Islet Insulin Secretion, β-Cell Mass, and Energy Balance in a Polygenic Mouse Model of Type 2 Diabetes With Obesity

Xia Mao, MD, PhD, Kristy D. Dillon, BS, Michael F. McEntee, DVM, Arnold M. Saxton, PhD, and Jung Han Kim, PhD

Abstract
Type 2 diabetes (T2D) and obesity are polygenic metabolic diseases, highly prevalent in humans. The TALLYHO/Jng (TH) mouse is a polygenic model of T2D and obesity that encompasses many aspects of the human conditions. In this study, we investigated the key metabolic components including β-cell physiology and energy balance involved in the development of diabetes and obesity in TH mice. Glucose-stimulated insulin secretion from freshly isolated islets was significantly enhanced in TH mice compared with normal C57BL/6 (B6) mice, similar to the compensated stage in human T2D associated with obesity. This increased glucose responsiveness was accompanied by an increase in total β-cell mass in TH mice. Energy expenditure and locomotor activity were significantly reduced in TH mice compared with B6 mice. Food intake was comparable between the two strains but water intake was more in TH mice. Together, obesity in TH mice does not appear to be due to hyperphagia, and TH mice may be a genetic model for T2D with obesity, allowing study of the important signaling or metabolic pathways leading to compensatory increases in insulin secretion and β-cell mass in insulin resistance.

Keywords
genetics, type 2 diabetes, obesity, β-cell mass, energy balance, insulin secretion, mice

Introduction
Type 2 diabetes (T2D) is the most common form of human diabetes, accounting for more than 90% of all cases and often coexists with obesity.1–3 The prevalence of T2D is growing worldwide, and per American Diabetes Association, the number of Americans with diagnosed and undiagnosed diabetes would soar from 23.7 million to 44.1 million by 2034. This increase may reflect current obesity epidemics since obesity is a major risk factor for T2D.5

The etiology of T2D involves multiple factors, including multiple susceptibility genes and environmental factors such as sedentary lifestyle and high-calorie diets.6 A substantial genetic contribution to human T2D has been appreciated, and supporting evidence for heritability includes the pronounced ethnic differences in T2D prevalence and strong familial aggregation of the disease.7 Most common forms of T2D in humans follow polygenic inheritance.8 Approximately 70% of all instances of T2D are associated with obesity, although the role of obesity in the etiology of T2D is complex.9

The pathogenesis of T2D involves a combination of insulin resistance in the target tissues including adipose tissue, liver, and muscle and failure of insulin secretion from pancreatic β cells.10 In most cases, prior to eventual failure, the β cells compensate for the insulin resistance for a long period of time via two primary mechanisms, increasing insulin secretion capacity and increasing β-cell mass.11

Animal models that accurately represent the complex nature of human T2D with obesity are valuable in providing resources to investigate the pathogenic mechanisms and function of genes underlying T2D and obesity.12 The TALLYHO/Jng (TH) mouse is a polygenic model for T2D, which encompasses many aspects of the human T2D conditions, including obesity and insulin resistance.13 To gain better understanding of the...
pathophysiologic characteristics of TH mice, here we extended our investigation to β-cell physiology including glucose-stimulated insulin secretion from isolated islets and β-cell mass and the regulation of energy balance in TH mice.

**Materials and Methods**

**Animals**

TALLYHO/Jng mice used in this study were from our breeding colony that has been maintained since 2001. C57BL/6 (B6) mice were purchased from The Jackson Laboratory (Bar Harbor, Maine) and bred time to time in our facility. Because of polygenic inheritance of T2D in TH mice, a precise genetic control strain does not exist for TH mice as is the case for all other polygenic models. We therefore used the B6 inbred strain for comparison since B6 does not spontaneously develop diabetes when fed standard laboratory chow. The B6 strain is also one of the most commonly used strains in diabetes and obesity research and has been used as the control in our previous studies and by others. All mice were maintained on standard rodent chow (Purina 5001; PMI Nutrition, Brentwood, Missouri) ad libitum with free access to water in a temperature- and humidity-controlled room with a 12-hour light–dark cycle. The dietary energy contents of macronutrients are presented in Table 1. All animal studies were carried out with the approval of Marshall University Animal Care and Use Committee. Mice were euthanized by CO2 asphyxiation followed by exsanguination.

**Isolation of Pancreatic Islets and Glucose-Stimulated Insulin Secretion**

Islets from pancreata of mice were isolated by collagenase digestion and Ficoll density gradient centrifugation as described in Zhang et al., with modification. Briefly, male B6 and TH mice were killed at 10 and 24 weeks of age and pancreata were infused with collagenase P solution (1.5 mg/mL; 249-002, Roche; Mannheim, Germany) via the common bile duct. Islets were digested at 37°C for 15 minutes, washed, and separated using discontinuous Ficoll (F9378; Sigma-Aldrich, St Louis, Missouri) in Euro-Collins solution. Islets were then incubated overnight in Roswell Park Memorial Institute (RPMI) 1640 medium that contained 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and 11.1 mmol/L glucose. The next day, the islets were handpicked, washed, and incubated (10 islets per well in 24-well culture plate) for 1 hour in fasting RPMI 1640 medium containing low glucose (1.1 mmol/L) and FBS (0.1%), and insulin content in the media was determined (baseline measurement). The glucose concentration in the media was then increased either to 11 or to 27.5 mmol/L, followed by 1-hour incubation, and insulin content in the media was measured. Insulin secretion was then calculated (difference from the baseline measurement) and normalized by islet DNA content. For each sample, triplicate measurements were performed and the average was used for data analysis. Insulin content was measured using an enzyme-linked immunosorbent assay (ELISA) kit (90080; Crystal Chem, Downers Grove, Illinois) and DNA content using Cyquant cell proliferation assay kit (C7026; Invitrogen, Eugene, Oregon).

**Immunohistochemical Staining for Insulin**

Pancrea from male TH and B6 mice at 10 and 24 weeks of age were dissected, fixed in 10% neutral-buffered formalin (245-684; Fisher Scientific, Kalamazoo, Michigan), routinely processed into paraffin, and sectioned at 4 μm. The sections were heat pretreated with citrate buffer as described previously, incubated overnight at 4°C with anti-insulin antibody (1:400, C14, Cell Signaling, Danvers, Massachusetts), washed, and incubated with anti-rabbit secondary antibodies conjugated with peroxidase. The sections were then color developed with diaminobenzidine and counterstained with hematoxylin.

**Morphometric Analysis of β-Cell Mass**

Pancrea from male TH and B6 mice at 19 weeks of age were dissected, weighed, fixed, paraffin embedded, and sectioned. Sections with maximum footprint were immunostained for insulin as described earlier. The areas of β cells, defined as the insulin-positive cell area, were quantified using the image analysis software (ImageJ; http://imagej.nih.gov/ij/). Total β-cell mass was then assessed by multiplying the ratio of the β-cell area to the total tissue area on the entire section by pancreas weight as described in Maier et al.

**Indirect Calorimetry, Locomotor Activity, and Food and Water Intake**

Heat production, respiratory exchange ratio (RER), food intake, water intake, and locomotor activity were measured in male B6 and TH mice at 17 to 19 weeks of age, using an 8-chamber Comprehensive Laboratory Animal Monitoring System (CLAMS; Columbus Instruments, Columbus, Ohio) as described previously. All mice were acclimatized to monitoring cages for 24 hours prior to an additional 48 hours of recordings under the regular 12-hour light–dark cycle. In this system, heat production (kcal/h) is calculated by multiplying the calorific value (CV = 3.815 + [1.232 × RER]) by the observed oxygen consumption per unit time (VO2; Heat = CV × VO2). Heat production was then normalized by body weight to calculate energy expenditure (EE) (kcal/kg/h). The RER is the ratio between the VCO2 and VO2 (RER = VCO2/VO2). Locomotor activity was determined as ambulatory.

**Table 1. Energy Contents of Diet.**

<table>
<thead>
<tr>
<th></th>
<th>g%</th>
<th>kcal%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>23.9</td>
<td>28.5</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>48.7</td>
<td>58.0</td>
</tr>
<tr>
<td>Fat</td>
<td>5.0</td>
<td>13.5</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Quantitative analysis of β-cell mass revealed considerable increases in total β-cell mass in TH mice compared with B6 mice (Figure 2).

### Indirect Calorimetry, Locomotor Activity, and Food and Water Intakes

The 2-day averages of EE, RER, locomotor activity, food intake, and water intake, presented in Table 3, are used to investigate the regulation of energy balance in TH mice. The calculated EE (heat production normalized by body weight) over a 24-hour period was significantly lower in TH mice than that in B6 mice. This trend in EE was consistent both during light and dark phases. The RER over a 24-hour period was slightly, but significantly, lower in TH mice than that in B6 mice, mainly during the dark phase.

Locomotor activity, determined by the ambulatory count, over a 24-hour period was significantly reduced in TH mice compared with B6 mice, mainly during the dark phase. Food intake, determined as gram consumed, was not different between TH and B6 mice over a 24-hour period. Interestingly, TH mice showed slightly, but significantly, greater food intake during light phase. At the same time, during dark phase decreased percentage of food consumed was observed in TH mice compared with B6 mice, mainly during the dark phase.

### Discussion

In this study, we demonstrated that glucose-stimulated insulin secretion increased in islets isolated from TH mice compared with B6 mice as was previously observed during an intraperitoneal glucose tolerance test. Along with hyperinsulinemia, this increased acute glucose-stimulated insulin secretion is commonly found in insulin resistance related to obesity in humans and represents compensation for the disease. The increase in insulin secretion possibly results from an increase in β-cell mass or enhanced secretory capacity per cell or both. In the present study, we identified an increase in total β-cell mass in TH mice, which may, in part, allow TH mice to increase glucose responsiveness. The compensatory β-cell mass expansion in response to increasing systemic insulin demand could be caused through enhanced proliferation of existing β cells, neogenesis, or decreased β-cell apoptosis. More work is required to evaluate the mechanism underlying the increase in β-cell mass in TH mice.

It was somewhat surprising that TH mice preserved the excessive glucose-stimulated insulin secretion at 24 weeks of age when they were expected to develop full-blown diabetes. As it is generally accepted that diabetes reflects a failure of both insulin secretion and action, we expected to observe a diminished glucose-stimulated insulin secretion from isolated islets in TH mice at this age. Previously, we have observed that
the extent and onset of diabetes in TH mice vary from litter to litter in our colony, and it is possible that the mice used in this study may represent the late developers. It is also possible that the onset of diabetes in our colony of TH mice has become delayed over time, with continued inbreeding without further phenotypic selection for diabetes. More studies using mice at older ages are warranted. Nevertheless, the marked response to glucose in islets of TH differentiates this model from other obese diabetic models, such as NZO mice in which the response to an acute insulinotropic stimulus is impaired and \(\beta\)-cell mass is greatly reduced. Compensatory insulin secretion in response to insulin resistance is known to be highly influenced by the genetic background. Therefore, TH mice could be a genetic model of T2D with obesity that allows study of the critical signaling or metabolic pathways leading to a compensatory increase in insulin secretion, coupled with \(\beta\)-cell mass, in insulin resistance.

Identification of altered elements in regulating energy balance provides an important insight into the pathogenesis of obesity. Here we report that significantly reduced ambulatory activity emerged in TH mice compared with B6 mice.
Table 3. Indirect Calorimetry, Locomotor Activity, and Food and Water Intake of B6 and TH Mice (males; 17-19 weeks of age).\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>B6 (n = 7)</th>
<th>TH (n = 9)</th>
<th>Light Phase</th>
<th>Dark Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight, g</td>
<td>27.1 ± 0.8(^b)</td>
<td>37.6 ± 0.7(^c)</td>
<td></td>
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<tr>
<td>24 Hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>EE, kcal/kg/h</td>
<td>16.50 ± 0.39(^c)</td>
<td>14.36 ± 0.34(^b)</td>
<td>15.07 ± 0.40(^b)</td>
<td>13.56 ± 0.35(^b)</td>
</tr>
<tr>
<td>RER</td>
<td>0.926 ± 0.005(^c)</td>
<td>0.907 ± 0.005(^b)</td>
<td>0.887 ± 0.006(^c)</td>
<td>0.884 ± 0.006(^c)</td>
</tr>
<tr>
<td>Ambulatory count</td>
<td>12535 ± 728(^c)</td>
<td>10206 ± 642(^b)</td>
<td>2834 ± 333(^c)</td>
<td>2906 ± 294(^c)</td>
</tr>
<tr>
<td>Food, g</td>
<td>3.79 ± 0.28(^c)</td>
<td>4.34 ± 0.24(^c)</td>
<td>0.95 ± 0.20(^b)</td>
<td>1.65 ± 0.18(^b)</td>
</tr>
<tr>
<td>Water, mL</td>
<td>3.23 ± 0.29(^b)</td>
<td>4.92 ± 0.26(^c)</td>
<td>0.72 ± 0.12(^b)</td>
<td>1.77 ± 0.11(^c)</td>
</tr>
</tbody>
</table>

Abbreviations: B6, C57BL/6; TH, TALLYHO/Jng; EE, energy expenditure; RER, respiratory exchange ratio; SEM, standard error of the mean.\(^a\) Data are means ± SEM. Strain means labeled with different letters are significantly different for each phase (\(P < .05\)).

Concomitant reduction in EE was also seen in TH mice. Currently, the mechanism by which decreases in locomotor activity in TH mice compared with B6 mice is unknown. One notable early alteration in TH mice is an increase in plasma leptin levels as well as insulin resistance.\(^26\) Hyperleptinemia has been identified as a profound metabolic confounder with regard to insulin resistance in obese mice and humans.\(^26\) Sartorius et al.\(^27\) reported that administration of insulin into the cerebrospinal fluid significantly increased locomotor activity in mice and leptin abolished this insulin action. The authors previously found that obese mice develop insulin resistance in the brain that is accompanied by physical inactivity.\(^28\) With this knowledge, they concluded that a crossstalk of leptin and insulin in the brain might represent a molecular mechanism in obesity to inhibit locomotion. This mechanism may be applicable to TH mice in reducing the desire to move, but it remains highly speculative and serves as a hypothesis to be tested. It is also possible that the presence of physical limitations due to the increased body weight may cause hypoactivity in TH mice.

The overall daily food intake was comparable between TH and B6 mice. Despite the comparable food intake, TH mice have significantly greater body weight and fat mass than B6 mice.\(^29\) This may suggest that TH mice have a higher energy retention efficiency than B6 mice.

Although the biological relevance remains unclear, there were 2 interesting observations in TH mice; one was a greater food intake during the daytime, when mice normally sleep, and the other was a mildly reduced RER. In humans and animals, obesity is often concomitant with alterations in the diurnal regulation of feeding behavior. For instance, the Zucker obese rats carrying a leptin receptor mutation exhibit more daytime food intake and decreased percentage of food consumed at night compared with lean controls.\(^30–32\) In humans, obese patients showed a tendency for daily food intake later in the day than in lean cohorts.\(^33,34\)

It appears that there are some discrepancies in metabolic phenotypes of TH mice compared with previous reports. Sung et al.\(^35\) reported that glucose-stimulated insulin secretion from isolated islets is comparable between male TH and B6 mice at 4 weeks of age. Also, Rhee et al.\(^36\) previously reported that compared with B6 mice male TH mice have hyperphagia at 4 weeks of age. These results are contradictory to our observations. The causes for these discrepancies are currently unknown, but they might reflect the age differences in mice between the studies. Alternatively, they might reflect environmental effects on metabolic phenotypes in TH mice at different locations, but these remain to be confirmed.

In summary, we present data showing that glucose-stimulated insulin secretion in freshly isolated islets is enhanced in TH mice accompanied by an increase in total β-cell mass. We also report alterations in energy balance in TH mice. Our findings will contribute to the development of a valid research model for T2D and obesity, which are serious public health problems in humans.

Declaration of Conflicting Interests
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