Bioequivalence Evaluation Between Acarbose and Metformin Fixed-Dose Combination and Corresponding Individual Components in Healthy Chinese Male and Female Subjects

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Abstract
Acarbose and metformin have been recommended both as monotherapy and add-on therapy in type 2 diabetes mellitus. A novel fixed-dose combination (FDC) of acarbose and metformin has been developed to improve compliance and patient adherence to therapy. The current study investigated the bioequivalence (BE) between acarbose/metformin FDC (50 mg/500 mg) with corresponding loose combination of individual components under fasting conditions in healthy Chinese male and female subjects, using a randomized, 2-period, 2-way crossover study design. Pharmacodynamic parameters of serum glucose ratio between treatment day and baseline (ratio of maximum concentration \([C_{\text{max}}]\), day 1/\(C_{\text{max}}\), day \(-1\) and ratio of area under the concentration-time curve \([AUC]\) from time 0 to 4 hours, day 1/\(AUC\) from time 0 to 4 hours, day \(-1\)) were used as the primary variables to evaluate BE of acarbose. Pharmacokinetic parameters \(C_{\text{max}}\), \(AUC\) from time 0 to the last data point greater than the lower limit of quantification, and \(AUC\) were used to evaluate BE of metformin. The results showed that the 90% confidence intervals of the ratios of all primary target variables including ratio of \(C_{\text{max}}\), day 1/\(C_{\text{max}}\), day \(-1\) and ratio of \(AUC\) from time 0 to 4 hours, day 1/\(AUC\) from time 0 to 4 hours, day \(-1\) for acarbose, and \(C_{\text{max}}\), \(AUC\) from time 0 to the last data point greater than the lower limit of quantification, and \(AUC\) for metformin all fell within the acceptance limits of 0.8 to 1.25. Thus, BE between 50-mg acarbose and 500-mg metformin as an FDC and loose combination was established. Furthermore, different kinds of exploratory pharmacodynamic parameters (based on either serum glucose or insulin) including several newly proposed parameters were also investigated for acarbose BE evaluation in this study, and inconsistent results were observed.

Keywords
acarbose, bioequivalence, fixed-dose combination, metformin, type 2 diabetes mellitus

Diabetes mellitus is a complex, chronic illness requiring continuous medical care. The management of type 2 diabetes mellitus (T2DM) involves various approaches such as changes in the lifestyle and use of pharmacologic interventions for maintaining glycemic control and long-term complications.¹ The initiation of met-
formin as first-line treatment in individuals with T2DM was recommended when change in lifestyle fails to achieve blood glucose target.2

Since T2DM is a progressive disease in many patients, maintenance of glycemic targets with monotherapy is often possible for only a few years, after which combination therapy is needed. This necessitates a combination therapy with drugs having complementary mechanisms of action known as fixed-dose combination (FDC) for the successful management of T2DM.3 FDCs have the potential to increase treatment adherence in patients because of reduced pill burden along with improved compliance and fewer disease management costs.5

Previous studies reported that treatment with acarbose and metformin has higher efficacy with regard to hemoglobin A1C, fasting blood glucose, and 2-hour postprandial blood glucose (PPG) and insulin levels than their monotherapy.6-9 Acarbose, an oral alpha-glucosidase inhibitor, is a complex oligosaccharide that reduces glucose absorption from the distal small intestine and lowers PPG levels in patients with T2DM10 and is associated with lower rates of the development of T2DM.11 Metformin, an insulin-sensitizing biguanide,12 lowers blood glucose levels, ameliorates insulin resistance, improves lipid profiles, prevents vascular complications, and lowers the potential for hypoglycemia.13 The estimated oral absolute bioavailability of metformin is approximately 50% to 60% in healthy subjects. This might be attributed to the fact that metformin exhibits a nonlinear relationship between the glucose-lowering effect and PK parameters with a significant inverse trend at high metformin exposure.14 From the pharmacologic perspective, when metformin monotherapy cannot achieve glycemic control, acarbose is preferred as a second antidiabetic drug due to the synergistic mechanism of actions of these 2 drugs.

As acarbose acts locally in the gastrointestinal tract with very low bioavailability (<2% of the dose), glucose-related pharmacodynamic (PD) end points rather than pharmacokinetic (PK) parameters were used in the acarbose bioequivalence (BE) evaluation. Previous studies usually used a 2-way crossover design recommended by the Food and Drug Administration, with an oral administration of 75 g of sucrose on day −1 of each period (serum glucose baseline), followed by coadministration of 75 g of sucrose/acarbose on day 1. Blood samples were collected throughout the 4 hours after dosing for analysis of serum glucose concentration on day −1 and day 1. The alteration of the area under the concentration-time curve (AUC; ΔAUC from time 0 to 4 hours [ΔAUC(0–4h)]) and maximum plasma concentration (C_{max}; ΔC_{max} from time 0 to 4 hours [ΔC_{max}(0–4h)]) after acarbose administration vs baseline levels were considered as effective PD metrics.

However, ΔAUC(0–4h) and ΔC_{max}(0–4h) were found to be unsuitable for BE testing mainly because of the negative values during data processing, with the proportion of negative values reaching up to 30%.15 So far, various studies have explored further PD parameters that could establish the BE of acarbose.16-19 A ratio approach was recommended and applied in a previous BE study.20 The exploration of scientific and reasonable PD metrics for evaluating the BE of acarbose tablets in vivo is necessary and highly desirable.

Various studies have indicated that FDCs of acarbose and metformin have similar efficacy and tolerability compared with their individual dose administration.8,9,20,21 However, the BE between the FDC and coadministration of individual drugs of acarbose and metformin in the Chinese population under fasting conditions is unknown. The objective of this study was to investigate the BE of this novel acarbose/metformin FDC (50 mg/500 mg) with corresponding loose combination (LC) of acarbose (50 mg) and metformin (500 mg) tablets, in which several PD parameters for acarbose BE evaluation were tested. Safety and tolerability of each treatment were also investigated.

Methods

Study Design and Subjects

The study protocol and informed consent were reviewed and approved by the institutional review board. The study (CTRI Number: NCT04065581) was conducted in accordance with the Declaration of Helsinki and the International Council for Harmonization guideline E6: Good Clinical Practice. All subjects provided written informed consent before study entry.

This was a single-center, randomized, nonblinded, 2-period, 2-way crossover study conducted among 24 healthy Chinese subjects at Zhongshan Hospital, Fudan University, China. Both male and nonpregnant, nonlactating female subjects aged ≥18 years, with a body mass index ≥19 to <28 kg/m², body weight ≥50 kg, hemoglobin A1C value within the normal range, fasting plasma glucose <110 mg/100 mL and 2-hour plasma glucose after glucose load test <140 mg/100 mL were considered eligible. Screening examinations were used to confirm the health of subjects, including medical interview, physical examination, vital signs, laboratory tests, and 12-lead electrocardiogram. Subjects with abnormal results of screening examinations, family history of diabetes, or history of known hypersensitivity or drug allergy were excluded.

Study Procedure

Eligible and consenting subjects were randomized into 2 groups in a ratio of 1:1 to receive study drugs on 2 treatment periods separated by a washout interval.
In each treatment period, the subjects received either treatment A, a single oral dose of acarbose/metformin FDC (50 mg/500 mg), or treatment B, oral LC dose of 50-mg acarbose and 500-mg metformin according to the randomization plan. Using a crossover method, the study treatment was given either following the sequence: Treatment A-Treatment B or Treatment B-Treatment A on each day 1. The 2 treatments were separated by a 7-day washout interval:

Group 1: Treatment A – washout – Treatment B
Group 2: Treatment B – washout – Treatment A

The complete study was divided into several sections: screening, admission, treatment period 1, washout phase, treatment period 2, and follow-up. Screening started with the subject’s signature on the informed consent form and ended with the eligibility assessment before first sucrose load. Subjects were admitted to the hospital on day −3 (admission day, 2 days before the first sucrose load test) to standardize lifestyle. There were 2 treatment periods, each of which consisted of 2 treatment days (day −1 and day 1). On each day −1, a sucrose load test was conducted to obtain baseline glucose/insulin profiles by administering 75 g of sucrose dissolved in 225 mL of water only 10 ± 1.0 minutes after intake of 240 mL of nonsparkling water. On the next day (each day 1), the study drug was administered, and the same sucrose load test was repeated 10 ± 1.0 minutes after respective study drug administration. On day −1 and day 1, serum glucose and serum insulin concentrations were determined for the next 4 hours at 15, 30, 45, 60, 90, 120, 180, and 240 minutes after sucrose load. On day 1, blood samples were also taken for metformin PK analysis immediately before drug administration, and then at 30-minute intervals for the first 4 hours after drug administration, and at 5, 6, 8, 10, 12, 15, and 24 hours after dosing. Subjects continued fasting until at least 4 hours after the sucrose load.

Further, the subjects were monitored for adverse events (AEs) and concomitant medication. All post-treatment examinations were conducted on day 2 of each treatment period, and all subjects were followed up telephonically 8 days after the last discharge from the ward. An overview of the study design is presented in Figure 1.

**Pharmacodynamic/Pharmacokinetic Analysis**

The analysis of PD and PK parameters were conducted in a blinded manner. For the determination of PD parameters of acarbose, serum glucose and serum insulin relevant parameters were compared between the treatments (FDC and LC). The primary PD parameters were ratio of maximum concentration (Cmax), day 1/Cmax,day −1(RatioCmax) and ratio of area under the concentration-time curve from time 0 to 4 hours (AUC(0-4h),day 1/AUC(0-4h),day −1(RatioAUC(0-4h))) for serum glucose between the 2 study days (day 1 and day −1). The other exploratory parameters assessed included: (1) Cmax, AUC from time 0 to 2 hours (AUC(0-2h)) and AUC(0-4h) for serum glucose and serum insulin on both day 1 and day −1; (2) predose single point baseline-corrected (bc) Cmax, AUC(0-2h),bc and AUC(0-4h),bc for serum glucose and serum insulin on both day 1 and day −1; (3) RatioCmax and RatioAUC(0-4h) for serum glucose and serum insulin of the 2 study days; (4) ΔCmax and ΔAUC(0-4h) for serum glucose and serum insulin between the 2 study days; (5) % Cmax inhibition, % AUC(0-2h) inhibition, % AUC(0-4h) inhibition for serum glucose and serum insulin between the 2 study days; (6) RatioCmax,bc, RatioAUC(0-2h),bc, and RatioAUC(0-4h),bc for serum glucose and serum insulin between the 2 study days. The various definitions of the parameters are given in Table S1.

The PK parameters of metformin were calculated from plasma concentration-time data. The primary PK parameters of metformin mainly were Cmax,
AUC\textsubscript{(0–t\textsubscript{last})}, and AUC. The bioanalysis was performed by Covance Pharmaceutical R&D (Shanghai) Co., Ltd, China. The PK parameters were calculated using the model-independent compartment-free method using the PK software Phoenix (Version 8.1; Certara, Princeton, New Jersey).

Laboratory assays were conducted in a blinded manner. Serum glucose was measured with a glucose colorimetric detection kit (Invitrogen, Carlsbad, California) with a calibration range of 9.00 mg/100 mL (lower limit of quantification [LLOQ]) to 432 mg/100 mL. A beta-D-glucose standard was provided to generate a standard curve for the assay, and all samples should be read off the standard curve. Samples were mixed with the colorimetric substrate and horseradish peroxidase and the reaction initiated by addition of glucose oxidase. The reaction was incubated at room temperature for 30 minutes. The glucose oxidase reacted with glucose to produce hydrogen peroxide, which, in the presence of horseradish peroxidase, reacted with the colorimetric substrate to convert the colorless substrate into a pink-colored product. The pink product was read at 560 nm. Serum insulin was measured with an insulin enzyme-linked immunosorbent assay kit (ALPCO, Salem, New Hampshire) with a calibration range of 7.81 pmol/L LLOQ to 2000 pmol/L. The 96-well microplate was coated with a monoclonal antibody specific for insulin. The standards, controls, and samples were added to the microplate wells with the detection antibody. The microplate was then incubated on a microplate shaker at 700 to 900 rpm. Once the first incubation was complete, the wells were washed with wash buffer and blotted dry. TMB (3,3′,5,5′-tetramethylbenzidine) substrate was added, and the microplate was incubated a second time on a microplate shaker at 700 to 900 rpm. Once the second incubation was complete, Stop Solution was added, and the optical density was measured by a spectrophotometer at 450 nm. The intensity of the color generated was directly proportional to the amount of insulin in the sample. The plasma concentration of metformin was measured by high-performance liquid chromatography–tandem mass spectrometry with a calibration range of 2.00 (LLOQ) to 2000 ng/mL. Metformin-d\textsubscript{6} was used as internal standard. Briefly, concentrations of metformin were determined after the protein precipitation using acetonitrile followed by separation by high-performance liquid chromatography–tandem mass spectrometry. Chromatography was performed on an Epic Silica column (50 × 3.2 mm, 3μ; ES Industries, West Berlin, New Jersey) at a flow rate of 1.00 mL/min using the mobile phase A: 10 mM ammonium formate:formic acid (100:0.1) solution and mobile phase B: acetonitrile:formic acid (100:1) solution. Metformin was monitored in positive mode with the transition of m/z 130.0→71.0, and metformin-d\textsubscript{6} was monitored with the transition of m/z 136.0→77.0. The analyte response (peak area) at the LLOQ in the calibration standard sample was >5 times the blank analyte response and meets the acceptance criteria for the sensitivity experiment. The interday precision (between-day coefficient of variation [CV]) of quality control samples was within 3.3%, and the interday accuracy ranged between 96.0% and 102.3%. The within-day precision (within-day CV) of quality control samples was within 3.7%, and the within-day accuracy ranged between 94.7% and 105.1%.

**Bioequivalence Assessments**

BE assessments were performed for acarbose and metformin separately. For metformin, the PK parameters C\textsubscript{max}, AUC from time 0 to the last data point greater than the LLOQ (AUC\textsubscript{(0–t\textsubscript{last})}) and AUC were chosen as primary PK parameters. For acarbose, the following study design and statistical analysis were applied: C\textsubscript{max} and AUC\textsubscript{(0–4h)} of the serum glucose profile were assumed to be log-normal distributed, and 2 serum glucose profiles were taken (one was after sucrose challenge only at day –1, and the other was after administration of both investigated drugs and sucrose at day 1) into consideration. Statistically, it was assumed that a ratio of 2 log-normal distributed variables will follow log-normal distribution as well; therefore, Ratio\textsubscript{C\textsubscript{max}} and Ratio\textsubscript{AUC(0–4h)} of serum glucose were determined as primary PD parameters for this study.

Furthermore, regarding previous PD parameters for acarbose BE evaluation, that is, the difference of C\textsubscript{max} and AUC\textsubscript{(0–4h)} between day –1 and day 1, there were internal discussions among industry, academia, and regulatory agencies due to negative value issue detected in this difference approach,\textsuperscript{15} based on which a few new parameters were proposed. As suggested in the investigations by Huang et al\textsuperscript{18} and Chen et al,\textsuperscript{19} in which C\textsubscript{max} from time 0 to 2 hours and AUC\textsubscript{(0–2h)} at day 1 after baseline correction were recommended after a comprehensive evaluation of different parameters, and where the predosing glucose concentration at day 1 was regarded as baseline, an exploratory BE assessment for acarbose was also conducted on these newly proposed PD parameters in this study as well. In addition, other kinds of PD parameters such as % C\textsubscript{max} inhibition, % AUC\textsubscript{(0–2h)} inhibition, % AUC\textsubscript{(0–4h)} inhibition, Ratio\textsubscript{C\textsubscript{max}}, Ratio\textsubscript{AUC(0–2h)}, and Ratio\textsubscript{AUC(0–4h)} for serum glucose, together with corresponding insulin-related parameters were also explored.

**Safety Assessments**

Subjects who received at least 1 dose of the study medication were included for safety evaluation. The safety assessment included physical examination of vital organs, vital signs, laboratory parameters (blood and
urine analysis), and 12-lead electrocardiograms. Information on AEs was collected throughout the study until the follow-up. AEs was considered treatment-emergent AEs (TEAEs) if the start time was at or after the time of study drug administration and no later than 48 hours after the time of study drug administration and was assigned to the corresponding treatment administered before start of this AE. The incidence of TEAE was summarized by treatment.

**Statistical Analysis**

Demographics and subject characteristics at baseline were summarized by arithmetic mean and standard deviation. The concentration-time courses of serum glucose, serum insulin, and plasma metformin were tabulated separately by treatment. Arithmetic mean and standard deviation were calculated for each of the sampling points. For the calculation of the arithmetic mean value, data below LLOQ were entered as 0. The pre-dose metformin concentration was below LLOQ and was also entered as 0. PD characteristics for glucose and insulin, and PK characteristics (time to reach C_{max} excluded) for metformin were summarized by the statistics mentioned above. Time to reach C_{max} was described using minimum, maximum, and median.

Regarding BE evaluation, the logarithmized values of primary PK/PD parameters mentioned above were analyzed using traditional analysis of variance (ANOVA) model with sequence, subject (sequence), period, and treatment as factors. When 90% confidence intervals (CIs) of the ratios (FDC/LC) of all primary PK/PD parameters derived from ANOVA model fall into BE limits (0.8-1.25), the BE of FDC vs LC was considered to be established. Other exploratory PD parameters were analyzed by the same ANOVA model as above.

All the statistical evaluations were performed using the software package SAS release 9.2 (SAS Institute Inc., Cary, North Carolina).

**Sample Size Determination**

Based on a previous study, the sample size for 20 subjects was estimated. Assuming the point estimate for Ratio_{C_{max}} of serum glucose is 1.1 and CV is 0.167, 20 subjects (10 subjects per sequence) are ensured to have at least 95% power to detect bioequivalence among various parameters between FDC and LC. Thus, a sample size of 24 subjects (12 subjects per sequence) was determined, taking into account possible dropouts.

**Results**

**Subject Demographics**

In total, 24 subjects were randomized and completed the study as planned. Twelve male and 12 female Chinese subjects were included, with a mean age of 27.1 (range, 21-33) years, a mean height of 163.5 (range, 149-188) cm, a mean weight of 60.0 (range, 51-73), and a mean body mass index of 22.5 (range, 20-26) kg/m². The demographic characteristics are described in detail in Table 1.

**PD Results**

Acarbose PD results based on arithmetic mean glucose and insulin concentrations following administration of acarbose/metformin FDC and LC are presented in Figures 2 and 3. The mean glucose concentration curves were similar following administration of both the formulations, with a mean reduction of 23.2 and 26.8 mg/100 mL, respectively, in peak concentration after study treatment. Similar reduction in mean insulin concentration curves were observed, with a reduction of about 206.7 and 186.2 pmol/L, respectively, following administration of FDC and LC (Table 2).

**Table 1. Demographics and Subject Characteristics at Baseline**

|               | FDC-LC | LC-FDC | Total |
|---------------|--------|--------|-------|
| **Sex**       |        |        |       |
| Male          | 6 (50.0) | 6 (50.0) | 12 (50.0) |
| Female        | 6 (50.0) | 6 (50.0) | 12 (50.0) |
| **Age, y**    |        |        |       |
| Male          | 28.0 ± 3.5 | 26.3 ± 3.8 | 27.1 ± 3.7 |
| Female        | 28.0 ± 3.0 | 27.0 ± 3.9 | 27.5 ± 3.4 |
| **Weight, kg**|        |        |       |
| Male          | 60.4 ± 8.4 | 59.7 ± 4.8 | 60.0 ± 6.7 |
| Female        | 62.7 ± 9.1 | 62.3 ± 4.2 | 62.5 ± 6.8 |
| **BMI, kg/m²**|        |        |       |
| Male          | 167.3 ± 7.8 | 169.1 ± 9.5 | 168.2 ± 8.3 |
| Female        | 160.3 ± 5.5 | 157.2 ± 4.6 | 158.7 ± 5.1 |

BMI, body mass index; FDC, fixed-dose combination; LC, loose combination.

Data are presented by arithmetic mean ± standard deviation or n (%).

Summary statistics reported that the arithmetic mean values of AUC(0-2h) and AUC(0-4h) of glucose were similar between FDC and LC on day 1 (182.6 mg • h/100 mL vs 178.5 mg • h/100 mL for AUC(0-2h), and 328.6 mg • h/100 mL vs 326.8 mg • h/100 mL for AUC(0-4h), respectively). Arithmetic mean values of C_{max} for glucose were also similar between 2 formulations after administration (107.9 mg/100 mL vs 102.9 mg/100 mL). There was a clear decrease in AUC(0-2h), AUC(0-4h), and C_{max} of glucose and insulin following administration of acarbose/metformin FDC and LC (Table 2).
**Figure 2.** Concentration-time curve for serum glucose concentrations following an oral sucrose load without (day –1) or with (day 1) concomitant study drug treatment of acarbose/metformin FDC or corresponding individual components (arithmetic mean ± standard deviation). FDC, fixed-dose combination.

**Figure 3.** Concentration-time curve for serum insulin concentrations following an oral sucrose load without (day -1) or with (day 1) concomitant study drug treatment of acarbose/metformin FDC or corresponding individual components (arithmetic mean ± standard deviation). FDC, fixed-dose combination.

**Pharmacokinetic Results**

There was no significant difference observed between the 24-hour plasma concentration and time curves of metformin following both the acarbose/metformin FDC and LC (Figure 4). Following administration of acarbose/metformin FDC and LC, the arithmetic mean peak plasma concentration was reached 4 hours after administration (592.8 μg/L vs 602.3 μg/L). Summary statistics showed that arithmetic mean values of AUC, AUC(0-tlast), and Cmax of
metformin were comparable between FDC and LC (Table 2).

Bioequivalence Evaluation
With respect to RatioC<sub>max</sub> and RatioAUC(0-4h) values of serum glucose for acarbose, geometric mean ratios, and 90% CIs were entirely contained within the 0.8 to 1.25 BE limits (Table 3). Similarly, the 90% CIs for the geometric mean ratios of AUC, AUC(0-last), and C<sub>max</sub> of metformin following administration of acarbose/metformin FDC vs acarbose/metformin LC were well within the BE range (Table 3).

However, the BE for most of exploratory PD parameters of acarbose could not be established as 90% CI did not meet the BE criterion (0.8-1.25). Especially for those recently recommended side effect of metformin and acarbose, especially when of serum glucose on day 1, wide confidence limits were observed between 2 treatment with the upper limit of the CI falling outside the predefined limit of 1.25 (Table 3, Figures 5, and 6). Further evaluation was conducted on those recommended PD parameters to explore the effect of sex on BE evaluation. It was observed that point estimate for women was better than men for BE evaluation. However, the within-subject variations in women for these parameters were found to be much higher than men (Table 4).

Adverse Events
In total, 19 of 24 (79.2%) subjects had at least 1 TEAE: 16 after receiving FDC and 13 after receiving LC. The most frequently occurring TEAE was diarrhea (16 after FDC and 12 after LC). Diarrhea is a well-known side effect of metformin and acarbose, especially when

### Table 2. Key Pharmacodynamic/Pharmacokinetic Parameters Following an Oral Sucrose Loading Without (Day –1) or with (Day 1) Concomitant Study Drug Treatment of FDC or LC

| Analyte | Parameter, Unit | Day | Acarbose | Metformin FDC | Acarbose | Metformin LC |
|---------|-----------------|-----|----------|--------------|----------|--------------|
| Glucose | C<sub>max</sub>, mg/100 mL | –1 | 131.1 (15.8) | 129.7 (15.5) | 6.2 (2.9) | 6.2 (2.9) |
|         |                  | 1  | 107.9 (10.3) | 102.9 (12.9) | 107.9 (10.3) | 102.9 (12.9) |
|         | AUC<sub>(0-2h)</sub>, mg h/100 mL | –1 | 209.0 (26.9) | 209.7 (30.0) | 78.5 (29.7) | 78.5 (29.7) |
|         |                  | 1  | 182.6 (14.1) | 178.5 (18.1) | 182.6 (14.1) | 178.5 (18.1) |
|         | AUC<sub>(0-6h)</sub>, mg h/100 mL | –1 | 349.7 (44.1) | 349.4 (46.3) | 349.7 (44.1) | 349.4 (46.3) |
|         |                  | 1  | 332.9 (27.1) | 328.6 (31.0) | 332.9 (27.1) | 328.6 (31.0) |
|         | C<sub>max,bc</sub>, mg/100 mL | –1 | 57.2 (15.9) | 56.9 (13.1) | 57.2 (15.9) | 56.9 (13.1) |
|         |                  | 1  | 31.7 (13.0) | 27.9 (12.1) | 31.7 (13.0) | 27.9 (12.1) |
|         | AUC<sub>(0-2h),bc</sub>, mg h/100 mL | –1 | 61.3 (24.1) | 64.5 (26.3) | 61.3 (24.1) | 64.5 (26.3) |
|         |                  | 1  | 30.6 (12.0) | 28.9 (14.5) | 30.6 (12.0) | 28.9 (14.5) |
|         | AUC<sub>(0-4h),bc</sub>, mg h/100 mL | –1 | 70.5 (33.9) | 74.9 (34.7) | 70.5 (33.9) | 74.9 (34.7) |
|         |                  | 1  | 36.3 (18.5) | 36.5 (22.0) | 36.3 (18.5) | 36.5 (22.0) |
| Insulin | C<sub>max</sub>, pmol/L | –1 | 443.1 (203.4) | 389.6 (190.1) | 443.1 (203.4) | 389.6 (190.1) |
|         |                  | 1  | 236.4 (114.3) | 203.4 (92.0) | 236.4 (114.3) | 203.4 (92.0) |
|         | AUC<sub>(0-2h)</sub>, pmol h/L | –1 | 528.4 (210.6) | 490.2 (210.4) | 528.4 (210.6) | 490.2 (210.4) |
|         |                  | 1  | 301.9 (125.2) | 280.8 (112.3) | 301.9 (125.2) | 280.8 (112.3) |
|         | AUC<sub>(0-6h)</sub>, pmol h/L | –1 | 660.1 (254.3) | 622.5 (250.9) | 660.1 (254.3) | 622.5 (250.9) |
|         |                  | 1  | 444.3 (167.3) | 424.5 (151.7) | 444.3 (167.3) | 424.5 (151.7) |
|         | C<sub>max,bc</sub>, pmol/L | –1 | 395.5 (196.1) | 346.1 (180.9) | 395.5 (196.1) | 346.1 (180.9) |
|         |                  | 1  | 178.7 (107.4) | 147.9 (87.9) | 178.7 (107.4) | 147.9 (87.9) |
|         | AUC<sub>(0-2h),bc</sub>, pmol h/L | –1 | 431.5 (193.9) | 401.1 (192.6) | 431.5 (193.9) | 401.1 (192.6) |
|         |                  | 1  | 185.9 (108.6) | 168.7 (103.0) | 185.9 (108.6) | 168.7 (103.0) |
|         | AUC<sub>(0-6h),bc</sub>, pmol h/L | –1 | 495.7 (220.1) | 471.7 (224.5) | 495.7 (220.1) | 471.7 (224.5) |
|         |                  | 1  | 230.9 (139.0) | 218.2 (129.1) | 230.9 (139.0) | 218.2 (129.1) |
| Metformin | AUC<sub>0-6h</sub>, μg h/L | –1 | 14820.6 (1216.2) | 46039 (1155.8) | 14820.6 (1216.2) | 46039 (1155.8) |
|         |                  | 1  | 4689.1 (1208.1) | 4493.9 (1130.3) | 4689.1 (1208.1) | 4493.9 (1130.3) |
|         | C<sub>max</sub>, μg/L | –1 | 592.8 (140.3) | 602.3 (112.0) | 592.8 (140.3) | 602.3 (112.0) |
|         |                  | 1  | 4.9 (1.2) | 4.9 (0.8) | 4.9 (1.2) | 4.9 (0.8) |
|         | t<sub>max</sub>, h | –1 | 4.0 (1.0-6.0) | 4.0 (0.5-5.0) | 4.0 (1.0-6.0) | 4.0 (0.5-5.0) |

All parameters (except t<sub>max</sub>) are presented by arithmetic mean (SD). T<sub>max</sub> is presented by median (minimum-maximum).
AUC, area under the concentration versus time curve from zero to infinity after single (first) dose; AUC<sub>(0-2h)</sub>, AUC from time 0 to 2 hours; AUC<sub>(0-6h)</sub>, AUC from time 0 to 4 hours; AUC<sub>(0-2h),bc</sub>, baseline-corrected AUC from time 0 to 2 hours; AUC<sub>(0-6h),bc</sub>, baseline-corrected AUC from time 0 to 4 hours; AUC<sub>(0-last)</sub>, AUC from time 0 to the last data point greater than lower limit of quantification; C<sub>max</sub>, maximum observed analyte concentration in measured matrix after a single dose administration; C<sub>max,bc</sub>, baseline-corrected C<sub>max</sub>; FDC, fixed-dose combination; LC, loose combination; t<sub>1/2</sub>, half-life associated with the terminal slope; t<sub>max</sub>, time to reach C<sub>max</sub> (in case of 2 identical C<sub>max</sub> values, the first t<sub>max</sub> was used) after single (first) dose.
Figure 4. Concentration-time curve for plasma metformin following an oral sucrose load without (day –1) or with (day 1) concomitant study drug treatment of acarbose/metformin FDC or corresponding individual components, semilogarithmic scale (arithmetic mean ± standard deviation). FDC, fixed-dose combination.

Table 3. Key Parameters for the Bioequivalence Evaluation (FDC/LC)

| Analyte   | Parameter          | Day | N  | Point Estimate | Lower CI | Upper CI | Geometric CV (Within Subject) |
|-----------|--------------------|-----|----|----------------|----------|----------|-------------------------------|
| Glucose   | Ratio_{C_{max}}    | 1/-1| 24 | 1.0391         | 0.9992   | 1.0805   | 0.079                         |
|           | Ratio_{AUC(0-4h)}} | 1/-1| 24 | 1.0126         | 0.9716   | 1.0553   | 0.084                         |
|           | C_{max,bc}        | 1/24|    | 1.1504         | 1.0016   | 1.3213   | 0.285                         |
|           | AUC_{(0-2h),bc}   | 1/24|    | 1.1252         | 0.9304   | 1.3608   | 0.398                         |
|           | AUC_{(0-4h),bc}   | 1/24|    | 1.0914         | 0.8636   | 1.3793   | 0.500                         |
| Metformin | C_{max}           | 1/24|    | 0.9763         | 0.9173   | 1.0391   | 0.126                         |
|           | AUC               | 1/24|    | 1.0475         | 1.0143   | 1.0818   | 0.065                         |
|           | AUC_{(0-tlast)}   | 1/24|    | 1.0427         | 1.0086   | 1.0779   | 0.067                         |

AUC_{(0-2h),bc}, baseline-corrected AUC from time 0 to 2 hours; AUC_{(0-4h),bc}, baseline-corrected AUC from time 0 to 4 hours; AUC_{(0-tlast)}, AUC from time 0 to the last data point greater than lower limit of quantification; AUC, area under the concentration versus time curve from time 0 to infinity after single (first) dose; CI, confidence interval; C_{max}, maximum observed analyte concentration in measured matrix after a single dose administration; C_{max,bc}, baseline-corrected C_{max}; CV, coefficient of variation; FDC, fixed-dose combination; LC, loose combination; Ratio_{AUC(0-4h)}, ratio of AUC_{(0-4h)}, day 1/AUC_{(0-4h)}, day –1; Ratio_{C_{max}}, ratio of C_{max}, day 1/C_{max}, day –1.

people first start taking these drugs. None of the subjects were prematurely withdrawn from the study due to a TEAE. All TEAEs were mild in intensity and were completely resolved at the end of the study (Table 5).

Discussion
The BE of the primary PD parameters Ratio_{C_{max}} and Ratio_{AUC(0-4h)} for acarbose was demonstrated following administration of a single dose of acarbose/metformin FDC compared with their individual formulations in healthy Chinese subjects under fasting conditions. Further, BE on the basis of PK parameters including C_{max}, AUC_{(0-tlast)}, and AUC of metformin was also established. Thus, this FDC formulation is expected to deliver the same therapeutic effect as coadministration of the individual drugs and could be targeted in Chinese patients already taking 2 separate tablets.

The purpose of this study was carried out specifically for the development of FDC of acarbose and...
metformin for assessing BE in Chinese subjects. Kim et al. demonstrated that although there was a reduction in C\textsubscript{max} by 24% and AUC by 26% of metformin when given in combination with acarbose, this reduction was considered as clinically irrelevant, presumably due to the complementary mechanism of action according to the literature. The C\textsubscript{max} of glucose after an oral sucrose load was reduced on average by about 17%, 22%, and 21% after treatment with acarbose/metformin FDC, acarbose/metformin LC, and acarbose alone, whereas no effect was seen for metformin alone. There was a minor reduction in AUC(0-4h) for PPG by about 3% after intake of the acarbose-containing drugs, compared with 1% after metformin alone. Considering that metformin will show similar minor decrease on glucose levels in both FDC and LC, glucose-related PD parameters after sucrose load can be considered an appropriate PD end point to demonstrate BE of acarbose between FDC and LC. 

Due to the combined effect of fluctuations of blood glucose levels and human internal retroaction regulation via insulin, in practice, the negative values derived from the difference between day -1 and day 1 was commonly observed in about 30% subjects. These negative values could not be analyzed in the log-transformation process for BE evaluation. Therefore, we defined the ratios of C\textsubscript{max} and AUC(0-4h) as the primary PD parameters for the BE evaluation of acarbose. In summary, the key reason to select the ratio approach was to avoid a negative value in difference approach. Furthermore, compared to the difference approaches, the ratio approach will follow log-normal distribution statistically. Several deficiencies were identified in previous parameters of ΔAUC(0-4h) and ΔC\textsubscript{max}(0-4h) for acarbose BE evaluation in vivo. First, due to the negative feedback regulation of serum glucose itself, the serum glucose concentration was lower, 2 to 4 hours after sucrose administration alone than at the baseline level,
Figure 6. Forest plot of bioequivalence evaluation on pharmacodynamic parameters of insulin for acarbose through point estimates and 90% confidence intervals. Reference line with value 0.8, 1, and 1.25. AUC, area under the concentration versus time curve from time 0 to infinity after single (first) dose; AUC$_{0-2}$, AUC from time 0 to 2 hours; AUC$_{0-4}$, AUC from time 0 to 4 hours; C$_{max}$, maximum observed analyte concentration in measured matrix after a single dose administration; C$_{max,bc}$, baseline-corrected C$_{max}$; AUC$_{0-2,bc}$, baseline-corrected AUC from time 0 to 2 hours; AUC$_{0-4,bc}$, baseline-corrected AUC from time 0 to 4 hours; LCL, lower confidence limit; PE, point estimates; Ratio$_{C_{max},C_{max}}$, day 1/C$_{max}$, day –1; Ratio$_{AUC_{0-4},AUC_{0-4}}$, day 1/AUC$_{0-4}$, day –1; Ratio$_{C_{max,bc},C_{max,bc}}$, day 1/C$_{max,bc}$, day –1; Ratio$_{AUC_{0-2,bc},AUC_{0-2,bc}}$, day 1/AUC$_{0-2,bc}$, day –1; UCL, upper confidence limit; % C$_{max}$ inhibition, (1 – C$_{max,bc}$, day 1/C$_{max,bc}$, day –1) *100; % AUC$_{0-2}$ inhibition, (1 – AUC$_{0-2,bc}$, day 1/AUC$_{0-2,bc}$, day –1) *100; % AUC$_{0-4}$ inhibition, (1 – AUC$_{0-4,bc}$, day 1/AUC$_{0-4,bc}$, day –1) *100.

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 with ΔAUC$_{0-4h}$ was observed that could not be analyzed through logarithmic conversion. Second, as the effect of acarbose is not dose dependent from 2 to 4 hours, prolongation of the time of blood sample collection 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, new PD parameters were proposed on the basis of the serum glucose concentration from 0 to 2 hours (C$_{max(0–2h)}$ and AUC$_{0–2h}$). Huang et al and Chen et al considered these parameters more sensitive than PD parameters of ΔAUC$_{0-4h}$ and ΔC$_{max(0-4h)}$ for acarbose BE, and could distinguish the hypoglycemic effects of different doses of acarbose. We also applied this single-point baseline-corrected approach as exploratory PD parameters. However, the BE for these PD parameters of acarbose in the current study could not be established as the 90%CI did not meet the BE criterion of 0.8 to 1.25 with the upper limit falling outside the predefined limit of 1.25. Thus, more subjects might be needed to establish acarbose BE due to the higher within-subject variability observed between the 2 treatments. Further, during the data analysis process, negative values were still observed which resulted in different BE evaluation results by either using 0 to replace value below baseline or by keeping negative values for AUC calculation. On the other hand, different from the study design considering both pretreatment day (day –1) and treatment day (day 1), the new method only took the data from 1 treatment day into account for BE evaluation, without considering the internal glucose fluctuation and/or insulin regulating effect in the study design. Thus, these parameters might require further validation for BE evaluation and relevant study design needs to be researched.
Table 4. Exploratory Parameters of Serum Glucose for the Bioequivalence Evaluation on Gender (FDC/LC)

| Gender | Parameter       | N  | Point Estimate | 90%CI Lower | 90%CI Upper | Geometric CV (Within Subject) |
|--------|-----------------|----|----------------|-------------|-------------|-----------------------------|
| Male   | C_{max,bc}      | 12 | 1.144          | 0.967       | 1.354       | 0.231                       |
|        | AUC(0-2h),bc    | 12 | 1.178          | 1.016       | 1.367       | 0.202                       |
|        | AUC(0-4h),bc    | 12 | 1.191          | 1.035       | 1.372       | 0.192                       |
| Female | C_{max,bc}      | 12 | 1.157          | 0.896       | 1.494       | 0.356                       |
|        | AUC(0-2h),bc    | 12 | 1.011          | 0.624       | 1.637       | 0.727                       |
|        | AUC(0-4h),bc    | 12 | 1.063          | 0.718       | 1.573       | 0.570                       |

AUC, area under the concentration versus time curve from time 0 to infinity after a single (first) dose; AUC(0-2h),bc, baseline-corrected AUC from time 0 to 2 hours; AUC(0-4h),bc, baseline-corrected AUC from time 0 to 4 hours; CI, confidence interval; C_{max}, maximum observed analyte concentration in measured matrix after a single dose administration; C_{max,bc}, baseline-corrected C_{max}; CV, coefficient of variation; FDC, fixed dose combination; LC, loose combination.

Table 5. Number of Subjects With Treatment-Emergent Adverse Events by Primary System Organ Class, Preferred Term

| Primary System Organ Class | Preferred Term | Acarbose Metformin FDC | Acarbose Metformin LC | Total |
|----------------------------|----------------|------------------------|-----------------------|-------|
| MedDRA version 22.1, N (%) |                | 24 (100)               | 24 (100)              | 24 (100) |
| Number (%) of subjects with at least 1 such adverse event | 16 (66.7) | 13 (54.2) | 19 (79.2) |
| Cardiac disorders, n (%) | 0              | 1 (4.2)                | 1 (4.2)               |       |
| Nodal rhythm, n (%)      | 0              | 1 (4.2)                | 1 (4.2)               |       |
| Gastrointestinal disorders, n (%) | 16 (66.7) | 12 (50.0) | 18 (75.0) |
| Diarrhea, n (%)          | 16 (66.7)      | 12 (50.0)              | 18 (75.0)             |       |
| Investigations, n (%)    | 0              | 1 (4.2)                | 1 (4.2)               |       |
| Blood uric acid increased, n (%) | 0      | 1 (4.2)               | 1 (4.2)               |       |

FDC, fixed-dose combination; LC, loose combination; MedDRA, Medical Dictionary for Regulatory Activities.

To our knowledge this is the first study that includes both healthy men and women for metformin/acarbose BE evaluation. Based on our results, further investigations focusing on the newly proposed predose bc parameters and potential role of sex on BE of the FDC and LC might be needed. An interesting observation was noted that the within-subject variability was higher in women than in men when PD parameters C_{max,bc}, AUC(0-2h),bc, and AUC(0-4h),bc of serum glucose were evaluated. However, this aspect of potential sex difference needs to be further explored via sucrose challenge model for confirmations of findings. In addition, the BE evaluation was also performed for other exploratory PD parameters of glucose and insulin. As shown in the forest plots (Figures 5 and 6), most exploratory PD parameters did not meet the criterion for BE, which might be due to either high variability with too low values after baseline correction or insufficient sample size in this study.

Conclusion
The BE of acarbose/metformin FDC (50/500 mg) and corresponding LC could initially be established on the basis of the primary target variables. Both FDC and LC were safe and well tolerated in healthy Chinese male and female subjects. However, inconsistent results were observed when other exploratory PD parameters were used to evaluate the BE of acarbose. More research is needed to validate which parameters are more suitable for acarbose BE evaluation.

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Conflicts of Interest
C.L., F.Y., L.S., and X.L. have no conflict of interests with regard to the content of this article. A.C. and Y.S. are employees of Bayer. Y.L. and J.L. are former employees of Bayer.
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**Data Accessibility**
The data set(s) supporting the conclusions of this article are available from the corresponding author on reasonable request.

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**Supplemental Information**

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