Title
Dehydration in Laboratory Mice

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Publication Date
2021-08-13

EFFECTS OF DEHYDRATION ON VOLUNTARY WHEEL-RUNNING BEHAVIOR AND BODY MASS IN LABORATORY HOUSE MICE

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A capstone project submitted for Graduation with University Honors

May 06, 2021

University Honors
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Abstract

Since 1993, the Garland laboratory has selectively bred replicate lines of mice for high voluntary exercise on wheels. After more than 90 generations of artificial selection, mice from the four High Runner lines now run approximately three times as many revolutions/ day compared to mice from four non-selected Control lines. High Runner mice require more food and water and weigh less compared to mice from Control lines. Various studies have demonstrated adverse effects of dehydration on human athletic and cognitive performance, but few have studied effects in rodents, and none have studied the High Runner mice, which are both highly athletic and highly motivated to exercise on wheels. The purpose of this study was to evaluate the effects of dehydration on wheel running and body mass in laboratory house mice.

We weaned mice at 21 days of age and let mice acclimate to 6 days of wheel running. At the end of day 6, we weighed all mice and removed water bottles for ½ of all mice for 24 hours. After the 24-hours, we weighed mice again to determine any changes in body weight; we also calculated the change of revolutions run. Due to the complex interaction between wheel running, food consumption, body mass, and water restriction, we developed two hypotheses: 1) mice from High Runners lines would significantly decrease revolutions/ day run; 2) all mice without water would decrease in body mass.

Body mass decreased in all mice without water. Wheel running did not significantly change in water-deprived mice from the Control lines, but water-deprived High Runner females and males significantly increased the number of revolutions run during the 24-hour period. Wheel running is done voluntarily because it provides a rewarding experience; therefore, the significant increase in running after water removal in High Runner mice may be attributed to "reward substitution," in which mice replace the satisfaction provided by drinking water with the

rewarding experience from running. Alternatively, mice may have wanted to forage for resources, but only mice from High Runner lines have evolved elevated non-opioid exercise-induced analgesia that allowed them to increase their running.

Acknowledgments

I thank my mentor, Dr. Theodore Garland, Jr. who took me under his guidance and patiently introduced me to the world of research, and who without this project would not have been possible.

I also thank members of the Garland Lab, especially Nicolas Schwartz, for conducting the experiment and gathering the data. I regret that I was unable to be in the lab due to the COVID-19 pandemic restrictions.

And to my parents and brothers, who have given me everything I need so that I could achieve my goals and dreams. I couldn’t have made it this far in my academic career without them.

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Introduction

Dehydration, the process of losing water without sufficient replacement (Maughan and Shirreffs 2010), is a ubiquitous process affecting species ranging from human athletes to mice scurrying away from predators. Dehydration can be caused by a variety of mechanisms, such as low water availability, extreme heat, kidney failure, age, and exercise (Maughan and Shirreffs 2010; Bennett 2000). The body maintains homeostasis, but how does it respond to extreme dehydration prompted by endurance exercise, such as in extreme athletes? How does a species' physiology and behavior change during and after a period of dehydration? Similar to extreme athletes, the High Runner mice exercise on wheels for long intervals (Swallow et al. 2005). In our study, we used laboratory house mice to evaluate the effects of wheel running and body mass due to a period of dehydration.

Since 1993, Dr. Theodore Garland's laboratory has selectively bred four replicate lines of mice for high voluntary exercise on wheels (Swallow et al. 1998, 2009). Mice that ran more wheel revolutions per day were bred to produce High Runner mice, whereas four non-selected Control lines have been bred without regard to wheel running. After more than ninety generations of artificial selection, mice from High Runner lines now run approximately three times as many revolutions per day compared to mice from the Control lines (Careau et al. 2013; Claghorn et al. 2017).

Water restriction may impact mice from High Runner and Control lines in many ways. Mice from High Runner lines run approximately three times more revolutions per day than mice from Control lines, so they require more food and water for energy expenditure (Swallow et al. 1998, 2005; Thompson et al. 2018). High Runners also have a smaller body mass compared to mice from Control lines (Thompson et al. 2018), and larger animals may need more food. 24-hour water restriction causes dehydration, which reduces body mass and leads to a decrease in food consumption, which also reduces body mass (Bekkevold et al. 2013). Dehydration also impacts normal skeletal muscle function because less blood is available for blood flow and muslce perfusion, which negatively affects “muscle performance” (Cleary et al. 2006).

Given the complexity of the interactions between wheel running, food consumption, body mass, and water restriction, and the fact that we are directly removing water and indirectly reducing food consumption, we have developed two hypotheses: 1) mice from High Runner lines would significantly decrease revolutions per day run while mice from Control lines would possibly not change much from baseline; 2) all mice undergoing water restriction would experience a decrease in body mass.

Literature Review

Past experiments analyzing behavioral and physiological responses of water restriction have been performed on rats and humans, but few studies have analyzed mice (Bekkevold et al. 2013). The general conclusions from these studies were that dehydration decreases total body mass. One study determined that the total body mass of the rats decreased when deprived of water, and the longer a rat was deprived of water, the lower the chances it had to regain the mass lost (Armstrong et al. 1980). When rats were completely deprived of water for 96 hours, food *ad lib*, there was a 16% decrease of food intake compared to the baseline period, and rats lost 12.7% of their original body mass (Armstrong et al. 1980). Bekkevold et al. noticed that mice completely deprived of water for a period of 24 and 48 hours decreased body weight by an absolute average of 12% and 18%, respectively, due to a phenomenon termed “dehydration anorexia” (2013), which is characterized by a reduction of food intake when water was completely removed, similar to what was observed in rats (Armstrong et al. 1980).

Interestingly, in the aforementioned experiment performed in rats, water was an essential component for recovering body mass lost in the immediate post deprivation period, demonstrating that food intake may rely, in part, on the amount of water intake, as suggested by Bekkevold et al. (2013; Armstrong et al. 1980). Also of interest, researchers observing neonatal rats during a 12-hour milk deprivation period noticed a significantly larger decrease of body mass in the older groups compared to the younger groups, indicating that age may also affect body mass (Anzai and Kawahara 1998).

Regarding rodent's activity, studies observing changes in activity in response to food or water deprivation have been conflicting. In one study, a Doppler radar was used to measure rat's cage activity, but not wheel running, of 6 male rats in response to 96 hours of food deprivation and found that there was a total daily increase of activity by 33% on the 4th day; researchers noted that a similar study observed an increase in activity in wheel running when food or water was deprived (Armstrong et al. 1980). However, when researchers studied 6 male rat's activity during a 96-hour period of water deprivation, food *ad lib,* they saw that activity remained the same as pre-deprivation until the 4th day, where a decrease of 44% of activity was observed only in the nocturnal period (Armstrong et al. 1980). In concurrence, when 192 mice were tested in a similar experiment of water deprivation for 24 and 48 hours (food *ad lib)*, their "attitudes," measured by levels of activity and appearance, decreased exponentially after 24 hours (Bekkevold et al. 2013). As the time of water deprivation increased, mice appeared more distressed and experienced more of a decrease in their alertness and activity; however, wheel running was not measured (Bekkevold et al. 2013).

Physiologically, mice deprived of water for 24 hours had an increase of plasma osmolarity, plasma cortisone, and hematocrit, the volume percentage of red blood cells in blood (Bekkevold et al. 2013). No studies have been found measuring VO_2 max.

Previous studies involving human subjects measuring water removal were highly monitored and restricted but also had similar findings as in mice and rats. Water deprivation longer than 1.5 hours with continual exercise may decrease body weight by up to 2-5%, which can be detrimental to both health and athletic performance, such as the amount of running, cognitive function, and accuracy in a task, such as kicking a ball (Finn and Wood 2004; Maughan and Shirreffs 2010; Castro-Sepulveda et al. 2015; Trangmar and González-Alonso 2019). Researchers submitted 14 college athletes to a 45-minute period of exercise without water access and separated them into two groups: one that underwent a rehydration period after the exercise, and one who did not (Castro-Sepulveda et al. 2015). Athletes who did not undergo the rehydration period showed a significant decrease in body mass and a reduction in heart rate variability compared to athletes who did have a rehydration period (Castro-Sepulveda et al. 2015).

Bongers et al. acknowledged possible kidney damage during a dehydration period but underscored the lack of research conducted on the kidney to determine the effects of dehydration on them (2018). Researchers suggest that prolonged exercise leads to a secretion of vasopressin, a peptide hormone crucial for maintaining osmolality, and an increase of biomarkers for kidney injury (Bongers et al. 2018). These findings can possibly be more dire in the elderly. As proposed by Jill Bennet, a registered nurse, total body water decreases with age because aging kidneys are less able to concentrate urine (Bennett 2000).

Materials and Methods

Mouse model

We used laboratory house mice from generation 94 of an ongoing artificial selection experiment for high voluntary wheel-running behavior (Swallow et al. 1998). Each generation, mice are weaned at 21 days of age and housed in a 4/cage. At approximately 6-8 weeks of age, mice are housed individually with access to a Wahman-type activity wheel (1.12-m circumference) with *ad lib* food and water. Over a period of six days, wheel revolutions are recorded in 1-minute intervals for a period of ~23 hours, beginning at noon. For the four replicate High Runner lines, the highest-running male and female from each of 10 families are chosen as breeders for the next generation, with no sibling pairings allowed. For the four replicated Control lines, breeders are chosen without regard to wheel running.

Water restriction

To determine the effects of water restriction on voluntary activity behavior, all mice were allowed an additional 7th day of wheel access. At the end of the 6th day of wheel access, the water bottles were removed from ½ of all mice, with the other ½ retaining *ad lib* water. Both groups retained *ad lib* food. In addition to collecting wheel-running data, body mass was recorded on the day wheel access began, after six days (as part of routine selection procedures), and after the 7th day of wheel access. After the 24-hour period, we weighed the mice once again to determine the weight lost during the dehydration period. We analyzed the number of revolutions run on the wheel during the 24 hours. Water deprivation lasting longer than 24 hours was not recommended (Bekkevold et al. 2013).

Statistical analyses

Males and females were analyzed separately because of several known sex differences, such as body size (Garland et al. 2011). High Runner and Control lines were compared by a two-way mixed-model with linetype and water restriction as fixed effects, line nested within linetype as a random effect, and body mass and/or age as covariates (SAS Proc Mixed v15). Outliers were removed when the standardized residual exceeded ~3.

Results

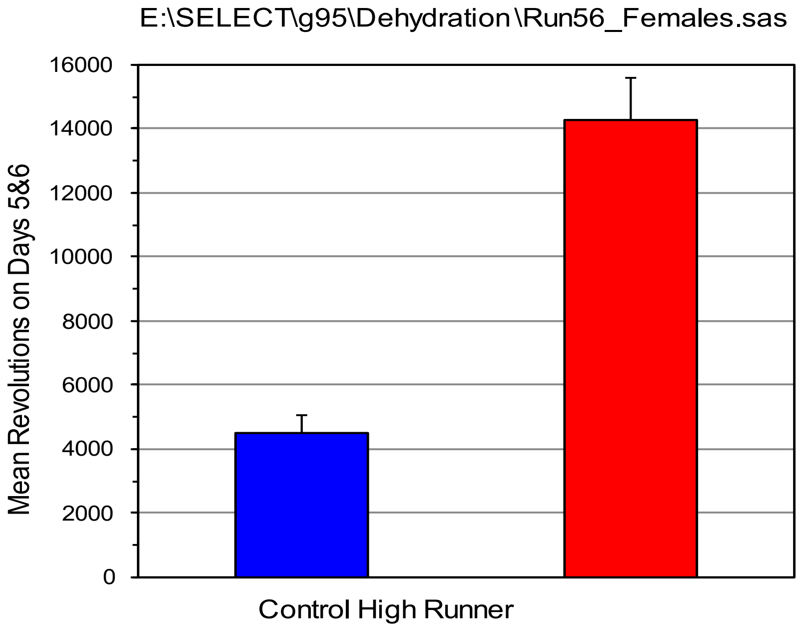
The average revolutions/ day run was roughly 3x greater in both male and female High Runner mice compared to the mice from non-selected Control lines; on average, High Runner females ran more than the males (Fig. 1).

For females, both groups of High Runner mice (with water and without water) tended to increase running from days 5 & 6 to day 7 (Table 1, linetype P = 0.0915), with no significant overall effect of water deprivation (P = 0.3278) and no water X linetype interaction (P = 0.7382). Only the change in running for water-deprived High Runner females was statistically significant, indicating that they increased their running by ~2000 revolutions (P = 0.0340, Fig 2).

For males, we observed a water X linetype interaction (Table 1, P = 0.0385), indicating that High Runners increased their running by ~4,000 (P = 0.0021) revolutions during the 24-hour period of dehydration (Table 1, Fig. 2). None of the other groups showed a significant change, although Control males increased by ~700 revolutions (P = 0.0762).

The High Runners had less body mass compared to mice from the Control (Fig 3). ~4.5 grams and ~5 grams were lost in all females and males, respectively, from days 5 & 6 to day 7 when deprived of water (Fig 4, Table 2).

Figure 1. High Runner (red) and Control (blue) males and females. Mean revolutions run during days 5 and 6. The High Runners ran roughly 3x as the Control mice.



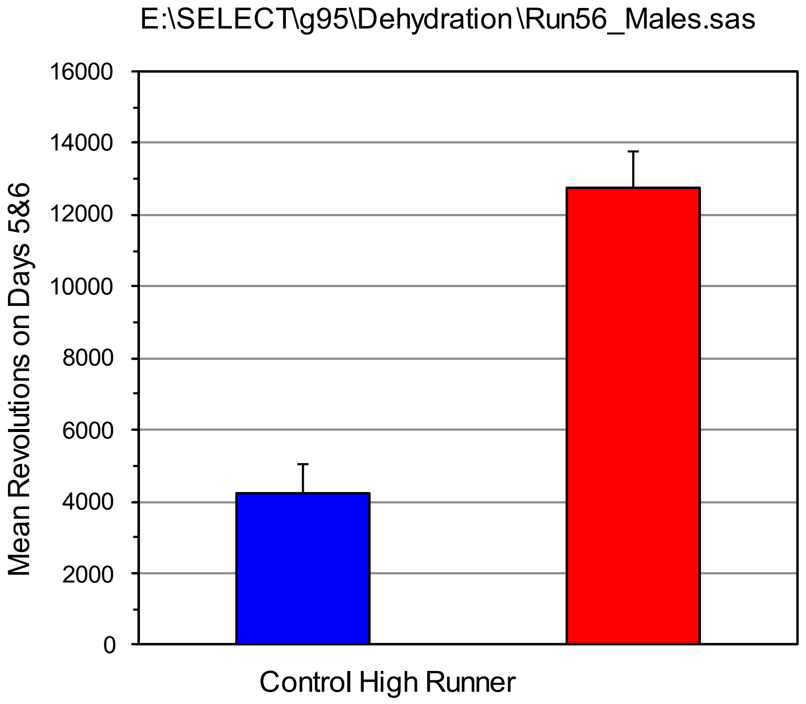


Table 1. Statistical analyses of the change in running (revolutions/day) from days 5 & 6 to day 7.

Water = 0 indicates water bottles were removed. Linetype= 1 indicates High Runners.

Females (N = 96)

Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Water	1	6	1.13	0.3278
Linetype	1	6	4.03	0.0915
Water*Linetype	1	6	0.12	0.7382
Age	1	28	1.18	0.2847
Wheel Freeness	1	28	0.59	0.4468

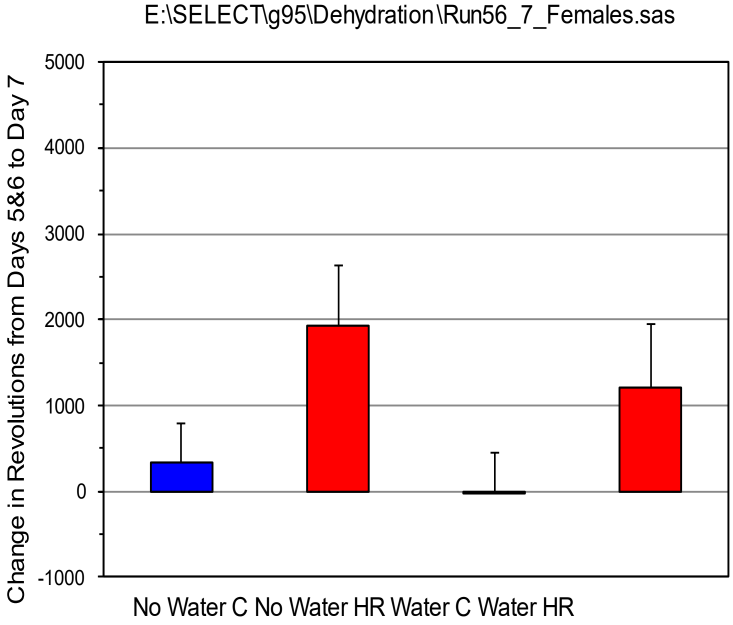
Effect	**Water**	**Linetype**	**Estimate**	**StdError**	**DF**	**t Value**	**P**
Water	0		1134.60	416.93	6	2.72	0.0346
Water	1		597.26	437.71	6	1.36	0.2214
Linetype		0	163.62	397.91	6	0.41	0.6952
Linetype		1	1568.25	569.79	6	2.75	0.0332
Water*Linetype	0	0	343.82	455.17	6	0.76	0.4786
Water*Linetype	0	1	1925.38	704.00	6	2.73	0.0340
Water*Linetype	1	0	-16.59	475.59	6	-0.03	0.9733
Water*Linetype	1	1	1211.12	739.40	6	1.64	0.1525

Males (N = 87)

Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Water	1	6	20.73	0.0039
Linetype	1	6	6.57	0.0428
Water*Linetype	1	6	6.97	0.0385
Age	1	28	0.45	0.5087
Wheel Freeness	1	28	0.49	0.4894

Effect	**Water**	**Linetype**	**Estimate**	**StdError**	**DF**	**t Value**	**P**
Water	0		2312.54	413.64	6	5.59	0.0014
Water	1		-157.76	444.88	6	-0.35	0.7350
Linetype		0	219.94	270.99	6	0.81	0.4480
Linetype		1	1934.85	610.17	6	3.17	0.0193
Water*Linetype	0	0	737.22	344.58	6	2.14	0.0762
Water*Linetype	0	1	3887.87	754.24	6	5.15	0.0021
Water*Linetype	1	0	-297.35	331.38	6	-0.90	0.4041
Water*Linetype	1	1	-18.18	827.11	6	-0.02	0.9832

Figure 2. Change in daily revolutions run from days 5 & 6 to day 7. The males and females from Control running lines (blue) did not significantly change when water was retained or removed. In contrast, High Runner (red) males and females significantly increased their running on day 7 when water was removed, especially High Runner males.



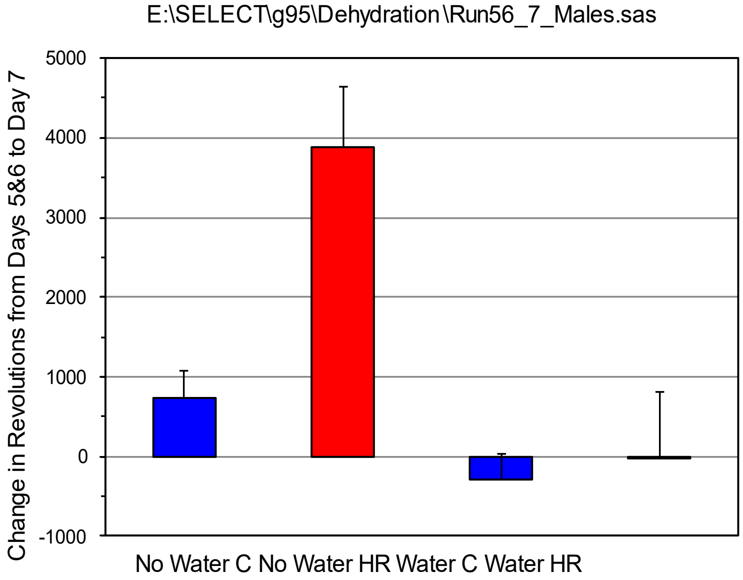
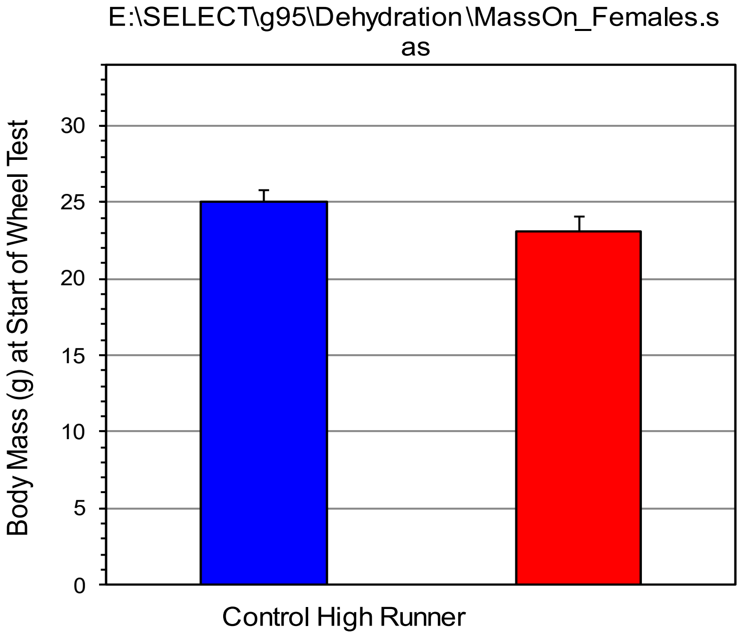


Figure 3: Female and male Control (blue) and High Runner (red) body mass at the start of wheel testing, before water removal. High Runners had a slighly smaller body mass.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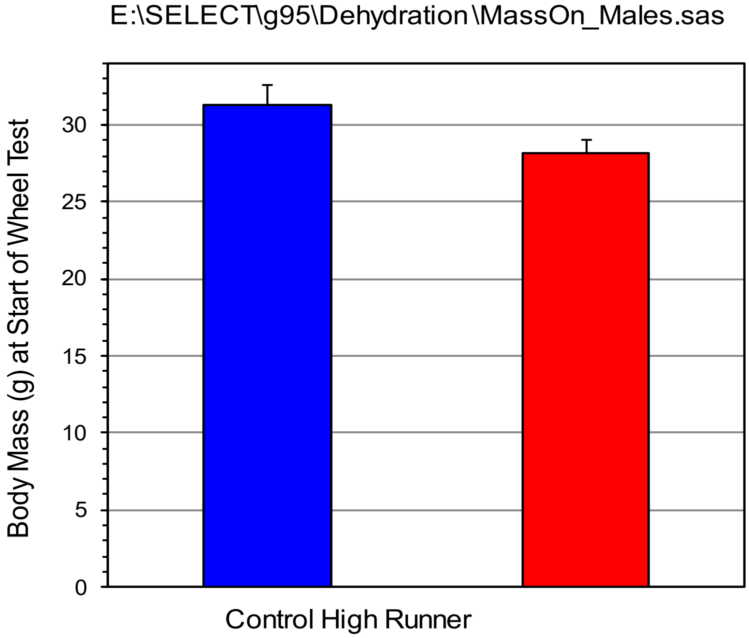
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Figure 4: Female and male change in Body Mass from days 5 &6 to day 7. Mice without water had a large decrease in body mass compared to mice retaining water.

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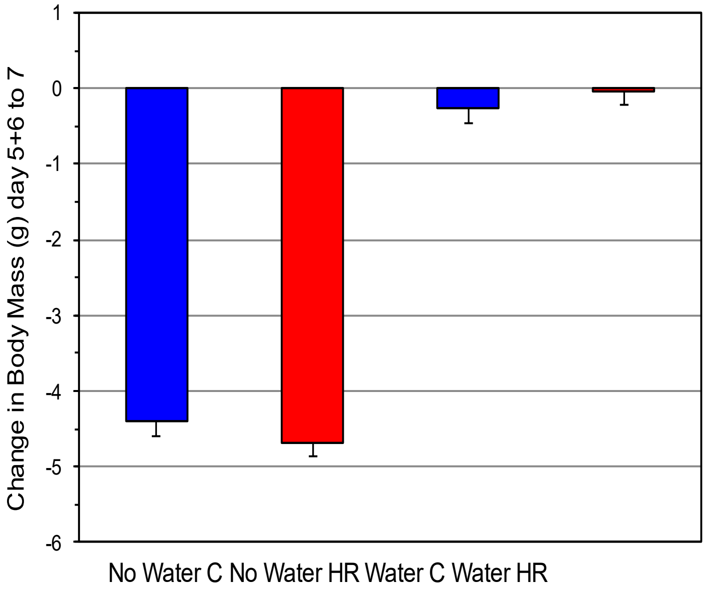

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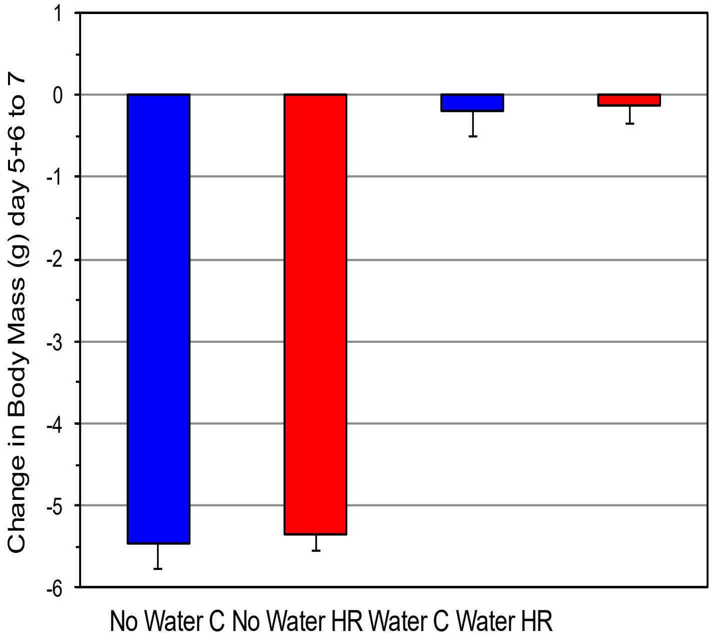

Table 2: Statistical analyses of the change in body mass from days 5 & 6 to day 7.

Water= 0 indicates water bottles were removal; Linetype= 1 indicates High Runners.

Females
N = 129

Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Water	1	6	750.80	0.0000
Linetype	1	6	0.03	0.8774
Water*Linetype	1	6	2.56	0.1607
WHLSTAGE	1	53	4.08	0.0483

Effect	**Water**	**Linetype**	**Estimate**	**StdError**	**DF**	**t Value**	**P**
Water	0		-4.54	0.13	6	-34.66	0.0000
Water	1		-0.15	0.13	6	-1.14	0.2963
Linetype		0	-2.33	0.14	6	-16.29	0.0000
Linetype		1	-2.36	0.15	6	-15.50	0.0000
Water*Linetype	0	0	-4.39	0.20	6	-22.29	0.0000
Water*Linetype	0	1	-4.68	0.17	6	-27.11	0.0000
Water*Linetype	1	0	-0.26	0.20	6	-1.33	0.2332
Water*Linetype	1	1	-0.04	0.18	6	-0.23	0.8281

Males
N = 123

Effect	Num DF	Den DF	F Value	Pr > F
Water	1	6	551.67	0.0000
Linetype	1	6	0.09	0.7724
Water*Linetype	1	6	0.01	0.9131
WHLSTAGE	1	47	5.43	0.0241

Effect	Water	Linetype	Estimate	StdError	DF	t Value	P
Water	0		-5.40	0.18	6	-29.19	0.0000
Water	1		-0.16	0.18	6	-0.89	0.4058
Linetype		0	-2.83	0.22	6	-12.56	0.0000
Linetype		1	-2.74	0.19	6	-14.56	0.0000
Water*Linetype	0	0	-5.45	0.31	6	-17.80	0.0000
Water*Linetype	0	1	-5.34	0.21	6	-25.78	0.0000
Water*Linetype	1	0	-0.20	0.30	6	-0.64	0.5428
Water*Linetype	1	1	-0.13	0.21	6	-0.64	0.5451

Discussion

Dehydration, the process of losing water without sufficient replacement, is a ubiquitous process affecting numerous species (Maughan and Shirreffs 2010). The purpose of the present study was to evaluate the effects of dehydration on voluntary exercise of mice, as measured by voluntary wheel-running behavior, and on body mass.

We allowed 6 days of wheel access, and at the end of the 6th day, water bottles for ½ of the mice were removed and the other ½ retained their water bottles. All mice retained food *ad lib* food consumption. Mice without water bottles underwent a 24-hour period of dehydration. After the period of water deprivation, change of revolutions run and body mass were calculated. We hypothesized that (1) mice from selectively bred High Runner lines would significantly decrease revolutions/ day run while mice from Control lines would possibly not change much from baseline and (2) all mice undergoing water restriction would experience a significant decrease in body mass.

Results did not support our first hypotheses. Rather than decreasing in running, High Runner females and males increased their running ~2,000 ($P = 0.0340$) and ~4,000 ($P = 0.0021$) revolutions/day, respectively, during the 24-hour period of dehydration (Table 1, Fig. 2). None of the other groups showed a statistically significant change, although males from Control running lines increased by ~700 revolutions ($P = 0.0762$). However, as also hypothesized, when water was removed, both Control and High Runner lines lost body mass (~4.5 grams and ~5 grams were lost in females and males, respectively, from days 5 & 6 to day 7: Table 2, Fig 4).

One possible explanation for the increased running in mice from High Runner lines (and the tendency for males from Control running lines to increase their running as well) after water removal is an overwhelming desire to escape their cages and forage for water, which may be an evolutionary mechanism that aids their survival during resource shortages. A study of rats deprived of food for 5 days reported an increase in their cage activity, and the authors noted that previous studies had observed increases in wheel running when rats were deprived of water, although similar studies have been conflicting (Armstrong et al. 1980). Armstrong et al. (1980) also suggested that the increase of activity may be attributed to the rat’s desire to escape their cages and forage for resources. The mice from non-selected Control lines may not have significantly increased their running because the High Runners have evolved elevated non-opioid exercise-induced analgesia that allowed them to increase their running.

Non-opioid exercise-induced analgesia, a mechanism that may rely on the endocannabinoid system (ECS) to alleviate pain and stress, such as that caused the 24-hour water restriction, is activated during exercise (Da Silva Santos and Galdino 2018). As mentioned before, a 24-hour period of water restriction leads to dehydration in mice (Bekkevold et al. 2013), and dehydration is known to negatively affect muscle performance (Cleary et al. 2006), and cause stress, which may lead to pain. Previous studies suggest that the opioidergic system has not evolved in High Runner mice (Li et al. 2004), but the ECS has evolved in the High Runner mice (Keeney et al. 2012; Thompson et al. 2018). The endocannabinoid system is active during aerobic exercises, such as acute running (Keeney et al. 2012), and may even participate in exercise-induced analgesia as shown when the analgesic effect produced by running were reversed in rats when treated with an endocannabinoid receptor antagonist (Da Silva Santos and Galdino 2018).

An alternative explanation for the significant increase in running after water removal in High Runner mice is "reward substitution," in which mice replace the satisfaction provided by drinking water with the rewarding experience from running. Wheel running is known to be a self-rewarding behavior (Sherwin 1998; Novak et al. 2012). In addition, a study conducted in the wild showed that wheel running is an elective, voluntary behavior exhibited by wild mice, frogs, rats, shrews, and even slug (Meijer and Robbers 2014). All species, especially mice, entered the cage and ran on the wheel even when no food was used as an attractor into the cage, and wild mice ran as much as 200-day old laboratory mice, demonstrating that wheel running is not a stereotypic behavior, but rather a voluntary behavior (Meijer and Robbers 2014).

Furthermore, the reward system of High Runner mice is known to be altered (Rhodes et al. 2005). For example, Thomson et al. (2018) observed a significantly smaller increase in consumption of artificial sweetener blends by High Runner mice when given access to wheels, but not when housed without wheels. This difference may indicate that the reward attained from running was stronger and preferred relative to the reward obtained from consuming artificial sweet blends (Thompson et al. 2018). Moreover, mice from High Runner lines developed an enhanced preference for western diets (high fat and sugar, with lower protein), which may be attributable to an alteration in the reward system or a stronger need for lipids for their energy expenditure that may help them increase their running and attain a rewarding response from running (Acosta et al. 2017).

As expected, a decrease in body mass was observed in all mice (Control and High Runner) when deprived of water, indicating that the amount of food intake is reliant, in part, on water availability, reinforcing the idea of “dehydration anorexia” (Bekkevold et al. 2013). Because mice from High Runner lines run more revolutions/day and consume more food than mice from Control lines (Swallow et al. 1999, 2005; Thompson et al. 2018), it might have been expected that High Runners deprived of water would experience more body mass loss compared to mice from Control lines deprived of water, but this was not the case. Instead, mice from both High Runner and

Control lines experienced a body mass decrease of ~4.5 grams and ~5 grams in females and males, respectively, from days 5 & 6 to day 7 when deprived of water (Table 2, Fig 4).

Conclusion and Future Directions

The purpose of this experiment was to test the effects of dehydration on voluntary exercise of mice, as measured by voluntary wheel-running behavior, and effects on body mass. We observed a statistically significant increase of running in High Runner females and males when deprived of water, which may be attributable to a “reward substitution.” Alternatively, mice may have wanted to forage for resources, and only mice from High Runner lines have evolved elevated non-opioid exercise-induced analgesia that allowed them to increase their running. All mice deprived of water experienced a decrease in body mass.

It should be noted that one of the computers recording wheel data crashed, decreasing our sample size by 50 mice. In addition, due to the restrictions imposed by COVID-19, we were unable to repeat the experiment to test the reproducibility of our results. Given the strong seasonal variation in running by Control and especially High Runner mice (Careau et al. 2013), it would be prudent to repeat the experiment in a different season.

Further advancements to this study may focus on measuring blood or urine osmolarity, hematocrit, and VO_2max levels to determine the effects of dehydration on physiology during a 24-hour period of water deprivation. Future studies may be aimed at analyzing the effects of dehydration on kidneys and the association between exercise and dehydration in older mice. Additionally, future studies may be aimed at determining how dehydration affects other behaviors, such as mating or maternal care.

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