Analyses of gut microbiota and plasma bile acids enable stratification of patients for antidiabetic treatment

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Analyses of gut microbiota and plasma bile acids enable stratification of patients for antidiabetic treatment

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Antidiabetic medication may modulate the gut microbiota and thereby alter plasma and faecal bile acid (BA) composition, which may improve metabolic health. Here we show that treatment with Acarbose, but not Glipizide, increases the ratio between primary BAs and secondary BAs and plasma levels of unconjugated BAs in treatment-naive type 2 diabetes (T2D) patients, which may beneficially affect metabolism. Acarbose increases the relative abundances of Lactobacillus and Bifidobacterium in the gut microbiota and depletes Bacteroides, thereby changing the relative abundance of microbial genes involved in BA metabolism. Treatment outcomes of Acarbose are dependent on gut microbiota compositions prior to treatment. Compared to patients with a gut microbiota dominated by Prevotella, those with a high abundance of Bacteroides exhibit more changes in plasma BAs and greater improvement in metabolic parameters after Acarbose treatment. Our work highlights the potential for stratification of T2D patients based on their gut microbiota prior to treatment.
Host-gut microbiota interaction is central in bile acid (BA) metabolism and signalling, and it is essential for the maintenance of metabolic health. Additionally, compared to healthy individuals, alterations in the levels and compositions of plasma BAs have been observed in patients with diabetes or obesity, further highlighting a possible involvement of BA metabolism in the pathogenesis of metabolic diseases. Finally, an animal study transferred a gut microbiota with a higher baseline abundance of *Bacteroides* to recipient mice, suggesting that BA signalling is required for mediating the therapeutic effects of bariatric surgery.

Acarbose, a pseudo-tetrasaccharide produced by *Actinoplanes* species and an alpha-glycosidase inhibitor, is recommended in China and other Asian countries as an alternative to Metformin. Although solid proof of a causal link between gut microbial dysbiosis in T2D patients and the intestinal microbiota. We identify drug-dependent metabolic parameters, including plasma BAs, and the intestinal microbiota. We identify drug-dependent alterations in the microbiome and plasma BAs that correlate with clinical measurements and outcomes, including body mass index (BMI), blood glucose, glycated haemoglobin (HbA1c), lipid profiles, insulin secretion and resistance status. Of interest, T2D patients can be stratified into two groups based on their individual baseline microbiomes. Patients harboring a gut microbiota with a higher baseline abundance of *Bacteroides* have lower levels of secondary BAs and exhibit more beneficial therapeutic responses to Acarbose treatment, including reduced BMI, and improved insulin resistance status and lipid profile, suggesting that baseline metagenome signatures may be used to stratify T2D patients prior to treatment.

## Results

### Baseline characteristics of patients and clinical outcomes

To characterise the clinical effects of Acarbose and Glipizide, we compared the effects of two drugs on metabolic parameters, including plasma BAs, and the intestinal microbiota. We identify drug-dependent alterations in the microbiome and plasma BAs that correlate with clinical measurements and outcomes, including body mass index (BMI), blood glucose, glycated haemoglobin (HbA1c), lipid profiles, insulin secretion and resistance status. Of interest, T2D patients can be stratified into two groups based on their individual baseline microbiomes. Patients harboring a gut microbiota with a higher baseline abundance of *Bacteroides* have lower levels of secondary BAs and exhibit more beneficial therapeutic responses to Acarbose treatment, including reduced BMI, and improved insulin resistance status and lipid profile, suggesting that baseline metagenome signatures may be used to stratify T2D patients prior to treatment.

### Table 1 Clinical parameters in the pre-treatment and post-treatment samples in the two treatment arms

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>P value (Pre vs Post)</th>
<th>P value</th>
<th>Acarbose</th>
<th>Glipizide</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic characteristics</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>53 ± 7</td>
<td>54 ± 7</td>
<td>0.497</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Gender (male/total)</td>
<td>34/51</td>
<td>24/43</td>
<td>0.387</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>74.7 ± 10.3</td>
<td>71.5 ± 10.7</td>
<td>0.067</td>
<td>0.190</td>
<td>2.36E-07</td>
<td>0.138</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.3 ± 3.2</td>
<td>26.0 ± 3.4</td>
<td>0.265</td>
<td>0.945</td>
<td>3.48E-07</td>
<td>0.113</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>91.5 ± 8.5</td>
<td>90.7 ± 8.4</td>
<td>0.527</td>
<td>0.870</td>
<td>3.84E-04</td>
<td>0.144</td>
</tr>
<tr>
<td>WHR</td>
<td>0.92 ± 0.06</td>
<td>0.92 ± 0.05</td>
<td>0.485</td>
<td>0.779</td>
<td>0.005</td>
<td>0.019</td>
</tr>
<tr>
<td><strong>Glycaemic characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.5 ± 0.8</td>
<td>7.7 ± 0.9</td>
<td>0.541</td>
<td>0.508</td>
<td>1.45E-09</td>
<td>1.82E-08</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>7.6 ± 1.4</td>
<td>7.9 ± 1.4</td>
<td>0.291</td>
<td>0.665</td>
<td>3.58E-07</td>
<td>2.48E-06</td>
</tr>
<tr>
<td>PPG (mmol/L)</td>
<td>14.3 ± 2.7</td>
<td>15.0 ± 2.8</td>
<td>0.164</td>
<td>0.902</td>
<td>5.30E-10</td>
<td>6.29E-10</td>
</tr>
<tr>
<td>AUC blood glucose (mmol/L)</td>
<td>2241 ± 263</td>
<td>2381 ± 351</td>
<td>0.077</td>
<td>0.002</td>
<td>5.30E-10</td>
<td>6.98E-11</td>
</tr>
<tr>
<td>AUC Insulin (pmol/L)·min</td>
<td>5981 ± 3340</td>
<td>5460 ± 2507</td>
<td>0.697</td>
<td>1.62E</td>
<td>2.40E-06</td>
<td>0.019</td>
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<tr>
<td>AUC C Peptide (ng/ml·min)</td>
<td>528 ± 210</td>
<td>501 ± 154</td>
<td>0.978</td>
<td>1.94E</td>
<td>1.94E-08</td>
<td>0.002</td>
</tr>
<tr>
<td>HOMA-IR (μIU·mmol⁻¹)</td>
<td>3.7 ± 2.3</td>
<td>3.6 ± 2.1</td>
<td>0.868</td>
<td>0.638</td>
<td>0.002</td>
<td>0.050</td>
</tr>
<tr>
<td><strong>Other clinical indices</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>127 ± 16</td>
<td>131 ± 18</td>
<td>0.431</td>
<td>0.122</td>
<td>0.022</td>
<td>0.043</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>82 ± 8</td>
<td>81 ± 10</td>
<td>0.675</td>
<td>0.703</td>
<td>2.94E-05</td>
<td>0.013</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>2.5 ± 2.1</td>
<td>2.1 ± 1.2</td>
<td>0.459</td>
<td>0.093</td>
<td>2.39E-05</td>
<td>0.382</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.1 ± 1.2</td>
<td>4.9 ± 1.0</td>
<td>0.696</td>
<td>0.712</td>
<td>0.007</td>
<td>0.228</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>1.2 ± 0.4</td>
<td>1.2 ± 0.4</td>
<td>0.500</td>
<td>0.864</td>
<td>0.276</td>
<td>0.5355</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>3.1 ± 1.0</td>
<td>3.1 ± 0.8</td>
<td>0.492</td>
<td>0.514</td>
<td>0.484</td>
<td>0.0357</td>
</tr>
<tr>
<td>FLI</td>
<td>334 ± 554</td>
<td>237 ± 341</td>
<td>0.352</td>
<td>0.400</td>
<td>1.92E-09</td>
<td>0.013</td>
</tr>
</tbody>
</table>

1/ WC, waist circumference; WHR, waist-hip circumference ratio; HbA1c, glycated haemoglobin; FPG, fasting plasma glucose; PPG, postprandial plasma glucose; AUC, area under the curve; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; HDL, high-density lipoprotein; FLI, fatty liver index. FLI = \((e^{0.053 \cdot \log_e[\gamma GT]} + 0.193 \cdot BMI + 0.718 \cdot \log_e[\gamma GT] + 0.053 \cdot WC - 15.745)/e^{0.953 \cdot \log_e[\gamma GT]} + 0.193 \cdot BMI + 0.718 \cdot \log_e[\gamma GT] + 0.053 \cdot WC - 15.745) + 100 \cdot \gamma GT\), gamma-glutamyltransferase.

Data are presented as mean ± SD.
recruited patients from five centres in Shanghai, China. A total of 171 treatment-naive T2D patients were screened and based on inclusion and exclusion criteria (Supplementary Table 1; registered in ClinicalTrials.gov. NCT01758471), 106 patients were enrolled and 1:1 randomised into the two treatment arms (Supplementary Methods). There was no difference between the two arms in relation to gender, age and BMI (Table 1). Ninety-four of the enrolled patients completed the study and were included in the analyses, while 5 in the Acarbose group and 7 in the Glipizide group were not included in the analysis (flow chart shown in Supplementary Fig. 1). The goal of the treatment was to achieve a generally recommended targeted level of HbA1c below 7.0%22, 23 in both arms within 3 months, indicative of improved glycaemic control. Faecal and plasma samples were collected at baseline and after 3 months of treatment. There were no significant differences between anthropomorphic, metabolic, biochemical parameters and HbA1c levels in the two groups at baseline (Wilcoxon rank-sum test, P > 0.05, Table 1).

Fig. 1 Major clinical outcomes in patients after 3 months of treatment with Acarbose or Glipizide. a Effect on glyced haemoglobin A1c (HbA1c), in the Acarbose (brown) and Glipizide (white) arms, Wilcoxon rank-sum test. b Effects on fasting blood glucose concentration (FBG), postprandial blood glucose concentration (PBG) in the Acarbose (brown) and Glipizide (white) treatment arms; paired Wilcoxon rank-sum test, *P < 0.01. c Effects on body weight (BW) and body mass index (BMI) in the Acarbose (brown) and Glipizide (white) treatment arms. d Blood glucose excursion curve and e plasma insulin release curve during the meal tests in the two treatment arms, paired Wilcoxon rank-sum test, *P < 0.01, +P < 0.05, pre-treatment vs. post-treatment with Acarbose. f Effects on HOMA-IR, g Total cholesterol (TC) and triglycerides (TG) in the two treatment arms; paired Wilcoxon rank-sum test, *P < 0.01. n = 51 in Acarbose pre and Acarbose post-treatment, n = 43 in Glipizide pre and Glipizide post-treatment.
At the end of the 3-month treatment, the reductions in HbA1c levels, and fasting blood glucose (FBG) and postprandial blood glucose (PBG) levels were significant in both groups (paired Wilcoxon rank-sum test, \( P < 0.01 \) between baseline and treatment, Table 1), and both arms reached the targeted level of glycaemic control that did not differ significantly (Wilcoxon...
rank-sum test, \( P > 0.05 \) between groups, Fig. 1a, b). However, the reductions in body weight (BW) and BMI were more pronounced in the Acarbose arm than in the Glipizide arm after 3 months of treatment (Fig. 1c). The area under the curve (AUC) value of insulin release was significantly reduced after Acarbose treatment, but increased as expected after Glipizide treatment during a carbohydrate meal test containing 100 g flour as described previously\(^2\), (1029.9 ± 575.2 pmol/L/min vs. 637.1 ± 342.6 pmol/L/min, Acarbose pre vs. post-treatment, \( P = 2.41E−06; 940.2 ± 431.7 \) pmol/L/min vs.1139.6 ± 608.9 pmol/L/min, Glipizide pre vs. post-treatment, \( P = 0.019; \) paired Wilcoxon rank-sum test; Fig. 1d, e). In addition, patients receiving Acarbose but not...
Glipizide showed a significant improvement in clinical parameters that are major risk factors for metabolic comorbidities and cardiovascular complications of T2D, such as the homeostasis model assessment of insulin resistance (HOMA-IR), total cholesterol (TC), triglyceride (TG) and fatty liver index (FLI)\(^{25}\). Of note, neither Acarbose nor Glipizide treatment increased, but rather decreased plasma FGF19 levels, a key factor produced in the intestine and shown to be of importance for metabolic health\(^{26,27}\), indicating that improvement in HbA1c and FBG and PBC levels was not dependent on FGF19, (Table 1; Fig. 1f, g, all clinical parameters are given in Supplementary Table 2). Considering the known Acarbose-induced reduction of carbohydrate absorption in the small intestine, we next investigated to what extent Acarbose-induced changes in the gut microbiota might contribute to the observed additional metabolic benefits besides the hypoglycaemic effect.

**Changes in plasma BAs correlate with therapeutic benefits.**

Alterations in the composition of the BA pool in humans can reflect intraluminal changes in the gut modulating gut-derived signals that regulate host metabolism and alteration in plasma BAs have been linked with metabolic disorders\(^{12}\). Thus, the level of taurine-conjugated BAs (TaurineBA, the sum of all taurine-conjugated BAs) is elevated in T2D\(^{15}\) and the ratio of 12-α OH to non-12α OH BAs (12-α OH/non-12α OH BA ratio) in the total BA pool has been linked to insulin resistance\(^{14}\). In our study, we assayed 15 BA species using high-performance LC-MS/MS on fasting plasma samples. At baseline, no differences in plasma BA levels were observed between the treatment arms (Fig. 2a). After treatment, plasma BA composition was substantially altered solely in the Acarbose arm with the level of total BAs remaining unchanged (Fig. 2b, Supplementary Table 3, paired Wilcoxon rank-sum test, \(P<0.05\)). Acarbose treatment decreased the levels of plasma secondary BAs (SBAs), mainly conjugated deoxycholic acids (DCAs). Due to the increased levels of cholic acid (CA) and chenodeoxycholic acid (CDCA), we observed an increased overall level of unconjugated primary BAs (PBAs). Further, the ratios of PBAs to SBAs (PBA/SBA ratio) and unconjugated to conjugated BAs (UnconBA/ConBA ratio) increased, whereas the levels of TaurineBA and the 12-α OH/non-12α OH BA ratio decreased after Acarbose treatment (Supplementary Table 3, paired Wilcoxon rank-sum test, \(P<0.05\)).

A multivariate correlation analysis based on the generalised estimated equation (GEE) revealed that the Acarbose-induced improvements of glycaemic control and other metabolic parameters were correlated with the changes in plasma BA composition (Fig. 2c; Supplementary Table 4). The significant increases in the UnconBA/ConBA ratio and PBA/SBA ratio correlated with improvements in HbA1c, BW, FLI, blood pressure and lipid profile (Fig. 2c, Supplementary Table 4). Via deconjugation and the sequential dehydroxylation of PBAs to produce SBAs, the gut microbiome is crucial for regulating BA diversification\(^{27}\). The increased UnconBA/ConBA and PBA/SBA ratios after Acarbose treatment indicated that Acarbose treatment might alter two key steps of bacterial BA biotransformation.

**Acarbose modulates the composition of the gut microbiota.**

We next performed whole-metagenome sequencing on the 188-paired faecal samples from the patients and generated 1.42 Tb of high-quality sequencing data with an average of 7.7 Gb per sample to investigate the differential responses of the gut microbiome to the two types of antidiabetic treatment. Genes were identified by aligning the reads to the 9.9 M integrated reference catalogue of genes in the human gut microbiome\(^{28}\). On average, 80.5% of the reads in each sample were mapped (Supplementary Table 5). Metagenomic analyses demonstrated that Acarbose treatment significantly altered the relative abundances of 141,382 microbial genes (paired Wilcoxon rank-sum test, \(q<0.01\)), whereas no significant changes in relative gene abundances were observed following Glipizide treatment (Supplementary Table 5). Rarefaction analyses revealed that the number of genes in the microbiomes of patients in the Acarbose arm surprisingly was significantly decreased, whereas no changes were observed in the Glipizide arm (Fig. 3a, b). In line with this observation, both gene count and Shannon index decreased after Acarbose treatment, but not after Glipizide treatment (Fig. 3c, d). Analysis of species-level molecular operational taxonomic unit (mOTU) profiles demonstrated that Acarbose led to significant changes in the relative abundances of 69 mOTUs (paired Wilcoxon rank-sum test, \(q<0.01\); Fig. 3e; Supplementary Table 6); Glipizide treatment did not affect the relative abundances of any mOTUs at this significance level. At baseline, there were no significant differences in relation to gene rarefaction, gene richness and gut microbiome alpha diversity or taxonomic richness between the two arms (Supplementary Fig. 2, Fig. 3c, d). Of note, despite of the comparable hypoglycaemic effect of Glipizide and Acarbose treatment, the gut microbiome alteration after Glipizide treatment was small, suggesting that changes in blood glucose levels were not a primary driver of the changes in the distal gut microbiota. Unlike Glipizide that regulates the ATP-sensitive potassium (K\(_{ATP}\)) channel activity of pancreatic β cells, Acarbose impedes the hydrolysis and absorption of carbohydrates in the small intestine, which in turn changes the abundances of substrates for fermentation by the microbiota in the distal intestine, thereby allowing saccharolytic Lactobacillus and Bifidobacterium species to thrive and depleting the original distal gut-residing, putrefactive species of Bacteroides, Allisites and Clostridium (Fig. 3e, paired Wilcoxon rank-sum test, \(q<0.01\)).

**Plasma BA changes, metabolic benefits and gut microbiota.**

A mutual interaction between host BAs and the gut microbiome modulates the microbial biotransformation of BAs\(^{29}\). In the untreated T2D patients at baseline, permutation multivariate analysis of variance (PERMANOVA) analysis revealed that plasma levels of the SBAs, ursodeoxycholic acid (UDCA), glycocholodeoxycholic acid (GUDCA), lithocholic acid (LCA) and

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*Fig. 3* Acarbose elicits stronger impact on the gut microbiota than Glipizide. Effects of Acarbose and Glipizide treatments on gene rarefaction, \(n=51\) in Acarbose pre and Acarbose post-treatment, \(n=43\) in Glipizide pre-treatment and Glipizide post-treatment. All bar plots are shown as mean ± S.D. The number of genes in each group was calculated after 100 random samplings with replacement. The effects of treatment in both arms on gene richness \(c\) and Shannon index \(d\) of the gut microbiome, \(n=51\) in Acarbose pre and Acarbose post-treatment, \(n=43\) in Glipizide pre and Glipizide post-treatment. e mOTUs significantly changed in abundance by Acarbose treatment (left panel), Benjamini-Hochberg q-value < 0.01, presented in box-plots to illustrate the relative abundances of the different groups. The mOTUs are listed in the order of their relative abundances pre-treatment. mOTUs in red represent mOTUs that decreased in abundance are after Acarbose treatment, whereas mOTUs that increased in abundance are given in green. The phyllum colour code is indicated to the left of the mOTU annotation. The same mOTUs did not change after Glipizide treatment (right panel of e). All plotted boxes are interquartile ranges. Dark lines in the boxes indicate medians, the lowest and highest values within 1.5 times IQR from the first and third quartiles. Outliers are shown as circles beyond the whiskers. *Bifidobacterium catenulatum*–Bpc: *Bifidobacterium catenulatum*-Bifidobacterium pseudocatenulatum complex
DCA correlated with changes in the composition of the gut microbiota (Bray–Curtis distance, $q < 0.01$; Supplementary Table 7). Interestingly, 58 out of the 69 mOTUs that exhibited significant changes in relative abundance in response to Acarbose treatment also correlated with Acarbose-induced alterations in plasma BA composition (GEE adjusted for age, sex and BMI, $q < 0.05$; Supplementary Data 1), pointing to a tight link between Acarbose-dependent alterations of the gut microbiota and plasma BA composition.

Many of the Acarbose-induced species have been classified and used as probiotics, and the increase in the relative abundance correlated with improvements in metabolic parameters (GEE adjusted for age, sex and BMI, $q < 0.05$; Supplementary Fig. 3). Thus, Acarbose-induced increases in Lactobacillus gasseri and Bifidobacterium longum correlated inversely with changes in BW and HbA1c. These correlations are consistent with those observed in mice treated with VSL3* (a probiotic supplement containing strains of Lactobacillus, Bifidobacterium and Streptococcus)\(^3^0\) and...
with the effect of microbial overexpression of the gene encoding bile salt hydrolase (Bsh) which also increased the UnconBA/ConBA ratio in mice\(^\text{31}\). The reduced plasma DCA levels and reduced BW gain after Acarbose treatment, correlated with the reductions of Acarbose-depleted species, such as *Bacteroides plebeius*, *Bacteroides dorei/vulgatus* and *Clostridium bolteae*. These species harbour a multi-gene BA-inducible (*bai*) operon encoding genes involved in 7-αβ-dehydroxylation of BAs, reported not to be present in probiotic bacteria\(^{27,32}\).

**Acarbose treatment alters gut bile acid composition.** To explore the possible impact of Acarbose on gut bacterial BA metabolism, we performed functional annotations of genes using a manually built reference library which includes 1011 annotated amino acid sequences mapped to 16,102 genes implicated in bacterial BA metabolism (Supplementary Table 8). The relative abundances of genes encoding enzymes involved in bacterial BA modification, including BA deconjugation, BA transport, hydroxysteroid dehydrogenation and 7αβ-dehydroxylation were thus calculated and compared between pre-treatment and post-treatment samples (Supplementary Table 8). The relative abundances of *baiE* (encoding BA 7α-dehydroxylase, the rate-limiting enzyme for 7αβ-dehydroxylation) and *baiL* (encoding BA 7β-dehydroxylase) were significantly decreased after Acarbose treatment, while the *baiG* (encoding BA transporter) and genes encoding 7β-Hsdh (7β-Hsdh) showed significant enrichment after Acarbose treatment (paired Wilcoxon rank-sum test, *P < 0.05*; Fig. 4a; Supplementary Table 9). None of these genes changed significantly in relative abundance after Glipizide treatment (paired Wilcoxon rank-sum test, *P > 0.05*; Fig. 4a; Supplementary Table 9).

The taxa distributions of genes involved in BA metabolism (Supplementary Data 2) in baseline samples revealed that *B. plebeius*, *B. vulgatus/dorei* and *B. dorei* largely accounted for the abundances of *bsh*, *baiG* and *baiE*. This suggested that these *Bacteroides* species were mainly responsible for both BA deconjugation and subsequent dehydroxylation in the distal gut microbiota of treatment-naive T2D patients. After treatment, the relative abundances of these genes were reduced due to the loss of their dominant contributor species, or the dominant contributors of some genes were replaced by other species. For example, the dominant contributors of the genes encoding Bsh changed from *B. plebeius* and *B. vulgatus/dorei* to *B. longum*, and *Lactobacillus amylovorus* (Fig. 4b). The *Bifidobacterium* and *Lactobacillus* genera have been reported to possess high Bsh activity, but are lacking the *bai* genes\(^23,34\).

We investigated if the changes in the bacterial potential for BA metabolism induced by Acarbose treatment were reflected in the BA composition in the faecal samples. The total level of faecal BAs was significantly decreased after Acarbose treatment (Supplementary Fig. 4a, b, Supplementary Table 10). Actually, the faecal levels of the primary BAs, CA and CDCA, increased following Acarbose treatment, but this was more than counteracted by the large decrease in the levels of the secondary BAs, DCA and LCA (Supplementary Fig. 4a). Overall, these changes resulted in an increased faecal PBA/SBA ratio in response to Acarbose treatment (Supplementary Fig. 4d). The faecal UnconBA/ConBA ratio was unchanged (Supplementary Fig. 4c). These changes mirrored the changed relative abundance of *bai* genes with an enrichment in a relative abundance of *bsh* genes following Acarbose treatment (illustrated in Fig. 4c), but do not explain the significant increase in the plasma UnconBA/ConBA ratio after Acarbose treatment.

In addition to altering BA metabolism, bacterial functional pathway analysis showed that Acarbose treatment also increased the potential for glutamine synthesis, decreased the potential for glycosaminoglycan degradation, and decreased the potential for Fatty acid and cholesterol degradation (Supplementary Data 3), all associated with host-gut barrier integrity and T2D risks\(^35-38\).

Finally, both antidiabetic treatments reduced the abundances of genes involved in type III secretion and LPS biosynthesis that are linked to insulin resistance\(^39,40\), suggesting the existence of certain common routes in the response of the gut microbiome to antidiabetic treatments despite the use of different medical agents.

**Microbiota composition predicts the response to Acarbose.** Studies have suggested a stratification of individuals by clustering algorithms based on the genus composition of the intestinal microbiota\(^41,42\), but the concept of the existence of discrete enterotypes has also been questioned, suggesting a continuous distribution of enterotypes in an individual\(^43\). In our study, the baseline microbiomes of patients could be clearly separated into two enterotype-like clusters, one driven by *Bacteroides* (Cluster B) and the other driven by *Prevotella* (Cluster P) shown by PCA analysis (Supplementary Fig. 5a), network analysis (Supplementary Fig. 5b) and abundance box plot (Supplementary Fig. 5c). In agreement with previous reports, anthropomorphic, metabolic and biochemical parameters did not differ between patients from the two clusters\(^41\) (Wilcoxon rank-sum test, *P > 0.05*, Supplementary Table 11). However, already at baseline patients in Cluster B had lower levels of LCA and DCA, and higher levels of UDCA in the BA pool both in plasma and faeces (Wilcoxon rank-sum test, *P < 0.05*, Fig. 5a; Supplementary Tables 12, 13). Patients in Cluster B also exhibited a higher plasma PBA/SBA ratio and lower 12α-OH/non-12α-OH BA ratio compared to...
patients in Cluster P (Wilcoxon rank-sum test, \( P < 0.05 \), Fig. 5a; Supplementary Table 12). Further, patients in Cluster B exhibited significantly decreased relative abundance of \( \text{baiG} \), but increased relative abundances of \( \text{bsh} \), and \( 7\beta\)-\( \text{hsdh} \), whereas \( \text{baiE} \) and \( \text{baiI} \) exhibited similar relative abundances in both clusters (Wilcoxon rank-sum test, \( P < 0.05 \), Supplementary Table 14). Interestingly, the clinical effects of Acarbose were more pronounced in patients belonging to Cluster B than in patients belonging to Cluster P. Despite a similar improvement in HbA1c levels by Acarbose treatment, patients in Cluster B exhibited significantly greater improvements in G0 (FBG), insulin, C peptide levels and, therefore, HOMA-IR over baseline levels than patients in Cluster P (Fig. 5b; Supplementary Fig. 6a; Wilcoxon rank-sum test, \( P < 0.05 \), see also Supplementary Table 15 for all results). We observed the same tendency in the Glipizide arm. The compositions of the gut microbiome of patients in Cluster B changed significantly with depletion of the dominant \( \text{Bacteroides} \) genus and the increase of the \( \text{Bifidobacterium} \) genus after treatment, whereas fewer changes were observed in the gut microbiomes of patients belonging to Cluster P (Supplementary Fig. 6b; Wilcoxon rank-sum test, \( P < 0.01 \)). Further, Acarbose treatment led to a decreased abundance of \( \text{baiE} \) and an increased abundance of \( 7\beta\)-\( \text{hsdh} \) and \( \text{baiG} \), exclusively in patients in Cluster B (Supplementary Fig. 6c). Together, these results suggest that some of the therapeutic effects of Acarbose-induced modulation of the gut microbiota are related to bacterial BA metabolism and, therefore, BA signalling.

**Discussion**

This study compared the effects of two antidiabetic drugs on the gut microbiota, plasma BAs and their relations with clinical outcomes in a clinical trial-based cohort. A key finding of this study is that the baseline composition of the gut microbiota may be used for stratification of patients prior to initiation of treatment.

The significant reductions in BW with targeted glycaemic control after Acarbose treatment is in line with earlier studies\(^{44, 45}\). Further, we demonstrate that patients receiving Acarbose, but not Glipizide showed a significant improvement in the HOMA-IR, lipid profile and FLI. The results of plasma BA profiling and the multiple correlations between changes of plasma BAs and clinical parameters, including BW, HOMA-IR, lipid profile and FLI, indicate that the modulation of BA signalling may contribute to the unique metabolic effects of Acarbose. The increased levels of plasma CDCA and an increased UnconBA/ConBA ratio induced by Acarbose may enhance in vivo FXR activity\(^{46}\) and change the faecal BA composition, resulting in decreased intestinal lipid absorption and reduced hyperglycaemia\(^{47, 48}\). Interestingly, plasma UnconBA/ConBA and PBA/SBA ratios were also shown to increase after biliopancreatic diversion surgery\(^{13}\), which in a 2-year follow-up study was reported to result in superior improvements in hyperglycaemia and dyslipidaemia compared to the Roux-en-Y bypass and conventional medical therapy\(^{49}\). The notion that similar changes in BA composition and correlations between metabolic traits and plasma BAs after both Acarbose treatment and gut reconstruction surgeries, despite differences in populations, sample size and follow-up time, points to the importance of BA signalling in metabolic control in T2D treatments.

Acarbose treatment led to increased abundances of species possessing high Bsh activity, mainly \( \text{B. longum} \), \( \text{L. gasseri} \) and reduced abundance of the SBA producers, \( \text{B. plebeius} \), \( \text{B. vulgatus/dorei} \) and \( \text{C. bolteae} \). Further functional analysis showed decreased abundances of genes encoding the rate limited enzyme for \( 7\alpha/\beta \) dehydroxylation and a switch in the main contributors of BA deconjugation enzymes from \( \text{Bacteroides} \) spp. to \( \text{Lactobacillus} \) and \( \text{Bifidobacterium} \) spp., which rarely contain \( \text{bsh} \) genes. Thus, the metagenomics analyses indicated lower capacity for BA \( 7\alpha/\beta \) dehydroxylation after Acarbose treatment, which is in keeping with the observed increased plasma and faecal PBA/SBA ratios.

Interestingly, the increased ratio of UnconBA/ConBA was observed in plasma, but not in faecal samples, and the abundances of bacterial \( \text{bsh} \) genes did not change either. This would argue against an increased potential of bacterial deconjugation in response to Acarbose treatment, even in patients harbouring higher abundances of species such as \( \text{B. longum} \) and \( \text{L. gasseri} \) which have been reported to possess high Bsh activity. However, deconjugation occurs in the upper part of intestine and most of unconjugated PBA are reabsorbed in the ileum, and thus, to
what extent the UnconBA/ConBA ratio in the faecal sample represents a valid proxy for in vivo bacterial deconjugation capacity needs to be further investigated. It is possible that the significantly reduced level of FGF19, which conveys the main inhibitory intestinal BA signal for hepatic BA neo-synthesis, or an altered intestinal BA reabsorption after Acarbose treatment may contribute to the discrepancy between the UnconBA/ConBA ratios in plasma and faecal samples. However, taken together our results clearly point to the importance of Acarbose-induced changes in the composition of the gut microbiome and the observed alterations in BA profiles.

With the increased attention on the metabolic significance of BA signalling, the discovery of an Acarbose-gut microbiota-BAs axis may aid the identification of novel modalities for therapeutics intervention targeting the gut microbiome or plasma BAs. The implication of gut microbiota-plasma BAs axis underlying therapeutic effects of Acarbose is further supported by the finding of more pronounced changes in plasma BA composition and clinical benefits of Acarbose treatment in patients where the baseline microbiomes belonged to an enterotype-like cluster driven by Prevotella (Cluster B) vs. those belonging to an enterotype-like cluster driven by Bacteroides (Cluster B) according to the 1999 World Health Organization criteria with fasting plasma glucose ≥ 7 mmol/L and/or an oral glucose tolerance test (OGTT) 2 h ≥ 11.1 mmol/L and/or a previous diagnosis of type 2 diabetes. The study was approved by the Institutional Review Board at each hospital and is registered at ClinicalTrials.gov (NCT01758471). Written informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Methods

Trial design. This study was a randomised, open-label, two-arm, multicentre clinical trial. Five centres participated in this study: Ruijin Hospital, Renji Hospital, Xinhua Hospital, all affiliated to Shanghai Jiaotong University Medical School, Shanghai General Hospital affiliated to Shanghai Jiaotong University and Shanghai Hospital of Minhang District affiliated to Fudan University Medical School. The study was approved by the Institutional Review Board at each hospital and is registered at ClinicalTrials.gov (NCT01758471). Written informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Randomisation and treatment. After administration of a glucose tolerance test and evaluation of other baseline characteristics, eligible patients were diagnosed according to the 1999 World Health Organization criteria with fasting plasma glucose ≥ 7 mmol/L and/or an oral glucose tolerance test (OGTT) 2 h ≥ 11.1 mmol/L and/or a previous diagnosis of type 2 diabetes. Informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Sample preparation. One day before the first visit, the enrolled patients were confined in the hospital and served the same meals for lunch and dinner. Faeces and urine were collected in dispensable sterile containers. Samples were kept on dry ice before being stored at −80°C. Patients were fasted overnight. At 0800 hours, the patients ingested a carbohydrate meal containing 100 g of flour as an oral glucose load35. Faeces and urine were collected and stored at −80°C. After 3 months of treatment, all patients were again confined in the hospital as for the run-in period, served the same meals for lunch and dinner as during the first visit, fasted overnight and given an oral glucose load the next morning. Blood samples, faeces and urine were collected as described above (more details are provided in the Supplementary Methods, Screening, recruitment, dietary information and follow-up).

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Analysis of richness and biodiversity. Rarefaction and α-diversity analysis (within-sample diversity) were performed as described by Li et al57. The gene counts in each faecal sample were determined as previously reported57. Rarefaction analysis was performed to assess gene richness in the T2D patients before and after the patients had been treated with drugs for 3 months. For the rarefaction analysis, we performed random sampling 100 times in the cohort with replacement and estimated the total number of genes that could be identified from these samples using the Chaos richness estimator. To minimise erroneous identification, only the genes with ≥2 pair of mapped reads were considered to be present in a sample.

Correlations between microbial genes and plasma BA species. The interactions between gene abundance profiles and each of the 15 plasma BA species were assessed by PERMANOVA with the Bray–Curtis distance. The number of permutations was 9999 (performed using the vegan package in R 3.3.1).

Multivariate regression analysis. A GEE analysis (corrected for age, sex and BMI) was performed to explore Acarbose-induced longitudinal associations between changes in (1) BA species and clinical parameters, (2) BA species and the mOTUs significantly altered in relative abundance by Acarbose and (3) the mOTUs significantly altered in relative abundance by Acarbose and clinical parameters. The P value of each regression coefficient was calculated, and an adjusted P value of <0.05 was considered significant (performed using the gee package in R 3.3.1).

All regression analyses between BW and other traits were conducted after adjustment for age and sex, but not BMI.

KEGG enrichment analysis. Differentially enriched KO modules were identified according to their reporter scores from the Z-scores of individual KOs as described previously58. An absolute value of reporter score of 1.95 or higher was used as the detection threshold for significance.

Clustering of microbial community types. Samples were clustered based on the genus profiles. Two enterotype-like clusters were identified in our samples using the method described previously59. In this study, samples were clustered using Jensen–Shannon distance and presented by a PCA (Principal Component Analysis) graph implemented in the ade4 package in R.

Identification of microbial genes involved in BA biotransformation. We retrieved 11 enzymes involved in microbial BA metabolism from UniProt (Uni-Prot Protein Resource) protein database. After removing redundant sequences with greater than 90% identity, a total of 1011 amino acid sequences were obtained.
and used as the reference database for the blast search. Detailed information, including the Enzyme Commission number (EC number), KO number, protein name and search keywords are provided in Supplementary Table 8. The 1011 amino acid sequences were then aligned with the predicted amino acid sequences translated from the 9.9 M gene catalogue using NCBI-BLASTP (E-value < 1E−5 and bit score > 60). In total, 16,102 genes potentially encoding enzymes involved in BA metabolism were identified. Additionally, we assessed the 16,102 potential-hits against KEGG using BlastKOALA (KEGG Orthology And Links Annotation) algorithm and compared the best hits with UniProt based BLASTP results. Accordingly, only genes exhibiting highly consistent annotation (bsh, baE, batG, baiG and 7Fl-hisH) have been included in the analyses (for more detailed description and discussion, see Supplementary Methods, Functional annotation of genes involved in BA synthesis based on BLASTP and BlastKOALA, and Supplementary Data 4–7).

To identify dominant species harbouring BA-metabolising enzymes, we summed the total relative abundances of genes with a potential to encode enzymes involved in BA metabolism and calculated the cumulative relative abundances of these genes according to the contribution of each species harbouring the genes: (relative gene X abundance in one species/total relative abundances of gene X) × 100%. If the species contributed more than 0.5% of the total pool, this species was considered as a dominant contributor.

Statistical analyses. Paired Wilcoxon rank-sum tests were conducted to detect differences in clinical parameters, BAs, genes and mOTUs pre-treatment and post-treatment. P values were adjusted by the Benjamini–Hochberg correction. A P value or an adjusted P value of less than 0.05 was considered significant.

Data availability. Metagenomic sequencing data for all samples have been deposited in the European Bioinformatics Institute (EBI) database under accession code PRJEB12124. All other data are available upon request.

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Additional information

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