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ORIGINAL ARTICLE

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Pharmacokinetic and pharmacodynamic equivalence of Biocon's biosimilar Insulin-R with the US-licensed Humulin® R formulation in healthy subjects: Results from the RHINE-1 (Recombinant Human INsulin Equivalence-1) study

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Abstract

Aim: To establish equivalence in the pharmacokinetic (PK) and pharmacodynamic (PD) endpoints between proposed biosimilar Insulin-R (Biocon's Insulin-R) and Humulin® R using the euglycaemic clamp technique in healthy subjects.

Materials and Methods: In this phase-1 automated euglycaemic glucose clamp study, 42 healthy subjects were randomized (1:1) to receive a single dose of 0.3 IU/kg of Biocon's Insulin-R and Humulin-R. Plasma insulin concentrations and glucose infusion rates (GIRs) were assessed over 12 hours. Primary PK endpoints were area under the insulin concentration-time curve from 0 to 12 hours ($AUC_{ins.0\text{-}12h}$) and maximum insulin concentration ($C_{ins.max}$). Primary PD endpoints were area under the GIR time curve from 0 to 12 hours ($AUC_{GIR.0\text{-}12h}$) and maximum GIR (GIR_{max}).

Results: Equivalence was demonstrated between Biocon's Insulin-R and Humulin-R for the primary PK and PD endpoints. The 90% confidence intervals were within 80.00% to 125.00% limits. The PK and PD profiles were comparable. There were no significant differences in the safety profiles of the two treatments, and no serious adverse events were reported.

Conclusion: PK and PD equivalence was demonstrated between Biocon's Insulin-R and Humulin-R in healthy subjects. Treatment with Biocon's Insulin-R and Humulin-R was well tolerated.

KEYWORDS

basal insulin, biosimilar insulin, pharmacodynamics, pharmacokinetics, type 1 diabetes, type 2 diabetes

1 | INTRODUCTION

Insulin therapy is indispensable to patients with type 1 diabetes (T1D) and advanced type 2 diabetes (T2D) to effectively maintain glycaemic control and prevent further complications.[1] While the number of people who need insulin has increased exponentially over the years, insulin access in many countries is inadequate, mostly because of unaffordable prices.[2-4] The direct annual cost of diabetes treatment globally has increased to US$825 billion[5] and, in countries like the United States, insulin prices tripled from 2002 to 2013.[2] The insulin expenditure for underinsured or uninsured patients results in non-adherence or rationing of insulin, leading to diabetes complications with early and increased morbidity and mortality.[6,7]

The World Health Organization (WHO) launched the first-ever insulin prequalification programme in 2019 to boost access by increasing the flow of quality-assured products in the international market, providing countries with a greater choice and patients with lower prices.[8] The WHO Steering Group strongly recommends the use of human insulin to manage blood glucose (BG) in adults with T1D and T2D,[9-11] and the recombinant human insulins (rHIs; neutral protamine Hagedorn, regular and premix) represent an economical alternative to analogues.[12] Several reference-listed rHI drugs are available 'over-the-counter' in the United States,[13] implying that in the real-world scenario patients may be switching freely between these marketed rHI products. The introduction of biosimilar rHIs can extend access to people in need (90% T2D and 10% T1D)[14] by increasing available choices and ensuring affordability. Recently, the Endocrine Society made strong recommendations to expedite the approval of insulin biosimilars as one of the policies to increase access to life-saving insulins for patients with diabetes in the United States.[15]

Development of Biocon's Insulin-R (regular insulin; produced in the cell line *Pischia pastoris*[16]), as a proposed biosimilar to US-approved Humulin® R (henceforth referred to as Humulin-R; Eli Lilly and Company, IN), is an endeavour to increase patient access. Regular insulin is a short-acting, prandial insulin indicated to improve glycaemic control in adult and paediatric patients with diabetes.[17]

Stringent regulatory requirements, including multiple orthogonal analytical methods to evaluate similarity in structure and function, as well as pharmacokinetic (PK)/pharmacodynamic (PD) equivalence, ensure the similarity of the proposed product to the reference product.[18] When assessing the biosimilarity of insulins, a surrogate PD marker (glucose infusion rate [GIR]) exists and correlates with clinical efficacy. As per recent guidance from the Food and Drug Administration (FDA) for biosimilar insulins,[18] if comparative analytical assessment using state-of-the-art technology demonstrates 'high similarity' for a proposed biosimilar, it can be considered that there would be little or no residual uncertainty regarding immunogenicity.

Biocon's Insulin-R is similar to Humulin-R as assessed through a rigour of physicochemical analyses and non-clinical studies. The current study was designed to demonstrate equivalence in the PK/PD endpoints between Biocon's Insulin-R and Humulin-R using the euglycaemic clamp technique in healthy subjects.

2 | MATERIALS AND METHODS

2.1 | Study design

In this phase-1, single-centre, randomized, double-blind, single-dose, two-treatment, two-period, two-sequence, crossover, 12-hour automated euglycaemic glucose clamp study (EudraCT: 2018-003217-18; Clinicaltrial.gov: NCT04022317), eligible subjects were randomly allocated to a sequence of single doses of Biocon's Insulin-R and Humulin-R (Figure 1). Subjects had fasted for at least 10 hours prior to the dosing. Single doses of 0.3 IU/kg of Biocon's Insulin-R (Biocon Limited, India) and Humulin-R (US-sourced), both 100 IU/ml, were administered subcutaneously into a lifted skin fold of the abdominal wall into the peri-umbilical area using a standard skin-fold technique. Insulin was administered at two different abdominal quadrants (left lower quadrant and right lower quadrant) with a BD Micro-fine +0.5 ml U100 syringe fitted with a 0.30 mm (30G) × 8 mm needle. Blood was collected predose and postdose at prespecified intervals until 12 hours for BG, insulin, and C-peptide measurement. There was a washout period of 5-7 days between dose administrations to avoid any carryover effect before the crossover. This study was conducted at Profil Mainz GmbH & Co., KG, Germany.

2.2 | Study subjects

The study population included healthy subjects aged 18-55 years (both inclusive), with a body mass index (BMI) of 18.5-29.0 kg/m^2 (both inclusive) and fasting plasma glucose (FPG) concentration of 100 mg/dl or less. Major exclusion criteria included receipt of any medicinal product

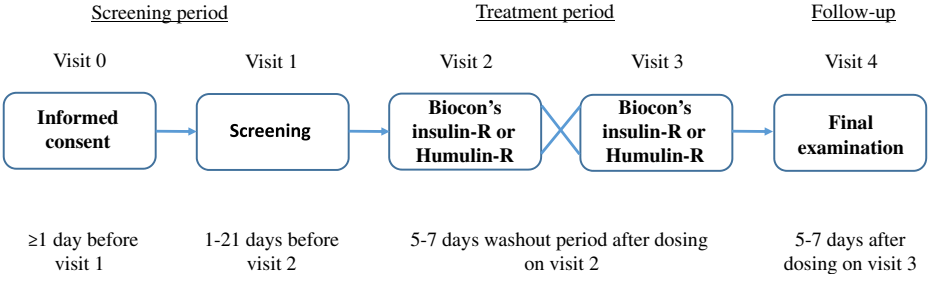

FIGURE 1 Study design

in clinical development within 30 days or five times its half-life (whichever was longer) before being randomized into this trial, any history or presence of a clinically relevant co-morbidity (as judged by the investigator), systolic blood pressure less than 95 or greater than 140 mmHg and/or diastolic blood pressure less than 50 or greater than 90 mmHg after resting for at least 5 minutes in the supine position, and a pulse rate at rest outside the range of 50-90 beats/min.

2.3 | Ethics

This study was conducted in accordance with Good Clinical Practice and conformed to the ethical principles of the Declaration of Helsinki and all local and federal laws and regulations. The study was approved by the ethics committee and Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM) before study initiation. Subjects provided written informed consent prior to initiation of the study.

2.4 | Euglycaemic glucose clamping

Euglycaemic glucose clamping was performed using a glucose clamp device (ClampArt®; Profil Institut für Stoffwechselforschung, Neuss, Germany). The quality of the clamp data was reviewed on a regular basis by the investigator and clamp supervisors. The quality of clamps was evaluated[19] based on all BG measurements during the clamp procedure, where GIR was greater than 0 mg/kg/min, as follows:

- Precision measured as clamp coefficient-of-variation (CV%), derived as

$$\frac{100 * (\textit{standard deviation of BG}) \textit{ measured by ClampArt}}{\text{mean BG measured by ClampArt}}.$$

- Control deviation, measured as clamp deviation from the target (DFT), derived as

$$\text{Mean}\,(\text{BG measured by ClampArt} - \text{clamp level}).$$

The mean clamp CV was required to be less than 15% and the mean DFT was required to be within the range of ±10 mg/dl after dosing until the end of the clamp. After the investigational medicinal product was administered (time zero), the clamp device-controlled variable glucose infusion was initiated at the time of onset of action (when BG had dropped by 5 mg/dl from baseline as measured by ClampArt). The glucose clamp device automatically kept the subjects' glucose concentration at a target clamp level of 81 mg/dl with minimal deviations. It automatically calculated appropriate adjustments of the intravenous GIR using an algorithm based on the difference between the actual BG values and the predefined target level, the slope of the BG values (in the preceding 5 minutes), and weighted area under the preceding GIR curve. The GIR necessary to keep the BG concentration at the target level was recorded every minute throughout the glucose clamp duration. BG was analysed at the study site using a Super GL glucose analyser (Dr. Müller Gerätebau GmbH, Freital, Germany) for verification of measurements of ClampArt. Please refer to Appendix S1 (Glucose Clamp Procedures) for further details.

2.5 | PK sampling

Blood samples were taken for PK (plasma insulin and serum C-peptide levels) analysis at predefined time intervals.

2.6 | Bioanalytical methods

A validated ultra-performance liquid chromatography with tandem mass spectrometry detection was used to analyse the study samples. Insulin concentrations in plasma were measured using liquid chromatography-tandem mass spectroscopy. The lower and upper limits of quantification (LLOQ and ULOQ, respectively) of this method were 50 and 8000 ng/L, respectively. The C-peptide levels in serum were measured using a validated electrochemiluminescence immunoassay test kit (Roche Diagnostics, Switzerland) with the LLOQ and ULOQ being 0.2 and 32.0 ng/ml, respectively. All validation and sample quantification runs met the prespecified acceptance criteria, including incurred sample reproducibility.

2.7 | PK assessments

The primary PK parameters included $AUC_{0\text{-}12h}$ (area under insulin concentration-time curve from 0 to 12 hours) and $C_{ins.max}$ (maximum insulin concentration). Other parameters included $AUC_{ins.0\text{-}2h}$, $AUC_{ins.0\text{-}6h}$, $AUC_{ins.6\text{-}12h}$, $AUC_{ins.0\text{-}\infty}$ (areas under insulin concentration-time curve in the indicated time intervals), $t_{ins.max}$ (time to maximum insulin concentration), $t_{50\%\text{-}ins(early)}$ (time to half-maximum insulin concentration before $C_{ins.max}$), $t_{50\%\text{-}ins(late)}$ (time to half-maximum insulin concentration after $C_{ins.max}$), $t_{½}$ (terminal elimination half-life), and λ_z (terminal elimination rate constant). The values of all individual PK parameters were calculated using non-compartmental methods in Phoenix WinNonlin v. 8.0 (Certara, NJ).

The primary PK analysis was conducted using Owen's method for correction of endogenous insulin secretion using the C-peptide–based correction formula.[20] Exogenous insulin (Insulin EXOG) concentration was calculated as per the formula:

Insulin EXOG = observed plasma insulin concentration – (mean of insulin/C-peptide conc. ratios at −30, −15 and 0 minutes) × observed serum C-peptide concentration.

A sensitivity analysis of the primary PK endpoints was performed using the same mixed model as described for the primary analysis with uncorrected (i.e. without applying Owen's correction for C-peptide)

insulin concentrations. C-peptide–based exclusion rules (described in the next section for the primary PD variables) were applied for the PK sensitivity analysis.

2.8 | PD assessments

Primary PD parameters included $AUC_{GIR.0\text{-}12h}$ (area under GIR time curve from 0 to 12 hours) and GIR_{max} (maximum GIR). Other parameters included $AUC_{GIR.0\text{-}2h}$, $AUC_{GIR.0\text{-}6h}$, $AUC_{GIR.6\text{-}12h}$ (area under GIR time curve in the indicated time interval), $t_{GIR.max}$ (time to maximum GIR), $t_{50\%\text{-}GIR(early)}$ (time to half-maximum GIR before GIR_{max}), $t_{50\%\text{-}GIR(late)}$ (time to half-maximum GIR after GIR_{max}), and the onset of action.

Primary PD analysis was conducted using C-peptide–based exclusion of profiles. For this, C-peptide concentration-time profiles were inspected during the blinded data review meeting to identify and exclude profiles as predefined for the study.

To account for meaningful fluctuations that can reflect changes in endogenous insulin concentration during the clamp period, profiles meeting the predefined criteria were excluded from the primary PD analysis set.

Sensitivity analysis for the PD data was conducted using all profiles without applying any C-peptide–based exclusion criteria.

2.9 | Safety assessments

All adverse events (AEs) were evaluated in terms of intensity, duration, severity, outcome, and relationship to study medication throughout the study. Other safety parameters included injection-site reactions, local tolerability, hypoglycaemic episodes (classification and recording of hypoglycaemia in the trial were performed according to the guidelines of the American Diabetes Association[21,22]; refer to Appendix S2), vital signs, physical examinations, 12-lead electrocardiograms (ECGs), and standard laboratory safety tests.

2.10 | Statistical analysis

All statistical analyses were performed using SAS® v. 9.4 (SAS Institute Inc., NC). Equivalence between Biocon's Insulin-R and Humulin-R was considered demonstrated if the 90% confidence interval (CI) for the primary PK endpoints, $AUC_{ins.0\text{-}12h}$-ratio and $C_{ins.max}$-ratio, and the primary PD endpoints, $AUC_{GIR.0\text{-}12h}$-ratio and GIR_{max}-ratio, lay within an acceptance interval of 80.00%-125.00%.

2.10.1 | Sample size

Based on the intra-subjectn variability observed in earlier studies,[23,24] CV of PK/PD parameters for Biocon's Insulin-R and Humulin-R was not expected to exceed 25%. Based on this CV% and an assumed ratio of 0.95 between reference and test insulin, a sample size of 38 subjects was considered necessary to establish equivalence with sufficient power of at least 90% (sample size calculation based on $\alpha = .05$ and 90% CIs in the range of 80.00%-125.00%). To account for potential dropouts during the study, 42 subjects were planned to be randomized.

2.10.2 | PK and PD endpoints

The per-protocol population (PPP) for PK/PD included all randomized subjects who completed the trial without any important protocol deviation. Single profiles of subjects who did not provide evaluable PK data were excluded from the PPP for PK if less than 50% of concentration measurements were above LLOQ or zero postdosing (i.e. 11 of 24 measurements). Single profiles of subjects who did not meet the clamp-quality criteria were excluded from the PPP for PD analysis.

For analysis of the primary PK/PD endpoints, data were logarithmically transformed as these parameters were assumed to follow a log-normal distribution. Logarithm-transformed endpoints were analysed using analysis of variance with sequence, period, and treatment as fixed effects and subject within the sequence as a random effect. The least-square (LS) mean for each treatment, a difference of LS means between treatment groups, and corresponding 90% CI was calculated, exponentially back-transformed, and multiplied by 100 to find the estimated ratio percentage of responses between the insulin formulations and the corresponding 90% CI.

Secondary PK/PD AUC endpoints were compared using the same statistical approach as the primary endpoints. Time-related PK/PD endpoints were analysed using descriptive statistics by treatment only.

2.10.3 | Safety

Analysis of safety endpoints was based on the safety analysis set (SAS), which included all randomized subjects who had received at least one dose of the study treatment. Safety data were summarized by treatment using descriptive statistics.

3 | RESULTS

3.1 | Subject disposition and baseline characteristics

Of the 75 male subjects screened, 42 were randomized to one of the two treatment sequences. Forty-one subjects completed the study, and one subject withdrew consent after the first dose of Humulin-R. The age, BMI, and FPG ranged from 19 to 54 (mean 33.4) years, 19.9 to 28.7 (mean 24.45) kg/m^2, and 74 to 99 (mean 87.7) mg/dl, respectively. Demographic characteristics were similar for the two treatment sequences. The disposition, demographics, and baseline characteristics of the subjects are presented in Table S1.

TABLE 1 Primary PK and PD endpoints (PP population)

Endpoint	Biocon's Insulin-R		Humulin-R		Geometric LS-mean ratio Biosimilar Insulin-R/Humulin-R (90% CI)	Intra-subject CV%	Power (%)
	N	LS-mean	N	LS-mean			
PK endpoints							
$AUC_{ins.0\text{-}12h}$ (h*ng/L)	41	11 058.46	41	11 127.01	99.38 (97.02; 101.81)	6.5	>99
$C_{ins.max}$ (ng/L)	41	1977.859	41	2142.127	92.33 (87.34; 97.61)	15.0	>99
PD endpoints							
$AUC_{GIR.0\text{-}12h}$ (mg/kg)	39[a]	3201.511	38[a]	3249.590	98.52 (92.63; 104.79)	15.7	>99
GIR_{max} (mg/kg/min)	39[a]	8.952	38[a]	9.384	95.40 (89.46; 101.74)	16.4	>99

Abbreviations: $AUC_{ins.0\text{-}12h}$, area under the insulin concentration curve from 0 to 12 hours; $AUC_{GIR.0\text{-}12h}$, area under the glucose infusion rate curve from 0 to 12 hours; CI, confidence interval; $C_{ins.max}$, maximum insulin concentration; CV%, percentage coefficient of variation; GIR_{max}, maximum observed glucose infusion rate; LS mean; least square mean; PD, pharmacodynamics; PK, pharmacokinetics; PP, per protocol.
[a]Five profiles (two Biocon's Insulin-R and three Humulin-R) were excluded based on C-peptide exclusion rules.

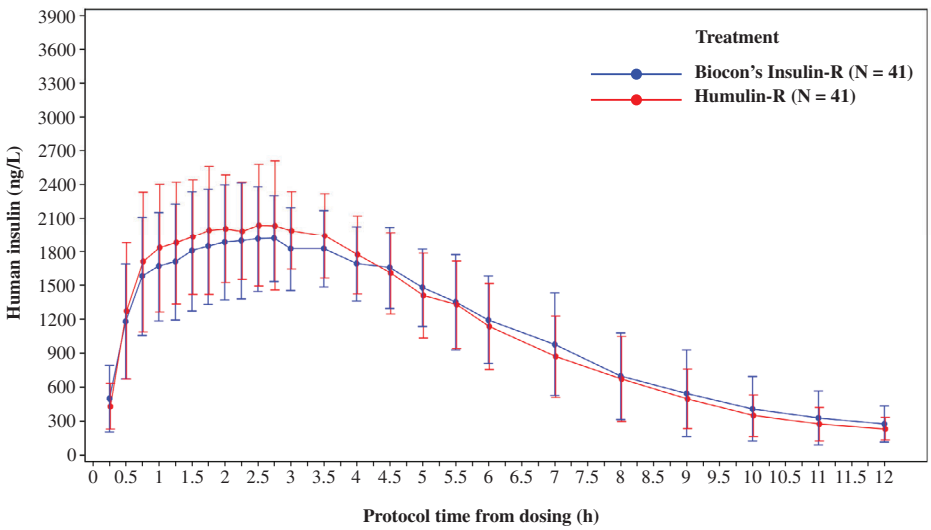

FIGURE 2 C-peptide–corrected mean insulin profiles linear scale (PP population for PK). PK, pharmacokinetics; PP, per protocol

There were no significant protocol deviations and none of the profiles were excluded from the PPP for PK because of non-evaluable data and from the PPP for PD because of non-fulfilment of any of the defined clamp-quality criteria. The PPP for PK/PD comprised 41 subjects, whereas the SAS included 42 subjects.

3.2 | Pharmacology

3.2.1 | PK analyses

For the primary analysis, the 90% CIs for geometric mean ratios (Biocon's Insulin-R/Humulin-R) were within 80.00% and 125.00% limits for both primary PK endpoints, $AUC_{ins.0\text{-}12h}$ and $C_{ins.max}$ (Table 1). Mean C-peptide–corrected plasma insulin concentration-time profiles (Owen's method) showed close similarity between Biocon's Insulin-R and Humulin-R (Figure 2).

Results of the sensitivity analysis based on uncorrected data applying the C-peptide–based exclusion rules were similar to the primary analysis ($AUC_{ins.0\text{-}12h}$: 90% CI, 97.56%, 102.71%; $C_{ins.max}$: 90% CI, 88.46%, 98.90%; both within 80.00% and 125.00% limits), thus indicating the robustness of the study.

Secondary endpoint analyses showed the mean values of the secondary PK endpoints to be comparable between Biocon's Insulin-R and Humulin-R (Table 2). The secondary endpoints—$AUC_{ins.0\text{-}2h}$, $AUC_{ins.0\text{-}6h}$, and $AUC_{ins.0\text{-}\infty}$—met the bioequivalence criteria.

3.2.2 | PD endpoints

For the primary analysis, 90% CIs for the geometric mean ratios (Biocon's Insulin-R/Humulin-R) were within 80.00% and 125.00% limits for both primary PD endpoints, $AUC_{GIR.0\text{-}12h}$ and GIR_{max} (Table 1). The mean GIR profiles were similar between Biocon's Insulin-R and Humulin-R (Figure 3).

Results of the sensitivity analysis, without applying any C-peptide–based exclusion rules, were similar to the primary analysis ($AUC_{GIR.0\text{-}12h}$: 90% CI, 92.99%, 104.03%; GIR_{max}: 90% CI, 89.71%, 101.54%; both within 80.00% and 125.00% limits), thus indicating the robustness of the study.

TABLE 2 Secondary PK and PD endpoints (PP population)

Endpoint	Biocon's Insulin-R		Humulin-R		LS-mean ratio Biocon's Insulin-R/ Humulin-R (90% CI)	Intra-subject CV%	Power (%)
	N	LS-mean	N	LS-mean			
PK endpoints							
$AUC_{ins.0\text{-}2h}$ (h*ng/L)	41	2365.172	41	2585.301	91.49 (85.44; 97.96)	18.5	95
$AUC_{ins.0\text{-}6h}$ (h*ng/L)	41	8417.183	41	8790.505	95.75 (92.20; 99.44)	10.2	>99
$AUC_{ins.6\text{-}12h}$ (h*ng/L)	41	2031.112	41	1806.684	112.42 (100.51; 125.75)	30.8	47
$AUC_{ins.0\text{-}\infty}$ (h*ng/L)	40[a]	11 386.45	41	11 313.22	100.65 (98.27; 103.08)	6.4	>99
$t_{ins.max}$ (h)[b]	41	2.75	41	2.50	-	-	-
$t_{50\%\text{-}ins(early)}$ (h)[b]	41	0.53	41	0.55	-	-	-
$t_{50\%\text{-}ins(late)}$ (h)[b]	41	6.48	41	6.23	-	-	-
λ_z (1/h)[b]	40[a]	0.5249	41	0.5373	-	-	-
$t_{1/2}$ (h)[b]	40[a]	1.32	41	1.29	-	-	-
PD endpoints							
$AUC_{GIR.0\text{-}2h}$ (mg/kg)	39[c]	335.953	38[c]	370.057	90.78 (81.69; 100.89)	27.2	66
$AUC_{GIR.0\text{-}6h}$ (mg/kg)	39[c]	2007.125	38[c]	2102.477	95.47 (89.59; 101.73)	16.1	>99
$AUC_{GIR.6\text{-}12h}$ (mg/kg)	39[c]	1093.324	38[c]	1052.520	103.88 (93.14; 115.85)	28.4	88
$t_{GIR.max}$ (h)[b]	39[c]	4.60	38[c]	4.15	-	-	-
$t_{50\%\text{-}GIR(early)}$ (h)[b]	39[c]	1.53	38[c]	1.33	-	-	-
$t_{50\%\text{-}GIR(late)}$ (h)[b]	39[c]	7.37	38[c]	7.03	-	-	-
Onset of action (min)[b]	39[c]	27.0	38[c]	27.5	-	-	-

Abbreviations: CI, confidence interval; CV%, percentage coefficient of variation; $AUC_{GIR.0\text{-}2h}$, area under the glucose infusion rate curve from 0 to 2 hours; $AUC_{GIR.0\text{-}6h}$, area under the glucose infusion rate curve from 0 to 6 hours; $AUC_{GIR.6\text{-}12h}$, area under the glucose infusion rate curve from 6 to 12 hours; $AUC_{ins.0\text{-}2h}$, area under the insulin concentration-time curve from 0 to 2 hours; $AUC_{ins.0\text{-}6h}$, area under the insulin concentration-time curve from 0 to 6 hours; $AUC_{ins.6\text{-}12h}$, area under the insulin concentration-time curve from 6 to 12 hours; $AUC_{ins.0\text{-}\infty}$, area under the insulin concentration-time curve from 0 to infinity; GIR_{max}, maximal glucose infusion rate; λ_z, terminal elimination rate constant of insulin; LS mean, least square mean; PD, pharmacodynamic; PK, pharmacokinetic; $t_{50\%\text{-}GIR(early)}$, time from dosing to the first time point where the GIR was greater than or equal to $GIR_{max}/2$; $t_{50\%\text{-}GIR(late)}$, time from dosing to the first time point after $t_{GIR.max}$ where the GIR was less than or equal to $GIR_{max}/2$; $t_{50\%\text{-}ins(early)}$, time from dosing to the first time point where the concentration was greater than or equal to $C_{ins.max}/2$; $t_{50\%\text{-}ins(late)}$, time from dosing to the first time point after $t_{ins.max}$ where the concentration was less than or equal to $C_{ins.max}/2$; $t_{GIR.max}$, time to maximum glucose infusion rate; $t_{1/2}$, terminal elimination half-life; $t_{ins.max}$, time to maximum observed insulin concentration.

[a]Adjusted R-square value of the regression lines was not greater than or equal to 0.7 for one subject.

[b]Median values are presented.

[c]Baseline C-peptide less than or equal to 0.5 nmol/L and postdosing C-peptide concentration increased to 1 nmol/L in one profile from Biocon's Insulin-R; baseline C-peptide greater than 0.5 nmol/L and postdosing C-peptide concentration increased by at least 100% of baseline in one profile each from Biocon's Insulin-R and Humulin-R; and increase of greater than 0.5 nmol/L in C-peptide concentration from one postbaseline sample time point to the next sample time point in one profile each from Biocon's Insulin-R and Humulin-R.

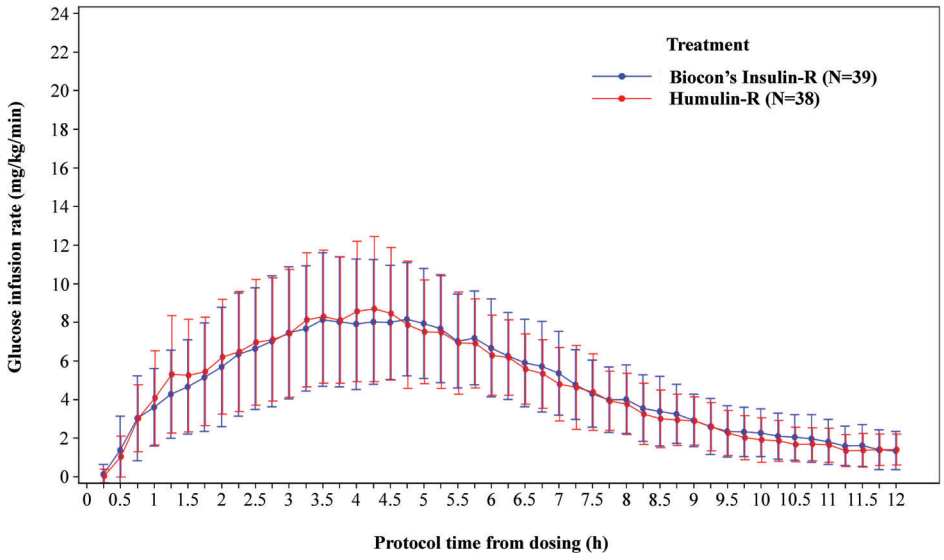

FIGURE 3 Mean GIR profiles (PP population for PD). GIR, glucose infusion rate; PD, pharmacodynamics; PP, per protocol

Mean values of the secondary PD endpoints were overall comparable for Biocon's Insulin-R and Humulin-R (Table 2). Although secondary endpoints were not expected to meet bioequivalence criteria, all PD endpoints met the criteria.

3.2.3 | Clamp performance

Mean precision variability (CV%) was observed to be less than 6% for both treatments. The mean deviation from the clamp target was 0.191 and 0.241 mg/dl after dosing with Biocon's Insulin-R and Humulin-R, respectively. Based on the fulfilment of acceptability criteria, the clamp quality was considered as good and comparable between the treatments.

3.3 | Safety

Overall, 39 treatment-emergent AEs (TEAEs; Biocon's Insulin-R: 22 AEs [12 subjects]; Humulin-R: 17 AEs [14 subjects]) were reported during the study. The most frequently reported AEs were headache (eight events in seven subjects with Biocon's Insulin-R and eight events in eight subjects with Humulin-R) and injection-site reactions (four AEs in four subjects with Biocon's Insulin-R and four AEs in three subjects with Humulin-R). No serious AEs, deaths, or discontinuations for safety/tolerability reasons occurred in the study. Most AEs (17 and 15 events with Biocon's Insulin-R and Humulin-R, respectively) were mild in intensity. Three clinically significant hypoglycaemic episodes were observed in the study: two episodes in two subjects with Biocon's Insulin-R and one episode in one subject with Humulin-R. Two documented symptomatic hypoglycaemic episodes (one each with Biocon's Insulin-R and Humulin-R) were reported. These were of moderate severity, were considered related to the treatment administration, and were resolved.

No clinically significant changes in vital signs, physical examinations, or ECGs were observed. One case of transient elevation of creatine kinase (274.9 U/L) at follow-up was considered clinically significant in the Humulin-R arm. The event was of mild severity, unlikely to be related to the treatment, and was resolved. There were no clinically significant findings in haematology, biochemistry, or urinalysis laboratory tests that were assessed throughout the study.

4 | DISCUSSION

The principal goal of this study was to demonstrate PK and PD equivalence between Biocon's proposed biosimilar (Biocon's Insulin-R) and Humulin-R in healthy subjects. The results showed a similarity in the rate and extent of absorption of the two formulations, as depicted by the $C_{ins.max}$ and $AUC_{ins.0\text{-}12h}$. The results also demonstrated a similar glucose-lowering activity of the two formulations, as indicated by a comparable $AUC_{GIR.0\text{-}12h}$ and GIR_{max}. The statistical assessment of PK/PD similarity is based on the 90% CI for the ratio of test and reference products being contained within the predefined acceptance limits of 80.00%-125.00%.[25] The results of the secondary endpoints were similar between the two treatments.

The FDA and the European Medicines Agency[25-27] recommend the use of the euglycaemic glucose clamp technique to assess the exposure and activity of biosimilar insulin products.[25-29] The automated glucose clamp technique with continuous BG measurements and minute-by-minute adaptations of GIR helps to achieve the highest clamp quality possible, while also minimizing the risk of any drug-induced hypoglycaemia.[30] The quality of the clamp performance is critical for the interpretation of the data.[26] In this study, both precision and DFT data demonstrate that the clamp performance was excellent and comparable between the two treatments. A clamp duration of 12 hours was considered as a clinically meaningful treatment duration to assess the complete PK/PD profiles of a single dose of study insulins.

The study was conducted in accordance with regulatory guidelines[25,26] in healthy subjects as they are homogenous and insulin-sensitive. No eligible female subjects were identified to be enrolled in the trial taking into consideration the inclusion and exclusion criteria, especially the requirement of the female subjects to be postmenopausal and with an upper age limit of 55 years. The study design did not exclude female subjects, per se, and, in future trials, eligible female subjects may be included. Healthy subjects exhibit lower intra-individual variability compared with subjects with T1D. Methods inherent to the clamp technique, when implemented appropriately, enable suppression of the endogenous insulin, thereby minimizing its potential interference with the PK/PD results. The following measures were implemented to this effect: (a) a dose of 0.3 IU/kg was selected to enable suppression of the endogenous insulin secretion; this dose is toward the higher end of the recommended range for insulin doses in the clamp studies; (b) a BG clamp target of 81 mg/dl ± 10% was selected to facilitate the suppression of endogenous insulin, while avoiding induction of hypoglycaemia/counter-regulatory hormones at the lowest end of the target range; (c) C-peptide levels were determined in parallel with insulin concentrations to identify the subjects whose endogenous insulin production potentially interfered with the insulin PK/PD measurements; (d) C-peptide–based correction methods[20] were employed for the primary analyses of PK/PD parameters to further rule out any impact of the endogenous insulin on PK/PD outcomes; and (e) all predose insulin concentrations were targeted to be within the reference range for fasting insulin in healthy subjects.

Both Biocon's Insulin-R and Humulin-R were generally well tolerated with no significant safety issues. Thirty-nine TEAEs were reported, and the most prevalent AE was headache, which has been commonly reported in numerous other glucose clamp studies.[31-33] Clinically significant and documented symptomatic hypoglycaemic events that occurred during the glucose clamp procedure were transient and resolved with intravenous glucose infusion. Overall, the number of hypoglycaemic events was comparable between the two treatment groups. No clinically relevant differences were observed in the safety profiles between the study drug

formulations concerning the type, frequency, and severity of AEs, local tolerability, vital signs, physical examination, ECG, and clinical laboratory results.

rHI is the standard of care in the management of diabetes.[10,34] Introduction of Biocon's Insulin-R can ensure reliable and affordable access, potentially bringing better management of diabetes and its complications, and reducing the subsequent financial burden in the United States and globally.

In conclusion, this study has demonstrated equivalence between Biocon's Insulin-R and Humulin-R when administered as a single subcutaneous injection for the primary PK and PD endpoints. The study also demonstrated equivalence for secondary PK endpoints ($AUC_{ins0\text{-}2}$, $AUC_{ins0\text{-}6}$, $AUC_{ins0\text{-}\infty}$) and all the secondary PD endpoints between the two treatments. Both insulin preparations were well tolerated and had similar safety profiles.

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CONFLICT OF INTEREST

GS, SMNM, AM, JP, SL, and SNA are employees of Biocon Biologics Ltd. LP-M has received speaker honoraria and travel grants from Eli Lilly and Company and Novo Nordisk. GS, AM, JP, SL, and SNA hold stocks in Biocon.

AUTHOR CONTRIBUTIONS

Design: SNA, SL, AM, and SMNM. Conduct/data collection: LP-M, GS, and JP. Analysis: GS, SL, AM, and SMNM. Writing and review of manuscript: all authors. All authors read and approved the final version of the manuscript. SNA, as the guarantor of this work, takes full responsibility for the work, including the study design, access to data, and the decision to submit and publish the manuscript.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. American Diabetes Association. 9. Pharmacologic approaches to glycemic treatment: standards of medical care in diabetes-2020. *Diabetes Care*. 2020;43(suppl 1): S98-S110.
2. Cefalu WT, Dawes DE, Gavlak G, et al. Insulin access and affordability working group: conclusions and recommendations. *Diabetes Care*. 2018;41(6):1299-1311.
3. World Health Organization. WHO launches first-ever insulin prequalification programme to expand access to life-saving treatment for diabetes 2019. https://www.who.int/news/item/13-11-2019-who-launches-first-ever-insulin-prequalification-programme-to-expand-access-to-life-saving-treatment-for-diabetes. Accessed December 2, 2021.
4. Ewen M, Zweekhorst M, Regeer B, Laing R. Baseline assessment of WHO's target for both availability and affordability of essential medicines to treat non-communicable diseases. *PLoS One*. 2017;12(2):e0171284.
5. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet*. 2016;387(10027):1513-1530.
6. Herkert D, Vijayakumar P, Luo J, et al. Cost-related insulin underuse among patients with diabetes. *JAMA Int Med*. 2019;179(1):112-114.
7. Nyenwe EA, Kitabchi AE. The evolution of diabetic ketoacidosis: an update of its etiology, pathogenesis, and management. *Metabolism*. 2016;65(4):507-521.
8. World Health Organisation. WHO pilot procedure for prequalification of biotherapeutic products: human insulin 2020. https://www.who.int/medicines/regulation/prequalification/01_Pilot_PQ_procedure_insulin_Feb2020.pdf. Accessed December 2, 2021.
9. Lipska KJ, Parker MM, Moffet HH, Huang ES, Karter AJ. Association of initiation of basal insulin analogs vs neutral protamine Hagedorn insulin with hypoglycemia-related emergency department visits or hospital admissions and with glycemic control in patients with type 2 diabetes. *JAMA*. 2018;320(1):53-62.
10. World Health Organization. Guidelines on second- and third-line medicines and type of insulin for the control of blood glucose levels in non-pregnant adults with diabetes mellitus. World Health Organization 2018. https://apps.who.int/iris/bitstream/handle/10665/272433/9789241550284-eng.pdf?sequence=1&isAllowed=y. Accessed December 2, 2021.
11. Luo J, Khan NF, Manetti T, et al. Implementation of a health plan program for switching from analogue to human insulin and glycemic control among Medicare beneficiaries with type 2 diabetes. *JAMA*. 2019;321(4):374-384.
12. Health Action International. Comment from Health Action International to the Expert Committee on the application to include long-acting analogue insulins in the WHO Model List of Essential Medicines for type 1 diabetes 2019. https://www.who.int/selection_medicines/committees/expert/22/applications/s18.5_insulin_analogues_HAI.pdf. Accessed December 2, 2021.
13. LaMattina J. Human insulin is available for as little as $24.88 - but it's not for everyone. Forbes Magazine 2021. https://www.forbes.com/sites/johnlamattina/2020/01/30/human-insulin-is-available-for-as-little-as-2488but-its-not-for-everyone/?sh=6ae2e46e5c48. Accessed December 2, 2021.
14. IDF Diabetes Atlas. https://diabetesatlas.org/. Accessed December 2, 2021.
15. Endocrine Society. Addressing insulin access and affordability: an Endocrine Society position statement. *J Clin Endocrinol Metab*. 2021;106(4):935-941.
16. Safder I, Khan S, Islam I-u, Kazim M. Pichia pastoris expression system: a potential candidate to express protein in industrial and biopharmaceutical domains. *Biomed Lett*. 2018;4(1):1-13.
17. *Humulin R [package insert]*. Indianapolis, IN: Eli Lilly and Company; 2019.
18. U.S. Food and Drug Administration. Clinical immunogenicity considerations for biosimilar and interchangeable insulin products: guidance

for industry 2019. https://www.fda.gov/media/133014/download. Accessed December 2, 2021.
19. Benesch C, Heise T, Klein O, Heinemann L, Arnolds S. How to assess the quality of glucose clamps? Evaluation of clamps performed with ClampArt, a novel automated clamp device. *J Diabetes Sci Technol*. 2015;9(4):792-800.
20. Owens DR. *Human Insulin: Clinical Pharmacological Studies in Normal Man*. . New York: Springer Publishing; 1986.:
21. American Diabetes Association Workgroup on Hypoglycemia. Defining and reporting hypoglycemia in diabetes: a report from the American Diabetes Association workgroup on hypoglycemia. *Diabetes Care*. 2005;28(5):1245-1249.
22. International Hypoglycaemia Study Group. Glucose concentrations of less than 3.0 mmol/L (54 mg/dL) should be reported in clinical trials: a joint position statement of the American Diabetes Association and the European Association for the study of diabetes. *Diabetes Care*. 2017;40(1):155-157.
23. Heinemann L, Weyer C, Rauhaus M, Heinrichs S, Heise T. Variability of the metabolic effect of soluble insulin and the rapid-acting insulin analog insulin aspart. *Diabetes Care*. 1998;21(11):1910-1914.
24. Ziel F, Davidson M, Harris M, Rosenberg C. The variability in the action of unmodified insulin is more dependent on changes in tissue insulin sensitivity than on insulin absorption. *Diabet Med*. 1988;5(7):662-666.
25. US Food and Drug Administration. Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product Guidance for Industry 2016. https://www.fda.gov/media/88622/download. Accessed December 2, 2021.
26. European Medicines Agency. Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues 2015. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-non-clinical-clinical-development-similar-biological-medicinal-products-containing_en-0.pdf. Accessed December 2, 2021.
27. US Department of Health and Human Services FaDA, Center for Drug Evaluation and Reserach (CDER), Center for Biologics Evaluation and Reserach (CBER). Clinical pharmacology data to support a demonstration of biosimilarity to a reference product guidance to industry 2016. https://www.fda.gov/media/88622/download. Accessed December 2, 2021.
28. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol*. 1979;237(3):E214-E223.
29. Heinemann L, Anderson JH Jr. Measurement of insulin absorption and insulin action. *Diabetes Technol Ther*. 2004;6(5):698-718.
30. Liu D, Moberg E, Kollind M, Lins PE, Adamson U, Macdonald IA. Arterial, arterialized venous, venous and capillary blood glucose measurements in normal man during hyperinsulinaemic euglycaemia and hypoglycaemia. *Diabetologia*. 1992;35(3):287-290.
31. Ocheltree S, Hompesch M, Wondmagegnehu E, Morrow L, Win K, Jacober S. Comparison of pharmacodynamic intrasubject variability of insulin lispro protamine suspension and insulin glargine in subjects with type 1 diabetes. *Eur J Endocrinol*. 2010;163(2):217-223.
32. Wolzt M, de la Peña A, Berclaz PY, Tibaldi FS, Gates JR, Muchmore DB. AIR inhaled insulin versus subcutaneous insulin: pharmacokinetics, glucodynamics, and pulmonary function in asthma. *Diabetes Care*. 2008;31(4):735-740.
33. Heise T, Donnelly C, Barve A, Aubonnet P. Pharmacokinetic and pharmacodynamic bioequivalence of proposed biosimilar MYL-1501D with US and European insulin glargine formulations in patients with type 1 diabetes mellitus. *Diabetes Obes Metab*. 2020;22(4):521-529.
34. Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of diabetes. *Diabetes Care*. 2015;38(1):140-149.

SUPPORTING INFORMATION

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