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Author for correspondence:
Walter F. Eanes
e-mail: walter.eanes@stonybrook.edu

[†]Present Address: Department of Biology, University of Louisiana at Monroe, Monroe, LA, USA.
[‡]Present Address: Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY, USA.
[§]Present Address: Biological, Environmental and Climate Sciences Department, Brookhaven National Labs, Upton, NY, USA.

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Genetic perturbation of key central metabolic genes extends lifespan in *Drosophila* and affects response to dietary restriction

Matthew E. Talbert[†], Brittany Barnett, Robert Hoff[‡], Maria Amella, Kate Kuczynski[§], Erik Lavington, Spencer Koury, Evgeny Brud and Walter F. Eanes

Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY 11794, USA

There is a connection between nutrient inputs, energy-sensing pathways, lifespan variation and aging. Despite the role of metabolic enzymes in energy homeostasis and their metabolites as nutrient signals, little is known about how their gene expression impacts lifespan. In this report, we use P-element mutagenesis in *Drosophila* to study the effect on lifespan of reductions in expression of seven central metabolic enzymes, and contrast the effects on normal diet and dietary restriction. The major observation is that for five of seven genes, the reduction of gene expression extends lifespan on one or both diets. Two genes are involved in redox balance, and we observe that lower activity genotypes significantly extend lifespan. The hexokinases also show extension of lifespan with reduced gene activity. Since both affect the ATP/ADP ratio, this connects with the role of AMP-activated protein kinase as an energy sensor in regulating lifespan and mediating caloric restriction. These genes possess significant expression variation in natural populations, and our experimental genotypes span this level of natural activity variation. Our studies link the readout of energy state with the perturbation of the genes of central metabolism and demonstrate their effect on lifespan.

1. Introduction

In their lifetime, all organisms experience environments that change both temporally and spatially in their nutrient availability and energy content. The optimal utilization and storage of available energy is a physiological challenge that shapes variation in life-history phenotypes, and in principle sets the trade-off between lifespan and reproduction [1]. Failing to allocate energy optimally to the changing availability of nutrients would be expected to have significant fitness costs. As a consequence, nutrient sensing and response networks are strongly conserved pathways [2]. Since there are optimal physiological responses that reset internal energy balance for different environments, we should expect genetic variation associated with these responses.

As potential modifiers of cell energy state through their action on metabolite levels, the genes of central metabolism are potential sources for this genetic variation. The response to changing nutrient input results in intercellular signals that drive a cascade of downstream gene transcription shifts that facilitate energy utilization and storage [3]. The proximal signal is generally derived from specific metabolite levels as they change under shifting nutrient load [4]. There is considerable precedent for this general mechanism; for example, the well-known action of glucose on insulin secretion in vertebrates [5], and the signals associated with the secretion and action of adipokinetic hormone (AKH) in insects [6]. The effect of energy balance on longevity in yeast is well known [7,8]. Since metabolite concentrations act as proximal signals and also appear to correlate with the associated gene expression levels of the

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component steps in metabolism [9–11], this relationship clearly implicates the natural genetic variation in expression (or activity) of the central metabolic genes as potential sources of genetic variation in setting metabolite levels, and thus play a role in sensing and setting responses. Moreover, we expect specific enzymes will emerge as the targets of natural selection where genetic expression or activity level will act as an 'energy-stat'. Different genotypes will bracket and set the nutrient levels involved in triggering downstream responses that affect traits associated with fitness, such as lifespan variation and fecundity.

The well-established observation that nutritional or dietary restriction (DR) extends lifespan in many species [12,13] is mechanistically related to energy-state signalling [14]. In *Drosophila*, many studies have shown DR to extend lifespan [15–19], and using genetic manipulation, many of the signalling pathways have been identified that extend lifespan and thus effectively mimic DR [2,20]. Experimental work has shown connections between energy-signalling steps and other energy correlated phenotypes such as starvation resistance, nutrient storage and stress resistance that have connections to DR [21–24].

In both plants and animals, it is becoming apparent that many metabolic genes and their enzyme products are associated with roles other than simple processing of metabolites [25–29]. Based on evidence from RNAi reduction screens of central metabolic genes in *Caenorhabditis elegans*, it is estimated that as much as 25% of the genes screened implicate metabolism in longevity extension [30,31]. RNAi knockdown of expression of several genes in the mitochondrial respiratory chain has been shown to extend lifespan in *Drosophila* [32]. In yeast, the overexpression of several of the genes involved in cofactor shuttles actually extends lifespan [7]. Despite its importance as a model in lifespan studies, and the connection between metabolism and lifespan seen in other models, no studies in *Drosophila* have examined the mutational perturbation of central metabolic gene activity and their effects on longevity.

Natural populations of *Drosophila melanogaster* vary in both average lifespan and response of lifespan to dietary challenge [19,33]. In *D. melanogaster*, the genes of the central metabolic pathway harbour considerable sequence and expression variation [34–36], which often shows change with latitude and season [37–39]. In this regard, unlike the other experimental models used in aging studies, *Drosophila* offers the unique opportunity to associate aging and signalling pathways with their population genetics [40,41].

The long-term goal of our studies is to integrate geographical and seasonal variation in metabolic genes with life-history phenotypes, such as longevity and its fitness correlates [42]. In this report, we use matched sets of P-element excision alleles in *D. melanogaster* to create genotypes that possess modest reductions in gene expression and subsequently examine the impact of these perturbations on lifespan under normal and restricted diets. We study seven metabolic genes: *Idh*, *Mdh2*, *Hex-C*, *Hex-A*, *Gpdh*, *Gdh* and *Men*. These enzymes involve possible signalling via glucose, ATP/ADP, NAD/NADH and NADP/NADPH ratios, citrate, pyruvate, malate and glutamate, and they also involve genes with primary expression restricted to the cytoplasm or the mitochondria. We observe a range of effects on lifespan, from none at all to very significant increases in lifespan that can depend on diet.

2. Material and methods

(a) Lifespan design

Under any diet, each genotype was represented by 20 vials; each experiment (genotype and diet) consisted of a total of 80 vials. Each vial has 20 five-day-old virgin females, for a total of 1600 flies phenotyped for each enzyme examined. Flies were transferred to fresh media every 3–4 days and surviving individuals counted at 1–3-day intervals. To make all results comparable with other published studies, food treatments were matched with the dietary formulae used by Min *et al*. [43]. Normal or high-nutrient food (normal diet; DN) consisted of 16 g yeast (SAF Dry Autolyzed), 16 g sucrose and 5.2 g cornmeal. Low-nutrient (DR) food consisted of 4 g yeast (SAF Dry Autolyzed), 4 g sucrose and 5.2 g cornmeal per 100 ml. Vials were not seeded with live yeast. All lines and lifespan studies were maintained at 25°C, and all genotype and dietary experiments for each gene were carried out concurrently.

All experiments were carried out using paired P-element excision lines. All genetic backgrounds were replaced as described in Merritt *et al*. [44] and therefore each paired set differs only in the gene of interest [44–46]. The P-element insertions used to generate the excision allele pairs were: *Idh*, Idh^{EP3729}; *Mdh2*, $Mdh2^{EY01940}$; *Gdh*, $Gdh^{KG00965}$; *Men*, $Men^{BG02790}$; *Hex-A* (*20817*) [*EP(X)352*]; *Hex-C*, $CG8079^{KG09154}$. *Hex-A*, *Idh* and *Gpdh* alleles have been described previously [44,46,47]. In each gene, we have an excision allele with impaired activity and an excision allele with normal activity. Enzyme activities were assayed as in earlier work [44,46–49] and are shown in units of change in optical density per minute (ΔOD). The Men^{9D} allele is a perfect excision. The $Idh^{\Delta 1}$ allele had three single base substitutions in the first 100 nucleotides of the transcript and is an *Idh* knockout [47]. The Men^{7SC2} allele possessed a small 14-nucleotide retention of the 3′ end of the GT element at the insertion site, and acquired a 788-nucleotide deletion 120 nucleotides up-stream of the insertion site. The $Gdh^{9.3}$ allele is a perfect excision. The $Gdh^{24.1}$ allele is an imperfect excision that retains a large portion of the original P-element. The $Mdh2^{20B1}$ excision allele was homozygous lethal and possessed a large 986-nucleotide deletion that removed part of exon 1.

The survival data were analysed using a Cox proportional-hazards model. Individual flies were counted for survival. These observations were used in a Cox proportional-hazards regression against the covariates of genotype and replicate vial for each diet separately. Conversely, diet effects were tested for each genotype separately using the covariates of diet and vial. The relative risk associated with each of the covariates is evaluated statistically as a log-likelihood test with corresponding χ^2 and probability. These were carried out in JMP v. 11.2.0 (SAS Institute Inc.).

(b) Hexokinase activities

Determining the relative activity reductions associated with *Hex-A* and *Hex-C* genotypes is complex since whole fly hexokinase assays combine the activities for both HEXA and HEXC enzymes. The *Hex-A* gene is primarily expressed in the nervous tissue and muscle. The $Hex\text{-}A^{74}$ allele possesses an eight-nucleotide deletion that results in a truncated protein and should be a complete knockout of HEXA. *Hex-C* is largely fat body expressed [50], and the $Hex\text{-}C^{35}$ allele possesses a large deletion that removes all of the 3′UTR and much of the exon and should be a knockout. We estimated the activities associated with the combination of the genotypes for alleles $Hex\text{-}A^{79}$ and $Hex\text{-}A^{74}$, and $Hex\text{-}C^{4}$ and $Hex\text{-}C^{35}$ (figure 1), all in *6326* backgrounds. The genotypes were produced by the four crosses (1) $Hex\text{-}A^{74}$/*6326;6326* females X *6326/Y*; $Hex\text{-}C^{4}$/*6326* males, (2) $Hex\text{-}A^{79}$/*6326;6326* females X *6326/Y*; $Hex\text{-}C^{4}$/*6326* males, (3) $Hex\text{-}A^{79}$/*6326;6326* females X *6326/Y*; $Hex\text{-}C^{35}$/*6326* males and (4) $Hex\text{-}A^{74}$/*6326;6326* females X *6326/Y*;

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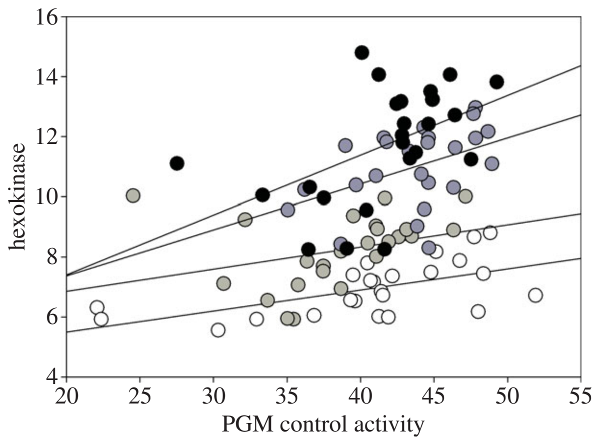

Figure 1. Hexokinase activity of progeny genotypes. The genotypes were produced by the four crosses: (1) *Hex-A*^{74}*/6326;6326* females X *6326/Y*; *Hex-C*^{4}*/6326* males, (2) *Hex-A*^{79}*/6326;6326* females X *6326/Y*; *Hex-C*^{4}*/6326 males*, (3) *Hex-A*^{79}*/6326;6326* females X *6326/Y*; *Hex-C*^{35}*/6326* males and (4) *Hex-A*^{74}*/6326;6326* females X *6326/Y*; *Hex-C*^{35}*/6326 males*. Black = *Hex-A*^{79}*/6326*; *Hex-C*^{4}*/6326*. Dark grey = *Hex-A*^{79}*/6326;6326/Hex-C*^{35}. Light grey = *Hex-A*^{74}*/6326*; *Hex-C*^{4}*/6326*; white = *Hex-A*^{74}*/6326*; *Hex-C*^{35}*/6326*. (Online version in colour.)

Table 1. Two-way ANOVA of hexokinase activities of the *Hex-A* and *Hex-C* genotypes.

genotype	estimate	s.e.	*t*-ratio	*p*
Hex-A^{74}	−1.716	0.133	−12.82	<0.0001
Hex-C^{35}	−0.609	0.133	−4.64	<0.0001
Hex-A^{74}X *Hex-C*^{35}	−0.141	0.129	−1.09	0.2782
PGM control activity	0.1055	0.0241	4.38	<0.0001

Hex-C^{35}*/6326* males. The two-way analysis-of-variance (ANOVA) showed statistically significant reduction in activity associated with each allele (table 1). We estimated that, compared with the presumed full-activity *Hex-A*^{79}*/6326* and *Hex-C*^{4}*/6326* genotypes, the *Hex-A*^{74}*/6326* genotype reduced overall whole-body hexokinase activity by 27%, and the *Hex-C*^{35}*/6326* genotype reduces it by 8% (table 2).

However, the tissue-specific reduction of hexokinase activity associated with each gene and not whole-body activity is the relevant phenotype and its estimation depends on the relative contribution of each tissue to whole-body HEX activity, as well as the activities of the *Hex-A* and *Hex-C* alleles on the *X* and second *6326* chromosomes. The relative contribution of the two enzymes estimated from the isoelectric focusing data [50] is a notably larger contribution of the *Hex-A* gene to whole-body HEX activity. This would appear to explain the smaller reduction for the *Hex-C*^{35}*/6326* genotype. If, as the data suggest, that HEX-C is about 25% of whole-body activity, then it is predicted that the 8% reduction associated with *Hex-C*^{35}*/6326* translates into a 45% HEX reduction in *Hex-C* tissues. The overall analysis supports the expectation that the tissue-associated activities have reduced the knockout heterozygotes by about 50% of normal activity.

3. Results

Idh (isocitrate dehydrogenase (cytosolic)). The results are shown in figure 2*a*. The *Idh*$^{\Delta 1}$*/6326* genotype has a 50%

Table 2. Mean hexokinase activities of the *Hex-A* and *Hex-C* genotypes.

genotype	hex activity	s.e.
Hex-A^{79}*/6326*; *Hex-C*^{4}*/6326*	11.65	0.258
Hex-A^{79}*/6326*; *Hex-C*^{35}*/6326*	10.71	0.265
Hex-A^{74}*/6326*; *Hex-C*^{4}*/6326*	8.49	0.265
Hex-A^{74}*/6326*; *Hex-C*^{35}*/6326*	7.00	0.258

activity reduction in IDH activity (figure 2*a*, inset) [47]. While there is an overall highly significant DR effect in both genotypes (*Idh*$^{\Delta 1}$*/6326*, $\chi^2 = 45.50$, $p < 0.0001$; *Idh*$^{\Delta 4}$*/6326*, $\chi^2 = 65.53$, $p < 0.0001$), there are no significant *Idh* genotype effects on lifespan on either diet.

Men (malic enzyme (cytosolic)). The *Men*^{7SC2}*/6326* genotype has 52.3% of the activity of the *Men*^{9D}*/6326* genotype ($F_{1,28} = 37.6$, $p < 0.0001$). The results are shown in figure 2*b*. There is a highly significant diet effect, for the high-activity *Men* genotype (*Men*^{9D}*/6326*, $\chi^2 = 288.28$, $p < 0.0001$), but not the low-activity *Men*^{7SC2}*/6326* genotype (less than 1 day, $\chi^2 = 1.15$, $p < 0.2837$). Under DR, the *Men* genotypes had significantly different lifespans ($\chi^2 = 11.19$, $p < 0.0008$), where the normal activity genotype showed a large and highly significant extension of lifespan compared with the reduced activity genotype (more than 12 days; $\chi^2 = 461.41$, $p < 0.0001$).

Hex-A (hexokinase-A). The results for *Hex-A* are summarized in figure 3*a*. Both genotypes responded to DR significantly (*Hex*^{79}*/6326*, $\chi^2 = 35.08$, $p < 0.0001$; *Hex*^{74}*/6326*, $\chi^2 = 6.924$, $p < 0.0085$, less than 1 day). There was a significant genotype difference under DN diet ($\chi^2 = 67.92$, $p < 0.0001$), with the lower-activity *Hex-A*^{74}*/6326* genotype living 10% longer (more than 4 days). There was no significant difference between genotypes under DR ($\chi^2 = 0.0036$, $p < 0.952$)

Hex-C (hexokinase-C). Both diet and *Hex-C* genotype had effects on lifespan (figure 3*b*). There was a DR effect for both the normal full-activity *Hex-C*^{4}*/6326* genotype ($\chi^2 = 116.5$, $p < 0.0001$) and *Hex-C*^{35} ($\chi^2 = 48.1$, $p < 0.0001$) genotypes. There were significant extensions of lifespan (more than 6 days) for the low *Hex-C*^{35}*/6326* genotype compared with the normal activity *Hex-C*^{4}*/6326* genotype under both diets, although the DR genotype effect was small (DN, $\chi^2 = 50.58$, $p < 0.0001$; DR, $\chi^2 = 12.41$, $p < 0.0004$).

Gdh (glutamate dehydrogenase (mitochondrial) (figure 4*a*)). The *Gdh*$^{24.1}$*/6326* genotype had 71% of the activity of the normal *Gdh*$^{9.3}$*/6326* genotype ($F_{1,87} = 364.2$, $p < 0.0001$). Both genotypes showed an effect of DR on lifespan (*Gdh*$^{24.1}$*/6326*; $\chi^2 = 145.70$, $p < 0.0001$; *Gdh*$^{9.3}$*/6326*, $\chi^2 = 43.48$, $p < 0.0001$). The normal and reduced *Gdh* genotypes had identical lifespans under DN diet ($\chi^2 = 0.461$, $p < 0.4970$). Under DR, the *Gdh*$^{24.1}$*/6326* genotype shows a significantly longer lifespan (more than 14 days; $\chi^2 = 89.37$, $p < 0.0001$).

Mdh2 (malate dehydrogenase (mitochondrial) (figure 4*b*)). About 85% of the malate dehydrogenase activity in the whole body is MDH2 (mitochondrial) in origin [51]. *Mdh-2*^{20b1}*/6326* had 56% of the activity of the normal *Mdh*^{21w1}*/6326* genotype ($F_{1,27} = 99.75$, $p < 0.0001$). For both *Mdh2* genotypes, there was a large DR effect (*Mdh*^{21w1}*/6326*, $\chi^2 = 138.84$, $p <$ 0.0001; *Mdh-2*^{20b1}*/6326*, $\chi^2 = 259.62$, $p < 0.0001$). The low-activity *Mdh2*^{20B1}*/6326* genotype possessed a significantly longer lifespan than the higher-activity genotype under DR

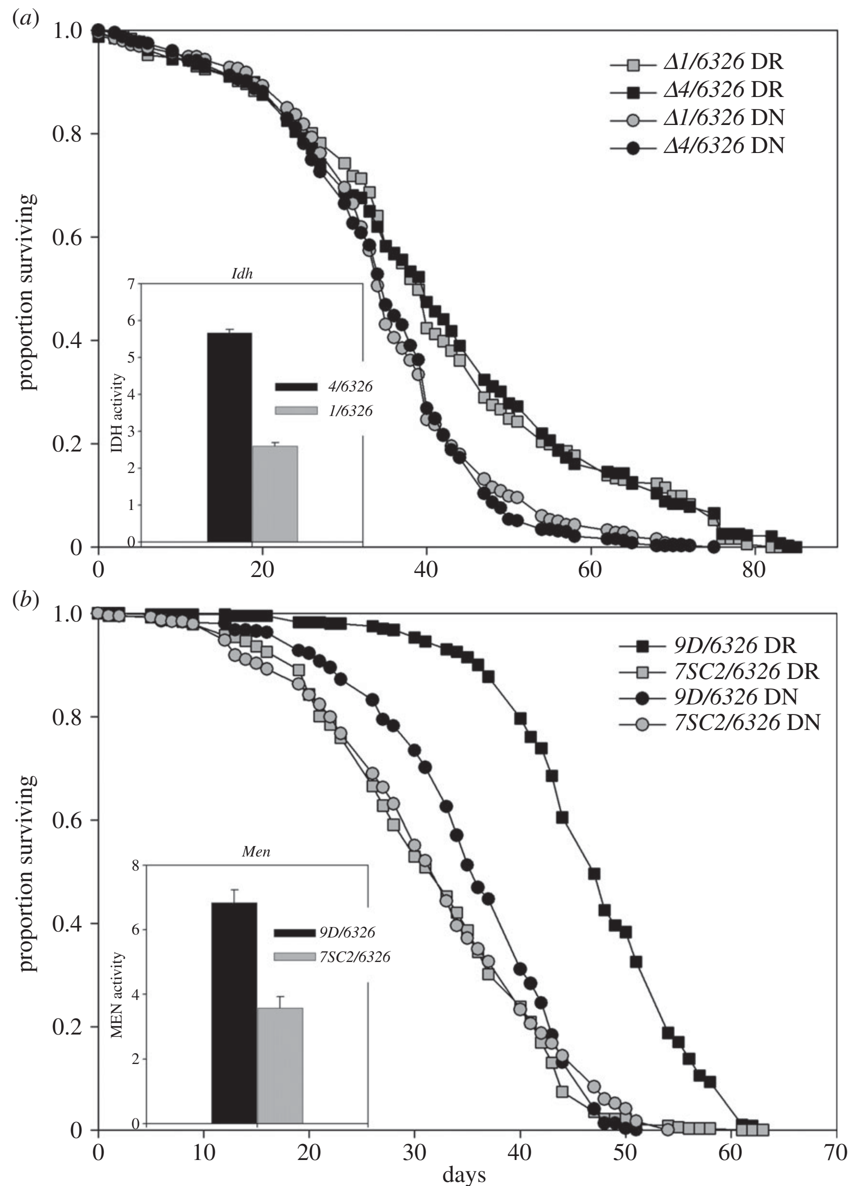

Figure 2. The survival of *Idh* and *Men* genotypes of female *D. melanogaster* under normal and DR. (*a*) *Idh* genotypes *IdhΔ1/6326* (grey) and *Δ4/6326* (black) under normal (DN, circles) and DR (squares). Relative *Idh* genotype activities are shown in inset. (*b*) *Men* genotypes *Men7SC2/6326* (grey) and *9D/6326* (black) under normal (DN, circles) and DR (squares). Relative *Men* genotype activities are shown in inset.

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($\chi^2 = 12.68$, $p < 0.0004$), but not under DN ($\chi^2 = 0.48$, $p < 0.4894$)

Gpdh (glycerol-3-phosphate dehydrogenase (figure 5)). The $Gpdh^{9.2}$ and $Gpdh^{10.3}$ alleles were intercrossed to produce the test genotypes. The $Gpdh^{9.2}$ allele is a full knockout and the $Gpdh^{9.2}/Gpdh^{10.3}$ genotype had 59.6% normal activity ($F_{1,69} = 339.5$, $p < 0.0001$). The *Gpdh* genotypes individually showed no effect of DR ($Gpdh^{10.3}/Gpdh^{10.3}$, $\chi^2 = 2.22$, $p < 0.1359$; $Gpdh^{9.2}/Gpdh^{10.3}$, $\chi^2 = 0.49$, $p < 0.4831$). There was a large and highly significant difference in the effect of *Gpdh* genotype on lifespan seen at both diets (DN, $\chi^2 = 28.39$, $p < 0.0001$; DR, $\chi^2 = 40.37$, $p < 0.0001$), with the lower-activity $Gpdh^{9.2}/Gpdh^{10.3}$genotype possessing the significantly longer average lifespan under both diets (5.5 days or 20% extension in lifespan).

4. Discussion

Energy homeostasis is intimately connected to the signals of nutrient sensing, and it is expected that energy balance ultimately sets the life-history trade-offs that establish longevity. In *D. melanogaster*, we observe that the genetic perturbation of central metabolic genes has significant effects on lifespan in our experimental setting. Moreover, the general observation is that low-activity genotypes show extension of lifespan. Perhaps not surprisingly, some genotype effects are also diet dependent. Some genes (*Idh*, *Mdh2*, *Hex-C*) show an increase in lifespan under DR, but no effect of genotype, while others show a strong (*Gdh*, *Men*) genotype dependence in their response to DR. Finally, *Gpdh* shows a strong dependence on genotype activity, yet no effect of DR. These differences are expected because our observations represent seven enzymes that act on different metabolites and cofactors, and are limited to mitochondrial or cytosolic function.

In our work, we cannot strictly compare experimental outcomes across genes. First, while we are studying single gene effects within identical genetic backgrounds, the backgrounds differ across the seven gene sets. The P-element progenitor lines differ in the type of element used in the excision series (e.g. KG versus EP), and while the replacement backgrounds possess some chromosomes in common (often the *6326* chromosomes),

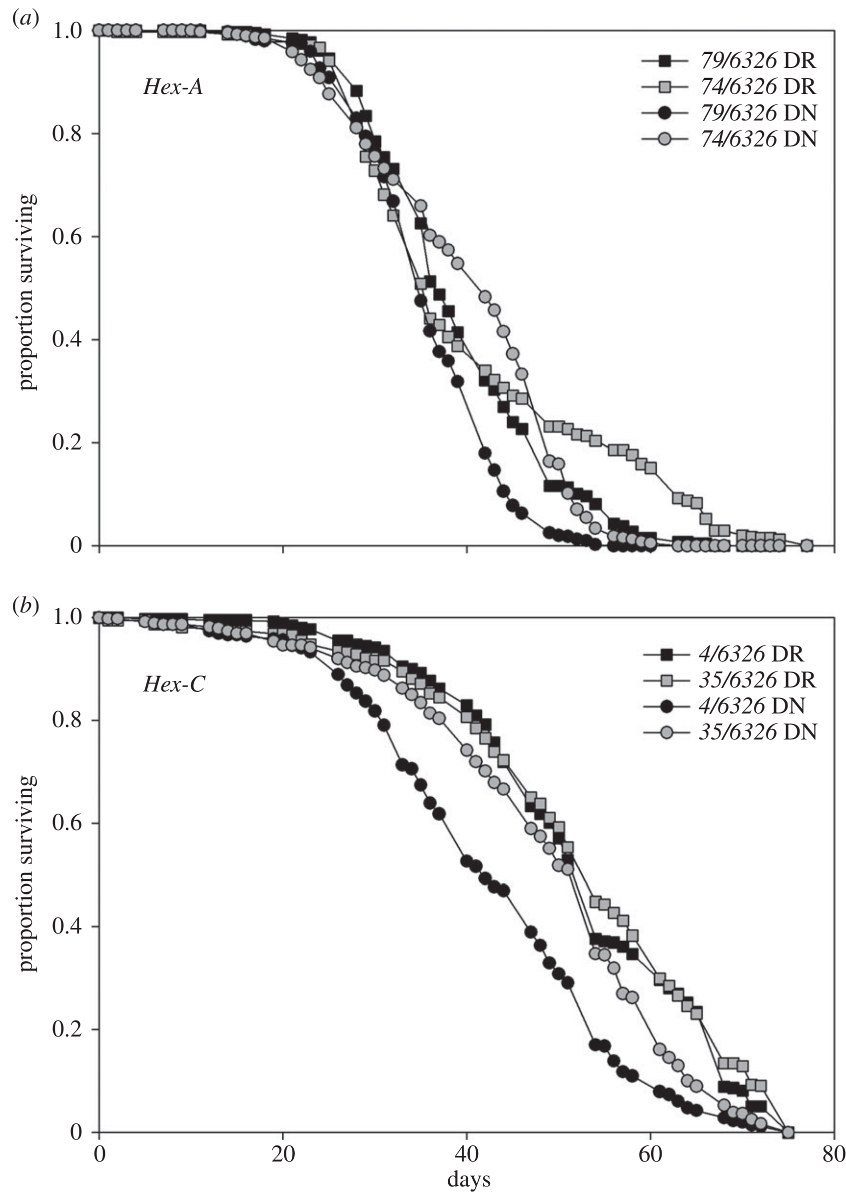

Figure 3. The survival of *Hex-A* and *Hex-C* genotypes of female *D. melanogaster* under normal and DR. (*a*) *Hex-A* genotypes *74/6326* (grey) and *79/6326* (black) under normal (DN, circles) and DR (squares). (*b*) *Hex-C* genotypes *35/6326* (grey) and *4/6326* (black) under normal (DN, circles) and DR (squares). Relative activities are discussed in text.

they generally differ in others. Second, while all of our genotype comparisons involve reductions of activity of 50% or less, we should not expect the same level of flux control across enzymes [42]. Thus, cytosolic IDH may possess little flux control over NADPH/NADP levels, while MEN may exercise greater control over these metabolites, especially at reduced nutrient levels. It should be pointed out that in the Raleigh population the cytosolic *Idh* gene bears little molecular polymorphism and the few SNPs seen show little *cis*-based expression effect or clinal change [38].

It should also be emphasized that unlike many gene-targeted lifespan studies, we are not using full knockout genotypes, or genotypes where the relative functional reduction is unknown, and we have precise estimates of genotype activity. Moreover, these activity differences are representative of the range of much of the *cis*-associated SNP expression variation seen in natural populations [34,38]. The observation that metabolic genes, when perturbed modestly in activity, have an effect on lifespan is certainly relevant to discussions of the maintenance of genetic variation in these genes in natural populations, especially as nutrient levels vary geographically and seasonally [38,39].

Gpdh, *Gdh* and *Hex-A* were highlighted in our study of clinal SNP expression variation in the pathway [38]. These genes, among others, showed significant changes in gene transcript expression with latitude. Our observations here add a fitness component to the causes of genetic expression variation of metabolic genes in natural populations of *Drosophila*. Also, the expectation that the gene-specific extension of lifespan can depend on dietary level adds complexity, since we expect nutritional background to shift locally and seasonally in this species. The effect of reduced activity is incrementally small in terms of daily survival, but when integrated over the average lifespan of a fly this can be very significant. This relationship is in contrast to flight metabolism, where similar activity changes have no effects on flight performance [46].

Our enzymes were targeted because they act on different metabolites and cofactors, and are limited to either mitochondrial or cytosolic function. Both IDH and MEN are cytosolic enzymes and NADPH dependent, and, along with the

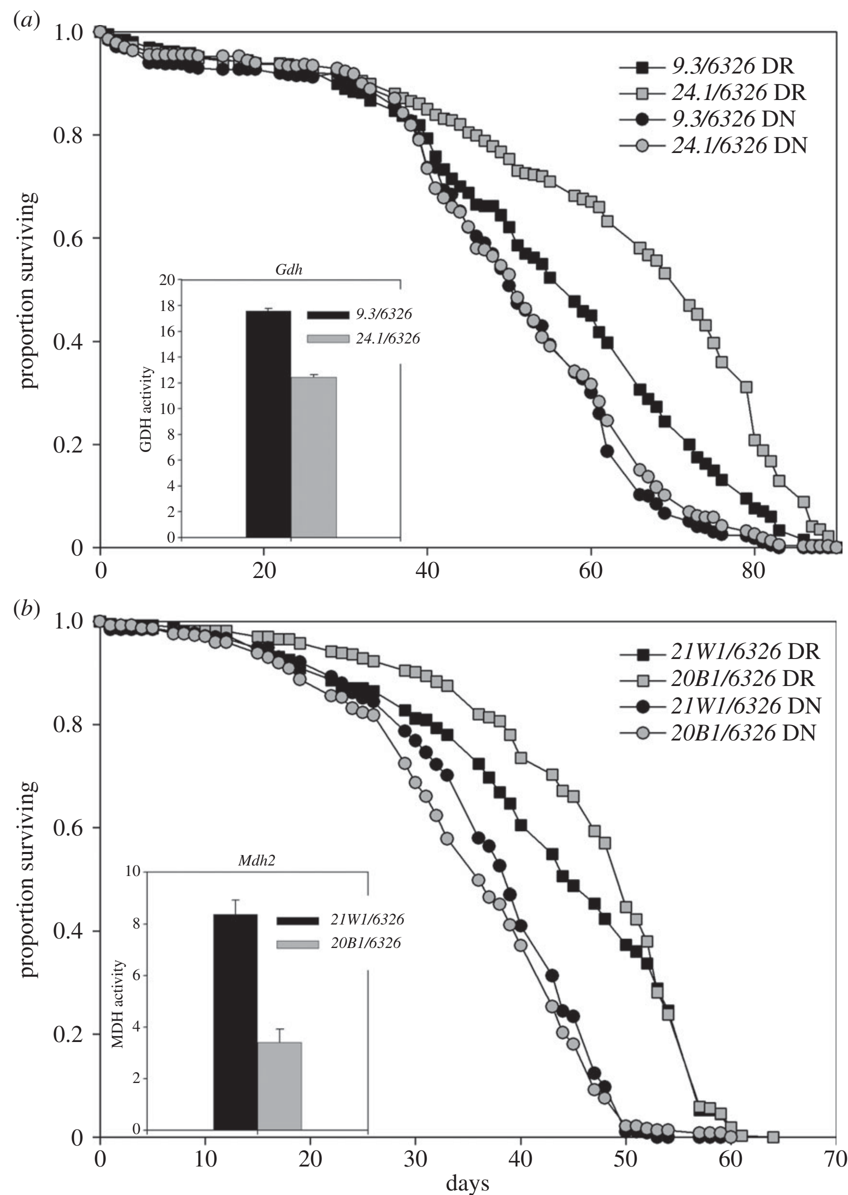

Figure 4. The survival of *Gdh* and *Mdh2* genotypes of female *D. melanogaster* under normal and DR. (*a*) *Gdh* genotypes *24.1/6326* (grey) and *9.3/6326* (black) under normal (DN, circles) and DR (squares). Relative *Gdh* genotype activities are shown in inset. (*b*) *Mdh2* genotypes *20B1/6326* (grey) and *21W1/6326* (black) under normal (DN, circles) and DR (squares). Relative *Mdh2* genotype activities are shown in inset.

pentose shunt enzymes, provide a significant contribution to the NADPH/NADP pool [52]. Both glutamate (GDH) and malate dehydrogenase (MDH2) are limited to mitochondrial function and, like GPDH, are dependent on NAD/NADH, and will impact that redox balance and its effect on signalling and aging [53]. The two hexokinases, HEX-A and HEX-C, potentially affect the ADP/ATP ratio and have different tissue expressions. They could vary the ADP/ATP content, and in that fashion set energy-state response via regulating AMPK signalling [54–56]. This AMP-activated kinase is a sensor that has effects in *Drosophila* [57,58], which provides a link between lifespan and caloric restriction [59].

Our observations for the *Gpdh* and *Gdh* genes implicate mitochondrial function and the redox balance with lifespan extension. GPDH is part of the essential mitochondrial phosphoglycerol shuttle in insects and is often considered a point of ROS production [60]. The NAD/NADH redox balance is emerging as an important element of lifespan extension in yeast [8,61] and is often considered a direct readout of metabolic state. Moreover, the lifespan extension associated with these genes might also act through the Sir-like enzymes, which are NAD-dependent histone deacetylases that silence chromatin and thus control transcription in a fashion directly coupled to energy-state imbalance [62]. This relationship is important because starvation in *Drosophila* has been clearly shown to significantly raise the NAD/NADH ratio [63], although the role of *Sir2* in lifespan extension in *Drosophila* has been questioned [45]. GDH is also limited to mitochondrial function, and potentially affects the redox balance and NAD/NADH ratio. It connects glutamate, a key energy-state signalling molecule, to metabolic control, and sits at the important crossroads of carbohydrate and amino acid metabolism [64,65]. As well, glutamate is at the hub of connectivity in the large central metabolic network [66]. Both of these enzymes show significant extension of lifespan with only 40% reductions in whole-body activity. However, all mitochondrial or NAD-dependent genes are not similar in affecting lifespan; comparable activity changes in mitochondrial and NAD-dependent MDH (*Mdh2*) have little effect on lifespan in either dietary condition.

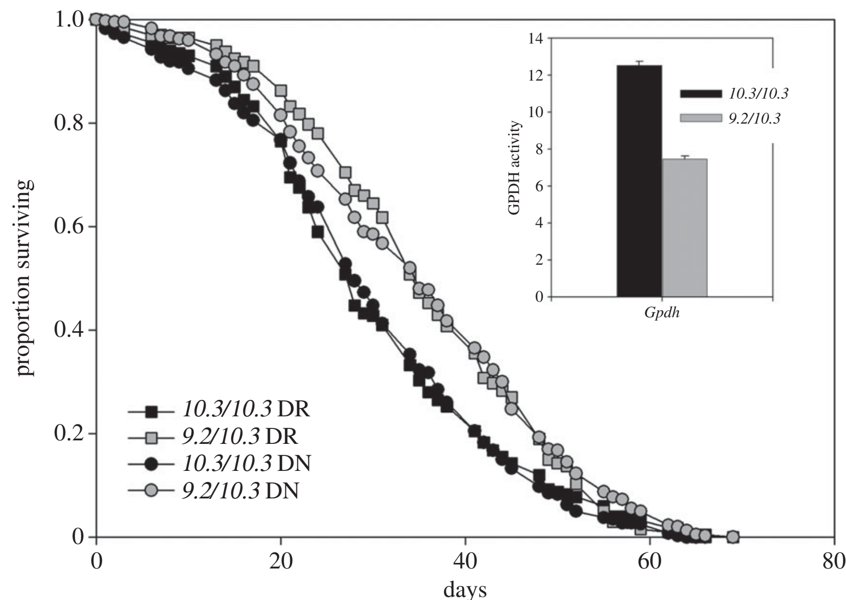

Figure 5. The survival of *Gpdh* genotypes of female *D. melanogaster* under normal and DR. *Gpdh* genotypes *9.2/10.3* (grey) and *10.3/10.3* (black) under normal (DN, circles) and DR (squares). Relative *Gpdh* genotype activities are shown in inset.

The dependence of our results on diet is important. Over the past two decades, DR has been shown to impose a trade-off where it extends lifespan and reduces reproduction fecundity [1]. It has gained prominence because of its association with aging research in general [12], but the phenomenon of DR has obvious relevance to studies of life-history evolution because it will be associated with plastic responses to nutritional challenges in nature, and the potential maintenance of genetic variation. Studies on model organisms have led to the discovery of many genes where mutational perturbation extends lifespan and thus mimics DR restriction [12]. This is most notable in the parallel effects of disruption of genes specifically associated with energy-sensing pathways and signalling of dietary state and DR [12]. Despite its general occurrence in *D. melanogaster* [19], we see that an effect of DR on lifespan is not seen in some of our experimental lines. Two (*Gpdh*, *Hex-A*) of the seven genes show no DR effect in general. For *Gdh*, DR is seen just for the low-activity genotype. This different response to DR is suggested by studies where line-by-diet interactions are noted [67], but is shown more definitively in studies in mice [68] and yeast, where it becomes clear that genetic background affects the response to DR [69,70]. In Schleit *et al*. [70], 166 single, non-essential genes were made deficient in yeast, and a large proportion of genes showed loss of DR extension capability, as well as an enhanced DR response. Clearly, DR response can be modified genetically and it would not be surprising if genetic variation in natural populations were to reflect this observation.

Does the failure of some expression modified genotypes to respond to DR imply a mechanistic connection to the signalling associated with DR? An interaction between genotype and diet would suggest that the lifespan responses to DR may be coupled to metabolic signals associated with these enzymes or pathways. For example, the full-activity *Men* genotype shows a significant DR lifespan response, yet the *Men* low-activity genotype appears resistant to lifespan extension under DR. This may suggest that the NADPH/NADP ratio in the case of MEN is a signal associated with DR. However, the NADPH-dependent IDH shows no genotypic effect on DR, which may contradict this suggestion or simply be because IDH possesses low control over cofactor pool levels. Conversely, the *Gdh* normal activity genotype shows no DR response, while a genotype reduction in *Gdh* activity strongly enhances a DR response. Perhaps the reduction in amino acids that is associated with DR in *Drosophila* [71,72] is *enhanced* by reduction of *Gdh* activity, since glutamate and GDH sit at the crossover of carbohydrate and amino acid metabolism in the mitochondria [65]. However, this interaction must be interpreted with caution, since tests of genotype dependence of DR should be tested by using a range of diet changes [73].

Where might the mechanism of action reside that extends lifespan or is associated with a genotype-dependent response to DR for these metabolic genes? Discussions of energy-signalling pathways typically start with the statement that nutrient levels are first 'sensed' and then the pathway of interest is addressed (e.g. the insulin receptor insulin/TOR pathway in *Drosophila* [74]). Presumably, this initial sensing must emanate from direct immediate readouts of the cell's metabolic state (i.e. metabolites). In *Drosophila*, dietary sugars induce significant metabolite changes [4]. These metabolite levels changes trigger the secretion of neuropeptides from specialized neurosecretory cells [6]. This model of sensing is similar to the regulation of glucagon in mammalian pancreatic cells, and a similar case has been made by Kim & Rulifson [75] for the sensing and regulation of AKH by the corpora cardiac cells in *Drosophila*. It is possible that either these cell-specific or just systemic metabolite levels initiate the signalling process. Genetic variation will regulate these metabolite levels in conjunction with nutrient inputs. In this sense, lifespan extension by some metabolic genes is top-down.

Over the past two decades, a large number of genes in several model species have been observed to extend lifespan when mutated. In *Drosophila*, most studies have emphasized the signalling cascades emanating from neurosecretory cells [6]. However, the most proximal steps of central metabolism must set the signalling environments because their metabolite levels respond immediately to nutrient inputs. Here, we place the metabolic pathway in the discussion of energy signalling and look at the impact of genetic perturbation of some key genes on lifespan. The outcome is that there are numerous examples where reductions in activity extend lifespan. Perhaps

not surprisingly, this extension depends on the nutrient environment. We propose that this setting of lifespan response to gene expression variation also provides a selective context for naturally segregating metabolic gene variation, and moreover may contribute to unravelling the patterns of genetic variation observed in natural populations.

Data accessibility. The raw data for all the lifespan experiments is deposited in Dryad under http://dx.doi.org/10.5061/dryad.r620q.

Authors' contributions. M.E.T., B.B. and R.H. carried out the lifespan experiments. M.E.T. and W.F.E. designed and conceptualized the project. W.F.E. and E.B. drafted the document. W.F.E and E.L. carried out the data analysis. S.K. and E.B. characterized the sequence changes. M.E.T., B.B., M.A. and K.K. recovered the mutant alleles and measured activities.

Competing interests. We declare we have no competing interests.

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