Phenotypic characterization of polygenic type 2 diabetes in TALLYHO/JngJ mice

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Abstract

The TALLYHO/JngJ (TH) strain is a newly established, polygenic mouse model for type 2 diabetes (T2D) and obesity, and we have previously reported some key physiological features of this model after the overt onset of diabetes. In the present work, we conducted a comprehensive phenotypic characterization of TH in order to completely characterize this new and relevant model for human T2D and obesity. We monitored the development of obesity and diabetes starting at 4 weeks of age by measuring body weight, glucose tolerance, and plasma levels of insulin, glucose, and triglyceride. Additionally, histological alterations in the pancreas and glucose uptake and glucose transporter 4 (GLUT4) content in soleus muscle were also examined. Compared with age- and sex-matched C57BL/6J (B6) mice, both male and female TH mice were significantly heavier, hyperleptinemic, and hyperinsulinemic at 4 weeks of age, without glucose intolerance or hyperglycemia. TH mice maintained higher body weights throughout the study period of 16 weeks. The hyperinsulinemia in TH mice

worsened with age, but to a lesser degree in females than in males. Both the male and the female TH mice had enlarged pancreatic islets. Male TH mice showed impaired glucose tolerance at 8 weeks that became more prominent at 16 weeks. Plasma glucose levels continuously increased with age in male TH mice resulting in frank diabetes, while female TH mice remained normoglycemic throughout the study. Impaired glucose tolerance and hyperglycemia in male TH mice were accompanied by impaired 2-deoxyglucose uptake in the soleus muscle at basal and insulin-stimulated states, but without any reduction in GLUT4 content. Interestingly, male TH mice exhibited a drastic elevation in plasma triglyceride levels in the pre-diabetic stage that was maintained throughout the study. These findings suggest that obesity and insulin resistance are an inherent part of the TH phenotype and glucose intolerance is evident preceding progression to overt diabetes in male TH mice.

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Introduction

The prevalence of diabetes is growing worldwide and it is estimated that 366 million people will be affected by the year 2030 (171 million in the year 2000; Wild *et al.* 2004). Diabetes is associated with an increased risk for mortality as well as morbidity. In 2002, it is estimated that 186 000 deaths in US were attributed to diabetes (Hogan *et al.* 2003). Type 2 diabetes (T2D) is the most common form of human diabetes, accounting for approximately 90% of cases and is typically associated with obesity (Gannon 2001, Expert Committee 2003). The frequent concurrent incidence of diabetes and obesity even led one to coin the term ‘diabesity’, originally describing the associated symptoms of adult-onset obesity and diabetes (From the NIH 1980).

The etiology of T2D involves genetic predisposition and non-genetic risk factors such as high calorie diets and

reduced physical activity (Florez *et al.* 2003, Leahy 2005, O’Rahilly *et al.* 2005). Most common forms of T2D in humans follow polygenic inheritance, i.e. multiple genes are involved in the development of the disease. Further, it is thought that T2D is genetically heterogeneous and various pathologic pathways underlie the disease in different affected individuals (Leahy 2005, O’Rahilly *et al.* 2005). Possibly owing to the etiological complexity, no common molecular/cellular pathogenesis for human T2D is yet known. Understanding the pathogenesis is necessary to identify therapeutic targets as well as to generate prognostic information, which ultimately should lead to improved outcomes in affected individuals (O’Rahilly *et al.* 2005).

Genetic animal models have been valuable resources for T2D research, but few polygenic rodent models have been developed (Rees & Alcolado 2005). These models include the Goto-Kakizaki (GK), the Otsuka Long-Evans Tokushima

Fatty (OLETF) rats, the Spontaneously Diabetic Torii (SDT) rats, the Nagoya-Shibata-Yasuda (NSY) mice, the Tsumura-Suzuki-Obese-Diabetes (TSOD) mice (Kim 2004), and the New Zealand Obese (NZO)/non obese non diabetic (NON) F1 hybrid mice (Leiter & Reifsnnyder 2004). Of these, the GK and SDT rats develop T2D without developing obesity and the NSY mice develop diabetes very late in life without either extreme obesity or severe hyperinsulinemia (Ueda *et al.* 1995, 2000, Kim 2004).

Recently, we have developed a new polygenic mouse model for T2D, TALLYHO/JngJ (TH; formerly named TallyHo), by selective breeding for hyperglycemia from an outbred colony of mice (Kim *et al.* 2001). TH mice exhibited obesity, hyperinsulinemia, hyperglycemia (males), and hyperlipidemia at 26 weeks of age, and genetic analysis revealed major loci and epistatic interactions accounting for the diabetes in TH mice (Kim *et al.* 2001). In the present study, we have conducted a more comprehensive phenotypic characterization of TH mice from an early age. Understanding the underlying metabolic characteristics of TH mice will provide the basis for new insights into the molecular pathogenesis of T2D in this mouse strain as well as contribute to further understanding of T2D in general.

Materials and Methods

Animals

The initial establishment of the TH strain has been detailed in our previous report (Kim *et al.* 2001). TH mice from the F6NE4F8, F6NE4F18 (maintained at The Jackson Laboratory, Bar Harbor, ME, USA, stock no. 005314), and the F6NE4F8+8~+10 (maintained at The University of Tennessee, Knoxville, TN, USA) generations were used in this study. Measurements of body weights (bw) and plasma levels of glucose, insulin, and triglyceride with age were from the F6NE4F8 and F6NE4F8+8~+10 generations. Histological examination, tissue glucose uptake, glucose tolerance, and plasma leptin levels were obtained from F6NE4F8+8~+10 mice. Since the diabetic trait in TH mice is polygenic, a simple genetic control of non-diabetic mice does not exist. Therefore, C57BL/6J (B6) inbred mice were used as arbitrary control, since B6 is one of the most commonly used strains in diabetes and obesity research and does not develop diabetes when fed standard laboratory chow. The B6 strain was also used as control in our previous study (Kim *et al.* 2001) and by others (Sung *et al.* 2005). Mice were maintained on standard rodent chow with 4% fat (5K54, LabDiet, St. Louis, MO, USA or Harlan Teklad Rodent Diet (W) 8604, Madison, WI, USA) *ad libitum* with free access to water (HCl acidified, pH 2.8–3.2) under controlled temperature and humidity with a 12-h light and darkness cycle. All animal studies were carried out with the approval of either The University of Tennessee or The Jackson Laboratory Animal Care and Use Committee. Mice were euthanized by CO₂ asphyxiation.

Plasma glucose, triglyceride, insulin, and leptin levels

For all studies, blood was drawn in the morning from the retro-orbital plexus via heparinized capillary tubes and plasma was obtained by centrifugation (1200 g) at 4 °C. Plasma levels of glucose (635–100, Sigma or TR15103/1530–500, Thermo Electron, Louisville, CO, USA), and free and total glycerol (337, Sigma) were determined using commercial colorimetric assays. Plasma triglyceride concentrations were estimated by subtraction of free glycerol from total glycerol. Plasma insulin and leptin levels were determined using RIA (RI-13K, Linco Research, St. Charles, MO, USA) and ELISA kits (90030, Crystal Chem Inc., Downers Grove, IL, USA) respectively.

Other blood assays

Plasma was obtained as above and all assays were performed using a Beckman Coulter Synchron CX5 Delta chemistry analyzer (Beckman Coulter, Inc., Diagnostic Division, Brea, CA, USA). This instrument allows automated measurements of total cholesterol, HDL-cholesterol, lactate dehydrogenase, creatine kinase, alkaline phosphatase, pancreatic lipase, T4, total protein, albumin, total bilirubin, urea nitrogen, phosphorous, and calcium in the plasma.

Intraperitoneal glucose tolerance test

Mice were fasted overnight and injected intraperitoneally with glucose (1 mg/g bw) in saline. Blood was collected via the retro-orbital plexus using a heparinized microcapillary tube at 0, 15, 30, 60, and 120 min after injection. Plasma was obtained and plasma glucose and insulin levels were determined as above.

Tissue glucose uptake in vivo

Mice were fasted overnight and injected intravenously through the tail vein with a bolus of 2-deoxy-D-glucose 1, 2-³H (N) (2-DG) (D-4539, Sigma; 0.5 µCi/g bw) in saline either with or without insulin (I-5523, Sigma; 1 U/ml). Mice were euthanized by CO₂ asphyxiation 30 min after injection. The soleus muscle was collected, washed, blot dried, weighed, and dissolved in SOLVABLE (6NE9100, PerkinElmer, Boston, MA, USA) at room temperature. The incorporated radioactivity was counted in a scintillation counter (LS3801, Beckman, Fullerton, CA, USA). The 2-DG uptake was expressed as counts per minute divided by tissue weight (Hom *et al.* 1984).

Histological examination

Mice were euthanized by CO₂ asphyxiation. Pancreas was dissected, fixed in 10% neutral-buffered formalin, routinely paraffin embedded, sectioned at 3 µm and the sections were stained with aldehyde fuchsin, using an orange-G

counterstain, and hematoxylin and eosin (H&E) for morphologic evaluation. Aldehyde fuchsin- or H&E-stained sections were used to measure islet size using ImageJ morphometric software (Abramoff *et al.* 2004). All islets were photographed at 100 $\times$ magnification (digital camera). Each islet in these images was manually outlined and individual islet areas (in square pixels) were recorded.

Western blot analysis

Protein extracts were prepared from the soleus muscle of B6 and TH mice using Tri Reagent (Sigma). Total proteins (40 μ g) from each sample were separated on 10% SDS-PAGE gels and transferred to nitrocellulose using a mini trans-blot apparatus (Bio-Rad). The blots were blocked in 5% non-fat milk dissolved in Tris-buffered saline/0.1% Tween for 1 h at room temperature, then probed either with anti-glucose transporter 4 (GLUT4; ab654, Abcam Ltd, Cambridge, MA, USA) followed by incubation with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (ab6721, Abcam Ltd), or with HRP-conjugated glyceraldehyde-3-phosphate dehydrogenase (GAPDH; sc-25778, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 90 min at room temperature. Bound antibody was visualized using the SuperSignal West Pico Chemiluminescent Substrate system (34077, Pierce Biotechnology, Rockford, IL, USA).

Statistical analysis

Data analysis was conducted by ANOVA with StatView 5.0 (Abacus Concepts, Berkeley, CA, USA). All data are presented as means $\pm$ S.E.M.

Results

Longitudinal measurements of body weights and plasma levels of glucose, insulin, and triglyceride

At 4 weeks of age, TH mice of both sexes were significantly heavier than the age- and sex-matched B6 mice (16 ± 0.4 ($n=12$) vs 12 ± 1.8 ($n=5$), $P=0.01$, female; 18 ± 0.5 ($n=14$) vs 15 ± 1.2 ($n=13$), $P=0.01$, male; g). TH mice of both sexes were also hyperleptinemic at 4 weeks compared with B6 mice (8.2 ± 0.9 ($n=6$), vs 2.9 ± 0.9 ($n=4$), $P=0.003$, female; 7.5 ± 0.8 ($n=6$) vs 2.3 ± 0.6 ($n=6$), $P=0.0001$, male; ng/ml). Higher mean body weights in TH mice of both sexes were maintained throughout the study period of 16 weeks (Fig. 1). At 4 weeks, TH mice of both sexes were also hyperinsulinemic compared with B6 mice (2.0 ± 1.1 ($n=7$) vs 0.9 ± 0.8 ($n=6$), $P=0.03$, female; 2.2 ± 0.9 ($n=6$) vs 0.9 ± 0.6 ($n=6$), $P=0.01$, male; ng/ml). While the level of hyperinsulinemia remains relatively stable in female TH mice, it increased in male TH mice at puberty reaching their maximal levels between 8 and 12 weeks (Fig. 2A). Both pre-pubertal male and female TH mice were normoglycemic at

Figure 1 Body weight changes in TALLYHO/IngJ (TH) and C57BL/6J (B6) mice from 4 to 16 weeks of age. Open and filled symbols represent B6 and TH respectively. Squares and circles represent male (M) and female (F) respectively. Data are means $\pm$ S.E.M. B6-F ($n=5-13$), B6-M ($n=7-14$), TH-F ($n=15-24$), and TH-M ($n=12-19$).

4 weeks (Fig. 2B). However, in post-pubertal males, plasma glucose levels continuously increased with age, reaching full-blown diabetes levels (300–400 mg/dl, non-fasting) by 14 weeks. While TH males are consistently hyperglycemic, the extent and onset of hyperglycemia varies from litter to litter. On the other hand, female TH mice were normoglycemic throughout the study period (Fig. 2B). Development of hypertriglyceridemia was also notable in TH mice; male TH mice exhibited a striking rise in plasma triglyceride levels between 6 and 10 weeks and maintained high levels throughout the study (Fig. 2C). However, female TH mice did not exhibit the triglyceride spike as shown in TH males, although they were hypertriglyceridemic compared with B6 mice (Fig. 2C).

We also measured other blood parameters to determine additional metabolic differences between TH and B6 mice (Table 1). Compared with male B6 mice, male TH mice were hypercholesterolemic. In addition, notable were significantly lower activity of pancreatic lipase and alkaline phosphatase, and lower levels of T4, total bilirubin, and urea nitrogen in male TH compared with B6 mice (Table 1); however, these were within normal ranges for male mice (unpublished The Jackson Laboratory reference data for retired breeders from strains B6, C3H/HeJ, and BALB/cJ).

Histology of pancreatic islets

At both pre- (4 weeks) and post-diabetic (16 weeks) stages, the mean size of the islets was significantly larger in TH mice than in age- and sex-matched B6 mice, as measured by morphometry (Fig. 3). This suggests the presence of continuous potent stimuli for insulin secretion in TH mice. H&E-stained pancreatic islets were also examined for

Figure 2 Changes in plasma levels of (A) insulin, (B) glucose, and (C) triglyceride in TH and B6 mice from 4 to 16 weeks of age (non-fasting). Open and filled symbols represent B6 and TH mice respectively. Squares and circles represent male (M) and female (F) respectively. Data are means $\pm$ S.E.M. B6-F ($n=5-16$), B6-M ($n=5-15$), TH-F ($n=6-20$), and TH-M ($n=6-31$).

histologic abnormalities. Islet cells in 4-week-old TH males, 16-week-old TH females, and 16-week-old B6 males were all morphologically similar with relatively abundant and uniform cytoplasmic granulation and scattered mitoses, particularly in younger mice (Fig. 4). In contrast, islets in 16 week-old TH males often had evidence of degeneration characterized by cells with reduced amounts of cytoplasm, granulation (often sequestered in a perinuclear location), occasional vacuolation, scattered apoptotic and/or necrotic cells and, in some instances, separation of cells by a fine eosinophilic stroma (mild fibrosis).

Figure 3 The average area of islets was determined morphometrically from digital images in female and male TH and B6 mice at (A) 4 weeks and (B) 16 weeks. Open bars represent B6 and filled bars TH mice respectively. Data are means $\pm$ S.E.M. from 36 to 45 islets (as indicated in each bar) obtained from 4 to 7 mice for each group. * $P<0.01$ and † $P<0.001$ vs B6 mice.

Intraperitoneal glucose tolerance test (IPGTT)

Male TH mice were glucose tolerant at 4 weeks of age, but exhibited impaired glucose tolerance at 8 weeks that further deteriorated at 16 weeks (Fig. 5A). As glucose intolerance can be attributable to insulin resistance and/or defects in insulin secretion, we measured the changes in plasma insulin levels during IPGTT to evaluate the contribution of insulin secretory defects. At 8 weeks of age, fasting and post-glucose challenge insulin levels were significantly higher in male TH mice than B6 males (Fig. 5A), indicating presence of insulin resistance in TH. At 16 weeks of age, the fasting insulin levels were higher in TH males, but total insulin secretion in response to glucose, as assessed by the area under the curve of insulin in IPGTT, was not significantly different between TH and B6 mice (Fig. 5A). On the other hand, female TH mice showed normal glucose tolerance at all ages determined (Fig. 5B). However, their fasting and post-glucose challenge insulin levels were significantly higher than those of B6 mice at 8 and 16 weeks of age (Fig. 5B).

Glucose uptake

Glucose uptake, the first step of glucose metabolism in the body was tested *in vivo* in the insulin sensitive soleus muscle of male TH and B6 mice, using 2-DG. TH mice exhibited significant reductions in both basal and insulin-stimulated 2-DG uptake compared with the age- and sex-matched B6 mice (Fig. 6).

GLUT4 content in soleus muscle

GLUT4 mediates the rate-limiting step of insulin-responsive glucose transport in skeletal muscle (Bell *et al.* 1990). The abundance of GLUT4 protein in soleus muscle was not significantly different between B6 and TH mice (Fig. 7).

Discussion

Previously, we reported the physiological features of TH mice at 26 weeks of age after the overt onset of diabetes (Kim *et al.*

Table 1 Blood parameters in 8 week-old C57BL/6J and TALLYHO/JngJ mice (males, non fasting). Values are means $\pm$ S.E.M.

	C57BL/6J (n=10)	TALLYHO/JngJ (n=6)	P value
Total cholesterol (mg/dl)	99.7 $\pm$ 1.7	144.7 $\pm$ 8.6	<0.0001
HDL-cholesterol (mg/dl)	69.8 $\pm$ 1.6	105.9 $\pm$ 7.8	<0.0001
Lactate dehydrogenase (IU/l)	585.9 $\pm$ 197.7	494.0 $\pm$ 154.3	0.73
Creatine kinase (IU/l)	2056 $\pm$ 968	1842 $\pm$ 1086	0.9
Alkaline phosphatase (IU/l)	174 $\pm$ 12	127 $\pm$ 6	0.01
Pancreatic lipase (U/l)	123 $\pm$ 8	65 $\pm$ 1	<0.0001
T4 (μ g/dl)	7.57 $\pm$ 0.38	4.70 $\pm$ 0.47	0.0004
Total protein (g/dl)	5.7 $\pm$ 0.34	6.0 $\pm$ 0.24	0.19
Albumin (g/dl)	2.9 $\pm$ 0.14	2.5 $\pm$ 0.06	0.06
Total bilirubin (mg/dl)	0.8 $\pm$ 0.05	0.6 $\pm$ 0.11	0.04
Urea nitrogen (mg/dl)	30.9 $\pm$ 0.9	24.2 $\pm$ 1.2	0.0006
Phosphorus (mg/dl)	8.3 $\pm$ 0.3	8.1 $\pm$ 0.5	0.75
Calcium (mg/dl)	10.3 $\pm$ 0.2	10.6 $\pm$ 0.2	0.1

Figure 4 Representative photomicrographs of islets in H&E-stained sections from (A) 4-week-old TH male, (B) 16-week-old TH male, (C) 16-week-old TH female, and (D) 16-week-old B6 male mice. Islet cells in all but the 16-week-old TH males contained granulated cytoplasm, whereas the older TH male islets contained smaller cells with less cytoplasmic granulation, scattered apoptotic or necrotic cells (arrow heads), and occasional evidence of fibrosis. Mitotic activity (arrows) was most evident in younger mice and not apparent in older TH males (all images 400 $\times$ original magnification).

2001). In the present study, we conducted a more comprehensive characterization from an early age.

Normal blood enzyme levels indicate that no major organ damage is evident at the 8-weeks time point. However, plasma pancreatic lipase activity was significantly lower in TH male mice than in B6 controls. It is interesting to note that TH mice also have significantly lower gene expression of pancreatic lipase related protein 1 (Brown *et al.* 2005), a secreted protein that can function as a lipase (Grusby *et al.* 1990). Whether this gene expression difference can account for the reduced lipase levels observed in the plasma remains to be determined. It is also noted that plasma thyroid hormone (T4) levels were found to be significantly lower in TH than in B6 mice, perhaps indicative of a lower metabolic rate in TH

mice (Sane & Taskinen 1993). It has also recently been shown that hypothyroidism is associated with decreased peripheral glucose utilization in rats (Cettour-Rose *et al.* 2005).

For both male and female TH mice, the metabolic aberrations shown at weaning age included increased mean body weights and plasma insulin levels (Figs 1 and 2A). TH mice were also hyperleptinemic at this age. Since plasma leptin levels are positively correlated with adiposity (Leibel 2002) and hyperinsulinemia reflects pancreatic attempts to overcome peripheral insulin resistance (Lebovitz 2001), obesity and reduced sensitivity to insulin action appear to be primary features in TH mice. However, the obesity in TH mice is not as severe as that in some other polygenic T2D models including NZO (43 g, male, 8 weeks) (Leiter &

Figure 5 (continued)

Figure 5 Intraperitoneal glucose tolerance (IPGTT) in (A) male and (B) female TH and B6 mice at 4, 8, and 16 weeks of age ($n=5$ for each group). Mice were fasted overnight and injected intraperitoneally with a bolus of glucose (1 mg/g body weight) in saline. Blood was obtained at times 0, 15, 30, 60, and 120 min after injection, and plasma glucose and insulin levels were measured. Area under the curve (AUC) of glucose and insulin levels during the IPGTT was also calculated. Open and filled symbols represent B6 and TH respectively. Data are means $\pm$ S.E.M. * $P<0.01$, † $P<0.001$, and ‡ $P<0.0001$ vs B6 mice.

Reifsnyder 2004) and TSOD (45 g, male, 8 weeks) mice (Suzuki *et al.* 1999).

The hyperinsulinemia in TH mice was accompanied by hypertrophied islets, possibly reflecting the β -cell adaptation to the increased loads caused by the body's insulin resistance (Fig. 3). With disease progression in TH males, there was also histologic evidence of islet degeneration (Fig. 4) presumably reflecting chronic over-stimulation of β -cells, although β -cell mass was adequate to maintain elevated levels of circulating insulin. The cellular pathobiology responsible for this change

and characterization of further deterioration with age remains to be determined.

Impaired glucose tolerance is well recognized as a precursor to the development of T2D in human populations including Pima Indians, in which subjects with impaired glucose tolerance are at a higher risk than those with normal tolerance (Knowler *et al.* 1990). The progress from glucose intolerance to diabetes is known to be accompanied by failure of insulin secretion in response to glucose (Knowler *et al.* 1990, DeFronzo 1997, Lebovitz 2001). The high risk of developing

Figure 6 *In vivo* 2-deoxy-D-glucose 1, 2-³H (N) (2-DG) uptake in the soleus muscles of TH and B6 mice at 6 weeks of age (males). Mice were fasted overnight and injected intravenously through the tail vein with a bolus of 2-DG (0.5 µCi/g body weight) in saline without (basal) or with insulin (insulin) (1 U/ml), and tissue was harvested 30 min after injection. The 2-DG uptake was expressed as counts per minute (CPM) normalized by tissue weight (mg). Open bars represent B6 (basal, *n*=6; insulin, *n*=6) and closed bars represent TH mice (basal, *n*=5; insulin, *n*=6) respectively. Data are means ± S.E.M. **P*=0.02, †*P*=0.004, and ‡*P*=0.0001.

T2D correlates with reduced β-cell function relative to the degree of insulin sensitivity (Kahn *et al.* 1993, 2001). Early alterations in both insulin sensitivity and secretion were observed in individuals, who progress from normal glucose tolerance to impaired glucose tolerance in a longitudinal study of a Pima Indian population (Weyer *et al.* 1999). We found that glucose intolerance was accompanied by increased insulin secretion during IPGTT in male TH mice at 8 weeks

Figure 7 Western blot analysis for glucose transporter 4 (GLUT4) in the soleus muscles of TH and B6 mice at 6–10 weeks of age (males, *n*=4 for each group). Mean GLUT4 content of soleus muscle homogenates determined by densitometric analysis of autoradiographs are displayed as arbitrary units after normalization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) content levels in the same blot. Data are mean ± S.E.M.

of age (Fig. 5A), possibly via a compensatory hypersecretion mechanism in response to diminished insulin action. However, this compensatory increase of insulin secretion during IPGTT was not profound in TH males at 16 weeks of age when their glucose intolerance became severe (Fig. 5A). Therefore, it appears that insulin resistance is a primary defect of TH mice and with disease progression insufficient β-cell compensation leads to glucose intolerance and diabetes in male TH mice. Sung *et al.* (2005) recently reported mild glucose intolerance, reduced insulin secretion during IPGTT, and elevated leptin expression in male TH mice at 4 weeks of age with little sign of insulin resistance. These results are contradictory to our observations, but higher dose of glucose load (2 g/kg bw) in IPGTT for their study could possibly explain the glucose intolerance. Sung *et al.* (2005) have proposed that hyperleptinemia may cause the inhibition of insulin secretion in TH mice via direct effects of leptin on the pancreas. However, in our study, there was no evidence of insulin secretion defects during IPGTT in TH mice at 4 weeks of age, despite their hyperleptinemia. In addition, Sung *et al.* (2005) did not observe obesity, hyperinsulinemia, or hypertriglyceridemia in TH mice compared with B6 at a young age, unlike in our study. Presently, it is unknown what causes these discrepancies in the observations. It is conceivable that environmental factors may be involved in different phenotypic expressions in TH mice at different laboratories, but this remains to be confirmed.

Sex dimorphism for diabetes is commonly observed in T2D rodent models including NSY, TSOD, *Lepr^{db}*, and *Lepr^{ob}* mice, and in OLETF rats (Leiter & Chapman 1994, Hirayama *et al.* 1999, Rees & Alcolado 2005). In *Lepr^{db}* and *Lepr^{ob}* mutant mice, the protection from diabetes in female mice can be attributed to low hepatic estrogen sulfotransferase activity, which prevents virilization of liver metabolism (Leiter & Chapman 1994). It has also been demonstrated that estrogen increases the density of insulin receptor in the hepatocyte membrane (Krakower *et al.* 1993), perhaps offsetting post-receptor insulin resistance in female rodents.

In support of glucose intolerance and hyperglycemia in male TH mice, soleus muscle in TH males exhibited lower basal and insulin-stimulated 2-DG uptake than in B6 mice even at a young age of 6 weeks (Fig. 6), stressing insulin resistance as a primary defect of TH mice. Reduced glucose uptake in skeletal muscle has also previously been reported in other animal models of obesity and T2D, including NZO mice (Veroni *et al.* 1991) and OLETF rats (Sato *et al.* 1995). In addition to skeletal muscle, a classic insulin target is adipose tissue (Biddinger & Kahn 2006). Indeed, insulin insensitivity on glucose uptake in adipocytes from diabetic humans as well as animals has been demonstrated (Hjollund *et al.* 1985, Ikeda 1994). Previously, we reported a reduced insulin-stimulating glucose uptake in adipose tissue of congenic mice carrying an obesity quantitative trait locus (*tabu2*) derived from TH mice (Kim *et al.* 2005). Congenic mice represent a simplified system that allows one to tease out polygenic traits (Lyons *et al.* 2000). Like the *tabu2* congenic mice, the parental TH mice might have diminished glucose

uptake in adipose tissue, but this remains to be tested. In addition, future study is needed to identify the major tissues that are defective in TH mice, in order to understand the causative mechanism(s) underlying T2D in this model.

A major mechanism accounting for impaired glucose uptake in skeletal muscle is decreased GLUT4 content and/or its altered translocation to the plasma membrane (Angel *et al.* 1996, Kelley *et al.* 1996, Toide *et al.* 1997, Miura *et al.* 2001). Although significantly suppressed GLUT4 expression was exhibited in skeletal muscle of insulin resistant rodent models (Kahn & Pedersen 1993, Machado *et al.* 1993), there was no evidence of reduced GLUT4 content in soleus muscle from TH male mice (Fig. 7). GLUT4 protein is usually sequestered into specialized vesicles within the cell at basal conditions and translocates to the plasma membrane following activation of insulin signal transduction when post-prandial glucose levels rise (Watson & Pessin 2006). Blunted GLUT4 translocation to the plasma membrane from the intracellular storage vesicles has been reported in insulin resistant states (Shao *et al.* 2000, Miura *et al.* 2001). Since B6 and TH mice exhibit comparable GLUT4 levels, it is possible that GLUT4 translocation is the defective step in glucose transport in TH mice.

Insulin resistance was profoundly associated with hypertriglyceridemia in male TH mice (Fig. 2C). Roden *et al.* (1996) demonstrated that an i.v. infusion of a triglyceride emulsion into healthy individuals decreased muscle glycogen synthesis by 50% and the rate of whole-body glucose uptake by 46%. Dresner *et al.* (1999) also demonstrated that lipid infusion abolished insulin signaling. Family studies indicate that patients with hypertriglyceridemia are at increased risk of developing T2D and hypertriglyceridemia served as a risk marker of glucose intolerance and T2D in such families (Sane & Taskinen 1993). Normalization of triglyceride levels lowered fasting plasma glucose levels more than three times and improved insulin sensitivity in subjects with extreme hypertriglyceridemia and overt diabetes (Mingrone *et al.* 1999). It may be speculated that the severe hypertriglyceridemia preceding the overt hyperglycemia might contribute to the development of diabetes in male TH mice, but this remains to be tested.

In summary, this study reveals that obesity and insulin resistance emerge early in the development of T2D in TH mice. Severe hypertriglyceridemia and impaired glucose tolerance precede overt hyperglycemia in male TH mice. The impaired glucose tolerance and hyperglycemia are in part attributed to reduced glucose uptake in muscle. This new mouse model recapitulates many of the metabolic abnormalities observed in human T2D and will be a valuable tool for identification of the underlying molecular defects.

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