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The Effects of Body Weight, Food Intake, Activity, and Anxiety in Female Rats

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The Effects of Zolpidem on Body Weight, Food Intake, Activity, and Anxiety in Female Rats

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Abstract:

Zolpidem is a nonbenzodiazepine sedative-hypnotic drug. It is placed in the imidazopyridine class of drugs, which are gamma aminobutyric acid (GABA)-A receptor agonists due to their effect on the alpha-1 GABA-A receptor subunit. The present study focused on differences observed during administration of zolpidem to female rats as compared to a previous study performed on male rats. Female rats receiving zolpidem did not differ significantly from those rats not receiving zolpidem; whereas, male animals were shown to be more affected by zolpidem causing increased food intake, more positive feed efficiency, higher relative food intake, lowered activity levels, increased anxiety, and increased visceral adiposity. Female rats exhibited little change during the withdrawal period compared to the experimental period; while, during the withdrawal period, male rats previously receiving zolpidem quickly returned to levels observed during the habituation period. This suggests that the effects of zolpidem are longer lasting in the female body than the male body. The differences between male and female rats observed in these experiments may be useful in making dosage recommendations for human patients being administered zolpidem.

Introduction:

Zolpidem, most commonly known as Ambien, has, in recent years, been one of the most commonly prescribed medications in America (University of Maryland Medical Center, 2012). Zolpidem was approved by the Food and Drug Administration (FDA) in 1992 and is marketed as a short-term solution to combat insomnia to be taken on an as-needed basis to initiate sleep (Food and Drug Administration, 2008). It is a nonbenzodiazepine and a sedative-hypnotic drug

and is placed in the imidazopyridine class of drugs, which are gamma aminobutyric acid (GABA)-A receptor agonists (Holm & Goa, 2000). GABA-A receptor modulators are one of the most common types of drugs used as sedative-hypnotics. Specifically, the sedative/hypnotic effects of zolpidem are due to its effect on the alpha-1 GABA-A receptor subunit (Crestani et al., 2000). This action depresses the central nervous system; thereby, slowing down the brain processes and allowing sleep to occur (Cubala & Gabrielsson, 2014).

Though zolpidem is effective, there are many side effects to the drug, some of which can be serious and dangerous. The most common side effects of zolpidem are drowsiness, dizziness, diarrhea, and a groggy or drugged feeling (Ambien Medication Guide, 2013). Side effects can also occur after a person stops taking zolpidem, such as trouble sleeping, panic attacks, and generalized anxiety (Ambien Medication Guide, 2013). More serious side effects associated with walking, eating, or even driving while not completely awake have been reported while taking the drug (Ambien Medication Guide, 2013). It has been reported that even when feeling fully awake, decreased mental alertness may be present while taking Zolpidem (U.S. Food and Drug Administration, 2013). Zolpidem can also be associated with abnormal and more aggressive behavior than is normal (Ambien Medication Guide, 2013).

Because of the side effects associated with zolpidem, in 2013, the Food and Drug Administration (FDA) lowered the recommended dosage of immediate-release zolpidem from a 10 mg before-bed dose to a 5 mg before-bed dose for many patients (U.S. Food and Drug Administration, 2013). The FDA stated that new data suggested that, in some patients, the blood levels of zolpidem remained high until the morning after use and that this effect could

impair alertness leading to workplace and driving dangers. Women have been shown to be more susceptible to next morning risks associated with zolpidem because of lower body weight and eliminating zolpidem at a lower rate than men (U.S. Food and Drug Administration, 2013). Therefore, the change in dosage is more often applied to women than to men (U.S. Food and Drug Administration, 2013).

Due to increased reports of dangerous side effects associated with zolpidem - especially in women - including sleep walking, sleep eating, and sleep driving; many recent studies have been conducted regarding zolpidem and its side effects. One such study, conducted by Cubala and Gabrielsson (2014), presented data that showed that out of 47 patients who experienced sleep-related amnestic behavior after taking a regular recommended dose of zolpidem, 27 of the patients were women. Their study also showed that even with a reduced dosage to 5 mg of zolpidem, as recommended by the FDA, some behavioral side effects were still being reported. A lower level of testosterone is a possible explanation as to why women are more susceptible to sleep related amnestic behavior with zolpidem than men (Olubodun et al., 2003). In the study conducted by Olubodun et al. (2003), it was shown that higher levels of free serum testosterone led to higher clearance of zolpidem from the body, suggesting that testosterone plays a role in the degradation of zolpidem. Cubala and Gabrielsson (2014) indicated that the pharmacokinetics of zolpidem are tied to CYP3A enzymes and stated that lower levels of testosterone in women are thought to reduce CYP3A activity. Low levels of testosterone, as well as CYP3A enzymes, slow down the processing of the drug, allowing zolpidem to remain in the female body longer, causing negative side effects during sleep and, for a considerable amount of

time, after waking (Cubala & Gabrielsson, 2014).

The finding that zolpidem affects females differently than males has not been the only recent finding in the field of medicine in which there are differences based upon gender. For example, it has recently been found that women are more susceptible to multiple sclerosis and are less sensitive to statins used to lower cholesterol (Flam, 2014). Due to these discoveries, the exclusion of female animals and female cells in research has come under scrutiny. Kelly and Baumans (2015) reported on a "60 Minutes" program that only 3% of biomedical researchers included female animals and cells in their research and that 34% of research studies did not report the gender of the research animals or cells. In research studies, the use of female animals has lagged behind the use of male animals due to the presence and cycling of hormones in the female body (Clayton & Collins, 2014). Despite these possible hormonal issues with female research animals, in October 2014, the National Institutes of Health began taking steps toward instituting a policy to balance male and female animals and cells used in research studies (Clayton & Collins, 2014).

Concerning the role of zolpidem on food intake, there are conflicting results. An increase in food intake was observed in some studies (Stanhope et al., 1993; Mitchell et al., 2004; Murphy et al., 2011); whereas, in another study, no effect on food intake was noted in relation to the administration of the drug (Lobarinas & Falk, 2000). Stanhope et al. (1993) suggested that zolpidem increased the intake of palatable fluid. In this study, it was stated that differences in results related to increased food intake after the administration of zolpidem could result from differing experimental methods and lengths of experiments, as well as differences in palatability

of the fluids used in each experiment. In the study conducted by Mitchell et al. (2004), it was stated that zolpidem increased food consumption at first but, as more zolpidem was administered, food consumption decreased due to the sedative effects of the medication. In the study conducted by Murphy et al. (2011), it was shown that rats being administered zolpidem had a significantly higher food intake than rats in the control group, and that the experimental animals had a significantly higher food intake during the administration of zolpidem than they did during the habituation and withdrawal periods of the experiment. In contrast to the above mentioned studies, the experiment performed by Lobarinas and Falk (2000) showed no significant relationship between the administration of zolpidem and food intake on either food-deprived or non-food-deprived rats.

Studies related to the anxiolytic or anxiogenic effects of zolpidem have also produced inconsistent results. The elevated plus maze (EPM) is one of the most widely used tests for measuring anxiety levels in rats (Komada et al., 2008). The EPM consists of two open arms and two closed arms, with avoidance of open arms suggesting anxiety (Komada et al., 2008). In one study on male rats conducted by Cui et al. (2006), it was demonstrated that zolpidem increased time spent in the open arms of the EPM, showing that zolpidem had an anxiolytic effect. In two other studies (Huang et al., 2010; Murphy et al., 2011) the exact opposite was shown. In the study conducted by Huang et al. (2010), at low doses of 1 mg and 3 mg of zolpidem, anxiogenic effects were observed in the EPM, so male rats spent less time in the open arms. At a higher dose of 10 mg, however, results were fairly inconclusive, since, at high doses, animals had a decreased ability to walk and move due to the sedative effects of the drug. In the study

conducted by Murphy et al. (2011), it was shown that male rats receiving zolpidem spent less time in the open arms of the EPM, suggesting that zolpidem had anxiogenic effects.

The purpose of the current experiment was to test the effects of zolpidem on female rats. The data generated in female rats was then compared to historical data obtained in male rats in studies conducted in the same laboratory (Murphy et al., 2011). The zolpidem was administered daily to experimental rats via a condensed milk "treat" and the effects of zolpidem on body weight, food intake, water intake, anxiety, activity levels, and visceral adiposity were recorded and analyzed. Food and water intake, as well as body weight of the rats, was measured daily and adiposity was measured at the conclusion of the experiment. An EPM was used to measure anxiety in the rats, with avoidance of open arms indicating anxiety. Wheel revolutions were also recorded to track activity levels of the rats.

It was hypothesized that similar results would be found in the female rats as compared to male rats during administration of the drug, including an increase in body weight, an increase in food intake, a decrease in locomotor activity, and an increase in anxiety. It was also hypothesized that, while these results would be similar, there would be more extreme differences between the experimental and control groups since zolpidem has been shown to have greater effects in females than in males (U.S. Food and Drug Administration, 2013) at the same dosage. It was hypothesized that results would differ during the withdrawal period from those found in the study conducted using male rats, since it has been suggested that zolpidem is broken down more slowly in the female body and that it may have longer lasting effects. Therefore; it was hypothesized that results during the withdrawal period for female rats as

compared to male rats would be similar to the results seen during the experimental period since the effects of the drug may persist longer in the female body.

Materials and Methods:

All procedures were approved by the local Institutional Animal Care and Use Committee and followed the guidelines set by the National Institute of Health Guide for the Care and Use of Laboratory Animals. Twelve female Long-Evans rats (Harlan, Inc., Indianapolis, IN), weighing approximately 100g at the beginning of the habituation period, were placed into individual cages, each containing a monitored running wheel which recorded daily activity using a magnetic switch, which in turn sent information every five minutes to a multiplexer, which then sent the information to a computer for storage. The rats were kept on a 12-hour light/12-hour dark cycle with a constant room temperature ranging from 20°C – 22°C. Each day at the beginning of the dark cycle, the rats were removed from their cages and placed in a weighing chamber and their daily body weight was recorded and the remaining food and water was collected, measured, and recorded. The cages were then cleaned. Following cleaning, each rat was given a 500 microliter "treat" of sweet condensed milk, which was presented for 15 minutes following cleaning and was then removed. All twelve rats consumed the entire treat within the 15 minute period during which the treat was presented. After treat removal, rats were presented with new food (rodent chow #5001, PMW International, LLC, Brentwood, MO) and water, which were available ad libitum for the remainder of the 24 hour cycle.

Following a 7-day habituation period, the rats were equally divided into two groups, six in an experimental group and six in a control group. The six rats in the experimental group were

given a 10 mg/kg dose of zolpidem (Sanofi-Aventis, Bridgewater, NJ) in the condensed milk "treat" and the six rats in the control group received only water (the vehicle for zolpidem) in the condensed milk "treat" for three weeks. A 3-day withdrawal period from the drug for the experimental group occurred following the experimental phase in which the "treat"' was administered without zolpidem present. During the withdrawal period, the control group continued to receive water in the condensed milk "treat" as they had during the experimental period

An EPM was utilized, which was 44" wide by 44" deep by 33.5" tall. Each of the four arms arm was 4.25" wide and 19.75" long with an intersection in the center which was 4.25" by 4.25". The two closed arms were enclosed by walls which were 5.75" tall. Testing trials using the EPM occurred two hours into the dark cycle and took place three times during the experiment: 2 days before the end of the habituation period, 2 days before the end of the experimental period, and 2 days into the withdrawal period. Rats were placed in the center of the EPM and the amount of time they spent in the closed and open arms was recorded for a five minute period. Following use by each rat, the arms of the EPM were wiped down using a sponge and water.

After the withdrawal period, the rats in both groups were sacrificed using a method approved by the IACUC. At that time the visceral adiposity in the inguinal, renal, and mesentery areas were visually observed and ranked on a subjective scale ranging from 0 – 3 (Murphy et al., 2011). The significance of the rankings are: 0 – no visible fat; 1 – "normal" amounts of fat; 2 – greater fat than 1, but less than 3 amounts of fat; 3 – excessive amounts of fat. All results were

statistically analyzed using SPSS software. A 2 (group) x 3 (period) repeated measures ANOVA was employed to measure statistical significance in data regarding body weight, food intake, feed efficiency (the ratio of food consumed to weight gained), relative food intake (the ratio of food consumed to current body weight), activity, and anxiety levels. Post-hoc testing used Bonferroni tests, and a p value of <0.05 was considered significant. An independent samples t-test was used to measure statistical significance regarding adiposity levels.

Results:

Figure 1 presents the mean body weight for C and Z rats on the last day of the habituation, experimental, and withdrawal periods. An ANOVA test showed that there was a main effect for period $F(2,20) = 551.205$, $p<0.001$; no main effect for group $F(1,10) = 0.046$, $p > 0.05$; and no significant interaction between period and group $F(2,20) = 1.817$, $p > 0.05$. The body weights of the rats in both groups were significantly higher during the experimental period as compared to the habituation period and were significantly higher during the withdrawal period than during the experimental and habituation periods.

Figure 2 presents the mean food intake for C and Z rats on the last day of the habituation, experimental, and withdrawal periods. An ANOVA test showed that there was a main effect for period $F(2,20) = 69.627$, $p < 0.001$; no main effect for group $F(1,10) = 1.189$, $p > 0.05$; and no significant interaction between period and group $F(2,20) = 0.974$, $p > 0.05$. Both groups of rats consumed significantly more food during the experimental period and withdrawal periods than during the habituation period.

Figure 3 presents the mean water intake for C and Z rats on the last day of the

habituation, experimental, and withdrawal periods. An ANOVA test showed that there was a main effect for period $F(2,20) = 30.089$, $p < 0.001$; no main effect for group $F(1,10) = 0.027$, $p > 0.05$; and no significant interaction between period and group $F(2,20) = 1.022$, $p > 0.05$. Both C and Z rats consumed significantly more water during the experimental period than during the habituation period. The Z rats consumed significantly less water during the withdrawal period than during the experimental period.

Figure 4 presents the mean feed efficiency for C and Z rats on the last day of the habituation, experimental, and withdrawal periods. An ANOVA test showed that there was a main effect for period $F(2,20) = 115.282$, $p < 0.001$; no main effect for group $F(1,10) = 0.485$, $p > 0.05$; and no significant interaction between period and group $F(2,20) = 2.292$, $p > 0.05$. Both groups of rats had a statistically significant decrease in feed efficiency between the habituation period and the experimental period and withdrawal period.

Figure 5 presents the mean relative food intake for C and Z rats on the last day of the habituation, experimental, and withdrawal periods. An ANOVA test showed that there was a main effect for period $F(2,20) = 11.927$, $p < 0.001$; no main effect for group $F(1,10) = 3.420$, $p > 0.05$; and no significant interaction between period and group $F(2,20) = 3.295$, $p > 0.05$. The mean relative food intake for the Z rats was significantly lower during the experimental and withdrawal periods as compared to the habituation period. The mean relative food intake for the C rats was significantly lower during the withdrawal period compared to the habituation period.

Figure 6 presents the mean number of running wheel revolutions for C and Z rats on the

last day of the habituation, experimental, and withdrawal periods. An ANOVA test showed that there was a main effect for period $F(2,20) = 15.834$, $p < 0.001$; no main effect for group $F(1,10) = 2.512$, $p > 0.05$; and no significant interaction between period and group $F(2,20) = 1.069$, $p > 0.05$. The amount of running wheel revolutions was significantly higher during the experimental and withdrawal periods as compared to the habituation period for both groups of rats.

Figure 7 presents the mean amount of time spent in the open arms of the EPM for C and Z rats during the habituation, experimental, and withdrawal periods. An ANOVA test showed that there was a main effect for period $F(2,20) = 8.761$, $p < 0.05$; no main effect for group $F(1,10) = 0.203$, $p > 0.05$; and no significant interaction between period and group $F(2,20) = 0.034$. $p > 0.05$. The amount of time that both groups of rats spent in the open arms was significantly higher during the experimental period as compared to the habituation period.

Figure 8 presents the mean amount of times spent in the closed arms of the EPM for C and Z rats during the habituation, experimental, and control periods. An ANOVA test showed that there was a main effect for period $F(2,20) = 6.329$, $p < 0.05$; no main effect for group $F(1,10) = 0.070$, $p > 0.05$; and no significant interaction between period and group $F(2,20) = 0.718$, $p > 0.05$. The amount of time spent in the closed arms for both groups of rats was significantly lower during the experimental period than during the habituation period.

Figure 9 presents the mean relative visceral adiposity of the C and Z rats at the conclusion of the experiment. A t-test showed no significant difference between the two groups [$t(10) = 7.045$, $p > 0.05$].

Discussion:

In regard to body weight in the current experiment, there was no significant difference between the body weights of the C and Z female animals during any part of the experiment. There was significance when comparing the different periods of the experiment, in which a steady increase in body weight was seen for both groups of female rats as the experiment progressed. In the study performed by Murphy et al. (2011) using male rats, there was no significant difference observed in the weights of the C and Z animals during any period, which is consistent with the results found in the present experiment. One notable difference, however, is the fact that in the study with male rats, the Z animals showed a significant decrease in body weight during the withdrawal period as compared to the experimental period. This suggests that male rats stopped experiencing the effect of the drug soon after it was no longer being administered, indicating that it was quickly eliminated from the body during the withdrawal period.

Regarding food intake in the current experiment, the only statistically significant difference was in the comparison of the three periods, while there was no statistical difference between the amount of food consumed by the C and Z female rats. The same was found for water consumption in the current experiment. The results differ from those obtained previously in male rats. In the study conducted by Mitchell et al. (2004), the administration of zolpidem to male rats originally led to increased food consumption, but as more of the drug was administered, it led to decreased consumption of food, possibly due to the sedative effects of the medication. In the study conducted by Murphy et al. (2011), it was shown that male rats

receiving zolpidem consumed significantly more food than rats not receiving the drug. In both studies, the administration of zolpidem had significant effects on the consumption of food in the male rats; whereas, in the current study, there was no significant difference in the amount of food consumed by female rats receiving zolpidem compared to female rats not receiving the drug.

Mean feed efficiency was also analyzed in the present experiment. For both the C and Z female rats, the feed efficiency was significantly lower during the experimental period than during the habituation or withdrawal periods. There was no significant difference between the C and Z female rats during any period of the experiment and both groups had a positive feed efficiency throughout the experiment. These results differed from those obtained in the study performed by Murphy et al. (2011) using male rats. In that experiment, the Z rats had a significantly more positive feed efficiency than the C rats during the experimental period. Thus, the drug caused a change in the feed efficiency of male rats, while no change was seen in female rats. The male Z rats also had a negative feed efficiency during the withdrawal period while the C rats continued to have a positive feed efficiency. This indicates that during the withdrawal period from zolpidem, effects of the drug were lost suggesting quick elimination of zolpidem by male rats.

The mean relative food intake observed in this study showed that for both groups of female rats, mean relative food intake was significantly lower during the withdrawal period than during the habituation period and, for Z rats, the mean relative food intake was significantly lower during the experimental period as compared to the habituation period as well. This could

suggest that food consumption by the Z rats was slightly elevated in comparison to food consumption by the C rats, despite their very similar body weights, though the difference in food consumption was not significant. There was no significant difference between the two groups of rats during any period of the experiment. In the study conducted by Murphy et al. (2011) using male rats, Z rats were shown to have had higher mean relative food intake than C rats during the experimental period. In addition, Z rats were shown to have a higher mean relative food intake value during the experimental period as compared to the habituation period and had lower relative food intake during the withdrawal period than during the experimental period. These results again show that the drug had a greater effect on male rats during its administration, as well as quick elimination during the withdrawal from the drug.

In the current study using female rats, there was no significant difference between the C and Z rats in regards to activity levels recorded using the mean number of running wheel revolutions. Both groups of rats engaged in significantly more activity during the experimental and withdrawal periods as compared to the habituation period. The study conducted by Murphy et al. (2011) using male rats, showed that Z animals engaged in less activity during the experimental period. In that same study, it was also shown that the number of revolutions for Z animals increased during the withdrawal period as compared to the experimental period, indicating that administration of the drug had a greater effect on male rats than on female rats, and that the drug was quickly eliminated during the withdrawal period by male rats.

In regard to the anxiolytic or anxiogenic effects of zolpidem, utilizing the EPM, the only statistically significant result was that rats spent more time in the open arms of the EPM during

the experimental and withdrawal periods than they did during the habituation period suggesting that the anxiety of the rats decreased with familiarity to the maze during the later trials. This finding is in opposition to the finding in a study conducted by Cui et al. (2006) using male rats, which showed that zolpidem increased time spent in the open arms of the EPM, suggesting an anxiolytic effect. The opposite effect was shown in a study conducted by Huang et al. (2010) using male rats and showed that at low doses of 1 mg and 3 mg of zolpidem, rats spent less time in the open arms suggesting anxiogenic effects. When the rats were given 10 mg of zolpidem, the rats had a decreased ability to walk and move due to the sedative effects of the drug, so results were inconclusive. In the study conducted by Murphy et al. (2011) using male rats, Z rats spent significantly less time in the open arms than C rats, also suggesting that zolpidem had anxiogenic effects. In both cases of anxiolytic effects or anxiogenic effects, male rats were more effected by zolpidem in relation to anxiety than were female rats utilized in the present study.

At the conclusion of the experiment following sacrifice, the visceral adiposity of the rats was examined and was shown have an identical mean value for the C and Z animals. This is different than the study conducted by Murphy et al. (2011) using male rats where Z rats were observed to have a significantly higher visceral adiposity than the C rats. This again suggests that male rats were more affected by zolpidem than female rats regarding food intake and body weight.

It was hypothesized that similar results would be found in the female rats as compared to male rats during administration of the drug, including an increase in body weight, an increase

in food intake, a decrease in locomotor activity, an increase in anxiety, and that there would be more extreme differences between the experimental and control groups since zolpidem has been shown to have greater effects in female rats than in male rats at the same dosage. It was hypothesized that results would differ during the withdrawal period from those found in the study conducted using male rats, since it has been suggested that zolpidem is broken down more slowly in the female body and that it may have longer lasting effects.

The current experiment with female rats did not fully support the hypotheses. One portion of the hypothesis that was supported was the fact that the effects of the drug were eliminated quickly in the male rats and may persist longer in the female rats. In the male rats, changes were seen during the withdrawal period suggesting that the drug was quickly eliminated from the body in that food intake, feed efficiency, relative food intake, and activity levels returned to levels similar to the habituation period during withdrawal. In the female rats, this return to “normal” was not observed. The reason for this possible expedited elimination of zolpidem from the male body was indicated by Cubala and Gabrielsson (2014) when they stated that the pharmacokinetics of zolpidem are tied to CYP3A enzymes which have reduced activity in females due to the lower levels of testosterone present in the female body. These low levels of testosterone, as well as CYP3A enzymes, slow down the processing of the drug, allowing zolpidem to remain in the female body longer which could be a reason why male rats would return more quickly to a 'normal' level during withdrawal from zolpidem.

The results obtained in this experiment suggest that male rats were more affected by zolpidem than female rats, which is in opposition to the recommendation by the FDA to lower

dosages for females, since they have been shown to have more negative side effects from the drug (U.S. Food and Drug Administration, 2013). Since current research on the effects of zolpidem has been performed on male rats, further research using female rats could reveal whether these results are unique or whether they would be replicated in repeated trials. Further research could assist in prescribing the correct dosage of zolpidem for human usage.

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Figure Legends:

Figure 1. Mean body weight (+SEM) for C and Z rats on the last day of the H period (day 7), E period (day 28), and W period (day 31). The # indicates that the body weight of the C rats was significantly higher during the E period than during the H period. The ## indicates that the body weight of the C rats was significantly higher during the W period than during the H and E periods. The * indicates that the body weight of the E rats was significantly higher during the E period than during the H period. The ** indicates that the body weight of the E rats was significantly higher during the W period than during the H and E periods.

Figure 2. Mean food intake (+SEM) for C and Z rats on the last day of the H period, E period, and W period. The # indicates that food intake of the C rats was significantly greater during the E and W periods as compared to the H period. The * indicates that food intake of the Z rats was significantly greater during the E and W periods as compared to the H period.

Figure 3. Mean water intake (+SEM) for C and Z rats on the last day of the H period, E period, and W period. The # indicates that water intake of the C rats was significantly greater during the E period than during the H period. The * indicates that the water intake of the Z rats was significantly greater during the E period than during the H period. The ^ indicates that the water intake of the Z rats was significantly lower during the W period than during the E period.

Figure 4. Mean feed efficiency (+SEM) for C and Z rats on the last day of the H period, E period, and W period. The + indicates that the feed efficiency of the C rats during the E period was significantly lower as compared to the H period and W period. The ^ indicates that the feed efficiency of the Z rats during the E period was significantly lower as compared to the H period and the W period.

Figure 5. Mean relative food intake (+SEM) for C and Z rats on the last day of the H period, E period, and W period. The + indicates that the mean relative food intake of the C rats during the W period was significantly lower as compared to the H period. The ^ indicates that the mean relative food intake of Z rats during the E and W periods was significantly lower as compared to the H period.

Figure 6. Mean number of running wheel revolutions (+SEM) for C and Z rats on the last day of the H period, the last day of the E period, and the last day of the W period. The # indicates that the number of running wheel revolutions for C rats was significantly higher during the E and W periods as compared to the H period. The * indicates that the number of running wheel revolutions for Z rats was significantly higher during the E and W periods as compared to the H period.

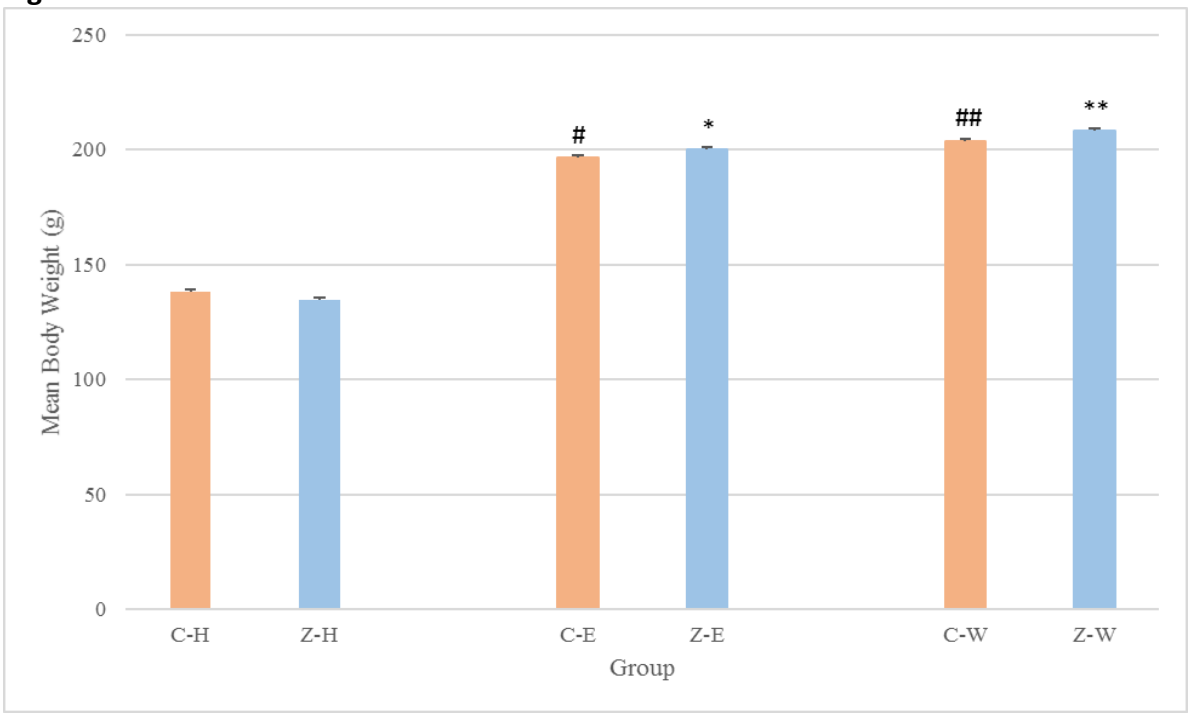
Figure 7. Mean amount of time spent in the open arms of the EPM (+SEM) for C and Z rats during the H, E, and W periods. The # indicates that the amount of time spent in the open arms for C rats was significantly higher during the E period as compared to the H period. The *

indicates that the amount of time spent in the open arms for Z rats was significantly higher during the E period as compared to the H period.

Figure 8. Mean amount of time spent in the closed arms of the EPM (+SEM) for C and Z rats during the H, E, and W periods. The + indicates that the mean amount of time spent in the closed arms for C rats during the E period was significantly lower as compared to the H period. The ^ indicates that the mean amount of time spent in the close arms for Z rats during the E period was significantly lower as compared to the H period.

Figure 9. Mean relative visceral adiposity (+SEM) of C and Z rats at the end of the experiment.

Figure 1:



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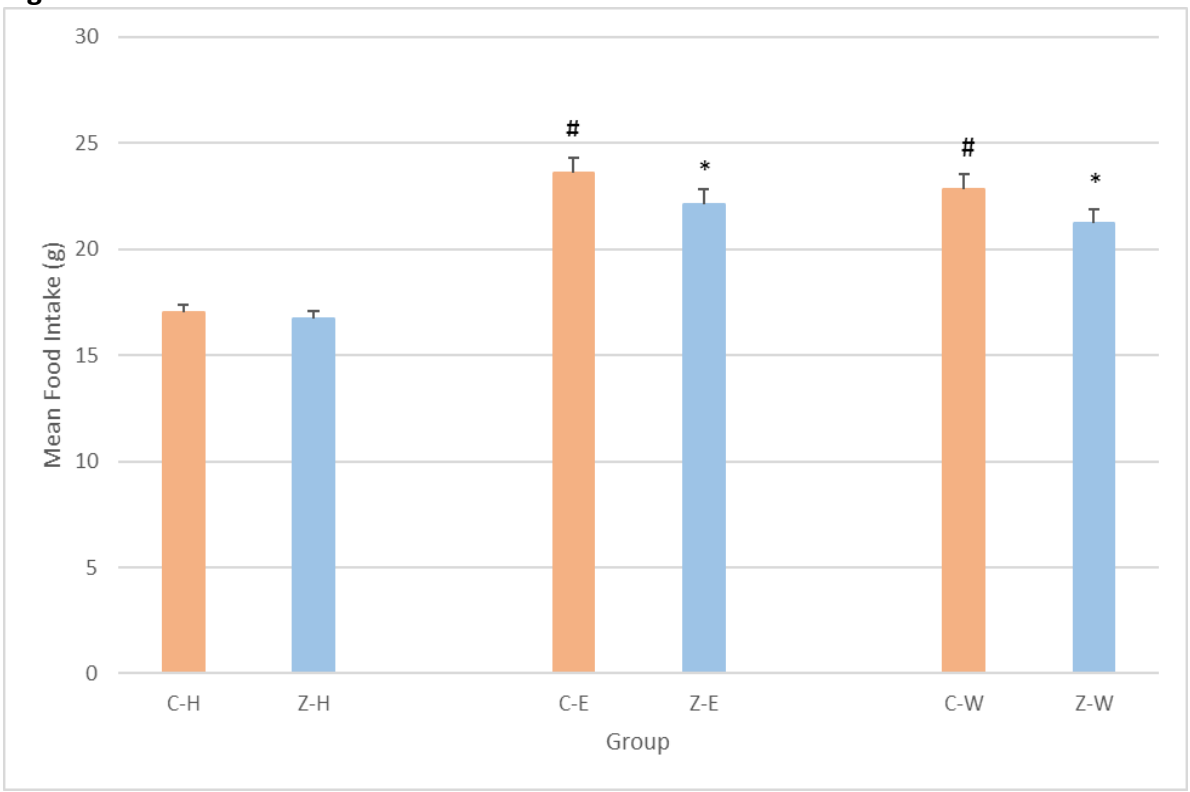

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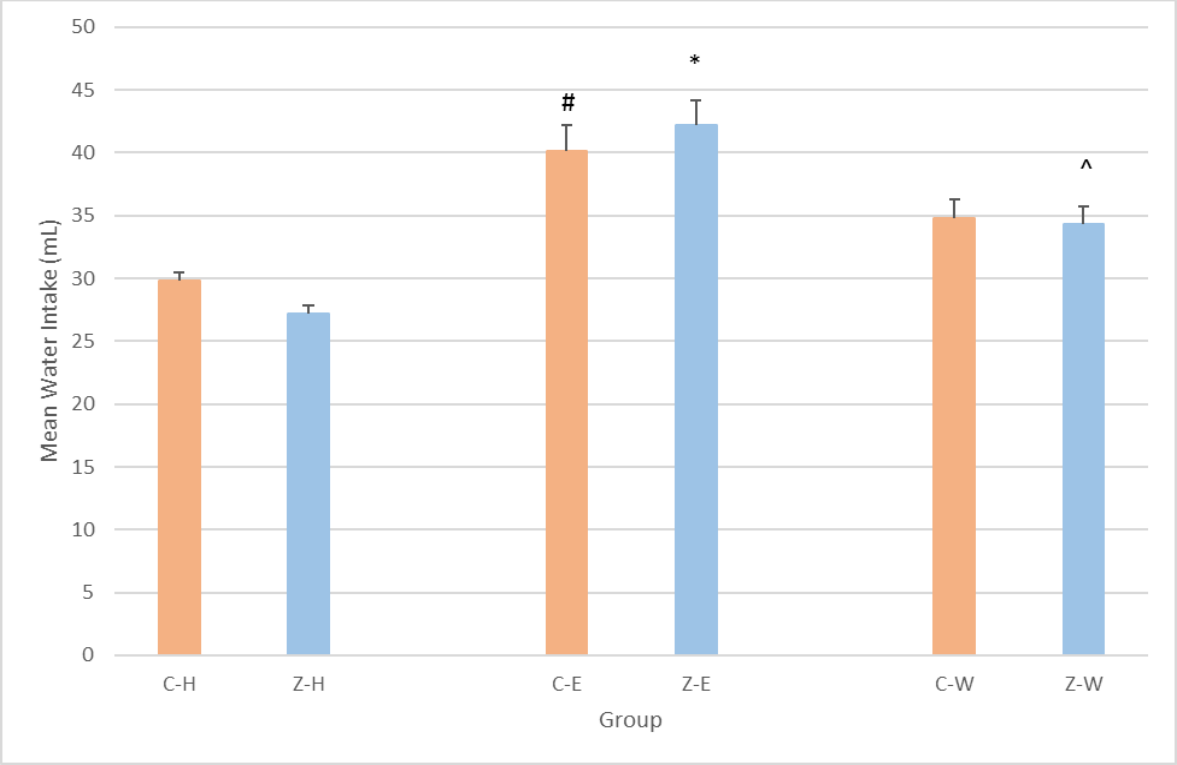

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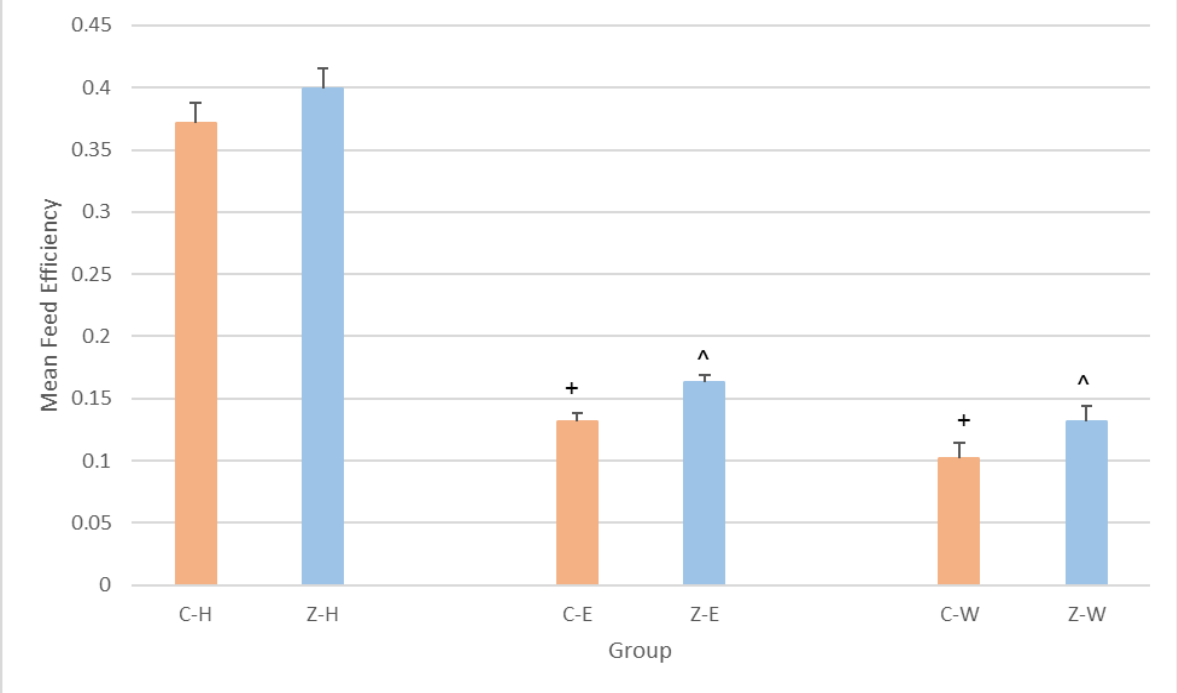

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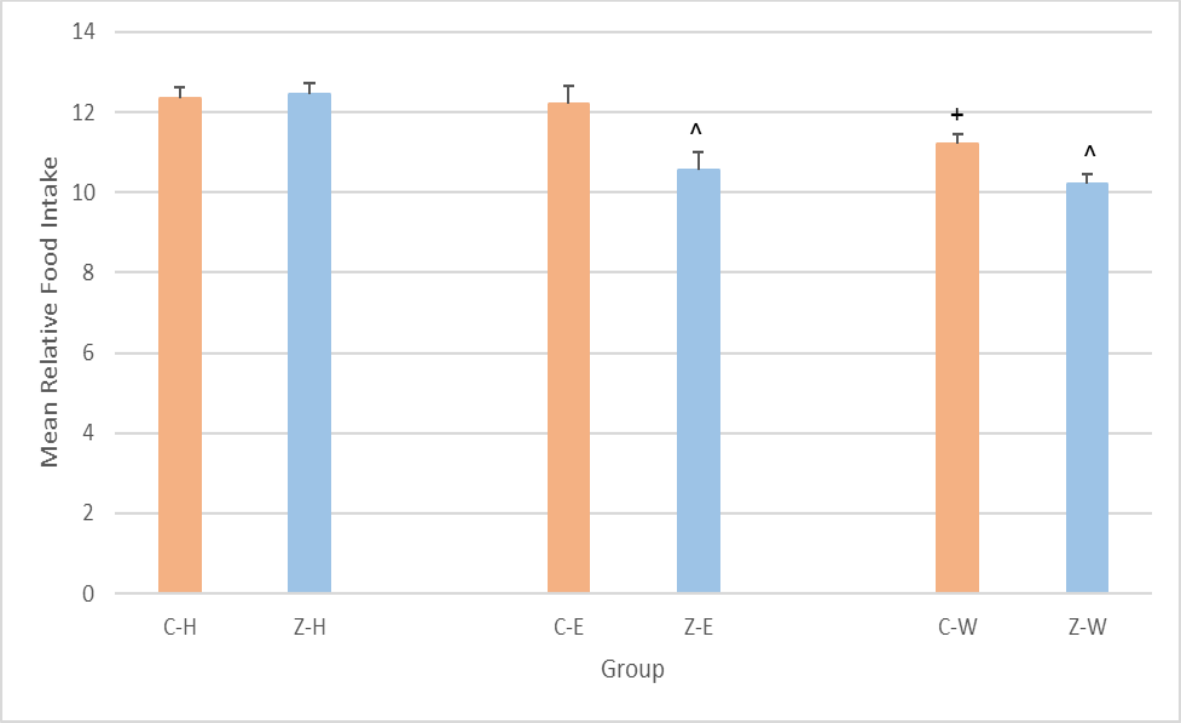

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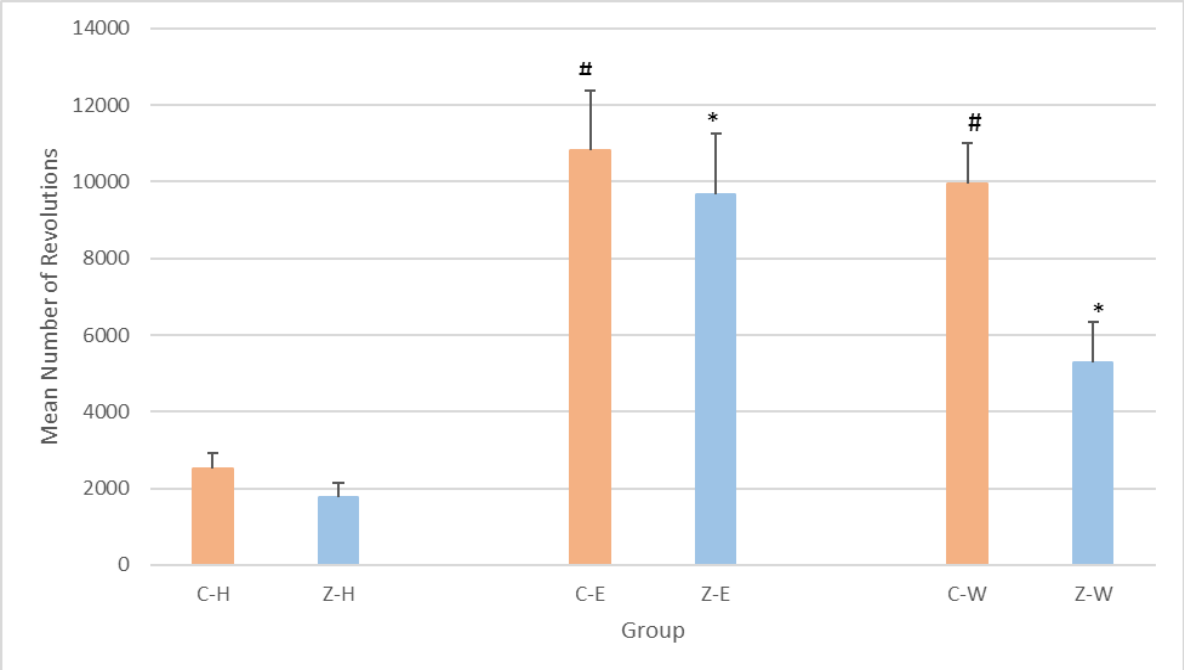

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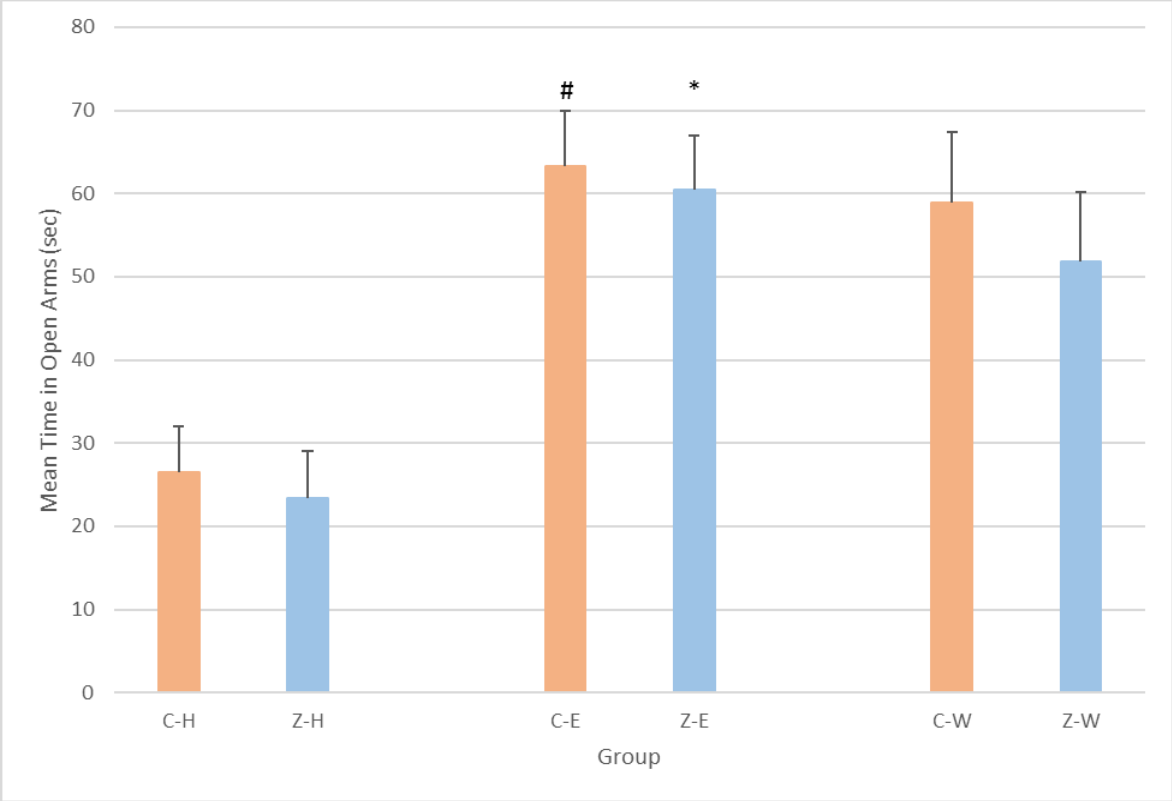

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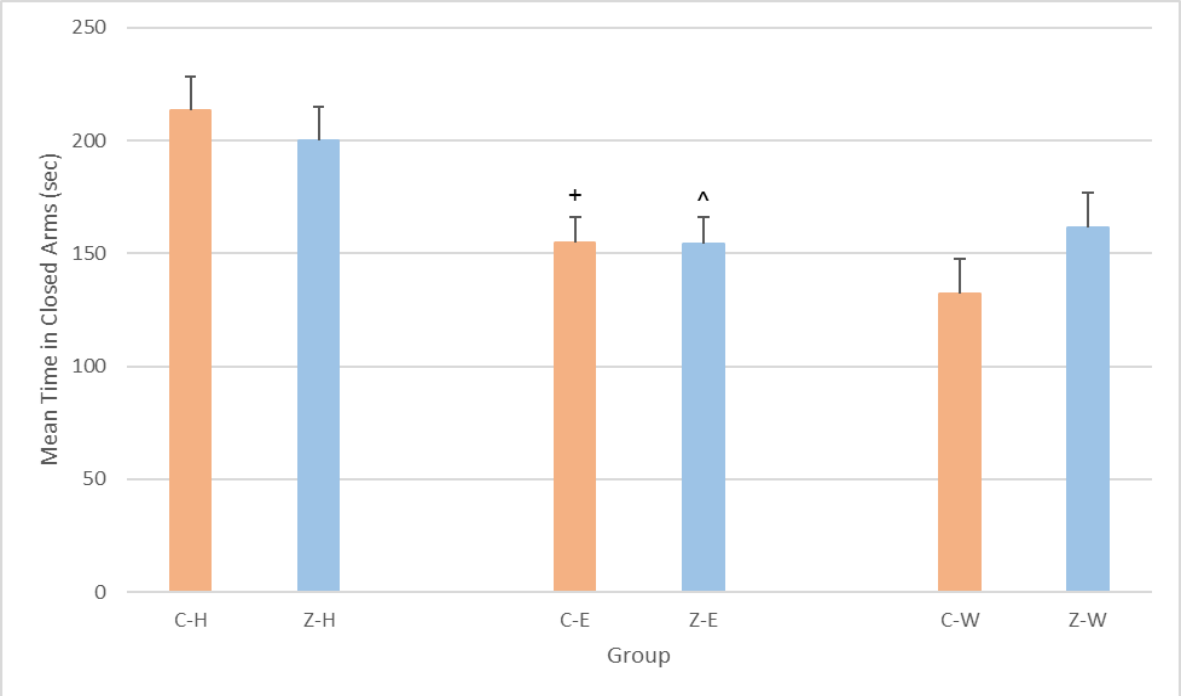

Figure 9: