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**Metformin stimulates intestinal glycolysis and lactate release: A single-dose study of metformin in patients with intrahepatic portosystemic stent.**

Nikolaj Rittig (PhD)\(^1,2\), Niels K Aagaard (associate professor)\(^3\), Elias Sundelin (PhD)\(^1,2\), Gerda E Villadsen (associate professor)\(^3\), Thomas D Sandahl (associate professor)\(^3\), Jens J Holst (professor)\(^4\), Bolette Hartmann (associate professor)\(^4\), Kim Brøsen (professor)\(^5\), Henning Grønbæk (professor)\(^3\), Niels Jessen (professor)\(^1,6,7\)

\(^1\) Steno Diabetes Center Aarhus, Aarhus University Hospital, 8200 Aarhus N, DK
\(^2\) Department and laboratories of Diabetes and Hormone diseases, Aarhus University Hospital, 8200 Aarhus N, DK
\(^3\) Department of Hepatology & Gastroenterology, Aarhus University Hospital, 8200 Aarhus N, DK
\(^4\) Department of Biomedical Sciences and Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, 2100 Copenhagen, DK
\(^5\) Department of Public Health, Clinical Pharmacology, Pharmacy and Environmental Health, University of Southern Denmark, 5000 Odense, DK
\(^6\) Department of Biomedicine, Aarhus University, 8000 Aarhus C, DK
\(^7\) Department of Clinical Pharmacology, Aarhus University Hospital, 8200 Aarhus N, DK

**Corresponding author:**
MD, professor Niels Jessen
Steno Diabetes Center Aarhus (SDCA), Hedeager 3, 2'nd Floor, 8200 Aarhus N, Denmark
Niels.jessen@biomed.au.dk

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The authors declared no competing interests for this work.

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**Keywords:** metformin; lactate; gut; portal vein; GDF15; human

**Abbreviations:**
EGP = endogenous glucose production

18F-FDG = 18F-flourdeoxyglucose

GDF15 = growth differentiation factor 15

GI = gastrointestinal tract

GLP-1 = glucagon-like peptide-1

LC-MS/MS = liquid chromatography and tandem mass spectrometry

MRI = magnetic resonance imaging

PET/CT = positron emission tomography-computed tomography

T2DM = type 2 diabetes mellitus

TIPS = transjugular intrahepatic portosystemic stent
The pharmacodynamic effects of metformin remain elusive, but several lines of evidence suggest a critical role of direct effects in the gastrointestinal (GI) tract. We investigated if metformin stimulates intestinal glucose metabolism and lactate release in the prehepatic circulation. We included eight patients with transjugular intrahepatic portosystemic stent (TIPS) in an open label study. Portal and arterialized peripheral blood was obtained before and 90 minutes after ingestion of 1000 mg metformin. Metformin increased lactate concentrations by 23% (CI95%:6-40) after 90 minutes in the portal vein. The plasma concentration of glucose, insulin, and C-peptide was higher in the portal vein compared with arterialized blood (p<0.05, all) and was lowered at both sampling sites following metformin ingestion (p<0.01, all). Plasma concentration of GLP-1 was 20% (CI95%:2-38) higher in the portal vein at baseline and metformin increased the concentration with 11% (1.5 pmol/l, p=0.05). The median concentration of growth differentiation factor 15 was 10% (CI95%:1-19) higher in the portal vein compared with arterialized blood. Ninety minutes after metformin administration, the median portal vein concentration increased to around 3000 ng/ml with a mean portal/arterial ratio of 1.5 (95%CI: 1.2 to 1.8). Non-targeted metabolomics showed that metformin acutely affected benzoate-hippurate metabolism. A single-dose of metformin directly affects substrate metabolism in the upper GI tract in humans with direct stimulation of non-oxidative glucose metabolism. These data suggest glucose lowering effects of metformin can be intrinsically linked with the GI tract without hepatic uptake of the drug.
(EudraCT ID 2017-001132-19)
Introduction

Metformin is an effective oral glucose-lowering drug that has been in clinical use for over 60 years, and it is today the first-line treatment of type 2 diabetes mellitus (T2DM) worldwide. Metformin effectively lowers plasma glucose and has an impressive safety record. Effects of metformin beyond its original indication are actively pursued, but its mechanism of action is incompletely understood and this complicates a more expansive use of the drug. Metformin does not interact with extracellular receptors and needs to be transported into cells to exert its effects. Uptake of the drug is dependent on specific transporter proteins and assumedly metformin actions require uptake in target organs.

In humans metformin uptake is most pronounced in liver, kidney and the intestine. In poorly controlled T2DM patients, metformin may reduce endogenous glucose production (EGP), but this is not a consistent finding. In T2DM patients with moderate hyperglycemia, metformin reduces plasma glucose but is not associated with reduced EGP. Metformin treatment of newly diagnosed T2DM and healthy individuals increased EGP, and it is therefore evident that reduction in EGP alone cannot account for the glucose lowering effect by metformin.

A single oral dose of metformin can reduce blood glucose levels but not after intravenous administration of equal doses of the drug. Intestinal effects of metformin may therefore be important for drug-efficacy. Metformin treatment significantly increases uptake of 18F-flourdeoxyglucose (18F-FDG) in positron emission tomography-computed tomography (PET/CT) examinations of the gastrointestinal (GI) tract. However, it is unclear whether this is associated with increased glucose metabolism in the enterocytes. In humans, 18F-FDG PET-MRI images show 18F-FDG uptake in the intestinal wall after metformin treatment, but glucose also appears in the lumen, suggesting that glucose is transported into the luminal space during metformin treatment. Alternatively, glucose in the enterocytes may undergo glycolysis, resulting in release of lactate as supported by animal models and in vitro studies where supra-pharmacological doses of metformin increased non-oxidative glucose metabolism and elevated lactate production in the GI tract.

The clinical effects of metformin are not limited to glucose lowering, but include beneficial effects on appetite regulation and body weight, and adverse effects like nausea and
These pleiotropic effects of metformin may also, at least in part, be mediated through direct effects of metformin on the GI tract. The inaccessibility of the portal vein precludes direct investigations of the intestinal effects of metformin in humans. Instead, evidence is predominantly obtained from animal and in vitro models. Unfortunately, the pharmacokinetic properties of metformin are species dependent and in rodent and in vitro studies metformin concentrations that are many fold higher than clinical levels are often used. We therefore designed this human clinical study aimed at i) establishing whether a single-dose of metformin increases non-oxidative glucose metabolism in enterocytes (primary endpoint), ii) assessing metformin concentrations in the portal vein during pharmacological dosing, iii) investigating acute gut-derived effects of metformin by applying non-targeted metabolomics analysis, and iv) performing hypothesis-driven investigations of metformin-associated release of GI peptides (e.g. glucagon-like peptide-1 (GLP-1), growth differentiation factor 15 (GDF15) and glucagon).
Material and Methods

Volunteers and location
Volunteers, >18 years of age, were eligible for inclusion in the study if they had cirrhosis and were treated with a transjugular intrahepatic portosystemic stent (TIPS) to reduce portal pressure and ameliorate complications of portal hypertension. Volunteers were not allowed to participate if they had a Child Pugh score ≥ 12, did not speak or understand Danish, had ongoing signs of infection, had an eGFR < 30 ml/min, were pregnant/nursing, or were already on Metformin treatment. All volunteers gave an informed oral and written consent before inclusion in the study. Volunteers were recruited by letter, during visits to the outpatient clinic, or during admission to the Department of Hepatology and Gastroenterology, Aarhus University Hospital, Denmark (single-centre study). The study was carried out in the period January 2018 to January 2020. During this time period we screened a total of 13 patients, and nine of these were recruited for the trial. Three out of the 13 patients did not want to participate after reading the study information brochure, one patient was diagnosed with hepatocellular carcinoma shortly after screening and withdrew his consent.

Ethics
The study was performed in accordance with the Helsinki Declaration and was monitored by the Danish Unit for Good Clinical Practice (GCP-unit Aarhus/Aalborg). The study was registered at the European Medicines Agency (EudraCT ID 2017-001132-19), approved by the Central Denmark Region Ethics Committee (Ethical approval ID 1-10-72-67-17), and registered at the Danish Data Protection Agency.

Study design
Nine patients with cirrhosis who had received or were about to receive a TIPS were included in this single-armed, intra-individual before-and-after trial. One patient was excluded due to unsuccessful blood sampling from the portal vein, and data are therefore based on the eight patients who successfully completed the study. Three volunteers were enrolled prior to the TIPS procedure, which allowed us to leave a catheter in the portal vein after having performed the TIPS procedure. Following a 2-hour standard observation period after anaesthesia, these volunteers entered the trial and followed the same protocol.
as the other volunteers. The five outpatient volunteers have had their TIPS procedure performed at least one year before entering the trial. Patients arrived at the Department of Hepatology and Gastroenterology at Aarhus University Hospital, Denmark, at around 07.30 AM following an overnight fast. A Swan-Ganz 5F (Cardinal Health, US) catheter was via the right internal jugular vein X-ray guided through the TIPS with the tip of the catheter placed in the portal vein. Blood was obtained from the portal vein approximately one hour (time = -60 minutes) before metformin administration. An intravenous catheter was then placed in peripheral vein (dorsal hand) and covered by a heating blanket in order to arterialize the blood. Baseline blood samples were obtained from the portal- and peripheral vein (time = 0 minutes). All volunteers ingested 1000 mg metformin crushed and diluted in 50 ml of tap water. Blood samples were drawn simultaneously and consecutively from both sampling sites every 15 minutes for the following 90 minutes.

Blood sample analysis
The oxygen saturation and blood concentrations of lactate and glucose were immediately (within 15 minutes) measured following blood sampling using an ABL800 (Radiometer Medical ApS, Denmark). Plasma and serum concentrations of GDF-15 (ELISA, Sigma-Aldrich, USA), insulin (ELISA kit from Dako A/S, Denmark), C-peptide (ELISA kit from ALPCO, USA), glucagon (RIA kit from EMD Millipore, Germany), and GLP-1 (cat no: 10-1278-01, Mercodia, Uppsala, Sweden) were measured using commercially available kits, following the manufacturers’ guidelines and recommendations.
Metformin concentrations in plasma were determined using liquid chromatography and tandem mass spectrometry (LC-MS/MS) as previously described.
A non-targeted metabolomics approach was applied in order to investigate relative changes in metabolites at baseline vs. 90 minutes following metformin treatment in arterialized and portal vein blood samples as previously described (Metabolon Inc, USA).

Statistics
All baseline data are presented as means ± standard deviation (SD) and graphs as means ± standard error of mean (SEM) unless otherwise specified. Data were compared using repeated measure two-way ANOVA analysis and post-hoc t-tests (Student-Newman
Keuls’s method for multiple testing) and p-values < 0.05 were considered significant. The interaction is reported as time x sampling site and as main effects of time or sampling site. The non-targeted metabolomics data were compared within (before and after metformin) and between sampling sites (arterialized and portal vein blood) using paired t-tests and estimates of the false discovery rate (q-value) to take multiple testing into account. A pre-study power calculation was performed using a α-value of 0.05 (risk of type 1 error), a β-value of 0.8 (risk of type 2 error), an expected relative increase in blood lactate of around 12% with a standard deviation of 9% in the portal vein, which is in line with similar reports in rodents. This equalled a total of nine volunteers. ArrayStudio was used to perform principal component analysis. SigmaPlot (version 14, Systat Inc, USA) was used to perform all targeted analyses and create graphs.

Data and resource availability.
The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.
Results

Volunteers and safety
The patient characteristics are listed in table 1. Eight volunteers completed the study. Two volunteers experienced mild and transient abdominal discomfort following the TIPS procedure but without relation to metformin consumption. No severe adverse events were recorded during the trial.

Lactate concentrations
At baseline the mean concentration of lactate was 0.9 ±0.1 mM in the portal vein and 1.2 ±0.1 mM in arterialized blood (difference 0.3 mM, 95%CI: 0.1 to 0.5). Lactate increased by 23% (CI95%: 6 to 40) in the portal vein and by 2% (CI95%: -15 to 19) in arterialized blood following metformin ingestion (time x sampling site, p=0.002, figure 1).

Oxygen saturation and concentrations of glucose, insulin, c-peptide, glucagon, GLP-1, and GDF15.
The mean oxygen saturation at baseline was 83 ± 6% in the portal vein and 93 ± 2% in arterialized blood and the saturations were stable throughout the study period (data not shown).
At baseline the mean concentration of glucose was 6.8 ± 0.8 mM in the portal vein and 6.6 ± 0.8 mM in arterialized blood (figure 2A). Metformin reduced glucose concentrations to 6.4 ±0.7 mM in portal vein blood and 6.1 ± 0.7 mM in arterialized blood at 90 minutes (time x sampling site, p= 0.74, and main effect of time, p<0.001).
At baseline the mean plasma concentration of insulin was 41% (95%CI: 7 to 75) and c-peptide 15% (95%CI: 8 to 22) higher in the portal vein blood compared with arterialized blood. Metformin reduced (main effect of time) the concentration of insulin (p=0.01) and c-peptide (p=0.009) similarly at both sampling sites (time x sampling site, p=0.37 and p=0.29, respectively).
The mean concentration of glucagon was 55% (95%CI: 15 to 95) higher in the portal vein compared with arterialized blood at baseline and increased by 5 pmol/l (time, p=0.08) following metformin administration (figure 2D).
At baseline the mean plasma concentration of GLP-1 was 20% (CI95%: 2 to 38) higher in the portal vein compared with arterIALIZED blood (figure 2E), and metformin elevated GLP-1
concentrations similarly (time x sampling site, p = 0.90) with a 1.2 pmol/l increase in arterialized blood (paired t-test, p<0.05) and a 1.5 pmol/l increase in the portal vein (paired t-test, p=0.05).

At baseline the median plasma concentration of GDF15 was 10% (95%CI: 1 to 19) higher in the portal vein compared with arterialized blood (figure 2F). Plasma GDF15 concentrations rose (time, p=0.11) at both sampling sites throughout the trial and with no difference between groups (sampling site, p=0.13, figure 2F).

**Metformin concentrations**

As expected, metformin was undetectable at both sampling sites at baseline (figure 3). The median concentration of metformin increased to ~3000 ng/ml in the portal vein and ~1500 ng/ml in arterIALIZED blood (figure 3A). There was a high degree of interindividual variation in metformin concentrations and peak values ranged from 181 ng/ml to 7242 ng/ml in the portal vein (figure 3, B and C). The mean portal/arterial ratio 90 minutes following metformin ingestion was 1.5 (95%CI: 1.2 to 1.8).

**Metabolomics**

A total of 993 named compounds were detected. As expected, the inter-individual variability dominated principal component analysis of the non-targeted metabolomic data. This was followed by time of sampling/treatment (baseline or 90-minutes post metformin) which dominated over site of sampling (portal vs arterial) (Figure S1). Metformin lowered glucose- and elevated 3-phosphoglycerate levels in the portal vein 90 minutes following consumption of the drug (Figure S2) which together with increases in lactate levels demonstrates that metformin increases intestinal glycolysis. Metabolomics determinations of lactate levels followed the pattern of the levels determined immediately after sampling, but the metabolomics determinations did not reach statistical significance (Figure S2). The porto-arterial ratio (portal/arterialized) of glucose was >1 and substrates typically used for gluconeogenesis (pyruvate, lactate, glutamine, glycerol, and alanine) were all <1 at baseline (Figure S2). Metformin treatment increased circulating levels of long-chain fatty acids in arterIALIZED blood but not in the portal vein. Numerous microbiome derived metabolites linked to the benzoate and hippurate metabolism decreased 90 minutes following metformin consumption at both sampling sites (Figure S2). Median
scaled values from the complete non-targeted metabolomic dataset are shown as a heatmap in Figure S2.

*Supplementary material.*

Graphs showing individual results for patients with acute and chronic TIPS, diabetes and non-diabetes are shown in Figure S3.
Discussion

This is to our knowledge the first clinical study with portal vein blood sampling investigating the effects of a single oral clinically relevant dose of metformin and provide evidence for direct stimulation of gastrointestinal glycolysis by metformin in humans. Lactate levels in portal vein blood increased by >20% already 90 min after ingestion of the drug. At this time-point, the intestinal exposure to metformin is limited to the upper intestine\textsuperscript{4}, and \textsuperscript{18}F-FDG PET-MRI images indicate that metformin-induced glucose uptake is most prominent in colon. Therefore, our results likely represent an underestimation of the potential lactate levels in the portal vein when the whole GI tract is saturated. This corroborates suggestions of a prominent role of the GI tract in metformin action\textsuperscript{18, 19}, where the GI tract constitutes a significant glucose sink during metformin treatment.

Ingestion of a single dose of 1000 mg metformin typically leads to peak systemic concentrations of metformin within the first 2-4 hours and in the range of ~1000 to 5000 ng/ml (~10 to 40 µM) with large interindividual variance\textsuperscript{29}. We found a median portal concentration of metformin around 3000 ng/ml (~18 µM) that seemed to have already peaked (plateau between 60 and 90 minutes), but notably with a large interindividual range from ~181 ng/ml to 7242 ng/ml (~1 µM and 44 µM). Portal vein levels are higher compared with systemic circulation, but only 1.5 fold and not 2-3 fold as previously suggested\textsuperscript{30}.

Metformin treatment was accompanied by parallel reductions in plasma glucose, insulin and C-peptide levels in both portal and systemic blood, while plasma concentration of glucagon tended to increase during the trial period, which aligns with previous studies\textsuperscript{9, 12, 31}. It is highly conceivable that the metformin-induced intestinal glucose uptake reduced blood glucose levels and subsequently reduced insulin secretion while increasing glucagon secretion. The fasting conditions under which the study was conducted may have contributed, and the absence of a placebo-control precludes firm conclusions on whether this was caused by metformin per se.

At baseline, we found 33% higher lactate concentrations and slightly lower glucose concentrations in arterialized blood compared with blood from the portal vein. These findings are in line with a previous clinical study obtaining blood from the portal vein of cirrhotic patients with TIPS also showing a slightly negative (near null) arterio-portal concentration of glucose (indicating net glucose release from the GI tract) combined with
positive arterio-portal concentrations (indicating GI tract uptake) of potential gluconeogenic substrates such as lactate, pyruvate, glycerol, and especially glutamine. These observations are in line with mouse studies with intestinal gluconeogenesis observed during fasting conditions. However, a limitation of our study design (i.e. lack of portal blood flow measurements and labelled substrates) preclude us from concluding there is glucose release from the GI tract. Our group has recently shown how metformin lowers the portal venous pressure gradient without affecting hepatic blood flow.

Lactate concentration is affected by oxygen availability, but we did not find evidence of artefacts caused by the use of the heated hand vein method used in this study. The observed oxygen saturation of ~83% in portal vein blood and ~93% in arterialized blood aligns with previous observations and oxygen saturation in arterialized- and portal vein blood remained stable without change during time (data not shown). Increased lactate levels following metformin consumption were also detected in our non-targeted metabolomic analyses although the increase did not reach statistical significance in this analysis. This is most likely due to the difference in methods and sample handling (i.e. immediate measurement on blood sampled with arterial blood gas syringe vs. sampled in EDTA tubes and freezer stored) used to estimate the two independent lactate levels.

GLP-1 is an incretin hormone stimulating insulin secretion from the beta-cells of the pancreas, but also affecting gastrointestinal motility and appetite, at least partly, through neural signals within the portal system. Portal concentrations of GLP-1 plays a key role for its glucose-lowering effects, and animal studies indicate that these effects are insulin-independent; and may be due to GLP-1 receptors different from those in pancreatic beta-cells. In the present study, we observed 20% higher GLP-1 levels in the portal vein compared with arterialized blood and metformin treatment elevated plasma concentration of GLP-1 at both sampling sites in line with previous human studies.

Metformin increases circulating GDF15 in humans and GDF15 action is required for the weight loss effects of metformin in mice. We found GDF15 levels in TIPS patients to be around 10-fold higher than concentrations reported in healthy individuals in line with human investigations of patients with cirrhotic liver disease. The three patients who had TIPS performed acute before this study had the highest baseline GDF15 concentrations (Figure S3), emphasizing that GDF15 is released upon stress. Under these conditions, we did not detect statistically significant increases in GDF15 levels.
during the 90 min of metformin exposure where plasma was sampled, but plasma concentrations of GDF15 were 10% higher in the portal vein compared with arterialized blood suggesting that the elevated GDF15-levels are not due to production in the cirrhotic liver per se but also includes GDF15 release from the GI tract. Thus, these results support that the GI tract produces GDF15 and it cannot be excluded that it also plays an important role in the elevated concentrations associated with metformin use.

Our investigations also comprised an exploratory part where metabolites in portal vein blood were determined in a non-targeted analysis with detection of 993 named compounds. There were no inconsistencies in directions of changes between targeted analysis and the non-targeted metabolomics. At a statistical significance cut-off of p<0.05, 50 differences can be expected between groups by random chance. The only comparison that was near this level was arterial vs portal vein at baseline (65 differences, likely representing inherent differences between the two sources of blood). The pre- and post-metformin comparisons at each location, as well as the arterial vs portal at 90 minutes post-metformin showed numbers of changes that were ~2-3 fold above the random chance level (114-147 differences). This suggests that the treatment with metformin causes detectable changes in the metabolic profile in both portal and arterial blood, and that differences between the sampling sites are present after treatment.

Grouping the metabolites allowed for identification of hypothesis generating metformin signatures. The reduced glucose levels and increased lactate levels determined by targeted analysis were followed by increases in fumarate and malate levels (either significant, trending, or not statistically significant) at 90 minutes in blood from both sources. This could suggest that increased TCA cycle activity occur due to the elevated tissue glycolysis. Concentrations of several long chain fatty acids increased in the general circulation but not in the portal vein. This could be explained by decreased insulin levels and a subsequent increase in lipolysis, but tracer experiments are needed to test this hypothesis. In the portal vein, a marked reduction of metabolites associated with benzoate-hippurate metabolism was noted. Dietary aromatic compounds such as polyphenols, purines, aromatic acids, and amino acids undergo microbial degradation and produce benzoate. Benzoate is absorbed and conjugated with glycine to produce hippurate trough the formation of benzoyl CoA within mitochondria of the liver and kidneys. Recently, it was shown that months of metformin treatment affects the metabolic
functions of the microbiota, which may favour certain bacterial strains and may help explain the changes in microbiota associated with metformin treatment. The short duration of exposure in these metformin-naive patients makes it unlikely that the reductions in benzoate-hippurate associated metabolites are direct drug-microbiota interaction; in part because metformin has not reached the colon at this time-point. Instead, we speculate that metformin may affect transport and local metabolism of metabolites produced by the intestinal microbiota. However, firm conclusions cannot be drawn from our study and this remains to be investigated in other research designs.

A pre-study power calculation showed that nine volunteers were to be recruited. Unfortunately, one volunteer was excluded due to unsuccessful blood sampling. The requirement for inclusion of volunteers with a TIPS limited recruitment, which was expected and the main reason why we chose a single-armed design. A randomized placebo-controlled parallel or crossover design would have strengthened the study, but significantly limited recruitment and the number of volunteers in each group. We investigated the acute effects of metformin in the GI tract by including patients with cirrhotic liver disease. Therefore, extrapolation of our results to other patient groups (e.g. T2DM patients) and longer treatment exposure should be done cautiously. Two out of the eight patients in our study had T2DM, but we did not detect differences in the response to metformin among patients with or without diabetes in this small cohort. Noteworthy, the two patients with T2DM had markedly higher insulin concentrations in the portal vein throughout the study, but had comparable systemic concentrations of insulin (Figure S3). GI symptoms are common in patients with cirrhosis and we cannot rule out that results were affected by GI dysfunctions. Each study was limited to a 90-minute period following metformin consumption due to ethical considerations (mainly the risk of portal vein thrombosis), and we are therefore unable to explore the effects beyond this time-point. Also, TIPS is associated with ~30% porto-systemic shunting of blood, which may lead to an underestimation of porto-systemic differences. Despite these limitations we feel that our study provides new and important insight to the acute effects of metformin treatment in humans and clearly shows that metformin has significant effects on the GI tract.

In conclusion, we demonstrate that a single dose of metformin stimulates intestinal glycolysis and lactate release and that this is associated with altered release of insulin and GLP-1 in patients with cirrhosis. We characterize metformin absorption and distribution in
the portal vein system, and describe the relative changes in metabolites in the portal vein during metformin treatment. Together, these results provide further experimental evidence for the importance of metformin effects in the GI tract for the beneficial effect of one the most widely prescribed drugs world-wide.

What is already known about this subject?
- The pharmacodynamic and -kinetic properties of metformin translate poor from animal models to humans
- In humans, metformin increases intestinal uptake of glucose tracers, but the fate of glucose is unknown

What is the key question?
- Does metformin increase intestinal glycolysis in humans?

What are the new findings?
- Metformin acutely increases portal vein lactate levels by 23% and GLP-1 levels with 11%.
- Metformin acutely increases circulating metabolites associated with intestinal glycolysis while reducing microbiome derived metabolites.
- Metformin levels in the portal vein showed large interindividual variation (up to 35-fold difference).

How might this change clinical pharmacology or translational science?
These findings demonstrate direct effects of metformin on intestinal glucose metabolism and gut hormone secretion. This provides evidence of the intestines as a direct target of metformin action in human, and will help facilitate an optimized use of metformin in established and putative treatment indications.

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Author Contributions
NR and NJ wrote the manuscript; NR, NKA, ES, HG, and NJ designed the research; NR, NKA, HG, GEV, TDS, KB, BH, and JJH performed the research; All authors analyzed the data.
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**Table 1. Baseline characteristics of the volunteers.**

Data are shown as median [range] or number of volunteers (n). Abbreviations: BMI= body mass index, INR= international normalized ratio, CRP= C-reactive protein, FPG = fasting plasma glucose, NASH= non-alcoholic steatohepatitis.

**Figure legends.**

**Figure 1. Relative change in lactate.**

The mean relative change ±SEM in lactate concentration at baseline (time = 0 minutes) and 90 minutes following consumption of 1000 mg metformin is shown for blood sampled...
as arterialized blood (open circles) and from the portal vein (closed circles). A two-way repeated measurement ANOVA analyses showed a time x sample site interaction, p = 0.002, and post hoc (Student-Newman Keuls method) t-tests are shown for differences between sample sites, * = p<0.05. N = 8.

Figure 2. Portal and arterialized blood concentrations of glucose, C-peptide, insulin, glucagon, GDF15, and GLP-1.
The blood concentration of glucose (A), insulin (B), C-peptide (C), glucagon (D), GLP-1 (E), and GDF15 (F) is shown for arterialized blood (open circles) and blood from the portal vein (closed circles) before and following consumption of 1000 mg metformin. Data are shown as means ±SEM with the exception of GDF15, which is shown as median ±SEM due to an unequal distribution of data. Comparisons were performed using two-way repeated measurement ANOVA analyses. A paired students t-test (# = p<0.05) was performed to compare sampling sites at baseline (time = 0 minutes). N = 8. GDF15 = growth differentiation factor 15, GLP-1 = glucagon-like peptide-1.

Figure 3. Metformin concentrations.
The median ±SEM concentration of metformin is shown (A) in arterialized blood (open circles) and in blood from the portal vein (closed circles) before and after consumption of 1000 mg metformin. A two-way repeated measurement ANOVA analyses showed a time x sample site interaction, p<0.001, and post hoc (Student-Newman Keuls method) t-tests are shown for differences between sample sites, * = p<0.05. The individual portal vein metformin concentrations (B) and the metformin concentration in arterialized blood (C) are shown. N = 8.

Supplemental Information
Figure S1
Figure S2
Figure S3