Biochemical and Biophysical Research Communications 456 (2015) 676–682

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Overexpression of malic enzyme in the larval stage extends *Drosophila* lifespan

Gye-Hyeong Kim [1], Young-Eun Lee [1], Gun-Ho Lee, Youn-Ho Cho, Young-Nam Lee, Yeogil Jang, Donggi Paik, Joong-Jean Park *

Department of Physiology, College of Medicine, Korea University, 73 Inchon-ro, Seongbuk-gu, Seoul 136-705, Republic of Korea

ARTICLE INFO

Article history:
Received 2 December 2014
Available online 13 December 2014

Keywords:
Malic enzyme
Pyruvate
Longevity
ROS
Drosophila

ABSTRACT

Metabolic modifications during the developmental period can extend longevity. We found that malic enzyme (*Men*) overexpression during the larval period lengthened the lifespan of *Drosophila*. *Men* overexpression by S106-GeneSwitch-Gal4 driver increased pyruvate content and NADPH/$NADP^+$ ratio but reduced triglyceride, glycogen, and ATP levels in the larvae. ROS levels increased unexpectedly in *Men*-overexpressing larvae. Interestingly, adults exposed to larval *Men*-overexpression maintained ROS tolerance with enhanced expression levels of glutathione-S-transferase D2 and thioredoxin-2. Our results suggest that metabolic changes mediated by Men during development might be related to the control of ROS tolerance and the longevity of *Drosophila*.

1. Introduction

Metabolism is the major regulator of longevity. Reduced metabolism through inhibition of insulin/IGF-1 signaling (IIS) or proper calorie restriction (CR) extends the lifespan in many organisms [1–3]. Intriguingly, timing may be important for the metabolic changes to extend the lifespan. For example, the beneficial effects of CR on lifespan are found mostly in the adult period [3,4]. The lifespan of *Caenorhabditis elegans* was lengthened only when IIS was reduced during the adult period [5]. In rhesus monkeys, a significant decrease in age-related mortality or the incidence of age-related diseases by CR was observed in the adult period [3,6].

In addition to the metabolic benefits in adults, metabolic modifications during the developmental period also control the adult lifespan. Lowering mitochondrial activity through the inhibition of electron chain complex III or V during the larval period reduced ATP production and lengthened the lifespan of nematodes [7]. However, the same treatment during the adult period did not affect the lifespan. One characteristic of developmental metabolic effects on longevity is that the metabolic stimuli remain in the organisms until the adult period; however, such mechanisms remain unclear.

Through a large-scale screen for novel longevity genes, we isolated malic enzyme (Men, CG10120), a homolog of human ME1 (EC 1.1.1.40), and reported that ubiquitous and constitutive overexpression of Men lengthened the lifespan of *Drosophila melanogaster* significantly [8]. Men oxidizes malate to pyruvate in the cytoplasm to produce CO_2 and NADPH. ME1 is involved in the regulation of the cellular pyruvate pool and lipid metabolism [9–12]. NADPH generated by Men is an essential cofactor of several ROS-scavenging enzymes [13–15]. Thus, Men controls both energy metabolism and cellular ROS levels. In the present study, we report that *Men* overexpression during only the larval period extends the adult lifespan. Surprisingly, larval *Men* overexpression by the inducible *S106*-Gal4 driver induced adaptive responses. Without further *Men* overexpression in the adult period, the enhanced ROS tolerance was partially maintained, which might be related to an extension in *Drosophila* lifespan.

2. Materials and methods

2.1. Drosophila stock

Flies were obtained personally or from public stock centers: w^{CS10} wild-type [16], *S106*-GS-Gal4 [17], UAS-*mCD8::GFP*, and UAS-*nls.GFP* (GFP stocks from Bloomington *Drosophila* Stock Center, Bloomington, IN, USA). We established UAS-*Men* flies (described in Supplementary materials and methods). All flies used in this study were isogenized by backcrossing to w^{CS10} 6–8 times. Flies were maintained on the standard fly food at 25 °C, 50% relative humidity, and a 12:12 light/dark cycle [8].

* Corresponding author. Fax: +82 925 5492.
E-mail address: parkjj@korea.ac.kr (J.-J. Park).
[1] Contributed equally on this work.

http://dx.doi.org/10.1016/j.bbrc.2014.12.020
0006-291X/

2.2. Longevity assay

A cohort of 80–140 male or female flies, 1–3 days after eclosion, were grown in an aging chamber [8]. Dead flies were counted, when fresh food was supplied every 2–3 days. To control *Men* overexpression during the larval period, the larvae of *S106*-GS-Gal4/+>UAS-*Men*/+ were given food containing 0, 2.5, 5, or 10 μg/mL RU486 (all chemicals were purchased from Sigma–Aldrich Corp., Louis, MO, USA, if not specified) for the entire larval period.

2.3. Real-time PCR

cDNA was synthesized by reverse transcription with total RNA and oligo-dT. PCR was carried out using a cDNA template and various pairs of primers (Suppl. Table 2) mixed with SYBR Premix EX-Taq™II(Takara Bio Inc., Otsu, Japan). The ABI prism 7000 Sequence Detection System was used to determine real-time amplification curves (Life Technologies Corp., Waltham, MA, USA).

2.4. Measurement of malic enzyme activity

Men activity was measured, as described previously [18]. Flies were homogenized in PBS. The supernatant was mixed with 100 mM triethanolamine hydrochloride, 100 mM malic acid, 20 mM $NADP^+$, and 20 mM $MnCl_2$. Rates of NADPH production were determined by measuring the change in absorbance at 340 nm for 10 min using a microplate reader (Spectramax, Molecular Devices, Sunnyvale, CA, USA).

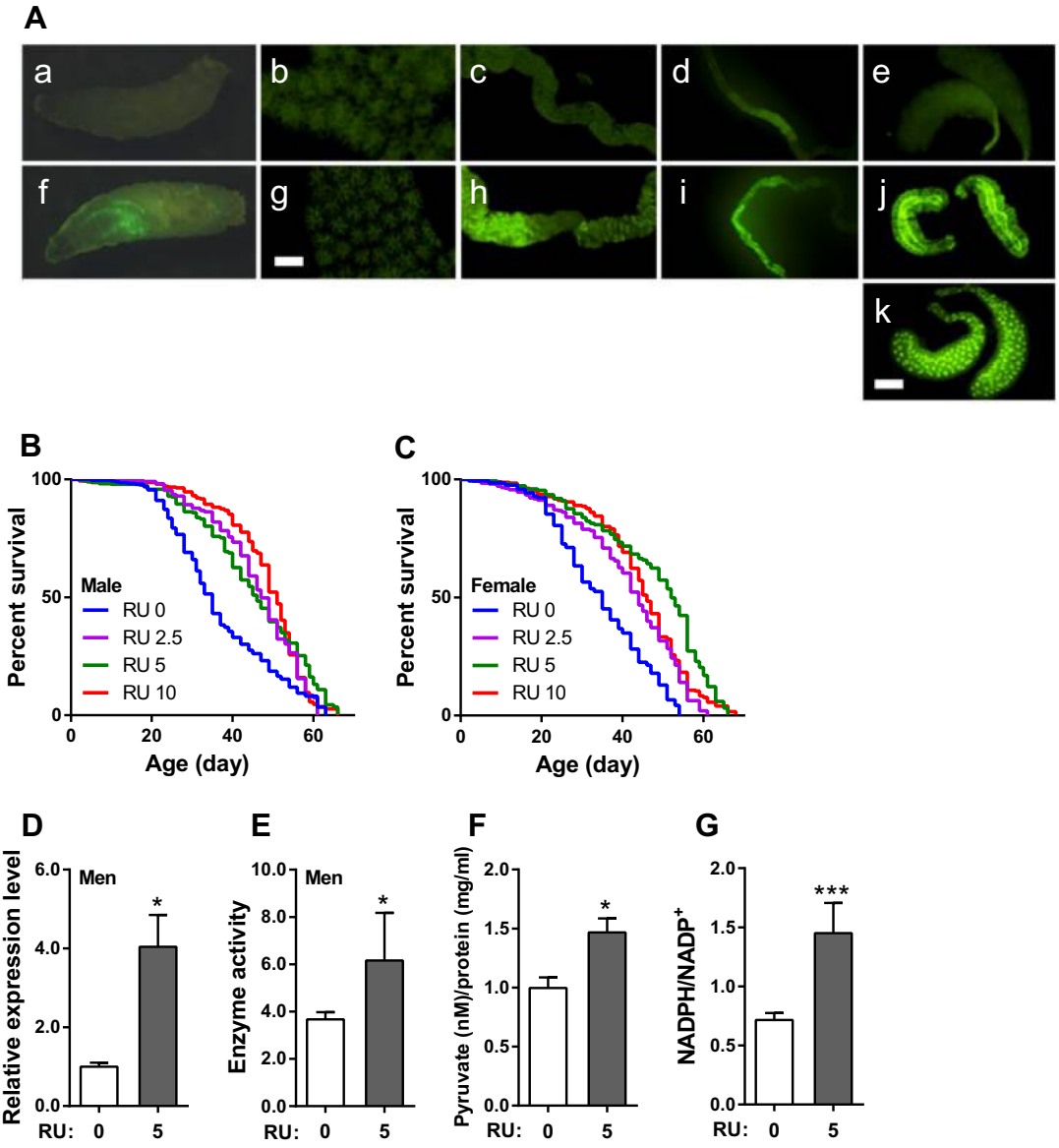

Fig. 1. *Men* overexpression during the larval period extends adult lifespan. The larvae of *S106*-GS-Gal4/+>UAS-*GFP* were fed either 0 (A, a–e) or 5 μg/mL RU486 (A, f–k). Using the cell membrane GFP reporter (UAS-*mCD8::GFP*), expression of *S106*-GS-Gal4 was observed in the salivary gland (j), a part of the gut (h), and Malpighian tubules (i). Expression of *S106*-GS-Gal4 in the salivary gland was reconfirmed by the nuclear GFP reporter (UAS-*nls.GFP*; k). *Men* was overexpressed during the larval period by feeding larvae with food containing various doses of RU486 (0, 2.5, 5, or 10 μg/mL). Larval *Men* overexpression by *S106*-GS-Gal4 significantly lengthened lifespan in males (B) and females (C). *Men* mRNA levels (D) and Men activity (E) were significantly increased in the larvae fed food containing RU486. *Men* overexpression resulted in a significant increase in the pyruvate level (F) and the ratio of NADPH/$NADP^+$ (G). The scale bar, 100 μm in Ag applied to Ab and g; the scale bar, 200 μm in Ak applied to Ac–e and h–k. RU, RU486; $^*p < 0.05$; $^{***}p < 0.001$.

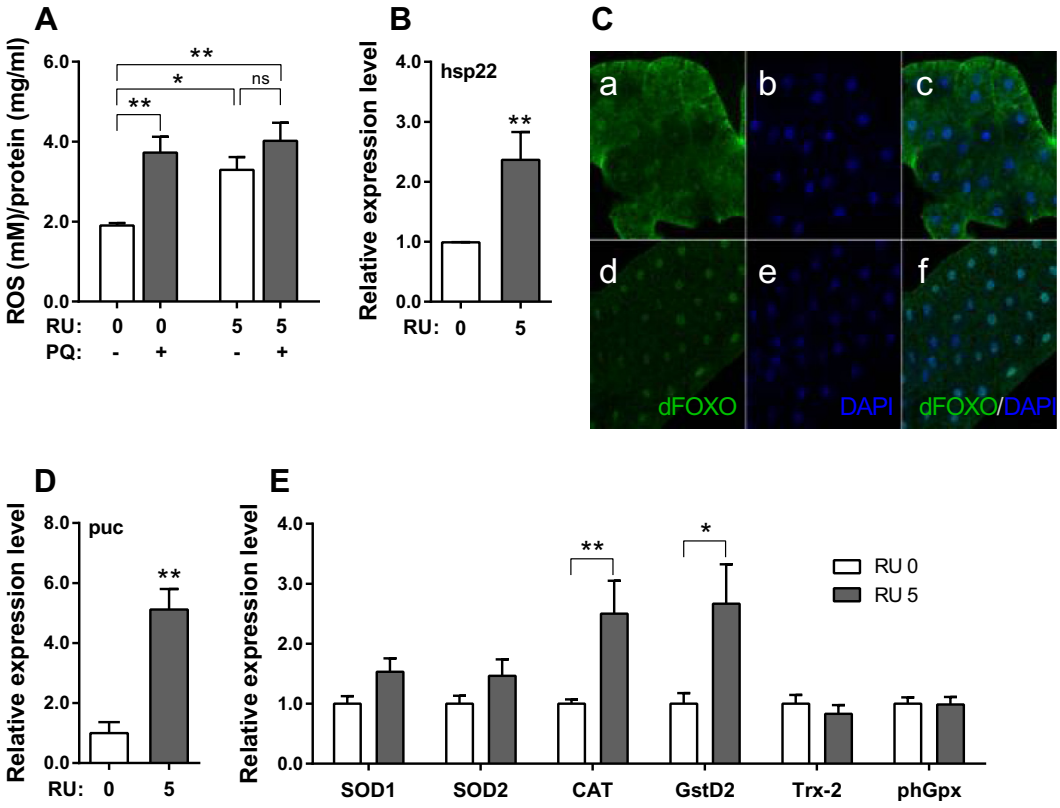

Fig. 2. *Men* overexpression during the larval stage induced ROS production and an adaptive response. ROS levels were increased in RU486-fed larvae, compared to those of the control larvae (A). The expression level of *hsp22* mRNA, a mitochondrial oxidative response gene, was also increased in RU486-fed larvae (B). Feeding 10 mM PQ did not cause any further increase in ROS levels in *Men*-overexpressing larvae (A). Such conditions coincided with the nuclear translocation of dFOXO (C). While the fat body from control animals (a–c) showed diffused cytoplasmic distribution of the dFOXO-immunoreactive (IR) signal (a and c), those of *Men*-overexpressing larvae (d–f) exhibited a nuclear localization of the dFOXO-IR signal (d and f). Cellular nuclei were visualized by DAPI. The expression level of *puc*, a JNK target gene, was increased in *Men*-overexpressing larvae (D). Larval *Men* overexpression was related to the significant increase of *CAT* and *GstD2* expression (E). RU, RU486; PQ, paraquat; DAPI, 4,6-diamidino-2-phenylindole; $^{*}p < 0.05$; $^{**}p < 0.01$.

2.5. Immunohistochemistry and imaging

Larval fat bodies were fixed in 4% paraformaldehyde, blocked with 3% bovine serum albumin, and incubated with an antibody raised against *Drosophila* forkhead box O (dFOXO, 1:500, a gift from Dr. Y. Kwon and Dr. M. Tatar) overnight at 4 °C, followed by incubation with the secondary anti-rabbit IgG antibody conjugated with Alexa Fluor 594 (1:200, Molecular Probes, Eugene, OR, USA) for 3 h. Larval expression patterns of *S106*-GS-Gal4 were examined using GFP reporter flies. Fluorescent images were taken using the LSM 510 laser scanning confocal microscope (Carl Zeiss AG, Oberkochen, Germany).

2.6. Measurements of eclosion rate, developmental time, and fecundity

To calculate eclosion rate, eggs (*S106*-GS-Gal4/+>UAS-*Men*/+) laid by 10 *S106*-GS-Gal4 females were counted and cultured on food containing 5 μg/mL RU486. Then emerged adults were counted every 24 h for 5 days. To measure developmental time, eggs (*S106*-GS-Gal4/+>UAS-*Men*/+) were collected for 3 h and grown on the RU486-containing food. Again, emerged adults were counted every 24 h for 7 days. For fecundity, 10 females (*S106*-GS-Gal4/+>UAS-*Men*/+) fed RU486 during the larval period were mated with 5 wild-type males. Eggs laid by these females were counted every 24 h.

2.7. Assays for stress tolerance of adults

Resistance to various stresses was evaluated [19]. To estimate ROS tolerance, ~20 adult males were allocated to individual food vials containing 18 mM PQ. Dead flies were counted every 3 h for 48 h. Feeding only water in agar vials, starvation resistance was measured by counting dead flies every 3 h for 36 h. To examine heat tolerance, food vials containing ~20 flies were placed in 37 °C incubator. Paralyzed flies were counted every 5 min.

2.8. Statistics

Survival data were plotted to Kaplan–Meier survival plots and MLS was calculated. Differences between genotypes were analyzed by the Log-Rank test using Prism v6.05 (GraphPad Software, La Jolla, CA, USA). Among the cases with one factor, RT-PCR data were analyzed by paired *t*-test, and others by unpaired *t*-test. In the cases with two factors, data were analyzed by nested 2-way analysis of variance (ANOVA), and followed by Tukey's multiple comparisons test. Data from developmental time and fecundity were analyzed by 2-way repeated measure ANOVA. A *p* value less than 0.05 was considered to indicate statistical significance. Data are presented as Mean ± SEM.

Detailed methods for longevity assay, measurement of ROS, and biochemical assay are described in Supplementary materials and methods.

3. Results

3.1. Men overexpression during the larval period extends adult lifespan

Ubiquitous and constitutive overexpression of *Men* extends the lifespan of fruit flies [8]. While searching for the organs mediating

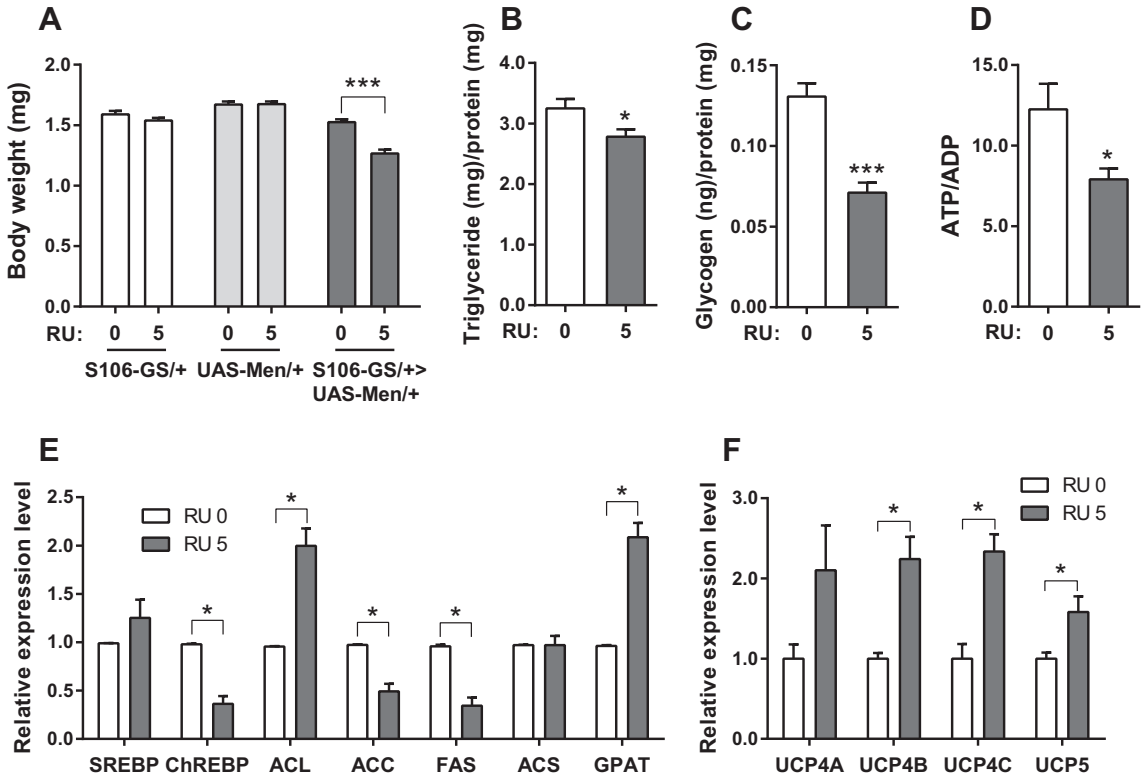

Fig. 3. *Men* overexpression during the larval period alters energy metabolism. *Men* overexpression significantly reduced body weight of third instar larvae relative to the controls, but feeding RU486 did not change the body weight within controls (A). Triglyceride, glycogen levels, and the ATP/ADP ratio in the whole body were significantly reduced by *Men* overexpression (B–D). Gene expression of *ChREBP*, *ACC*, and *FAS* was significantly reduced, while that of *ACL* and *GPAT* was increased in larvae fed RU486 (E). No expressional difference was detected in the mRNA expression of *SREBP* and *ACS* (E). But gene expression of mitochondrial *UCP4B*, *UCP4C*, and *UCP5* was significantly induced (F). RU, RU486; $^{*}p < 0.05$; $^{***}p < 0.001$.

these Men effects, we found that constitutive *Men* overexpression by *S106*-GS-Gal4 increases the lifespan. *S106*-GS-Gal4 is commonly used as an abdominal fat body-specific Gal4 driver in adults [17,20]. In larvae, however, we observed *S106*-GS-Gal4 expression in the salivary gland (Fig. 1A, j and k), a part of the gut (Fig. 1A, h), and Malpighian tubule (Fig. 1A, i), but not in the fat body (Fig. 1A, g), as Shen et al. reported [21]. Normally Men is expressed in the larval fat body [10], salivary gland, gut, and Malpighian tubule [22], which are mostly overlapped with the tissues where *S106*-GS-Gal4 is expressed.

Lifespan was significantly extended in flies overexpressing *Men* by feeding various doses of RU486 (2.5, 5, or 10 μg/mL) during the larval period, relative to the control group (Fig. 1B for males; Fig. 1C for females; Suppl. Table 1A). Larval *Men* overexpression by feeding 5 μg/mL RU486 caused a 24% increase of MLS in males (36.1 vs. 44.8 days) and 39% in females (34.2 vs. 47.5 days), compared to the negative controls (Fig. 1B and C; Suppl. Table 1A). Since the maximum MLS was attained when *Men*-overexpressing larvae were fed 5–10 μg/mL RU486 (Suppl. Table 1A), we used 5 μg/mL RU486 in later experiments. *Men* overexpression in the adult abdominal fat body with *S106*-GS-Gal4 did not affect the lifespan (Suppl. Fig. 1D–F).

Both *Men* mRNA level and Men enzyme activity in the larvae fed RU486 were significantly higher than those of the controls (Fig. 1D and E). *Men* overexpression resulted in an increase in pyruvate concentration and NADPH/$NADP^+$ ratio in the larvae (Fig. 1F and G). However, there was no significant difference in the NADH/NAD^+ ratio or amount of lactate between these groups (Suppl. Fig. 1G and H).

3.2. Men overexpression during the larval stage induces the adaptive response

When *Men*-overexpressing larvae were exposed to PQ, main effects of *Men* overexpression ($F_{(1,24)} = 6.081$, $p = 0.0212$) and PQ feeding on ROS production ($F_{(1,24)} = 13.84$, $p < 0.001$) were found (Fig. 2A). Basal ROS levels were significantly higher in *Men*-overexpressing larvae than in the controls (Fig. 2A). Concurrently, heat shock protein 22 (*hsp22*), a mitochondrial oxidative response gene [23], was significantly elevated (Fig. 2B). PQ administration caused an increase of ROS in control larvae, whereas further ROS increase was not found in *Men*-overexpressing larvae (Fig. 2A). In the fat body of control larvae, dFOXO was distributed mostly in the cytoplasm (Fig. 2C, a and c). However, dFOXO was translocated into the nuclei of the fat cells in the *Men*-overexpressing larvae (Fig. 2C, d and f). *Men*-overexpressing larvae exhibited increased mRNA levels of *puckered* (*puc*), a target gene of Jun N-terminal kinase (JNK) (Fig. 2D). Finally, *Men*-overexpressing larvae expressed substantially higher levels of catalase (*CAT*) and glutathione S transferase D2 (*GstD2*) mRNAs than the controls (Fig. 2E). Thus, the larval *Men* overexpression by *S106*-GS-Gal4 may cause an adaptive response through the JNK signaling pathway.

3.3. Men overexpression during the larval period alters energy metabolism

Men-overexpressing larvae showed significantly lower body weight than the controls (Fig. 3A), without reducing food ingestion

(Suppl. Fig. 1K). Feeding RU486-containing food did not affect the body weights of control flies (Fig. 3A).

A significant reduction in the amount of triglyceride (TG) was detected in *Men*-overexpressing larvae, compared to the controls (Fig. 3B). Among the lipogenic enzymes, expression levels of acetyl-CoA carboxylase (*ACC*) and fatty acid synthase (*FAS*) were significantly reduced, but those of ATP-citrate lyase (*ACL*) and glycerol-3-phosphate acyltransferase (*GPAT*) were significantly increased (Fig. 3E). In response to larval *Men* overexpression, the expression of sterol regulatory element binding protein (*SREBP*), which is implicated in lipid homeostasis, was not changed (Fig. 3E).

Compared to the controls, levels of glycogen and carbohydrate response element binding protein (*ChREBP*) and the ratio of ATP/ADP were also significantly reduced in *Men*-overexpressing larvae (Fig. 3C, D and E). But mRNA expression of uncoupling protein 4B (*UCP4B*), *UCP4C*, and *UCP5* was significantly enhanced (Fig. 3F). Glucose and trehalose levels in the larval hemolymph were not different between the two groups (Suppl. Fig. 1I and J).

3.4. Adult flies that overexpressed Men during the larval period exhibit tolerance to ROS

Compared to those of control animals, no differences in eclosion rate, developmental time, and fecundity were detected in the flies that overexpressed *Men* only during the larval period (Fig. 4A–C). However, these flies showed significant resistance to PQ (Fig. 4D). Such tolerance was not observed in starvation or heat stress tests (Figs. 4E and F). Expression levels of *hsp22* or *puc* mRNA were not different between the two groups (Fig. 4G and H), suggesting no induction of ROS or JNK signaling in the adults that overexpressed *Men* during the larval period. However, among the ROS-scavenging enzymes, mRNA expression of *GstD2* and thioredoxin-2 (*Trx-2*) was significantly increased in these flies (Fig. 4I).

4. Discussion

We expected that increased NADPH production due to *Men* overexpression would enhance the activity of ROS-scavenging

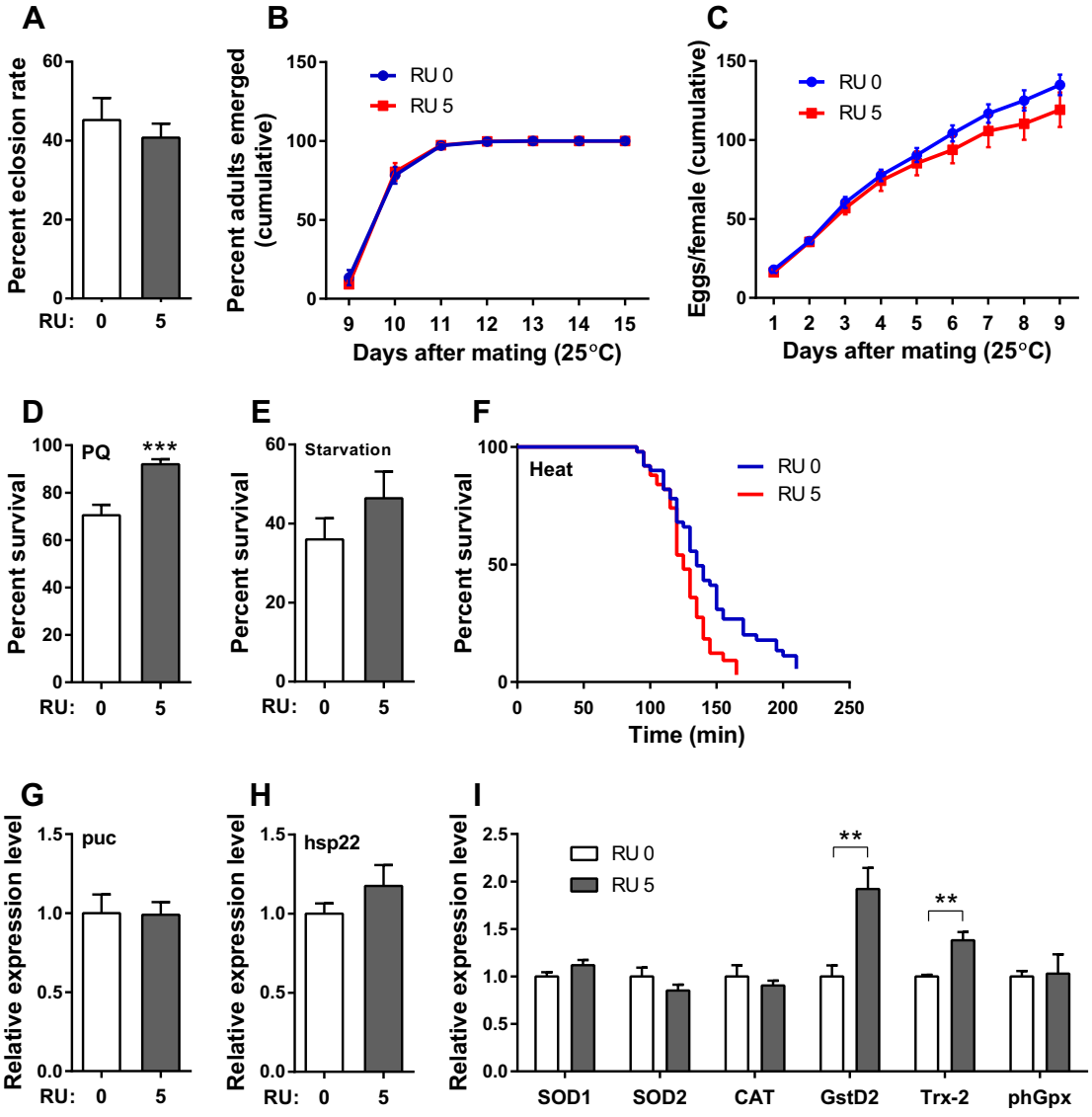

Fig. 4. Adult flies that overexpressed *Men* during larval period exhibit tolerance to ROS. No significant difference was detected in eclosion rate (A), developmental time (B), and fecundity of females (C) between control and adult flies fed 5 μg/mL RU486 during the larval period. Adult flies in which *Men* was overexpressed during the larval period did not show any resistance to starvation (E) or heat stress (F), but did exhibit significantly higher tolerance to 18 mM PQ after 48 h (D). Relative expression levels of *hsp22* (G) and *puc* (H) were not different between these two groups. However, mRNA expression levels of *GstD2* and *Trx-2* among ROS-scavenging enzymes were significantly higher in the adults in which *Men* was overexpressed during the larval period (I). RU, RU486; $^{**}p < 0.01$; $^{***}p < 0.001$.

enzymes to reduce cellular ROS, which might contribute to the extended lifespan. Surprisingly, *Men* overexpression by *S106*-GS-Gal4 increased ROS content (Fig. 2A and B) and induced the adaptive response, possibly through the JNK pathway (Fig. 2C–E) [24].

Mild induction of mitochondrial ROS seems necessary for a longer lifespan. Recent studies suggest that low ROS levels inhibit aging processes [25,26]. In *Drosophila*, RNAi inhibition of mitochondrial respiratory complexes I, III, IV, and V also resulted in lifespan extension [27]. In *C. elegans*, inhibition of mitochondrial respiration induced ROS production, and the mild increase in ROS activated hypoxia-inducible factor, which then promoted expression of longevity genes [28].

More recently, it was suggested that metabolic effects on longevity are coupled with mitochondrial ROS production. That is, a reduction in glucose availability or acute impairment of insulin signaling augmented ROS production, CAT activity, resistance to oxidative stress, and finally lengthened the worm lifespan [29]. Mutations in a monocarboxylate transporter also caused pyruvate imbalance, ROS production, and adaptive response [30]. Thus, similar to these observations in worms, the pyruvate imbalance in *Drosophila*, caused by *Men* overexpression, is likely related to ROS production and induction of the adaptive response.

Men is known as a lipogenic enzyme [31]. In our experiments, however, *Men* overexpression caused a decrease in TG levels, which may be related to the lower expression of *ACC* and *FAS*, the rate-limiting enzymes in lipogenesis (Fig. 3B and E) [32]. Such conditions might also enhance fatty acid synthesis and lipid oxidation [33], though this possibility was not explored in our study. In addition, glycogen levels were also significantly decreased in *Men*-overexpressing larvae (Fig. 3C) without altering lactate levels (Suppl. Fig. 1H). Despite the conditions of reduced anabolism and enhanced catabolism for carbohydrates and lipids, the ATP/ADP ratio was still significantly reduced (Fig. 3D) and expression of most UCPs was increased (Fig. 3F). Thus, some surplus energy substrates should contribute to the production of mitochondrial ROS in *Men*-overexpressing larvae.

We have tried to understand how larval *Men* overexpression could extend the adult lifespan. Eclosion rate and developmental time are not different from those of control flies, indicating that there is no selection process during development (Fig. 4A and B). Moreover, no effect of larval *Men* overexpression on the fecundity of females was detected (Fig. 4C). However, without induction of *puc* and *hsp22* expression (Fig. 4G and H), the adult flies exposed to larval *Men* overexpression were more resistant to PQ and showed increased *GstD2* and *Trx-2* expression (Fig. 4D and I).

Nonetheless, the causes of lifespan extension by the larval *Men* overexpression should not be limited only in the control of ROS. For example, mammalian triiodothyronine and insect juvenile hormone (JH) regulate expression of Men [18,34]. The promoter region of human *ME1* contains two thyroid hormone response elements to which the heterodimeric complex of thyroid hormone receptor and retinoid X receptor bind, resulting in enhanced ME1 expression [35,36]. JH, together with ecdysone, reciprocally control *Men* transcription and translation [18,37]. Intriguingly, ovarian ecdysone production is reduced in insulin receptor mutants [38], and ecdysone controls growth rate through the fat body [39], implying that Men may be connected to IIS via ecdysone and JH. In mammals, ME1 controls pyruvate cycling to affect glucose-stimulated insulin secretion in pancreatic β-cells [12]. Thus, it would be interesting to determine whether hormones such as JH, ecdysone, and insulin can modulate the effect of Men on longevity.

How developmental stimuli can be conveyed to adult animals remains unclear. Recently, a handful of genes regulated by DNA methylation and histone modification under CR conditions were reported as epigenetic targets for metabolic reprogramming [40]. In addition, acetyl-CoA produced by ACL is necessary for histone acetylation that is activated by growth factors during differentiation [41]. Therefore, we can speculate that *Men* overexpression during the larval period might also participate in epigenetic reprogramming in the systems eventually to extend the lifespan.

We report that *Men* overexpression during the larval stage extends the lifespan of *Drosophila*, and this Men effect might be linked to the control of ROS. Further studies are required to understand how the effects of a metabolic enzyme expressed during the developmental period are transmitted to the later stages of life in *Drosophila*.

Acknowledgments

This work was supported by grants from the Basic Science Research Program (2009-0070901) and the Bio and Medical Technology Development Program (2012055351) through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology and by a grant from the Eco Health Action Project (H112-00135-0011-0) funded by the Ministry of Environment of Korea. This research was also supported by private funds: the Gil Jung Hee Memorial Fund and the Chung-Yang Young Sun Cha Memorial Fund. We thank Dr. M. Tatar and Dr. K. Yu for dFOXO antibodies.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.12.020.

References

[1] L. Guarente, C. Kenyon, Genetic pathways that regulate ageing in model organisms, Nature 408 (2000) 255–262.
[2] L. Bordone, L. Guarente, Calorie restriction, SIRT1 and metabolism: understanding longevity, Nat. Rev. Mol. Cell Biol. 6 (2005) 298–305.
[3] R.J. Colman, R.M. Anderson, S.C. Johnson, E.K. Kastman, K.J. Kosmatka, T.M. Beasley, D.B. Allison, C. Cruzen, H.A. Simmons, J.W. Kemnitz, R. Weindruch, Caloric restriction delays disease onset and mortality in rhesus monkeys, Science 325 (2009) 201–204.
[4] D.E. Harrison, R. Strong, Z.D. Sharp, J.F. Nelson, C.M. Astle, K. Flurkey, N.L. Nadon, J.E. Wilkinson, K. Frenkel, C.S. Carter, M. Pahor, M.A. Javors, E. Fernandez, R.A. Miller, Rapamycin fed late in life extends lifespan in genetically heterogeneous mice, Nature 460 (2009) 392–395.
[5] A. Dillin, D.K. Crawford, C. Kenyon, Timing requirements for insulin/IGF-1 signaling in *C. elegans*, Science 298 (2002) 830–834.
[6] J.A. Mattison, G.S. Roth, T.M. Beasley, E.M. Tilmont, A.M. Handy, R.L. Herbert, D.L. Longo, D.B. Allison, J.E. Young, M. Bryant, D. Barnard, W.F. Ward, W. Qi, D.K. Ingram, R. de Cabo, Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study, Nature 489 (2012) 318–321.
[7] A. Dillin, A.L. Hsu, N. Arantes-Oliveira, J. Lehrer-Graiwer, H. Hsin, A.G. Fraser, R.S. Kamath, J. Ahringer, C. Kenyon, Rates of behavior and aging specified by mitochondrial function during development, Science 298 (2002) 2398–2401.
[8] D. Paik, Y.G. Jang, Y.E. Lee, Y.N. Lee, R. Yamamoto, H.Y. Gee, S. Yoo, E. Bae, K.J. Min, M. Tatar, J.J. Park, Misexpression screen delineates novel genes controlling *Drosophila* lifespan, Mech. Ageing Dev. 133 (2012) 234–245.
[9] E. Heart, G.W. Cline, L.P. Collis, R.L. Pongratz, J.P. Gray, P.J. Smith, Role for malic enzyme, pyruvate carboxylation, and mitochondrial malate import in glucose-stimulated insulin secretion, Am. J. Physiol. Endocrinol. Metab. 296 (2009) E1354–1362.
[10] B.W. Geer, D. Krochko, J.H. Williamson, Ontogeny, cell distribution, and the physiological role of NADP-malic enzyme in *Drosophila melanogaster*, Biochem. Genet. 17 (1979) 867–879.
[11] K. Uyeda, J.J. Repa, Carbohydrate response element binding protein, ChREBP, a transcription factor coupling hepatic glucose utilization and lipid synthesis, Cell Metab. 4 (2006) 107–110.
[12] C. Guay, S.R. Madiraju, A. Aumais, E. Joly, M. Prentki, A role for ATP-citrate lyase, malic enzyme, and pyruvate/citrate cycling in glucose-induced insulin secretion, J. Biol. Chem. 282 (2007) 35657–35665.
[13] H.N. Kirkman, G.F. Gaetani, Catalase: a tetrameric enzyme with four tightly bound molecules of NADPH, Proc. Natl. Acad. Sci. U.S.A. 81 (1984) 4343–4347.
[14] E.S. Arner, A. Holmgren, Measurement of thioredoxin and thioredoxin reductase, Curr. Protoc. Toxicol. (2001). Chapter 7, Unit 7.4.
[15] G. Wu, Y.Z. Fang, S. Yang, J.R. Lupton, N.D. Turner, Glutathione metabolism and its implications for health, J. Nutr. 134 (2004) 489–492.
[16] D. Yamazaki, J. Horiuchi, Y. Nakagami, S. Nagano, T. Tamura, M. Saitoe, The *Drosophila* DCO mutation suppresses age-related memory impairment without affecting lifespan, Nat. Neurosci. 10 (2007) 478–484.

[17] D.S. Hwangbo, B. Gershman, M.P. Tu, M. Palmer, M. Tatar, *Drosophila* dFOXO controls lifespan and regulates insulin signalling in brain and fat body, Nature 429 (2004) 562–566.
[18] R. Farkas, P. Danis, L. Medved'ova, B.M. Mechler, J. Knopp, Regulation of cytosolic malate dehydrogenase by juvenile hormone in *Drosophila melanogaster*, Cell Biochem. Biophys. 37 (2002) 37–52.
[19] M.C. Wang, D. Bohmann, H. Jasper, JNK signaling confers tolerance to oxidative stress and extends lifespan in *Drosophila*, Dev. Cell 5 (2003) 811–816.
[20] S. Libert, Y. Chao, X. Chu, S.D. Pletcher, Trade-offs between longevity and pathogen resistance in *Drosophila melanogaster* are mediated by NFkappaB signaling, Aging Cell 5 (2006) 533–543.
[21] J. Shen, C. Curtis, S. Tavare, J. Tower, A screen of apoptosis and senescence regulatory genes for life span effects when over-expressed in *Drosophila*, Aging (Albany NY) 1 (2009) 191–211.
[22] V.R. Chintapalli, J. Wang, P. Herzyk, J.A.T. Dow, FlyAtlas: Survey of Adult and Larval Expression, 2010.
[23] G.N. Landis, D. Abdueva, D. Skvortsov, J. Yang, B.E. Rabin, J. Carrick, S. Tavare, J. Tower, Similar gene expression patterns characterize aging and oxidative stress in *Drosophila melanogaster*, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 7663–7668.
[24] M.C. Wang, D. Bohmann, H. Jasper, JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling, Cell 121 (2005) 115–125.
[25] S.S. Lee, R.Y. Lee, A.G. Fraser, R.S. Kamath, J. Ahringer, G. Ruvkun, A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity, Nat. Genet. 33 (2003) 40–48.
[26] X. Liu, N. Jiang, B. Hughes, E. Bigras, E. Shoubridge, S. Hekimi, Evolutionary conservation of the clk-1-dependent mechanism of longevity: loss of mclk1 increases cellular fitness and lifespan in mice, Genes Dev. 19 (2005) 2424–2434.
[27] J.M. Copeland, J. Cho, T. Lo Jr., J.H. Hur, S. Bahadorani, T. Arabyan, J. Rabie, J. Soh, D.W. Walker, Extension of *Drosophila* life span by RNAi of the mitochondrial respiratory chain, Curr. Biol. 19 (2009) 1591–1598.
[28] S.J. Lee, A.B. Hwang, C. Kenyon, Inhibition of respiration extends *C. elegans* life span via reactive oxygen species that increase HIF-1 activity, Curr. Biol. 20 (2010) 2131–2136.
[29] T.J. Schulz, K. Zarse, A. Voigt, N. Urban, M. Birringer, M. Ristow, Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress, Cell Metab. 6 (2007) 280–293.
[30] L. Mouchiroud, L. Molin, P. Kasturi, M.N. Triba, M.E. Dumas, M.C. Wilson, A.P. Halestrap, D. Roussel, I. Masse, N. Dalliere, L. Segalat, M. Billaud, F. Solari, Pyruvate imbalance mediates metabolic reprogramming and mimics lifespan extension by dietary restriction in *Caenorhabditis elegans*, Aging Cell 10 (2011) 39–54.
[31] E.M. Wise Jr., E.G. Ball, Malic enzyme and lipogenesis, Proc. Natl. Acad. Sci. U.S.A. 52 (1964) 1255–1263.
[32] W.E. Donaldson, Regulation of fatty acid synthesis, Fed. Proc. 38 (1979) 2617–2621.
[33] S.D. Katewa, F. Demontis, M. Kolipinski, A. Hubbard, M.S. Gill, N. Perrimon, S. Melov, P. Kapahi, Intramyocellular fatty-acid metabolism plays a critical role in mediating responses to dietary restriction in *Drosophila melanogaster*, Cell Metab. 16 (2012) 97–103.
[34] A.G. Goodridge, Regulation of malic enzyme synthesis by thyroid hormone and glucagon: inhibitor and kinetic experiments, Mol. Cell. Endocrinol. 11 (1978) 19–29.
[35] C. Gonzalez-Manchon, N. Butta, M. Ferrer, M.S. Ayuso, R. Parrilla, Molecular cloning and functional characterization of the human cytosolic malic enzyme promoter: thyroid hormone responsiveness, DNA Cell Biol. 16 (1997) 533–544.
[36] E. Jeannin, D. Robyr, B. Desvergne, Transcriptional regulatory patterns of the myelin basic protein and malic enzyme genes by the thyroid hormone receptors alpha1 and beta1, J. Biol. Chem. 273 (1998) 24239–24248.
[37] R. Farkas, J. Knopp, Ecdysone-modulated response of *Drosophila* cytosolic malate dehydrogenase to juvenile hormone, Arch. Insect Biochem. Physiol. 35 (1997) 71–83.
[38] T. Flatt, M.P. Tu, M. Tatar, Hormonal pleiotropy and the juvenile hormone regulation of *Drosophila* development and life history, BioEssays 27 (2005) 999–1010.
[39] R. Delanoue, M. Slaidina, P. Leopold, The steroid hormone ecdysone controls systemic growth by repressing dMyc function in *Drosophila* fat cells, Dev. Cell 18 (2010) 1012–1021.
[40] Y. Li, M. Daniel, T.O. Tollefsbol, Epigenetic regulation of caloric restriction in aging, BMC Med. 9 (2011) 98.
[41] K.E. Wellen, G. Hatzivassiliou, U.M. Sachdeva, T.V. Bui, J.R. Cross, C.B. Thompson, ATP-citrate lyase links cellular metabolism to histone acetylation, Science 324 (2009) 1076–1080.