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Characterization and Validation of a Streptozotocin-Induced Diabetes Model in the Vervet Monkey

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Abstract

Streptozotocin (STZ), preferentially toxic to pancreatic beta cells, is commonly used to model type 1 diabetes mellitus (DM) in numerous species, including nonhuman primates. We induced DM in twenty vervet monkeys (*Chlorocebus aethiops*) by intravenous administration of either 45 (n=8, STZ-45) or 55 mg/kg STZ (n=12, STZ-55); ten control (CTL) monkeys received saline. Overall there was 15% mortality, likely secondary to renal toxicity. Twice-daily insulin therapy was initiated to maintain comparable glycemic control, confirmed by comparable glycated hemoglobin levels. Exogenous insulin requirements increased rapidly for 4 weeks; STZ-45 insulin doses stabilized thereafter while STZ-55 doses continued to increase through 16 weeks. Glucose tolerance testing and arginine-stimulated insulin secretion confirmed 80–90% reduction in pancreatic beta cell function in both groups. Body weight was reduced in all STZ monkeys, with return to baseline only in STZ-45 at 16 wks. Elevated blood urea nitrogen (BUN) and creatinine were noted in the STZ-55 group. Alkaline phosphatase (ALKP) was also increased with STZ-55 ($p<0.05$ vs. CTL) whereas STZ-45 ALKP elevation resolved by study end. Red cell parameters were reduced in all STZ monkeys, but more severely in the STZ-55 group. We have demonstrated that a model of DM can be induced and maintained in vervets with a single dose of STZ. The lower dose of STZ (45mg/kg) significantly improved the toxicity profile without altering efficacy in inducing DM. Finally, sufficient time following induction is recommended to allow transient renal, hepatic and hematologic parameters to resolve.

Keywords

diabetes; insulin; streptozotocin; monkey; vervet; methods

Introduction

While the increasing incidence in Type 2 DM is well described, there is also a rising incidence of Type 1 Diabetes Mellitus (T1DM) in most countries in the world that is cause for concern [1]. There has been an average annual increase in T1DM of 3.4% in Europe for children between the ages of 0–14 and a 6.3% increase in incidence for the 0–5 year old age range [2]. The move towards a younger age of onset and overall increase in incidence has

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been hypothesized to result from overfeeding; increased growth rates lead to pancreatic hyperfunction and increased presentation of autoantigens from the β -cells, potentially accelerating an autoimmune response [1].

This growing prevalence of T1DM imparts greater urgency for the development of animal models sufficiently close to humans in which T1DM pathogenesis and complications can be adequately studied. Preclinical models of DM have been developed in numerous species, including mouse [3], rat [4], canine [5] and nonhuman primates [6]. In addition to their underlying anatomic and phylogenetic relationships to humans [7], nonhuman primates are a preferred model as they are of larger size and longer lifespan than most laboratory species, allowing for longitudinal studies. Even more important is their clinical relevance, as diabetic monkeys present with pancreatic islet cell pathology and chronic diseases, such as cardiovascular complications, similar to those observed in humans with T1DM [8, 9]. To create an animal model of T1DM, total or partial pancreatectomy has been used; however, pharmacologically-induced loss of pancreatic β -cell function using streptozotocin (STZ) is far more common [10]. STZ (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose), a product of the bacteria *Streptomyces achromogenes*, is toxic to β -cells and has been used to reliably produce diabetes in non-human primates [11] as well as other animal models. Monkeys with STZ-induced DM present with hyperglycemia, elevated hemoglobin A1c (HbA1c), elevated plasma lipid concentrations and other clinical complications characteristic of diabetic humans [6, 9].

While macaques have been widely used as a model for the study of DM [6, 9, 12, 13], their use incorporates significant biosafety issues. Macaques are the natural reservoir of *Cercopithecine herpesvirus-1* (CHV-1 or herpes B) [14]. CHV-1 is a nonhuman primate herpes virus known to demonstrate disease in humans and if untreated, usually results in meningoencephalitis and mortality rate greater than 80% [15]. Moreover, surveys of captive macaques estimate that 40% [16] to nearly 100% [17] test positive for CHV-1.

Such biosafety risks emphasize the importance of alternative nonhuman primate models to study DM. Non-macaques such as the vervet monkey are not carriers of CHV-1, and have commonly been used to study atherosclerosis [18]. While the use of STZ to destroy β -cells in recipients of pancreatic allografts has been documented in vervets [19], to our knowledge a working model of STZ-induced insulin-dependent DM has not been thoroughly developed in vervets. The goals of the present study were to induce DM in vervet monkeys to assess their usefulness, potential STZ toxicity, and implications for experimental use.

Methods

Study Animals

All experimental procedures involving animals in this study were approved and complied with the guidelines of the Institutional Animal Care and Use Committee of Wake Forest University Health Sciences.

Thirty mature male vervets (*Chlorocebus aethiops*, also known as African Green monkeys), ranging in age from six to twelve years (mean 7.1 ± 0.3 years) and in weight from 5.9 to 10.0 kg (mean 7.5 ± 0.2 kg), were included in the study. Monkeys were stratified into two treatment groups (Control, CTL and streptozotocin-induced diabetic, STZ-DM) based on age, body weight, and glycemic control (HbA1c), as well as rate of glucose disposal (K_g) and glucose and insulin areas-under-the-curve (AUC) derived from baseline glucose tolerance testing. All animals were fed a standard monkey chow diet (PMI Nutrition International, Brentwood, MO)

Monkeys were fasted overnight prior to sedation with ketamine hydrochloride 15 mg/kg intramuscularly (IM). After recording body weights, blood samples were collected via percutaneous femoral venipuncture for clinical chemistry and hematology panels. A 21G butterfly catheter was used to infuse STZ (Zanosar, SICOR Pharmaceuticals, Irvine, CA) 100 mg/mL in normal saline at a dose of 55 mg/kg (STZ-55, n=12), while ten control (CTL) monkeys were administered an equivalent volume of saline. After two STZ-55 monkeys died within two months of STZ administration, the STZ induction dose was reduced to 45 mg/kg for the remaining STZ-DM animals (STZ-45, n=8) with the goal of reducing toxicity. In all STZ-DM animals, glucose concentrations in blood from conscious tail sticks were then measured daily using a glucometer (MediSense Precision Xtra, Abbott Laboratories, Abbott Park, Illinois). Once hyperglycemia was confirmed (typically 2 days after STZ), monkeys were started on insulin therapy, receiving twice-daily insulin injections (70% intermediate-acting [NPH], 30% short-acting [regular insulin], Novolin 70/30, Novo Nordisk USA, Princeton, NJ). Insulin doses were adjusted based on whole blood glucose measurements determined via glucometer measured approximately 3-hours following insulin dosing and feeding (post-prandial) at least twice weekly, in order to avoid diabetic ketoacidosis.

Blood Sampling

Every four weeks, all monkeys were sedated and blood samples were collected as above in order to quantitate glycemic and lipid indices, and to run standard hematology and serum biochemistry panels. STZ treated monkeys had insulin therapy withheld for 18 hours prior to any blood sampling. Aliquots of serum and EDTA-treated whole blood were shipped overnight for CBC and chemistry analyses by a commercial laboratory (Antech Diagnostics, Memphis TN). Whole blood samples in EDTA-treated tubes were placed on ice immediately after collection, were centrifuged within 1–2 hours and the resulting plasma removed and stored at -80°C . Glycemic indices analyzed include plasma glucose, insulin, c-peptide, HbA1c and fructosamine concentrations. Plasma lipid measures include total plasma cholesterol (TPC) and triglycerides (TG).

Glucose Tolerance Test

Baseline intravenous glucose tolerance tests (IVGTTs) were performed on all study animals to stratify monkeys into treatment groups prior to induction. Under ketamine sedation (15 mg/kg IM, supplemented at 3–5 mg/kg as necessary), two baseline blood samples were collected via percutaneous femoral venipuncture. After drawing the second baseline blood sample, a 21G butterfly catheter was used to infuse 50% dextrose (750 mg/kg) via the saphenous vein, followed by saline flush. Blood samples were subsequently collected at 5, 10, 20, 30 and 60 minutes post-dextrose into EDTA-treated tubes and placed on ice. Samples were centrifuged, and plasma stored at -80°C until analysis for glucose, insulin and c-peptide concentrations. Calculated parameters include AUC for glucose and insulin and glucose disappearance rate (K_g).

Glucose Tolerance Test with Arginine Stimulation

Three weeks after study start and prior to each animal's necropsy, a modified IVGTT was performed in order to further characterize pancreatic β -cell secretory capacity. An intravenous arginine challenge, directly stimulating β cell insulin secretion, was added to the IVGTT protocol to estimate residual β -cell mass [20]. The IVGTT was performed as described above with 67 mg/kg arginine (R-Gen 10, 10% arginine hydrochloride) injected via the saphenous vein after the 30 minute time point. Blood samples were subsequently collected at 1, 3, 5, 7 and 10 min post-arginine. Parameters measured include AUC for glucose and insulin during both the glucose- and arginine-clearance phases, as well as c-peptide AUC following arginine stimulation.

Clinical Analyses

Plasma glucose (Sigma-Aldrich, St. Louis, MO) and fructosamine (Roche Diagnostics, Mannheim, Germany) were assayed by enzymatic colorimetric methods. The inter-assay and intra-assay coefficients of variation (CV) % are <5% for glucose and <10% for fructosamine. Insulin and c-peptide were determined using ELISA (Mercodia, Uppsala, Sweden) with inter-assay and intra-assay CV being <10%. Glycated hemoglobin percentages were measured by HPLC (Primus PDQ, Primus Diagnostics, Kansas City, MO).

Statistical Analysis

All results are reported as mean $\pm$ standard error of the mean (SEM). Statistical analyses were performed using Statistica 6 (StatSoft Inc., Tulsa, OK). Log transformation of variables (AST, ALT, ALKP, TG, c-peptide) was performed when normality assumptions were not met. Baseline intergroup comparisons were performed on all variables by one-way ANOVA. IVGTT parameters were then assessed by analysis of covariance, using baseline values as covariate. Repeated measures ANOVA was used in analysis of all other parameters. In either case, when an overall significant group effect or group by time interaction was indicated by $p < 0.05$, Tukey's HSD was used in post hoc testing to determine specific differences.

Results

Streptozotocin was generally well-tolerated at both the 45 and 55 mg/kg doses. The most common side effect observed was emesis (15% of STZ administrations) upon STZ infusion and/or transient inappetence upon recovery. After administering STZ, 19 out of 20 monkeys became consistently diabetic with average blood glucose concentration over 200 mg/dL within two days. Three animals who received STZ died within 8 weeks of induction and were excluded from all analyses (15% mortality). Two of the animals had received 55 mg/kg, and the third received 45 mg/kg STZ. One animal that received 45 mg/kg STZ did not exhibit hyperglycemia. An additional dose of 35 mg/kg STZ administered one week later to that animal also failed to induce fasting hyperglycemia, and it was also excluded from all analyses. Thus, the final study groups included in all analyses consisted of ten CTL animals, ten STZ-55 animals and six STZ-45 animals.

Body Weight

Baseline body weights were similar between the control and both STZ-DM groups (Table 1). The CTL group's average weight did not change throughout the 16 week period. Although both STZ-DM groups exhibited decreases in average body mass, vervets treated with the lower 45 mg/kg STZ dose took longer to decrease significantly compared to baseline and their weights recovered by the end of study, while STZ-55 body weight remained significantly reduced through the 16 weeks of evaluation.

Glycemic Indices / Exogenous Insulin Requirements

There were no differences between study groups at baseline among the measurements used to characterize glycemic control (Table 2). Average weekly post-prandial glucose concentrations were controlled during study with insulin and did not differ between the two doses of STZ. HbA1c (Figure 1) and plasma fructosamine concentrations (Table 2), long- and medium-term indicators of glycemic control, respectively, confirm comparable average glucose concentrations. HbA1c percentages, fructosamine and post-prandial glucose concentrations were significantly elevated in all STZ-DM monkeys independent of STZ dose. Exogenous insulin requirements necessary to maintain post-prandial glucose levels in the two groups of STZ-DM monkeys are illustrated in Figure 2. Exogenous insulin

requirements increased rapidly over the first 4 weeks post-STZ; while remaining relatively constant thereafter in the STZ-45 group, average insulin required to maintain similar glucose levels continued to increase over the next 12 weeks in the STZ-55 monkeys. There were no differences between STZ-45 and STZ-55 animals in plasma insulin or endogenous c-peptide concentrations (Figure 3). Plasma insulin was significantly and persistently attenuated in all the STZ-DM monkeys (Figure 3A, $p < 0.05$ all measures post-induction). Likewise, plasma c-peptide remained significantly decreased at all measures post-STZ (Figure 3B $p < 0.05$ vs. baseline). Monkeys received no morning insulin prior to blood sampling to minimize erroneous insulin measures; therefore, similarly reduced c-peptide concentrations suggest that the measured insulin was endogenous. Together these data indicate some residual β -cell activity after either dose of STZ.

Glucose Tolerance Test with Arginine Challenge

Glucose and insulin responses to the IVGTT/arginine challenge are presented in Table 2 and Figure 4. Area under the glucose excursion curve during the first 30 min following intravenous dextrose administration was significantly increased in all STZ-DM monkeys ($p < 0.01$ vs. CTL and vs. baseline). Conversely, there was virtually no insulin response to the glucose challenge (Figure 4 and Table 2, $p < 0.01$ vs. both CTL and baseline). Because of inability of the STZ-DM pancreas to respond to glucose, the rate of glucose disappearance (K_g) measured during IVGTT was also significantly reduced in both STZ-45 and STZ-55 animals compared to both CTL ($p < 0.01$) and to baseline ($p < 0.01$). Figure 4 illustrates that insulin secretion in all STZ-DM vervets is virtually absent in response to either glucose challenge or direct stimulation via arginine. There were no significant differences between STZ-45 and STZ-55 in glucose-, arginine-, or insulin-stimulated release of insulin or c-peptide (Figure 4 and Table 2). There were no significant differences or change in arginine-stimulated release of insulin or c-peptide in diabetic animals at the necropsy time point (data not shown) indicating an irreversible and stable diabetic state.

Clinical Chemistry and Hematology

Table 3 contains selected clinical chemistry results. There were no differences at baseline between groups in any of the measured parameters, with the exception of red blood cell count (RBC). Renal function indices included blood urea nitrogen (BUN) and plasma creatinine concentrations. There was a significant overall increase in BUN only in the STZ-55 group compared to the CTL animals ($p = 0.04$), and creatinine was significantly increased after 12 weeks in the STZ-55 group relative to its baseline value ($p = 0.011$) and compared to the control group, together suggesting greater renal toxicity with the greater STZ dose. Hepatic function was assessed using alkaline phosphatase (ALKP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations. While plasma concentrations of all three were increased in all STZ-DM monkeys, significance ($p < 0.05$ vs. CTL) of ALKP (Figure 5) and ALT elevations was again attained only in the STZ-55 group. The initial hepatic enzyme increases were generally resolved more rapidly in the STZ-45 animals than in those receiving 55 mg/kg STZ. Pancreatic exocrine function is reflected by circulating lipase and amylase concentrations. Transient elevation of lipase suggestive of pancreatitis was seen between 8 and 12 weeks only in the STZ-55 group (Table 3). Plasma triglycerides in the STZ-55 vervets were significantly elevated compared to baseline ($p < 0.05$ at every post-induction timepoint) and to CTL ($p < 0.05$ at 3, 12 and 16 weeks). While plasma triglycerides were also elevated in STZ-45 monkeys, they were significantly different than baseline only after 16 weeks. Likewise, total cholesterol in the STZ-55 group was significantly ($p < 0.05$) higher than CTL at 3 weeks and 8 weeks post-induction and significantly elevated compared to baseline ($p < 0.01$) at every post-induction time point.

Hematology results are shown in Table 4. Red cell counts were reduced to a greater magnitude and through the entire study in the STZ-55 group ($p < 0.05$ vs. baseline at 12 and 16 wks) while STZ-45 values recovered by 12 weeks. RBC in the STZ-55 monkeys was also significantly attenuated ($p < 0.05$) compared to CTL at 16 weeks. Between 8 and 16 weeks post-STZ, hemoglobin concentrations and hematocrit values were significantly reduced from baseline only in the STZ-55 group.

Discussion

White et al described the first documented use of STZ in vervets [19]. They induced DM in five monkeys with 60 mg/kg STZ; one of the five vervets died within a week, however it appears that exogenous insulin was not administered to control hyperglycemia. They reported neither clinical chemistry nor hematology parameters, and the only data reported other than fasted blood glucose and glucose response to IVGTT was the presence of trace amounts of glucose, blood, and protein in the monkeys' urine. They concluded that STZ is an effective diabetogenic agent in vervets, but provide no evidence regarding safety concerns or longevity of the model for study of mechanisms or therapeutic approaches to DM.

In the current study we describe in detail for the first time the development of a model of STZ-induced insulin-dependent diabetes in vervets. We conclude that a single dose of STZ induced insulin-dependent diabetes with 95% efficacy in vervets without recovery of β -cell function. Vervets could be maintained at relatively stable levels of blood glucose control with twice-daily insulin therapy for at least four months. Additionally we show that relatively small reduction in STZ dose (18%) significantly improves the toxicity profile with little change in efficacy. Finally, investigators that employ STZ T1DM models need to remain cognizant of the time required for the condition to stabilize (as indicated by insulin requirements) and for the STZ-related adverse effects on multiple organ systems to subside. In this vervet model, 16 weeks following a single 45 mg/kg dose of STZ is recommended as this combination resulted in monkeys that were not different in bodyweight or clinical pathology as compared to controls.

There is well-known species variation in STZ dose required to induce diabetes in animal models, and equally large variability exists in the literature regarding only macaque models of STZ-DM [11]. Single STZ doses of 30 mg/kg [10, 21] or even 50–60 mg/kg [12, 22, 23], have proven unreliable in effective or reproducible induction of insulin-dependent DM in macaques (i.e., conversion of 50% or fewer animals); conversely, Koulmanda et al. [13] demonstrated 100% efficaciousness with a single 55 mg/kg dose of STZ in the same species. Others have employed single STZ doses between 100 and 150 mg/kg [22, 24, 25] or combined serial STZ doses [12, 21, 26] totalling up to or over 100 mg/kg in order to effect DM. Such high doses of STZ may be efficacious or purportedly safe in younger monkeys [13, 24], but may effect acute kidney and liver tissue damage [13], renal failure [26] or even death [25] in older monkeys. Rood et al. [22] even showed that different types of STZ vary in both efficacy and toxicity. Thus, we erred on the side of caution in choosing single doses of 45 mg/kg and 55 mg/kg to induce DM in mature adult vervets. There is a documented correlation between amount of STZ administered and development of hepatic and/or renal morbidity [13]. In the current study our primary objective was the establishment of insulin-dependent DM using a single STZ dose while incurring minimal adverse side effects. We therefore felt it important to maintain uniformity amongst test subjects regarding dosing of STZ, especially in order to assess potential extra-pancreatic toxicity in our model, as a function of time and STZ dose, via regular clinical pathology analyses.

Both 45 mg/kg and 55 mg/kg STZ proved equally efficacious in producing DM characterized by hyperglycemia and insulin dependence. Blood glucose levels and exogenous insulin requirements increased steadily in all monkeys during the first 4 weeks after STZ. While similar levels of glycemic control were maintained independent of STZ dose, we observed a trend toward lower exogenous insulin requirements in monkeys receiving 45 mg/kg STZ, suggesting that reduction in STZ dose might lead to less β -cell destruction. On the other hand, we found similar residual insulin and c-peptide production, both under basal conditions and after stimulation by glucose tolerance testing or arginine challenge, independent of STZ dose. Insulin and/or c-peptide responses to arginine-mediated direct stimulation of β -cells via arginine challenge are reflective of functional β -cell mass [20]. While basal insulin and c-peptide concentrations averaged 10–20% of baseline, we documented no appreciable release of either insulin or c-peptide in response to arginine challenge in any STZ-DM vervets, indicative that a single dose of either amount was sufficient to destroy pancreatic β -cell reserve capacity. Koulmanda et al. (2003) report no difference in blood glucose in macaques two days after receiving either 55 mg/kg STZ or 100 mg/kg STZ, but no subsequent documentation of glycemic control is provided. Aside from Litwak et al. (1998), who reported serial measurements of glycated hemoglobin up to 6 months after STZ, reporting of glycemic control parameters in primate STZ-DM is scarce.

While pancreatic endocrine function was markedly suppressed in STZ-DM vervets, exocrine function, as assessed by plasma amylase and lipase concentrations, was not diminished at any timepoint in either dose group. As sometimes seen in human insulin-deficient DM, serum triglyceride and cholesterol levels were both significantly elevated in the 55 mg/kg treated STZ-DM vervets. STZ-induced DM has led to only minimal increases in lipid concentrations but greater atherogenesis in macaques [8]; however Jonasson et al. [27] found no evidence of atherosclerotic changes after ten years in STZ-DM rhesus macaques. Since the vervet has been shown to be an appropriate model for human atherosclerosis [18, 28], further long term follow-up may prove the STZ-DM vervet even more valuable for the study of diabetic atherogenesis.

In many macaque STZ-DM reports, there is little [13, 22, 24, 26] or no documentation [21, 27] of toxicity in other organ systems, particularly renal or hepatic. Wijkstrom et al. [25] reported elevation of liver enzymes, but in only half of macaques that received 100–150 mg/kg STZ. Likewise, Theriault et al. [24] reported acute transient elevation of hepatic enzymes that apparently returned to normal only 72 hours after STZ in juvenile macaques. Assessment of toxicity in both of these studies, however, may be confounded by administration of immunosuppressive therapy and/or islet transplantation only weeks after STZ. Of note, commonly studied complications of DM include examination of multiple organs; thus, knowing whether abnormalities may be DM- or STZ-related is highly pertinent. In mice, hepatic changes including lipid peroxidation, mitochondrial swelling, peroxisome proliferation and inhibition of hepatocyte proliferation may appear even before STZ-induced hyperglycemia, indicating hepatotoxicity due directly to STZ and not secondarily to DM [29]. Furthermore, direct STZ hepatotoxicity via the upregulation of apoptosis-related genes has also been shown in primary cultured hepatocytes [30]. Primates are less sensitive to STZ hepatotoxic effects than other species [31], however we document significant elevation of liver enzymes in STZ-DM vervets by 3 weeks post-STZ that appears not to resolve until at least 16 weeks. Three weeks after STZ, circulating alkaline phosphatase and alanine transferase were 100% and 400% higher than baseline, respectively. Elevation of hepatic enzymes also appeared to be STZ-dose related, since alkaline phosphatase was statistically different only in the STZ-55 group, and levels of both alkaline phosphatase and alanine transferase returned toward baseline more rapidly in monkeys receiving 45 mg/kg STZ than in those receiving 55 mg/kg.

Despite nephropathy being noted in macaque STZ-DM models, either concomitant with [25] or in absence of elevated liver enzymes [22], only transient significant differences in BUN and creatinine between control and STZ-55 vervets were found, suggesting minimal adverse renal effects due to either STZ or to chronic hyperglycemia; however urinalysis was not available for a complete assessment. All three animals that did not survive the protocol exhibited excessive plasma concentrations of BUN (74 – 105 mg/dL) and creatinine (1.5 – 2.4 mg/dL). All three presented clinically with severe inappetence and lethargy, and upon necropsy all appeared emaciated with total body mass loss ranging from 28% to 50%. It is not clear whether the primary cause of the animals' poor condition was extensive loss of functional pancreatic tissue, which may have subsequently led to a more rapid decline into iatrogenic type I diabetes and pathologic metabolic dysfunction despite insulin therapy, or if STZ directly caused acute nephropathy. While rapid excessive loss of body mass and extensive liberation of fatty acids might have directly cause renal tubular lipidosis (observed histopathologically; data not shown) in these three monkeys, others have associated nephrotoxicity, including proteinuria, squamous metaplasia, tubular abnormalities/lesions and even acute renal failure with STZ administration [26, 31, 32]. While others report mortality rates associated with STZ-induced DM in macaques as high as 43% [12] and 67% [25], those in the current study were considerably lower.

One of the more notable observations in the current study is the apparently STZ dose-related significant attenuation of hemoglobin, hematocrit, and red blood cell count, beginning as early as 8 weeks after induction. Changes were not related to phlebotomy since blood collection was consistent amongst all groups and our control animals exhibited no changes in any red cell parameter. Although mention or measure of hematological indices is absent from most macaque STZ-DM reports [8, 12, 21, 22, 24, 33], Wijkstrom et al [25] similarly document anemia in cynomolgus macaques as early as two weeks after receiving STZ doses 2–3 times higher than those in the current study. It is unclear in either case whether the anemia is a direct STZ effect or a byproduct of the STZ-induced hyperglycemia, but the accelerated time course with the higher STZ dose suggests the former. Elevated glycated hemoglobin levels do correlate significantly with attenuation of erythrocyte life span, suggesting that sustained high blood glucose levels such as those measured in these vervets leads to rapid removal of red blood cells from the circulation [34]. Also diabetic humans present with increased erythrocyte lipid peroxidation [35], which has been shown *in vitro* to result in increased cell fragility [36]. Glycemic control was independent of STZ dose in the current study, and greater attenuation of red blood cell count and hemoglobin in animals receiving 55 mg/kg STZ than in those dosed with 45 mg/kg, offers support that a direct STZ effect is more likely.

This study establishes the STZ-induced diabetic vervet as a viable animal model and, owing to its phylogenetic similarity to humans, as a potentially valuable tool in furthering our understanding of mechanisms behind T1DM complications, diabetic atherogenesis and therapeutic approaches to treatments. As detailed here, caution is warranted in implementation of this as well as other animal models of STZ-induced diabetes, especially when interpreting results or attributing pathologies to hyperglycemia or to side effects of therapeutic interventions. Furthermore, as age has been shown to be a factor in susceptibility to the primary and/or secondary effects of streptozotocin, it is important to emphasize that most vervets in this study were young adults; the recommendations we make may not be applicable to very young or old vervet monkeys. At a minimum, regardless of animal model, sufficient time should be allowed for stabilization of glycemic control/insulin requirements as well as resolution of potential direct STZ-mediated side effects before introduction of further intervention.

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Figure 1.

Glycated hemoglobin (HbA1c), a long-term indicator of glycemic control, was increased by over 100% in both STZ-45 and STZ-55 monkeys and maintained at this elevated level from 8 to 16 weeks post-induction. Insulin was administered twice daily to diabetic monkeys to control blood glucose to comparable concentrations in both groups of STZ monkeys.

Figure 2.

Exogenous insulin requirements increase rapidly for all animals in the 4 weeks immediately following STZ administration. Insulin doses remained relatively constant after 4 weeks in STZ-45 monkeys while continuing to steadily increase in the STZ-55 group, despite comparable glycemic control between the two groups.

Figure 3. Plasma levels of insulin (A) and c-peptide (B) were reduced by 80–90%, indicating destruction of the vast majority of pancreatic β -cells in STZ-DM monkeys. A trend towards dosedependent reductions in β -cell function as evidenced by c-peptide and insulin is suggested.

Figure 4. Following challenges with both intravenous glucose and arginine, there was negligible stimulated insulin release in either STZ-45 or STZ-55 monkeys.

Figure 5.

Hepatic alkaline phosphatase (ALKP) levels throughout 16 weeks of study. ALKP was significantly different from Control only in the STZ-55 group. Though ALKP in STZ-45 monkeys did not differ statistically from Control, it did not return to baseline levels until 16 weeks post-induction, while STZ-55 levels of ALKP remain elevated. * $p < 0.05$ overall group difference STZ-55 vs. Control.

Table 1

Body weight

	Baseline	+3 weeks	+8 weeks	+12 weeks	+16 weeks
Body Weight (kg)					
Control	7.31 ± 0.27	7.36 ± 0.28	6.93 ± 0.30	6.95 ± 0.41	7.15 ± 0.56
STZ-45	7.38 ± 0.37	6.90 ± 0.39	6.72 ± 0.37 *	6.87 ± 0.31 *	7.16 ± 0.29
STZ-55	7.60 ± 0.27	6.66 ± 0.23 *	6.41 ± 0.24 *	6.36 ± 0.27 *	6.50 ± 0.28 *

* p<0.05 vs. Baseline

Table 2

Glycemic and IVGTT indices

	Baseline	+3 weeks	+8 weeks	+12 weeks	+16 weeks	Group p-value vs. Control
Post-prandial Glucose (mmol/L)						
Control	2.89 ± 0.17	3.27 ± 0.17	3.50 ± 0.28	3.39 ± 0.33	3.11 ± 0.22	
STZ-45	2.89 ± 0.17	12.88 ± 2.22	12.27 ± 2.05	11.71 ± 4.27	8.49 ± 3.77	0.01
STZ-55	2.77 ± 0.17	15.43 ± 1.22	12.65 ± 2.05	12.88 ± 2.33	11.99 ± 1.39	0.003
Fructosamine (mEq/L)						
Control	163 ± 5	163 ± 8	158 ± 7	165 ± 10	168 ± 14	
STZ-45	157 ± 9	343 ± 18 *	358 ± 23 *	340 ± 49 *	397 ± 66 *	0.001
STZ-55	159 ± 8	391 ± 20 *	446 ± 19 *	464 ± 34 *	462 ± 33 *	0.0002
IVGTT Glucose AUC						
Control	7444 ± 231	6889 ± 383				
STZ-45	6675 ± 265	14026 ± 863 *				0.0001
STZ-55	7734 ± 234	15109 ± 652 *				0.0001
IVGTT Insulin AUC						
Control	3233 ± 298	2921 ± 268				
STZ-45	2476 ± 322	221 ± 52 *				0.0001
STZ-55	2685 ± 416	109 ± 32 *				0.0001
IVGTT K_g (%/min⁻¹)						
Control	3.47 ± 0.28	3.57 ± 0.45				
STZ-45	3.40 ± 0.51	1.08 ± 0.12 *				0.0004
STZ-55	3.17 ± 0.35	1.06 ± 0.10 *				0.0001
Arginine-Stimulated Insulin AUC						
Control		1108 ± 172				
STZ-45		111 ± 24 †				0.0002
STZ-55		48 ± 15 †				0.0001
Arginine Stimulated C-peptide AUC						
Control		7547 ± 1248				

	Baseline	+3 weeks	+8 weeks	+12 weeks	+16 weeks	Group p-value vs. Control
STZ-45		819 ± 268 [‡]				0.0002
STZ-55		586 ± 93 [‡]				0.0001

* p<0.05 vs. Baseline

[‡] p<0.05 vs. CTL

Table 3

Clinical chemistry

	Baseline	+3 weeks	+8 weeks	+12 weeks	+16 weeks	Group p-value vs. Control
Amylase (U/L)						
Control	393 ± 18	407 ± 23	382 ± 22	380 ± 27	389 ± 31	
STZ-45	409 ± 20	406 ± 17	410 ± 33	422 ± 55	404 ± 37	NS
STZ-55	391 ± 25	357 ± 27	352 ± 30	335 ± 23	333 ± 28	NS
Lipase (U/L)						
Control	85 ± 10	96 ± 11	95 ± 12	91 ± 10	98 ± 18	
STZ-45	88 ± 8	94 ± 9	101 ± 8	83 ± 10	107 ± 12	NS
STZ-55	70 ± 9	89 ± 14	103 ± 13 *	107 ± 17 *	87 ± 15	NS
BUN (mmol/L)						
Control	5.96 ± 0.25	5.50 ± 0.21	5.07 ± 0.36	5.32 ± 0.43	6.07 ± 0.21	
STZ-45	5.82 ± 0.25	6.18 ± 0.25	6.78 ± 0.68	8.50 ± 2.64	7.50 ± 0.71	0.24
STZ-55	5.96 ± 0.18	6.89 ± 0.36	7.71 ± 0.61	8.39 ± 0.54	9.39 ± 1.11	0.04
Creatinine (mmol/L)						
Control	59.5 ± 3.81	56.4 ± 2.29	53.4 ± 4.58	53.4 ± 2.29	51.9 ± 5.34	
STZ-45	67.1 ± 2.29	64.8 ± 1.53	64.8 ± 5.34	76.3 ± 12.2	76.3 ± 6.86	0.047
STZ-55	58.7 ± 2.29	64.1 ± 1.53	67.1 ± 3.05	64.1 ± 3.05	81.6 ± 9.91 * [†]	0.07
ALT (U/L)						
Control	67 ± 6	83 ± 12	84 ± 14	69 ± 13	106 ± 27	
STZ-45	60 ± 10	441 ± 295 *	170 ± 87	133 ± 24	127 ± 35	0.63
STZ-55	59 ± 5	394 ± 78 * [†]	313 ± 144 *	168 ± 27	153 ± 24	0.03
AST (U/L)						
Control	46 ± 3	42 ± 3	52 ± 13	42 ± 6	69 ± 23	
STZ-45	41 ± 2	140 ± 74	52 ± 12	51 ± 10	83 ± 14	NS
STZ-55	38 ± 2	165 ± 52 *	149 ± 74	85 ± 18	67 ± 14	NS
Triglycerides (mmol/L)						
Control	0.41 ± 0.03	0.41 ± 0.02	0.43 ± 0.05	0.49 ± 0.09	0.37 ± 0.05	
STZ-45	0.45 ± 0.06	0.89 ± 0.11	1.21 ± 0.49	1.28 ± 0.63	1.27 ± 0.31 *	0.027

	Baseline	+3 weeks	+8 weeks	+12 weeks	+16 weeks	Group p-value vs. Control
STZ-55	0.47 ± 0.05	2.23 ± 0.36 ^{*,†}	1.65 ± 0.46 [*]	1.95 ± 0.42 ^{*,†}	1.98 ± 0.41 ^{*,†}	0.001
Total Cholesterol (mmol/L)						
Control	3.78 ± 0.16	3.86 ± 0.18	3.73 ± 0.23	3.60 ± 0.21	3.52 ± 0.36	
STZ-45	4.33 ± 0.34	6.19 ± 0.65	5.39 ± 0.49	5.02 ± 0.85	5.62 ± 0.85	0.02
STZ-55	4.09 ± 0.18	6.97 ± 0.49 ^{*,†}	6.24 ± 0.34 ^{*,†}	5.75 ± 0.31 [*]	5.65 ± 0.26 [*]	0.004
WBC (×10⁶/L)						
Control	5.5 ± 0.6	5.6 ± 0.5	4.8 ± 0.6	5.2 ± 0.7	4.8 ± 0.9	
STZ-45	4.1 ± 0.4	4.4 ± 0.4	3.6 ± 0.5	3.5 ± 0.5	3.1 ± 0.3	NS
STZ-55	4.3 ± 0.3	4.8 ± 0.6	3.5 ± 0.4	3.6 ± 0.5	3.5 ± 0.4	NS
RBC (×10¹²/L)						
Control	6.9 ± 0.1	6.9 ± 0.1	6.4 ± 0.2	6.5 ± 0.3	6.9 ± 0.2	
STZ-45	7.3 ± 0.2	6.9 ± 0.2	6.0 ± 0.3 [*]	6.3 ± 0.3	6.3 ± 0.4	0.88
STZ-55	6.6 ± 0.2 [#]	6.5 ± 0.1	5.5 ± 0.2	5.2 ± 0.3 [*]	5.2 ± 0.3 ^{*,†}	0.03

BUN: blood urea nitrogen; ALT: alanine aminotransferase; AST: aspartate aminotransferase;

^{*} p<0.05 vs. Baseline;

[†] p<0.05 vs. CTL; NS indicates no significant overall group difference by repeated measures ANOVA

Table 4

Clinical hematology

	Baseline	+3 weeks	+8 weeks	+12 weeks	+16 weeks	Group p-value vs. Control
WBC ($\times 10^6/L$)						
Control	5.5 $\pm$ 0.6	5.6 $\pm$ 0.5	4.8 $\pm$ 0.6	5.2 $\pm$ 0.7	4.8 $\pm$ 0.9	
STZ-45	4.1 $\pm$ 0.4	4.4 $\pm$ 0.4	3.6 $\pm$ 0.5	3.5 $\pm$ 0.5	3.1 $\pm$ 0.3	NS
STZ-55	4.3 $\pm$ 0.3	4.8 $\pm$ 0.6	3.5 $\pm$ 0.4	3.6 $\pm$ 0.5	3.5 $\pm$ 0.4	NS
RBC ($\times 10^{12}/L$)						
Control	6.9 $\pm$ 0.1	6.9 $\pm$ 0.1	6.4 $\pm$ 0.2	6.5 $\pm$ 0.3	6.9 $\pm$ 0.2	
STZ-45	7.3 $\pm$ 0.2	6.9 $\pm$ 0.2	6.0 $\pm$ 0.3 *	6.3 $\pm$ 0.3	6.3 $\pm$ 0.4	0.88
STZ-55	6.6 $\pm$ 0.2 #	6.5 $\pm$ 0.1	5.5 $\pm$ 0.2	5.2 $\pm$ 0.3 *	5.2 $\pm$ 0.3 *†	0.03
Hct (%)						
Control	51.9 $\pm$ 0.8	52.7 $\pm$ 1.0	47.9 $\pm$ 2.5	50.5 $\pm$ 2.2	49.0 $\pm$ 3.1	
STZ-45	54.6 $\pm$ 1.7	52.8 $\pm$ 2.5	46.7 $\pm$ 2.3	46.3 $\pm$ 2.9	47.1 $\pm$ 0.0	0.87
STZ-55	50.1 $\pm$ 1.3	47.5 $\pm$ 0.8	37.3 $\pm$ 1.6 *	38.7 $\pm$ 2.2 †	38.0 $\pm$ 1.0 *†	0.01
Hb (g/L)						
Control	166 $\pm$ 3	171 $\pm$ 3	160 $\pm$ 9	168 $\pm$ 8	167 $\pm$ 9	
STZ-45	177 $\pm$ 5	166 $\pm$ 5	154 $\pm$ 8	156 $\pm$ 10	149 $\pm$ 0	0.91
STZ-55	161 $\pm$ 4	157 $\pm$ 3	126 $\pm$ 6	126 $\pm$ 7 †	127 $\pm$ 5 *†	0.007

WBC: white blood cells; RBC: red blood cells;

* p<0.05 vs. Baseline;

† p<0.05 vs. CTL; NS indicates no significant overall group difference by repeated measures ANOVA