ORIGINAL ARTICLE

Exploration of suitable pharmacodynamic parameters for acarbose bioequivalence evaluation: A series of clinical trials with branded acarbose

Jie Huang[1] | Wen-yu Liu[1] | Jing-jing Yu[1] | Jin-bo Yang[2] | Min Li[2] |
Chan Zou[1] | Cheng-xian Guo[1] | Xiao-yan Yang[1] | Shuang Yang[1] | Jin-lian Xie[1] |
Zhi-jun Huang[1] | Hui Chen[3] | Qi Pei[4] | Guo-ping Yang[1,4,5] 🆔

[1]Center for Clinical Pharmacology, the Third Xiangya Hospital, Central South University, Changsha, Hunan, China

[2]Centre for Drug Evaluation, National Medical Products Administration, Beijing, China

[3]Clinc Lab Center, The Third Xiangya Hospital of Central South University, China

[4]Department of Pharmacy, the Third Xiangya Hospital, Central South University, Changsha, Hunan, China

[5]Research Center for Drug Clinical Evaluation of Central South University, Changsha, Hunan, China

Correspondence
Guo-ping Yang, PhD, Center for Clinical Pharmacology, the Third Xiangya Hospital, Central South University, 138 TongZiPo Road, Changsha, Hunan, 410013, China; Research Center of Drug Clinical Evaluation of Central South University, 138 TongZiPo Road, Changsha, Hunan, 410013, China.
Email: ygp9880@126.com

Qi Pei, PhD, Department of Pharmacy, the Third Xiangya Hospital, Central South University, 138 TongZiPo Road, Changsha, Hunan, 410013, China.
Email: peiqi1028@126.com

Aims: To determine deficiencies in the Food and Drug Administration (FDA)'s guidance for assessing acarbose bioequivalence (BE) and to explore optimal pharmacodynamic (PD) metrics for better evaluation of acarbose BE.

Methods: Three clinical trials with branded acarbose were conducted in healthy subjects, including a pilot study (Study I, $n = 11$, 50 and 100 mg), a 2×2 crossover BE study (Study II, $n = 36$, 100 mg) and a 4×4 Williams study (Study III, $n = 16$, 50/100/ 150 mg). Serum glucose concentrations were measured by the glucose oxidase method.

Results: In Study I, compared with 50 mg acarbose, only 100 mg acarbose had a significantly lower $C_{max0-4h}$ than that of sucrose administration alone (7.96 ± 0.83 mmol/L vs 6.78 ± 1.02 mmol/L, $P < .05$). In Study II, the geometric mean ratios of the test formulation to the reference formulation (both formulations were the branded drug) for FDA PD metrics, $\Delta C_{max0-4h}$ and ΔAUC_{0-4h}, were 0.903 and 0.776, respectively, and the 90% confidence intervals were 67.44–120.90 and 53.65–112.13, respectively. The geometric mean ratios (confidence interval) for possible optimal evaluation PD metrics ($C_{max0-2h}$ and AUC_{0-2h}) were 1.035 (94.23–112.68) and 0.982 (89.28–107.17), respectively. Further, $C_{max0-2h}$ and AUC_{0-2h} also met the sensitivity requirements for BE evaluation in Study III.

Conclusion: Considering the mechanisms of action of acarbose, the PD effect was shown to be dose independent during the 2–4 hours postadministration of acarbose. Hence PD metrics based on the serum glucose concentration from 0 to 2 hours ($C_{max0-2h}$ and AUC_{0-2h}) are more sensitive than the FDA-recommended PD metrics for acarbose BE evaluation from 0–4 hours ($\Delta C_{max0-4h}$ and ΔAUC_{0-4h}).

1 | INTRODUCTION

As the largest and among the most respectable drug review and regulatory agencies, the Food and Drug Administration (FDA) has a tremendous influence on drug development both in the USA and throughout the world. To improve the quality and efficiency of drug development, the FDA has developed and published many guidance documents to guide and accelerate the development of new drugs, making substantial contributions to innovation in drug development and to the availability of drug use globally. Therefore, the scientificity and feasibility of the published guidance have received a great deal of attention.

Acarbose is an alternative drug to metformin, which is the first-line treatment drug for type 2 diabetes.[1-3] As a prototypical α-glucosidase inhibitor, acarbose acts on the brush border of small intestinal mucosal epithelial cells by reversibly binding to α-glucosidase, competitively inhibiting the activity of the enzyme and delaying the breakdown of starch into glucose, thereby reducing the absorption of glucose in the intestine to reduce postprandial hyperglycaemia.[4,5]

Because of the extremely low bioavailability of acarbose due to poor absorption in the gastrointestinal tract, the main site of acarbose action, bioequivalence (BE) testing of generic acarbose formulations cannot be based on a pharmacokinetic endpoint. In 2009, the FDA released a draft guidance for the BE assessment of acarbose tablets based on pharmacodynamic (PD) parameters and revised the guidance in 2017.[6] The guidance recommended that generic products should be demonstrated to be therapeutically equivalent by conducting equivalence studies either in vitro or in vivo. The prerequisite for implementation of an in vitro BE study is that the generic product has exactly the same formulation as the branded product; however, the formulation of the branded product was undisclosed. Thus, the choice for most generic pharmaceutical companies is to conduct BE studies in vivo. Based on the FDA's guidelines, a 2-way crossover design was used; generally, 75 g of sucrose was orally administered on Day 1 of each period (serum glucose baseline), followed by coadministration of 75 g sucrose/acarbose on Day 2. Blood samples were collected throughout the 4 hours after dosing for analysis of serum glucose concentration on Day 1 and Day 2. The alteration of the area under the serum curve (AUC; ΔAUC_{0-4h}) and maximum plasma concentration (C_{max}; $\Delta C_{max0-4h}$) after acarbose administration vs baseline levels are considered to be effective PD metrics. Thus far, owing to the importance of the FDA's guidance in drug research and development,

What is already known about this subject

- The guidance issued by the Food and Drug Administration (FDA) helps the development of new drugs in many countries. Unfortunately, previous studies[7-11] found that the FDA guidance for evaluating the bioequivalence (BE) of acarbose tablets appeared to be inappropriate in practice.

What this study adds

- Compared with $\Delta C_{max0-4h}$ and ΔAUC_{0-4h} proposed by the FDA, we proved that $C_{max0-2h}$ and AUC_{0-2h} exhibited superior applicability and sensitivity in the evaluation of acarbose BE in healthy subjects. $C_{max0-2h}$ and AUC_{0-2h} could be superior pharmacodynamic metrics for evaluating acarbose BE in the future.

multiple countries have carried out acarbose BE studies with reference to these guidelines.[7-11] However, both of these parameters are unsuitable for BE testing mainly because of the negative values of ΔAUC_{0-4h} and $\Delta C_{max0-4h}$ during data processing, and the proportion of negative values can reach up to 30%. To guide BE studies, several new PD metrics have been proposed by different groups,[7-11] but the applicability, sensitivity and scientificity of these new PD metrics still need further confirmation because the effects of the formulation on acarbose BE cannot be ruled out. Therefore, an exploration of scientific and reasonable PD metrics for evaluating the BE of acarbose tablets in vivo is necessary and highly sought after.

Using the branded drug, we conducted 3 studies, including a pilot study exploring the minimum appropriate dosage in 11 healthy volunteers at 2 doses of acarbose (50 and 100 mg), a 2×2 crossover BE study in 36 healthy volunteers at a single dose (100 mg), and a 4×4 Williams study in 16 healthy volunteers at 3 doses (50, 100 and 100 mg). The aim was to determine whether the FDA's guidance for assessing acarbose BE is appropriate and identify what the possible deficiencies are. We also attempted to explore optimal PD parameters by thorough analysis of the mechanism of action of acarbose together with related clinical data for better evaluation of acarbose BE in the future (Table 1).

TABLE 1 The objective and design of each study

	Study I (n = 12)	Study II (n = 36)	Study III (n = 16)
Objective	Preliminary dose exploration	PD metrics exploration for BE evaluation	Dose–response relationship and sensitivity of PD metrics
Study design	Randomized, 2×2 crossover design	Randomized, 2×2 crossover design	Randomized, 4×4 Williams design
Dose administration	Day 0 (baseline)	Day 1 (75 g of sucrose)	Day 1 (75 g of sucrose)
	Day 1 (75 g of sucrose)		Day 2 (75 g of sucrose
	Day2(75 g of sucrose	Day2(75 g of sucrose	+50 mg (A) or 100 mg (B_3) or 100 mg (B_4) or 150 mg (C) acarbose
	+50 mg (A) or 100 mg (B) acarbose	+100 mg (B_1) or 100 mg (B_2) acarbose	
Sequence	AB, BA	B_1B_2, B_2B_1	AB_3CB_4, B_3B_4AC, B_4CB_3A, CAB_4B_3
Wash-out day	7 wash-out days	7 wash-out days	7 wash-out days
Sample collection times	0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4 h	0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4 h	0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4 h

Note: The letters A, B and C represent treatments A, B and C are acarbose doses of 50, 100 and 150 mg, respectively; the numbers after the same letter indicate the replicate treatments with same acarbose dose. The sample size mentioned in the table was the scheduled sample size.

2 | METHODS

2.1 | Study design

Three independent studies were included: Study I was a pilot study using a 2×2 crossover design, exploring the lowest significantly effective dose of acarbose tablets in a healthy Chinese population and providing a theoretical basis for dose optimization in subsequent studies. Study II was an applicability 2×2 crossover BE study using the same branded acarbose, assessing the current PD metrics for BE evaluation of acarbose tablets in healthy Chinese volunteers and defining which PD metrics recommended by the FDA apply to the Chinese population. Study III was a dose–response relationship analysis study using a 4×4 Williams design to evaluate the sensitivity of the PD metrics for acarbose tablets, verify the sensitivity of the PD metrics and assess whether a dose–response relationship exists. The detailed design for each study is elaborated in Table 2.

For all the studies, eligible subjects were selected from healthy Chinese male or female volunteers aged ≥18 years with a body weight ≥ 50 kg and body mass index 19–26 kg/m². A full medical examination was performed on all subjects including a physical examination, medical history, biochemical tests and electrocardiogram. An oral glucose tolerance test was also performed to ensure normal oral glucose tolerance. Subjects were admitted to the clinical trial centre on the day before the first treatment for each period. Subjects maintained alimentary abstinence for over 10 hours prior to each treatment and continued for 4 hours after dosing. Diet and exercise were strictly controlled, and any excessive exertion or lying supine for long periods were not allowed.

We assure that the work described has been carried out in accordance with The Declaration of Helsinki. The study was approved by the medical ethics committee of the Third Xiangya Hospital of Central South University, and the ethical approval number was R17005.

2.2 | Study drug

Acarbose tablets: Bayer; main components: acarbose; specifications: 50 mg per tablet; batch number: BJ34593; usage: oral; dose: a single dose according to the protocol.

2.3 | Sample processing and detection

After the blood sample was collected, it was mixed 5 or 6 times. After coagulation, the blood was centrifuged at 2301 g at 4°C for

TABLE 2 Characteristics of healthy volunteers in Studies I, II and III

	Study I (n = 11)	Study II (n = 36)	Study III (n = 16)
Sex (male/female)	11/0	36/0	8/8
Age (y)	24 ± 3	25 ± 5	26 ± 6
Height (m)	1.71 ± 0.07	1.71 ± 0.06	1.63 ± 0.05
Weight (kg)	63.5 ± 5.0	64.3 ± 7.4	57.0 ± 7.9
BMI (kg/m²)	21.7 ± 2.0	22.0 ± 2.2	21.3 ± 2.0

Note: Values of age, height, weight and body mass index (BMI) are the mean ± standard deviation. Twelve subjects participated in Study I, of whom 11 completed the study (1 individual withdrew from the study due to personal reasons was unrelated to the study); 36 subjects participated in and completed Study II; and 16 subjects participated in and completed Study III. The sample size mentioned in the table was the actual sample size.

10 minutes, and the upper serum layer was transferred to a new tube and stored at 2 to 8°C for use. The backup serum tubes were pre-frozen, stored at −20 ± 5°C and transferred to −80°C at the end of each cycle. The serum samples were sent to the clinical laboratory of the Third Xiangya Hospital of Central South University for analysis by the glucose oxidase method after the end of the daily testing, and the backup serum samples, if needed, were measured within 48 hours.

The serum glucose concentration in this research was measured by the glucose oxidase method using a Hitachi 7170 automatic analyser (Hitachi Co., Tokyo, Japan) in central laboratory of the Third Xiangya Hospital of Central South University, according to the standard laboratory manual for glucose determination. All results of glucose concentration were obtained with the same automatic analyser. Quality control was performed on the automatic analyser at the beginning and the end of the glucose testing using the manufacturer's control solution according to the manufacturer's instructions to ensure ongoing accuracy. Two levels of control (known normal and abnormal glucose values) were run to monitor the validity of the reaction. The precision and accuracy of quality control should state a coefficient of variation <5%.

2.4 | Statistical analysis

2.4.1 | BE candidate PD metrics calculation

All candidate BE PD metrics were analysed using R software (version number: 3.3.2). In our research, the $\Delta C_{max0-4h}$ was the maximum difference between the baseline glucose profile from 0 to 4 hours determined on the day prior to drug treatment (Day 1) and the glucose profile from 0 to 4 hours determined on the day of drug treatment (Day 2), $\Delta C_{max0-4h} = C_{max0-4h, \, Day1} - C_{max0-4h, \, Day2}$; the ΔAUC_{0-4h} was the difference in postprandial area under the curve from 0 to 4 hours (AUC_{0-4h}) for blood glucose following sucrose load without (Day 1) and with acarbose (Day 2), $\Delta AUC_{0-4h} = AUC_{0-4h, \, Day1} - AUC_{0-4h, \, Day2}$. AUC_{0-2h} and $C_{max0-2h}$: the AUC and C_{max} of the glucose profile after each time point minus the baseline glucose concentration (0 h) on the day of drug treatment (Day 2). The other 14 PD metrics are detailed in the supplement.

2.4.2 | BE evaluation

The BE evaluation was performed using Phoenix WinNonlin software (version number: 6.1) in Study II for BE parameter exploration and Study III for BE parameter sensitivity. When the 90% confidence interval of the ratio of the geometric mean of the PD metrics was between 80.00 and 125.00%, the 2 sets of parameters were considered equivalent. The data from Study II were used to screen for candidate PD metrics that met the BE criteria. The sensitivity of each candidate was evaluated by equivalence testing of parameters between different dose groups in Study III (e.g. 50 mg [A] vs 100 mg [B_3]; 50 mg [A] vs 100 mg [B_4]; 50 mg [A] vs 150 mg [C]; 100 mg [B_3]

vs 150 mg[C]; and 100 mg [B_4] vs 150 mg [C]). Note that, according to actual conditions, negative concentrations were considered to be zero when calculating the 2 PD metrics $C_{max0-2h}$ and AUC_{0-2h}. For the 2 FDA-recommended parameters, $\Delta C_{max0-4h}$ and ΔAUC_{0-4h}, negative values were not included in the BE analysis.

2.4.3 | Dose–effect relationship of PD metrics

For a PD endpoint BE study, the FDA recommends that the dose should be on the linear portion of the PD dose–response curve.[12] Therefore, the linear dose–response relationship of PD metrics was evaluated in R by using the lm() function.

2.5 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.

## 3 | RESULTS

3.1 | Study population

Twelve subjects participated in Study I, of whom 11 completed the study (1 individual withdrew from the study due to personal reasons unrelated to the study); 36 subjects participated in and completed Study II; and 16 subjects participated in and completed Study III. The demographic characteristics of subjects are detailed in Table 2. Subjects who completed the study per protocol were included in the statistical analysis.

3.2 | Postprandial high-sugar model and acarbose hypoglycaemic effect in the human body

In the pilot study, we initially explored the effective dose of acarbose tablets. The $\Delta C_{max0-4h}$ of coadminstration of 50 mg of acarbose/sucrose was (0.42 ± 1.13) mmol/L, and no significant difference in $\Delta C_{max0-4h}$ was found between subjects receiving acarbose tablets vs control at a dose of 50 mg ($P > .05$). However, the $\Delta C_{max0-4h}$ of 100 mg acarbose was (1.17 ± 0.79) mmol/L, which was much higher than the $\Delta C_{max0-4h}$ of 50 mg (Figure 1). Therefore, 100 mg of acarbose showed a significant hypoglycaemic effect compared to 50 mg and should subsequently be used as the optimal dose.

In Study I, the profiles of serum glucose levels from 0 to 4 hours on Day 0 without administration of sucrose or acarbose were essentially the same, with no significant differences between the 2 periods ($P > .05$), indicating that healthy volunteers had fewer fluctuations in serum glucose during fasting (Figure 1). The curve of serum glucose

FIGURE 1 Serum glucose concentration–time curve of the administration of 75 g sucrose in the absence or presence of acarbose. Serum glucose concentration are represented as mean ± standard deviation and shown with the error bars. Jittering were used to avoid overlapping of the points and whiskers. Day 0: no administration of sucrose or acarbose; day 1: administration of sucrose (75 g); day 2: sucrose in addition of acarbose. The treatments in terms of A, B (B_1, B_2) and C represent that the investigated dose acarbose in each treatment arm was 50, 100 and 150 mg, respectively, and B_1 and B_2 arm indicate the replicate treatments with same acarbose dose. (A, B) Serum glucose profiles receiving treatments A and B in Study I, which was a preliminary dose-exploration study using a 2×2 crossover design (AB and BA). (C) Serum glucose concentration–time curves receiving the treatment B_1 and B_2 in study II for the purpose of pharmacodynamic metric exploration with a 2×2 crossover design (B_1B_2 and B_2B_1). (D) Serum glucose profiles of receiving acarbose from 50 mg to 150 mg in study III, which adopted a 4×4 Williams design (AB_3CB_4, B_3B_4AC, B_4CB_3A and CAB_4B_3). To show the dose–response relationship of acarbose, 2 groups (B_3, B_4) were combined in (D)
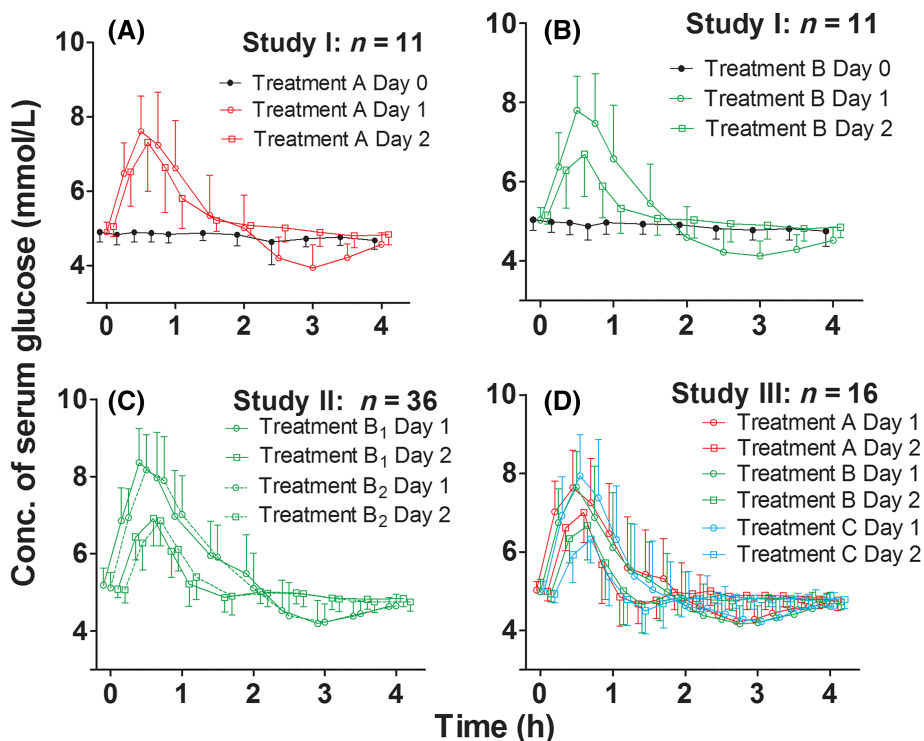
concentration over time after sucrose administration alone was revealed to be an approximate sinusoid. During the 2 hours postdosing, serum glucose levels rapidly rose to the highest level, followed by a continuous decrease to even lower than baseline, but finally returned to the initial baseline level between 2 and 4 hours (Figure 1). In contrast, the extent of serum glucose reduction was less and was almost stable near the baseline level in the 2–4 hours after coadministration of acarbose and sucrose. Since Studies I and II were densely sampled only during the initial hour, Study III was conducted and intensively sampled the entire 4 hours period to better characterize the curve, and the curves for these 3 studies demonstrated similar features (Figure 1D).

3.3 | Defects of the current PD metrics proposed by the FDA

Guidance published by the FDA requires logarithmic transformation of ΔAUC_{0-4h} and $\Delta C_{max0-4h}$ before performing BE analysis, but we found that 11.1% of the ΔAUC_{0-4h} values were negative and thus impossible to convert logarithmically. We evaluated the applicability of the 2 FDA PD metrics ($\Delta C_{max0-4h}$ and ΔAUC_{0-4h}) with B_1 as the

reference formulation and B_2 as the test formulation (both B_1 and B_2 were the branded product); both $\Delta C_{max0-4h}$ and ΔAUC_{0-4h} failed to meet the BE criteria (as shown in Table 3). In addition, Study III (the dose–effect relationship of acarbose) showed that the concentration–time curves from 2 to 4 hours nearly overlapped after administration of 50, 100 or 150 mg acarbose, and the effect of acarbose is not dose dependent from 2 to 4 hours. Further, ΔAUC_{0-4h} recommended by the FDA[11] has not significantly different between different doses of acarbose ($P = .588$, Figure 2). Therefore, the PD metrics proposed by the FDA are defective and unable to evaluate acarbose BE in humans.

3.4 | PD metric candidates

A total of 18 PD metrics was evaluated for applicability and sensitivity of acarbose BE study. Some PD metrics were selected according to the FDA guidance and literatures, and the remains were first proposed based on the mechanisms of action of acarbose. The applicability and sensitivity were evaluated based on the results of Study II (refer to Table S1) and Study II (refer to Table S1), respectively, and summarized in Table S2. Eleven PD metrics satisfied the criteria of applicability evaluation; however, only C_{max0_2h}, AUC_{0-2h} and ΔAUC_{0-2}

TABLE 3 Bioequivalence evaluation of 2 pharacodynamic (PD) metrics recommend by the Food and Drug Administration ($\Delta C_{max0-4h}$ and ΔAUC_{0-4h}) based on serum glucose concentrations for study II (n = 36)

	Mean ± SD				
PD metrics	B$_1$ (n = 36)	B$_2$ (n = 36)	Ratio	90% CI	CV%
$\Delta C_{max0-4h}$ (mmol/L)	1.56 ± 0.14	1.42 ± 0.15	90.30	(67.44,120.90)	82.36
ΔAUC_{0-4h} (h*mmol/L)	1.56 ± 0.23	1.35 ± 0.18	77.56	(53.65,112.13)	106.23

$\Delta C_{max0-4h}$: the maximum difference between the baseline glucose profile from 0 to 4 hours determined on the day prior to drug treatment (Day 1) and the glucose profile from 0 to 4 hours determined on the day of drug treatment (Day 2). $\Delta C_{max0-4h} = C_{max0-4h, Day1} - C_{max0-4h, Day2}$; ΔAUC_{0-4h}: the difference in postprandial area under the curve from 0 to 4 hours (AUC_{0-4h}) for blood glucose following sucrose load without (Day 1) and with acarbose (Day 2). $\Delta AUC_{0-4h} = AUC_{0-4h, Day1} - AUC_{0-4h, Day2}$.

SD, standard deviation; CI, confidence interval; CV, coefficient of variance
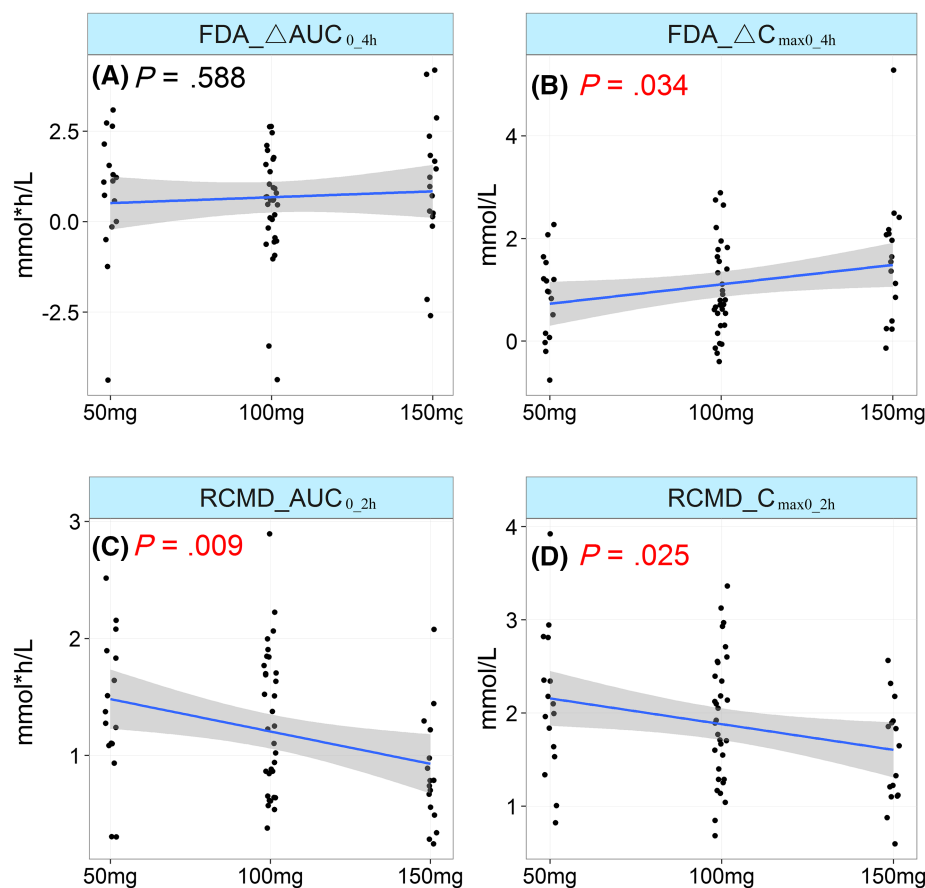
FIGURE 2 Dose–effect relationship of Food and Drug Administration pharmacodynamic (PD) metrics (ΔC_{max} and ΔAUC_{0-4}) and recommended PD metrics (C_{max0_2h} and AUC_{0-2h}). The linear dose–response relationship of PD metrics was evaluated in R using the lm() function. The black dots represent each calculated specific PD metric. The blue lines mean the linear regression lines and the shadow parts represent the 95% confidence interval around the regression lines. P < .05 means significant linear dose–response relationship of PD metrics. RCMD: recommended

satisfied the further sensitivity evaluation. Some PD metrics were calculated by ratio method (Ratio$_{Cmax}$ and RatioAUC$_{0-4}$), or without considering the baseline ($C_{max-Day2}$ and $AUC_{0-4-Day2}$). Obviously, these approaches were too insensitive to be considered as the PD metric candidates for BE of acarbose. In addition, several PD metrics have only satisfied the sensitively evaluation for some but not all groups (Av- ΔAUC, GE-Day2, GE'-Day2 and fAUC-Day2), and also cannot be considered to be appropriate PD metric candidates. Taken together, we proposed C_{max0_2h} and AUC_{0-2h} as the optimal PD metrics to evaluate the BE of acarbose. Table 4 presents that these parameters met the applicability and sensitivity requirements for BE evaluation.

4 | DISCUSSION

We conducted an optimal dose-exploration study, a BE study and a 3-dose self-controlled study in healthy volunteers using branded acarbose, and the data indicated that the FDA-recommended PD metrics ($\Delta C_{max0-4h}$ and ΔAUC_{0-4h}) for acarbose BE evaluation in vivo are defective in terms of both mechanism and practice. The mechanism of action of acarbose and observations of the activity of acarbose at 2–4 hours were basically consistent among different doses, which was indicated by the similar serum glucose response curves of the 50, 100 and 150 mg doses at the later time point after coadministration

TABLE 4   Bioequivalence and sensitivity analysis of C_{max0_2h} and AUC_{0-2h} calculated based on serum glucose concentrations

Study	Data	$C_{max0-2h}$ (mmol/L)			AUC_{0-2h} (h*mmol/L)		
		Ratio	90% CI (%)	CV%	Ratio	90% CI (%)	CV%
Study I (n = 11)	50 (A)-100 (B) [b]	150.90	(127.71–178.29)	21.49	185.88	(157.08–219.96)	21.70
Study II (n = 36)	100 (B₁)-100 (B₂) [a]	103.45	(94.23–112.68)	20.52	98.23	(89.28–107.17)	22.35
Study III (n = 16)	50 (A)-100 (B₃) [b]	107.98	(90.92–128.24)	26.29	100.60	(75.80–133.51)	41.43
Study III (n = 16)	50 (A)-100 (B₄) [b]	108.58	(89.66–131.49)	31.48	111.74	(92.91–134.37)	30.28
Study III (n = 16)	50 (A)-150 (C) [b]	135.17	(112.19–162.85)	30.60	161.61	(126.85–205.90)	40.40
Study III (n = 16)	100 (B₃)-150 (C) [b]	79.50	(65.72–96.18)	31.30	58.99	(45.56–76.38)	40.27
Study III (n = 16)	100 (B₄)-150 (C) [b]	80.33	(68.79–93.80)	25.28	67.56	(52.05–87.69)	30.54
Study III (n = 16)	100 (B₃)-100 (B₄) [a]	98.97	(81.62–120.02)	31.71	90.96	(69.87–118.41) [c]	44.32

[a]Applicability analysis;

[b]Sensitivity analysis; the 90% confidence interval (CI) of the ratios for $C_{max0-2h}$ and AUC_{0-2h} falling within 80.00–125.00% was considered indicative of equivalence for 2 formulations. $C_{max0-2h}$: the maximum glucose concentration of the glucose profile after each time point minus the baseline glucose concentration (0 h) on the day of drug treatment (Day 2); AUC_{0-2h}: the postprandial area under the curve from 0 to 2 hours for blood glucose after each time point minus the baseline glucose concentration (0 h) on the day of drug treatment (Day 2).

[c]The AUC_{0-2h} of 100 (B₃)-100 (B₄) in Study III failed for applicability analysis, this may largely be attributed to the small sample size.

CV, coefficient of variance

---

of sucrose and acarbose (e.g. 2–4 h). These results led us to focus on the other 2 parameters, $C_{max0-2h}$ and AUC_{0-2h}, based primarily on the 0–2 hours effect of acarbose, which are relatively optimal PD metrics for the evaluation of acarbose BE in vivo.

4.1 | The main reasons for the deficiencies in FDA parameters in acarbose BE evaluation

In comparison with sucrose alone, the serum glucose concentration–time curve in most of the healthy volunteers after the simultaneous administration of sucrose and acarbose tablets showed a significant reduction in serum glucose peak, along with an increase in the serum valley, presenting a trend of an initial increase followed by a dramatic decrease to the approximate baseline levels (Figure 1). This phenomenon could be partially explained by the mechanism of action of acarbose. Acarbose binding to α-glucosidase leads to a reduction in carbohydrate absorption in the upper part of the duodenum and jejunum, resulting in a decreased peak blood glucose value compared to that of sucrose administration. Thereafter, unabsorbed carbohydrates would be absorbed in the lower and middle parts of the small intestine or even the colon, and then slightly increase valley blood glucose values. In addition, the administration of acarbose attenuates a rapid increase in blood glucose concentrations in response to a corresponding decrease in insulin secretion, which may somehow prevent and control a possible overreaction of insulin in healthy subjects. This phenomenon not only is consistent with previous reports[4] but also can explain other clinical experiences, such as 3 hours after having a carbohydrate-based breakfast in the morning, a group of people have experienced extreme hunger for approximately 30 minutes.

The main reasons for the deficiencies in FDA parameters in acarbose BE evaluation in vivo may include the following. First, due to the negative feedback regulation of serum glucose itself, the serum

glucose concentration was lower 2–4 hours after sucrose administration alone than at the baseline level, whereas after coadministration of sucrose and acarbose, the serum glucose values from 2 to 4 hours were similar to the baseline levels. As a result, some negative values of ΔAUC_{0-4h} are present and cannot be analysed through logarithmic conversion. Second, as the effect of acarbose is not dose dependent from 2 to 4 hours (Figure 1D), prolongation of the blood sample collection time to 4 hours for PD analysis partially diluted the PD effect that occurs during the first 2 hours, reducing the sensitivity of the evaluation. Thus far, the European Medicines Agency and Pharmaceuticals and Medical Devices Agency have not yet released acarbose-specific BE guidance. However, in the overview of the marketing application information of acarbose, we found that some acarbose BE studies have used the ratio method of PD parameters for BE evaluation when applying to the European Medicines Agency market, and some studies have used baseline-corrected PD parameters when applying to the Pharmaceuticals and Medical Devices Agency market.

4.2 | The applicability and sensitivity of PD metrics

In fact, the findings of several recent acarbose BE studies[7-11] performed in different countries indicated challenges in applying the current FDA-recommended BE evaluation method, and some researchers attempted to explore and propose new PD metrics, such as PD metrics reflecting the fluctuation of blood glucose.[7] However, all the PD metric exploration was based on the assumption that the reference and test formulations were bioequivalent, while in fact the premise was uncertain, so the applicability, sensitivity and scientificity of these new PD metrics still need further confirmation. Considering these limitations, our research was the first to determine the appropriateness of the FDA's guidance for assessing acarbose BE using branded drugs and to apply a more systematic assessment method to explore optimal

PD metrics. The current study analysed the applicability and sensitivity of 18 PD metrics, most of which met the requirements for BE evaluation but did not show significant dose-dependent differences at doses of 50, 100 and 150 mg, making it impossible to distinguish the differences in hypoglycaemic efficacy between different treatment groups (50 vs 100 mg or 100 vs 150 mg), and indicating insufficient sensitivity of these PD metrics (Tables S1 and S2, Figure S3).

In our research, Study I was a preliminary dose exploration that showed that healthy volunteers had fewer fluctuations in serum glucose during fasting without administration of sucrose or acarbose, and the fluctuations in serum glucose after administration of 75 g sucrose alone have no significance between periods. Compared to 50 mg, 100 mg of acarbose showed a significant hypoglycaemic effect and 100 mg was selected as the optimal dose for Study II. Study II was a PD metrics exploration for BE evaluation using the repeated 100 mg branded acarbose. The dose selection of Study III was based on the data of Studies I and II, and 150 mg was added to further verify whether 100 mg had reached the dose saturation point. To characterize a more remarkable profile of serum glucose level, the PD sampling were designed denser compared to Study I and Study II. The 2 100 mg treatments designed in Study III could also verify the rationale of Study II. In Study III, some PD metrics were applicable with 100 (B_4) but not with 100 (B_3). This may be attributed that the PD metrics were calculated based on the glucose concentrations which affected by a number of factors, such as the intrinsic and extrinsic factor. Here, the Study III has a relative long clinical trial span (24 days) that could increase the intrasubject variability between periods. Since the PD effect was dose independent during the 2–4 hours period postadministration of acarbose, we speculate that the prolongation of blood sample collection time to 4 hours may partially eliminate the PD effects in the first 2 hours and result in a reduction in sensitivity. Therefore, we recommended 2 new PD metrics: $C_{max0-2h}$ and AUC_{0-2h}, which are baseline corrected PD parameters obtained from analysis of blood samples collected between 0 and 2 hours after administration of sucrose together with acarbose. Through the correction of the zero point, lowering the blood sugar value (i.e. $C_{max0-2h}$) and area under the serum curve (i.e. AUC_{0-2h}) increases the sensitivity of the parameters and shows a good dose–effect relationship. The accepted criterion for showing BE for $C_{max0-2h}$ and AUC_{0-2h} is that the 90% confidence interval of the B_1/B_2 ratio falls within 80.00–125.00%, while ensuring that all of the primary PD parameters of the generic product as well as the branded product show statistically significant differences compared to sucrose alone.

4.3 | The potential factors of individual differences in response to acarbose treatment

Individual differences in response to acarbose treatment were observed; 13% of the subjects failed to exhibit hypoglycaemic effects after administration of acarbose tablets. To our knowledge, no related literature or relevant data associated with genetic polymorphisms encoding glycosidase genes have been reported. In contrast, several groups[13–15] focused on the interaction between acarbose and the intestinal flora. Gu et al[13] found that acarbose significantly changed the relative abundance of some of the intestinal microbial genes and mOTUs in the gastrointestinal tract with regard to the improvement in fasting serum glucose, insulin secretion, C-peptide levels and insulin resistance. The efficacy of acarbose was more intensive in patients with type B diabetes than in those with type P diabetes. Hereby, we conducted a preliminary intestinal microbiological study through the analysis of faecal samples and found differential expression of 8 intestinal flora between 2 different hypoglycaemic groups (Figure S1, S2, Table S3). Therefore, we speculated that differential expression of intestinal flora among individuals may be an important factor leading to the variation in efficacy of acarbose tablets.

4.4 | Limitations

Potential limitations exist in this study. First, due to the absence of the specifications of 25-mg acarbose tablets, the dose–effect relationship of acarbose (indicating the sensitivity of the PD metrics) was specified at 3 doses, 50, 100 or 150 mg. We supposed that screening for more sensitive PD metrics with acarbose 75, 100 and 125 mg would be a good choice. Second, the negative concentration considered to be zero for calculating the 2 PD metrics ($C_{max0-2h}$ and AUC_{0-2h}) could create a certain degree of bias. However, compared with the baseline glucose concentration level (5.02 ± 0.23 mmol/L), the proportion of absolute values of negative concentrations from 0 to 2 hours (0.43 ± 0.35 mmol/L) to the baseline was approximately 8.6%. Therefore, the possible bias caused by negative concentrations could have a minor effect on the result of PD metrics. Third, in our research, there was no strict sample size calculation criteria. Study I was a pilot trial, a small sample size (n = 12) was adequate to achieve the purpose. The sample size of Study II was calculated based on the results of Study I as well as literature. Based on the purposes of Study III, a relatively small sample size could better reflect the sensitivity of PD metrics. Comprehensive consideration of the 4×4 Williams design, 16 subjects was finally selected. Additionally, as the studies were conducted only in a Chinese population, the outcome may be different between different populations.

5 | CONCLUSIONS

Considering the mechanisms of action of acarbose, the PD effect was shown to be dose independent manner during the 2–4 hours period postadministration of acarbose, so the PD metrics calculated based on the serum glucose concentration from 0 to 2 hours are more sensitively than that derived from 0–4 hours. Meanwhile, The FDA-recommended PD metrics ($\Delta C_{max0-4h}$ and ΔAUC_{0-4h}) for acarbose BE evaluation in vivo are insufficient in terms of both mechanism and practice. Similarly, the PD metrics calculated by ratio

method or without considering the baseline were too insensitive to be considered as the PD metric candidates. Collectively, $C_{max0-2h}$ and AUC_{0-2h} could be optimal PD metrics for evaluating acarbose BE in the future.

CONTRIBUTORS

The principal investigator of the three clinical trials was Dr. Guo-ping Yang. Guo-ping Yang, Jin-bo Yang and Min Li conceived and designed the study; Jie Huang, Wen-yu Liu, Jing-jing Yu, Xiao-yan Yang, Shuang Yang, Jin-lian Xie and Zhi-jun Huang implemented the clinical trials; Hui Chen and Cheng-xian Guo assessed the biological samples; Qi Pei, Jie Huang, Wen-yu Liu and Jing-jing Yu analysed and interpreted the data; Jie Huang, Wen-yu Liu and Chan Zou wrote the paper; Guo-ping Yang and Qi Pei reviewed the paper.

COMPETING INTERESTS

There are no competing interests to declare.

FULL DISCLOSURE

We confirm that the manuscript has not been previously published in any language anywhere and that it is not under simultaneous consideration by another journal.

DATA AVAILABILITY STATEMENT

The authors of this study are willing to share data. If readers are interested in the relevant data mentioned in this paper, they can send an email to ygp9880@126.com, provide the confidentiality agreement and instructions for use, and we will consider data sharing.

ORCID

Guo-ping Yang https://orcid.org/0000-0001-5930-586X

REFERENCES

1. Weng J, Ji L, Jia W, et al. Standards of care for type 2 diabetes in China. *Diabetes Metab Res Rev*. 2016;32(5):442-458.
2. Ko SH, Kim SR, Kim DJ, et al. 2011 clinical practice guidelines for type 2 diabetes in Korea. *Diabetes Metab J*. 2011;35(5):431-436.
3. Zhang JP, Wang N, Xing XY, Yang ZJ, Wang X, Yang WY. Efficacy of acarbose and metformin in newly diagnosed type 2 diabetes patients stratified by HbA1c levels. *J Diabetes*. 2016;8(4):559-567.
4. Jenkins DJ, Taylor RH, Goff DV, et al. Scope and specificity of acarbose in slowing carbohydrate absorption in man. *Diabetes*. 1981;30 (11):951-954.
5. Rosak C, Mertes G. Critical evaluation of the role of acarbose in the treatment of diabetes: patient considerations. *Diabetes Metab Syndr Obes*. 2012;5:357-367.
6. Food and Drug Administration (FDA). Guidance on Acarbose 2017. Available at https://www.accessdata.fda.gov/drugsatfda_docs/psg/Acarbose_oral%20tablet_NDA%2020482_final%2008-17.pdf. (last accessed 2 March 2019).
7. Zhang M, Yang J, Tao L, Li L, Ma P, Fawcett JP. Acarbose bioequivalence: exploration of new pharmacodynamic parameters. *AAPS J*. 2012;14(2):345-351.
8. Bayer HealthCare. Clinical Study Synopsis 2011. Available at http://trialfinder. bayerscheringpharma.de/html/pdf/15624_Study_Synopsis_CTP.pdf. (last accessed 2 March 2019).
9. Medicines and Healthcare products Regulatory Agency (MHRA). Acarbose 50 mg and 100 mg tablets 2010. Available at http://www.mhra.gov.uk/home/groups/par/documents/websiteresources/con108742.pdf. (last accessed 2 March 2019).
10. Sawai Pharmaceutical Co., Ltd. The Biological Equivalence Test Data of Acarbose Tablets 100mg 2009. Available at https://med.sawai.co.jp/file/pr15_1258.pdf. (last accessed 2 March 2019).
11. Lee S, Chung JY, Hong KS, et al. Pharmacodynamic comparison of two formulations of Acarbose 100-mg tablets. *J Clin Pharm Ther*. 2012;37(5):553-557.
12. Yu LX, Li BV (Eds). *FDA Bioequivalence Standards*. New York: Springer; 2014.
13. Gu Y, Wang X, Li J, et al. Analyses of gut microbiota and plasma bile acids enable stratification of patients for antidiabetic treatment. *Nat Commun*. 2017;8(1):1785.
14. Zhang X, Fang Z, Zhang C, et al. Effects of Acarbose on the gut microbiota of Prediabetic patients: a randomized, double-blind, controlled crossover trial. *Diabetes Ther*. 2017;8(2):293-307.
15. Su B, Liu H, Li J, et al. Acarbose treatment affects the serum levels of inflammatory cytokines and the gut content of bifidobacteria in Chinese patients with type 2 diabetes mellitus. *J Diabetes*. 2015;7(5): 729-739.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.