Published in final edited form as:

Horm Metab Res. 2009 January ; 41(1): 1–4. doi:10.1055/s-0028-1087209.

Noninvasive Monitoring of Changes in Pancreatic Beta-cell Mass by Bioluminescent Imaging in MIP-*luc* Transgenic Mice

S.-Y. Park and G. I. Bell

Departments of Medicine and Human Genetics, The University of Chicago, Chicago, Illinois, USA

Abstract

We have generated a transgenic mouse model (MIP-*luc*) that allows real-time imaging of insulin-secreting pancreatic beta cells in living mice. The beta cells of MIP-*luc* transgenic mice emit a light signal that can be visualized externally by bioluminescent imaging using specialized equipment. In order to determine whether the intensity of the bioluminescent signal accurately reflects changes in beta-cell mass rather than simply transcriptional modulation of the mouse insulin I promoter–luciferase transgene, we examined the correlation between the bioluminescent signal and the beta-cell mass in MIP-*luc* mice fed a regular or high-fat Western diet. Male MIP-*luc* mice were fed a standard rodent diet (5% of calories from fat) or a high-fat Western diet (42% from fat) beginning at 4 weeks of age. The bioluminescent signal and beta-cell mass were measured after 6 and 10 weeks on each diet. The body weight, beta-cell mass, and bioluminescent signal increased with age and increased further in mice fed a high-fat diet. There was a statistically significant correlation between beta-cell mass and bioluminescent signal ($r^2 = 0.660$, $p = 0.00137$). Thus, *in vivo* bioluminescent imaging can be used to noninvasively monitor changes in beta-cell mass in living MIP-*luc* mice, and it complements other approaches for monitoring beta-cell mass in states of insulin resistance, obesity, and diabetes.

Keywords

diabetes; firefly luciferase; high-fat diet; imaging; insulin; pancreatic beta cell; transgenic mice

Introduction

Pancreatic beta-cell mass is not static but changes in response to changes in the demand for insulin under physiological (e.g., pregnancy) and pathophysiological conditions (e.g., obesity and insulin resistance) [1,2]. Diabetes results from a loss of beta cells due to immunological or other mechanisms as well as from failure of the beta-cell mass to compensate for increased insulin demand [3,4]. The ability to noninvasively monitor changes in beta-cell mass in humans and animal models could provide new insight into its regulation in normal and disease states [5]. Recent studies have shown that positron emission tomography [6], nuclear magnetic resonance imaging [7], and optical imaging [8,9] can be used to “visualize” the beta-cell mass *in vivo*, although it is still not possible to visualize individual pancreatic islets, the unit of beta-cell mass. We recently described a transgenic mouse model in which firefly luciferase is specifically expressed in beta cells using the mouse insulin I promoter (MIP-*luc*), and we showed that the beta cells in this model can be visualized using bioluminescent imaging [8]. However, the MIP-*luc* transgene represents an indirect imaging agent, with the luciferase activity reflecting the number of cells expressing the transgene as well as the transcriptional

modulation of the insulin I promoter in expressing cells. Thus, it is unclear whether the signal is correlated with beta-cell mass or metabolic status of the animal. Here we show that bioluminescent signal intensity in MIP-*luc* transgenic mice is correlated with beta-cell mass and thus can be used to noninvasively monitor changes in beta-cell mass over time in individual animals.

Materials and Methods

Male MIP-*luc* transgenic mice on a CD-1 background were maintained in a specific pathogen-free environment on a 12 h light/dark cycle. The MIP-*luc* transgene was always kept in a hemizygous state, and genotyping was carried out as described previously [8]. Mice were housed five per cage in a specific pathogen-free facility and allowed free access to food and water. They were fed either a regular diet (Teklad Irradiated Global 18% Protein Diet, Harlan Teklad, Madison, Wis.) or a high-fat Western diet (Teklad Adjusted Calories Diet [42% fromfat]) beginning at weaning (4 weeks of age). This study was approved by the University of Chicago Institutional Animal Care and Use Committee.

The mice were weighed weekly. Fasting blood glucose and serum insulin were determined after a 4 h fast [10] starting at about 8:00 AM. Blood was obtained from the tail vein. Glucose levels were measured using a Precision Q.I.D. Glucometer (MediSense, Waltham, Mass.). At the same time, blood was collected into a microtainer (BD Microtainer SST; BD, Franklin Lakes, N.J.). The blood sample was centrifuged at 7000×g for 10 min at 4°C, and serum insulin concentration was determined using a rat insulin ELISA kit (Crystal Chem Inc., Downers Grove, Ill.).

Beta-cell mass was determined as described previously following a commonly used protocol [11]. Mice were sacrificed by cervical dislocation and pneumothorax. The pancreas was removed, weighed and embedded in Paraplast. Each block was serially sectioned (5 μm thickness) at 100-μm intervals. The sections were immunostained for insulin using a guinea pig polyclonal antiporcine insulin antibody (diluted 1:200; Dako, Carpinteria, Calif.) for 1 h at room temperature. The primary antigen was visualized with biotinylated secondary antibody (HistoMouse-Plus; Zymed, San Francisco, Calif.). The sections were then counterstained with hematoxylin. Quantitative evaluation was performed with a Nikon Eclipse E800M microscope (Nikon Instruments, Melville, N.Y.) with an Olympus Q-Color3 video camera (Optical Analysis Corp., Nashua, N.H.). The area of insulin-positive cells and total pancreatic area were evaluated in each stained section (five sections per mouse). Overall beta cell–relative volume in each mouse was determined as the mean of the ratio of the area occupied by insulin-positive cells to the area occupied by total pancreatic cells in each section multiplied by section thickness. Total beta-cell mass per pancreas was derived by multiplying the total pancreatic weight and relative beta-cell volume.

Bioluminescent optical imaging was performed with an IVIS 200 imaging system (Caliper Life Sciences, Hopkinton, Mass.) using a standard protocol as previously described [8]. Mice were fasted for 4 h and then anesthetized with isoflurane. Following an i.p. injection of D-luciferin (150 mg/kg) in sterile PBS (15 mg/ml), mice were placed on their right side on the imaging stage with their left side facing the camera, and a series of 1 min exposures beginning 5 min after injection of D-luciferin was used to generate the bioluminescent image. There was no difference in the intensity of the bioluminescent signal in the 5–10 min period following luciferin injection. An overlay image was taken with a light inside the imaging chamber turned on to illuminate the mice. Image processing including quantitation was carried out using Living Image v. 2.05 software (Caliper Life Sciences). We observed no toxic effects of repeated injection of luciferin on beta-cell function or on the general health of the transgenic mice. We imaged the same mice daily without any evidence of adverse effects.

Data are expressed as mean $\pm$ SEM. We used ANOVA to compare group differences (Table 1; StatView, SAS Institute, Cary, N.C.) and linear regression (Fig. 2 and Fig. 3; Excel, Microsoft, Redmond, Wash.) to test the correlation between variables. The p-values are two-tailed, and $p < 0.05$ was considered significant.

Results

Body weight, beta-cell mass, and bioluminescent signal increased in MIP-*luc* mice with age and increased further on a high-fat diet (Table 1). Fig. 1 shows the increase in bioluminescent signal in two mice at different ages and on different diets, highlighting the utility of this model and in vivo bioluminescent imaging for obtaining repeated physiological measures on the same animal. As expected, there was a highly significant correlation between body weight and beta-cell mass ($r^2 = 0.716$, $p = 2.6 \times 10^{-6}$; Fig. 2a). In addition, there was a strong correlation between bioluminescent signal and beta-cell mass ($r^2 = 0.660$, $p = 0.00137$; Fig. 2b).

We also compared the relationship between fasting serum insulin levels and beta-cell mass or bioluminescent signal within each dietary group (Fig. 3). For the group fed a regular diet, we observed a trend of insulin levels being related to beta-cell mass and bioluminescent signal ($r^2 = 0.296$, $p = 0.104$ and $r^2 = 0.266$, $p = 0.086$, respectively). However, in the group fed a high-fat diet, we observed a stronger correlation between insulin levels and beta-cell mass and bioluminescent signal ($r^2 = 0.543$, $p = 0.015$ and $r^2 = 0.800$, $p = 0.0005$, respectively). We also examined the relationship between fasting blood glucose levels and beta-cell mass or bioluminescent signal (Fig. 3c). There was a weak correlation between fasting plasma glucose levels in the mice fed a regular diet ($r^2 = 0.303$, $p = 0.099$). This correlation was significant in the mice fed a high-fat diet ($r^2 = 0.408$, $p = 0.047$).

Discussion

In vivo bioluminescent imaging allows repeated measurements of beta-cell mass in living mice (e.g., Fig. 1). We have shown here that it can be used to monitor changes in beta-cell mass with age as well as the increase in beta-cell mass that occurs when mice are fed a high-fat diet. In mice fed a high-fat diet, fasting serum insulin and beta-cell mass are well correlated (Fig. 3a). Thus, measuring the bioluminescent signal complements fasting serum insulin levels and provides an independent assessment of beta-cell mass. However, under normal conditions (e.g., mice fed a regular diet and in the absence of beta-cell compensation), serum insulin levels are not well correlated with beta-cell mass, and assessment of beta-cell mass by bioluminescent imaging adds an important additional dimension to the study. The MIP-*luc* model and in vivo bioluminescent imaging allow repeated measures of beta-cell mass and/or function in living mice and may thereby reduce the cost and improve the efficiency of studies in mice.

Acknowledgements

This work was funded in part by USPHS grant DK-20595 to the University of Chicago Diabetes Research and Training Center and a gift from the Kovler Family Foundation. We thank Dr. M. Geoffrey Hayes for his help with the statistical analyses and Drs. Kenneth S. Polonsky and Louis H. Philipson for their helpful comments on the studies and this manuscript.

References

1. Bouwens L, Rومان I. Regulation of pancreatic beta-cell mass. *Physiol Rev* 2005;85:1255–1270. [PubMed: 16183912]
2. Montanya E, Nacher V, Biarnés M, Soler J. Linear correlation between beta-cell mass and body weight throughout the lifespan in Lewis rats: role of beta-cell hyperplasia and hypertrophy. *Diabetes* 2000;49:1341–1346. [PubMed: 10923635]

3. Gottlieb PA, Eisenbarth GS. Diagnosis and treatment of pre-insulin dependent diabetes. *Annu Rev Med* 1998;49:391–405. [PubMed: 9509271]
4. Prentki M, Nolan CJ. Isletbeta cell failure in type 2 diabetes. *J Clin Invest* 2006;116:1802–1812. [PubMed: 16823478]
5. Souza F, Freeby M, Hultman K, Simpson N, Herron A, Witkowsky P, Liu E, Maffei A, Harris PE. Current progress in non-invasive imaging of beta cell mass of the endocrine pancreas. *Curr Med Chem* 2006;13:2761–2773. [PubMed: 17073627]
6. Souza F, Simpson N, Raffo A, Saxena C, Maffei A, Hardy M, Kilbourn M, Goland R, Leibel R, Mann JJ, Heertum RV, Harris PE. Longitudinal non-invasive PET-based beta cell mass estimates in a spontaneous diabetes rat model. *J Clin Invest* 2006;116:1506–1513. [PubMed: 16710474]
7. Evgenov N, Medarova Z, Dai G, Bonner-Weir S, Moore A. In vivo imaging of islet transplantation. *Nat Med* 2006;12:144–148. [PubMed: 16380717]
8. Park SY, Wang X, Chen Z, Chen Z, Powers AC, Magnuson MA, Head WS, Piston DW, Bell GI. Optical imaging of pancreatic beta cells in living mice expressing a mouse insulin 1 promoter-firefly luciferase transgene. *Genesis* 2005;43:80–86. [PubMed: 16108006]
9. Smith SJ, Zhang H, Clermont AO, Powers AC, Kaufman DB, Purchio AF, West DB. In vivo monitoring of pancreatic beta-cells in a transgenic mouse model. *Mol Imaging* 2006;5:65–75. [PubMed: 16954020]
10. Marshall BA, Tordjman K, Host HH, Ensor NJ, Kwon G, Marshall CA, Coleman T, MacDaniel ML, Semenkovich CF. Relative hypoglycemia and hyperinsulinemia in mice with heterozygous lipoprotein lipase (LPL) deficiency: Islet LPL regulates insulin secretion. *J Biol Chem* 1999;274:27426–27432. [PubMed: 10488074]
11. Johnson JD, Ahmed NT, Luciani DS, Han Z, Tran H, Fujita J, Misler S, Edlund H, Polonsky KS. Increased islet apoptosis in Pdx1^{+/-} mice. *J Clin Invest* 2003;111:1147–1160. [PubMed: 12697734]

Fig. 1.

Bioluminescent imaging of pancreatic islets in male *MIP-luc* mice fed a regular or high-fat diet for 4 or 10 weeks beginning at 4 weeks of age. The images show the same animal imaged at the start of the study (4 weeks of age) and at 8 and 14 weeks of age. The signal intensity is expressed as photons (p) per second per cm² per steradian (sr, a unit of solid angle). There is interindividual variation in the bioluminescent signal even among littermates of the same age. Mice were randomly assigned to the regular or high-fat diet.

Fig. 2. Correlation between beta-cell mass and body weight (**A**) or bioluminescent signal (**B**) in male MIP-*luc* mice fed a regular diet (RD) or high-fat diet (HFD) for 6 or 10 weeks beginning at 4 weeks of age (each group, n=5).

Fig. 3. Correlation between fasting serum insulin and beta-cell mass (**A**) and bioluminescent signal (**B**) or fasting blood glucose and bioluminescent signal (**C**) in male MIP-*luc* mice fed a regular diet (RD) or high-fat diet (HFD) for 6 or 10 weeks beginning at 4 weeks of age (each group, $n = 5$).

Characteristics of male MIP-*luc* transgenic mice fed a regular or high-fat diet for 6 or 10 weeks beginning at 4 weeks of age (n = 5 per group).

	6 weeks			10 weeks		
	Regular diet	High-fat diet	p	Regular diet	High-fat diet	p
body weight ^a (g)	37.0±1.1	43.6±1.5	0.005	47.8±0.6	55.0±2.1	0.0025
fasting ^b blood glucose (mg/dl)	187.2±12.6	178.4±10.2	0.61	161.8±12.9	210.2±11.3	0.011
fasting serum insulin (ng/ml)	0.89±0.07	1.13±0.18	0.22	0.68±0.01	1.51±1.18	0.0005
pancreatic weight (g)	0.31±0.02	0.29±0.03	0.45	0.37±0.004	0.38±0.004	0.50
beta-cell mass (mg)	1.16±0.18	2.74±0.72	0.15	3.40±0.61	6.24±1.06	0.012
bioluminescent signal (photons/s)	283 800±27 490	664 200±191 765	0.34	1 009 800±128 258	1 902 200±501 593	0.036

^aThere was no difference in body weight between transgenic and nontransgenic mice on the different diets

^bMice were fasted 4 h before study [10]. A 4–6 h fast is recommended for mice ([10] and Animal Models of Diabetic Complications www.amdccc.org), as overnight fasting induces nonphysiological changes in glucose metabolism