

Reevaluating the Role of Megalin in Renal Vitamin D Homeostasis Using a Human Cell-Derived Microphysiological System

Supplementary Data

Supplementary materials

Validation of DBP as a delivery vehicle for vitamin D metabolites in the PT-MPS

After 5 days under standard culture conditions, 1 μM 25OHD₃ was added to the PT-MPS media perfusate with either the previously established delivery vehicle, 2% FBS, (Weber et al., 2016) or the experimental delivery vehicle, 3 μM purified human DBP. Media exiting the PT-MPS luminal path was collected over 24-hour intervals, for 3 days. All samples were stored at -80°C until they could be prepared for LC/MS-MS analysis using a previously established method (Weber et al., 2016, Wang et al., 2011). Quadruplicate PT-MPS (technical replicates) were used for each of the kidney tissue donors evaluated (biological replicates, n = 3). Some 24,25(OH)₂D₃ contaminant was present in both the FBS and the plasma-derived human DBP. As such, net appearance rate, rather absolute appearance rate of 24,25(OH)₂D₃ in the efflux media was used for the assessment of vitamin metabolite disposition in the PT-MPS (Equation S1).

$$\text{Net Efflux Appearance Rate}_{24,25(\text{OH})_2\text{D}_3} = \frac{[24,25(\text{OH})_2\text{D}_3]_{\text{outflow}} - [24,25(\text{OH})_2\text{D}_3]_{\text{input}}}{\text{Duration of Collection Interval} / \text{Volume Collected}} \quad (\text{S1})$$

The mean and standard deviation of net efflux appearance rate were calculated for each treatment (DBP or FBS) at each time point and two-way ANOVA was performed. A post-hoc Bonferroni procedure was used to make pairwise comparisons. Results are presented in Figure S1.

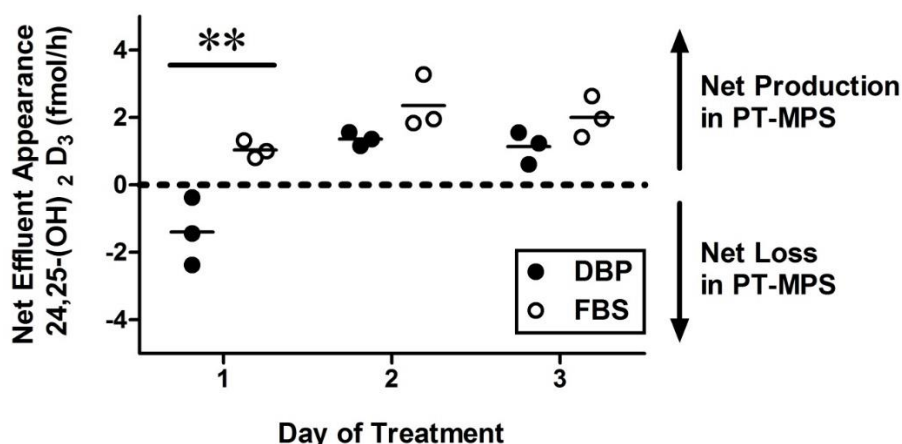


Fig. S1: Validation of DBP as a carrier protein for vitamin D metabolites in the PT-MPS

Cells cultured in the PT-MPS with either 2% FBS or 3 μM DBP as delivery vehicle for vitamin D metabolites exhibited net 24,25(OH)₂D₃ formation during the 24-48 h and 48-72 h collection intervals. Unlike with FBS, cells cultured with DBP exhibited a net loss of 24,25(OH)₂D₃ to the PT-MPS system during the 0-24 h collection interval. The difference in net 24,25(OH)₂D₃ formation rate between the FBS- and DBP-treated cells was significant for the 0-24 h collection interval. **Very statistically significant, p < 0.01. These results demonstrate that purified human DBP (which provides a more controlled culture milieu than 2% v/v FBS) is an effective vehicle for the delivery of vitamin D metabolites to the PT-MPS.

Tab. S1: Antibody information

RRID	Protein target	Antigen sequence	Name of antibody	Manufacturer, Catalog No.	Species raised in, monoclonal or polyclonal	Dilution used
AB_2640319	Recombinant protein corresponding to human CYP24A1	unknown	CYP24A1 Polyclonal Antibody	Thermo-Fisher, PA5-54579	Goat, polyclonal	1:200
AB_10673466	Synthetic peptide within human megalin 4600 to C-terminus	unknown	Anti-Lrp2 / Megalin Antibody	Abcam, ab76969	Rabbit, polyclonal	1:25
AB_306023	Full length native purified protein rabbit alpha 1 sodium potassium ATPase	unknown	Anti-alpha 1 Sodium Potassium ATPase Antibody	Abcam, ab7671	Mouse, monoclonal	1:100

Tab. S2: Kidney tissue donor characteristics in 25OHD₃ uptake/loss experiment

Donor No.	Age	Sex	Ethnicity	Pre-existing conditions	Reason for nephrectomy	Final pathology
1	58	Male	White	HTN, sleep apnea	Renal mass (bilateral)	Papillary RCC, multiple papillary adenomas
2	56	Male	White	None reported	Renal mass (left)	Clear cell RCC
3	58	Male	White	HTN, hernia	Renal mass (right)	Unclassified RCC with clear cell features
4	58	Male	White	None reported	Renal mass (right)	Clear cell RCC
5	40	Male	African American	Methamphetamine use, deep vein thrombosis	Renal mass (left)	Clear Cell RCC

Tab. S3: Kidney tissue donor characteristics in 1 α ,25(OH)₂D₃-mediated induction experiment

Donor No.	Age	Sex	Ethnicity	Pre-existing conditions	Reason for nephrectomy	Final pathology
1	65	Male	White	None reported	Renal mass	Not available
2	49	Male	White	Clear cell RCC	Renal mass	Not available
3	68	Male	White	HTN, GERD, Aortic Aneurysm, Diabetes Mellitus 2	Renal mass (left)	Clear cell RCC
4	62	Female	White	HTN, GERD, previous cervical, thyroid and breast carcinoma	Renal mass	Not available
5	58	Male	White	HTN, sleep apnea	Renal mass (bilateral)	Papillary RCC, multiple papillary adenomas