Comparative Studies on Plasma Vitamin D Binding Protein

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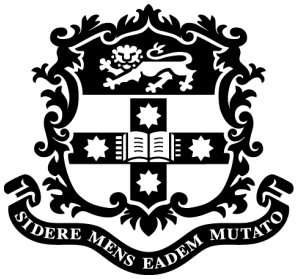

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University of Sydney (Australia)

A thesis submitted in fulfillment of the requirements for the degree of

Doctor of Philosophy

March, 2000

declaration

Apart from the assistance mentioned in the Acknowledgements, the studies contained within this thesis were planned and executed by the author, and have not been previously submitted or any degree to a University.

All experiments complied with the Australian Code of Practice for Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council et al., 1990).

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March, 2000

acknowledgements

First and foremost, I would like to thank my supervisor Professor David Fraser, who offered advice, guidance, encouragement, and friendship. His time and effort have been selflessly given, and have been so important at all stages during my candidature.

I would also like to thank Associate Professor Grant Stone, and Associate Professor Chis Maxwell who, as Heads of the Department of Animal Science, made the facilities of the Department available and allowed me the opportunity to complete my studies.

I would like to express thanks to Dr Glenn Shea, who shared the supervisory load, and was extremely generous in donating his time and efforts in helping me collect blood samples, and in reviewing and commenting upon my thesis manuscript.

I would like to gratefully acknowledge the technical assistance of Angelika Trube, whose continued willingness to help with experiments in a variety of capacities was indispensable. I would also like to thank the other members of staff in the Department of Animal Science who made my time here seem to go so quickly! Irene Van Ekris, Kerrie Murdoch, Zung Doan, Kim Heasman, Lyn Hicks, Joy Gill, Melinda Hayter, Darren Mulley, Noelene West, Carol Butler, Andrew Souter, and Margaret Byrne provided much assistance throughout my candidature. I would like to express my gratitude to Dr Michelle Hyde for her invaluable advice, help and friendship. The generosity of Associate Professors Frank Nicholas, Chris Moran, and Grant Stone, who allowed me access to the expertise of their laboratories, are gratefully acknowledged. Thanks also to Associate Professor Wayne Bryden for his advice and assistance.

I would also like to thank Professor Michael Bryden, Department of Veterinary Anatomy and Pathology, who provided me with the opportunity of working in practical classes during my candidature. The encouragement of all of the academic staff of the Faculty is gratefully acknowledged, and in particular, that of Dr Richard Malik, Dr Geraldine Hunt, and Dr Jill Maddison.

The postgraduates within the Faculty who shared with me so much, and with whom I have established friendships, have been an important part of my postgraduate experience. In particular I would like to thank Janine Aldred, Xueqin Du, Geoffrey Dutton, Lindsay Gillan, Mark Krockenberger, Jun Heon Lee, Seung Soo Lee, Margaret Lowe, Sally McEwan, Anthony Preshaw, Justine O'Brien, Andrew Spanner, Samantha Whitton, and the other members of the "Magnificent Seven": Merran Govendir, Joanne Connolly, and Michael Noad.

I would like to thank the following people for their kind and patient assistance in the collection of various blood plasma samples for this project: Dr Jack Giles, Dr Larry Vogelnest, Dr Frances Hulst, Vanessa DiGiglio, Michael Muscat, Chris Hibbard, and all the staff at **Taronga Park Zoo**, New South Wales. Dr Helen McCracken, Dr Michael Lynch, Margaret Webb, Jon Birkett, and all the staff at **Royal Melbourne Zoo**, Victoria. John Weigel, and Rob Porter, and the staff of the **Australian Reptile Park**, NSW. Dr Rosie Booth, Nicki Markus and the staff of **Currumbin Sanctuary**, Queensland. Dr Adrian Gallagher and staff, (**Brighton Veterinary Clinic**, Queensland), Dr Michael Ball and Narelle Halcrow (**Ball and Cannon Veterinary Clinic**, NSW), and Dr Ross Perry and staff (**Homebush Animal Hospital**, NSW). Tim Jessop and Professor Gordon Grigg (**Department of Zoology, University of Queensland**) who provided blood plasma samples from sea turtles. Nick Mooney (**Parks and Wildlife Service, Tasmania**), and Dr Kim Barrett who donated samples from various raptors, and Mr John Houston (**Pepe's Ducks Pty Ltd**, NSW) who donated blood samples from ducks. Dr Alison Cree, **Department of Zoology, University of Otago**, New Zealand, who provided plasma samples from tuatara. Dr Charlie Manolis and Adam Britton (**Wildlife Management International Pty Ltd**, Northern Territory) who provided blood samples from saltwater crocodiles. Dr Michelle Hyde, Dr Janine Aldred, and Associate Professor Wayne Bryden, **Department of Animal Science, University of Sydney**, and Dr Glenn Shea, **Australian Museum, NSW** were also of great assistance in the collection of various blood samples for this project.

I have been lucky to have the support of friends throughout. Although I won't list them by name, I would like to thank them all for their continued encouragement (and occasional bad influences!). Finally I would like to thank Mum, Dad and Marina for their love, support and infinite patience.

summary

The plasma vitamin D binding protein (DBP) is an α-glycoprotein, synthesised and secreted by the liver, which binds specifically vitamin D and its metabolites. The DBP molecule, has a single high affinity binding site for its ligands, and is present in blood in concentrations about 1000-fold greater than the sum of all its vitamin D ligands. Previous studies have not found any change in the concentration of DBP related to various derangements in mineral homeostasis. Therefore the general view is that DBP has a passive role in the physiology of vitamin D and its metabolites, and simply acts to solubilise and transport these hydrophobic ligands in the aqueous extracellular fluid. However, differences which have been described in its affinity for various vitamin D metabolites suggest that there have been evolutionary influences on the properties of this protein. Furthermore, plasma DBP concentration has been found to change in response to a number of physiological factors, such as changing sex steroid hormone secretion. The aim of the studies presented in this thesis was to investigate variation in the plasma concentration of the DBP in a range of vertebrate species, and in response to a variety of physiological factors. The results suggest that DBP may have an active role in regulating the bioavailability, and hence the utilisation and metabolism of its ligands.

DBP concentration has traditionally been measured using immunological techniques. These techniques, although fast and simple, have a number of draw-backs which can be overcome by the use of assays which rely upon functional aspects of the DBP. A saturation binding assay was modified from those described previously. Using this technique, it was found that both the circulating concentration of the DBP and its affinity for 25-hydroxyvitamin D_3 ($25(OH)D_3$) varied significantly among a wide range of species of reptiles and birds. This variation did not reflect phylogenetic relationships among the study species, suggesting that the variation was more likely to be the result of selective pressure in response to individual ecological or physiological circumstance, rather than to random mutation. In support of this, both the plasma concentration of DBP, and its affinity for $25(OH)D_3$ were significantly associated with a number of ecological

factors which might be considered to have some significance to vitamin D and calcium homeostasis. In addition, comparative binding data suggests that the ability of the DBP to bind 25-hydroxyvitamin D_2 with equal affinity to 25(OH)D_3 is an evolutionary innovation of mammalian vertebrates. In order to extend the idea of genetic variation in the concentration and affinity of plasma DBP, two strains of broiler (meat-type) chickens were studied. It was found that both the concentration and the affinity of plasma DBP for 25(OH)D_3 was characteristic for each strain, emphasising the sensitivity of DBP to genetic variation.

A number of factors have been found to modulate the genetically determined plasma concentration of DBP. Deficiencies of dietary protein and dietary energy, and variation in concentrations of sex steroids were found to affect the circulating concentration of DBP. However, species differences were still apparent, suggesting that the sensitivity of DBP to these physiological modifiers may have developed independently in different species, and may be secondary to genetic determinants of DBP properties.

The plasma DBP concentration and specific binding affinity both determine the availability of its ligands for cellular uptake. It is likely that this process is complex, and involves a combination of protein mediated and non-mediated uptake events. This makes DBP a potentially important determinant of the biological actions of its ligands. The studies in this thesis have produced two main lines of argument supporting an active role for DBP in the regulation of vitamin D metabolism and utilisation. The first is that genetic variation in the properties of plasma DBP appears to be genetically determined, and is selected for, both at the between-species, and the within-species level, than it is to random mutation. Secondly, the ability of physiological and environmental factors to modify the circulating concentration of DBP suggests that this protein is responsive to homeostatic processes. It is proposed that DBP is an active regulator of the physiological economy of vitamin D and its metabolites by being itself regulated by a number of genetic and non-genetic factors.

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BA	barbital acetate (buffer, pH 8.6)
BA/G	barbital acetate-gelatin (buffer, pH 8.6, 0.1% gelatin)
CBG	corticosteroid-binding globulin
DBP	vitamin D-binding protein (Gc)
dpm	disintegrations per minute
Gc	group-specific component (DBP)
^3H	tritium
1α(OH)D	1α-hydroxyvitamin D*
1,25$(OH)_2$D	1,25-dihydroxyvitamin D*
24,25(OH)D	24,25-dihydroxyvitamin D*
25(OH)D	25-hydroxyvitamin D*
K_A	binding association constant
K_D	binding dissociation constant (reciprocal of K_A)
LWI	liver weight index (indicating liver weight independent of body weight)
NSB	non-specific binding
PTH	parathyroid hormone
RBP	retinol-binding protein
SHBG	sex hormone-binding globulin
TBG	thyroid-binding globulin
TD	tibial dyschondroplasia
TPP	total plasma protein
TTR	transthyretin (formerly thyroid binding pre-albumin)
UV	ultraviolet
VDR	vitamin D receptor

* D_3 or D_2 indicate specifically metabolites of vitamin D_3 and vitamin D_2