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Danielle Amaral

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Danielle Amaral is a senior majoring in Biology with a minor in Chemistry. This research was conducted under the mentorship of Dr. Joseph Seggio (Biology) and was funded by a 2013 Adrian Tinsley Summer Research Grant. Danielle presented this research at the 2013 ATP Summer Research Symposium and for the International Behavioral and Neural Genetics Society in Chicago. Danielle plans to attend medical school.

Type 2 diabetes mellitus is a chronic disease that affects the lives of millions. A type 2 diabetic is unable to properly produce insulin, a hormone that helps glucose enter the cells. As a result, there are high levels of glucose in the bloodstream, which can lead to heart disease, kidney and nerve damage and loss of eyesight. It is well known that some individuals are genetically prone to the disease, and studies have shown that a disrupted sleep/wake cycle can increase an individual's chance of developing diabetes. Insulin is secreted in a predictable daily (i.e., circadian) pattern from the pancreas, and a functional biological clock is necessary for proper insulin release. In addition, studies have shown that diabetes affects some the genes which regulate the circadian rhythm, such as period, clock, *and* bmal1. *Given that there is a relationship between circadian rhythms and diabetes, this study investigates the selectively bred TALLYHO/Jng (TH) mice which develop type 2 diabetes at ten weeks of age, mimicking human diabetes symptoms such as hyperglycemia, hyperinsulinemia, obesity, and enlargement of the islets of Langerhans in the pancreas. Eight male TH mice running-wheel turn activity was observed under constant darkness for the course of several weeks. Their free running rhythms were observed pre-and-post onset of diabetes. TH displayed less activity but more frequent bouts per day than the wild type mouse C57BL/6J. TH mice with access to a running-wheel were significantly lighter when compared to studies done by Kim et al, 2006 and Steward et al, 2010, in which TH mice did not have access to running-wheels. Since there is such a weight difference among the mice from different studies, the blood glucose levels were measured for running and non-running mice. Access to running-wheel cage shows to cause long term reduction of diabetes symptoms.*

Introduction

Type 2 diabetes mellitus (T2DM) is the most common form of human diabetes affecting 23.2 million Americans. As of 2012, the total cost of treating people diagnosed with diabetes in the United States is approximately 245 billion dollars according to the American Diabetes Association. Type 2 diabetes occurs when the body is unable to produce enough insulin or else the insulin does not work properly. Therefore, insulin is unable to assist glucose in entering cells and it cannot be used as an energy source. Long-term complications from high blood sugar include heart disease, diabetic retinopathy, kidney failure and poor blood circulation which may lead to amputations. External factors such as obesity, diet and life style contribute to the occurrence and maintenance of the disease. The importance of a proper sleep cycle is

fundamental for glucose homeostasis, because sleep allows the body to restore proper metabolic and hormonal (i.e., insulin) processes (Tsumura et al., 1999). A normal glucose tolerance depends on the pancreatic beta cells to efficiently produce insulin, and a type 2 diabetic also develops insulin resistance which can lead to higher blood sugar levels. As a result, patients suffer from insulin resistance, improper insulin secretion or a combination of both.

The pancreas releases insulin in a daily rhythm, i.e, in a circadian pattern (Peschke et al., 1998). A circadian rhythm is an approximate 24 hour long daily rhythm that controls our physiological and behavioral processes that synchronizes to environmental cues, such as light. Once an organism is isolated from an environmental factor (i.e., light) a free-running rhythm emerges, which is usually 30 minutes longer or shorter than the average 24 hour daily cycle. A stimulus that directly affects the free-running period affects the biological clock. In mammals, the suprachiasmatic nuclei (SCN), is responsible for synchronizing all the biological processes in response to daily events. The SCN maintains the circadian rhythms as hierarchically organized oscillators, including the pancreas. One of the most well known ways to investigate a circadian rhythm is through analyzing the sleep-wake cycle. Physiological and behavioral alterations have been recorded in mice and humans who experience a disrupted sleep cycle. Perturbations to this cycle lead to an increase in appetite, weight gain and increased chances of developing type 2 diabetes (Kawakami et al., 2004, Morikawa et al., 2005). Therefore an organism that suffers from type 2 diabetes has an impaired daily insulin secreting cycle, which can result in glucose intolerance (Pesche et al., 1998). The lack of a functioning pancreatic circadian rhythm leads to altered insulin production by the beta cells of the islets of langerhans, which are critical for glucose homeostasis (Sadacca et al., 2010). In addition, glucose tolerance has been found to be lower in patients who are sleep deprived (Spiegel et al., 1999). Studies have also shown that diabetes affects critical genes, which regulate the circadian rhythm, such as "*clock*", "*bmal1*" and "*period"* (Marcheva et al., 2010). Since there is a connection between diabetes and the biological clock, our study aims to uncover the physiological and behavioral aspects of the circadian rhythm in a selectively-bred, non-insulin dependent type 2 diabetes mellitus mouse model, TALLYHO/JnJ (TH).

TH mice show a comparable genetic basis to diabetic humans, as they develop hyperglycemia, hyperinsulinemia, obesity, and enlargement of the Islets of Langerhans in the pancreas, at ten weeks of age (Kim et al., 2001, Kim et al., 2006, Stewart et al., 2010). While there are many studies showing the connection between the circadian rhythm and diabetes, few studies have investigated the free-running rhythm of diabetic organisms. Since diabetic humans may show altered sleep cycles, the study explored whether type 2 diabetes affects the sleep and wake cycles (Spiegel et al., 1999). It investigated the free-running rhythm, daily activity levels pre-and post-onset of diabetes in TH mice and other circadian parameters compared to wild type mouse C57BL/6J. It further explores whether access to a running-wheel cage alleviates diabetes symptoms such as body mass and glucose levels.

Methods

Eight, five-week old, male TallyHo/JngJ (TH) mice and fifteen male C57BL/6J (B6) mice were purchased from Jackson Laboratories (Bar Harbor, ME) and housed individually in running wheel cages (Minimitter, Bend, OR, diameter 16.5 cm). Running-wheel activity was monitored using the Vital View Interface System (Minimitter) and analyzed using ClockLab analysis software (Acitmetrics, Wilmette, IL). All mice were given water and standard mouse chow (Lab Diet 5001) ad libitum. Food and water consumption, and changes in body mass were recorded weekly.

The mice were initially maintained on a 12:12 Light:Dark (LD) cycle from 0600 to 1800 hours for 3-weeks to determine the entrainment and activity profiles of the TH mice. After those 3-weeks, the animals were placed into constant darkness (DD). As TH mice develop the symptoms of Type-2 Diabetes at 10-weeks of age, the first 2-weeks of DD were used to calculate the free-running period (i.e., the behavioral circadian rhythm) before the onset of the disease. The second 2-week epoch (10-12 weeks of age) was used to determine if any changes in the behavioral free-running period emerges after the onset of diabetes. Circadian rhythm parameters (free-running period, circadian activity profiles) were determined for each of the experimental epochs. Free-running circadian period was determined using the x^2 periodogram analysis, a well-established method in the ClockLab analysis routines. The total number of daily wheel turns was also determined for each animal and for each epoch of the experiment. A bout analysis for both LD and DD was conducted for all genotypes. An activity bout was defined as being greater than or equal to the average size of activity counts across the day, separated by at least ten minutes of inactivity. The mean length of time (minutes), beam crosses per bout, and number of bouts per day were analyzed. At 26 weeks of age, four mice were moved to a non running-wheel cage while four mice remained in running-wheel cages. Blood was obtained by snipping the tail with a 25 gauge needle and collecting 50 µl with a test strip. Glucose levels of weeks 28 and 30 were obtained using One-Touch Ultra Glucose Meters.

Statistical analyses were performed to calculate differences in

the circadian (period, wheel turns) and physiological (food, water, body mass, blood glucose) parameters between the two genotypes as well as throughout the course of the study. The experimental procedures described in this report were reviewed and approved by the Bridgewater State University's Institutional Animal Care and Use Committee (IACUC 2013-08).

Results

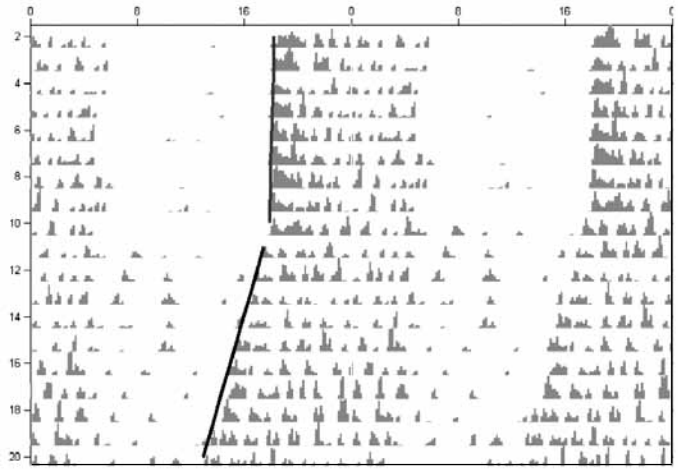
Free Running Period and Activity Profile

TallyHo (TH) mice are able to successfully entrain to an LD cycle and show a robust circadian activity rhythm in DD (Figure 1). A paired t-test revealed that there is no significant change in the average free running period lengths pre- (23.72 h) and post- (23.75 h) the onset of diabetes (p=0.50 – Figure 2). In addition, there are no significant differences (all p>0.10) between pre- and post-diabetic TH mice, respectively, regarding average wheel turns per day (28.62, 24.16), average counts per bout (586.63, 442.93), average time per bout (46.49, 40.36), and number of bouts per day (8.30, 8.67).

Comparison of TallyHo/Jng and C57BL/6J

When compared to C57BL/6J mice (49.36), TallyHo mice (28.62) exhibit significantly lower locomotor activity in terms of average of number of wheel turns per day (p=0.016). Additionally, TH mice, when compared to B6 mice respectively, display more bouts per day (8.30, 5.11; p=0.015), but significantly shorter average bout length (46.49, 96.58; p<0.001) and reduced wheel turns per bout (586.63, 1402.72; p=0.004 – Figure 3). Thus, it appears that B6 mice have higher and more concentrated levels of activity compared to TH mice. There were no differences found between TH mice and B6 mice, respectively, regarding average free running circadian activity period in DD from weeks 8 through 10 (23.72, 23.72; p=0.97).

Figure 1.



This actogram represents the locomotor activity of a pre-diabetic TallyHo mouse. It demonstrates a stable entrainment to a light-dark cycle (days 1-11) and free-running period rhythmicity under constant darkness (days 12-20). The vertical line in the chart calculates the period length to be 24 hours long and the angled line indicates the period length to be 23.79 h.Y axis represents days and X axis represents time of day.

Food Intake and Body Mass

TallyHo mice food intake significantly increased from week 6 to 7 (p=0.025); however, paired t-tests with Bonferroni Correction for multiple comparisons revealed that food intake leveled off as they aged (all p>0.10 – Figure 4). As expected the body mass increased; however, there is no correlation (Pearson's Correlation – all p>0.10) between food intake and weight gain during the course of the experiment. A paired t-test showed

Figure 2. Pre Diabetes

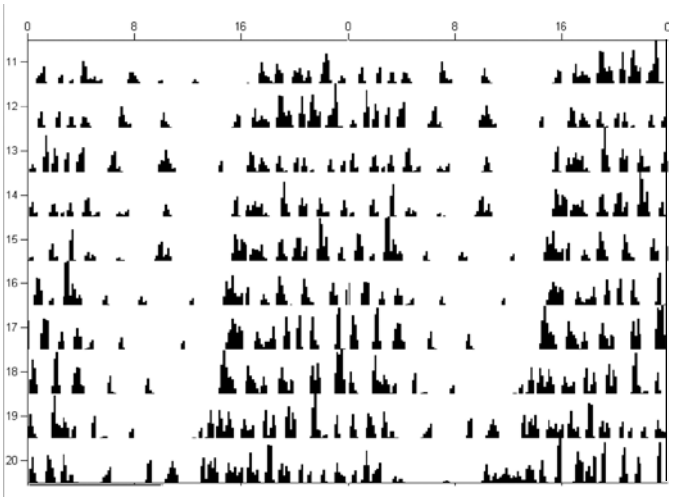
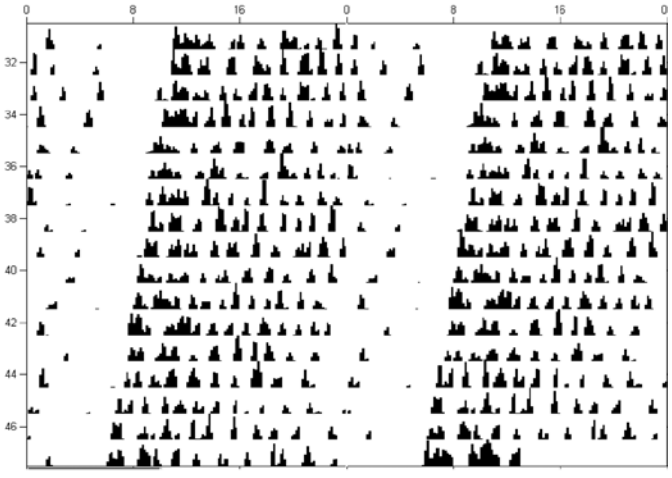


Figure 2. Post Diabetes



Segments of double plotted TallyHo free running activity records from pre-diabetic mice (left) and diabetic mice (right). There are no differences between the behavioral circadian rhythm and activity profile from before the onset of diabetes to afterward.

Figure 3. TallyHo/Jng

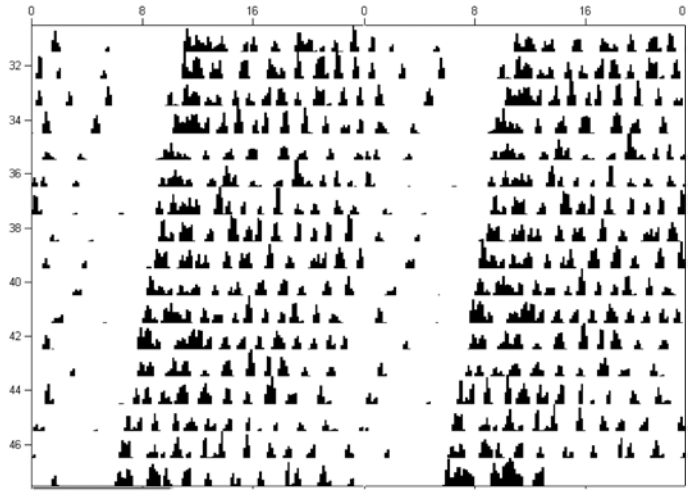
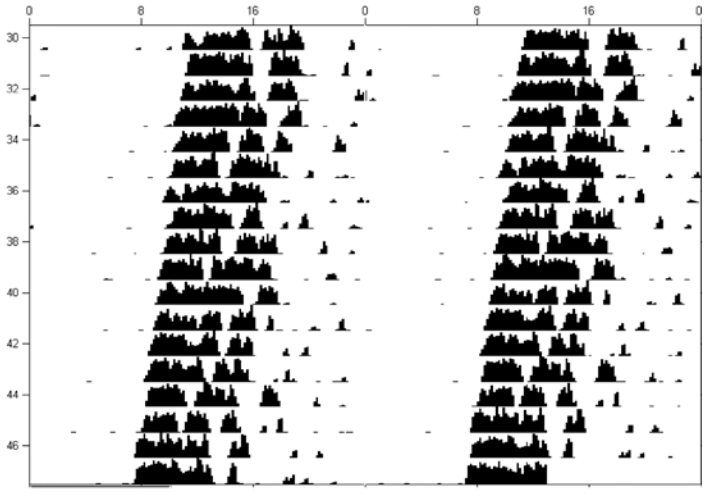
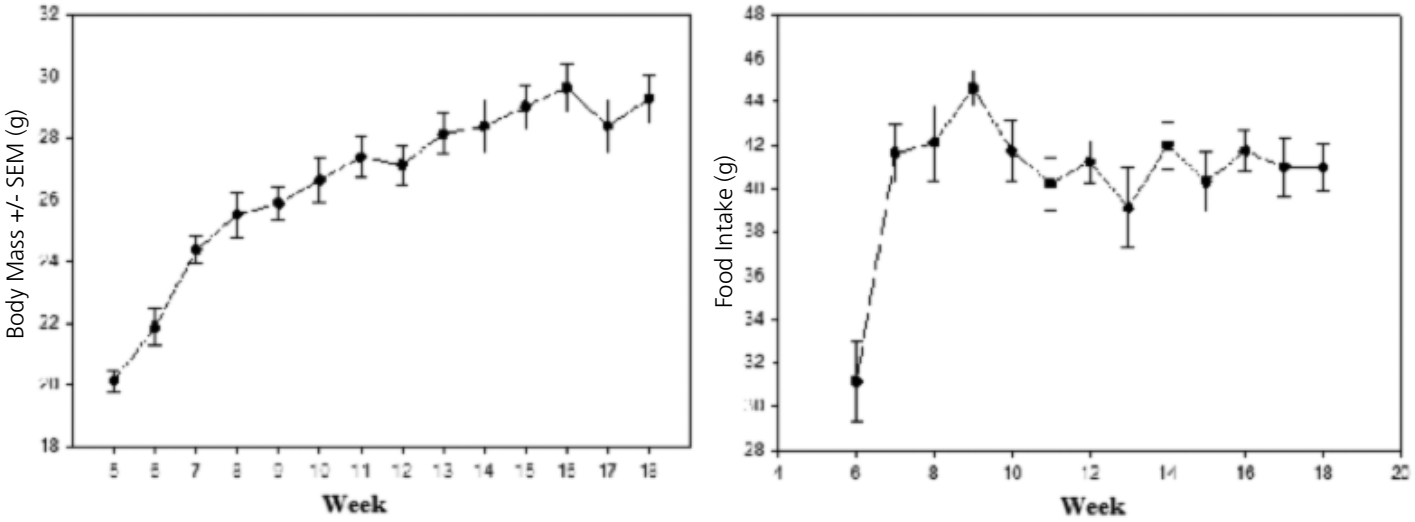


Figure 3. C57Bl/6



Double plotted free running activity profiles of diabetic TallyHo/ Jng (TH) (left) and C57BL/6J (B6) (right). B6 mice show increased wheel turns per day and increased bout length, but reduced bouts per day than TH mice.

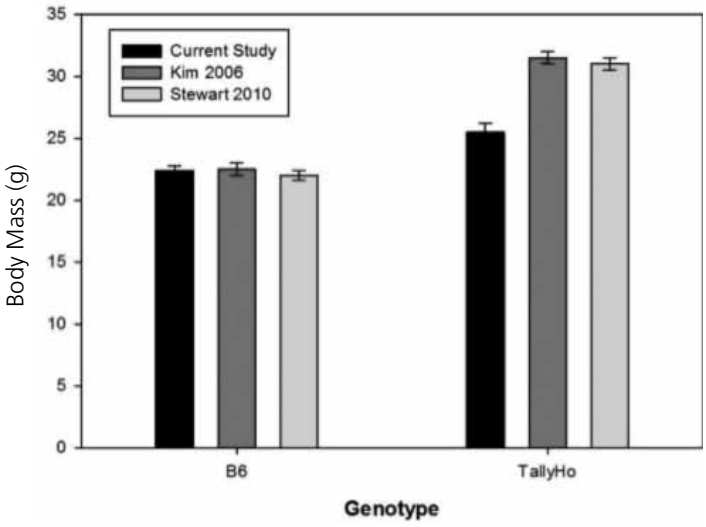
Figure 4.



Graph on the left displays the food intake from week 6 through week 18. Graph on the right illustrates the body mass increase as the mice aged. There is no correlation between food intake and increased body weight for any of the weeks tested.

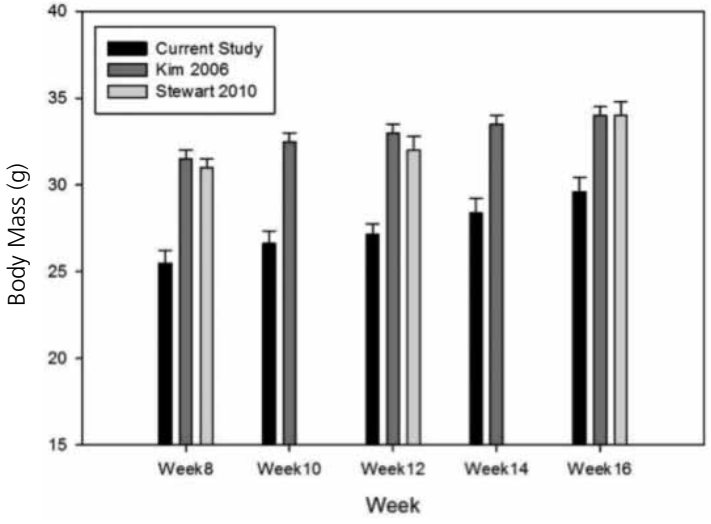
that weeks 5, 6 and 7 are significantly different from each other (all $p<0.05$); however, no differences were found from week 8 onward ($p>0.10$). An independent t-test revealed that our TallyHo is significantly heavier than our age matched C57BL/6J ($p=0.003$), which confirms results from previous experiments showing that TallyHo mice are consistently heavier than C57BL/6J mice. Additionally, TallyHo mice from the current experiment were noticeably lighter than TallyHo from previous studies (Kim et al., 2006, Stewart et al., 2010) at 8-weeks of age (Figure 5). Further investigation showed that the body mass reduction in the current study persisted over the next eight weeks (Figure 6). This result is different than what Rhee et al., 2010 found, where obesity in TH mice was directly correlated with increased food intake.

Figure 5.



The bar graph represents the body mass difference between C57BL/6J and TallyHO along three different studies at 8 weeks of age. TH mice are significantly heavier in all three studies compared to B6 mice. Additionally, access to wheel running from current study caused the TH mice to show reduced body mass compared to previous studies where TH mice had no access to wheel running. B6 mice from current study were purchased at 8 weeks of age and had no access to running -wheels.

Figure 6.

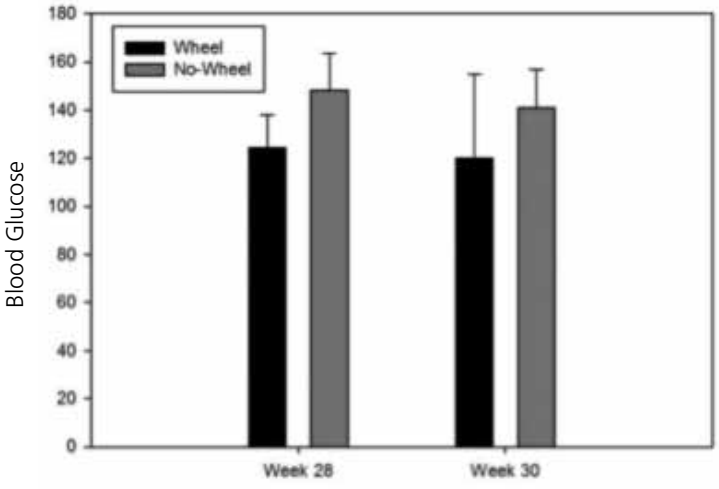


Bar graph displays TallyHO body mass from three different studies along the course of eight weeks. Wheel running access produced a reduction in body mass compared to two previous studies. While the TH mice from current study continued to gain weight over the course of the study, this reduction in body mass compared to previous studies persisted for eight weeks with continuous access to running -wheels.

Blood Glucose Levels

The blood glucose levels are significantly lower than TH mice from previous studies (Figure 7). For the current experiment blood glucose levels for TH mice, weeks 28 and 30 were not significantly different between running and non-running mice. Upon removal from running-wheel the body mass increased; TH mice without access to running-wheel showed a higher body mass compared to mice kept in running-wheel cages (P= 0.039) (Figure 8). These results suggest that exercise in the form of wheel-running leads to long term alleviation of diabetes symptoms, including obesity and glucose levels, but upon cessation of exercise, only blood glucose levels maintained stable while body mass significantly increased.

Figure 7A.



Bar graph on above represents a 4-hour fasting glucose levels in male TH mice compared among two other studies and current study, which shows a large difference in blood glucose levels. Bottom graph shows TH mice blood glucose for running and non running mice. No differences were found; however, a small sample was used.

Figure 7B.

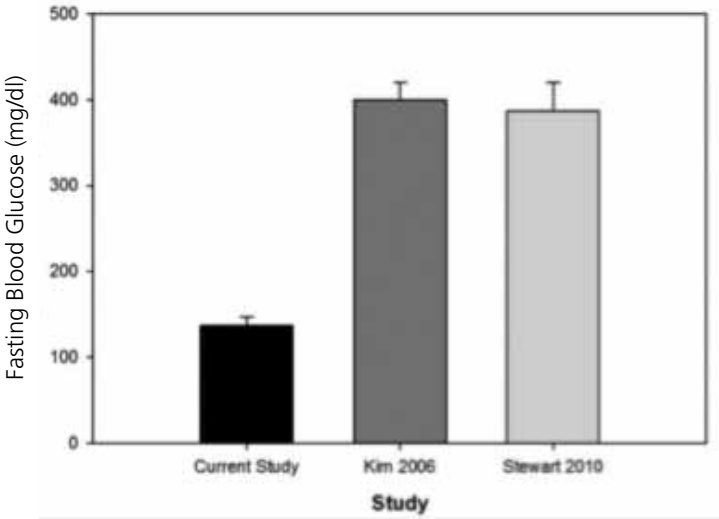
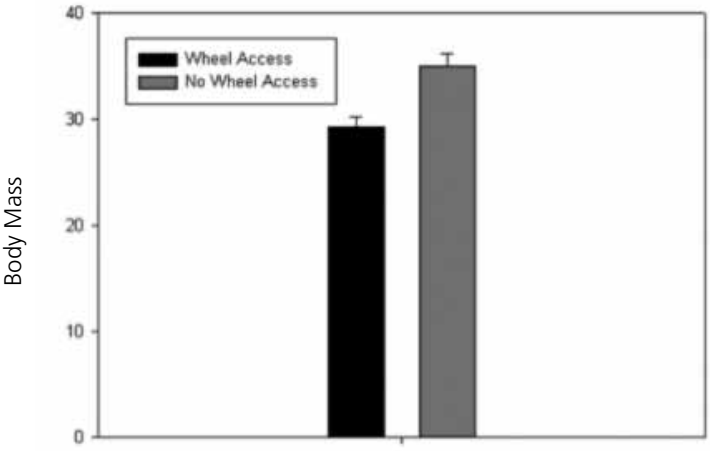


Figure 8.



TallyHo average body mass during wheel access (black) and no wheel access (red) for weeks 28-30.

Discussion

The present study in TallyHo mice found that there are no significant differences pre-and post-onset of diabetes in terms of the behavioral circadian activity rhythm or activity profile, as TallyHo mice did not display significant alterations in activity levels and free running circadian period after the development of Type 2 diabetes. These findings suggest that diabetes does not directly affect the behavioral clock. It also suggests that the behavioral free-running period should be considered independent of the diabetic phenotype, whenever studies investigate this particular mouse model.

A detailed investigation of wheel-running behavior during DD revealed that TallyHo, when compared to sex and age match C57BL/6J mice, displayed significantly less locomotor activity. As TallyHo mice are consistently heavier than B6 mice throughout the courses of this and previous experiments (Kim et al., 2006, Stewart et al., 2010), the increased body mass may be having an impact on the ability of TH mice to run on the running-wheel, causing their activity to be reduced overall, but also more "choppy" with increased number of bouts per day, but each bout having reduced number of wheel turns and time. Still, the TH mouse was developed on a different background strain (Swiss) than the B6 mouse. As the B6 mouse is considered a "high-locomotor activity" mouse strain (Jackson Labs), differences in background strain cannot be ruled out as a possibility for the source of the activity level differences found between the two strains.

Our TallyHo are significantly lighter than mice from previous experiments (e.g., Kim 2006 and Stewart 2010); these results suggest that an accessibility to a running-wheel affects body mass. In addition, mice see wheel running as a rewarding activity, as voluntary wheel running increases the dopaminergic system pathway (O'Dell et al., 2007). Previous studies have shown that increased body weight due to fat deposits can decrease dopamine signaling within the brain in humans (Wang et al., 2001). Additionally, both insulin and leptin are inhibitors of dopamine (Palmiter, 2007), while application of dopamine agonists can induce a decrease in body weight in rodents (Chen et al., 2001). As TH mice show increased fat deposits, and increased leptin and insulin, and decreased efficacy of insulin (Kim et al., 2006), the body weight reduction observed in the current experiment can also be due to increased dopamine signaling in the brain, or a combination of the increased voluntary exercise and dopamine signaling. Ongoing study is determining the effects of wheel running on glucose levels in TH mice. Current results indicate that accessibility to running-wheel cage reduced diabetes symptoms such as high blood glucose and obesity. Our current experiment supports previous findings (Fig 7, 8). Exercise stimulates the glucose uptake, and there is an increase in insulin regulatable glucose transporters (IRGT), which induce glucose to enter cells (Vannucci et al, 1998). Additionally, cholecystokinin (CCK) inhibits neuropeptide Y (NPY) which increases insulin secretion. NPY has shown to increase adipose tisse. Access to running-wheels have shown to bypass CCK signal and directly inhibit NPY expression, which leads to a decrease adipose tissue (Bi et al., 2003). Thus, upon removal of the running wheel, body mass increases.

As there is a correlation between body weight and plasma glucose levels, future experiments will further analyze blood plasma insulin levels and blood glucose levels at different circadian times between B6 and TH mice, with and without access to running-wheels. One of the most important physiological adaptations is the variation of the daily glucose tolerance and how it disrupts the circadian oscillation of glucose uptake. Studies suggest that metabolic and circadian mechanisms are directly connected (Marcheva et al., 2010). Thus, future studies will aim to uncover differences in the circadian rhythms of physiological processes that underlie metabolism and diseases that affect metabolism, such as Type II diabetes.

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References

Bi, S., Robinson, B. M., & Moran, T. H. (2003). Acute food deprivation and chronic food restriction differentially affect hypothalamic NPY mRNA expression. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *285*(5), R1030-R1036.

Chen TY, Duh SL, Huang CC, Lin TB, Kuo DY. (2001). Evidence for The Involvement Of Dopamine D(1) and D(2) Receptors in Mediating The Decrease of Food Intake During Repeated Treatment with Amphetamine. *J Biomed Sci*. 8:462-466.

Kim JH, Sen S, Avery CS, Simpson E, Chandler P, Nishina PM, Churchill GA, Naggert JK. (2001). Genetic Analysis of a New Mouse Model for Non-Insulin-Dependent Diabetes. *Genomics*. 74:273-286.

Kim JH, Stewart TP, Soltani-Bejnood M, Wang L, Fortuna JM, Mostafa OA, Moustaid-Moussa N, Shoieb AM, McEntee MF, Wang Y, Bechtel L, Naggert JK. (2006). Phenotypic Characterization of Polygenic Type 2 Diabetes in TALLYHO/JngJ Mice. *J Endocrinol*. 191:437-446.

Marcheva B, Ramsey KM, Buhr ED, Kobayashi Y, Su H, Ko CH, Ivanova G, Omura C, Mo S, Vitaterna MH, Lopez JP, Philipson LH, Bradfield CA, Crosby SD, JeBailey L, Wang X, Takahashi JS, Bass J. (2010). Disruption of The Clock Components Clock and BMAL1 Leads to Hypoinsulinaemia and Diabetes. *Nature*. 466:627-631.

Morikawa Y, Nakagawa H, Miura K, Soyama Y, Ishizaki M, Kido T, Naruse Y, Suwazono Y, Nogawa K. (2005). Shift Work and The Risk of Diabetes Mellitus Among Japanese Male Factory Workers. Scand J *Work Environ Health*. 31:179-183.

Muhlbauer E, Wolgast S, Finckh U, Peschke D, Peschke E. (2004). Indication Of Circadian Oscillations in The Rat Pancreas. *FEBS Lett.* 564:91-96.

O'dell, S. J., Gross, N. B., Fricks, A. N., Casiano, B. D., Nguyen, T. B., & Marshall, J. F. (2007). Running Wheel Exercise Enhances Recovery From Nigrostriatal Dopamine Injury Without Inducing Neuroprotection. *Neuroscience*, *144*(3), 1141-1151.

Peschke E, Peschke D. (1998). Evidence for a Circadian Rhythm of Insulin Release from Perifused Rat Pancreatic Islets. *Diabetologia*. 41:1085-1092.

Rhee SD, Sung YY, Lee YS, Kim JY, Jung WH, Kim MJ, Lee MS, Lee MK, Yang SD, Cheon HG. (2010). Obesity of TallyHO/JngJ Mouse Is Due to Increased Food Intake with Early Development of Leptin Resistance. *Exp Clin Endocrinol Diabetes*. 119:243-251.

Sadacca LA, Lamia KA, deLemos AS, Blum B, Weitz CJ. (2010). An Intrinsic Circadian Clock of The Pancreas is Required for Normal Insulin Release And Glucose Homeostasis In Mice. *Diabetologia*. 54:120-124.

Spiegel K, Leproult R, Van Cauter E. (1999). Impact of Sleep Debt on Metabolic and Endocrine Function. *Lancet*. 354:1435-1439.

Stewart TP, Kim HY, Saxton AM, Kim JH. (2010). Genetic and Genomic Analysis of Hyperlipidemia, Obesity And Diabetes Using (C57BL/6J x TALLYHO/JngJ) F2 Mice. *BMC Genomics*. 11:713.

Tsumura K, Hayashi T, Suematsu C, Endo G, Fujii S, Okada K. (1999). Daily Alcohol Consumption and The Risk Of Type 2 Diabetes in Japanese Men: The Osaka Health Survey. *Diabetes Care*. 22:1432-1437.

Vannucci, S. J., Maher, F., & Simpson, I. A. (1997). Glucose Transporter Proteins in Brain: Delivery of Glucose to Neurons And Glia. *Glia*, *21*(1), 2-21.

Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, Netusil N, Fowler JS. (2001). Brain Dopamine and Obesity. *Lancet*. 357:354-357.