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A large prospective trial

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Using a limited sampling strategy (LSS) to investigate the interindividual pharmacokinetic variability in metformin – a large prospective trial

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Keywords limited sampling strategy, metformin, pharmacokinetics, OCT1, OCT2, MATE1

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What is already known about the subject

- We have previously reported an almost 80-fold interindividual difference in the mean trough steady state concentration of metformin in patients with type 2 diabetes mellitus (T2D).
- Neither we nor others have been able to find robust associations between variation in genes coding for metformin transporters and the pharmacokinetics and -dynamics of metformin.
- Recently we have shown in a retrospective study that the LSS method gave accurate predictions of the AUC_{0-24h} of metformin.

What this study adds

- We confirm in a large prospective pharmacogenetic trial in healthy subjects that the LSS approach is feasible for metformin pharmacokinetic assessment, thus saving costs of analytical analysis and time for both subjects and investigators.

Abstract

Aim

Recently a limited sampling strategy (LSS) for determination of metformin´s pharmacokinetics was developed. The LSS utilizes the plasma concentration of metformin 3- and 10-hours after oral intake of a single dose to estimate the area under the concentration-time curve ($AUC_{0\text{-}24h}$). The main purpose of this study was to support the feasibility of this strategy in a large prospective trial.

Methods

Volunteers orally ingested two 500 mg tablets of metformin hydrochloride. A blood sample was drawn three and ten hours after the ingestion. Urine was collected for 0-10 and 10-24 hours and urine volumes recorded. The $\mathrm{AUC}_{0-24\mathrm{h}}$ was calculated using the equation $AUC_{0\text{-}24h}$ = 4.779*C3 + 13.174*C10. Additionally, all participants were genotyped for the single-nucleotide polymorphism (SNP) A270S in *OCT2*, g.-66T>C in *MATE1*, R61C, G465R, G401S and the deletion M420del in *OCT1*.

Results

212 healthy volunteers participated. The median (25^{th} – 75^{th} IQR) $\mathrm{AUC}_{0-24\mathrm{h}}$, $\mathrm{CL}_{\mathrm{renal}}$, C_3 and C_{10}, were 10600 (8470 – 12500)ng*hr*mL^{-1} , 29 (24 – 34) L*$hour^{-1}$, 1460 (1180-1770)and 260 (200 – 330)ng*mL^{-1} , respectively, which is in agreement with our previous results. GFR_i was correlated with metformin AUC and $\mathrm{CL}_{\mathrm{renal}}$ (P=<0.001). As expected, we found a great pharmacokinetic interindividual variability among the volunteers and no effect of the *OCT1* genotype on the CL_{renal} and the AUC_{0-24h}. We were unable to reproduce our previous finding of a gene-gene interaction (*OCT2* and *MATE1*) effect on CL_{renal} in this cohort.

Conclusion

This study further supports the use of the two-point LSS algorithm in large pharmacokinetic trials.

Introduction/background

Approximately half a billion people are living with diabetes worldwide and the number is increasing (1). The vast majority of patients with diabetes mellitus suffer from type 2 diabetes (T2D), which is mainly caused by lifestyle. Accordingly, T2D can be treated with weight loss, physical exercise and glucose-lowering drugs. Metformin is the glucose lowering drug of choice in T2D, because it is safe, cheap, effective and most importantly because it reduces cardiovascular death in T2D (2). Presently there are nearly 1000 ongoing clinical trials on metformin (3)

The mechanism of action of metformin is still not fully understood. It lowers both basal and postprandial plasma glucose through a reduction in gluconeogenesis in the liver, but there is increasing evidence that the gut is also a target in metformin action (4). The metformin molecule contains two basic nitrogen atoms and it therefor exists in its mono protonated form at physiological pH. Thus, it is hydrophilic and is therefore highly dependent on drug transporters mainly OCT1 in the liver and OCT2 and MATE 1/2-K in the kidney. After oral ingestion, metformin is absorbed in a dose dependent and saturable manner from the small intestine via the plasma monoamine transporter (PMAT) and the organic cation transporter 3 (OCT3), both of which are localized to the luminal surface of enterocytes (2,5). Recent studies have suggested the serotonin reuptake transporter (SERT), the Thiamine transporter 2 (THTR-2) and the carnitine/organic cation transporter (OCTN1), may contribute to the uptake of metformin across the apical membrane in the small intestine (5). Transport from the enterocyte and into the bloodstream is thought to occur via organic cation transporter 1 (OCT1) (2). However, more recent reports place OCT1 on the apical surface of intestinal epithelial cells (6). After oral intake of immediate-release metformin in humans, the absorption is incomplete and approximately 70% of the dose is absorbed with the remainder excreted in faeces (7). In the liver, both OCT1 and OCT3 seem to be in charge of metformin uptake into hepatocytes though, at least in mice, OCT1 appears to be the major force (2). Metformin is not metabolized or protein bound and is excreted in urine unchanged by both glomerular filtration and active tubular secretion, facilitated by the organic cation transporter 2 (OCT2) and the multidrug and toxin extrusion transporters *1* (MATE1) and 2 (MATE2-K) (5,7).

Around one third of all patients have insufficient response to metformin as monotherapy (8). A minor group suffer severe unacceptable gastrointestinal side effects and has to discontinue the

drug (9). The large interindividual pharmacokinetic variability of metformin may partially explain this. Although, human pharmacogenetic studies of the aforementioned transporter genes have indicated that they do not appear to be critical to the pharmacodynamics of the drug (8), there is still a need to study the influence of other genes on the pharmacokinetics of metformin, and this requires DNA and pharmacokinetic data from large cohorts of subjects. Thus, pharmacokinetic data from one of our previous trials (10) was used developed a limited blood sampling strategy (LSS) based on the measurement of the plasma concentration of metformin after 3 and 10 hours, C_3 and C_{10}, and this retrospective approach led to $AUC_{0\text{-}24}$ determinations, which correlated excellently with the results of traditional multiple blood- and urine sampling pharmacokinetic studies, (11). The purpose of the present study was to demonstrate the feasibility of the LSS approach applied prospectively in healthy volunteers. As a secondary aim we wanted to investigate the pharmacogenetics of metformin.

Material and methods

Study participants

The volunteers were mainly recruited among students at the University of Southern Denmark. All volunteers were healthy with a body mass index (BMI) below 29,9kg/m², there were no pregnant or breastfeeding women, none took any medication; prescription, over the counter, supplements or herbal medicine (birth control pills and regular vitamin supplements were accepted). None had a history of alcohol abuse or hypersensitivity towards metformin. Before the administration of study medication, all women were tested negative for pregnancy. The renal function was assessed by plasma creatinine and eGFR and both had to be within normal range or clinically insignificantly deviate from it. HbA1c was also measured and was required to be within normal range. Verbal and written informed consent were obtained from all volunteers included in the study.

Study design

It was an open label, non-randomized study. The participants fasted from midnight and ingested two tablets each of 500 metformin hydrochloride (Aurobindo)" corresponding to 390 mg of free metformin base at 5:00 AM. Right before ingestion of metformin the participants emptied the bladder. Urine was collected from the time of metformin ingestion and for the following 24 hours. If necessary, participants received a new urine container ten hours after the ingestion of metformin. A blood sample was drawn three and ten hours after the ingestion. These were immediately centrifuged and kept at −20 °C until drug analysis. The 0 -24 hour or 0-10-hour and 10-24-hour urine volumes were determined, and an aliquot kept at −20 °C until analysis.

Study procedures

The study was registered in the European Clinical Trial Database (EudraCT no.: 2017-003857-40), OPEN at the University of Southern Denmark (no: OP_510) and approved by the Danish Health and Medicines Authority (J. no: S-20170166), and the Danish Data Protection Agency (J. no. 2012-58-0018). The trial was registered at www.clinicaltrials.gov (NCT03335423). The study was conducted in accordance with the Helsinki Declaration and Good Clinical Practice (GCP) and monitored by the GCP unit, Odense University Hospital, Odense, Denmark.

Analytical methods

The concentration of metformin in plasma and urine samples were determined at the Department of Clinical Pharmacology and Pharmacy, Institute of Public Health, University of Southern Denmark, by use of liquid chromatography and tandem mass spectrometry (LC-MS/MS). The LC-MS/MS system consisted of an Ultimate 3000 UHPLC system connected to a TSQ Quantiva Triple Quadropole Mass Spectrometer with heated electrospray ionization (Thermo Scientific, San Jose, CA). Data acquisition was performed in single reaction monitoring (SRM) mode. Metformin was quantitated by positive ionisation at the transition from (m/z) 130.4 – 71.1, and with (m/z) 130.4 – 60.1 as a qualifier trace. Metformin-d6 (internal standard) was monitored from (m/z) 136.4 – 77.1. The analytical separation was performed using hydrophilic interaction chromatography as described by Nielsen et al and McCreight et al (12,13).

The sample preparation of the plasma samples consisted of a single protein precipitation step. To a 100µL plasma sample, 10 µL 25 ug/mL metformin-d6 (internal standard), 20 µL 0.53M ammonium acetate and 390 µL acetonitrile were added. The sample was vortex mixed for 30 sec and centrifuged at 3.000g for 15 minutes. The urine samples were diluted (1:50) before use but were otherwise treated as the plasma samples. A volume of 10 µL of the supernatant was injected onto the LC-MS/MS system. Calibration curves, as well as quality control samples, were prepared and included in each batch of analysis. The intra- and inter-day variability was < 8%. The limit of detection (LOD) for the method was 1 ng/mL and limit of quantification (LOQ) was 10 ng/mL.

Genotyping

Genomic DNA was extracted from an aliquot of venous blood using the Maxwell 16 Blood DNA Purification Kit (Promega Corporation, Madison, WI, USA). Selected single nucleotide polymorphisms (SNPs) in *OCT1* (rs12208357; rs34059508; rs72552763; rs34130495), *OCT2* (rs316019), and *MATE1* (rs2252281) were genotyped as previously described (MM 2011)(14). Briefly, rs72552763 (M420del) and rs34130495 (G401S) were genotyped by Sanger sequencing. The rs2252281 SNP was genotyped using File-builder primers and probes while rs12208357

(R61C), rs34059508 (G465R), and rs316019 (A270S) were genotyped using predesigned TaqMan SNP genotyping assays on a StepOne Plus real-time instrument (Applied Biosystems, Thermo Fisher Scientific, Foster City, CA) according to the manufacturer's protocol.
Assay numbers and sequence of primer and probes used for genotyping are summarized in Supplementary Table S1. All variants were in Hardy–Weinberg equilibrium.

Statistical analysis and considerations

The estimated glomerular filtration rate (eGFR) is a compound variable consisting of sex, age, and plasma creatinine levels. The individual glomerular filtration rates (GFR_i) were calculated using eGFR according to the Modification of Diet in Renal Disease formula adjusted for the body surface area (BSA). Thus, GFR_i=(eGFR * BSA)/1.73 m^2.

BSA, body surface area= $weight(kg)^{0.425} \times height(cm)^{0.725} \times 0.007184$

The descriptive data are presented as medians and range. The pharmacokinetic data are presented as medians with 25th and 75th interquartile range (IQR). Before statistical analysis, visually guided by $\mathrm{Q_{norm}}$ plots, metformin AUC was logarithm transformed to create a Gaussian distribution. The impact of genotypes and gender on metformin $\mathrm{AUC_{0-24h}}$ and $\mathrm{CL_{renal}}$ was investigated using linear regression.

The CL_{renal} depends on glomerular filtration, and thus the $\mathrm{CL_{renal}}$ results are adjusted for the GFR_i. As detailed above GFR_i is a compound variable consisting of weight, height, sex, age, and serum creatinine levels. CL_{renal} has an impact on metformin AUC and a linear regression was performed to investigate the correlation between metformin AUC and GFR_i.

A P value less than 0.05 was considered statistically significant. All statistical analyses were performed using Stata Statistical Software: Release 16. College Station, Texas: StataCorp LP.

Pharmacokinetics
The area under the plasma concentration-time curve of metformin (0-24h) was calculated as follows:

$4.779 * \mathrm{C_3} + 13.174 * \mathrm{C_{10}}$, where $\mathrm{C_3}$ and $\mathrm{C_{10}}$ is the concentration of metformin in plasma three and ten hours after ingestion of metformin.

Metformin $\mathrm{CL_{renal}}$ was calculated as follows:

$$\mathrm{CL_{renal}} = \frac{\text{amount of metformin in urine}_{0-24\mathrm{h}}}{\mathrm{AUC_{0-24h}}}$$

Diplotype interference

The diplotypes of the four RF alleles in *OCT1* was inferred using the software package PHASE, version 2.1.1 (University of Washington, Seattle, Washington, USA) by Stephens et al. (15). The haplotypes were phased seven times with random seeds to ensure a stabile diplotype result.

Sample size

We did not perform a priori power calculations because the main purpose of the present study was to confirm prospectively that the limited sampling strategy was applicable in a prospective design and that the obtained pharmacokinetic results ($\mathrm{CL_{renal}}$ and $\mathrm{AUC_{0-24h}}$) was within the expected range. A secondary purpose was to recruit healthy volunteers of known *OCT1* and CYP2D6 genotype for a subsequent metformin-codeine interaction study. We aimed to screen 300 subjects however when we passed 212 included subjects, we stopped inclusion since we had achieved a large enough sample to draw subjects from.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.

Results

236 healthy volunteers gave written consent to participate in the trial. One of the volunteers got sick and failed to participate before the trial ended, another was excluded due to technical difficulties and 22 volunteers choose to withdraw their consent before the start of the trial. Ultimately, 212 healthy volunteers (133 women and 79 men) completed the study (see demographics in table 1).

Metformin pharmacokinetics and interindividual variability

Pharmacokinetic parameters from all 212 participants are summarized in table 3 and figure 1 and 2. Six participants were excluded from the $\mathrm{CL_{renal}}$ estimation due to problems with the urine

collection (Four participants forgot to use the urine container and urine samples were missing for two participants). The median (25^{th} – 75^{th} IQR) $\mathrm{AUC_{0-24h}}$, $\mathrm{CL_{renal}}$, C_3 and C_{10}, were 10600 (8470 – 12500)ng*hr*mL^{-1} , 29 (24 – 34) L*hour^{-1} , 1460 (1180-1770)and 260 (200 – 330)ng*mL^{-1} All parameters showed substantial interindividual variation, with a five-fold difference detected in AUC, a seven-fold variation in CL_{renal} and five respectively, seven- fold variation in metformin plasma concentration, three and ten hours after ingestion. GFR_i was highly correlated with both metformin AUC and $\mathrm{CL_{renal}}$ (P=<0.001).

The LSS algorithm

When comparing our obtained pharmacokinetic results ($\mathrm{CL_{renal}}$ and $\mathrm{AUC_{0-24h}}$) with results previously reported by our group (10) the parameters are within the expected range.

Gene evaluation

The genotype characteristics for the 212 healthy participants is summarized in table 2. The results of the *OCT1, OCT2* and *MATE1* gene-analysis are detailed in supplementary file 2. We found no effect of the *OCT1* genotype and the AUC_{0-24h}, and were unable to reproduce our previous finding of a gene-gene (OCT2-MATE 2-K) interaction effect on $\mathrm{CL_{renal}}$ (10).

Discussion

We have for the first time applied the two-point limited sampling algorithm to a large prospective clinical pharmacogenetic study. All pharmacokinetic parameters were within the expected range and in line with previous research. As expected, $\mathrm{GFR_i}$ was highly correlated with metformin $\mathrm{CL_{renal}}$ and $\mathrm{AUC_{0-24h}}$. This has also been confirmed in one of our previous studies (10). We found a considerable interindividual variability in the pharmacokinetics of metformin (Table 3). Our group has previously reported an 80-fold variability in the trough steady-state metformin plasma concentration among 159 T2D patients treated with the same dose (14). However, in several subsequent pharmacokinetic studies performed in healthy participants, including this one, we were unable to detect similar variability in the pharmacokinetics of metformin (16).

The strength of this study is the large sample size. To our best knowledge this is the largest prospective pharmacokinetic metformin trial to this day. However, the study also has some limitations. We cannot be sure if the LSS over- or underestimates the AUC_{0-24h}. The ideal way to investigate the correctness of the LSS approach would be to do a full pharmacokinetic study in a large sample of subjects and confirm the two-point LSS strategy prospectively. For several reasons: ethics, safety, economy, time consumption, we do not believe that such a study ever would be undertaken. Rather we took the pragmatic approach of demonstrating that the two-point LSS can

be applied in real life, and the fact that the mean pharmacokinetic values were almost identical to those obtained in our previous studies further supports that the two-point LSS method at the population level doesn't give a systematic error or bias.

Since the urine collection was not observed we had to trust that instructions were followed as agreed. Not all blood samples were taken at precise three and ten hours after metformin ingestion why the concentrations used in the LSS not all match C_3 and C_{10} perfectly. Due to metformins long absorption time we do not believe this has a major impact on the results of the trial.

The impact of *OCT2* c.808 (G>T) on metformin AUC and CL_{renal} have previously been investigated in human studies (10,17–24), with conflicting results. Our study population is the largest to this day, and our CL_{renal} results are in line with previous studies made on Caucasians(10,23) where the *OCT2* genotypes do not have an effect on the CL_{renal}.

Numerous studies have been conducted on the importance of genetic variation in *OCT1* and the effect on the pharmacokinetics and dynamics of metformin. While some studies have reported a positive impact of genetic variants in OCT1 on the metformin AUC (19,25–27), several others have reported negative findings on pharmacokinetic parameters (10,25,28) and the pharmacokinetics and pharmacodynamics of metformin might not be as tightly genetically controlled as first assumed (8,29). In this study we were unable to detect an impact of *OCT1* genetic variants on metformin AUC_{0-24h}.

Genetic variations in the promoter region of MATE1 (g.-66T>C, rs2252281) were mainly analyzed to try and reproduce our previous finding of a gene-gene interaction effect on CL_{renal}, however we were unable to replicate these findings. The alternative allele in both *OCT2* c.808 (G>T) and MATE1 (g.-66T>C) are quite rare in Caucasians (30) and even more so in combination. Our study was not powered to detect a possible impact of a gene-gene interaction on metformin pharmacokinetics and we did not have enough volunteers with the rarer gene combinations for it to be acceptable to draw any conclusions from the results. Especially when the renal clearance of metformin shows such a great interindividual variance.

Conclusion: This study further supports the use of the "Limited Sampling Strategy"- method in large pharmacogenetic trials, saving both time and money for future metformin studies.

Legends to figures

Figure 1: Histogram of metformin $AUC_{0\text{-}24h}$ in 212 healthy subjects.

Figure 2: Histogram of metformin renal clearance in 206 healthy subjects.

Reference-list

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, m.fl. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. november 2019;157:107843.

2. Florez JC. The pharmacogenetics of metformin. Diabetologia. september 2017;60(9):1648–55.

3. ClinicalTrials.gov - metformin [Internet]. www.clinicaltrials.gov; Tilgængelig hos: https://clinicaltrials.gov/ct2/results?term=metformin&Search=Apply&recrs=h&r

4. Foretz M, Guigas B, Viollet B. Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. Nat Rev Endocrinol. oktober 2019;15(10):569–89.

5. Liang X, Giacomini KM. Transporters Involved in Metformin Pharmacokinetics and Treatment Response. J Pharm Sci. 8. maj 2017;

6. Han TK, Everett RS, Proctor WR, Ng CM, Costales CL, Brouwer KLR, m.fl. Organic cation transporter 1 (OCT1/mOct1) is localized in the apical membrane of Caco-2 cell monolayers and enterocytes. Mol Pharmacol. august 2013;84(2):182–9.

7. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 3. august 2017;

8. Dujic T, Zhou K, Yee SW, van Leeuwen N, de Keyser CE, Javorský M, m.fl. Variants in pharmacokinetic transporters and glycemic response to metformin: A metgen meta-analysis. Clin Pharmacol Ther. 10. november 2016;

9. McCreight LJ, Bailey CJ, Pearson ER. Metformin and the gastrointestinal tract. Diabetologia. marts 2016;59(3):426–35.

10. Christensen MMH, Pedersen RS, Stage TB, Brasch-Andersen C, Nielsen F, Damkier P, m.fl. A gene-gene interaction between polymorphisms in the OCT2 and MATE1 genes influences the renal clearance of metformin. Pharmacogenet Genomics. oktober 2013;23(10):526–34.

11. Santoro AB, Stage TB, Struchiner CJ, Christensen MMH, Brosen K, Suarez-Kurtz G. Limited sampling strategy for determining metformin area under the plasma concentration-time curve. Br J Clin Pharmacol. oktober 2016;82(4):1002–10.

12. McCreight LJ, Stage TB, Connelly P, Lonergan M, Nielsen F, Prehn C, m.fl. Pharmacokinetics of metformin in patients with gastrointestinal intolerance. Diabetes Obes Metab. 2018;20(7):1593–601.

13. Nielsen F, Christensen MMH, Brøsen K. Quantitation of metformin in human plasma and urine by hydrophilic interaction liquid chromatography and application to a pharmacokinetic study. Ther Drug Monit. april 2014;36(2):211–7.

14. Christensen MMH, Brasch-Andersen C, Green H, Nielsen F, Damkier P, Beck-Nielsen H, m.fl. The pharmacogenetics of metformin and its impact on plasma metformin steady-state levels and glycosylated hemoglobin A1c. Pharmacogenet Genomics. december 2011;21(12):837–50.

15. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet. april 2001;68(4):978–89.

16. Stage TB, Wellhagen G, Christensen MMH, Guiastrennec B, Brøsen K, Kjellsson MC. Using a semi-mechanistic model to identify the main sources of variability of metformin pharmacokinetics. Basic Clin Pharmacol Toxicol. januar 2019;124(1):105–14.

17. Chung H, Oh J, Yoon SH, Yu K-S, Cho J-Y, Chung J-Y. 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.

18. Hou W, Zhang D, Lu W, Zheng T, Wan L, Li Q, m.fl. Polymorphism of organic cation transporter 2 improves glucose-lowering effect of metformin via influencing its pharmacokinetics in Chinese type 2 diabetic patients. Mol Diagn Ther. februar 2015;19(1):25–33.

19. Yoon H, Cho H-Y, Yoo H-D, Kim S-M, Lee Y-B. Influences of organic cation transporter polymorphisms on the population pharmacokinetics of metformin in healthy subjects. AAPS J. april 2013;15(2):571–80.

20. Song IS, Shin HJ, Shim EJ, Jung IS, Kim WY, Shon JH, m.fl. Genetic variants of the organic cation transporter 2 influence the disposition of metformin. Clin Pharmacol Ther. november 2008;84(5):559–62.

21. Wang Z-J, Yin OQP, Tomlinson B, Chow MSS. OCT2 polymorphisms and in-vivo renal functional consequence: studies with metformin and cimetidine. Pharmacogenet Genomics. juli 2008;18(7):637–45.

22. Chen Y, Li S, Brown C, Cheatham S, Castro RA, Leabman MK, m.fl. Effect of genetic variation in the organic cation transporter 2 on the renal elimination of metformin. Pharmacogenet Genomics. juli 2009;19(7):497–504.

23. Tzvetkov MV, Vormfelde SV, Balen D, Meineke I, Schmidt T, Sehrt D, m.fl. The effects of genetic polymorphisms in the organic cation transporters OCT1, OCT2, and OCT3 on the renal clearance of metformin. Clin Pharmacol Ther. september 2009;86(3):299–306.

24. Moon SJ, Oh J, Lee SH, Choi Y, Yu K-S, Chung J-Y. Effect of plasma membrane monoamine transporter genetic variants on pharmacokinetics of metformin in humans. Transl Clin Pharmacol. juni 2018;26(2):79–85.

25. Santoro AB, Botton MR, Struchiner CJ, Suarez-Kurtz G. Influence of pharmacogenetic polymorphisms and demographic variables on metformin pharmacokinetics in an admixed Brazilian cohort. Br J Clin Pharmacol. 2018;84(5):987–96.

26. Shu Y, Brown C, Castro RA, Shi RJ, Lin ET, Owen RP, m.fl. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. Clin Pharmacol Ther. februar 2008;83(2):273–80.

27. Naja K, El Shamieh S, Fakhoury R. rs622342A>C in SLC22A1 is associated with metformin pharmacokinetics and glycemic response. Drug Metab Pharmacokinet. februar 2020;35(1):160–4.

28. Christensen MMH, Højlund K, Hother-Nielsen O, Stage TB, Damkier P, Beck-Nielsen H, m.fl. Steady-state pharmacokinetics of metformin is independent of the OCT1 genotype in healthy volunteers. Eur J Clin Pharmacol. juni 2015;71(6):691–7.

29. Stage TB, Damkier P, Pedersen RS, Christensen MMH, Christiansen L, Christensen K, m.fl. A twin study of the trough plasma steady-state concentration of metformin. Pharmacogenet Genomics. maj 2015;25(5):259–62.

30. https://www.ncbi.nlm.nih.gov/snp/.

Table 1. Demographic information for the 212 healthy volunteers.

Demographic information	Median	Range	Frequency %
Age at inclusion (years)	24	18 - 60	
BMI (kg/m^2)	23.2	18.5 – 29.8	
Plasma creatinine (µmol/L)	72.5	36 - 113	
BSA (m^2)	1.8	1.4 – 2.5	

HbA1c (mmol/mol)	32	25 – 39
eGFR (ml/min)	90	65 - 90

Ethnicity	
European	86.3
African	2.8
Asian	5.66
South American	5.2
Other*	
Gender n: women 133, men 79	

BMI= weight (kg)/height (m) ^{2}
Ethnicity was self-reported
*6 participants came from the Middle East, 3 were 50% European 50% African and 2 were 50% European and 50% Asian
BSA, body surface area= $weight(kg)^{0.425} \times height(cm)^{0.725} \times 0.007184$ (10)

Table 2. Genotype characteristics of 212 healthy subjects

					Genotyped (n)		
Gene	**dbSNP ID**	**Coding position**	**Nucleotide change**	**Amino acid change***	**WT/WT**	**WT/V**	**v/v**
OCT2	rs316019	c.808	G>T	A270S(Ala-Ser)	153	51	8
OCT1	rs12208357	c.181	C>T	R61C (Arg – Cys)	184	25	3
	rs72552763	c.1260	GAT>del	M420del (Met – del)	144	63	5
	rs34130495	c.1201	G>A	G401S (Gly -Ser)	194	17	1
	rs34059508	c.1393	G>A	G465R (Gly -Arg)	199	13	0
MATE1	rs2252281	g.-66	T>C	- (promotor)	86	102	24

dbSNP ID: Single nucleotide polymorphism database identification. * Single letter nomenclature amino acid substitution. WT: wildtype. V: the genetic variant. Ala: Alanine. Arg: Arginine. Cys: Cystein. Gly: Glycine. Ser: Serine. Del: deletion.

Table 3. Metformin pharmacokinetics determined in 212 healthy subjects using the limited sampling strategy

Parameter	~ Median (p25 – 75)	Range
$AUC_{0-24h}(\frac{ng*h}{mL})$	10600 (8470 – 12500)	4500 - 22800
CL_{renal} (L/h)	29 (24 – 34)	9 - 66
C_3 (ng/mL)	1460 (1180-1770)	600-3000
C_{10}(ng/mL)	260 (200 – 330)	90-680

Data is presented as medians with the 25th – 75th interquartile range and a minimum to maximum range.