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Anoxia elicits the strongest stimulatory protective response in insect low-oxygen hormesis

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Abstract

The manipulation of oxygen to trigger the stimulatory response known as hormesis is an area of interest in insects that was born almost fifty years ago. Varying low-oxygen treatments have been investigated many times since with differing responses found; some hormetic/some harmful. In this review, we summarize the recent advancements in low-oxygen hormesis with a focus on severe hypoxia and anoxia. These two low-oxygen treatments fall below the critical partial oxygen pressure (PO_2, often referred to as P_{crit}), the oxygen level where metabolism is impaired, for insects and represent the most robust forms of this type of hormesis, yielding the largest protective responses recorded in insects. We introduce six factors that influence the effectiveness of low-oxygen hormesis: oxygen content, length of and age at treatment, treatment method, sex, and genetic background. Additionally, we present a glimpse at the known mechanism of this type of hormesis.

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Cross tolerance, Hypoxia, Oxidative stress, Preconditioning.

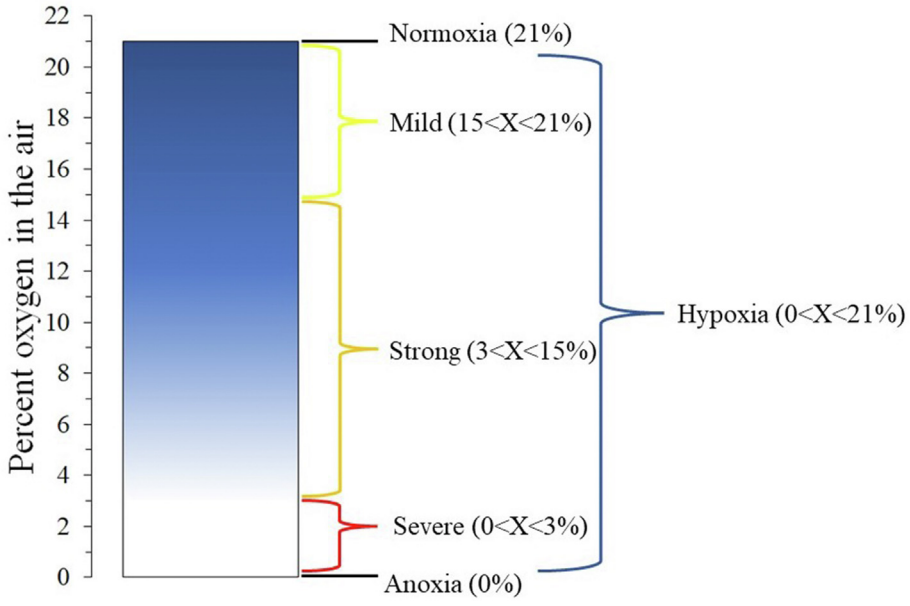
Introduction

The dose response phenomenon that results in low dose stimulatory and protective effects, where high doses are inhibitory and detrimental, is known as hormesis [1], and is often referenced as cross-tolerance, preconditioning, or postconditioning. Hormesis in response to numerous abiotic and biotic stimuli yields improvements in performance in the individuals experiencing the conditioning and their offspring, both in the short- and long-term therefore increasing fitness [2,3,4]. One of the crucial aspects of hormetic responses is the exposure that triggers it and how changes in concentration/dose and length of exposure can quickly turn a potential beneficial response into a harmful one [1,5]. Such is the case of anoxia hormesis, where an oxygen-free environment triggers a strong protective response, but the response is weakened if small amounts of oxygen are present (hypoxia; Figure 1). In recent years, there has been a progression in our understanding of this complex physiological response [3]. The inspiration for much of this recent work traces back nearly fifty years to experiments manipulating oxygen environments to trigger protection from free radical damage. This seminal work showed that anoxia prevented sterilization of moths, and higher doses were required to achieve radiation-induced sterilization in oxygen-free environments [6]. Given the importance of insects as agricultural pests, we focus this review on the use of low-oxygen hormesis to increase the efficacy of radiation-based pest control treatments. For more than 60 years, radiation has been used for pest suppression and these practices remain popular today due to the low environmental impact they offer in comparison to pesticide use. It is in this area of research where advances in our understanding of low-oxygen hormesis are rapidly occurring. Since the hormesis response starts at the cellular level, it is likely conserved and similar across all animals, providing a framework that goes beyond agriculture and potentially into biomedicine.

Anoxia hormesis

More recently, low-oxygen hormesis investigations have focused on the use of hypoxia (low oxygen) or anoxia (no oxygen) to protect against a second detrimental stressor. A recent review highlights how anoxia hormesis increases survival to high temperatures in locust and low temperatures in flies and moths [7]. Soil-dwelling insects likely experience hypoxia/anoxia during development and while overwintering in cavities in the ground. In this context, exposure to anoxia primes the insects to successfully manage future bouts of anoxia by allowing for faster recovery without depletion of their limited energy reserves [8]. This hormesis protection is robust enough to improve bee performance following a long (9–10 month) overwintering period, wherein anoxia exposure directly after overwintering provided positive lifelong consequences [5].

Figure 1



Normal concentration of oxygen in the air (normoxia) is 21 kPa and referred to as 21% oxygen content. Any decrease in oxygen from normoxia is considered hypoxia. Hypoxia ranges from 21 to 0 kPa (or 0%) that is considered anoxia. For the purposes of this review, we are defining mild hypoxia as 15–21%, strong hypoxia as 3–15%, and severe hypoxia (near anoxia hypoxia) as less than 3% but more than 0%.

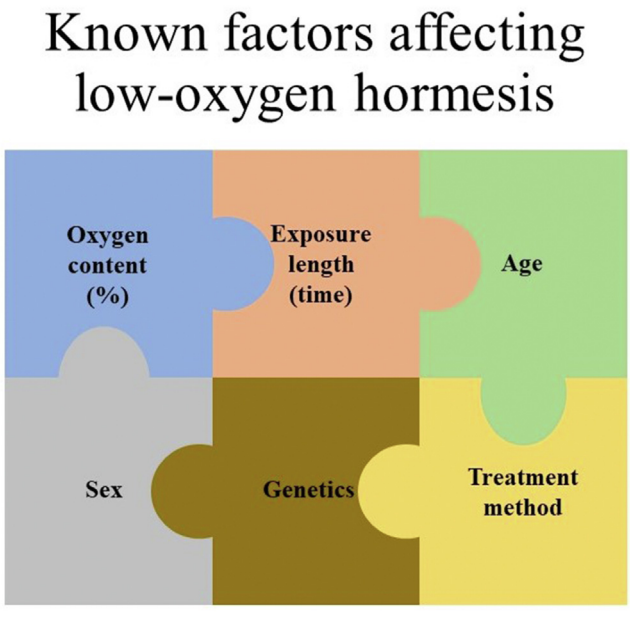
Outside of that handful of reports, the bulk of the research into low-oxygen hormesis is in response to irradiation. Ionizing radiation (gamma, X-rays, or E-beam) is used in several environmentally friendly pesticide-free pest control approaches known as the sterile insect technique (SIT [9]; and phytosanitary/quarantine irradiation (PI; [10]. The protection that low-oxygen hormesis confers during sterilization of insects is desirable as the insects accumulate lower oxidative damage and performs better, therefore maximizing the invasive pest control effort. In fact, this model of low-oxygen protection has been successfully tested many times in the framework of the sterile insect technique where the outcome is reduced oxidative damage leading to improved post-irradiation performance in insects given low-oxygen conditioning [11,12]. Flies treated with a 1-h anoxia conditioning treatment prior to irradiation had higher flight ability, starvation survival, longevity, and mating success at youth (10 days post treatment) and old age (30 days post treatment; reviewed by Berry et al. [3]. Similar improvements in laboratory-based performance assays were also found in moths [13], and that protection was extended outside the lab when those anoxia-irradiated moths were released into an infested field [14]. The benefits of anoxia hormesis are dramatic enough to partially rescue radiation-induced sterility and thus increasing the fitness of otherwise sterile moths [15]. In the context of phytosanitary irradiation, low-oxygen environments are used to promote the longevity of the fresh fruit or vegetable being treated and radiation is used to rid the produce of unwanted pests [16]. This low-oxygen conditioning of produce may lead to unwanted hormetic effects in the accompanying pests and a problematic outcome is surviving pests, resulting in higher doses of radiation required to prevent any survival [17]. This area of hormesis research continues to grow because of the interest in improving the performance of sterilized insects by lowering radiation off-target damage (SIT) and the concern that low-oxygen might prevent death in sanitary treatments (PI).

Factors influencing the hormetic response

The importance of dose and exposure are fundamental to hormesis and even mild changes to these can have dramatic impacts on the outcome. For anoxia, insects are exposed to pure nitrogen (dose) and exposure is represented by the time spent in anoxia. The protection following anoxia conditioning can quickly become harmful when the dose extends beyond the typical 1–3-h range [5]. These two factors, oxygen concentration and length of treatment, might be the most studied and understood components of low-oxygen hormesis but there are multiple additional components that also affect the response. Age at treatment is instrumental in achieving a robust hormetic response and must be considered because doses that are hormetic at one age can be detrimental earlier or later in life (Visser at al. 2018, [3,5]. In *Drosophila*, anoxia tolerance declines with age [18], adult flies are more tolerant to anoxia than

larvae [19], and the effect of low-oxygen hormesis on sexual competitiveness is dependent on stress experienced during development [20]. This is the way in which the low-oxygen conditioning will affect the response and indicates the importance of the age effect (Figure 2). Some studies use pure nitrogen to replace oxygen and induce anoxia, while others may use specific concentrations (2, 5, or 10% oxygen) to induce hypoxia by either constantly flushing with that concentration or sealing the insects in that atmosphere. Once sealed in a particular oxygen concentration, insects will continue to consume oxygen and produce carbon dioxide until the concentration is low enough–likely below the critical partial pressure of oxygen impairing their metabolism—to force them into a low-oxygen comma [21]. Out of all these factors, the two most poorly understood at this time are genetic background and sex. There is a connection between the genetic background of the insect and the hypoxia conditioning response, but little is known beyond that [22,23]. On the sex effect, male flies that experience severe hypoxia conditioning required higher doses of irradiation than females to achieve the same level of sterility [24]. And there are multiple reports showing that males may benefit more from low-oxygen hormesis (reviewed by Berry et al. [3] but this pattern may not be universal [5].

Figure 2



We have identified six factors known to be critical for successful hormesis responses to occur. While we represent these factors as equally contributing to the effectiveness of hormesis, current data suggest that oxygen content (concentration) and length of exposure may play a bigger role than age, treatment method, and sex. There is not enough data on age at receiving low dose stimulation to conclude whether the effect of age is a minor component. Little is known about how additional factors in treatment methodology (i.e., hypercapnia) and genetic background affect responses, but what we present here impacts hormesis responses and merits additional investigation.

Hypoxia hormesis

There is modest recent work into the effects of age and how hypoxia exposure is generated and applied (treatment method) on the hormesis response, and so we will focus on concentration (dose) and time, and their effect on hypoxia hormetic responses. Unlike anoxia, hypoxia has a much broader range of oxygen concentrations. A hypoxic environment is defined as one where the partial pressure of oxygen is lower than normoxia (21 kPa; which we practically refer to as 20−21% oxygen in air), but higher than 0 kPa (0% oxygen, Figure 1). With such a broad range of doses, we would expect very different responses when insects are treated to ranging concentrations (mild, strong, or severe hypoxia) and published reports agree with that assertion. When a series of low-oxygen doses, ranging from 0 to 8% oxygen content, were used to test for radioprotection in flies, the results were mixed. Lower doses (0 and 2%) were protective, while 4, 6, and 8% led to mortality similar to normoxia controls [25]. In this experiment, the conditioning period was 15 min, and it is possible that the exposure length (Figure 2) is the additional factor that requires parameterization before the effectiveness of hypoxia hormesis can be fully assessed. Still, 15 min of anoxia or severe hypoxia (2%) was effective in providing some form of protection, indicating that even brief exposures can lead to hormesis once the appropriate dose is found. Similarly, severe hypoxia (0.3%) conditioned flies required a higher dose of radiation to achieve sterility, yet those individuals surviving after low-oxygen hormesis did not perform better than normoxia controls [9]; this response represents a fraction of other recorded responses and is likely due to other factors (Figure 2). A similar effect was recorded in mosquitoes where higher radiation doses were required for full sterilization under severe hypoxia [26]. Also in mosquitoes, strong hypoxia conditioned (< 5%) individuals had the same level of sterility but higher hatching rate, indicating that there was a partial benefit to hypoxia conditioning [27]. The benefits of near anoxia/severe hypoxia are strong enough that at the 0.4% level there was a very similar protective effect to that seen in anoxia [28]. Perhaps, one explanation for similar responses between anoxia and severe hypoxia is related to the animals' critical partial pressure of oxygen. One recent study linked increased radiation resistance to individuals treated with oxygen levels below their critical PO_2 [29]. Beyond dose, when we consider time as a factor, looking at the time spent in hypoxia, insects treated for less than a day in hypoxia had higher emergence and flight activity than those treated for more than a day [30,31].

In the context of phytosanitary irradiation, where the goal is eradication, a hormetic response in the insect is undesirable; therefore, the goal is to find the goldilocks low-oxygen treatment—one that doesn't stimulate the insect but protects the commodity from radiation damage [32]. Here, commonly used hypoxia doses ranged from 3 to

10% and the duration of the exposure also ranges from a day to just minutes prior to irradiation. The reasons for the lack of protective response of hypoxia are related to all six factors previously mentioned (Figure 2). The concentration and length of exposure seem to be essential here but experiments with conditioning lasting for hours sometimes lead to no stimulatory effects; the reasons for the lack of stimulatory response may be carbon dioxide toxicity (treatment method). Hypercapnia, or elevated environmental CO_2, often accompanies hypoxia in contained/sealed spaces and the response to CO_2 toxicity may counteract/influence the hypoxic response. Hypoxia hormesis does not typically occur when hypoxia is accompanied by hypercapnia (8–26% CO_2) and instead higher mortality is recorded [33]. Thus, the lack of clear hormetic responses here is likely related to treatment method and emphasizes the critical need for experiments that manipulate or control for all current known hormesis-influencing factors (Figure 2). Despite that, near anoxia hypoxia provides some protection during phytosanitary irradiation; four cosmopolitan fly species showed increased radiation resistance following a severe hypoxia exposure, conforming that low-oxygen hormesis is dose–concentration dependent even as an unwanted byproduct in PI [32].

The mechanism of low-oxygen hormesis

The mechanism of low-oxygen, specifically anoxia, hormesis remains largely unknown at present time, but multiple studies have identified key players. The hormetic benefits that accompany anoxia exposure may be in part rooted in the preparation for oxidative stress (POS) hypothesis [34,35]. Under POS, upon entering a hypoxic environment, the mitochondria upregulate a suite of protective genes in preparation for oxygen reperfusion and the damage that accompanies the resumption of normal mitochondrial function [36]. Thus, our current working model for low-oxygen hormesis suggests that this increase in protection likely ameliorates damage from oxygen reperfusion and the excess gene products lower/prevent additional damage following reperfusion. This protection may be what allows the animal to go beyond recovery and into life history trait improvements and increased fitness [4]. A series of reports from snakes, frogs, turtles, fish, and insects over the last twenty years revealed that antioxidant enzymes play a role in anoxia responses [3]. Superoxide dismutase, catalase, and several glutathione-based enzymes are upregulated as a result of anoxia conditioning and are involved in lowering oxidative damage accumulated following reperfusion or when challenged with a second stressor (i.e., ionizing radiation). Antioxidant enzymes also increase in response to hypoxia [3,37]. Additionally, reports from fish and insects indicate that low-oxygen hormesis improves metabolism [38], to the point that no energetic cost to hormesis was detected [5,8]. Despite these efforts, the exact mechanisms responsible for low-oxygen hormesis remain elusive and warrant further investigation.

Conclusive remarks

The benefits of low-oxygen hormesis are linked to the concentration of oxygen (%) used, the length of the treatment (time), the age of the treated individual, how low-oxygen was generated and applied (treatment method), their genetic background, and sex. However, the exact mechanism of this hormetic response remains unclear and may be connected to the preparation for oxidative stress hypothesis. This indicates that oxygen reperfusion may play a big role here and likely other protective mechanisms (i.e., heat shock proteins and DNA repair [39]; are also involved. The regulatory role of target of rapamycin (TOR) on cell signaling and the hormetic response [40] has shed light on new mechanistic areas of exploration. Given that low-oxygen hormesis promotes neural stem cell proliferation and differentiation [41], transcription factors, like Nrf2 [42], may be the master regulators triggering the responses recorded to date.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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