Effects of Unsuppressed Endogenous Insulin On Pharmacokinetics And/Or Pharmacodynamics of Study Insulin In The Healthy: A Retrospective Cohort Study

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Research Article

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Abstract

Background: C-peptide, a marker of endogenous insulin, should be consistently inhibited during euglycemic clamping, while an elevated postdosing C-peptide ($CP_{postdosing}$) is not an occasional phenomenon. This study aimed to describe the effects of insufficient suppression of endogenous insulin on estimates of the pharmacokinetics and pharmacodynamics of subcutaneously injected insulin.

Methods: This was a retrospective cohort study that included 33 males who underwent a manual euglycemic clamp with a subcutaneous injection of insulin aspart (IAsp). Time-profiles of whole blood glucose, human insulin, glucose infusion rate (GIR), and C-peptide were recorded. The subjects were divided into two groups at a ratio of 2:1: group A [$(CP_{postdosing})_{max} > CP_{baseline}$], group B [$(CP_{postdosing})_{max} \leq CP_{baseline}$]. The endogenous insulin was approximately equal to the measured value of human insulin or calculated from the C-peptide.

Results: The basal glucose, $CP_{baseline}$, basal human insulin, HOMA-IR, IAsp dose, and demographic statistics were all comparable between the two groups except the 'clamped' glucose. The 'clamped' glucose was 99.7±7.1% (group A) and 94.9±5.1% (group B) of baseline. After correction for 'clamped' glucose, $AUC_{GIR,0\text{-}8h}$ was higher in group A ($P < 0.05$) under comparable IAsp exposure. $AUC_{endogenous\ insulin,0\text{-}8h}$ calculated from C-peptide was different from that measured from human insulin in group A ($P < 0.05$), whereas no significant difference between these measures was observed in group B.

Conclusions: Blood glucose should be controlled below baseline to ensure the inhibition of endogenous insulin. Unsuppressed endogenous insulin may contribute to observed GIR, and the endogenous-insulin-corrected pharmacokinetics estimated by C-peptide may be inaccurate with insufficient endogenous insulin suppression.

Introduction

In euglycemic clamp studies aimed at evaluating the pharmacokinetics (PK) and pharmacodynamics (PD) of new insulin preparations in healthy subjects, 'clamped' blood glucose levels have been found to be below the subjects' basal glucose (e.g., 10% or 5 mg/dL lower than fasting glucose levels)[1–5] or to remain around baseline[6–13]. A priming dose of rapid-acting insulin followed by a basal rate of iv insulin infusion (e.g., 0.1–0.15 mU/min/kg)[14–17] can be used, but the latter has been largely abandoned since the finding that the effect of such infusion increases over time[18]. The sufficient inhibition of endogenous insulin in such studies is of paramount importance, and C-peptide should always be measured in parallel to exogenous insulin concentration to estimate the extent and consistency of the suppression of endogenous insulin throughout the experiment. During euglycemic clamp procedures that utilize an insulin analog, if PK-specific assays for the analog are lacking, the insulin to C-peptide ratio before dosing can be used to distinguish it from native human insulin in serum assays[4, 6–7, 19]. However, we noticed

that C-peptide levels can be higher than the baseline values after the administration of exogenous insulin during the clamp procedure. Other researchers have reported similar observations[8, 9].

Here, we report the time-profiles of insulin aspart (IAsp), human insulin, and C-peptide after a subcutaneous injection of IAsp during an 8-h manual euglycemic clamp procedure conducted in healthy Chinese male volunteers. The main aim was to determine the effects of elevated postdosing C-peptide on PD and/or PK (when using C-peptide to correct endogenous insulin in the absence of PK-specific assay) of the insulin analog. The second aim was to explore the consistency of the changes in C-peptide and endogenous insulin levels during the euglycemic clamp.

Methods

The present study was a retrospective cohort study of healthy males enrolled between 2016 and 2018 in a euglycemic clamp study conducted to evaluate the PK/PD of IAsp. The subjects were divided into two groups according to the relationship of postdosing C-peptide ($CP_{postdosing}$) to basal C-peptide ($CP_{baseline}$): group A, $(CP_{postdosing})_{max} > CP_{baseline}$; and group B, $(CP_{postdosing})_{max} \leq CP_{baseline}$. The ratio of the sample size of group A to that of group B was 2:1.

2.1 Participants

Healthy Chinese males between 18 and 45 years of age with a body mass index (BMI) between 18 and 24 kg/m^2 were considered eligible. All participants were nonsmokers and without a family history of diabetes mellitus or hypertension. Their blood glucose concentrations were within a normal range (fasting glucose level<6.1 mmol/L, 2-h 75-g OGTT<7.8 mmol/L and HbA1c<6.1%/43.1 mmol/mol), and no abnormalities were found in ECG, a complete blood count and urinalysis, or liver and renal function.

The trial procedures were carried out in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. All participants provided informed consent, and the study was approved by the ethics committee of West China Hospital of Sichuan University.

2.2 Clamp procedure

From 3 days before the dosing day on, participants were instructed to abstain from drinking alcohol, smoking tobacco, engaging in exercise and ingesting caffeine. Participants came to the research center at approximately 18:00 the day before drug administration to ensure an overnight fasting condition of 10–12 h the next morning. Throughout the clamp, the subjects remained fasting and in a supine position. A 20-gauge polyethylene cannula was inserted into an antecubital vein for infusion of 20% dextrose, and a second 18-gauge catheter was inserted retrogradely into a wrist vein on the dorsum of the hand to draw blood. The hand for blood drawing was maintained continuously in a heated blanket at 55–65°C, allowing sampling of arterialized venous blood. After recording the basal blood glucose level (defined as the mean of the glucose measurement at -30, -20, and − 10 min), the subjects received a 0.2 IU/kg dose of NovoRapid (Novo Nordisk, Denmark) by s.c. injection into a lifted abdominal skin fold. Blood samples

were obtained at bedside for immediate determination of whole blood glucose concentrations every 5 min from 0 to 240 min and every 10 min from 240 to 480 min. During the manual euglycemic clamp procedure, the glucose infusion rate (GIR) was adjusted based on the obtained glucose measurements to maintain blood glucose around or 0.28 mmol/L below baseline. A 4-mL blood sample was collected at each of the following points for analysis of C-peptide, human insulin and IAsp levels: -30, 0 (before dosing), 10, 20, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, 420, and 480 min. The baselines for each of C-peptide, human insulin, and IAsp were defined as the mean of the − 30 min and 0 min (before dosing) measurements.

2.3 Bioanalytical methods

Whole blood glucose concentrations were tested with a glucose analyzer (Biosen C_line GP+, Neckar Healthcare, Co., Ltd., Magdeburg, Germany) using an automated glucose oxidase technique. Human insulin levels were determined using an ultrasensitive enzyme-linked immunosorbent assay (ELISA; Cat. No. 10-1132-01; Mercodia, Uppsala, Sweden) with monoclonal antibodies that had a cross-reactivity of less than 0.006% with IAsp. C-peptide levels were analyzed using an ELISA (Cat. No. 80-CPTHU-E01.1; ALPCO, Salem, NH) method, and IAsp concentrations were assessed by means of an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method[20–21] at Covance Laboratories in Shanghai that had no detectable cross-reactivity with human insulin. The ranges of quantification were 15–850 ng/L for human insulin, 6.7–1000 ng/L for C-peptide, and 200–1000 ng/L (0.2–10 ng/mL) for IAsp. Values of IAsp below the lower limit of quantification were set to zero.

2.4 Sample sizes and statistical methods

The sample sizes of 22 for group A and 11 for group B yielded 91% power to detect a difference of 489.0 mg/kg in the area under the curve (AUC) of glucose infusion during the clamp ($AUC_{GIR,\,0-8\,h}$) with known group standard deviations of 551.0 and 306.0, respectively, and a type I error of 5%. The time profiles of GIR and blood glucose were recorded during each clamp for individuals following the administration of IAsp. The GIR-time profiles were used to calculate $AUC_{GIR,\,0-8\,h}$, maximum GIR (GIR_{max}), and time of GIR_{max} ($tGIR_{max}$) as the PD parameters. The PK parameters included maximum plasma concentration of IAsp ($C_{IAsp,\,max}$), time of $C_{IAsp,\,max}$ ($T_{IAsp,\,max}$), and the AUC of IAsp concentration from time zero to 8 h ($AUC_{IAsp,0-8h}$). The parameters mentioned above were calculated with PKsolver (version 2.0)[22]. HOMA-IR was calculated as fasting glucose (mmol/L)×fasting insulin (mU/L)/22.5[23]. The endogenous insulin predicted by C-peptide was determined based on Owen's method[19] using the following equation: [endogenous insulin] = *F*×C-peptide, where *F* was the average of the ratio of insulin to C-peptide at baseline. The clamp statistics included the coefficient of variance in blood glucose (CVBG), basal and 'clamped' glucose, and the AUC of glucose excursion above and below the baseline.

The results were expressed as the mean ± SD or median (25% percentile, 75% percentile) for normally distributed and nonnormally distributed data, respectively. Normality was examined with Q-Q plots. Unpaired Student's t-test or the Mann-Whitney U test was used to assess differences between groups A and B. Paired Student's t-test was used to detect differences between the two methods for predicting

endogenous insulin secretion, i.e., the C-peptide and the human insulin methods. Spearman rank correlation analysis was used to determine the relationship between CVBG and the highest rate of increase of C-peptide from baseline after injection of NovoRapid. Comparison of proportion of cases whose overall ‘clamped’ glucose was below baseline was evaluated by Fisher’s exact method. Since the two groups significantly differed in ‘clamped’ glucose (P<0.05, Table. 1), an analysis of variance of $AUC_{GIR,0-8h}$ using ‘clamped’ glucose as a covariate was conducted. A significance level of 5% (two-sided) was used. All the data were analyzed by SPSS 22.0 or GraphPad Prism 8.4.2 software.

Results

3.1 Demographic and disposition data

Thirty-three healthy male volunteers (22 in group A and 11 in group B) undergoing a manual euglycemic clamp study were enrolled in this study. The demographics of the subjects are presented in Table 1. No significant difference was detected in age, weight, height, BMI, the dose of IAsp, or HOMA-IR between groups A and B. There was no adverse reaction in any subject after injection of IAsp, and no adverse events were observed during the clamp procedures or the follow-up period.

Table 1
Subject demographics and statistics of the euglycemic clamp

Items	Group A	Group B	*P*
Number of subjects	22	11	-
Age (year)[a]	23.7 ± 2.0	25.1 ± 2.0	0.07
Height (cm)[a]	173.1 ± 6.0	171.0 ± 4.4	0.30
Weight (kg)[a]	66.0 ± 8.4	61.6 ± 7.6	0.15
BMI (kg/m^2)[a]	21.9 ± 1.8	21.0 ± 1.8	0.16
Basal C-peptide (ng/L)[b]	923 (832,1155)	899 (803,1245)	0.74
Basal human insulin (ng/L)[b]	164 (134,205)	184 (178,275)	0.25
Basal glucose (mmol/L)[a]	4.48 ± 0.34	4.54 ± 0.26	0.63
Clamped glucose (mmol/L)[a]	4.46 ± 0.26	4.27 ± 0.25	<0.01
Proportion of euglycemic clamps whose overall 'clamped' BG lower than baseline (%)	22.73	90.91	<0.01
CVBG (%)[a]	4.31 ± 0.99	4.26 ± 1.01	0.90
HOMA-IR (mmol/L×mU/L)[a]	0.80 ± 0.29	0.97 ± 0.34	0.16
IAsp dosage (IU)[a]	13.1 ± 1.75	12.4 ± 1.36	0.21
BMI, body mass index; CVBG, coefficient of variation of blood glucose; HOMA-IR, homeostasis model assessment of insulin resistance.			
[a] Mean ± SD			
[b]Median (25% Percentile, 75% Percentile)			

3.2 Clamp statistics in the two groups

As shown in Table 1, basal blood glucose and CVBG were comparable between groups A and B. The measured blood glucose during the clamp was higher in group A than in group B (4.46 ± 0.26 *vs.* 4.27 ± 0.25 mmol/L, $P<0.01$). The overall 'clamped' glucose concentrations in groups A and B were 99.7 ± 7.1% and 94.9 ± 5.1%, respectively, of baseline ($P<0.01$; Fig. 1A). The median AUCs of glucose excursion above baseline were 496 and 86.6 %×min in groups A and B, respectively. The median AUCs of excursion under baseline were 843 and 2460 %×min in groups A and B, respectively. As a result, nearly 37% (496/1339) of glucose excursions were above baseline in group A, whereas fewer than 4% (86.6/2546.6) of glucose excursions were above baseline in group B.

3.3 Pharmacokinetic and pharmacodynamic data of IAsp

Regarding the PD parameters (Fig. 1B), there were no differences in GIR_{max} (8.46 ± 2.43 *vs.* 7.90 ± 1.68 mg/kg/min, $P = 0.50$) and $tGIR_{max}$ (111 ± 40.6 *vs.* 113 ± 33.9 min, $P = 0.92$) between the groups, whereas $AUC_{GIR,0-8h}$ was slightly higher in group A than that in group B (1815 ± 551 *vs.* 1327 ± 306 mg/kg, $P<0.05$). After correction for the overall 'clamped' glucose, $AUC_{GIR,0-8h}$ remained higher in group A than in group B (1789 ± 107 *vs.* 1380 ± 157 mg/kg, $P = 0.048$). $AUC_{GIR,\ 0.5-2h}$ after correction for 'clamped' glucose from 0.5 to 2 h was 596 ± 38 and 520 ± 56 mg/kg in groups A and B, respectively. Regarding the PK parameters (Fig. 1C), $C_{IAsp,\ max}$ (4.72 ± 1.46 *vs.* 5.53 ± 1.61 ng/mL, $P = 0.16$), $T_{IAsp,\ max}$ (44.8 ± 11.0 *vs.* 47.3 ± 11.0 min, $P = 0.54$), and $AUC_{IAsp,0-8h}$ (527 ± 97.1 *vs.* 543 ± 97.2 ng/mL×min, $P = 0.66$) were comparable between groups A and B.

3.4 C-peptide and human insulin levels

Neither C-peptide level nor human insulin level at baseline significantly differed between the two groups (Table 1). The C-peptide levels after dosing are shown in Fig. 1D. Endogenous insulin secretion predicted by C-peptide and human insulin are shown in Fig. 2A&2B. The AUC of endogenous insulin predicted by C-peptide from 0 to 8 h was 80.6 ± 24.2 and 54.0 ± 21.1 ng/mL×min in groups A and B, respectively, and that predicted by human insulin was 91.0 ± 28.4 and 63.0 ± 27.3 ng/mL×min in groups A and B, respectively. The AUC_{0-8h} of endogenous insulin predicted by C-peptide was different from that predicted by human insulin ($P = 0.033$) in group A, whereas no significant difference between the methods was detected in group B ($P = 0.14$).

Of the 561 pairs of simultaneous serum C-peptide and human insulin measurements collected after dosing, 316 had values that were both equal to or lower than their corresponding baselines, 138 had values that both higher than baseline, and the remaining had values that were inconsistent with each other (Table 2). Therefore, in 85.2% (138/162) of cases where C-peptide level was elevated, human insulin was also increased, and in 79.2% (316/399) of cases where C-peptide level was inhibited, human insulin was also suppressed.

Table 2
Frequencies of different patterns of baseline-postdosing relationships of human insulin and C-peptide levels

Items	$CP_{postdosing} > CP_{baseline}$	$CP_{postdosing} \leq CP_{baseline}$	Total
$HI_{after\ dosing} > HI_{baseline}$	138	83	221
$HI_{after\ dosing} \leq HI_{baseline}$	24	316	340
Total	162	399	561
HI: human insulin; CP: C-peptide; $CP_{postdosing}$: C-peptide after dosing; $CP_{baseline}$: basal C-peptide			

3.5 Effects of blood glucose fluctuation on the levels of C-peptide and human insulin

The relationship between CVBG and the probability of C-peptide levels being above baseline during the euglycemic clamp is shown in Fig. 2C. All $CP_{postdosing}$ values were below baseline when CVBG was less than 2%. Interestingly, as CVBG increased, the probability of $CP_{postdosing}$ being higher than baseline increased, and there was a positive correlation between the two measures ($r = 0.51$, $P = 0.012$). The relationship between the AUC of clamped blood glucose higher than the basal blood glucose per hour and the AUC of human insulin higher than baseline per hour is shown in Fig. 2D. As the AUC of blood glucose above baseline decreased, the AUC of human insulin higher than baseline also decreased, with the two parameters exhibiting the same trend.

Discussion

The euglycemic glucose clamp technique has been regarded as the best available method for the assessment of PK/PD values of study insulin and its analogs[24]. Sufficient suppression of endogenous insulin secretion in such assessment is of considerable importance as it affects the precision of PK/PD assessments of new insulin preparations in the healthy. C-peptide, cleaved from the proinsulin molecule in islet cells, is released into the circulation in amounts equimolar to insulin, and the hepatic extraction of C-peptide is negligible[25]. Therefore, C-peptide is usually measured in parallel to insulin concentrations during euglycemic clamp studies to evaluate whether endogenous insulin secretion is inhibited. The half-life of C-peptide[26] is longer than that of human insulin such that it cannot correct for rapid changes due to poor clamp technique[27]. In the present study, human insulin was measured by a reliable ELISA method with a cross-reactivity of less than 0.006% with IAsp, allowing the interference of IAsp in the analysis of human insulin to be ruled out. Therefore, the endogenous insulin represented by human insulin might be more similar to the true situation. We considered human insulin to be roughly equivalent to endogenous insulin secretion. The study revealed that the method using C-peptide to predict endogenous insulin secretion had a sensitivity of 85.2% and a specificity of 79.2%. Increased C-peptide level can thus be regarded as a marker of insufficient inhibition of endogenous insulin.

In general, clamp studies aimed at evaluating short-acting insulin preparations require that the CVBG not exceed 10%, whereas those aimed at evaluating long-acting insulin preparations require that it not exceed 5%[28]. The results of the present study showed that as the CVBG increased, the distance of C-peptide above baseline increased (Fig. 2C), and there was a positive correlation between the two measures. These findings indicate that large fluctuations in blood glucose may be one factor responsible for insufficient suppression of endogenous insulin secretion. However, since the overall CVBG values of group A and group B were comparable in this study, we speculate that there may be other factors associated with uninhibited endogenous insulin secretion. According to the European Medicines Agency (EMA) guidelines[29], the blood glucose is recommended to be clamped below the subject's fasting glucose in healthy volunteers, and the blood glucose should be controlled to within ± 10% of the target value. Other

researchers have reported that blood glucose could be clamped at the subject's own basal level[6–13]. Based on the AUCs of glucose excursion in our study, nearly 37% of excursions were above baseline when the ‘clamped’ glucose was maintained around baseline (group A), whereas fewer than 4% of glucose excursions were above baseline when the ‘clamped’ glucose was approximately 5% below baseline (group B). ‘Clamped’ glucose around baseline increased the possibility of over-baseline glucose excursion, which might stimulate endogenous insulin secretion.

Insufficient information is available to evaluate the accuracy of insulin PK/PD data obtained in the clamp in the context of an elevated postdosing C-peptide level. Some researchers have suggested if the C-peptide level is increased by > 200 pmol/L from baseline after dosing, the data are not suitable for analysis[30]. One possible reason for the difference in $AUC_{GIR,\,0-8h}$ after correction for ‘clamped’ glucose between the two groups in this study is the difference in C-peptide level between the two groups. Another possible explanation is the unequal extent of suppression of hepatic glucose production (HGP) between the groups due to the absence of a continuously high blood insulin level. Much stronger, almost complete, suppression of HGP has been observed at serum insulin levels higher than 40 uU/mL[28]. We calculated the AUC of GIR for the period from 0.5 to 2 h, when insulin level was high, to minimize the interference due to HGP. The $AUC_{GIR,\,0.5-2h}$ after ‘clamped’ glucose correction differed by approximately 14.6% between the two groups. This finding indicates that if a subject’s endogenous insulin secretion is not sufficiently inhibited, it will contribute to the observed GIR. Furthermore, we found that elevated postdosing C-peptide level was a marker of inappropriately excessive glucose infusion.

EMA guidelines state that the C-peptide correction method can be considered in the absence of endogenous insulin suppression or when commercially specific assays for insulin preparations are restricted[29]. The calculation is based on the consistency of the ratio between human insulin and C-peptide after dosing and the initial basal period (*F* value)[19]. In a research where specific assays for the tested insulin are lacking, the primary PK parameter, total exogenous insulin exposure, can be calculated using the following equation: [AUC_{0-t} of exogenous insulin]=[AUC_{0-t} of observed insulin]-[AUC_{0-t} of endogenous insulin]. The results of the present study showed that in the absence of endogenous insulin inhibition (group A), the value of total endogenous insulin predicted using C-peptide was significantly different from that predicted by human insulin, whereas no such significant difference was detected when postdosing C-peptide was inhibited (group B). Therefore, estimates of the total exogenous insulin exposure might be inaccurate when C-peptide is used to correct endogenous insulin in a euglycemic clamp in the absence of suppressed endogenous insulin. This finding conflicts with the EMA guidelines mentioned above.

To our knowledge, this study is the first to quantitatively evaluate the effect of unsuppressed endogenous insulin on PD and to reveal the inaccurate results of the C-peptide correction method in the absence of endogenous insulin suppression. This study only included young healthy males, which limits the extrapolation of the results to subjects with diabetes with and without some preserved insulin secretion

and to females. Further study may be needed to detect that to what extent $CP_{postdosing}$ increasing significantly affect the accuracy of the C-peptide correction method.

Conclusions

To enhance the success of manual euglycemic clamps, blood glucose levels should preferably be controlled below baseline (e.g., 5% below), and rapid, frequent fluctuations should be avoided to prevent over-baseline glucose excursions that could stimulate endogenous insulin secretion. Unsuppressed endogenous insulin may contribute to observed GIR, and insulin PK values estimated from the C-peptide correction method (when PK-specific assays are unavailable) may not be accurate in the absence of endogenous insulin suppression.

Abbreviations

CP: C-peptide; IAsp: insulin aspart; GIR: glucose infusion rate; PK: pharmacokinetics; PD: pharmacodynamics; CVBG: the coefficient of variance in blood glucose; EMA: European Medicines Agency; HGP: hepatic glucose production; HOMA-IR:homeostasis model assessment of insulin resistance.

Declarations

Ethics approval and consent to participate

All participants provided informed consent, and the study was approved by the ethics committee of West China Hospital of Sichuan University.

Consent for publication

Not applicable.

Availability of data and material

The data are available on reasonable request from the corresponding author.

Competing interests

The authors declared that have no competing interest.

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Author contributions

Y.Y. designed the study. H.L., H.Y., L.S., J.Q., J.L., H.T. performed the experiments. H.L. drafted the first version of the manuscript. H.L., Y.Y. interpreted the data, edited the paper, and all authors approved the final version of the paper.

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Figures

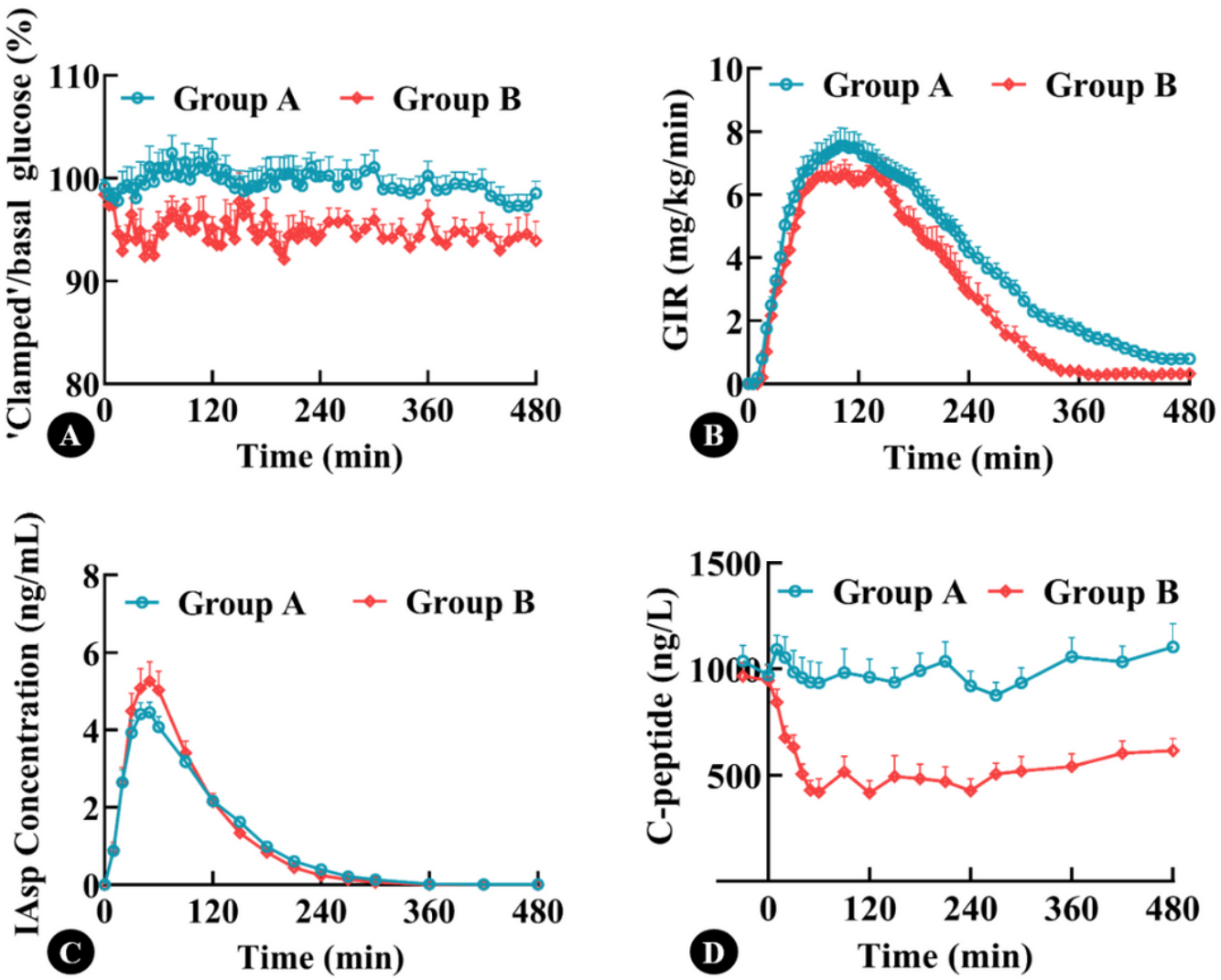

Figure 1

Time profiles of the ratio of ‘clamped’ blood glucose to basal blood glucose (A), GIR level (B), insulin aspart (IAsp) concentration (C), and C-peptide level (D) during the euglycemic clamp (Mean±SE).

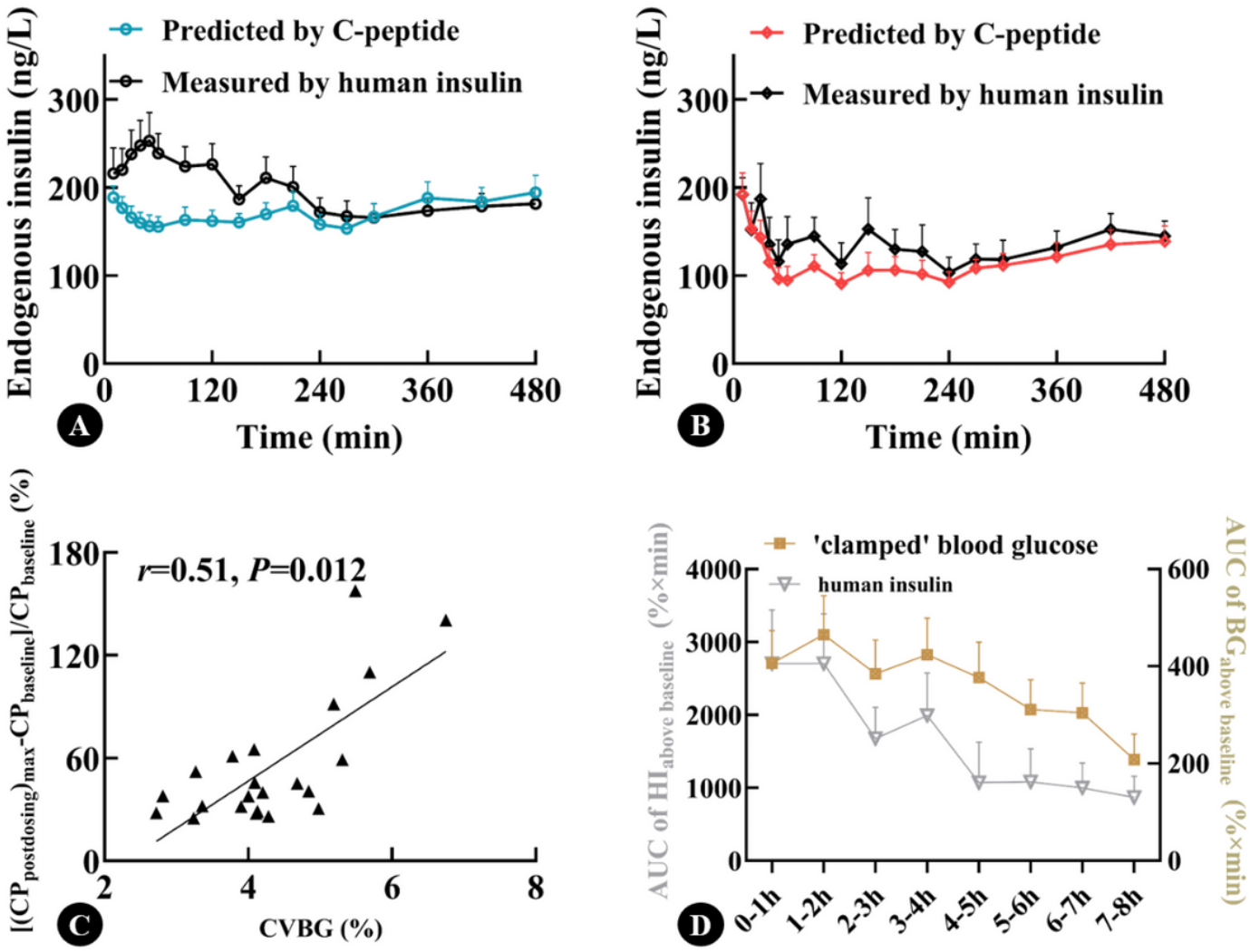

Figure 2

(A, B) Time profiles of endogenous insulin measured by human insulin or calculated by C-peptide during the euglycemic clamp in groups A and B, respectively; (C) relationship between CVBG and the highest rate of increase of C-peptide after dosing (CPpostdosing) from basal C-peptide (CPbaseline); (D) changes of the AUCs of increases of blood glucose (BG) and human insulin (HI) from their baselines per hour (Mean±SE).