Brief communication

MOLECULAR METABOLISM

# Defective insulin secretory response to intravenous glucose in C57Bl/6J compared to C57Bl/6N mice

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## ABSTRACT

**Objective:** The C57Bl/6J (Bl/6J) mouse is the most widely used strain in metabolic research. This strain carries a mutation in nicotinamide nucleotide transhydrogenase (*Nnt*), a mitochondrial enzyme involved in NADPH production, which has been suggested to lead to glucose intolerance and beta-cell dysfunction. However, recent reports comparing Bl/6J to Bl/6N (carrying the wild-type *Nnt* allele) under normal diet have led to conflicting results using glucose tolerance tests. Thus, we assessed glucose-stimulated insulin secretion (GSIS), insulin sensitivity, clearance and central glucose-induced insulin secretion in Bl/6J and N mice using gold-standard methodologies.

**Methods:** GSIS was measured using complementary tests (oral and intravenous glucose tolerance tests) and hyperglycemic clamps. Whole-body insulin sensitivity was assessed using euglycemic-hyperinsulinemic clamps. Neurally-mediated insulin secretion was measured during central hyperglycemia.

**Results:** Bl/6J mice have impaired GSIS compared to Bl/6N when glucose is administered intravenously during both a tolerance test and hyperglycemic clamp, but not in response to oral glucose. First and second phases of GSIS are altered without changes in whole body insulin sensitivity, insulin clearance, beta-cell mass or central response to glucose, thereby demonstrating defective beta-cell function in Bl/6J mice.

**Conclusions:** The Bl/6J mouse strain displays impaired insulin secretion. These results have important implications for choosing the appropriate test to assess beta-cell function and background strain in genetically modified mouse models.



**Keywords** Beta-cell; Insulin secretion; Insulin sensitivity; Genetic background; Mouse strain

## 1. INTRODUCTION

In the past twenty years, numerous transgenic and gene knock-out mouse models have been generated to assess the molecular and cellular mechanisms regulating beta-cell function and to better understand the process involved in defective insulin secretion and the etiology of type 2 diabetes (T2D) (reviewed in Ref. [1]). The most commonly used mouse strains to generate genetic models are inbred and include FVB, 129, DBA and C57Bl/6. The methodology required to produce these models often results in animals with mixed genetic background. However, it is well established that the background strain has a strong influence on glucoregulatory responses and beta-cell function [2–4]. For instance, DBA mice show a strong insulin secretion in response to glucose compared to C57Bl/6 [5] or FVB mice [4]. As a result, single strain or backcrossing has been used as a way to circumvent confounding effect of the background on processes involved in the control of glucose homeostasis. In metabolic studies, the C57Bl/6 strain has been widely used as a control strain mainly because of its high susceptibility to develop obesity and hyperglycemia when fed with a high-fat diet compared to other strains [3,6]. However, the C57Bl/6 mouse exhibits glucose intolerance compared to other strains even when fed on a regular chow diet [7–9]. Most importantly, it was recently established that C57Bl/6 mice supplied by the Jackson Laboratory (Bl/6J) carry a five-exon deletion in *Nnt* which encodes the

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*Abbreviations:* DI, disposition index; FSIVGTT, frequently sampled intravenous glucose tolerance test; GIR, glucose infusion rate; GSIS, glucose-stimulated insulin secretion; IDE, insulin degrading enzyme; IVGTT, intravenous glucose tolerance test; MI, insulin sensitivity index; NNT, nicotinamide nucleotide transhydrogenase; OGTT, oral glucose tolerance test

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nicotinamide nucleotide transhydrogenase (NNT), a mitochondrial enzyme involved in NADPH production [5,9]. In contrast, C57Bl/6 supplied by Taconic or Charles River (Bl/6N) do not harbor the mutation. The *Nnt* mutation has been associated in some studies with impaired glucose-stimulated insulin secretion (GSIS) and glucose intolerance compared to mouse strains carrying the wild-type *Nnt* [5,9]. In addition, transgenic expression of the wild-type *Nnt* gene in Bl/6J rescues beta-cell function and glucose tolerance [10]. While these findings strongly support the role of NNT in insulin secretion, recent studies have led to conflicting results showing that GSIS and glucose tolerance during glucose tolerance tests are similar in Bl/6J compared to Bl/6N [11,12]. While the reasons for this discrepancy are not clear, it is important to mention that none of these studies used the hyperglycemic clamps, the gold-standard methodology to measure beta-cell function [13]. In addition, it is still unclear whether impaired insulin secretion in Bl/6J mice involves changes in pancreatic beta-cell mass and/or insulin sensitivity. Finally, although NNT is expressed at high levels in other organs including the brain, the impact of the *Nnt* mutation on central glucose sensing has not been investigated.

Based on these conflicting results and the important implications of this issue for choosing the appropriate background strain in genetically modified mouse models, we assessed beta-cell function using complementary tests as well as beta-cell mass, insulin sensitivity and central glucose-induced insulin secretion in the Bl/6J vs. N mice.

## 2. METHODS

### 2.1. Animals

Male C57Bl/6 mice (12–14 weeks old) were purchased from the Jackson Laboratory (Bl/6J) and Charles River (Bl/6N). Animals were housed on a 12-h light/dark cycle at 21 °C with free access to water and standard chow diet for at least ten days before starting the experimentation. All procedures using animals were approved by the institutional animal care and use committee (Comité Institutionnel de Protection de Animaux, protocol #An12012TArs) of Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM) and the animal experimentation committee of Université de Bourgogne (protocol #105, C2EA, Dijon, France).

### 2.2. DNA extraction and genotyping

The presence of the NNT mutation was verified by PCR performed on DNA extracted from the liver using the protocol and primers described on the Jackson Laboratory website: http://jaxmice.jax.org/protocolsdb/f?p=116:2:0::NO:2:P2_MASTER_PROTOCOL_ID,P2_JRS_CODE:7470,012371.

PCR products were subjected to electrophoresis using 2% agarose gel.

### 2.3. Oral (OGTT) and intravenous (IVGTT) glucose tolerance tests

Oral glucose tolerance was assessed in overnight-fasted mice by measuring tail blood glucose 0, 15, 30, 45, 60, 90, and 120 min after oral administration of 2 g/kg glucose by gavage. Plasma samples were collected at 0, 15, 30 and 60 min for insulin measurement.

Intravenous glucose tolerance tests were performed in conscious, free-moving mice using modifications of a protocol previously described [14]. Briefly, a catheter was inserted into the right jugular vein under general anesthesia. Animals were allowed to recover for 5–6 days. Insulin secretion in response to intravenous glucose (0.75 g/kg) was measured at 0, 2.5, 5, 10, 15 and 30 min in mice fed *ad libitum* before the test. Plasma insulin was measured using a bead-based AlphaLISA insulin immunoassay kit (Perkin Elmer, Waltham, MA).

### 2.4. Assessment of insulin secretion and sensitivity by hyperglycemic and euglycemic-hyperinsulinemic clamps

One-step hyperglycemic clamps were performed on conscious animals (fed *ad libitum* before the clamp) as described [15]. A 20% dextrose solution was infused through the jugular vein to clamp plasma glucose at 320 mg/dl for 70 min and was adjusted based on glucose measurements (Roche Accu-Check; Roche, Indianapolis, IN). At 60 min, an arginine bolus injection was performed (1 mmol/kg; Sandoz Canada) to assess the maximal insulin response. Plasma samples were collected from the tail at several time points during the clamp for insulin measurements using the AlphaLISA kit. Plasma samples for C-peptide measurements were collected at 45 min and analyzed using a mouse C-peptide ELISA kit (Alpco Diagnostics). Two-hour hyperinsulinemic-euglycemic clamps were performed in 4 h food-restricted mice as previously described [15]. Briefly, following a 1-min bolus insulin infusion (85 mU/kg; Humulin R), insulin was infused at 8 mU/kg/min. Twenty percent dextrose was infused starting 5 min after the insulin infusion to clamp glycemia at ~120 mg/dl. Insulin levels during the steady state were measured at 90 and 120 min using the AlphaLISA kit. The insulin sensitivity index ($M/I$) was calculated as the glucose infusion rate ($M$) divided by the average insulinemia during the last 30 min of the clamp ($I$). The Disposition Index (DI) was calculated by multiplying the insulin sensitivity during the euglycemic hyperinsulinemic clamp by insulin secretion during the hyperglycemic clamp (AUC insulin 0–60 min). Insulin clearance was calculated by dividing the insulin infusion rate by the $\Delta$ increase in circulating insulin levels during the steady state of the hyperinsulinemic euglycemic clamp as described previously [13].

### 2.5. Beta-cell mass

Whole pancreata were dissected and placed in ice-cold PBS. After removing all the surrounding fat, lymph nodes, connective tissue and excess buffer, pancreata were weighted and fixed in freshly prepared 10% formalin in PBS at room temperature for 24 h, followed by embedding in paraffin blocks. Cross pancreatic sections were cut at 5-μm thickness and collected at 50 μm intervals. At least 6 slides from each pancreas were processed for beta-cell mass measurement. Paraffin sections were rehydrated, heated at 95 °C in 10 mmol/l citrate (pH 6) for 45 min, blocked, immunostained with anti-guinea pig insulin antibody (DAKO) and anti-guinea pig alkaline phosphatase conjugated secondary antibody (Jackson Immunoresearch), and finally developed with the Vector Red alkaline phosphatase substrate kit (Vector Laboratories). Harris-modified hematoxylin was used for counter-staining before mounting the slides with Vectamount medium (Vector Laboratories). The slides were scanned using a Super Cool Scan 9000 scanner (Nikon) and the images were analyzed using the image processing program Image J (National Institutes of Health) to assess the beta-cell area and the whole pancreas area.

### 2.6. Central glucose-induced insulin secretion

The test was adapted from a protocol previously developed in rats [16,17]. Briefly, a sylastic catheter was implanted into the left carotid artery in the cranial direction of anesthetized mice (pentobarbital, 60 mg/kg) and secured in place with sutures. Fifteen minutes after the surgery, a bolus of glucose (25 mg/kg in 30 μl, osmolarity adjusted to 300–310 mOsM with NaCl) was administered through the catheter over 30 s. Blood samples were collected from the tail vein 0, 1, 3 and 5 min post-injection to measure blood glucose (Roche Accu-Check) and plasma insulin levels (AlphaLISA kit).

 