INTRODUCTION

With the epidemic of obesity and type 2 diabetes, patients today frequently require large doses of insulin to achieve optimal glycaemic control because of their severe insulin resistance. Such patients often require multiple injections exceeding 100 units at a time, which can cause discomfort and reduced adherence to therapy. Additionally, a large depot of subcutaneous insulin may delay its absorption, which compromises...
the ability of the insulin to achieve optimal glycaemic control. Many such patients have been transitioned to the more concentrated formulation of U-500 regular insulin because they are able to deliver the same amount of insulin in a much smaller volume which in turn increases patient satisfaction, adherence and overall glycaemic control.2

Until recently, the dosing regimens recommended for U-500 insulin were based on pharmacokinetic and pharmacodynamic studies in healthy volunteers, which may not reflect how the insulin works in the obese and insulin resistant patients with diabetes for which it is typically prescribed. In obese nondiabetic subjects, de la Pena et al3 found that the peaks of the concentration and action profiles for U-500 were blunted and that the glucose lowering effect after the peak was prolonged as compared to identical doses of U-100. The data collected in this study were pooled with data collected from healthy normal weight subjects without diabetes and with normal weight subjects with type 1 diabetes to develop a model to describe the pharmacokinetic and pharmacodynamics parameters of U-500 insulin.4 The model demonstrated that steady state pharmacokinetics was reached by 24 hours after the first dose in regimens that included once, twice and thrice daily dosing. At steady state, the once daily dosing showed the greatest fluctuations in pharmacodynamics whereas thrice daily dosing was maintained throughout the dosing interval. Based on these findings, Hood et al5 performed a randomized trial that compared twice daily dosing to thrice daily dosing of U-500 in obese subjects with type 2 diabetes. They found that both regimens were effective in reducing haemoglobin A1c, albeit with an incidence of symptomatic hypoglycaemia that exceeded 90% in each group. Such a high rate of hypoglycaemia suggests that there may be some unpredictability in the glucose lowering action of the U-500 treatments applied to these subjects.

To directly assess the pharmacokinetics and pharmacodynamics of U-500 insulin in obese subjects with type 2 diabetes, we designed a double-blind, crossover euglycaemic clamp study using clinically relevant doses to determine duration of action and rate of onset of U-500 insulin. Our research goal was to develop more effective U-500 treatment regimens for the obese and insulin resistant patients who are most commonly prescribed U-500 insulin.

2 | MATERIAL AND METHODS

This was a single centre double-blind, crossover study in which subjects were randomly assigned to receive 100 or 200 units of U-500 insulin on two separate occasions separated by a minimum of 2 weeks. Inclusion criteria included type 2 diabetes diagnosed on clinical grounds, age between 30-65 years, haemoglobin A1c between 53-80 mmol/mol (7.0%-9.5%) in the month before enrollment, BMI between 27 and 38 kg/m², on an insulin regimen that included more than 100 units/d, willingness to discontinue oral/ injectable noninsulin medications one week before the study and basal insulin for a minimum of 15 hours prior to the expected U-500 dose administration, and willingness to avoid exercise for 48 hours before the study. Exclusion criteria included treatment with systemic corticosteroids in the preceding 3 months, alcohol consumption of >21 drinks/wk for men or >14 drinks/wk for women, unwillingness to stop alcohol consumption for 24 hours before each study visit, pregnant or actively trying to conceive, current diagnosis of active infection, cancer (other than basal cell carcinoma), vascular disease, organ failure or transplant. The study protocol was approved by the Institutional Review Board at the University of Minnesota and was registered in ClinicalTrials.gov (NCT02148250).

At the screening visit, informed consent was obtained and subjects were provided with individualized directions for how to manage their diabetes medications prior to the day of study with the intention to eliminate circulating home medications during the study period. Subjects were admitted to the Clinical Research Unit after dinner on the night (between 8 and 10 pm) before the study. They were maintained on intravenous insulin overnight to maintain blood glucose between 100 and 150 mg/dL. The insulin infusion was discontinued no later than 6:30 AM. At 7 AM, the following day subjects were given a blinded dose of either 100 or 200 units of U-500 regular insulin subcutaneously according to a randomization schema managed by the investigational pharmacy. Blood glucose was measured every 15-60 minutes using a bedside glucose analyzer after administration of U-500 insulin and variable infusion of 20% dextrose were given to maintain euglycaemia (100 mg/dL target). Meals containing 30 g of carbohydrate were provided at hours 4 (11 AM), 9 (4 PM), 13 (8 PM), 26 (9 AM day 2) and 30 (1 PM day 2). Samples for subsequent measurement of total insulin and C-peptide by chemiluminescent immunoassay (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA) and serum glucose (Vista Analyzer, Siemens Healthineers, Tarrytown, NY, USA) were collected at every 60 minutes until the subject could maintain euglycaemia without a glucose infusion for at least 10 hours or until the subject experienced hyperglycaemia (BG > 150 mg/dL) for two hours after discontinuation of the glucose infusion irrespective of total duration of glucose infusion. At the end of the study, the subject was scheduled to return no sooner than 2 weeks to repeat the study with the dose of U-500 they did not receive the first time.

To compare the responses to the two insulin doses, the U-500 onset was determined by measuring the area under the curve (AUC) of IV glucose given between hours 0-4, the peak infusion rate achieved during hours 0-4, and the time at which IV glucose was started following administration of the U-500 insulin. Duration of action was determined by measuring the time IV glucose was required to maintain euglycaemia. The effect of each dose on glucose lowering was determined by measuring the AUC of the IV glucose given to maintain euglycaemia for the entire study.

3 | RESULTS

Seventeen subjects were enrolled in the study with 12 (3F, 9M; mean age 54 ± 5 years, mean BMI 36.0 ± 3.3 kg/m²) completing both study parts (Table 1). Reasons for not completing the study included loss
of intravenous access, cancellation after screening visit and inability to schedule a second visit. The completers had a mean HbA1c of 67 ± 5.3 mmol/mol (8.3 ± 0.7%) and took 174 ± 40 units of insulin per day on average. All subjects were taking more than one medication to treat their diabetes. Eleven subjects were treated with lispro or aspart by injection or pump, and one subject was treated with U500 insulin by injection. Nine subjects were also treated with basal insulin (one with degludec, one with U300 glargine, one with NPH, and six with U100 glargine). Eight subjects were also treated with metformin. Liraglutide was used by one subject, and dulaglutide was used by one other subject.

Blood glucose concentrations were maintained at similar levels following administration of both doses of U-500 (Figure 1A), both before and after meals were ingested. Serum insulin concentrations were the same at the time of administration of both doses of U-500 and nearly doubled within 4 hours before the first meal (Figure 1B). After adjusting for the time since administration of the dose, insulin concentration following the 200-unit dose of U-500 was significantly higher than that achieved following administration of the 100-unit dose (P = 0.008). Serum C-peptide levels were the same at time 0 and decreased similarly during the first 4 hours after administration of both doses of U-500 (Figure 1C), but no significant differences were found in C-peptide concentrations measured after administration of each dose. The change in insulin concentration before and after the second meal was significantly higher following the 100 vs the 200 unit dose (P = 0.039), but no differences were noted before and after the first meal following administration of either dose.

There was no difference in the outcome measures used to assess onset of action between the doses but the duration of action, as measured by the number of hours the glucose infusion was required to maintain euglycaemia was significantly longer following the 200-unit dose than the 100-unit dose (Table 2). Despite this difference, there was no difference between the doses in the amount of glucose that was necessary to maintain euglycaemia for the duration of the study.

4 | CONCLUSIONS

In patients with type 2 diabetes, obesity and insulin resistance, we found that the time during which intravenous glucose was

TABLE 1 Participant characteristics at enrolment

| Gender  | 3 F, 9 M |
| Age    | 54 ± 5 y |
| BMI    | 36.0 ± 3.3 kg/m² |
| Total daily insulin dose | 174 ± 40 units |
| Haemoglobin A1c | 67 ± 5.3 mmol/mol |
|         | 8.3 ± 0.7% |

FIGURE 1 (A) Glucose concentrations following administration of each dose. (B) Insulin concentrations following administration of each dose. (C) C-peptide concentrations following administration of each dose. Data collected after 9 h are censored for subjects who met stopping rules for the glucose infusion.
TABLE 2 Pharmacodynamics of 100 vs 200 units of U-500 regular insulin in obese subjects with type 2 diabetes

| Dose of U-500 | 100 units | 200 units |
|---------------|-----------|-----------|
| Total glucose given 0-4 h after U-500 dose (mg/kg) | 5.3 ± 6.5 | 4.7 ± 5.6 |
| Peak infusion rate achieved 0-4 h after U-500 (mg/kg/min) | 5.3 ± 3.8 | 4.2 ± 1.9 |
| Time following injection the glucose infusion was started to maintain EU (hours) | 2.6 ± 1.3 | 2.2 ± 1.0 |
| Duration of action (hours) | 11.0 ± 5.6 | 16.5 ± 6.4* |
| Total glucose required to maintain euglycaemia (mg/kg) | 18.5 ± 20.7 | 20.1 ± 13.3 |

*P = 0.031.

required to maintain euglycaemia following a 200-unit dose of U-500 insulin was significantly greater (16.5 hours) than the time following a 100-unit dose (11.0 hours). Interestingly, no differences were found in measures related to the rate of onset or in the total amount of intravenous glucose required to maintain euglycaemia for the duration of the study. These observations demonstrate that the duration of action of U-500 insulin increases as the dose is increased in subjects with type 2 diabetes, obesity and insulin resistance as has already been demonstrated in obese subjects without diabetes.3 These findings have implications for U-500 treatment regimens and suggest that dosing intervals might have to be extended as the dose of U-500 is increased. We also found that patients remained euglycaemic without the infusion of intravenous glucose for about 2.5 hours after administration of both doses of U-500. The fall in serum glucose from ~135 to 140 mg/dL at the time of dosing to ~110 mg/dL when intravenous glucose was begun shows that each dose exerted an effect on glucose homeostasis during the first 2 hours after injection, but the magnitude of this effect was small. These findings suggest that U-500 should not be used as a premeal bolus insulin with the expectation that it will work rapidly enough to prevent carbohydrate-induced hyperglycaemia 1-2 hours after meals.

U-500 insulin is being used more frequently as patients with diabetes require larger and larger doses of insulin to achieve glycaemic control. The dosing and titration recommendations of the manufacturer are based on open label study of patients with poorly controlled type 2 diabetics randomized to treatment regimens with twice daily or thrice daily dosing.2 In this titration-to-target study, similar A1c reductions were obtained using both dosing schedules, although participants in the twice daily dosing had more hypoglycaemia. Since total daily dose in the two groups were similar, the higher dose in twice daily dosing could explain, in part, the increased frequency of hypoglycaemia in this group.

Strengths of our study include the randomized crossover design and enrolment of study participants that resemble many of the patients who are prescribed U-500 insulin. We also studied clinically relevant doses of U-500 insulin. Weaknesses include our small sample size and an inability to determine how much of the circulating insulin present 4 hours after administration consisted of U-500 or endogenous insulin. Nonetheless, the fact that subjects developed hyperglycaemia many hours after U-500 administration at a time their serum insulin concentrations were back to baseline suggests that most of the rise in serum insulin concentrations was due to the U-500 administered. Ingestion of meals at hours 4, 9, 13, 26 and 30 likely increased endogenous insulin secretion and increased glucose entry into the blood, both of which could confound our measures of insulin action. However, the meals were each limited to 30 grams of carbohydrate and we found no differences in the insulin concentrations measured after the first meal in both dosing conditions. Based on our findings following a single injection of U-500, the glucose lowering effect of a dose exceeding 100 units per injection can last more than 11 hours. Future studies where subjects are given multiple doses over time will be necessary to calculate the precise U-500 dosing frequency necessary to optimize glycaemic control without causing hypoglycaemia.

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AUTHOR CONTRIBUTIONS

RTT collected data and reviewed the manuscript; AFK wrote the original protocol, collected data, and reviewed the manuscript; AT collected data and reviewed the manuscript; AAK revised the original protocol and reviewed the manuscript; AM participated in study design, collected data and reviewed the manuscript; EO analysed the data and reviewed the manuscript; LEE participated in study design, analysed data, and reviewed the manuscript; ERS participated in study design, wrote the original protocol, collected data, and drafted the manuscript.

CONFLICT OF INTEREST

RS, AFK, AT, AM, EO and LEE have no conflicts to disclose. ERS consults for Eli Lilly and Company, Sanofi, Novo Nordisk, Zucara Therapeutics, Inc. and has received research grants from Eli Lilly and Company.

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