Are TallyHo Mice A True Mouse Model for Type 2 Diabetes and Alzheimer’s Disease?

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Abstract. The purpose of our article is to critically assess if TallyHo mice are a true mouse model for type 2 diabetes and Alzheimer’s disease. Diabetes is a lifestyle condition that is characterized by elevated blood glucose due to either insufficient amount of insulin or the body’s inability to use the produced insulin efficiently. Diabetes occurs in multiple forms, including type 1, type 2, type 3, neonatal, and gestational. Type 2 diabetes covers 95% of overall diabetes, found in individuals 65 years of age and above. Both modifiable and non-modifiable factors are involved in developing diabetes. In patients with diabetes, increased blood glucose levels are reported to induce multiple complications, such as heart disease, stroke, kidney failure, foot ulcers, and damage to the eyes. However, the molecular basis of diabetes is not completely understood. Further, there are no accurate animal model(s) that mimic both type 1 and type 2 diabetes of humans. Multiple polygenic models are being used, including the Goto-Kakizaki rat, the Otzhka Long-Evans Tokushima Fatty rat, the Nagoya Shibata Yasuda mouse, the New Zealand obese mouse, the Tsumura-Suzuki obese diabetes mouse, leptin deficient ob/ob and the leptin receptor deficient db/db mouse models. In 2001, Kim and colleagues described the TallyHo mice that represent many features of type 2 diabetes of humans. Since then, several groups studied TallyHo mice. Only the male mice develop hyperglycemia and the females exhibit features of obesity. Thus, this model can be used to study both diabetes and obesity. The purpose of this article is to discuss recent developments in TallyHo mice research including diabetes onset and progression.

Keywords: Alzheimer’s disease, diabetes, gestational diabetes, modifiable factors, obesity, TallyHo mice

INTRODUCTION

Diabetes is a metabolic disorder characterized by hyperglycemia due to insufficient production of insulin or the inability of the body to use the available insulin efficiently that affects several millions of people worldwide. According to the U.S. Centers for Disease Control and Prevention, more than 30
million Americans have diabetes and of these, type 2 diabetes (T2D) or non-insulin dependent diabetes mellitus (NIDDM) accounts for 90 to 95% of these cases. There is a major health care burden due to severe complications arising from the disease resulting in greater risk for mortality and morbidity. The detection of T2D is a challenge for clinicians, because of the lack of early diagnostic symptoms and features.

There are many factors associated with the onset of diabetes, some of which are modifiable and some which are not. Modifiable factors include diet, exercise, smoking, excessive alcohol, and insufficient sleep. Non-modifiable factors include sex, age, and ethnicity, and changes in genomes are major non-modifiable factors. Further, individuals with inherited DNA changes in the genome are susceptible to diabetes. In addition, ethnicity plays a large role in development of pre-diabetes. Some ethnic groups such as Africans, Alaskan Natives, American Indians, Asians, Latinos, and individuals of Pacific Islander descent have high risk of developing T2D [1].

The molecular basis and cellular changes involved in disease progression of T2D are not completely understood. Unlike Alzheimer’s disease (AD), currently, there are no rodent models that precisely mimic cellular changes in progression and pathogenesis of diabetes. However, there are some rodent models available for the study of both T1D and T2D. Polygenic models representative of NIDDM in rodents include the Goto-Kakizaki (GK) rat, the Otzhka Long -Evans Tokushima Fatty (OLETF) rat, the Nagoya Shibata Yasuda mouse, the New Zealand obese mouse and the Tsumura-Suzuki obese diabetes mouse, leptin deficient ob/ob and the leptin receptor deficient db/db mouse models [2–4]. Kim et al., 2001 first reported the TallyHo (hereafter abbreviated as TH) mouse model which is the fourth model that is similar to human NIDDM syndrome [5]. The purpose of this review is to discuss the use of this novel TH model in T2D research.

The TH mouse strain is a polygenic model established in 2001 for T2D with obesity. These mice exhibit insulin resistance, hyperinsulinemia, hyperglycemia, obesity, and dyslipidemia associated with increased triglyceride, free fatty acid, and HDL cholesterol levels. The female mice are not hyperglycemic although they are obese. Except for the high plasma HDL cholesterol levels which is a characteristic of the obese mouse models, all other abnormalities are commonly found in human NIDDM. This model was derived from two male mice showing polyuria and glucosuria that were identified as deviants in a colony of Theiler original mice in 1992. These developed late onset hyperglycemia at approximately 26 weeks of age. An inbred strain named TH was then established by selective breeding based on the hyperglycemia phenotype of this original deviant stock [5, 6].

**GENETIC FACTORS ASSOCIATED WITH DIABETES IN TH MICE**

Quantitative Trait Locus (QTL) mapping analysis has been used as a powerful tool to study the genetic factors associated with diabetes in TH mice. Details of QTL are given in Table 1.

**Hyperglycemia**

Kim et al. [5] identified the location of a major QTL Tanidd1 that mapped to Chromosome 19. Additional independent QTL was detected on Chromosome (Chr) 13 which was designated Tanidd 2. They also found gene-gene interactions between Tanidd1 and a locus on Chr 18 and Tanidd2 and a locus on Chr 16. These also contributed to hyperglycemia in TH mice 5.

**Obesity**

Kim et al. [7] mapped a major QTL related to obesity tabw2 on Chr6 in TH mice and confirmed on the congenic B6 background.

**Triglyceride**

Stewart et al. [8] identified four significant QTLs for plasma triglyceride level. They found hypertriglyceridemia from TH genome. Chr 11 and Chr 8 QTLs were dominant while Chr 1 and Chr 4 were recessive.

**Cholesterol**

A significant QTL on Chr1 was linked to plasma total cholesterol levels. The hypercholesterolemia was identified from TH genome. Another QTL on Chr3, also responsible for increased cholesterol, was found to be recessive.

Parkman et al. [9] generated congenic mouse strains that carry the Chr 1 QTLs derived from TH on a B6 background, B6.TH-Chr1-128Mb (128 Mb in size) and B6.TH-Chr1-92Mb. They characterized these congenic mice on chow and high fat diets. They
Table 1
Summarizes the QTLs identified so far for the genetic factors along with the markers in TH mice

<table>
<thead>
<tr>
<th>Genetic Factor</th>
<th>Locus</th>
<th>Chromosome</th>
<th>Closest Marker</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperglycemia</td>
<td>Tanidd1</td>
<td>19</td>
<td>D19Mit103</td>
<td>Kim et al. 2001 [5]</td>
</tr>
<tr>
<td></td>
<td>Tanidd2</td>
<td>13</td>
<td>D13Mit148</td>
<td></td>
</tr>
<tr>
<td>Body Weight</td>
<td>Tabw</td>
<td>7</td>
<td>D7Mit231</td>
<td></td>
</tr>
<tr>
<td>Fat Pad</td>
<td>Tafat</td>
<td>4</td>
<td>D7Mit312</td>
<td></td>
</tr>
<tr>
<td>CAST Cross</td>
<td>Tanidd1</td>
<td>19</td>
<td>D19Mit108</td>
<td>Kim et al. 2001 [5]</td>
</tr>
<tr>
<td></td>
<td>Tanidd3</td>
<td>16</td>
<td>D16Mit129</td>
<td></td>
</tr>
<tr>
<td>Body Weight</td>
<td>Tabw</td>
<td>7</td>
<td>D7Mit191</td>
<td></td>
</tr>
<tr>
<td>Dietary Obesity</td>
<td>Tabw2</td>
<td>6</td>
<td>D6Mit102</td>
<td>Kim et al. 2005 [7]</td>
</tr>
<tr>
<td>Triglyceride</td>
<td></td>
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<td>Stewart et al. 2010 [8]</td>
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<td>Cholesterol</td>
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<tr>
<td>Glucose</td>
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<td>Stewart et al. 2010 [8]</td>
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<td>Body Weight</td>
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<tr>
<td>Carcass Weight</td>
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found that B6.TH-Chr1-128Mb exhibited increased plasma cholesterol levels compared to B6.TH-Chr1-92Mb and B6 control mice. Thus, Chr 1 QTLs largely conferred obesity and hypercholesterolemia in TH mice.

Glucose

A QTL near distal Chr 4 was found to be associated with plasma glucose levels. The TH alleles were associated with elevated glucose levels at this locus and the inheritance was found to be dominant (Table 1).

Insulin

No significant changes were found for insulin levels in plasma.

Body weight

Two significant QTLs linked to body weight mapped to Chr11 and Chr1. TH alleles contributed to increased body weights and this was dominant (Table 1).

Fat pad weight

A QTL near Chr1 was linked to fat pad weight. The TH allele was associated with increased fat pad weights. The allele was dominant for all except epididymal fat pad weights (Table 1).

Carcass weights

Two QTLs on Chr11 and Chr14 were linked to carcass weights. The TH alleles and also the wild type (WT) B6 mice alleles were associated with higher carcass weights at these loci (Table 1).

PHENOTYPIC CHARACTERISTICS OF TH MICE

Brown et al. [10] showed that TH mice have significantly lower gene expression of pancreatic lipase related protein 1. These lipases are known to hydrolyze triglycerides to free fatty acids in the small intestines. Further research is still needed to understand the molecular insights between low levels of lipases and conversion of triglycerides to free fatty acids in the small intestines.
Sung et al. [11] examined the pathophysiology of diabetes mellitus in TH mice at a prediabetic age of 4 weeks. They found glucose intolerance with reduced glucose stimulated insulin secretion. There was an increase in the plasma leptin concentration, adipocytic expression of leptin, and pancreatic expression of leptin receptor in the TH mice. Their results indicated that increased plasma leptin concentrations might be a primary factor for the impaired insulin secretion in the TH mice. They did not observe hyperinsulinemia at a young prediabetic age [11].

Kim et al. [12] conducted an extensive phenotypic characterization of the TH mice with a goal to provide new insights into molecular pathogenesis of T2D in this mouse model. No major organ damage was evident at the 8-week time-point. The plasma pancreatic lipase activity was significantly lower in TH mice compared to the B6 controls. They also found plasma thyroid hormone (T4) levels to be lower in TH mice compared to the controls. Both male and female TH mice showed an increase in body weights and plasma insulin levels, at weaning age besides being hyperleptinemic and hyperinsulinemic [12]. Thus, the primary features in TH mice include obesity and reduced sensitivity to insulin action. In TH males, islet degeneration was also observed histologically, as the disease progressed. One of the precursors of T2D is glucose intolerance. The male TH mice at 8 weeks were found to demonstrate glucose intolerance and Intraperitoneal Glucose Tolerance Test revealed increased insulin secretion, characteristic of the disease progression. However, this increased insulin secretion was not profound at 16 weeks in these mice, when glucose intolerance was severe. In addition, they found that soleus muscle exhibited lower basal and insulin-stimulated 2-DG uptake compared to the B6 controls, even at a young age of 6 weeks. Based on these observations, insulin resistance may be one of the primary defects of TH mice [12].

Unlike the observations made by Sung et al. [11], Kim et al. [12] did not observe any insulin secretion defects in TH mice at 4 weeks of age, despite their hyperleptinemia. This study also has made important observations such as obesity, hyperinsulinemia, and hypertriglyceridemia in TH mice compared to B6 control mice at young age [12]. Interestingly, similar to the study by Sung et al. [11], preliminary studies conducted in our laboratory do not show hyperinsulinemia at the weaning age of 5 weeks, while we did see increase in the blood glucose levels. The insulin levels were undetectable at the sensitivity of the ELISA kit used. But the insulin was detected around 8 weeks of age. Hyperglycemia was detected with low levels of insulin and hyperinsulinemia was detected in animals that were not hyperglycemic [data unpublished].

**Kidney phenotype**

Nakamura [13] studied the kidney phenotype in TH mice. Even at a prediabetic age, the kidney weight/10 g body weight was found to be increased, Arginine vasopressin receptor 2 (AVPR2) protein expression level was reduced, and kidney aldehyde dehydrogenase (ALDH) activity was decreased, suggesting that the kidney phenotype occurred before the onset of T2D. Interestingly, this phenotype was found only in male TH mice, similar to hyperglycemia [13].

**Insulin resistance**

A characteristic of T2D is accelerated muscle loss during aging, decreased muscle function, and increase in disability as a result of progression of the disease to a pathological state called sarcopenia [14–17]. The Streptozotocin-induced type 1 diabetic (T1D) mouse model is one of the best understood models for muscle atrophy. In this model, increased muscle proteasomal protein degradation was observed as a result of pharmacologically induced protein degradation [18]. However, this is a T1D model which accounts for only 5–10% of diabetes cases. Several different models exist to study muscle atrophy in T2D. But these models show lot of variations in relation to muscle size, Insulin receptor substrate IRS-1/IRS-2 knockout, and muscle insulin receptor knockout. Most studies of T2D muscle atrophy have focused on the leptin deficient ob/ob and the leptin receptor deficient db/db mouse models.

Ostler et al. [19] studied the muscle weight, exercise capacity, and biochemistry in the well-established db/db mice and in the TH model at both prediabetic and matured diabetic ages. The TH model was chosen because it is a polygenic model of T2D with intact leptin signaling. These mice develop diabetes at a mature age. This allows to investigate the impact of insulin resistance and hyperglycemia on mature muscle, which is more clinically relevant rather than investigating the juvenile onset of
diabetes. Moreover, it was found that while the \( \text{db/db} \) model already showed reduced skeletal muscle size and decreased running ability at 5 weeks of age, before the onset of hyperglycemia, the TH model did not show any indication of muscle loss. The muscle weight increased with age and the exercise capacity was maintained at both prediabetic, 8-week-old and overtly diabetic, more than 6 months of age [19].

An assessment of the muscle weight and histology in \( \text{db/db} \) mice revealed reduction in hind limb muscle size at 6 to 12 weeks. No increase in centrally located nuclei suggesting muscle injury was evident [19]. Wet muscle mass was decreased in predominantly fast-type skeletal muscle from prediabetic 6-week-old mice and more so in overtly diabetic 12-week-old mice. No significant change was seen in tibia length, cardiac weight, or soleus weight in these mice, compared to their WT controls. Average muscle fiber cross-sectional area was found to be reduced in fast-type muscle (tibialis anterior) but not in slow oxidative muscle (soleus). On the other hand, TH skeletal and cardiac muscle weight and tibia length increased from 9 weeks of age to 24 weeks of age despite glucose intolerance and hyperglycemia. Average muscle weight and fiber cross-sectional area of both predominantly slow muscle (soleus) and fast muscle (tibialis anterior) were increased compared to B6 WT controls [19].

The TH mice demonstrated reduced serum corticosterone levels compared to both control and \( \text{db/db} \) mice. A corresponding decrease was seen in the serum levels for C5a, IL-4, CXCL-9, and TNF-\( \alpha \). TH mice had a significant reduction in all the 39 inflammatory cytokine markers compared to the \( \text{db/db} \) and control WT mice [19].

Fluitt et al. [20] characterized the physiologic response of mice with pre-existing insulin resistance to infused insulin in an attempt to study the factors related to BP control. They infused TALLYHO/Jng (TH) mice with insulin (50 U/kg bw/d) for two weeks. They found TH mice had modestly higher (10–15 mm Hg) basal BP. Heart rate and pulse pressure (a measure of vascular compliance) were also higher in the TH, and other classic signs indicative of cardiovascular and endothelial dysfunction were also seen. It is interesting to note that many of these features are observed in humans with metabolic syndrome. After insulin infusion for two weeks, they did not find any major effect on blood pressure or heart rate. However, they also observed several changes in the kidney and blood chemistry [20].

### MOLECULAR MECHANISMS FOR REDUCED GLUCOSE UPTAKE AND INSULIN RESISTANCE

One of the characteristic phenotypes of T2D is insulin resistance. During normal glucose homeostasis, insulin suppresses hepatic glucose production and increases glucose uptake in muscle and adipose tissues. Glucose transporter 4 (GLUT4) is the major insulin responsive transporter. When insulin binds to its receptor, it initiates a cascade of events resulting in translocation of GLUT4 from an intracellular compartment and insertion into the plasma membrane [21]. In T2D, insulin resistance causes diminishing of the insulin triggered glucose uptake resulting in decreased GLUT4 expression [22].

Wang et al. [21] examined GLUT4 protein levels, translocation, and localization in adipose tissue, as well as components of the insulin signaling pathway. They found that GLUT4 translocation was dysregulated in TH mice as in other models, previously reported. In addition, they found a defect in phosphoinositide (PI) 3-kinase activation and low insulin receptor substrate 1 (IRS1) levels. Thus, in this study, IRS1 degradation played a role in insulin resistance in TH mice [21].

**Molecular mechanisms in bladder dysfunction**

T2D is well known to cause urological complications such as diabetic nephropathy and bladder dysfunction [28]. Tomechko et al. studied the molecular mechanisms involved in these urological complications using the TH mice model and its control SWR/J mice. Their study indicated that diabetes induced extensive changes in the detrusor muscle and urothelium. In the detrusor muscle, diabetes was found to affect pathways associated with muscle contraction such as actin cytoskeleton and Rho family GTPase signaling pathways. Altered oxidative stress response was observed in both detrusor muscle and in the urothelium. Their study also indicated that in the urothelium, the diabetes associated phosphorylation was significantly decreased as compared to the detrusor muscle [29].

**Metabolism of bile acids in TH mice**

Since the TH mice exhibit hypercholesterolemia at an early age before they become hyperglycemic...
[5], Lee et al. hypothesized that the increased plasma cholesterol should be related to bile acids (BAs) metabolism because cholesterol is the precursor of BAs. They analyzed the BAs pool size, BAs composition, and expression levels of several proteins that have key roles in BAs synthesis, excretion, and reabsorption in TH mice. They found that the TH mice exhibited an increased total BAs pool size with a high expression level for the cholesterol 7 alphahydroxylase (Cyp7a1) gene resulting from the disrupted feedback inhibition of the Fxr/Fgf15/Fgfr4 pathway. In TH mice, the increase of the BAs pool size promoted biliary cholesterol secretion (Abcg5), suppressed intestinal BAs absorption (Asbt and Ost), and increased the hepatic absorption of BAs (Ntcp). Thus, this study suggested that induction of Cyp7a1 expression by hypercholesterolemia might be a main cause of the increased BAs pool size in TH mice [30].

**Leptin as an obesity factor in TH mice**

Obesity is considered as a major health concern as it is associated with T2D, cardiovascular diseases, and various other chronic diseases [23]. Leptin is a hormone secreted by the white adipose tissue and its expression increases during adipogenesis. Zhang et al. initially cloned a mutant gene responsible for extreme obesity in the ob/ob mice and found that it encoded for leptin [27]. Hence, the levels of leptin in plasma have been studied as a marker of obesity. Sung et al. [11] studied the diabetic pathophysiology of TH mice at pre-diabetic age. They found that the mice exhibited impaired glucose tolerance and reduced glucose-stimulated insulin secretion and increased plasma leptin concentration. They treated the mice intravenously with anti-leptin antibody and found that the plasma insulin levels increased with a decrease in the plasma glucose concentration. Chronic treatment of mice with leptin potentially inhibited the glucose-stimulated insulin secretion in islets of TH mice. Expression of insulin secretion related genes was similarly regulated in whole pancreas and leptin treated islets of TH mice. Their findings suggested that increased plasma leptin concentrations might be a primary factor for the impaired insulin secretion in the TH mice. As a result, the young mice demonstrated low plasma insulin concentration. This possibly may be a factor for diabetes development later in the adult TH mice as decreased insulin secretion is associated with the risk of T2D. Studies conducted in transgenic mice overexpressing leptin have indicated less body weights, lower plasma insulin concentrations, and lower food intake rates than their WT controls [24, 25]. However, the findings in TH mice indicate that increased leptin also increases obesity [11].

Rhee et al. [26] studied the developmental mechanism of obesity in TH mice in comparison to the B6 control animals. The obesity was observed at 4 weeks in TH mice with increase in food intake. However, no change was observed in energy expenditure factors such as body temperature, cold-induced thermogenesis, oxygen consumption rate, and spontaneous locomotor activity between the two groups of animals. Hence, the obesity was not a result of decreased energy expenditure [26]. They also found both fasting and nonfasting plasma leptin levels increased in TH mice compared to the B6 WT controls, similar to the observations made by Sung et al. [11]. Thus, the obesity of TH mice seems to be mainly induced by increased food intake rate, resulting from hypothalamic leptin resistance.

Kim et al. [31] used primary preadipocytes prepared from the subcutaneous fat of TH mice to study the effect of leptin on adipocyte differentiation. They used rosiglitazone to induce adipocyte differentiation with increased peroxisome proliferator-activated receptor γ (PPARγ), which is a key adipogenic transcription factor. They found that the expression of leptin was elevated seven times in TH mice compared to Ob mice in their mRNA level and three times in their protein level similar to previous findings by Sung and colleagues [11]. Kim and colleagues found that leptin has an inhibitory effect on the rosiglitazone-induced adipogenesis in primary adipocytes isolated from the subcutaneous fat of TH mice [31].

Louden et al. [32] used the TH mice model which is a genetic mouse model instead of using a diet induced obesity model to demonstrate that maternal obesity, characterized by insulin resistance and elevated fatty acids, results in an abnormal reproductive phenotype, specifically a smaller litter size. They also determined that blastocyst stage embryos from these mice experienced decreased insulin-stimulated glucose uptake and decreased fatty acid oxidation leading to increased lipid droplet accumulation at this stage of embryo development. This study indicated that the reduced litter size could be due to the combination of decreased insulin stimulated glucose uptake and abnormal fatty acid oxidation resulting in increased blastocyst apoptosis [32].
Vascular dysfunction in TH mice

Vascular complications have been reported in diabetes patients. Previous studies have been conducted in Ob/Ob and db/db mice to study the vascular complications in T2D. However, compared to these animal models, the TH model represents a polygenic model of human T2D and is more representative of the polygenic nature of T2D in humans. Dijon et al. [33] studied the vascular responses in TH mice. They examined whether superoxide played any role in alterations of vascular function in this model. They found that endothelium-dependent relaxation is impaired in carotid arteries and cerebral microvessels in TH mice [33].

Cheng et al. [34] examined the contribution of endothelium-derived contractile factors in endothelial dysfunction in TH mice. They found that blood glucose and serum lipid profiles were increased in TH mice. The aortae of TH mice demonstrated a significant increase in superoxide generation. Endothelium-dependent relaxation was impaired while endothelium-dependent contraction to acetylcholine was enhanced in the aortae of TH mice [34]. Interestingly, increased superoxide generation in diabetes has been suggested to be related to hyperglycemia, obesity, hyperlipidemia, and hyperinsulinemia [35–38].

Perivascular vascular tissue has been recognized as a regulator of vascular function because of its release of adipocyte-derived relaxing factors (ADRFs) that diminish the contractile actions of vasoconstrictors [39–44]. Li et al. [45] evaluated ADRF release in the TH mice because these strains exhibited an obese insulin-resistant phenotype along with hyperglycemia [5] and endothelial-vascular dysfunction [34]. Their study showed that the obese hyperglycemic phenotype of the TH mice is not directly linked to an ADRF-related mechanism [45].

Bone density

T2D has been associated with osteoporosis because of insulin resistance and hyperglycemia in these patients. Won et al. [46] studied the molecular links between T2D and osteoporosis using the TH mouse model. They observed that TH mice developed severe osteoporosis with male predominance. The bone mineral density was decreased in these mice while there was an increase in osteoclastogenic factors such as IFN-γ, IL-6, and RANKL that are markers of bone density loss, in the blood and bone marrow. They also found that the TH mice showed a decrease in bone hormone osteocalcin (OCN), a hormone that induces insulin production and sensitivity [47]. Thus, OCN deficiency mediates insulin resistance as seen in T2D. Previous studies have indicated that decreased bone density and increased fracture risk are closely associated with diabetes [48, 49]. Hence, bone homeostasis is affected by insulin insufficiency or resistance under diabetic conditions [41].

Devlin et al. [50] studied the effect of early onset T2D on body composition and bone properties in young TH male mice from 4 weeks of age to 8 and 17 weeks in comparison to the control SWR/J mice. They found that the TH mice had a severe trabecular bone deficit and lower cortical porosity in the distal femur with thicker cortices at 8 weeks of age, compared to the control mice. However, this pattern deviated from adult humans in whom high cortical porosity has been usually seen in individuals with T2D and a prior history of fracture. TH also exhibited lower whole-body bone mineral density from 4 to 17 weeks, suggesting impaired bone mass acquisition. These mice also exhibited increased cortical bone fraction and thickness at both 8 and 17 weeks. Their study suggested that defect in TH bone formation is cell-independent and likely due to diabetes metabolism. Hence, TH mice is an important model to understand the effect of T2D on bone cells [50].

In an attempt to determine whether TH mice can be a model of diabetic bone disease, representative of the disease in humans, Creecy et al. [51] analyzed bones from both TH and SWR/J mice at 16 weeks and 34 weeks of age. This study found that the bone fracture resistance from TH mice did not progressively worsen with the duration of T2D. In these mice, the low cortical bone toughness continued to exist at skeletal maturity (16 weeks) and in adulthood (34 weeks). However, the cortical porosity and trabecular number and thickness in long bones was lower in the TH mice, while diabetic humans can have elevated cortical porosity with otherwise normal trabecular architecture. Advanced glycation end-products did not accumulate in the bone of TH mice with diabetic progression as seen in diabetic humans. According to this study, the variability in hyperglycemia in the TH model could be the reason why the fracture resistance in this model did not decrease with increasing duration of hyperglycemia [51].
Response of TH mice to hypoxia

Hong et al. [52] established an in vitro culture model of mature adipocytes from both db/db mice and TH mice by enclosing them in a hyaluronan-based hydrogel to study their role in response to stress such as hypoxia. T2D is a high risk factor for wound infections. Adipocytes are important parts of injured tissue subject to hypoxia which is insufficient to cause full necrosis. Therefore, understanding the proliferation and differentiation of adipocytes is crucial for the mechanism to study wound repair. They showed that WT mice responded to hypoxia. In contrast, mature adipocytes of diabetic db/db and TH mice did not efficiently respond to hypoxia. Thus, their study suggested that mature adipocytes are functionally active cells, and their abnormal function to hypoxia can be one of underlining mechanisms in T2D [52].

Sherwani et al. [53] studied the effects of intermittent hypoxia (IH) on pancreatic function in the presence of diabetes. They examined the effects of IH on glucose tolerance in TH mice. Their study revealed that IH exposure worsened glucose tolerance in the male TH mice. This was associated with impaired pancreatic β-cell function as shown by the reduction in insulin, impaired insulin secretion, and increased apoptosis in pancreatic islet cells. There was an increase in the non-esterified fatty acids both in circulation and pancreatic tissue which resulted in β-cell dysfunction due to a shift in the composition of fatty acids toward long-chain saturated fatty acid in pancreatic tissue. Thus, this study revealed that IH exacerbated pancreatic dysfunction in TH mice [53].

TALLYHO MICE, TYPE 2 DIABETES, AND ALZHEIMER’S DISEASE

As described above, TallyHo male mice are a good model to understand several aspects of T2D, including hyperglycemia, obesity, cholesterol, kidney phenotype, and insulin resistance. There are no published studies available on oxidative stress, mitochondrial function/dysfunction, mitochondrial biogenesis, mitochondrial dynamics, and synaptic damage in the brain and peripheral tissues in TallyHo mice. Further, there are no published time-course studies that describe how diabetes can be progressed to AD features such as the formation of amyloid-β and phosphorylated tau similar to humans. Careful time-course investigations of brain tissue and peripheral tissues will provide new information about molecular links between diabetes and AD.

Diabetic periodontitis

Periodontitis is one of the most common lytic disease of bone and both T1D and T2D are high-risk factors for this disease. The disease is characterized by loss of supporting structure for the tooth consisting of connective tissue attachment and bone. The disease is caused by bacteria capable of initiating an inflammatory response and thereby resulting in destruction of tissue [54]. Li et al. [55] induced Porphyromonas gingivalis infection in TH mice to investigate the influence of periodontitis in T2D. They infected the mice before they developed diabetes. Their study indicated that while diabetes increased the risk for periodontal disease induced by P. gingivalis, there seemed to be no significant difference in the mice with periodontal and the control group [55]. Periodontal infection did not seem to influence the onset of diabetes in this study even though previous studies had showed that periodontal infection may enhance the onset of severe insulin resistance and impaired glucose homeostasis in a high-fat food induced diabetes rat model [56].

Wang et al. [57] compared the extent of periodontitis in two different mice models for T2D. They used db/db mice from the start of their experiment, the TH mice developed hyperglycemia around 8 weeks. The db/db mice exhibited more alveolar bone loss than those of the TH mice. The TH mice exhibited mild periodontitis [57]. This is similar to the findings from the study conducted by Li et al. [55]. Thus, hyperglycemia may be a factor responsible for extensive periodontitis.

Chronic wound infections

Chronic wound infections are one of the severe complications of diabetes that pose a financial and health burden. These are commonly known as diabetic foot ulcers and they exhibit difficulties in healing. They do not respond to antibiotic therapy and often result in surgical intervention. Wound infections involve colonization by many different bacteria with Staphylococcus aureus being the most common. These bacteria survive in the wound infections and become resistant to therapy, through formation of biofilms. Nguyen et al. [58] studied the host responses in biofilm impaired wounds using the TH mice. They found that diabetic biofilm-containing wounds
had significantly less TLR 2, TLR 4, interleukin-1β, and tumor necrosis factor-α expression compared to the control group. Diabetic wounds also had less neutrophil oxidative burst activity. Thus, this study suggested that impaired recognition of bacterial infection via the TLR pathway may be a potential mechanism underlying diabetic susceptibility to wound infection [58].

Wagner and colleagues [59] studied the effect of obesity on wound healing in the TH female mice model, which is a non-diabetic obese model in comparison to the SWR/J control mice which are non-diabetic and non-obese. They created 6 mm stented wounds on these mice to mimic wound infections in human beings. They quantified the peripheral blood mouse (PC) and analyzed the wounds. Their results suggested that obesity impairs BM-derived vasculogenic PC response to peripheral injury and this, in turn, impaired the wound closure [59].

Buck et al. [60] investigated wound healing impairments in the TH mice model using three different validated wound healing models: an incisional model, a splinted excisional model, and a cutaneous ischemia-reperfusion injury model. They observed wound healing deficits in all the three models, using the TH mice model. Although, the monogenic db/db mice is a widely used model to study T2D, polygenic models are more representative of the physiologic environment of diabetes in humans, particularly of T2D. This study used TH mice model to study wound healing because the db/db mice were significantly more obese than the TH mice. Db/db mice exhibited a significant wound healing impairment with excisional wound models. However, according to the authors, this impairment may be due to an “intrinsic skin tension” as a result of its thin skin and extreme obesity and may not be due to the hyperglycemia and diabetic phenotype [60].

The TH mice diabetic wound model has been successfully used by Burand et al. to develop a low-cost portable microscope that would help track cells in vivo, thereby, eliminating the need to transport the animals to an imaging facility [61].

TREATMENT STRATEGIES FOR DIABETES

The main strategy in therapy for diabetes is achieving optimal glycemic control in early T2D. Metformin has been used as a pharmacotherapy for a long time. Metformin is known to increase insulin sensitivity, reduce hepatic glucose production, and enhance peripheral glucose uptake. However, it has been reported that metformin by itself fails to maintain glycemic control. Several researchers have evaluated the efficacy of metformin alone and in combination with other drugs [62]. One such approach is combining metformin with a sodium glucose cotransporter 2 inhibitor (SGLT2I). It has been reported that combination of metformin with an SGLT2I more efficiently counteracted hyperglycemia in patients with inadequately controlled T2D than metformin alone [63]. Neschen et al. [64] investigated the physiological mechanism by which by which metformin and SGLT2I lower the blood glucose concentration in diabetic mice. They conducted studies in both db/db mice and the TH mice. Although improved glycemic control was seen with both types of mouse models, vehicle-treated mice exhibited strain specific differences probably due to early onset diabetes phenotype of db/db mice compared with TH mice. The Db/db mice also exhibited less food intake behavior compared to the TH mice [64].

Franko and colleagues (2017) reported that bezafibrate (BEZ) improved glucose metabolism and diabetes in insulin-deficient streptozotocin-treated mice [65]. They used the TH mice model to study whether BEZ also improved glucose metabolism in the fatty liver and T2D model. Their study indicated that BEZ treated late onset mice diabetic mice were protected against diabetes and the already established diabetic mice in the early onset group reverted upon treatment. Thus, BEZ could prevent the progression of a prediabetic state to clinical diabetes and even revert an established diabetic state [66]. Since obesity is a high risk for cardiovascular diseases and diabetes, a novel strategy to combat these diseases is to treat obesity. Accordingly, Chen et al. [67] investigated the effect of altering the gut microbiota on obesity. They used the obese TH female mice and focused on N-acyl-phosphatidylethanolamines (NAPEs), the immediate precursors of N-acylethanolamides, a family of the potent anorexigenic lipids [67]. NAPEs are synthesized in the small intestine in response to feeding and this is impaired by a high fat diet. They administered E. coli (pEc) or transformed E. coli (pNAPE-Ec) into TH female mice and the B6 control mice. The mice were put on a high fat diet. The gut microbiota provided sustained attenuation of weight gain for up to 6 weeks after ending the treatment. The TH mice maintained total body weight and adiposity lower than that of control-treated animals even 12 weeks after ending the treatment. [62].
Rapamycin has been investigated as a potential treatment for several chronic diseases including cancers and autoimmune diseases. However, rapamycin treatment has been shown to promote insulin resistance and hence has negative implications on patients with T2D. Reifsnyder et al. [68] analyzed the effects of rapamycin on 5 different models of obesity and T2D (diabesity), including the TH mouse model. Encapsulated rapamycin was administered through the diet in these mice. The treatment did not exacerbate insulin resistance, glucose intolerance, and circulating lipids in any of these 5 models. Rapa-treatment diminished obesity, weight gain, and adipose tissue weight and improved insulin sensitivity in 3 of the models including the TH mice. Interestingly, of all the 5 models studied, the pancreatic insulin content was significantly reduced to the lowest levels in the TH mice [68].

CONCLUSIONS AND FUTURE DIRECTIONS

This article has provided a review of the use of TH mice as a model for T2D. King discussed the use of various animal models in diabetes research including factors to consider in choosing the appropriate animal model for research [69]. Since TH is a polygenic model, it provides a more accurate model of diabetes in humans. While only the male mice develop hyperglycemia in this model, the females do exhibit obesity and the female TH mice have been used to study non-diabetic obesity [59, 67]. Thus, this model can be used in both diabetes and obesity research. Although this seems to be an excellent model for T2D research, some of the limitations of this model include the great variation between animals with respect to the hyperglycemia and hyperinsulinemia as the disease progresses, which is difficult to control. Since only the male mice are diabetic, this model is not useful to conduct studies involving gender differences. Most importantly, since the strains are maintained through inbred sibling matings, and several genetic factors are involved in the diabetic phenotype, it is difficult to assess whether new litters obtained by cross mating these mice with a different strain carries the TH transgene. Overall, this TH model is useful and provides new insights to understand T2D in humans.

Although this TH model was originally described in 2001, progress is limited thus far in terms of oxidative stress, mitochondrial function/dysfunction, mitochondrial biogenesis, mitochondrial dynamics, and synaptic damage. Further, it is unclear whether diabetes progression to AD in features such as the formation of amyloid-β and phosphorylated tau is similar to humans. In addition, analysis of plasma insulin levels in obese B6 X BTBR intercross mice led to the identification of the Alzheimer gene, APP, as a candidate regulator of insulin secretion by Tu et al. [70]. They identified that APP knockout mice exhibited increased insulin in response to glucose. This suggests similarities between diabetes and neurodegenerative diseases such as AD [4, 70]. Hence, it will be interesting to study such diseases in the TH model. Further research is still needed to understand molecular links between diabetes and inflammation in the TH model.

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