Effect of Metformin on Bile Salt Circulation and Intestinal Motility in Type 2 Diabetes Mellitus

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Gastrointestinal symptoms can be a limiting factor in optimizing metformin therapy, particularly at the onset of treatment. The underlying cause remains unclear. We have investigated whether metformin changes oral-caecal transit and if it causes bile salt malabsorption using the lactulose breath test and orally administered ^{14}C-glycocholate followed by breath $^{14}CO_2$ measurement over 6 h and stool collection for 72 h, respectively. Twenty-four diet and/or sulphonylurea treated patients underwent 7 days of baseline investigations before entering a randomized double-blind crossover study of 21 days duration with either metformin (850 mg bd) or placebo. No difference was observed in the oral-caecal transit time but a change in fasting plasma glucose was observed of 2.6 mmol l^{-1} (95 % CI 1.3, 3.8). Significant increases in percentage $^{14}CO_2$ breath elimination were observed during treatment with metformin (9.7 ± 6.3) compared with placebo (3.1 ± 1.9) $p = 0.020$. In addition, percentage faecal ^{14}C bile salt excretion was increased with metformin (17.2 ± 9.9 vs 10.1 ± 6.9) $p = 0.037$. A significant association ($p = 0.002$) emerged for stool bile salt content and liquidity of the stool. We conclude that metformin may cause gastrointestinal disturbances by reducing ileal bile salt reabsorption leading to elevated colonic bile salt concentrations. © 1998 John Wiley & Sons, Ltd.

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Introduction

Metformin has been used in the treatment of Type 2 diabetes since 1957 and its effectiveness as an antihyperglycaemic agent has been extensively reviewed.[1–3] Some patients experience gastrointestinal side-effects which can limit its clinical application, especially at high doses.[4,5] In clinical trials up to 5 % of patients have to be withdrawn from therapy because of gastrointestinal (GI) symptoms, despite following the standard recommendations to take metformin with food and to increase the dose gradually.[6] While upper GI symptoms are reported by some patients descriptive studies list diarrhoea as the most frequent GI disturbance.[7,8]

Few studies have examined the effects of metformin on gut physiology.[9] It remains unclear whether lower GI symptoms are due to changes in intestinal transit, bacterial colonization of the small bowel, or interruption of the entero-hepatic circulation of bile salts. This study seeks to examine the effects of metformin on small bowel transit and bile salt absorption in Type 2 subjects.

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Patients and Methods

Patients

Twenty-four patients with Type 2 diabetes (16 male) within the age range 40–70 years were recruited from the Diabetes Centre at the North Staffordshire Hospital. Eighteen patients were managed by dietary modification alone and the remaining six patients were controlled by sulphonylurea agents. All patients selected for the study gave written informed consent in accordance with the study protocol approved by the Ethical Committee of the North Staffordshire Health Authority. Patient entry was restricted to those with a history of diabetes of greater than 3 months, fasting glucose control in the range 7–14 mmol l^{-1} and with no history of gut surgery, autonomic neuropathy, gastrointestinal symptoms or taking medication known to interfere with gut function. Patients were excluded from entry if they had been treated with metformin in the last 28 days before enrolment or had a recognized contraindication to metformin therapy.

Study Design

Each subject attended 7 study visits over a period of 35 days (Figure 1). At enrolment (visit 1), a medical history

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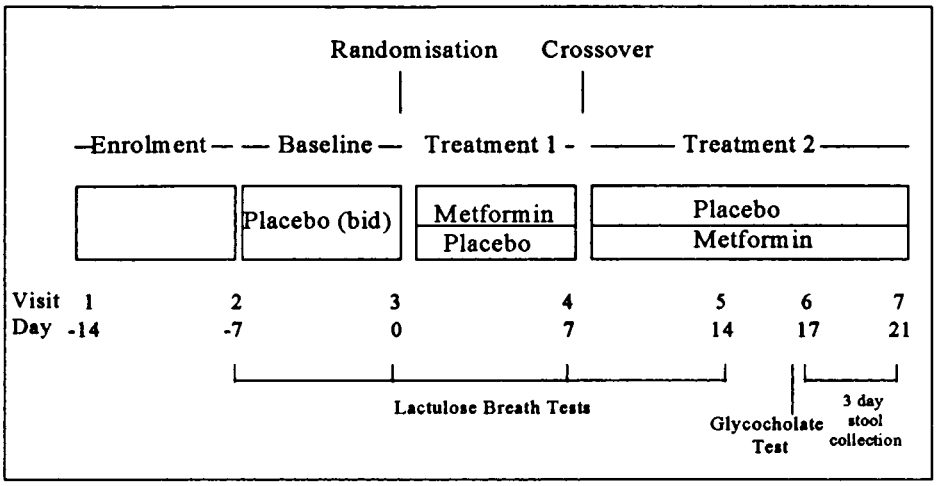

Figure 1. Study flow chart

was taken, concomitant medication noted, and blood samples taken for biochemical and haematological screens. Lactulose breath tests were performed at the second and third visits in order to exclude subjects with either very early breath hydrogen excretion, indicative of bacterial overgrowth, or those with delayed hydrogen production, suggesting prolonged small bowel transit. During this phase, patient evaluation took place under single blind conditions with subjects being given twice daily placebo. Patients meeting the acceptance criteria for motility measurements based on an endpoint measurement of between 40 and 180 min for the lactulose breath test result, were randomly allocated under double-blind conditions to either placebo or metformin (850 mg twice daily) for 1 week after which they were crossed over to the alternative regimen. A lactulose breath test was repeated on completion of 7 days treatment on either placebo or metformin (visits 4 and 5).

As part of the design, patients then entered an extended 7-day period on the second treatment for the purpose of a single measurement of bile salt circulation. At visit 6, patients underwent a 6 h oral ^{14}C glycocholate test and were asked to collect and return stool specimens over the next 72 h to the clinic at visit 7. The test was not performed twice on each patient because of the concerns of radiolabel exposure over the short duration of the study.

At each clinic visit fasting plasma glucose, concomitant medication, and symptoms reported by the patient were recorded. Treatment compliance was assessed by tablet counts.

Assessment of Small Bowel Motility

Investigations of small bowel transit time followed the method of Bond and Levitt[10] as modified by Staniforth.[11] The technique was based on the oral administration of lactulose and the collection of breath hydrogen, an end product liberated from the metabolism of lactulose by colonic bacteria. In brief, after an overnight fast, subjects were instructed to take their morning dose of metformin or placebo and then asked to take up a semi-recumbent position for a period of 4 h. Breath samples were taken at −60, −30, and 0 min before lactulose administration and every 10–20 min for 3 h afterwards. Lactulose was administered as a 20 ml lactulose solution BP (3.35 g 5 ml^{-1}) admixed with 200 g rice pudding. Breath samples were taken at end expiration and analysed for hydrogen content using an electrochemical technique (Hydrogenmeter Gas Measurements Ltd, Renfrew, UK). Oral-caecal transit time was taken as the elapsed time between ingestion of the lactulose and the first sustained increase in hydrogen concentration by at least 15 ppm or more above baseline concentration.

Assessment of Bile Acid Metabolism

Bile acid absorption and excretion were measured using the oral ^{14}C-glycocholate test as described by Fromm and Hofman[12] with the modifications of Scarpello and Sladen.[13] The technique involved the measurement of breath $^{14}CO_2$ excretion over 6 h and faecal ^{14}C excretion over 72 h to distinguish ileal malabsorption of bile salts from bile acid deconjugation arising from bacterial overgrowth in the upper small intestine.

On the day of the ^{14}C-glycocholate test patients were instructed to take their morning dose of metformin or placebo 1 h before the procedure. Subjects were then given 5 μCi of ^{14}C-glycocholate (Radiochemical Centre, Amersham, UK) and unlabelled glycocholate (0.1 mmol) as carrier in 50 ml of aqueous solution. Copper thiocyanate (2.5 mmol) was coadministered as a non-absorbed faecal marker to correct for non-recovery of ^{14}C. Ten minutes later subjects were given a standard test meal and duplicate breath samples were collected every 60 min over 6 h by direct exhalation into liquid scintillation vials containing methyl benzethonium hydroxide (1 mmol) in 2 ml ethanol with phenolphthalein as indicator. They were counted for ^{14}C activity and converted to disintegrations per minute (dpms) by external standardiz-

ation. Breath $^{14}CO_2$ excretion was expressed as a percentage of administered radiolabel.

Subjects were given three weighed containers for 24 h stool collection over the next 3 days. The daily stool output was weighed, qualitatively assessed, and homogenized with a known volume of methanol. All faecal samples requiring hydration with less than 100 ml methanol were assessed as having a liquid composition. Faecal assessments were undertaken by a technician blinded to the treatment code. Duplicated wet samples (0.6 g) were combusted (Packard B306 sample oxidizer) and ^{14}C activity counted (Wallac 1409 liquid scintillation counter) and converted to dpms.

Recovery values for copper content in the samples were measured using atomic absorption spectrometry. All results were expressed as a percentage of the administered dose with correction for the non-recovery of the copper marker.

Statistical Analysis

Results are expressed as mean ± standard deviation or with 95 % confidence intervals (CI), unless indicated otherwise. Measurements of ^{14}C recovery in the breath and faeces in the case of the ^{14}C-glycocholate test were expressed as a percentage of dose administered. Comparative data on small bowel motility for metformin and placebo were analysed by ANOVA for a two-way crossover study. The existence of carry over effects was evaluated using the method described by Hills and Armitage.[14] ^{14}C recovery data in the breath and faeces from the glycocholate test were analysed by a two sample Student *t*-test for unpaired parallel comparisons, after log transformation, or non-parametrically by the Mann–Whitney U-test. Differences were judged to be significant at $p < 0.05$.

Results

Thirty-one patients were enrolled into the study, but allowing for 7 baseline withdrawals (3 severe hyperglycaemia, 1 unstable medical condition, 2 unco-operative, 1 negative lactulose breath test) a total of 24 patients were randomized into the double-blind treatment phase. One patient was withdrawn prematurely due to flatulence and general malaise while on metformin, leaving 23 evaluable patients.

Demographic characteristics

The general characteristics of the study population are given in Table 1. All patients except for one were overweight (BMI >25 kg m^{-2}).

Small Bowel Motility

Comparative data for the time taken for breath hydrogen to exceed 15 ppm above baseline or to peak following lactulose administration are shown for the pre-randomization baseline period and after 7 days of treatment on either placebo and metformin in Figure 2. Considerable variation was observed in repeated measurements of breath hydrogen excretion but the response to metformin treatment remained unchanged in terms of onset or magnitude compared to placebo or pre-randomization baseline values for the lactulose breath test. Neither the net difference in oral-caecal transit time between metformin and placebo (41.3 min CI – 9.2, 91.9 $p = 0.104$) nor the net difference in time to peak on the hydrogen excretion curve (11.3 min CI – 47.9, 25.4 $p = 0.530$) reached statistical significance.

Table 1. Demographic characteristics (mean ± SD)

Gender (M/F)	15/8
Age (yr)	54.0 ± 6.7
BMI (kg m^{-2})	29.7 ± 4.4
Duration of diabetes (yr)	5.9 ± 6.1
Diet/sulphonylurea treated	18/5
Fasting plasma glucose (mmol l^{-1})	11.6 ± 2.6
HbA_{1C} (%) (normal <6.5 %)	10.1 ± 1.1

Bile Salt Metabolism

No significant difference in the mean cumulative excretion of $^{14}CO_2$ in the breath was observed for the first 2 h. By contrast, subsequent breath $^{14}CO_2$ excretion was significantly raised for metformin compared to placebo at each time point for the remainder of the study (Figure 3). At 6 h, the total percentage $^{14}CO_2$ breath elimination was 9.7 ± 6.3 % for metformin and 3.1 ± 1.9 for placebo ($p = 0.020$).

Results for the 72 h faecal collection (Figure 4) showed that mean corrected faecal ^{14}C recovery was increased from 10.1 ± 6.9 to 17.2 ± 9.9 ($p = 0.037$) in response to metformin therapy. While changes in faecal weight remained unchanged ($p = 0.850$), measurements of faecal consistency for all patients showed a strong association with ^{14}C faecal recovery ($p = 0.002$) (Figure 5). Liquid to semi-liquid stools were reported in all patients except one with high faecal ^{14}C recovery compared with healthy volunteers.[13] Eight out of the 13 patients (62 %) with watery stools were on metformin treatment. No significant correlation was observed between stool consistency and the reporting of symptoms of diarrhoea.

Glucose

The mean fasting plasma glucose fell after metformin treatment leaving a mean net difference compared to placebo of 2.59 mmol l^{-1} (95 % CI 1.33, 3.84 $p < 0.001$).

Gastrointestinal Side-effects

A total of 12 patients on metformin and 9 patients on placebo reported side-effects during the course of the

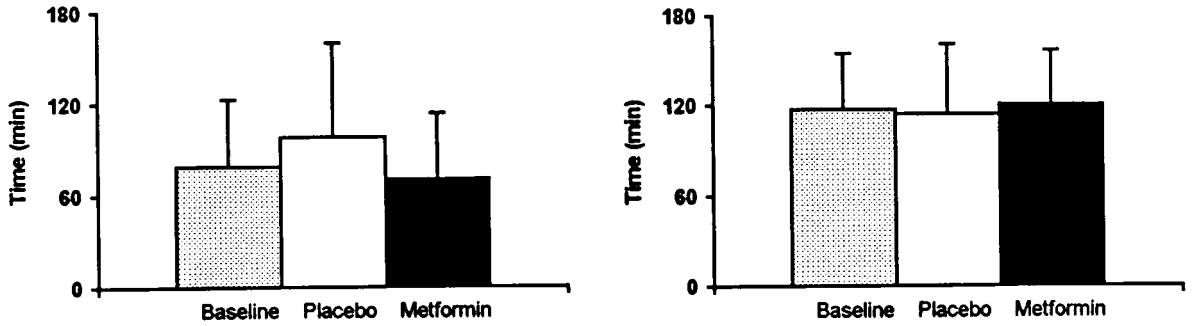

Figure 2. Mean (± SD) for (a) the oral caecal transit time and (b) time to peak hydrogen excretion at the end of the 7-day pre-randomization baseline period ▒ and after 7 days treatment with metformin ■ or placebo □. Transit times were non-significantly different between treatments and unchanged from the 7-day pre-randomization baseline result

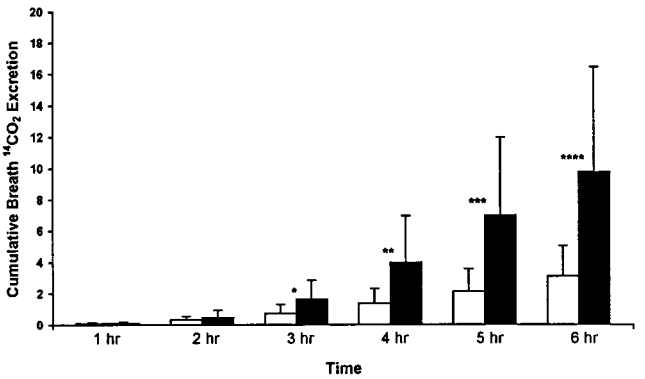

Figure 3. Pattern of deconjugation of ^{14}C-glycocholate after metformin ■ (n = 11) and placebo treatment □ (n = 11). Values are mean ± SD. *p = 0.049, **p = 0.018, ***p = 0.017, **** p = 0.020 Mann–Whitney U-test

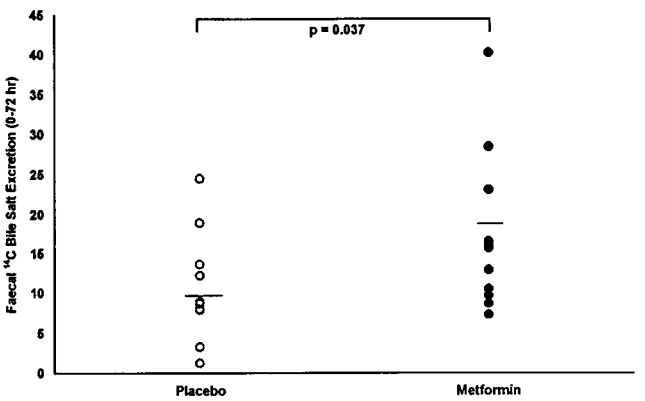

Figure 4. Faecal ^{14}C bile salt excretion values as a percentage of the administered dose after metformin (n = 11) and placebo (n = 11)

study. Symptoms were mainly gastrointestinal, with diarrhoea of greater than 12 h duration being the most frequently reported for metformin (37.5 % of patients versus 12.5 % on placebo).

Discussion

Good glycaemic control is now considered to be the therapeutic aim in patients with Type 2 diabetes. Side-

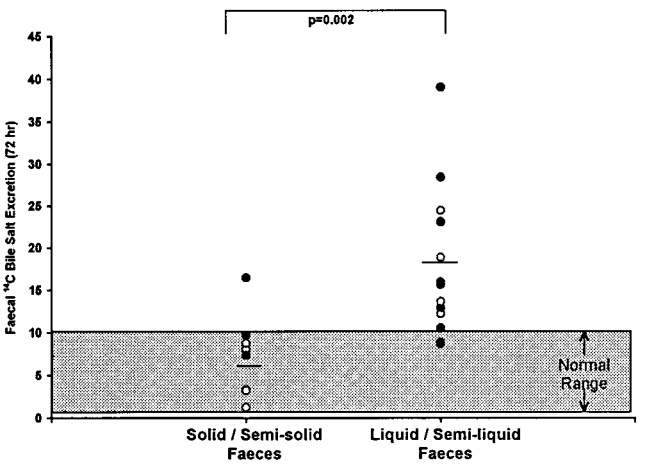

Figure 5. Relationship between faecal bile salt excretion and faecal consistency. Data given for patients on metformin ● and placebo ○ (n = 22)

effects which lead to poor compliance or submaximal dosing can make this strategy difficult to accomplish. For most patients tolerance to metformin is good, especially where the dose is submaximal. A minority of patients complain of gastrointestinal symptoms which limits metformin treatment.

In this study we report the effects of metformin on two potential sources of gut disturbance, altered small bowel transit and interference with bile salt absorption. No effect was observed on small bowel motility. This confirms previous findings in mice,[15] in which metformin had no effect on the transit time of a dye-marked meal. Changes in gastric emptying can affect the oral-caecal transit time. Most studies of gastric emptying in diabetes point towards a delayed response due either to hyperglycaemia or the presence of autonomic neuropathy[16] but this is not a universal finding particularly in the obese subject.[17,18] Previous studies with metformin have shown no effect on gastric emptying in both diabetic[19] and normal subjects.[20]

In contrast to the motility findings, we provide evidence that metformin treatment leads to disturbances in the entero-hepatic circulation of bile salts. The presence of elevated breath $^{14}CO_2$ and ^{14}C faecal excretion point

towards ileal malabsorption of bile salts. Bile salt deconjugation in the small bowel is unlikely since the increase in breath $^{14}CO_2$ was not seen until 3 h and the finding of increased faecal ^{14}C excretion.

Considerable variation in ^{14}C faecal bile salt excretion was observed after metformin and matching placebo treatments. In both cases the variation was as much as 8–10-fold, reflecting significant intersubject differences in bowel behaviour. In contrast to the placebo responses, over 80 % of metformin treated subjects had values in excess of the upper limit of normal for quoted values in non-diabetics subjects.[13]

The finding in all patients of an association between ^{14}C faecal recovery and stool liquid consistency was not unexpected, given the recognized link between faecal bile salts and watery stool formation from the increased osmotic burden. The lack of agreement between stool liquid consistency and self reporting of diarrhoea is surprising but others have reported a poor correlation between GI disturbances and symptoms in diabetic patients.[21]

Effects on bile salt metabolism by metformin have been reported by only one other group.[22] Caspary and colleagues, also using the ^{14}C-glycocholate test, showed in a group of 10 Type 2 patients that 4 days treatment with metformin gave rise to a 5-fold increase in bile acid deconjugation. By contrast with the present study, they found a decrease in faecal bile acid excretion. As deconjugation could be partly reversed by the coadministration of a broad spectrum antibiotic, Caspary concluded that metformin treatment gave rise to bacterial overgrowth in the small bowel. This hypothesis was not confirmed in patients with subclinical vitamin B12 deficiency on chronic metformin therapy.[23] In this case reversal was only possible by drug withdrawal and not tetracycline treatment.

Support for our findings has been demonstrated in animal studies when metformin has been shown to increase faecal bile salt excretion.[24] Oral metformin caused significantly more malabsorption than parenteral metformin. This may in part be due to tissue distribution of metformin since kinetic studies have shown higher sustained drug levels in the intestinal wall after oral therapy compared to the intravenous route.[25]

The aetiology of metformin-induced bile salt malabsorption is unclear. Bile salt transport has been examined by Caspary *et al*.[26] and the results from tissue uptake studies in rat jejunum and ileum suggest that metformin inhibits active transport systems in the ileum with no effect on passive diffusion in the jejunum. As therapeutic concentrations of metformin do not measurably alter the ATP content or redox state of cells[27] and the binding affinity of metformin for mitochondrial membranes is low further studies are warranted to elucidate the effect of metformin on the bile salt transporter.

The incidence of gastrointestinal symptoms was high during both phases of the study but, apart from two patients on metformin, these were mild to moderate in severity, intermittent, and did not give rise to premature withdrawal. Both the intense nature of the study design and the use of lactulose were viewed as possible explanations for the level of adverse event reporting.

The observed improvement in glycaemia was expected since previous studies have shown the acute response to metformin after short-term exposure.[28]

It is clear from the present study that metformin treatment is associated with disturbances in bile salt metabolism. This might provide an explanation for the cholesterol lowering action of the drug. While the underlying cause of increased deconjugation of bile acids remains unknown, the results eliminate changes to small bowel motility as a possible explanation. The increased osmotic burden in the colon is a possible explanation for watery stool formation in patients on metformin particularly at treatment onset. Further studies are warranted to define the causative factors in metformin-induced diarrhoea with a view to improving treatment.

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