Table 1. Vitamin D effect on RAS components in different tissues and/or conditions

Condition / Tissue	Treatment / experimental model	Outcome	References
Lung tissue	Intraperitoneal VD to mice	VD treatment significantly upregulated *Ace2* mRNA expression by more than six folds, while downregulated the expression of *Tmprss2* by 2.2 folds.	[1]
SARS-CoV-2 inhibitor screening kit. Cultured HaCaT keratinocytes	Evaluation of VD hydroxymetabolites ability to inhibit SARS-CoV2-ACE2 interaction and their effect on *TMPRSS2* and *ACE2* gene expression	The binding between SARS-CoV2 RBD with human ACE2 was significantly inhibited by various VD derivatives, including calcitriol, as showed by using a SARS-CoV-2 inhibitor ELISA screening kit. The gene expression of *ACE2* and *TMPRSS2* was significantly inhibited by various VD derivatives, including calcidiol and calcitriol.	[2]
MetS Liver tissue	VD supplementation to rats with MetS	VD-treatment significantly reduced SBP, DBP, and hepatic *ACE1* gene expression, while upregulated that of *ACE2*, in MetS affected rats.	[3]
Diabetes Serum Renal cortex TECs	Paricalcitol supplementation to diabetic rats and treatment in cultured TECs	The VD analog Paricalcitol reduced Ang II levels and ACE2 activity in rat serum, while increased *ACE2* expression in the renal cortex and in cultured TECs. There was no effect of paracalcitol on serum renin activity or blood pressure, but a high dose inhibited *REN* gene expression.	[4]
Diabetes	Calcitriol supplementation in diabetic rats	Calcitriol reduced ACE1 levels and ACE1/ACE2 ratio, while increased ACE2 levels in diabetic rats.	[5]
Hypertensive rats	Calcitriol treatment to hypertensive rats	Calcitriol modulated brain RAS in spontaneously hypertensive rats. Calcitriol enhanced ACE2/Ang(1–7)/MasR axis *in vivo* and *in vitro*, reduced Ang II formation and enhanced expression of ACE2, MasR and Ang(1–7) generation in microglial cells.	[6]
ALI PMVECS Lung tissue	Calcitriol administration to LPS-challenged rats	Calcitriol reversed the LPS-dependent ACE1 increased and ACE2 decreased gene expression in cultured PMVECS. Calcitriol downregulated the LPS-induced *REN* gene expression and Ang II release into the culture media. Calcitriol reduced *ACE1* and AT1R expression, while increased that of *ACE2* in LPS-treated rat lung tissue.	[7]
VDR KO mice	VDR Deletion in vivo Calcitriol supplementation to VDR-KO mice. Calcitriol treatment to As4.1 mouse kidney epithelial cells.	In VDR-null mice, *REN* expression and plasma Ang II production were increased, causing hypertension, cardiac hypertrophy, and increased water intake. In WT mice, calcitriol inhibition increased *REN* expression, while calcitriol injection suppressed it. In cultured As4.1 cells, calcitriol markedly suppressed *REN* transcription by a VDR-mediated mechanism.	[8]
As4.1 and HEK293 cells	Calcitriol effects in vitro on REN gene promoter by luciferase reporter assays.	Calcitriol-VDR blocked the binding of CREB to the CRE by interacting with CREB, thus blocking the formation of CRE-CREB-CBP complex at the *REN* promoter.	[9]

Normotensive individuals in high sodium balance	Analysis of the relationship between plasma calcidiol and RAS components	Individuals with VD insufficiency/deficiency had significantly higher circulating Ang II levels and blunted renal plasma flow responses to infused Ang II. Plasma renin activity was higher among individuals with VD insufficiency, although not significantly.	[10]
Normotensive and hypertensive individuals	Circulating calcitriol levels and plasma renin activity	A negative correlation was found between plasma calcitriol levels and renin activity in all patients.	[11]
Computational study	Molecular docking, molecular dynamics simulations and binding free energy analyses	VD active hydroxyderivatives showed to be capable of binding ACE2 and TMPRSS2, affecting their ability to recognize and prime SARS-Cov2 spike protein. Also, docking studies identified VD derivatives as potential ligands of the free fatty acid pocket of the Spike, capable of stabilizing the locked conformation of the spike, thus inhibiting viral entry.	[12,13]
In silico analysis	Genomics-Guided tracing of SARS-CoV2 targets in human cells to identify potential activators and repressors of the *ACE2* gene	The VDR gene was identified as a putative repressor of *ACE2* gene expression. Databases from gene expression profiling experiments of WT and VDR-KO bone marrow-derived macrophages demonstrated increased expression of the *ACE2* gene in VDR-KO cells. Same in human bronchial smooth muscle cells.	[14]
RNA-seq analysis of gene signatures	Global gene expression study in calcitriol/inecalcitol treated breast cells	In breast cancer cells, *TMPRSS2* gene expression was significantly upregulated by calcitriol. Inecalcitol was more potent to induce *TMPRSS2*.	[15-18]

Acute Lung Injury (ALI), Angiotensin II (Ang II); Angiotensin II type 1 receptor (AT1R); cAMP response element-binding protein (CREB); cAMP response elements (CRE); Diastolic blood pressure (DBP); Lipopolysaccharide (LPS), Mas receptor (MasR); Metabolic Syndrome (MetS) ; Pulmonary microvascular endothelial cells (PMVECS), Renin (REN), Receptor binding domain (RBD); Systolic blood pressure (SBP); Tubular epithelial cells (TECs); Wild type (WT); Vitamin D (VD); Vitamin D receptor (VDR) ; Vitamin D receptor knockout (VDR-KO)

References

1. M, A.A.; Jarrar, Y. Effects of vitamin C and D on the mRNA expression of angiotensin converting enzyme 2 receptor, cathepsin L, and transmembrane serine protease in the mouse lungs. Libyan J Med 2022, 17, 2054111, doi:10.1080/19932820.2022.2054111.

2. Qayyum, S.; Slominski, R.M.; Raman, C.; Slominski, A.T. Novel CYP11A1-Derived Vitamin D and Lumisterol Biometabolites for the Management of COVID-19. Nutrients 2022, 14, doi:10.3390/nu14224779.

3. Mohater, S.; Qahtan, S.; Alrefaie, Z.; Alahmadi, A. Vitamin D improves hepatic alterations in ACE1 and ACE2 expression in experimentally induced metabolic syndrome. Saudi Pharm J 2023, 31, 101709, doi:10.1016/j.jsps.2023.101709.

4. Riera, M.; Anguiano, L.; Clotet, S.; Roca-Ho, H.; Rebull, M.; Pascual, J.; Soler, M.J. Paricalcitol modulates ACE2 shedding and renal ADAM17 in NOD mice beyond proteinuria. Am J Physiol Renal Physiol 2016, 310, F534-546, doi:10.1152/ajprenal.00082.2015.

5. Lin, M.; Gao, P.; Zhao, T.; He, L.; Li, M.; Li, Y.; Shui, H.; Wu, X. Calcitriol regulates angiotensin-converting enzyme and angiotensin converting-enzyme 2 in diabetic kidney disease. Mol Biol Rep 2016, 43, 397-406, doi:10.1007/s11033-016-3971-5.

6. Cui, C.; Xu, P.; Li, G.; Qiao, Y.; Han, W.; Geng, C.; Liao, D.; Yang, M.; Chen, D.; Jiang, P. Vitamin D receptor activation regulates microglia polarization and oxidative stress in spontaneously hypertensive rats and

angiotensin II-exposed microglial cells: Role of renin-angiotensin system. Redox Biol 2019, 26, 101295, doi:10.1016/j.redox.2019.101295.

7. Xu, J.; Yang, J.; Chen, J.; Luo, Q.; Zhang, Q.; Zhang, H. Vitamin D alleviates lipopolysaccharide-induced acute lung injury via regulation of the renin-angiotensin system. Mol Med Rep 2017, 16, 7432-7438, doi:10.3892/mmr.2017.7546.

8. Li, Y.C.; Kong, J.; Wei, M.; Chen, Z.F.; Liu, S.Q.; Cao, L.P. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest 2002, 110, 229-238, doi:10.1172/JCI15219.

9. Yuan, W.; Pan, W.; Kong, J.; Zheng, W.; Szeto, F.L.; Wong, K.E.; Cohen, R.; Klopot, A.; Zhang, Z.; Li, Y.C. 1,25-dihydroxyvitamin D3 suppresses renin gene transcription by blocking the activity of the cyclic AMP response element in the renin gene promoter. J Biol Chem 2007, 282, 29821-29830, doi:10.1074/jbc.M705495200.

10. Forman, J.P.; Williams, J.S.; Fisher, N.D. Plasma 25-hydroxyvitamin D and regulation of the renin-angiotensin system in humans. Hypertension 2010, 55, 1283-1288, doi:10.1161/HYPERTENSIONAHA.109.148619.

11. Resnick, L.M.; Muller, F.B.; Laragh, J.H. Calcium-regulating hormones in essential hypertension. Relation to plasma renin activity and sodium metabolism. Ann Intern Med 1986, 105, 649-654, doi:10.7326/0003-4819-105-5-649.

12. Song, Y.; Qayyum, S.; Greer, R.A.; Slominski, R.M.; Raman, C.; Slominski, A.T.; Song, Y. Vitamin D3 and its hydroxyderivatives as promising drugs against COVID-19: a computational study. J Biomol Struct Dyn 2022, 40, 11594-11610, doi:10.1080/07391102.2021.1964601.

13. Shoemark, D.K.; Colenso, C.K.; Toelzer, C.; Gupta, K.; Sessions, R.B.; Davidson, A.D.; Berger, I.; Schaffitzel, C.; Spencer, J.; Mulholland, A.J. Molecular Simulations suggest Vitamins, Retinoids and Steroids as Ligands of the Free Fatty Acid Pocket of the SARS-CoV-2 Spike Protein*. Angew Chem Int Ed Engl 2021, 60, 7098-7110, doi:10.1002/anie.202015639.

14. Glinsky, G.V. Tripartite Combination of Candidate Pandemic Mitigation Agents: Vitamin D, Quercetin, and Estradiol Manifest Properties of Medicinal Agents for Targeted Mitigation of the COVID-19 Pandemic Defined by Genomics-Guided Tracing of SARS-CoV-2 Targets in Human Cells. Biomedicines 2020, 8, doi:10.3390/biomedicines8050129.

15. Murray, A.; Madden, S.F.; Synnott, N.C.; Klinger, R.; O'Connor, D.; O'Donovan, N.; Gallagher, W.; Crown, J.; Duffy, M.J. Vitamin D receptor as a target for breast cancer therapy. Endocr Relat Cancer 2017, 24, 181-195, doi:10.1530/ERC-16-0463.

16. Goeman, F.; De Nicola, F.; D'Onorio De Meo, P.; Pallocca, M.; Elmi, B.; Castrignano, T.; Pesole, G.; Strano, S.; Blandino, G.; Fanciulli, M.; et al. VDR primary targets by genome-wide transcriptional profiling. J Steroid Biochem Mol Biol 2014, 143, 348-356, doi:10.1016/j.jsbmb.2014.03.007.

17. Sheng, L.; Anderson, P.H.; Turner, A.G.; Pishas, K.I.; Dhatrak, D.J.; Gill, P.G.; Morris, H.A.; Callen, D.F. Identification of vitamin D(3) target genes in human breast cancer tissue. J Steroid Biochem Mol Biol 2016, 164, 90-97, doi:10.1016/j.jsbmb.2015.10.012.

18. Simmons, K.M.; Beaudin, S.G.; Narvaez, C.J.; Welsh, J. Gene Signatures of 1,25-Dihydroxyvitamin D3 Exposure in Normal and Transformed Mammary Cells. J Cell Biochem 2015, 116, 1693-1711, doi:10.1002/jcb.25129.