First

Unique

Automated

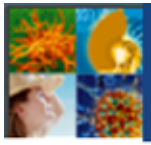
LIAISON®

1,25 Dihydroxyvitamin D

First fully automated, extraction free immunoassay for the accurate detection of 1,25 Dihydroxyvitamin D

DiaSorin

The Diagnostic Specialist



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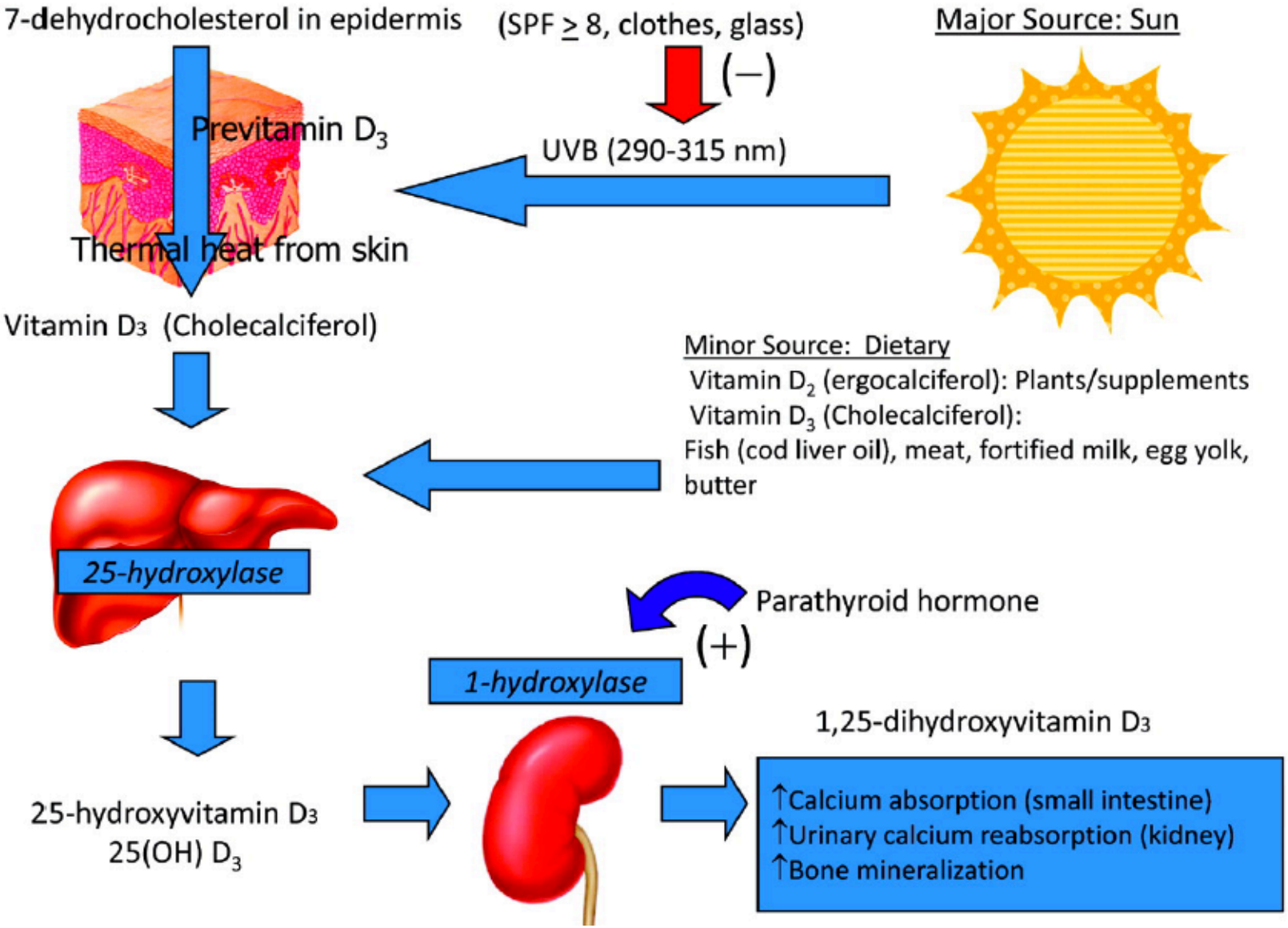
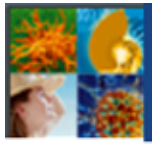
維生素D的合成

Synthesis of Vitamin D





Physiology of $1,25(OH)_2$ D

- skin , food ,liver, parathyroid gland, kidney, bone, and small intestine all play a role
- The major form of Vitamin D, 25 (OH) Vitamin D(Calcidiol), has a limited biological activity.
- $1,25(OH)_2$ Vitamin D(Calcitriol) is a biologically active form
- 1,25 (OH)2 vitamin D controls calcium homeostasis in body by targeting intestines and bones

Physiology of $1,25(OH)_2$ D

Targeting :

Intestine : 腸道： 增加腸道對鈣和磷酸鹽的吸收

increase absorption of calcium and phosphate from the intestine

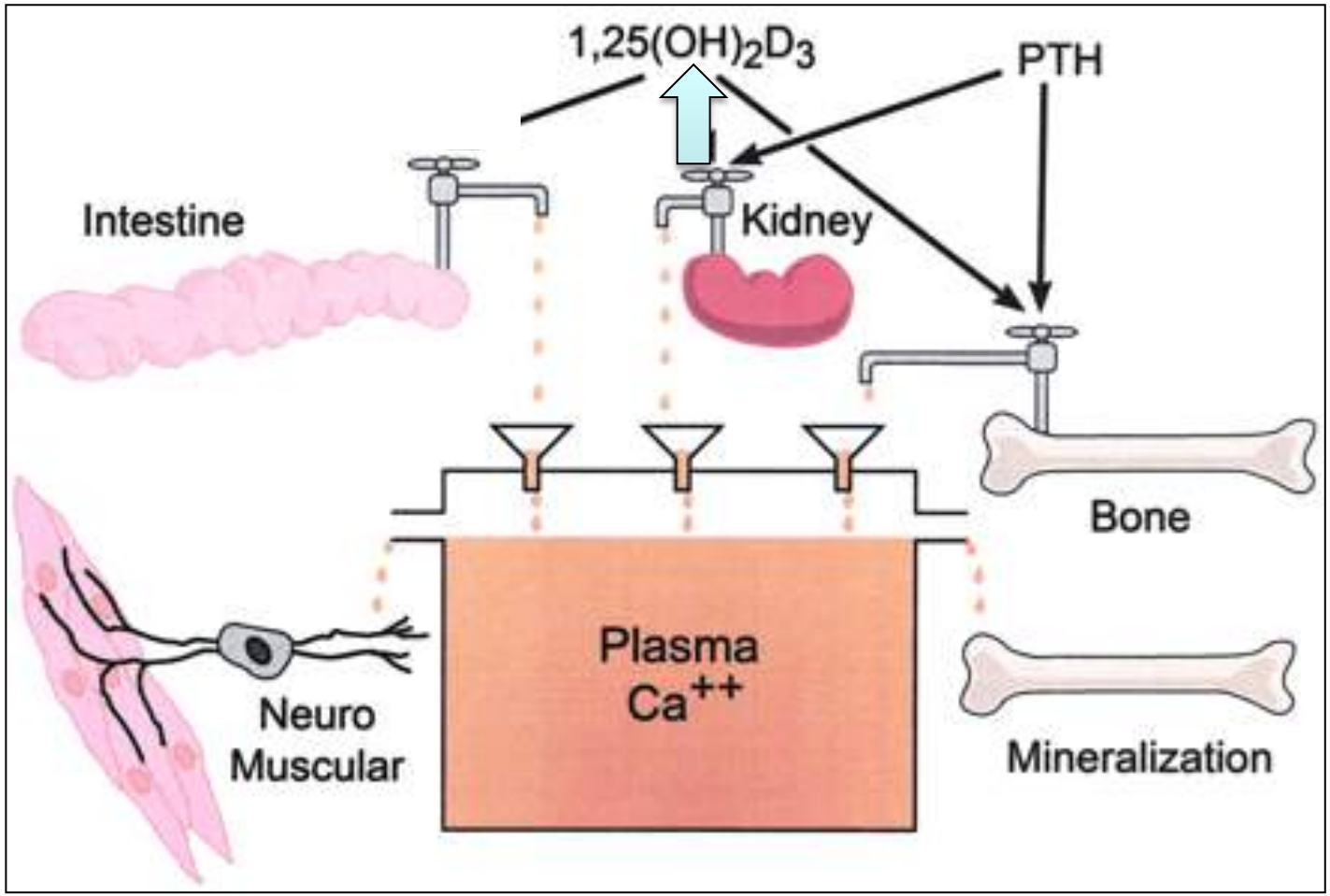
Bone : 骨頭： 增加鈣和磷酸鹽的骨吸收

increase bone resorption of calcium and phosphate

Regulation : 調節 : PTH功能可以增加血清鈣，但會減少血清磷酸鹽

recall PTH functions to increase serum calcium , but decrease serum phosphate

Physiology of 1,25(OH)$_2$ D



DiaSorin The Diagnostic Specialist

1,25(OH)$_2$ D regulates PTH secretion from the parathyroid gland through negative feedback control

Calcium homeostasis

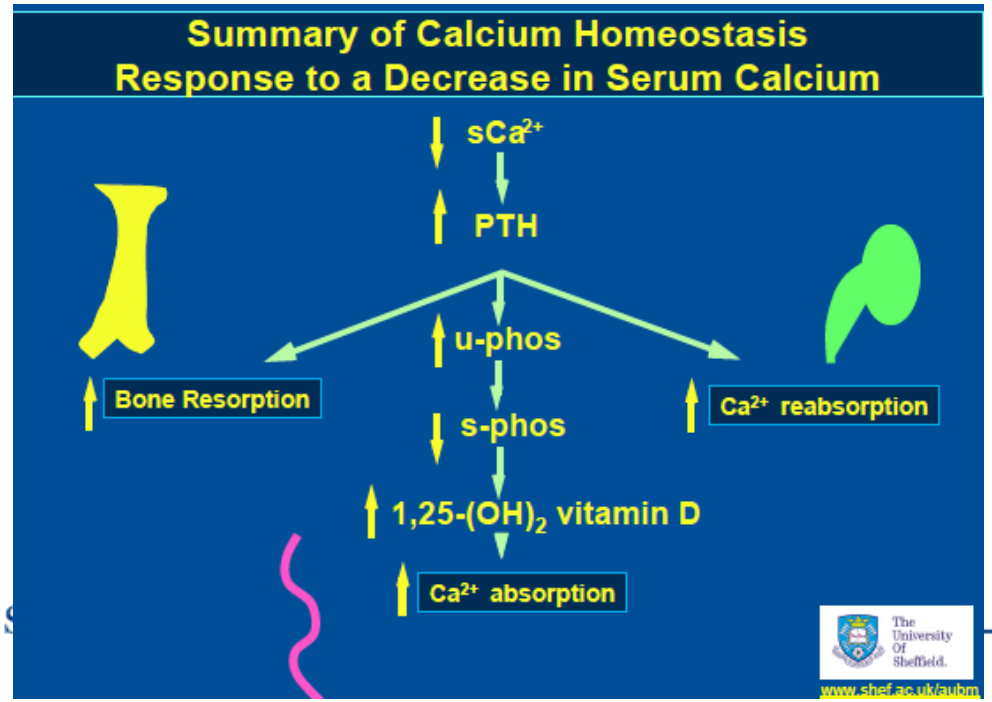
Parathyroid hormone (**PTH**)

- released by low plasma calcium
- stimulates bone resorption 刺激骨吸收
  (PTH receptor is on the osteoblasts which secretes IL-1 to activated osteoclasts)
- prevents calcium excretion by kidneys.
- stimulates calcitriol synthesis.

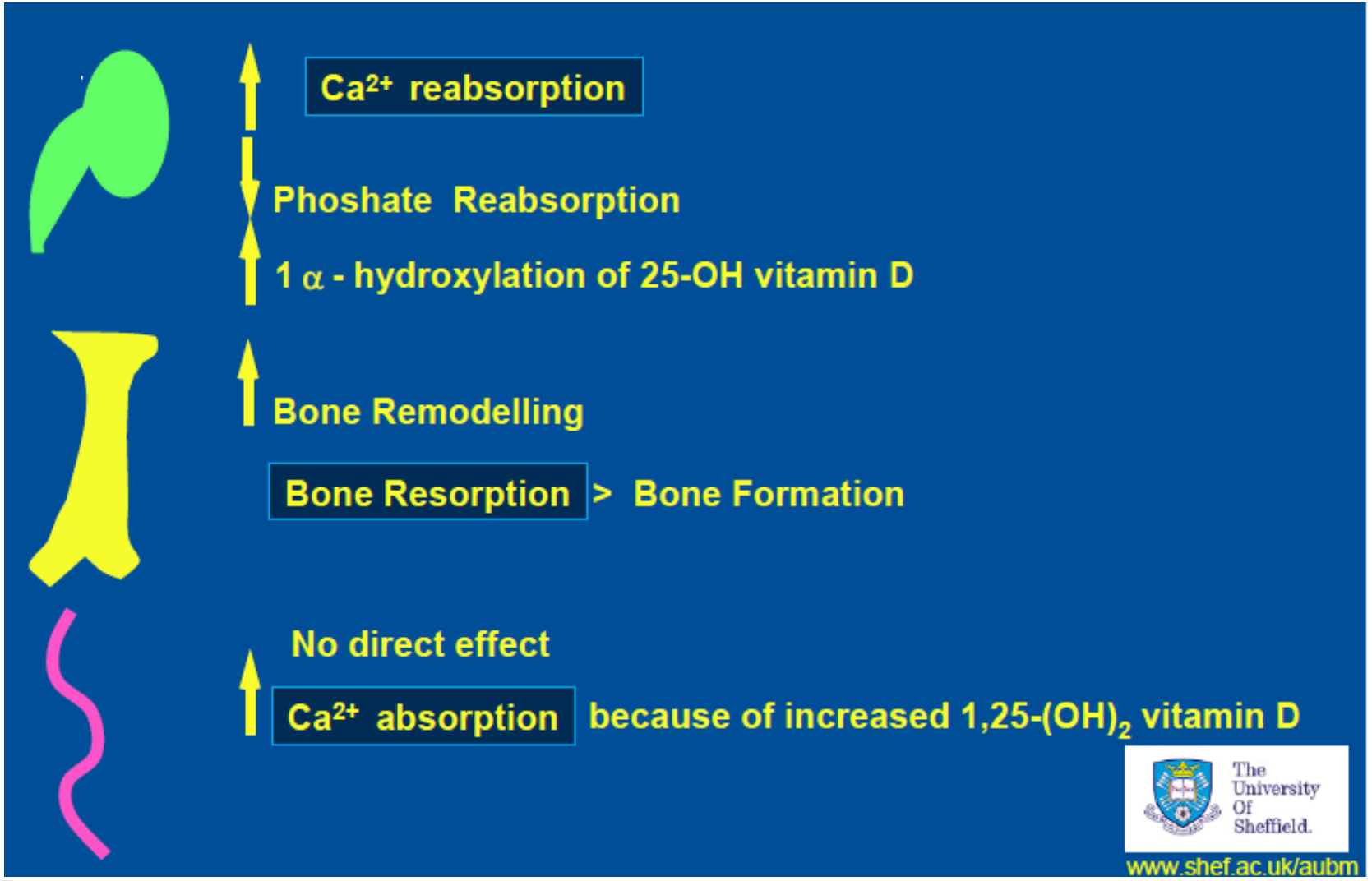
1,25-(OH)2-Vit. D (**Calcitriol**)

刺激骨吸收 - >骨形成

- stimulates bone resorption-> bone formation
- stimulates intestinal calcium absorption. 刺激腸道鈣吸收



Physiology of PTH



Phosphate homeostasis

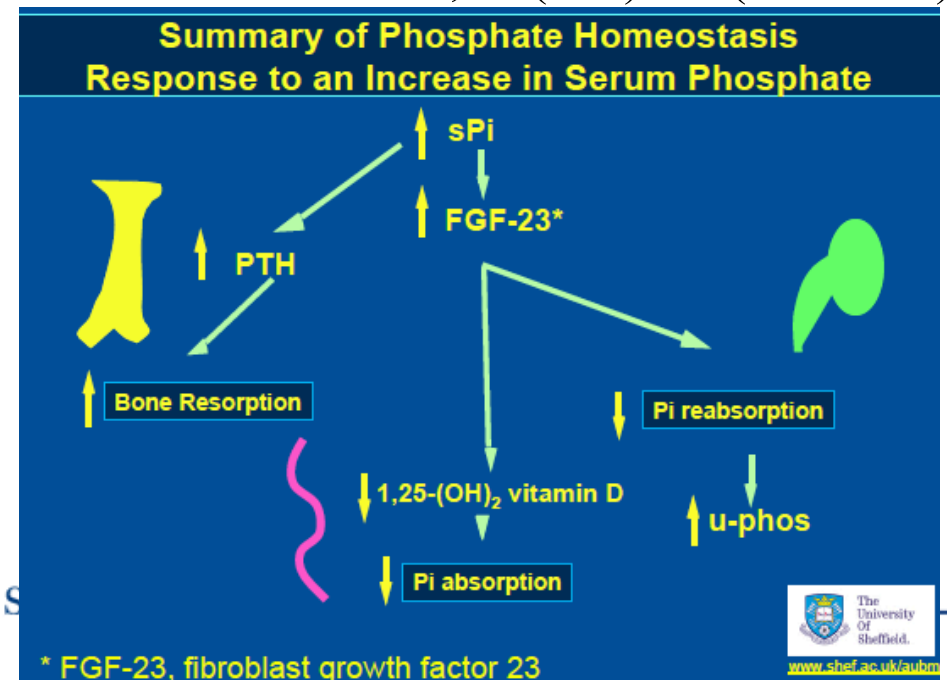
Parathyroid hormone (**PTH**)

- inhibits phosphate reabsorption in proximal tubular cell 抑制近端小管的磷酸鹽再吸收

Fibroblast growth factor 23 (**FGF23**)

LIAISON® FGF 23 (REF 318700)

- polypeptide , synthesized by the osteoblasts
- involved in the calcification of bone matrix
- acts on the kidney
- decreases serum inorganic phosphate by inhibiting renal phosphate reabsorption and 1,25.(OH)2 D (calcitriol) production



Chronic Kidney Disease (CKD)

FGF-23在慢性腎衰竭導致之續發性副甲狀腺機能亢進中所扮演的角色

內科學誌　2012：23：199-205

江銘彥　黃志強　呂宜樺　吳佳純　簡志強
甘偉志　王憲奕　鄭高珍[1]

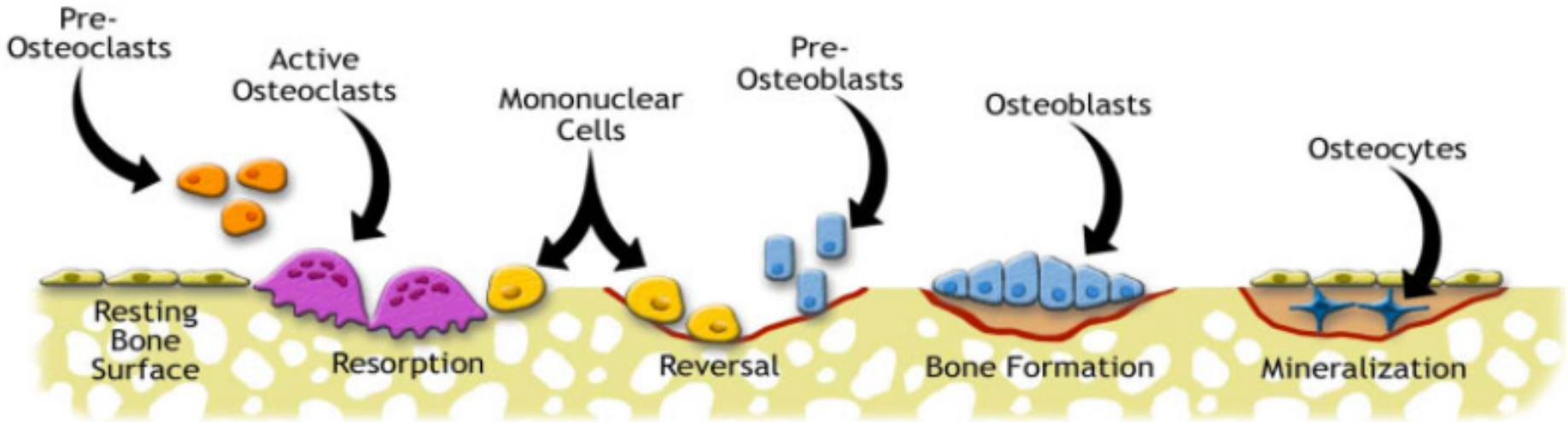
奇美醫療財團法人奇美醫院　[1]內科部　內科部腎臟科

Markers	Effect
PTH	✓decrease phosphate reabsorption 減少磷酸鹽再吸收 ✓increase calcium reabsorption 增加鈣再吸收
1,25 (OH)2 Vitamin D	✓increase intestinal absorption of calcium and phosphate ✓suppress PTH production 抑制PTH的產生
FGF-23	✓facilitate excretion of phosphate ✓promote calcitriol deficiency

增加腸道對鈣和磷酸鹽的吸收

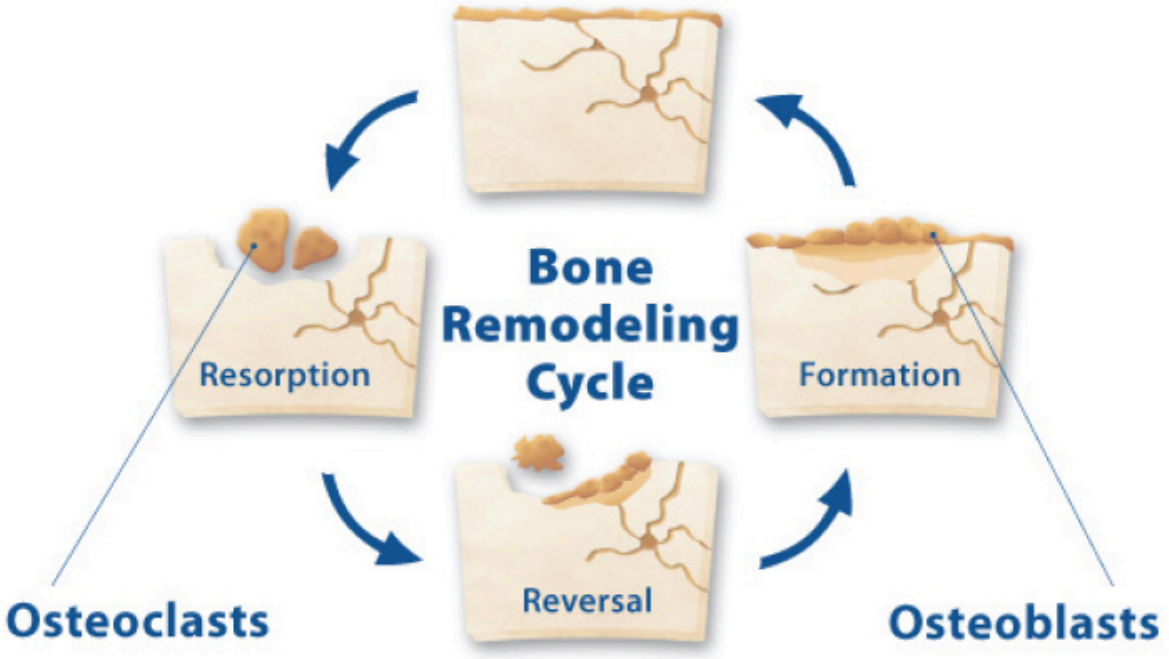
Bone Remodel

Bone resorption begins when **RANKL** on the Osteoblasts membrane activates the **RANK** protein on the cell membrane of the Pre-Osteoblasts



Bone Remodeling Cycle

Bone resorption and bone formation are
Not separated, Not independently regulated process.



Osteoclasts

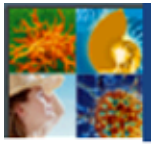
Bone Resorption

Bone resorption begins when osteoclasts remove a portion of the bone to be replaced later by the action of osteoblasts. This is a vital step for signaling bone formation.

Osteoblasts

Bone Formation

Osteoblasts lay down collagen and mineral deposits over the area previously remodeled by osteoclasts. Osteoblast activity is vital for maintaining bone mineral density and bone strength.



Metabolic bone disorders

- Normal bone
  - bone resorption or degradation is balanced by bone formation

- **Osteoporosis** (low bone mass and abnormal bone microarchitecture) 骨質疏鬆症（低骨量和異常的骨微結構）
  - the rate of resorption exceeds the rate of foemation

Causes:

high bone turnover / endocrine disorder(primary and secondary hyperparathyroidism) 高骨轉換/內分泌紊亂（原發性和繼發性甲狀旁腺功能亢進）
osteomalacia / renal failure / gastrointestinal disease(malabsorption syndrome) /
long-term corticosteroid therapy /multiple myoloma / cancer metastatic to the bones
骨軟化 /腎功能衰竭/ 胃腸疾病（吸收不良綜合徵）/ 長期皮質類固醇治療/ 多發性骨髓瘤/ 癌症轉移到骨骼

- **Paget's disease** (a condition of abnormal bone formation)

Causes:

excessive rates of bone remodeling , results in
local lesions of abnormal bone matrix which results in fractures or neurological involvement. 骨質重塑率過高，導致骨基質異常的局部病變導致骨折或神經系統疾病

Metabolic bone disorders

佝僂病（導致兒童骨骼軟弱或軟骨的病症）

- **Rickets** (a condition that results in weak or soft bones in children)
  - the most common cause is vitamin D deficiency

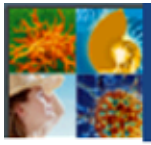
VDDR I :
a deficiency of the renal 25-hydroxyvitamin D (25(OH)D)-1 alpha-hydroxylase.

VDDR II :
a spectrum of intracellular vitamin D receptor (VDR) defects

低磷血症性佝僂病（X連鎖低磷血症性佝僂病）

- **Hypophosphatemic rickets** (X-linked hypophosphatemic rickets)
  - a form of rickets that is characterized by low serum phosphate levels and resistance to treatment with ultraviolet radiation or vitamin D ingestion

- circulating FGF-23 concentrations have been shown to be 5 times higher in XLH patients, resulting in significant phosphaturia.

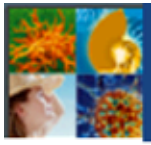


Metabolic bone disorders

Disorders caused by drug therapies:

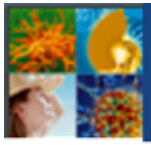
藥物治療引起的疾病

- immunosuppressive drugs for treating cancer and organ transplants
- heparin, used in kidney dialysis
- phenytoin (Dilantin.) for epilepsy
  (phenobarbital / rifampicin which induce hepatic P450 enzyme to accelerate the catabolism of Vitamin D)
- glucocorticoids (corticosteroids) for rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and asthma
- aluminium-containing antacids



Drug therapies for metabolic bone diseases

Anti-resorptive agents:	Formation stimulating agents:	Agents inhibiting resorption and stimulating formation:
Estrogen (hormone replacement)	Sodium fluoride	Strontium ranelate / Inhibits bone resorpti
Phytoestrogen (hormone replacement)	Parathyroid hormone (human recombinant PTH (1-34))	PTH initially stimulates bone formation and later increases bo remodeling; increases spinal BMD. Suggested for treatment patients with persistent osteoporosis after prior alendronate treatment. (Teriparatide (Forteo))
Calcium	Growth factors	Growth hormone therapy is used (and FDA approved) in the treatment of hypo-pituitarism and somatotropin deficiency c children and adults.
Selective estrogen receptor modulators		
Bisphosphonates	Prevent bone loss and increase BMD ((Alendronate (Fosamax); Risedronate (Actonel)). Rigid administration is a disadvantage.	
Calcitonin	Treatment of osteoporosis and Paget's disease, considered not as effective as bisphosphonates. Decreased tolerance with long-term use.	
Vitamin D	Active form of vitamin D given to post-menopausal women who have osteoporosis in the spine.	



Clinical background of 1,25 Dihydroxyvitamin D

1,25 $(OH)_2$ D is the **active form** of Vitamin D, its production is tightly regulated through concentration of serum calcium, phosphorus and PTH.

- Low levels can be found in CKD, Vit D dependant rickets type 1, hypophosphatemic rickets, hypoparathyroidism

- High levels in Vit D dependant rickets type 2, Sarcoidosis, RA, IBD, primary hyperparathyroidism

Challenges in 1,25 Dihydroxyvitamin D measurement

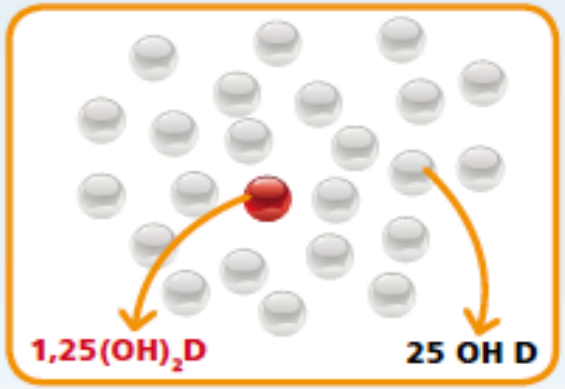
Until now, all assays required a long, manual, operator dependent pre-analytical step due to the following facts:

- The molecule circulates in **low amounts**

  The blood levels of 1,25(OH)2 D being 100 to 1000 less than 25 OH D.
  (pg/mL concentration vs ng/mL concentrations)

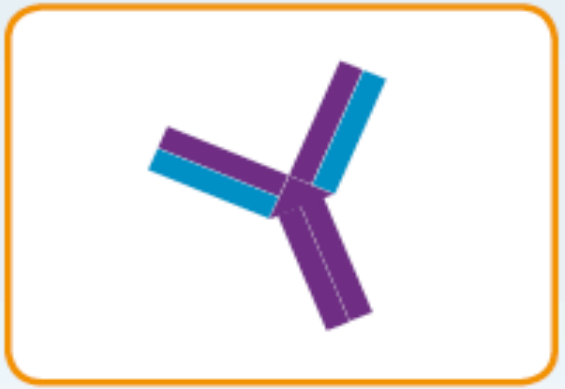
- **Similarity with** its metabolic precursor, **25-OH Vitamin D**

Novel Assay format

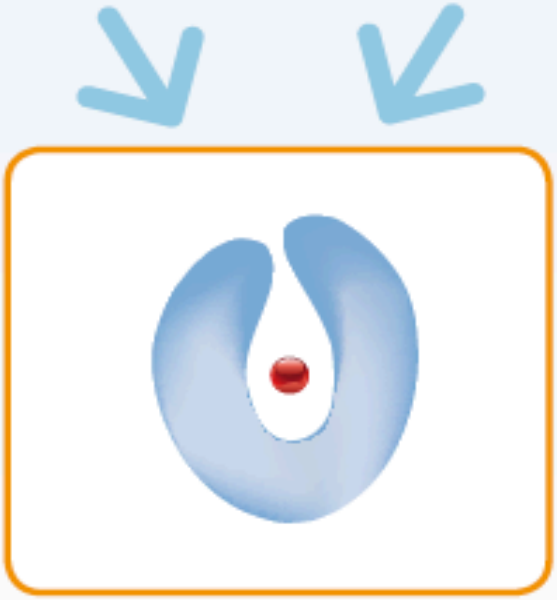


Concentrations of ***1,25(OH)$_2$D*** are normally about 1000-fold lower than the precursor compound 25(OH)D

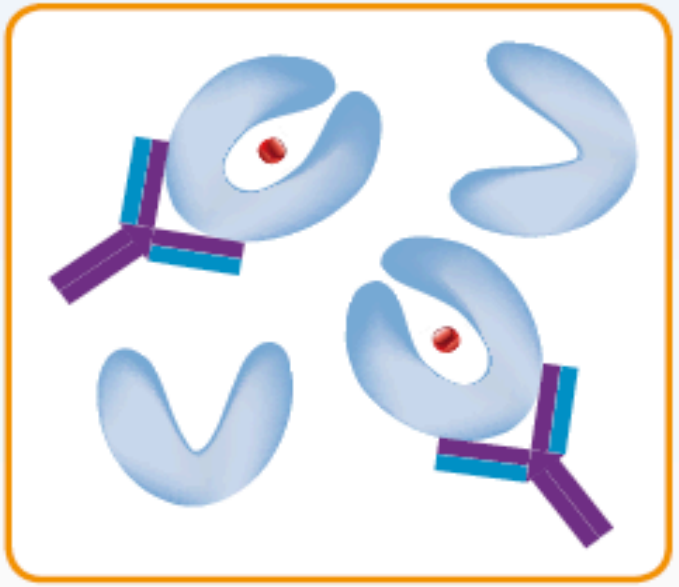
Recombinant Fusion Protein **(RFP)**



Specific murine monoclonal antibody ***(MAB)*** which only recognizes the RFP Complex



RFP changes conformation after capturing 1,25(OH)$_2$D and forms the ***RFP Complex***



RFP Complex is selectively recognized by the MAB

LIAISON® XL 1,25 Dihydroxyvitamin D – A paper

DE GRUYTER Clin Chem Lab Med 2017; aop

Katharina Spanaus* and Arnold von Eckardstein

Evaluation of two fully automated immunoassay based tests for the measurement of 1α,25-dihydroxyvitamin D in human serum and comparison with LC-MS/MS

DOI 10.1515/cclm-2016-1074
Received November 25, 2016; accepted January 10, 2017

Key messages

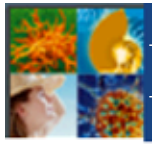
PRECISION The DiaSorin test is very precise: total imprecision between 3.1 and 5.2%

DiaSorin test measured 1,25$(OH)_2$ vitD with high accuracy. **ACCURACY**

LC-MS/MS CORRELATION The DiaSorin measurement results showed stronger correlations with the LC-MS/MS results ($r = 0.852$ vs. $r = 0.967$).

Nearly complete cross-reactivity with 1,25$(OH)_2$ Vit.D2. **VIT.D2 & D3 MEASUREMENT**

Due to its high sensitivity, low imprecision, broad measurement range, and good agreement with LC-MS/MS, the DiaSorin test is a valuable analytical option for the determination of 1,25$(OH)_2$ Vit.D.



LIAISON® XL 1,25 Dihydroxyvitamin D – Benefits

- First fully automated, extraction free
- First result in just 65 minutes
- Low sample volume (75 µL)
- More test from the same patient tube (eg 25-OH Vitamin D, PTH)

Bone and Mineral panel

The LIAISON® Bone & Mineral panel also includes:

LIAISON® 25 OH Vitamin D TOTAL Assay (Code 310600)
LIAISON® 1-84 PTH (Code 310630)
LIAISON® N-TACT® PTH Gen II (Code 317910)
LIAISON® BAP OSTASE® (Code 310970)
LIAISON® Osteocalcin (Code 310950)

LIAISON® XL BAP OSTASE

骨特異性鹼性磷酸酶（BAP）

- **B**one-specific **a**lkaline **p**hosphatase (**BAP**) , a glycoprotein that is found on the surface of osteoblasts.

- Reflects the biosynthetic activity of these bone-forming cell.

  反映骨形成細胞的生物合成活性

- Has shown to be a sensitive and reliable indicator of bone metabolism.

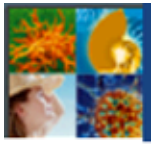
  已被證明是一種敏感而可靠的骨代謝指標

LIAISON® XL BAP OSTASE

- Increased serum levels of BAP : BAP 上升
  (in conditions characterized by excessive bone turnover)
  - postmenopausal women/ osteoporosis/ Paget's disease/ thyrotoxicosis / hyperparathyroidism / metastatic cancer, and are associated with rapid bone loss
  停經後婦女/ 骨質疏鬆症/ 佩吉特病/ 甲狀腺毒症/ 甲狀旁腺功能亢進

- BAP levels decrease following anti-resorptive therapy in a dose-dependent manner. BAP 下降 抗癲癇治療

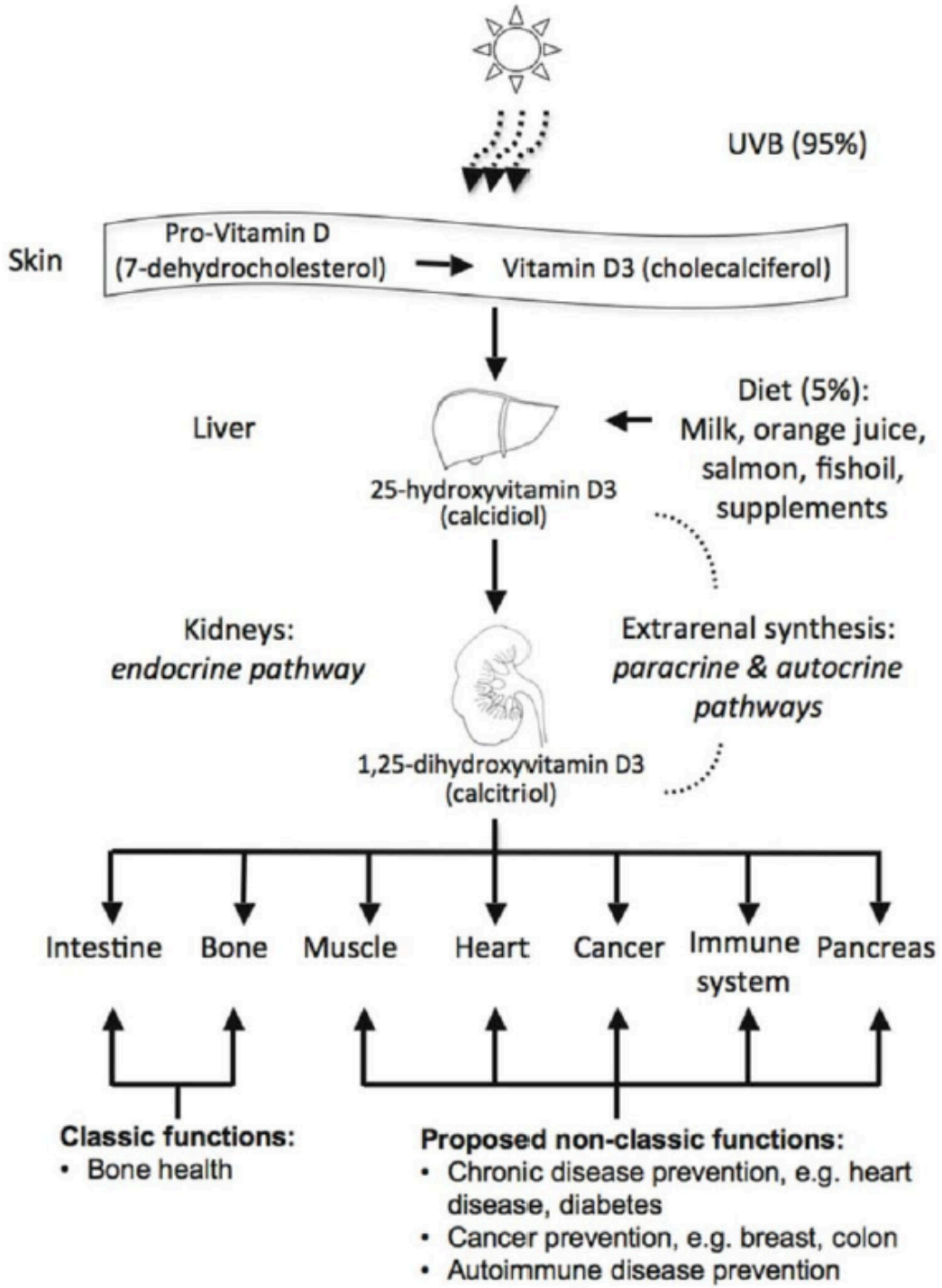
- BAP identifies rapid bone losers, and accurately monitors the efficacy of hormone replacement-, bisphosphonate-, PTH analogue- and growth hormone-therapies



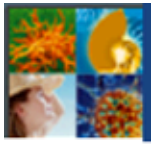
Advantage of Bone turnover marker

- To rapidly identify therapy responders and non-responders
  (detectable and significant changes in bone mineral density (BMD) take 18 to 24 months to develop, bone turnover marker takes 3-6 months after starting anti-resorptive therapy)
- To assess therapy efficacy and to determine the optimal therapy and dose of treatment.
- Biochemical bone marker reflect the whole-body rates of bone turnover, the combined measurement of bone marker and BMD provides more information on overall bone loss than BMD measurement at specific skeletal sites alone.

VDR



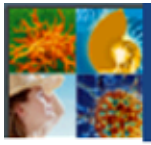
The cellular effect of vitamin D is mediated through the intra-nuclear vitamin D receptor (**VDR***)*



Pandemic of Vitamin D deficiency

Vitamin D deficiency related diseases : 維生素D缺乏相關疾病

- Rickets in Children 兒童佝僂病
- Osteoporosis, Osteomalacia 骨質疏鬆症
- Cancer
- Type II Diabetes 第II型糖尿病
- Cardiovascular disease 心血管疾病
- Auto Immune Diseases 自體免疫疾病
- Parkinson's disease 帕金森氏病
- *Reproductive system* 生殖系統

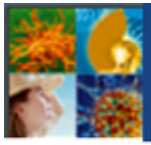


Vitamin D and Reproductive system

- In the last few years , many researchers have studied the association of Vitamin D and reproductive health but there is still **no single consensus** on its influence in reproductive health.

- While it is s general observation that **optimal** level of Vitamin D is essential in **PCOS**, **Endometriosis**, **Male infertility** and **IVF technique.**

- but there has been no significant correlation between Vitamin D level and ovulation stimulation or embryo development.



Vitamin D and Female Reproduction

PCOS

(Polycystic ovary syndrome)

多囊卵巢症候群

- Inverse association of serum Vit D and circulating androgens and insulin resistance in women with PCOS.

- Vit D supplementation improves menstrual frequency and metabolic syndromes. 維生素D補充可改善月經頻率和代謝症候群。

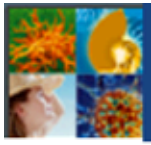
PCOS: 高雄激素 多毛症 卵巢和月經不調 胰島素抵抗
hyper-androgenism / hirsutism / ovalatory and menstrual irregulations /insulin resistence / low pregnancy success rate / obesity / elevated cardiovascular disease risk
低妊娠成功率 肥胖 心血管疾病風險升高

Endometriosis

子宮内膜異位症

- Higher 25(OH)D levels in women with endometriosis than control.

  - extrarenal site of Vit D synthesis and action : endometrial tissue
  - similar VDR polymorphism genotype



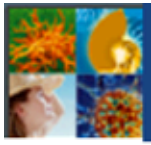
Vitamin D and Male Reproduction

Male infertility 男性不育

- Vitamin D metabolism enzymes (CYP24A1) are describes in the human testis, the ejaculatory tract , mature spermatozoa and in the Leydig cells.

- Observed significantly reduced CYP24A1-expressing spermatozoa in the subfertile man compared with the healthy group.($P<0.001$)

- Man with Vit D deficiency displayed a lower percentage of motile and morphologically normal sperm compared with Vit D sufficient subjects. 維生素D缺乏的人 精子表現出較低的運動百分比和正常的精子形態



Vitamin D and Female Reproduction

IVF

(In Vitro Fertilization) 體外受精

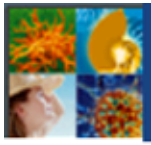
- High 25(OH)D levels are associated with higher clinical pregnancy rate.
- No significant difference.
- High follicular fluid 25(OH)D levels : lower clinical pregnancy rate.

Vitamin D and Female Reproduction

AMH 抗穆勒氏管荷爾蒙

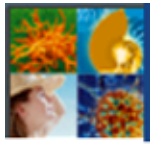
- Premenopausal women where divided in 3 groups: age < 35 , age 35-39 , age > 40. For the youngest women AMH was negatively correlated with Vit D, whereas for the oldest women the relationship was reversed. The mean age at which the relationship was reversed was 35.

- AMH levels exhibited seasonal variation in women , with an 18% decrease in AMH levels in winter compared with summer. Vit D prevented seasonal AMH change. Vit D may be a positive regulator of AMH production in adults.



Vitamin D and Reproductive system

Large studies including all ethnic and racial groups would be required to proclaim the role of Vitamin D in infertility .



Thank you for tour attention