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Reduced activity without hyperphagia contributes to obesity in Tubby mutant mice

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Abstract

The *Tub* gene was originally identified as a spontaneous mutation in C57Bl/6J mice, and associated with adult-onset obesity (*Tub* MUT mice). Although the original *Tub* MUT mouse was identified over 15 years ago, there have been few reports on the animal's food intake, body fat percentage or energy expenditure. In this study, we report food intake, body weight from 5–20 weeks, body fat, body temperature and three different measures of physical activity behavior. *Tub* MUT mice display reduced food intake, uncharacteristic of many obese mouse models, and reduced voluntary wheel running with normal home cage ambulatory behavior. We conclude that motivation for food and exercise is an underlying defect in *TUB* MUT mice.

Keywords

locomotor activity; food intake; body temperature; wheel running; body fat; obesity

1. Introduction

Tubby mutant mice were originally identified in a colony of mice at The Jackson Laboratories and exhibit adult-onset obesity starting between 9–12 weeks of age [1]. When the *Tub* gene was originally cloned, it lacked any strong homology to other proteins [2,3]. Subsequently, other proteins have been identified constituting the Tubby-like or TULP family (for a review [4]). All family members have a highly conserved C-terminal region with a positively charged groove and a central hydrophilic β barrel structure [4]. Based on this observation and the results of DNA binding and transactivation experiments, Boggon and coworkers proposed that TULP proteins are a unique family of transcription factors [5]. Another group has shown that Tubby protein can interact with G-protein coupled receptors like the serotonin receptor, suggesting that Tubby functions as a signaling molecule [6]. To date the function of Tubby as a

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transcription factor or as a cytoplasmic-to-nucleus signaling factor remains unclear; neither a sequence specific DNA binding motif nor any Tubby-dependent genes have been identified.

Tubby protein and *Tub* mRNA are expressed throughout the hypothalamus, including the arcuate nucleus, ventral medial hypothalamus, and paraventricular nucleus. Within both the ventral and dorsomedial hypothalamus, *Tub* mRNA levels are mildly elevated in hypothyroid animals [7]. This is the expected direction for a gene that might negatively regulate energy balance and suggests that the *Tub* gene may be a target of thyroid hormone receptors. Tubby protein is phosphorylated in response to insulin and IGF-1 in cell lines, although insulin-mediated phosphorylation of Tubby has not been demonstrated in animals or hypothalamic extracts [8].

NPY and POMC mRNA levels in the hypothalamic arcuate nucleus of Tubby mutant mice are reduced when compared to normal mice [9]. While POMC reduces food intake, NPY has the opposite effect and enhances food intake [10]. NPY expression is elevated to the point of being misexpressed in non-arcuate areas of the hypothalamus of the mutant mice, leading one to speculate that increased NPY in areas outside of the arcuate nucleus combined with reduced NPY and POMC in the arcuate nucleus could contribute to the obesity phenotype of the mutant mice.

The *TUB* gene is located on human chromosome 11 at p15.5. Three different studies have found linkage of BMI, abdominal fat and leptin levels to 11p15 in humans [11], and polymorphisms within the C-terminus of the Tubby protein are linked to increased body mass index in humans [12]. In *C. elegans*, the *Tub* homolog, *tubby*, is involved in fat storage [13]. *TULP* family members have been implicated in human syndromes of retinal degeneration [4], but these syndromes do not include obesity as one of the phenotypes.

Our laboratory began studying the Tubby mutant mice as these animals represent one of the very few mouse models of adult-onset obesity. In the early stages of our study, we realized that the mechanism of weight gain of Tubby mutant animals was not extensively described. Earlier reports on the Tubby mutant animals showed that slight obesity is evident by 10 weeks of age. By 24 weeks there is only a modest obesity averaging about 46 grams in *TUB* MUT males [1]. Hyperinsulinemia and mild hypoglycemia is present in older obese *TUB* MUT mice and is accompanied by hypertrophy of the pancreatic islets [1]. A more recent report measured home cage activity, respiration and carbohydrate metabolism, and concluded that defects in respiration and carbohydrate metabolism may contribute to obesity in this mouse model [14]. These authors did not find any differences in home cage activity. These data suggested that energy expenditure may be defective in *TUB* MUT animals, but since food intake was not measured, nor body temperature or spontaneous wheel running it was not clear if these behaviors also contributed to the development of obesity. It was also not clear if any of the other reported phenotypes of *TUB* MUT animals, such as retinal and cochlear degeneration, would also contribute to any of their behavioral differences. In this paper, we report the results of a long-term study of food intake, body temperature and activity measurements, with a specific focus on balance and locomotion in *TUB* MUT animals.

2. Methods and Materials

2.1 Animal maintenance and breeding

All animal work was done in compliance with the Institutional Animal Care and Use Committee at the University of Massachusetts. A heterozygous breeder pair and a homozygous $\times$ heterozygous breeder pair were purchased from The Jackson Laboratories (Bar Harbor, ME) and used to start the colony. Breeding colony maintenance and weaning have been described elsewhere [15]. Genotype analysis by PCR restriction fragment length polymorphism using

SmL1 was done according to the protocol provided by The Jackson Laboratories. Tub homozygous (MUT), Tub heterozygous (HET) and normal (WT) mice were maintained in 12:12 hour light: dark conditions (7 AM to 7 PM) with *ad lib* food (PMI Laboratory Rodent Diet 5001, 4.5% crude fat) and water.

2.2 Body weight growth curve determination

Animals were weighed weekly from 5 weeks to 20 weeks of age using an Ohaus portable electronic scale. Animals were maintained in hanging cages (food intake study) or cages with suspended wire floors (wheel running study). The data are reported as the genotype mean $\pm$ SEM. N = 10 for each genotype in the analysis. These mice were also used in the food intake and body temperature studies. Power analysis indicated power = 0.9838 for this study.

2.3 Body Composition Analysis

Animals were euthanized by CO₂ asphyxiation and weighed. For carcass analysis, animals were decapitated and brains used for other experiments. Weight of the body minus the head was recorded. The difference in weight between the fresh and dried carcass was used to determine the percent water. This value was then used to calculate the percent body fat using the equation: $(-1.272 \times \% \text{ water}) + 95.56 = \% \text{ fat}$ [16]. Total fat in grams was calculated using the equation: $(\% \text{ fat} \times \text{fresh carcass weight (grams)}) / 100 = \text{fat (grams)}$. Grams of lean body mass was calculated using the equation: $\text{Final dried weight (grams)} - \text{fat (grams)} = \text{lean body mass (grams)}$. Details of the procedure and calculations can be found elsewhere [17]. The data are reported as the genotype mean $\pm$ SEM. Age group 1 = 5–10 weeks, age group 2 = 11–20 weeks and age group 3 = older than 20 weeks. For WT mice, N = 6, age group 1; N = 4, age group 2; and N = 15, age group 3. For HET mice, N = 3, age group 1 and N = 5, age group 3. There were no HET mice in age group 2 category. For *TUB* MUT mice, N = 10, age group 1; N = 4, age group 2; and N = 20 age group 3. Power analysis indicated power = 0.9895 with the average sample size of 22, and power = 0.6557 with the lowest genotype group size of 8 (HET mice).

2.4 Food intake measurements

WT, HET and MUT mice were individually housed in hanging wire-bottom cages from 5-weeks to 20-weeks of age. Food (PMI Laboratory Rodent Diet 5001, 4.5% fat) intake was measured three times per week using an OHAUS LS2000 scale with a sensitivity of 0.1 grams. Spillage (both crumbs and food nuggets) was collected on paper towels that were placed beneath the wire-bottom cages. Total spillage minus fecal material was collected and weighed three times weekly. The amount was added to the total of food remaining in the bin, giving the total food remaining value. This value was subtracted from the total food received to obtain the total food intake for that period. The data are reported as grams eaten per day over each week as the genotype mean $\pm$ SEM. Animals in the food intake study were weighed once weekly and this data used for body weight analysis. N = 10 for each genotype in the analysis. These mice were also used in the body weight and body temperature studies. Power analysis indicates power = 0.9965.

2.5 Core body temperature measurements

Core body temperatures were determined using a Thermalert TH-5 mouse rectal probe attached to a Physitemp (Clifton, NJ) digital thermometer. The probe was washed with 70% alcohol between animals and lubricated with mineral oil prior to insertion. The probe was inserted into the mouse's rectum to a depth of two centimeters and held in place until a constant reading was obtained for 15 seconds. The probe was withdrawn, and once ambient temperature was reached, a second temperature reading was taken. If the variation between the two readings was greater than 0.5°C, a third reading was taken. The readings for each animal were averaged

and the data reported as the genotype mean $\pm$ SEM. The ambient temperature in the room was similar each day (average room temperature $21.3 \pm 0.5^{\circ}\text{C}$). The data are reported as the genotype mean $\pm$ SEM. $N = 10$ for each genotype in the analysis. These mice were also used in the body weight and food intake studies. Power analysis indicates power = 0.9904.

2.6 Daily spontaneous activity

Pre-obese *TUB* MUT animals and their WT and HET littermates (5–7 weeks of age at the start of the experiment) were used to eliminate problems caused by the heavier body weight of the *TUB* MUT mice at older ages, as it has been reported that obesity affects exercise [18–20].

Male mice ($N = 3$ per genotype) were placed in the cages equipped with computer-monitored running wheels (Mini-Mitter, Bend, OR). To ensure that the novelty of the wheel apparatus did not confound the activity measurements, the first thirty-six hours of activity were not analyzed. The animals were monitored in the cages for four weeks. During those weeks, the total number of wheel rotations per 20-minute bin was collected over each 24-hour period using VitalView Software (Mini-Mitter). Food intake and body weight were monitored as described above. Food intake is not being reported in this study as power analysis indicated that the power was too low (0.4046) to be evaluated. Data were reported as the total number of wheel rotations per day (genotype mean $\pm$ SEM). The total number of wheel rotations per 20-minute bin over 24-hours of time during both lights on and lights off phases were also plotted to analyze the circadian pattern of wheel running. Power analysis indicates that power = 0.4046 for total spontaneous physical activity and power = 0.5593 for body weight during wheel experiment. Although these experiments had low power, statistically significant results were obtained, indicating that the differences were strong. For other measures, power = 0.9960 for average total activity in light and dark periods, power = 0.5439 for activity during light phase and power = 0.9951 for activity during dark phase.

2.7 Optical Sensor Analysis of Voluntary Activity

Male mice between 16 and 22 weeks of age were placed in wire top plastic cages. An optical sensor (Mini-Mitter, Bend, OR) was attached to each cage lid in such a manner as to record any movement in the cage by the mouse. Any break in the optical beam in a 20-minute bin was recorded as positive activity in that period. This voluntary activity movement was recorded using VitaView Software (Mini-Mitter). Voluntary activity was recorded in three 24-hour periods after which time the mice were returned to normal cages. $N = 4$ WT, 3 HET, and 4 *TUB* MUT mice. Power analysis indicates that power = 0.8203.

2.8 Rota-Rod analysis

Animals were tested on a Columbus Instruments (Columbus, OH) Economex Rota-Rod apparatus. Age-matched WT, HET and MUT mice (aged 5–10 weeks) were tested four times each day, between 11 AM and 1 PM for four consecutive days from 0 rpm to a maximum speed of 20 rpm with an acceleration slope of 2.65%. The first day of the test the animals were trained on the rota-rod with one test using a portion of the rod that had been modified with bathroom grip tape. They then had three additional tests on the standard rod. None of the data from the first day of testing is included in the analysis. For all days of testing, animals were tested for a maximum of five minutes per test or until the animals fell off the device. The Economex Rota-Rod apparatus is equipped with a pressure sensitive landing area so that the time spent on the rotating rod is automatically recorded when the animal falls. Animals were given between five and fifteen minutes of rest between trials. The data are reported as the genotype mean $\pm$ SEM of the length of time spent on the Rota-Rod device before falling off. $N = 8$ WT mice, 8 MUT mice and 4 HET mice. Power analysis indicates power = 0.9951.

2.9 Statistical analysis

Statistical analyses were performed using either SPSS (Windows, version 13.0, Chicago, IL) or MiniTab (Windows, Version 13.0, State College, PA) software. We used ANOVAs with two between-subjects effects (age group and genotype) to analyze the results for the dependent variables percent fat and grams lean mass, and repeated measures, and ANOVAs with one within-subjects effect (either week or day) and one between-subjects effect (genotype) to analyze the results from the dependent variables body temperature, body weight, food intake, wheel running activity and Rota-Rod activity. Post-hoc pairwise comparisons were done when the overall tests of between- or within-subjects effects were significant to determine where the differences existed. All pairwise comparisons were based on estimated marginal means and are described further in the results section. Significance was accepted at $P \leq 0.05$. For all figures $P \leq 0.05$ is indicated by a single asterisk (*), while $P \leq 0.01$ is indicated by a double asterisk (**). Power analysis was performed using Java applets with online software (<http://www.stat.uiowa.edu/~rlenth/Power>) [21]. This power analysis is based on the “unrestricted” parameterization of the balanced mixed ANOVA model. Where necessary, error terms are constructed using linear combinations of mean squares, and the degrees of freedom for the denominator of the approximate F test are computed using the Satterthwaite method [21,22].

3. Results

3.1 Daily Food Intake

Food intake was measured on single-housed animals from age 5 weeks to age 20 weeks (Fig. 1). This age range was chosen because it allowed us to analyze food intake in young, pre-obese *TUB* MUT mice as well as older obese MUT animals. Statistical analysis revealed that the effect of genotype was highly significant ($F_{(2,27)} = 4.645$, $P < 0.02$). Surprisingly, post-hoc tests revealed that both the *TUB* MUT ($P < 0.01$) animals and the *TUB* HET animals ($P < 0.05$) were not hyperphagic and actually ate *less* than WT animals (mean 3.93 grams of food/day MUT, 4.00 grams/day HET, 4.29 grams/day WT, Fig. 1). There was no effect of week of testing on food intake, indicating that the animals performed the same regardless of age or body weight at the time of measurement. A subsequent analysis of food intake during the early 5–10 week age period revealed that even young *TUB* MUT mice had significantly reduced food intake compared to WT mice (mean WT = 4.30 ± 0.14 grams/day compared to mean *TUB* MUT = 3.92 ± 0.11 grams/day, $P = 0.01$). Young MUT mice also showed a reduction in daily food intake, but the difference did not reach statistical significance (mean HET = 4.00 ± 0.11 grams/day, $P = 0.061$ compared to WT).

3.2 Body weight and carcass fat content

Body weight was measured during the food intake experiment. All genotypes showed the expected increase in the body weight during the 15 week period ($F_{(15,405)} = 4.986$, $P < 0.001$). A significant week * genotype interaction effect emerged by 11 weeks of age ($F_{(30,405)} = 4.049$, $P < 0.001$) with *TUB* MUT mice weighing significantly more than WT ($P < 0.05$) and HET animals ($P < 0.01$, Fig. 2A). This is despite the fact that *TUB* MUT mice were consuming less food than the other two genotypes during this same time period.

Carcass analysis was done on three age groups of animals: pre-obese (5–10 weeks of age, Group 1), newly obese (11–20 weeks of age, Group 2) and obese (>20 weeks of age, Group 3). There was a significant effect of genotype on carcass fat content ($F_{(2,59)} = 8.208$, $P < 0.001$, Fig. 2B), but not lean mass (data not shown). Post-hoc tests revealed that *TUB* MUT mice had significantly higher % fat than both WT ($P < 0.001$) and HET ($P < 0.05$) mice. While there was no significant genotype * age group effect ($F_{(3,59)} = 2.385$, $P = 0.078$), a trend was revealed through post-hoc analysis, which showed that *TUB* MUT mice had increased percent body fat

percentage in the newly obese and obese age groups (Fig. 2B) compared to WT mice. In fact, *TUB* MUT mice had more than twice the % body fat of WT animals in the newly obese age group (WT: 13.0 ± 0.8 percent fat versus MUT: 27.7 ± 1.7 percent fat) and 50–60% more fat than either WT or HET animals in the obese age group (WT: 20.8 ± 3.1 percent fat, HET: 17.6 ± 1.2 percent fat, MUT: 32.1 ± 2.0 percent fat)

3.3 Body Temperature

Animals in the food intake study were studied at 5 weeks of age and 20 weeks of age to analyze differences in pre-obese (5 weeks) or obese (20 weeks) *TUB* mutant mice. While there was the expected significant difference in body temperature between the two age groups ($F_{(1,27)} = 68.958$, $P < 0.001$), this difference did not vary with genotype (Fig. 3), indicating that lowered body temperatures do not contribute to the observed obesity phenotype.

3.4 Physical activity behavior

Voluntary activity was measured in animals from 5–10 weeks of age (pre-obese for *TUB* MUT) to prevent any confounding effect of obesity on the ability to perform the wheel running activity. Activity level was significantly different by genotype ($F_{(2,6)} = 19.222$, $P < 0.01$) with pre-obese *TUB* MUT mice running significantly less than HET ($P < 0.01$) or WT ($P < 0.01$) animals in voluntary running wheel activity (Fig. 4A). HET mice show a similar level of voluntary activity compared to WT.

To determine the circadian pattern in wheel running activity, data from the activity experiments were plotted in one-hour time bins over a 24 hour day to analyze the circadian running pattern. Figure 4B shows the average daily activity levels for lights on and lights off periods, while Figure 5 shows average wheel running patterns for Wt (Fig. 5A), HET (Fig. 5B) and MUT (Fig. 5C) mice. Although wheel running activity was lower overall for *TUB* mice, there was no apparent difference in the timing of activity, as all mice performed significantly more wheel running activity during the dark phase (lights off) of the 24-hour cycle compared to the light phase ($F_{(1,6)} = 235.157$, $P < 0.001$). Furthermore, while there was no difference due to genotype in wheel running activity during the light phase (lights on), there was a significant light phase*genotype interaction ($F_{(2,6)} = 15.848$, $P < 0.01$) with *TUB* MUT mice running significantly less than both WT ($P < 0.01$) and HET ($P < 0.01$) mice during the dark phase.

Body weight of the mice was measured during the five week voluntary activity trial. As shown in Figure 4C, there was a significant week * genotype interaction for effect on body weight during the wheel running experiment ($F_{(8,24)} = 7.749$, $P < 0.001$). Post-hoc pairwise comparisons revealed that there were no significant differences in body weight due to genotype at the start of the trial. However, there was a marginally significant difference at 6, 7, and 9 weeks of age with MUT mice weighing marginally more than WT mice ($P = 0.060$, $P = 0.051$, and $P = 0.052$, respectively). Power in this study was low (0.5593) indicating that additional animals in each of these groups may have yielded significant differences at ages other than 8 weeks. Furthermore, at week 8, MUT mice weighed significantly more than WT mice ($P < 0.05$). These data are consistent with the animal's age (9 weeks old) at the end of the trial, which is prior to the age of adult-onset obesity in this line, but show a trend towards increased weight despite access to running wheels.

To test whether reduced voluntary activity was due to a physical condition that affected balance or locomotion, animals were tested on an accelerating Rota-rod apparatus using a forced movement paradigm (Fig. 6A). The effect of genotype was significant ($F_{(2,17)} = 10.812$, $P \leq 0.001$) and there was a significant effect of day of testing ($F_{(3,51)} = 7.124_{15} = 6.240$, $P = 0.001006$) but there was no interaction of genotype with day of testing, indicating that all of the animals showed improvement of the task during the four days of testing. Post hoc analysis

demonstrates that *TUB* mutant animals perform worse than both WT and HET animals in the rota-rod test ($P \leq 0.001$ versus WT and $P = 0.006$ versus MUT). The comparison between WT and MUT showed no significant difference ($P = 0.606$).

Wheel running measurements can be affected in animals with alterations in brain reward systems as wheel running behavior is considered to be a highly motivated behavior [23–25]. A combination of wheel running measurements with home cage activity measurements can determine if physical activity in general or motivated reward-generated activity is altered. Previous work by Wang and colleagues had demonstrated no difference in activity level in 7–8 week old animals [14]. This age is prior to major degenerative retinal changes that could affect activity level. Thus we measured home cage activity using optical sensors to detect beam breaks over three, 24-hour periods in mice aged 16–22 weeks, just prior to the age of maximal retinal degeneration for these mutants. There was no difference between MUT and WT animals on optical beam breaks during any of the days tested (Fig. 6B), indicating that reduced activity in *TUB* mutants was only detectible when spontaneous wheel running behavior was measured.

Discussion

While the Tubby mutant animals were first reported in 1980 and were shown have an adult-onset obesity, this is the first comprehensive analysis of body weight gain, food intake, body temperature, wheel running, and home cage ambulatory activity in these animals. Recently it was reported that Tubby mutant mice have defective carbohydrate utilization with lower oxygen consumption during daily activity [14].

The analysis of food intake in *TUB* mutant and heterozygous mice revealed a significant reduction in daily food intake in these two genotypes compared to WT animals. This was a surprising result, as *TUB* MUT, but not HET become obese. However, the HET mice exercise at normal levels which could prevent weight gain. It is interesting that reduced food intake has never before been reported for these obese animals. This may be due to the use of a mix of WT and HET mice as controls in many studies. In previous reports [1,14], *TUB* MUT animals with adult-onset obesity were considered one grouping, while all other animals, which included both HET and WT mice, were considered the other grouping. As can be seen with our data, *TUB* HET mice show a similar food intake phenotype to *TUB* MUT animals. If HET animals were grouped with WT mice in our reported data, the phenotype of reduced food intake would have been obscured. In the report by Wang and colleagues, mice in the food intake study were grouped as *tub/tub* and $+/?$. The question mark designation indicates that phenotypic determination of genotype were performed, and animals with the designation of $+/?$ could be either of the WT or HET genotype. While Wang et al. saw a slight reduction in food intake, the data did not reach statistical significance [14]. Interestingly, our data suggest that the *TUB* mutation is a dominant allele with respect to food intake, as *TUB* HET mice display this reduced food intake phenotype.

Differences in food intake could be caused by differences in taste perception. In addition to the adult-onset obese phenotype, *TUB* mutants display retinal and cochlear degeneration which ultimately leads to blindness and deafness in this model [4,26–29]. While sensory defects in taste have not been noted in *TUB* MUT animals, it is possible that these animals have an overall deficiency in taste perception or taste bud development. In *TUB* mice, the photoreceptor cells and the organ of Corti undergo premature cell death, similar to humans with Usher's syndrome [28,29]. Taste buds have a rapid turnover rate, with 11% of them being replaced every day and mice with a deletion of the pro-apoptotic *bax* gene have longer-lived and larger buds [30]. Thus it is possible that mutations in genes such as *TUB* could affect longevity of taste receptors. The reduced food intake phenotype in *TUB* MUT mice should be further analyzed with respect to taste perception in these mutants. This is especially important given

the elevated dorsal medial and ventromedial hypothalamic NPY levels in *TUB* mutant mice which should act to increase food intake [9]. Interestingly, in the ARC, both NPY and AGRP levels are reduced which could contribute to reducing food intake in these animals [9,31]. These authors claim that *TUB* mice are hyperphagic, but only measured older and already obese animals.

Reduced food intake in light of ensuing obesity suggests two things: First, energy expenditure must be altered in these animals for body weight gain to occur, and second, that the energy balance systems in *TUB* animals may be attempting to compensate for the increased body weight by reducing food intake. In the results presented here, food intake was measured three times weekly for 16 weeks (aged 5 weeks to 20 weeks). Breaking down the analysis to look at food intake in just the young pre-obese animals still reveals a significant reduction in food intake in the *TUB* MUT animals, although the reduction in HET animals is marginal (data not shown). This could suggest that the rewarding or motivating aspect of food [32] was not detected by the mutant animals. Interestingly, *TUB* MUT mice increase their food intake when given access to a running wheel to compensate for the increased expenditure of the activity, with no significant difference between daily food intake between the three genotypes but an overall average increase in food intake of 0.45 grams (118%) per day in animals with wheels compared to those in hanging wire cages (data not shown). These data support motivated food reward as a possible defect in the *TUB* MUT animals, as normal, required increases in food intake can and do occur in the mutant mice to compensate for increased energy expenditure.

Despite reduced food intake, *TUB* MUT animals showed increased body weight and body fat beginning around 11 weeks of age. As indicated above, these data suggest that some aspect of energy expenditure or energy utilization by the body is altered resulting in an excess of available energy relative to what is needed. In testing measures of energy expenditure in these animals, we found that body temperature was not reduced in MUT compared to either WT or HET animals at any age, although there was a significant decrease in body temperature at older ages in all genotypes. Body temperature is one way to measure energy expenditure by animals. Another method is to assess oxygen consumption, as was done by Wang et al. [14]. *TUB* MUT mice have lower oxygen consumption. Combined with the lower CO₂ production in the MUT, respiratory quotient, which is the ratio of CO₂ production to oxygen consumption, was also lower. Further studies by this group indicate that defective carbohydrate metabolism in *TUB* MUT mice results in effects on several metabolic pathways [14]. These changes could account for increased body weight in *TUB* MUT mice and warrant further investigation.

We have shown that spontaneous physical activity in a wheel running task is reduced in *TUB* MUT mice, and that the phenotype was only prevalent during dark phase high activity levels. Total physical activity can contribute as much as 12.5% of total energy expenditure in animals, and animals with genetic obesity often show reduced activity levels [33,34]. Interestingly for *TUB* MUT mice, home cage ambulatory activity was not different compared to WT animals, and the ability to run on a Rota-Rod, which measures balance and running ability was slightly, although not significantly reduced in these mutants. These were important measurements to do in *TUB* MUT mice given their phenotype of retinal degeneration beginning around 1 month of age with maximal degeneration by 6 months of age [28,29]. Reduced wheel running activity in *TUB* MUT mice, accompanied by a significant reduction in Rota-Rod activity, but with normal home cage ambulatory activity implies several possible mechanisms. First, retinal degeneration in these mutants could interfere with their ability to use the running wheel and/or Rota-Rod. While this seems plausible at first, a study by McFadyen and colleagues using C3H/HeJ mice with retinal degeneration showed that the sight-impaired animals were better at the Rota-Rod task than animals with normal eyesight [35]. In addition, animals blinded at birth or housed in complete darkness have no deficit in wheel running, indicating that good visual sense is not required for normal wheel running [25]. A second

possibility is that known degeneration of other sensory neurons, most notably progressive deterioration of inner ear sensory cells could impair Rota-Rod activity. Unfortunately, there has been little published on inner ear degeneration and Rota-Rod activity. One study used the mouse model of the human condition, Mucopolysaccharidosis (MPS) IIIB (Sanfilippo Syndrome type B). This condition is caused by a deficiency in the lysosomal enzyme N-acetylglucosaminidase (Naglu) and leads to sensory neuron loss due to lysosomal inclusions. The mouse model of MPS displays reduced nighttime motor activity but no difference in behavior on a standard accelerating Rota-Rod apparatus in either young or old mice. The authors were only able to show impairment when they used a rocking Rota-Rod testing method which employed a shift in the direction of the rod with each turn [36]. The mouse model of MPS leads one to postulate that the sensory defects alone would not impair behavior on a standard accelerating Rota-Rod apparatus. A third possibility is that *TUB* MUT mice have reduced *motivated* running behavior with normal ambulatory behavior. Wheel running is self-reinforcing and is even subject to a so-called “rebound” effect following deprivation, suggesting that the motivation to run on a wheel (unlike home cage ambulatory behavior) is strong [25]. In animals, serotonin has been shown to play an important role in initiating movement. Treatment of animals with m-chlorophenylpiperazine (mCPP), a serotonin receptor agonist, leads to hypoactivity in normal mice [37], while mice with a targeted deletion of the 5-HT_{2c} serotonin receptor display hyperactivity following treatment with mCPP [38]. Tubby protein has been shown to interact with G-coupled receptors, including the 5-HT_{2c} receptor, where tubby is sequestered in the cytoplasm until released following ligand binding [6]. 5-HT_{2c} knockout mice display hyperactivity [39], which could be the result of overactivated tubby protein in these animals. It is possible that tubby protein is involved in the motivated response to serotonin. In *TUB* MUT mice, lack of tubby protein may lead to lack of positive reinforcement and motivation for running, leading to reduced overall running wheel and Rota-Rod activity behavior.

Overall, food intake and voluntary wheel running, two behaviors associated with motivation and reward mechanisms, are reduced in *Tub* MUT mice. More work in specifically studying motivated behavior in *Tub* MUT mice, such as experiments to quantify motivation to perform wheel running [25] or involvement of dopamine for food reward [32,40,41] could define whether the *TUB* mutation leads to a motivational behavior defect phenotype. These information could provide interesting correlates to human obesity, where sedentary lifestyles are one culprit in the obesity epidemic [42]. Although a recent cohort of individuals was found to have a mutation in *TUB* no assessment of their level of physical activity was made [12]. Other studies focusing on genetic contributions to physical activity levels in humans did not use rare individuals with *TUB* mutations as these had not yet been identified when the study was conducted [43]. In light of our findings, translational work on humans with *TUB* polymorphisms should examine relative activity levels and general metabolic rate to assess if humans with *TUB* mutations display a similar phenotype to the mouse model of the disease.

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Fig. 1.

Food intake in WT, HET and *TUB* MUT mice. Food intake on standard rodent chow was measured for 20 weeks. Data is reported as the average daily intake $\pm$ SEM in grams for each genotype. ** $P \leq 0.01$, * $P \leq 0.05$ compared to WT.

Fig. 2. Body weight and percent fat in WT, HET and *TUB* MUT mice. A. Animals in the food intake study were used for the body weight analysis. Animals were weighed once weekly and the data is reported as the mean $\pm$ SEM in grams for each genotype. B. Mean percent carcass fat $\pm$ SEM for each genotype is reported for three age groups. Note that no HET animals were available for age group 2. ** $P \leq 0.01$, * $P \leq 0.05$.

Fig. 3. Body temperature in WT, HET and *TUB* MUT mice. Two age groups were used for each genotype. Body temperature is reported as mean °C $\pm$ SEM. ** $P \leq 0.01$.

Fig. 4. Activity, body weight and food intake for animals given access to a voluntary wheel apparatus. A. Average total daily wheel revolutions $\pm$ SEM were reported for mice during the 5 week collecting period. B. Data from graph A was separated into lights on (6 AM – 5:50 PM) and lights off periods (6 PM – 5:50 AM) and reported as above. C. Body weight at each week of the activity testing is reported as mean grams $\pm$ SEM. $**P \leq 0.01$.

Fig. 5.

Circadian rhythm analysis in mice given access to a voluntary wheel apparatus. Average wheel rotations for 20 minute bins were averages for WT (A), HET (B) and *TUB* MUT (C) mice and plotted. Open bars on x-axis indicate light phase, while dark bars indicate dark phase. Standard error bars were left off this figure for simplicity, but are similar in magnitude to those in Figure 4.

Fig. 6. Rota-Rod and home cage ambulatory activity for WT, HET and *TUB* MUT mice. A. Animals were tested four times per day for four days on a Rota-Rod. The mean length of time performing the task (in seconds) $\pm$ SEM is reported. B. Total optical beam breaks $\pm$ SEM is reported for each genotype. *** $P \leq 0.001$