Responses of *Drosophila melanogaster* to atypical oxygen atmospheres

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**ARTICLE INFO**

Article history:
Received 4 November 2010
Received in revised form 2 January 2011
Accepted 10 January 2011
Available online 15 January 2011

Keywords:
Aging
Hyperoxia
Hypoxia
Life span
Water loss
Negative geotaxis
Phenotype
Metabolic depression
Oxidative damage
Reversible damage

**ABSTRACT**

We examined physiological phenotypes of *Drosophila melanogaster* in hypoxic to hyperoxic atmospheres. We performed measurements on life span or behavioural function in 5, 21, 40, 60, and 80% O₂, and combined this with literature data for 2% and 100% O₂ incubation resulted in a concentration-dependent reduction of life span in both hypoxia and hyperoxia, though different measures of life span were affected differently. We also examined how behavioural and metabolic functions were affected by exposure to hyperoxia (up to 60% O₂). Climbing behaviour was measured as a fast (4 s) and slow (55 s) response in a negative geotaxis assay. In normoxia, both measures of climbing response declined exponentially until disappearing completely. Interesting, survivorship was very high until the loss of climbing ability, after which it dropped rapidly. This pattern appeared accelerated in 40% O₂. However, while flies in 60% O₂ also apparently lost their fast climbing ability immediately prior to the drop in survivorship, they maintained considerable climbing ability over the longer trial. Metabolism, measured by CO₂ release, did not change with age in normoxic flies, but was significantly lower in flies exposed to hyperoxia, particularly as the flies aged. There was, however, a slight increase in water loss rate with age in normoxia, while in hyperoxia, water loss was reduced. Uniquely, the water loss rates of flies in 60% O₂ doubled immediately prior to the end of their life span. Because ageing results in generally irreversible functional declines, we examined if functional declines in hyperoxia (80% O₂) were also irreversible, or whether some functioning could recover after a return to normoxia. After 7 days of recovery, water loss rates decreased, CO₂ exhalation slightly increased, and climbing ability was partially recovered. Therefore, the effect of O₂ on *D. melanogaster* function is non-linear, may be reversible, and may include unique phenotypes that arise at some O₂ concentrations, and nor others.

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1. Introduction

During the course of normal ageing, DNA (Hamilton et al., 2001), proteins (Levine and Stadtman, 2011), and cellular structures (Miquel et al., 1975) can become damaged by reactive oxygen species. This damage may impair metabolism and neural function, and contribute to mortality in adults (e.g., Martin et al., 2009a; Wicks et al., 2009). Frequently, model organisms are exposed to hyperoxic atmospheres to simulate or accelerate pathologies associated with oxygen damage (e.g., Rebrin and Sohal, 1991; Walker and Benzer, 2004). In the modern literature, hyperoxia (normoxia = 21% O₂) is usually synonymous with 100% O₂, but a paucity of information on the effects of moderate hyperoxia impedes our understanding of the mode of oxygen toxicity (Harrison et al., 2006). It is generally assumed that the loss of function in hyperoxia is a linear, graded function of oxygen content. This does indeed suggest that a pure oxygen atmosphere will result in the most pronounced loss of function, but this dependence on linearity assumes that novel or exaggerated phenotypes do not emerge at different oxygen concentrations.

The literature supporting a linear impact of oxygen content on *Drosophila melanogaster* function is conflicting, and largely relies on measured life spans. Baret et al. (1994) concluded that life span of *D. melanogaster* was linearly related to O₂ concentrations from 20% to 50% O₂; however, in hyperbaric hyperoxic atmospheres, the effect of O₂ concentration was distinctly non-linear (Fenn et al., 1967). There is generally little information on the effect of intermediate hyperoxia (in this text, 22–100% O₂) on decay of function or tissue damage in fruit flies (Harrison et al., 2006). At 50% O₂, the decay of locomotor function was accelerated compared to normoxia, which supported the view that O₂ damage caused the behavioural deficits in ageing flies (Miquel et al., 1975). Besides this single exposure, intermediate normobaric O₂ concentrations are poorly described, so it remains an open question to what degree variation in O₂ reduces life span and alters behavioural performance. Moreover, a consideration of the graded effects of O₂ would benefit from more data on long-term exposures to hypoxia (<21% O₂).

The effect of hyperoxia on *D. melanogaster* physiology may indeed be simply to accelerate the onset of the normal ageing
phenotype. Overexpression and deletion of antioxidant genes, which alter endogenous levels of oxidative stress, have provided results consistent with this interpretation. For example, expression of SOD1 (Sun and Tower, 1999) or SOD2 (Sun et al., 2002) transgenes extends D. melanogaster lifespan, while deletion or loss of function mutations of Sod1 or Sod2 shortens lifespan (e.g., Martin et al., 2009a,b). Some phenotypes are strikingly similar between hyperoxia and senescence, such as the onset of skeletal muscle mitochondrial whorls (Sacktor and Shimada, 1972; Walker and Benzer, 2004). Nonetheless, the physiological bases for the shared phenotypes or functional declines may differ. For instance, Miquel et al. (1975) compared flies in normoxia and 50% O2, and noted that while many tissue-level degenerative phenotypes are shared, the phenotypes in hyperoxia are frequently more pronounced than in senescence. At the cellular level, the redox state (as measured in the glutathione system) of ageing flies gradually and consistently becomes more oxidised, but flies exposed to 100% O2 show no obvious trend towards oxidation (Rebrin and Sohal, 2006). On the other hand, protein carbonylation rates are far greater in hyperoxia and hypoxia than in normoxia (Rascón and Harrison, 2010).

In the transcriptome, aged and 100% O2-exposed flies exhibit a 40% overlap, with one key difference being a downregulation of metabolic enzymes during senescence (Landis et al., 2004). While exposure to hyperoxia leads to marked destruction in the neural system (Miquel et al., 1975), where apoptotic bodies and neurodegeneration are readily induced (Gruenewald et al., 2009), caspase activity, a marker of apoptosis, is absent from senescent flies' heads (Zheng et al., 2005). Similarly, Kloek et al. (1978) observed that vacuolation was absent from senescent flies' brains, but widespread in brains of flies exposed to some hyperoxic conditions. Interestingly, they observed a threshold of >33% O2 for brain vacuolation, and increasing amounts of vacuolation thereafter (up to 55% O2). Together, these data suggest that, although O2 contributes to normal senescence, there may be fundamental differences in the functional phenotypes which result from chronic exposure to low O2 doses (in this case, normoxia) and those which result from acute exposure to massive doses.

We raised flies in hypoxic and hyperoxic conditions to examine how life span, metabolism, and locomotor function are altered, compared to normoxia. We report two indices of life span, the ages at which 10% or 100% of the population has died. We monitored locomotor function using negative geotaxis, both as the number of flies that climbed to a threshold height within 4 s, and the number which climbed within 55 s. We then measured flies' metabolic activities by their carbon dioxide release rate and the rate of water vapor loss. Water vapor loss is commonly used to indicate spiracular opening frequency (e.g., Lighton and Schilman, 2007). Finally, we examined whether the flies’ hyperoxic phenotypes were reversible if the flies were removed from hyperoxia and allowed to recover in normoxia.

2. Materials and methods

2.1. Fly breeding

Breeding populations of flies (strain Oregon-R) were raised in ambient laboratory conditions, on a cornmeal/yeast/agar diet. Male flies <24 h old were collected and sorted on a chill table between −2 °C and −4 °C degrees (BioQuip Products, Inc); cold anaesthetisation was used to prevent confounding effects of anoxia (Rascón and Harrison, 2010). Flies were placed on a Kimwipe to prevent direct contact with the metal surface, and sorted within 15–20 min; in no case did flies die after cold exposure. The flies were allowed to recover from the cold for 2 h, and then were placed into hypoxia (5% O2), normoxia (~21% O2), or hyperoxia (40%, 60%, or 80% O2) in 75 mL bottles. For each O2 condition, the experiment was replicated with 9–11 groups of 45–55 flies, with the exception of the hypoxic treatment, which was replicated in three groups of 28–37 flies. Flies from each breeding population were distributed throughout the treatments to account for any breeding effects. We were limited to three incubators (see Section 2.2), so flies in 20–60% were tested together, and flies in 5% and 80% O2 were tested separately. Only life span was determined for flies in 5% and 80% O2.

2.2. Incubation and lifespan

Beverage coolers were modified into incubators, and O2 concentration was controlled by solenoid switch-based set-point regulation (5%, 40%, 80% O2; Pro-Ox 110, BioSpherix, Ltd.; 60% O2; ROXY-1, Sable Systems International). Each incubator is slightly leaky, allowing gas to leak from the system. The regulator injects O2 into the incubator to maintain the setpoint, but the leakiness maintains equilibrium with atmospheric pressure. Relative humidity was maintained at >90% by bubbling the incoming gas through a water-filled beaker. Chamber temperature was maintained at 26–26.5 °C by heated-water pipes connected to a temperature-controlled water circulator.

Adults’ feed medium was changed each 2–3 days, and survivorship recorded as the number of dead flies in the medium. Two indices of life span are reported: the age at which 90% of the population was surviving (LS90), and the maximum life span, at which the entire population had died (LS). Because deaths in the population may subselect cohorts based on differential resistance to stress, behavioural and metabolic measurements were performed during the LS90 at 6, 11, 18, 24, 30, and 36 days incubation.

2.3. Recovery from hyperoxia

We subsequently tested flies’ ability to recover from exposure to 60% O2. For this experiment, all measurements were performed on the same flies. Ten groups of 50–60 flies were placed in 60% O2 for 11 days, and then removed to normoxia. Their recovery was tested 6 days later at 18 days old. Geotaxis and respiration were examined at 6, 11, and 18 days.

2.4. Negative geotaxis

Flies were tested for negative geotactic response in an apparatus based on the column design of Gargano et al. (2005). We counted the number of flies climbing over 46 mm (55% of column height) 4 s and 55 s after flies were dropped to the floor of the column by banging the apparatus down. Negative geotaxis was assayed three times for each group, at 1 min intervals, and we report the mean of the three trials. Following geotaxis trials, flies were replaced into incubation until respirometry was performed, up to 3 h later.

2.5. Metabolic function

An entire group of flies (45–55 individuals) was assayed together in a 50 mL respirometer chamber, in total darkness. The chamber was cradled by an infrared activity detector (AD-1, Sable Systems International) inside a thermally insulated styrofoam box, which was temperature-controlled by a water pipe in series with the incubators. Bev-A-Line low-permeability tubing was used for all gas flows in the system, to minimise errors from H2O and CO2 absorbance. Ambient room air (normoxic) was scrubbed of CO2 and H2O in a drierite–soda lime–drierite column and then regulated at 50 mL/min with a needle-valve. The air was then pushed into the styrofoam box using a gas subsampler unit (Version 2.0, Sable Systems International), and about 1 m of tubing was coiled inside.
the respirometer box to thermally equilibrate the incoming air with the chamber. Air excurrent from the chamber was pushed first through a relative humidity meter (RH-200, Sable Systems International), then dried and pushed through a CO₂ analyser (CA-2A, Sable Systems International).

For each respirometry session, we first collected a 15 min baseline of gas flowing through the empty respirometry chamber, and then the flies were immediately assayed for 15 min. Respirometric data was obtained from the final 5 min of the recording, to allow flies to settle. The baseline was subtracted from the recording to obtain the carbon dioxide release rate ($V_{CO2}; \mu L CO_2 h^{-1} fly^{-1}$) and the water loss rate ($V_{H2O}; \mu g H_2O h^{-1} fly^{-1}$). After respirometry, groups of flies were weighed to 0.1 mg; the flies’ masses were $0.75 \pm 0.07$ mg, and did not differ across treatments ($F_{5,117} = 0.422, p = 0.83$).

2.6. Data analysis

$LS_{90}$ and $LS_{0}$ at each age and O₂ incubation are reported as the mean and standard deviation of the $LS_{90}$ and $LS_{0}$ of each replicate population in the study. To compare across O₂ concentrations, $LS_{90}$ and $LS_{0}$ were included from studies of Oregon-R flies in the literature (Baret et al., 1994; Miquel et al., 1975; Rascón and Harrison, 2010; Rebrin and Sohal, 2006; Wicks et al., 2009). In the study of Baret et al. (1994), flies in 40% O₂ exhibited a clear sub-cohort effect, which confounded the estimation of $LS_{90}$; it was therefore omitted. For each study, $LS_{90}$ and $LS_{0}$ were digitised from life span figures in the respective texts, using the DigiEyes package in ExpeData software (Sable Systems International). $LS_{90}$ and $LS_{0}$ from each study were standardised to the life span in normoxia in that study. Comparisons across O₂ concentrations were made on the basis of the mean observed response.

All statistical analyses were performed with R software (v2.8.1, R Development Core Team, 2009). Significance was tested with linear models (ANOVA, regression), and the F-statistic, with degrees of freedom in subscript, is reported alongside the p-value.

In all cases, O₂ content and age were treated as fixed effects. Changes in life spans with O₂ or log O₂ were tested by regression. Metabolic and behavioural data were normally distributed, and the variance in metabolic data, but not behavioural, was homoscedastic. No transformation of the behavioural data was adequate to meet the assumption of homoscedasticity, and therefore the behavioural data was ranked. The raw or transformed data was then tested in a two-way ANOVA. Significant differences among group means were examined post hoc with Duncan’s test.

3. Results

3.1. Life span reduction by both hypoxia and hyperoxia

In our experiments, flies’ $LS_{90}$ were the same in hypoxia (5% O₂) and normoxia, though $LS_{90}$ was shorter in hypoxia; both $LS_{90}$ and $LS_{0}$ were decreased in hyperoxia (Fig. 1). We compared our data to published life spans in hypoxia and hyperoxia, and the results were similar (Fig. 2). $LS_{90}$ was maximum in normoxia, and significantly reduced by incubation in hypoxia or hyperoxia (Fig. 2a). $LS_{0}$ instead peaked at 10% O₂, as per the observations of Rascón and Harrison (2010; Fig. 2b). The available data support this extension of life span, since neither we nor Wicks et al. (2009) found a decrease in life span at 5% O₂ (Fig. 1). However, Wicks et al. (2009) reported they did not find any extension of $LS_{0}$ in preliminary examinations of flies’ life spans in hypoxic atmospheres (down to, but not including, 2.5% O₂; 2.5% O₂ decreased life span).

We used our data and those from published studies to examine whether the reduced life spans in non-normoxic atmospheres are...
best described by linear or logarithmic functions (shown by lines of best fit in Fig. 2). From 21 to 100% O2, LS90 was better explained by log O2 concentration than O2 concentration directly (O2: \( F_{1,8} = 177.20, p < 0.001 \); log O2: \( F_{1,8} = 363.24, p < 0.001 \)). The LS90 outlier of Baret et al. (1994) at 40% O2 is shown in Fig. 2a, although it was omitted for statistical comparisons; however, including the datum did not affect the trend’s overall significance (not shown). From 2% to 21% O2, log O2 concentration nearly significantly predicted LS90, while O2 did not (O2: \( F_{1,3} = 4.01, p = 0.14 \); log O2: \( F_{1,3} = 9.41, p = 0.055 \)). The nearly significant result occurred because Wicks et al. (2009) found very little reduction in LS90 at 5% O2. LS0 in hyperoxia was modelled from 10% to 100% O2, since it peaked at 10% O2, LS0 in hyperoxia was better explained by log O2 concentration than by O2 concentration directly (O2: \( F_{1,9} = 79.6, p < 0.001 \); log O2: \( F_{1,9} = 121.23, p < 0.001 \)). The prediction of hypoxic (≤10% O2) LS0 from log O2 concentration approached significance (O2: \( F_{1,2} = 3.82, p = 0.19 \); log O2: \( F_{1,2} = 14.61, p = 0.06 \).

3.2. Age- and O2-dependent decline in geotaxis

Both the fast and slow geotactic response of flies in normoxia decayed exponentially with age, but was more variable at younger ages (Fig. 3; 4 s response: \( F_{1,64} = 404.25, p = 0.001 \); 55 s response: \( F_{1,64} = 267.09, p < 0.001 \)). This decline in function was strongly dependent on the O2 incubation condition (interaction of age \( \times \) O2 incubation on 4 s response: \( F_{2,125} = 41.16, p < 0.001 \); significant differences in group means are presented in Fig. 3 with different letters). We observed that in normoxia and 40% O2, both the 4 s and 55 s climbing response was almost completely lost by the end of the LS90 (36 and 24 days old, respectively). Conversely, while flies in 60% O2 also showed a near total loss of climbing ability within 4 s at the end of their LS90 (11 days old), the longer climbing response was still present.

3.3. Age- and O2-dependence of respiratory function

Respiration patterns with age and incubation are shown in Fig. 4. There was no consistent directional change with age of VCO2 of normoxia-incubated flies (\( F_{1,41} = 0.60, p = 0.44 \)). However, there was a strong interaction between the O2 incubation and the age-specific pattern of change in respiration (significant differences in the mean between all groups are shown in Fig. 4 with different letters). Flies in 60% O2 showed an immediate significant decrease in VCO2. In 40% O2, VCO2 decreased with age (\( F_{1,41} = 9.25, p = 0.004 \)), though the difference in group means became significant only at 24 days. At the end of the LS90, and therefore
immediately prior to the rapid decrease in survivorship, VCO₂ was lowest in flies in 40% or 60% O₂ and highest in normoxia. It is possible that these differences were caused by reduced behavioural activity within the respirometry chamber. We qualitatively assessed flies’ activity within the respirometry chamber using an infrared activity detector, but the differences in VCO₂ between O₂ incubation were greater than could be accounted for by any difference in behavioural activity (not shown).

VH₂O increased over the life span of flies in normoxia (F₁,₆₄ = 19.42, p < 0.001), though the most significant pairwise comparisons were between young (≤11 days old) and 30 day old flies. There was no such change in VH₂O of flies in 40% O₂ (F₁,₄₁ = 0.12, p = 0.73). In 60% O₂, VH₂O was depressed by 6 days in incubation, compared to both normoxia and 40% O₂-raised flies, but surprisingly, at 11 days old, flies’ VH₂O nearly doubled.

Although VCO₂ and VH₂O did not significantly differ between flies in normoxia and 40% O₂ at most time points individually, overall the mean response of flies in 40% O₂ was lower (Fig. 4). Flies between the ages of 6 and 24 days were compared in a two-way ANOVA, with age and O₂ incubation as predictors. Overall, VCO₂ of flies in 40% O₂ was significantly reduced (O₂ incubation: F₍₁,₁₈₎ = 14.70, p < 0.001; Age × O₂ incubation: F₍₁,₈₂₎ = 8.71, p = 0.004). VH₂O, too, was depressed in flies in 40% O₂ (O₂ incubation: F₍₁,₈₂₎ = 7.80, p = 0.007; Age × O₂ incubation: F₍₁,₈₂₎ = 2.46, p = 0.12).

3.4. Recovery after hyperoxia

Separately from the previous experiments, flies were exposed to 60% O₂ for 11 days, and then allowed to recover in normoxia for 7 days before testing. Flies exposed to 60% O₂ in this experiment showed the same behavioural and metabolic patterns as previously. The fast – and slow – geotactic responses declined significantly between the sixth and 11th day of incubation (Fig. 5). Post hoc comparisons picked out significant differences between each of the three ages in the fast and slow climbing response (Fig. 5), indicating a substantial recovery of climbing ability in that trial. Geotactic ability recovered to levels comparable to or greater than that of normoxic flies of the same age (4 s climbing did not differ: F₍₁,₁₀₎ = 0.32, p = 0.32; 55 s climbing was greater in recovered flies: F₍₁,₁₀₎ = 8.96, p = 0.007).

Metabolic patterns, most notably water loss rates, were also altered by removal from hyperoxia (Fig. 5). Recovered flies’ VCO₂ was significantly greater than that of day old flies (F₍₁,₁₈₎ = 7.73, p = 0.01), and approached a significant difference from 11 day old flies (F₍₁,₁₈₎ = 3.67, p = 0.07). The VCO₂ of recovered flies was borderline lower than normoxic flies of the same age, but also more variable (F₍₁,₁₀₎ = 4.17, p = 0.06). As seen in the previous experiment, VH₂O nearly doubled at 11 days in incubation, but after recovery it significantly decreased again, though not to a level as low as 6 day old flies in 60% O₂ (from 6 to 11 d: F₍₁,₁₈₎ = 48.03, p < 0.001; 11–18 d: F₍₁,₁₈₎ = 35.28, p < 0.001; 6–18 d: F₍₁,₁₈₎ = 46.53, p < 0.001). The recovered VH₂O was lower than normoxic 18 day old flies (F₍₁,₁₀₎ = 6.13, p = 0.02).

4. Discussion

4.1. The effect of O₂ on life span

In general, our observations confirm the reduction of D. melanogaster life span in both hypoxic and hyperoxic oxygen atmospheres (Fig. 2; Rascón and Harrison 2010). Our analysis of original and published data suggests that the effects of O₂ atmospheres on life span, from extreme hypoxia (2% O₂) to extreme hyperoxia (100% O₂), are best modelled by non-linear equations:

\[ L_{90} = \frac{0.28 \ln O_2 + 0.19}{-0.58 \ln O_2 + 2.76} \]

\[ L_0 = \frac{0.59 \ln O_2 - 0.04}{-0.52 \ln O_2 + 2.52} \]

The logarithmic relationships imply a concentration-dependence of life span on O₂. L₉₀ and L₀ were similarly affected by hyperoxia, suggesting that high O₂ concentrations affected each part of the life span proportionately. Indeed, if hyperoxic L₀ was predicted only from data ≥21% O₂, the equations became more similar still (L₀ = −0.61 ln O₂ + 2.91). This is comparable to the bell effect on life span that Fenn et al. (1967) observed in hypo- and hyperbaric oxygen atmospheres.

Although the reduction of life span in hyperoxia is well established, the effect of hypoxia on life span is controversial. While Rascón and Harrison (2010) found that it was extended at 10% O₂, Wicks et al. (2009) commented that they did not find any extension of life span at any hypoxic O₂ concentration (unpublished, preliminary experiments). Similarly, while this study and Rascón and Harrison (2010) found a reduction of L₉₀ in hypoxia, Wicks et al. (2009) found little or none. It may be that this reflects differences in population heterogeneity between the studies, and subpopulations that respond to stresses differently; the popula-

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**Fig. 5.** Regain of negative geotactic behaviour (panel a) and metabolic function (panel b) in Drosophila melanogaster recovering after exposure to 60% O₂. Flies were removed from hyperoxia at 11 days of age. Differences were tested post hoc by treating each age of each incubation as separate groups, and groups which are not significantly different share at least one letter. Asterisks in each panel indicate that means of the respective groups have not been compared to each other (i.e., VCO₂ and VH₂O were not compared). Error bars show the standard deviation of 10 replicates.
tions of Wicks et al. may be relatively homogenous. Rascón and Harrison (2010) observed a ‘crossover’ point between flies in 10% O₂ and normoxia: though flies in hypoxia initially experienced a greater number of deaths, the surviving flies outlasted those in normoxia. They suggest that hypoxia may have acted as a hormetic stressor that produced a beneficial response in the surviving individuals. This could also apply to the experiment of Baret et al. (1994), who observed a clear sub-cohort effect in one hypoxic condition. Future studies might examine differences in the responses of subcohorts.

It may also be the case that even within the Oregon-R strain, genetic divergence has led to different O₂ tolerances. Surprisingly, most studies on the effect of intermediate hyperoxia have been conducted in this strain, but there are indications that other strains show different responses to oxygen. In contrast to Fig. 2, Vigne and Frelin (2007) examined life spans of the w¹¹¹B strain in 5% O₂, and generally found that life span was drastically reduced. Rebrin and Sohal (2006) found different life spans and responses to 100% O₂ exposure between yw and Oregon-R. Strehler (1977) found an increase in life span at 1% and 2% O₂, also in contrast to Fig. 2, although the strain and raising conditions were not reported. Kloek et al. (1976) examined wild-caught flies, and found no significant differences in life span from 9% to 33% O₂, though life span was reduced at 49% O₂. Recently, Zhou et al. (2007) and Zhao et al. (2010) have selected for flies that live and reproduce in 4% and 90% O₂ respectively. The selection experiments revealed specific genetic targets, reinforcing the role of genetic background on demo- graphics in atypical O₂ environments. While the response of all fruit fly strains to O₂ is likely to be similar in form to Fig. 1, we suggest that a systematic survey of strains’ responses to hypoxic and hyperoxic atmospheres may yield important insights. In particular, it may be that some strains have a shifted optimal O₂ condition, and some strains may have narrower or broader ranges of tolerable O₂ atmospheres.

4.2. The effect of age and O₂ on climbing behaviour

The patterns of decline in climbing ability were mediated by O₂ concentration, which is similar to the decline in locomotive function due to increased oxidative stress in cytosolic (Sod1) and mitochondrial (Sod2) superoxide dismutase knockdown flies (Martin et al., 2009a,b). In all incubations, we found flies’ complete loss of fast (4 s) climbing ability immediately predicted the end of the LSP, and a rapid increase in death rates. However, in the case of flies in 60% O₂, this observation masked the fact that the longer-term climbing ability was not completely lost (Fig. 3). The different observed climbing behaviours may be an example of similar phenotypes with different underlying causes. A qualitative explanation is that flies in 60% O₂ were apparently stunned by the banging in the geotaxis assay. After recovering from this stunning, they resumed climbing, albeit slowly (DAS, pers. obs.). An increased bang sensitivity might suggest that neural damage underlies their reduced responsiveness, since it has been suggested that the neural system may be especially susceptible to oxidative damage in hyperoxia (Miquel et al., 1975). In particular, a general sponginess of the brain is much more pronounced in hyperoxia than in senescent flies (Miquel et al., 1975).

Mutant fly models do not conclusively support or refute this hypothesis. While life span does not seem to be affected by targeted overexpression of SOD1 or Sod1 to skeletal muscle (Martin et al., 2009a; Phillips et al., 2000), there is evidence both for and against an extension of life span by its targeted overexpression in fly motorneurons (Martin et al., 2009a; Parkes et al., 1998). The conflicting results may be due to differences in the background oxidative stress of the flies’ genotype, since expression of SOD1 in the motorneurons of otherwise Sod-null flies extends life span (Parkes et al., 1998). On the other hand, Martin et al. (2009b) observed that targeted silencing of Sod2 in the nervous system does reduce locomotor function and life span, but much less dramatically than silencing Sod2 in muscle. This suggests that the loss of locomotor function and life span in hyperoxia could be mediated especially by damage to the musculature, and to the mitochondria specifically.

4.3. The effect of age and O₂ on metabolic activity

Over the life span of flies in normoxia, there was no significant change in VCO₂ (Fig. 4a), but the general impact of age on flies’ standard metabolic rates is unclear. Marden et al. (2003) found it increased between middle- and old-aged flies; Arking et al. (1988) found O₂ consumption peaked at midlife, and declined thereafter; Fenn et al. (1967) found it increased in young flies to a plateau in older flies; Hulbert et al. (2004) found no differences; Mockett et al. (2001) showed patterns suggestive of a mid-life peak; Promislov and Haselkorn (2002) found a slight increase with age; Van Voorhies et al. (2003) found metabolic rate dropped from 5 days to 16 days and remained constant thereafter, though Van Voorhies et al. (2004) found no correlation at all. However, fly muscle is designed to accommodate flight metabolic rates at least 10-fold greater than standard metabolic rates (based on Lehmann, 2001). Indeed, the respiratory capacity of isolated mitochondria declines with age, even though citrate synthase, a marker of mitochondrial abundance, does not (Ferguson et al., 2005). Consequently, standard metabolic rates may only be able to conclude that energetic requirements of non-flying Drosophila do not differ over the life time of the fly.

On this basis, the reduced VCO₂ in hyperoxia may actually underestimate the extent of damage to the mitochondria, if the metabolism reflects both damage to the unutilised portion of bioenergetic capacity (i.e., the metabolic scope) and to energetic requirements (difference between normoxia and hyperoxia exposures). VCO₂ of flies in 60% O₂ was 15% lower than flies in normoxia or 40% O₂ by just 6 days of incubation, after which it did not change. Flies in 40% O₂ exhibited a progressive reduction in VCO₂ with age, also to 15% less than their initial VCO₂. This is consistent with the decrease in the volume of CO₂ release in response to oxygen reperfusion damage (Lighton and Schilman, 2007).

Water loss rates are usually taken to signify changes in spiracular opening frequency, and so are another measure of respiratory function in different O₂ atmospheres. They can also be a measure of a fly’s health, since Lighton and Schilman (2007) found that water loss rates increased as a result of increasing oxygen reperfusion damage. Over the life span of flies in normoxia, water loss rates slightly increased (Fig. 4b), which is consistent with accruing damage in the ageing flies. On the other hand, VH₂O was depressed in 6 day old flies in 60% O₂, and over the life time of flies in 40% O₂. Lighton et al. (2004) showed that flies acutely exposed to 100% O₂ shut their spiracles, a response hypothesised to have evolved to limit O₂ toxicity (Hetz and Bradley, 2005). Therefore, the trends in VH₂O over the different O₂ incubations are consistent both with oxidative damage and behavioural modifications to limit oxidative damage.

Surprisingly, water loss rates of 11 day old flies in 60% O₂ were nearly double those of 6 day old flies. A number of mechanisms could be involved. Flies might lose control of their excretory system and defeate more water, or the ganglia controlling spiracular closer muscles may be damaged, causing the spiracles to remain open. It is also possible that severe hypoxia may mimic hypoxia, since hyperoxia impairs mitochondrial function and therefore the organism’s ability to generate ATP, which might
trigger an internal, hypoxia-driven ventilatory response. Similarly, both hyperoxia and severe hypoxia result in elevated protein carbonylation (a marker of oxidative stress, Rascón and Harrison, 2010), and so they may involve similar signalling cascades. In either scenario, although the hyperoxic fly is unable to metabolise any faster, it opens its spiracles and only succeeds in flooding its cells with more high \(O_2\), hastening its own death. Moreover, because the change in water loss is so drastic, this may contribute to fly mortality at the end of the LSape, separately from factors seen at other \(O_2\) concentrations (though humidity in the incubation chambers was high).

4.4. Recovery from hyperoxia

The recovery experiments may offer insight to the sources of damage. The reduced metabolism, elevated water loss, and decay in locomotor function were all somewhat reversible if the flies were allowed to recover in normoxia (Fig. 5). Baret et al. (1994) also performed recovery experiments, and found that life spans of hyperoxia-exposed flies were extended if they were returned to normoxia. Fenn et al. (1967) exposed flies to intermittent or continuous bouts of hyperoxia (same amount of time in each), and found that the former case, which allowed for some recovery, significantly extended life spans. In our experiment, flies’ life spans were extended at least 6 days longer when they were recovered, and they also regained some locomotor function and metabolism. The ability to reverse the hyperoxic phenotypes could mean they were actually a sign of acclimation, rather than damage. However, neither Fenn et al. (1967) nor Lighton et al. (2004) observed that acute exposure of flies to pure \(O_2\) resulted in sustained changes to metabolic rate, and our metabolic results are consistent with the responses of flies to oxygen perfusion injuries (Lighton and Schilman, 2007).

Wicks et al. (2009) recently exploited the reduced oxidative stress of hypoxic atmospheres to selectively rescue \(Sod\)-null mutants of flies. When \(Sod2\) mutants are removed from normoxia to 5% \(O_2\), they recover a life span similar to normoxic controls. Conversely, the damage to \(Sod\) mutants is irreversible, and they can only be rescued if they spend their whole lives in 5% \(O_2\). Their results suggest the mechanism of oxidative damage in hyperoxia in the current experiment. Since the flies recovered after removal from hyperoxia, as in the \(Sod2\)-null mutants of Wicks et al. (2009), the damage may have been localised to the mitochondria. After the damage was repaired, metabolic rate and locomotor capacity recovered, which is consistent with mitochondrial impairment. A corollary of this result is Rebrin and Sohal’s (2006) observation that the cellular redox state, as indicated by the glutathione system, was largely unaffected by exposure to hyperoxia. As suggested previously, damage to the mitochondria would also be consistent with the drastic increase in water loss rates in 60% \(O_2\), which suggested that the flies were ‘gasping’ for air even while the hyperoxic atmosphere was causing cellular destruction. After repairing the mitochondria, their breathing rates returned to normal.

4.5. Conclusions

Overall, we find evidence of an \(O_2\) concentration-dependent decrease in life span of flies in both hypoxia and hyperoxia. Hyperoxia led to a marked decrease in the rapid, and to a lesser extent prolonged, climbing behaviour, though only flies in moderate hyperoxia (40% \(O_2\)) showed a decrease that paralleled flies in normoxia. Metabolism and water loss were reduced in hyperoxia, and showed different age-specific trends from normoxia. Surprisingly, 11 day old flies in the highest \(O_2\) concentration nearly doubled their water loss rates, which was not seen in the other cases. Much of the behavioural and metabolic dysfunction caused by hyperoxia was reversible by rescuing flies from hyperoxia, although by definition, senescence is not reversible. Consequently, although the decay of some traits, such as climbing behaviour, may be accelerated by exposure to higher \(O_2\) concentrations, the physiological basis of their loss may be different in hyperoxia than normoxia.

Acknowledgements

We gratefully acknowledge the emotional, intellectual, nutritional, and technical support provided by our colleagues Viviana Cadena, Marc Charette, Charles Darveau, Danielle Levesque, Melissa Page, Ellen Robb, Kurtis Salloway, and Brent Wiens. We also gratefully acknowledge two anonymous referees whose comments substantially improved and clarified the manuscript. This research was supported by Natural Sciences and Engineering Research Council of Canada grants to GJT and JAS.

References


