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Original Research

Comparison of Insulins Glargine and Degludec in Diabetic Rhesus Macaques (*Macaca mulatta*) with CGM Devices

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Treating and monitoring type 2 diabetes mellitus (T2DM) in NHP can be challenging. Multiple insulin and hypoglycemic therapies and management tools exist, but few studies demonstrate their benefits in a NHP clinical setting. The insulins glargine and degludec are long-acting insulins; their duration of action in humans exceeds 24 and 42 h, respectively. In the first of this study's 2 components, we evaluated whether insulin degludec could be dosed daily at equivalent units to glargine to achieve comparable blood glucose (BG) reduction in diabetic rhesus macaques (*Macaca mulatta*) with continuous glucose monitoring (CGM) devices. The second component assessed the accuracy of CGM devices in rhesus macaques by comparing time-stamped CGM interstitial glucose values, glucometer BG readings, and BG levels measured by using an automated clinical chemistry analyzer from samples that were collected at the beginning and end of each CGM device placement. The CGM devices collected a total of 21,637 glucose data points from 6 diabetic rhesus macaques that received glargine followed by degludec every 24 h for 1 wk each. Ultimately, glucose values averaged 29 mg/dL higher with degludec than with glargine. Glucose values were comparable between the CGM device, glucometer, and chemistry analyzer, thus validating that CGM devices as reliable for measuring BG levels in rhesus macaques. Although glargine was superior to degludec when given at the same dose (units/day), both are safe and effective treatment options. Glucose values from CGM, glucometers, and chemistry analyzers provided results that were analogous to BG values in rhesus macaques. Our report further highlights critical clinical aspects of using glargine as compared with degludec in NHP and the benefits of using CGM devices in macaques.

Abbreviations: BG, blood glucose; CGM, continuous glucose monitoring; FBG, fasted blood glucose; HbA1c, glycosylated hemoglobin; T2DM, type 2 diabetes mellitus

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Diabetes is a group of metabolic diseases that cause hyperglycemia secondary to deficient insulin response, secretion, or both.[4] Diabetes is categorized by the American Diabetes Association into 4 types: 1) type 1 diabetes mellitus, in which the pancreas is unable to produce insulin for glucose absorption; 2) type 2 diabetes mellitus (T2DM), when the body does not use insulin correctly; 3) gestational diabetes, in which the body is insulin-intolerant during pregnancy (or is first discovered then); and 4) other specific forms of diabetes in which the patient is particularly predisposed to becoming diabetic due to various comorbidities or to inadvertent induction caused by some medications.[4] In 2018, 34.2 million (10.5%) Americans of all ages were diagnosed with diabetes.[22,23,30] Approximately 90% to 95% of Americans with diabetes have T2DM,[24] making T2DM the most common form of diabetes diagnosed in humans.

T2DM is a multifactorial disease primarily determined by genetics, behavioral and environmental factors (for example, age, diet, sedentary lifestyle, obesity).[4,46,50,74] As a consequence of these factors, the pancreas increases insulin secretion to maintain normal glucose tolerance.[74] Over time, the high insulin demand causes pancreatic β-cell destruction, resulting in reduced production of insulin.[39,50,74] As β-cell destruction increases, hyperglycemia and T2DM develop. Insulin resistance and hyperglycemia are tolerated for a period of time[19,82,83] before clinical signs associated with T2DM develop (e.g., polydipsia, polyuria, polyphagia with concurrent weight loss).[4] Once clinical signs develop, T2DM is most commonly diagnosed as a fasting blood glucose level (FBG) of 126 mg/dL or greater,[2,4] 2-h plasma glucose value of 200 mg/d or greater during a 75-g oral glucose tolerance test,[2,4] and/or glycosylated hemoglobin (HbA1c) of 6.5% or greater.[2,4] Depending on the FBG, oral glucose tolerance test, and HbA1c results, various treatment options are recommended by the American Diabetes Association. Most importantly, lifestyle changes, including diet and exercise, are recommended as the first line of treatment, along with oral antihyperglycemic drugs such as metformin.[5,25,46] Treatment efficacy is evaluated with self-monitoring blood glucose or continuous glucose monitoring (CGM) devices.[3] Human patients using CGM devices have achieved considerable reductions in HbA1c compared with patients not using them.[3] As CGM devices have become more readily available, user friendly, and affordable, they have become an essential tool in managing T2DM.

Similar to humans, most NHP affected by diabetes are diagnosed with T2DM.[80,83] NHP are predisposed to similar genetic, behavioral and environmental factors (e.g., age, diet,

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sedentary lifestyle, obesity);[6,18,19,37,44,52,82,83] have similar pathophysiology;[38,81-83] are diagnosed via FBG,[39,83] HbA1c,[21,31,49,56] fructosamine,[20,83,87] and weight loss;[49,80,83,86] and are treated with exercise and diet modifications as a first line of treatment.[11,19,39,53,79] Although the human and NHP conditions are similar, the treatment and management of T2DM is somewhat different, especially when NHP have restricted physical activity due to housing constraints.

Previous studies indicate that daily dosing with insulin glargine achieves appropriate glycemic control in NHP.[48] Therefore, we implemented glargine, along with some diet modification, to improve glycemic control in our diabetic colony. Other noninsulin therapies, such as metformin, had been used, but compliance was low (for example, due to large pill size, unpleasant taste, etc.). However, achieving glycemic control using diet modification, insulin glargine treatment, monthly scheduled FBG, quarterly HbA1c, and regular weight monitoring was challenging in a large colony. Monthly FBG and fructosamine testing were performed due to affordability and practicality for NHP in a research setting. Given that fructosamine levels correlate with BG concentrations for the preceding 2 to 3 wk and HbA1c percentages relate to BG concentration over 1.5 to 3 mo,[49,87] HbA1C was selected over fructosamine for T2DM management in our colony. Determining which T2DM treatment and diagnostics are most effective can be difficult in large colonies of NHP. Therefore, improved treatment and management strategies would help to manage T2DM in NHP more efficiently.

Insulin glargine is a long-acting insulin, with a half-life of 12 h and duration of action of 12 to 24 h in humans[40,55] and 12 h in dogs.[34,43,60] Once injected subcutaneously, insulin glargine forms a microprecipitate in the neutral pH environment, which delays and prolongs absorption in subcutaneous tissues.[12] Insulin degludec is a newer form of long-acting insulin, with a half-life of 25 h[41,63,62,77] and duration of action that exceeds 42 h in humans.[40,41,68,77] Insulin degludec forms a soluble and stable dihexamer in the pharmaceutical formulation, which includes phenol and zinc.[63,78] The phenol diffuses away, leading to the formation of a soluble depot in the form of long multihexamer chains in which zinc slowly diffuses from the end of the multihexamers, causing a gradual, continuous, and extended-release of monomers from the depot of the injection site.[63,78] Pharmacodynamic studies in humans, demonstrate that the "glucose-lowering effect" of insulin degluc[40] is evenly distributed over 24 h, allowing a more stable steady-state and improved wellbeing.[78] This approach could potentially reduce the number of hypoglycemic events and provide a less rigid daily injection schedule,[58] thus potentially making insulin degludec—compared with insulin glargine—a safer, alternative diabetes therapy.

In addition to medical intervention, glycemic control is achieved through regular management and monitoring of BG. Self-monitoring blood glucose checks in humans[3,5] and glucose curves in animals[10] are some of the management tools used to determine or evaluate therapy for T2DM patients. Telemetry systems like CGM devices are used to monitor interstitial glucose and have been used extensively in humans[3,17,33] and animals[16,27,36,42,47,84,85] to monitor BG in real-time. Using CGM devices 1) reduces or eliminates the number of blood draws needed to collect FBG,[61] 2) accurately assesses insulin therapy via a real-time glucose curve,[72,84,85] 3) allows patients and clinicians to titrate treatment[61,73] as indicated, and 4) obtains continuous glucose data with reduced manipulation and subsequent decreased stress.[72,84,85] Therefore, CGM devices can be a safe and informative tool in monitoring spontaneous T2DM in NHP.

Between 2015 and 2030, the prevalence of diabetes is predicted to increase by 54% to more than 54 million Americans affected by diabetes (i.e., diabetes mellitus types 1 and 2).[70] NHP are an essential model for human T2DM because of their similar pathophysiology, diagnostics, treatment, and management. As more people develop diabetes, novel therapies will continue to be developed. Studying new treatments and management tools in NHP can further human and NHP T2DM research to prevent the progression of T2DM and hopefully diminish projections for the number of future diabetes cases. Human medical literature, American Diabetes Association, and drug manufacturers all recommend giving equal doses (i.e., number of units/day) of long-acting insulins when changing from one long-acting insulin to degludec.[26,63,67] Therefore, we hypothesized that insulin degludec would provide effective glycemic control for rhesus macaques with T2DM when dosed at equivalent doses (that is, the same number of units/day) as insulin glargine. In addition, we hypothesized that CGM devices would provide accurate BG readings as compared with chemistry analyzer and glucometer BG readings, making it a more efficient and effective tool for measurement of BG levels in rhesus macaques with T2DM.

Materials and Methods

Animals and animal care. Six adult rhesus macaques, two adult male (age, 14.0 ± 4.0 y; weight, 12.4 ± 0.4 kg) and four adult female rhesus macaques (age, 20.0 ± 4.0 y; weight, 8.5 ± 1.7 kg), with naturally occurring T2DM were born and housed at the California National Primate Research Center (Davis, CA) and were used in this pharmacodynamics and efficacy study. Prior to this study, diabetic animals were managed by administering insulin glargine at varying units/day. Doses differed significantly between animals based on each animal's individual response to therapy (Table 1). The wellbeing of each animal was ensured by obtaining a clinical history, physical examination, CBC count, and serum biochemical profile prior to and at the conclusion of each CGM device application and removal. All animals were free of *Macacine herpesvirus 1*, simian retrovirus type D, SIV, and simian T-lymphotropic leukemia virus. The macaques were tested twice annually for tuberculosis, and all remained negative. During the study, animals were housed individually in stainless steel cages, pair-housed when compatible or provided with visual and auditory contact with conspecifics. Cage sizes were based on weight per the USDA Animal Welfare Act.[6] Cages were cleaned daily and complete cage changes were performed every 2 weeks. Animals were fed a commercial diet twice daily (LabDiet Monkey Diet 5047, Purina Mills International, St Louis, MO), offered water ad libitum, and supplemented with vegetables (instead of fruits and vegetables). Toys and coconuts were supplied as manipulanda. Macaques were maintained on a 12:12-h light:dark cycle. The cages were kept at a constant temperature (typically 23 ± 3 °C) and humidity (30% to 70%). All animal procedures in this study were approved by the University of California—Davis (UC Davis) IACUC prior to implementation. Animals were maintained in accordance with the USDA Animal Welfare Act,[6] Animal Welfare Regulations[7] and the Guide for the Care and Use of Laboratory Animals.[45] The animal care and use program of UC Davis is fully AAALAC-accredited, is USDA-registered, and maintains a Public Health Services assurance.

Application and calibration of CGM device. The CGM device system has 3 parts: a sensor that collects interstitial glucose data from a small needle inserted into the skin; a transmitter that wirelessly transfers glucose readings from the sensor to

Table 1. Demographics of rhesus macaques included in the study

Animal	Weight (kg)	Body condition score (1–5)	Sex	Age (y)	Housing condition	Fasting serum glucose (mg/dL)[a]	HbA1c (%)[b]	Insulin dose (units/day)
MMU01	6.74	2.5	Female	24	Paired	172	10.3	7
MMU02	10.21	2.5	Female	22	Unpaired	170	9.7	1
MMU03	6.88	2	Female	20	Paired	179	10.2	8
MMU04	6.92	2	Female	16	Unpaired	82	9.8	5
MMU05	12.32	2.5	Male	18	Paired	91	7.3	2
MMU06	12.4	3	Male	10	Paired	482	15.7	7

[a]Fasting serum glucose measured via chemistry analyzer at the beginning of the study, while animals received glargine
[b]Measured at enrollment into study

a receiver; and a receiver that displays the glucose readings. As directed in the manufacturer's instructions, the receiver (model G6, Dexcom, San Diego, CA) was fully charged 1 d prior to study start; the time, date, transmitter ID, and glucose alert range were then set on day 1 of the study. For application and removal of the CGM device, each macaque was fasted for approximately 10 h and then injected with ketamine (5 to 30 mg/kg IM; MWI, Boise, ID) and dexmedetomidine (0.0075 to 0.015 mg/kg IM; Orion Pharma, Espoo, Finland) for sedation on day 1 of the study. Once the animal was sedated, the skin around the animal's upper back was shaved and aseptically prepped with alternating betadine and 70% ethanol. The skin was allowed to air dry before sensor insertion by using an injection device. Next, the sensor was fitted with a transmitter that snapped in place. The fabricated edges of the transmitter–sensor combination were affixed to the skin surface with skin glue and a bandage was applied (Figure 1). Once the CGM device application was finished, the animal was returned to its cage, and anesthesia reversed by using intramuscular atipamezole (Orion) at an appropriate dose.

The CGM device detects interstitial tissue fluid glucose levels via a tiny glucose sensor inserted under the skin. A transmitter attached to the sensor calibrates for 2 h and displays its first reading 2 h after initial placement of the device. After calibration, the CGM device wirelessly sends readings every 5 min to a receiver or smart device located within 20 ft of the animal. If only a receiver is used, live data are displayed on its screen and saved for future download. If a smart device is used, the CGM device wirelessly sends values to the individual's Dexcom account. This feature allows monitoring of glucose values remotely on an additional smart device or computer. Once all glucose readings for both glargine and degludec were collected for each animal, the values were downloaded to an Excel (Microsoft, Redmond, WA) spreadsheet format from the Dexcom website or software.

Dosing with glargine and degludec. Once a macaque was returned to its cage after CGM device placement and recovery, glargine (Sanofi-Aventis, Bridgewater, NJ) was administered subcutaneously at the previously prescribed dose for each animal. Doses of glargine were not changed for at least 4 wk prior to study start. Glargine was administered once daily at 0800 ± 15 min. On subsequent days, clinical observations confirmed CGM placement and glucose readings. At the end of 1 wk, the animal was sedated to remove the CGM device, and glargine treatment continued to be administered until the next CGM application and concurrent switch to degludec, 1 to 8 wk later.

Each animal randomly received a 1- to 8-wk break from the CGM device before reapplication in order to accommodate other activities, such as a pair mate's involvement on research projects and routine cage changes. During this time, the animal continued to receive glargine. After a 1- to 8-wk break from the previous CGM device application, a new CGM device was placed. After CGM placement and recovery, degludec (Novo Nordisk, Bagsvaerd, Denmark) was administered subcutaneously at the same daily dose as glargine. This dose reflected both the manufacturer[63] and American Diabetes Association[26] recommendations and a study[67] conducted in humans. Degludec was administered once daily at 0800 ± 15 min. Daily clinical observations confirmed CGM placement and glucose readings. At the end of 1 wk, the animal was sedated with ketamine for removal of the CGM device, and degludec continued to be administered after study completion.

Comparing BG values between CGM, chemistry analyzer, and glucometer devices. To compare glucometer (One Touch Ultra, LifeScan, Milpitas, CA), chemistry analyzer, and CGM device glucose values simultaneously, fasted BG was determined at the beginning and end of each CGM device application and removal. Prior to study start, positive reinforcement techniques were used to train macaques to present the left arm for blood collection. After CGM device application and a 2 h calibration period, blood samples were collected from the cephalic vein. Once blood was obtained, the sample was time-stamped and immediately tested by using a glucometer device. Within 10 to 15 min of collection, the remaining blood was centrifuged at 3,000 x *g* for 15 min inside of a refrigerated centrifuge. The serum was separated immediately, and glucose analysis was performed by using a chemistry analyzer (model AU480, Beckman Coulter, Brea, CA). Once glucometer and chemistry analyzer BG values were obtained, they were compared with the time-stamped CGM device glucose reading to assess the CGM device's accuracy. At the time of CGM device removal from the ketamine-sedated animal, readings were also taken from the CGM device, chemistry analyzer, and glucometer. Blood was analyzed via glucometer and chemistry analyzer as described previously.

Statistical analysis. Summary data are expressed as the average ± SE. Statistical analysis was performed in the R programming environment (R Core Team, Vienna, Austria). All plots were created using ggplot2. To evaluate the effects of the insulin analogs on the CGM measured glucose levels, we used linear mixed-effects regression as provided by the lmer command in the lme4 package.[7] We fitted models with the experimental drug as the fixed effect variable. Animal subject and study time point (longitudinal time scale) were used as the random-effect covariates. To use maximum likelihood for the estimation, restricted maximum likelihood was set to 'false.' To compute confidence intervals (lower 5% and higher 95%) for model parameters, we used the confint function with the fitter model as the object. The estimation was done by using bootstrapping method (n = 1000 simulations) for the parametric bootstrap intervals.

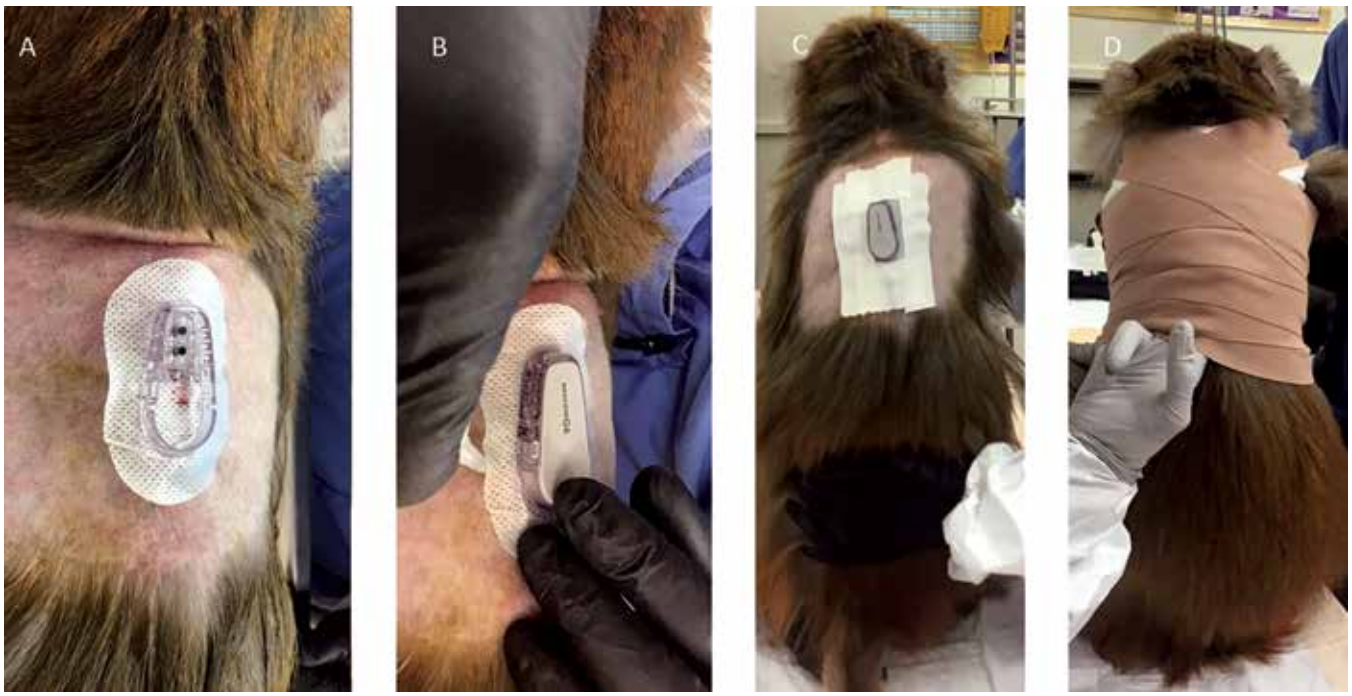

Figure 1. CGM application. (A) After the macaque's back was shaved and aseptically prepped, the sensor was applied to dried skin by using the applicator device. (B) The transmitter was snapped into the sensor, and glue was applied to the sensor's fabricated edges. (C) Medical tape was affixed to the sensor's fabricated edge and animal's skin. (D) A bandage was applied over the CGM device to prevent its removal by the macaque or its cage mate.

Results

Equivalent dosing of glargine and degludec. The CGM devices recorded 21,637 glucose data points from 6 diabetic rhesus macaques, with an average number of recorded data points of 1791 ± 80 for glargine and 1796 ± 79 for degludec. The data were then used to assess whether glargine and degludec could be dosed at equivalent numbers of units daily. Because of the severe hyperglycemia on the first day of CGM device recordings for animal MMU06, we increased the insulin glargine dose from 5 U to 7 U. To incorporate 7 full days of administration of the same dose of glargine, which would subsequently also be used for degludec, we excluded the first day of readings from the analysis for this animal and used the CGM device for a total of 8 d. The summary of the mixed-effects model of the longitudinal data collected by using the CGM device demonstrated that glargine lowered the glucose value on average by 29 ± 1 mg/dL ($P < 2.2 \times 10^{-16}$) as compared with degludec (Figure 2). The 95% CI for the proposed difference was computed as 28 to 31 mg/dL.

Comparison of BG values according to measurement method. Interstitial glucose values were recorded by using the CGM device and were compared with chemistry analyzer or glucometer BG values collected at the same time points from unsedated animals. BG values were measured by using the chemistry analyzer and glucometer at 2 separate time points for each type of insulin ($n = 22$). These values were compared with those simultaneously collected from the CGM device. One CGM device was removed prematurely by a cage mate, and another CGM device stopped recording glucose values at day 7 of the study, just prior to sedation. Thus, simultaneous CGM, chemistry analyzer and glucometer glucose comparisons were not available at 2 time points. Nonetheless, linear regression fit found that significant amounts of the variation in CGM measures could be explained by glucometer glucose readings ($R^2 = 0.831$; $P \leq 0.0001$) and the chemistry analyzer glucose readings ($R^2 = 0.810$; $P \leq 0.0001$; Figure 3).

Discussion

This study demonstrates for the first time that the same doses of glargine and degludec result in differing glucose levels and that subcutaneous CGM application can be used successfully in rhesus macaques. In our colony of rhesus macaques with naturally occurring T2DM, doses for glargine were taken from a published study that used drug-induced T2DM NHP.[48] Although both induced and spontaneous T2DM have similar pathologies, they differ in origin. This could possibly cause differences in insulin absorption and/or secretion. No pharmacokinetic studies of insulin glargine have specifically been conducted in rhesus macaques with spontaneous T2DM. Further research is needed to determine the pharmacokinetics of glargine in rhesus macaques with spontaneous T2DM. According to human literature, degludec has a longer (>42 h) duration of action than does glargine (up to 24 h).[40,41,77] However, our current study revealed that glucose values after glargine were lower overall than were values after degludec given at the same dose and frequency. Our results demonstrate that the efficacy of degludec in NHP differs from that of glargine, contrary to our hypothesis. Human studies show that degludec significantly decreases HbA1c as compared with glargine and had less day-to-day variability.[40,58] Additional research is needed to determine whether degludec at higher doses can achieve lower HbA1c values and better glycemic control compared with glargine in macaques. Our study confirmed that CGM devices are a reliable tool to monitor glucose values in rhesus macaques, as suggested by previous studies in other species.[61,72,84]

Diagnosis of T2DM in our rhesus macaque colony typically occurs when animals undergo routine physical exams or blood work or have unexpected weight loss. In addition, once considered geriatric (19 y or older), our animals receive an HbA1c blood test biannually. NHP are diagnosed with T2DM when fasting blood glucose is greater than or equal to 126 mg/dL[11,80] and HbA1c is greater than or equal to 10%. NHP are diagnosed

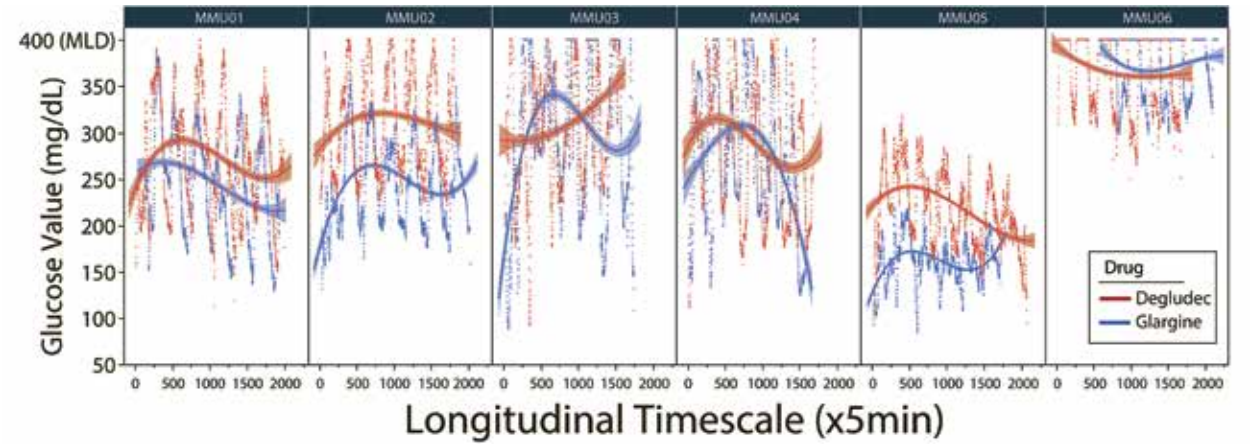

Figure 2. Longitudinal changes in the BG level. CGM devices were implanted subcutaneously and used to record the BG concentration every 5 min (*x* axis) while diabetic macaques received glargine or degludec at the same dose. On average, the BG value was 29 mg/dL lower when macaques received glargine. The curves shown are elements (cubic spline with a lambda of 0.05); the increase in BG shown is highly significant in mixed effect regression ($P = 2.2 \times 10^{-16}$). The shaded area around the splines are the 95% CI for the fitted values. The maximum level of detection (MLD) of the device was 400 mg/dL.

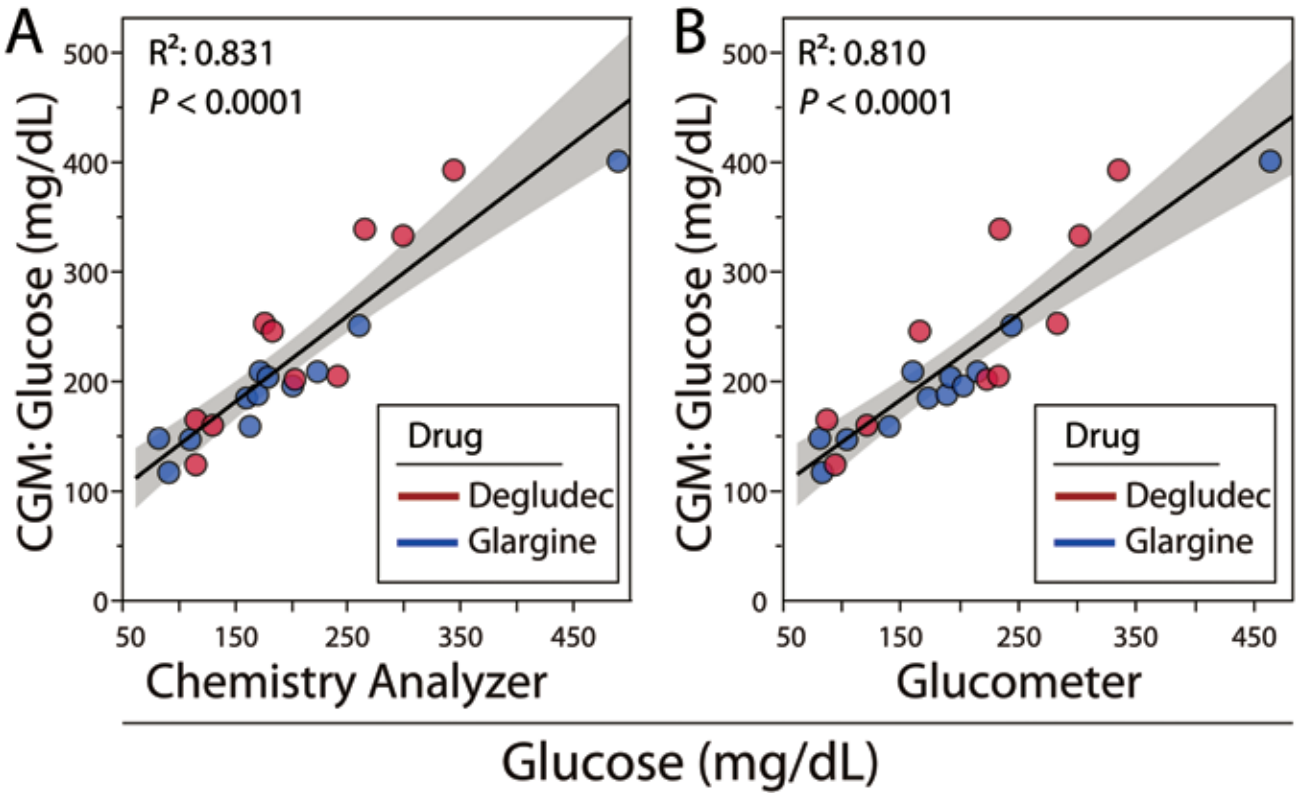

Figure 3. Relationship between BG measurement by using chemistry analyzer or glucometer and CGM. (A and B) BG measurement by using a (A) chemistry analyzer method or (B) glucometer device was highly correlated with the BG recorded via CGM method. Note that maximum level of detection of the CGM device is 400 mg/dL. R^2, adjusted r-square. Gray shading indicates the 95% CI for the predicted value (fit).

with prediabetes when they have a fasted BG of 100 to 125 mg/dL[11] and HbA1c of 5% to 10%. Animals with metabolic syndrome are typically obese with a high normal FBG, low HDL cholesterol, and elevated triglycerides.[80] All nondiabetic macaques in our facility receive 7 chow biscuits twice daily, with daily forage and vegetables and fruits twice weekly. Diets for diabetic animal diets are the same, except their fruit is replaced with extra vegetables. Once diagnosed with T2DM, glycemic control was monitored through monthly FBG and quarterly HbA1c levels. FBG provides a snapshot of BG levels, whereas HbA1c approximates BG levels over the previous 3 mo period. However, the CGM device allows the creation of a continuous glucose curve. Five of the 6 animals had elevated glucose levels with either type of insulin. This elevation in glucose despite treatment was abnormal and was attributed to insulin resistance and/or poor insulin absorption.

In addition to obesity,[83] NHP have demonstrated insulin resistance as a result of feeding a high-fructose diet[13] and inherited genes related to impaired glucose transport into skeletal muscle,[75] defective insulin action on cAMP-dependent protein kinase activity,[64] adipose tissue glycogen synthase,[65] and skeletal muscle glycogen synthase.[66] These varied etiologies

of insulin resistance complicate the appropriate treatment of T2DM. T2DM in NHP has been managed with insulin and noninsulin therapies that include glargine,[43] Humulin,[11,47,48] metformin,[32,86] rosiglitazxone,[35] pioglitazone,[51] dietary restriction,[11,19,39,53,79] and unrestricted activity.[11] Metformin has historically been used in NHP and is also the first line of treatment in humans. However, a difficulty with metformin is the inability to administer the drug properly to NHP. Metformin in whole pill-form has an unpleasant taste, large pill size, gastrointestinal side effects, and requires multiple doses daily. The drug has been administered to chair-trained NHP via oral gavage or physical manipulation of the pill (for example, cutting or crushing).[32,86] According to manufacturers, this manipulation can change the pharmacokinetic properties and lead to unwanted adverse effects.[1,14,28,57,69] Metformin and other insulin regimens (besides glargine) also require multiple daily treatments, potentially leading to more stress.

Advantages to using the CGM device include 1) performing fewer venipunctures, 2) reduced animal handling, 3) easy application, 4) low cost, 5) glucose recordings every 5 min, 6) wireless glucose readings that can be viewed remotely, and 7) acquiring a glucose curve. Disadvantages of a CGM device as compared with other glucose recording methods potentially include the need to replace the CGM device every 7 to 10 d,[84,85] and fluctuations in glucose values secondary to pressure and temperature changes, as previously reported in pigs and humans.[42,59,76] Encapsulation has also occurred in subcutaneous tissues of animals with a CGM device,[16,36] which requires more frequent replacement of CGM devices as compared with other telemetry devices. Future studies are needed to evaluate alternative T2DM managing devices in rhesus macaques, such as longer acting CGM devices, and also using CGM devices concurrently with insulin pumps.

Sex-associated differences were not evaluated in the current study because unequal numbers of females and males were enrolled. As a breeding colony, females are overrepresented due to management design. Relatively few naturally occurring T2DM animals are present in the colony, again with females overrepresented. Endogenous progestins impair insulin sensitivity, but endogenous estrogens and androgens have minimal effects on glucose regulation.[15,83] In addition, fasting insulin levels and insulin response to glucose challenge are highly correlated with body fat.[15] Therefore, sex-associated differences in insulin sensitivity may be associated more strongly with body composition and fat distribution.

Although most cross-over studies involve a washout period between drug administrations, we did not include a washout period because the study animals had spontaneous T2DM and required daily insulin therapy. Degludec steady state is reached in humans after 3 to 4 d.[63] Despite allowing degludec 3 to 4 d to equilibrate, the initial BG, as determined by the CGM device, was not significantly different from that measured at the end of the week. As previously mentioned, the efficacy between degludec differed between humans and rhesus macaques. Thus, the steady state may also differ between species. The American Diabetes Association recommends in emergency situations that when switching from degludec to another long-acting insulin, including glargine, the human dose (in units/day) should be decreased by 20% when less than 80 units/day.[54] Thus, the efficacy of glargine may change when switching back from degludec.

Following the same instructions described in the methods section of CGM manual, we initially attempted to place the CGM device on the arm of a female macaque. However, once the animal recovered from sedation, she quickly removed the bandage and CGM device. Because of this episode, the remaining CGM devices were applied on the upper back with an overlying bandage to decrease the likelihood of premature removal. Nevertheless, with a cage mate's help, one animal (MMU03) removed its CGM device with bandage intact while receiving degludec. Thus, chemistry analyzer and glucometer BG measurements were not obtained for this animal at the end of the study because it had no CGM reading for comparison. This demonstrates that maintaining CGM devices on rhesus macaques and other NHP can be challenging. Other studies have placed CGM devices on the back and covered it with a monkey vest[84,85] to maintain placement. However, animals in that study were singly housed, so access to the CGM device was more limited than in this study.

In one animal (MMU06), the CGM device stopped emitting glucose readings despite correct placement while the animal was receiving degludec. Glucose readings stopped at day 7, just prior to CGM device removal. Thus, chemistry analyzer nor glucometer BG values were not obtained at the end of the study for this animal because a CGM reading was not available for comparison. Removing the CGM device revealed no external trauma to the device or sensor needle. Other studies have shown that sensors have a short lifespan secondary to temperature and pressure changes,[42,59,76] deposits adhering to the sensor affecting its performance,[88] and encapsulation.[16,36] Any one of these factors could have caused the sensor to prematurely stop emitting glucose values despite being labeled to remain functional for as long as 10 d.[29]

The primary purposes of this study were to determine whether insulins glargine and degludec could be effectively administered at the same doses (in units/day) and to test whether CGM devices accurately assessed BG values as compared with values from a standard chemistry analyzer and a glucometer device. Our study also identified important shortcomings in existing T2DM management, as shown by hyperglycemic values recorded by the CGM devices. Previous research has shown that despite insulin therapy, hyperglycemia could be related to insulin resistance and insulin deficiency as occurs in diabetic humans.[71] The hyperglycemia ultimately led us to change T2DM diagnosis and treatment within our NHP colony. These changes included performing HbA1c tests if high-normal or high FBG readings are found in undiagnosed animals, adding oral hypoglycemics to recently diagnosed T2DM animals, replacing glargine with degludec for T2DM animals, and using higher doses of glargine or degludec. Previously, we were hesitant to increase insulin doses for animals with low FBG and high HbA1c for fear of causing hypoglycemia. However, the CGM device revealed that FBG was not an accurate representation of an animal's glycemic control. Although degludec did not achieve similar glucose levels at the same dose as glargine in our study, degludec has less day-to-day variability and fewer hypoglycemic events than does glargine.[58,62,67,78] These properties allowed us to safely increase degludec doses to achieve better glycemic control. These recent changes have allowed us to diagnose more NHP with T2DM, and treated animals have had significant improvement in glycemic control. We did not anticipate these findings, but they demonstrate the advantage of CGM devices for T2DM diagnosis and maintenance.

In summary, degludec was not as effective in lowering glucose at the same units/day dose as glargine nor did it seem to have the same duration as described in humans. However, neither insulin therapy achieved glycemic control in most of our T2DM animals. More research is needed to determine the appropriate doses of both degludec and glargine. CGM devices are

reliable tools in analyzing glucose values in rhesus macaques and have the benefit of providing remote access for monitoring glucose levels by using smart devices or computers. These new T2DM treatment and management tools can be safely used to treat and manage diabetic rhesus macaques. Appropriately treating T2DM in NHP will not only improve the health of these animals but also will aid in longitudinal studies with aged macaques that naturally develop T2DM, thus ultimately prolonging their lives and their research contributions.

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