






ORIGINAL ARTICLE

Initiation of glucose-lowering drugs reduces the anticoagulant effect of warfarin—But not through altered drug metabolism in patients with type 2 diabetes

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Aims: Drug metabolism might be altered in patients with type 2 diabetes. We aimed to evaluate if initiation of glucose-lowering drugs impacts warfarin efficacy and drug metabolism.

Methods: First, we conducted a register-based self-controlled cohort study on Danish and Scottish warfarin users. Warfarin efficacy (international normalized ratio [INR]) was compared before and after initiation of glucose-lowering drugs. Second, we conducted a clinical pharmacokinetic trial comprising treatment-naïve type 2 diabetes patients. Patients ingested probe drugs for drug-metabolizing enzymes (the Basel Cocktail) before initiating glucose-lowering treatment, and after 3 and 12 weeks of treatment. Drug metabolism, glycaemic control, and inflammation were assessed on each visit.

Results: In the Danish and Scottish cohorts ($n = 982$ and $n = 44$, respectively), initiating glucose-lowering drugs reduced warfarin efficacy. INR decreased from 2.47 to 2.21 in the Danish cohort (mean difference -0.26 ; 95% CI -0.35 ; -0.17) and from 2.33 to 2.13 in the Scottish cohort (-0.21 ; 95% CI -0.52 ; 0.11) after initiation of glucose-lowering treatment. This impact on INR was more pronounced among individuals with stronger effects of glucose-lowering treatment. In the clinical pharmacokinetic trial ($n = 10$), initiating metformin did not affect drug metabolism after 3 weeks (geometric mean ratio of CYP3A metabolic ratio: 1.12 [95% CI: 0.95; 1.32]) or 12 weeks of metformin treatment. Glycaemic control improved during treatment, while inflammation remained low and unchanged during treatment.

Registry number: [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT04504045) identifier NCT04504045.

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Conclusions: In conclusion, initiation of glucose-lowering drugs among chronic warfarin users seems associated with a reduction in INR, particularly among individuals with a large decrease in HbA_{1c}. This effect seems unrelated to CYP enzyme activity and warfarin drug metabolism.

KEYWORDS

drug–drug interaction, glucose-lowering drugs, metformin, pharmacokinetics, translational research, type 2 diabetes, warfarin

1 | INTRODUCTION

Type 2 diabetes is a chronic metabolic disease caused by a combination of insulin deficiency and insulin resistance and affects more than 400 million individuals worldwide.¹ Pharmacotherapy is the cornerstone of diabetes management, including glucose-lowering drugs to prevent diabetes-related comorbidities. The most widely used drugs are metabolized by **cytochrome P450** (CYP) enzymes,² and potential drug–drug interactions are of utmost importance as altered drug metabolism may affect clinical outcomes.

The recent introduction of novel glucose-lowering drugs³ has changed the landscape of diabetes management. However, metformin remains the first-line treatment for type 2 diabetes.^{4,5} Metformin is not metabolized by CYP enzymes and is excreted unchanged renally.⁶ Due to these properties, metformin rarely causes drug–drug interactions.⁷ However, some studies have shown that initiation of metformin caused a decreased efficacy of the anticoagulant vitamin K antagonists warfarin and phenprocoumon.^{8,9} Surprisingly, warfarin's decreased efficacy was also observed following the initiation of insulin and sulfonylureas.⁸ The observed effect across drug classes is counterintuitive since the glucose-lowering drugs are metabolized and excreted through different pathways. Warfarin is mainly metabolized by CYP2C9 and CYP3A4 with contribution from multiple other CYP enzymes.¹⁰ Together, glucose-lowering drugs are not expected to affect warfarin metabolism through direct drug–drug interactions. This surprising finding led us to hypothesize that the glucose-lowering effect leads to altered activity of drug-metabolizing CYP enzymes.

Diabetes development and progression are associated with inflammation,¹¹ and inflammation is associated with decreased activity of drug-metabolizing enzymes.¹² Patients with type 2 diabetes have reduced activity of drug-metabolizing enzymes compared to non-diabetic individuals.^{13–15} Still, it is unknown if the activity of CYP enzymes is altered by the initiation of glucose-lowering drugs in patients with type 2 diabetes.

We aim to evaluate the impact of initiating glucose-lowering drugs on drug metabolism. First, we assessed if glucose-lowering therapy affects warfarin efficacy in a register-based study of two cohorts. Second, we aimed to establish the impact of this proposed interaction in a clinical pharmacokinetic trial, comparing the activity of drug-metabolizing enzymes before and after initiation of metformin in treatment-naïve patients with type 2 diabetes.

What is already known about this subject

- Initiation of glucose-lowering therapy has previously been linked to reduced efficacy of warfarin.
- Patients with type 2 diabetes might have reduced capacity of drug-metabolizing enzymes. Glucose-lowering drugs might reverse this suppression and normalize drug metabolism.

What this study adds

- Anticoagulant efficacy of warfarin decreases after initiation of glucose-lowering drugs, particularly among individuals with a substantial glycaemic response in register-based studies.
- Drug metabolism is not affected by initiation of metformin among patients with treatment-naïve type 2 diabetes in a clinical pharmacokinetic trial.

2 | METHODS

2.1 | Register-based study

2.1.1 | Data sources and setting

Warfarin users were identified using two independent data sources. The Danish cohort was based on the Copenhagen Primary Care Laboratory (CopLab) database, covering 1.3 million individuals from 2000 to 2015.¹⁶ Data were linked to the Danish Prescription Registry¹⁷ using the unique individual identification number.¹⁸ The Scottish cohort was based on laboratory and point-of-care data that covered approximately 400 000 individuals from the areas of Tayside (1992–2021) and Fife (2005–2021). Data were linked to prescription encashment data from Tayside and Fife using the NHS unique individual Community Health Index (CHI) identifier.¹⁹

2.1.2 | Population

Among warfarin users, we identified individuals with the first prescription of a glucose-lowering drug, defined as the index date. Ongoing long-term treatment was assured by a filled warfarin prescription 180 days before the index date and measurement of INR in the range of 8 weeks before to 12 weeks after the index date. We assessed glucose-lowering drugs within each drug class, for example, insulins, biguanides, sulfonylureas, thiazolidinediones, dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide-1 (GLP-1) agonists, and sodium-glucose co-transporter 2 (SGLT2) inhibitors. The Danish cohort did not include users of thiazolidinediones or SGLT-2 inhibitors due to a scarce number of prescriptions during the available study period. We excluded individuals with prescriptions for the same glucose-lowering drug within 2 years of the index date to ensure initial use. We also excluded individuals initiating another glucose-lowering drug within 120 days of the index date to avoid conflating effects of individual glucose-lowering drugs. Individuals must be over 18 years of age at the index date.

2.1.3 | Outcome

In a self-controlled design, we compared the INR levels 1–4 weeks after (short-term effect) and 5–7 weeks (long-term effect) after the index date, with the last INR measurement within 8 weeks before the index date. We assessed the proportion of individuals with one INR measurement below the therapeutic limit ($\text{INR} < 2$) 1–6 weeks before compared to 5 weeks after the index date. We conducted several sensitivity analyses in the Scottish cohort. First, we included only individuals at the first-ever prescription of any glucose-lowering drug in the study period. Second, we assessed INR levels in different time ranges before and after the index date. Lastly, we assessed potential confounding by warfarin indication or concomitant use of potential inhibitors (amiodarone, fluconazole, erythromycin, ciprofloxacin and metronidazole).

We assessed the relationship between INR difference and the last glycated haemoglobin (HbA_{1c}) measurement within 1 year of the index date. Pre-index HbA_{1c} levels were categorized into four groups: <48 mmol/mol, 48–57 mmol/mol, 58–75 mmol/mol and >75 mmol/mol. Furthermore, we assessed the change in HbA_{1c} following the initiation of a glucose-lowering drug; we compared the last measurement within 1 year of the index date with the first measurement within 2–6 months after the index date. The change in HbA_{1c} was categorized into three groups: an increase (difference ≥ 0), a small decrease (0 to 10 mmol/mol) and a large decrease (more than 10 mmol/mol).

2.1.4 | Ethics

The Danish cohort was exempt from ethical approval according to Danish law. The Scottish cohort was covered by general ethics and data protection approvals for anonymized record linkage studies, which were obtained for projects hosted by the Dundee Health Informatics Centre.

2.1.5 | Statistics

Differences in INR were tested by paired *t*-test and non-parametric Wilcoxon signed-rank test for small samples. Only results with $n > 5$ are shown to limit random effects by low power.

2.2 | Clinical pharmacokinetic trial

We conducted a self-controlled, clinical pharmacokinetic trial to assess the impact of initiating metformin treatment on the activity of drug-metabolizing enzymes in patients with treatment-naïve type 2 diabetes.

2.2.1 | Trial participants

Patients with type 2 diabetes were recruited at multiple general practices across the region of Funen, Denmark, from December 2020 to May 2022. Eligible patients were aged 18–75 years with a body mass index (BMI) ≤ 40 kg/m² and no current use of glucose-lowering treatment. Individuals were excluded if they suffered from self-reported inflammatory diseases or cancer, had excessive alcohol consumption defined according to national guidelines, or took medication deemed to affect the safety of the individual or the outcome of the trial. A blood sample was conducted before inclusion to confirm $\text{HbA}_{1c} \geq 48$ mmol/mol and glutamic acid decarboxylase (GAD-65) antibody, liver and kidney function within normal range.

2.2.2 | Trial medication and dose

The patients self-administered 500 mg metformin twice daily on Days 2–8. On Day 9 and the remaining trial period, the administered dose was increased to 1000 mg metformin twice daily (Figure S1). Both amounts are used in routine clinical practice. Compliance was assessed by interviewing and counting tablets, and at least 70% compliance was required to be included in the data analysis. The Basel cocktail was administered orally on the three trial days and consisted of 100 mg caffeine, 50 mg efavirenz, 12.5 mg losartan, 10 mg omeprazole, 12.5 mg metoprolol and 2 mg midazolam. Complete manufacturing details are provided in the Supporting Information. The Basel cocktail has previously been validated,^{20,21} and all medications were available in Denmark.

2.2.3 | Trial days

Patients participated in three trial days: before metformin (baseline, Day 1), after 3 weeks of metformin treatment (Day 22 ± 3 days), and after 12 weeks of metformin treatment (Day 85 ± 3 days) (Figure S1). The patients fasted, except for water, for 12 h before the trial days,

and fasting was continued until 3 h after ingestion of the Basel cocktail. In addition, 48 h before each trial day, the patients were restricted from consuming bitter oranges, grapefruit, alcohol, caffeine and theobromine. The Basel cocktail was administered with 75 g glucose in an oral solution to perform an oral glucose tolerance test (OGTT). Blood samples were collected at 0 (before administration of the Basel cocktail), 0.5, 1, 1.5, 2, 4 and 6 h (Figure S1). Urine was collected from 0 to 6 h.

2.2.4 | Trial approvals and registrations

The trial was approved by the Regional Scientific Ethics Committee of Southern Denmark (identifier S-20200014), the Danish Medicines Agency (identifier 2019111719), and registered in the EudraCT database (identifier 2020-000162-42) and [ClinicalTrials.gov](https://clinicaltrials.gov) database (identifier NCT04504045). The trial was conducted according to the Helsinki Declaration and Good Clinical Practice (GCP) and monitored by the GCP unit at Odense University Hospital. Trial subjects were included after providing written, informed consent.

2.2.5 | Analytical methods

Analyses for drug and metabolite concentrations were conducted at the University of Southern Denmark. All samples were analysed at the end of the trial and blinded to clinical data. Drugs and metabolites were analysed in ethylenediaminetetraacetic acid (EDTA) plasma and urine after a sample pretreatment procedure with enzyme deglucuronidation followed by protein precipitation with standard internal solutions. We used high-performance liquid chromatography (LC) and high-resolution mass spectrometry (HR-MS) following an approach previously described with minor modifications to fit HR-MS.^{20,22–24} We used quality control (QC) samples, blanks and calibration curves in each batch to assess precision and accuracy. The within-batch and between-batch precision (coefficient of variation [CV]) and accuracy (bias) were <15% for all compounds. The calibration curves were linear with $r^2 > 0.99$. The limit of detection (LOD) ranged from 0.1 to 1 ng/mL, and the limit of quantification (LOQ) ranged from 0.5 to 5 ng/mL (Supporting Information).

Glucose and HbA_{1c} were analysed continuously during the trial as a part of the daily routine at the Odense University Hospital. Samples were analysed using Cobas 8000 Roche and TOSOH G8, Alere, respectively. Insulin and C-peptide were analysed at the end of the trial using Cobas E411.

Analyses for hsCRP, IL-6, IL-1 β , TNF- α and IFN- γ were performed at Lillebælt Hospital. hsCRP was analysed using Roche/Hitachi Cobas c701/702. IL-6 was measured using Cobas e801. Commercially available kits for the Simoa HD-X analyser (Quanterix®, Billerica, MA, USA) were used to quantify IL-1 β , TNF- α and IFN- γ in plasma

according to the manufacturer's procedure. Plasma samples were analysed blinded to clinical data. Quality control was performed using two controls prepared from commercially available control material provided by the manufacturer. The analytical variations were calculated to be <16% in the IL-1 β assay, <13% in the TNF- α assay and <6% in the IFN- γ assay.

Genotyping was conducted using a previously validated 5' nuclease real-time polymerase chain reaction (PCR) test panel²⁵ to identify the most common CYP2C9 alleles (*1, *2, *3), CYP2C19 alleles (*1, *2, *3, *4 and *17) and CYP2D6 alleles (*1, *2, *3, *4, *5, *6, *9, *10, *17 and *41 or gene duplicates).

2.2.6 | Statistics and pharmacokinetic analyses

We aimed to include 12 patients in the trial to detect a $\geq 35\%$ change in midazolam metabolic ratio with 80% power, a two-sided significance level of 5%, and allowing for a 20% drop-out rate. Recruitment was delayed due to the COVID-19 pandemic, and the trial ended prematurely on 5 May 2022 following the completion of trial subject number 10. Statistical and pharmacokinetic analyses were conducted as previously described.²⁶ In brief, demographic data and pharmacokinetic parameters are shown as medians with interquartile ranges (IQR; 25th–75th percentiles) or geometric mean ratios (GMR) with 95% confidence intervals. Non-compartmental analysis was computed using the R package PKNCA.²⁷ AUC_{inf} is only presented when the extrapolated AUC percent is below 25%. Post-hoc analysis of efavirenz elimination half-life was calculated with non-compartmental analysis using the concentration from 0 h at 3 weeks as a data point for the baseline analysis and time-after-dose was calculated based on days between visits. In the primary analysis, the metabolic ratio was calculated as [drug]/[metabolite] at the time points previously shown to have the highest correlation to the AUC ratio.²¹ The formation clearances (CL_f) were estimated as [amount of metabolite in urine_{0–6h}] over [AUC of substrate_{0–6h}], and renal clearances (CL_R) were calculated as [amount of substrate in urine_{0–6h}] over [AUC of substrate_{0–6h}]. AUC was determined for glucose, insulin and C-peptide using the same methods as the pharmacokinetic parameters.²⁶

To assess the random effects of baseline HbA_{1c} and change in HbA_{1c}, we used a longitudinal mixed-effect model with the metabolic ratio as the outcome and a fixed interaction term between the variable and time. Change in HbA_{1c} was grouped in the same ranges as used in the register-based study.

2.3 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22.²⁸

3 | RESULTS

3.1 | Register-based study

The Danish and Scottish cohorts included 1026 individuals (982 and 44, respectively). The median age was 73 years (IQR: 65–80) and 74 years (IQR: 67–79), respectively. Males comprised 56% of the Danish and 52% of the Scottish cohorts.

In the Danish cohort, initiation of any glucose-lowering drug caused a mean change in INR of -0.22 (95% CI: -0.30 ; -0.13) 1–4 weeks after the index date. A similar change was observed 5–7 weeks after the index date (Table 1). Analysis of the individual drug classes showed similar effects for insulin, metformin and sulphonylureas, while data on DPP-4 inhibitors and GLP-1 receptor agonists did not affect warfarin efficacy (Table 1). Data from the Scottish cohort

showed no effect for all glucose-lowering drugs 1–4 weeks after the index date (Table 1), whereas 5–7 weeks after the index date, the mean INR changed by -0.21 (95% CI: -0.52 ; -0.11). A sensitivity analysis, including only the first-ever prescription of any glucose-lowering drug in the Scottish cohort, provided comparable estimates for metformin between the two cohorts (mean INR difference -0.18 (95% CI: -0.69 ; 0.33 ; $n = 13$) 1–4 weeks after the index date and -0.32 (95% CI: -0.84 ; 0.20 ; $n = 14$) 5–7 weeks after the index date). The remaining sensitivity analyses did not yield different results than the main estimates (data not shown).

Baseline HbA_{1c} did not affect the difference in INR levels following the initiation of glucose-lowering treatment (Figure 1A,C). However, the change in HbA_{1c} levels after initiation of glucose-lowering therapy compared to baseline is associated with the degree of INR reduction. Patients with a large decrease in HbA_{1c} (more than

TABLE 1 The anticoagulant efficacy of warfarin is reduced among patients initiating glucose-lowering treatment in the Danish and Scottish cohorts.

	Danish cohort			Scottish cohort		
	<i>n</i>	Mean INR Before/after	Mean difference (95% CI)	<i>n</i>	Mean INR Before/after	Mean difference (95% CI)
All glucose-lowering drugs						
1–3 weeks after	677	2.49/2.27	-0.22 (-0.30 ; -0.13)	32	2.55/2.50	-0.05 (-0.36 ; 0.26)
5–7 weeks after	539	2.47/2.21	-0.26 (-0.35 ; -0.17)	31	2.33/2.13	-0.21 (-0.52 ; 0.11)
INR < 2	540	28.3%/39.3%	-	30	45.5%/43.3%	-
Insulin						
1–3 weeks after	192	2.44/2.08	-0.36 (-0.53 ; -0.19)	17	2.52/2.96	0.45 (-0.69 ; 1.58)
5–7 weeks after	156	2.44/2.26	-0.18 (-0.39 ; 0.02)	15	2.66/2.76	0.11 (-0.58 ; 0.79)
INR < 2	170	41.8%/55.3%	-	16	66.7%/25.0%	-
Metformin						
1–3 weeks after	626	2.48/2.30	-0.18 (-0.26 ; -0.09)	24	2.38/2.50	0.12 (-0.25 ; 0.49)
5–7 weeks after	523	2.42/2.20	-0.22 (-0.30 ; -0.14)	21	2.32/2.25	-0.08 (-0.46 ; 0.31)
INR < 2	496	28.5%/36.1%	-	22	39.1%/36.4%	-
Sulphonylureas						
1–3 weeks after	329	2.45/2.28	-0.17 (-0.28 ; -0.06)	23	2.62/2.72	0.10 (-0.28 ; 0.48)
5–7 weeks after	244	2.45/2.27	-0.18 (-0.31 ; -0.05)	22	2.41/2.51	0.12 (-0.50 ; 0.74)
INR < 2	267	29.9%/41.9%	-	21	40.9%/47.6%	-
DPP-4 inhibitor						
1–3 weeks after	138	2.38/2.31	-0.08 (-0.28 ; 0.12)	-	-	-
5–7 weeks after	103	2.39/2.42	0.03 (-0.20 ; 0.26)	-	-	-
INR < 2	113	34.9%/31.9%	-	-	-	-
GLP-1 receptor agonist						
1–3 weeks after	32	2.26/2.32	0.06 (-0.11 ; 0.22)	-	-	-
5–7 weeks after	26	2.37/2.72	0.35 (-0.02 ; 0.72)	-	-	-
INR < 2	30	17.2%/30.0%	-	-	-	-

Note: Warfarin efficacy was assessed by the international normalized ratio (INR) in patients treated with warfarin before and after initiation of glucose-lowering treatment. In the Danish cohort, more patients experience INR below the therapeutic range (INR < 2) 1–3 weeks after initiating glucose-lowering treatment compared to 2–4 weeks before.

Abbreviations: CI, confidence interval; DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide 1; INR, international normalized ratio.

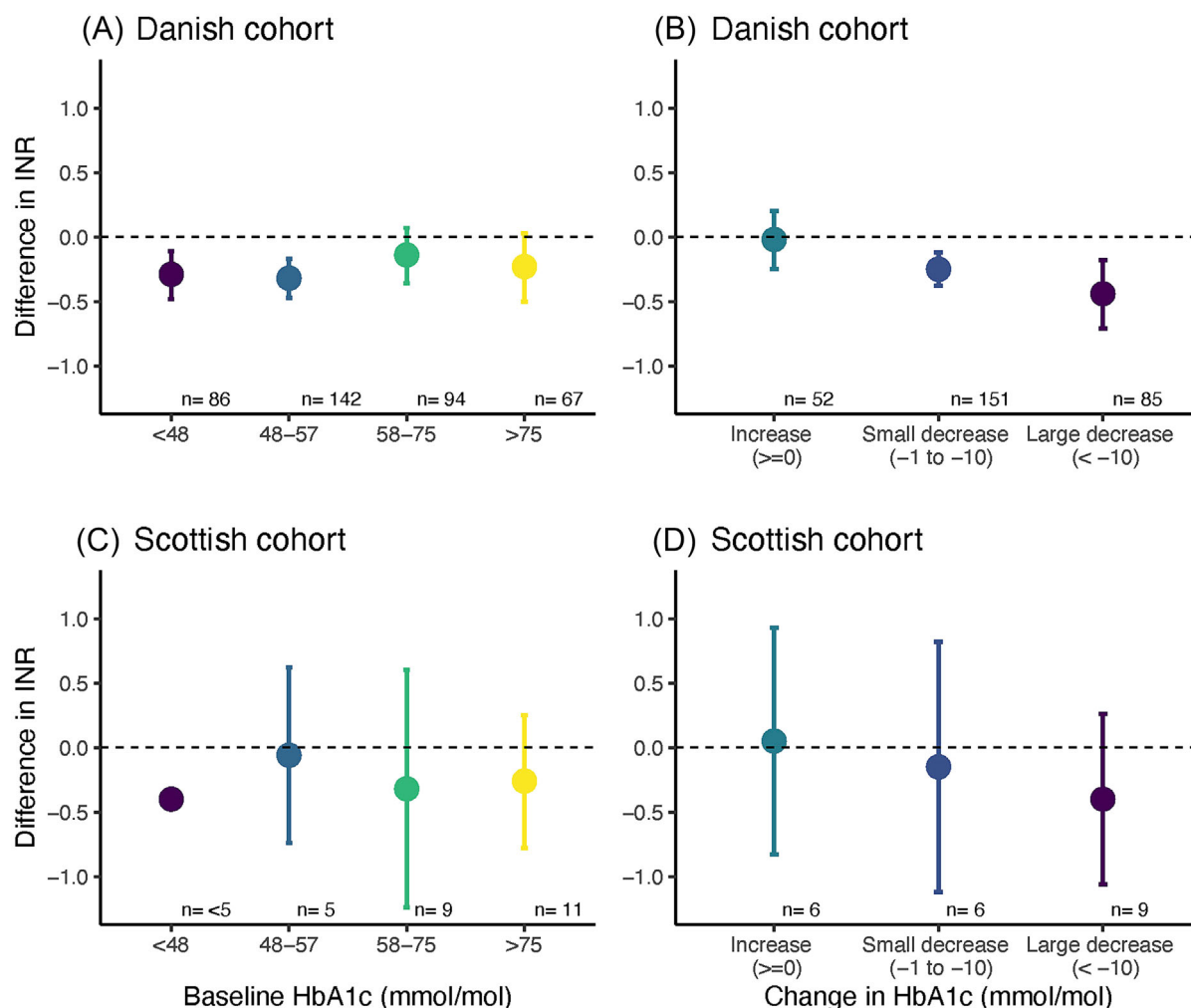


FIGURE 1 Initiation of glucose-lowering treatment reduces the anticoagulant efficacy of warfarin and the effect is associated with the change in glycated haemoglobin (HbA_{1c}) during treatment. (A) and (C) Association between baseline HbA_{1c} and change in INR before and 5–7 weeks after initiation of glucose-lowering therapy in the Danish and Scottish cohorts. (B) and (D) Association between change in HbA_{1c} from before to 2–6 months after initiation of glucose-lowering treatment and change in INR before and 5–7 weeks after initiation of glucose-lowering therapy in the Danish and Scottish cohorts.

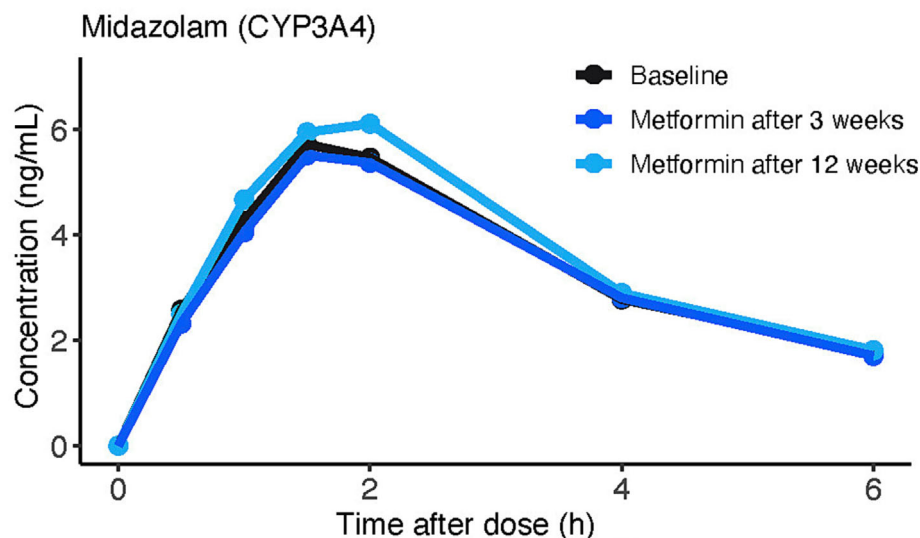


FIGURE 2 The pharmacokinetics of midazolam (CYP3A) is unaffected by 3 and 12 weeks of metformin treatment in patients with treatment-naïve type 2 diabetes. The concentration–time curves illustrate mean plasma concentrations. Data are based on nine subjects.

10 mmol/mol) had a more pronounced reduction in INR, mean difference -0.44 (95% CI -0.71 ; -0.18) and -0.40 (95% CI -1.06 ; 0.26), in the Danish and Scottish cohort, respectively (Figure 1B,D).

3.2 | Clinical pharmacokinetic trial

We screened 20 individuals for eligibility and included 10 patients with treatment-naïve type 2 diabetes (Figure S1). Their median HbA_{1c} was 56 mmol/mol (range 48–102) at inclusion. The median age at inclusion was 61 years (IQR: 59–67 years), all were of white descent, and 90% were male. Two patients were smokers. The median number of concomitant drugs was two (IQR: 2–5); most prevalently used were lipid-modifying drugs (60%), thiazides (40%), calcium channel

blockers (40%) and ACE inhibitors (40%). Two patients did not receive any concomitant treatment before the study. During the trial, seven patients experienced a total of nine adverse events; four experienced gastrointestinal adverse events related to metformin, two experienced drowsiness associated with midazolam and three reported unrelated adverse events. One patient started treatment with an SGLT2 inhibitor after the Baseline visit (Day 2) due to severe symptomatic hyperglycaemia.

All patients were compliant, with metformin intake >90% of expected. Complete pharmacokinetic data were obtained for midazolam (CYP3A) due to the short elimination half-life. Compared to baseline, 3 and 12 weeks of metformin treatment did not result in clinically or statistically significant changes in midazolam pharmacokinetics (Figures 2 and 3 and Table S1). The metabolic ratio of caffeine

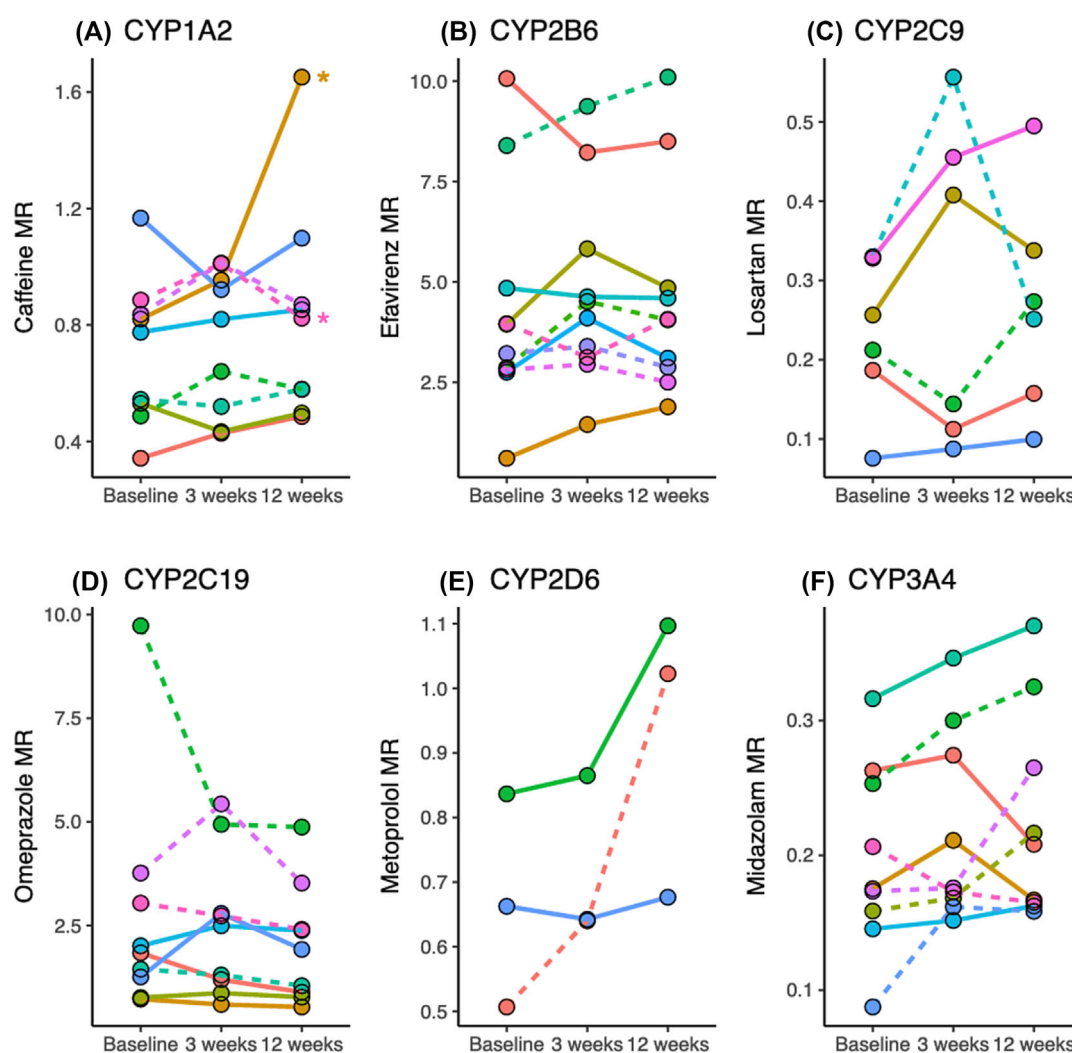


FIGURE 3 The activity of drug-metabolizing enzymes is unaffected by 3 and 12 weeks of metformin treatment in patients with treatment-naïve type 2 diabetes. Individual metabolic ratios (MR) for each of the six probe drugs are illustrated, and each patient is illustrated with the same colour as in Figures 4 and 5. Patients with a large decrease in HbA_{1c} (> -10 mmol/mol) after 12 weeks are shown as dashed lines. (A) Metabolic ratio [caffeine]/[paraxanthine] at 4 h ($n = 9$). * indicates the two patients who were smoking before and during the trial. (B) Metabolic ratio [efavirenz]/[8-hydroxyefavirenz] at 6 h. (C) Metabolic ratio [losartan]/[E3174] at 6 h ($n = 6$). (D) Metabolic ratio [omeprazole]/[hydroxyomeprazole] at 4 h ($n = 9$). (E) Metabolic ratio [metoprolol]/[5-hydroxymetoprolol] at 6 h ($n = 3$). (F) Metabolic ratio [midazolam]/[α -hydroxymidazolam] at 2 h ($n = 9$). Abbreviations: CYP, cytochrome P450 enzymes; MR, metabolic ratio.

(CYP1A2), efavirenz (CYP2B6), losartan (CYP2C9), omeprazole (CYP2C19), metoprolol (CYP2D6) and midazolam did not reveal any changes following metformin treatment (Figure 3 and Table S1). The patients showed considerable interindividual variation in drug pharmacokinetics (Figure 3).

All patients had detectable efavirenz plasma concentrations in the sample for 0 h at 3 weeks, approximately 21 days after the first trial day (baseline). We included this sample in a post-hoc non-compartmental analysis for efavirenz at baseline and determined a median elimination half-life of 96.87 (IQR: 86.11–106.32) for efavirenz ($r^2 > 0.98$).

Following genotyping, one patient was identified as a CYP2C9-poor metabolizer, and one patient was identified as a CYP2D6-ultrarapid metabolizer. Neither of these patients was included in the data analysis for other reasons (Table S1). Besides these two, the other patients were found to have normal metabolic activity of CYP2C9, CYP2C19 and CYP2D6.

To assess if a reduction in HbA_{1c} impacts the change in drug metabolism, we applied the same ranges for change in HbA_{1c} as in the register-based study. The magnitude of the change in HbA_{1c} at 12 weeks compared to baseline (low [<10 mmol/mol] vs. high [>10 mmol/mol]) did not affect the metabolic ratios of any of the six probe drugs ($P > .05$ for all six probe drugs) (Figure 3).

Bodyweight and BMI were reduced during the trial and were significantly lower at the follow-up after 12 weeks of metformin treatment (Table 2). Two patients (20%) obtained a clinically relevant weight loss of 5% or more. Similarly, the median waist circumference was lower after 12 weeks of metformin treatment (Table 2).

All patients improved their glycaemic control after 3 and 12 weeks of metformin treatment (Table 2). The median change in HbA_{1c} after 3 and 12 weeks of metformin treatment was -4 mmol/mol (IQR: -6 ; -3 mmol/mol) and -10 mmol/mol (IQR: -18 ; -5 mmol/mol), respectively. After 12 weeks, five patients (50%) had obtained a reduction in HbA_{1c} > 10 mmol/mol. At the end of the trial,

TABLE 2 Clinical characteristics and glycaemic control improved in 10 patients with treatment-naïve type 2 diabetes following 3 weeks and 12 weeks of metformin treatment compared to baseline.

Parameter	Baseline (Day 1) Median (IQR)	3 weeks (Day 22) Median (IQR)	3 weeks/baseline GMR (95% CI)	12 weeks (Day 85) Median (IQR)	12 weeks/baseline GMR (95% CI)
Clinical characteristics					
Weight (kg)	94.2 (90.1–114)	93.2 (88.3–113.2)	0.99 (0.98–1)	92.6 (86.8–108.4)	0.97 (0.94–0.99)
BMI (kg/m ²)	32 (30–36)	31 (30–36)	0.99 (0.98–1)	30 (29–36)	0.97 (0.94–0.99)
Waist circumference (cm)	114 (108–125)	112 (108–123)	0.99 (0.98–1)	111 (107–122)	0.98 (0.95–1.00)
Glycaemic control					
HbA _{1c} (mmol/mol)	58 (50–65)	54 (46–58)	0.89 (0.83–0.96)	47 (43–48)	0.77 (0.65–0.91)
Fasting glucose (time 0) (mmol/L)	8.6 (7.2–10.9) ^a	7 (6.2–9.1) ^a	0.82 (0.77–0.87)	6.7 (6.3–8.9) ^a	0.81 (0.74–0.87)
Glucose (time 2 h) (mmol/L)	17.4 (11.6–21.6)	15.1 (13.8–17.3)	0.85 (0.7–1.03)	15.2 (12.9–17.7)	0.87 (0.71–1.07)
Glucose AUC _{total} (mmol*min L ⁻¹)	1818 (1471–2069) ^a	1539 (1425–1754) ^a	0.87 (0.82–0.92)	1550 (1448–1731) ^a	0.85 (0.75–0.96)
Glucose AUC _{incremental} (mmol*min L ⁻¹)	709 (551–927) ^a	764 (430–812) ^a	0.93 (0.81–1.08)	754 (548–968) ^a	0.90 (0.72–1.13)
C-peptid AUC (×10 ⁻³ min*pmol/L)	3376 (1977–4048)	3639 (2509–4242)	1.17 (0.97–1.42)	3429 (2476–3496) ^b	1.14 (0.91–1.44) ^b
Insulin AUC (×10 ⁻³ min*pmol/L)	528 (225–712)	554 (344–855)	1.27 (0.98–1.65)	459 (273–702) ^b	1.14 (0.91–1.44) ^b
HOMA IR	8.0 (7.2–14.7) ^a	6.4 (5.7–9.1) ^a	0.8 (0.59–1.08)	5.8 (5.6–7.6) ^c	0.71 (0.50–1.00) ^c
HOMA-β	120 (40–129) ^a	121 (70–193) ^a	1.39 (1.08–1.80)	128 (55–170) ^c	1.32 (1.08–1.60) ^c
Inflammation					
hsCRP (mg/L)	2.1 (1.2–2.9)	1.2 (1–2.1)	0.8 (0.45–1.39)	1.5 (0.9–2.6)	0.89 (0.64–1.22)
IL-6 (pg/mL)	2.4 (2.1–3.2)	2.4 (2.3–3.5)	1.09 (0.75–1.59)	2.6 (2.4–2.9)	1.04 (0.8–1.37)
IL-1β (pg/mL)	0.7 (0.5–0.8)	0.7 (0.5–1.3)	1.15 (1–1.32)	0.8 (0.6–1.3)	1.29 (1.12–1.49)
TNF-α (pg/mL)	2.2 (2–2.7)	2.2 (2.1–2.5)	0.99 (0.92–1.08)	2.4 (2–2.8)	1.02 (0.89–1.17)
IFN-γ (pg/mL)	0.4 (0.3–0.8)	0.6 (0.3–0.6)	1.08 (0.86–1.35)	0.4 (0.3–0.6)	1.01 (0.64–1.6)

Note: Inflammation remained low and unchanged during the course of metformin treatment. $N = 10$ unless otherwise stated. Some patients were excluded from analysis due to sample coagulation or haemolysis.

Abbreviations: AUC, area under the plasma concentration–time curve; CI, confidence interval; GMR, geometric mean ratio; HbA_{1c}, haemoglobin A1c (glycated haemoglobin); HOMA IR, homeostasis model assessment for insulin resistance; HOMA-β, homeostasis model assessment for β-cell function; hsCRP, high-sensitivity C-reactive protein; IFN, interferon; IL, interleukin; IQR, interquartile range; TNF, tumour necrosis factor.

^a $n = 8$.

^b $n = 9$.

^c $n = 7$.

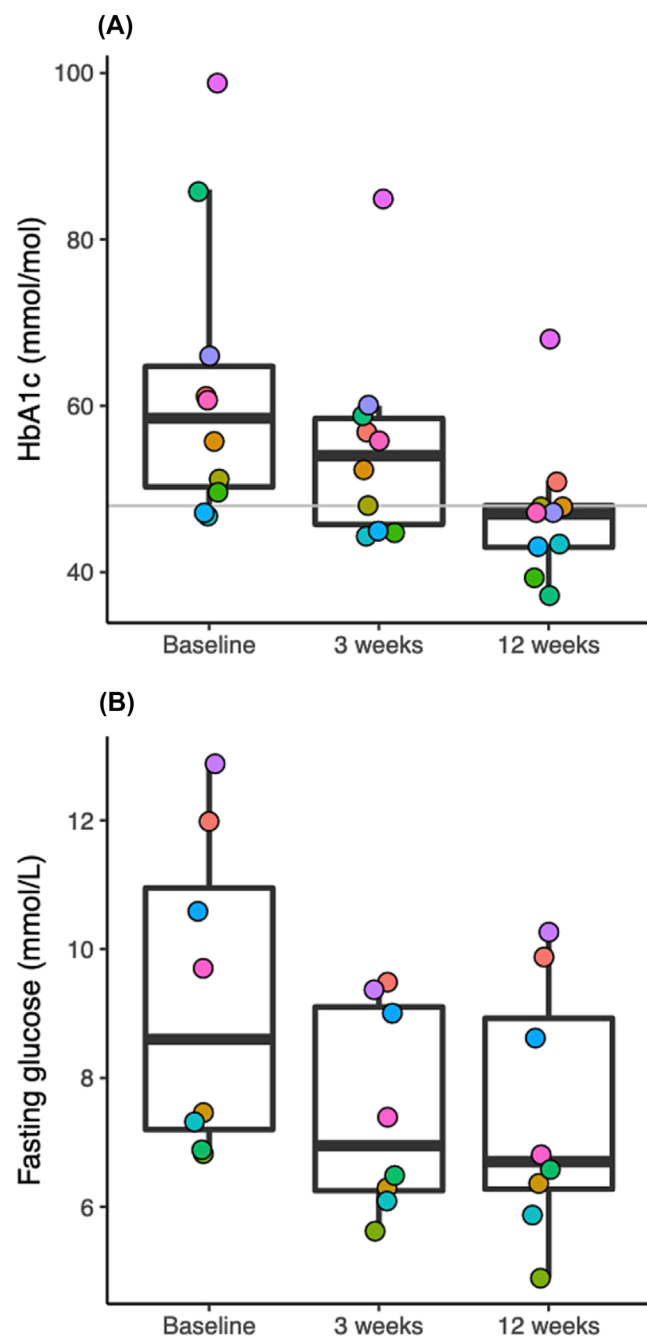


FIGURE 4 All 10 treatment-naïve patients with type 2 diabetes responded to metformin treatment assessed by glycated haemoglobin (HbA_{1c}) (A) and fasting glucose (B) after 3 and 12 weeks of treatment with metformin, compared to baseline. Boxes are median and interquartile ranges. Each patient is illustrated with the same colour as in Figures 3 and 5. Two patients had incomplete sampling due to sample coagulation and are not shown ($n = 8$). (A) HbA_{1c} (mmol/mol), the grey horizontal line marks 48 mmol/mol, the diagnostic limit and standard therapeutic goal of type 2 diabetes. Note that two patients had a slight decrease in HbA_{1c} from inclusion (inclusion criteria HbA_{1c} \geq 48 mmol/mol) to the baseline visit (B) fasting plasma glucose (mmol/L), time 0.

60% of the patients reached the therapeutic goal of HbA_{1c} $<$ 48 mmol/mol (Figure 4). The total exposure to glucose reduced following metformin treatment; fasting glucose and glucose AUC_{0-2 h} were significantly lower after 3 weeks and 12 weeks of treatment than baseline. However, mean fasting glucose at 2 h remained unchanged at 11 mmol/L. Surrogate measures of insulin sensitivity and secretion improved in response to metformin treatment and glycaemic control (Table 2).

All patients had detectable levels of the inflammatory marker hsCRP and the pro-inflammatory cytokines IL-6, IL-1 β , TNF- α and IFN- γ . IL-1 β increased slightly after 12 weeks of metformin treatment, but levels remained at a clinically insignificant low level. Throughout the trial, the levels of hsCRP, IL-6, TNF- α and IFN- γ remained low and unchanged following metformin treatment and improved glycaemic control (Table 2 and Figure 5).

4 | DISCUSSION

This translational study demonstrates that chronic warfarin users risk decreased warfarin efficacy when initiating glucose-lowering treatment in a register-based study. Furthermore, we show that this is not caused by altered drug metabolism in a clinical pharmacokinetic trial. Initiating metformin in patients with treatment-naïve type 2 diabetes did not affect the activity of drug-metabolizing enzymes (CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3A) following 3 and 12 weeks of metformin treatment, compared to baseline. This is despite a marked improvement in glycaemic control following metformin treatment. The level of inflammation remains low and unchanged during metformin treatment.

In this study, we confirm the previous findings that warfarin efficacy is reduced following the initiation of glucose-lowering treatment.^{8,9} Moreover, we demonstrate that patients with a strong effect of glucose-lowering therapy have a more pronounced reduction in warfarin efficacy. Several studies have found lower CYP activity, most importantly CYP3A4, in patients with type 2 diabetes compared to healthy controls.¹³⁻¹⁵ We speculated that the initiation of glucose-lowering therapy might reverse the diabetes-induced suppression of drug-metabolizing enzymes. As observed in our register-based studies, this would lead to increased warfarin metabolism and reduced therapeutic efficacy. However, our clinical pharmacokinetic trial does not support this hypothesis, as we see no changes in the activity of CYP enzymes involved in warfarin metabolism. From a clinical perspective, the results are reassuring, and the observed effect seems limited to warfarin. With frequent monitoring of INR after initiation of glucose-lowering treatment, this effect may be mitigated by dose adjustment of warfarin. Vitamin K is the primary target of warfarin, and glucose-dependent effects on the vitamin K pathway or warfarin sensitivity are potential mechanisms for the observed effect of glucose-lowering drugs. Further exploratory studies are warranted to understand the mechanism leading to decreased warfarin efficacy after initiating glucose-lowering drugs.

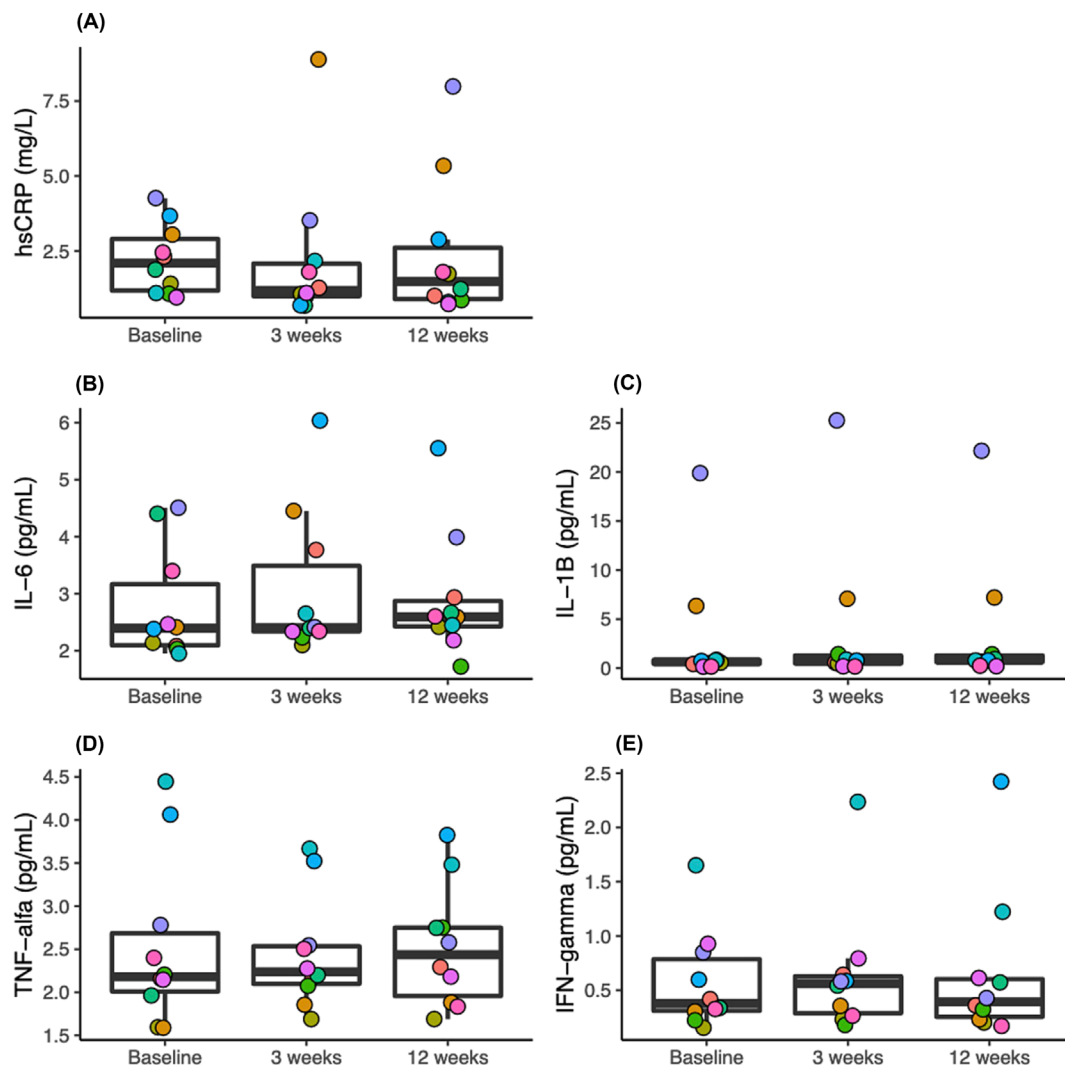


FIGURE 5 Inflammation remained low and unchanged in 10 patients with treatment-naïve type 2 diabetes following 3 and 12 weeks of metformin treatment compared to baseline. Boxes mark the median and interquartile ranges. Each patient is illustrated with the same colour as in Figures 3 and 4. (A) High-sensitivity C-reactive protein (hsCRP) (mg/L). (B) Interleukin 6 (IL-6) (pg/mL). (C) Interleukin 1beta (IL-1β) (pg/mL). (D) Tumour necrosis factor alpha (TNF-α) (pg/mL). (E) Interferon-gamma (IFN-γ) (pg/mL).

This is the first self-controlled clinical pharmacokinetic trial assessing the impact of initiating a glucose-lowering drug on the activity of multiple drug-metabolizing enzymes. Previous studies have focused on the drug-metabolizing activity¹³⁻¹⁵ and the content of drug-metabolizing enzymes^{15,29} in patients with type 2 diabetes compared to healthy individuals. In line with previous studies,^{13-15,30} our results suggest that the drug-metabolizing activity of CYP2C19 and CYP3A is decreased, and CYP1A2 activity is increased in patients with type 2 diabetes compared to healthy individuals (manuscript in the draft, [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT04840641) identifier NCT04840641). Further studies are needed to assess the mechanism behind this difference.

We observed efavirenz to have an elimination half-life far exceeding the expected level^{31,32}; all patients had detectable plasma concentrations of efavirenz 21 days after taking a single dose. In the product labels, the single dose elimination half-life is reported to be 52–76 h,^{31,32} and 21 days should be sufficient to clear efavirenz after

administration. A study assessing the steady-state pharmacokinetics of efavirenz reported an elimination half-life ranging from 27–136 h following discontinuation of steady-state treatment.³³ We find elimination half-lives from 78 to 147 h in patients with type 2 diabetes; this estimate might even be too low as efavirenz elimination is biphasic, and more data points are required to determine the terminal elimination. Based on the observation of prolonged elimination half-life among patients with type 2 diabetes, we hypothesize that the mechanisms underlying this potential impactful regulation of CYP2B6 and efavirenz metabolism may be caused by the pathophysiological properties of type 2 diabetes. Further studies are warranted to address this.

The main strength of this study is the translational approach. The register-based study allows us to evaluate the impact on a population level, while the clinical pharmacokinetic trial provides mechanistic insight into this potential interaction by glucose-lowering drugs. We

replicated the population-level data in two geographically distinct cohorts with many chronic warfarin users. Using a self-controlled design reduces the confounding effects of interindividual variability and provides more accurate estimates. The primary limitation is the lack of clinical endpoints related to decreased INR, for example, death and thromboembolic events. Furthermore, we interpreted redeeming a prescription as the initiation of a glucose-lowering drug. Thus, ingestion of the medication is not guaranteed. HbA_{1c} data suggest that some individuals are non-responsive, non-compliant or had an increase in HbA_{1c} in the time window between the last HbA_{1c} and the index date. Exposure misclassification of these individuals, with little or no change in INR, might underestimate the impact on warfarin efficacy.

The design of the clinical trial includes several strengths. We included males and females with a medical indication for treatment with metformin and with a broad range of ages and comorbidities based on concomitant medication. The significant heterogeneity among trial subjects is accounted for in the self-controlled design, leaving the estimates unaffected by interindividual differences. Lastly, we have limited the sampling time to 6 h, ensuring the inclusion of a representative proportion of treatment-naïve patients with type 2 diabetes and avoiding a potential socioeconomic bias in the patients included. However, the shorter sampling time is also the main limitation of the clinical pharmacokinetic trial. Complete pharmacokinetics of all six probe drugs would require 72 h of sampling and allow assessment of all pharmacokinetic parameters rather than metabolic ratios. The Basel cocktail and the metabolic ratios were validated in a cohort of healthy volunteers,^{20,21} but it is unknown if this correlates to drug exposure in patients with type 2 diabetes. Only data from six individuals were included in the data analysis of losartan (CYP2C9), as four individuals had a chronic intake of losartan and concentrations above the limit of detection at baseline. As such, there is a risk that the CYP2C9 assessment is underpowered. Another limitation of the trial is the lack of metformin concentrations in the trial subjects. Thus, metformin exerts some of its glucose-lowering effects in the intestine,⁶ and the correlation between metformin exposure and response is limited.³⁴ Lastly, we observed inflammation to be low and unchanged after metformin treatment. However, inflammation might be different in patients with longer diabetes duration, which could potentially impact CYP activity. We did not require the trial subjects to have a reduction in blood glucose during the trial, which could maximize the observed impact. Introducing such restrictions in the trial design would decrease the number of trial subjects available for analysis. We do not observe the reduction in HbA_{1c} to affect the drug-metabolizing activity.

In conclusion, initiation of glucose-lowering drugs in patients with chronic warfarin use seems to be associated with a reduced anticoagulant efficacy of warfarin that needs special clinical attention and frequent monitoring to avoid potential thrombotic complications. We show that this is not caused by altered drug metabolism in treatment-naïve patients with type 2 diabetes that initiates glucose-lowering therapy. Further studies are needed to understand whether the

reduction in warfarin efficacy leads to an increased risk of clinical endpoints such as stroke and death. Finally, additional work is required to understand the underlying mechanisms of this effect.

AUTHOR CONTRIBUTIONS

Ann-Cathrine Dalgård Dunvald, Anton Pottgård and Tore B. Stage designed the research. All authors participated in obtaining data sources or conception and design of the studies. Ann-Cathrine Dalgård Dunvald performed the research. Flemming Nielsen, Dorte Aalund Olsen and Jonna Skov Madsen contributed new reagents/analytical tools. Ann-Cathrine Dalgård Dunvald, Martin Thomsen Ernst, Louise Donnelly, and Enrique Soto-Pedre conducted the data analysis. All authors revised the manuscript critically for important intellectual content, and all authors have read and approved the final version to be published.

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All statistical analysis is conducted with Stata 15.1 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC) and RStudio Version 1.3.1056 (RStudio Team [2020]. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA). Figure 1 is created with Biorender.com.

CONFLICT OF INTEREST STATEMENT

A.D. has given paid lectures for Astellas Pharma. F.P. has served as a consultant, on advisory boards, or as educator for AstraZeneca, Novo Nordisk, Boehringer Ingelheim, Sanofi, Mundipharma, MSD, Novartis, Amgen and has received research grants to institution from Novo Nordisk, Boehringer Ingelheim, Amgen and AstraZeneca. J.S. has participated on advisory boards for AstraZeneca, Roche, Novo Nordisk and has participated in research funded by GSK and AstraZeneca. E.P. has received honoraria from Lilly, Illumina and Sanofi. A.P. reports participation in research projects funded by Alcon, Almirall, Astellas, Astra-Zeneca, Boehringer-Ingelheim, Novo Nordisk, Servier and LEO Pharma, all regulator-mandated Phase IV studies, all with funds paid to the institution where he was employed (no personal fees). T.S. has given paid lectures for Pfizer and Eisai and consulted for Pfizer. All are unrelated to work reported in this paper. F.N., D.A.O., M.T.E., L.D., E.S.P., M.R.K., J.S.N., K.H. and J.S.M. report no conflicts of interest.

DATA AVAILABILITY STATEMENT

Individual-level data from the register-based study and the clinical pharmacokinetic trial are not publicly available.

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REFERENCES

- World Health Organization. Diabetes [Internet]. <https://www.who.int/health-topics/diabetes>. Accessed June 23, 2022.
- Iversen DB, Andersen NE, Dalgård Dunvald AC, Pottegård A, Stage TB. Drug metabolism and drug transport of the 100 most prescribed oral drugs. *Basic Clin Pharmacol Toxicol*. 2022;131(5):311-324. doi:10.1111/bcpt.13780
- Christensen DH, Rungby J, Thomsen RW. Nationwide trends in glucose-lowering drug use, Denmark, 1999–2014. *Clin Epidemiol*. 2016;8:381-387. doi:10.2147/CLEP.S113211
- American Diabetes Association. 8. Pharmacologic approaches to glycemic treatment. *Diabetes Care*. 2017;40(Supplement 1):S64-S74. doi:10.2337/dc17-S011
- Buse JB, Wexler DJ, Tsapas A, et al. 2019 update to: Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia*. 2020;63(2):221-228. doi:10.1007/s00125-019-05039-w
- Foretz M, Guigas B, Viollet B. Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. *Nat Rev Endocrinol*. 2019;15(10):569-589. doi:10.1038/s41574-019-0242-2
- Stage TB, Brøsen K, Christensen MMH. A comprehensive review of drug–drug interactions with metformin. *Clin Pharmacokinet*. 2015;54(8):811-824.
- Stage TB, Pottegård A, Henriksen DP, et al. Initiation of glucose-lowering treatment decreases international normalized ratio levels among users of vitamin K antagonists: a self-controlled register study. *J Thromb Haemost*. 2016;14(1):129-133. doi:10.1111/jth.13187
- Wijnen JCF, van de Riet IR, Lijfering WM, van der Meer FJM. Metformin use decreases the anticoagulant effect of phenprocoumon. *J Thromb Haemost*. 2014;12(6):887-890. doi:10.1111/jth.12578
- Warfarin Pathway, Pharmacokinetics [Internet]. PharmGKB. <https://www.pharmgkb.org/pathway/PA145011113>. Accessed January 4, 2023.
- Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol*. 2011;11(2):98-107. doi:10.1038/nri2925
- Dunvald AD, Järvinen E, Mortensen C, Stage TB. Clinical and molecular perspectives on inflammation-mediated regulation of drug metabolism and transport. *Clin Pharmacol Ther*. 2022;112(2):277-290. doi:10.1002/cpt.2432
- Gravel S, Chiasson J, Turgeon J, Grangeon A, Michaud V. Modulation of CYP450 activities in patients with type 2 diabetes. *Clin Pharmacol Ther*. 2019;106(6):1280-1289. doi:10.1002/cpt.1496
- Marques M, Coelho E, Dos Santos N, Geleilate T, Lanchote V. Dynamic and kinetic disposition of nisoldipine enantiomers in hypertensive patients presenting with type-2 diabetes mellitus. *Eur J Clin Pharmacol*. 2002;58(9):607-614. doi:10.1007/s00228-002-0528-4
- Dostalek M, Court MH, Yan B, Akhlaghi F. Significantly reduced cytochrome P450 3A4 expression and activity in liver from humans with diabetes mellitus: downregulation of P450 3A4 by diabetes. *Br J Pharmacol*. 2011;163(5):937-947. doi:10.1111/j.1476-5381.2011.01270.x
- Department of Public Health, University of Copenhagen. CopLab—The Copenhagen Primary Care Laboratory Database [Internet]. <https://publichealth.ku.dk/research/databases-for-collaboration/coplab/>. Accessed October 4, 2022.
- Pottegård A, Schmidt SAJ, Wallach-Kildemoes H, Sørensen HT, Hallas J, Schmidt M. Data resource profile: the Danish National Prescription Registry. *Int J Epidemiol*. 2017;46(3):798-798f. doi:10.1093/ije/dyw213
- Schmidt M, Pedersen L, Sørensen HT. The Danish Civil Registration System as a tool in epidemiology. *Eur J Epidemiol*. 2014;29(8):541-549. doi:10.1007/s10654-014-9930-3
- Morris AD, Boyle DI, Macalpine R, et al. The diabetes audit and research in Tayside Scotland (DARTS) study: electronic record linkage to create a diabetes register. *BMJ*. 1997;315(7107):524-528. doi:10.1136/bmj.315.7107.524
- Donzelli M, Derungs A, Serratore MG, et al. The Basel cocktail for simultaneous phenotyping of human cytochrome P450 isoforms in plasma, saliva and dried blood spots. *Clin Pharmacokinet*. 2014;53(3):271-282. doi:10.1007/s40262-013-0115-0
- Derungs A, Donzelli M, Berger B, Noppen C, Krähenbühl S, Haschke M. Effects of cytochrome P450 inhibition and induction on the phenotyping metrics of the Basel cocktail: a randomized cross-over study. *Clin Pharmacokinet*. 2016;55(1):79-91. doi:10.1007/s40262-015-0294-y
- Stage TB, Graff M, Wong S, et al. Dicloxacillin induces CYP2C19, CYP2C9 and CYP3A4 in vivo and in vitro. *Br J Clin Pharmacol*. 2018;84(3):510-519. doi:10.1111/bcp.13467
- Puris E, Pasanen M, Gynther M, et al. A liquid chromatography-tandem mass spectrometry analysis of nine cytochrome P450 probe drugs and their corresponding metabolites in human serum and urine. *Anal Bioanal Chem*. 2017;409(1):251-268. doi:10.1007/s00216-016-9994-x
- Tanaka S, Uchida S, Inui N, Takeuchi K, Watanabe H, Namiki N. Simultaneous LC-MS/MS analysis of the plasma concentrations of a cocktail of 5 cytochrome P450 substrate drugs and their metabolites. *Biol Pharm Bull*. 2014;37(1):18-25. doi:10.1248/bpb.b13-00401
- Borggaard Larsen J, Rasmussen JB. Pharmacogenetic testing revisited: 5' nuclease real-time polymerase chain reaction test panels for genotyping CYP2D6 and CYP2C19. *Pharmacogenomics Pers Med*. 2017;10:115-128. doi:10.2147/PGPM.S131580
- Dunvald AD, Iversen DB, Svendsen ALO, et al. Tutorial: statistical analysis and reporting of clinical pharmacokinetic studies. *Clin Transl Sci*. 2022;15(8):1856-1866. doi:10.1111/cts.13305

27. PKNCA.pdf [Internet]. [<https://cran.r-project.org/web/packages/PKNCA/PKNCA.pdf>]. Accessed July 14, 2022.
28. Alexander SPH, Fabbro D, Kelly E, et al. The Concise Guide to PHARMACOLOGY 2021/22: Enzymes. *Br J Pharmacol*. 2021;178(S1):S313-S411.
29. Sotaniemi EA, Pelkonen O, Arranto AJ, Tapanainen P, Rautio A, Pasanen M. Diabetes and elimination of antipyrine in man: an analysis of 298 patients classified by type of diabetes, age, sex, duration of disease and liver involvement. *Pharmacol Toxicol*. 2002;90(3):155-160. doi:10.1034/j.1600-0773.2002.900308.x
30. Kvitne KE, Åsberg A, Johnson LK, et al. Impact of type 2 diabetes on in vivo activities and protein expressions of cytochrome P450 in patients with obesity. *Clin Transl Sci*. 2022;15(11):2685-2696. doi:10.1111/cts.13394
31. stocrin-epar-product-information_en.pdf [Internet]. https://www.ema.europa.eu/en/documents/product-information/stocrin-epar-product-information_en.pdf. Accessed August 26, 2022.
32. SUSTIVA (efavirenz) capsules and tablets [Internet]. https://www.accessdata.fda.gov/drugsatfda_docs/label/2005/020972s026,021360s013lbl.pdf. Accessed August 26, 2022.
33. Trancart S, Charreau I, Marchou B, et al. Presence of lamivudine or emtricitabine is associated with reduced emergence of nonnucleoside reverse transcriptase inhibitor mutations in an efavirenz-based intermittent antiretroviral treatment regimen. *Antimicrob Agents Chemother*. 2012;56(3):1655-1657. doi:10.1128/AAC.05452-11
34. Chung H, Oh J, Yoon SH, Yu KS, Cho JY, Chung JY. A non-linear pharmacokinetic-pharmacodynamic relationship of metformin in healthy volunteers: an open-label, parallel group, randomized clinical study. *PLoS ONE*. 2018;13(1):e0191258.

SUPPORTING INFORMATION

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

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