Supplementary information

Proteomics-informed prediction of rosuvastatin plasma profiles in patients with a wide range of body weight

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Supplementary Methods

Patient inclusion and data exclusion

The day before surgery, 57 patients undergoing either gastric bypass surgery or cholecystectomy were enrolled in the COCKTAIL study for the pharmacokinetic analysis and biopsy sampling. Three of the 57 patients were excluded from the analysis of the model developed for predicting rosuvastatin plasma profiles. For one patient, plasma samples were only collected during the first three hours giving an incomplete pharmacokinetic (PK) profile. For the other two patients, either proteomics or PK data was not collected. After excluding the data from these patients, matching PK and proteomics and PK data were obtained from 36 patients undergoing gastric bypass and 18 patients undergoing cholecystectomy.

Measuring concentration-time profile of rosuvastatin *in vivo*

A tablet of 20 mg rosuvastatin (Crestor®, AstraZeneca) was given to the patients as a cocktail, together with tablets of losartan (Cozaar®, MSD 25 mg), omeprazole (Losec®, AstraZeneca 20 mg), and digoxin (Digoxin® Takeda 0.5 mg), and oral syrup of midazolam (Midazolam HCL, Roxane Laboratories 1.5 mg). Blood samples were collected at different time points from 0 to 24 hours. Blood samples were immediately placed on ice, followed by centrifugation for 10 min at 4 °C at 1 800 × g. Plasma was decanted into Cryovials pre-filled with matching volume of 0.1 M sodium acetate buffer solution and frozen at -70 °C within one hour. Rosuvastatin was measured by Covance Laboratories, as previously described [1]. In brief, buffered plasma samples treated with lithium heparin anticoagulant were extracted by supported liquid extraction (SLE). After evaporation, the residue was reconstituted and analyzed with LC-MS/MS. The analyte was separated on a C_{18}-column (Aquasil) with a gradient mobile phase of acetonitrile and 0.1% formic acid using a LC system from Thermo Electron Corporation. Rosuvastatin was analyzed by MS/MS using a Sciex API 5500 with positive electrospray ionization, monitoring the m/z 482.2 to 258.2 transition. The standard curve ranged from 0.04 to 40 ng/ml, using a human plasma sample volume of 0.1 mL. The assay coefficient of variations of the rosuvastatin analysis were 7.1%, 4.4% and 4.5% at 0.12 ng/mL, 2 ng/mL, and 20 ng/mL (n=130), respectively.

Cell culture and transport experiments

Mock-transfected HEK Flp-In-293 cells and cells stably expressing either OATP1B1, OATP1B3, OATP2B1, or NTCP [2, 3] were cultivated in DMEM (Dulbecco's Modified Eagle Medium, Gibco) supplemented with 10% FBS (Fetal Bovine Serum), 1% L-glutamate, and 75 µg/mL hygromycin B. Two days before the experiment, the cells were seeded in 24-well CellBind plates (Corning) at a density of 600 000 cells per well, in culturing medium without hygromycin B and phenol red. For the uptake assays, cells were washed twice with prewarmed HBSS (Hank's Balanced Salt Solution, Gibco), pH 7.4, followed by incubation with prewarmed rosuvastatin at varying concentrations in HBSS for 2 min at 37 °C. The incubation was terminated by adding ice-cold DPBS (Dulbecco's Phosphate-Buffered

Saline, Gibco), followed by two washes with ice-cold DPBS. The accumulated drug was extracted with ice-cold acetonitrile/water (60:40) with 50 nM as internal standard. Samples were centrifuged for 20 min at 2465 × g and the rosuvastatin in the supernatant was determined with UPLC-MS/MS, consisting of a Waters Acquity UPLC coupled to a Waters Xevo TQ MS with electrospray ionization. Rosuvastatin was eluted with a 2 min gradient of acetonitrile and 0.1% formic acid (flow rate of 0.5 mL/min) on a Waters BEH C_{18} column, 2.1 × 50 mm (1.7 µm) at 60 °C, and rosuvastatin was analyzed by monitoring m/z 482.2 to 258.0 transition. The amount of total protein in the incubation was determined using the BCA Protein Assay Reagent Kit (Thermo Fisher Scientific Inc). All transport experiments were run in duplicate on at least two separate occasions.

Genotyping

Analysis of SLCO1B1 (rs4149056; 521C>T) variant alleles were performed using Taqman-based real-time polymerase chain reaction assays implemented for routine pharmacogenetic analyses at the Center for Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway.

Protein quantification

Liver biopsies were homogenized and lysed in 100 mM Tris-HCl-buffer, pH 7.4, containing 2% SDS and 50 mM DTT with a homogenizer (IKA, T10 basic). Proteins were denatured at 95 °C. Samples were prepared for proteomic analysis using the multi-enzyme digestion filter-aided sample preparation (MED-FASP) protocol, where proteins were digested with LysC and trypsin [4]. Protein and peptide amounts were determined based on tryptophan fluorescence [5]. Aliquots of 1 µg peptide were separated on a 50 cm column with 75 µm inner diameter packed C_{18} material, using a 2 hour acetonitrile gradient in 0.1% formic acid at a flow rate of 300 nL/min. The LC was coupled to a Q Exactive HF or Q Exactive HF-X (Thermo Fisher Scientific), operating in a data dependent mode with survey scans at a resolution of 60 000, AGC target of 3×10^6, and maximum injection time of 20 ms. The top 15 most abundant isotope patterns were selected from the survey scan with an isolation window of 1.4 m/z and fragmented with nCE at 27. The MS/MS analysis was performed with a resolution of 15 000, AGC target of 1×10^5, and maximum injection time of 60 ms. The resulting MS data was processed with MaxQuant (Version 1.6.0.16) [6], where proteins were identified by searching MS and MS/MS data of peptides against the human UniProtKB (UP000005640). Carboamidomethylation was set as fixed modification and protein discovery rates were specified as 0.01. Spectral raw intensities were normalized with variance stabilization (vsn) [7], and were subsequently used to calculate the protein concentrations using the Total Protein Approach [8]. Batch effects were removed by geometric mean centering of the proteins in samples analyzed at different time points.

Since only the individual overexpressed transporters (OATP1B1, OATP1B3, OATP2B1, and NTCP) were of interest from the HEK293 cells, these were quantified using targeted proteomics. The protein

quantification have previously been shown comparable with the label-free method used for the liver biopsies [9]. Cells were lysed in 100 mM Tris-HCl-buffer, pH 7.4, containing 2% SDS and 50 mM DTT, and proteins were denatured at 95 °C. For targeted proteomics, a modification of the FASP protocol was used [9, 10], using trypsin alone as digestion enzyme. The targeted proteomics LC-MS/MS analysis was performed as previously described [9]. In short, peptides were separated on a C_{18} column (Acquity UPLC BEH, 2.1 x 100 mm, 1.7 µm), with a 13 min gradient of 2-30% acetonitrile, 0.1 % formic acid at a flow rate of 500 µL/min using an Agilent 1290 LC-system. Peptides were quantified using a QTRAP 6500 (Sciex), with a source temperature of 500 °C, source voltage of 5500 V, run in scheduled multiple reaction monitoring (sMRM)-mode. Data acquisition was performed with a target scan time of 0.5 s and MRM detection window of 60 s. Three transitions per surrogate peptide were monitored for quantification in MRM-mode. More detailed description of the parameters can be found in Table S1. Data was processed using MultiQuant (Version 3.0.5373.0, AB Sciex). Protein concentrations were calculated by the peak area ratios of the internal standard peptide and the sample peptide transitions.

Supplementary Tables and Figures

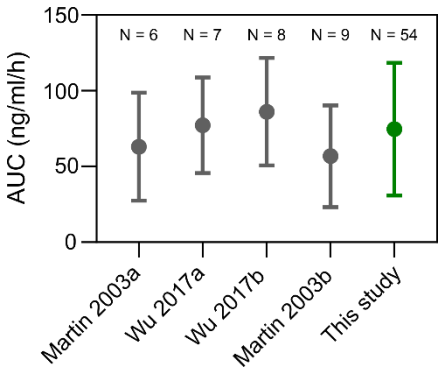

Figure S1. Inter-individual variability of rosuvastatin AUC from different studies ([11-13]) and this study, displaying mean values and standard deviation. N denotes number of subjects included in each study.

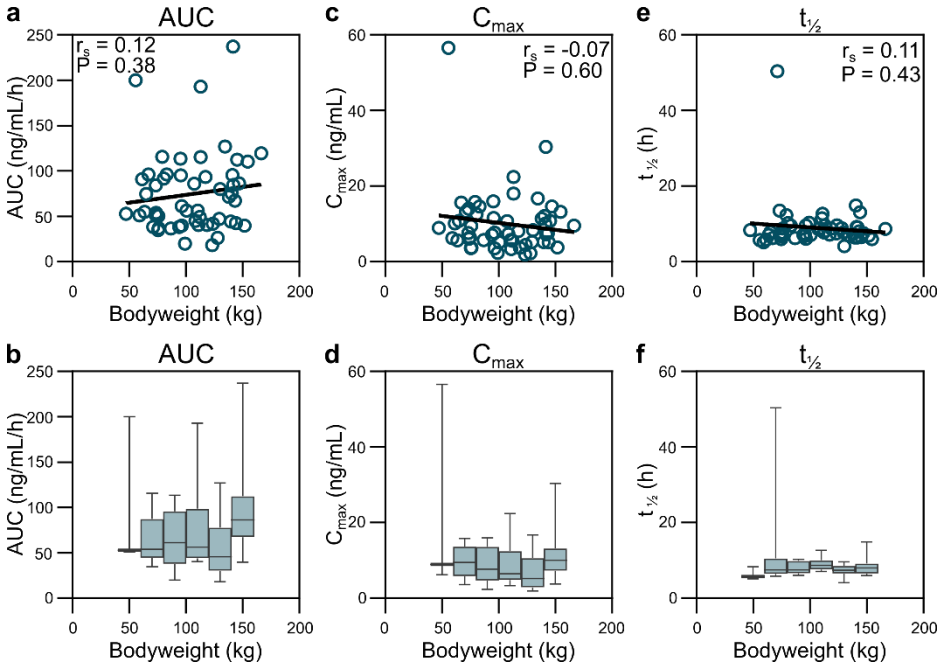

Figure S2. (**a**) AUC, (**c**) peak plasma concentrations (C_{max}), and (**e**) terminal half-life ($t_{½}$) from each of the 54 patients, correlated with their corresponding bodyweight. Distribution of (**b**) AUC, (**d**) C_{max}, and (**f**) $t_{½}$ from each patient across their bodyweight range, data divided into 20 kg bins. r_s, Spearman's rank correlation coefficient; P, two-tailed p-value.

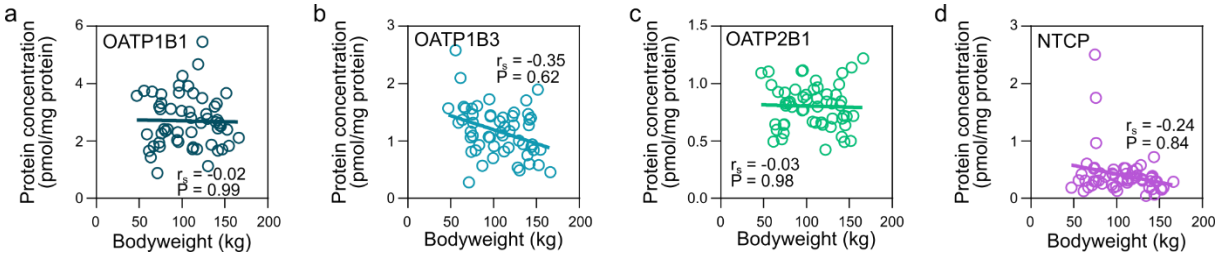

Figure S3. (**a**) Protein levels correlated with body weight for OATP1B1, (**b**) OATP1B3, (**c**) OATP2B1, and (**d**) NTCP in 54 patients with obesity from gastric bypass and without obesity from cholecystectomy; r_s, Spearman's rank correlation coefficient; P, two-tailed p-value.

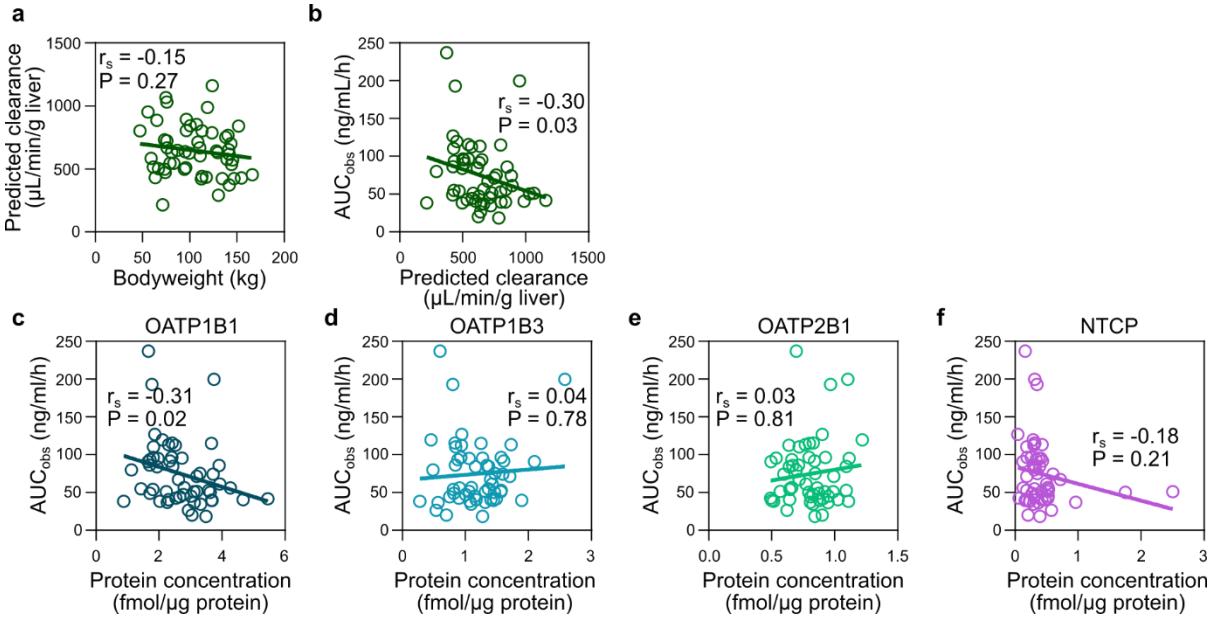

Figure S4. (a) Predicted hepatic uptake clearance of rosuvastatin, obtained from clearance estimates in HEK293 cells and protein concentration in cells and liver biopsies in the 54 patients correlated with bodyweight (kg). **(b)** Observed AUC (ng/mL/h) for each patient correlated with each patients corresponding predicted hepatic uptake clearance of rosuvastatin (µl/min/g liver). (**c-f**) Observed AUC for each patient correlated with corresponding protein concentrations of OATP1B1, OATP1B3, OATP2B1, and NTCP, respectively, in liver biopsies obtained from each patient. r_s, Spearman's rank correlation coefficient; P, two-tailed p-value.

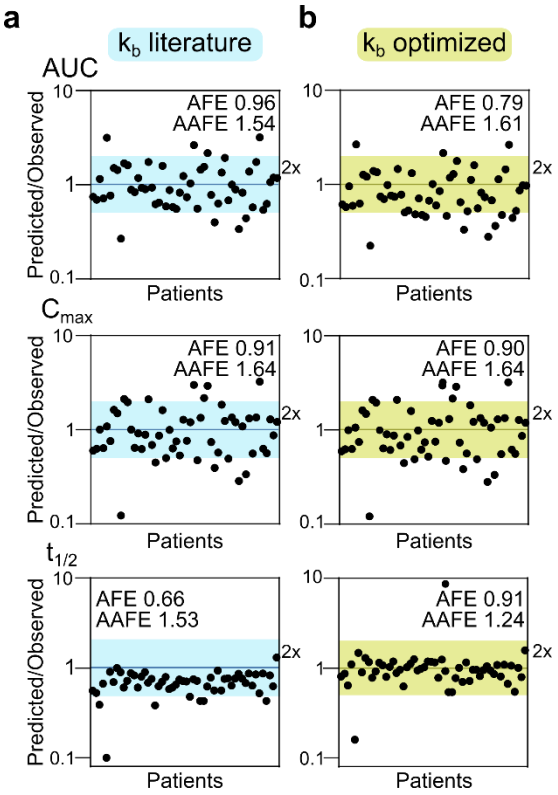

Figure S5. Fold values from predicted and observed AUC, C_{max}, and $t_{1/2}$ of rosuvastatin from the 54 patients, (**a**) using Model 1, with literature gallbladder emptying rate [14] and (**b**) using Model 2 with optimized, reduced, gallbladder emptying rate. AUC (ng/mL/h); C_{max} (ng/mL); $t_{1/2}$ (h).

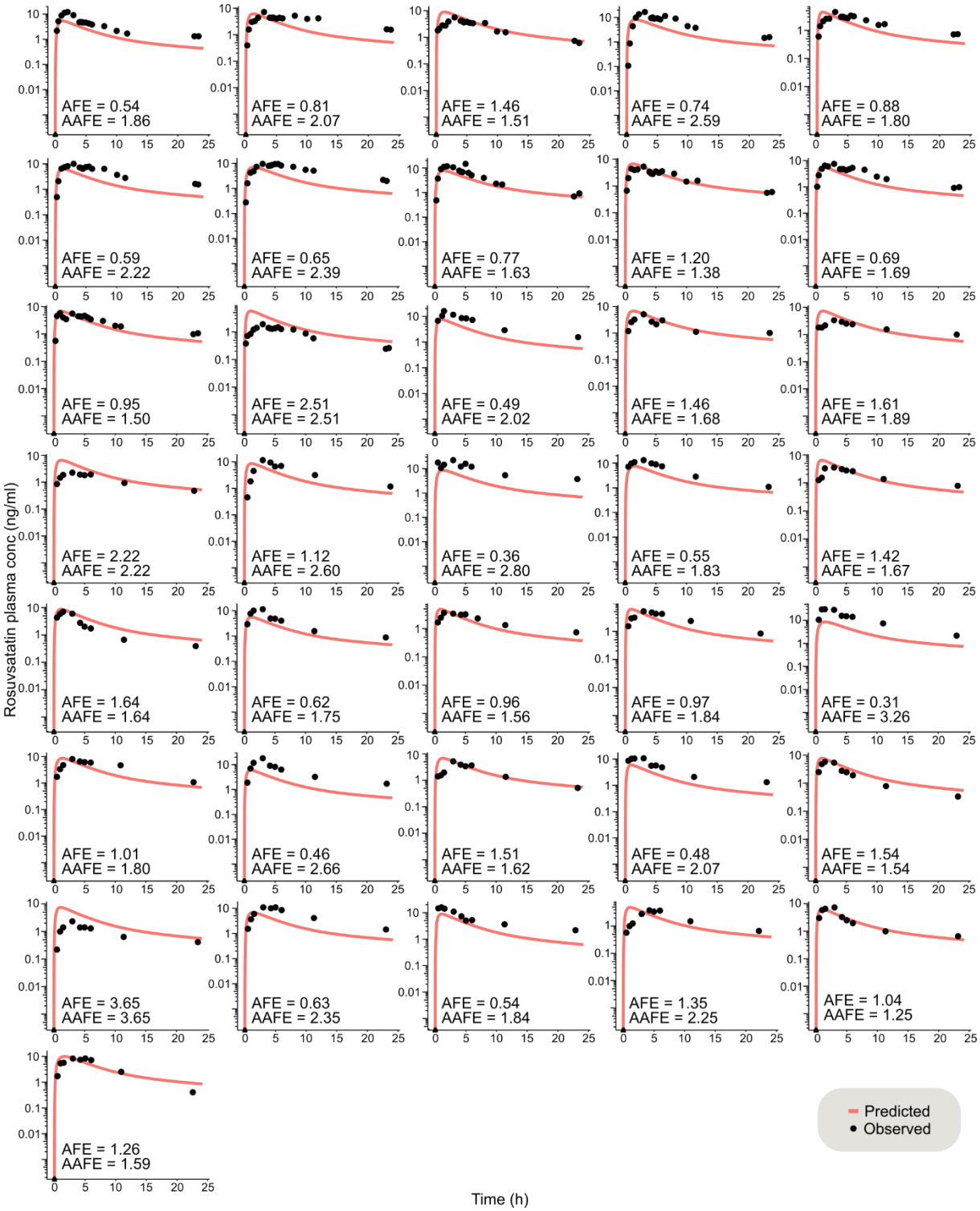

Figure S6. Comparison between predicted (Model 2) and observed rosuvastatin disposition in the 36 patients undergoing gastric bypass surgery. AFE and AAFE – average fold error and absolute average fold error between predicted and observed values for each time point.

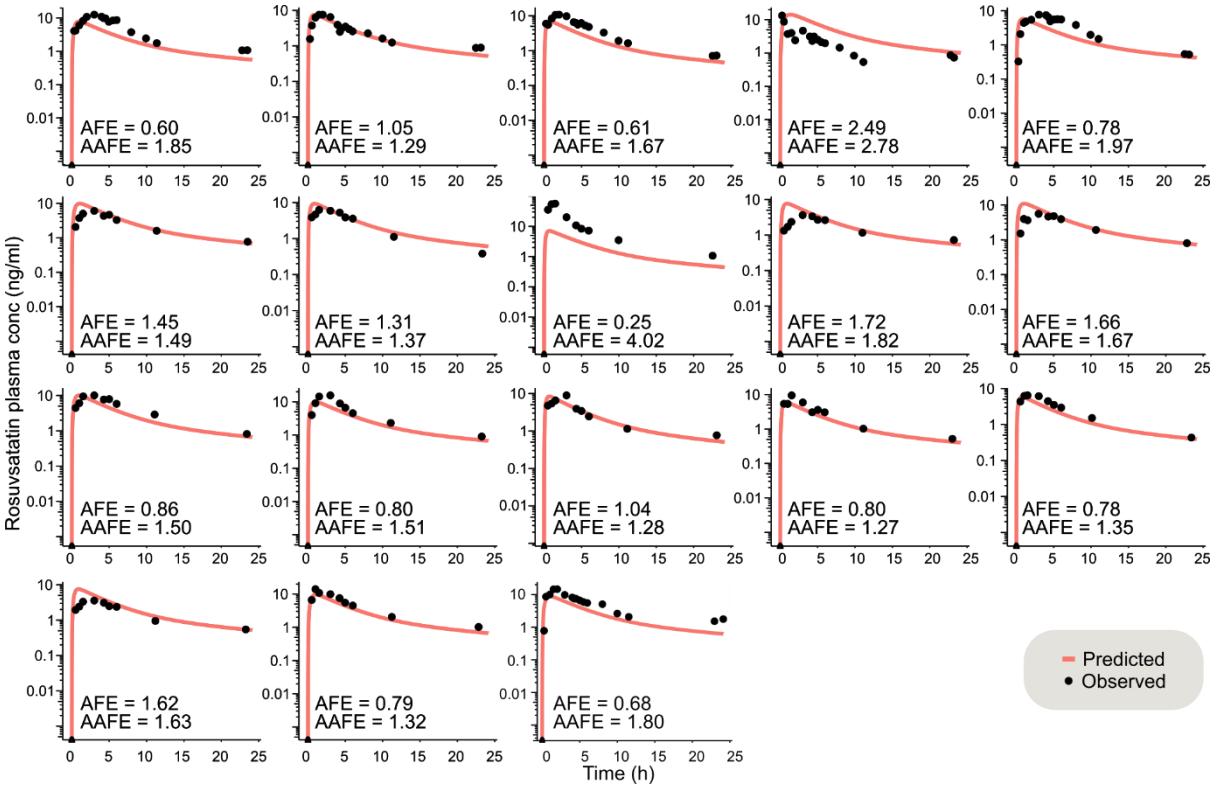

Figure S7. Comparison between predicted (Model 2) and observed rosuvastatin disposition in the 18 patients undergoing cholecystectomy. AFE and AAFE – average fold error and absolute average fold error between predicted and observed values for each time point.

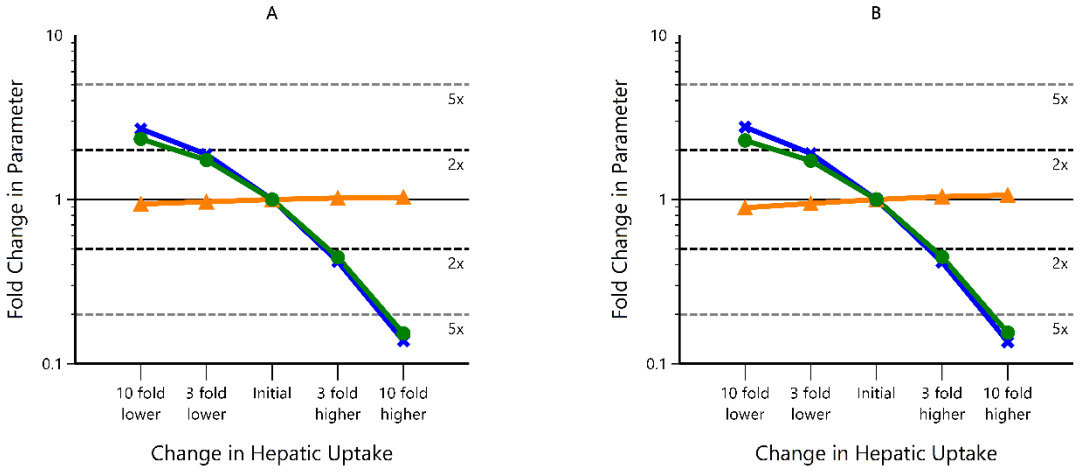

Figure S8. Sensitivity analysis on PK parameters: C_{max} (green), AUC (blue) and $t_{1/2}$ (orange) for hepatic uptake clearance in (**a**) the prediction Model 1 with k_b from literature [14] and b) Model 2 with optimized k_b.

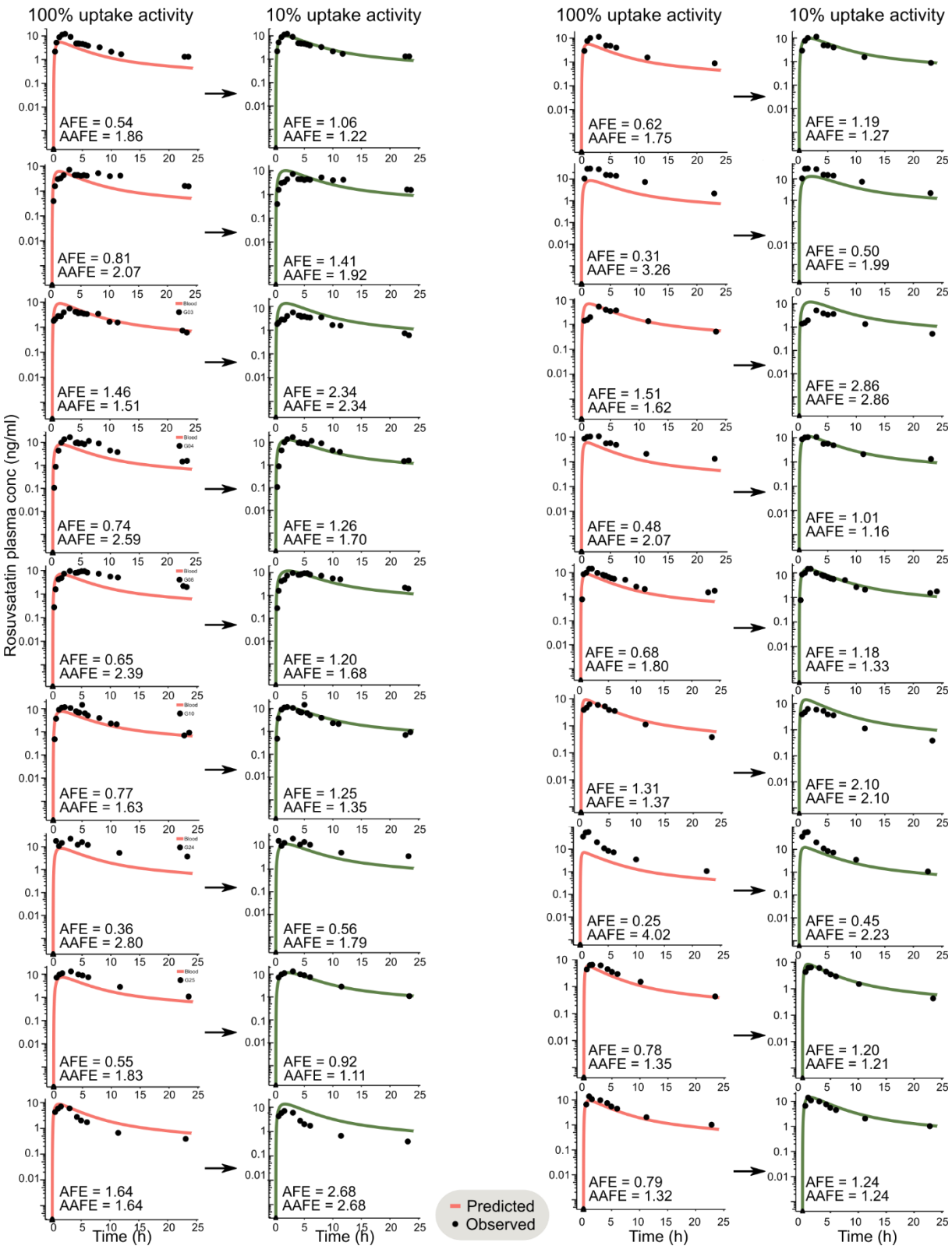

Figure S9. Comparison between predicted and observed rosuvastatin disposition in the 18 patients having the *OATP1B1* 521C allele, before (red; Model 2) and after (green; Model 3) adjusting for 10% uptake activity. AFE and AAFE – average fold error and absolute average fold error between predicted and observed values for each time point.

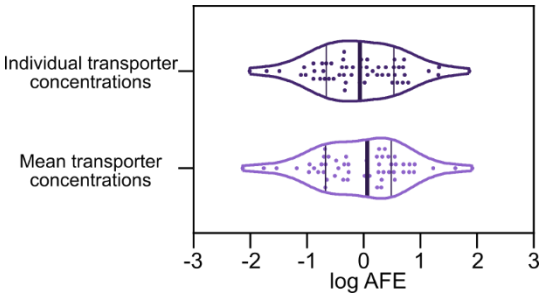

Figure S10. Average fold error (AFE) of predicted rosuvastatin disposition compared to that observed in the 54 patients after using individual transporter concentrations for each patient or using mean transporter concentrations from all patients in the predictions.

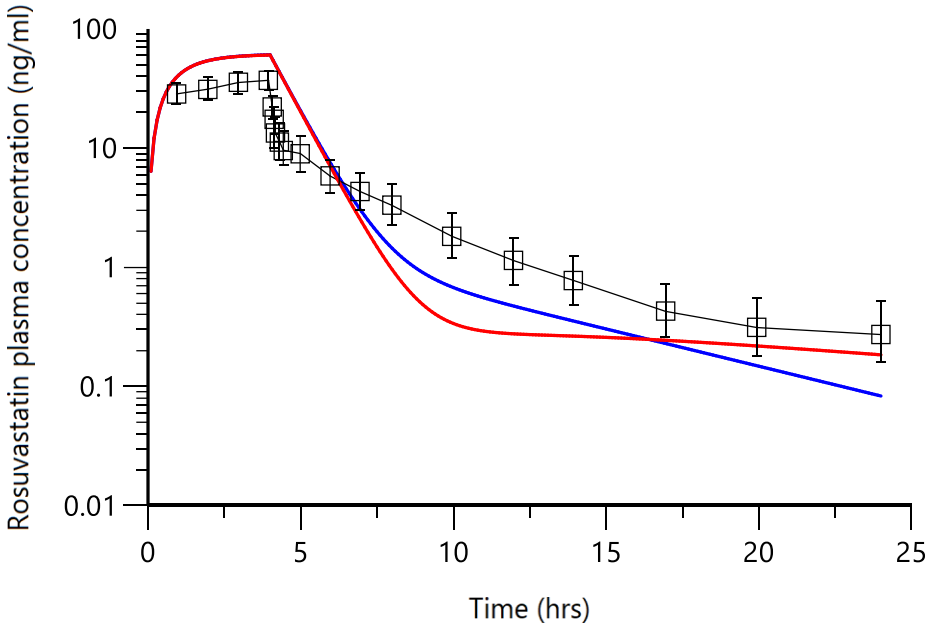

Figure S11. Geometric mean rosuvastatin plasma pharmacokinetic profile (black line, and squares) after an 8 mg of 4 hour intravenous infusion. Data were digitized from Martin et al 2003[15]. The error bars corresponds to standard deviation calculated on log-scale and back-transformed. The red and blue line corresponds to predictions using Model 1 and 2.

Table S1. Mass-spectrometry parameters for targeted proteomics

		Q1	Q3.1	Q3.2	Q3.3	Collision Energy [V]	Declustering Potential [V]
OATP1B1	NVTGFFQSFK	587.9	961.4	860.4	656.4	24/24/27	70
	NVTGFFQSFK*	591.8	969.5	868.4	664.3	24/24/27	70
OATP1B3	NVTGFFQSLK	570.9	927.5	826.4	622.6	21/23/27	56
	NVTGFFQSLK*	574.5	934.5	833.5	629.3	21/23/27	56
OATP2B1	SSPAVEQQLLVSGPGK	799.1	712.1	445.2	1155.6	35/35/35	96
	SSPAVEQQLLVSGPGK*	803.7	716.7	453.1	1165.3	35/35/35	96
NTCP	GIYDGDLK	440.9	710.5	547.2	355.9	17/20/19	45
	GIYDGDLK*	444.9	718.4	555.4	359.9	17/20/19	45

* $^{13}C^{15}N$ labeling

Table S2. Parameters for mechanistic modeling

Parameter	Description	Value	Reference
k_a	Absorption constant	0.244 h^{-1} (0.004 min^{-1})	Internal AZ pop-PK modelling
F	Gastrointestinal absorption (bioavailability)	0.5	[15]
$Liver_{cellcount}$	Number of hepatocytes per g liver	120 x 10^6 hepatocytes per g liver	[16]
$Liver_{protein}$	Amount of protein per g liver	88 mg per g liver	[3]
W_{liver}	Weight of the liver	704.06 + 8.52 x bw + 1.63 x age + 123.38 x gender	[17] (bw in kg, Age in year, gender (M=1, F=0))
$Liver_{fraction}$	Fractional liver volume of total body weight	0.021 L/kg body weight	[18]
V_{liver}	Volume of liver	$bw*Liver_{fraction}*10^6$ µl	
V_{blood}	Volume of distribution	0.227 L/kg	Estimated based on [19, 20]
CL_{renal}	Renal clearance	13.6 L/h	[15]
CL_{met}	Metabolism clearance	3.52 L/h	[15]
$CL_{passive}$	Passive permeability to liver	0.71 pmol/min/mg protein	Own generated data
CL_{uptake}	Total CL uptake by transporters	Calculated from V_{max}/K_m *in vitro* data	Own generated data
CL_{efflux}	Total efflux from liver to blood	0	
CL_{bile}	Biliary clearance	1.23 uL/min/10^6 hepatocytes	[21, 22]
k_b	Emptying rate of Gallbladder	0.0013 min^{-1}	Based on [14] and optimized

Table S3. Comparison of K_m values

Transporter	K_m (µM)			
	This study[a]	[23][a]	[24][b]	[25][a]
OATP1B1	9.54 +/- 1.9	13 +/- 0.4	4-7.3	0.8 +/- 0.27
OATP1B3	17.2 +/- 2.9	16.5 +/- 2.1	9.8 +/- 0.1	14.2 +/- 2.8
OATP2B1	14.7 +/- 2.2	26.1 +/- 8.0	2.4 +/- 1.9	6.42 +/- 1.03
NTCP	192 +/- 25.6		65 +/- 40	

[a]*Transfected HEK293-cells,* [b]*Vaccinia infected HeLa cells*

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Table S4. Comparison of inter-individual variability of rosuvastatin AUC across studies

Rosuvastatin	Dose	Mean	SD	CV%	N	Ref	Ref
AUC_{0-24} (ng-h/ml)	20 mg	63.1*	35.7	56.6	6	Martin 2003a	[13]
AUC_{0-48} (ng-h/ml)	20 mg	77.2	31.5	40.8	7	Wu 2017a	[11]
AUC_{0-48} (ng-h/ml)	20 mg	86.2	35.5	41.2	8	Wu 2017b	[11]
AUC_{0-30} (ng-h/ml)	20 mg	56.8*	33.5	58.9	9	Martin 2003b	[12]
AUC_{0-24} (ng-h/mL)	20 mg	74.64	43.8	58.7	54	This study	

**Geometric mean*

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Table S5. Comparison of intravenous data from Martin *et al.* with the presented model

Parameter	Observed (Martin *et al*.)	Model 1[1]	Model 2[2]
AUC (ng/mL/h)	164	250	247
C_{max} (ng/mL)	37	61	60
$t_{1/2}$ (h),$_{10\text{-}24\ hrs}$	5.0	4.8	20

1. *Literature data for bile emptying rate (k_b) [14]*
2. *Optimized k_b based on the observed plasma concentration profile of rosuvastatin in the COCKTAIL study (patients to undergo gastric bypass or cholecystectomy surgery)*

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209 **Supplementary Data S1**

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Donor	Bodyweight	Age	Gender	OATP1B1 (pmol/mg protein)	OATP1B3 (pmol/mg protein)	OATP2B1 (pmol/mg protein)	NTCP (pmol/mg protein)
Chol1	83.2	43	F	2.40	0.93	0.80	0.26
Chol2	80.7	29	M	2.34	1.61	0.84	0.43
Chol3	73.4	62	F	3.20	1.06	0.57	0.52
Chol4	65.2	56	F	3.72	1.59	0.60	0.60
Chol5	71.3	36	F	0.88	0.28	0.51	0.22
Chol6	96.5	42	M	3.93	1.43	0.77	0.50
Chol7	73.9	50	F	1.80	0.81	0.65	0.53
Chol8	59.1	63	F	2.24	1.32	0.99	0.42
Chol9	55.8	26	F	3.75	2.58	1.10	0.31
Chol10	76	41	M	2.26	1.32	0.81	0.96
Chol11	63.6	25	F	1.43	1.37	0.64	0.31
Chol12	61.5	23	F	1.65	2.10	0.50	0.12
Chol13	67	19	F	1.82	1.57	0.93	0.19
Chol14	47.4	27	F	3.58	1.57	1.09	0.19
Chol15	75.8	50	F	3.62	1.46	0.80	1.75
Chol16	74.8	58	F	3.11	1.56	0.92	2.51
Chol17	75.7	59	F	3.33	1.09	0.83	0.29
Chol18	73.5	50	F	1.94	1.16	0.64	0.30
GBP1	140.4	52	M	3.30	1.47	0.85	0.35
GBP2	141.9	54	M	2.43	1.37	1.09	0.39
GBP3	112.3	55	F	1.72	0.82	0.87	0.27
GBP4	134.5	45	F	1.86	0.94	0.90	0.04
GBP5	124	49	F	5.46	1.49	0.91	0.52
GBP6	141.1	29	F	2.73	1.27	0.54	0.37
GBP7	166.4	48	F	2.11	0.45	1.22	0.29
GBP8	147.1	47	M	1.70	0.84	0.71	0.29
GBP9	129.6	38	F	2.78	1.01	0.83	0.33
GBP10	143.4	49	M	2.62	1.40	0.72	0.72
GBP11	110.6	42	F	2.80	1.09	0.66	0.52
GBP12	123.4	44	M	3.49	1.28	0.84	0.40
GBP13	95.2	46	F	2.31	1.72	0.77	0.43
GBP14	144.2	51	M	2.54	0.90	0.49	0.06
GBP15	111.1	49	F	2.30	1.41	1.03	0.43
GBP16	128.2	58	F	2.94	0.54	0.62	0.58
GBP17	95.7	40	F	1.96	1.06	1.12	0.41
GBP18	113.2	48	F	1.77	0.80	0.97	0.35
GBP19	154.7	36	M	1.83	0.84	0.72	0.19
GBP20	96.6	32	F	3.69	1.45	0.88	0.18
GBP21	94.3	56	F	2.03	1.07	1.11	0.23
GBP22	138.1	23	M	3.20	1.71	0.99	0.19
GBP23	118.7	50	M	4.67	1.17	0.64	0.48

Donor	Bodyweight	Age	Gender	OATP1B1 (pmol/mg protein)	OATP1B3 (pmol/mg protein)	OATP2B1 (pmol/mg protein)	NTCP (pmol/mg protein)
GBP24	100.6	53	F	4.27	0.91	0.86	0.13
GBP25	141.7	48	F	1.66	0.60	0.69	0.16
GBP26	117.4	43	F	1.72	0.88	0.66	0.33
GBP27	113.1	53	F	3.67	1.25	0.80	0.31
GBP28	139.6	34	F	2.66	0.75	0.81	0.30
GBP29	107.4	47	F	3.91	1.34	0.67	0.32
GBP30	87	47	F	3.11	0.57	0.95	0.37
GBP31	99.4	44	F	3.04	0.70	0.90	0.21
GBP32	145.2	25	F	2.52	0.94	0.64	0.30
GBP33	79.2	54	F	2.41	0.83	0.83	0.31
GBP34	151.7	50	M	3.66	1.90	0.50	0.16
GBP35	108.5	63	M	3.02	1.15	0.95	0.53
GBP36	130.4	38	F	1.13	0.49	0.70	0.31

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References

1. Braamskamp, M.J.A.M., et al., *Efficacy and safety of rosuvastatin therapy in children and adolescents with familial hypercholesterolemia: Results from the CHARON study.* Journal of Clinical Lipidology, 2015. **9**(6): p. 741-750.
2. Karlgren, M., et al., *Classification of Inhibitors of Hepatic Organic Anion Transporting Polypeptides (OATPs): Influence of Protein Expression on Drug–Drug Interactions.* Journal of Medicinal Chemistry, 2012. **55**(10): p. 4740-4763.
3. Vildhede, A., et al., *Hepatic Uptake of Atorvastatin: Influence of Variability in Transporter Expression on Uptake Clearance and Drug-Drug Interactions.* Drug Metabolism and Disposition, 2014. **42**(7): p. 1210.
4. Wiśniewski, J.R. and M. Mann, *Consecutive Proteolytic Digestion in an Enzyme Reactor Increases Depth of Proteomic and Phosphoproteomic Analysis.* Analytical Chemistry, 2012. **84**(6): p. 2631-2637.
5. Wiśniewski, J.R. and F.Z. Gaugaz, *Fast and Sensitive Total Protein and Peptide Assays for Proteomic Analysis.* Analytical Chemistry, 2015. **87**(8): p. 4110-4116.
6. Tyanova, S., T. Temu, and J. Cox, *The MaxQuant computational platform for mass spectrometry-based shotgun proteomics.* Nature Protocols, 2016. **11**: p. 2301.
7. Huber, W., et al., *Variance stabilization applied to microarray data calibration and to the quantification of differential expression.* Bioinformatics, 2002. **18**(suppl_1): p. S96-S104.
8. Wiśniewski, J.R. and D. Rakus, *Multi-enzyme digestion FASP and the 'Total Protein Approach'-based absolute quantification of the Escherichia coli proteome.* Journal of Proteomics, 2014. **109**: p. 322-331.
9. Wegler, C., et al., *Variability in Mass Spectrometry-based Quantification of Clinically Relevant Drug Transporters and Drug Metabolizing Enzymes.* Molecular Pharmaceutics, 2017. **14**(9): p. 3142-3151.
10. Wiśniewski, J.R., et al., *Universal sample preparation method for proteome analysis.* Nature Methods, 2009. **6**: p. 359.
11. Wu, H.F., et al., *Rosuvastatin Pharmacokinetics in Asian and White Subjects Wild Type for Both OATP1B1 and BCRP Under Control and Inhibited Conditions.* J Pharm Sci, 2017. **106**(9): p. 2751-2757.
12. Martin, P.D., et al., *A double-blind, randomized, incomplete crossover trial to assess the dose proportionality of rosuvastatin in healthy volunteers.* Clinical Therapeutics, 2003. **25**(8): p. 2215-2224.
13. Martin, P.D., et al., *Metabolism, excretion, and pharmacokinetics of rosuvastatin in healthy adult male volunteers.* Clin Ther, 2003. **25**(11): p. 2822-35.
14. Vezina, W.C., et al., *Increased volume and decreased emptying of the gallbladder in large (Morbidly obese, tall normal, and muscular normal) people.* Gastroenterology, 1990. **98**(4): p. 1000-1007.
15. Martin, P.D., et al., *Absolute oral bioavailability of rosuvastatin in healthy white adult male volunteers.* Clinical Therapeutics, 2003. **25**(10): p. 2553-2563.
16. Sohlenius-Sternbeck, A.K., et al., *Evaluation of the human prediction of clearance from hepatocyte and microsome intrinsic clearance for 52 drug compounds.* Xenobiotica, 2010. **40**(9): p. 637-649.
17. Mathuramon, P., et al., *Correlation of internal organ weight with body weight and length in normal Thai adults.* Medical journal of the Medical Association of Thailand, 2009. **92**(2): p. 250.
18. Jones, H.M. and K. Rowland-Yeo, *Basic Concepts in Physiologically Based Pharmacokinetic Modeling in Drug Discovery and Development.* CPT: Pharmacometrics & Systems Pharmacology, 2013. **2**(8): p. 63.
19. Rodgers, T. and M. Rowland, *Physiologically based pharmacokinetic modelling 2: Predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions.* Journal of Pharmaceutical Sciences, 2006. **95**(6): p. 1238-1257.
20. Rodgers, T. and M. Rowland, *Mechanistic Approaches to Volume of Distribution Predictions: Understanding the Processes.* Pharmaceutical Research, 2007. **24**(5): p. 918-933.
21. Abe, K., A.S. Bridges, and K.L.R. Brouwer, *Use of sandwich-cultured human hepatocytes to predict biliary clearance of angiotensin II receptor blockers and HMG-CoA reductase inhibitors.* Drug metabolism and disposition: the biological fate of chemicals, 2009. **37**(3): p. 447-452.
22. Jamei, M., et al., *A mechanistic framework for in vitro–in vivo extrapolation of liver membrane transporters: prediction of drug–drug interaction between rosuvastatin and cyclosporine.* Clinical pharmacokinetics, 2014. **53**(1): p. 73-87.
23. Bosgra, S., et al., *Predicting carrier-mediated hepatic disposition of rosuvastatin in man by scaling from individual transfected cell-lines in vitro using absolute transporter protein quantification and PBPK modeling.* European Journal of Pharmaceutical Sciences, 2014. **65**: p. 156-166.
24. Ho, R.H., et al., *Drug and bile acid transporters in rosuvastatin hepatic uptake: function, expression, and pharmacogenetics.* Gastroenterology, 2006. **130**(6): p. 1793-1806.
25. Kitamura, S., et al., *Involvement of Multiple Transporters in the Hepatobiliary Transport of Rosuvastatin.* Drug Metabolism and Disposition, 2008. **36**(10): p. 2014.