**Title page**

**Title:** MRI measurement of drug-induced inhibition of liver function

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**Abbreviated title page**

Title of the manuscript: MRI measurement of drug-induced inhibition of liver function

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A summary statement:

**MRI measurements of gadoxetate liver uptake and biliary excretion rates can confidently detect drug-induced inhibition of hepatocellular function.**

Key Results

* In healthy volunteers, a clinical dose of rifampicin induces a strong reduction in hepatocellular uptake (-94%) and biliary excretion (-44%) of gadoxetate.
* The same results can be obtained with a shorter 25-minute acquisition feasible in clinical routine.
* Routine bilirubin levels also respond to rifampicin administration, but the effect is only detectable after 4 hours and is an order of magnitude weaker.

List of all abbreviations

DILI: Drug-induced liver injury

DDI: Drug-drug interaction

DCE-MRI: Dynamic Contrast-Enhanced MRI

CI: Confidence interval

MOLLI: modified Look-Locker sequence

khe: Hepatocellular uptake rate

kbh: Biliary excretion rate

**Abstract (<300) words**

Background

Effective management of drug-induced liver injury and drug-drug interactions in drug development and in clinical practice requires better non-invasive assessment of hepatocellular function. Gadoxetate-enhanced MRI may provide a solution, but it is not known whether it is sufficiently sensitivity to detect drug-induced inhibition of liver function in humans.

Purpose

The aim of this study was to investigate if liver uptake and excretion rates of gadoxetate, as measured with dynamic contrast enhanced MRI (DCE-MRI) in healthy volunteers, can detect changes in hepatocellular function by a known inhibitor.

Materials and Methods

This prospective study (2022-2023) recruited 10 healthy volunteers that were assessed on two separate visits. On the second visit, a known inhibitor drug (rifampicin, 600mg) was administered orally one hour before the start of the scan. DCE-MRI was performed over two separate scans, one hour apart, to ensure the slow excretion of gadoxetate after inhibition of liver function was captured. DCE was acquired with a fast (2.5 sec) 3D free-breathing protocol collecting data continuously for 50 minutes. Gadoxetate was injected at 1/8th of a clinical dose, escalated to 1/4th after the first 3 volunteers. Liver uptake and excretion rates were derived using pharmacokinetic modelling, and the effect of rifampicin was determined with a paired t-test on pre- and post rifampicin data. The significance level was set at p<0.01.

Results

Eight out of the 10 participants completed both visits (age xx, gender xx). Rifampicin reduced the hepatocellular uptake rate with 94% (p<0.001), and the effect was consistent between volunteers (95%CI 1.0%). The biliary excretion rate was reduced by a smaller amount (44%, p=0.01) and the effect was more variable (95%CI 35%). Both rate constants reduced in each subject, except for one excretion rate which was low at baseline.

Conclusion

MRI measurements of gadoxetate liver uptake and biliary excretion rates can confidently detect drug-induced inhibition of hepatocellular function.

**Introduction**

Drug induced liver injury (DILI) can be caused by numerous drugs in humans and may result in a spectrum of clinical presentations from asymptomatic to life-threatening acute liver failure. DILI may present as hepatocellular (i.e. affecting hepatocytes), cholestatic (affecting bile flow and the biliary system) and mixed [David2010]. Hepatobiliary transport inhibition by drugs is thought to be the one of the main mechanisms related to drug induced cholestasis [Wagner2009]. Drugs impair bile flow from hepatocytes by inhibiting the activities of hepatic efflux transporter proteins (BSEP, MRP2, OATP1B and others).

In addition, numerous clinically relevant drug-drug interactions (DDIs) arise via inhibition or induction of hepatic uptake or excretion of victim drugs, which may impact on their efficacy and toxicity. DDIs are assessed during drug development by a combination of in-vitro and in-silico methods, but predictions are difficult to verify clinically – especially when DDIs arise via inhibition of the liver’s excretory function. Consequently, clinical trials risk either underestimating DDIs, and so potentially harming study subjects, or overestimating DDI’s and consequently failing to show efficacy. Managing these risks effectively requires more specific and noninvasive methods to determine drug exposure within the tissue of interest in human subjects.

A potential solution is the widely available quantitative biomarker modality of dynamic gadoxetate enhanced MRI (gadoxetate DCE-MRI). The contrast agent gadoxetate is a substrate of the liver uptake and biliary efflux transporters OATP1B1, OATP1B3, NTCP, MRP2 and MRP3 [Tsuboyama 2010, Nassif 2012]. Gadoxetate DCE-MRI is used routinely in the clinic to detect and characterize liver lesions in adults with known or suspected focal liver disease [Poetter-Lang 2020]. While visual assessment or simple signal-based metrics have proven useful in clinical practice, the data can also be used to measure gadoxetate liver uptake- and excretion rates directly [Sourbron2012, Georgiou2017]. This is appealing in the context of drug development as these data can be directly integrated with evidence based on preclinical- and in-vitro assays. In clinical practice such absolute values have the benefit of providing absolute benchmarks of liver function that are independent of the specific acquisition method used.

Preclinical interventional studies in rats have shown that drug-induced inhibition of liver uptake and excretion can be measured reproducibly with gadoxetate DCE-MRI [Ulloa 2013, Karageorgis 2018], and can distinguish OATP1B inhibitors from non-inhibitors [Melillo2023]. The aim of this study was to test whether this finding translates to humans, by measuring the change in liver gadoxetate uptake and excretion after administration of a known inhibitor drug in healthy volunteers. Rifampicin was selected as a test drug since the preclinical studies revealed a strong inhibition of biliary efflux in addition to the expected inhibition in OATP1B activity.

**Materials and Methods**

Study design

The study was approved by the local institutional review board and prospectively recruited 10 healthy volunteers, who gave informed consent. Exclusion criteria beyond standard MRI exclusions were previous history of liver- or kidney disease and regular prescribed medication including the contraceptive pill.

MRI was performed on two separate days (Fig 1). The only difference between the two MRI visits was the oral administration of 600mg rifampicin one hour before the start of MRI scanning on visit 2. This timing was chosen so that peak inhibition would occur during the scan. Since excretion of gadoxetate after inhibition of liver function is slow, the measurement on each day was split over two different scans, separated by an hour where the subject left the scanner and rest. In each of the two scans, DCE-MRI was performed continuously for 50 minutes. Given the length of the acquisitions and the fact that scans couldn’t be always scheduled for early morning, participants were allowed to eat and drink before the study in moderation.

The study design included a gadoxetate dose escalation strategy, in order to ensure that no higher gadoxetate dose was used than strictly necessary. Initial dosage was one eighth of a standard clinical dose in each of the 4 MRI’s. The data were reviewed after two participants completed both visits and investigators were allowed to increment the dose at this stage if this was deemed necessary to guarantee the data integrity.

Blood samples were taken before and after each scan and analysed in the local clinical lab with standard liver function tests. Part of the of sampled blood was sent to a central study lab and analysed for Coproporphyrin, which acts as an endogenous biomarker of OATP1B activity [Takita 2022].

MRI protocol

MRI was performed in a single site on a 3T Siemens Prisma. The protocol included localizers, high-resolution T2-weighted axial and coronal scans, transverse T2\* mapping, transverse T1-mapping with a modified Look-Locker sequence (MOLLI), B1-mapping, variable flip angle T1-mapping (VFA) and free-breathing DCE-MRI. The first MRI of each day concluded with a post-contrast MOLLI T1. T2\*, B1 and VFA T1 were acquired for secondary objectives and are not reported in this paper.

MOLLI was acquired with 5 slices, 5 mm thick, FA=12 deg, TR/TE = 423/2.75 ms, 7(2)4 scheme with simulated ECG trace set at 80 bpm. The DCE sequence was a coronal 3D FLASH with a time resolution of 2.3sec, 36 slices, FOV 450mm, TR 4.93ms, TE 1.23ms, FA 15deg, voxel size 4.7x4.7x5mm, base resolution 96 and 3D GRAPPA factor 2. Gadoxetate was injected at 1mL/sec and followed by a 20mL saline flush at the same rate. Prior to injection, a baseline of 5 mins was acquired on the first scan, and 10 min on the second.

MRI processing

DICOM images were anonymised, exported and uploaded on a central server and further analysed using in-house software, Voxelflow. The axial MOLLI T1 maps calculated by the scanner software (syngo MR E11) were manually segmented to extract median whole liver and aorta T1 values. Whole liver regions were drawn in parenchyma avoiding major blood vessels. Breathing motion and patient positioning between scans was corrected using 3D rigid and deformable motion correction [Avants2011]. The whole liver and aorta were segmented manually on a peak enhancement map. Signal-time curves for both scans were concatenated and processed off-line using automated python scripts.

DCE modelling

Gadoxetate concentrations in aorta and liver were modelled as shown in Figure 2. In the liver model, uptake and excretion rates khe and kbh were varied linearly over time to allow for changes in liver function during the day.

Concentrations were converted to longitudinal relaxation rates using the precontrast MOLLI T1 and literature values for the Gadoxetate relaxivity in liver in blood at 3T [Ziemian 2021]. Relaxation rates were converted to signals using the standard model for a spoiled gradient echo sequence in the steady state [Buxton 1987].

Model fitting was performed using the python package scipy. The aorta signal model was fitted first, aorta concentrations were derived from the optimal parameters, and these were used as input for fitting the liver signal model.

Data analysis

Effect sizes were calculated for each participant and each parameter p as (p\_rifampicin-p\_baseline) / p\_baseline. Mean values and 95% confidence intervals on the mean (95%CI) were calculated for all parameters at baseline and after rifampicin, and for their effect sizes. A paired t-test with a significance level of p<0.01 was used to test for differences between baseline and rifampicin values.

The primary outcome markers were the hepatocellular uptake rate khe and biliary excretion rate kbh, averaged over the duration of the acquisition. Other markers were also tested for rifampicin effect as secondary aims. For comparison with non-imaging pharmacokinetic studies, the MRI-measured liver volumes were used to derive whole liver clearance (mL/min).

As a secondary aim it was tested to what extent the scan time could be reduced without affecting the main results. Only the data from the first scan were used for this, and full results were rederived after reducing the total acquisition time from 45 min to 5 min in steps of 5 min.

**Results**

Ten volunteers were recruited and underwent the baseline scan (table 1). Liver function tests before the first scan confirmed that all participants had normal liver function.

Eight volunteers completed the second day scanning. The fifth subject could not complete the second visit because of difficulties in accessing an adequate vein. In the first volunteer, the gadoxetate at baseline was mistakenly administered at 10x the planned dose. Since the dose in this volunteer was prescribed to be 1/8th of a clinical dose for each scan, the volunteer received 2.5x a standard clinical dose on the first day and was withdrawn from the study. The volunteer was followed up clinically and no adverse events were reported.

After inspection of the data from the first two complete baseline/rifampicin studies it was decided to escalate the dose of gadoxetate per scan from 1/8th to 1/4th of a clinical dose to boost the enhancement-to-noise level.

A visual inspection of the data confirms that the models fit the data well, and qualitatively shows kinetic differences in the liver signals after rifampicin, consistent with reduced uptake and delayed excretion (Fig 3). At baseline the extracellular phase of the signal is masked by a rapid uptake which peaks after approximately 25 minutes (Fig 3a). After rifampicin, uptake is much reduced with no obvious hepatobiliary peak concentration - exposing an early peak in concentration consistent with the extracellular contribution (Fig 3b). The second injection shows a similar pattern as the first. No obvious qualitative differences between baseline and rifampicin data are observed in the aorta signal (Fig 3 c, d).

Figure 4 illustrates the results for the primary outcomes graphically, and table 2 shows numerical results for all liver biomarkers. On average, rifampicin reduced the hepatocellular uptake rate khe with 94% (p<0.001), and the effect was consistent between volunteers (95%CI 1.0%). The biliary excretion rate kbh was reduced by a smaller amount (44%, p=0.01) and the effect was more variable (95%CI 35%). Both rate constants reduced in each subject with the exception of one kbh value which was already low at baseline (Fig4 c, red line).

Of the secondary liver parameters shown in table 2, the extracellular volume fraction (p=0.002) and mean transit time (p=0.01) increased after rifampicin. The total liver clearance mirrors the changes seen in the hepatocellular uptake rate (p<0.001). The MOLLI parameters T1 and ΔR1 at 45mins and 120mins after contrast agent administration also showed significant changes after rifampicin (p<0.001).

Table 3 verifies that rifampicin does not affect the systemic parameters derived from the aorta model (all p>0.01). At a weaker significance level (p<0.05), the extraction fractions into the leakage space and out of the body are reduced - indicating that the impaired liver function is not fully compensated by an increased extraction through the kidneys.

Figure 5 shows that the effect of rifampicin can be measured with equal accuracy and precision in a single scan with a 25-minute DCE-MRI. This is a substantial reduction compared to the full 2-scan, 4-hour protocol, and is feasible within routine clinical scan sessions.

Figure 6 shows the measured variability in liver uptake and excretion rates during the day, both at baseline and after rifampicin. At baseline, average variability of the uptake rate and biliary excretion rate during the experiment is 43% and 41%, respectively. The variability is not consistent between individuals, except for khe after rifampicin which shows a small increase during the day in each subject, consistent with reduced concentration of the drug.

Table 4 shows the results from the routine liver function tests for all visits and time points. Values at baseline visit 1 show a small reduction in albumin (-6.4%, p=0.002) and ALP (-10%, p=0.003) over 2 hours but there is no change between both visits. Rifampicin does not have a measurable effect on blood tests at 2 hours. 4 hours after administration, bilirubin is increased (140%, p<0.001) whereas albumin (-5.4%, p=0.001) and ALP (-7.4%, p=0.004) show a small reduction.

**Discussion**

Effective management of DILI and DDI in drug development and in clinical practice requires better non-invasive methods to determine the effect of drugs on hepatocellular function. The aim of this study was to investigate if liver uptake and excretion rates of gadoxetate, as measured with MRI in healthy volunteers, can detect changes in hepatocellular function by a known inhibitor. Results showed a strong and consistent reduction in liver uptake (-94%) and biliary excretion (-44%) of gadoxetate after rifampicin administration, indicating a potential role of this assay in drug safety assessment.

The result is qualitatively and quantitatively in line with findings in a previous preclinical study in healthy rats, acquiring the same biomarkers using similar acquisition and modelling strategies [Melillo 2023]. The study found that rifampicin induced a -90% reduction in liver uptake and -43% in biliary excretion, in close numerical agreement with our observations in healthy volunteers. The rat study also found a similar effect in another test drug (ciclosporin) whereas other test drugs affected either uptake (ketoconazole) or excretion (pioglitazone and asunaprevir), but not both. Such experiments with multiple test drugs have not yet been done in humans, but the current proof of concept using rifampicin provides a strong rationale for more in-depth validation in human subjects.

The results also confirmed preclinical observations that inhibition of biliary excretion by rifampicin is weaker and more variable than inhibition of hepatocellular uptake. This may reflect a weaker interaction between the drug and biliary excretion transporters of gadoxetate. However, it may also be due to the drastic inhibition of uptake. This reduces the drug concentration inside the cell, causing a more limited exposure to excretion transporters. Additionally, excretion after strong inhibition is likely to be a very slow process, most likely reducing the precision of the measurement even with the extended acquisition times employed in this study.

The aorta was modelled in this study to allow extrapolation of concentrations in the gap between the two scans, and to improve the robustness of the input to the liver model – which is critical for the stability of the liver results. The systemic biomarkers derived from the aorta model are therefore a spin-off result that does not contribute to the main aim of the study, but they are nevertheless useful as an additional sanity check. Measured systemic parameters such as cardiac output are in the expected range for human adults in rest, and are not affected by rifampicin – as expected from known pharmacokinetic properties of the drug.

The assay used in this study was designed for use in early-stage drug development, where it is intended to inform critical decisions on whether a novel drug should be developed further. Elaborate designs such as the 2-scan protocol deployed in this study are justifiable at this stage of drug development, but they are unlikely to be widely applicable in clinical routine. This is a major limitation considering the wide range of potential applications of this method in evaluating liver function in chronic liver disease [Poetter-Lang 2020]. The secondary finding that the protocol can be shortened to 25min without impact on the main observations is in that sense highly significant. Future studies will have to investigate whether these shorter acquisitions are sufficient to provide more fine-grained differentiation between varying levels of inhibition, which may be critical in a context of chronic liver disease.

The results have also demonstrated that the effect of rifampicin can be detected using much simpler MRI biomarkers, in particular T1 at 45 minutes after injection, or the change in relaxation rate at this time. These biomarkers they do not require elaborate modelling or image analysis and they are available via in-line postprocessing of product sequences on most scanners. Similar approaches have been studied in chronic liver disease [Obmann 2022] A downside is that they do not distinguish between uptake and excretion, cannot be compared to findings with non-imaging assays, and their numerical values depend on variables settings such as contrast agent dose and injection rate. Careful consideration of the specific requirements of individual clinical applications is warranted to decide to what extent these trade-offs are beneficial.

Analysis of the blood samples has shown that the effect of rifampicin can also be detected from bilirubin levels using routine liver function tests. However, these are less sensitive to inhibition. Bilirubin does not show a measurable effect after 2 hours, and at 4 hours is increased with a factor 2.4. The intracellular uptake rate, on the other hand, is reduced with a factor 15 during the first scan at 1 hour after rifampicin administration. Like the T1-based imaging biomarkers, blood tests are also less specific in that they cannot distinguish inhibition of uptake and excretion.

A fundamental limitation of the MRI assay is that it only probes the transporters that show an affinity with gadoxetate, notably organic-anion-transporting polypeptide 1B1 (OATP1B1) and multidrug resistance-associated protein 2 (MRP2). In particular, this means that specific inhibition of the bile salt export pump (BSEP) cannot be detected [Kubitz 2012]. Inhibition of BSEP is considered important because genetically inherited defects in BSEP expression have been found to cause severe cholestatic liver injury and because many drugs that cause human idiosyncratic DILI inhibit BSEP activity [Wagner2009].Invitro drug testing for BSEP inhibition has been recommended by European Medicine Agency [EMA 2012]. Despite this, the Food and Drug Administration (FDA) has indicated an interest in developing the gadoxetate-based assay by accepting it into their biomarker qualification program [FDA 2021].

Study limitations: analysis of the data revealed substantial variations in liver function during the course of the experiment, particularly between the first and second scan on the same day. The changes were not consistent between volunteers, except after rifampicin where a small but systematic increase in uptake was observed – consistent with a decaying plasma concentration of the drug after administration. The study design did not standardize preparation of the participants by controlled diet or fluid intake before the visit, and participants were not given any dietary instructions between the two scans. It is not clear whether differences in intake explain the variability observed, but it appears prudent for future validation studies to include some level of standardization in the diet and the timing of the scan.

In conclusion, this study showed that MRI measurements of gadoxetate liver uptake and biliary excretion rates can confidently detect drug-induced inhibition of hepatocellular function. Future studies should investigate whether these findings can be replicated with a wider range of test drugs and evaluate the effect of drug-induced inhibition in patients with impaired liver function.

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**Figure legends**

A diagram of a patient's schedule

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**Figure 1**: flow diagram showing the study design and assessments performed at each visit. Visit 0 was a screening visit to assess eligibility for the study, take informed consent and collect key data. Visit 1 was the baseline visit, no more than 60 days after consent, and involved two MRI scans at least 2 hours apart, and blood tests before and after the scans. The post-treatment visit 2 took place between 2 and 4 weeks after the baseline visit, and involved blood sampling before oral rifampicin administration, and then after 1 hours a repeat of the same protocol performed at visit 1.

A diagram of a heart and lungs model

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**Figure 2:** Diagrams of the kinetic models used to model the aorta concentrations (a, top) and liver concentrations (b, bottom). (a) The aorta model is an 8-parameter simplified model of the circulation, assuming a stepwise injection that arrives in the bloodstream after a time BAT (bolus arrival time, sec). Bolus delay and dispersion through heart and lungs is modelled by a plug flow system with transit time delay TTDhl (sec) and a compartment with mean transit time MTThl (sec). Passage through the other organs of the body is modelled by a two-compartment exchange system with intravascular mean transit time MTTo (sec), leakage fraction El, and mean transit time MTTl (sec) of the leakage space. Extraction out of the body by kidney and liver combined is quantified by the extraction fraction E and the blood flow through the body is the cardiac output CO (L/min). The aorta concentrations are taken at the outlet of the heart-lung system and fed as input to a 4-parameter liver model shown in (b). Passage through the vasculature and extracellular space of the gut and liver is modelled by a plug flow system (extracellular transit time delay TTDe, sec) and a compartment with mean transit time MTTe (sec). The liver tissue (grey rectangle) is modelled with a two-compartment filtration model. The first liver compartment is a fraction ve (extracellular volume fraction) of the extracellular compartment. From there Gadoxetate is transported at a rate khe (mL/min/100mL) into a hepatocyte compartment with mean transit time MTTh (sec). From there the agent is excreted into the bile at a rate khe (mL/min/100mL) which is derived from the other parameters as (1-ve)/MTTh.

A group of graphs showing different data

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**Figure 3**: Signal-time curves for the two scans performed on the same day for one volunteer at baseline (a, c) and after rifampicin (b, d). The top row shows the data in the liver (blue, a, b), the bottom row shows the data in the aorta (red c, d). In each case the model fit is superposed on the data as a smooth continuous line. The MOLLI data used as check points are plotted with a black symbol.

A diagram of different colored lines

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**Figure 4**. Plots of the main outcome measures showing: (a) the effect size for the uptake rate khe and the excretion rate kbh across the population as box plots; and the individual values for khe (b) and kbh (c) at baseline (left of plot) and after rifampicin (right of plot). Colored lines in (b,c) connect values in the same volunteer. The baseline values of the 2 volunteers that did not have a second visit are also shown on the plots in (b,c).

A graph of different sizes and numbers

Description automatically generated with medium confidence

**Figure 5**: Effect of reducing the acquisition time on hepatocellular uptake rate (khe, top row) and biliary excretion rate (kbh, bottom row). Each plot shows the effect size for the whole population as a box plot, for acquisition times from 5 minutes (left) up to 40 minutes (right). The single boxplots on the right show the reference values measured over the full acquisition window of 3-4hrs.

A group of graphs with different colored lines

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**Figure 6**: Intra-day changes in hepatocellular uptake (khe, top row - a, b) and biliary excretion (kbh, bottom row - c, d) of Gadoxetate at baseline (left column - a, c) and after administration of rifampicin (right column - b, d). Subjects are color coded and full lines connect values in the same subject and on the same day.

**Tables**

A table with numbers and letters

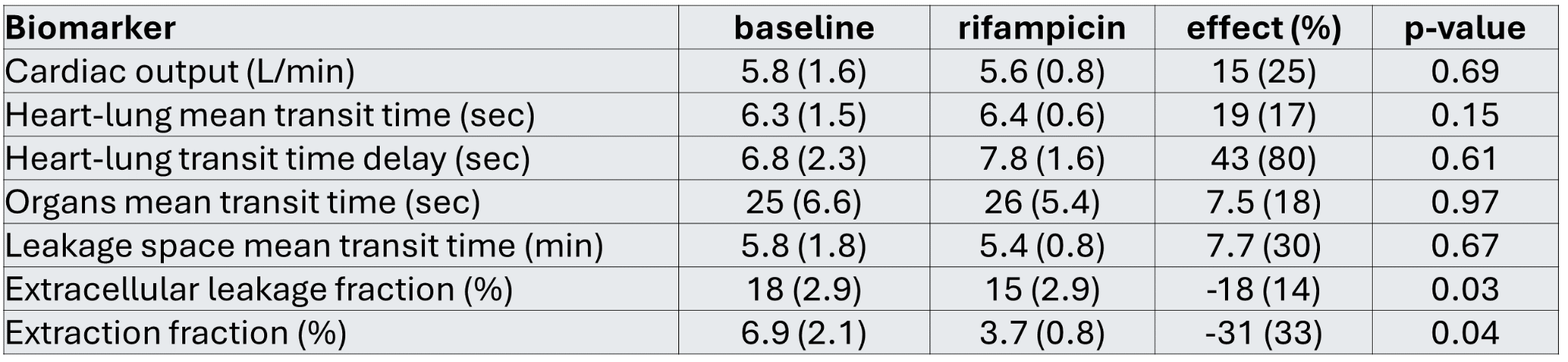
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**Table 1**: Demographics and clinical characteristics of the 10 participants recruited into the study. 3 of the participants (30%) were female.

A table with numbers and symbols

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**Table 2**. Numerical values for all liver biomarkers including, in the top two rows, the primary outcome markers khe (hepatocellular uptake rate) and kbh (biliary excretion rate). For each biomarker, the table shows mean (95% CI) across the population at baseline (column 1) and after rifampicin (column 2). The third column shows the mean (95% CI) of the individual effect sizes in %, and the 4th column shows the p-value of the paired t-test between baseline and rifampicin values.



**Table 3**. Numerical values for all parameters derived from the aorta model fit. For each biomarker, the table shows mean (95% CI) across the population at baseline (column 1) and after rifampicin (column 2). The third column shows the mean (95% CI) of the individual effect sizes in %, and the 4th column shows the p-value of the paired t-test between baseline and rifampicin values.

**A close-up of a table

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**Table 4**: Results from routine blood tests at both visits, including a comparison of values between both visits. Values for both visits show mean (95% CI) across the population for all markers, as well as effect sizes (%) and p-values for paired t-test between values at the start of the visit and those at 2hrs and 4hrs (on the rifampicin visit).