

SUPPLEMENTARY INFORMATION

Is the Protein-Mediated Uptake Of Drugs By OATPs A Real Phenomenon Or An Artifact?

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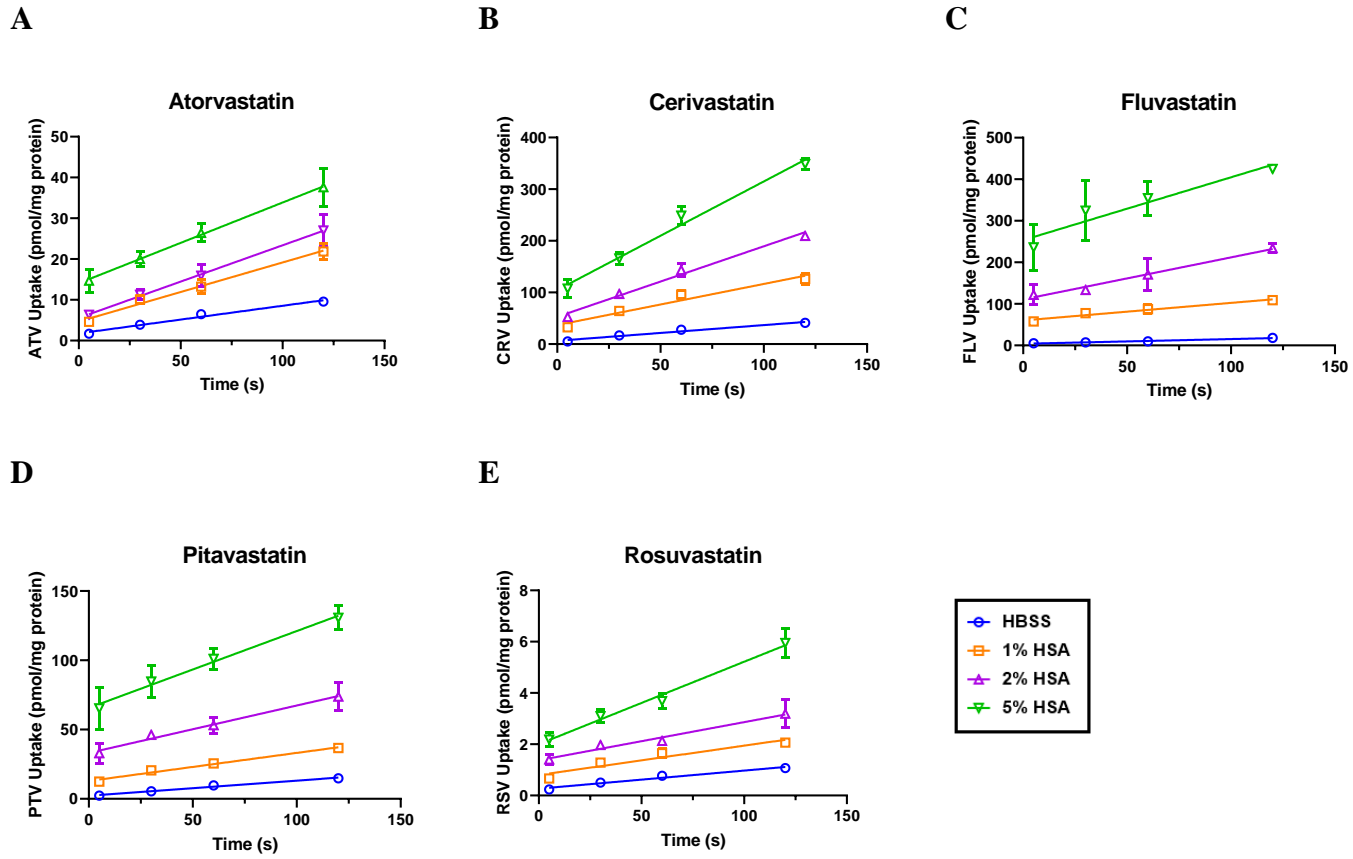
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Supplementary Figures

Supplementary Fig. 1. The statin uptake-time profiles for OATP1B1_RIF cells. The increase in the slope of the uptake curves in the presence of HSA *vs.* HBSS suggests a PMUE on the passive uptake of the statins. The increased intercept in the presence of HSA suggests NSB of statin-HSA complex to the cells/labware. Data shown are mean \pm standard deviation (SD) of statin uptake (normalized to 1 μ M unbound concentration) and are representative of three independent experiments, each conducted in triplicate at each time point.

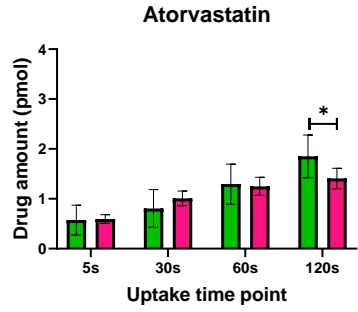
Supplementary Fig. 2. Comparison of the amount of statin non-specifically bound to the cells (as statin-HSA complex; pink bars) and the increase in statin taken up by OATP1B1_RIF cells in the presence of 5% HSA *vs.* HBSS (green bars). The increase in the uptake of ATV, FLV, PTV and RSV in the presence of 5% HSA (*vs.* HBSS) can be completely explained by the NSB of the stain-HSA complex to the OATP1B1_RIF cells. However, this was not the case for CRV. The uptake data shown have been corrected for small differences in unbound concentration and total protein content between each experiment. Data shown are mean \pm SD of three independent experiments, each conducted in triplicate. Statistical comparison between the increase in statin uptake in the presence of 5% HSA and NSB was performed using the Student's t test (* $p < 0.05$; ** $p < 0.01$).

Supplementary Fig. 1.

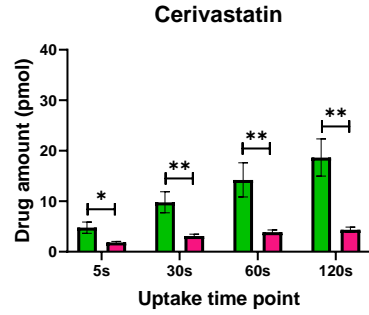


Supplementary Fig. 2.

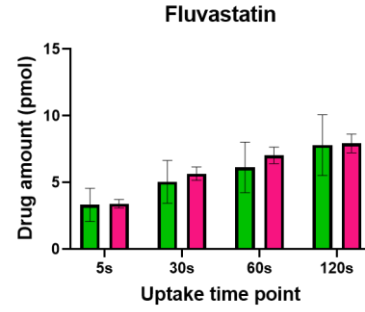
A



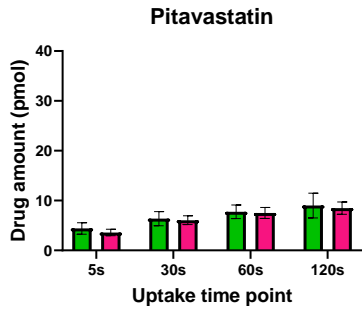
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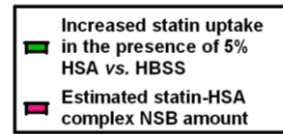
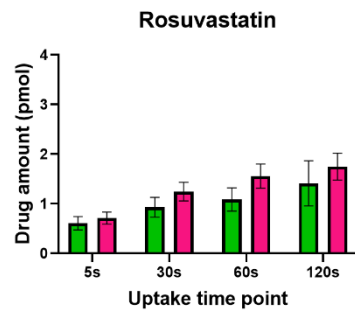
C



D



E



Supplementary Tables**Supplementary Table. 1 LC-MS/MS conditions****LC method for quantification of the 5 statins**

Time	Flow Rate	%A	%B
Initial	0.5	90	10
0.5	0.5	90	10
0.7	0.5	5	95
2.2	0.5	5	95
2.3	0.5	90	10
3	0.5	90	10

A: 0.1% formic acid in water; B: 0.1% formic acid in acetonitrile

MS conditions for quantification of the 5 statins

Compound ID	ESI Mode	Q1 Mass (Da)	Q3 Mass (Da)	DP (volts)	CE (volts)	CXP (volts)
Atorvastatin	Positive	559	250.1	60	60	10
Cerivastatin	Positive	460.3	356.3	100	40	10
Fluvastatin	Positive	412	224.3	60	41	15
Pitavastatin	Positive	422.2	290.2	90	35	17
Rosuvastatin	Positive	482.3	258.2	80	30	10
Diclofenac (IS)	Positive	295.82	213.9	40	45	9

ESI, electrospray ionization; DP, declustering potential, CE, collision energy; CXP, collision cell exit potential.

LC method for quantification of HSA and Na⁺K⁺ATPase

Time	Flow Rate	%A	%B
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Initial	0.3	97.0	3.0
3.00	0.3	97.0	3.0
10.00	0.3	87.0	13.0
20.00	0.3	75.0	25.0
22.00	0.3	66.7	33.3
22.90	0.3	50.0	50.0
23.00	0.3	20.0	80.0
24.10	0.3	20.0	80.0
24.20	0.3	97.0	3.0
28.00	0.3	97.0	3.0

A: 0.1% formic acid in water; B: 0.1% formic acid in acetonitrile

MS conditions for quantification of HSA and Na⁺K⁺ATPase

Compound ID	Peptide Sequence	ESI Mode	Q1 Mass (Da)	Q3 Mass (Da)	DP (volts)	CE (volts)	CXP (volts)
HSA F1	VFDEFKPLVEEPQNLIK (unlabeled)	Positive	682.65	712.65	170	45	12
HSA F2		Positive	682.65	970.75	170	45	12
HSA IS F1	VFDEFKPLVEEPQNLIK (labeled)	Positive	685.35	712.65	170	45	12
HSA IS F2		Positive	685.35	978.75	170	45	12
Na ⁺ K ⁺ ATPase F1	AAVPDAVGK (labeled)	Positive	414.235	685.388	61.3	18	12
Na ⁺ K ⁺ ATPase F2		Positive	414.235	586.320	61.3	18	12
Na ⁺ K ⁺ ATPase IS F1		Positive	418.242	693.402	61.3	18	12
Na ⁺ K ⁺ ATPase IS F2		Positive	418.242	594.334	61.3	18	12

ESI, electrospray ionization; DP, declustering potential, CE, collision energy; CXP, collision cell exit potential; F1/2, fragment 1/2. **Bolded letters** indicate the labeled amino acids.

Supplementary Table. 2 In vitro apparent hepatic uptake intrinsic clearance of the 5 statins into OATP1B1-expressing or MOCK cells for the HBSS and HSA (1%, 2% and 5%) conditions

Drug	HBSS				1% HSA				2% HSA				5% HSA							
	CL _{int,tota} [#] (μ /min/ mg protein)	CL _{int,passive} [%] (μ /min/mg protein)		CL _{int,OATP1B1} [*] (μ /min/mg protein)		CL _{int,tota} [#] (μ /min/ mg protein)	CL _{int,passive} [%] (μ /min/mg protein)		CL _{int,OATP1B1} [*] (μ /min/mg protein)		CL _{int,tota} [#] (μ /min/ mg protein)	CL _{int,passive} [%] (μ /min/mg protein)		CL _{int,OATP1B1} [*] (μ /min/mg protein)						
		Based on passive uptake in		Based on passive uptake in			Based on passive uptake in		Based on passive uptake in			Based on passive uptake in		Based on passive uptake in						
		MOCK	OATP1B1_RIF	MOCK	OATP1B1_RIF		MOCK	OATP1B1_RIF	MOCK	OATP1B1_RIF		MOCK	OATP1B1_RIF	MOCK	OATP1B1_RIF	MOCK	OATP1B1_RIF			
Atorvastatin	43.2 (35.8)	2.1 (24.2)	3.7 (24.2)	41.1	39.5	76.7 (9.5)	3.6 (17.2)	6.6 (29.1)	73.1	70.1	101.5 (26.9)	4.3 (17.3)	9.6 (20.5)	97.2	91.9	94.9 (30.2)	7.8 (30.4)	13.3 (39.5)	87.1	81.6
Cerivastatin	39.2 (18.3)	15.0 (22.1)	17.5 (22.8)	24.2	21.7	84.5 (29.5)	40.1 (41.9)	58.3 (23.4)	44.4	26.1	161.5 (26.4)	72.5 (10.5)	100.1 (24.2)	88.9	61.4	199.2 (34.2)	121.8 (9.4)	159.8 (28.9)	77.4	39.4
Fluvastatin	32.1 (20.6)	8.6 (27.7)	7.5 (30.1)	23.5	24.6	95.1 (21.2)	23.3 (6.9)	23.2 (18.4)	71.7	71.9	151.8 (16.5)	43.2 (31.4)	43.8 (33.6)	108.6	108.0	224.4 (12.9)	123.5 (7.5)	116.1 (47.2)	100.9	108.3
Pitavastatin	79.2 (28.9)	3.7 (41.6)	7.4 (23.0)	75.5	71.9	264.7 (19.9)	9.7 (33.2)	11.4 (7.3)	255.0	253.3	285.2 (17.4)	18.9 (42.9)	26.2 (31.4)	266.4	259.1	294.7 (24.2)	45.0 (29.6)	39.1 (11.1)	249.7	255.7
Rosuvastatin	15.3 (40.0)	0.15 (33.4)	0.48 (15.4)	15.1	14.8	15.7 (42.9)	0.30 (40.2)	0.58 (2.8)	15.4	15.1	15.3 (16.2)	0.57 (33.1)	0.94 (38.1)	14.8	14.4	16.3 (27.0)	1.79 (25.9)	1.62 (31.4)	14.5	14.7

CL_{int,tota} - Apparent total uptake clearance into OATP1B1-expressing HEK293 cells. % CL_{int,passive} - Apparent passive uptake clearance into either MOCK or OATP1B1_RIF HEK293 cells. *CL_{int,OATP1B1} = mean CL_{int,tota} - mean CL_{int,passive} where the CL_{int,passive} measured in the MOCK cells or the OATP1B1_RIF cells was used. Data shown are mean (CV%) values of three to five independent experiments.