

## Longitudinal evaluation of myocardial glucose metabolism and contractile function in obese type 2 diabetic db/db mice using small-animal dynamic $^{18}\text{F}$ -FDG PET and echocardiography

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**Keywords:** type 2 diabetes mellitus (DM), db/db mice, small animal imaging, positron emission tomography (PET),  $^{18}\text{F}$ -fluorodeoxyglucose ( $^{18}\text{F}$ -FDG)

**Received:** June 22, 2017

**Accepted:** August 17, 2017

**Published:** September 23, 2017

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### ABSTRACT

**The aim was to evaluate sequential changes of myocardial glucose utilization and LV systolic function in db/db mice.**

**Eight db/db and eight wild-type mice underwent plasma substrate analysis and dynamic  $^{18}\text{F}$ -FDG PET at week 8 (W8), W10, W12, W14, and W16.  $^{18}\text{F}$ -FDG uptake constant  $K_i$  and the rate of myocardial glucose uptake (MRGlu) were derived via Patlak graphic analysis. Another 8 db/db and 8 wild-type mice received echocardiography at W8, W12, and W16 and LV structure and function were measured.**

**The db/db mice showed increased weights and glucose levels as they aged. The index of homeostasis model assessment-estimated insulin resistance, insulin, and free fatty acid concentrations were higher in db/db mice compared with wild-type. MRGlu of db/db mice across all time points was markedly higher than that of wild-type. An age-dependent elevation of MRGlu was observed in db/db mice.  $K_i$  and MRGlu of db/db mice showed negative correlation with triglyceride levels. When two groups were pooled together,  $K_i$  and MRGlu were significantly proportional to glucose levels. No significant difference in LV structure and function was noted between db/db and control mice.**

**In conclusion, we demonstrated altered myocardial glucose utilization preceding the onset of LV systolic dysfunction in db/db mice.**

## INTRODUCTION

Cardiovascular disease has been recognized as a major complication of type 2 diabetes mellitus (DM). Accumulating data have shown that DM results in cardiac functional and structural changes. The changes are independent of hypertension and coronary artery disease, thereby supporting the existence of diabetic cardiomyopathy [1]. It is important to note that diabetes-associated changes are amplified by the existence of co-morbidities which can augment development of left ventricular (LV) hypertrophy, increase susceptibility of the heart to ischemic injury, and increase the overall likelihood of developing heart failure [2]. Although several mechanisms, including myocardial fibrosis, small vessel disease, autonomic dysfunction and insulin resistance, have been put forth to explain diabetic cardiomyopathy, growing evidence suggests that the condition is partly a consequence of severe alteration in myocardial energy metabolism [3-6] and the diabetic heart primarily relied on fatty acid utilization for energy production.

Positron emission tomography (PET) has been used to quantify myocardial glucose use noninvasively in humans [7-9]. Small-animal PET systems have been introduced, allowing for high-resolution preclinical small-rodent PET imaging [10, 11]. Compared with qualitative interpretation in static imaging, dynamic  $^{18}\text{F}$ -FDG-PET imaging offers more detailed information on myocardial metabolism, including absolute quantification of the myocardial metabolic rate of glucose utilization. The latter is achieved through analysis by compartmental modeling.

Several applications of dynamic small animal  $^{18}\text{F}$ -FDG PET have been proposed. Yamane et al. [12] have developed a novel assay adapted to available small-animal PET for reliable quantification of myocardial tracer kinetics. Dynamic small-animal PET has helped to clarify mechanisms responsible for the metabolic alterations that occur in various diseases and pathologic processes, including diabetes [13, 14] and LV hypertrophy [15, 16]. The cited studies highlight advances in small-animal PET technology that enable quantification of metabolic parameters and exemplify the translational capabilities of metabolic PET.

Consequently, the aim of this study was to evaluate non-invasively the time course of myocardial glucose utilization and LV systolic function in C57BL/KSJ-lepr<sup>db</sup>/lepr<sup>db</sup> (*db/db*) mice, which has a mutation in the leptin receptor. Lepr<sup>db</sup> mutation is a recessive mutation on chromosome 4 that occurred in C57BLKS/J inbred strain in 1966. The Lepr<sup>db</sup> mutation was subsequently transferred to the C57BLKS/J inbred strain by backcrossing [17, 18]. It was a well-established animal model displaying type 2 DM characteristics, such as hyperglycemia, insulin resistance, obesity due to hyperphagia, and reduced energy expenditure [19-21]. Evidence of diabetic cardiomyopathy has also been reported in *db/db* mice. Systolic and

diastolic dysfunction were detected by echocardiography [22]. With the use of ex vivo perfused hearts from *db/db* mice, augmented LV end-diastolic pressure and reduced cardiac output and cardiac power have also been observed [23]. Early alteration of substrate metabolism might develop without contractile dysfunction [23, 24]. To our knowledge, there was no longitudinal in-vivo evaluation of myocardial metabolic alterations of *db/db* mice using dynamic  $^{18}\text{F}$ -FDG PET. We hypothesize that altered myocardial glucose utilization occurs in the early stage of diabetic cardiomyopathy, prior to LV structure change and contractile dysfunction.

## RESULTS

The fasting glucose levels of *db/db* mice were significantly greater than those of age-matched controls (all  $P < 0.05$ ). In the *db/db* group, body weight increased as animals got older, but wild type mice showed only a mild age-dependent increase in body weight (Table 1, 2). Body weight and blood glucose were positively correlated with age in *db/db* mice (Figure 1); there was no correlation of body weight and glucose levels in the control group.

The echocardiographic measurements are summarized in Table 2. Comparing *db/db* and their age-matched controls, there was no significant difference in LV volumetric data, including wall thickness, systolic and diastolic dimensions, and fractional shortening and LVEF. Neither *db/db* nor control mice displayed a significant change in LV parameters with age. In the end of the experiments (16-17 weeks of age), there were no significant differences in LV weight (LVW) and heart weight (HW) between the two groups (Table 1, 2); while *db/db* mice had significantly higher body weight, resulting in a significantly lower HW/BW ratio.

Circulating substrate values, including glucose, FFA, TG, insulin, and the HOMA-IR index are summarized in Table 1. Glucose, FFA levels, and HOMA-IR index of the *db/db* group were significantly higher compared with age-matched controls. When all time points were combined between W8 to W16, insulin levels in *db/db* mice were higher than in wild-type mice. Between 8 to 12 weeks, *db/db* mice tended to have higher TG levels, which declined at ages 14 and 16 weeks, with no statistical differences.

Dynamic PET parameters are depicted in Table 1. The *db/db* mice had significantly higher glucose uptake rates ( $\text{MRGl}_{\text{mean}}$ ,  $\text{MRGl}_{\text{max}}$ ) compared with age-matched controls (Table 1, Figure 2). At W12, either *db/db* mice or wild-type mice tended to have higher TG as compared with other time points which might resulted relatively lower  $\text{MRGl}$ . Although the variability in maximum and mean values of  $\text{MRGl}$  was observed, similar trends of myocardial glucose uptake rates described above existed in all-time points. In *db/db* mice, there were mild correlations between age and  $\text{MRGl}_{\text{mean}}$  ( $R^2 = 0.142$ ,  $P = 0.047$ ), or  $\text{MRGl}_{\text{max}}$  ( $R^2 = 0.198$ ,  $P = 0.017$ ), and  $\text{MRGl}$  values were

**Table 1: Clinical characteristics, plasma substrate levels, HOMA-IR index and estimated metabolic glucose utilization using FDG PET of wild type and db/db animal models**

Mice group (n)	Weight (g)	TG (nmol/ $\mu$ l)	FFA (nmol/ $\mu$ l)	HOMA-IR			MRGlu (umol/min/100g)		Isolated Heart		
				Glucose (mg/dl)	Insulin ( $\mu$ g/l)	Index	Max	Mean	LVW (g) (n=4/group)	HW (g) (n=4/group)	HW/BW (% (n=4/group))
<b>W8</b>											
wild-type (4)	19.99 $\pm$ 2.59	0.57 $\pm$ 0.15	0.36 $\pm$ 0.07	98.50 $\pm$ 16.62	0.06 $\pm$ 0.01	0.45 $\pm$ 0.01	14.15 $\pm$ 5.76	2.43 $\pm$ 1.41			
db/db (7)	36.17 $\pm$ 1.84*	0.69 $\pm$ 0.23	4.27 $\pm$ 2.14*	318.25 $\pm$ 54.84*	0.08 $\pm$ 0.01	1.89 $\pm$ 0.07*	79.64 $\pm$ 27.10*	35.95 $\pm$ 19.70*			
<b>W10</b>											
wild-type (6)	20.85 $\pm$ 2.84	0.54 $\pm$ 1.68	0.38 $\pm$ 0.04	120.33 $\pm$ 19.33	0.06 $\pm$ 0.01	0.54 $\pm$ 0.02	24.00 $\pm$ 7.32	7.25 $\pm$ 3.71			
db/db (4)	40.25 $\pm$ 1.17**	0.79 $\pm$ 0.10	2.31 $\pm$ 2.47*	354.75 $\pm$ 50.10*	0.11 $\pm$ 0.01	2.84 $\pm$ 0.03*	87.24 $\pm$ 25.61*	45.12 $\pm$ 31.40*			
<b>W12</b>											
wild-type (4)	23.26 $\pm$ 2.42	1.20 $\pm$ 0.31	0.44 $\pm$ 0.01	112.00 $\pm$ 38.20	0.09 $\pm$ 0.04	0.72 $\pm$ 0.11	11.03 $\pm$ 6.21	0.88 $\pm$ 0.43			
db/db (5)	43.48 $\pm$ 2.47**	2.10 $\pm$ 0.25	1.47 $\pm$ 0.51*	372.12 $\pm$ 49.25*	0.11 $\pm$ 0.02	2.97 $\pm$ 0.08**	77.69 $\pm$ 34.88*	19.28 $\pm$ 15.96*			
<b>W14</b>											
wild-type (7)	22.86 $\pm$ 2.36	0.81 $\pm$ 0.34	0.46 $\pm$ 0.10	104.12 $\pm$ 20.53	0.05 $\pm$ 0.01	0.44 $\pm$ 0.02	12.27 $\pm$ 6.83	3.66 $\pm$ 1.85			
db/db (5)	45.38 $\pm$ 2.76**	0.61 $\pm$ 0.23	2.78 $\pm$ 3.05*	414.25 $\pm$ 49.10**	0.08 $\pm$ 0.02	2.29 $\pm$ 0.06**	165.80 $\pm$ 87.93**	76.66 $\pm$ 65.46**			
<b>W16</b>											
wild-type (7)	23.09 $\pm$ 2.23	0.80 $\pm$ 0.28	0.46 $\pm$ 0.05	93.50 $\pm$ 14.00	0.06 $\pm$ 0.01	0.43 $\pm$ 0.01	16.58 $\pm$ 5.81	3.63 $\pm$ 3.60	0.08 $\pm$ 0.04	0.11 $\pm$ 0.05	0.48 $\pm$ 0.16 <sup>‡</sup>
db/db (7)	47.03 $\pm$ 4.03**	0.55 $\pm$ 0.20	1.15 $\pm$ 0.42*	469.75 $\pm$ 76.86**	0.07 $\pm$ 0.01	2.35 $\pm$ $\pm$ 0.06**	129.13 $\pm$ 48.49**	71.90 $\pm$ 40.37**	0.08 $\pm$ 0.03	0.12 $\pm$ 0.03	0.26 $\pm$ 0.06

TG, triglyceride. FFA, free fatty acid. HOMA-IR, homeostasis model assessment-estimated insulin resistance. MRGlu, rate of myocardial glucose uptake. LVW, left ventricular weight. HW, heart weight. BW, body weight.

\*Significantly higher than wild-type group. \*\*Significantly higher than baseline W8 values. †Significantly higher than db/db group.  $P < 0.05$  was considered significant.

significantly higher than baseline after age of 14 week. Regarding the relationship between  $K_i$ , MRGlu, and glucose level, no significant correlation was observed in neither db/db nor wild-type mice group (Figure 3). When both groups were pooled together,  $K_i$  ( $R^2 = 0.13$ ,  $P = 0.004$ ) and MRGlu ( $R^2 = 0.38$ ,  $P < 0.0001$  for MRGlu<sub>mean</sub>;  $R^2 = 0.55$ ,  $P < 0.0001$  for MRGlu<sub>max</sub>) depended on glucose level modestly. On the other hand, in db/db mice, we observed that  $K_i$  ( $R^2 = 0.38$ ,  $P = 0.002$ ) and MRGlu ( $R^2 = 0.44$ ,  $P = 0.0007$  for MRGlu<sub>mean</sub>;  $R^2 = 0.31$ ,  $P = 0.006$  for MRGlu<sub>max</sub>) were both inversely proportional to TG (Figure 4).

## DISCUSSION

Fatty acid oxidation is the predominant source (60-90%) of adenosine triphosphate in the normal heart,

with glucose oxidation and lactate contributing the rest [25]. In the diabetic heart, the contribution of glucose to total overall energy expenditure is diminished such that the heart muscle relies almost entirely on fatty acids for energy [26]. Ours is the first study to address the early metabolic alterations of LV myocardium in the obesity, type 2 DM mouse model using small animal PET/CT. Based on our findings, metabolic changes appear to precede morphological and mechanical changes of LV related to circulating substrate shifts.

PET imaging of the heart has been used to evaluate the myocardial metabolic pathways in diabetes in animal models as well as humans [13, 27-33]. In the present study, we demonstrated that before development of LV hypertrophy, db/db mice with preserved LV systolic function had enhanced myocardial glucose utilization in

**Table 2: Clinical and echocardiographic characteristics in wild type and db/db mice**

Parameters	Wild-type (n)			db/db (n)		
	W8 (8)	W12 (7)	W16 (7)	W8 (7)	W12 (7)	W16 (7)
<b>UCG</b>						
IVS, mm	0.68 ± 0.15	0.64 ± 0.08	0.60 ± 0.10	0.75 ± 0.93	0.68 ± 0.05	0.68 ± 0.06
PW, mm	0.72 ± 0.73	0.70 ± 0.11	0.63 ± 0.11	0.62 ± 0.11	0.69 ± 0.07	0.70 ± 0.08
EDD, mm	3.94 ± 0.42	4.11 ± 0.48	3.88 ± 0.48	3.57 ± 0.52	3.82 ± 0.30	3.84 ± 0.25
ESD, mm	2.47 ± 0.58	2.82 ± 0.47	2.60 ± 0.44	2.51 ± 0.37	2.62 ± 0.27	2.46 ± 0.44
EF, %	74.13 ± 8.26	66.51 ± 7.85	68.18 ± 6.41	65.66 ± 6.34	67.08 ± 0.18	71.25 ± 9.28
FS, %	38.20 ± 8.10	32.25 ± 5.38	33.00 ± 4.70	31.00 ± 4.41	31.96 ± 3.76	36.18 ± 8.83
<b>Weight, g</b>	23.65 ± 2.50	26.45 ± 2.87	28.08 ± 2.82	39.06 ± 2.74*	45.28 ± 3.02**	50.98 ± 2.49**
<b>Isolated heart (n= 5 for wild-type; n= 7 for db/db)</b>						
LVW, g			0.12 ± 0.03			0.13 ± 0.03
HW, g			0.15 ± 0.03			0.16 ± 0.03
HW/BW (%)			0.60 ± 0.01†			0.31 ± 0.06

LVW, left ventricular weight. HW, heart weight. BW, body weight. UCG, ultrasound cardiogram. IVS, interventricular septum.

PW, posterior wall, EDD, end diastolic dimension. ESD, end systolic dimension. EF, ejection fraction. FS, fractional shortening.

\*Significantly higher than wild-type group.

†Significantly higher than db/db group.

‡Significantly higher than baseline W8 values.

P<0.05 was considered significant.

the early stage of type 2 diabetes and obesity, associated with circulating substrates shifting. Contradictory to our study, previous small animal studies have demonstrated reduced myocardial glucose uptake or utilization in the diabetic heart, suggesting abnormalities in glucose transport. In four dynamic animal PET studies (Table 4), designs of the diabetic animal model, animal age and time points of PET imaging were different from those in our experiment. Two did not provide LV function data. On the other hand, two db/db mice studies [27, 31] reported diminished myocardial glucose uptake. Yue et al. [27] showed that LV mass and wall thickness were significantly increased in db/db mice group. Abdurrachim et al. [31] found diabetic mice had normal cardiac function, higher myocardial TG (~50%) and lower <sup>18</sup>F-FDG uptake (44%), and which the values of glucose uptake were seemed to be normalized upon pressure overload. The major difference was static <sup>18</sup>F-FDG PET with semi-quantitative method. MRGlu provided a more accurate evaluation of glucose consumption as compared with semi-quantitative method [34]. In addition, baseline characteristics including age, circulating substrate levels other than plasma glucose, and control group (wild-type vs. heterozygous control), were different from our study, which might influence the myocardial glucose utilization. In human research, Kim et al. [32] found that myocardial glucose uptake on static

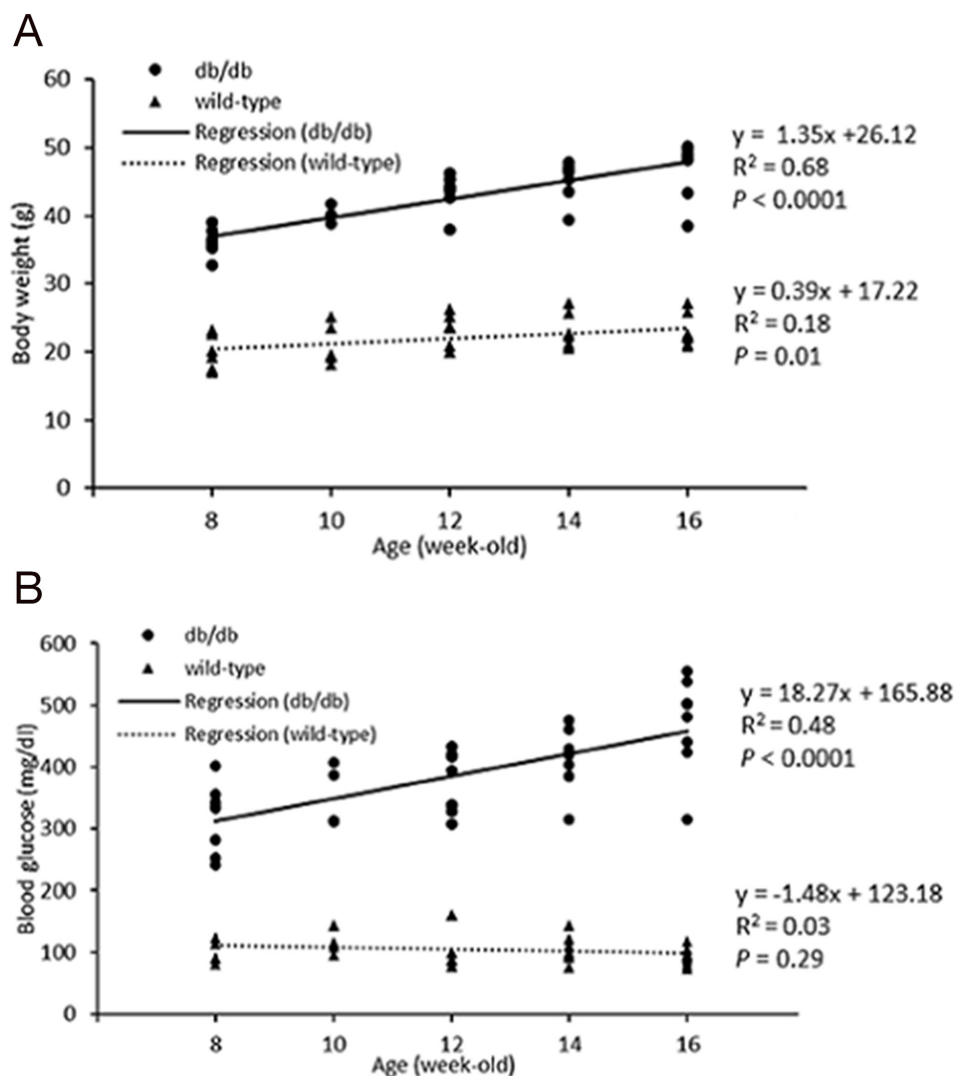
<sup>18</sup>F-FDG PET was significantly decreased in subjects with type 2 diabetes compared to the normal glucose tolerance or prediabetes groups. On the other hand, one quantitative study with <sup>18</sup>F-FDG under hyperinsulinemic-euglycemic clamping demonstrated that young male patients with less than a five-year history of insulin-dependent diabetes had similar insulin-stimulated myocardial glucose uptake compared with healthy controls [33]. The inconsistent findings might be related to study design, methodologies and most human studies on <sup>18</sup>F-FDG PET entailing a long history of type 2 DM plus multiple comorbidities and heterogeneous medications which these factors confound assessment of myocardial glucose utilization.

The natural history of db/db mice exhibited a distinct pattern [35, 36]. In the beginning, peripheral insulin resistance could be overcome by increased insulin secretion. Hyperglycemia developed when enhanced insulin secretion could no longer compensate for insulin resistance. Body weights of db/db mice increase progressively with greater lipolysis of endogenous adipose stores, leading to increased levels of circulating FFAs. Concordant to our findings, obesity, hyperglycemia, and elevated HOMA-IR index were present in db/db mice. The former two factors became progressively obvious as aging, in comparison with their genetic controls. Additionally, db/db mice tended to have higher circulating FFA and

insulin levels, albeit non-significantly as compared with wild-type mice.

The most important finding of this study was that db/db mice had a progressively increased rate of LV myocardial glucose uptake compared with wild-type mice, without significant changes on LV volumetric data, systolic function and mass, till 16 weeks of age. <sup>18</sup>F-FDG myocardial uptake varies greatly, because cardiac myocytes can derive energy from various competitive substrates, including FA, glucose, lactate, pyruvate, and ketones, depending on various physiologic and pathologic conditions. Glucose uptake is dependent on the transmembrane glucose gradient and density of glucose transporters and increased circulating glucose concentration and decreased FA levels could enhance glucose uptake [13, 28, 37]. Our data pointed out the trends that K<sub>t</sub> and myocardial MRGlu values correlated

with glucose concentration positively and with TG levels negatively. Taken together, it was speculated that early metabolic remodeling, which could be considered as adaptive, might precede the onset of contractile dysfunction in DM. Interestingly, Zhong et al. [15] have found a similar early adaptive response, enhanced myocardial glucose uptake, in a mouse model of pressure overload LV hypertrophy *in vivo*. Longitudinal evaluation of myocardial glucose metabolism and contractile function using dynamic <sup>18</sup>F-FDG PET and echocardiography allow us to track the footprints of deranged metabolic activity, LV structure and function in the diabetic heart. Although the mechanistic details leading to diabetic cardiomyopathy remain unclear, restoring cardiac metabolism seem to be a cornerstone. In light of the data provided here, a prospective therapeutic strategy focused on correcting the early imbalance of cardiac metabolism may be outlined



**Figure 1:** Body weight (A) and blood glucose (B) of wild type and db/db mice at 8, 10, 12, 14, and 16 weeks of ages. Body weight and glucose levels in db/db demonstrated moderate age-dependent increases. Body weight, not glucose, in wild type mice increased only minimally with age. *P* value of less than 0.05 was considered significant.

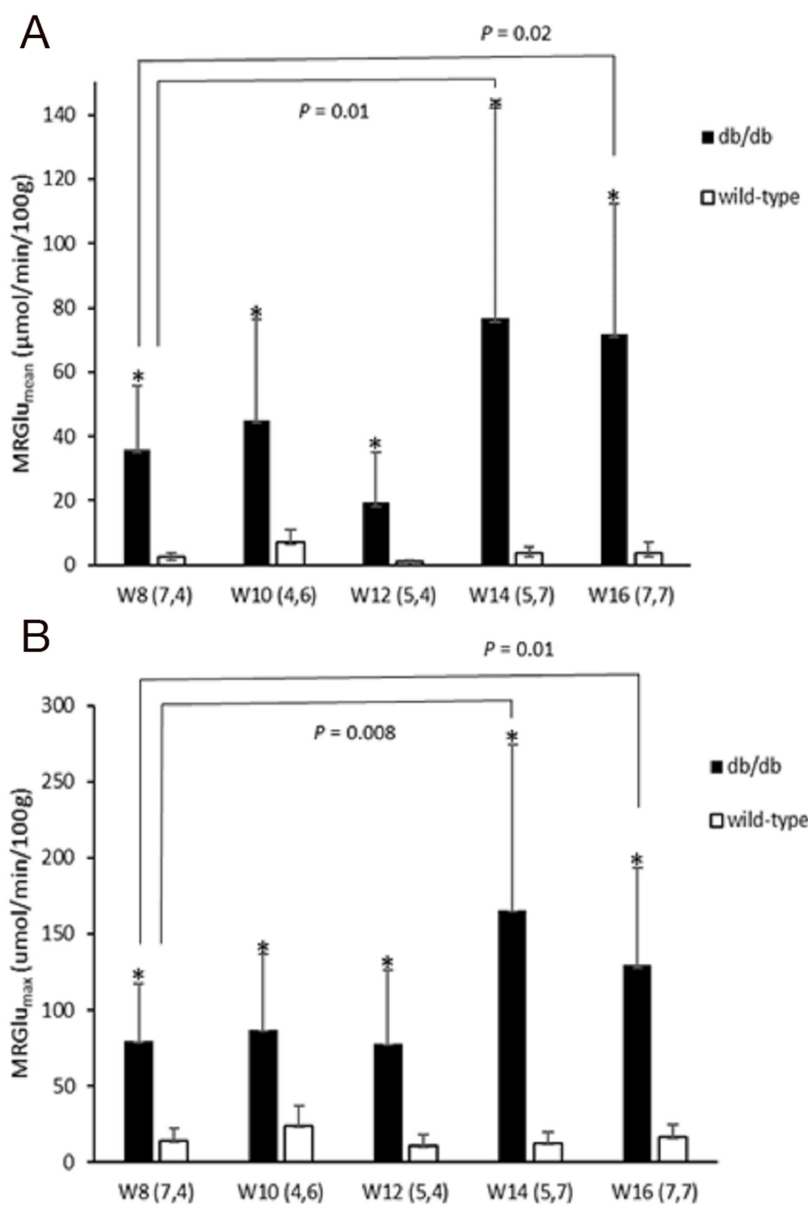


**Table 3: Experimental designs**

Mice models	Experiments	Time point (age in week)
1. db/db vs. wild-type (8/group)	NPO FDG PET (MRGlu) HOMA-IR, glucose, TG, FFA	8, 10, 12, 14, 16
2. db/db vs. wild-type (8/group)	Echocardiography	8, 12, 16

MRGlu, rate of myocardial glucose uptake. NPO, nil per os. HOMA-IR, homeostasis model assessment-estimated insulin resistance.

TG, triglyceride. FFA, free fatty acid.



**Figure 2:** Metabolic glucose utilization of the myocardium [MRGlu<sub>mean</sub> (A) and MRGlu<sub>max</sub> (B)] in wild type and db/db mice at 8, 10, 12, 14, and 16 weeks of age. Significantly higher MRGlu values were observed in db/db mice as compared with age-matched controls (all  $P < 0.05$ ). A statistically significant trend was also seen with time. Significance values are denoted above bar plots.

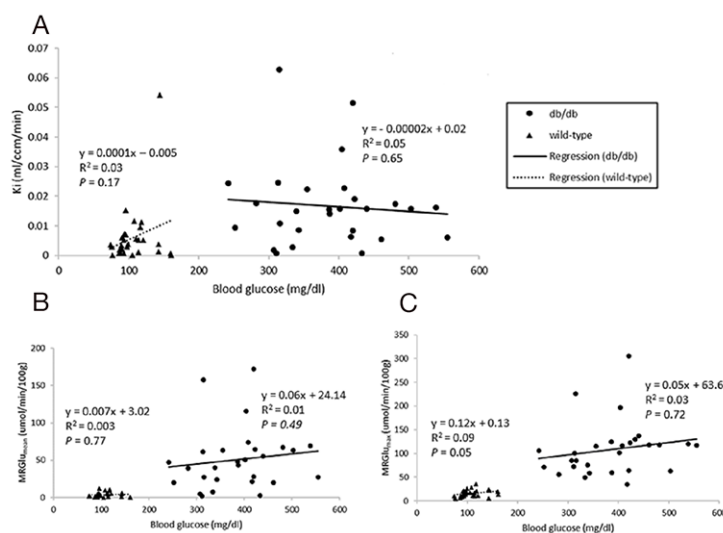
and may prevent cardiac malfunction and lethal coronary events. Additionally, the emergence of new heart-specific drugs for type 2 DM that target specific metabolic processes is creating a need to direct and monitor metabolic response. This is paving a path for personalized therapy directed by metabolic imaging [38].

Several issues should be considered when interpreting our results. Although db/db mice or wild-type mice had the relatively lower MRGlu at W12 which might be resulted from increased TG, the trend of myocardial glucose uptake rate described above existed in all-time points. The transient cause of plasma TG elevation was not clearly understood, possibly related to dietary condition or fasting hours. The significant correlation between glucose level and myocardial glucose uptake rate was only seen in pooled data. The reason might be due to that plasma glucose levels were significantly different between db/db and wild-type groups, while the range of plasma glucose levels within group was rather small, thus the statistical power was not enough in small sample size of each group.

Dietary conditions affect  $^{18}\text{F}$ -FDG PET imaging and it has been known that myocardial glucose uptake is diminished by fasting [39, 40]. Although some studies have used glucose load conditioning, fasting could make metabolic conditions uniform and produce similar imaging quality [15]. Because our purpose was to determine the rate of myocardial glucose uptake, a reasonable contrast between LV cavity and myocardial tissue was enough for us to draw regions of interest. Hyperinsulinemic-euglycaemic clamp was regarded as the gold standard in human  $^{18}\text{F}$ -FDG PET imaging. However, it was difficult to adopt in the rodent experiments (Table 4). Because glucose levels may affect myocardial glucose utilization, we performed HOMA-IR and multiple regression analysis

to evaluate the effects of circulating substrate levels on the rate of myocardial glucose uptake. However, we did not validate our results based on circulating substrate analysis, echocardiography, and PET measurement of glucose utilization by correlation to histological, tissue substrate, genetic or protein expression studies.

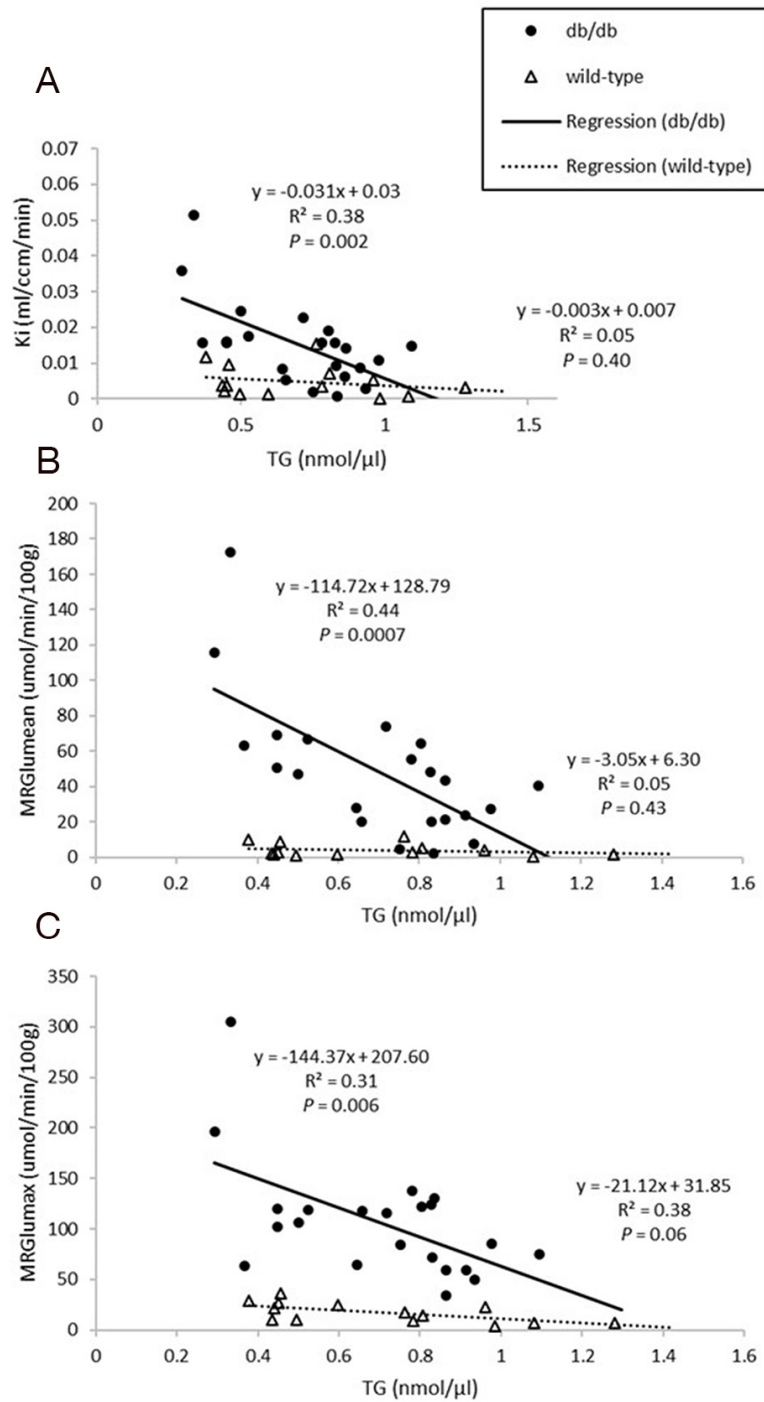
There were some methodologic limitations. We utilized intraperitoneal  $^{18}\text{F}$ -FDG injection instead of tail-vein injection. Although tail-vein injection is probably the route of choice for administrating radiopharmaceuticals or drugs to rodents in dynamic study, it is not easy to perform on mice. Additionally, reproducible intravenous injection is not possible for longitudinal studies. Intraperitoneal injection appears to be an alternative and has been proven to be reproducible, thereby reducing stress to the animal [41, 42]. Wong et al. 2011 [39] reported that both intraperitoneal and tail-vein routes of administrations provided equivalent pharmacokinetic parameters and comparable  $^{18}\text{F}$ -FDG biodistribution within 60 minutes after injection. Invasive nature and limited blood volume in mice precludes repeated blood sampling rendering imaged-derived input function desirable for quantification of glucose uptake. In our study, the LV cavity was used to measure  $^{18}\text{F}$ -FDG input function. However, in small animals, this image-derived volume might be less effective because limited spatial resolution and partial-volume effects result in spill-over from the myocardial tissue in later frames [28, 29, 43]. In our study, 17.5 % of PET images were uninterpretable, mostly because of non-, scarce or heterogeneous myocardial  $^{18}\text{F}$ -FDG uptake. Although several methods measuring the input function, such as a hybrid modeling approach [44] and an inferior vena cava time-activity curve [45, 46], have been proposed, further validations should be undertaken. The LC used to account for differences in transport and phosphorylation rates between  $^{18}\text{F}$ -FDG and glucose is



**Figure 3:** Relationships between blood glucose levels and  $K_i$  (A),  $\text{MRGlu}_{\text{mean}}$  (B),  $\text{MRGlu}_{\text{max}}$  (C) in the myocardium.  $P$  value of less than 0.05 was considered significant.

an important parameter in the determination of MRGlu in the dynamic PET [47, 48]. However, this value may vary, depending on circulating substrate and hormonal conditions. In the current work, the constant applied (LC = 0.67) was originally suggested for rabbits and modified for mice and rats [29, 46, 49]. The LC proportionally

affected the precision of the MRGlu value, but not the relative difference between the two groups. PET allows simultaneous measurements of radiotracer uptake and tissue kinetics in multiple organs [50-52]. However, we did not compare skeletal muscle due to higher background activity after intraperitoneal  $^{18}\text{F}$ -FDG injection.



**Figure 4:** Relationships between plasma triglyceride (TG) levels and Ki (A), MRGlu<sub>mean</sub> (B), MRGlu<sub>max</sub> (C) in the myocardium. Ki and MRGlu were both inversely proportional to TG in db/db mice (all  $P < 0.05$ ).



**Table 4: Previous FDG PET studies of animal heart *in vivo* for assessment of myocardial glucose utilization**

Reference	Animal models	FDG PET	Time points	Injection route	Glucose clamp	glucose uptake/MRGlucose	LV function
Yue et al., 2007 [27]	db/db and db/+ mice	Static	W9	IV	Insulin tolerance test	↓glucose uptake	Preserved EF
Shoghi et al., 2008 [13]	Type 2 diabetic ZDF rat	Dynamic	W14, W19	IV	No	↓MRGlucose, no difference in aging	Preserved FS
Ménard et al., 2010 [28]	Diet-induced Type 2 diabetic rat	Dynamic	W14	IV	Yes	↓MRGlucose	↓EF
Thorn et al., 2013 [29]	Type 1 diabetic STZ mice	Dynamic	Baseline, 2wk after STZ	IV	No	↓MRGlucose	No mention
Nemanich et al., 2013 [30]	Type 2 diabetic ZDF rat	Dynamic	W14	IV	No	↓MRGlucose	No mention
Abdurrachim et al., 2017 [31]	db/db and db/+ mice	Static	W10	IV	No	↓glucose uptake	Preserved EF

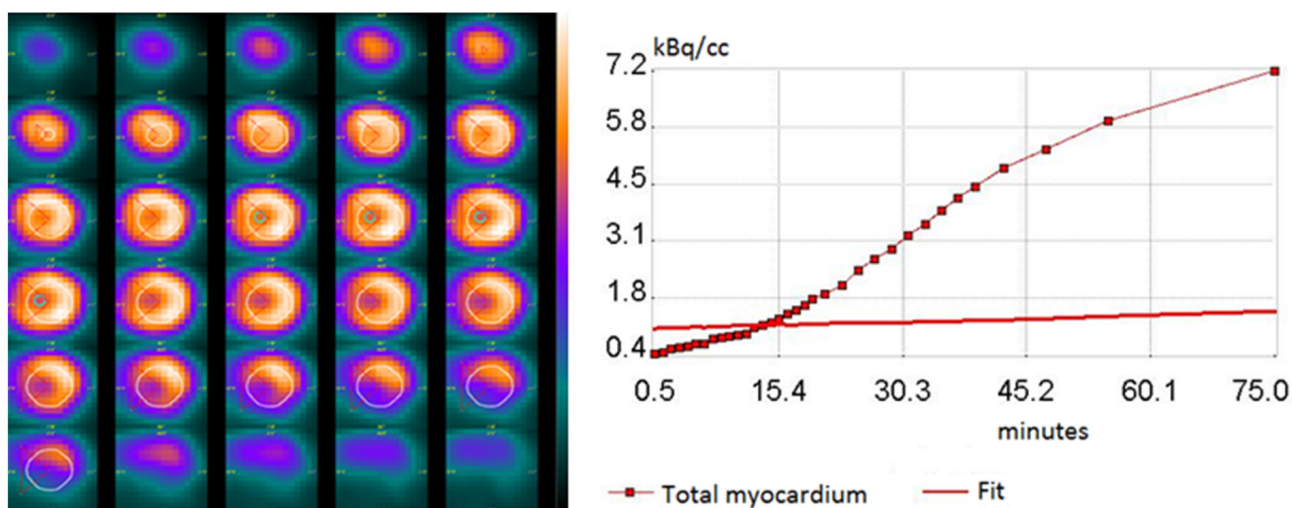
ZDF, Zucker Diabetic Fatty. STZ, streptozotocin. IV, intravenous. MRGlucose, rate of myocardial glucose uptake. FS, fractional shortening. EF, ejection fraction.

↑and↓stand for increased and decreased effect, respectively.

For reducing the physical and psychological stress from the anesthesia procedure, we designed two experimental models instead of one group receiving both PET study and echocardiographic measurement. Therefore, the animals were not exactly the same in two experiments. Moreover, due to unexpected death from the anesthesia procedure in mice, experiments could not be performed on all mice. Further work using finite

element mechanical model to determine ventricular deformation directly from nuclear imaging data will be considered [53].

Finally, isoflurane anesthesia is known to affect myocardial glucose uptake [54, 55]. However, this limitation is unlikely to influence the inter-group comparison, because all animals were exposed to the same anesthesia.



**Figure 5: Representative of myocardial time-activity curve together with the <sup>18</sup>F-FDG kinetics fitted model curve (right), and its transverse PET images in fasting mice after intraperitoneal injection of <sup>18</sup>F-FDG (left). The highlighted circle and radius demonstrated the circular volume-of-interest (VOI) which has a 60° angle indicating the septum location.**

## MATERIALS AND METHODS

### Animals used in this study

All experiments were performed in compliance with experimental animal care guidelines, and were conducted under protocols approved by the Institutional Animal Care and Use Committee at National Taiwan University College of Medicine and Far Eastern Memorial Hospital. Adult male db/db mice (BKS.Cg-Dock 7m +/+ Leprdb/JNarl) and wild-type C57BL mice (C57BL/6JNarl, used as non-diabetic controls) were obtained from the Laboratory Animal Center at National Taiwan University College of Medicine (Taipei, Taiwan). Experiments began when animals were 8 weeks of age. As shown in Table 3, in order to reduce the stress to animals and death from the anesthesia procedure, two protocols for PET and echocardiography were designed [27, 56, 57]. Sixteen mice (8 db/db, 8 control) underwent PET imaging and plasma subject analysis, including homeostasis model assessment-estimated insulin resistance (HOMA-IR), in 2-week intervals. A second group of eight db/db mice and 8 control (wild type) mice received echocardiography in 4-week interval.

### Animal preparation

The animals were freely fed standard laboratory diet and water in temperature-controlled facilities with a 12-hour light, 12-hour dark cycle (lights on at 6:00AM). Only before PET imaging, food was taken away at least 12 hours while water was given *ad libitum* in the evening before the studies. For PET and echocardiographic studies, mice were anesthetized via inhalational isoflurane and medical-grade oxygen. The gases were administered, via an induction chamber and custom-designed nose cone, at 2-2.5% isoflurane for induction and 1-1.5% isoflurane for maintenance throughout the imaging session. After completion of all experiments, anaesthetized animals were sacrificed and the heart was excised and weighed.

### Small-animal PET imaging protocol and imaging analysis

Small-animal PET was performed on the Argus PET-CT scanner (SEDECAL, Madrid, Spain). Prior to administration of  $^{18}\text{F}$ -FDG, each mouse received a scout scan for localization. Dynamic PET imaging was started immediately after  $^{18}\text{F}$ -FDG ( $15.0 \pm 3.0$  MBq) injection into the peritoneal cavity and continued for 90 minutes (20 x 60, 10 x 120, 2 x 300, 1 x 600, 1 x 1,800-s) in prone position [39]. After the PET scan, a CT study was acquired and the list-mode PET data were reconstructed using a filtered back projection algorithm with CT-based attenuation correction. Images were analyzed in a blinded manner using PMOD 3.6 software (PMOD Technologies Ltd., Zürich, Switzerland), which provides the quantification of dynamic PET of the

heart with a broad range of tracers in small animals including rodents [46, 58, 59]. In brief, volumes of interest (VOI) were drawn semi-automatically by use of a center-line within the myocardium in the projection of three dimensions (short-axis, vertical long, horizontal-long). To generate blood pool time-activity curves, VOI were placed within the LV cavity with care taken to avoid the boundary of myocardium and LV cavity and to reduce the partial volume effect. Time-activity curves were fitted to a Patlak kinetic model (Figure 5). The rate of myocardial glucose uptake (MRGlu) was calculated as  $\text{MRGlu} = \text{KiCglu}/\text{LC}$ , where Ki is the graphically defined Patlak slope, Cglu is the mean plasma glucose concentration at the start and end of the scan, and LC is the lumped constant, which is assumed to be 0.67 as estimated for rodents. The data of MRGlu were summarized by volume-weighting averaging to a 17-segment model based on the American Heart Association classification. To exclude the potentially confounding effects from averaging multiple segments ( $\text{MRGlu}_{\text{mean}}$ ), values in the maximal segment of each subject ( $\text{MRGlu}_{\text{max}}$ ) were also calculated.

### Circulating substrate analysis and HOMA-IR Index

On the day of the PET study, whole blood samples were drawn from the retro-orbital venous sinus for substrate analysis. Plasma glucose levels were measured by placing whole blood on a glucose test strip for immediate analysis, using a plasma blood glucose analyzer [Accu-Chek; Roche Diagnostics, Inc.]. Serum samples were separated from whole blood samples by centrifuging at 13,000 rpm for 20 min (Axygen® 1.5mL MaxyClear Snaplock Microcentrifuge Tube, Polypropylene, Red, Nonsterile, Product #MCT-150-R) and placed in a -20°C freezer prior to analysis. Triglyceride (TG) and free fatty acid (FFA) levels were measured using Quantification Colorimetric/Fluorometric Kits (BioVision, CA, USA). Insulin levels were measured using a mouse insulin ELISA test kit (Mercodia, Uppsala, Sweden). All kits were commercially available, and used well-documented methods that have been validated in small animals [60], and all measurements had an SD of  $\leq 5\%$ .

HOMA-IR, developed by Matthews *et al.* [61] has been used widely in research for estimation of insulin resistance. The value was calculated by multiplying fasting plasma insulin (mU/L) by fasting plasma glucose (mmol/L), then dividing by the constant 22.5. The substrate values reported in the study correspond to values obtained at the baseline of PET, just before  $^{18}\text{F}$ -FDG injection.

### Echocardiography measurement

Echocardiography on mice was performed using a GE Vivid 7 Ultrasound machine (General Electric Company, Fairfield, CT, USA) with GE I13L probe (5.8 to 14.0 MHz) at ages 8, 12, and 16 weeks. Animals were

anesthetized via isoflurane inhalation and secured on an imaging platform in the supine position, then the anterior chest was shaved and ultrasonic coupling gel was applied. Care was taken to maintain adequate contact and to avoid excessive pressure on the chest. Complete 2-dimensional and M-mode examinations were performed from multiple views. Mean fractional shortening (FS), left ventricular ejection fraction (LVEF), end-diastolic dimension (EDD), and end-systolic dimension (ESD) were obtained after repeated measurement for three times by two operators (Liu CW, Wu YW) in consensus [62].

## Statistics

Data processing and statistical analysis were performed using either Microsoft Excel or Medcalc Statistical software (Brunswick, Maine, USA). Continuous variables were expressed as mean±SD. The two-tailed student t-test or Mann-Whitney rank sum test were performed to test for significant difference between and within groups. Regression analysis was performed in the two groups to characterize the dependence of blood glucose, body weight, MRGlu on age and Ki, MRGlu on circulating substrate levels. The significance level was set at 0.05.

Regarding sample size, power analysis (type 1 error: 5%; power: 80%; two-tailed test; t-test for difference of myocardial glucose utilization between control and diabetic mice) and the information of effect size was taken from previously studies [13, 16]. The result was 7 animals per group with expecting 10% attrition, and final sample size 8 animals per group.

## CONCLUSION

In our study, noninvasive serial imaging of the diabetic heart using dynamic PET has revealed that enhanced uptake rate of <sup>18</sup>F-FDG by the myocardium in type 2 DM and obese mice precedes the onset of LV hypertrophy and contractile dysfunction.

## Author contributions

Conception or design of the work: Ko, Wu  
Data collection: Ko, Wu, Liu, Cheng, Yen, Yang  
Data analysis and interpretation: Ko, Wu, Liu, Cheng, Yen, Yang  
Drafting the article: Ko  
Critical revision of the article: Ko, Wu  
Final approval of the version to be published: All authors

## ACKNOWLEDGMENT AND FUNDING

We gratefully acknowledge the assistance of Ms. Yu-Shuan Hung in the lab work, and Mr. Pei-Yao Lin and Chi-

Han Wu in small animal PET/CT studies. This work was supported in part by the Small Animal PET/CT Core Facility of Molecular Imaging Center, National Taiwan University, and Animal Research Center of Far Eastern Memorial Hospital.

## CONFLICTS OF INTEREST

The authors have declared that no Conflicts interests exist.

## FUNDING

This work was supported by research grants from the Ministry of Science and Technology of Taiwan (MOST 101-2314-B-418-012-MY3, 104-2314-B-418-008 and 105-2628-B-418-002-MY2). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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