Supplementary methods.

**Replication study group.**

In Slovakia the study was conducted in a university hospital setting. T2D was diagnosed in patients according to the criteria of the American Diabetes Association. Louis Pasteur University Hospital Review Board gave ethical approval for this study. All participating subjects gave a written consent to be included in the study. 148 patients of Caucasian origin were recruited from three out-patient clinics. Patients with malignancies, another endocrine disorders, chronic kidney disease stage 3-5, severe liver disease and systemic inflammatory disease were excluded. Only drug-naïve patients with HbA1c in the range of 6.5-11\% [48-97 mmol/mol] were included. Baseline HbA1c measurement was done within one week prior the treatment initiation and second measurement after 6 months of metformin monotherapy. 131 patients were further included in the study as they had HbA1c measurements in the corresponding time period (101.5±20.6 days).

**Pharmacokinetics group and estimation of metformin levels.**

35 volunteers were included in study. All participants have signed informed consent and study was approved by Committee of Ethics (PSCUH, Nr.3000610-18L). To be eligible for this study, subjects confirmed not using any medications other than vitamins. Level of liver enzymes of participants (alanine aminotransferase, g-glutamyltransferase) were less than double of respective normal value, none had renal failure (mean serum creatinine 76.7±12.3 mmol/l).Women were asked to provide a urine sample to confirm a negative pregnancy test before the study. Venous blood samples (0, 1, 2, 3, 4, 6, 10, 24 h after oral administration of metformin) and urine samples (4, 6, 10, 24 h after oral administration of metformin) were taken within 24 hours. Whole blood samples were centrifuged immediately and the pellet was washed three times with 0.9% sodium chloride. All plasma, urine and pellet samples were stored at -20°C until determination of metformin. After acetonitrile-induced protein precipitation of the biological samples, metformin and phenformin (internal standard, IS) were analyzed on hydrophilic interaction liquid chromatography (HILIC) column to obtain better specificity in respect to metformin. Metformin and IS were detected using multiple reaction monitoring (MRM) in positive ion electrospray mode. The assay was validated for quantitative determination of metformin in human RBC and plasma samples. The obtained calibration curves were linear over the concentration range of from 5ng/ml to 500 ng/ml for erythrocytes, from 5ng/ml to 1500 ng/ml for blood plasma and from 2,5μg/ml to 250 μg/ml for urine samples. Creatinine clearance (Clcr) was calculated from creatinine measurements from 24 hours urine and corrected for body surface area (BSA) by using the Mosteller formula. Ten individuals with creatinine clearance values outside a reference range (≥80 and ≤140 mL/min/BSA) were excluded from further study.

Altogether 9 men and 16 women were investigated after a single dose of 500 mg of metformin (Metforal Berlin Chemie). The maximum observed concentration (C_{max}) and the time point of observed
Cmax (tmax) were both obtained directly from the measured data. Estimation of area under the curve (AUC0-24) was calculated by trapezoid method and corrected for infinity using the rate constant of the last exponential phase (k). Extrapolation to infinity was computed \( AUC_{\infty} = AUC_{0-24} + C_{\text{last}}/k \). Ratio of AUC0-24 to \( AUC_{\infty} \) is < 3%. The k value was calculated from final 2 concentrations of the concentration time-curve \( k = (\ln C_{10h} - \ln C_{24h})/t \). The elimination half-life (t1/2) was calculated as 0.693/k. Clearance/bioavailability (CL/F) was calculated as dose/AUC\(_{\infty}\), where bioavailability was estimated as 50% as reported previously. Volume of distribution/bioavailability (V/F) was estimated by dividing CL/F by k. Pharmacokinetic research was performed in framework of VPP Biomedicine and has permission from Committee of Ethics (PSCUH) Nr. 2012.1212 - 10L.

**Genotyping in replication and pharmacokinetics groups.**

In the patient group from Slovakia OCT2 rs7757336 and OCT2-OCT3 rs2481030 polymorphisms were analyzed by high-resolution melting analysis after real-time PCR in the presence of LCGreen Plus dye and an unlabeled probe on Eco Real-Time PCR System (Illumina, Inc., San Diego, CA, USA). Genotypes were identified using Eco™ Software 4.1. Genotyping success rate for all examined variants was 100% and duplicate genotyping concordance was 100% (15 samples for each SNP. Where available, 5 samples for each genotype were used).

Genetic testing of rs2481030 and rs7757336 in pharmacokinetic group was carried out using the Applied Biosystems TaqMan SNP (Applied Biosystems, Foster City, California, USA). Genotyping assay with a modified protocol using 4.75 ml TaqMan Genotyping Mix, 0.25 ml SNP genotyping assay, and 5 ml Millipore H\(_2\)O on a 7500 Real-Time PCR system (Applied Biosystems). AutoCaller 1.1 (Applied Biosystems) software was used to assign genotype calls for all samples simultaneously (Genotype call rate was 100%, concordance rate –100%).

**Data quality analysis.**

Tidwell-Box linearity test, standartized residual values, standard errors (SE) of independent variables and Pearson’s r were obtained using SPSS 13.0 (Standard version, Chichago, IL, USA) to analyse quality of data and confirm the use of samples in logistic and linear regressions performed. Durbin-Watson test, Kalmogorov-Smirnov test and Shapiro-Wilkson test, VIF and tolerance, standardized residuals were obtained using SPSS 13.0 (Standard version, Chichago, IL, USA) to analyse quality of data retrieved from pharmacokinetic study.