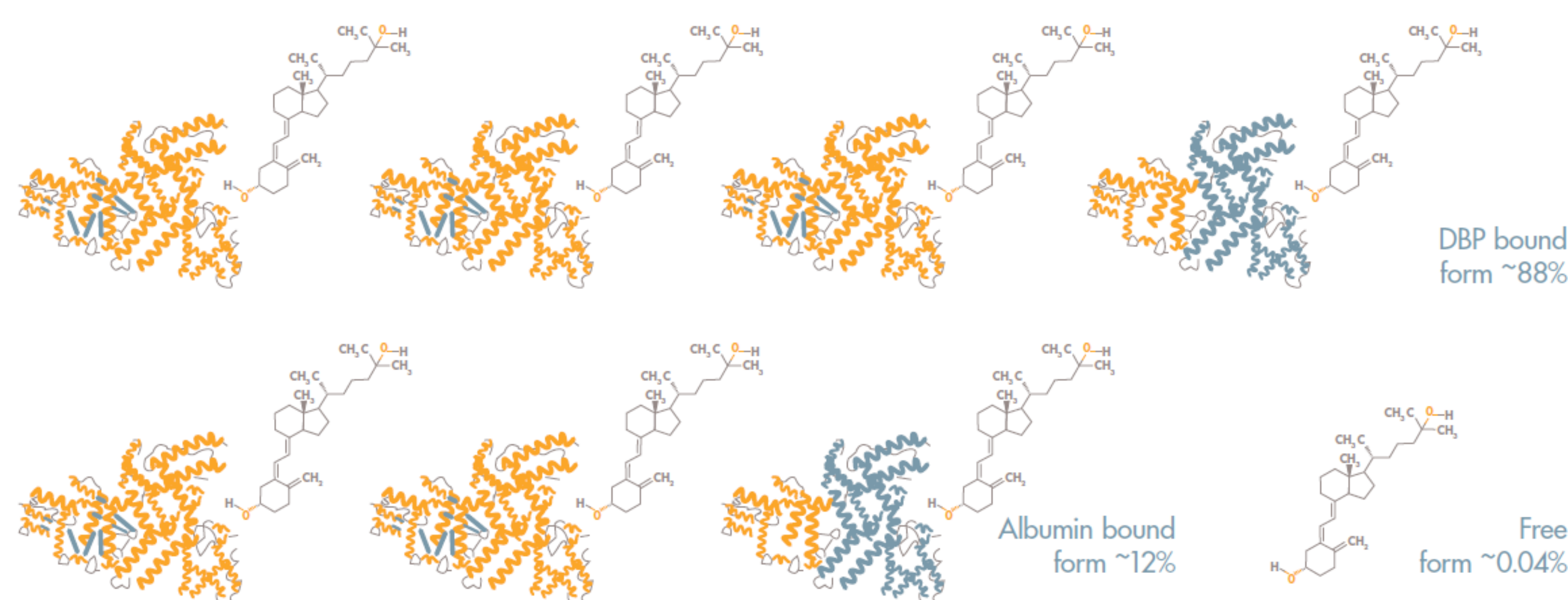


# FREE 25OH VITAMIN D – A REVIEW OF EVIDENCE

N. Heureux\* DIAsource Immunoassays, Louvain-la-Neuve, Belgium

## > Free 25OH Vitamin D represents 0.04% of the total 25OH Vitamin D

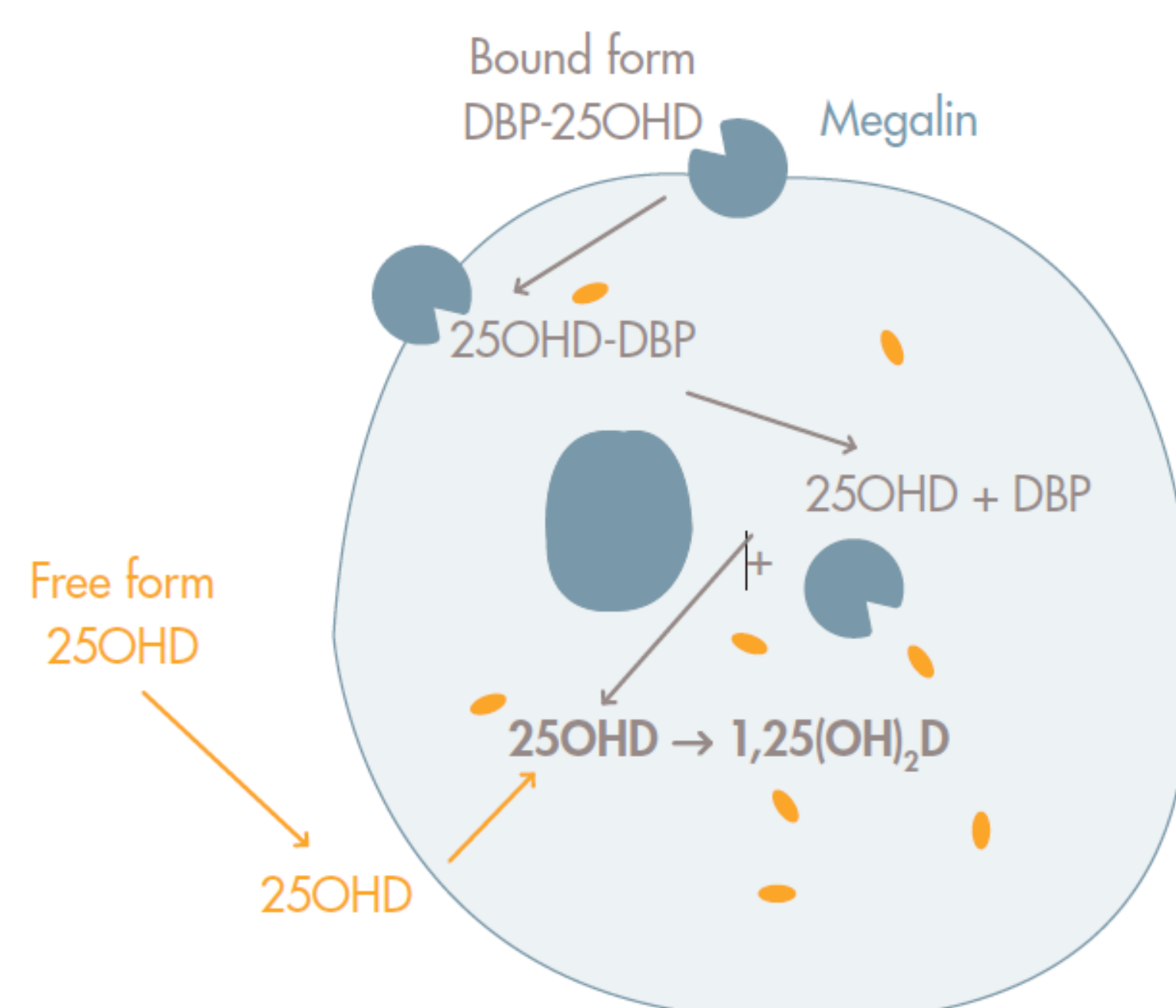
Almost all circulating 25OH Vitamin D in serum is bound to Vitamin D Binding Protein DBP (88%) and Albumin (12 %). A very small fraction, approximately 0.04 % of the 25OH Vitamin D, circulates in the free, non-protein bound form.



## > The free hormone hypothesis

The free hormone hypothesis states that the biological activity of a given hormone is affected by its free rather than protein-bound concentration in the plasma. The conversion of 25OH Vitamin D into the biologically active  $1,25(\text{OH})_2$  Vitamin D requires the transport of 25OH Vitamin D into the cells. Mainly two transport mechanisms are involved : **megalyn-mediated endocytic uptake of the DBP bound form** and **passive diffusion of the free ligand**.

The predominant mechanism depends on the tissue and still needs to be fully elucidated. In case of passive diffusion of the free ligand the concentration of free 25OH Vitamin D is directly linked to the intracellular concentration of  $1,25(\text{OH})_2$  Vitamin D and may therefore better represents the physiological activity of Vitamin D than the total concentration.



## > Conditions affecting the free 25OH Vitamin D concentration

The concentration of DBP is not constant and can be influenced by a number of factors including

- ⊗ Obesity
- ⊗ Pregnancy
- ⊗ The use of oral contraceptives
- ⊗ Hormone replacement therapy
- ⊗ Liver disease
- ⊗ Renal disease
- ⊗ Proteinuria
- ⊗ Intensive care

In case of elevated concentration of DBP the % of free 25OH Vitamin D is decreased. In case of decreased concentration of DBP the % of free 25OH Vitamin D is increased.

## > Supplementation with Vitamin D2 or D3?

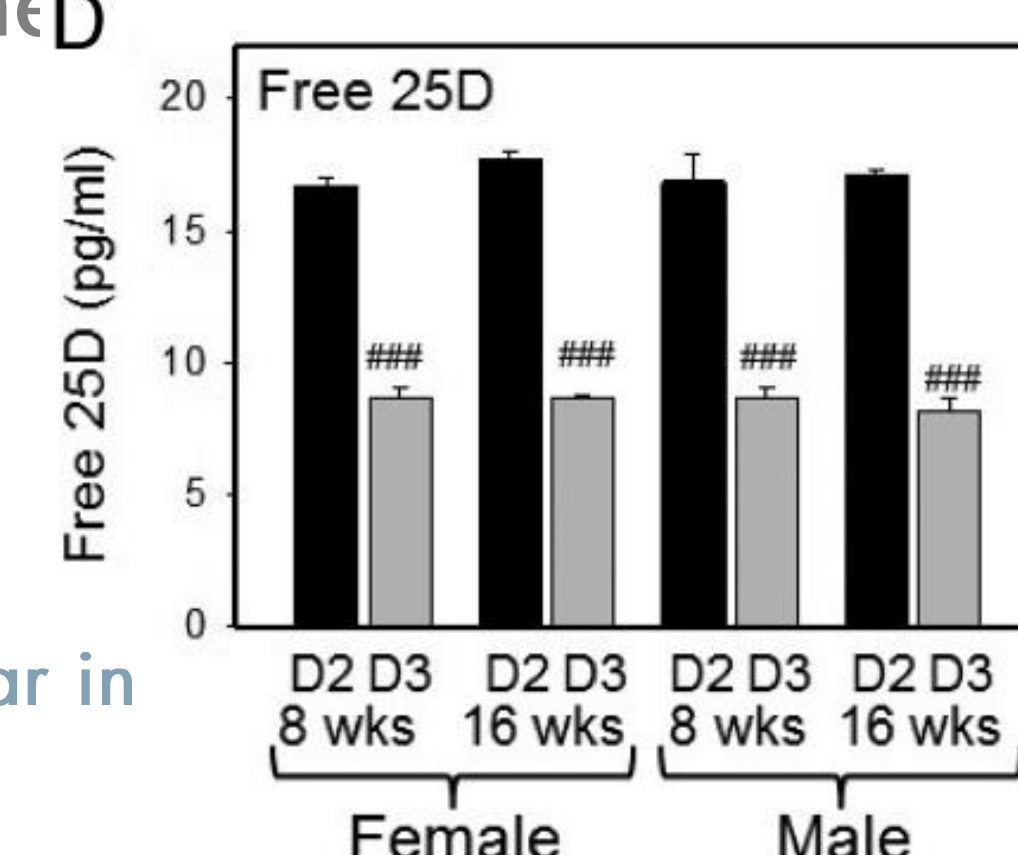
⊗ **Hypothesis:** Vitamin D2 promotes higher serum levels of free 25OHD, with different functional responses, relative to Vitamin D3.

⊗ **Method:** 3-week old male and female mice were placed on diet supplemented with either 1000 IU/kg Vitamin D2 or Vitamin D3, until euthanasia at wk8 or wk16 of age.

⊗ **Results and discussion:** The data support that 25OHD2 binds to DBP with lower affinity than 25OHD3, and therefore exhibits greater bioavailability. Serum free 25OHD concentrations were significantly higher

- Free 25OHD at 8 wks in
- D2 mice:  $16.75 \pm 0.65$  pg/ml
  - D3 mice:  $8.40 \pm 0.63$  pg/ml
- Free 25OHD at 16 wks in
- D2 mice:  $17.38 \pm 0.43$  pg/ml
  - D3 mice:  $8.43 \pm 0.44$  pg/ml

The concentration of total 25OHD were similar in both the D2 and D3 groups.



Chun R.F. *et al.* Endocrinology. 2016 Sep;157(9):3420-30. Differential Responses to Vitamin D2 and Vitamin D3 Are Associated With Variations in Free 25-Hydroxyvitamin D.

## > Vitamin D status amongst ethnical groups

⊗ **Hypothesis:** Free 25OHD is a better marker of Vitamin D status than total 25OHD amongst ethnical groups.

⊗ **Study 1:** Total 25OHD is lower in black than white Americans, free 25OHD is almost identical. The appropriateness of using free 25OHD to assess vitamin D status in African Americans merits further study.

⊗ **Study 2:** Irrespective of race, geographical region, or GC genotype, free 25OHD concentrations mirror total 25OHD concentrations.

⊗ **Discussion:** Despite using the same method for the measurement of free 25OHD, opposite results have been obtained. This field requires further study.

Bouillon R. *et al.* J. Clin. Endocrinol. Metab. 2016 May; 101(5): 2226–2234. Free 25-Hydroxyvitamin D: Impact of Vitamin D Binding Protein Assays on Racial-Genotypic Associations.

Aloia J. *et al.* J. Clin. Endocrinol. Metab. 2015 Sep;100(9):3356-63. Free 25(OH)D and the Vitamin D Paradox in African Americans.

## > Free Vitamin D during CKD

⊗ **Hypothesis:** Because Vitamin D binding is altered during CKD, it has been proposed that serum free 25OHD better reflects Vitamin D metabolism than total 25OHD.

⊗ **Method:** Serum free 25OHD and total 25OHD were measured in 34 CKD patients.

⊗ **Results and discussion:** The ratio free/total 25OHD strongly decreases with the decline of kidney function ( $r=0.55$   $p<0.001$ ). Free 25OHD may therefore represent a new target for treatment adaptation.

Bouchara A. *et al.* J. Am. Soc. Nephrol. 27: 2016 TH-PO510. Free vitamin D may be altered during chronic kidney disease.

> Other fields of interest include liver failure, pregnancy, obesity, bone metabolism, respiratory diseases, critical illness, and others