension, whereas health and longevity benefits may be obtained with less cost by agents like aspirin that *regulate* geroprotective pathways.

Keywords Metformin · Aspirin · *Acheta domesticus* · Life extension · Growth · Trade-offs

Introduction

Dietary restriction obtains life extension across broad phylogenies, but substantial gains require reductions in calories of 25 to 40 % that are impractical for humans. Consequently, attention has turned to identifying agents that mimic dietary restriction to obtain benefits without drastic dieting (Ingram et al. 2006). We developed the cricket *Acheta domesticus* as a model suitable for testing nutra/pharmaceuticals for geroprotective qualities. We previously showed that dietary restriction and dilution extended cricket longevity (Lyn et al. 2011) and carried out >40 studies of geroprotective treatments. To date, we have extended maximal longevity of crickets from control levels by 210 % (Lyn et al. 2012).

A meta-analysis of studies from our laboratory found a unifying association of delayed maturation and reduced growth rate with life extension (Lyn et al. 2012). We also found that stress sufficient to induce higher mortality (particularly of juveniles) imparted life

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extension beyond control levels in survivors. This is consistent with a general trade-off between production (growth and reproduction) versus stress resistance (Rollo 2010, 2012). This may be the most fundamental of life history trade-offs and the best understood at hormonal, cellular, and molecular levels (see Rollo 2010, 2012; Bartke et al. 2013).

The production side of this trade-off is regulated by insulin in insects and/or the growth hormone (GH) axis (insulin-like growth factor (IGF-1)) in vertebrates. IGF-1 and insulin activate the switch element protein kinase B (PKB/Akt) in the phosphatidylinositol 3-kinase (PI3K) pathway. Activated PKB drives protein synthesis and cell proliferation via the cellular effector, the target of rapamycin (TOR). When PKB is inactive, forkhead transcription factors (FOXO) translocate to the nucleus and orchestrate generalized stress resistance (Rollo 2010, 2012). The TOR-FOXO signaling system is conserved in crickets (Dabour et al. 2011). TOR-FOXO balance responds to the energy sensor, AMP-activated protein kinase (AMPK).

Metformin and aspirin both increase AMPK activity (signaling energy stress) and provide anticancer and survivorship benefits (Zhou et al. 2001; Onken and Driscoll 2010; Algra and Rothwell 2012; Din et al. 2012; Rothwell et al. 2011, 2012; Hawley et al. 2012; Galluzzi et al. 2013). Aspirin regulates AMPK via direct phosphorylation (Hawley et al. 2012; McCarty 2014), whereas metformin indirectly activates AMPK by reducing mitochondrial complex I and energy production (El Mir et al. 2000; Owen et al. 2000; Viollet et al. 2012). Martin-Montalvo et al. (2013) suggest, however, that mice may adapt to this impact of metformin.

Stress resistance is generally associated with life extension, so “longevity assurance” mechanisms likely fall in this realm (see Cutler 1984a, b). The trade-off between production and stress resistance/longevity assurance also underpins Kirkwood and Holliday’s disposable soma theory (see Kirkwood 2008). Holliday (1989) argued that the dietary restriction response is a form of adaptive plasticity, and regulation of this reaction norm traces to TOR-FOXO antagonism conserved from yeast to vertebrates (Rollo 2010, 2014). In this framework, our results suggest that metformin acts as a hormetic stressor, whereas the active derivative of aspirin, salicylic acid, may act as a regulator of conserved elements in animal longevity assurance pathways. In either case, we expected that life extension would be associated with trade-offs in growth and

maturation age. Here we report that metformin and aspirin both provide geroprotection, but their life history impacts (especially production) strongly differ. Implications for trade-off theory central to evolutionary ecology and life span are discussed.

Materials and methods

Breeding colony

Crickets (*A. domesticus*) were generated from a large breeding colony established with genetically heterogeneous stock. Breeding crickets were housed in an acrylic terrarium (93 cm×64.2 cm×46.6 cm) encased in 1.5 cm thick Durofoam® insulation and maintained at 31±1 °C on a 12-h light/12-h dark photoperiod. The terrarium was covered with fine mesh screening that prevented escape while providing ventilation. Cardboard egg cartons provided shelter. Water was provided ad libitum by moistened cellulose sponges and a dish of agar (made with 6.2 g of agar per 1 L of distilled water). The colony was sprayed with distilled water, sponges were moistened, and agar was changed daily. Breeding crickets were fed chicken feed (Quick Feeds®) distributed in plastic dishes throughout the terrarium. The colony was cleaned daily. Potting soil was provided in plastic dishes as oviposition medium. The soil was moistened daily and the dishes were removed every 2 days. Eggs were incubated in their dishes at 31±1 °C in a 12-h light/12-h dark photoperiod and soil was moistened daily. Hatchlings were removed daily to obtain cohorts of known age and were provided with chicken feed, water-soaked sponges, and shredded paper strips that were sprayed with distilled water daily.

Experimental animals

One hundred 14-day-old crickets were assigned to each treatment: controls, metformin, high-dose aspirin [high aspirin], low-dose aspirin [low aspirin], and metformin/high-dose aspirin combined [MetAsp]. Ten animals were housed to a transparent plastic box (18×10×7 cm). Each box had a ventilated lid and contained an egg carton shelter, a tray with a moistened sponge, and an ad libitum supply of respective diet. The environment was maintained at 31±1 °C with a photoperiod of 12 h light/12 h dark. Crickets were checked for deaths and provided with fresh diet and distilled water daily. Crickets express a visible ovipositor before maturation, allowing us to

separate immature genders and prevent mating. Oviposition medium was withheld, preventing oviposition.

Diets

Diets were based on Living World Extrusion® guinea pig food (protein 16 %, fat 3.5 %, fiber 12.5 %, ash 6 %, calcium 0.8 %, phosphorus 0.6 %, copper sulfate 15 mg/kg, vitamin A 6000 IU/kg, vitamin C 300 mg/kg, vitamin D₃ 520 IU/kg, vitamin E 24 IU/kg). The control diet was made with 30 g of Living World Extrusion® guinea pig food, 2.07 g of agar, and 300 mL of distilled water. This yielded food with a consistency readily ingested by crickets. Agar was added to boiling water and the mixture was thoroughly stirred and then allowed to cool to room temperature. The guinea pig food was then added, the mixture thoroughly blended in. This was then set in a refrigerator for 24 h before use. Doses for metformin and aspirin were derived from recommended human doses adjusted for size and metabolic rate (Appendix).

Food consumption and palatability

Distasteful nutra/pharmaceuticals can reduce feeding and potentially activate dietary restriction pathways independently of their medicinal qualities. Alternatively, dietary restriction mimetics may impact TOR-FOXO pathways and reduce feeding via reductions in metabolic rate, growth rate, and body mass rather than palatability.

To assess palatability, we quantified food consumption over the first 2 days of exposure for separate samples of adult crickets of both genders. Experimental boxes (18×10×7 cm) with ventilated lids, egg carton shelters, and moistened sponges housed four crickets of a single gender each. We employed groups of crickets because this reflected the experimental design and isolated animals may behave differently. Diets (control, high aspirin, metformin, and MetAsp) were prepared as described above. Low aspirin was not assessed since high aspirin would most effectively detect distaste.

Each dietary treatment was assigned three boxes of each gender (i.e., 12 crickets of each gender per treatment). To measure consumption, we estimated the initial dry weight of wet food provided based on samples dried to constant weight at 60 °C. The food remaining after 24 h of consumption was similarly dried. The dry weight eaten was calculated from the estimated original dry weight of initial food minus the actual remaining dry

food. Consumption was analyzed as milligram of dry food/cricket/day.

Feeding was again measured following feeding for 21 days using adult females (low aspirin was also added and examined at 21 days). The longer term study was intended to detect whether treatments might alter consumption via physiological and/or regulatory impacts independently of palatability. Feeding was measured similarly to the palatability study.

Longevity, growth, and mature mass

Mortality was recorded daily. For juveniles, samples of ten animals were weighed weekly. When genders became recognizable by the presence of an ovipositor, five of each sex were weighed weekly. Maturation age (days) and mature mass (mg) were recorded at the mature molt (recognizable by the presence of mature genitalia and wings). Growth rate was calculated by dividing maturation mass by maturation age to obtain an estimate of gain (mg/day) across the juvenile period. Adult insects were also weighed at 100 days of age as they can grow into their expanded exoskeleton, accumulate eggs, and store lipids.

Hydrogen peroxide assay

A hydrogen peroxide assay was applied to assess oxidative status associated with metformin and aspirin. We examined last instar male nymphs fed the respective diets for 30 days prior to sacrifice (~5 per group). Heads were used for the bioassay to avoid gut contents. Crickets were killed by freezing and then decapitated. Individual heads were weighed and mechanically homogenized on ice. H₂O₂ levels in whole head homogenates were assayed using the Amplex Red® hydrogen peroxide/peroxidase colorimetric assay kit (Molecular Probes®, Eugene, OR, USA, Catalog# A22188). Estimates were corrected for mass.

Statistical analyses

General survivorship was assessed using Kaplan-Meier (log rank) survival analysis. Maximal longevity was statistically assessed using the longevity of the ten oldest animals in each treatment. Analysis of variance (ANOVA) was applied to various yield variables (mean and maximal longevity, growth rate, maturation mass, mature mass at 100 days, maturation age, hydrogen peroxide measures, and food consumption). Post hoc

comparisons were tested with Student-Newman-Keuls (SNK), Bonferroni, or Duncan's tests.

Results

Food consumption

Water content of food was ~85 % on average. No significant differences in food consumption among controls and treatments were resolved in the short-term palatability study ($p=0.85$), but females ate ~15 % more than males ($p<0.002$). Thus, palatability was not an issue upon initial exposure. Unlike the short-term palatability study, long-term feeding (21 days) by females showed strong divergence from controls. On average, controls ate 98.8 ± 5.7 mg dry food/adult cricket/day ($\pm$ SE). Low-aspirin crickets consumed 128.5 ± 3.6 (~130 % of controls, $p<0.005$) and high-aspirin crickets ate 103.8 ± 3.0 (~105 % of controls, $p=0.5$). Alternatively, the metformin group ate 81.9 ± 4.9 (~83 % that of controls, $p=0.057$), and MetAsp crickets consumed 78.0 ± 7.3 (~79 % that of controls, $p<0.03$). The difference between the highest and lowest consumption rates (i.e., low aspirin versus MetAsp) amounted to ~39 % (50.5 mg, $p<0.0003$). Remarkably, these results show that low aspirin altered cricket physiology such that food intake was *increased* over controls. Metformin impacts showed a marginally significant trend for reduced feeding, whereas MetAsp significantly reduced feeding.

Mean longevity and survivorship

Figure 1 shows survivorship curves for controls and all treatments. Kaplan-Meier (log rank) analysis found highly significant elevation ($p<0.0002$ or better) of general survivorship for crickets on all treatments compared to controls. All groups displayed relatively steep juvenile mortality in the first 40 days which is typical of this species (Lyn et al. 2011). Only 25 % of crickets fed the control diet survived past 40 days of age. By comparison, 61 % of crickets on low aspirin lived >40 days. The increase in mean longevity for low aspirin over controls (41.4 versus 96.9 days) was 234 % (Table 1). Both aspirin treatments dramatically improved survivorship, but this was largely lost when combined with metformin (Fig. 1). The percentage of animals living beyond 40 days in the other treatments was 57 % for high aspirin, 58 % for metformin, and 54 % for MetAsp (i.e., >2-fold better than

Fig. 1 Survivorship curves for crickets in various treatments ($n=496$). The low-aspirin treatment achieved the greatest mean longevity followed by high-aspirin, metformin, and the metformin-aspirin combination. All curves significantly differed from controls using the log rank test ($p<0.0002$ or better). In fact, comparisons of each curve against all others were significantly resolved (log rank test: $p=0.01$ or better) except for the high- and low-aspirin groups. Although increases in maximal life span were impressive, impacts on overall survivorship (health span) were even greater, mean survivorship of crickets treated with high- and low-aspirin being more than double that of controls (Tables 1 and 2)

controls). The age at which 75 % of the population had died was 40 days for controls, but this was remarkably higher for both the high- and low-aspirin groups (~170 days) (Fig. 1). By this benchmark, the aspirin-treated groups survived 4-fold better than controls.

Table 1 provides the mean longevity and upper 95 % confidence intervals for each treatment with differentiation by ANOVA and post hoc Bonferroni analyses. All treatments differed from controls ($p<0.05$) except for the MetAsp combination. As clearly shown in Fig. 1, the percent improvement in mean longevity for both metformin (179 %) and aspirin (high 211 %, low 234 %) was considerably greater relative to extension of maximal longevity (Table 2). Moreover, the upper 95 % confidence limits for aspirin treatments were also >200 % of controls.

Maximal longevity

Whereas mean longevity and survivorship are indicative of "health span," maximal longevity best reflects aging rates. The oldest living cricket (194 days) was obtained with high aspirin (143 % of controls) with metformin and low aspirin achieving maximal life spans of 188 and

Table 1 Mean longevity and upper 95 % confidence interval (IC) for survivorship of the cricket, *Acheta domesticus*, treated with metformin and/or aspirin

	Treatment	Gender	Mean longevity (days)	Percentage of controls	Upper 95 % CI	Percentage of controls
1	Control	All	41.4 (4, 7, 10)		47.6	
2		Female	97.9 (5, 8, 11)		119.0	
3		Male	88.9 (9, 12)		110.8	
4	Metformin	All	74.1 (1, 10)	179	84.7	178
5		Female	143.7 (2)	147	159.9	134
6		Male	123.2 (9)	139	107.9	97
7	Aspirin high	All	87.4 (1, 13)	211	100.4	211
8		Female	145.8 (2, 14)	149	160.9	135
9		Male	157.7 (3, 6)	177	175.8	159
10	Aspirin low	All	96.9 (1, 4, 13)	234	109.9	231
11		Female	149.2 (2, 14)	152	162.4	137
12		Male	148.8 (3)	167	162.9	147
13	Metformin and aspirin	All	60.5 (7, 10)	146	68.9	145
14		Female	110.2 (7, 10)	113	126.0	106
15		Male	125.3 ns	141	110.4	100

Results for “All” include juveniles and adults, whereas gender results refer only to mature animals. Numbers enclosed in parenthesis represent statistical resolution as determined by ANOVA ($F(4, 426)=8.538, p<0.0000001$) and Bonferroni differentiation

ns not significant

187 days (138 % of controls) (Table 2). The MetAsp combination lived 163 d (120 % of controls). We

statistically tested maximal life span using the oldest ten crickets in each treatment (Table 2). All treatments

Table 2 Maximal longevity of the cricket *Acheta domesticus* (oldest animal and oldest ten animals) treated with metformin and/or aspirin

	Treatment	Gender	Oldest animal (days)	Percentage of controls	Oldest 10 animals (days)	Percentage of controls
1	Control	All	136		122.8 (4, 7, 10, 13)	
2		Female	130		97.0 (5, 8, 11)	
3		Male	136		96.4 (6, 9, 12, 15)	
4	Metformin	All	188	138	170.1 (1, 7, 10, 13)	139
5		Female	188	145	163.0 (2, 14)	168
6		Male	183	135	155.9 (3, 9)	162
7	Aspirin high	All	194	143	188.8 (1, 4, 13)	154
8		Female	188	145	172.9 (2, 14)	178
9		Male	194	143	186.6 (3, 6, 15)	194
10	Aspirin low	All	187	138	184.0 (1, 4, 13)	150
11		Female	186	143	177.7 (2, 14)	183
12		Male	187	138	176.9 (3, 15)	184
13	Metformin and aspirin	All	163	120	147.9 (1, 4, 7, 10)	120
14		Female	161	124	118.6 (5, 8, 11)	122
15		Male	163	120	143.2 (3, 9, 12)	149

Longest lived were aspirin-treated crickets followed by metformin and a metformin-aspirin combination. All groups lived significantly longer than controls except for females on the metformin-aspirin combination. Percentages compare control longevity to respective treatments. Maximal longevity for the ten longest lived animals included both genders. Numbers enclosed in parenthesis represent statistical resolution as determined by ANOVA ($F(4, 45)=28.07, p<0.000001$) and Bonferroni differentiation

differed significantly from controls ($p<0.0003$ for all), and generally, the percent increase resolved was greater than obtained by comparing the longest lived cricket. It is unusual for control crickets to live more than ~120 days, and the 136 days maximum achieved here reflected a few exceptional individuals. The maximal longevity of controls based on the oldest ten animals (123 days) was closer to expectation. Maximal longevity of the last ten high-aspirin crickets (188.8 days, 154 % of controls) significantly exceeded longevity of those on metformin (170.0 days, 139 % of controls) ($p<0.05$). The oldest ten males on high aspirin obtained life extension 194 % that of controls (Table 2).

Maturation mass

No significant reduction in body size relative to controls was resolved for aspirin-treated crickets, and both genders showed a trend for larger size on low aspirin (Table 3, ns). Crickets on low aspirin were 135 % the size of metformin-treated animals for females and 160 % for males ($p<0.05$ in both cases). Metformin-treated females were only 81 % of the mass of controls, and for males, this was 74 % ($p<0.05$ in both cases). Males and females on the MetAsp treatment were not statistically resolved from those on metformin alone.

Postmaturation mass

We weighed crickets at 100 days of age to assess the degree of postmaturation gain compared to their

maturation mass (Table 4). Insects grow into their exoskeleton following a molt, allowing some weight gain, and the abdomen has a telescoping structure that allows expansion. Males made relatively small gains (103 to 117 %) compared to those of females (155 to 198 %). Our virgin crickets retain their eggs so mature ovary mass is a good estimate of reproductive allocation (King et al. 2011). The majority of gain in females reflected development of eggs so postmaturation mass provides a biomarker of reproduction.

ANOVA resolved that all treatments differed from one another except for metformin and the MetAsp combination ($p<0.000001$). There was also a significant treatment by gender interaction ($p<0.00009$). Males were significantly smaller than females ($p<0.00001$). Using Student-Newman-Keuls test, it was found that males on low aspirin were significantly larger than those on metformin ($p<0.05$), but otherwise, variation among treatments for males was not significant.

Among females, those on low aspirin achieved a size of 852 mg—significantly larger than controls ($p<0.03$) and all other treatments. Females on high aspirin were nearly identical in size to controls (i.e., no trade-off). Thus, for aspirin, a trade-off between life extension and reproduction was absent judging by postmaturation gain, and for low aspirin, this was significantly enhanced. Females on the metformin and MetAsp were only 66 % the size of controls ($p<0.0003$) and 58 % the size of females on low aspirin ($p<0.0001$). The MetAsp treatment was very similar to metformin alone.

Table 3 Impacts of metformin and aspirin on maturation mass, maturation age, and growth

	Treatment	Gender	Maturation mass (mg±SE)	Percent	Maturation age (days±SE)	Percent	Growth rate (mg/day±SE)	Percent
1	Control	Female	396.1±16.63 (3, 9)		46.2±0.9 (3, 5, 7, 9)		8.55±0.2 (3, 5, 9)	
2		Male	338.2±10.6 (4, 10)		47.4±0.7 (4, 6, 8, 10)		6.61±0.6 (4, 10)	
3	Metformin	Female	319.2±15.3 (1, 5, 7)	81	64.8±1.4 (1, 5, 7)	140	4.99±0.28 (1, 5, 7)	58
4		Male	250.1±13.5 (2, 6, 8)	74	69.0±1.6 (2, 6, 8)	145	3.67±0.21 (2, 6, 8)	56
5	Aspirin high	Female	391.3±7.1 (3, 9)	99	53.8±0.48 (1, 3, 9)	117	7.29±0.15 (1, 3, 7, 9)	85
6		Male	346.4±14.2 (4, 10)	102	55.7±1.12 (2, 4, 10)	118	6.2±0.21 (4, 8, 10)	94
7	Aspirin low	Female	431.0±11.0 (3, 9)	109	53.6±0.58 (1, 3, 9)	116	8.08±0.24 (3, 5, 9)	85
8		Male	401.2±14.3 (4, 10)	119	56.0±0.69 (2, 4, 10)	118	7.15±0.21 (4, 6, 10)	108
9	Metformin and aspirin	Female	300.2±12.9 (1, 5, 7)	76	67.7±2.1 (1, 5, 7)	147	4.48±0.23 (1, 5, 7)	52
10		Male	262.7±10.5 (2, 6, 8)	78	70.7±1.9 (2, 6, 8)	149	3.74±0.16 (2, 6, 8)	57

Percent values were calculated relative to controls. Numbers enclosed in parenthesis indicate treatments that differed significantly via ANOVA ($p<0.000001$ for maturation mass, maturation age, and growth rate) with Bonferroni differentiation ($p<0.05$)

Table 4 Impacts of aspirin and metformin on maturation mass and postmaturation mass (100 days) in *Acheta domesticus*

	Treatment	Gender	Maturation mass (mg)	Mass at (100 days) (mg±SE) ^a	Percentage of controls	Percent gain since maturation
1	Control	Female	396.1	740.0±33 (3, 7, 9)		186.8
2		Male	338.2	373.2±33		110.3
3	Metformin	Female	319.2	495.0±26 (1, 5, 7)	66.8	155.1
4		Male	250.1	276.7±26 (8)	74.1	110.6
5	Aspirin high	Female	391.3	741.3±41 (3, 7, 9)	100.2	189.4
6		Male	346.4	373.1±29	100.0	107.7
7	Aspirin low	Female	431.0	851.5±33 (1, 3, 5, 9)	115.1	197.6
8		Male	401.2	415.8±33 (4)	111.4	103.6
9	Metformin and aspirin	Female	300.2	488.7±26 (1, 5, 7)	66.0	162.7
10		Male	262.7	306.8±24	82.2	116.7

Maturation mass is provided here for comparison from Table 3. ANOVA detected significant differences among treatments ($F(4, 67)=27.89$, $p<0.00001$)

^aNumbers enclosed in parenthesis denote treatments that differed significantly for mass at 100 days (Student-Newman-Keuls). Males and females consistently differed statistically so this has not been indicated by numbers in parenthesis ($p<0.00001$)

Maturation age

Maturation age of crickets on both the low- and high-aspirin treatments were nearly identical and significantly delayed relevant to controls (~117 % for both treatments, Table 3). However, aspirin-treated crickets matured significantly earlier than those on metformin. Relative to controls, the maturation age of metformin-treated crickets was 140 % for females and 145 % for males (Table 3). Maturation age was the feature most altered by treatments (especially metformin). As with maturation mass, maturation age of males and females on MetAsp was similar to metformin alone and did not differ statistically.

Growth rate

Females in high aspirin grew significantly slower than controls, but males and both genders on low aspirin did not differ statistically from controls. Aspirin-treated males had a slightly higher growth rate than controls (108 %, ns). Alternatively, metformin severely reduced the growth rate of both females (58 %) and males (56 %) relative to controls (Table 3). The metformin and MetAsp treatments did not differ significantly.

Hydrogen peroxide

Levels of H_2O_2 in whole head homogenates for control, metformin, aspirin, and MetAsp treatments

are shown in Fig. 2. All treatments (other than high aspirin) expressed significantly lower H_2O_2 than controls (ANOVA, post hoc Duncan's test, $p<0.05$ in all cases). Reductions of H_2O_2 in the low-aspirin, metformin, and MetAsp treatments ranged from ~30 to 40 % (Fig. 2).

Fig. 2 Concentrations of H_2O_2 in head homogenates of the cricket *Acheta domesticus* in controls (Cont) and those treated with high (AspH) and low (AspL) doses of aspirin, metformin (Met), and a combination of metformin and aspirin (MetAsp). ANOVA detected a significant difference among treatments ($F(4, 18)=3.167$, $p=0.0389$). Other than for high-dose aspirin, all treatments significantly differed from controls (post hoc Duncan's test: $p<0.05$)

Discussion

Mean longevity and survivorship

Control crickets expressed a type III survivorship curve, whereas a type I curve was obtained with aspirin (Fig. 1). Aspirin dramatically extended life span (Tables 1 and 2) and achieved a profile approaching the “square survivorship curve” (live long and healthy, then die quickly). A 23 % life extension by aspirin was reported for *Caenorhabditis elegans* (Ayyadevara et al. 2013; Wan et al. 2013). This was obtained via upregulation of FOXO and associated stress resistance. Hochschild (1971) found that aspirin increased maximal longevity of adult *Drosophila* by 18 % in females and 27 % in males. A mixture of aspirin and salicyl-salicylic acid extended male maximal longevity by 18 % and female maximal longevity by 42 %. This was attributed to stabilization of membranes. Others failed to extend longevity of adult *Drosophila* with aspirin (Massie et al. 1985). Inconsistent results for aspirin and metformin (see below) with *Drosophila* may reflect that studies are generally limited to adults and bypass growth and maturation stages. Aspirin extended survivorship of male mice (Strong et al. 2008) but not maximal longevity. Aspirin also delayed onset of motor defects in a mouse model of amyotrophic lateral sclerosis without improving survival (Barneoud and Curet 1999).

Activity of aspirin is mediated by its metabolite, salicylic acid, a plant hormone mediating diverse stress responses (e.g., pathogens, drought, temperature, toxins, osmotic stress) (Verhage et al. 2010; Rivas-SanVicente and Plasencia 2011). Salicylic acid may act via conserved regulators of stress resistance in animals. Besides activating AMPK in animals (conserved in all eukaryotes including fungi and plants; Hardie 2005; Hardie et al. 2012), salicylic acid modulates antioxidants and prostaglandins, inhibits NF κ B, and binds to heme-containing enzymes such as catalase and peroxidase (Ruffer et al. 1995; Shimamura et al. 2003; Torres et al. 2006; Koornneef et al. 2008; Yan and Dong 2014). Antioxidant actions may be mediated via nitric oxide and inhibition of NAD(P)H oxidase (Wu et al. 2002; Grosser and Schroder 2003).

We obtained nearly equivalent maximal longevity for metformin and aspirin although enhancement of early survivorship by metformin was half that of aspirin (Tables 1 and 2, Fig. 1). It is not possible to judge which drug might be most effective overall as we do not have

full dose-response curves. Although Jafari (2010) suggested that metformin reduced mortality of *Drosophila*, Slack et al. (2012) found that metformin was ineffective. Our results appear to be the first to document life extension by metformin in an insect. Onken and Driscoll (2010) found that metformin extended mean longevity of *C. elegans* by upregulating AMPK and antioxidants. Midlife survivorship and locomotor activity in older ages were improved, but maximal longevity was not extended nor did metformin extend chronological life span of yeast (Choi et al. 2013).

Metformin extended longevity and reduced cancer in mice (Anisimov et al. 2005a, b, 2008, 2010, 2011; Arkadieva et al. 2011), including a model of Huntington's (Ma et al. 2007). Hou et al. (2010a) found that metformin protected mice from metabolic syndrome associated with a high-carbohydrate and high-fat diet. In rats, metformin failed to improve survivorship at any age (Smith et al. 2010). Wang et al. (2012) found that metformin promoted neurogenesis and enhanced spatial memory, suggesting potential protection against neurodegeneration. Given the hormetic mechanism of metformin, high variation among studies may partly reflect dosages. We found that metformin strongly extended mean and maximal longevity of crickets although improvement in health span/survivorship was far less than for aspirin (Tables 1 and 2, Fig. 1).

The MetAsp treatment was very similar to that of metformin alone (especially for maturation age, size, and growth rates), but survivorship and maximal life span were less (Tables 1 and 2, Fig. 1). Thus, the survivorship benefits seen with aspirin were largely lost when combined with metformin and were even lower than for metformin alone. The MetAsp dosage may have exceeded optimal (Fig. 1). Metformin and aspirin are toxic at high doses, and results with the combination could reflect convergence on a common mechanism such as mitochondrial function (see El Mir et al. 2000; Gupta et al. 2013). The fact that metformin largely masked the impacts of aspirin suggests that a lower dose of metformin might obtain greater benefits.

Maturation age

Results with metformin are consistent with a trade-off between life extension and both maturation age and growth. For aspirin, however, maturation age was the key factor impacted (Table 3). Metformin results strongly resemble outcomes obtained with dietary restriction

(delayed maturation, small size, and extended longevity). Delayed maturation with aspirin agreed with predictions, but remarkably, life extension by aspirin had little impact on mature body size. Even for maturation age, the delay in the aspirin treatments was smaller (116–117 % relative to controls) than for metformin (140–149 %) (Table 3).

Delayed maturation may be the primary response to stressors that restrict performance because this allows compensation and costs can be spread over time. Indeed, in a treatment combining dietary restriction and nutritional supplements, crickets that were juveniles at 160 days of age had already outlived adult controls by 133 % (Lyn et al. 2012).

Growth rate

Reduction in growth rate by metformin (Table 3) is consistent with inhibition of mitochondrial complex I (El-Mir et al. 2000) leading to elevated AMPK signaling and consequent activation of stress/dietary restriction pathways. Energy shortfalls would explain delayed maturation, reduced growth rates, and smaller mature sizes. Metformin also reduces TOR activity via inhibition of PKB/Akt (Zakikhani et al. 2010). Thus, inhibition of growth by metformin may reflect both reduced energy and downregulated TOR.

Although aspirin/salicylic acid directly activates AMPK, it can also uncouple mitochondria and reduce ATP (Xie and Chen 1999; Hawley et al. 2012). Wan et al. (2013) detected reductions in energy in aspirin-treated *C. elegans* suggesting that metformin and aspirin share a common mechanism in this regard. Regardless, benefits obtained by aspirin with only minor impacts (or significant enhancement) on growth indicate that energy was not strongly inhibited in crickets (Table 3). Remarkable life extension with aspirin likely involved modulation of regulatory mechanisms (such as FOXO) downstream of AMPK.

Maturation mass

Lack of significant reductions in body mass by aspirin reflects that maturation age was significantly delayed and growth rates were reduced, but not significantly. For males on low aspirin, *both* maturation age and growth rates were greater, resulting in larger adults than in other treatments (Table 3, ns). Although low aspirin slightly slowed the growth of females, delayed maturation was

sufficient to achieve larger adults than in any other treatment (~109 % of controls). Lyn et al. (2012) previously recognized that maturation age was delayed in defense of body size. Overcompensation could explain the trend for increased maturation size. For metformin, impacts on body mass were relatively less than on growth rate since body size was buffered by delayed maturation. Regardless, reduced maturation size reflected a significant cost.

Postmaturation mass

If extended longevity can be obtained with little cost, the question becomes why this is not constitutively elevated. One possibility is that trade-offs emerge in other dimensions. Aspirin inhibits prostaglandins/eicosanoids which serve diverse regulatory roles in insects, including reproduction and immunity. Growth can be supported or even exaggerated by inhibition of reproduction, and a trade-off between reproduction and life span permeates the literature (Edward and Chapman 2011). High survivorship on aspirin, however, suggests that any costs associated with immunity might only emerge under challenge.

We prevented oviposition but females still developed and carried eggs. Analysis of mass at 100 days showed small gains in males, but females expressed substantial gains that dissection showed was mainly associated with eggs (Table 4). The weight gain of females on metformin was significantly lower than controls indicative of a trade-off with both growth and reproduction. Females on low aspirin, however, exceeded the mass of controls ($p < 0.02$), whereas those on high aspirin were statistically equivalent to controls. Thus, reproduction shows the same pattern as growth—significant costs associated with metformin and lack of any trade-off with aspirin. This is consistent with elevated feeding on low aspirin.

Hydrogen peroxide

Both aspirin and metformin ameliorate free radical stress (Bonnetfont-Rousselot et al. 2003; Phillips and Leeuwenburgh 2004; Onken and Driscoll 2010; Ayyadevara et al. 2013). AMPK also downregulates NF κ B via phosphorylation of I κ B, thereby reducing NAD(P)H oxidase activity (McCarty 2014). Besides regulating stress resistance, salicylic acid can act as an antioxidant and chelator to inhibit iron-mediated generation of hydroxyl radical (Yang et al. 2004). Whether or

not free radicals constitute a cause of aging remains controversial.

We previously found that longevity of mice was negatively associated with oxidative and nitrosative stress (Rollo et al. 1996; Aksenov et al. 2013; Long et al. 2012). Lipid peroxidation in the heart or brain of transgenic growth hormone mice expressing doubled growth rates explained ~89 % of variation in maximal longevity associated with genotype, sex, and diet (Rollo et al. 1996). If free radicals do not impact aging, some are strongly associated. Low free radicals may reflect reduced metabolic rates and/or elevated stress resistance associated with life extension.

We found dramatic reduction in H_2O_2 in the heads of metformin-treated crickets (Fig. 2). Strongly reduced growth with metformin suggests that reduced metabolic rate may have contributed. This would not apply to low aspirin that strongly reduced H_2O_2 with little reduction in growth or feeding. No reduction in H_2O_2 by high aspirin was surprising. It seems likely that the dosage exceeded optimal, and indeed, health span was reduced compared to low aspirin. The high dose of aspirin in the MetAsp treatment also did not augment reduction of free radicals by metformin. The association of significant life extension (slightly longer than low aspirin) without reduced H_2O_2 suggests that high aspirin extended maximal longevity via a mechanism independent of free radicals. Confirmation would require examination of other free radicals and associated damage.

Aspirin extended longevity of *C. elegans* by up to 23 % in association with upregulated superoxide dismutases, catalase, and glutathione S transferases (Ayyadevara et al. 2013). This required DAF-16/FOXO and AMPK, supporting aspirin as a dietary restriction mimetic (Ayyadevara et al. 2013; Wan et al. 2013). Both aspirin and metformin activate AMPK which can mediate sirtuin/FOXO-driven stress resistance and longevity assurance (Canto et al. 2009). Onken and Driscoll (2010) suggest that metformin activates antioxidant defenses via a FOXO-independent mechanism, but in human tissue, metformin upregulates oxidative stress resistance by eliciting the antioxidant thioredoxin via an AMPK-FOXO3 mechanism (Hou et al. 2010b).

Trade-offs and life extension

Failure to detect a trade-off between production and longevity with aspirin was surprising given that such antagonism is phylogenetically conserved from yeast to

vertebrates (see Rollo 2010, 2012; Bartke et al. 2013). Failure to detect trade-offs can reflect numerous causes. Where individuals vary in fitness, those of higher quality may do everything better (i.e., no trade-offs). This extends to variation associated with density, nutrition, or pathology (van Noordwijk and de Jong 1986; King et al. 2011). An expected trade-off between longevity and factors like growth and reproduction may be obscured by a trade-off in a third dimension (e.g., immunity) or where trade-offs involve different currencies (e.g., energy versus risk). Trade-offs between antagonistic circuitry regulating aging (e.g., TOR-FOXO) may also be avoided by temporal compartmentalization (Rollo 2010, 2012).

Trade-offs can be ameliorated by increasing feeding and metabolic rate, or by utilizing storage reserves. Compensatory increases in assimilation efficiency allowed caterpillars to maintain growth despite reduced foraging under predation risk (but costs emerged at later ages; Thalera et al. 2012). Organisms have considerable “compensatory scope” to increase feeding and metabolism to offset elevated costs (Rollo 1994). We could not identify a cost associated with life extension on low aspirin other than a slight delay in maturation. Increased feeding (130 % of controls, $p < 0.005$) likely supported costs of longevity assurance mechanisms. Notably, this “eat more, live longer” paradigm differs radically from the reigning dietary restriction paradigm.

Saul et al. (2013) suggest that life extension via hormetic agents is reliably associated with fitness costs, but Lopez-Martinez and Hahn (2014) found that exposure of fly pupae to hormetic levels of anoxia derived lifelong benefits. Such “conditional hormesis” likely involves epigenetic alterations (Constantini et al. 2010). These authors also suggested that elevated feeding could offset hormetic costs. Indeed, a trade-off between exaggerated growth and accelerated aging in transgenic growth hormone mice appeared to reflect a failure to elevate feeding sufficiently to pay for their upregulated growth (Rollo et al. 1999). Indeed, providing *more* calories via sucrose supplements significantly extended life span of females (Rollo et al. 1996).

Resource allocation to production versus stress resistance may be altered by geroprotective *regulators* such as salicylic acid. Our analysis of crickets (and present results) shows that compensation by delayed maturation can buffer targeted body mass (Table 3; Lyn et al. 2012). Metformin results suggest that such compensation has limitations. Growth rate better predicts longevity than mature mass, but maturation age explains most variation

in maximal life span (Lyn et al. 2012). As fecundity is positively associated with female size, delaying maturation to achieve larger adults reflects an indirect investment in reproduction. Larger size also improves competitive ability, predator defense, and heat and water balance. Although the larger size of females on low aspirin (109 %) was not significant at maturation (Table 3), by 100 days, they exceeded controls by 115 % ($p < 0.03$, Table 4).

Flatt (2011) and Edward and Chapman (2011) suggest that although reproduction is generally traded off against survivorship, these can be decoupled. Leroi (2001) and Braendle et al. (2011) suggest that trade-offs may not always reflect resource competition but might simply reflect altered regulatory signaling. Flies with mutated ecdysone receptors or decreased ecdysone obtained extended longevity and resistance to oxidative stress, heat, and starvation with no trade-offs in activity or reproduction (Simon et al. 2003). A trade-off between reproduction and longevity did not emerge when *C. elegans* was subjected to condition-dependent rather than random mortality (Chen and Maklakov 2012). In *Drosophila* selected for extended longevity, females had similar or even higher fecundity throughout life, and stress resistance, metabolic rate, maturation time, and body size also had no correlation with life span (Wit et al. 2013). Similarly, exceptional longevity of Rottweiler dogs was not limited by female reproductive effort (Kengeri et al. 2013).

Wood et al. (2004) found no suppression of reproduction with life extension by resveratrol in *Drosophila* and *C. elegans*. Resveratrol upregulates effectors of dietary restriction like sirtuins and AMPK (Baur et al. 2006; Burkewitz et al. 2014). Grandison et al. (2009) found that dietary restriction extended life span and reduced reproduction of *Drosophila*, but augmenting methionine restored reproduction without reducing life extension. Constantini et al. (2010) noted that simultaneous increases in survivorship and reproduction may be obtained by mild hormetic stressors. Walker et al. (2000) found that a mutation conferring life extension in *C. elegans* had no costs when food was abundant, but fitness declined under recurrent starvation.

The “principle of allocation” is a guiding paradigm for evolutionary ecology. In a previous test of this principle, we found that expected trade-offs were absent (Rollo 1986). In two sibling cockroach species with nearly identical morphology, *Periplaneta brunnea* produced oothecae 180 % the mass of those of *Periplaneta*

americana (24 versus 16 eggs) and did so in 30 % less time. However, reducing resource quality caused 100 % mortality in *P. brunnea* when only 16 % of “low rate” *P. americana* had died. This suggests an evolutionary framework based on ensured *persistence* rather than maximized *production* (i.e., reproduction and other costs are physiologically tuned to resource reliability). This framework also allows for compensatory scope that could ameliorate expression of trade-offs. We addressed compensatory scope in two freshwater snails, *Stagnicola elodes* and *Physella gyrina*, using diets diluted with cellulose. Remarkable compensatory abilities indicated that indeed these snails normally operated at *submaximal* rates and that compensation could ameliorate resource limitations (Rollo and Hawryluk 1988). Such responses could alleviate trade-offs within the evolved safety margin.

Classes of geroprotectors

As outlined above, appropriate nutrition can allow compensation and amelioration of trade-offs (Rollo 1986; Rollo et al. 1996; Walker et al. 2000; Wood et al. 2004; Grandison et al. 2009). As a direct activator of AMPK, aspirin may alter the set point for energy metabolism (such as elevation of FOXO in its circadian window of activity, without inhibiting TOR; see Rollo 2010, 2012). Thus, geroprotectors acting via AMPK might both signal and/or cause low energy states, but this could be offset by elevated feeding to a new set point. For regulators that elevate AMPK when there is no energy deficit (i.e., aspirin), set point elevation could allow hyperperformance as well as increased feeding and life span. Alternatively, life extension via factors that modulate dietary restriction pathways (i.e., regulation or induced energy shortfalls) may not be detected if dietary restriction is imposed (e.g., Wood et al. 2004).

This discussion suggests that geroprotective agents fall into at least three major classes: (1) *hormetic stressors or toxins* that are beneficial at appropriate doses (e.g., metformin, rapamycin, dietary restriction); (2) *geroprotective augmentors* that provide agents, precursors, or cofactors supporting longevity assurance mechanisms (e.g., antioxidants, chelators, vitamins, minerals, or nutrients supporting repair and regeneration); or (3) *geroprotective regulators* that alter signaling in stress resistance/aging pathways (e.g., stress hormones, certain neurotransmitter precursors, aspirin, melatonin?). Mutations in regulators are also likely to cause dysregulation. Since dietary restriction itself is hormetic,

rather than pursuing stressors we might be better served to identify geroprotectors associated with minimal costs.

Both metformin and aspirin were derived from phytochemicals. Inhibition of mitochondrial complex I in animals is consistent with a defensive function for metformin. Alternatively, salicylic acid is a crucial plant stress hormone that may act as a geroprotective regulator of conserved elements in animals. Wood et al. (2004) suggest a similar role for resveratrol. Since many regulators of stress resistance are highly conserved, plant-derived regulatory agents are a promising source of geroprotectors that may obtain health benefits with minimal detriment.

Because TOR is associated with increased production and decreased longevity, suppressing its activity is of considerable interest as a way to extend longevity (Johnson et al. 2013). Given the decline in the growth hormone axis and rise in the stress hormone axis with age, however, interventions that exacerbate TOR-FOXO distortion may be misdirected. It may be better to maintain youthful levels of GH (and TOR) functions while elevating stress resistance and survival mechanisms (FOXO). We argued that expression of growth (production) and stress resistance pathways in separate temporal windows abrogates the *necessity* of trade-offs (Rollo 2010, 2012). In this context, mutations in mice that disrupt the growth hormone axis may obtain extended longevity, but this is traded off against growth and reproduction (Bartke et al. 2013).

Results for aspirin suggest that agents that have regulatory impacts may be better candidates for human life extension than hormetic stressors as they may activate antiaging mechanisms without reducing energy metabolism or other functions. This could then allow greater elevation of FOXO-mediated stress resistance functions than possible under conditions of reduced energy (hormetics, dietary restriction) while maintaining TOR-mediated anabolism. Indeed, the relative ablation of aspirin benefits when combined with metformin suggests antagonism. Alternatively, the fact that most results with the combination were otherwise nearly identical to metformin suggests that the dosage of metformin was sufficient to obscure contributions of aspirin.

Food consumption

There was no evidence of palatability issues for any of our treatments, but longer term feeding indicated a significant increase on low aspirin and reductions

associated with metformin and MetAsp. Over the life span, reduced growth rate and body size in metformin and MetAsp treatments would undoubtedly reduced feeding further. This could reflect reduced energy associated with mitochondrial inhibition by metformin.

Palatability is a critical issue since taste aversion can obtain dietary restriction benefits. Imposing limitations on food to induce hunger, however, excludes possible compensatory feeding that might otherwise offset trade-offs induced by hormetic agents. Indeed, Martin-Montalvo et al. (2013) obtained late-life benefits of metformin in ad libitum fed C57BL/6 mice but not in B6C3F1 mice where both treated and control groups were limited to 13.3 kcal of food per day (see also Spindler et al. 2013a, b). Based on the information in Pugh et al. (1999), rations of these mice were reduced by ~10–12 %. Although much milder than typical dietary restriction (i.e., >25 %), even this degree of underfeeding may be sufficient to obscure impacts of treatments (especially where controls are similarly limited) (see Duffy et al. 2004; Wood et al. 2004; Spindler 2010; Rocha et al. 2012; Colman et al. 2014).

Conclusions

Although metformin and aspirin obtained similar degrees of life extension in crickets, metformin severely reduced productivity and delayed maturation, whereas aspirin had little negative impact (and significantly enhanced feeding and adult weight of females). Geroprotective agents that upregulate antiaging pathways (like aspirin) may generally have low costs compared to hormetic agents that activate these pathways via low-grade stress (metformin). This implies that low-cost life extension might be obtained by geroprotective *regulators* targeting various elements of the antiaging/stress resistance circuitry. Concentrations of hormetics where trade-offs can be offset by compensation might also be considered.

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Conflict of interest The authors declare no conflicts of interest.

Appendix

Determining doses of metformin and aspirin was guided by several goals. Starting with human doses, we

adjusted for body mass and metabolic rates to scale cricket doses roughly to that respective to humans. This was intended to identify a range likely tolerable for crickets. It was logistically intractable to match doses to growing crickets, particularly since males and females differ in mass and treatments were anticipated to diverge with time as well. Compensatory feeding or reductions associated with reduced growth could not be anticipated. We aimed to maintain a single dose for each treatment and across ages to provide a unified experimental framework. We also wanted to ensure that the doses were high enough to strongly impact crickets as there was uncertainty of biological differences between species (i.e., higher than the conservative human doses). We calculated the dose such that a juvenile cricket eating 30 mg of food would obtain that scaled equivalent of a human (i.e., adjusted for mass and metabolic rate). This ensured that the experiment began with a relatively high dose. Initial pilot studies did not detect any tolerance issues and results illustrate that all treatments obtained increased survivorship over controls across all ages (Fig. 1).

Diets for all treatments were prepared similarly. Aspirin and metformin were obtained from Sigma-Aldrich. Doses of drugs were based on recommendations for humans adjusted for differences in the mass and metabolic rate of crickets. The mass ratio of crickets (0.5386 g) to humans (~80 kg) was 6.7325×10^{-6} . The ratio of the metabolic rate of crickets (14.38 ml O₂/g/day; Hack 1997) to humans (4.62 ml O₂/g/day; Nieman et al. 2003; Rizzo et al. 2005) was 3.11. Combining these conversion factors obtained a global conversion factor of 2.09×10^{-5} . For metformin, the maximum recommended human dose is ~2.55 g/day (FDA guidelines). Applying the conversion factor yields a rough estimate of $2.09 \times 10^{-5} \times 2.55 = 5.3294 \times 10^{-5}$ g of metformin/cricket. A juvenile cricket eats ~30 mg of food/day so the concentration of metformin in food that would deliver a high dose was 5.329×10^{-5} g metformin/0.03 g food or 1.78×10^{-3} g metformin/g diet. Anisimov (2013) reviewed dosages used in studies of metformin. The dose of metformin obtaining health and survival benefits in C57BL/6 mice was 0.1 % (w/w) in food (e.g., 1.0×10^{-3} g/g) (Martin-Montalvo et al. 2013)—very close to ours.

The recommended high dose of aspirin for humans is 325 mg, and a low dose (25 %) is 81 mg (pharmacy products). The high dose per cricket obtained by applying the global conversion factor was $2.09 \times 10^{-5} \times 325 =$

6.79×10^{-3} mg/cricket. Given that a juvenile cricket eats ~30 mg of food/day, the concentration for the diet was $6.79 \times 10^{-6} / 0.03 = 2.263 \times 10^{-4}$ g aspirin/g food for the high dose. The low dose was 25 % of the high dose (5.65×10^{-5} g aspirin/g food). The metformin-aspirin combination was made with the above dosage of metformin and the high-dose aspirin.

Drugs were stirred into the mixture of agar and water after it had cooled well below 50 °C. Metformin is stable at temperatures as high as 70 °C (Sharma et al. 2010), but aspirin loses stability above 50 °C (Snaveley et al. 1993; Al-Gohary and Al-Kassas 2000). When the mixture reached room temperature, it was poured into a blender and 30 g of guinea pig food was thoroughly blended in. The agar was then set and stored in a dark refrigerator. New diet was prepared weekly.

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