

Comparative Studies on Plasma Vitamin D Binding Protein

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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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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 D₃ (25(OH)D₃) 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)D₃ 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 D₂ with equal affinity to 25(OH)D₃ 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)D₃ 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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list of abbreviations

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
$1\alpha(\text{OH})\text{D}$	1α -hydroxyvitamin D*
$1,25(\text{OH})_2\text{D}$	$1,25$ -dihydroxyvitamin D*
$24,25(\text{OH})_2\text{D}$	$24,25$ -dihydroxyvitamin D*
$25(\text{OH})\text{D}$	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
