Vitamin D and Calcium Metabolism In Horses in New Zealand

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy

in

Veterinary Science

at

Institute of Veterinary, Animal and Biomedical Sciences (IVABS), Massey University, Manawatū, New Zealand

Sara Azarpeykan

2016

Abstract

The physiology of vitamin D in horses has not been studied in great depth. Few studies on vitamin D metabolites ($25OHD_2$, $25OHD_3$, and $1,25(OH)_2D$) and their relationship to other serum analytes exist. In addition, some studies suggest that equine vitamin D physiology may be different from other species. This thesis aimed to investigate aspects of vitamin D metabolism in horses.

The effect of blanketing on vitamin D synthesis and its relationship with other analytes involved in calcium homeostasis, including vitamin D metabolites (25-hydroxyvitamin D_2 ($25OHD_2$), and 25-hydroxyvitamin D_3 ($25OHD_3$), 1,25-dihydroxyvitamin D ($1,25(OH)_2D$)), ionised calcium (iCa), total calcium (tCa), phosphorus (P), total magnesium (tMg) and parathyroid hormone (PTH) were studied in horses. Regardless of blanketing, $25OHD_3$ was undetectable in equine serum and $25OHD_2$ was the main form of 25OHD in circulation. A strong seasonal variation in serum $25OHD_2$, $1,25(OH)_2D$, iCa, tCa, P, tMg and PTH concentrations was detected, although no differences were seen between horses that were blanketed and those that were not. The circadian rhythms of serum vitamin D metabolites, iCa, tCa, P, tMg, and PTH concentrations in horses was studied over 48 h on the summer and winter solstices. A significant difference was seen between the serum concentrations of studied analytes between solstices, with no rhythm detected in winter. An *in vivo* study suggested that equine skin may be unable to convert 7-dehydrocholesterol (7-DHC) to vitamin D_3 after exposure to ultraviolet B (UVB) light. Quantitative PCR was performed on equine kidney to study the expression of vitamin D responsive and calcium transporting genes, which were then compared to genes in sheep and dogs.

The results suggested that TRPV6, $calD_{9k}$ /$calD_{28k}$, and PMCA were the main calcium transporting pathways in the kidney of these species, and there was a high correlation between VDR and other studied genes. It was concluded that $25OHD_2$ is the main metabolic precursor for $1,25(OH)_2D$ and should be considered the best available index of vitamin D status in unsupplemented horses, and that horses most likely rely on diet as their primary source of vitamin D.

Acknowledgements

I started this study three years ago as a fun horsey project, but it soon became a long battle against different analytes and genes. This thesis is the culmination of a long process, but it cannot fully speak of the many long days spent at the farm and in the lab, the careful preparation of experiments, the joy of good results and the disappointment of each failed attempt.

My deepest gratitude goes to my chief supervisor, Dr Keren Dittmer. I have been amazingly fortunate to have such a generous supervisor with her time and knowledge. Her patience and support helped me overcome many crisis situations and finish this thesis, not to mention her carefully reading and commenting on countless revisions of this manuscript.

My sincere thanks also go to my co-supervisors, Dr Erica Gee, Dr Els Acke, and Professor Keith Thompson, for their valuable guidance. I am extra thankful to Keith, for encouraging the use of correct grammar and consistent notation in my writings, and to Dr Jonathan Marshall for his statistical assistance and artwork!

I am also thankful to the laboratory staff at NZVP, Nutrition lab, and Canterbury Health lab, staff at VLATU and Massey University Veterinary Teaching Hospital, and Massey University veterinary students for their technical assistance. I am especially grateful to Mike Hogan and Craig Thomas at the Pathobiology post mortem room for their support throughout my study.

I also want to thank Debbie Hill, our lovely postgraduate and research administrator at IVABS, who has provided plenty of support, not only to me but to all postgrads, to produce and complete our theses.

I would like to acknowledge the Equine Trust and IVABS Postgraduate Student Research Fund, Institute of Veterinary, Animal and Biomedical Sciences, Massey University to provide funding for this body of work.

I have been blessed with a friendly and cheerful group of fellow students who have helped me stay sane these past three years. Your support, care, and friendship helped me overcome setbacks and stay focused as we deliberated on our problems and findings, and had broader discussion on life, the universe and all things in between. I greatly value your friendship.

Most importantly, none of this would have been possible without the love and patience of my partner, my Mom, my sisters and my brother. I would like to express my heart-felt gratitude to all of you and really thank you for supporting me through all my studies.

I would like to dedicate this dissertation to my beloved sister, Raha, who was a constant source of love, energy, concern, support and strength. You are forever in my mind and my heart.

Table of Contents

List of Figures

List of Tables

Glossary

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
18S	ribosomal RNA
25OHD	25-hydroxyvitamin D
$25OHD_2$	25-hydroxyvitamin D_2
$25OHD_3$	25-hydroxyvitamin D_3
28S	ribosomal RNA
7-DHC	7-dehydrocholesterol
B2M	ß-2-microglobulin
Ba^{2+}	Barium
BLAST	Basic Local Alignment Search Tool
BMD	bone mineral density
Ca^{2+}	calcium
Calbindin	calcium-binding protein
$CalbindinD_{9k}$	vitamin-D-dependent 9k, calcium-binding protein
$CalbindinD_{28k}$	vitamin-D-dependent 28k, calcium-binding protein
cAMP	cyclic 3',5'-adenosine monophosphate
CaSR	calcium sensitive receptors
C cells	parafollicular cells
cDNA	complementary DNA
CGRP	calcitonin gene-related peptide
Cl^-	chloride
CNT	connecting tubule
CYP24A1	24-hydoxylase
CYP27A1	cytochrome P450, family 27, subfamily A, polypeptide 1
CYP27B1	1α-hydroxylase
CYP2R1	cytochrome P450, family 2, subfamily R, polypeptide 1
DAG	diacylglycerol
DBP	vitamin D-binding protein
DCAD	dietary cation-anion difference
DCs	dendritic cells

DCT	distal convoluted tubule
DHCR1	7-dehydrocholesterol reductase 1
DNA	deoxyribonucleic acid
Fe^{2+}	iron
FGF23	fibroblast growth factor 23
FE_{Ca}	fractional urinary clearance of calcium
FE_{Mg}	fractional urinary clearance of magnesium
FE_{P}	fractional urinary clearance of phosphorus
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GC	glucocorticoids
GC content	guanine-cytosine content
GCs	glucocorticoid hormones
GH	growth hormone
GIT	gastrointestinal tract
HKG	housekeeping gene
HMBS	hydroxymethylbilane synthase
HPO_4^{2-} /$H_2PO_4^-$	phosphorus anions
HPLC	high-performance liquid chromatography
HPRT1	hypoxanthine phosphoribosyltransferase 1
iCa	ionised calcium
CI	confidence interval
CV	coefficient of variation
IGFs	insulin-like growth factors
IL-1	interleukin 1
IL-6	interleukin 6
IVABS	Institute of Veterinary, Animal and Biomedical Sciences
K^+	potassium
LC-MS	liquid chromatography–mass spectrometry
MAPK	mitogen-activated protein kinase
Mg^{2+}	magnesium
mgcv	mixed generalized additive models computation vehicle
MS	multiple sclerosis

Na^+	sodium
Na^+/Pi	sodium-dependent phosphate
NCBI	national centre for biotechnology information
NCX1	sodium calcium exchanger 1
NH_4^+	ammonium ions
NH_4Cl	ammonium chloride
NIWA	National Institute of Water and Atmospheric research
NRC	nutrient requirements of horses
NZVP	New Zealand Veterinary Pathology
OPG	osteoprotegerin
P	phosphorus
PCR	polymerase chain reaction
PGK1	phosphoglycerate kinase 1
PKC	protein kinase C
PLA2	phospholipase A2
PLC	phospholipase C
PLD	phospholipase D
PMCA	plasma membrane Ca^{2+}-ATPase
PTH	parathyroid hormone
PTHR1	parathyroid hormone receptor 1
PTHrP	parathyroid hormone -related peptide
RANK	receptor activator of the nuclear factor-kappaB
RANKL	receptor activator of the nuclear factor-kappaB ligand
RNA	ribonucleic acid
RPL13A	ribosomal protein L13a
RPL30	ribosomal protein L30
RPL32	ribosomal protein L32
RPS5	ribosomal protein S5
RPS19	ribosomal protein S19
RT-qPCR	real-time quantitative reverse transcriptase polymerase chain reaction
SCN	suprachiasmatic nucleus

SDHA	succinate dehydrogenase complex
SE	standard error
sFRP-4	secreted frizzled related protein-4
SLC17	type I sodium-phosphate co-transporters
SLC20/PiT2	type III sodium-phosphate co-transporters
SLC34	type II sodium-phosphate co-transporters
SLC34A1	type II sodium-phosphate co-transporters, member 1
SLC32A2	type II sodium-phosphate co-transporters, member 2
SLC34A3	type II sodium-phosphate co-transporters, member 3
SNPs	single nucleotide polymorphisms
SPF	sun protection factor
SST	serum separator tube
tCa	total calcium
TLR	toll-like receptors
tMg	total magnesium
TRPM	transient receptor potential cation channel, subfamily M
TRPM6	transient receptor potential cation channel, subfamily M, member 6
TRPM7	transient receptor potential cation channel, subfamily M, member 7
TRVP5	transient receptor potential cation channel, subfamily V, member 5
TRPV6	transient receptor potential cation channel, subfamily V, member 6
UBB	ubiquitin B
UV	ultraviolet
UVB	ultraviolet B
VDR	vitamin D receptor
VLATU	Veterinary Large Animal Teaching Unit
YWHAZ	zeta polypeptide