

Article

# Differential Effects of 25-hydroxyvitamin D<sub>3</sub> Versus 1 $\alpha$ , 25-dihydroxyvitamin D<sub>3</sub> on Adipose Tissue Browning in CKD-Associated Cachexia

Robert H. Mak <sup>1\*</sup>, Uwe Querfeld <sup>2</sup>, Alex Gonzalez <sup>1</sup>, Sujana Gunta<sup>1,3</sup>, Wai W. Cheung <sup>1</sup>

<sup>1</sup> Division of Pediatric Nephrology, Rady Children's Hospital, University of California, San Diego

92093-0831, USA; [romak@health.ucsd.edu](mailto:romak@health.ucsd.edu) (R.H.K.); [w5cheung@health.ucsd.edu](mailto:w5cheung@health.ucsd.edu) (W.W.C.); [alg022@health.ucsd.edu](mailto:alg022@health.ucsd.edu) (A.G.)

<sup>2</sup> Department of Paediatric Nephrology, Charité Children's Hospital, Augustenburger Platz 1, 13353 Berlin, Germany; [uwe.querfeld@charite.de](mailto:uwe.querfeld@charite.de) (U.Q.)

<sup>3</sup> Pediatric Services, Vista Community Clinic, Vista, CA 92084; [sujana.kaushik@gmail.com](mailto:sujana.kaushik@gmail.com) (S.G.)

\* Correspondence: [romak@health.ucsd.edu](mailto:romak@health.ucsd.edu); Tel.: +1-858-822-6717; Fax: +1-858-822-6776

**Supplemental information**

**Supplemental Table S1: Immunoassay information for blood and serum chemistry, muscle adenosine triphosphate content as well as muscle and adipose tissue protein analysis.**

<u>Blood &amp; Serum chemistry</u>	<u>Assay information</u>
Bicarbonate, BUN, Ca & Pi	VetScan Comprehensive Diagnostic Profile, Abaxix, 500-0038
Creatinine	LC-MS/MS method (reference # 20)
25(OH)D <sub>3</sub>	IDS, AC-35F1
1,25(OH) <sub>2</sub> D <sub>3</sub>	IDS, AC-62F1
PTH	Immutopics, 60-2305
VDBP	R&D Systems, MVDBPO

<u>Muscle &amp; adipose tissue</u>	<u>Assay information</u>
ATP Assay Kit (Colorimetric / Fluorometric)	Abcam, ab83355
Mouse UCP1 ELISA kits	Aviva Systems Biology, OKCD02970
Mouse UCP3 ELISA kits	Aviva Systems Biology, OKEH05259

**Supplemental Table S2: PCR primer information**

<b>Gene</b>	<b>Forward primer sequence</b>	<b>Reverse primer sequence</b>
Agt	TCTCCTTTACCACAACAAGAGCA	CTTCTCATTACAGGGGAGGT
Akt1	CTGCTCCTAGTCCACCACCT	AGAGACCTCCATTATCGCTACC
Atf3	GAGGATTTTGCTAACCTGACACC	TTGACGGTAACTGACTCCAGC
Atp2 $\alpha$ 2	GAGAACGCTCACACAAAGACC	CAATTTCGTTGGAGCCCCAT
Atrogin-1	CAGCTTCGTGAGCGACCTC	GGCAGTCGAGAAGTCCAGTC
Bmp7	ACGGACAGGGCTTCTCCTAC	ATGGTGGTATCGAGGGTGGAA
CD137	CGTGCAGAACTCCTGTGATAAC	GTCCACCTATGCTGGAGAAGG
Cidea	TGACATTCATGGGATTGCAGAC	GGCCAGTTGTGATGACTAAGAC
Cyfp2	ATGACCACCCACGTCACTTTG	CCTGTCCTCGAAGTTCGTGTC
Cox2	AACCCAGGGGATCGAGTGT	CGCAGCTCAGTGTTCGGGAT
Csrp3	GGGGGAGGTGCAAAATGTG	CAGGCCATGCAGTGGAACA
Ctgf	GTGGAATATTGCCGGTGCA	CCATTGAAGCATCTTGGTTCC
Dio2	AATTATGCCTCGGAGAAGACCG	GGCAGTTGCCTAGTGAAAGGT
Fhl1	GACTGCCGCAAGCCATAA	CCAAGGGGTGAAGGCACTT
Fos	TTGAGCGATCATCCGGTC	GCGTGAGTCCATACTGGCAAG
Gng2	ACCGCCAGCATAGCACAAG	AGTAGGCCATCAAGTCAGCAG
IL-1 $\alpha$	ACGGCTGAGTTTCAGTGAGACC	CACTCTGGTAGGTGTAAGGTGC
IL-1 $\beta$	GCAACTGTTCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
IL-6	TAGTCCTTCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
IL-13R $\alpha$ 2	ACCGAAATGTTGATAGCGACAG	ACAATGCTCTGACAAATGCGTA
Itpr1	CGTTTTGAGTTTGAAGGCGTTT	CATCTTGCGCCAATTCCTCG
Maff	AGGAGGAGGTATCCGACTG	CTTCTCGCTCTCCAGAATGTG
Murf-1	GTGTGAGGTGCCTACTTGCTC	GCTCAGTCTTCTGTCTTGGA
Myd88	TCATGTTCTCCATACCCTTGGT	AAACTGCGAGTGGGGTCAG
Myl2	ATCGACAAGAATGACCTAAGGGA	ATTTTTCACGTTCACTCGTCCT
Myod	CCACTCCGGGACATAGACTTG	AAAAGCGCAGGTCTGGTGAG
Myogenin	GAGACATCCCCCTATTTCTACCA	GCTCAGTCCGCTCATAGCC
Myostatin	AGTGGATCTAAATGAGGGCAGT	GTTTCCAGGCGCAGCTTAC
PAI-1	TCTGGGAAAGGGTTCACTTTACC	GACACGCCATAGGGAGAGAAG
Pax7	TCTCCAAGATTCTGTGCCGAT	CGGGGTCTCTCTCTTATACTCC
Pgc1 $\alpha$	TATGGAGTGACATAGAGTGTGCT	CAGGAGTTGATTCCAGACAGGTA
Pgf2 $\alpha$	CTGGACTCATCGAAACACAA	AGGAAGCCTTTGACTTCTGTCTA
Ppargc1 $\alpha$	AGAGCCCCATCTGTCTCTC	ACTGGTAGTCTGCAAAACCAA
Prdm16	CCCCACATTCCGCTGTGAT	CTCGCAATCCTTGCACTCA
Tbx-1	CTGTGGGACGAGTTCAATCAG	TTGTCATCTACGGGCACAAAG
Tgfa1	TGATACGCTGAGTGGCTGTCT	CACAAGAGCAGTGAGCGCTGAA
Tgif1	GAACACAGATACAACGCCTATCC	CCATCCTTTCTCAGCATGTCAG
Timp3	CTTCTGCAACTCCGACATCGT	GGGGCATCTTACTGAAGCCTC
Tlr2	GCAAACGCTGTTCTGCTCAG	AGGCGTCTCCCTCTATTGTATT
Tmem26	TTCTGTGTCATTCCCTGGTC	GCCGGAGAAAGCCATTTGT
Tnf- $\alpha$	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
Tnnc1	GCGGTAGAACAGTTGACAGAG	CCAGCTCCTTGGTGCTGAT
Tpm3	ACCACCATCGAGGCGGTAA	CCCTTTCCTCCGCATCATCA
Traf6	AAAGCGAGAGATTCTTTCCCTG	ACTGGGGACAATTCCTAGAGC
Smad3	AAAGCGAGAGATTCTTTCCCTG	ACTGGGGACAATTCCTAGAGC
Gapdh (internal control)	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA