

# **Diabetic Complications Consortium**

**Application Title:** “*Mouse with inducible and reversible T1D for studying metabolic memory*”

**Principal Investigator:** Michael Brownlee.

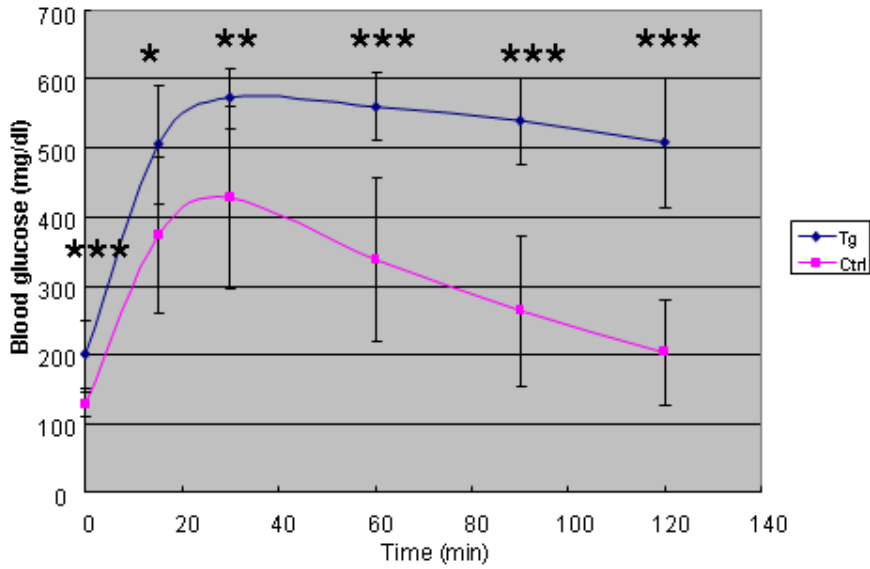
## **1. Project Accomplishments:**

We characterized a unique type 1 diabetes mouse model for studies of metabolic (hyperglycemic) memory that are impossible to do with current models. This model is based on the rare clinical syndrome of permanent neonatal diabetes most commonly caused by gain-of-function mutations in the inward rectifying potassium channel (Kir6.2) component of the  $\beta$ -cell ATP-sensitive potassium (KATP) channels. Heterozygous gain-of-function mutations in the *KCNJ11* gene encoding Kir6.2 impair the ability of ATP to close the channel, thus impairing glucose-stimulated insulin secretion. In our mouse model the expression of the mutant Kir6.2 is under the control of the tetracycline operator (tetO). rtTA expression is under the transcriptional control of the RIP-2 promoter to ensure  $\beta$ -cell specific expression of the mutant Kir6.2. Here we show that in these unique mice diabetes can be induced, completely reversed and successfully re-induced.

## **2. Specific Aim:**

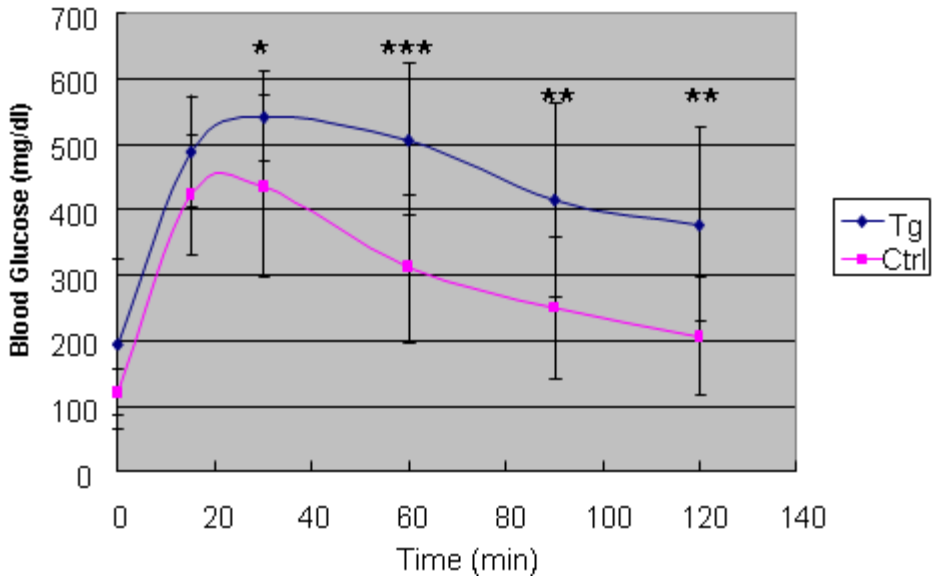
**Specific Aim.** To characterize the time course of doxycycline-dependent type 1 diabetes induction, reversal, and effect of duration on re-inducibility of diabetes in double transgenic mice expressing Kir 6.2 with the V59M gain-of function mutation.

**Results:** We performed intraperitoneal glucose tolerance test (2g glucose/kg body weight) on overnight fasted males double transgenic mice expressing Kir 6.2 with the V59M gain-of function mutation (*Tg*) and their non-double transgenic littermates (*Ctrl*) after 2 and 3 months of free access to water containing 400mg/dL doxycycline and to doxycycline food pellets. After 2 months of doxycycline treatment, Tg mice showed diabetic glucose tolerance curves compared to Ctrl mice (Fig.1).



**Fig 1: Intaperitoneal glucose tolerance test (GTT) after 2 months of doxycycline exposure.** (\*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ ; Tg mice  $n=10$ ; Ctrl  $n=9$ ). Data are expressed as mean  $\pm$  SD.

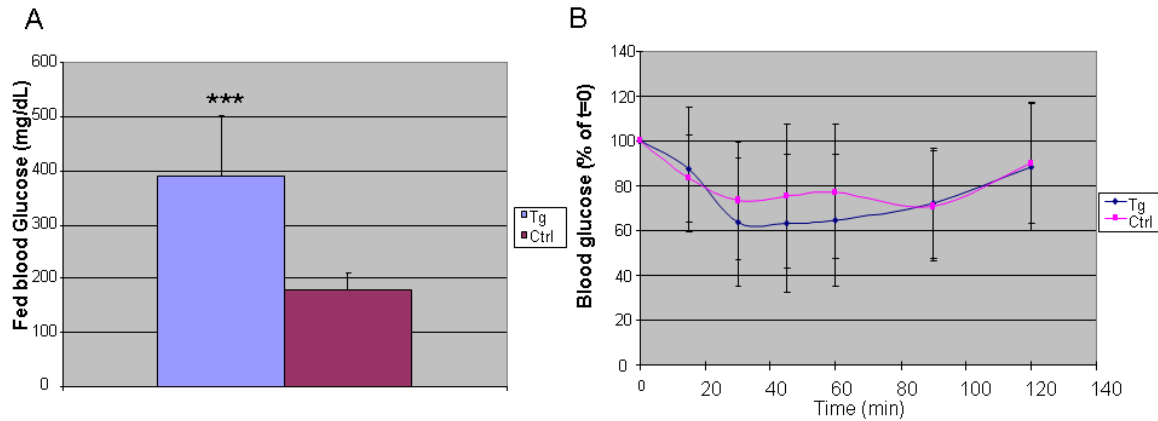
This defect remained after 3 months of treatment (Fig.2).



**Fig 2: Intaperitoneal glucose tolerance test (GTT) after 3 months of doxycycline exposure.** (\*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ ; Tg mice  $n=13$ ; Ctrl  $n=9$ ). Data are expressed as mean  $\pm$  SD.

Tg mice also showed a significant increase in fed blood glucose levels (Fig 3 A). Moreover, after 3 months of doxycycline exposure Tg mice had a normal blood glucose

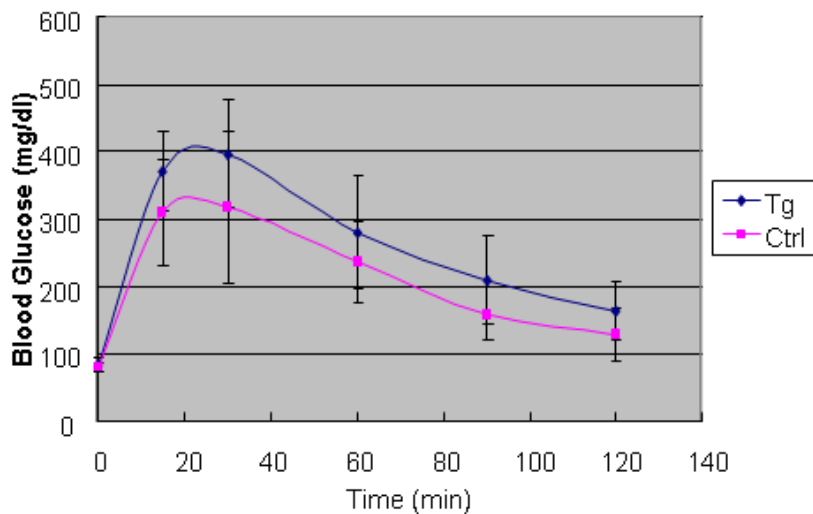
response to insulin during an intraperitoneal insulin tolerance test (0.75 insulin Units/kg body weight; Fig. 3 B).



**Fig 3: A. Random fed blood glucose levels and B. Intraperitoneal insulin tolerance test (ITT) after 3 months of doxycycline exposure.** ITT data are expressed as % of the initial blood glucose levels. ( \*\*\*  $p < 0.001$ ; Tg mice  $n=10$ ; Ctrl  $n=9$ ). Data are expressed as mean  $\pm$  SD.

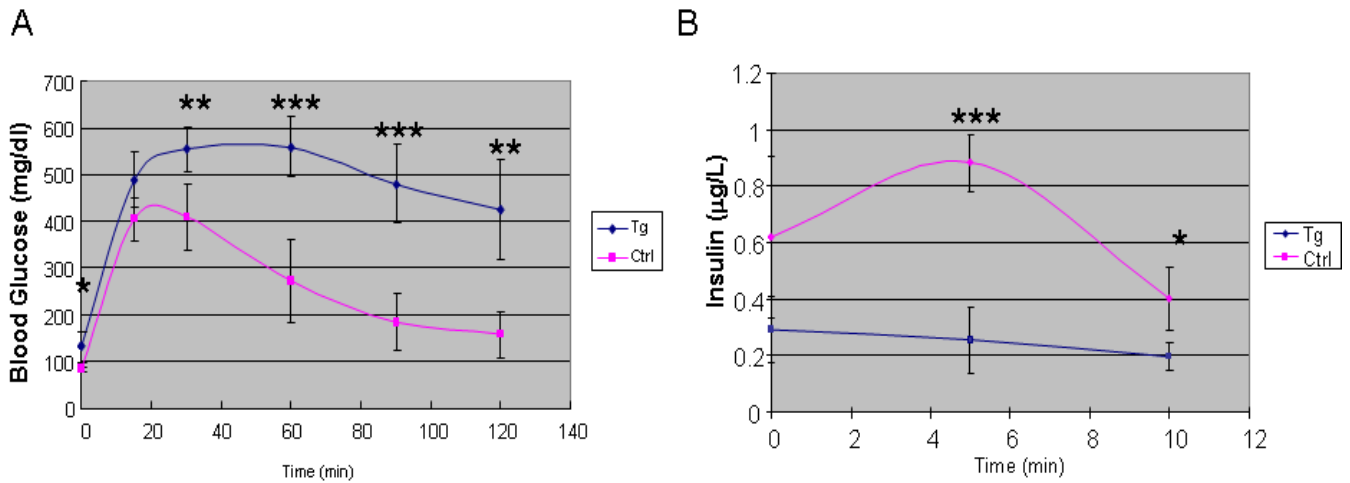
A small but significant body weight reduction was observed at 2 months of doxycycline exposure in Tg mice compared to Ctrl (Tg= $28 \pm 2.4$  g vs Ctrl  $31 \pm 1.2$  g). Three months of doxycycline exposure did not induce a change in blood pressure in Tg mice compare to Ctrl mice (Ctrl systolic=  $107 \pm 2$  mmHg, diastolic=  $80 \pm 3$  mmHg; Tg systolic=  $107 \pm 17$  mmHg, diastolic=  $77 \pm 12$  mmHg; Tg mice  $n=5$ ; Ctrl  $n=4$ ).

After 3 months of diabetes, we next showed that after doxycycline withdrawal, diabetes in Tg mice could be reversed. Three months after continuous treatment, doxycycline was withdrawn for a month. After doxycycline withdrawal, Tg mice showed a normal glucose tolerance compared to Ctrl mice (Fig. 4).



**Fig 4: Intraperitoneal glucose tolerance test (GTT) after 3 months of doxycycline exposure and 1 month withdrawal.** (Tg mice  $n=8$ ; Ctrl  $n=4$ ). Data are expressed as mean  $\pm$  SD.

Re-administration of doxycycline for a month after 3 months of continuous treatment and a month of withdrawal re-induced diabetic glucose tolerance (Fig. 5 A) and abolished first phase glucose-induced insulin secretion (Fig.5 B) in Tg mice.



**Fig 5: A. Intaperitoneal glucose tolerance test (GTT) and B. Glucose stimulate insulin secretion after 3 months of doxycycline exposure, 1 month withdrawal and 1 month re-exposure.** A: (\*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ ; Tg mice  $n=6$ ; Ctrl  $n=3$ ); B: Overnight-fasted animals were intraperitoneally injected with glucose (2g/kg body weight) and serum insulin levels were evaluated before and 5 and 10 minutes after glucose injection by ELISA. (\*  $p \leq 0.05$ , \*\*\*  $p \leq 0.001$ ; Tg mice  $n=4$ ; Ctrl  $n=3$ ). Data are expressed as mean  $\pm$  SD.

Taken together our data demonstrate that in our unique mouse model diabetes can be induced, completely reversed, and successfully re-induced.

**Statistics:** Data are expressed as mean  $\pm$  SD and were analyzed using student's t-test.