GLOOCOSE STUDY FINAL REPORT

A randomised controlled trial of the sulfonylurea **G**liclazide and the DPP-4 inhibitor **L**inagliptin **o**n the frequency of hyp**o**gly**c**aemia am**o**ng patients with Type 2 Diabete**s** and Chronic Kidn**e**y Disease (CKD) stage 3b and 4.
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JRCO reference: 16HH3133

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Joint Research Compliance Office (JRCO), Imperial College London and Imperial College Healthcare NHS Trust

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Table of Contents

1.0 GLOOCOSE Study

1.1 Study Set Up, Approvals and Funding

The GLOOCOSE study is an abbreviation for "A randomised controlled trial of the sulphonylurea **G**liclazide and the DPP-4 inhibitor **L**inagliptin **o**n the frequency of hyp**o**gly**c**aemia am**o**ng patients with Type 2 Diabete**s** and chronic kidn**e**y disease (CKD) stage 3b and 4." The GLOOCOSE study is a randomised controlled Phase IV trial, which is unblinded to the participant and study team. It aimed to recruit 50 participants with type 2 diabetes and CKD stage 3b or 4 who were currently taking Gliclazide, and randomised them to either continuation of their current Gliclazide dose or switching to Linagliptin 5 mg od for 8 weeks (25 participants each arm).

The study was first conceived in 2013, and study protocol and other pertinent trial documents were submitted via the Integrated Research Application System (IRAS) in 2015. Approvals were obtained from the Medicines and Healthcare products Regulatory Agency (MHRA), the London Brent Research Ethics Committee (REC), and trust research and development (R&D) management. The study was also registered with the European electronic database of clinical trials (EudraCT). The first patient was recruited on 21st September 2016. A summary of the regulatory approvals is outlined in Table 1.

Regulatory Approvals		
MHRA	Sought 19/08/2015	Obtained 27/08/2015
REC (Ref: 15/LO/1548)	Sought 26/10/2015	Obtained 30/11/2015
JRCO Sponsor		Obtained 13/01/2016
Trust R&D	Sought 23/03/2016	Obtained 10/06/2016
Study Start Approval		Obtained 23/06/2016
HRA (study processed through pre-HRA approval systems)		Obtained 12/12/2016
Supporting Departmental Approvals		
Tissue Bank		Obtained 09/12/2015
Pathology		Obtained 15/03/2016
ICRRU		Obtained 15/03/2016
Pharmacy		Obtained 11/04/2016

Table 1 GLOOCOSE regulatory approvals on study set up

The GLOOCOSE study was sponsored by the Joint Research Compliance Office (JRCO) of Imperial College London (ICL) and Imperial College Healthcare NHS Trust (ICHT), and

funded by the DIAMOND Imperial College Healthcare Charity (ICHC) grant and Boehringer Ingelheim (BI) Limited. All study visits for GLOOCOSE study participants were held at the Imperial Clinical Respiratory Research Unit (ICRRU) at St Mary's Hospital. Both paper and electronic case report forms (CRFs) were used to record patient data. Electronic CRFs and randomisation of patients were completed using the InFORM Integrated Trial Management (ITM) database system. The study was eligible for National Institute for Health Research Clinical Research Network (NIHR CRN) support and portfolio adoption.

1.2 Substantial Amendments

There has been a sharp increase in Linagliptin use in renal patients since the GLOOCOSE study was originally designed in 2013; and being on Linagliptin was an exclusion criteria. In addition, patients with type 2 diabetes who have had diabetes long enough, or poorly controlled enough to have progressed onto stage 3 CKD onwards were usually on insulin, or 3 or more hypoglycaemic agents, which were also exclusion criteria. Substantial amendments were made in response to these recruitment challenges. A notification of substantial amendment form was submitted in May 2017 and approved by the relevant regulatory authorities in June 2017 (Table 2).

The substantial amendments mainly pertained to broadening of the eligibility criteria and summarising the PIS length from 11 to 3 pages. The age limit of eligible study participants was extended to 21 to 80 years inclusive. The initial eligibility criteria stated that patients had to have type 2 diabetes of 10 years or more duration – this was removed, as duration of diabetes was not usually accurately coded or unavailable during searches, and also not considered to influence the study findings. The protocol was amended to include patients taking Gliclazide, either with or without Metformin, Pioglitazone and/or basal insulin. This change to the eligibility criteria intended to increase recruitment numbers while still allowing for comparison of study patients staying on their usual Gliclazide to those randomised to Linagliptin. The HbA1c cut off limit was raised from 65 mmol/mol (8%) to 75 mmol/mol (9%), with the aim of improving recruitment. Moreover, hypoglycaemia and glycaemic variability can still occur in patients at higher HbA1c levels. Duration of study recruitment was adjusted upward from two to three years, to allow for the slower recruitment rate.

Regulatory Approvals		
REC	Sought 10/05/2017	Obtained 17/05/2017
MHRA	Sought 10/05/2017	Obtained 06/06/2017
HRA	Sought 10/05/2017	Obtained 09/06/2017
Trust R&D & Sponsor	Sought 10/05/2017	Obtained 12/06/2017

Table 2 GLOOCOSE regulatory approvals for substantial amendment

1.3 Screening and Recruitment

Screening of eligible patients was undertaken within ICHT hospitals (Hammersmith, St Mary's and Charing Cross), primarily from diabetes and renal outpatient clinic lists and patients discharged to the renal shared care pathway. The GLOOCOSE study also received support from the NIHR CRN for primary care organisations under North West London, North Thames London and South London CCGs to act as Participant Identification Centres (PICs). These comprised of 24 GP PICs in North West London, 30 in North Thames and 26 in South London – a total of 80 primary care PIC sites where potential participants were identified by the named GP for that PIC. London North West Healthcare NHS Trust (LNWHT, encompassing Northwick Park, Central Middlesex and Ealing Hospitals) and Central London Community Hospitals (CLCH) were further added as PIC sites. All eligible patients were approached in person or invited by letter (with the PIS attached) to meet the research team at ICCRU or the PIC site itself to discuss the study. No research procedures were conducted at the PICs.

1.4 Study Documents

1.4.1. Study Protocol, Patient Information Sheet (PIS), and Informed Consent Form (ICF)

Study protocol Version 3.2, 25th April 2016 was used when the trial first opened, and was superseded by Version 4, 10th January 2017 after the substantial amendment was approved. PIS Version 3, 11th July 2016 was used at the start of the GLOOCOSE study, but was replaced by Version 4, 10th January 2017 after the substantial amendment was approved. The informed consent form was updated from Version 1, 19th May 2015 to Version 1.1, 13th June 2017.

1.4.2 Food Diary and DTSQ

The GLOOCOSE study used a 7-day food diary (Version 2, 13th April 2016), which was adapted from the Airwave Health Monitoring Study's food diary in conjunction with the dietetic staff at Imperial College London.

The Diabetes Treatment Satisfaction Questionnaire (DTSQ)[1] comprises of 8 questions pertaining to the patient's current diabetes treatment. Question 1 and questions 4 – 8 assessed overall satisfaction, convenience, flexibility, understanding of diabetes, willingness to recommend their current diabetes treatment to others, and inclination to continue with their present treatment. These questions were rated on a 7-point Likert scale[2], with scores ranging from 0 ("Very dissatisfied") to 6 ("Very satisfied"). Questions 2 and 3 on the DTSQ asked the patient to rate their own perception of hyper- and hypoglycaemia, with scores ranging from 0 ("None of the time") to 6 ("Most of the time"). The scores (excluding questions 2 and 3) are added up for the DTSQ total score, with a minimum of 0 and a maximum of 36. Lower total DTSQ scores signify lower treatment satisfaction, while higher total DTSQ scores indicate greater satisfaction.

1.4.3 Case Report Forms (CRFs)

Electronic CRFs were developed by the Imperial Clinical Trials Unit (ICTU) InFORM team, together with the GLOOCOSE study statistician and research team. Enrolment of each patient on the InFORM database was required to generate a unique study number, which was used as a patient identifier throughout the study. Electronic and paper CRFs were completed for each study visit (including the screening visit), extra visits outside of the study schedule, adverse events, concomitant medications and protocol deviations.

1.5 Study Aims and Outcome Measures

1.5.1 Hypotheses

1. Patients with type 2 diabetes and CKD stage 3b and 4 on Linagliptin have fewer hypoglycaemic episodes and spend less time in hypoglycaemia, compared to CKD stage 3b and 4 patients who are on Gliclazide
2. Patients on Linagliptin have less glycaemic variability when compared to patients on Gliclazide
3. Serum and urine biomarkers associated with advancing kidney disease, are lower in patients taking Linagliptin, compared to patients taking Gliclazide
4. Patients on Linagliptin are more satisfied with their diabetes treatment than patients on Gliclazide

1.5.2 Aims

1. To assess whether patients with type 2 diabetes and moderate to severe kidney disease have less hypoglycaemia when taking Linagliptin instead of Gliclazide
2. To assess whether these same patients have decreased glycaemic variability when taking Linagliptin compared to Gliclazide
3. To assess whether Linagliptin has any other advantages over Gliclazide by examining effects on serum and urine biomarkers associated with declining kidney function
4. To assess whether study participants are more satisfied on Linagliptin or Gliclazide

1.5.3 Outcome Measures

1. Number of hypoglycaemic episodes and percentage of time spent in hypoglycaemia, pre and post randomisation
2. Glycaemic variability as measured by CGM indices, pre and post randomisation
3. Levels of serum and urine biomarkers of inflammation and fibrosis (MCP-1 and TGFβ-1), pre and post randomisation
4. Patient satisfaction with their diabetic treatment as measured by the total DTSQ score, pre and post randomisation

1.6 Eligibility Criteria and Withdrawal Criteria

The study inclusion criteria for the GLOOCOSE study were:

1. Type 2 diabetes mellitus
2. Age between 21 and 80 years inclusive
3. eGFR of 15 to 45 ml/min/1.73 m^2
4. HbA1c less than 75 mmol/mol (< 9%)
5. Currently taking Gliclazide with or without Metformin, Pioglitazone and/or basal insulin
6. Stable diabetic control in the last 2 months prior to randomisation
7. Understands adequate written and verbal English

The exclusion criteria were:

1. Type 1 diabetes mellitus
2. Currently on rapid/short acting, intermediate acting or mixed insulin
3. Currently on other DPP-4 inhibitors or GLP-1 receptor agonists
4. Immunosuppressive therapy (excluding inhaled steroids) within the previous 6 months
5. Pregnant or lactating women
6. Current malignancy or other life threatening illness
7. Inability to give informed consent

All study participants could withdraw from the study at any time for any reason, without it affecting their usual medical care. Other withdrawal criteria included protocol violation by the patient, failure of the study participant to follow research procedures, and patient harm as a result of following study procedures.

1.7 Disadvantages, Risks and Potential Benefits

There are no serious risks to taking part in the GLOOCOSE study. The main disadvantage of taking part in the research study is the time commitment required by the patient. The patient may also experience minor bruising or bleeding from blood taking and CGM insertion, mild skin irritation from the CGM adhesive dressings and rarely, CGM site infection. If the CGM sensor is dislodged, the patient may need to remove it and CGM data would not be collected after sensor displacement. Any changes to the patient's usual diabetes medication carries a small risk of making their glycaemic control unstable, although the study team closely monitors each patient to minimise that risk.

The potential benefits for the patient are learning more about their diabetes control. Their CGM results would be discussed with them and the study team would advise if their diabetes treatment regimen should be changed, particularly in the case of unrecognised hypoglycaemia.

1.8 Sample Size and Power Calculation

A CGM study of a cohort of patients with Type 2 diabetes showed that the mean duration of asymptomatic hypoglycaemia was 89 +/- 14 minutes. The GLOOCOSE study aimed to detect a clinically meaningful difference of 20% in the duration of asymptomatic hypoglycaemia between the control group (patients continuing with Gliclazide) and the treatment group (patients randomised to Linagliptin). A higher standard deviation of 20% of the mean was assumed for a better safety margin in the power calculation. Therefore, a sample size of 36 patients (18 patients in each arm) was required for detecting a 20% difference in the time spent in hypoglycaemia between the 2 treatment arms (assuming SD of 20%, alpha a of 0.05 and power of 0.85). The Type 1 error probability is 0.05. A recruitment aim of 50 patients was set to allow for a 15% CGM device failure rate and 10% subject drop out/withdrawal.

1.9 Study Design and Study Visits

There were a total of 6 study visits (inclusive of the initial screening visit) over 11 weeks. Figure 1 outlines the research procedures undertaken at each visit.

At the screening visit (Visit 0), a member of the study team would go over the PIS and answer questions pertaining to the study. If the patient agreed to participate, the informed consent form would be signed. The patient's eligibility would be assessed by going over their past medical history and medication history. Blood tests (FBC, U&Es and HbA1c), weight and blood pressure would be obtained. If the blood results rendered the patient ineligible they would be informed and no further study visits would take place.

At their first visit after the screening visit (Visit 1), a Medtronic Minimed Enlite™ (MMT-7008) CGM sensor (Northridge CA, USA) would be inserted on the patient's cleaned abdomen using the sensor insertion device. A charged Medtronic iPro2 CGM device would then be connected to the sensor, and both sensor and device would be secured into place with adhesive dressings. The study patient would also be instructed at the same visit on how to obtain their capillary blood glucose (CBG) readings and complete their 1-week food diary. A GlucoMen Areo by A. Menarini Diagnostics glucometer, a Glucoject Dual PLUS 33G finger pricker, lancets and GlucoMen Areo sensor test strips were given to each study participant. They were required to take at least four CBG meter readings per day, for the duration of the study week, to enable calibration of the CGM data. Study participants were advised to follow their normal daily routine, but to record their meals, diabetes medication timings, and any exercise or strenuous activities in their food diary. Care instructions and contact numbers for the study team were provided.

At Visit 2, there would be a quick check of the CBG meter readings obtained and the diary entries recorded by the patient. If these were satisfactory, the glucometer containing the CBG readings and completed food diary would be collected, the CGM sensor and device would be removed from the patient, and the insertion site inspected. Weight and blood pressure were taken, and the patient would then be asked to complete the DTSQ. Study bloods (approximately 15 mls) for FBC, U&Es, HbA1c, serum MCP-1 and serum TGF-β1, as well as urine samples (approximately 30 mls) for albumin-creatinine ratio, protein-creatinine ratio, urine MCP-1, urine TGF-β1 and culture would also be obtained.

Randomisation occurred via the InFORM database; if the patient were randomised to Linagliptin 5mg od, they would be instructed on its potential side effects and asked to stop their usual Gliclazide whilst on the study drug Linagliptin (their other diabetes medications such as Metformin, Pioglitazone or basal insulin would be continued throughout the study). An 8-week supply of Linagliptin was prescribed by the research team and dispensed by ICHT pharmacy at St Mary's Hospital to patients randomised to Linagliptin. Otherwise, if the patient had been randomised to stay on their current treatment, then they would continue on their usual dose of Gliclazide alongside any other current medications.

The study team would phone the study participant (Phone Call 1) one week after Visit 2 to ask if they had had any symptoms or episodes suggestive of hypo- or hyperglycaemia, and if any adverse events or side effects had occurred. Visit 3 would be held two weeks following Phone Call 1, and the study patient would attend ICRRU to have their weight and blood pressure checked, with a brief enquiry into their wellbeing. Another short telephone consultation would occur two weeks after Visit 3 (Phone Call 2) for the same queries to the patient as in Phone Call 1.

At Visit 4 (two weeks after Phone Call 2), each study participant was required to wear the CGM sensor and device for another week. The same research procedures as in Visit 1 were carried out at Visit 4, with the patient briefed to record their CBG readings four times a day for one week, and to write down their food intake, exercise and medication timings.

The final study visit (Visit 5) occurred one week after Visit 4, and ten weeks after the initial screening visit. The research procedures carried out at Visit 5 were identical to those undertaken at Visit 2, with the exception of randomisation. Patients randomised to switch to Linagliptin at Visit 2 would have finished their supply of the trial medication, and all patients would revert back to their original dose of Gliclazide at the end of the trial. A member of the research team would meet with each study patient within a month of study end to go over his or her CGM results, and advise if their current diabetic treatment was satisfactory, or if it needed to be changed. A letter detailing their CGM results and the research team's recommendations would also be sent to the patients and their GPs.

Flowchart

A randomised controlled trial of the sulfonylurea Gliclazide and the DPP4 inhibitor Linagliptin on the frequency of hypoglycaemia among patients with Type 2 Diabetes and chronic kidney disease (CKD) stage 3b and 4.

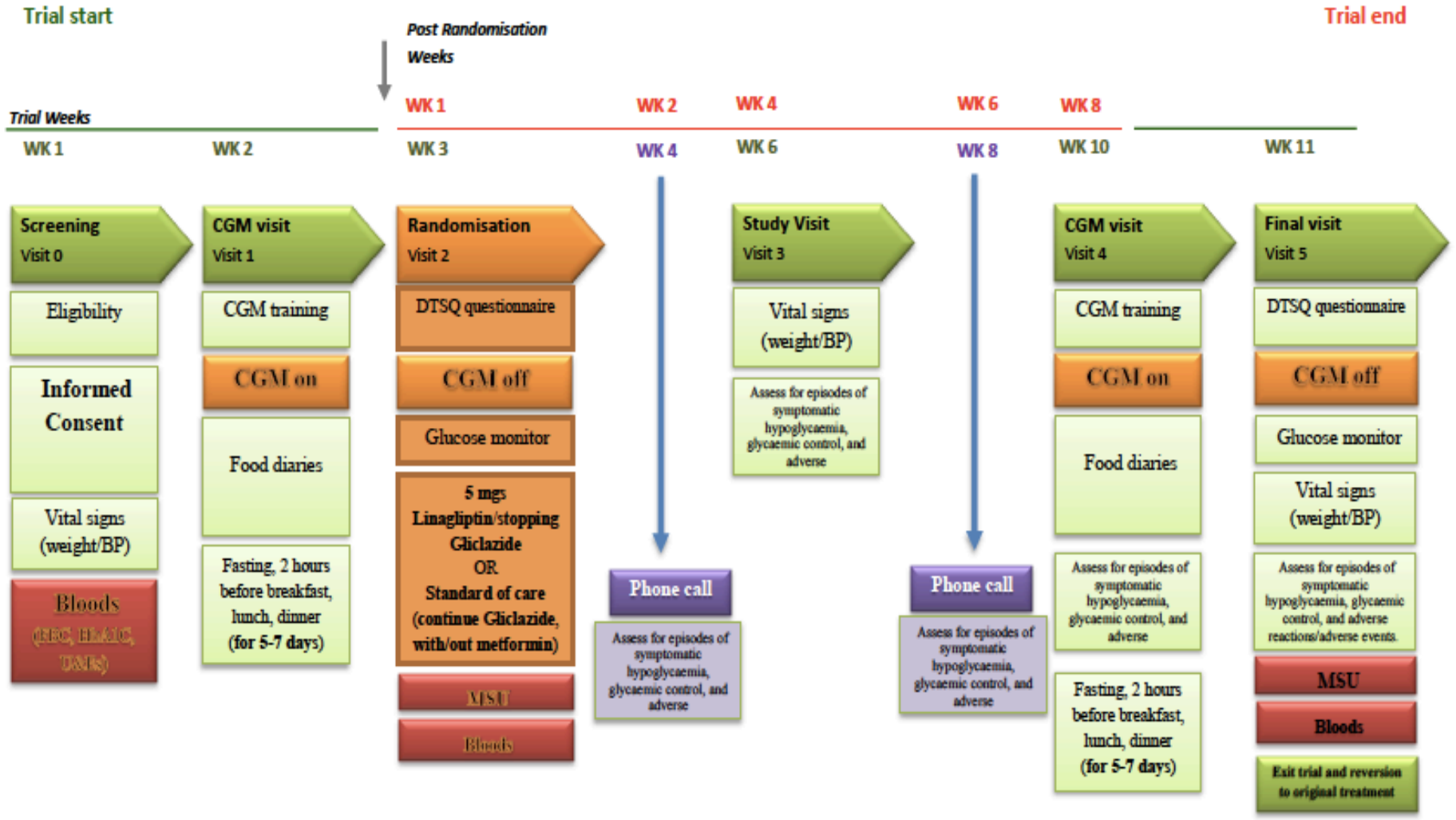

Figure 1 GLOOCOSE study trial flowchart

1.10 Study Management

1.10.1 Randomisation

Randomisation at Visit 2 was performed using the InFORM study database, which automatically allocated the next unused randomisation code. The randomisation lists were prepared before the start of the study by the senior statistician using randomised blocks of variable length, and assigning a study randomisation code number to each.

1.10.2 Confidentiality

Patient confidentiality was conducted in line with the Data Protection Act and regulated by current General Data Protection Regulation (GDPR). All patient information was kept confidential and only accessed by the research team, sponsor, and regulatory authorities. Patient identifiable data was kept secure on password protected NHS computers and within the locked research team office.

1.10.3 Safety Reporting

Any adverse events (AEs), serious adverse events (SAEs), suspected unexpected serious adverse reactions (SUSARs) and adverse events of special interest (AESIs) reactions, were recorded, and depending on the nature of the event, further reporting procedures to the sponsor and or the REC/MHRA were followed.

1.11 Study Monitoring

Annual progress reports (APRs) were submitted from 2015 to 2018, and annual developmental safety update reports (DSURs) from 2015 to 2019 to the sponsor and regulatory authorities. The GLOOCOSE study also had satisfactory trial monitoring visits in 2017 and 2018, and a satisfactory close out visit in September 2019.

1.12 Study Closure

The GLOOCOSE study recruited its final participant on 17th August 2018, and the last study visit took place on 19th October 2018. The official recruitment end date was on 8th June 2019, and formal notification of the end of the GLOOCOSE study was given on 10th June 2019 to the sponsor, MHRA, REC and HRA. The acknowledgements of the end of trial declaration forms were received from the REC and HRA on 14th June 2019, the MHRA on 18th June 2019, and the JRCO on 20th June 2019.

2.0 GLOOCOSE Study Methods

2.1 CGM Data Upload

The Medtronic iPro2 CGM does not make CGM data available to patients in real time, but only allows CGM data to be reviewed by the research team after the recording interval. After the CGM device was removed from the patient at Visit 2 and Visit 5 for the GLOOCOSE study and at Visit 2 for the LINDA-CKD study, it was cleaned with disinfectant alcohol, placed onto the iPro2 dock and connected via a USB cable to an NHS computer with CareLink iPro software installed. The CGM data for each patient for that visit was then uploaded by logging into the CareLink iPro website https://carelink.minimed.eu/ipro/hcp/login.jsf. The CGM data was labelled with that particular patient's unique study number and the dates of data collection. CBG readings from the patient's glucometer were entered manually onto the CareLink iPro Logbook for data calibration. The CGM report was then generated automatically in PDF format, and electronically saved onto the study research folder. Each CGM report was analysed together with the corresponding 7-day food diary for that patient.

Calibration errors, insufficient CBG readings (ie CBG readings that are more than 12 hours apart) and sensor displacement resulted in data gaps. A thicker, flat sensor trace at 2.2 or 22.2 mmol/L indicated that CGM values were outside these limits.

The EasyGV Calculator Version 10 Excel spreadsheet (Nathan R Hill, copyright University of Oxford 2010-2016) was used to calculate measures of glycaemic variability that were not generated in the Medtronic CGM output. These included continuous overall net glycaemic action (CONGA-1), mean absolute glucose (MAG), low blood glucose index (LBGI), high blood glucose index (HBGI), and a customisable percentage of time in range (%TIR), time below range and time above range.

2.2 Processing of blood and urine samples

Blood samples at Visit 2 and Visit 5 were collected in the following specimen tubes:

FBC; lavender EDTA tube 3mls

HbA1c; lavender EDTA tube 3mls

Glucose; grey fluoride tube 1mls

Renal function; yellow biochemistry SST tube 3mls

Serum cytokines MCP-1 and TGF-β1; red silicon coated tube 5mls

Blood samples for FBC, U&Es, HbA1c and glucose were sent to the ICHT haematology and biochemistry laboratory for usual processing and analysis.

Mid-stream urine specimens collected at Visit 2 and 5 were collected in plain universal specimen bottles, and sent to the ICHT biochemistry laboratory for urine ACR / PCR and to microbiology if the urine dipstick were suggestive of a urinary tract infection.

The remaining blood and urine samples for biomarkers MCP-1 and TGF-β1 obtained at Visit 2 and 5 were transported to the renal research laboratory at Hammersmith Hospital campus ICL. The blood samples were allowed to clot at room temperature (RT) for at least 30 minutes. Both blood and urine samples were then centrifuged at 4°C at 1000G / 2500 rpm for 10 minutes. The serum supernatant was aliquoted into at least 3 tubes of 1.5mls each, while the urine was aliquoted into 5 tubes of 1.5mls each. The remainder of the urine was aliquoted into 7ml universal containers. Each tube/container was labelled with the patient's study ID and visit number, and different coloured caps were used to distinguish pre from post randomisation samples. All samples were stored and frozen at -80°C within 8 hours of collection.

2.3 ELISA Methods

2.3.1 General Reagents and Buffers

Equipment used for the cytokine ELISAs included 96-well clear microplates, Eppendorf microcentrifuge polypropylene tubes, and clear adhesive plate sealers. Table 3 summarises the constituents of frequently used buffers and reagents.

Phosphate Buffered Saline (PBS)

NaCl	8	g/l
KCl	0.20	g/l
$Na_2HPO_4 2H_2O$	1.44	g/l
$K(HPO_4)_2$	0.20	g/l

pH adjusted to 7.4

Wash Buffer

0.05% Tween 20 in PBS

Block Buffer for MCP-1

1% Bovine Serum Albumin (BSA) in PBS

Block Buffer for TGF-β1

5% Tween 20 in PBS

Reagent Diluent for MCP-1

1% BSA in PBS

Reagent Diluent for TGF-β1

Reagent Diluent Concentrate 1 (R&D Systems, Catalog #DY997)	1.4	ml
0.05% Tween 20 in PBS	98.6	ml

Substrate Solution

Colour Reagent A (H_2O_2)
Colour Reagent B (Tetramethylbenzidine)
1:1 mixture

Stop Solution

2N H_2SO4

Table 3 General reagents and buffers for MCP-1 and TGF-β1 ELISAs

2.3.2 ELISA method for MCP-1

The human MCP-1 ELISA development kits were purchased from Peprotech (Catalog #900-T31 New Jersey, USA) and contained:

1. Capture antibody: 25µg rabbit anti-human MCP-1 + 0.5mg D-mannitol, reconstituted in 250µl sterile water for a concentration of 100µg/ml and aliquots stored at -80°C.
2. Human MCP-1 standard: 1µg recombinant human MCP-1 + 2.2mg BSA + 11.0mg D-mannitol, reconstituted in 1ml sterile water for a concentration of 1µg/ml and aliquots stored at -80°C.
3. Detection antibody: 25µg of biotinylated rabbit anti-human MCP-1 + 0.5mg D-mannitol, reconstituted in 250µl sterile water for a concentration of 100µg/ml and aliquots stored at -80°C.

4. Streptavidin-HRP conjugate: 17µl vial diluted upon receipt using 153µl of PBS for a total of 170µl at a concentration of 100µg/ml, and stored in the dark at 2-8°C.

The capture antibody was diluted with PBS to a concentration of 0.25µg/ml, and 50µl was added to each well. Each plate was sealed and incubated overnight at RT. The wells were aspirated and each plate washed 3 times using 150µl of wash buffer per well, then inverted and blotted on a paper towel to remove residual buffer. Block buffer 150µl was added to each well and incubated for a minimum of 1 hour at RT, then aspirated, washed and blotted. Standard was serially diluted 2-fold from 250pg/ml to zero in reagent diluent for a 7-point serial curve, and 50µl was added to the first two columns on each ELISA microplate. Serum/urine 50µl samples at 1:2 dilution with reagent diluent were then added in duplicate and incubated at RT for at least 2 hours. The aspiration and washing step was repeated, before 50µl per well of detection antibody (diluted in reagent diluent to a concentration of 0.25µg/ml) was added and the plate incubated at RT for 2 hours. Each plate was again aspirated and washed 3 times, and 50µl per well of Streptavidin-HRP was added (diluted beforehand in reagent diluent to a concentration of 0.05µg/ml) and incubated for 30 minutes at RT, avoiding direct light. The wells were aspirated and each plate washed for a final time, then 50µl of substrate solution was added to each well and incubated at RT for colour development, which usually happened within 20 minutes. At satisfactory colour change, 50µl of stop solution was added per well.

2.3.3 ELISA Method for TGF-β1

The human TGF-β1 DuoSet ELISA development kits were purchased from R&D Systems (Minneapolis, USA) and contained:

1. Capture antibody: 120µg mouse anti-human TGF-β1, reconstituted in 0.5ml of PBS for a concentration of 240µg/ml and aliquots stored at -80°C.
2. Human TGF-β1 standard: 95ng recombinant human TGF-β1, reconstituted in 0.5ml of reagent diluent for a concentration of 190ng/ml and aliquots stored at -80°C.
3. Detection antibody: 3µg of biotinylated chicken anti-human TGF-β1, reconstituted in 1ml of reagent diluent for a concentration of 3µg/ml and aliquots stored at -80°C.
4. Streptavidin-HRP B: 2ml vial stored undiluted in the dark at 2-8°C.

Capture antibody was diluted with PBS to a concentration of 2µg/ml, 50µl per well was added, and each plate was sealed and incubated overnight at RT. Each well was aspirated and all plates were washed 3 times using 200µl of wash buffer per well, then inverted and blotted on paper towels to remove residual buffer. Aspiration, wash and blotting were repeated between each ELISA step before the next diluent was added. Block buffer of 150µl per well was added and each plate was incubated at RT for at least 1 hour. A 7-point standard curve using 2-fold serial dilutions from 2000 pg/ml to zero was made using reagent diluent, and 50µl was pipetted into the first two columns on each ELISA plate. Serum/urine samples underwent an activation step to activate latent TGF-β1 to immunoreactive TGF-β1 as follows:

1. For serum TGF-β1 samples: 20µl of 1 N HCl was added to 40µl of serum, mixed well and incubated for 10 minutes at RT. The acidified sample was then neutralised by adding 20µl of 1.2 N NaOH / 0.5 M HEPES, and mixed well. Each activated sample was diluted 20-fold with reagent diluent before 50µl per well was pipetted in duplicate, therefore final dilution factor was 40.
2. For urine TGF-β1 samples: 40µl of 1 N HCl was added to 80µl of urine, mixed well and incubated for 10 minutes at RT. The acidified sample was then neutralised by adding 40µl of 1.2 N NaOH / 0.5 M HEPES, and mixed well. Then, 50µl per well of activated sample was added in duplicate, therefore final dilution factor was 2.

After the standards and samples were added, each ELISA plate was incubated at RT for at least 2 hours. The detection antibody was diluted with reagent diluent to a concentration of 50ng/ml, 50µl was then added to each well and plates were incubated for 2 hours at RT. Streptavidin-HRP B was diluted 40-fold with reagent diluent, before 50µl was added to each well and incubated for 20 minutes at RT, avoiding direct light. 50µl of substrate solution was pipetted into each well and again incubated for 20 minutes at RT. 25µl of stop solution was added to each well after satisfactory colour development had occurred.

2.3.4 Calculation of ELISA results

Each ELISA plate was read at wavelength 450nm to determine the optical density of each well. The Gen5™ software was used to calculate and average the duplicate readings for each standard and sample, and the average zero standard optical density was subtracted. A

standard curve was created for each ELISA plate by generating a four-parameter logistic curve fit. The concentration read off the standard curve was multiplied by the appropriate dilution factor.

2.3.5 ELISA Precision

Freeze-thaw cycles of patient samples were minimised to reduce variability in measured biomarker concentrations. The intra-assay coefficient of variability (CV) was assessed by repeating 10 random samples pipetted in different rows and columns on the same ELISA plate. The inter-assay CV was also calculated by running the ELISA for 10 random samples on a separate day after the first experiment, to measure plate-to-plate consistency. Intra and inter-assay percentage CVs for the MCP-1 and TGF-β1 ELISAs (Table 4) are overall below 10%, reflecting acceptable precision. The intra-assay %CV for urine TGF-β1 was zero as the majority of samples had concentrations <31.3 pg/ml, which was below the lower end of the standard curve. Inter-assay CVs for TGF-β1 are not displayed in Table 6 as repeat TGF-β1 ELISAs on serum and urine samples had marked variations in the measured TGF-β1 levels. This was the case after just 2 freeze-thaw cycles or being kept overnight in the cold room, and is likely due to TGF-β1 being particularly susceptible to denaturing from its activated immunoreactive form.

	Intra-Assay CV (%)	Inter-Assay CV (%)
Serum MCP-1	1.93	4.14
Urine MCP-1	7.17	7.87
Serum TGF-β1	4.96	N/A
Urine TGF-β1	0	N/A

Table 4 Intra and inter-assay percentage CVs for MCP-1 and TGF-β1 ELISAs

2.4 Statistical Methods

The IBM SPSS Statistics Version 25 software package was used to perform statistical analyses of patient data. The statistical tests used to analyse each outcome measurement are further detailed in the following results section.

3.0 GLOOCOSE Study Results

3.1 Study Recruitment and Progress

Patients were screened from Imperial and GP PIC sites, and Figure 2 outlines the number of patients consented from each site. As PIC site staff performed most of the screening at PICs, the exact numbers of patients screened from PICs were not available. More than 4500 patients were assessed for eligibility for the GLOOCOSE study, but more than 4200 were excluded (Figures 2 and 3), usually for having an eGFR that was above 45 ml/min/1.73m^2 or being on an excluding medication, typically a DPP-4 inhibitor. An excluded patient could have more than one reason why they were ineligible for the study. PIC site staff also highlighted patients that were not suitable for the study due to frailty, being housebound or had other medical or social reasons precluding them from participating in research.

Only a fraction of the total number of patients screened were eligible, and these 216 patients were invited to discuss the study further. The majority did not attend the informative visit, or declined to participate in the study when approached. Some patients who attended were later found to have an exclusion criterion at the informative appointment. Reasons for declining the study were not wishing to change medications, not liking the idea of wearing the CGM and testing their CBGs regularly, or not wanting to take part in research.

By the end of the recruitment period, 27 patients had been consented into the study (Figure 3). Four patients turned out to be screen failures after screening bloods and detailed medical history were taken, leaving 23 patients enrolled in the study. A further four patients were withdrawn from the study prior to randomisation due to the participants' failure to follow research procedures, although two of the four patients went on to be re-consented and randomised. Nineteen patients were randomised, with ten patients randomised to stay on Gliclazide and nine patients randomised to stop Gliclazide and switch to Linagliptin.

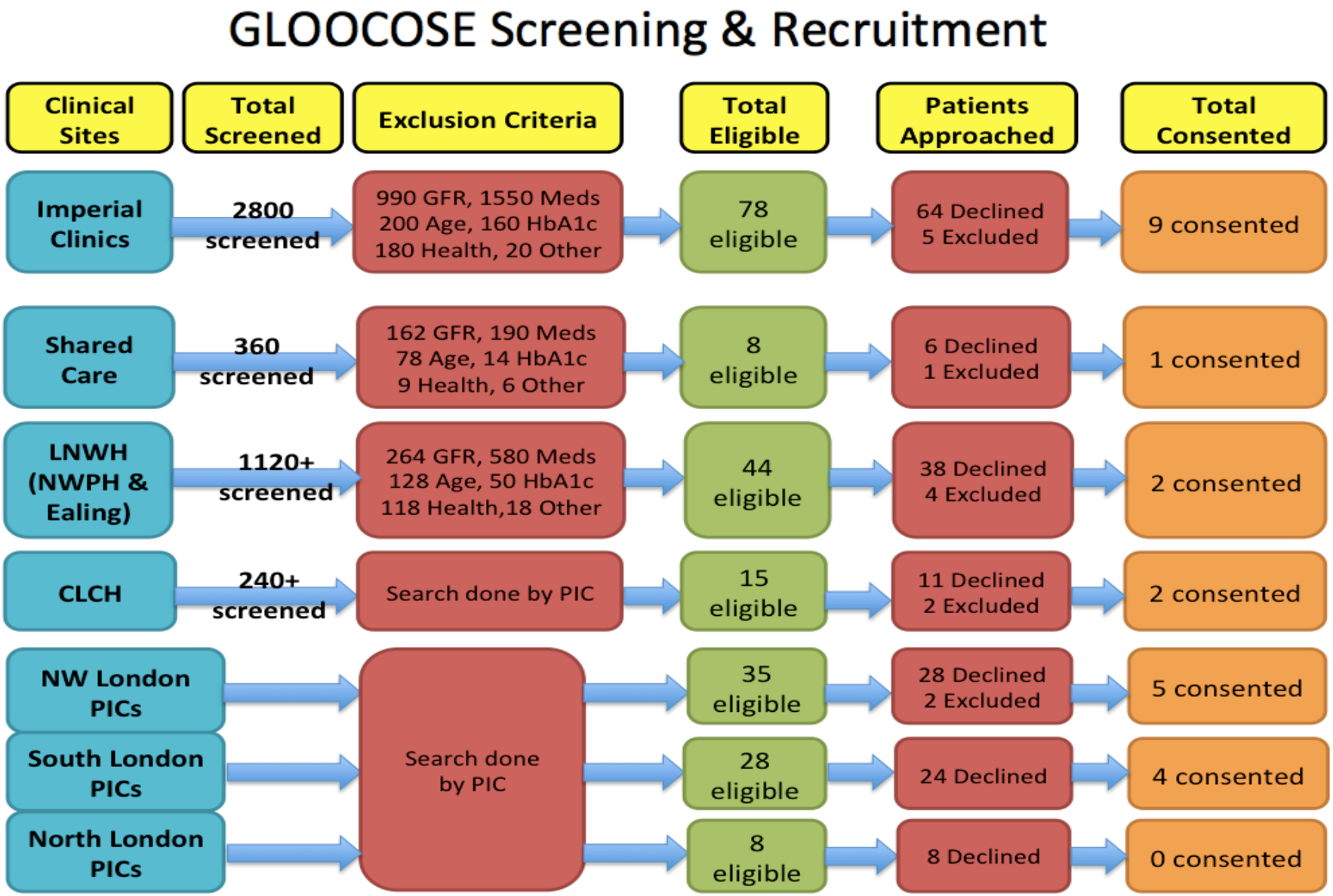

Figure 2 Number of patients screened, excluded and consented from Imperial and GP PIC sites

All 10 patients randomised to stay on Gliclazide had pre-randomisation CGM data, but only 9 patients had post-randomisation CGM data as 1 patient had CGM technical failure. Two patients were withdrawn from the study after randomisation; both had been randomised to Linagliptin. One study patient that had been randomised to Linagliptin had had no pre-randomisation CGM data due to technical failure (discovered after randomisation had been carried out), and was withdrawn from the study and re-consented. The other patient that had been randomised to Linagliptin was withdrawn for safety reasons because of newly deranged liver function tests. This was reported as an AESI; further investigations revealed that the likely reason was a passed gallstone and his liver function subsequently returned to its normal baseline. Overall, 23 patients were consented (after excluding screen failures), 19 patients were randomised and a total of 17 patients completed the study.

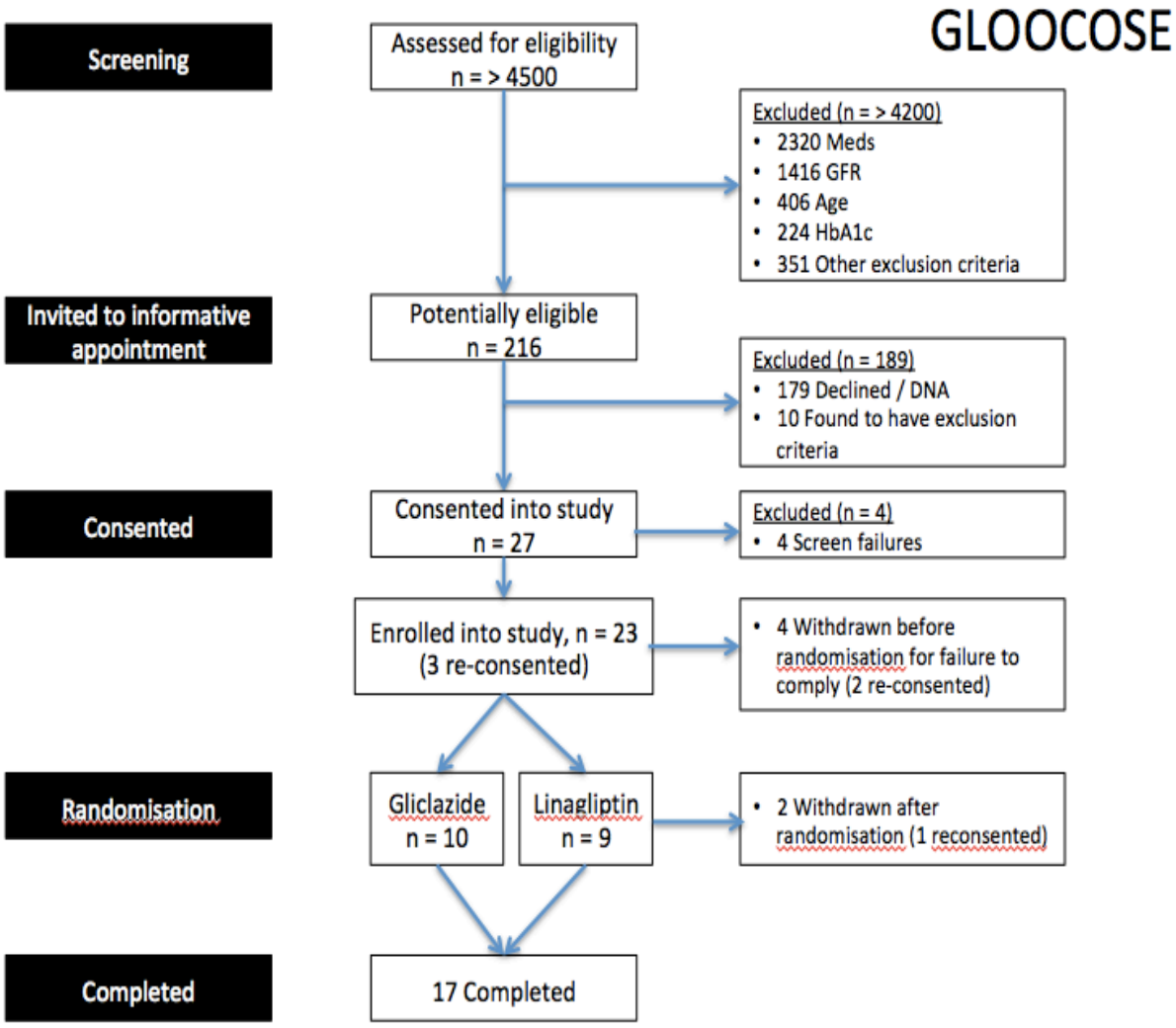

Figure 3 Number of patients screened, consented, randomised and completed study

3.2 Clinical Data

Baseline characteristics of all randomised study participants are collated in Table 5. The vast majority of study patients were male and of Caucasian ethnicity. The median age of 72 years for those randomised to Gliclazide was roughly the same as the median age of 71 years for those randomised to Linagliptin. Patients randomised to Gliclazide were more than 17kgs heavier than patients randomised to Linagliptin, and had a correspondingly higher BMI (33.1 kg/m^2 vs 29.4 kg/m^2).

Study participants randomised to Linagliptin had had diabetes for a little longer, and lower systolic blood pressure than those randomised to Gliclazide. Average baseline HBA1c, fasting CBG, eGFR, urine ACR and urine PCR was lower in the Linagliptin group, with range of data being wider for urine ACR / PCR in the Linagliptin group. Co-morbidities were similar between the two treatment arms.

Baseline Characteristics	Randomised to Gliclazide (n = 10)	Randomised to Linagliptin (n = 9)
Sex	Male 8 Female 2	Male 9 Female 0
Ethnicity	Caucasian 6 Afrocaribbean 1 Asian 2 Other/Mixed 1	Caucasian 6 Afrocaribbean 0 Asian 3 Other/Mixed 0
Age, y	72 (50 to 76)	71 (57 to 79)
Weight, kg	97.5 (60.8 to 116.6)	80.2 (64.8 to 103.8)
BMI, kg/m^2	33.1 (25.7 to 39.5)	29.4 (22.4 to 33.8)
Blood Pressure, mmHg	141 / 76 (99 to 173 / 59 to 91)	134 / 78 (94 to 153 / 56 to 94)
Duration of Diabetes, y	13 (6 to 23)	14 (3 to 30)
HbA1c, mmol/mol	55 (39 to 62)	52 (33 to 64)
Fasting CBG pre-randomisation, mmol/L	7.5 (5.6 to 10.3)	6.5 (4.7 to 10.9)
eGFR MDRD, ml/min/1.73m^2	37 (20 to 45)	32 (26 to 44)
Urine ACR, mg/mmol	35 (0 to 72)	6 (1 to 257)
Urine PCR, mg/mmol	58 (0 to 136)	16 (0 to 339)

Co-morbidities	Hypertension	10	Hypertension	9
	Hyperlipidaemia	9	Hyperlipidaemia	9
	Ischaemic Heart Disease	6	Ischaemic Heart Disease	6
	Heart Failure	1	Heart Failure	4
	Retinopathy	2	Retinopathy	1
	Neuropathy	0	Neuropathy	0

Results presented as Median with (Data Range: Minimum to Maximum)
Table 5 Baseline characteristics of all randomised patients, n = 19

Descriptive statistics were performed for all outcome data within both groups of the predictor variable of Gliclazide versus Linagliptin, along with histograms of each dependent outcome variable and standardised residuals, P-P plots, Q-Q plots and boxplots. As statistical tests of normality such as the Kolmogorov-Smirnov and Shapiro-Wilk tests accepts assumptions of normality too readily in smaller sample sizes, their results were considered in conjunction with visual assessments of normal distributions. The clinical pre and post randomisation outcomes in Table 6 did not fulfil assumptions of normality and/or homogeneity of variance, due to skewing from inherent biological variations and outlier data. Therefore the non-parametric independent samples Mann-Whitney statistical test was used for each outcome.

The median difference between the pre and post randomisation clinical measurements taken at Visit 2 and Visit 5 respectively (i.e. post-randomisation value – pre-randomisation value), were compared between both treatment arms (Table 6). The test statistic U, and exact two-tailed p-values are also quoted in Table 6. Each two-tailed probability p value at the chosen alpha a of 0.05, tests the non-directional null hypothesis that there is no difference in the two treatment groups for the change in clinical measurements. Therefore the null and alternative hypotheses are as follows:

1. Null hypothesis, H_0: There is no difference in the clinical outcomes (weight, blood pressure, HbA1c, fasting CBGs, eGFR, urine ACR and urine PCR) of patients randomised to Gliclazide and patients randomised to Linagliptin
2. Alternative hypothesis, H_1: There is a difference in the clinical outcomes of patients randomised to Gliclazide and patients randomised to Linagliptin.

If the probability p value is greater than 0.05, we do not have enough evidence to reject the null hypothesis that Linagliptin has no effect on the measured clinical outcomes.

Post randomisation value – Pre randomisation value	Randomised to Gliclazide (n = 10)	Randomised to Linagliptin (n = 7)	Test statistic U, p value
Change in weight, kg	+ 0.3 (-1.1 to +4.8)	- 0.5 (-3.8 to +0.1)	12.5, p = 0.025
Change in BMI, kg/m²	+ 0.1 (-0.4 to +1.7)	- 0.1 (-1.4 to 0.0)	13.0, p = 0.033
Change in BP, mmHg	+ 5.0 / + 0.5 (-29 to +22 / -10 to +14)	+ 3.0 / + 3.0 (-19 to +33 / -10 to +12)	37.0 / 43.5, p = 0.887 / p = 0.417
Change in HbA1c, mmol/mol	+ 1.5 (-2.0 to +11.0)	+ 8.0 (-2.0 to +18.0)	49.5, p = 0.161
Change in Fasting CBGs	+ 0.5 (-0.9 to +1.2)	+ 2.6 (+0.6 to +6.8)	65.5, p = 0.001
Change in eGFR MDRD	+ 1.0 (-2.0 to +9.0)	- 1.0 (-3.0 to +1.0)	15.0, p = 0.055
Change in Urine ACR	+ 3.1 (-19.1 to +130.2)	- 0.3[a] (-9.4 to +9.4)	21.0, p = 0.368
Change in Urine PCR	+ 8.0[b] (-22.0 to +157.0)	- 1.0[a] (-20.0 to +39.0)	20.5, p = 0.456

Results presented as Median with (Data Range: Minimum to Maximum)
[a] : Based on 6 patient samples, [b]: Based on 9 patient samples
Table 6 Change in clinical outcomes for participants randomised to Gliclazide or Linagliptin

Study participants randomised to Gliclazide had put on weight at the end of the study compared to participants randomised to Linagliptin who had lost weight; this difference in weight change between the treatment arms was significant (p = 0.025, Table 6). This was reflected in the change in BMI, where patients randomised to Gliclazide had a significantly higher BMI compared to patients randomised to Linagliptin (p = 0.033). Changes in systolic and diastolic blood pressures by study end between the two treatment groups were non-significant (systolic BP: p = 0.887; diastolic BP: p = 0.417).

Study patients randomised to switch to Linagliptin had a higher increase in fasting CBGs (p = 0.001) and a higher increase in HbA1c (p = 0.161) at the end of the study; however the difference in change of fasting CBGs between groups was significant but the difference in change of HbA1c was not (Table 6). This was accompanied by deterioration in eGFR in the Linagliptin group, although the difference between the treatment groups did not quite reach significance (p = 0.055). Urine ACR and urine PCR had risen in the Gliclazide group but dropped in the Linagliptin group by study end, however, differences between the groups were not significant (urine ACR: p = 0.368; urine PCR: p = 0.456).

3.3 Outcome 1: Hypoglycaemic Incidence and Severity

The primary outcome measures for the GLOOCOSE study were:

1. Hypoglycaemic frequency during each CGM period and;
2. The percentage of time spent in hypoglycaemia for each CGM period.

For the group randomised to continue on Gliclazide, there were 10 patients with pre-randomisation data and 9 patients with post-randomisation CGM data. For the 9 patients randomised to switch to Linagliptin, only 8 patients had pre-randomisation CGM data and 7 had post-randomisation CGM data (see section 3.1). Therefore:

1. Null hypothesis, H_0: There is no difference in the number of hypoglycaemic episodes / time spent in hypoglycaemia in patients randomised to Gliclazide and patients randomised to Linagliptin, i.e. they are equal.
2. Alternative hypothesis, H_1: There is a difference in the number of hypoglycaemic episodes / time spent in hypoglycaemia in patients randomised to Gliclazide and patients randomised to Linagliptin.

The level of hypoglycaemia that results in clinical symptoms and counter-regulatory responses depends on each individual patient's glycaemic control. Danne et al's[3] international consensus on the use of CGM recommended categorising hypoglycaemia into three levels as follows:

1. Level 1: Glucose values between 3.0 – 3.9 mmol/L, with or without symptoms; our studies define the level 1 hypoglycaemia threshold as 3.0 – 3.8 mmol/L
2. Level 2: Glucose values less than 3.0 mmol/L with or without symptoms
3. Level 3: Severe hypoglycaemia (not distinguished by a particular glucose value), with cognitive impairment requiring third party assistance

The total number of hypoglycaemic episodes and overall percentage of time spent below the CGM threshold of 3.9 mmol/L is reported first, before examining the number of level 1 and level 2 hypoglycaemic events and time spent in these thresholds respectively. Although level 2 hypoglycaemia is clinically important to all clinicians managing patients with diabetes, frequent or prolonged level 1 hypoglycaemia indicates higher risk of progressing onto level 2 or level 3 hypoglycaemia.

A hypoglycaemic episode was noted when the CGM sensor recorded a reading below 3.9 mmol/L for a minimum of 15 minutes. A level 1 hypoglycaemic episode was documented when sensor readings were between 3.0 to 3.8 mmol/L, and a level 2 hypoglycaemic episode noted when readings were below 3.0 mmol/L for at least 15 minutes. The hypoglycaemic event was considered to have ended when readings rose above each defined threshold for at least 15 minutes. The number of minutes and/or hours spent in each defined threshold was automatically calculated and converted into the percentage of time spent in each gradation of hypoglycaemia over the given CGM reporting period.

Histograms of both primary outcomes and their standardised residuals showed marked positive skew. This was due to the majority of patients having no hypoglycaemic episodes during the pre and post-randomisation CGM periods and consequently spending 0% time in hypoglycaemia, which is a convincing expectation of real life data. The non-parametric Mann-Whitney statistical test for independent samples was used to examine for each hypoglycaemic outcome.

3.3.1 Total number of Hypoglycaemic Episodes

The median pre-randomisation total number of hypoglycaemic episodes was the same in both treatment arms (1.0 hypoglycaemic episode). The median post-randomisation total number of hypoglycaemic episodes was zero in both treatment arms, but the range of values was wider in the Gliclazide group, suggesting that a small number of patients had frequent hypoglycaemic episodes (Table 7).

Patients randomised to continue Gliclazide had one fewer hypoglycaemic episode after randomisation, whereas patients who switched to Linagliptin had no change in the number of hypoglycaemic episodes. This difference in the pre and post randomisation total number of hypoglycaemic episodes between the two treatment groups was non-significant ($p = 0.918$, Table 8), therefore it appears that randomisation to Linagliptin did not significantly affect the total number of hypoglycaemic episodes.

3.3.2 Percentage of Time Spent in Hypoglycaemia

The percentage of time spent in hypoglycaemia (CGM reading below 3.9 mmol/L) was also recorded for each patient's CGM week (Table 7). These results were similar to that of number of hypoglycaemic episodes. The median amount of time spent in hypoglycaemia was 0% in both groups before and after randomisation, but there was more spread of data in the Gliclazide group post randomisation, indicating a small number of patients spent more time in hypoglycaemia .

The change in the percentage of time spent in hypoglycaemia after randomisation between the two treatment arms was not significant ($p = 0.470$, Table 8), therefore study patients randomised to switch to Linagliptin did not spend more or less time in hypoglycaemia, compared to study patients randomised to stay on Gliclazide.

3.3.3 Number of Level 1 Hypoglycaemic Episodes and Percentage of Time Spent in Level 1 Hypoglycaemia

Table 7 describes the median number and range of level 1 hypoglycaemic episodes and percentage of time spent in level 1 hypoglycaemia before and after randomisation for both treatment groups. Ranges of values for both level 1 hypoglycaemic outcomes were again wider in the Gliclazide group, especially after randomisation. There was no change in the number of level 1 hypoglycaemic events, or time spent in level 1 hypoglycaemia after randomisation in both treatment groups. Therefore the difference between treatment arms for the number of level 1 hypoglycaemic episodes ($p = 0.758$), and the amount of time spent in level 1 hypoglycaemia ($p = 0.837$) after randomisation were both non-significant (Table 8). Thus, switching to Linagliptin did not make a significant difference to the number of level 1 hypoglycaemic episodes or time spent in level 1 hypoglycaemia.

3.3.4 Number of Level 2 Hypoglycaemic Episodes and Percentage of Time Spent in Level 2 Hypoglycaemia

The median number of level 2 hypoglycaemic episodes in both treatment groups before and after randomisation was zero, and therefore 0% median time was spent in this hypoglycaemic threshold also (Table 7). Ranges of values for both level 2 hypoglycaemic outcomes post randomisation were wider in the Gliclazide group.

There was no change in the number of level 2 hypoglycaemic events, or time spent in level 2 hypoglycaemia after randomisation in both treatment groups. Thus there was no significant difference between the treatment groups for the change in number of level 2 hypoglycaemic events (p = 0.918), and time spent in level 2 hypoglycaemia (p = 0.351) after randomisation (Table 8). Hence participants randomised to Linagliptin did not have significantly less or more level 2 hypoglycaemic episodes / time spent in level 2 hypoglycaemia, compared to participants who continued on Gliclazide.

Hypoglycaemic Incidence & Severity	Randomised to Gliclazide	Randomised to Linagliptin
Total number of hypoglycaemic episodes		
Pre-randomisation[a]	1.0 (0.0 to 10.0)	1.0 (0.0 to 7.0)
Post-randomisation[b]	0.0 (0.0 to 26.0)	0.0 (0.0 to 1.0)
Total time spent in hypoglycaemia <3.9 mmol/L, (%)		
Pre-randomisation[a]	0.0 (0.0 to 8.0)	0.0 (0.0 to 6.0)
Post-randomisation[b]	0.0 (0.0 to 17.0)	0.0 (0.0 to 1.0)
Number of Level 1 hypoglycaemic episodes		
Pre-randomisation[a]	0.5 (0.0 to 9.0)	1.0 (0.0 to 6.0)
Post-randomisation[b]	0.0 (0.0 to 19.0)	0.0 (0.0 to 1.0)
Total time spent in Level 1 hypoglycaemia 3.0 – 3.8 mmol/L, (%)		
Pre-randomisation[a]	0.0 (0.0 to 8.0)	0.0 (0.0 to 2.0)
Post-randomisation[b]	0.0 (0.0 to 12.0)	0.0 (0.0 to 1.0)
Number of Level 2 hypoglycaemic episodes		
Pre-randomisation[a]	0.0 (0.0 to 2.0)	0.0 (0.0 to 2.0)
Post-randomisation[b]	0.0 (0.0 to 7.0)	0.0 (0.0 to 0.0)

Total time spent in Level 2 hypoglycaemia <3.0 mmol/L, (%)		
Pre-randomisation[a]	0.0 (0.0 to 1.0)	0.0 (0.0 to 5.0)
Post-randomisation[b]	0.0 (0.0 to 5.0)	0.0 (0.0 to 0.0)

Results presented as Median with (Data Range: Minimum to Maximum)
[a] : Based on 10 patients in the Gliclazide group, and 8 patients in the Linagliptin group
[b]: Based on 9 patients in the Gliclazide group, and 7 patients in the Linagliptin group
Table 7 Pre and post randomisation values of hypoglycaemic incidence and severity

Post randomisation value – Pre randomisation value	Randomised to Gliclazide (n = 9)	Randomised to Linagliptin (n = 7)	Test statistic U, p value
Change in total number of hypoglycaemic episodes	- 1.0 (-8.0 to +16.0)	0.0 (-6.0 to 0.0)	30.0, p = 0.918
Change in total time spent in hypoglycaemia <3.9 mmol/L, (%)	0.0 (-4.0 to +9.0)	0.0 (-5.0 to 0.0)	24.5, p = 0.470
Change in number of Level 1 hypoglycaemic episodes	0.0 (-7.0 to +10.0)	0.0 (-5.0 to 0.0)	28.0, p = 0.758
Change in total time spent in Level 1 hypoglycaemia 3.0 – 3.8 mmol/L, (%)	0.0 (-4.0 to +4.0)	0.0 (-2.0 to 0.0)	29.0, p = 0.837
Change in number of Level 2 hypoglycaemic episodes	0.0 (-2.0 to +6.0)	0.0 (-2.0 to 0.0)	30.0, p = 0.918
Change in total time spent in Level 2 hypoglycaemia <3.0 mmol/L, (%)	0.0 (-1.0 to +5.0)	0.0 (-5.0 to 0.0)	22.5, p = 0.351

Results presented as Median with (Data Range: Minimum to Maximum)
Table 8 Change in hypoglycaemic incidence and severity for participants randomised to Gliclazide or Linagliptin

3.4 Outcome 2: Glycaemic Outcomes

Secondary outcome measures for the GLOOCOSE study included glycaemic outcomes from CGM data. Patients with unavailable CGM data were excluded (see section 3.1). The glycaemic outcomes measured for each CGM study period (before and after randomisation) were:

1. Overall glycaemic control
    a. Mean CGM glucose
    b. Estimated CGM HbA1c
    c. Time spent in normoglycaemia 3.9 – 10.0 mmol/L
2. Glycaemic variability
    a. Percentage Coefficient of Variation (% CV)
    b. Standard deviation (SD)
    c. Continuous Overall Net Glycaemic Action (CONGA-1)
    d. Mean Absolute Glucose (MAG)
    e. Mean of Daily Differences (MODD)
    f. Mean Amplitude of Glucose Excursions (MAGE)
3. Time spent in hyperglycaemic ranges
    a. Time spent in hyperglycaemia >10.0 mmol/L
    b. Time spent in hyperglycaemia >13.9 mmol/L
4. Measures of risk
    a. Low Blood Glucose Index (LBGI)
    b. High Blood Glucose Index (HBGI)

The null and alternative hypotheses are as follows:

1. Null hypothesis, H_0: There is no significant difference in the glycaemic outcomes (outlined above in section 3.4) in patients randomised to Gliclazide and patients randomised to Linagliptin
2. Alternative hypothesis, H_1: There is a significant difference in the glycaemic outcomes in patients randomised to Gliclazide and patients randomised to Linagliptin.

The pre and post randomisation glycaemic outcomes did not fulfil assumptions of normality and/or homogeneity of variance, therefore Mann-Whitney statistical tests were performed.

3.4.1 Overall Glycaemic Control

Overall glycaemic control was determined by examining mean CGM glucose, estimated CGM HbA1c and percentage of time spent in normoglycaemic range (3.9 – 10.0 mmol/L). Although mean CGM glucose in both treatment groups prior to randomisation was similar, study participants randomised to Linagliptin had a slightly higher mean CGM glucose by study end (Gliclazide group median 8.5 mmol/L and Linagliptin group median 8.8 mmol/L, Table 9). The Linagliptin group had an increase of 1.5 mmol/L in mean CGM glucose by the end of the study, compared to the Gliclazide group, which had a smaller increase of 0.1 mmol/L (Table 10). This difference was significant ($p = 0.023$).

Estimated CGM HbA1c was higher in the Gliclazide group before randomisation (Gliclazide group median 53.0 mmol/mol, Linagliptin group median 51.5 mmol/mol), but higher in the Linagliptin group after randomisation (Gliclazide group median 52.0 mmol/mol, Linagliptin group median 55.0 mmol/mol, Table 9). The Linagliptin group had a 10 mmol/mol increase in estimated CGM HbA1c by study end, whereas there was no change in the Gliclazide group. This difference was significant ($p = 0.016$, Table 10).

Study patients in both treatment arms spent comparable median amounts of time in the normoglycaemic range before randomisation. After randomisation, the median time spent in normoglycaemia was higher in the Linagliptin group (Gliclazide group median 62.0%, Linagliptin group median 73.3%), however there was wide spread of data particularly in the Linagliptin group (Table 9). The change in pre and post-randomisation values was calculated for each patient to examine the effect of Linagliptin on time spent in normoglycaemic range. Patients randomised to Linagliptin spent less time in normoglycaemia after randomisation (-12%) compared to patients randomised to Gliclazide (-3%), but this difference between the treatment groups was not significant ($p = 0.299$, Table 10).

In summary, overall glycaemic control was poorer in patients randomised to Linagliptin, with these patients having a significantly higher mean CGM glucose and higher estimated CGM HbA1c after randomisation. Patients randomised to Linagliptin also spent less time in normoglycaemia by study end but this finding was non-significant.

Overall Glycaemic Control	Randomised to Gliclazide	Randomised to Linagliptin
Mean CGM glucose (mmol/L)		
Pre-randomisation[a]	8.5 (6.9 to 10.7)	8.4 (6.8 to 11.5)
Post-randomisation[b]	8.5 (5.9 to 11.0)	8.8 (7.6 to 15.4)
Estimated CGM HbA1c (mmol/mol)		
Pre-randomisation[a]	53.0 (42.0 to 67.0)	51.5 (41.0 to 73.0)
Post-randomisation[b]	52.0 (34.0 to 68.0)	55.0 (46.0 to 100.0)
Time spent in normoglycaemia 3.9 – 10.0 mmol/L, (%)		
Pre-randomisation[a]	70.8 (52.0 to 99.4)	74.9 (29.9 to 98.5)
Post-randomisation[b]	62.0 (48.8 to 100.0)	73.3 (0.0 to 88.3)

Results presented as Median with (Data Range: Minimum to Maximum)
[a] : Based on 10 patients in the Gliclazide group, and 8 patients in the Linagliptin group
[b]: Based on 9 patients in the Gliclazide group, and 7 patients in the Linagliptin group
Table 9 Pre and post randomisation values of overall glycaemic control

Post randomisation value – Pre randomisation value	Randomised to Gliclazide (n = 9)	Randomised to Linagliptin (n = 7)	Test statistic U, p value
Change in mean CGM glucose (mmol/L)	+ 0.1 (-1.1 to +1.1)	+ 1.5 (-0.4 to +6.8)	53.0, p = 0.023
Change in estimated CGM HbA1c (mmol/mol)	0.0 (-8.0 to +7.0)	+ 10.0 (-2.0 to +47.0)	54.0, p = 0.016
Change in time spent in normoglycaemia 3.9 – 10.0 mmol/L, (%)	- 3.2 (-12.9 to +6.1)	- 12.0 (-64.0 to +10.4)	21.0, p = 0.299

Results presented as Median with (Data Range: Minimum to Maximum)
Table 10 Change in overall glycaemic control outcomes for participants randomised to Gliclazide or Linagliptin

3.4.2 Glycaemic Variability

Several measures of glycaemic variability illustrating the frequency, degree and duration of blood glucose fluctuations were examined as outcome variables (see section 3.4).

3.4.2.1 Percentage coefficient of variation (% CV) and standard deviation (SD)

The standard deviation measures the spread, or variation of glucose readings around the mean glucose level. Stable diabetes control would mean minimal excursions into hypo or hyperglycaemic range readings, and therefore a lower SD. To interpret the SD relative to the mean glucose, the percentage coefficient of variation (% CV) is calculated as the ratio of standard deviation to the mean, and expressed as a percentage. Stable glucose levels with low variability (and therefore less dips into hypoglycaemia) is expressed as a CV of less than 36%, while unstable levels with high variability is described as a CV of more than or equal to 36%[4].

Both median SD and median %CV in the Gliclazide and Linagliptin groups before randomisation were similar (Table 11). The SD post randomisation was lower in the Linagliptin treatment arm (2.0 versus 2.5), with a correspondingly lower post randomisation %CV (18.3% versus 28.1%) given the higher mean CGM glucose in the Linagliptin group. Study participants randomised to Linagliptin had less glycaemic variability (SD -0.6, %CV -9.2) at a higher mean CGM glucose compared to participants

randomised to continue Gliclazide (SD 0.0, %CV -0.7), although this was not significant (change in SD p value = 0.606, change in %CV p value = 0.071, Table 12).

3.4.2.2 Continuous Overall Net Glycaemic Action (CONGA-1)

Continuous overall net glycaemic action (CONGA-1) is calculated as the standard deviation of the summated differences between a current observation and an observation *n* hours previously; here the default n = 1. It assesses intra-day glycaemic variability; therefore CONGA-1 evaluates glycaemic swings over 1-hour intervals.

Median CONGA-1 before randomisation was similar in both treatment groups, but after randomisation was a little lower in the Linagliptin group than in the Gliclazide group (1.8 versus 2.2 respectively, Table 11). There was no change in CONGA-1 in patients randomised to switch to Linagliptin, and a small decrease in CONGA-1 (signifying lower intra-day glycaemic variability) in patients randomised to continue Gliclazide. However, this difference between the treatment arms was not significant (p = 0.918, Table 12).

3.4.2.3 Mean Absolute Glucose (MAG)

Change in mean absolute glucose (MAG) is another measure of glycaemic variability first established to determine the associations between glycaemic variability with intensive care mortality[5]. It is calculated from 5-minute CGM sensor intervals, and gives an estimation of variability over time.

Median MAG pre and post randomisation were similar in both treatment groups (Table 11). Patients randomised to Linagliptin had a small increase in MAG (+0.1) by study end, whereas patients that continued on Gliclazide had a small decrease in MAG (-0.2). This difference between the treatment groups was non-significant (p = 0.681, Table 12).

3.4.2.4 Mean of Daily Differences (MODD)

Where CONGA-1 provides a measure of the intra-day variability, the mean of daily differences (MODD) gives a gauge of inter-day variability. MODD is calculated from the mean of the absolute differences between the glucose values at the same time point in two successive 24-hour periods. Study participants that switched to Linagliptin had a lower MODD by study end (-0.4) when compared to study participants that continued on

Gliclazide (-0.2). However, this difference between the two treatment arms was not statistically significant (p = 0.837, Table 12).

3.4.2.5 Mean Amplitude of Glucose Excursions (MAGE)

The mean amplitude of glucose excursions (MAGE) takes the average of glycaemic excursions above and below one SD of the mean glucose concentration over a 24-hour interval, and is another CGM index of glycaemic variability. MAGE levels have correlated well with progression of atherosclerosis and adverse cardiovascular events in patients with type 2 diabetes[6, 7]. Zhou et al's[8] study in normal healthy volunteers ascertained a cut-off value of 3.9 mmol/L for MAGE to differentiate high glycaemic variability (≥3.9 mmol/L) from normal glycaemic variability (<3.9 mmol/L).

Patients randomised to Linagliptin had a lower MAGE (-1.4), whereas patients that remained on Gliclazide had a small increase in MAGE (+0.3) by study end. Although this suggests that Linagliptin lowers glycaemic variability compared to Gliclazide, this should be interpreted in the setting of higher mean CGM glucose values in the Linagliptin group. This difference in MAGE between the two treatment groups was non-significant (p = 0.210, Table 12).

Glycaemic Variability	Randomised to Gliclazide	Randomised to Linagliptin
Co-efficient of Variation CV, (%)		
Pre-randomisation[a]	27.7 (14.1 to 38.9)	28.0 (18.9 to 38.7)
Post-randomisation[b]	28.1 (12.1 to 46.3)	18.3 (14.9 to 25.2)
Standard Deviation SD		
Pre-randomisation[a]	2.4 (1.0 to 4.0)	2.5 (1.3 to 2.9)
Post-randomisation[b]	2.5 (0.7 to 3.4)	2.0 (1.5 to 2.8)
Continuous Overall Net Glycaemic Action (CONGA-1)	2.0 (1.1 to 3.6)	2.1 (1.1 to 2.3)
Pre-randomisation[a]		
Post-randomisation[b]	2.2 (0.8 to 2.9)	1.8 (1.4 to 2.2)
Mean Absolute Glucose (MAG)		
Pre-randomisation[a]	1.4 (0.9 to 2.6)	1.5 (0.9 to 1.7)
Post-randomisation[b]	1.5 (0.7 to 2.1)	1.4 (1.1 to 1.8)
Mean of Daily Differences (MODD)		
Pre-randomisation[a]	2.0 (1.0 to 3.1)	2.3 (1.3 to 2.5)
Post-randomisation[b]	2.0 (0.7 to 2.8)	2.0 (1.5 to 2.1)
Mean Amplitude of Glucose Excursions (MAGE)	5.3 (0.0 to 9.6)	6.3 (2.9 to 6.9)
Pre-randomisation[a]		
Post-randomisation[b]	5.9 (1.6 to 9.7)	4.7 (3.5 to 6.7)

Results presented as Median with (Data Range: Minimum to Maximum)
[a] : Based on 10 patients in the Gliclazide group, and 8 patients in the Linagliptin group
[b]: Based on 9 patients in the Gliclazide group, and 7 patients in the Linagliptin group
Table 11 Pre and post randomisation values of glycaemic variability outcomes

Post randomisation value – Pre randomisation value	Randomised to Gliclazide (n = 9)	Randomised to Linagliptin (n = 7)	Test statistic U, p value
Change in Co-efficient of Variation CV, (%)	- 0.7 (-12.1 to +12.9)	- 9.2 (-15.7 to +6.3)	14.0, p = 0.071
Change in Standard Deviation SD	0.0 (-1.0 to +0.8)	- 0.6 (-0.9 to +0.6)	26.0, p = 0.606
Change in Continuous Overall Net Glycaemic Action (CONGA-1)	- 0.3 (-0.8 to +0.7)	0.0 (-0.7 to +0.7)	33.0, p = 0.918
Change in Mean Absolute Glucose (MAG)	- 0.2 (-0.4 to +0.5)	+ 0.1 (-0.5 to +0.5)	36.0, p = 0.681
Change in Mean of Daily Differences (MODD)	- 0.2 (-1.5 to +0.2)	- 0.4 (-0.7 to +0.3)	29.0, p = 0.837
Change in Mean Amplitude of Glucose Excursions (MAGE)	+ 0.3 (-1.9 to +3.3)	- 1.4 (-2.5 to +1.7)	19.0, p = 0.210

Results presented as Median with (Data Range: Minimum to Maximum)

Table 12 Change in glycaemic variability outcomes for participants randomised to Gliclazide or Linagliptin

3.4.3. Time Spent in Hyperglycaemic Ranges

The percentage of time spent in hyperglycaemia before and after randomisation was extracted from each CGM period for all the study patients. Hyperglycaemic threshold ranges were identified as:

1. Level 1: Glucose values above 10.0 mmol/L and;
2. Level 2: Glucose values above 13.9 mmol/L, where hyperglycaemia in this range is clinically significant and carries higher risk of progression into diabetic ketoacidosis or hyperosmolar hyperglycaemic state (HHS)

Median percentage of time spent in hyperglycaemic range >10.0 mmol/L appeared to be similar in both groups for pre and post-randomisation CGM periods, but the Linagliptin group had considerably more spread of values (Table 13). By the end of the study, patients that had been randomised to Linagliptin spent more time in both hyperglycaemic thresholds above 10.0 mmol/L (+11.3 versus +2.6) and above 13.9 mmol/L (+0.8 versus +0.6) compared to patients that had continued on Gliclazide. These differences between the treatment arms was non-significant for hyperglycaemic thresholds above 10.0 mmol/L ($p = 0.351$) and above 13.9 mmol/L ($p = 0.536$, Table 14).

3.4.4. Risk Indices

The low blood glucose index (LBGI) and high blood glucose index (HBGI) are measures of the risk of hypo and hyperglycaemia respectively, based on the number and amplitude of excursions into these threshold ranges. Median LBGI values were low in both groups before and randomisation, in keeping with the low rates of hypoglycaemia in the study. Median HBGI was the same in both treatment groups before randomisation, and higher in the Linagliptin group after randomisation (5.4 versus 5.1, Table 13). There was greater range of values for HBGI in the Linagliptin group as well.

Randomisation to Linagliptin was associated with a decrease in LBGI (-0.2 versus 0.0, $p = 0.114$) and an increase in HBGI (+1.7 versus +0.3, $p = 0.174$) but both these differences were not significant (Table 14), in keeping with the hypoglycaemic outcomes in section 3.3 and the hyperglycaemic outcomes in the previous section 3.4.3.

Time in Hyperglycaemic Ranges	Randomised to Gliclazide	Randomised to Linagliptin
Time spent in hyperglycaemia >10.0 mmol/L, (%)		
Pre-randomisation[a]	23.1 (0.5 to 46.6)	22.8 (1.2 to 67.3)
Post-randomisation[b]	23.1 (0.0 to 49.8)	23.7 (10.5 to 100.0)
Time spent in hyperglycaemia >13.9 mmol/L, (%)		
Pre-randomisation[a]	1.1 (0.0 to 19.1)	3.1 (0.0 to 16.5)
Post-randomisation[b]	3.3 (0.0 to 19.5)	1.9 (0.0 to 65.9)
Risk Indices	**Randomised to Gliclazide**	**Randomised to Linagliptin**
Low Blood Glucose Index (LBGI)		
Pre-randomisation[a]	0.1 (0.0 to 1.4)	0.3 (0.0 to 2.4)
Post-randomisation[b]	0.1 (0.0 to 3.6)	0.0 (0.0 to 0.1)
High Blood Glucose Index (HBGI)		
Pre-randomisation[a]	4.8 (0.9 to 11.7)	4.8 (1.1 to 13.4)
Post-randomisation[b]	5.1 (0.1 to 12.2)	5.4 (2.8 to 28.9)

Results presented as Median with (Data Range: Minimum to Maximum)

[a] : Based on 10 patients in the Gliclazide group, and 8 patients in the Linagliptin group

[b]: Based on 9 patients in the Gliclazide group, and 7 patients in the Linagliptin group

Table 13 Pre and post randomisation values of time spent in hyperglycaemic thresholds and risk indices

Post randomisation value – Pre randomisation value	Randomised to Gliclazide (n = 9)	Randomised to Linagliptin (n = 7)	Test statistic U, p value
Change in time spent in hyperglycaemia >10.0 mmol/L, (%)	+ 2.6 (-5.3 to +13.7)	+ 11.3 (-10.2 to +69.0)	41.0, p = 0.351
Change in time spent in hyperglycaemia >13.9 mmol/L, (%)	+ 0.6 (-10.5 to +4.6)	+ 0.8 (-2.7 to +61.9)	38.0, p = 0.536
Change in Low Blood Glucose Index (LBGI)	0.0 (-0.7 to +2.1)	- 0.2 (-2.3 to 0.0)	16.0, p = 0.114
Change in High Blood Glucose Index (HBGI)	+ 0.3 (-2.8 to +3.0)	+ 1.7 (-1.6 to +22.8)	45.0, p = 0.174

Results presented as Median with (Data Range: Minimum to Maximum)

Table 14 Change in time spent in hyperglycaemic thresholds and risk indices for participants randomised to Gliclazide or Linagliptin

3.5 Outcome 3: Serum and Urine Biomarkers

Blood and mid-stream urine for serum and urine MCP-1 and TGF-β1 were obtained from all 19 study participants before randomisation. All 10 patients randomised to Gliclazide had serum and urine biomarkers taken after randomisation, while only 7 of the patients randomised to Linagliptin had them taken (2 patients withdrawn from the study after randomisation).

The null and alternative hypotheses are:

1. Null hypothesis, H_0: There is no significant difference in serum and urine MCP-1 and TGF-β1 levels in patients randomised to Gliclazide and patients randomised to Linagliptin
2. Alternative hypothesis, H_1: There is a significant difference in serum and urine MCP-1 and TGF-β1 levels in patients randomised to Gliclazide and patients randomised to Linagliptin.

The pre and post randomisation serum and urine MCP-1 and TGF-β1 levels did not follow a parametric distribution or fulfil assumptions of homogeneity of variance. The same Mann-Whitney statistical tests were performed to examine the relationship of being on Gliclazide or Linagliptin for serum and urine MCP-1 and TGF-β1 levels.

3.5.1 Serum and Urine MCP-1

Median values for serum MCP-1, urine MCP-1 and urine MCP-1/creatinine ratio in both treatment groups pre and post-randomisation are summarised in Table 15. The median serum MCP-1 for the Linagliptin group was higher than that for the Gliclazide group, both before (154.8 pg/ml vs 141.1 pg/ml) and after randomisation (136.7 pg/ml vs 126.3 pg/ml). Study participants that had switched to Linagliptin had more reduction in serum MCP-1 levels compared to participants that had continued on Gliclazide (-18.1 versus -12.1), but the change in serum MCP-1 levels between the treatment arms was not significantly different ($p = 0.740$, Table 16).

Median urine MCP-1 levels pre-randomisation in Gliclazide and Linagliptin patient groups were similar, as were median urine MCP-1 values post randomisation in both groups (Table 15). Spread of urine MCP-1 values pre-randomisation was greater in the

Gliclazide group. Patients that continued on Gliclazide throughout the study had more reduction in urine MCP-1 levels compared to patients that had swapped over to Linagliptin (-15.9 versus -7.3), but this finding was non-significant (p = 0.536, Table 16).

As for urine MCP-1/creatinine ratio, median values in the Gliclazide group were higher than the Linagliptin group both before (14.8 versus 11.2) and after randomisation (10.7 versus 10.6, Table 15). Linagliptin was associated with an increase in the urine MCP-1/creatinine ratio whereas Gliclazide was associated with a reduction in the urine MCP-1/creatinine ratio; this difference was significant (p = 0.002, Table 16).

3.5.2 Serum and Urine TGF-β1

The median serum TGF-β1 was higher in the Linagliptin group both before (17.6 ng/ml versus 16.8 ng/ml) and after randomisation (20.8 ng/ml versus 18.2 ng/ml, Table 17). Patients randomised to continue Gliclazide had the same increase in serum TGF-β1 levels as patients randomised to switch to Linagliptin (+2.6), and so Linagliptin did not have any significant effect on serum TGF-β1 levels (p = 0.887, Table 18).

The median urine TGF-β1 level was zero in both Gliclazide and Linagliptin groups, before and after randomisation. This was also the case for median urine TGF-β1/creatinine levels (Table 17). There was no change in urine TGF-β1 levels or urine TGF-β1/creatinine levels between the treatment arms, therefore being on Linagliptin did not significantly affect urine TGF-β1 levels (p = 1.000) or the urine TGF-β1/creatinine ratio (p = 1.000, Table 18).

Serum and urine MCP-1	Randomised to Gliclazide	Randomised to Linagliptin
Serum MCP-1 (pg/ml)		
Pre-randomisation[a]	141.1 (115.8 to 190.6)	154.8 (128.0 to 241.3)
Post-randomisation[b]	126.3 (87.1 to 174.6)	136.7 (112.0 to 172.7)
Urine MCP-1 (pg/ml)		
Pre-randomisation[a]	106.5 (15.8 to 678.4)	100.5 (48.4 to 197.8)
Post-randomisation[b]	69.9 (0.0 to 314.9)	71.0 (46.8 to 202.8)
Urine MCP-1/Creatinine ratio		
Pre-randomisation[a]	14.8 (4.7 to 79.8)	11.2 (4.0 to 24.5)
Post-randomisation[b]	10.7 (0.0 to 49.2)	10.6 (4.4 to 19.6)

Results presented as Median with (Data Range: Minimum to Maximum)
[a] : Based on 10 patients in the Gliclazide group, and 9 patients in the Linagliptin group
[b]: Based on 10 patients in the Gliclazide group, and 7 patients in the Linagliptin group
Table 15 Pre and post randomisation values of serum MCP-1, urine MCP-1, and urine MCP-1/creatinine ratio

Post randomisation value – Pre randomisation value	Randomised to Gliclazide (n = 10)	Randomised to Linagliptin (n = 7)	Test statistic U, p value
Change in serum MCP-1 (pg/ml)	- 12.1 (-55.2 to +14.0)	- 18.1 (-47.5 to +20.9)	31.0, p = 0.740
Change in urine MCP-1 (pg/ml)	- 15.9 (-363.5 to +10.0)	- 7.3 (-123.8 to +154.4)	42.0, p = 0.536
Change in urine MCP-1/ creatinine ratio	- 4.4 (-30.6 to +1.5)	+ 3.4 (-1.0 to +6.1)	65.0, p = 0.002

Results presented as Median with (Data Range: Minimum to Maximum)
Table 16 Change in serum MCP-1, urine MCP-1 and urine MCP-1/creatinine ratio for participants randomised to Gliclazide or Linagliptin

Serum and urine TGF-β1	Randomised to Gliclazide	Randomised to Linagliptin
Serum TGF-β1(ng/ml)		
Pre-randomisation[a]	16.8 (13.4 to 25.2)	17.6 (9.9 to 22.3)
Post-randomisation[b]	18.2 (14.5 to 27.8)	20.8 (9.6 to 24.2)
Urine TGF-β1(pg/ml)		
Pre-randomisation[a]	0.0 (0.0 to 198.5)	0.0 (0.0 to 88.0)
Post-randomisation[b]	0.0 (0.0 to 72.6)	0.0 (0.0 to 1023.4)
Urine TGF-β1/Creatinine ratio		
Pre-randomisation[a]	0.0 (0.0 to 39.7)	0.0 (0.0 to 4.7)
Post-randomisation[b]	0.0 (0.0 to 5.3)	0.0 (0.0 to 47.6)

Results presented as Median with (Data Range: Minimum to Maximum)
[a] : Based on 10 patients in the Gliclazide group, and 9 patients in the Linagliptin group
[b]: Based on 10 patients in the Gliclazide group, and 7 patients in the Linagliptin group
Table 17 Pre and post randomisation values of serum TGF-β1, urine TGF-β1, and urine TGF-β1/creatinine ratio

Post randomisation value – Pre randomisation value	Randomised to Gliclazide (n = 10)	Randomised to Linagliptin (n = 7)	Test statistic U, p value
Change in serum TGF-β1 (ng/ml)	+ 2.6 (-1.9 to +7.9)	+ 2.6 (-0.3 to +6.0)	37.0, p = 0.887
Change in urine TGF-β1 (pg/ml)	0.0 (-198.5 to +72.6)	0.0 (-88.0 to +1023.4)	36.0, p = 1.000
Change in urine TGF-β1/ creatinine ratio	0.0 (-39.7 to +5.3)	0.0 (-4.7 to +47.6)	36.0, p = 1.000

Results presented as Median with (Data Range: Minimum to Maximum)
Table 18 Change in serum TGF-β1, urine TGF-β1, and urine TGF-β1/creatinine ratio for participants randomised to Gliclazide or Linagliptin

3.6 Outcome 4: Patient satisfaction as measured by the DTSQ scores

The DTSQ score (total score excluding the scores for question 2 and 3) before randomisation was obtained from all 19 GLOOCOSE study patients. The DTSQ score after randomisation was acquired from 17 patients, as 2 of the patients randomised to Linagliptin had been withdrawn from the study. The null and alternative hypotheses are:

1. Null hypothesis, H_0: There is no significant difference in the DTSQ scores in patients randomised to Gliclazide and patients randomised to Linagliptin
2. Alternative hypothesis, H_1: There is a significant difference in the DTSQ scores in patients randomised to Gliclazide and patients randomised to Linagliptin.

The pre and post randomisation DTSQ scores did not have a parametric distribution therefore Mann-Whitney statistical tests for independent samples were undertaken. The median DTSQ score before randomisation was higher in the Gliclazide group, but equal in both Gliclazide and Linagliptin groups after randomisation (Table 19). Study participants randomised to Linagliptin had a higher increase in their DTSQ scores (+2.0) compared to participants who continued on Gliclazide (+0.5), but this difference was not significant ($p = 0.536$, Table 20). This indicates that patients were equally satisfied whether they were on Gliclazide or Linagliptin.

Question 2 on the DTSQ examines the patient's own perception of frequency of hyperglycaemia. There was no change in the scores for Question 2 for both the Gliclazide and Linagliptin groups, thus this difference was not significant ($p = 1.000$, Table 20). However, it is interesting to note that although patients spent more time in hyperglycaemia after randomisation to Linagliptin, they did not perceive this to be the case, and overall were still satisfied with being switched to Linagliptin (as per the total DTSQ score). This may be that patients themselves are not aware of what constitutes "hyperglycaemia" and the risks associated with it, or perhaps were satisfied with its once daily dosing.

Question 3 on the DTSQ assesses the patient's perception of frequency of hypoglycaemia. Here, patients randomised to continue Gliclazide thought that they had more hypoglycaemia by the end of the study compared to the beginning of the study (+0.5), whereas patients randomised to switch to Linagliptin did not perceive any

difference in hypoglycaemia frequency by study end (0.0, Table 20). This small difference in the change in Question 3 scores between the two groups did not achieve significance (p = 0.193, Table 20).

DTSQ score	Randomised to Gliclazide	Randomised to Linagliptin
Overall DTSQ score		
Pre-randomisation[a]	33.0 (21.0 to 36.0)	30.0 (20.0 to 35.0)
Post-randomisation[b]	34.0 (25.0 to 36.0)	34.0 (22.0 to 36.0)
Question 2 DTSQ		
Pre-randomisation[a]	1.5 (1.0 to 5.0)	2.0 (0.0 to 4.0)
Post-randomisation[b]	1.0 (0.0 to 5.0)	2.0 (0.0 to 6.0)
Question 3 DTSQ		
Pre-randomisation[a]	0.0 (0.0 to 3.0)	0.0 (0.0 to 2.0)
Post-randomisation[b]	1.0 (0.0 to 3.0)	0.0 (0.0 to 1.0)

Results presented as Median with (Data Range: Minimum to Maximum)
[a] : Based on 10 patients in the Gliclazide group, and 9 patients in the Linagliptin group
[b]: Based on 10 patients in the Gliclazide group, and 7 patients in the Linagliptin group
Table 19 Pre and post randomisation values of DTSQ scores

Post randomisation value – Pre randomisation value	Randomised to Gliclazide (n = 10)	Randomised to Linagliptin (n = 7)	Test statistic U, p value
Change in overall DTSQ score	+ 0.5 (-6.0 to +4.0)	+ 2.0 (-6.0 to +10.0)	41.5, p = 0.536
Change in Question 2 DTSQ	0.0 (-3.0 to +1.0)	0.0 (-2.0 to +4.0)	35.5, p = 1.000
Change in Question 3 DTSQ	+ 0.5 (-1.0 to +1.0)	0.0 (-2.0 to +1.0)	21.5, p = 0.193

Results presented as Median with (Data Range: Minimum to Maximum)
Table 20 Change in DTSQ scores for participants randomised to Gliclazide or Linagliptin

3.7 Summary of GLOOCOSE Study Outcomes

Patients randomised to Linagliptin had lost significantly more weight ($p = 0.025$) and had a significantly lower BMI compared to patients that continued on Gliclazide ($p = 0.025$). There was no significant difference in hypoglycaemic incidence or time spent in hypoglycaemia between the two treatment groups. However, overall glycaemic control by the end of the study was poorer in study patients who had been randomised to switch to Linagliptin, with significantly higher fasting CBGs ($p = 0.001$), higher CGM mean glucose ($p = 0.023$) and higher estimated CGM HbA1c ($p = 0.016$). There was also a trend towards increased serum HbA1c ($p = 0.161$), less time spent in normoglycaemia ($p = 0.299$), and more time spent in hyperglycaemia >10.0 mmol/L ($p = 0.351$) by the end of the study in participants who had switched to Linagliptin, although all of these findings were non-significant. Interestingly, there was also a trend towards reduction in eGFR in patients randomised to Linagliptin, although again, this was non-significant ($p = 0.055$). There were no significant differences in CGM measures of glycaemic variability (SD, %CV, CONGA-1, MAG, MODD or MAGE) between the two treatment groups.

Urine MCP-1/creatinine ratio was significantly increased in patients randomised to switch to Linagliptin ($p = 0.002$); this is likely to be related to the poorer glycaemic control in this group. There were no significant differences for change in serum/urine MCP-1 and TGF-β1 levels between the two treatment arms. There was also no difference in patient satisfaction in patients randomised to continue Gliclazide or to switch to Linagliptin. Although patients randomised to Linagliptin had poorer glycaemic control, these patients did not view their control as suboptimal.

The small number of patients is a limitation of the GLOOCOSE study, which may mean the study is underpowered to find statistical differences. Additionally, the relatively short duration of randomisation to Linagliptin means maximum effect may not have been reached by 8 weeks, and limits the conclusions drawn by this study.

4.0 References

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