Supplementary Materials and Methods

Pravastatin LC-MS/MS analysis. *LC-MS/MS Analysis*: Pravastatin blood, plasma and liver concentrations were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a non-validated method. Following protein precipitation with acetonitrile, the supernatant was injected onto a Phenomenex Kinetex XB C18 column (30 × 2 mm, 2.6 µm particle size). A Shimadzu Sil-30AC autosampler linked to a Shimadzu CBM-20A controller with LC-30AD pumps (Shimadzu, Columbia MD), coupled with a ABSciex Qtrap5500 mass spectrometer (Applied Biosystems, Foster City, CA) were used for the LC-MS/MS assay. The aqueous mobile phase was water with 0.1% formic acid (A) and the organic mobile phase was acetonitrile with 0.1% formic acid (B). The gradient was as follows: 20% B for the first 0.3 minutes, increased to 30% B from 0.3 to 1min, maintained at 30% B to 2.4min, then increased to 85% B from 2.4 to 2.5 minutes, kept at 85% B till 2.6minutes and decreased to 20% B at 2.62min. The total run time was 3 min with flow rate at 0.5 ml/min and the ionization was conducted in the negative ion mode using the transition m/z 423.132 → 321.1 in ESI mode. An internal standard with the transition of 398.991 → 320.0 was used. The injection volume was 10 µL. The lower limit of quantitation of the assay was 0.00240 µM for blood and 0.00719 µM for plasma and liver.

Rosuvastatin LC-MS/MS analysis. Rosuvastatin concentration in plasma and urine was analyzed by LC-MS/MS as described previously (Hobbs et al., 2012).

Pitavastatin LC-MS/MS analysis. The analytical method was based on a protein precipitation to extract Pitavastatin from plasma or liver protein. An aliquot of plasma (50 µL, sample or spiked standard) was pipetted in a 96-well polypropylene plate and the proteins were precipitated by addition of 400 µL acetonitrile containing the internal standard (d5-Pitavastatin). For liver homogenate the weighed tissue was homogenized with 8 volumes of PBS. 100 µL homogenate were combined with 50 µL blank plasma (for standards blank

homogenate was used and mixed with spiked plasma calibration standards) and proteins were precipitated by addition of 400 µL acetonitrile containing the internal standard (d5-Pitavastatin). Following vortexing, the plate was centrifuged at 2000 rpm for 10 minutes (4 °C). In an automated manner (Hamilton Starlet), 200 µL of the supernatant was transferred to a clean 96-well plate. Samples were diluted by addition of 200 µL 0.1 % formic acid and injected (10 µL) on a Phenomenex Kinetex C18 30 x 2.1 mm, 2.6 µm column with an acetonitrile 0.1% formic acid/0.1% formic acid gradient at a flow rate of 0.90 mL/min. The column temperature was held at RT. Mass spectrometric detection was performed on a AB SCIEX Triple Quad™ 5500 with a turbo-ion spray interface by multiple reaction monitoring (MRM) in positive mode with the following mass transitions: m/z 422.1 to 274.1 for Pitavastatin and 427.1 to 295.1 Da for d5-Pitavastatin. Pitavastatin and d5-Pitavastatin peak areas were determined using Sciex Analyst™ software. Due to the design of the experiments, the PK parameters were determined as a group analysis of all data sets. Standard statistical evaluation of AUC and $t_{1/2}$ values is therefore not possible. Oral bioavailability and liver exposure were determined by comparing AUC0-t of the two compartments. Analysis was done through Microsoft Excel.

Supplementary Methods References

Hobbs M, Parker C, Birch H and Kenworthy K (2012) Understanding the interplay of drug transporters involved in the disposition of rosuvastatin in the isolated perfused rat liver using a physiologically-based pharmacokinetic model. *Xenobiotica* **42:**327-338.