Conditional tradeoffs between aging and organismal performance of *Indy* long-lived mutant flies

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Alterations that extend the life span of animals and yeast typically involve decreases in metabolic rate, growth, physical activity, and/or early-life fecundity. This negative correlation between life span and the ability to assimilate and process energy, to move, grow, and reproduce, raises questions about the potential utility of life span extension. Tradeoffs between early-life fitness and longevity are central to theories of the evolution of aging, which suggests there is necessarily a price to be paid for reducing the rate of aging. It is not yet clear whether life span can be extended without undesirable effects on metabolism and fecundity. Here, we report that the long-lived *Indy* mutation in *Drosophila* causes a decrease in the slope of the mortality curve consistent with a slowing in the rate of aging without a concomitant reduction in resting metabolic rate, flight velocity, or age-specific fecundity under normal rearing conditions. However, *Indy* mutants on a decreased-calorie diet have reduced fecundity, suggesting that a tradeoff between longevity and this aspect of performance is conditional, i.e., the tradeoff can occur in a stressful environment while being absent in a more favorable environment. These results provide evidence that there do exist mechanisms, albeit conditional, that can extend life span without significant reduction in fecundity, metabolic rate, or locomotion.

Drosophila melanogaster | mortality | life span | fecundity | fertility

Mutations, laboratory selection regimes, and environmental perturbations that produce longer life in animals typically involve decreases in metabolic rate, physical activity, and/or early-life fecundity (1–11). Examples in many types of animals and yeast show that modifications of insulin-signaling pathways affect life span in a coordinate fashion with growth, reproduction, and metabolism (1, 12). A well documented environmental perturbation, caloric restriction, also affects aging and may involve the modulation of Sir2-mediated and insulin-like signaling pathways (1, 13). All of these mechanisms generally are supportive of both antagonistic pleiotropy and life history optimization models that pose tradeoffs between longevity and fitness early in life. Reductions in the ability to be active and reproduce may be linked irrevocably to life span extension, in which case life span extension is unlikely to be generally useful in agriculture or medicine. Thus, for both our basic understanding of the mechanisms of aging and our outlook on the potential utility of life span extension, it is critical to determine whether life span extension necessarily has negative impacts on organismal performance and fecundity.

The rate of aging usually is assessed demographically. Interventions that primarily alter the rate of aging change the slope of the mortality curve and are thought to reflect modifications in the process of aging itself (5, 14). Ambient temperature and physical activity in poikilotherms, and caloric restriction in mammals, are examples of alterations that change life span and alter the slope of the mortality curve (14). Life-extending interventions in *Drosophila*, such as laboratory-selected, long-lived lines and mutations in *methuselah* (*mth*) and *Insulin-like Receptor* (*InR*), primarily change the intercept, decreasing the baseline mortality, with only minor effects on the mortality slope and, hence, the demographic rate of aging (11, 14, 15). Aside from caloric restriction in mammals, all known interventions that change the slope of the mortality curve also show an associated alteration in growth and/or metabolic rate (ambient temperature, physical activity, and mutations in *Hyperkinetic* and *Shaker*; refs. 16–18).

The *Indy* mutation causes an approximate doubling of average adult life span and an increase in lifetime fecundity in the long-lived *Indy* heterozygous animals without a reduction in the rate of development or growth (19). Life span extension of *Indy* mutant flies could arise from a change in either the intercept or the slope of the mortality curve, and the high lifetime fecundity could be because of reduced metabolic rates and fecundity early in life. Thus, knowledge of the time course of mortality, fecundity, and energy metabolism is critical. Alternatively to the expectation of tradeoffs, the *Indy* mutation may represent an interesting mechanism of life span extension in which normal, age-specific rates of energy processing take place with little measurable decrease in early fecundity or physical activity. Demonstration of a normal life history for metabolism, fecundity, and physical activity in these long-lived animals would indicate that life span extension need not require a reduction of these important elements of organismal performance or at least provoke a wider examination in *Indy* mutant flies of factors such as energy storage levels and stress resistance that might comprise a more subtle cost.

Materials and Methods

Life Span Studies and Mortality-Rate Analysis. Life span studies were performed according to ref. 19. All flies were kept in a temperature-controlled environmental chamber, 12-h light/12-h dark cycle at 25°C, and passed every 2 days into fresh food vials. Long-lived *Indy* mutants *Indy206* (independent replication of *Indy206* life span and mortality are designated *Indy206-1* and *Indy206-2*) and *Indy302* were compared with those from male normal-lived heterozygous control animals derived from the original mutagenesis (2216). All animals were made heterozygous by crossing to a Canton S-5 lab stock (CS-5).

Mortality curves were calculated for males by using 2-day intervals in the calculations according to ref. 20. Males were used to avoid the confounding elements associated with female reproduction. Briefly, a series of hypothesis tests based on the maximum likelihood approach was performed. This method identifies the best fitting model (within the Gompertz family of models) for a particular cohort, obtains maximum likelihood estimates of the parameters of that model (along with 95% confidence intervals), and provides a framework for tests of differences in parameter values between two or more cohorts. The results from *Indy206-1* and *Indy302* were best fit by using the

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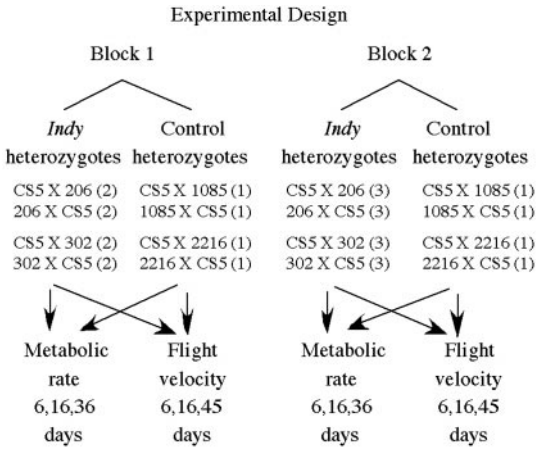

Fig. 1. Experimental design for crosses and physiological measurements. The numbers of replicate crosses are shown in parentheses. Days are the average number of days posteclosion on which physiological measurements were made.

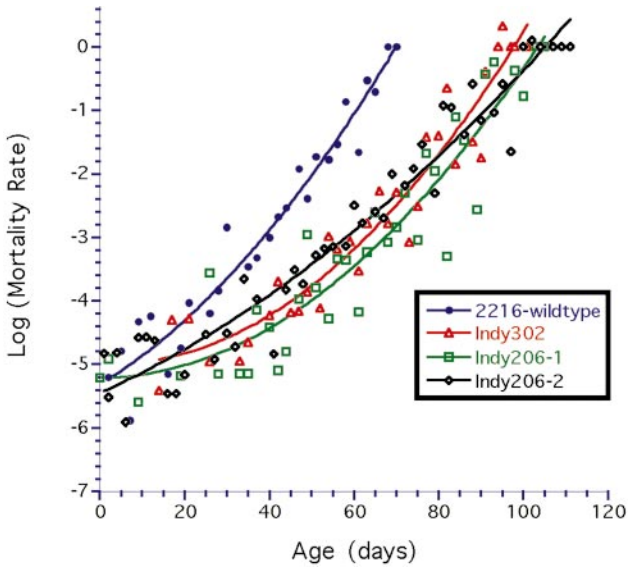

Fig. 2. *Indy* long-lived mutants show a decrease in the slope of the mortality curve. Mortality curves for males heterozygote for *Indy302* (red open triangle; $n = 149$), *Indy206–1* (green open square; $n = 183$), *Indy206–2* (black open diamond; $n = 500$), and 2216-control (blue solid circle; $n = 372$) at 25°C were calculated according to refs. 14 and 20. Days are number of days posteclosion. Curves were fit to the points by using a second polynomial equation.

Gompertz model, but *Indy206-2* and 2216 were best fit by using the Gompertz–Makeham model. Therefore, to perform comparisons, the Gompertz–Makeham model was used in all calculations (20). Further decomposition techniques were used to determine whether the life span differences seen between *Indy* and controls are because of the rate parameter (14).

Experimental Design for Metabolism and Flight Tests. To test for potential effects of the *Indy* mutation on metabolic rate and flight performance, we compared long-lived *Indy* heterozygous mutant animals from two different *Indy* alleles, *Indy206* (206) and *Indy302* (302), with normal-lived heterozygote control animals derived from the original mutagenesis (1085 and 2216; ref. 19). All animals were made heterozygous by crossing to a Canton S-5 lab stock (CS-5). For crosses involving the *Indy* mutation, we performed replicated crosses with different parental origin of the *Indy* mutation. For each cross, five virgin females and five virgin males were allowed to mate and produce eggs for 5 days. The crosses and physiological measurements were conducted in two replicate blocks, with crosses replicated within each block (Fig. 1). This experimental design used a high degree of replication, which allowed us to determine whether the results were repeatable across blocks and to detect any effects of parental and/or rearing condition.

Offspring were collected every other day, aged, and placed on fresh medium every 10 days. Males were collected by using light CO_2 anesthetization and allowed to recover for 48 h before measures of metabolic rate or flight performance. Measures of metabolic rate were made for males at 6, 16, and 36 days posteclosion; flight performance was measured in males at 6, 16, and 45 days posteclosion. Different individuals were used for the metabolic and flight measurements at the three ages.

Respirometry to Determine Resting Metabolic Rate. Metabolic rates were measured by using groups of five known-age male flies at rest within flow-through respirometry chambers. Dry, CO_2-free air was passed through the 10-ml respirometry chambers at 0.1 liter/min and then dried again and passed through a Li-Cor 6251 carbon dioxide analyzer (Lincoln, NE). Within each run, seven experimental chambers containing flies were sampled in a sequential fashion by using a computer-controlled valve system. Genotypes were assigned randomly to the chambers for each run. One additional chamber was kept empty and sampled before and after each experimental chamber to remove any baseline drift (see Fig. 3*A*). Temperature was measured by using a thermocouple within the empty chamber. Analog signals from the flow meter, carbon dioxide analyzer, and thermocouple were converted to digital and recorded on a computer (Sable Systems, Salt Lake City). The total sample size was 154 measures of metabolic rate.

Flight Performance. We used a three-dimensional tracking system to measure the velocity of individual flies during ≈0.2 s of free flight after takeoff. This is sufficient time for *Drosophila* to attain steady-state physiological conditions, and they show little or no acceleration after the first 0.05 s of flight (21). The flight-tracking system consisted of spatially calibrated digital cameras and video analyzers (Qualisys, Glastonbury, CT) that detect flies as dark spots moving against a bright background (details are given in ref. 21). Flies were allowed to take off from an open vial, whereupon the video tracking system collected three-dimensional coordinates at a rate of 60 samples per second. Each fly was tracked only once. A total of 8,615 coordinates from 652 flights were obtained. We determined the maximal velocity of each fly and used that value in our data analyses.

Fecundity and Eclosion. Age-specific fecundity was determined from daily counts of eggs produced by individual females from 21 replicate vials of each of the experimental and control lines. Heterozygote females (Indy206/CS-5, Indy302/CS-5, 1085/CS-5, and 2216/CS-5) along with males were placed, one pair each, into a plastic shell vial containing high-quality or reduced-calorie fly food according to refs. 19 and 22. Each day, they were passed into new vials, and the number of eggs present was recorded. Starting at 10 days and for an additional 8 days, the number of adults that eclosed from each vial also was recorded.

Results

Mortality Rates. Mortality curves for long-lived *Indy* mutants *Indy206* (replicates *Indy206-1* and *Indy206-2*) and *Indy302* along with the normal-lived heterozygous control, 2216, are shown in Fig. 2. The mortality curve slopes (Table 1) were similar among the *Indy* long-lived animals (0.146, 0.139, and 0.141, respectively

Table 1. Slope and intercept of male mortality rates

Genotype (no. of animals)	Slope estimate (95% confidence intervals)	Intercept estimate (95% confidence intervals)
Indy206-1 (183)	0.146 (0.122, 0.174)	0.00038 (0.0001, 0.0013)
Indy206-2 (500)	0.139 (0.124, 0.156)	0.0007 (0.0004, 0.0013)
Indy302 (149)	0.141 (0.123, 0.161)	0.0006 (0.0003, 0.0012)
2216 (372)	0.244 (0.213, 0.278)	0.00024 (0.0001, 0.0006)

Analysis as per refs. 14 and 20.

for *Indy206-1*, *Indy206-2*, and *Indy302*) and significantly different from the 2216 control (0.244). A likelihood ratio test for the equality of slopes between *Indy206-2* and 2216 yielded $P = 2.5 \times 10E^{-10}$, between *Indy206-1* and 2216, $P = 4.45 \times 10E^{-6}$, and between *Indy302* and 2216, $P = 2.93 \times 10E^{-6}$. The intercept estimates were quite similar among all of the lines. A test for equality of intercepts between *Indy206–2* and 2216 yields $P = 0.04$; between *Indy206–1* and 2216, $P = 0.5$; and between *Indy302* and 2216, $P = 0.19$. These statistical analyses demonstrate a significant difference in the slope of the mortality curves between long-lived *Indy* heterozygote and normal-lived 2216 heterozygote animals, with no evidence for consistent differences in intercept. Furthermore, decomposition techniques show that 100% of the life span difference between 2216 and *Indy206-2* is due to the rate parameter: the slope of the mortality curve (14).

***Indy* Long-Lived Animals Have Normal Metabolic Rates.** Metabolic rates were measured from the CO_2 emission of groups of five known-age male flies at rest within flow-through respirometry chambers (Fig. 3*A*). To test the hypothesis that metabolic rate is reduced in long-lived *Indy* heterozygotes, we first used a general linear model that examined effects of temperature, chamber, and body mass (total fresh mass of the five flies within each respirometry chamber) among our 154 replicate measures of metabolic rate. Residuals from that model then were examined for effects of block, parent of origin, age, genotype, and genotype by age interaction. The first model explained 35% of the total variation ($P < 0.0001$) and showed significant effects of temperature (24–27°C range; $P < 0.0001$), chamber ($P = 0.0001$), and body mass ($P < 0.0001$). The second model explained an additional 29% of the variation ($P < 0.0001$), primarily because of a significant increase in metabolic rate with increasing age ($P < 0.0001$). There were no significant effects of block ($P = 0.17$), parent of origin ($P = 0.83$), genotype ($P = 0.15$), or genotype by age interaction ($P = 0.45$). Thus, despite our ability to detect other effects, the two long-lived *Indy* heterozygote lines showed no significant difference in metabolic rate compared with normal-lived controls (Fig. 3*B*).

***Indy* Long-Lived Animals Have Normal Flight Velocity.** Flight velocity was measured from recordings of the three-dimensional path of flies during free flight (Fig. 4 *A* and *B*; ref. 21). A general linear model ($r^2 = 0.12$, $P < 0.0001$) showed no effect of experimental block ($P = 0.32$), but there were significant effects of parent of origin for the *Indy* mutation ($P = 0\ 017$), genotype ($P < 0.001$), age ($P = 0.04$), and genotype × age ($P = 0.0015$). However, these effects were not associated consistently with the *Indy* mutation, because the two long-lived *Indy* heterozygote lines showed no significant difference in maximum flight velocity compared with normal-lived controls ($P = 0.14$; Fig. 4*B*). All of these genotypes showed flight velocities close to or greater than velocities reported for lines established from wild-caught *Drosophila* (464–516 mm·s^{-1} at mean age of 7 days) that were tested by using identical methods (21).

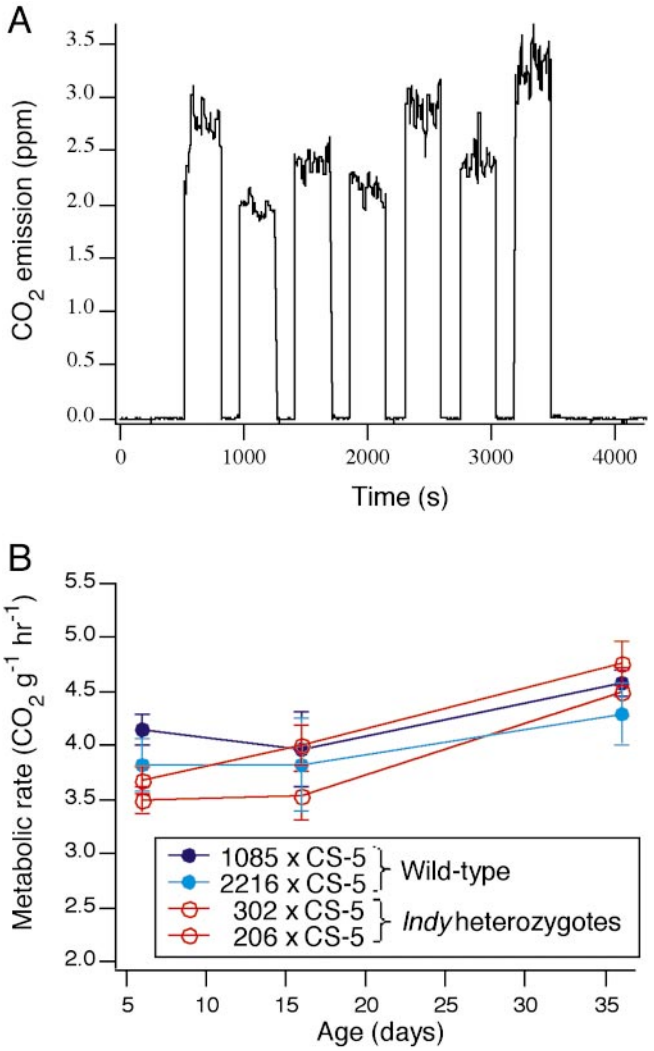

Fig. 3. *Indy* long-lived mutants have a normal resting metabolic rate. (*A*) Representative recording of CO_2 emission from groups of five male flies within each of seven respirometry chambers. A blank chamber was sampled before and after each experimental chamber to monitor and account for any shifts in the baseline. A 180-s period of wash-in/wash-out at each shift between the blank and each experimental respirometry chamber was omitted from the data collection; this omission accounts for the square-wave quality of the CO_2 emission trace. (*B*) All genotypes showed a significant increase in metabolic rate with increasing age, but there was no consistent difference in the metabolic rate of lines heterozygous for *Indy* mutations (302 × CS-5; 206 × CS-5) vs. control lines. (Bars, ±1 SE.) The total sample size is 154 measures of metabolic rate.

Age-Specific Fecundity in *Indy* Long-Lived Animals. Age-specific fecundity was determined from daily counts of eggs produced by individual females from 21 replicate vials of each of the experimental and control lines (19). Under normal rearing conditions (high-quality food; 25°C), *Indy* lines had higher fecundity early in life compared with normal-lived control lines ($P < 0.001$ for the effect of the *Indy* mutation on daily egg production over the first 25 days of life), and they had higher total lifetime egg production (Fig. 5 *Upper Left*). This changed markedly when the flies were reared on low-calorie food (22); in this case, *Indy* mutants showed a significant decrease in early fecundity ($P = 0.013$ for the effect of the *Indy* mutation on daily fecundity over the first 25 days of life) and lifetime fecundity compared with normal-lived controls (Fig. 5 *Upper Right*).

The rate of egg hatching, larval survival, and, ultimately, adult

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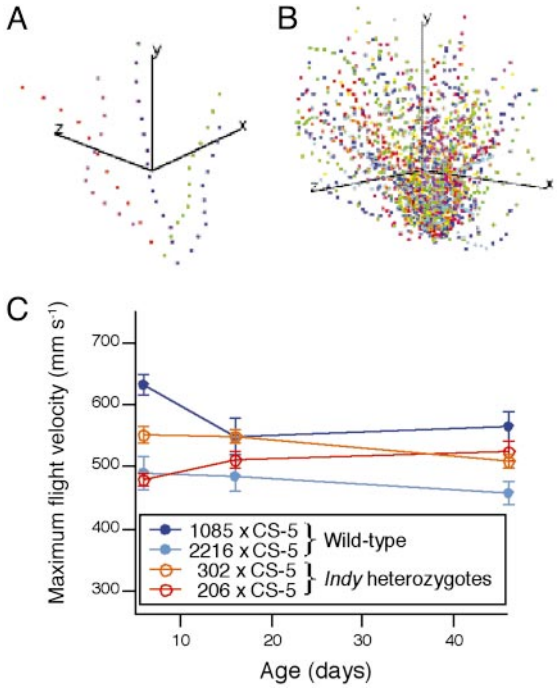

Fig. 4. *Indy* long-lived mutants have a normal maximal flight velocity as measured with a three-dimensional tracking system (21). (*A*) Representative plot of three-dimensional coordinates obtained from five flies during ≈0.2 s after takeoff from a point near the bottom of the figure. The axes labeled *x* and *z* are horizontal; the axis labeled *y* is vertical. The time interval between successive points is 0.017 s. (*B*) Three-dimensional coordinates for flights of all of the 652 flies sampled in this experiment. (*C*) There was no consistent difference in the maximum flight velocity of lines heterozygous for *Indy* mutations (302 × CS-5; 206 × CS-5) vs. control lines. (Bars, ±1 SE.)

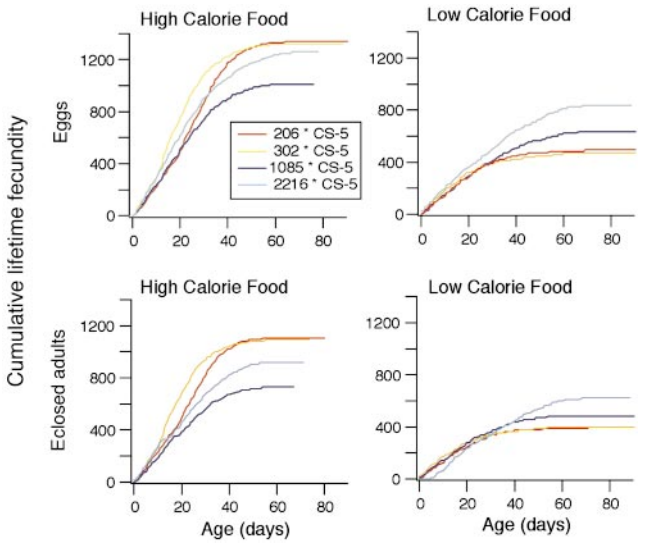

Fig. 5. Mean cumulative lifetime fecundity of female *Indy* long-lived mutants is not reduced. Lines heterozygous for the *Indy* mutation showed greater early egg production (≤25 days) and greater total lifetime egg production when reared on high-quality food (*Upper Left*) but showed reduced early (≤25 days) and total lifetime egg production when reared on low-calorie food (*Upper Right*; ref. 22). (*Lower*) Total production of adult offspring; patterns are essentially the same as for egg production. Error bars have been omitted from all of these plots for the sake of clarity.

eclosion over the first 40 days of adult life was 80% or more for each of the different female lines tested (87 ± 9; 82 ± 12; 82 ± 11; and 78 ± 17 SD for 206, 302, 2216, and 1085 heterozygotes, respectively, under high-quality food). Because of this homogeneity, measurement of lifetime fecundity as the production of adult offspring (Fig. 5 *Lower*) yielded essentially the same result that we obtained for egg production.

Discussion

These data show that the life-extending *Indy* mutation is associated with a decrease in the slope of the mortality curve, which implies that the *Indy* mutation causes a deceleration of the normal aging process, at least demographically. Unlike most other life-extending interventions that decrease the slope of the mortality curve (decreases in ambient temperature or physical activity in insects), *Indy* long-lived animals do not have a reduction in metabolic rate, maximal flight performance (a measure of maximal physical activity), or age-specific female fecundity. However, under conditions of nutritive stress, when the quality of food was significantly reduced, *Indy* mutant females were unable to maintain high reproductive rates. These results indicate that there is not necessarily a tradeoff between longevity and certain major features of organismal performance under all environmental conditions. Rather, we find that all ages of long-lived *Indy* mutant flies can perform as well (metabolism and locomotion) or better (female fecundity) than wild-type flies when the environment is nonstressful. This does not argue against evolutionary models that assume tradeoffs between longevity and age-specific organismal performance, because environmental variation and stress probably are ubiquitous in nature. In fact, our results provide insight regarding one of the possible mechanisms that maintains the wild-type *Indy* allele in nature, where the occurrence of nutritive stress presumably is common enough to counteract the heterozygote advantage that *Indy* mutant alleles display in the laboratory (likewise for other hypothetical alleles that create the same physiological effect even when homozygous). A similar result has been reported for the long-lived *Caenorhabditis elegans* mutation *age-1*, which is maintained in a mixed laboratory population with wild type when food supply is constant, but is lost rapidly from the population when subjected to periodic cycles of food deprivation (23).

Indy mutations consist of insertions of transposable elements in noncoding regions of the *Indy* gene (19), which cause a decrease in expression of the INDY protein that transports Krebs cycle intermediates through the epithelium of the gut and across cell plasma membranes into fat body and oneocytes, tissues important for intermediary metabolism in the fly (24, 25). Reduced expression of INDY protein is likely to change the uptake, utilization, or storage of metabolites but apparently not enough to affect metabolism, locomotion, or reproduction when food quality is high. Other *Drosophila* genotypes that have a reduced feeding rate show increased expression of free radical protective genes (8), and caloric restriction in mammals is associated with a reduction in the accumulation of oxidative damage with age (26). Thus, one hypothesis for the absence of decreases in metabolism, physical activity, or female fecundity of long-lived *Indy* heterozygotes reared on normal food is that their metabolite flux is altered in a way that is not sufficient to cause deficits in metabolic rate, locomotion, or fecundity but is sufficient to decrease oxygen radical formation and/or trigger pathways that up-regulate free radical or other damage protective genes. The only other known, life-extending intervention that decreases the slope of the mortality curve without an obvious decrease in metabolic rate is caloric restriction in mammals, with which *Indy* mutations may share some elements (19, 24, 27, 28).

In summary, our results are compatible with evolutionary models that predict tradeoffs (1, 2, 29–32), which we show exist when food quality is reduced. However, under normal food

conditions, *Indy* mutant animals live nearly twice as long as normal without reductions in metabolic rate, female reproduction, or motility. The decrease in the slope of the mortality curve suggests that the mechanisms by which *Indy* life span extension occurs are through a slowing in the normal rate of aging, implying that this mutation could provide an important model system for understanding the process of aging. Furthermore, these data suggest that there may exist interventions that extend life span without significant negative affects on key features of organismal performance. Conditions that create a proper dosage of protective effects in environments that maintain constant, favorable conditions may provide settings in which it is possible to extend life span without negatively impacting some of the major components of quality of life.

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