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Effects of Puberty Blocker Treatment on Voluntary Wheel Running Activity in Young Rats

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Abstract

Background: Barriers to physical activity in transgender youth often center on social and psychological factors, but the effects of puberty blocking treatment that some youth receive on physical activity have yet to be explored. This study investigated the effects of puberty blocking treatment on physical activity in young female and male rats. Methods: Four week old female (F, n=19) and male rats (M, n=19) received the gonadotropin releasing hormone agonist (GnRHa) triptorelin as a puberty blocker (F-PB, n=10; M-PB, n=10) or saline as a control (F-CON, n=9; M-CON, n=9) for 4 weeks. Animals were then housed in voluntary wheel running (VWR) cages and activity was recorded during treatment period. Results: Main drug ($p<0.0001$) and sex ($p=0.045$) effects with a significant drug x sex interaction ($p=0.0095$) was observed with total 4 week running distance with F-PB having a lower VWR activity than F-CON (240 ± 23 km vs 67 ± 9 km, respectively, $p<0.0001$) and M-PB having lower VWR activity than M-CON (165 ± 15 km vs. 77 ± 14 km, respectively, $p<0.001$). Significant differences in daily wheel running distance were first detected on day 5 for F-PB when compared to F-CON ($p=0.036$) and on day 11 for M-PB when compared to M-CON ($p=0.034$). Conclusions: Treatment with a GnRHa reduced voluntary running wheel activity in young female and male rats. Social and psychological factors are important variables impacting physical activity in transgender youth, but puberty blocker treatment may be an additional factor to consider when addressing physical activity.

Keywords: Gonadotropin Releasing Hormone Agonist, Physical Activity, Transgender, Youth

INTRODUCTION

The number of individuals identifying as transgender has been increasing in past years and is expected to continue increasing, [1] and it is reported that this growing transgender population makes up approximately 0.6% of the US population or 1.4 million US adults. [2] A great health disparity, however, exists in transgender individuals, and In 2011, The Institute of Medicine released a report addressing health disparities in the lesbian, gay, bisexual, and transgender (LGBT) community. [3] Although this report brought to light health issues facing the entire LGBT community, there seems to be even greater health inequality when considering transgender individuals specifically. These disparities include increased mental health concerns including higher rates of depression, anxiety, and suicide [4-8] and higher rates of physical health concerns including increased risk of cardiovascular disease and metabolic disease. [9-14]

Although the health of transgender individuals as a whole is a concern and is receiving attention, the number of children and adolescents (or youth) who identify as transgender also continues to grow, but at this point is not receiving ample attention with regards to health disparities. It is estimated that 1.4% of US youth ages 13-17 identify as transgender, [2] and the number of referrals for the medical treatment for transgender youth has been on the rise. [15, 16] Transgender youth may begin considering gender affirmation procedures during these ages, and clinical treatment for these individuals may involve gonadotropin releasing hormone agonist (GnRHa) treatment which suppresses sex hormone production thus halting the progression of puberty (i.e., puberty blocker). [17] This puberty blocker approach in transgender youth, also known as the "Dutch Model" or "Dutch Approach", [18] reduces the psychological and social distress that accompany developing secondary sex characteristics during puberty [19], and if transgender youth decide to continue with gender affirming procedures (i.e., cross-sex hormone treatment, surgery) later in life, pretreatment with GnRHa during puberty improves behavioral, psychological, and overall functioning outcomes and eases the transition into the gender role. [20, 21] An additional benefit of using GnRHa as a puberty blocker in transgender youth is that the mechanisms of sex hormone blockade are reversible since the GnRHa do not act directly on the gonads (ovaries or testes) but rather act on the pituitary (i.e., actions are "upstream" of the gonads). [22]

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Because the health of transgender individuals has been receiving increased attention, interventions to help improve transgender health have also emerged. These interventions and approaches range from improving social support [23, 24] to providing better education to health care providers; [25, 26] however, one intervention with potential to improve overall health in transgender individuals has yet to receive adequate attention. This intervention is increased physical activity. It is possible that low levels of physical activity may be contributing to some of the health disparities observed as it has been reported that transgender individuals are typically less physically active than cisgender individuals. [9, 11, 13] Transgender youth specifically also report low levels of physical activity, [27] and lack of physical activity has a detrimental effect on overall health during adolescence. [28] Increased physical activity has been shown to be important in managing depression and anxiety, [29] and physically active individuals are at a lower overall risk of cardiovascular disease, coronary heart disease, metabolic disorders, and certain cancers than sedentary individuals. [30-32] Consequently, many of the conditions that increased physical activity help to prevent or manage such as depression, anxiety, cardiovascular disease, and metabolic disease are prevalent in transgender individuals, [4-14] and in general, physical activity during childhood and adolescence is beneficial to health in adulthood. [33, 34]

Numerous barriers have been identified that contribute to lower physical activity levels in transgender individuals including social and psychological factors such as a more negative perception of physical self, lower self-efficacy for exercise behavior, and less social support for physical activity, [35] and many of these factors have also have also been identified in transgender youth [36, 37] which negatively influence physical activity levels. Very little attention, however, has been given to somatic factors that may impact physical activity levels in transgender youth. One such factor is sex hormone availability which is especially important in the context of puberty blockers (i.e., GnRHa). Although not all transgender youth are treated with GnRHa as a puberty blocker, their clinical use in treating youth with gender dysphoria is on the rise, [38] and the impact of sex hormones on physical activity should not be discounted.

Our laboratory has shown previously that GnRHa treatment in adult female and male rats reduces voluntary wheel running (VWR) activity, [39-41] but the impact that GnRHa treatment specifically during adolescence has on physical activity is unknown. The purpose of the study was therefore to explore the effects of GnRHa treatment in young female and male rats on physical activity using a VWR model. To our knowledge this is the first report exploring the effects of GnRHa used as a puberty blocker on physical activity, and in this context, understanding this effect will contribute to the overall understanding of factors that could affect physical activity in transgender youth going through the gender affirmation process.

MATERIALS AND METHODS

Animal and Animal Care

All procedures were approved by the Institutional Animal Care and Use Committee at the University of Northern Colorado and were in compliance with the Animal Welfare Act. Young (3-week old) female (F, n=19) and male (M, n=19) Sprague-Dawley rats were purchased from Envigo (Indianapolis, Indiana) and housed on a 12:12 light to dark cycle with water and rat chow provided *ad libitum*. The first week served as an acclimation period before the now 4-week old rats were randomly assigned to the puberty blocker (PB; F-PB, n=10; M-PB, n=10) or control (CON; F-CON, n=9; M-CON, n=9) groups.

Puberty Blocking Treatment

Starting at 4-weeks of age, F-PB and M-PB received daily 100 µg subcutaneous injections of the GnRHa triptorelin (100 µL of 1 mg/mL) while F-CON and M-CON received daily 100 µL subcutaneous injections of saline as a placebo. Immediately following the first injection, all animals were housed individually in cages equipped with voluntary running wheels to monitor physical activity as voluntary wheel running (VWR). Treatment with GnRHa or saline and wheel running continued for 4 weeks while daily wheel rotations were recorded (one wheel rotation being equal to one meter of distance). In addition to collecting VWR data, body mass was also monitored each week during treatment period. At the end of the 4 week treatment period, animals were euthanized with sodium pentobarbital (50 mg/kg) and skeletal muscle (soleus and extensor digitorum longus) were extracted and weighed.

Statistical analysis

Data are presented as mean ± standard error of the mean. A two factor (drug x sex) analysis of variance with Tukey's *post hoc* testing was performed on total VWR distance, weekly VWR distance, body mass, and muscle mass to assess main drug effects, sex effects, and drug x sex interactions. Student's t-tests were also performed on daily VWR distance to compare F-PB to F-CON and M-PB to M-CON. Significance was set at the α=0.05 level.

RESULTS

Total VWR activity throughout the 4 week treatment period is illustrated in Figure 1. There was significant main drug ($p < 0.0001$), sex ($p = 0.045$), and drug x sex interaction ($p = 0.0095$) for distance covered with F-PB running significantly less than F-CON ($p < 0.0001$) and M-PB running significantly less than M-CON ($p < 0.01$). Additional *post hoc* test results revealed that M-CON had lower VWR activity than F CON ($p < 0.05$), and F-PB and M-PB had lower VWR activity than M-CON and F-CON, respectively ($p < 0.001$ and $p < 0.0001$, respectively). No differences were observed, however, between F-PB and M-PB ($p > 0.05$).

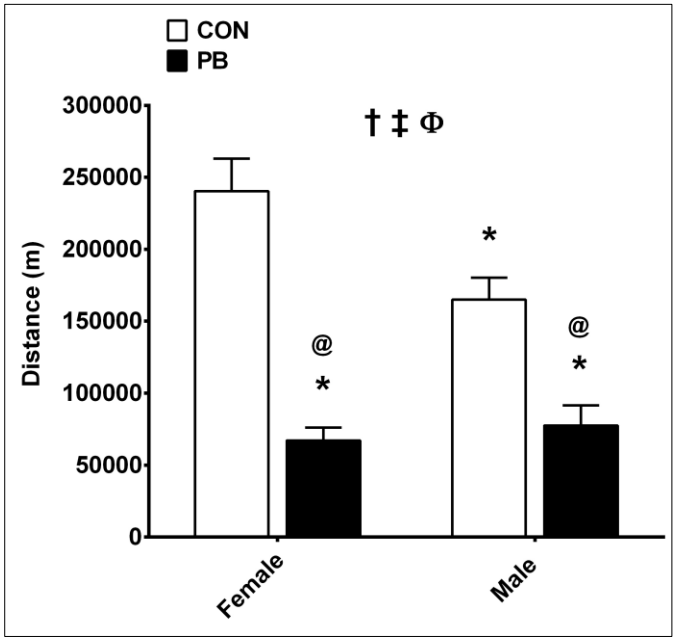

Figure 1: Total voluntary wheel running activity during 4 weeks of puberty blocker treatment. CON, control; PB, puberty blocker; † Main drug effect ($p < 0.0001$); ‡ main sex effect ($p = 0.045$); Φ sex x drug interaction ($p = 0.0095$); * $p<0.05$ vs Female CON; @ $p < 0.05$ vs Male CON.

With weekly VWR activity, a main drug effect was observed at week 1 (Figure 2A) with GnRHa-treated animals having less activity than control animals. At week 2, a main drug effect ($p < 0.0001$), sex effect ($p = 0.012$), and drug x sex interaction ($p = 0.006$) were observed with F-PB having lower VWR activity than F-CON ($p < 0.0001$), M-PB having lower VWR activity than F-CON ($p < 0.0001$), and M-CON having lower VWR activity than F-CON ($p < 0.01$, Figure 2B). No differences between F-PB and M-PB were observed at week 2. A main drug effect ($p < 0.0001$) and drug x sex interaction ($p = 0.006$) were observed at week 3 (Figure 3C) with both F-PB and M-PB having lower VWR activity than F-CON and M-CON ($p < 0.001$) and M-CON having lower VWR activity than F-CON. Figure 2D illustrates a main drug effect observed at week 4 ($p < 0.0001$) with F-PB and M-PB having lower VWR activity than F-CON and M-CON ($p < 0.001$). No differences were observed between F-CON and M-CON or F-PB and M-PB at week 4 ($p < 0.05$).

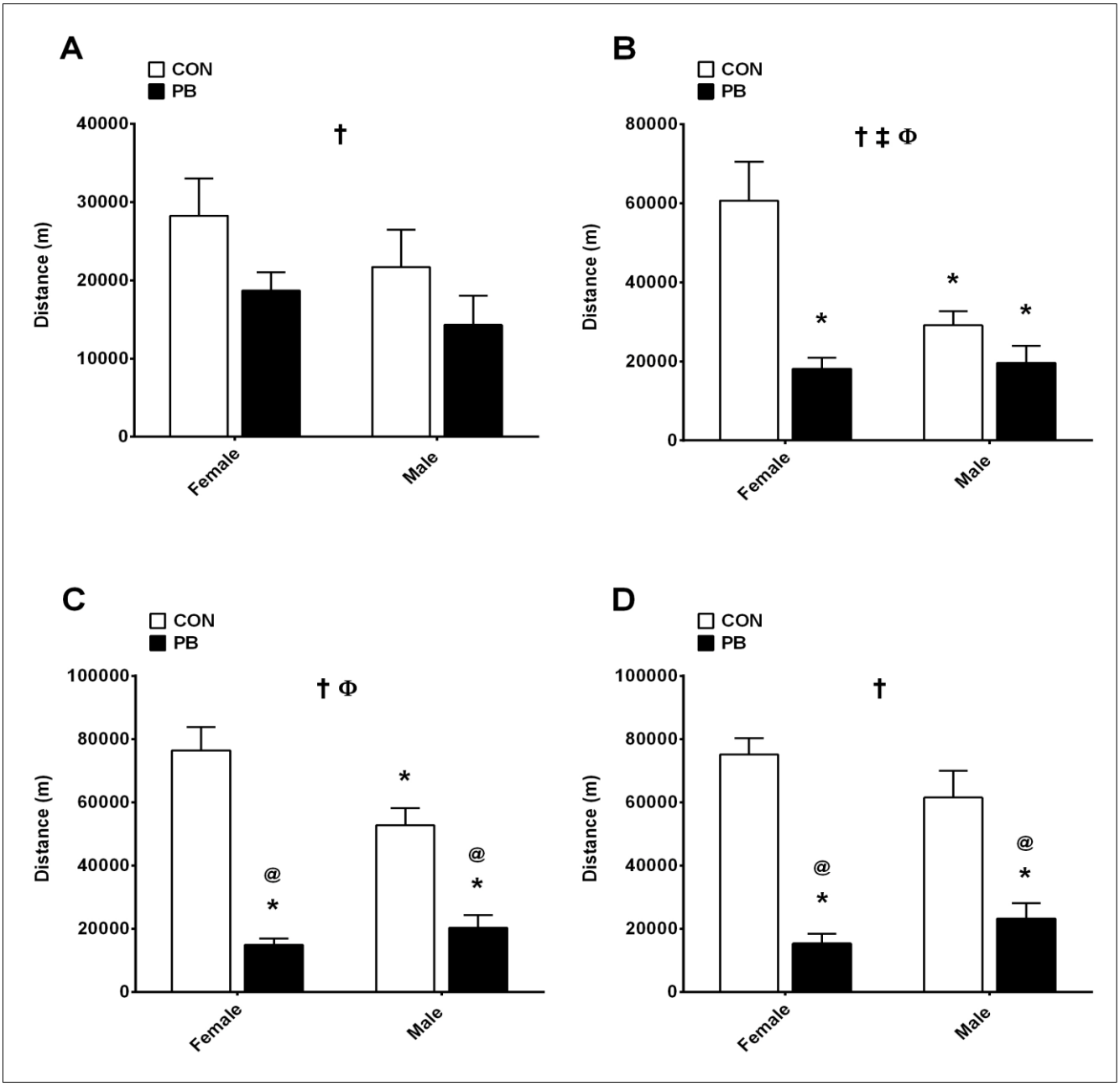

Figure 2: Weekly voluntary wheel running activity during 4 weeks of puberty blocker treatment. A, week 1; B, week 2; C, week 3; D, week 4; CON, control; PB, puberty blocker; † Main drug effect ($p < 0.05$); ‡ main sex effect ($p < 0.05$); Φ sex x drug interaction ($p < 0.05$); * $p < 0.05$ vs Female CON; @ $p < 0.05$ vs Male CON.

Daily VWR activity is illustrated in Figure 3. F-PB began running significantly less that F-CON at day 5, and this lower VWR activity continued throughout the treatment period ($p < 0.05$, Figure 3A). In males, P-PB ran significantly less that M-CON beginning at day 11, and this differences continued from day 13 to day 28 ($p < 0.05$, Figure 3B). In addition, animal body masses and skeletal masses are presented in Table 1. At the start of the treatment period, a main sex effects was observed with males weighing more than females ($p = 0.003$). At the end of the 28-day treatment period, a main drug effect ($p = 0.002$), sex effect ($p < 0.0001$), and drug x sex interaction $p = 0.01$) was observed, and F-PB had a significantly higher body mass than F-CON ($p < 0.001$). With change in body mass during the treatment period, a main drug ($p = 0.0009$) and main sex effect ($p = 0.0007$) with F-PB gaining more body mass than F-CON ($p < 0.05$). A main sex effect was observed for soleus mass ($p = 0.006$) with male animals having higher soleus mass than females, but no main drug effect or interaction was observed. In the EDL, however, a main drug effect ($p = 0.02$) and main sex effect ($p = 0.001$) were observed, but no drug x sex interaction was detected.

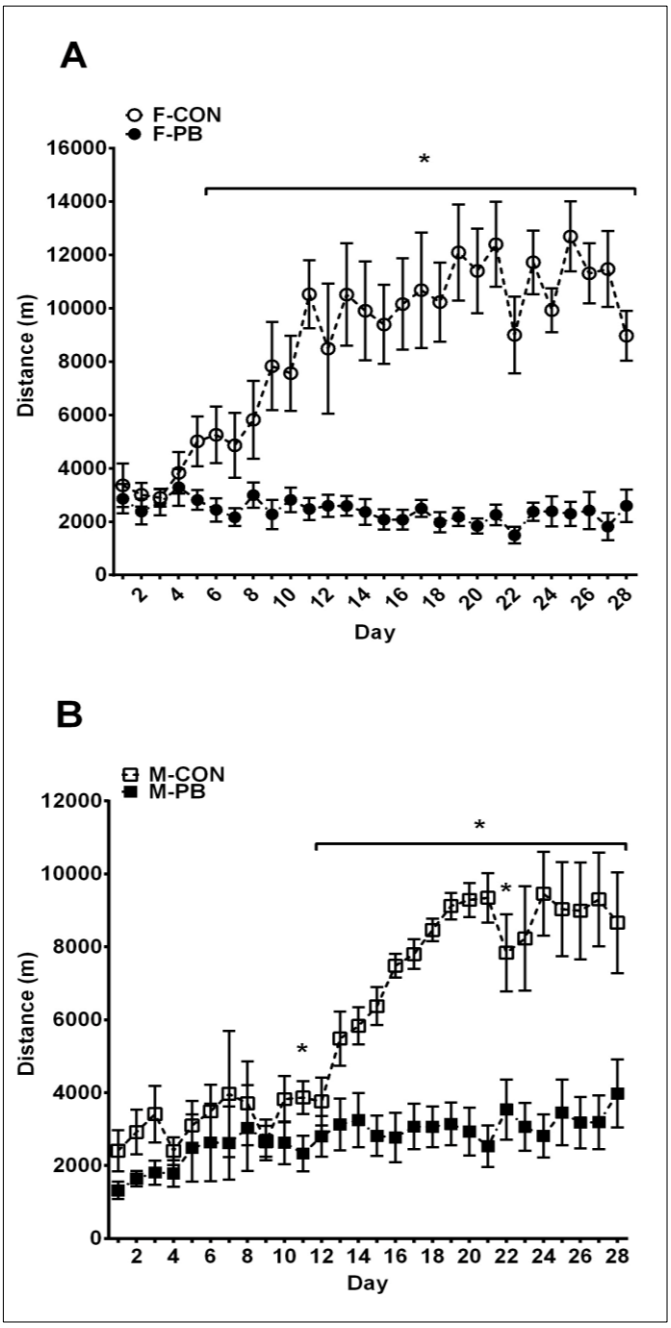

Figure 3: Daly voluntary wheel running activity during 4 weeks of puberty blocker treatment. A, Female voluntary wheel running activity; B, Male voluntary wheel running activity; F, female; M, male; CON, control; PB, puberty blocker; * significant difference between sex-matched control and puberty blocker ($p < 0.05$).

Table 1: Animal Characteristics

	F-CON	F-PB	M-CON	M-PB
Starting Body Mass (g) ‡	88 ± 6	87 ± 5	123 ± 12	106 ± 9
Final Body Mass (g) †‡Φ	194 ± 6	226 ± 4 @	263 ± 6	267 ± 5
Δ Body Mass (g) †	106 ± 7	140 ± 6 *	140 ± 10	161 ± 7
Soleus Mass (mg) ‡	85 ± 6	80 ± 3	100 ± 7	104 ± 9
EDL Mass (mg) †‡	72 ± 3	85 ± 3	91 ± 8	100 ± 4

Data are means ± SEM. F-CON, Female Control; F-PB, Female Puberty Blocker; M-CON, Male Control; M-PB, Male Puberty Blocker. † Main drug effect; ‡ main sex effect; Φ sex x drug interaction; @ $p < 0.001$ vs. F-CON; * $p < 0.05$ vs. F-CON.

DISCUSSION

The administration of the GnRHa triptorelin as a puberty blocker significantly decreased VWR activity in young female and male rats when compared rats receiving a placebo. Results of this study can be utilized to help understand some physiological and behavioral effects of reducing sex hormone production during puberty with a GnRHa that may lead to reduced physical activity. Recognizing these effects are essential in understanding contributors to lower physical activity levels observed in some transgender youth. If physical activity is to be addressed in transgender youth, the current study suggests that type of gender affirming care may need to be considered in order to effectively implement exercise interventions. There are a host of barriers to transgender physical activity which have also been shown to negatively impact transgender youth specifically which include inadequate locker rooms and changing spaces in facilities and transphobia in gyms and fitness centers. [42, 43] Furthermore, it has been reported that transgender youth have a more negative perceptions of physical self, lower self-efficacy for exercise behavior, less social support for physical activity transgender youth have a lower sense of [36, 37] which contributes to lower physical activity. Again, these particular barriers are indeed powerful and should not be overlooked. The current study merely adds another consideration for decreased physical activity for transgender youth so that health care professionals, exercise physiologists, physical activity leaders, and fitness professionals can better understand factors contributing to low physical activity in transgender youth. The findings of the current animal study are not meant to directly translate to the experiences and barriers that transgender youth face but rather the results are meant to increase awareness of the impact that puberty blockers may have on physical activity or willingness to be physically active.

It is know that reducing sex hormone levels reduces physical activity in animal models. with surgical gonadectomy (ovariectomy, orchiectomy) being shown to reduce VWR activity [44, 45] Furthermore, in models of surgical gonadectomy, administration of estradiol and testosterone restores physical activity [46, 47] indicating that sex hormone availability has a major influence on physical activity behavior. In the case of sex hormone disruption using GnRHa treatment, our laboratory reported previously that adult female and male rats receiving GnRHa treatment have lower VWR activity than control animals. [39-41] It is important to note that the nature of GnRHa suppressing sex hormone availability differs from that of surgical gonadectomy used in many studies in that administration of GnRHa often results in a "flare" effect where sex hormone level increases dramatically for a brief period of time as the GnRHa binds to the GnRH receptor first stimulating lutetinizing hormone (LH) and follicle stimulating hormome (FSH) production (and eventually increasing sex hormone synthesis). [48, 49] This "flare", however eventually subsides, and LH and FSH level plummet resulting in sex hormone levels consistent with surgical gonadectomy. To our knowledge, however, the current study is the first to explore GnRHa effects on VWR in young rats as a puberty blocking model.

Over the 28 day intervention, total VWR distance was significantly lower in young female and young male rats receiving the puberty blocker when compared to young female and male controls, respectively. Young female rats treated with triptorelin ran 72% less than control females, and young male rats receiving the puberty blocker ran 53% less than control males. The greater reduction in female wheel running suggests that blocking estradiol during puberty had a larger impact on physical activity than blocking testosterone during puberty. In general, however, female rodents have significantly higher VWR levels than male rodents [50] which is confirmed in the present study (significant sex effect with F-CON running more than M-CON); however, this same difference is not necessarily observed in humans. [51] The physiological or psychological mechanism behind the decreased physical activity in young rats treated with a puberty blocker

as well as the difference in how GnRHa treatment differentially effects female and male VWR activity requires further investigation.

The time point at which the GnRHa drug negatively impacts physical activity is another element to consider when constructing exercise prescriptions for youth receiving puberty blockers. In the current study, there was a significant reduction in physical activity of young females treated with the puberty blocker on day 5 which continued through day 28 when compared to F-CON. In young male rats, puberty blocker treatment significantly reduced wheel running on day 11 which continued through day 28. Young females treated with the puberty blocker presented an earlier reduction in physical activity than young males treated with the puberty blocker, and these differences may be important to consider when exploring the most effective timing of exercise interventions. Physical activity in general is important to metabolic and cardiovascular health, [52] and the low levels of physical activity in transgender youth may be linked to various health concerns. [53, 54] Puberty blocking treatment may lead to low physical activity levels which could contribute to some health concerns in transgender youth. Designing exercise programs that account for the potential blunted physical activity levels caused by puberty blocking treatment can help support the health and wellbeing of transgender youth.

The combined effects GnRHa treatment and VWR on body mass in female rats is also worthy of note. There was a significant drug x sex interaction with F-PB being significantly higher than F-CON, and this effect was not observed in male rats. Although not included in this project, our lab has observed that female rats without access to VWR cages (i.e., sedentary) did not have a significantly higher body mass than sedentary control female rats indicating that VWR promoted increased body mass during GnRHa treatment. Interestingly, no significant differences in skeletal muscle mass was detected between groups, indicating the greater body mass in F-PB could be due to increased fat mass, and this interaction warrants future investigation.

Increasing physical activity in transgender youth could improve the large health disparity between cisgender and transgender youth. [55] Transgender youth face many psychological and social barriers to exercise such as lack of appropriate changing facilities and social stigma, which contribute to low levels of physical activity. [56] The current study in rats eliminates these psychological and social barriers to exercise to focus on the physiological effects of puberty blocking treatment on physical activity. Both previous research in mature rats [57] and the present study suggest that the use of GnRHa treatment leads to reduced voluntary physical activity. The GnRHa treatment reduced voluntary wheel running in young female and young male rats. Body mass was also elevated in young female rats treated GnRHa when combined with exercise. The puberty blocking drug's impact on decreasing physical activity and potentially increasing body mass is important for individualizing exercise prescriptions for transgender youth. Future studies should explore if the effects of puberty blockers on physical activity are reversible once the treatment is stopped (i.e., detransition). In addition, a focus on examining interventions that aim to increase physical activity during puberty blocker treatment whould be explored (i.e., effect of increased physical activity BEFORE puberty blocker treatment).

CONCLUSION

This study can help understand potential factors that contribute to low physical activity reported in transgender youth. Young female and male rats treated with the puberty blocker drug triptorelin resulted in decreased physical activity. Transgender individuals face many psychological and social barriers that contribute to low physical activity levels. [58] The findings of this study suggest that treatment with a GnRHa to block puberty may also contribute to low physical activity levels. Transgender youth and their care givers may need to be aware that a decline in willingness to be physically active may be associated with puberty blocking treatment. Lower physical activity levels in youth may contribute to chronic health problems. It has been reported that transgender individuals report greater body mass, higher fasting glucose levels, elevated hemoglobin A1C, and greater insulin resistance when compared to cisgender individuals, [53, 54] that may be influenced by puberty blocking treatment decreasing physical activity. Exercise prescriptions and interventions could be implemented to address low physical activity in transgender youth with the understanding that suppressing sex hormone production during puberty maybe a factor to consider when employing such approaches.

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