Supplementary Data

Use of a mechanistic model to assess inter-individual and inter-species variability in active uptake in human and rat hepatocytes - Drug Metabolism and Disposition (DMD #46193)

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Table S1: Uptake clearance of 2 µM estrone-3-sulfate and demographic information on 28 human hepatocyte donors. Data were collated from the characterization spreadsheet supplied by BD Gentest between September 2008 and September 2011

Donor ID	Gender	Age (year)	Ethnicity	Estrone-3-sulfate uptake clearance (µL/min/10^6 cells)
HH120	Male	58	Caucasian	17.5
HH127	Male	5	Caucasian	13.5
HH139	Female	47	Caucasian	19.5
HH145	Male	69	Caucasian	29.5
HH157	Female	64	Caucasian	4.45
HH162	Male	42	African American	27
HH171	Male	52	Caucasian	35.5
HH193	Female	3	Native American	18
HH202	Male	0.9	Hispanic	11.5
HH206	Female	49	Caucasian	31
HH209	Male	14	Caucasian	27.5
HH215	Female	0.01	Caucasian	19.5
HH222	Female	48	Caucasian	12
HH225	Male	42	Caucasian	24
HH229	Male	56	Asian	30.5
HH243	Female	32	Caucasian	19.5
HH263	Male	2	Caucasian	26.5
HH266	Female	61	Caucasian	23.5
HH268	Female	54	Caucasian	22.5
HH269	Male	23	Caucasian	17.5
HH293	Female	61	Caucasian	32.5
HH304	Male	58	Caucasian	16
HH314	Male	26	African American	23
HH318	Female	64	Caucasian	35
HFC444	Female	53	Caucasian	30
HMC459	Male	53	Caucasian	14
HFC463	Female	70	Caucasian	4
HFC473	Female	57	Caucasian	14.5

Table S2: Literature data used for the comparison between observed and predicted hepatic clearance

Drug	CL_{tot} (mL/min/kg)	CL_r (mL/min/kg)	CL_{bile} (mL/min/kg)	fu_p
Bosentan	2.39[a]	0.0215[a]	0.0885[a]	0.02[b]
Pravastatin	13.5[c]	6.3[c]	3.04[d]	0.554[e]
Repaglinide	7.76[f]	0.621[f]	ND	0.036[g]
Rosuvastatin	11.6[h]	3.24[h]	3.12[h]	0.12[i]
Telmisartan	12.3[j]	0[k]	12.0[k]	0.005[j]
Valsartan	0.521[l]	0.148[l]	0.321[m]	0.059[n]

a: Weber C, Gasser R, and Hopfgartner G (1999) Absorption, excretion, and metabolism of the endothelin receptor antagonist bosentan in healthy male subjects. Drug Metab Dispos 27:810-815.

b: Blanchard N, Alexandre E, Abadie C, Lave T, Heyd B, Mantion G, Jaeck D, Richert L, and Coassolo P (2005) Comparison of clearance predictions using primary cultures and suspensions of human hepatocytes. Xenobiotica 35:1-15.

c: Singhvi SM, Pan HY, Morrison RA, and Willard DA (1990) Disposition of pravastatin sodium, a tissue-selective HMG-CoA reductase inhibitor, in healthy subjects. Br J Clin Pharmacol 29:239-243.

d: Everett DW, Chando TJ, Didonato GC, Singhvi SM, Pan HY, and Weinstein SH (1991) Biotransformation of pravastatin sodium in humans. Drug Metab Dispos 19:740-748.

e: Watanabe T, Kusuhara H, Maeda K, Kanamaru H, Saito Y, Hu Z, and Sugiyama Y (2010) Investigation of the rate-determining process in the hepatic elimination of HMG-CoA reductase inhibitors in rats and humans. Drug Metab Dispos 38:215-222.

f: Hatorp V, Oliver S, and Su CA (1998) Bioavailability of repaglinide, a novel antidiabetic agent, administered orally in tablet or solution form or intravenously in healthy male volunteers. Int J Clin Pharmacol Ther 36:636-641.

g: Marbury TC, Ruckle JL, Hatorp V, Andersen MP, Nielsen KK, Huang WC, and Strange P (2000) Pharmacokinetics of repaglinide in subjects with renal impairment. Clin Pharmacol Ther 67:7-15.

h: Martin PD, Warwick MJ, Dane AL, Hill SJ, Giles PB, Phillips PJ, and Lenz E (2003) Metabolism, excretion, and pharmacokinetics of rosuvastatin in healthy adult male volunteers. Clin Ther 25:2822-2835.

i: Regulatory documents.

j: Stangier J, Su CA, and Roth W (2000) Pharmacokinetics of orally and intravenously administered telmisartan in healthy young and elderly volunteers and in hypertensive patients. J Int Med Res 28:149-167.

k: Stangier J, Schmid J, Turck D, Switek H, Verhagen A, Peeters PA, van Marle SP, Tamminga WJ, Sollie FA, and Jonkman JH (2000) Absorption, metabolism, and excretion of intravenously and orally administered [14C]telmisartan in healthy volunteers. J Clin Pharmacol 40:1312-1322.

l: Flesch G, Muller P, and Lloyd P (1997) Absolute bioavailability and pharmacokinetics of valsartan, an angiotensin II receptor antagonist, in man. Eur J Clin Pharmacol 52:115-120.

m: Waldmeier F, Flesch G, Muller P, Winkler T, Kriemler HP, Buhlmayer P, and De Gasparo M (1997) Pharmacokinetics, disposition and biotransformation of [14C]-radiolabelled valsartan in healthy male volunteers after a single oral dose. Xenobiotica 27:59-71.

n: Colussi DM, Parisot C, Rossolino ML, Brunner LA, and Lefevre GY (1997) Protein binding in plasma of valsartan, a new angiotensin II receptor antagonist. J Clin Pharmacol 37:214-221.

Figure S1 Formation of M1 (○), M2 (●), M4 (□) and repaglinide glucuronide (■) in human hepatocytes over 15 min at a range of concentrations (0.1 - 100 µM). Metabolite concentrations were monitored in the cells. Data points represent the mean of duplicate measurements carried out in donor HU8089. A comparable profile was observed in donor HU4199.

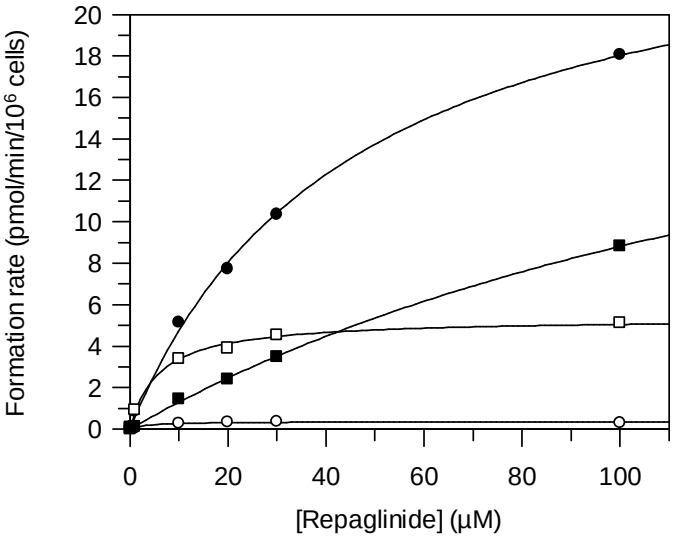
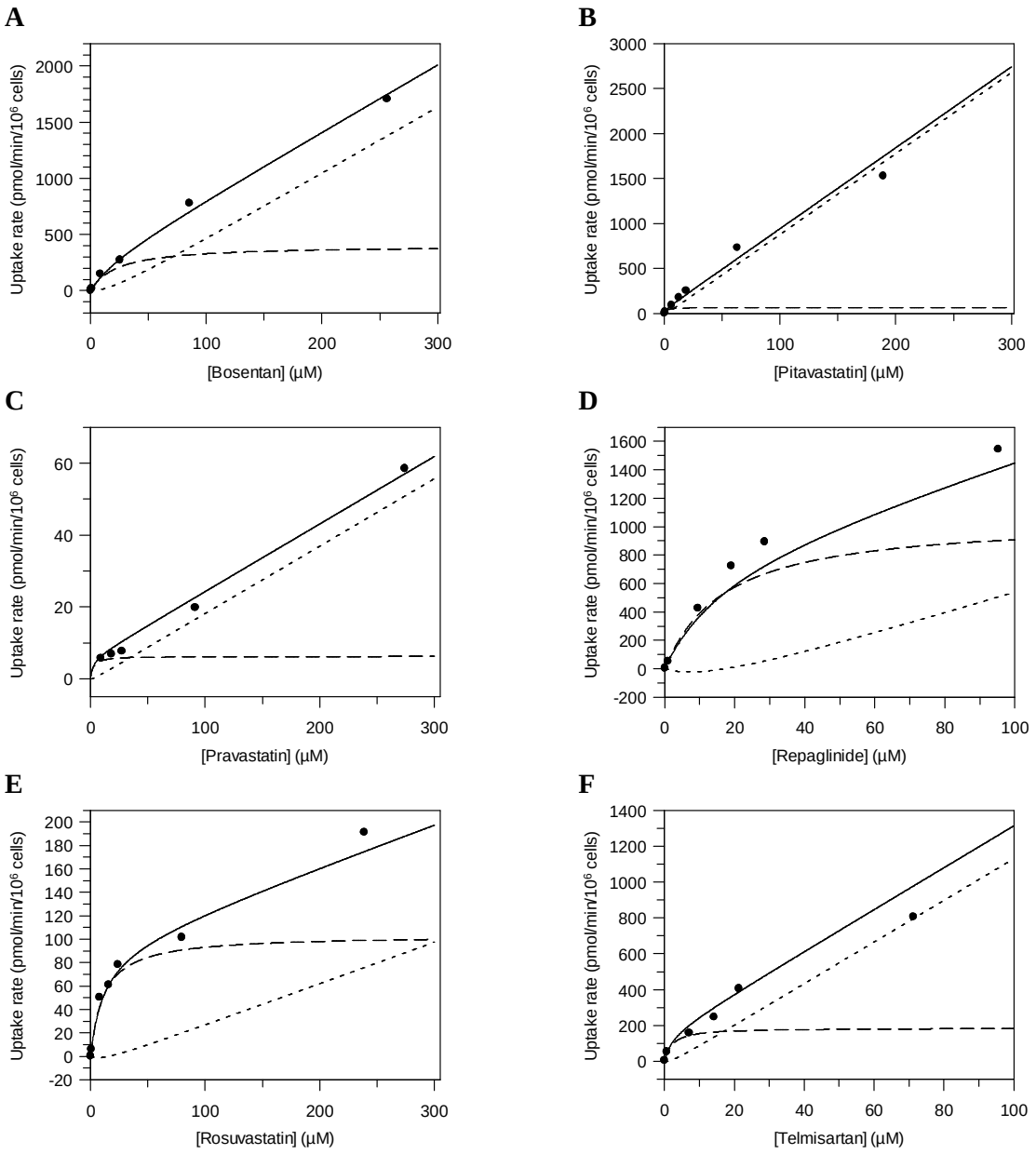


Figure S2: Representative uptake kinetic profiles after 2 min incubation for seven OATP substrates over a range of substrate concentrations based on the mechanistic modeling approach. Total uptake was obtained from measurements at 37ºC. Active transport and passive diffusion were delineated from Equation 2. Active uptake was expressed as the difference between total uptake and passive diffusion. Closed symbols and solid lines represent measured and predicted total uptake estimated from kinetic parameters obtained with each approach, respectively. Dashed and dotted lines represent cellular uptake due to active transport and passive diffusion, respectively. Data points are mean of duplicate measurements.



G

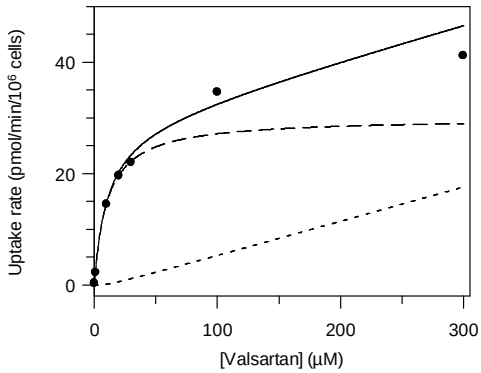

Uptake rate (pmol/min/10^6 cells)
40
20
0
0
100
200
300
[Valsartan] (µM)

Figure S3: Predicted and observed rosuvastatin (A) and repaglinide (B) cell concentrations when data were analyzed using a mechanistic two-compartment. Each plot represents cell concentrations measured in three experiments, over a range of concentrations (0.1 - 100 µM), in incubations carried out over 2 min for rosuvastatin and 15 min for repaglinide.

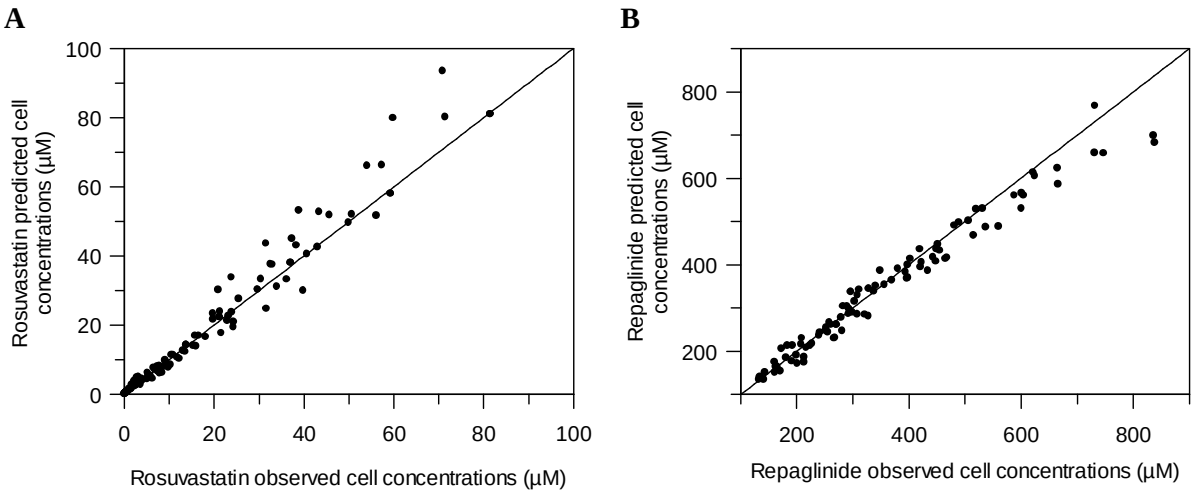


Figure S4: Relationship between uptake of estrone-3-sulfate, measured in 28 human hepatocyte donors and age and gender of the donors. Data were collated from BD Gentest characterization spreadsheets. Uptake was measured at 2 µM, over 3 min with 200,000 suspended hepatocytes per incubation. Open symbols are for females, closed symbols for males.