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Dysglycemia and Dyslipidemia Models in Nonhuman Primates: Part I. Model of Naturally Occurring Diabetes

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Abstract

Insulin-resistant diabetes (Type 2 diabetes mellitus, T2DM) is one of the main comorbidities of obesity and is the most common form of diabetes. T2DM and obesity dynamically influence each other and often escalate patients' other health issues. Cardiovascular, renal and other health consequences of obesity and diabetes have been studied for several decades. However, the underlying precise mechanisms and interactions of obesity and insulin-resistant diabetes have yet to be elucidated further. It has been recognized that sustained greater energy intake than expenditure is the main cause of obesity that can potentially lead to insulin resistance and diabetes due to excessive fat accumulation. To better understand the pathophysiology of human obesity and diabetes, nonhuman primate (NHP) models have been used for research to delineate molecular and cellular mechanisms because of the similarity of the metabolic diseases between NHP and humans. Also, NHP models have been well used for testing new novel therapies, which provides critical pre-clinic information for drug discovery. This article summarizes the data collected from a large scale of the naturally occurring diabetes monkeys housed in our facility. Manuscripts for other NHP models, such as diet-induced dyslipidemia and dysglycemia and streptozocin-induced diabetes, developed in our facility will follow lately.

Keywords: Nonhuman primate; Diabetes; Animal model; Insulin resistance; Nephropathy

Abbreviations: AAALA: Association for Assessment and Accreditation of Laboratory Animal Care; ACE: Angiotensin Converting Enzyme; ACR: Urine Albumin to Rrine Creatinine Ratio; ACTH - Adreno-Cortico-Trophic Hormone; AGM - African Green Monkey; ARB - Angiotensin II Receptor Antagonist; AUC - Area Under the Curve; BMI - Body Mass Index; BUN - Blood Urea Nitrogen; CRP - C-reactive Protein; CVD - Cardiovascular Disease; DBP - Diastolic Blood Pressure; DM - Diabetes Mellitus; eGFR - Estimated Glomerular Filtration Rate; HbA1c - Hemoglobin A1c; HDL-c - High Density Lipoprotein-Cholesterol; HR - Heart Rate; IACUC - Institutional Animal Care and Use Committee; ITT - Insulin Tolerance Test; ivGTT - Intravenous Glucose Tolerance Test; LDL-c - Low Density Lipoprotein-Cholesterol; MAP- Mean Arterial Pressure; MMTT - Mixed-Meal Tolerance Test; M rate - Glucose Handling Rate; NHP - Non-human Primate; oGTT - Oral Glucose Ttolerance Test; PD – Pharmacodynamics; PK – Pharmacokinetics; SBP - Systolic Blood Pressure; T2DM - Type 2 Diabetes Mellitus; TC - Total Cholesterol; TG - Triglycerides

Introduction

Obesity (body mass index >30 kg/m^2) becomes a serious epidemic issue in many developed and developing countries. Economic growth, urbanization, societal changes, modernization, and decreased physical activity have driven the obesity epidemic over recent decades. Globally, the estimation in 2008 was more than 1.5 billion overweight adults, of which approximately 500 million were obese [1,2]. Obesity can lead to several health problems, such as diabetes and cardiovascular diseases [3,4] and is one of the leading causes of morbidity and mortality. Obesity can result in insulin resistance and eventually develop to Type 2 diabetes mellitus (T2DM). Several hypotheses for insulin resistance have been postulated and documented. Among those are inflammation, mitochondrial dysfunction, hyperinsulinemia and lipotoxicity [5,6]. Others, such as genetic background, aging, fatty liver, endoplasmic reticulum stress, hypoxia and lipodystrophy are the areas under active research for understanding mechanisms of insulin resistance [5].

The evidence connected to obesity-associated health comorbidities has mainly been obtained from epidemiological analyses of human subjects. Evidence shows that dyslipidaemia accelerates the atherosclerotic process and its morbid consequences. Nutraceuticals and functional food ingredients may potentially be beneficial to vascular health and reduce the overall cardiovascular risk from dyslipidemia [7]. However, the precise molecular and cellular mechanisms of obesity-associated health problems, including diabetes, have not yet been fully elicited. Various animal models have been used in research for understanding obesity/diabetes mechanisms and discovery of novel therapies for the diseases [8-11]. Animal models can also be extended to determine if weight loss results from fat loss and/or from beneficial changes in key blood parameters (e.g. insulin). Typically, animal models have excellent predictive validity whereby drug-induced weight loss subsequently translates to weight loss in man [12,13]. Currently, more studies related to obesity/diabetes research are moving toward using non-human primates (NHPs) which have better patho-physiological metabolism similarities to humans [14,15]. NHP models also play an important role in the screening of novel compounds for regulation of food intake, blood glucose and/or body weight, which can result in discovery and validation of new mechanism and therapeutic strategy or target [16,17].

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This article introduces the characteristics of one NHP diabetic model, naturally occurring diabetes, which has been highly valuable for research and treatment of dyslipidemia and dysglycemia [14-17]. Other NHP models, such as diet-induced dyslipidemia and dysglycemia and streptozocin-induced diabetes, developed in our facility will be introduced lately via other manuscripts. While no single model is necessarily applicable to every dysmetabolic research program and drug discovery, it has been suggested that certain models have great validity. Therefore, if possible, selectively using of those preferable models to achieve the best potential outcome is recommended in order to address the very challenging diseases, obesity and diabetes. Normal and obese/diabetic monkeys housed in our animal center are collected periodically from the monkey farms in China and then raised in our own facility with a normal calorie monkey chow (Beijing Keao Xieli Feed Co., LTD, Beijing, China) enriched with seasonal fruits and vegetables in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) regulations and guidelines [18-20]. All the procedures for sample or data collection used in this study were approved by the Institutional Animal Care and Use Committee (IACUC) (Crown Bioscience, Inc., Taicang, Jiangsu province, The People's Republic of China) [18-20].

Animal grouping and their general characteristics

As humans and other great apes (Hominoidea) diverged from Old World monkeys (Cercopithecoidea) [21,22], obesity and diabetes occur in Old World monkeys, which would thus provide a good research model to study human obesity and diabetes [23]. Monkeys raised in indoor cages increase the chance to become obesity and facilitate their development to obesity-associated diseases in an age-dependent manner when given food ad libitum [22,24-26]. Like humans, these monkeys develop type 2 diabetes and diabetic complications, such as nephropathy, orthmopathy, neuropathy and cardiovascular complications [22,27].

In order to clarify the various groups, the criteria are set up for grouping the monkeys from normal to diabetes mainly based on consecutively fasted serum glucose levels. Three groups of cynomolgus macaques (Macaca fascicularis): normal, pre-diabetes and diabetes are listed in Table 1a. Experimental evidence clearly shows that normal fasting serum glucose concentrations for monkeys are about 30 mg/dL lower than for normal humans [14,16]. The monkeys housed in our facility with their fasting serum glucose ≤ 80 mg/dL (69 ± 1.5 mg/dL, mean ± SE, n=32, Table 2) were listed in the normal group. Those higher than 80 mg/dL and lower than 105 mg/dL were categorized into the pre-diabetes group (90 ± 1.3 mg/dL, n=38, Table 2, $p<0.05$, vs. Normal). Those animals showed their fasting serum glucose levels equal or higher than 105 mg/dL were placed in the diabetes group (207 ± 8.7 mg/dL, n=88, Table 2, $p<0.05$, vs. Normal and Pre-diabetes). In the mean time, blood insulin levels were also referenced for grouping the monkeys into the normal and diabetes groups (Table 1b). HbA1c level and assays, such as ivGTT (intravenous glucose tolerance test), ITT (insulin tolerance test) and glucose clamp (see the details in the section Diabetes validation methods below) were conducted to provide supplemental evidence for grouping an animal into which group.

The general metabolic characteristics of the grouped monkeys being housed in CrownBio animal facility are shown in Table 2. Clearly, the monkeys in the diabetes group were significantly older and showed obvious dysglycemia and dyslipidemia. The distribution of the monkeys was plotted against various serum glucose levels (Figure 1A). Among them 88 monkeys (11 insulin-dependent, 77 insulin-independent) were identified as having naturally occurring diabetes. Thirty-eight monkeys were pre-diabetic based on the criteria listed in Table 1a. Most of the studied monkeys are currently alive and housed in our animal facility for diabetes and dyslipidemia research. The collected diabetic monkeys were first noticed by elevated fasting blood glucose generally and then monitored closely and continuously for at least 3 months. The housed normal monkeys consuming a normal calorie chow diet showed their over-night fasted serum glucose concentrations less than 80 mg/dL, which was reported by others [14,16] and also by us (Table 2) [18-20].

Correlations between various glycemic and lipidemic parameters

In the experimental animals the age was not correlated well with the body weight, insulin, C-peptide, LDL-c and HDL-c, but was significantly correlated with the serum glucose, HbA1c, TG and TC ($p<0.001$, Table 3). However, the body weight was correlated well with the serum insulin, TC and LDL-c ($p<0.05$ or 0.001, Table 3), but not the glucose ($p>0.05$). The distribution of the serum insulin concentrations of individual monkeys was in a wide range, from 3 to 864 μIU/mL (Figure 1B) and no correlation was found between the serum glucose and insulin ($p>0.05$, inset in Figure 3B). However, the correlations between serum glucose and HbA1c (Figure 1C) and between serum C-peptide and insulin (Figure 1D) were significant ($p<0.001$).

The body mass index (BMI, body weight/Crown-rump length^2, kg/m^2) was measured in 62 monkeys and the distribution of the BMIs of individual monkeys is shown in Figure 2A. Interestingly, their body weights were highly correlated with their BMIs (n=62, $p<0.001$, Figure 2B). The other correlations of glycemic and lipidemic in the studied monkeys (n=158) were also tested (Table 3). Serum TG correlated well with TC ($p<0.001$, Figure 2C) and the correlation between serum

Group	Glucose (mg/dL)
Normal	≤ 80
Pre-diabetes	>80 - <105
Diabetes	≥ 105

Table 1a: Grouping the monkeys based on their fasting serum glucose levels.

	Normal	Diabetes						
		Mild			Moderate			Severe
Glucose (mg/dL)	<80	80–100	or	<80	100–126	or	≥126	≥150
Insulin (μIU/mL)	<90			≥90			≥90	<50

Table 1b: Grouping the monkeys based on their fasting serum glucose and insulin levels.

Parameter	Normal	Pre-diabetes	Diabetes
	n=32 (M/F, 27/5)	n=38 (M/F, 32/6)	n=88[$] (M/F, 60/28)
Age (y)	9.7 ± 1.0	12.3 ± 0.9	16.4 ± 0.4*#
Body weight (kg)	8.0 ± 0.5	8.8 ± 0.5	8.7 ± 0.3
Glucose (mg/dL)	69 ± 1.5	90 ± 1.3*	207 ± 8.7*#
Insulin (μIU/mL)	61 ± 11.5	91 ± 19	85 ± 12
C-peptide (nmol/L)	1.52 ± 0.17	1.61 ± 0.24	1.83 ± 0.15
HbA1c (%)	4.4 ± 0.1	5.2 ± 0.4	8.9 ± 0.5*#
TG (mg/dL)	69 ± 8	76 ± 7	324 ± 72*#
TC (mg/dL)	132 ± 21	120 ± 6	165 ± 14#
HDL-c (mg/dL)	53 ± 3	55 ± 4	48 ± 2
LDL-c (mg/dL)	42 ± 5	45 ± 3	58 ± 5

*, $p<0.05$; versus Normal. #, $p < 0.05$; versus Pre-diabetes. [$], Eleven insulin-dependent diabetes monkeys were included in the 88 diabetes ones

Table 2: General characteristics of the grouped animals.

glucose and TG was also significant (p<0.001, Figure 2D). In addition, serum TG and TC correlated well with LDL-c (p<0.001), but not well with HDL-c (p>0.05, Table 3).

C-reactive protein (CRP) produced by the cells in the liver is a biomarker of body inflammation. Its blood level may increase under inflammation and has been linked to vascular atherosclerosis which is thought to have an inflammatory component [28]. To look at the potential relationship between the inflammatory biomarker and dysmetabolism, the correlations between serum CRP and individual glycemic or lipidemic parameters were analyzed. It is interesting that the correlations between serum CRP and insulin and between serum CRP and TG were statistically significant (p<0.05, Table 3). However, the correlations between serum CRP and glucose, C-peptide or TC were not significant (p>0.05, Table 3). The correlations between serum CRP and age, body weight, HbA1c, LDL-c or HDL-c were also insignificant (data not shown).

Diabetes validation methods

There are several methods to help diagnosis of diabetes and validate the status/stages of the disease. Three commonly used methods, ivGTT, ITT and glucose clamp, were introduced in this section and tested in normal and diabetic NHPs. The results obtained from the tested animals varied among the groups. Other methods, such as oral glucose tolerance test (oGTT) and mixed-meal tolerance test (MMTT), are also commonly used in diabetic research and clinic tests, but not introduced and discussed here due to our intentionally condensing the article.

Intravenous glucose tolerance test (ivGTT)

ivGTT is a well-established method of measuring insulin resistance and β-cell insulin secretory response to hyperglycemia. The simplified ivGTT that lasts for 30 min was used in our study to evaluate the metabolic stages of the monkeys [29,30]. The data was analyzed via the comparison between the "strength" of the insulin response (the area under the curve, AUC) and the elimination kinetics of glucose (Kglucose). Forty nine monkeys, 23 females and 26 males, aged between 5 to 22 yrs (mean=14 yrs) with a body weight of 3-15 kg (mean=7 kg), were used for ivGTT according to the method reported previously [29,30]. The monkeys were objectively divided into 3 distinct groups based on the criteria (Table 1a), normal (N, n=11), pre-diabetic (Pre-DM, n=16) and overtly diabetic (DM, n=22). The animals were fasted for overnight 16 hrs and anesthetized with an initial dose of ketamine at 10 mg/kg intramuscularly (i.m.) and then with 5 mg/kg supplement if needed. The cephalic and/or saphenous veins were cannulated separately for glucose infusion and blood collection. Glucose (0.25 g/kg=0.5 ml/kg of 50% dextrose) was intravenously infused within a 30 second period and the system was flushed with 5 ml saline to remove residual glucose. Blood was collected immediately before and then at 3, 5, 7, 10, 15, 20, 30 min after glucose infusion. Collected blood samples were kept at room temperature (~21°C) for 30 min and then centrifuged at 3,500 rpm for 10 min to obtain the serum. Serum glucose was measured by the glucose oxidase method used in the hospital laboratory (Taicang 1st People's Hospital, Jiangsu, China). Serum insulin was measured using ELISA kits (Mercodia AB, Uppsala,

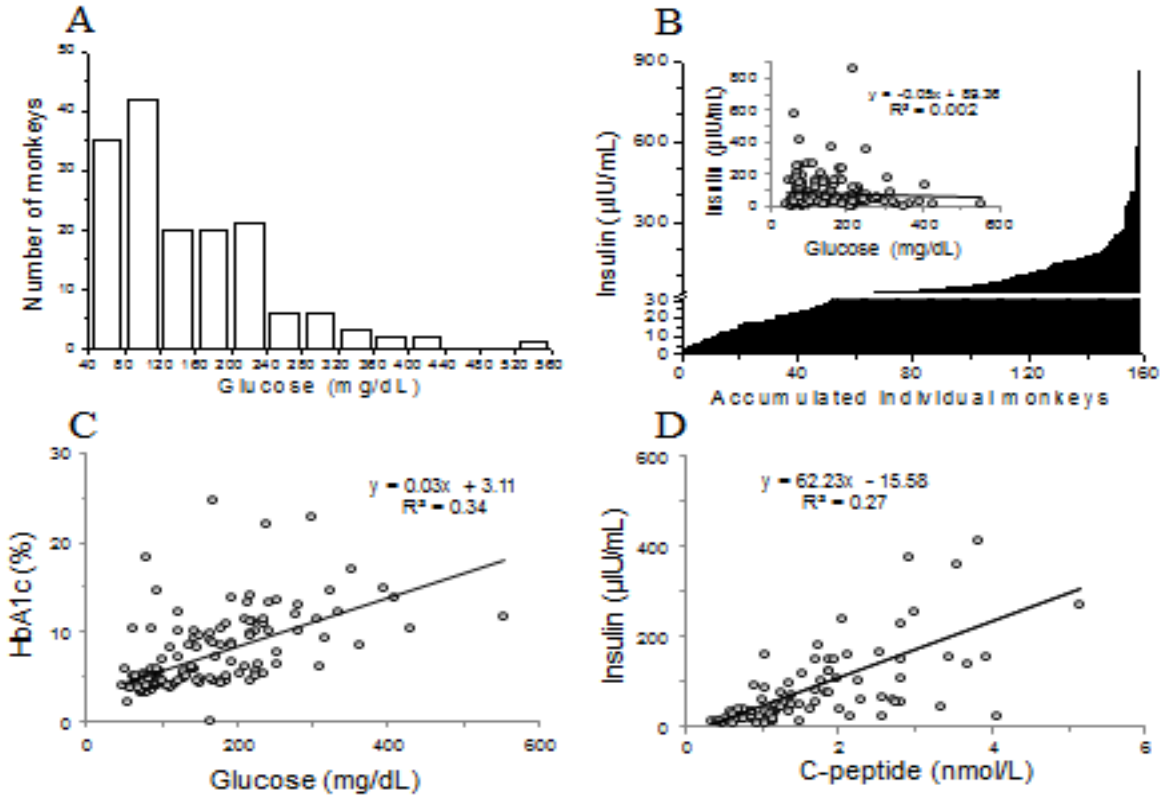

Figure 1: Distributions of fasting serum glucose (***A***) and insulin (***B***) concentrations for cynomolgus monkeys (n=158) and correlations between glucose and insulin (***B***-inset, p>0.05), glucose and HbA1c (***C,*** p<0.01), as well as C-peptide and insulin (***D,*** p<0.05).

Correlation	r^2	*p*
Age/BW	0.00	> 0.05
Age/Glucose	0.20	**< 0.001**
Age/Insulin	0.00	> 0.05
Age/C-peptide	0.00	> 0.05
Age/HbA1c	0.14	**< 0.001**
Age/TG	0.08	**< 0.001**
Age/TC	0.04	**< 0.001**
Age/LDL-c	0.01	> 0.05
Age/HDL-c	0.01	> 0.05
BW/Glucose	0.00	> 0.05
BW/Insulin	0.19	**< 0.001**
BW/C-peptide	0.11	> 0.05
BW/HbA1c	0.00	> 0.05
BW/TG	0.00	> 0.05
BW/TC	0.03	**< 0.05**
BW/LDL-c	0.04	**< 0.05**
BW/HDL-c	0.02	> 0.05
TG/LDL-c	0.16	**< 0.001**
TG/HDL-c	0.07	> 0.05
TC/LDL-c	0.55	**< 0.001**
TC/HDL-c	0.01	> 0.05
CRP/Glucose	0.00	> 0.05
CRP/Insulin	0.16	**< 0.05**
CRP/C-peptide	0.06	> 0.05
CRP/TG	0.21	**< 0.05**
CRP/TC	0.08	> 0.05

Table 3: Correlations among physiological and biochemical parameters.

Sweden). Glucose disappearance or clearance rate (*K*glucose) was expressed as the rate of glucose being removed from the circulation. Hansen introduced a method to calculate *K*glucose by the slop of 2 linear points selected from the glucose concentration decay curve, e.g., at 5 and 20 min, or other 2 time points [31]. This method is simple but its accuracy could be compromised by the random noise of the limited time points that may not represent the entire glucose decay curve. Like many small molecular compounds, the glucose distribution in the circulation following intravenous administration is fitting the one compartment kinetic model (glucose blood volume distribution/glucose concentration AUC). Based on this concept, the *K*glucose can be calculated by the slope of a linear trend best fitting the natural logarithm of the blood glucose concentrations of all the time points using the least square method with Microsoft Excel formula: LINEST (LN(blood glucose concentrations at each time point), 3, 5, 7, 10, 15, 20, 30 min) [19]. The *K*glucose values calculated by this method is very close to Hansen's method, however, with much less variability because it utilized all the data points from the glucose decay curve that significantly reduced the random errors from the limited time points used by Hanson's method.

The experimental data in Figure 3 clearly shows that compared to the normal group, the glucose clearance rates (*K*glucose) were significantly decreased in the animals of both pre-DM ($p<0.01$) and DM ($p<0.001$) groups (Fig. 3A, 3C). In contrast, the glucose AUC was significant lower in the normal NHPs than those in the pre-DM ($p<0.05$) and DM ($p<0.001$) animals (Figure 3D). The insulin AUC was the highest in the pre-DM monkeys and was the lowest in the

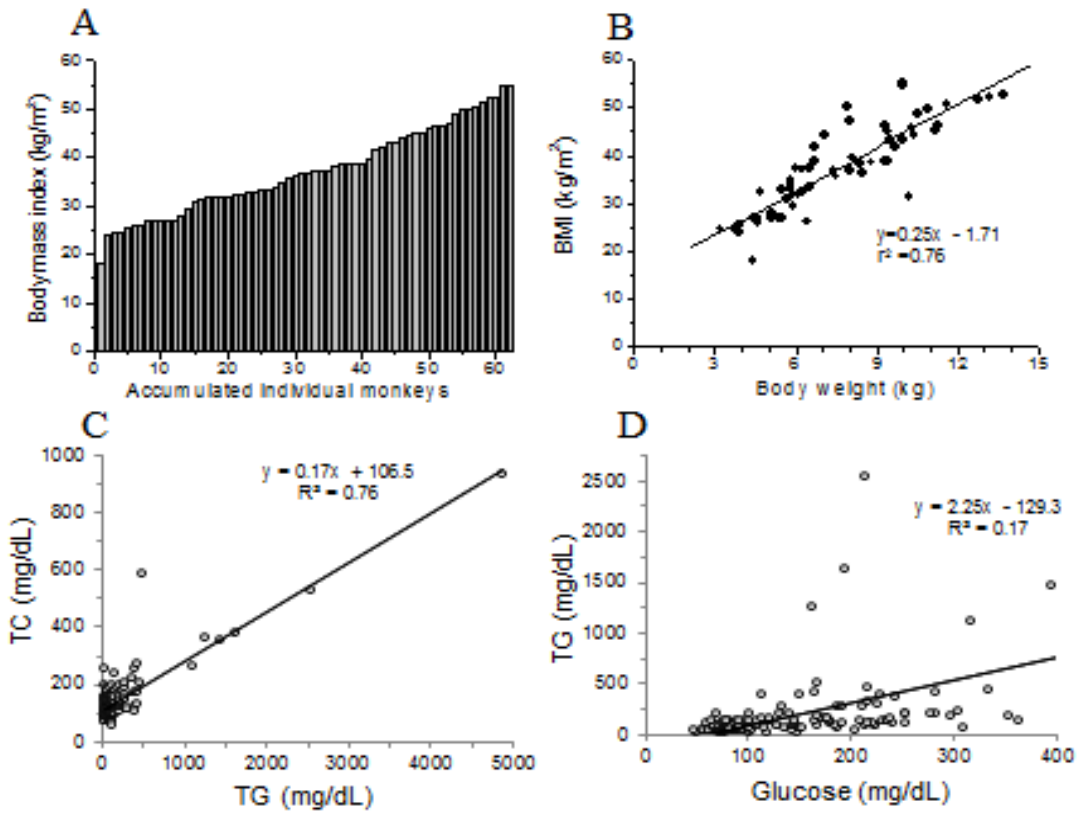

Figure 2: Distribution of body mass index (BMI) for cynomolgus monkeys (n=62, ***A***) and correlations between body weight and BMI (***B***), TG and TC (***C,*** $p<0.001$), as well as glucose and TG (***D,*** $p<0.001$).

DM group (Figures 3B and 3E). The data indicate that the capability of handling glucose metabolism was significantly reduced in both pre-DM and DM animals, but was more obvious and severe in the DM group due to impaired insulin production, secretion and utilization. Insulin production and secretion of the pre-DM animals were significantly enhanced, which mainly resulted from the compensation to the reduced sensitivity to insulin.

Insulin tolerance test (ITT)

The insulin tolerance test is to examine if the body is able to produce enough ACTH (adreno-cortico-trophic hormone) and growth hormone under stress. The 'stress' in the test is low blood sugar (hypoglycemia) by injected insulin under very controlled conditions. ACTH stimulates the adrenal glands to make cortisol which is a steroid hormone and has many functions, including balancing the effect of insulin in regulating blood glucose level. ITT was conducted in 16-hr-fasted monkeys (n=11, 7 male and 4 female with their ages from 7 to 19 yrs) under ketamine-anesthesia (10 mg/kg, i.m.). Cannulation of the cephalic and/or saphenous veins was conducted for insulin injection and blood sampling for glucose measurement. After the 1st blood glucose test insulin at a dose of 0.5 IU/kg was administered intravenously to each monkey to low its blood sugar. The blood glucose level was monitored continuously after insulin injection (Figure 4). The averaged fasting blood glucose level before insulin injection was 77 ± 5 mg/dL for the normal group (n=5) which was significantly lower than 206 ± 11 mg/dL for the DM group (n=6, $p<0.001$, Figure 4C). The blood glucose levels were decreased gradually in both normal and diabetes groups after insulin injection (Figure 4A), but the decrease in the DM group was obviously slower with a smaller K ($p<0.05$) and a larger $t_{1/2}$, but not significant ($p>0.05$, Figures 4B and 4D), compared with the normal group.

Glucose clamp

The hyperinsulinemic euglycemic glucose clamp is the "gold standard" for the validation of insulin production and sensitivity. Hyperinsulinemic-euglycemic clamp analysis was performed in overnight 16-hr-fasted monkeys (n=13) under ketamine-anesthesia (10 mg/kg, intramuscularly). Cannulation of the cephalic and/or saphenous veins was conducted for insulin and glucose infusion, and blood drawing for glucose measurement. Insulin (biosynthetic human insulin, Novo Nordisk, Denmark) was diluted to 300 mU/mL by isotonic saline to which 2 mL of the subject's blood per 50 mL were added in order to avoid adhesion of insulin to the syringe and infusion tube plastic surface. Insulin infusion at various rates was given during the 1st 10 min to quickly adjust blood glucose near a targeted level. The infusion rate for the hyperinsulinemic-euglycemic clamp was then maintained at 40 mU/m^2 Surface Area*min as reported previously [32].

A variable amount of 20% D-glucose was intravenously infused to maintain blood glucose levels in a euglycemic range. Blood samples were taken every 5 min and glucose was measured by a glucose analyzer (Accu-Chek Active, Roche Diagnostics, Indianapolis, IN, USA) to allow adjustment of the glucose infusion rate accordingly. When blood glucose was adjusted and balanced for approximately 90 to 150 min and then for a 40-min stable period of the glucose levels clamped in a range of 55 to 75 mg/dL under constant infusion of glucose. The difference between the glucose metabolic rates (M rates) in normal and diabetes

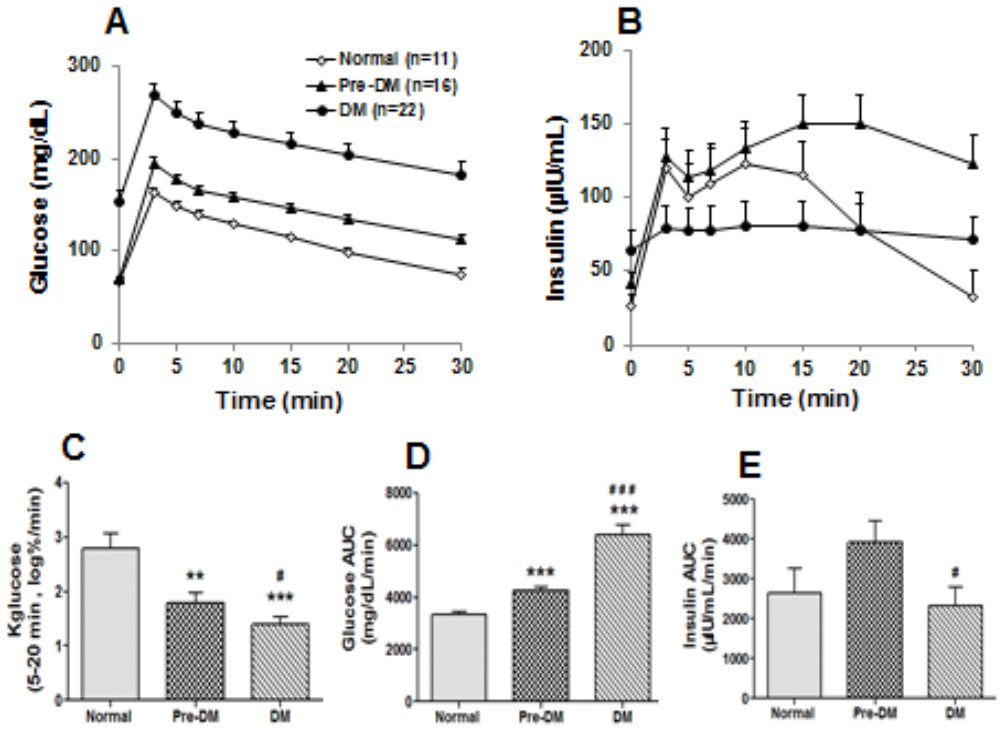

Figure 3: Intravenous glucose tolerance test (ivGTT) in normoglycemic, pre-DM and DM monkeys. Compared with the normal monkeys, the animals in pre-DM and DM groups showed significant insulin tolerance (***A***) with an increase in serum insulin level in pre-DM animals (***B***) and in contrast, a decrease in DM animals (***B***). The *K*glucose (***C***), glucose AUC (***D***) and insulin AUC (***E***) significantly increased in pre-DM and DM groups, compared with the normal animals. **, $p<0.01$; ***, $p<0.001$; vs. Normal. #, $p<0.05$; ###, $p<0.001$; vs. Pre-DM.

groups was calculated from the glucose infusion rates during the late stable clamp period. To reduce the variability of the M rate, the uptake of glucose during the clamp was corrected by the body weight (mg/kg/min, Figure 5). The experimental results (Figure 5A) clearly show that compared with the normal monkeys (n=7), the M rate of the DM group (n=6) was significantly decreased and was only around 1/3 of the M rate of the normal group (p<0.01). Again, the insulin sensitivity was significantly reduced in the animals suffered from diabetes.

The hyperglycemic clamp was conducted in another group of animals (n=15) after overnight fasting (16 hrs) and ketamine anesthesia (10 mg/kg, i.m.). The fasting blood glucose concentration at baseline was 85 ± 4 mg/dL for the normal group and 137 ± 20 mg/dL for the DM group. During the clamp, the blood glucose concentration was acutely raised to a fixed hyperglycemic plateau and maintained at that level for 2 hrs or longer. After the baseline blood samples were collected, the hyperglycemic clamp was then initiated by a 15 min "priming dose" of glucose infusion to raise the glucose concentration by 125 mg/dL. The dose of each animal was computed per square meter body surface area and adjusted empirically according to the baseline blood glucose level. Then, the glucose infusion rate was adjusted every 5 min based on the negative feedback principle to maintain blood glucose concentration at that high level. The infusion rate during the last 30 min was stabilized and was taken to represent the metabolism of glucose with a correction by the body weight (M rate, mg/kg/min, Fig. 5B). The experimental results clearly show that the M rate of the DM group (n=6) was significantly lower than that of the normal group (n=9) and was only less than 1/3 of the M rate of the normal group (p<0.01, Fig. 5B, low panel). These results demonstrate that the insulin production and sensitivity under the hyperglycemic clamp were also significantly impaired in the diabetes monkeys.

Metformin effects

To test glucose lowing effects in naturally occurring diabetes monkeys, metformin, the first-line drug of choice for the treatment of T2DM, was used for the treatment. Ten T2DM monkeys with either sex were enrolled and randomly divided into two groups (vehicle and metformin). The baseline levels of fasting serum glucose (Figure 6A, upper panel) and HbA1c (Figure 6A, low panel) were not significantly different between the vehicle (Vehicle, n=5) and metformin-treated group (Metformin, n=5). The animals were orally gavaged with 25 mg/kg metformin or vehicle twice daily for 30 days. Compared with the baseline levels, the fasting serum glucose level (upper panel) was significantly decreased on day 14 (p<0.05) and HbA1c level (low panel) was significantly reduced on day 14 and 30 (p<0.05) in the metformin-treated diabetes monkeys (Figure 6B). Compared with the control animals, the HbA1c level (low panel) was significantly lower on day 30 in the metformin-treated diabetes monkeys (Figure 6B). These results suggest that naturally occurring diabetes monkeys responded well to metformin, the first-line oral anti-diabetic drug in the biguanide class used widely in clinic for the treatment of T2DM.

Blood pressure and angiotensin pressor response

Hyperglycemia resulting from insulin resistance increases the risk of cardiovascular diseases (CVD) [28,33]. The risk of a CVD event is increased modestly during the pre-diabetic state. However, developed diabetes obviously increases the risk for CVD, as well as for other complications affecting the eyes, kidneys, and nervous system [34,35]. One recent review summarized the general perspective on the complex relationship between cardiovascular diseases onset, pre-diabetes and family history of diabetes [35]. Diabetes negatively influences vascular walls morphology and function and heart function with the potential so-called diabetic cardiomyopathy. Therefore, early detection of vascular impairment in subjects at risk of developing diabetes is of significance. Common carotid intima-media thickness, flow-mediated vasodilatation, pulse wave velocity are instrumental tools able to detect the early impairment in cardiovascular system and stratify cardiovascular risk of individuals [35]. These complications are related to diabetes duration, chronic level of glycemia, and other risk factors.

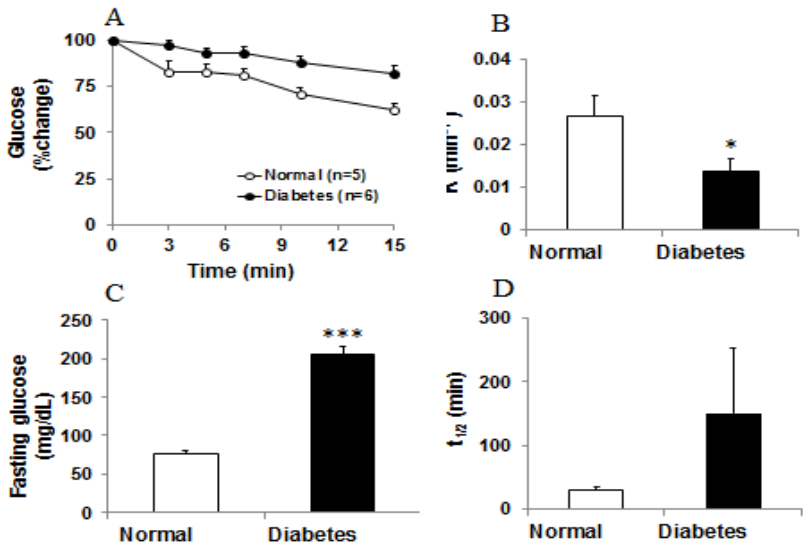

Figure 4: Insulin tolerance test (ITT) in normoglycemic and DM monkeys. ***A***, The time course of glucose changes (%) after insulin infusion. ***B***, The decay rate of the glucose percent changes (%) for the normal and DM groups. The mean rate for the diabetes animals was significantly slower than for the normal monkeys. ***C***, The serum glucose level was significantly higher in the diabetes group (n=6) than in the normal group (n=5). ***D***, Compared to the normal group, the $t_{1/2}$ of ITT in the diabetes group was prolonged, but not statistically significantly. *, p<0.05; ***, p<0.001; vs. Normal.

To look at the difference of blood pressure in normal, pre-DM and DM animals, 70 housed monkeys were trained in monkey chairs for 1 hr once every other day for at least 3 weeks. The tail cuff method was used to detect the animal blood pressure under conscious condition [36]. The results of measured blood pressure were repeatable and relatively stable during 11-week test (Figure 7A inset). The distribution of mean arterial pressure (MAP) measured in 70 conscious monkeys was shown with a range from 72 to 135 mmHg (Figure 7A). Obviously, blood pressure in some animals was much higher than others.

To look at the potential effects of blood glucose level on blood pressure, 3 animal groups were investigated (Figure 7). The averaged heart rates (HR) were 224 ± 6, 203 ± 4 and 199 ± 5 beats/min for the conscious normal (n=11), pre-DM (n=20) and DM (n=31) animals, respectively. The averaged systolic arterial pressures were 148 ± 4, 134 ± 4 and 132 ± 3 mmHg and diastolic pressures were 89 ± 4, 88 ± 4 and 86 ± 3 mmHg for the conscious normal, pre-DM and DM animals, respectively. It is interesting that the HR and systolic blood pressure measured under conscious condition were higher (p<0.05) in the normal group than those in the pre-DM and DM groups.

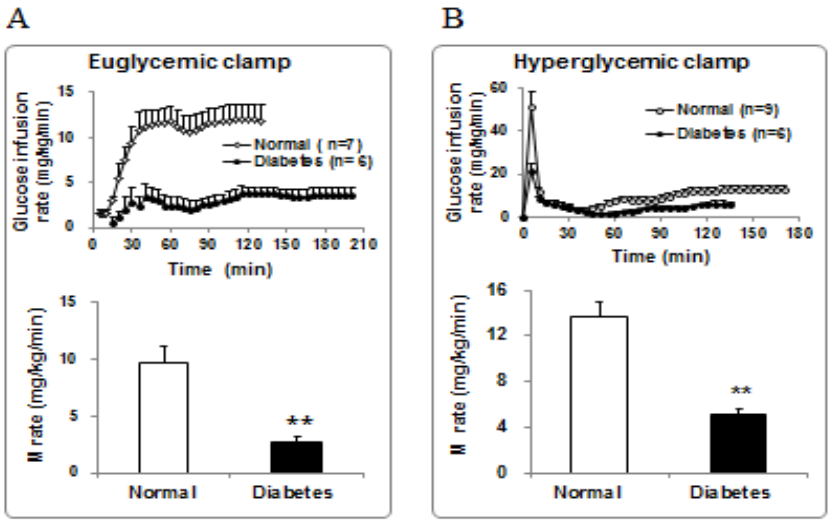

Figure 5: Euglycemic (***A***) and hyperglycemic (***B***) clamps in fasted normal and diabetes cynomolgus monkeys. ***A***, The time course of euglycemia against the glucose infusion rate (upper panel) is shown. Euglycemia in a range of 55 – 75 mg/dL was maintained by continuously intravenous glucose infusion for 130 min in the normal group (n=7) and for 200 min in the diabetes group (n=6). The glucose M-rate was significantly lower in the diabetes group than in the normal one (low panel). ***B***, The time course of hyperglycemia against the glucose infusion rate (upper panel) is shown. Hyperglycemia was maintained by continuously intravenous glucose infusion for 170 min in the normal group (n=9) and 135 min in the diabetes group (n=6). The glucose M-rate was significantly lower in the diabetes group than in the normal one (low panel). **, p<0.01; vs. Normal.

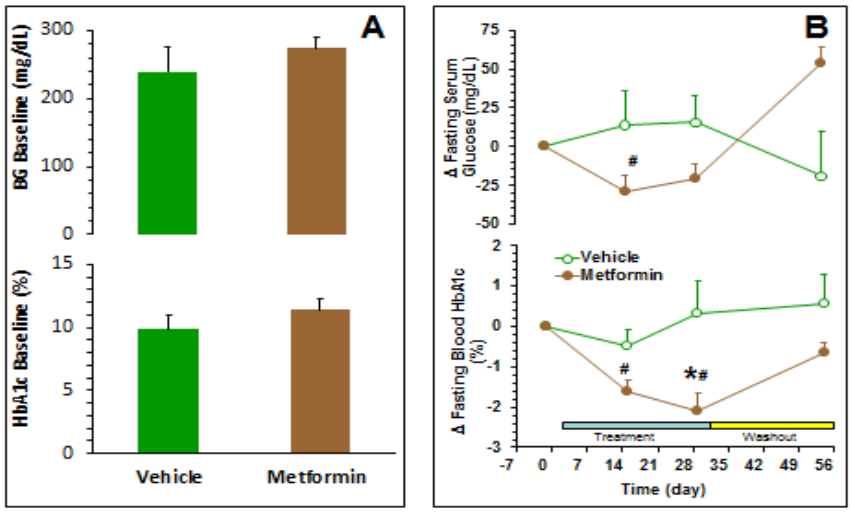

Figure 6: The effects of metformin on fasting serum glucose and HbA1c in diabetes monkeys. ***A***, The baseline levels of fasting serum glucose (upper panel) and HbA1c (low panel) in the experimental monkeys with (Metformin, n=5) and without (Vehicle, n=5) metformin treatment. ***B***, The delta changes of fasting serum glucose (upper panel) and HbA1c (low panel) with (Treatment) and without (washout) metformin treatment. Metformin (25 mg/kg) and vehicle was orally gavaged twice per day. *, p<0.05; vs. Vehicle; #, p<0.05; vs. baseline.

To understand if the differences might result from conscious measurement, the animals were anesthetized with ketamine (10 mg/kg, i.m.) and additional amount (5 mg/kg, i.m.) was given if needed. The blood pressure and HR were significantly lower under anesthesia in all of the three groups, compared to those measured under conscious condition (Figures 7B, 7C and7D). In addition, the systolic and diastolic arterial pressure and HR measured under anesthesia were almost the same among the three groups (p>0.05). The heart rates were 149 ± 9, 154 ± 6 and 154 ± 4 beats/min for the anesthetized normal, pre-DM and DM animals, respectively. The averaged SBPs were 107 ± 5, 110 ± 4 and 105 ± 4 mmHg and DBPs were 60 ± 3, 67 ± 4 and 62 ± 3 mmHg for the anesthetized normal (n=11, 9 male/2 female with the age of 6.6 ± 0.2 years), pre-DM (n=20, 14 male/6 female with the age of 15.2 ± 0.8 years) and DM (n=31, 21 male/9 female with the age of 17.9 ± 0.7 years) animals, respectively. The animals in the pre-DM and DM groups were significantly older than those in the normal group (p<0.05). Further analysis shows that there was no correlation between blood glucose levels and blood pressures in the studied monkeys with or without diabetes.

As the angiotensin system involves diabetic cardiovascular complications, angiotensin-II (Ang-II, Sigma, St. Louis, MO, USA) was administered intravenously to test the cardiovascular response in the NHPs (n=10, 5 male/5 female) with the age of 18 ± 1.5 years, body weight of 9 ± 1.2 kg and blood glucose of 169 ± 30 mg/dL. Ang-II at 20 μg/mL was prepared before an experiment in a stock solution containing 0.9% saline and 0.5% bovine albumin. Ang-II with a diluted testing dose was intravenously infused by an infusion pump (**Harvard Apparatus,** Holliston, MA, USA) at a rate of 1 mL/min with a volume of 0.1 mL/kg body weight to cause a pressor response. Blood pressure of each experimental conscious monkey was measured 2 times before dosing as the baseline and continuously for the 1st 10 min after dosing. The measurement was ceased when the blood pressure came back to its baseline and then the animal was returned to its cage. Clinical observation, such as animal activity and behavior, was performed during the procedure and the 1st hr after back to its cage. The pressor effect of Ang-II was in a dose-dependent manner (Figure 8A). The increase in blood pressure after intravenous administration of Ang-II 2 μg/kg was quick and reached the peak around 4 min after drug infusion (Figure 8B). The blood pressure then decreased gradually and returned to the pre-treatment level in 10 min. The HR was initially increased and reached the peak paralleling with the blood pressure. However, not like the blood pressure, the HR was returned to a level significantly lower than the pre-drug level at 7 min after Ang-II injection (Figure 7B), which most likely resulted from the baroreflex of Ang-II-induced pressor response. Then, the heart rate was back toward to the pre-drug level.

Diabetic nephropathy

Diabetic nephropathy is one of the complications of diabetes due to uncontrolled high blood sugar. It is a progressive kidney disease due to diffuse glomerulosclerosis because of angiopathy of capillaries in the glomeruli [28,33]. The disease manifests as a nephrotic syndrome, which may cause death in three years after the initial lesions. Currently, it is the leading cause of chronic kidney disease in many developed countries. The detectable change at the initial stage diabetic nephropathy is a thickening in the glomerulus. The diseased kidney leaks serum albumin, called microalbuminuria. When the

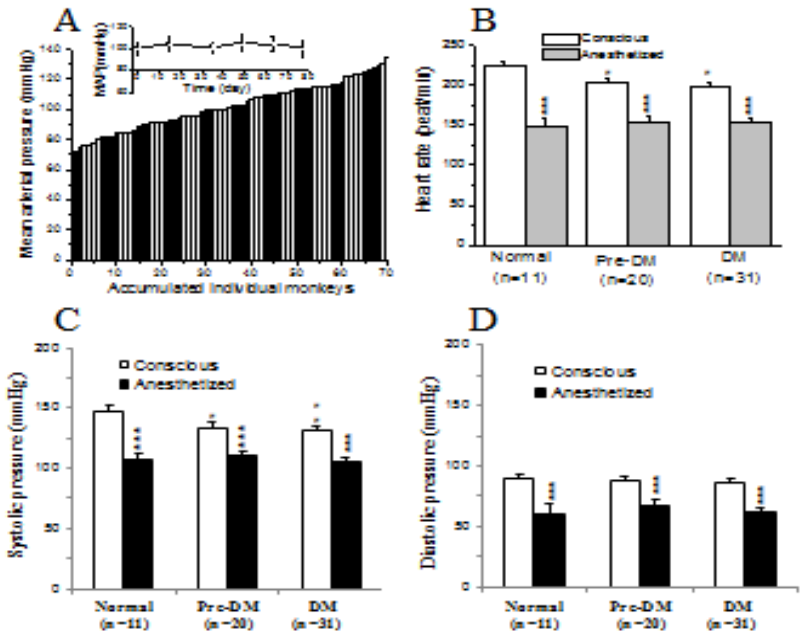

Figure 7: Accumulated distribution of mean arterial blood pressure (***A***) and effects of anesthesia on heart rate (***B***) and blood pressure (***C, D***). Blood pressure was measured via the tail cuff method in the animals with or without anesthesia (10 mg/kg ketamine, intramuscularly). The values of the mean arterial pressure (MAP) varied from 72 mmHg to 135 mmHg for individual monkeys (n=70, ***A***). The inset shows the repeatable measurements of MAP in conscious cynomolgus monkeys (n=6) during 11 weeks. ***B***, The heart rate was significantly faster for the normal conscious group (224 ± 6 beats/min, n=11) compared with the Pre-DM (203 ± 4 beats/min, n=20) and DM (199 ± 5 beats/min, n=31) ones. However, anesthesia significantly reduced the heart rates to the almost same levels (p>0.05) for all the three groups. ***C*** and *D*, The systolic blood pressure, but not diastolic one, was significantly higher (148 ± 4 mmHg) for the normal conscious group compared with the Pre-DM (134 ± 5 mmHg) and DM (132 ± 3 mmHg) ones. However, anesthesia significantly reduced both systolic and diastolic pressures to the almost same levels (p>0.05) for all the three groups (***C***, ***D***). ***, p<0.001; vs. conscious; #, p<0.05; ##, p<0.01; vs. Normal.

disease progresses more glomeruli are destroyed. Microalbuminuria becomes more severe. Kidney biopsy can confirm the diagnosis. The complications of diabetic nephropathy include arteriosclerosis and hypertension. Evidence suggests that early treatment delays or prevents the onset of diabetic nephropathy [37].

In the present study urine collection (24 hrs) was carried out in the monkeys housed individually in a stainless steel metabolic cage with a separation net being placed between the cage and urine collection tray (Suzhou Houhuang Animal laboratory Equipment Technology Co. Ltd, China) to prevent the urine from the contamination of the feces and residual food. Blood sample was taken from a cephalic vein on the next day after collection of 24-hr urine. Biochemistry measurements of the urine and blood samples were carried out by a clinical analyzer (Roche, Cobas 601, Taicang City Hospital Lab, Taicang, China). Renal function was evaluated by the clinically relevant surrogate markers, blood urea nitrogen (BUN) and serum creatinine (*S*creatinine) concentrations, as well as estimated glomerular filtration rate (eGFR) based on creatinine clearance with the equation of *U*creatinine/*S*creatinine x 1.73 / body weight$^{2/3}$ x 0.118, which is commonly used in the clinic as a "first line" test of kidney function [38-40].

The average age of the pre-DM monkeys (n=17) was slightly higher than that of the normal animals (n=23), but the animals in the DM group were significantly older than those in both normal and pre-DM groups (n=42, $p<0.05$, Table 4, Figure 9). Compared with the normal group, the serum glucose level was significantly higher in both pre-DM and DM groups ($p<0.05$). Other parameters with significant changes ($p<0.05$ or 0.01) in the DM group were blood HbA1c, serum creatinine, drinking volume, urine volume, urine glucose, urine albumin, urine total protein, ACR (the urine albumin to urine creatinine ratio) and eGFR (Table 4). It is interesting that compared with the normal group the serum creatinine level was lower in both pre-DM and DM groups. Based on the urine albumin level or ACR, the animals were characterized into several groups (Table 5).

To analyze the co-relationship of urine albumin with other major parameters measured, the age, body weight, glucose, HbA1c, insulin and urine total protein were plotted against urine albumin of the animals tested. It is interesting that neither the ages nor the body weights of the animals significantly correlated with the amounts of their 24-hr urine albumins (Figures 9A and 9B, $p>0.05$). The blood insulin levels also had no obvious correlation with the amounts of 24-hr urine albumins in the studied monkeys ($p>0.05$, Figure 9E). However, both the blood glucose and HbA1c levels of the experimental NHPs correlated well with the amounts of their 24-hr urine albumins (Figure 9C, 9D, $p<0.01$). In addition, the amounts of 24-hr urine total proteins also significantly correlated with the amounts of 24-hr urine albumins in the studied monkeys ($p<0.01$, Figure 9F). These results indicate that albuminuria seems most likely related to the degree of hyperglycemia and diabetes.

Discussion

The global prevalence of diabetes mellitus (DM) has increased considerably in recent two decades. Diabetes research and therapy can be markedly tardy because of lack of availability of good animal models, particularly for the study of comorbidities associated with diabetes. In this article the naturally occurring diabetes model in NHPs is introduced and characterized. The data obtained from our housed monkeys are analyzed and summarized. Here we proposed the criteria to group monkeys into the various groups based on their fasted serum glucose levels (Table 1) and some other information, such as blood HbA1c and glucose/insulin tolerance test (Table 2, Figures 3-5). Fasting serum glucose of our housed normal monkeys was 69 ± 1.5 mg/dL which is consistent with the range reported previously [14,16]. It has been known that fasting glucose concentrations are about 30 mg/dL lower in normal monkeys than in normal humans [14,16]. There is a lack of the standard criteria to define normal, pre-diabetes and diabetes for monkeys. In this article we proposed the fasting glucose levels for

Parameter	Normal	Pre-DM	DM
	23 (M/F, 17/6)	**17(M/F, 7/10)**	**42 (M/F, 17/25)**
Age (y)	12 ± 0.9	15 ± 1.3	**18 ± 0.6*$^{\#}$**
Body weight (kg)	8.3 ± 0.5	8.0 ± 0.6	7.4 ± 0.5
Serum glucose (mg/dL)	65 ± 2	**94 ± 4***	**197 ± 11*$^{\#}$**
Serum insulin (μIU/mL)	61 ± 9	110 ± 34	98 ± 22
Blood HbA1c (%)	4.8 ± 0.2	5.3 ± 0.3	**9.5 ± 0.6*$^{\#}$**
Serum Creatinine (μmol/L)	98 ± 7	**77 ± 5***	**61 ± 6***
Serum Cystatin C (mg/L)	1.3 ± 0.1	1.1 ± 0.1	1.3 ± 0.1
Serum BUN (mmol/L)	5.1 ± 0.4	6.3 ± 0.2	6.4 ± 0.4
Serum NAG (U/mL)	14.0 ± 3.6	10.3 ± 1.2	15.9 ± 2.5
Drinking volume (mL/24 hrs)	349 ± 61	539 ± 86	**768 ± 89***
Urine volume (mL/24 hrs)	265 ± 47	219 ± 43	**526 ± 64*$^{\#}$**
Urine glucose (mg/24 hrs)	204 ± 160	1470 ± 1132	**29906 ± 5108*$^{\#}$**
Urine total protein (mg/24 hrs)	16.3 ± 1.9	30.8 ± 15.0	**148.8 ± 35.1***
Urine albumin (mg/24 hrs)	4.9 ± 1.4	12.5 ± 7.1	**96.9± 27.0***
Urine creatinine (mmoL/24 hrs)	1.4 ± 0.2	1.4 ± 0.2	18.1 ± 16.0
ACR (mg/mmol)	7.9 ± 4.4	18.4 ± 8.8	**67.7 ± 14.1***
GFR (ml/min/1.73m^2)	32 ± 4	31 ± 5	**68 ± 8***
Urine SMAD1 (mg/24 hrs)	12.7 ± 2.8	4.4 ± 1.8	13.9 ± 3.8
Urine NGAL (mg/24 hrs)	1.3 ± 0.3	0.4 ± 0.2	1.23 ± 0.3
Urine MCP-1 (ng/24 hrs)	61.1 ± 11.3	21.2 ± 7.9	39.3 ± 8.6
*, $p < 0.05$; vs. normal. #, $p < 0.05$; vs. Pre-diabetes.			

Table 4: Comparison of kidney function among Normal, Pre-DM and DM monkeys.

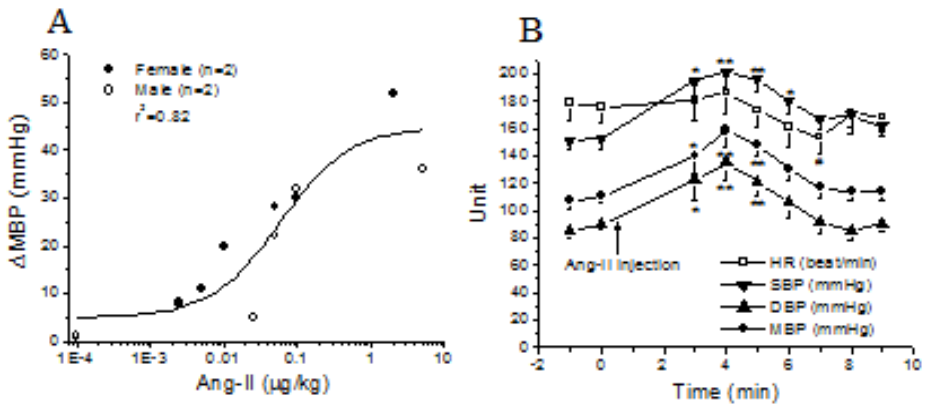

Figure 8: Dose-dependent response of heart rate and blood pressure to Ang-II (***A***) and time course of Ang-II-induced pressor response (***B***) in conscious cynomolgus monkeys. ***A***, Dose-response of Ang-II infusion with the various doses in two female and two male monkeys with their ages from 14 to 17 years old and body weights of 8.5 ± 0.9 kg. ***B***, The time course of the pressor response of Ang-II after intravenous injection at a dose of 2 μg/kg in conscious cynomolgus monkeys (n=10). *, $p<0.05$; **, $p<0.01$; vs. pre-Ang-II.

Data	n
Urine albumin (mg/24 hrs)	
≥300	5
≥100 - <300	6
≥50 - <100	6
≥20 - <50	7
<20	58
Urine ACR (mg/mmol)	
≥30	21
≥3.5 - <30	26
<3.5	35

Table 5: Distribution of the animals based on their urine albumin and ACR levels.

classifying monkeys into 3 groups, normal, pre-diabetes and diabetes (Table 1A). Additional criteria, such as insulin and HbA1c, and assays, such as glucose/insulin tolerance test and glucose clamp, are potentially needed for sorting out individual animals into which groups (Table 1B, Table 2, Figures 3 and 4).

The general metabolic characteristics of our housing monkeys show that the naturally occurring diabetes monkeys were significantly older than those normal and pre-diabetes animals ($p<0.05$, Table 2). Their ages were correlated well with the levels of blood glucose, HbA1c, TG and TC (Table 3, $p<0.001$). However, their ages had no significant correlations with their body weights and blood concentrations of C-peptide, insulin, LDL-c and HDL-c ($p>0.05$, Table 3). In addition, our data show that their body weights were correlated well with their insulin levels, total cholesterol, and LDL-c ($p<0.05$ or 0.001, Table 3), as well as with BMI (Figure 2B). Their TG/TC levels were correlated well with the levels of blood glucose and LDL-c ($p<0.001$, Table 3, Figures 2C and 2D). It is not surprised that the HbA1c concentrations of the monkeys correlated well with their blood glucose levels (Figure 1C) and their blood insulin concentrations correlated well with their C-peptide levels (Figure 1D). These results are consistent with the phenomena that T2DM can have a long period of moderate hyperglycemia with insulin resistance that is associated with obesity and dyslipidemia and with an initial increase in islet size and insulin content [14]. It is interesting that ages had no obvious correlations with body weight, C-peptide, LDL-c, and HDL-c. Also, body weights had no correlations with blood glucose, HbA1c, C-peptide, TG, and HDL-c. No correlation was found between TG and HDL-c and between TC and HDL-c (Table 3). These results are consistent with clinical findings that T2MD is often accompanied by change of inflammation markers and increase in serum LDL-c and cardiovascular risk, which is referred to as the metabolic syndrome [41]. Our data show that the naturally occurring diabetes monkeys increased their serum CRP levels which correlated well with their serum TG and insulin levels (Table 3). Its potential pathophysiological significance needs to be delineated.

Many methods have been used for clinical diagnosis of diabetes and diabetic animal research. Among them oGTT, ivGTT and ITT are commonly used for testing insulin sensitivity and secretion. The ivGTT technique requires a sufficient insulin response to the intravenous administration of glucose to obtain a reliable estimation of the parameters. Insulin resistance due to obesity is associated with an increased insulin secretion initially, which allows insulin sensitivity and secretion to move in opposite directions, so that the ability of the normal subject to dispose of glucose remains relatively constant [42]. In this article, we have presented the results obtained from the NHPs by using the most common methods, such as ivGTT and ITT, and shown the glucose tolerance, insulin sensitivity and beta cell function. Our data demonstrate that glucose tolerance and insulin tolerance were impaired in the pre-DM and DM monkeys (Figures 3 and 4). Insulin resistance was observed in pre-DM monkeys with hyperglycemia and hyperinsulimia by increase in insulin secretion and in DM monkeys with severe hyperglycemia and hypoinsulimia with decrease in insulin secretion (Figure 3).

The gold standard test for clinical diagnosis and for diabetic research is the glucose clamp [32]. This test provides an absolute index of insulin sensitivity, glucose handling rate (M rate), given by the glucose infusion rate when glucose concentration is kept at a constant steady state. The less glucose infusion is needed, the lower is the insulin action (insulin resistance) on glucose uptake by peripheral tissues. Our data obtained via either hyperglycemic clamp or euglycemic clamp demonstrate that the M rate was significantly reduced in the diabetes animals (Figure 5), which indicated the impaired glucose handling of their bodies due to insulin resistance.

Many diabetic patients during their life develop to high blood pressure (hypertension, defined as a blood pressure ≥ 140/90 mmHg) which is an extremely common comorbid condition of diabetes [43]. Having diabetes leads to hypertension and other heart and circulation

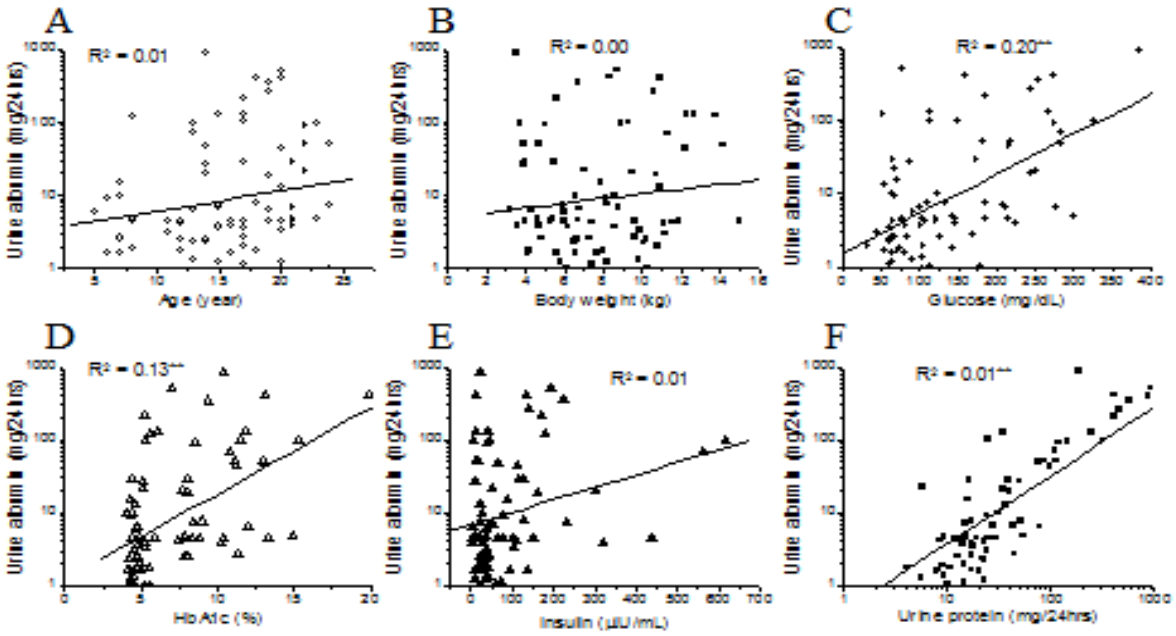

Figure 9: Correlations of multiple parameters in cynomolgus monkeys. ***A*** and B, The correlations between the ages and urine albumins and between the bodyweights and urine albumins were not significant ($p>0.05$). However, the correlations between the blood glucose levels and urine albumins (***C***) and between the blood HbA1c percentages and urine albumins (***D***) were statistically significant. The blood insulin levels had no obvious correlation with 24-hr urine albumins ($p>0.05$, ***E***), but the amounts of 24-hr urine total proteins significantly correlated with the urine albumins (***F***). **, $p<0.01$.

problems more likely, because diabetes damages arteries and makes them targets for hardening (atherosclerosis) [35]. Atherosclerosis can cause hypertension, which if not treated, can lead to blood vessel damage, stroke, heart failure, heart attack, or kidney failure [44-46]. Our data show that the mean arterial blood pressure (MAP) was above 100 mmHg in more than half of the conscious monkeys measured by the tail cuff method (Figure 7A). It is interesting that compared with the conscious pre-DM and DM monkeys, the systolic blood pressure (SBP) and heart rate are significantly higher in conscious normal monkeys (Figures 7B and 7C). A potential explanation for the unexpected differences is that the normal monkeys were relatively younger compared with those pre-DM and DM ones (Table 2). The younger ones were more active and potentially more being stressed during tail-cuff measurements under conscious condition. Therefore, anesthesia significantly reduced heart rate and blood pressure in all three experimental groups and in the meantime, eliminated the differences of heart rate and SBP among the conscious normal, pre-DM and DM monkeys (Figures 7B, 7C and 7D). Blood pressure and heart rate in conscious and anesthetized monkeys were reported previously [47-49]. SBP, DBP, and MAP and pulse rate were recorded from conscious cynomolgus monkeys by the tail cuff method [47-49]. The SBP, DBP, MAP, and pulse rate were approximately 121 ± 17, 60 ± 14, and 84 ± 17 mm Hg and 193 ± 18 pulses/min (mean ± SD), respectively. Treatment with isoproterenol, norepinephrine, and nitroprusside changed blood pressure to the same direction measured indirectly with the cuff on the tail or directly with an indwelling catheter in the descending thoracic aorta. The data suggest that tail cuff monitor was reliable and sufficiently accurate for evaluation of blood pressure parameters in conscious cynomolgus monkeys [47-49].

The African Green monkey (AGM), *Chlorocebus aethiops sabaeus,* is a highly translational non-human primate model of spontaneous hypertension. Blood pressure and heart rate were measured in freely moving, mature AGMs by the telemetry method [49]. Different recording parameters (n=4) yielded consistent blood pressures, in normotensive (MAP=104 ± 4.3 mmHg) and hypertensive (MAP=126 ± 7.5 mmHg) animals [49]. Also, using forearm plethysmography under ketamine sedation (~15 mg/kg), AGMs were categorized as hypertensive (SBP >140mmHg), borderline hypertensive (120 mmHg<SBP<140 mmHg), or normotensive (SBP<120 mmHg). Of the 264 animals phenotyped, 32% (85 of 264) were hypertensive (MAP, 108 ± 2.2 mmHg), 18% (49 of 264) were borderline hypertensive (MAP, 88 ± 2.0 mmHg), and 49% (130 of 264) were normotensive (MAP, 69 ± 1.3 mmHg). Hypertensive animals tend to be older than normotensive animals (12.4 ± 0.7 yrs vs. 8.7 ± 0.6 yrs; $p<0.05$) with elevated heart rates (134 ± 2.1 bpm vs. 121 ± 1.9 bpm, $p<0.05$), similar to human essential hypertension [49]. The criteria to define hypertension have not been fully established for monkeys. Therefore, it is hard to give the ratio of hypertension in our experimental monkeys, including those with pre-DM and DM. Based on our current data, it seems that the morbidity of hypertension was not higher in the DM or pre-DM monkeys than in the normal monkeys, because both systolic and diastolic blood pressures were almost the same in the three experimental groups under anesthesia and also because the systolic blood pressure was even significantly lower in the conscious pre-DM and DM monkeys than in the conscious normal ones (Figure 7).

Angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor antagonists (ARBs) can often be used as medications to treat high blood pressure for people with diabetes and also be used to prevent or slow kidney disease in diabetes patients. To test the response of the angiotensin system in cynomolgus monkeys, angiotensin II was injected intravenously. Blood pressure showed a dose-dependent increase after various doses of angiotensin injected (Figure 8A). The pressor response reached the peak within 5 min after intravenous injection of angiotensin and the heart rate was initially increased and then decreased due to the

reflex of the pressor response (Figure 8B). These results clearly indicate that the angiotensin system can regulate blood pressure in NHPs and may play an important role in the etiology of diabetic nephropathy which is a grave microvascular complication of diabetes. Indeed, one of our recent studies shows that in spontaneously diabetic cynomolgus monkeys oral treatment with the angiotensin receptor blocker, irbesartan (3 mg/kg/day achieved a plasma concentration at 70 ± 17 ng/mL at 24 hours) reduced urinary protein and albumin excretion as well as ACR significantly [50]. In a separate experiment in the same model, another angiotensin receptor blocker, losartan (1 mg/kg/day), resulted in a similar reduction of proteinuria in diabetic nephropathy monkeys [50]. After termination of the treatment, both urinary protein and albumin gradually returned towards the baseline. These results demonstrate for the first time that blockade of Ang-II receptors reduces proteinuria in diabetic nephropathy monkeys, which is consistent with the clinical observations in nephropathy patients. Thus, the monkey diabetic nephropathy model can be used as a translational model for studying pathophysiological mechanism(s) and for testing novel therapies of nephropathy [50].

Normally, albumin and other proteins are absent in the urine due to the kidney capability to retain them in the blood stream in a healthy body. Proteinuria is a term for the presence of urine albumin and/or other proteins due to the kidney leakage of protein into the urine. Human microalbuminuria can be diagnosed from a 24-hr urine collection (between 30–300 mg/24 hrs) for at least two positive measurements over 2 to 3 months [51]. Albumin above the microalbuminuria upper limit values is named "macroalbuminuria" [52]. ACR can also be used for evaluation of kidney function and likely to reduce variations due to using spot-check samples. Microalbuminuria in humans is defined as ACR ≥ 3.5 mg/mmol (female) or ≥ 2.5 mg/mmol (male) [53], which indicates an early stage of developing kidney disease. Macroalbuminuria indicates presence of a more severe kidney disease. Our data show that serum blood glucose and creatinine were significantly increased (p<0.05, Table 4) in the pre-DM monkeys accompanied with some level increases in urine glucose, albumin and total protein which are the important adverse predictors of glycemic outcomes associated with increased progression to diabetes. Therefore, in the DM monkeys all those parameters became obvious and significant (Table 4). Hence pre-DM and DM subjects with micro- or macro-albuminuria warrant more aggressive intervention not only for diabetes treatment but also for nephropathy therapy. Angiotensin II receptor antagonist, irbesartan or losartan, could reduce the severity of albuminuria in our diabetic monkeys [50].

Conclusion

In summary, our data demonstrate that as in humans, T2DM is the common form of diabetes in NHPs occurred more often in older dyslipidemic animals who show a metabolic progression from insulin resistance and impaired glucose tolerance to overt diabetes. Diabetic NHPs also have adverse changes in plasma lipid concentrations, inflammatory biomarker (CRP), and protein glycation (HbA1c) that can contribute to the numerous diabetic complications, such as nephropathy. As their clinical and pathologic characteristics are similar to humans, NHPs have been used for research of understanding the mechanisms of diabetes and other dysmetabolic diseases and for many pharmacological studies to assess new therapeutic agents. For these reasons, NHPs are particularly valuable for studying obesity and diabetes pathogenesis, risk factors, comorbidities, and therapeutic interventions. Although none of the models can completely capture the complexity of human metabolic syndrome, each model mimics certain aspects of the diseases. A naturally occurring diabetes model in NHPs is by far the most predictive animal model system for human metabolic syndrome, presenting complex symptoms such as loss of blood glucose control, diabetic nephropathy, diabetic retinopathy, and dyslipidemia. These features are especially critical since many next generation drugs for metabolic disease target diabetic complications rather than blood glucose control. Evaluation of new drugs, biologics or other novel therapeutic approaches in such model will offer tremendous value towards understanding efficacy, PK/PD (Pharmacokinetics/Pharmacodynamics) relationship, biomarker, and possible adverse effect by using the largest group in the world of our well-documented Cynomolgus and Rhesus monkeys with naturally occurring diabetes. Accompanied with the NHP model, our experienced scientific team will work closely with a collaborator to design a most relevant study plan and provide high quality work to advance the discovery process. Our expertise in diabetic NHPs provides the highest level of confidence for go/no go decision making in the drug discovery process. Such study can not only provide deep biological insights into the pharmacological mechanism of a test article, but also help to identify biomarkers important for designing a clinical trial.

Competing Interests

All of the authors are employees of Crown Bioscience, Inc.

Authors' contributions

XW performed the study, analyzed the data and prepared the tables and figures. BW, GS, JW, and LY conducted the study and collected the data. YXW and YFX designed the study, analyzed the data and drafted the manuscript. All authors read and approved the final manuscript.

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