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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.

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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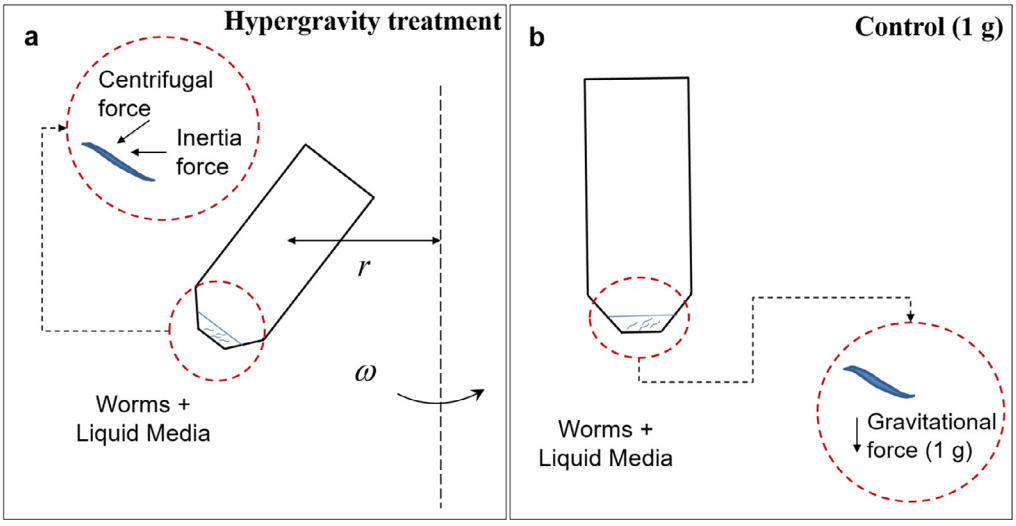

Fig. 1. Schematic of Experiment set-up. Panels A and B illustrate the experiment set-up for Hypergravity (a) and Control 1 g (b) treatments. In each flat bottomed tube, 10 L4-stage larvae were placed in 20 µl of liquid media. The tubes were then (a) spun in a centrifuge at 19–20 °C and set speeds, or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

motility assays, the worms from hypergravity or control treatments were first transferred from the CeMM onto NGM plates seeded with *E. coli* OP50 food. Individual animals were then randomly picked off these plates at each recovery interval (0 min, 30 min, 1 h, and 2 h) and inserted into chambers in the microfluidic chip (Fig. 2a) for recording, 1 animal per chamber. Following this, videos of worm movement in the device chambers were recorded at the rate of one image per second for a total of 1000 s. A custom worm tracking program was then used to analyze the videos (Carr et al., 2011; Parashar et al., 2011). Worm velocity was calculated from the tracking program's output using a custom Graphic User Interface (GUI) program. For statistical analyses, repeated measures ANOVAs were run using SAS software (version 9.4, SAS Institute, Cary, NC). The fixed effects included in the model were the main effect for treatment, recovery time, and their interaction. We accounted for correlation within each worm by including a random effect for worm identifier, where each worm tested had a unique identifier. We specified an AR(1) correlation structure.

2.5. Assaying capacity for self-reproduction

Self-fertile hermaphrodite *C. elegans* were initially placed on NGM plates with *E. coli* OP50 bacterial food after 2 or 12 h of hypergravity or control treatment. No progeny were produced in the liquid media during treatment. Individual animals were then picked onto new plates with food: 1 worm per plate. Animals were transferred onto fresh plates with food every 12 h, and the progeny left behind were counted. This process was continued until the animals laid only oocytes, signaling the end of their reproductive capacity. All plates were re-examined after 48 h, to count the number of animals that hatched and developed to adulthood. Statistical analyses using a generalized linear model were performed using SAS software (version 9.4, SAS Institute, Cary, NC). Poisson distribution was used for the total number of progeny laid and a binomial distribution for probability was employed for the probability of laying viable progeny as a function of the treatment.

2.6. Pharyngeal pumping rate

Animals were placed onto NGM plates with *E. coli* OP50 bacterial food after hypergravity or control treatment. A QICam 12-bit MonoFast 1394 cooled digital camera attached to a Leica MZ16 transmission stereozoom microscope linked with QCapture PRO software was used to record videos of the animals on the plates. Multiple videos of each animal were recorded, and the grinder movements were counted to obtain average pumps per minute. SAS software (version 9.4, SAS Institute, Cary, NC) was used to run repeated measures ANOVAs to analyze the data generated. The fixed effects were the treatment where treatment had 2 levels 1 g and 100 g. A random effect for worm identifier was also included and a compound symmetry structure was assumed.

2.7. Lifespan experiments

Lifespan assays were performed as described previously unless noted otherwise (Kenyon et al., 1993). Animals were placed onto NGM plates with *E. coli* OP50 bacterial food after hypergravity or control treatment. The plates did not contain FUdR or antibiotics. Three independent trials were conducted, and 45–60 animals were used in each trial. The experiments were conducted at 20 °C. Animals were transferred onto fresh plates every two days until they reached the end of their reproductive capacity, and every three days thereafter. Kaplan Meier survival curves were employed for non-parametric analyses. The Log-rank test was used to test for equality of survival distribution (SAS Software, version 9.4, SAS Institute, Cary, NC).

3. Results

Prior work has investigated the effects of specific hypergravity regimens on *C. elegans,* primarily in the range of 10 g to 200 g using a variety of media (Conley et al., 2001; Sasagawa et al., 2005; Kim et al., 2007). Building upon these studies, we sought to identify the liquid culture media that was best able to support *C. elegans* viability and health in our experiments. In these initial experiments we placed *C. elegans* L4-stage larvae in 1 g control conditions in various media for a duration of two hours. Further details on the experimental set-up are provided in the methods section on Hypergravity regimens. The media tested included CeMM, S basal, and M9 buffer supplemented with bacterial food (Szewczyk et al., 2003; Kim et al., 2007). M9 buffer and S basal are routinely used for *C. elegans* experiments (Stiernagle, 2006). CeMM is a chemically defined medium that was created to enable long term culturing of *C. elegans* in spaceflight experiments (Szewczyk et al., 2003). Following the 1 g control treatments, animal motility on NGM plates with food was visually monitored at 0, 30, 60, and 120 min. Further details about this assay are provided in the 'Materials and methods' section under the 'worm motility phenotypes' subheading. As seen in Table 1, CeMM was optimal, as assayed by motility following incubation at 1 g. In all other media a fraction of the worms remained non-motile through 2 h of recovery. CeMM has also been extensively studied as a nutrition source, and has been previously used for culturing worms under microgravity in space (Szewczyk et al., 2003; Szewczyk et al., 2006; Szewczyk et al., 2008). Therefore, this liquid media was used for all experiments that are subsequently described here.

3.1. Hypergravity treatments have transient effects on motility

Prior studies have found few detrimental effects of long-term hypergravity treatments on *C. elegans* motility (Conley et al., 2001; Kim et al., 2007). To confirm and extend these previous studies, we first monitored *C. elegans* movement on solid media plates following 2 or 12 h of treatment at 100 g. As shown in Table 2, treating L4 stage animals for 12 h at 100 g impaired movement in 70% of the animals, but they regained motility within 2 h of recovery on agar plates at 1 g.

We also quantitated the rate of *C. elegans* movement in a custom microfluidic device (Fig. 2a). While the data in Table 1 broadly classifies the movement of individual worms, the graphs in Fig. 2 show the impacts of 100 g treatments on the average velocity of animals at various times of recovery following treatment. In these assays, the animals subjected to hypergravity for 2 h did not show dramatic changes in velocity (Fig. 2c). As seen in Fig. 2d, animals that were exposed to hypergravity for 12 h recovered over a period of 2 h after treatment. Taken together, the results from these motility experiments are in agreement with results from prior work. *C. elegans* exposed to hypergravity treatments regain their motility (Conley et al., 2001; Kim et al., 2007).

3.2. The effect of hypergravity on pharyngeal pumping behavior

C. elegans pharyngeal pumping behavior and the resultant food intake rate is often altered by the animals' environment (Avery, 1993; Avery and You, 2012). Therefore, we next investigated the impacts of hypergravity on pharyngeal pumping behavior. The *C. elegans* pharynx is located near the beginning of the alimentary system, and it enables the animals to take in, grind, and ingest bacterial food. It is made up of the corpus, isthmus and a terminal bulb equipped with a grinder (Fig. 3a). The movements of this grinder can be counted as a representative measure of pharyngeal pumping rate (Avery and You, 2012; Raizen et al., 2012). Since the animals exposed to 2 h of hypergravity displayed normal

Table 1
Effects of alternative liquid media on *Caenorhabditis elegans* motility after 2 h treatments at 1 g. Experiments were performed to investigate the effects of various liquid media on *C. elegans* in 1 g control conditions. Most of the worms were motile after 2 h of recovery. However, a fraction of the animals were non-motile in all media tested except for CeMM. Hence, this media was used for the experiments described in this study. (n = number of worms tested).

Media	Recovery time on plates (min)	% Motile	% Limited motility	% Non- Motile
M9 buffer + OP50 bacterial food (n = 76)	0	78.9	21.1	0.0
	30	96.1	1.3	2.6
	60	97.4	0.0	2.6
	90	97.4	0.0	2.6
	120	97.4	0.0	2.6
S basal (n = 215)	Recovery time on plates (min)	% Motile	% Limited motility	% Non- Motile
	0	90.7	8.4	0.9
	30	96.7	2.3	0.9
	60	98.6	0.5	0.9
	90	98.6	0.5	0.9
	120	98.6	0.5	0.9
CeMM + OP50 bacterial food (n = 157)	Recovery time (min)	% Motile	% Limited motility	% Non-Motile
	0	23.6	45.9	30.6
	30	74.5	12.1	13.4
	60	89.2	1.9	8.9
	90	89.8	1.3	8.9
	120	91.1	0.0	8.9
CeMM (n = 84)	Recovery time (min)	% Motile	% Limited motility	% Non-Motile
	0	73.8	19	7.1
	30	96.4	3.6	0
	60	97.6	2.4	0
	90	97.6	2.4	0
	120	100	0	0

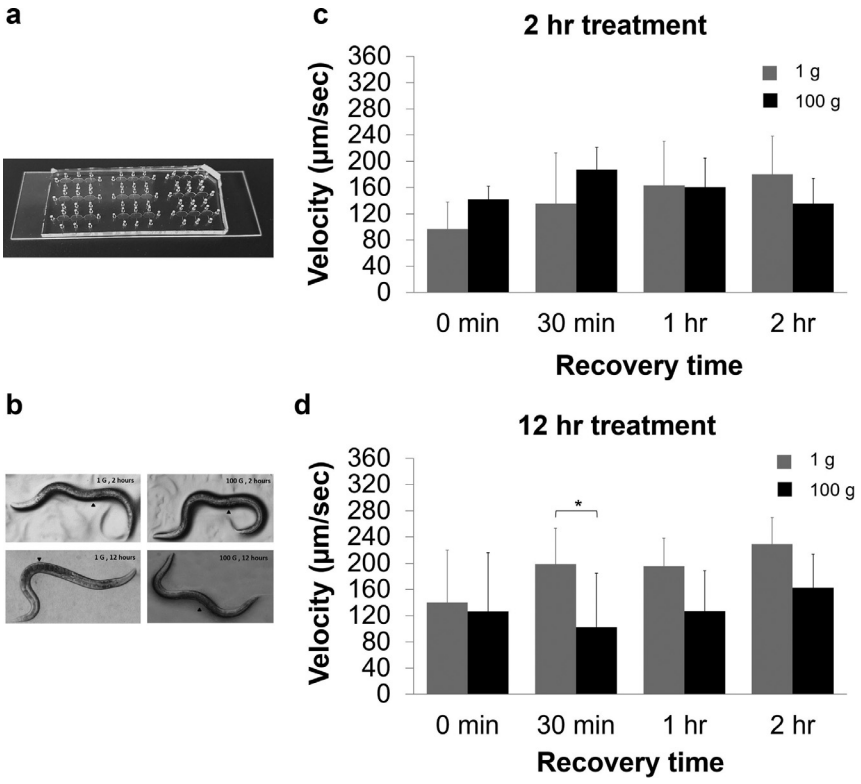

Fig. 2. The effect of hypergravity on worm velocity. Fig. a & b are photographs of (a) the microfluidic device used to assay velocity, and (b) images of the animals following each condition tested. (c-d) Animals were treated with 100 g hypergravity (black) or 1 g control (gray) conditions for 2 or 12 h. The animals' rate of movement was assayed at the recovery times shown. $* p < 0.05$, repeated measures ANOVA (Number of animals tested for each treatment are listed in supplementary Table S1, error bars indicate standard error).

Table 2
Effect of hypergravity on *C. elegans* motility. Animals were subjected to 100 g or maintained in 1 g control conditions in liquid culture for 2 h or 12 h, and then placed on NGM plates with food to recover. Individual animals were scored for the degree of mobility shown. (A) L4 stage animals exposed to hypergravity of 100 g or control conditions for 2 h showed some initial decrease in motility, but were able to recover within the first 30 min of treatment and were soon completely motile (100 g $n = 228$, 1 g $n = 257$). (B) After twelve hour treatments at 100 g, most of the animals regained motility in the first hour of recovery, with significant recovery seen within the first 30 min (100 g $n = 251$, 1 g $n = 243$). Three independent trials were performed for each treatment.

		Hypergravity			Control		
		100 g			1 g		
Time in CeMM	Recovery time (min)	% Motile	% Limited motility	% Non- Motile	% Motile	% Limited motility	% Non- Motile
A							
2 h	0	76.3	15.4	8.3	81.3	15.2	3.5
	30	90.8	9.2	0.0	97.7	2.3	0.0
	60	96.1	3.9	0.0	98.1	1.2	0.8
	90	96.1	2.2	1.8	98.4	0.8	0.8
	120	98.2	0.0	1.8	99.2	0.0	0.8
		Hypergravity			Control		
		100 g			1 g		
Time in CeMM	Recovery time (min)	% Motile	% Limited motility	% Non- Motile	% Motile	% Limited motility	% Non-Motile
B							
12 h	0	29.9	61.4	8.8	93.8	6.2	0.0
	30	70.1	29.1	0.8	97.1	2.9	0.0
	60	83.3	16.3	0.4	98.8	0.8	0.4
	90	95.2	4.4	0.4	99.2	0.8	0.0
	120	98.8	0.8	0.4	100.0	0.0	0.0

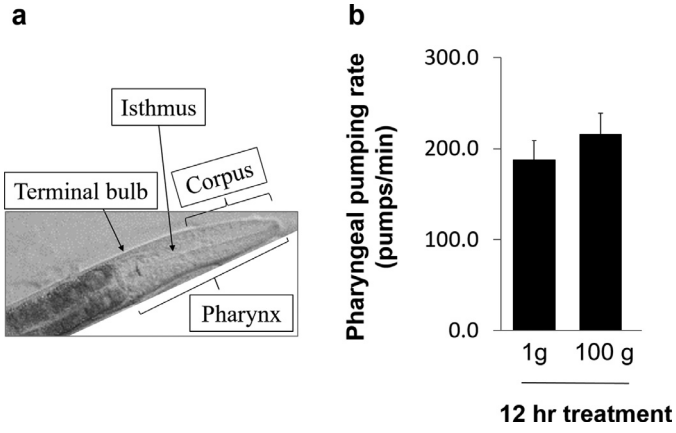

Fig. 3. Pharyngeal pumping rates in response to hypergravity treatments. (a) Representative image of the *C. elegans* pharynx, which consists of the corpus, isthmus and a terminal bulb. (b) Pumping rates after exposure to 12 h of 100 g hypergravity or 1 g control treatments. ($* p<0.005$) (n = 5–7, at least 3 recordings per animal).

movement (Table 2, Fig. 2) and feeding (data not shown), we decided to test the effects of 12 h of 100 g exposure on the animal's pharyngeal pumping behavior. As shown in Fig. 3, we determined that the rate of pharyngeal pumping in L4 stage animals exposed to 12 h of hypergravity (215.6 ± 23.1 pumps/min) was similar to *C. elegans* incubated in 1 g liquid culture control conditions for the same length of time (187.5 ± 21 pumps/min) (Fig. 3b). These results show that the effects of 12 h of hypergravity on pumping rates are not dramatic compared to 1 g controls.

3.3. Impact of hypergravity treatment on reproduction

Prior studies have shown that *C. elegans* can survive space flight and exposure to 100 g (Johnson and Nelson, 1991; Szewczyk et al., 2005; Kim et al., 2007; Adenle et al., 2009; Oczypok et al., 2012), but relatively little is known about the effects of short-term hypergravity exposure on the animal's reproductive capabilities and on the subsequent generations. We hypothesized that exposure to hypergravity might cause changes in the development or survival of the animals' progeny. To test this hypothesis, we first treated late stage larvae (L4) at 100 g for 2 h. As shown in Fig. 4a, hypergravity treatment for 2 h had little effect; animals that were incubated at 100 g for 2 h produced an average of 301 fertilized eggs, and 96% of these hatched (Fig. 4a, Table S2). In control experiments, the animals produced an average of 304 eggs, and 99.9% of the eggs hatched to yield viable larvae (Fig. 4a, Table S2). The results from these brood size experiments agree with prior reports of brood sizes for wild-type animals (Hodgkin and Barnes, 1991; Shen et al., 2006; Shao et al., 2010). To determine whether a longer hypergravity treatment impaired reproduction, we treated L4-stage larvae at 10, 55 or 112 g for 12 h. As seen in Fig. 2b, c and d, exposure to these hypergravity regimens did not dramatically alter the total number or viability of eggs that were laid by the animals.

3.4. The effects of short-term hypergravity exposure on worm lifespans

We considered the possibility that hypergravity treatments could injure the animals in ways that were not evident as changes in velocity or reproductive capacity, but could reduce lifespan. To investigate the effects of hypergravity, we treated L4 larvae for 2 h at 100 g and for 12 h at 112 g and then observed the effects on adult longevity. We found that treatment at 100 g in liquid CeMM for 2 h reduced the mean lifespan by 13% compared to 1 g controls (Fig. 5a, Table S3). Fig. 5 shows the combined data from 3 independent trails. By comparison, the animals exposed to 112 g for 12 h had lifespans similar to those of the 1 g controls (Fig. 5b, Table S3).

4. Discussion

C. elegans has proven to be a powerful model system for understanding biological responses to the rigors of space flight (Johnson and Nelson, 1991; Higashibata et al., 2007; Szewczyk et al., 2008; Higashitani et al., 2009; Qiao et al., 2013; Honda et al., 2014; Xu et al., 2014). Here we characterize the response of *C. elegans* to short-term hypergravity. Towards this goal, we first assessed different liquid media regimens and determined that CeMM was the best nutrition source for these experiments, when compared

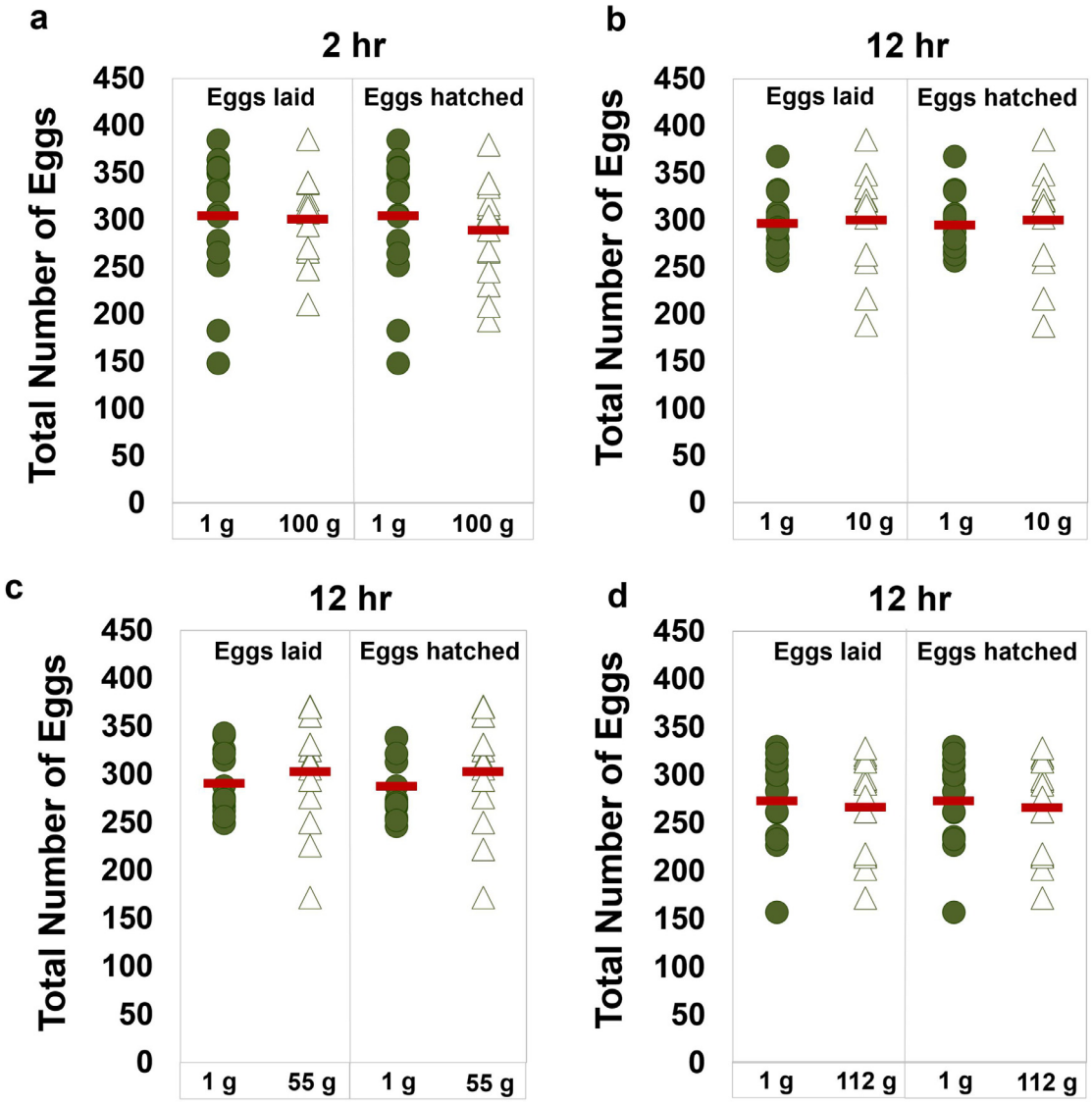

Fig. 4. The effect of hypergravity treatment on *C. elegans* brood size. Closed circles represent data from control treatments at 1 g and the open triangles represent data from hypergravity treatments. Average number of eggs laid are indicated by the red horizontal bars. (a-d) The average number of eggs laid did not differ very much after 2 or 12 h of hypergravity or control (1 g) treatments. 14–15 animals were assayed for each treatment. For details, see Table S1.

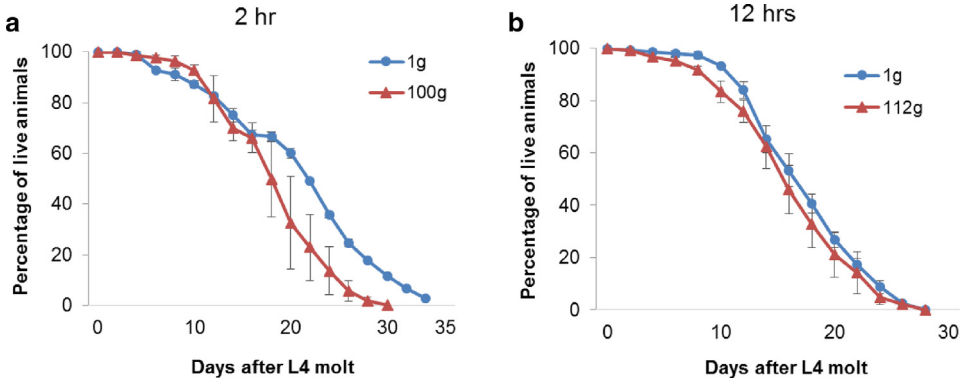

Fig. 5. Effects of 100 g exposure on *C. elegans* lifespan. The viability of *C. elegans* was assessed following 100 g hypergravity treatment for 2 h (a) or 112 g hypergravity treatment 12 h (b). Each graph illustrates the survival probability after exposure to hypergravity (red triangles) or control conditions (blue circles). The 100 g hypergravity treatment for 2 h reduced the mean lifespan of the animals ($p < 0.0001$, Log-rank test was used to test for equality of survival distribution). The lifespans of the animals exposed to 12 h of 112 g and 12 h of 1 g controls were similar ($p=0.1277$, Log-rank test). Further details are provided in Supplementary Table S3. Three independent trials were performed per treatment, and 45–60 animals were tested per trial. Error bars indicate standard error.

to other M9 or S basal media. In the studies reported herein, we show that *C. elegans* can recover well from short-term hypergravity treatments, as assayed by motility, pharyngeal pumping, reproduction, or lifespan.

4.1. Hypergravity and motility

Hypergravity can be a form of stress to a multicellular organism with the amount of g tolerated being related to the animal's size and weight (Le Bourg, 1999; vanLoon et al., 2005). Prior spaceflight based studies have reported changes in *C. elegans* muscle development; more specifically, decreases in myosin heavy chain gene expression in the worm's body wall and pharyngeal muscles have been observed (Higashibata et al., 2006). A 24 h treatment of 100 g in a specialized CD cultivation device has been shown to result in increased muscle sarcomere length (Kim et al., 2007). Animals cultivated at 10–50 g for a period of 4 days were reported to be immobile immediately after treatment, but recovered motility after a while (Conley et al., 2001). Building upon on these prior studies, we further examined the impacts of hypergravity treatments on worm movement, as assayed on plates or in microfluidic chambers. We found that the worms quickly regained their locomotive abilities while recovering on NGM plates with bacterial food (Fig. 2, Table 2). These data suggest that the worms are resistant to hypergravity exposure up to 100 g, and are capable of recovering motility after a period of recovery. In the motility assays we employed there is a possibility that the initial, small reductions in motility seen immediately after hypergravity or control treatments could have been the result of dietary restriction like conditions caused by CeMM or due to a shift from liquid CeMM to solid NGM media. It is worth noting that a more recent study has reported that *C. elegans* did not show major changes in locomotion when subjected to four days at low speeds of 10 g on standard NGM plates with OP50 bacterial food (Qiao et al., 2013).

4.2. Hypergravity and pharyngeal pumping

To better understand and describe the phenotypic and behavioral changes that occur in the animals immediately after hypergravity treatments we also measured their pharyngeal pumping behavior. While animals exposed to 2 h of 100 g actively foraged on the plates (data not shown), the animals exposed to 12 h of 100 g or 1 g displayed relatively similar pumping behavior (Fig. 3a). We recognize that the subtle decrease in pumping seen in the 1 g 12 h controls in Fig. 2a could have been a result of the shift from liquid CeMM to solid media. The data supports conclusions from a previous study that found no dramatic changes in animal feeding behavior 15–30 min into 100 g hypergravity exposure (Kim et al., 2007).

4.3. Hypergravity and reproduction

Fertilization and embryogenesis are complex processes, and hypergravity could disrupt any of several essential stages. Prior studies have shown that sea urchin sperm are sensitive to hypergravity, evidenced by decreases in sperm velocity and fertilization capabilities (Tash and Bracho, 1999; Tash et al., 2001). Sasagawa et al. (2005) examined the effects of 100 g treatments on oogenesis and fertilization in adult *C. elegans*, and they determined that in a low percentage of cases, hypergravity disrupted the final meiotic division and polar body formation upon fertilization. The experiments in our study show that exposure to 100 g for 2 h did not impact reproduction (Fig. 4, Table S2). We also tested the effects of 10 g, 55 g or 112 g for 12 h and found that these regimens have little effect on the total number and viability of the progeny produced, provided that the temperature is maintained at 19–20 °C throughout the hypergravity treatment (Fig. 4, Table S2). In a study concurrent to the work reported here, Qiao et al. (2013) investigated the effect of cultivating *C. elegans* at 10 g on NGM plates, and they did not find hypergravity-induced differences in brood size. The experimental conditions, culture media, and the developmental stages of the worms used in the 2005 and the 2013 studies were different from the ones described in this study. This can confound direct comparisons of the studies, but it also provides a richer understanding of how different hypergravity regimens can influence reproduction, development and survival.

4.4. Hypergravity and animal viability

Previous work has shown that worm growth and development is not affected dramatically by spaceflight (Johnson and Nelson, 1991; Adenle et al., 2009; Oczypok et al., 2012). Indeed, a recent gene expression study suggested that *C. elegans* experiencing microgravity could age slower (Honda et al., 2012). Mild hypergravity regimens on the other hand have been reported to have hormetic effects extending the lifespan of male *Drosophila melanogaster* (Le Bourg et al., 2000; Le Bourg et al., 2004; Minois, 2006; Le Bourg, 2011). Interestingly, in our experiments while 2 h of 100 g treatment mildly reduced the lifespan of the animals relative to the 1 g treatment, no dramatic differences were seen between the lifespans of the animals exposed to 12 h of hypergravity or control treatments (Fig. 5). The maximum lifespans of the animals treated with 100 g for 2 h fall well within the range of previously reported maximum lifespans of wild-type control animals (Lin et al., 2001; Zhang et al., 2009; Bansal et al., 2015). This indicates that the short-term hypergravity treatments tested in this study did not have significant detrimental effects on animal viability.

C. elegans undergo a number of metabolic and developmental changes from the four larval stages to adulthood (Corsi et al., 2015). Thus, the observed effects of hypergravity could depend upon the developmental stage of the animal being treated. Adult *C. elegans* should be tested in future studies to gain a more complete understanding of the effects of short-term hypergravity regimens. Also, insights into the effects of changes in media types will be provided by testing animals cultured for several generations in CeMM versus those raised on NGM plates with bacterial food. For long term experiments it is worth considering that the developmental stage of the animal at the times of acceleration and deceleration may influence fecundity and survival. Future studies are also needed to elucidate the roles of hypergravity on the developmental processes that underpin gametogenesis, fertilization, and embryogenesis in the worms.

In conclusion, we and others have shown that *C. elegans* can survive short-term, relatively intense hypergravity treatments. We found that initially, the animals were stunned by hypergravity treatments, but they recovered quickly, as assayed by velocity of movement or pharyngeal feeding behaviors. This is consistent with other analyses that have examined *C. elegans* motility or feeding behaviors following hypergravity treatments (Conley et al., 2001; Kim et al., 2007; Qiao et al., 2013). Also, relatively longer durations of hypergravity exposure had little effects on the animals' reproduction and lifespans. We expect that hypergravity response is multilayered and that an organism's response will be dependent on additional factors, such as age, temperature, nutrition or other stresses. These and other studies help to shape our understanding of the effects of increased gravitational forces on *C. elegans* lifespan, health, and behavior.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.lssr.2016.06.003.

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