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The effects of short-term hypergravity on *Caenorhabditis elegans*

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ABSTRACT

As we seek to recognize the opportunities of advanced aerospace technologies and spaceflight, it is increasingly important to understand the impacts of hypergravity, defined as gravitational forces greater than those present on the earth's surface. The nematode *Caenorhabditis elegans* has been established as a powerful model to study the effects of altered gravity regimens and has displayed remarkable resilience to space travel. In this study, we investigate the effects of short-term and defined hypergravity exposure on *C. elegans* motility, brood size, pharyngeal pumping rates, and lifespan. The results from this study advance our understanding of the effects of shorter durations of exposure to increased gravitational forces on *C. elegans*, and also contribute to the growing body of literature on the impacts of altered gravity regimens on earth's life forms.

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1. Introduction

Life forms on earth have evolved to maximize their fitness at 1 g which is earth's normal gravitational force. Processes from the molecular to the organismal are believed to all be impacted by the gravitational force of earth (Morey-Holton, 2003). Can multicellular organisms respond to changes in gravitational forces? And are they capable of adapting to gravitational forces higher than 1 g? To address these questions, previous work has investigated the abilities of diverse biological model organisms, such as *Escherichia coli, Arabidopsis thaliana, Drosophila melanogaster,* and mice, to withstand gravitational forces higher than the force of 1 g. Some studies have reported changes in muscle composition and architecture after organisms such as hamsters, mice, and developing chickens were treated at speeds of 2–5 g at durations ranging from 7 days to 14 weeks (Reviewed in VanLoon et al. (2005)). Mice exposed to 2 g or more during development have been shown to display alterations in some of their motor coordination features and reproductive functions (Ronca et al., 2001; Ronca, 2003; Bouët et al., 2004). Hypergravity exposure of 2.58–7.38 g for 12–24 days early in life has been found to confer increased longevity in male *Drosophila melanogaster* (Le Bourg et al., 2000). Low speed hypergravity regimens have also been tested on humans on a small scale in "human centrifuges" to determine whether these treatments can counter the effects of micro gravity on loss in bone density and circulation (Kourtidou-Papadeli et al., 2008; Blue et al., 2012; Porte and Morel, 2012). These and other studies highlight the differences seen in the responses to increased g-loading in various organisms based on size and physical contexts. However, they also demonstrate that a lot remains to be understood about the effects exerted by hypergravity on organismal physiology, functioning, reproduction, and behavior.

Studies have long focused on the effects of increased g forces on pilots, especially for those who perform maneuvers in combat or aerobatics (Tachibana et al., 1994; Convertino, 1998; Muller, 2002; Scott et al., 2007). Interest in the short-term and longer-tem effects of altered gravity on diverse organisms has also intensified in recent years with advances in interplanetary travel. Planets suitable to inhabitation by earth-based life are of particular interest, and most of them are predicted to have variable gravitational forces (Kalb and Solomon, 2007; Horneck, 2008; Oczypok et al., 2012). Additionally, astronauts and biological specimens sent into space are routinely subjected to hypergravity of 1.5–3.5 g when exiting the earth's atmosphere as well as on re-entering (Ashcroft, 2000; Hu et al., 2008, Wu et al., 2012). Research conducted in the field of space and gravitational biology and medicine has therefore been focused on actively investigating the effects of changes in gravitational forces on organismal health and survival (Clément and Slenzka, 2006). Studies conducted in model organisms can help bridge the gaps in our understanding of the consequences of gravitational forces greater than 1 g.

C. elegans is a microscopic nematode, and it has been established as a powerful model system for studying the effects of altered gravity and space travel. The animals are easy to cultivate,

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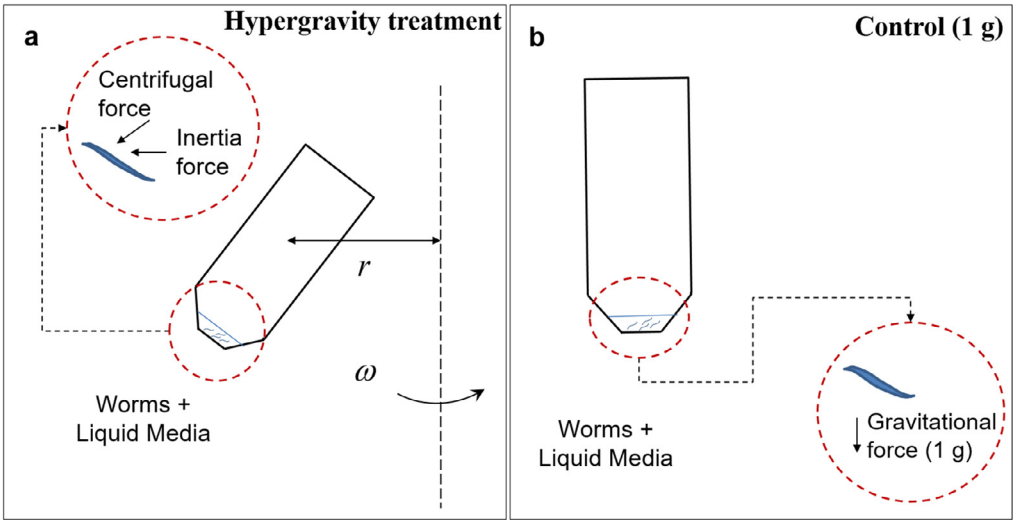

Fig. 1. Schematic of Experiment set-up. Panels A and B illustrate the experiment set-up for Hypergravity (a) and Control 1 g (b) treatments. In each flat bottomed tube, 10 L4-stage larvae were placed in 20 µl of liquid media. The tubes were then (a) spun in a centrifuge at 19–20 °C and set speeds, or (b) maintained at 1 g in a 20 °C incubator. Further details are provided in 'Materials and Methods- Hypergravity regimens.'

are amenable to genetic experimentation, and their genome, development, and anatomy have been extensively studied (Brenner, 1974; Kaletta and Hengartner, 2006; Powell-Coffman, 2010; Corsi et al., 2015). *C. elegans* have been sent into space and cultured aboard the International Space Station (Johnson and Nelson, 1991; Higashibata et al., 2007; Selch et al., 2008; Szewczyk et al., 2008; Adenle et al., 2009; Higashitani et al., 2009; Honda et al., 2012; Oczypok et al., 2012). Interestingly, while the effects of microgravity on *C. elegans* health has been studied intensively, much less is known about the effects of hypergravity. Conley et al. (2001) observed that *C. elegans* emerged immobile from 4 day long hypergravity treatments of 10 g to 50 g, but were able to survive this treatment and recovered mobility after approximately two hours of recovery at earth's normal gravity (Conley et al., 2001, Cited with author permission). Others have reported that the mechanical stress of 100 g hypergravity causes nuclear accumulation of the DAF-16 transcription factor and influences fat accumulation and muscle sarcomere elongation (Kim et al., 2007). While these previous studies on *C. elegans* provide important insights to the effects of altered gravity, they also demonstrate that there is still much to be learned. Some of the outstanding questions in the field include: How do the animals respond to and recover from short-term, intense increases in gravitational forces? What effects will these hypergravity treatments have on organismal physiology and behavior? What are the effects of intense and varying durations of hypergravity exposure? Does hypergravity exposure cause transgenerational effects? To extend previous work and to address some of these outstanding questions, we investigated the effects of hypergravity treatments ranging from 10 g to 100 g on *C. elegans.* The experiments described herein provide a better understanding of the effects of short, relatively intense hypergravity regimens the longevity and fecundity of this model organism.

2. Materials and methods

2.1. C. elegans strains and culture

N2 wild-type *C. elegans* used in this study were cultivated at 20 °C on standard Nutrient Growth Media (NGM) plates seeded with *E. coli* OP50 bacterial food, using previously described methods (Brenner, 1974).

2.2. Hypergravity regimens

The M9 buffer, S basal medium, and *C. elegans* maintenance medium (CeMM) liquid culture media were prepared using previously published methods (Szewczyk et al., 2003; Stiernagle, 2006). Fig. 1 shows a schematic of the experimental set up for hypergravity (Fig. 1a) or control 1 g (Fig. 1b) treatments. 10 L4-stage worms were added to 20 µl of liquid medium per flat bottomed tube. The worms were then spun in a tabletop, refrigerated centrifuge (Thermo Scientific Sorvall ST 16R) at a controlled speed for set periods of time. The 45° fixed angle rotor had a radius of 100 mm. The hypergravity treatments used in this study were 10 g (300 rpm), 55 g (700 rpm), 100–112 g (950–1000 rpm). The temperature within the centrifuge was maintained at 19–20 °C. Control worms were similarly set up in tubes kept at 20 °C at 1 g, which is the earth's gravitational field. All experiments that were 12 h or less were started with L4-stage animals.

2.3. Worm motility phenotypes

After the hypergravity or control treatments, the worms were transferred onto NGM plates seeded with *E. coli* OP50 food. Their motility was then monitored at intervals of 30 min for a total of two hours of recovery after treatment. Animals were classified as motile, having limited mobility or non-motile, based on the visual assessment of their relative motility on the plates, as described in our previous work (Saldanha et al., 2013). Briefly, 'motile' animals actively moved on the plates without any stimulus. Animals displaying relatively slow movement were classified as having 'limited motility'. Worms that were immobile and did not respond to any stimulus were classified as 'non-motile'. For statistical analysis, an ordered multinomial regression with a cumulative logit link was run using SAS software (version 9.4, SAS Institute, Cary, NC), that modeled the probability of a worm being in a lower movement category based on the treatment.

2.4. Microfluidic assay for worm motility

The microfluidic chip fabrication, system set-up and imaging, and data analyses were all performed as described in Saldanha et al. (2013). For the assays described in this paper, the microfluidic chip (Fig. 2a), was filled with 1X CeMM. Similar to the visual

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