Pharmacokinetics of metformin in patients with gastrointestinal intolerance

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Abstract

Aims:

Metformin intolerance symptoms are gastrointestinal in nature, but the underlying mechanism is poorly understood. The aim of this study was to assess potential causes of metformin intolerance including:

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altered metformin uptake from the intestine; increased anaerobic glucose utilisation and subsequent lactate production; altered serotonin uptake; and altered bile acid pool.

Methods:

This pharmacokinetic study recruited ten severely intolerant and ten tolerant individuals matched for age, sex and BMI. A single 500mg dose of metformin was administered, with blood sampling at eleven time points over 24 hours. Blood samples were analysed for metformin, lactate, serotonin, and bile acid concentrations and compared across the phenotypes.

Results:

The intolerant individuals were severely intolerant to 500mg metformin. No significant difference was identified between tolerant and intolerant cohorts in metformin pharmacokinetics: median C_{max} 2.1 (IQR 1.7 – 2.3) and 2.0 (IQR 1.8 – 2.2) mg/L respectively (p = 0.76); t_{max} 2.5 hours; median $AUC_{0\text{-}24}$ 16.9 (IQR 13.9 – 18.6) and 13.9 (IQR 12.9 – 16.8) (mg/L)*h respectively (p = 0.72). Lactate concentration peaked at 3.5 hours, with mean peak concentration of 2.4 mmol/L in both cohorts (95% CIs 2.0 – 2.8, and 1.8 – 3.0 mmol/L respectively), and comparable $iAUC_{0\text{-}24}$: tolerant 6.98 (3.03 –10.93) and intolerant 4.47 (-3.12 – 12.06) mmol/L*h, (p=0.55). Neither serotonin nor bile acid concentrations were significantly different.

Conclusions:

Despite evidence of severe intolerance in our cohort, there was no significant difference in metformin pharmacokinetics or systemic measures of lactate, serotonin or bile acids. This suggests that metformin intolerance may be due to local factors within the lumen or enterocyte.

Introduction

Despite affecting up to 20% of those treated, metformin intolerance is poorly understood[1]. Intolerance to metformin is usually characterised by gastrointestinal side-effects (GI SEs) of nausea, abdominal pain, bloating, or diarrhoea. Gradual up-titration of dose following introduction, or slow release preparations can, in some cases, attenuate symptoms of intolerance. However, in 5% of individuals exposed to metformin, the severity of the GI SE leads to discontinuation of treatment[1]. For others, metformin intolerance may result in sub-optimal dosing or poor compliance. These factors: delay optimal glycaemic control in the individual; result in the addition of, or switch to, alternative oral anti-hyperglycaemic agents; and as a result, potentially contribute to increased risk of microvascular complications of diabetes. Metformin is the first-line pharmaceutical treatment for type 2 diabetes recommended by the ADA-EASD guideline[2]. This, and other guidelines[3], recommends metformin based upon prospective[4-7] and retrospective[8] studies, which demonstrate an improved glycaemic profile with metformin treatment, reduction in cardiovascular mortality[4, 6-8], no associated hypoglycaemia[5], weight neutrality or weight loss[5]. These desirable characteristics, along with its low cost, explain metformin's status as the most extensively prescribed anti-hyperglycaemic agent worldwide. These same characteristics drive the need for ongoing research into the mechanisms underlying intolerance to metformin, aiming to prevent, modulate or treat intolerance. This would not only benefit the individual but could have significant implication for health economy.

Metformin has a complex relationship with the gastrointestinal tract[9]. It is predominantly absorbed from the small intestine, with a bioavailability of approximately 60%[10]. However, it also exerts many effects on the intestine as previously described[9]. Multiple hypotheses for the mechanism of GI intolerance to metformin have been proposed, including abnormal uptake, increased lactate production, accumulation of serotonin, histamine or bile acids.

Metformin uptake from the gut lumen is transporter-dependent[10, 11]. Genetic variation[12-15] in or inhibition[12, 14] of transporters, such as OCT1, could alter metformin uptake from the intestinal lumen to enterocytes, and subsequently affect efflux of metformin across the basolateral membrane to the systemic circulation. This would lead to changes in metformin concentration within the GI tract, enterocytes or systemic circulation.

Previous studies have shown that metformin concentration in enterocytes has been recorded at up to 300 times higher than the systemic concentration[16], and the variation in transporter activity described above could result in even greater differences in some individuals. Metformin is known to increase glucose uptake and anaerobic glucose utilisation in the intestine, resulting in increased lactate production[16-20]. In humans, there is a small but significant increase in systemic lactate when comparing those taking metformin to those who are not[20]. We suggest that metformin intolerance may be associated with an increased concentration of metformin in the intestine, or prolonged exposure of the enterocyte to metformin, leading to a greater increase in anaerobic glucose utilisation and lactate production, than in tolerant individuals. The increase in local lactate concentration may contribute to the intolerance to metformin. Intracellular lactate accumulation will lead to a subsequent increase in measurable serum lactate[20].

Metformin is known to stimulate the release of serotonin from enterochromaffin cells[21], and is a substrate for SERT (serotonin transporter) [14, 21, 22]. Metformin may inhibit the uptake of serotonin from the intestinal lumen, leading to accumulation of serotonin in the gut. Serotonin activates afferent

neurons of the enteric nervous system, and is responsible for peristaltic and secretory reflexes within the intestine, as well as information transmission to the central nervous system[23]. Known serotonergic effects on the gut include nausea, vomiting and diarrhoea[24], which are in-keeping with the GI SEs seen in metformin intolerance. Histamine also increases gut motility[25], and metformin may reduce the enterocytic metabolism of histamine by diamine oxidase[22].

It is recognised that metformin reduces ileal absorption of bile acid[26], leading to an increase in the bile acid pool and potential osmotic diarrhoea. Metformin could potentially alter the deconjugation of primary bile acids to secondary bile acids by bacterial 7α-dehydroxylase[27-29], due to the reduced diversity in the microbiome associated with metformin[30], specifically a reduction in the genera known to produce 7α-dehydroxylase.

This open-label pharmacokinetic study investigated these hypothesised mechanisms for metformin intolerance by studying how individuals tolerant to metformin differed from those who are intolerant. Plasma metformin and serum lactate concentrations were measured, along with targeted metabolomics, in the hours following the administration of a single dose of metformin IR 500mg.

Materials and Methods:

This study was conducted in the Clinical Research Centre (CRC) at Ninewells Hospital, in Dundee, between June 2015 and April 2016. It was co-sponsored by University of Dundee and NHS Tayside, and ethical approval was given by East of Scotland Research Ethics Committee. The study was conducted in accordance with the Good Clinical Practice guidelines, and the Declaration of Helsinki. The study is registered on the public database ClinicalTrials.gov, identifier NCT03361878. Formal written informed consent was obtained from each individual prior to inclusion.

Recruitment and study design

Individuals were recruited if they had type 2 diabetes (T2D), were white European, and met the criteria for tolerance or intolerance to metformin. Metformin intolerant individuals were defined as those who had previously been treated with a maximum of 1000mg metformin daily for a maximum of 8 weeks, and discontinued treatment due to GI upset (Criterion 1). Alternatively, intolerance was defined as inability to increase metformin dose above 500mg without experiencing GI SEs, despite having an HbA1c >53mmol/mol (Criterion 2). Tolerant individuals were defined as those taking 2000mg metformin daily in divided doses, with no GI SEs. Those taking metformin were asked to discontinue their metformin 72 hours prior to the study. The length of washout period was based on an estimated $t_{1/2}$ for plasma metformin of 5.7 hours[10]. Exclusion criteria were: inability to consent; age out with 18 – 90 years of age; eGFR <60 ml/min; pregnancy; history of gastric bypass; evidence of slowed gastric or intestinal motility. None of the patients included were treated with drugs known to affect the pharmacokinetics of metformin in vivo[31]: acarbose[32], cephalexine[33], cimetidine[34], dolutegravir[35], pyramethamine[36], ranolazine[37], trimethoprim[38] or tyrosine kinase inhibitors[39].

Ten metformin intolerant individuals were recruited from the DIRECT[40] cohort in Tayside, eight of whom met intolerance Criterion 1. Ten metformin tolerant individuals were then recruited from the GoDARTS[41] cohort, matching for gender, age, and BMI.

Participants attended the CRC at Ninewells Hospital fasted from midnight. At 0900 (time 0) a blood sample was obtained prior to administration of a single dose of oral metformin (IR) 500mg. Further blood samples were taken at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8 and 24 hours post-metformin. Urine was

collected over the 24 hours following administration of metformin. Participants were given breakfast two hours, and lunch five hours, post-metformin. Plasma metformin and lactate concentrations were measured at all time points, using plasma lactate concentration as a proxy of intestinal lactate production, secondary to metformin concentration within the enterocyte. Plasma lactate was measured using a lactate oxidase method; plasma and urine metformin concentrations were determined using liquid chromatography and tandem mass spectrometry (LC-MS/MS), and the limit of quantification (LOQ) was 0.01 mg/L. Histamine and serotonin, and bile acids were determined using the targeted metabolomic assays Biocrates Absolute*IDQ*™ p180 Kit and Biocrates™ Bile Acids Kit, respectively. Full descriptions of analytical methods are provided in the supplementary methods.

During the study, a Metformin Symptom Severity Score was completed by participants (supplementary methods). This questionnaire details the individual's maximum tolerated dose of metformin, identifies which GI SEs experienced while taking metformin, and scores the severity of the symptoms. This was completed to confirm the phenotype of the cohorts, and gather information as to the nature of the individuals' side effects. The questionnaire was not used as a diagnostic tool in this study, but as a means of characterising the intestinal intolerance experienced and the perceived severity of this. The "true diagnosis" of intolerance was based upon the inclusion criteria alone.

Statistical analysis

The primary endpoint was metformin pharmacokinetics as determined by the area under the curve (AUC) of metformin concentration over time. The study was powered to detect a 30% difference in $AUC_{0\text{-}24}$ of the metformin concentration-time curve, with 80% power, and significance of 5%. This value was chosen based on previous studies by Najib et al[42], and required a cohort of ten metformin-intolerant individuals plus ten metformin-tolerant individuals. The secondary objective of the study was to determine whether systemic lactate concentration, a surrogate for metformin concentration in the enterocyte, is associated with metformin intolerance. Additional objectives included the assessment of serotonin, histamine and bile acids concentrations in acute metformin dosing.

Pharmacokinetic data were analysed using non-compartmental analysis using the R package NCAPPC[43], in conjunction with the Department of Clinical Pharmacology and Pharmacy, Institute of Public Health, University of Southern Denmark. Pharmacokinetic endpoints are presented as median with interquartile range (IQR, 25th-75th percentiles) and geometric mean ratios with 95% confidence intervals. T_{max} was determined visually. Area under the plasma concentration-time curve (AUC) was estimated using the linear-up logarithmic-down method. Statistical significance was determined using unpaired t-test on log-transformed data and accepted at $p<0.05$. Half-life was estimated using the terminal slope ($-k_e$) of the log-transformed plasma metformin concentration-time curve, using the equation $T_{1/2} = \ln(2)/k_e$.

CL_R was estimated using the following equation:

$$CL_R = \text{Amount of substrate in urine}_{0\text{-}24} / \text{AUC of substrate}_{0\text{-}24}$$

CL/F, the apparent total clearance from plasma after oral administration, was calculated using:

$$CL/F = \text{Dose} / \text{AUC of substrate}$$

Bioavailability of metformin was not formally measured, as this requires quantification of faecal recovery of metformin, and stool samples were not obtained. However, estimated fractional drug

availability was calculated, by extrapolating our data to $AUC_{0\text{-}inf}$. By assuming that metformin is completely excreted by the kidneys, CL = CL_R, allowing the calculation of F by:

F = ($AUC_{0\text{-}inf}$ / $AUC_{0\text{-}24}$) x (Amount of metformin in urine $_{0\text{-}24}$ / dose)

Creatinine clearance was calculated using the Cockcroft-Gault equation using Ideal Body Weight (IBW), and corrected for Adjusted Body Weight (ABW = IBW + 0.4 x (actual body weight – IBW)) in those with BMI >25.

All other data were analysed using R studio, and were assessed for normality using Shapiro Wilks method. Those data with a normal distribution are expressed as mean +/- 95% CIs and were compared using unpaired t-test with two tails and unequal variance. Graphic data are plotted as mean ± SEM. Those data with non-normal distribution were expressed as median with IQR and compared using the non-parametric Mann Whitney U test.

Calculation of incremental area under the curve (iAUC) for lactate, serotonin and bile acids used the linear trapezoidal method. For the purpose of this study and to minimize multiple testing penalties, we analysed only serotonin and histamine from the Biocrates p180 panel, and accepted values of $p<0.05$ as statistically significant. For the analysis of the bile acids panel, adjusting for the Bonferroni correction, we accepted $p<0.0024$.

Results:

Baseline characteristics and effect of acute dosing

All 20 participants completed the study, with no withdrawals. The baseline characteristics are listed in Table 1. The cohorts were well matched for gender, age and BMI. There was no significant difference in creatinine clearance between the cohorts. HbA1c was different in the two cohorts: 60.4 (53.3 – 67.5) and 74.1 (69.0 – 79.2) mmol/mol in the tolerant and intolerant cohorts respectively, but this should not impact the pharmacokinetics of metformin. This difference is not surprising as the intolerant cohort have discontinued metformin, and their higher HbA1c may represent the difficulty in optimising their medical management. However, both cohorts had additional anti-hyperglycaemic medications prescribed, including SUs, TZDs, DPP4 inhibitors, GLP1 receptor agonists and insulin. Additional medication was administered two hours post-metformin dosing.

The Metformin Symptom Severity Score was completed by all participants, with a potential score ranging from 0 to 50. The intolerant cohort had a mean severity score of 30.4, much greater than the tolerant cohort's 1.9 ($p<0.0001$). Of the ten tolerant individuals, eight participants scored 0 for the severity score, with the two individuals who scored 8 and 11 having symptoms of IBS which preceded metformin and were unchanged by metformin treatment. Of the intolerant cohort, 70% of participants had previously experienced nausea with metformin, 50% described abdominal pain or bloating, and 50% suffered from diarrhoea.

During the 24 hour study, 9 of the 10 intolerant individuals experienced GI side effects after 500mg of metformin, while none of the tolerant cohort described any symptoms. Of the intolerant cohort, 50% suffered diarrhoea, 50% experienced nausea, with 30% describing abdominal pain, and 20% complaining of bloating (Figure 1; supplementary table 1). However, as this is an open-label study, it is susceptible to reporting bias in those expecting symptoms of intolerance with metformin, with a potential over-reporting of GI symptoms. It should also be noted that the intolerance seen in the 24 hour study period

is acute intolerance. We cannot comment on chronic intolerance, although our inclusion criteria identified individuals with true, chronic intolerance.

Metformin pharmacokinetics in intolerant and tolerant individuals

At time 0 hours (pre-metformin dose) the intolerant group had a plasma metformin concentration, as expected, under the limit of detection. The metformin tolerant group, despite 72 hours of metformin washout, had a detectable metformin concentration, median 0.067 (IQR 0.030 – 0.095) mg/L at baseline. Similarly, at 24 hours, median metformin concentration in the tolerant cohort was higher (0.085, IQR 0.066 – 0.135 mg/L) than the intolerant cohort (0.051, IQR 0.034 – 0.066 mg/L). Although the differences at baseline and at 24 hours post-metformin are significantly different from zero ($p < 0.001$; $p = 0.015$ respectively), the levels are small when compared to the peak metformin concentration after a 500mg of metformin. Peak concentration (C_{max}) for both cohorts was reached at 2.5 hours post-dose, with median C_{max} of 2.1 (IQR 1.7 – 2.3) and 2.0 (IQR 1.8 – 2.2) mg/L for tolerant and intolerant cohorts respectively ($p = 0.76$). The plasma metformin concentrations of the groups, over 24 hours post 500mg dose, were not significantly different, with median $AUC_{0\text{-}24}$ 16.9 and 13.9 (mg/L)*h in the tolerant and intolerant cohorts respectively ($p = 0.72$), as illustrated in Figure 2. The $t_{1/2}$ life of metformin was higher in the tolerant group (4.8 vs 4.1 hours, $p = 0.001$). However, the apparent oral volume of distribution (V/F), apparent total clearance from plasma after oral administration (CL/F), and renal clearance of metformin from the plasma (CL_r) did not differ between the tolerant and intolerant groups (Table 2).

Serum lactate and metformin intolerance

The lactate concentration increased post-metformin with the median time to peak 3.5h post-dose (figure 3). Mean peak lactate concentration was 2.4mmol/L for both groups (tolerant 95%CI 2.0 – 2.8 mmol/L) and (intolerant 95% CI 1.8–3.0mmol/L) groups. There was no significant difference in the incremental $AUC_{0\text{-}24}$ for lactate between the tolerant (6.98 mmol/L*h, 3.03–10.93) and intolerant (4.47 mmol/L*h, -3.12–12.06) groups, p=0.55.

Plasma Serotonin, histamine and bile acid concentrations

The incremental $AUC_{0\text{-}24}$ of the serotonin concentration–time curve did not differ between the cohorts (p = 0.529), and there was no apparent rise in plasma serotonin following metformin dosing in either group (supplementary figure 1). Histamine levels were below the lower limit of detection in the p180 panel for both cohorts.

The Biocrates bile acid panel measures the concentration of twenty different bile acids. There was no difference in incremental $AUC_{0\text{-}24}$ between the tolerant and intolerant cohorts for each individual bile acid, when corrected for multiple testing. Similarly, when considering the bile acids by class – primary, conjugated primary, secondary and conjugated secondary – no significant difference was identified (Supplementary table 2).

Discussion:

Metformin intolerance is a common and costly challenge in the management of type 2 diabetes. Despite metformin's status as first line medical treatment for T2DM, its mechanism of action is still debated. Although widely accepted that metformin acts in the liver to reduce gluconeogenesis[44], there is increasing evidence that metformin may exert some of its effect via the gastrointestinal tract[9], and it is unclear which of these potential mechanisms of action may be linked to metformin intolerance. In this

study of extreme intolerance, we have shown for the first time that metformin intolerance is unlikely to be mediated by differences in absorption, distribution or elimination of metformin. We also demonstrate that intolerance is not associated with lactate derived from anaerobic glucose metabolism in the gut, altered systemic bile acid or serotonin concentration.

Metformin uptake from the intestine is predominantly via three transporters: OCT1, PMAT and SERT. In observational studies using GoDARTS data, Dujic et al demonstrated increased risk of metformin intolerance in those with reduced function alleles for OCT1[12], and latterly SERT transporters[14]. Studies investigating the effect of OCT1 genotype on the pharmacokinetics of metformin have reported varying results. Shu et al show that, following acute dosing with metformin, the area under the plasma concentration–time curve (AUC) of metformin was significantly greater in those with OCT1 variants compared to those with wild type OCT1[45]. However, steady state pharmacokinetics of metformin appear to be independent of OCT1 genotype[46]. Christensen et al identified a number of SNPs in PMAT which were associated with reduced trough steady-state metformin concentrations, significant to p <0.05 level, but this result did not withstand multiple testing[15]. The above studies indicate that systemic metformin concentration may differ according to transporter genotype, and genotype has been associated with risk of intolerance, therefore we wanted to see if systemic metformin concentration was associated with intolerance. Our study shows that, despite a well-defined extreme intolerant phenotype, with 90% of the intolerant participants experiencing symptoms of metformin intolerance after a 500mg dose, neither the C_{max} nor t_{max} (and therefore absorption) of metformin, were significantly different between cohorts (table 2). The lack of association of metformin PK with severe intolerance suggests that the association reported of OCT1 and SERT variants altering metformin intolerance may reflect an impact of these transporter variants on local rather than systemic metformin concentrations.

We identified a surprising difference in baseline metformin concentration, resulting from detectable metformin in the plasma of the tolerant group after 72 hours washout. The detection of metformin after 72 hours washout may represent an improvement in metformin assay: from gas chromatography, to high-performance liquid chromatography and now liquid chromatography with tandem mass spectrometry. Results from the original pharmacokinetic studies of the 1970s would suggest 72 hours without metformin should result in complete washout[47]. The persistence of measurable plasma metformin at 72 hours is likely to be indicative of a two (or more) compartment model, with metformin taken up and released slowly, for example, by erythrocytes. The slow elimination phase of metformin from the erythrocyte compartment has a $t_{1/2}$ of 20 hours[10, 47, 48], compared to a plasma $t_{1/2}$ of 5.7 hours in subjects with normal renal function[10]. This is the likely cause of the difference in the calculated plasma $t_{1/2}$ of the two cohorts, as the tolerant cohort had been at steady state while on metformin and likely had higher metformin accumulation in secondary compartments. In contrast, the intolerant group have depleted secondary compartments, which are absorbing some of the excess metformin, and leading to a shorter elimination half-life.

Where transporter dysfunction may lead to reduced efflux and the systemic concentration of metformin, it may also lead to increased enterocytic or intraluminal metformin concentration. Cycling of metformin between lumen and enterocyte, or uptake to enterocyte with reduced efflux, could lead to increased local metformin concentration. The resulting increase in glucose uptake and anaerobic glucose utilisation, leads to a subsequent rise in intracellular lactate concentration[16-20]. As intracellular lactate rises, it is released into the systemic circulation. Therefore, measuring plasma lactate concentration can be used as a proxy measure of lactate production secondary to intestinal metformin

concentration. Serum lactate concentration was not significantly different between tolerant and intolerant cohorts, indicating that enterocyte metformin concentration was similar in both groups. Both groups did see a rise in lactate from 2 hours, peaking around 3.5 hours post-dose, at a mean maximum concentration of 2.4mmol/L, which is above the normal range in clinical practice. Portal venous sampling for lactate concentration may provide a more accurate measure of intestinal lactate production, when compared to peripheral concentrations, but this is extremely challenging to carry out in humans and beyond the scope of this pharmacokinetic study.

The use of metabolomics to measure serotonin and bile acids gave further insight to metformin intolerance. Serotonin was detectable using the Biocrates p180 panel, but metformin dosing did not increase serotonin concentrations. However, this does not rule out a local effect of metformin on serotonin uptake by SERT. Bile acid concentrations varied post-metformin dosing, however we did not identify a difference in systemic concentrations of the individual or grouped bile acid concentrations between tolerant and intolerant cohorts. There was a trend toward a lower total AUC for DCA (deoxycholic acid – a secondary bile acid from the conversion of cholic acid by 7α-dehydroxylase) in the intolerant group (p = 0.052). This is interesting as most bile acids are reabsorbed in the terminal ileum, whereas DCA is absorbed from the colon[26]. A reduced plasma concentration may indicate a reduced uptake of DCA, resulting in accumulation in the colon, which could potentially lead to bile acid diarrhoea. Further studies are required to investigate the role of the microbiome, and subsequent changes to bile acid metabolism, in metformin intolerance.

We acknowledge this study has a number of limitations. Firstly, the study had a small sample size, but was powered to detect a 30% change in metformin AUC between cohorts. We deemed a priori this would be a clinically important difference when comparing such extremes of intolerance. The similarity in the mean concentrations for the two groups, and overlap of the distributions of individual values, are not consistent with these parameters explaining the mechanism for the marked difference in tolerance seen in these two groups. However, the point estimates for some of the PK parameters and lactate do differ and this difference might achieve statistical significance if the sample size were much larger so it is possible that more subtle differences in metformin PK or the other measures evaluated do contribute to metformin intolerance. Secondly, we observed incomplete washout of metformin in the tolerant cohort which highlights the need for a longer washout in future studies, but as discussed above, the metformin level at baseline was very low when compared to the peak post-dose concentration and did not impact upon the parameters of metformin absorption. Thirdly, metformin is known to increase GLP1, and it is possible that this may lead to gastrointestinal symptoms in some cases. However, we were unable to measure GLP1 in our study cohort, due to the concurrent use of DPP4 inhibitors and GLP1 receptor agonists. Finally, serum lactate concentration increased 2 hours post-metformin dosing, but a potential confounding factor for this rise in lactate is the ingestion of a carbohydrate-rich meal at 2h post-metformin. However, previous studies in healthy volunteers indicate that the lactate concentrations increased transiently to a maximum at 90 minutes post mixed meal, returning to baseline by 180 minutes[49]. Participants in the study received a second carbohydrate-rich meal at 5 hours post-metformin dosing, which did not correspond with a further peak in serum lactate. This supports the conclusion that the rise in and peak lactate concentration is associated primarily with metformin dosing, as opposed to ingestion of a carbohydrate-rich meal.

In conclusion, in this pharmacokinetic study of well-defined extreme metformin intolerant and tolerant individuals, we ruled out multiple potential systemic effects of metformin that may have contributed to

metformin intolerance. We showed that the difference between tolerant and intolerant cohorts in the absorption, distribution or elimination of metformin, or in systemic lactate, serotonin or bile acid concentrations, were too small to be the mechanism of intolerance. It would be interesting to investigate further the link between transporter genotype, pharmacokinetics and tolerance of metformin, as genotype was not considered in this study. To do so, a large recruit-by-genotype study would be necessary. However, our results from this recruit-by-phenotype study suggest that metformin intolerance, is likely to be mediated by local factors within the lumen or enterocyte. There is, therefore, a need to undertake more mechanistic studies that investigate the local (luminal) environment, including the microbiome, in intolerant vs tolerant individuals.

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Abbreviations:

BMI	Body Mass Index
HbA1c	Glycated haemoglobin
OHA	Oral antihyperglycaemic agents
SU	Sulphonylurea
TZD	Thiazolidinedione
DPP4	Dipeptidyl peptidase 4
GLP1 RA	Glucagon-like peptide receptor agonist
MI	Metformin Intolerant
MT	Metformin Tolerant
GI	Gastrointestinal
SEs	Side effects
OCT	Organic cation transporter
PMAT	Plasma membrane monoamine transporter
SERT	Serotonin transporter
$AUC_{0\text{-}24}$	Area under the plasma concentration-time curve between time 0 and 24 hours
C_{max}	Peak concentration
t_{max}	Time to peak concentration
T2D	Type 2 diabetes
V/F	Volume of distribution
CL_r	Renal clearance of the drug from plasma
CL/F	Apparent total clearance of the drug from plasma after oral administration

Figure Legends:

Figure 1: Symptoms of metformin intolerance by phenotype, following a single dose of metformin, 500mg.

Figure 2: Plasma concentration of metformin over time, following a single dose of 500mg given at time 0 hr. Data points are mean ±SEM.

Figure 3: Mean lactate concentration over time, following a single dose of metformin, 500mg at time 0 hr. Data points are mean ±SEM.

Tables

Table 1: Baseline Characteristics

Characteristic	Metformin Tolerant	Metformin Intolerant	*p*
n	10	10	1.000
Female/Male	7/3	7/3	1.000
Age, yrs	67.5 (60.8 – 72.5)	71.0 (65.75 – 80.3)	0.307
Age at diagnosis, yrs	51.5 (51.0 – 58.0)	60.0 (57.3 – 61.8)	0.111
Diabetes duration, yrs	12.0 (9.0 – 15.5)	12.0 (7.5 – 14.8)	0.850
HbA1c,mmol/mol	60.0 (55.0 – 68.0)	72.0 (67.3 – 76.8)	**0.012**
Weight, kg	90.0 (79.0 – 97.2)	91.2 (79.6 – 104.0)	0.910
BMI	34.6 (26.3 – 38.3)	34.3 (29.5 – 38.5)	0.800
Creatinine Clearance	86.3 (76.6 – 107.3)	78.8 (68.3 – 93.2)	0.353
SU (n)	3	6	0.370
DPP4i (n)	3	1	0.582
GLP1 RA (n)	3	1	0.582
TZD (n)	0	2	0.474
Insulin (n)	4	4	1.000

Data are median (IQR); p value for Mann Whitney U test. For categorical data, p value for Fisher exact test. SU = sulphonylurea; GLP1 RA = GLP1 receptor agonist; DPP4i = dipeptidyl peptidase inhibitor; TZD = thiazolidinedione.

Table 2: Pharmacokinetic parameters after acute metformin dosing

	Intolerant, Median (IQR)	Tolerant, Median (IQR)	Geometric Mean Ratio (95% CIs)	P value (unpaired t-test)
AUC ((mg/L)*h)	13.9 (12.9 – 16.8)	16.9 (13.9 – 18.6)	0.95 (0.72 - 1.26)	0.72
C_{max} (mg/L)	2.0 (1.8 – 2.2)	2.1 (1.7 – 2.3)	1.04 (0.83 – 1.30)	0.76
$T_{1/2}$ (h)	4.1 (3.8 – 4.3)	4.8 (4.7 – 5.3)	0.82 (0.76 – 0.89)	<0.001
CL/F (L/h)	35.2 (29.4 – 38.1)	28.6 (25.8 – 34.6)	1.07 (0.81 – 1.43)	0.62
V/F (L)	211.4 (164.0 – 225.8)	197.3 (186.0 – 261.3)	0.88 (0.66 – 1.17)	0.36
CL_R (L/h)	17.6 (13.9 – 25.5)	20.5 (14.7 – 25.2)	0.88 (0.56 – 1.41)	0.59
F (%)	71 (62 – 84)	95 (56 – 101)	0.83 (0.53 – 1.27)	0.38

Figures

Figure 1

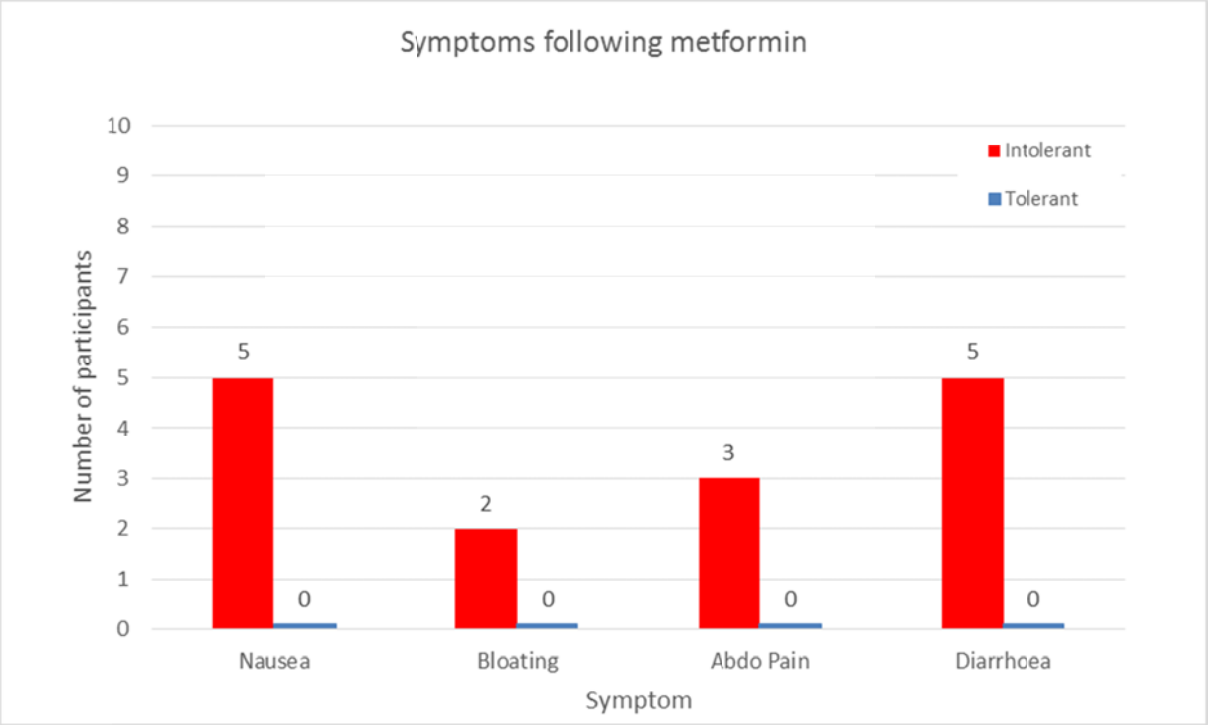

Figure 2

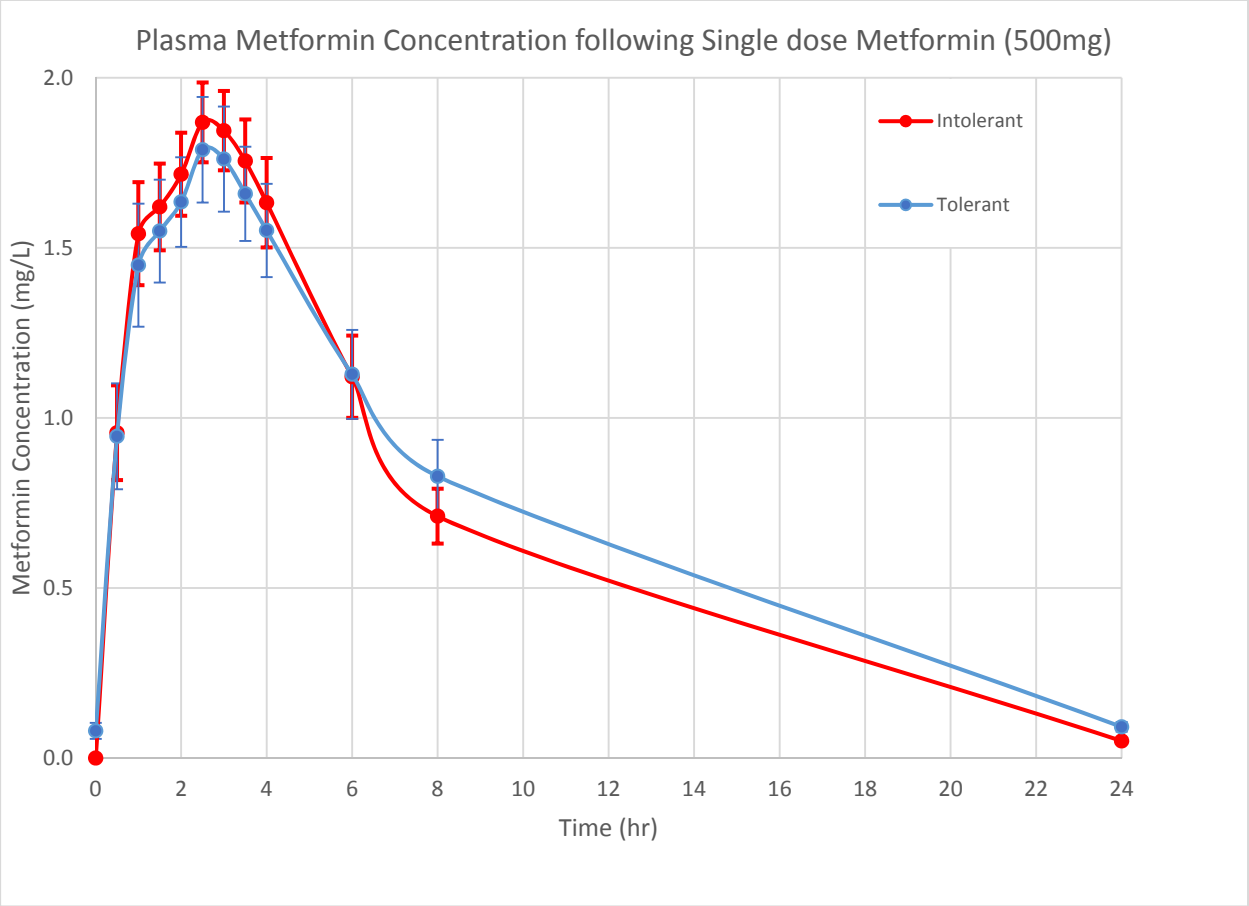

Figure 3