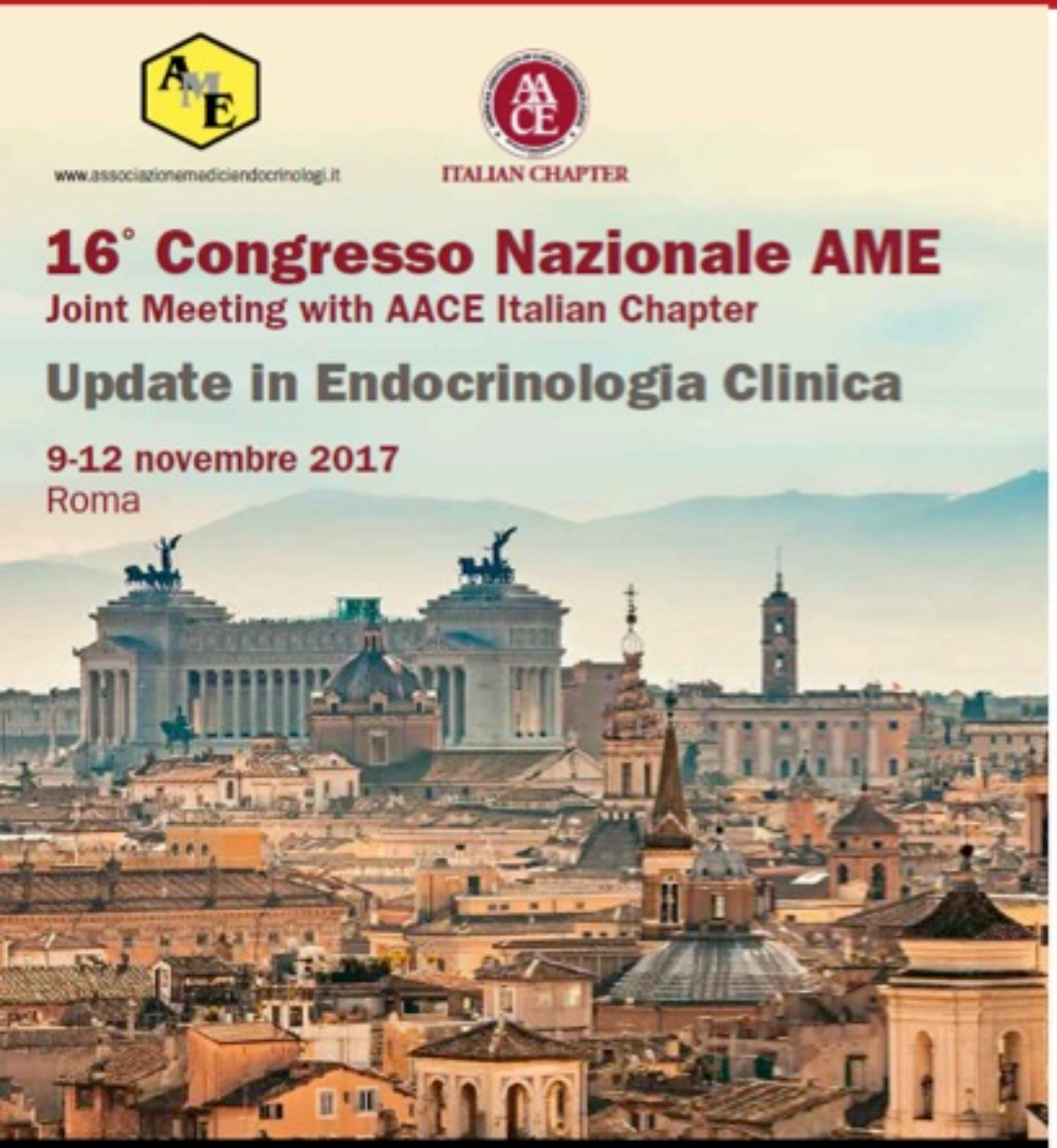
UNIVERSITÀ DEGLI STUDI DI PADOVA

DIPARTIMENTO DI MEDICINA
UOC Andrologia e Medicina della Riproduzione
Centro Regionale Specializzato di Crioconservazione dei Gameti Maschili
Centro Regionale Specializzato per la sindrome di Klinefelter
Direttore: Prof. Carlo Foresta



Vitamina D e obesità

Prof. Carlo Foresta

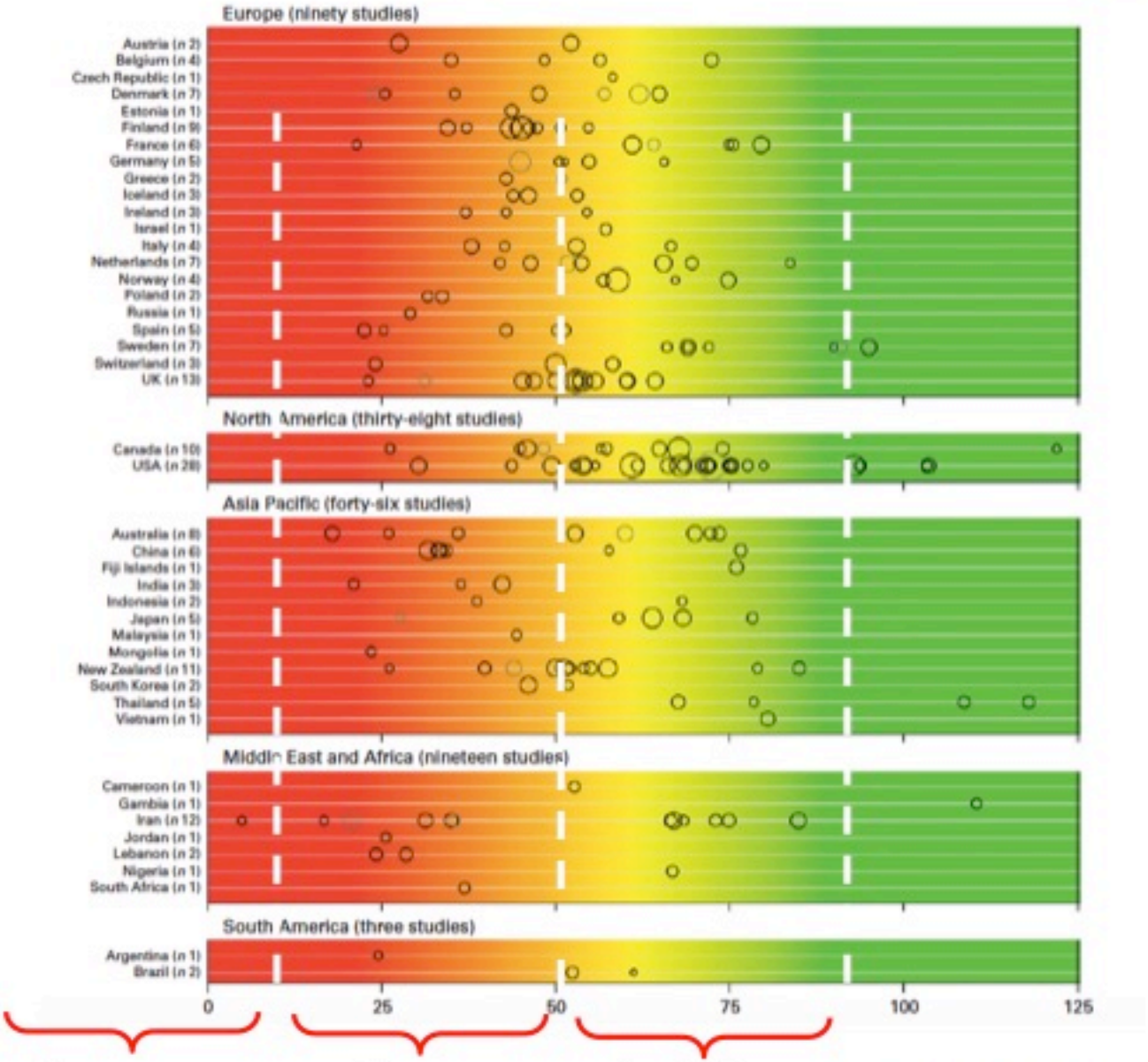
carlo.foresta@unipd.it

Prevalence of low vitamin D status worldwide

- Prevalence of low vitamin D status is a global problem in all age groups, even in countries with sun exposure all year round
- The problem is greater in the Middle East, particularly in girls and women
- There is a striking, and in most countries of South America and Africa
- Season appears to be a small component to the problem worldwide, as countries with long winters have less deficiency rates overall compared to sunny countries

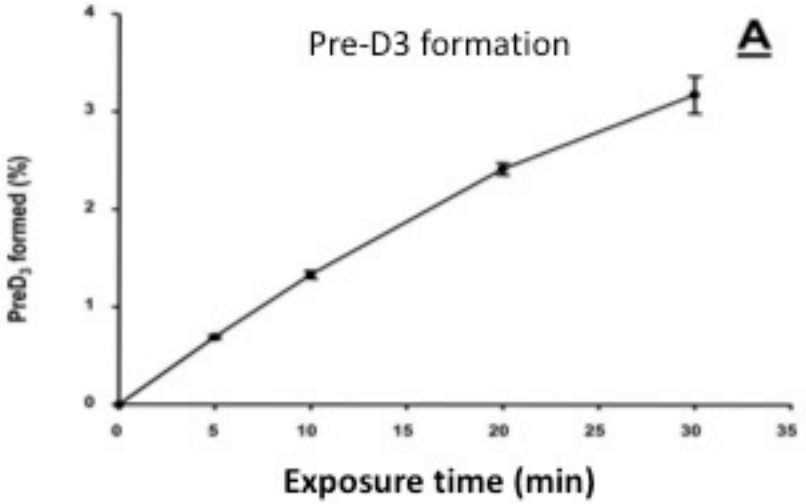
Palacios C et al. J Steroid Biochem Mol Biol (2013)

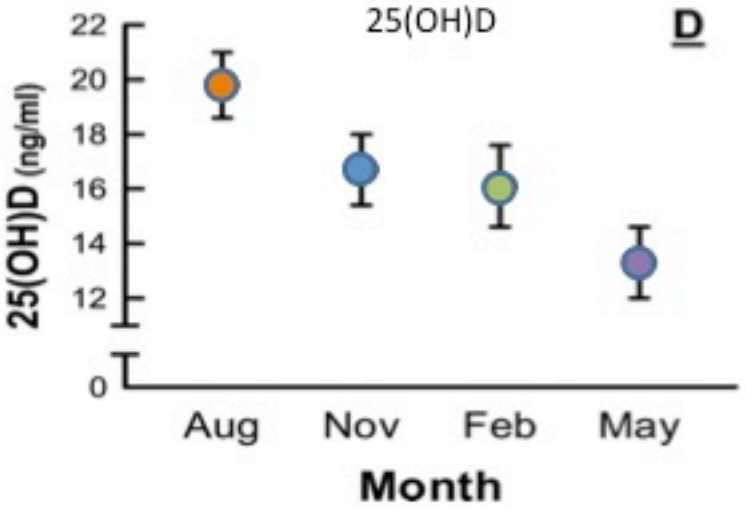
Systematic review (inclusive of *at risk* groups)

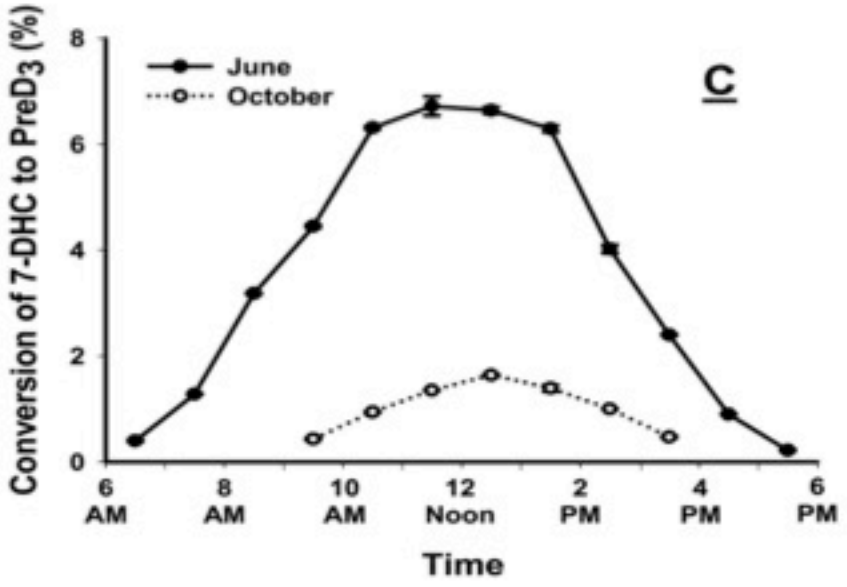


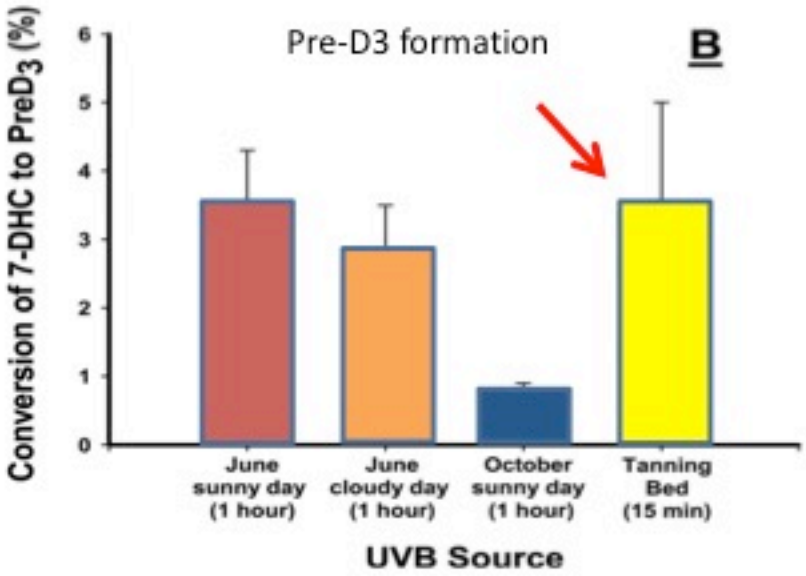
Hilger J et al. Br J Nutr 111:23-45 (2014)

Vitamin D synthesis and sun exposure

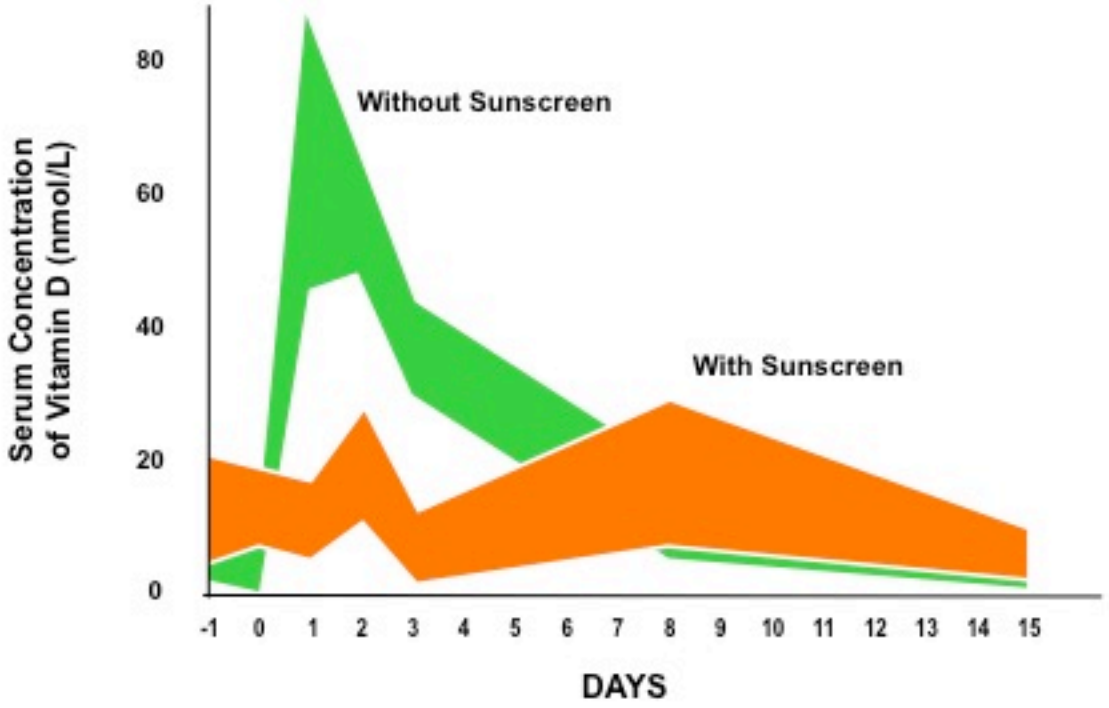






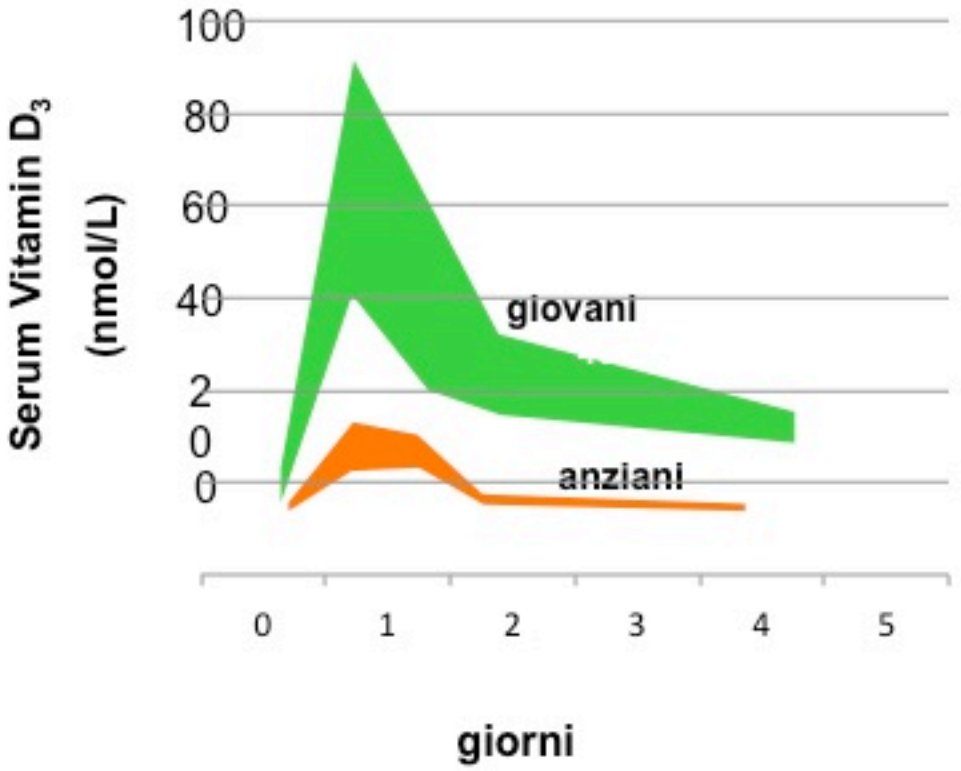


Circulating concentrations of vitamin D_3 in response to a whole-body exposure



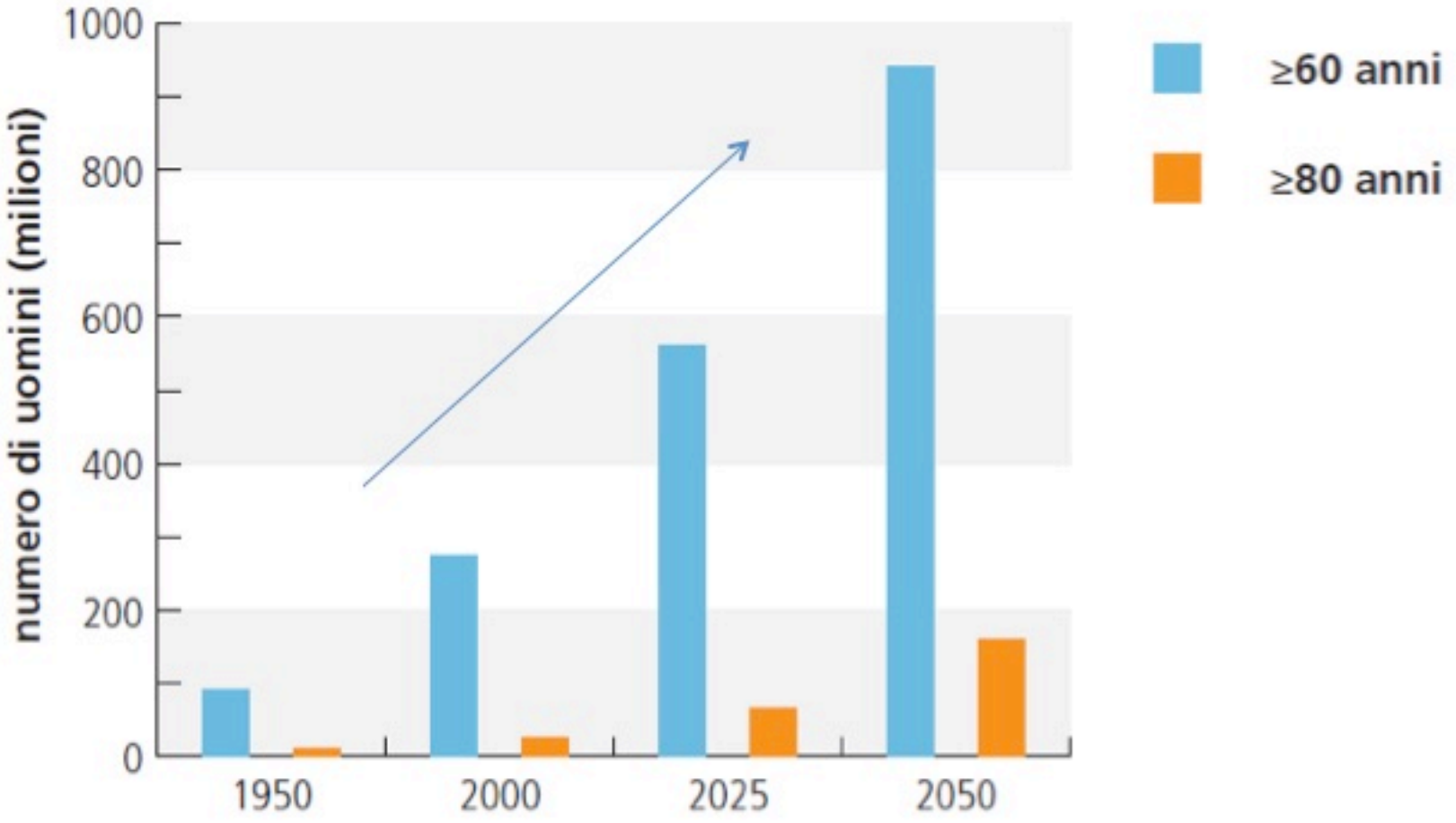
Holick MF et al., Am J Clin Nutr 1994

Concentrazioni sieriche di vitamina D_3 in risposta alla esposizione (whole-body) solare



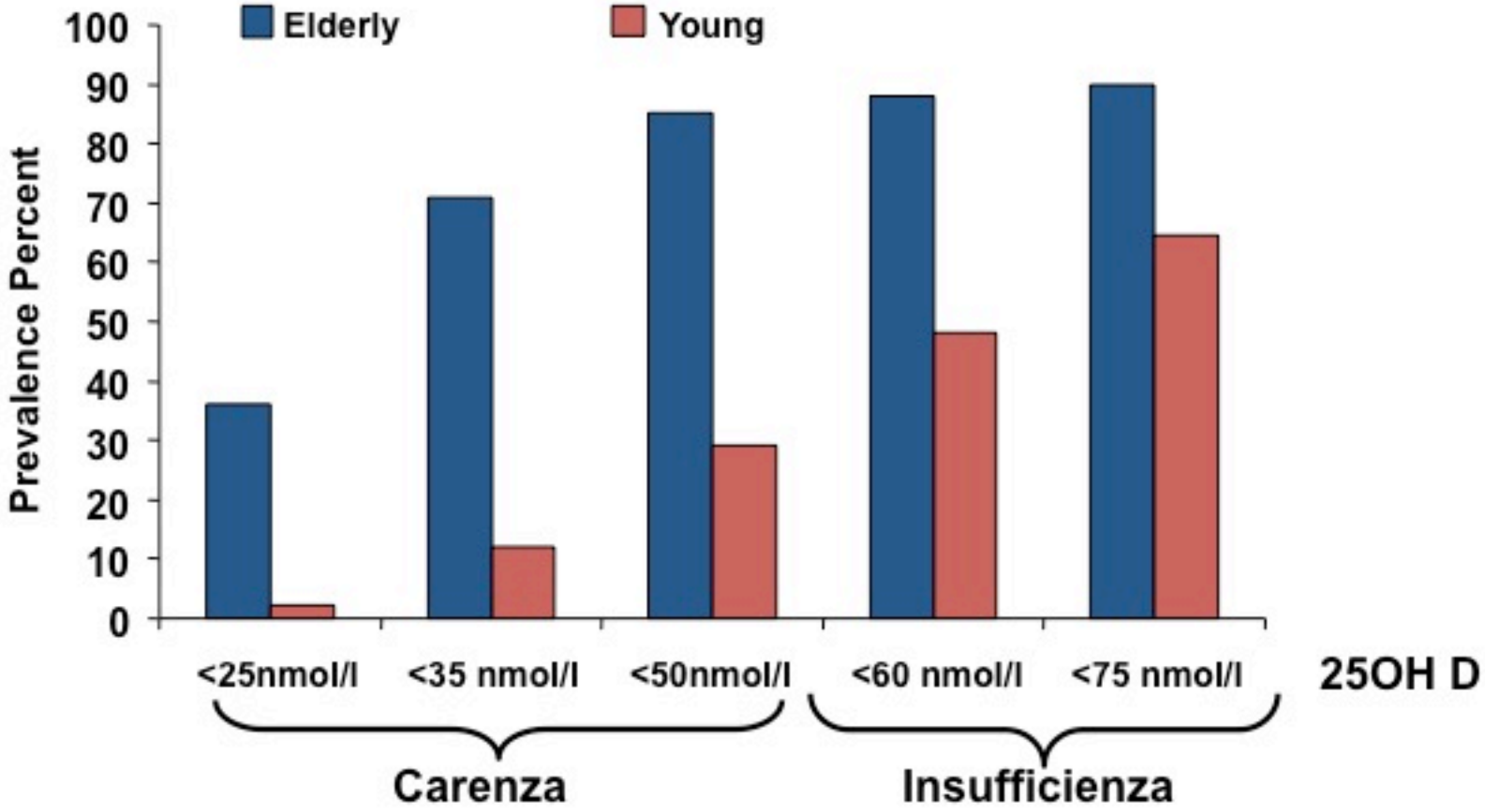
Holick MF et al, Am J Clin Nutr 1994

L'invecchiamento della popolazione maschile mondiale 1950-2050



What about Italy?

Prevalence of Vitamin D inadequacy in Italy

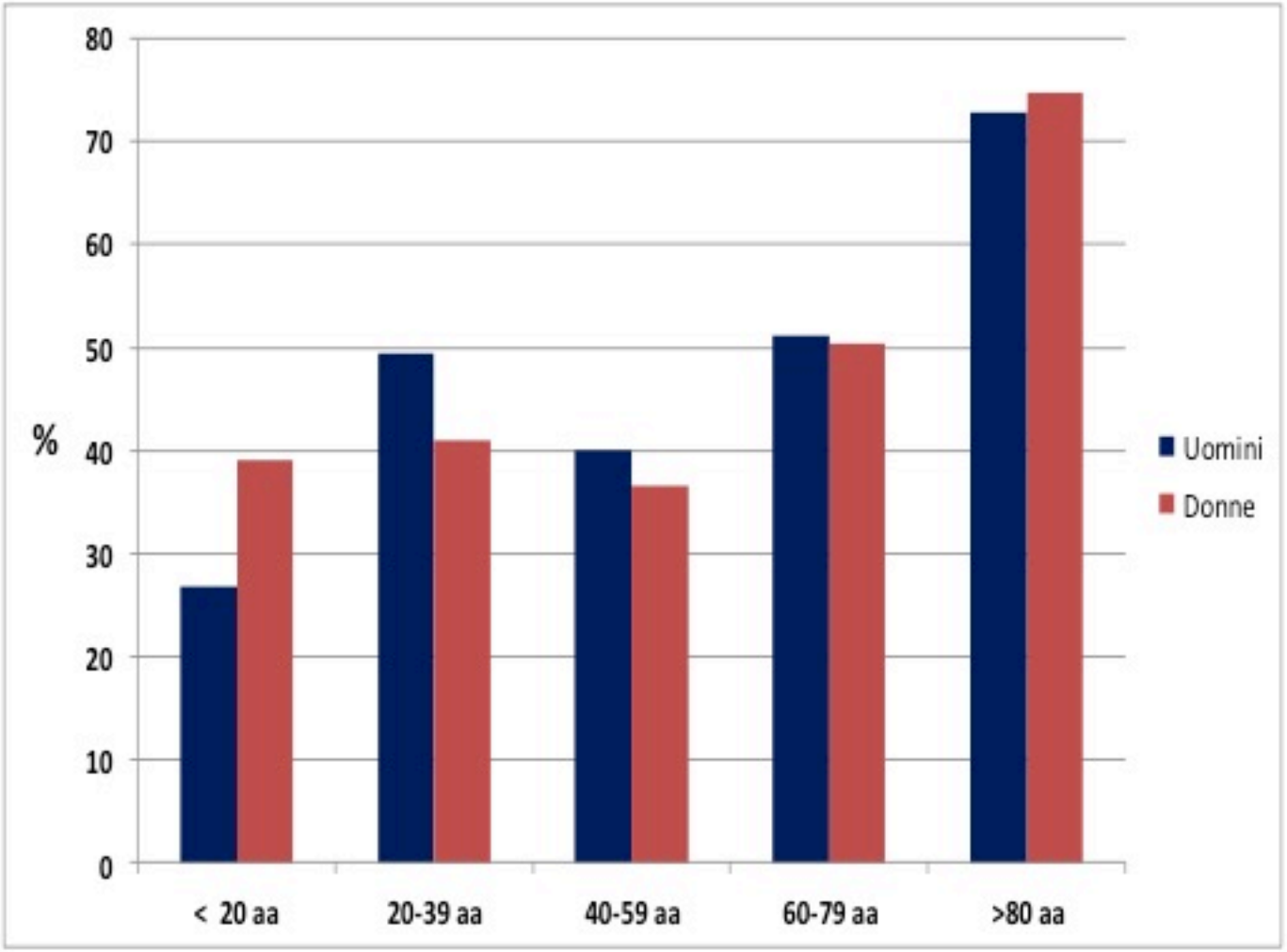


Severe Vit. D deficiency is found in > 50% of elderly subjects; inadequate levels in > 95%. Inadequate Vit D values are found in > 50% of young "healthy" subjects.

Isaia GC et al Osteoporos Int 2003
Adami S et al Bone 2009
Adami S et al Bone 2008

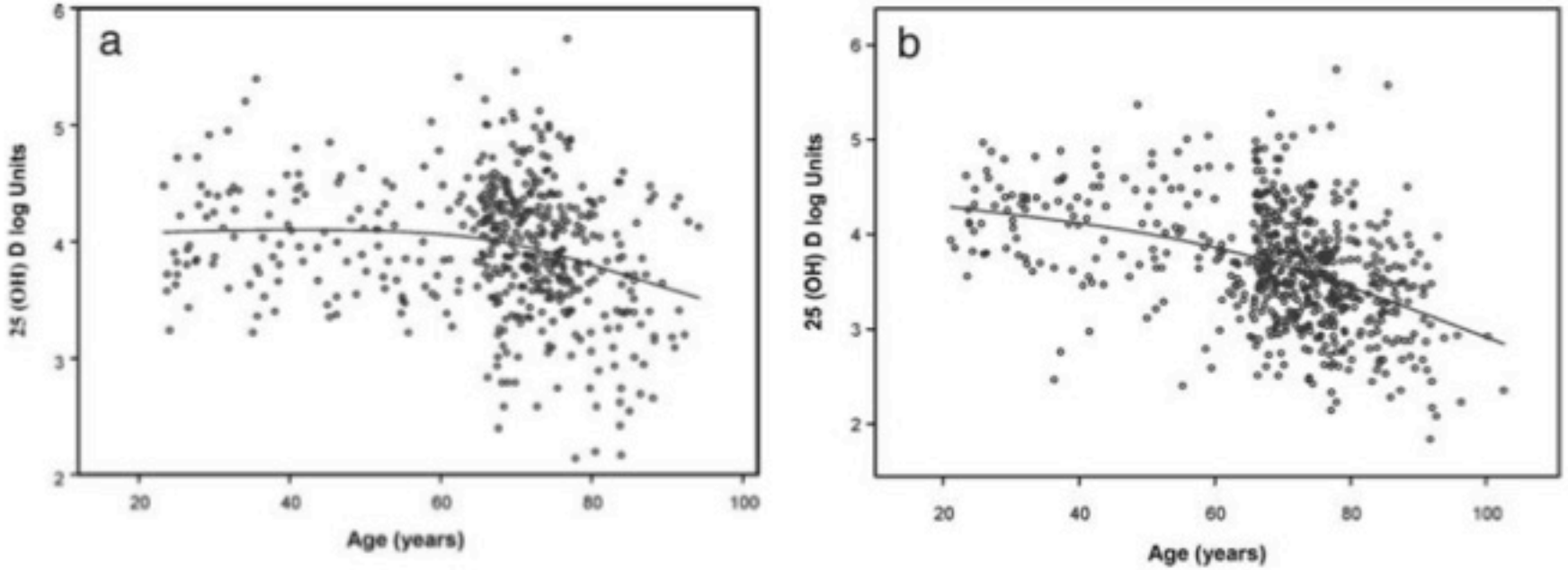
Prevalenza di ipovitaminosi D in Italia

6403 soggetti (1299 M, 5104 F); età 6 mesi-103 anni



Cigolini M et al, Clin Endocrinol, 2006

Hypovitaminosis D in Italy
InCHIANTI study

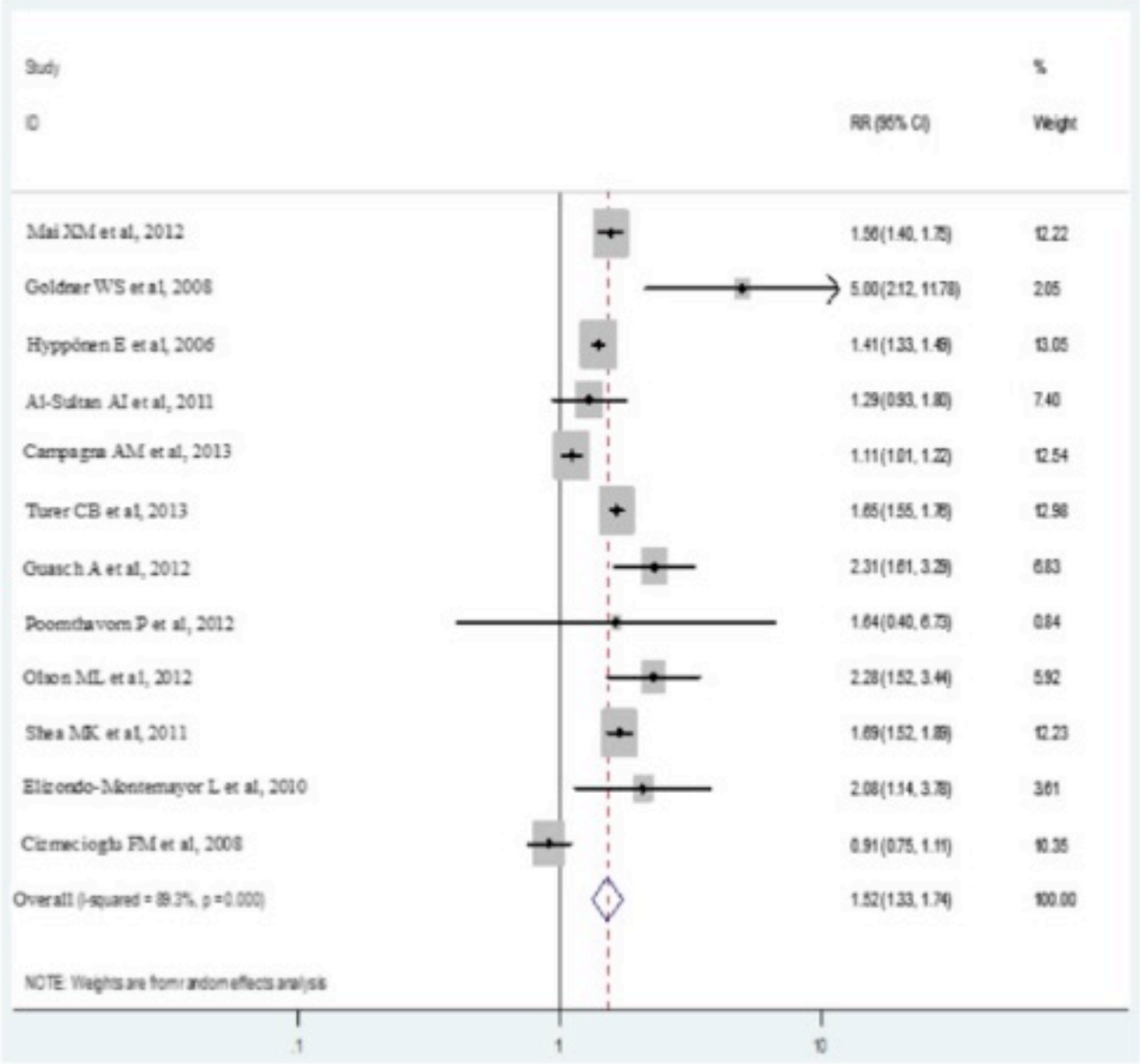


Maggio D. et al. J Genrontol A Biol Sci Med Sci 60:1414-1419. (2012)

Association between obesity and low vitamin D levels

- BMI > 30 kg/m^2
- 25(OH)D < 20 ng/ml (50 nmol/l)

In conclusion, our meta-analysis reports an inverse association between obesity and low vitamin D levels.

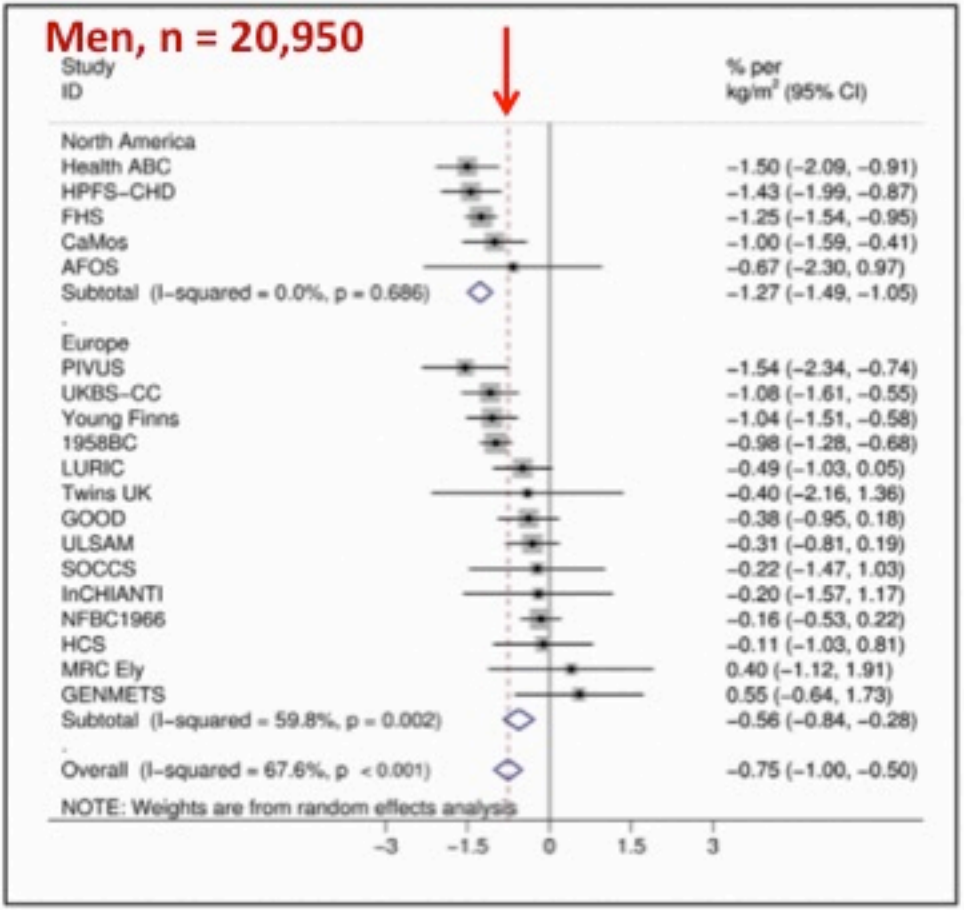


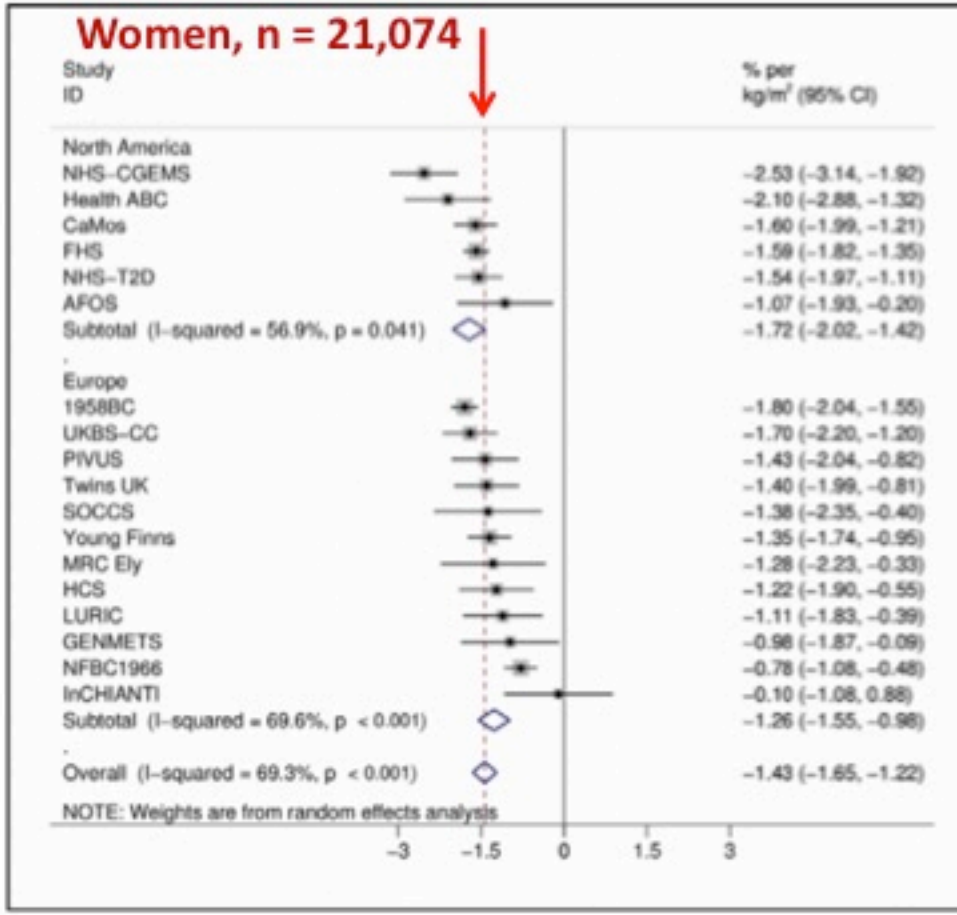
Thurkaa Shanmugalingam et al., BMC Cancer. 2014; 14(1): 712.

OPEN ACCESS Freely available online February 2013 | Volume 10 | Issue 2 | PLOS | MEDICINE

Causal Relationship between Obesity and Vitamin D Status: Bi-Directional Mendelian Randomization Analysis of Multiple Cohorts

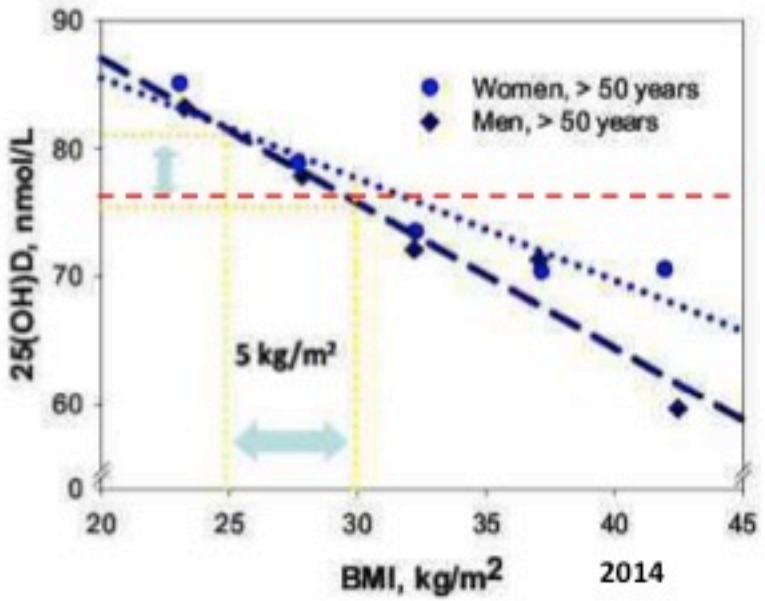
"These findings provide evidence for obesity as a causal factor in the development of vitamin D deficiency, but not for vitamin D deficiency as a causal factor in the development of obesity.»





Lagunova et al. Mol. Nutr. Food Res. 2010

Decay in serum 25(OH)D levels with increasing BMI



25 (OH)D decrease per each 5 kg/m² BMI increase

50 ng/ml

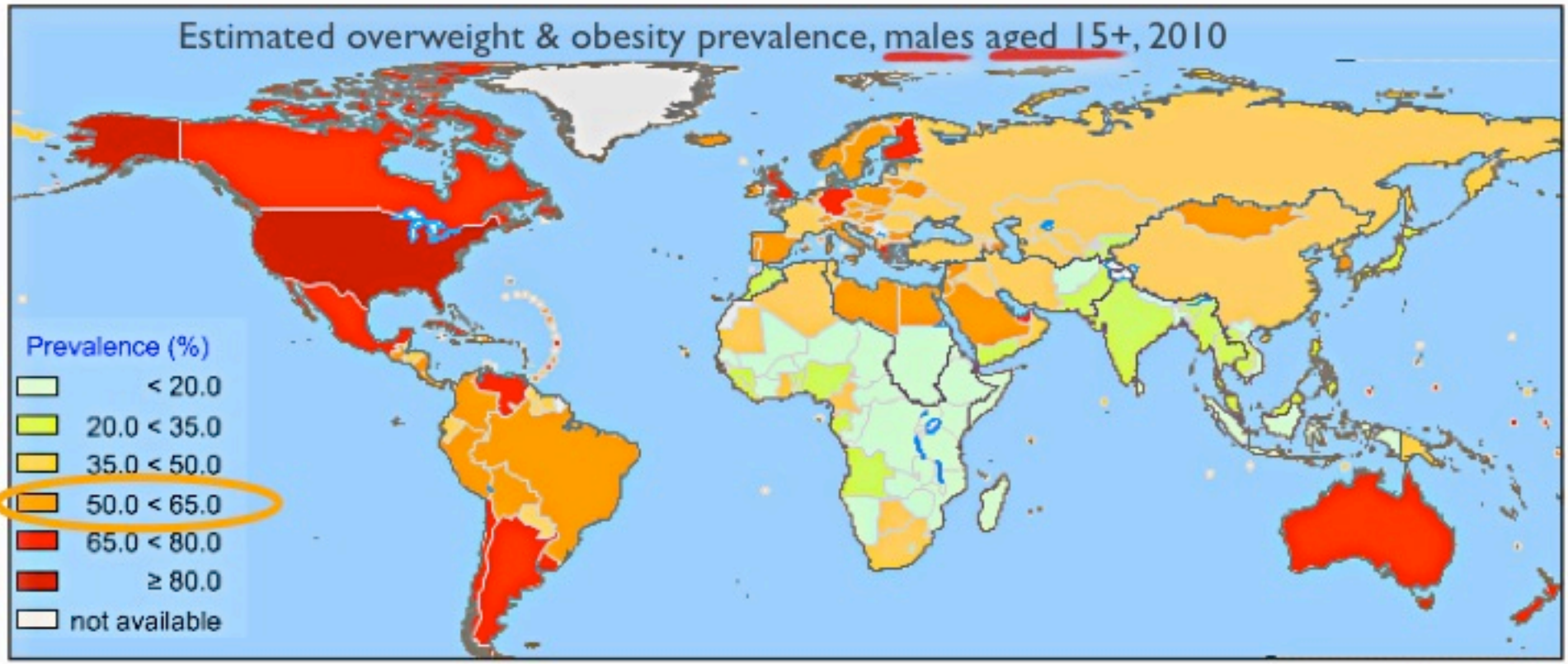
- Women: 4,5 nmol/L = 1,8 ng/ml
- Men: 5,5 nmol/L = 2,2 ng/ml
- All: 5 nmol/L = 2 ng/ml

How can we explain this negative correlation?

1. Reduced activation of vitamin D3 in obese subjects
2. Increased accumulation in adipose tissue of obese subjects

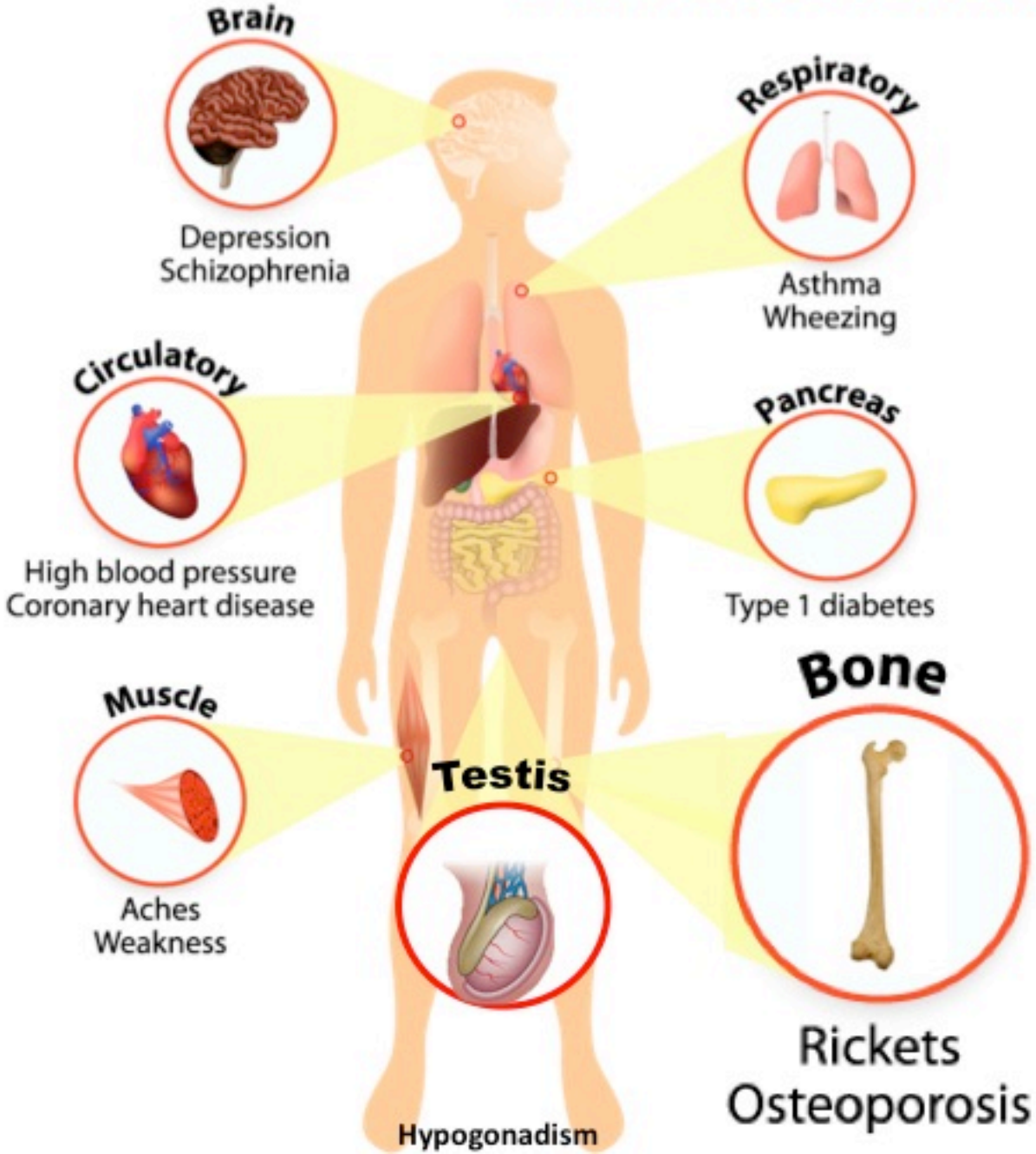
OBESITA'

L'obesità è stata definita dall'OMS come l'epidemia del XXI sec.

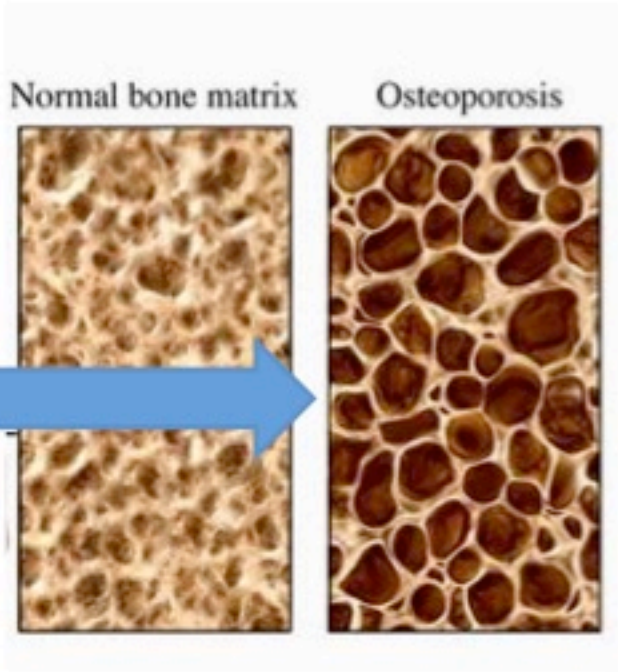


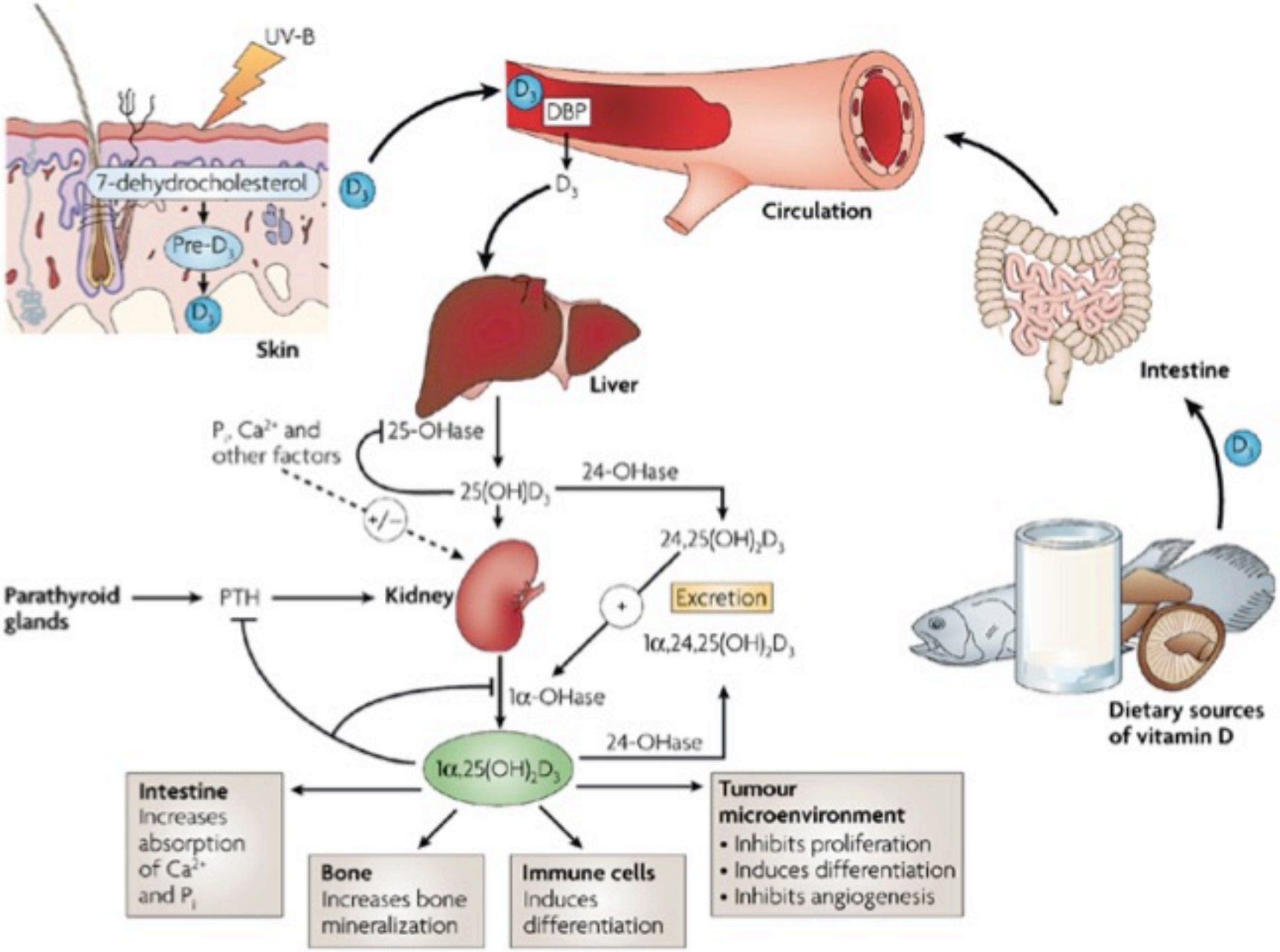
In Italia più del 50% degli uomini in età fertile sono in sovrappeso o obesi

Vitamin D deficiency

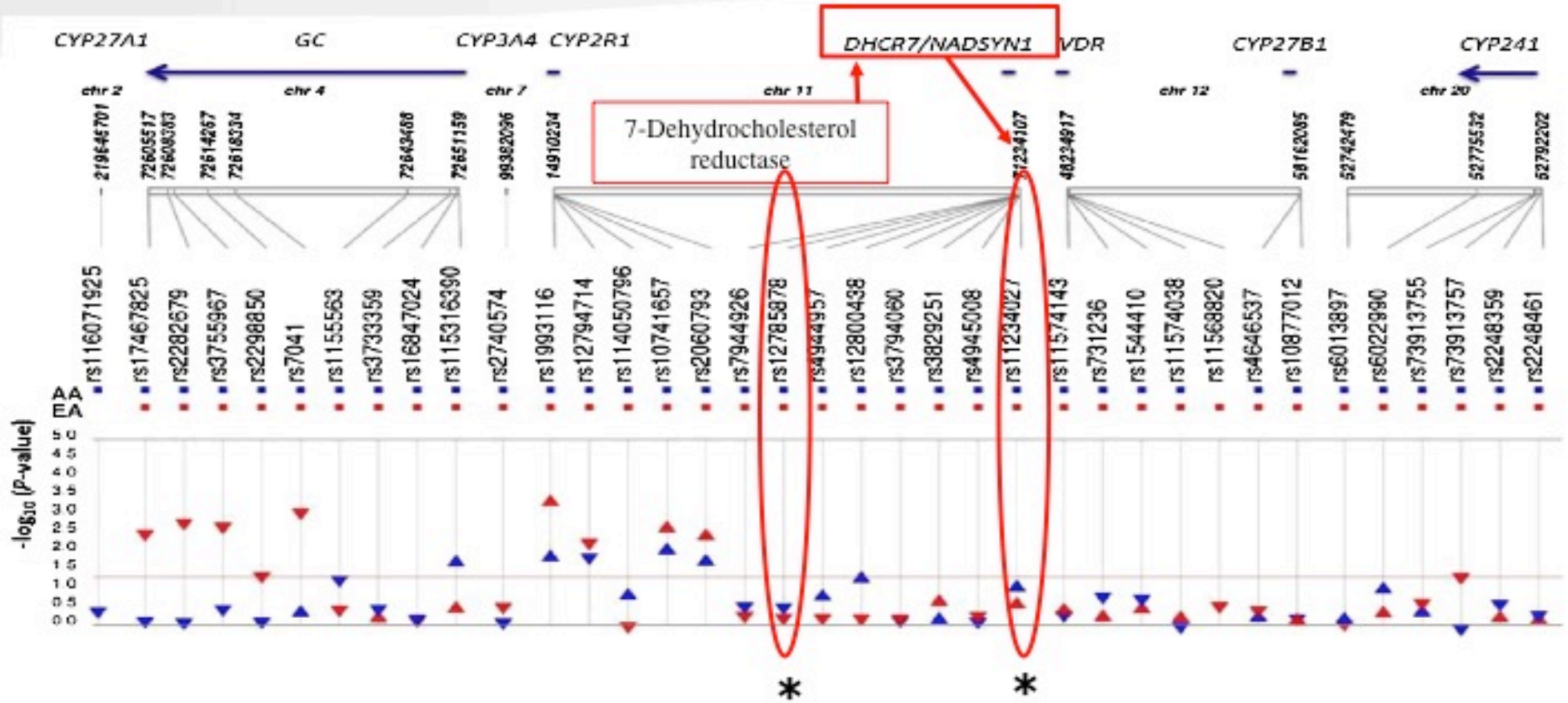


Vitamin D deficiency:
Not only a bone issue




UV-B
7-dehydrocholesterol
Pre-D3
D3
Skin
D3
DBP
D3
Circulation
Liver
Intestine
D3
P, Ca2+ and other factors
25-OHase
25(OH)D3
24-OHase
+/−
24,25(OH)2D3
Parathyroid glands
PTH
Kidney
+
Excretion
1α,24,25(OH)3D3
1α-OHase
24-OHase
1α,25(OH)2D3
Dietary sources of vitamin D
Intestine
Increases absorption of Ca2+ and Pi
Bone
Increases bone mineralization
Immune cells
Induces differentiation
Tumour microenvironment
• Inhibits proliferation
• Induces differentiation
• Inhibits angiogenesis

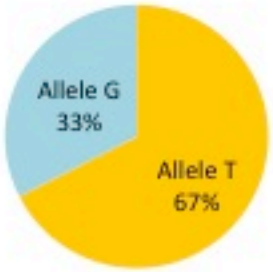
Genetic pattern of vitamin D function



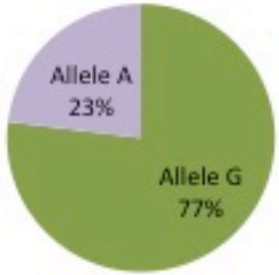
Frequenza polimorfismi (n=204) divisi tra osteoporotici/osteopenici e normali

Polimorfismi de-idro-colesterolo reduttasi

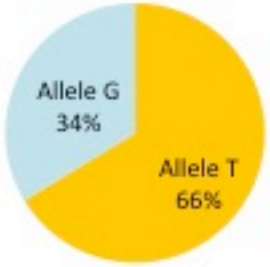
Normali (N=121)



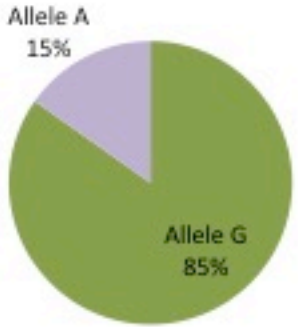
Normali (N=121)



Osteopenia-osteoporosi (N=83)



Osteopenia-osteoporosi (N=83)



L'80% **del fabbisogno di vitamina D** è garantito dall'**irradiazione solare UVB** (290-315 nm)

1

Ridotta formazione

Cute

7-deidrocolesterolo

Previtamina D3

Vitamina D3 (colecalciferolo)

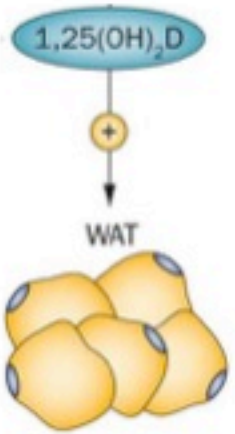
Alimentazione (20% del fabbisogno)

Vitamina D2 (calciferolo)

2

Sequestro di Vit D nel tessuto adiposo

Obesità



Testes

50%

Fegato
Vitamina D-25 idrossilasi

50%

25(OH)D (calcifediolo) principale forma circolante

Rene + Sistemico
25(OH)D-1-α-idrossilasi

1,25(OH)D (calcitriolo) Metabolita attivo

Scheletro

Intestino

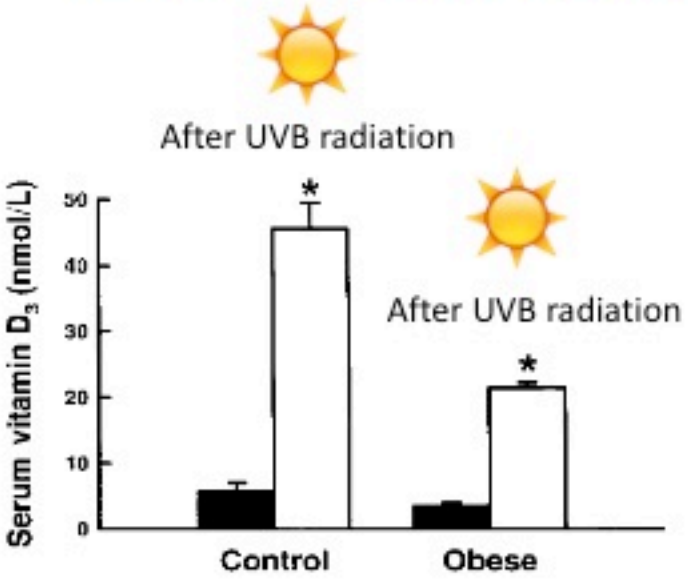
Mineralizzazione ossea

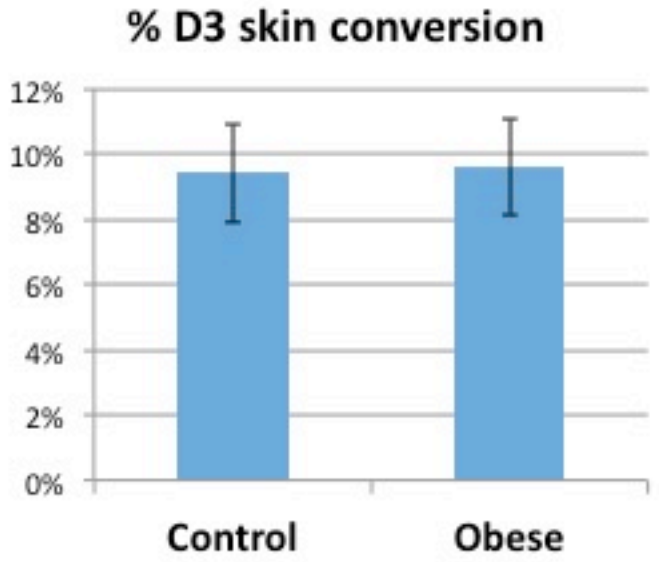
Assorbimento di calcio

Decreased bioavailability of vitamin D in obesity

Jacobo Wortsman, Lois Y Matsuoka, Tai C Chen, Zhiren Lu, and Michael F Holick

Am J Clin Nutr 2000;72:690–3.



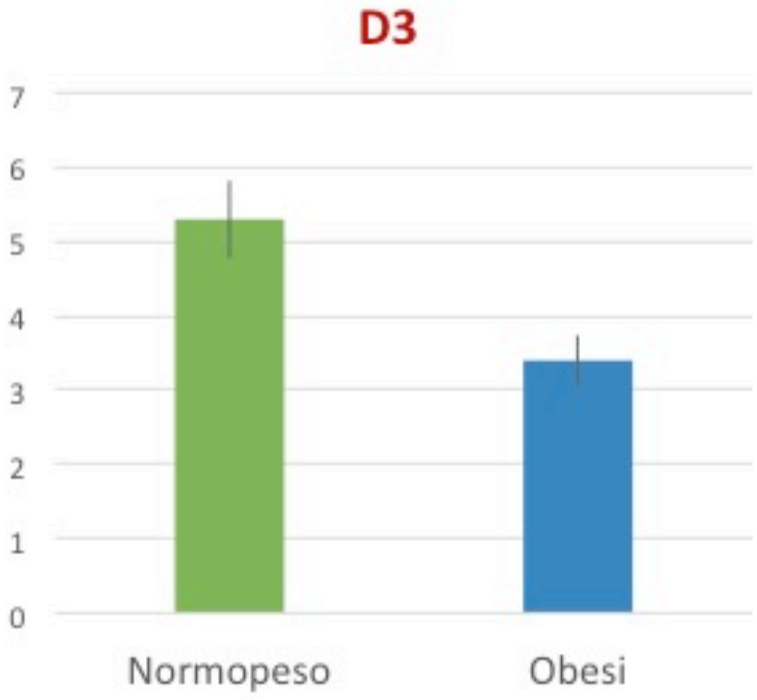


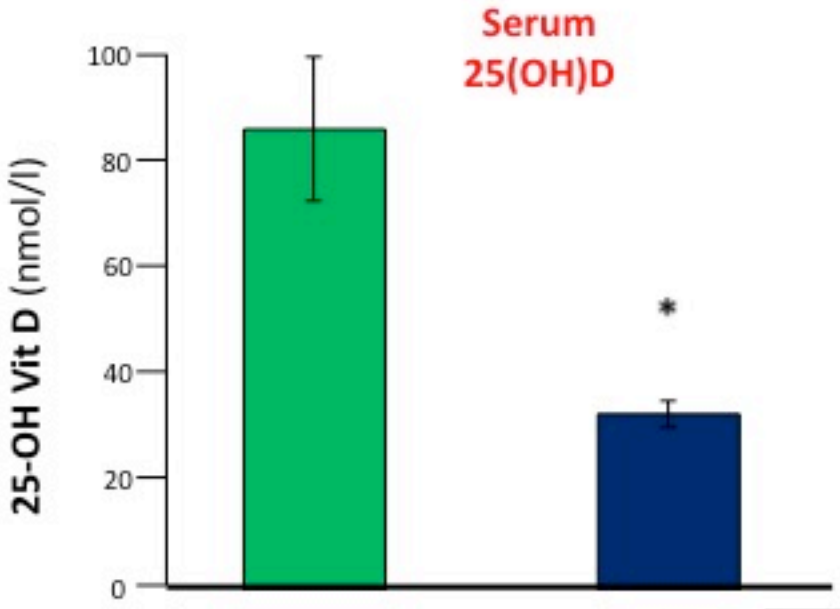
- The increase in blood vitamin D3 concentrations was 57% less in the obese

- The percentage of conversion to vitamin D3 was similar in both groups

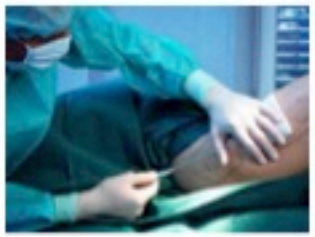
Vitamin D3 synthesis is not altered, but its concentration is reduced in obesity

	Vit D3 (nM)	25OH-D (nM)
Normopeso	5,3	84,9
Obesi	**3,4***	**29,4***

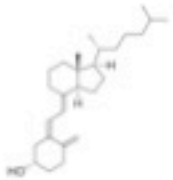




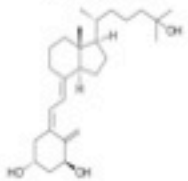
Tessuto adiposo sottocutaneo

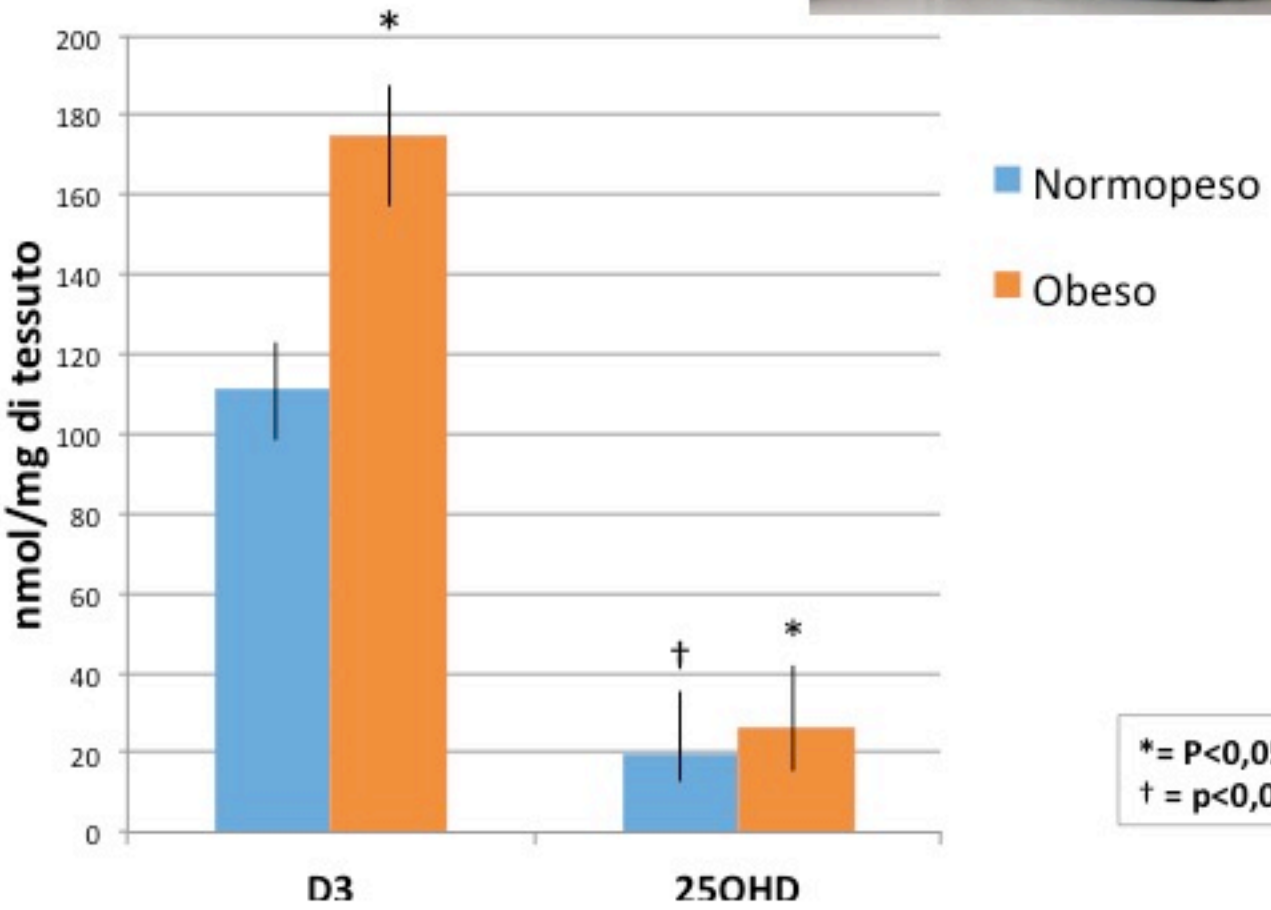


Vit D3



25OHD





*= P<0,05 vs Normo
† = p<0,05 vs D3

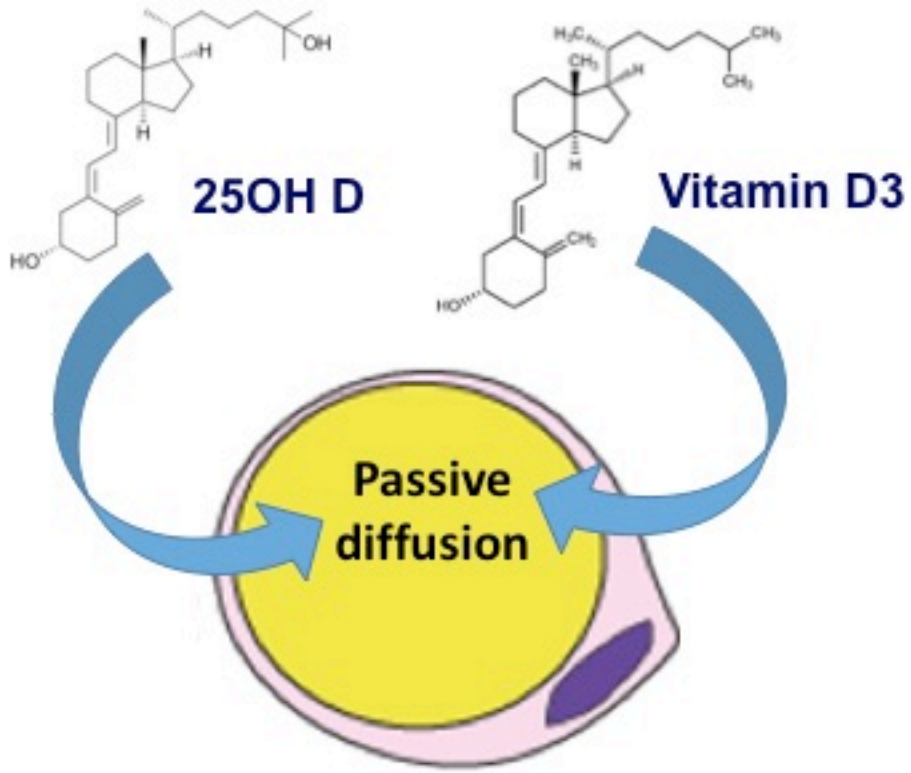
Foresta et al. JCEM 2017

Deposition in and Release of Vitamin D_3 from Body Fat: Evidence for a Storage Site in the Rat

SAUL J. ROSENSTREICH, CLAYTON RICH, and WADE VOLWILER

From the Department of Medicine, University of Washington School of Medicine, and the Veterans Administration Hospital, Seattle, Washington 98105

The Journal of Clinical Investigation Volume 50:679, 1971



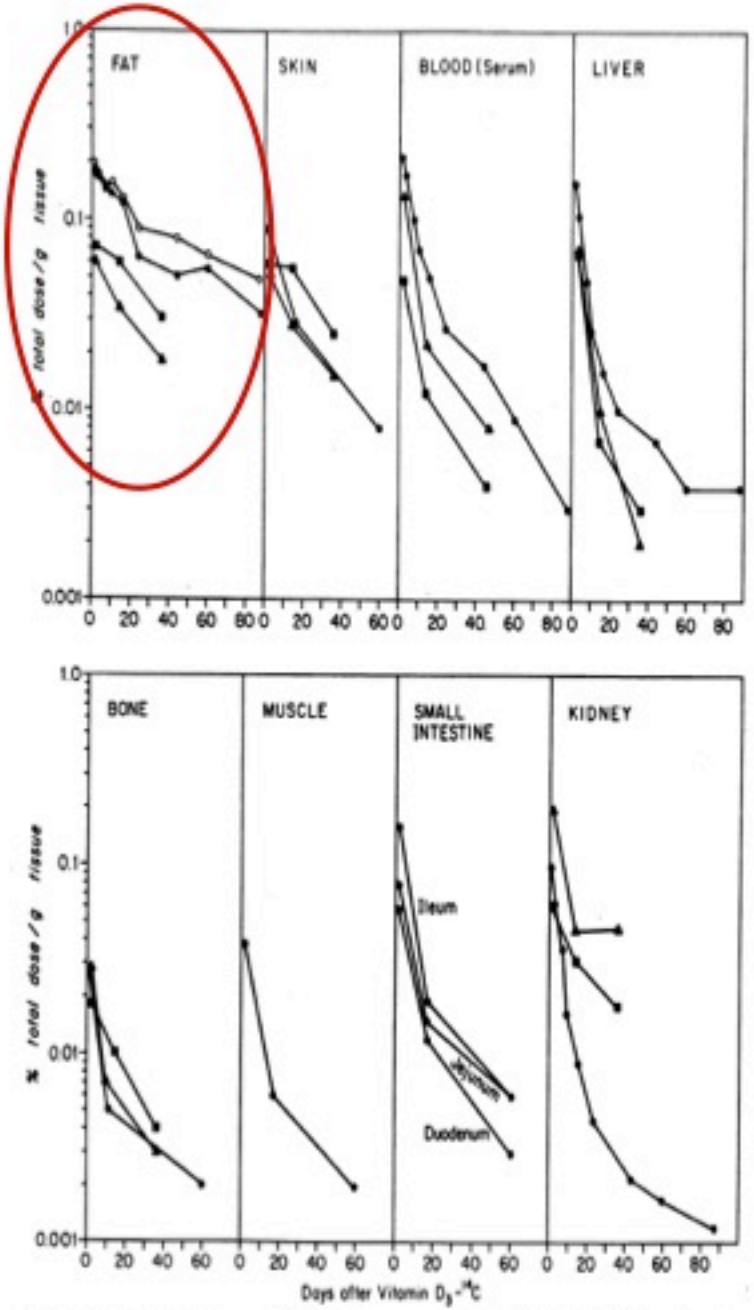
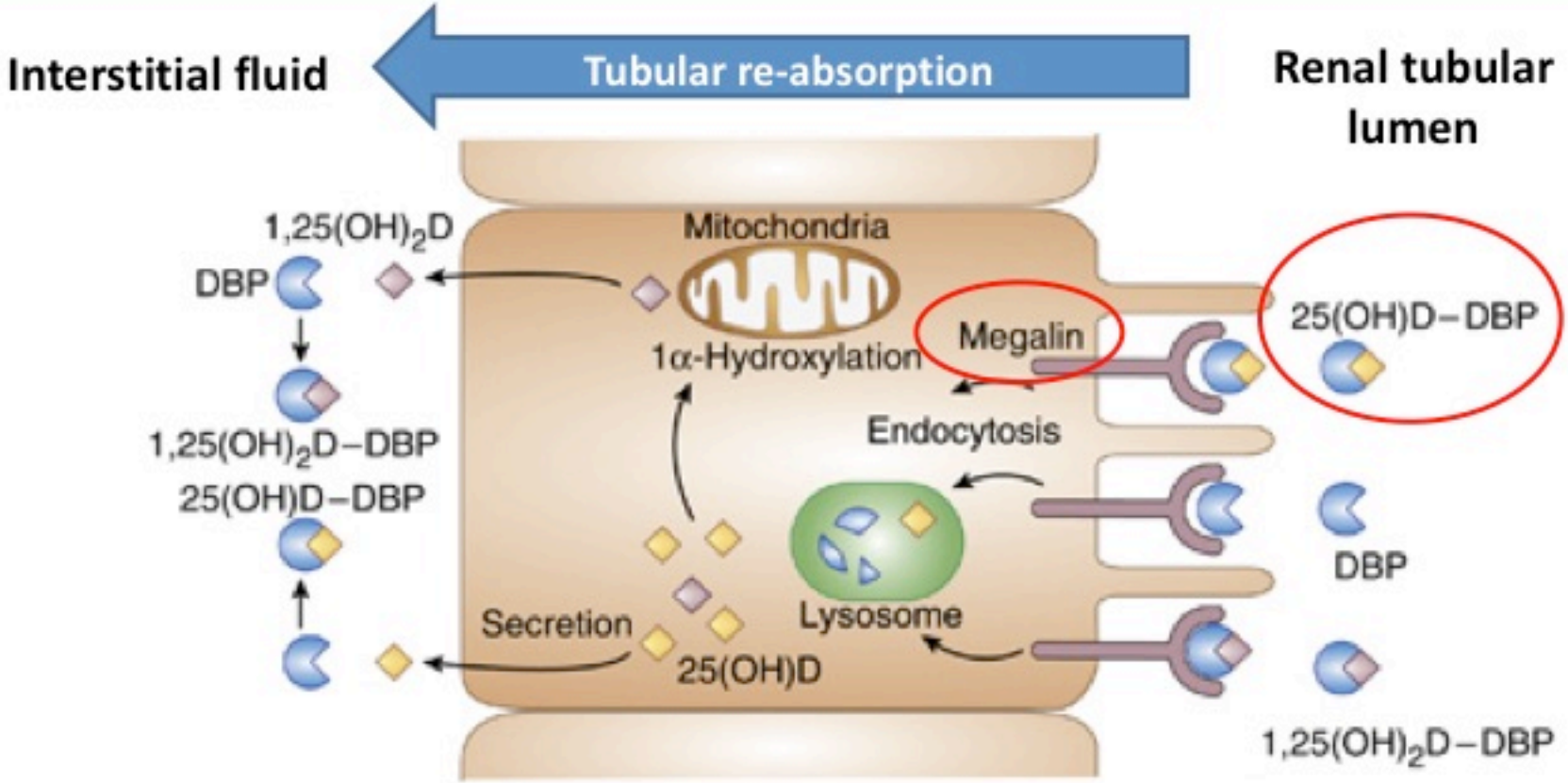


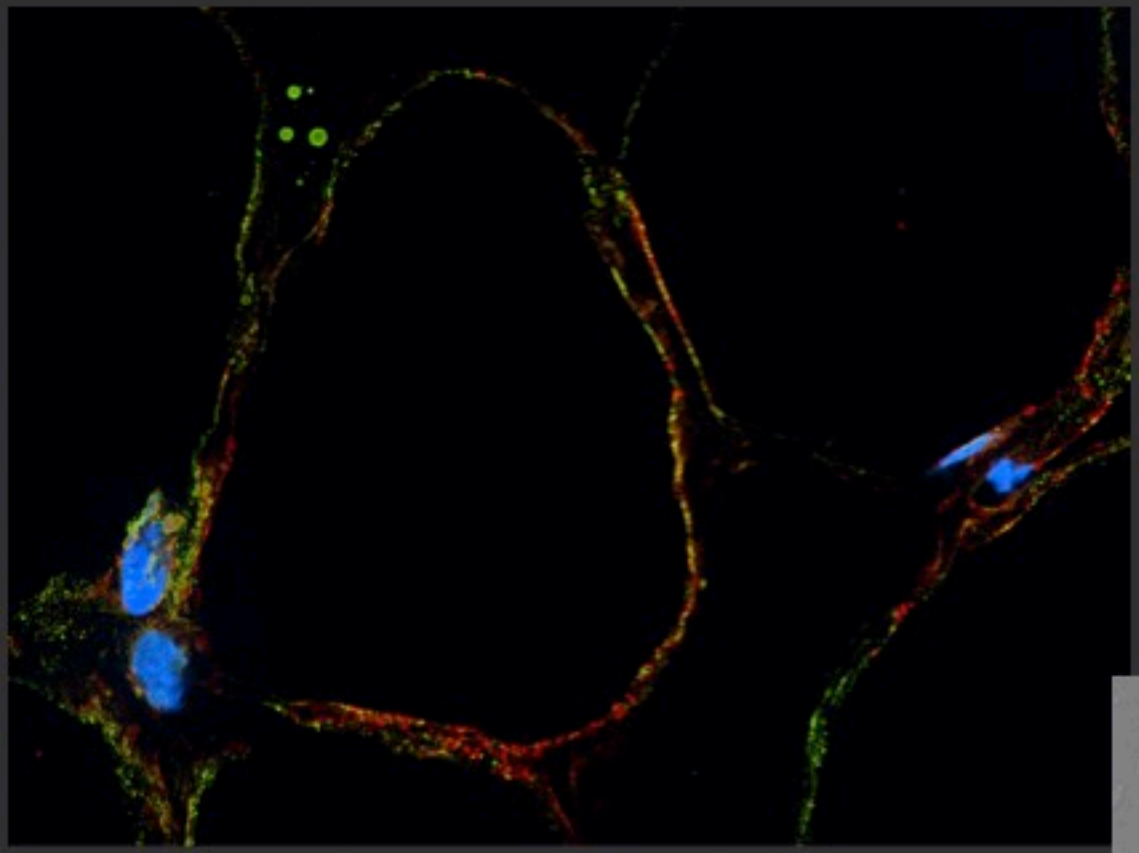
FIGURE 2 Decline in tissue radioactivity after completion of vitamin D_3-4-^{14}C supplementation. Group A = ▲; group B = ●; group C = ■. For Fat, group B animals, epididymal = ●, subcutaneous = ○.

25(OH)D internalization is mediated by megalin

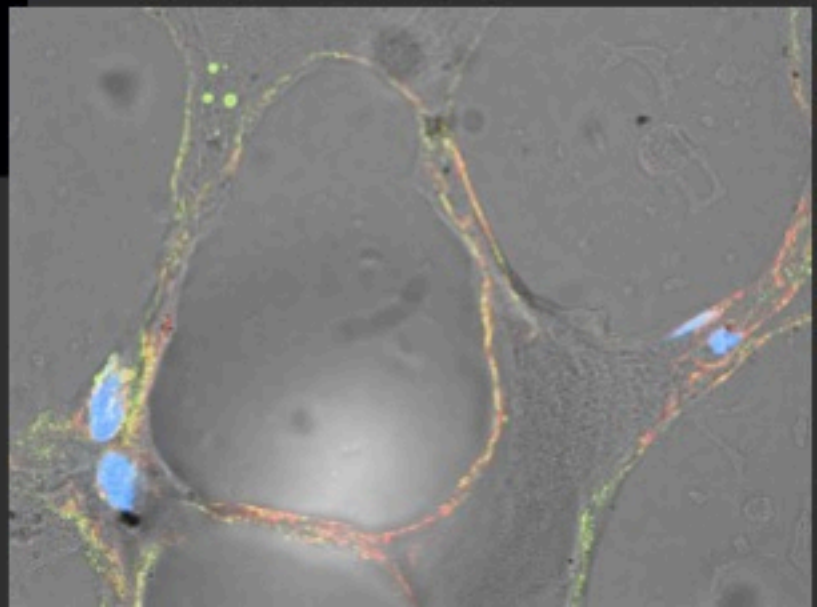
Internalisation of DBP-bound 25OHD is mediated by the megalin/ cubulin endocytic pathway in the proximal tubule epithelium. It is thought that other tissues (non-renal) which do not express megalin rely largely on the diffusion of free 25OHD through the plasma membrane.



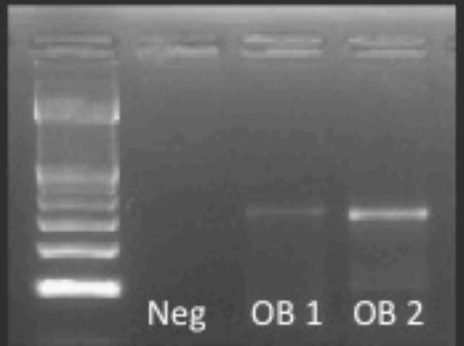
Megalin expression in fat cells



DAPI
MEGALIN
ABCA1



MEGALIN mRNA



J Am Coll Nutr. 2009 Jun;28(3):252-6.

Vitamin D3 distribution and status in the body.

Heaney RP[1], Horst RL, Cullen DM, Armas LA.

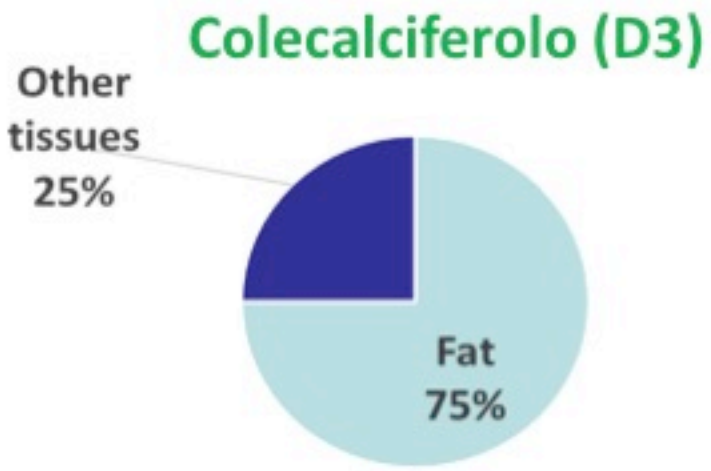
Abstract

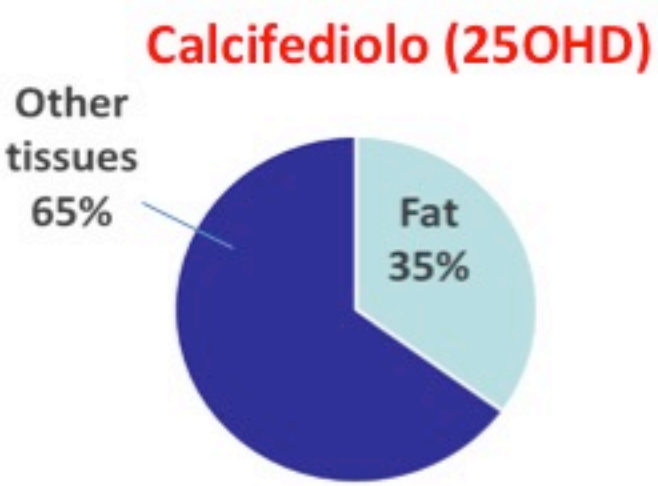
OBJECTIVE: To estimate the amount, type, and tissue distribution of vitamin D in the adult body under typical inputs.

METHODS: Review and reanalysis of published measurements and analysis of tissue samples from growing pigs raised in confinement on diets providing about 2000 IU vitamin D/day. Cholecalciferol and 25-hydroxyvitamin D [25(OH)D] concentration measured by HPLC.

RESULTS: Mean serum 25(OH)D in all studies combined was 45 nmol/L. At the level of vitamin D repletion represented by this concentration, total body vitamin D would be 14,665 IU for a 70 kg adult woman. 65% of this total was present as native cholecalciferol and 35% as 25(OH)D. Nearly three-quarters of the cholecalciferol was in fat, while 25(OH)D was more evenly distributed throughout the body (20% in muscle, 30% in serum, 35% in fat, and 15% in all other tissues). At the daily vitamin D consumption rates in these animals total body stores provided only a approximately 7-day reserve.

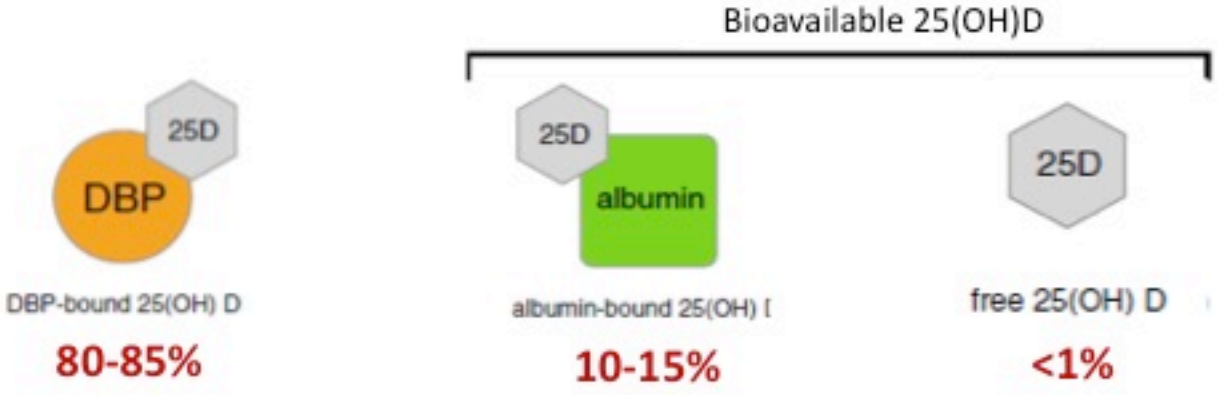
CONCLUSIONS: At total intakes on the order of 2000 IU/day, an adult has very little vitamin D reserve, despite intakes 10x the current recommendations. Those recommended inputs need to be increased by at least an order of magnitude. Food tables that fail to take into account 25(OH)D content of various meat products lead to underestimation of dietary vitamin D intake.





Why is D3 prefentially accumulated in adipose tissue?

The role of vitamin D binding protein **(DBP)**



DBP affinity:

- 25(OH)D: $7x10^8$ M^{-1}
- 1,25(OH)D: $5x10^7$ M^{-1}
- **Vitamin D3: $4x10^7$ M^{-1}** **-20X**

Liposolubilità (coefficiente di ripartizione acqua/n-ottanolo a 25°C)

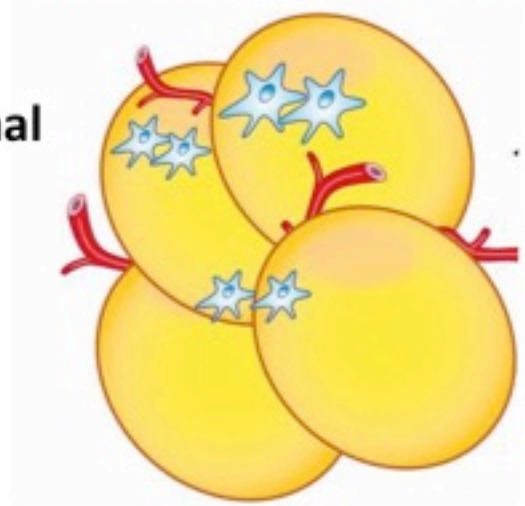
- **D3: $logK_{ow}$ = 10,2** **150X**
- 25(OH)D: $logK_{ow}$ = 8,43
- 1,25(OH)D: $logK_{ow}$ = 7,6

Vitamin D binding protein (DBP) binds vitamin d metabolites, but D3 has the lowest affinity

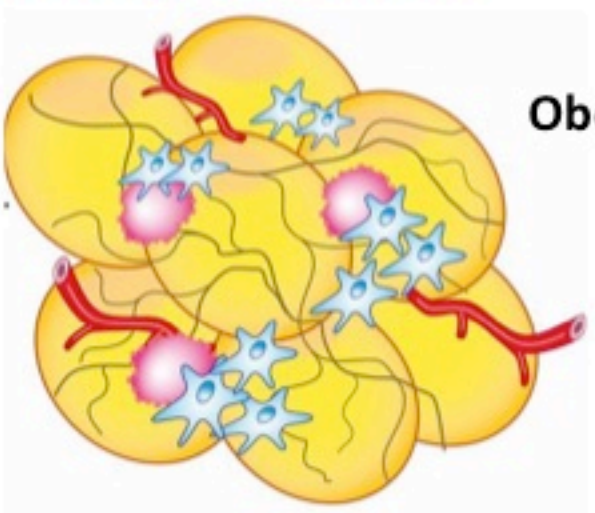
→ **More un-bound D3 can freely diffuse into adipose tissue given its higher lipo-solubility**

Why is D3 prefentially accumulated in adipose tissue?

Normal



Differential accumulation of D3 and 25(OH)D in normal and obese adipose tissue?



Obese

DBP affinity:

- 25(OH)D: $7x10^8$ M^{-1}
- 1,25(OH)D: $5x10^7$ M^{-1}
- **Vitamin D3: $4x10^7$ M^{-1}** **-20X**

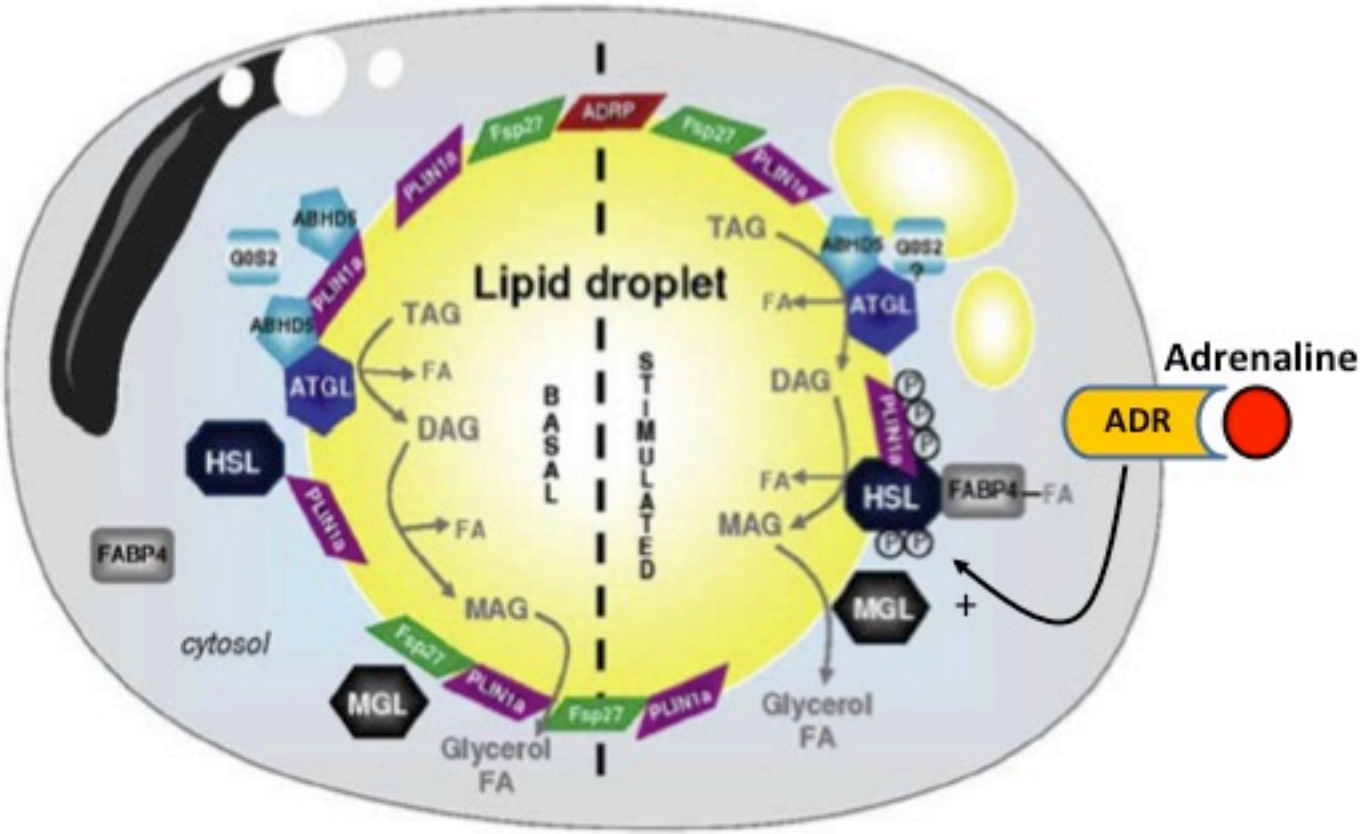
Liposolubilità (coefficiente di ripartizione acqua/n-ottanolo a 25°C)

- **D3: $logK_{ow}$ = 10,2** **150X**
- 25(OH)D: $logK_{ow}$ = 8,43
- 1,25(OH)D: $logK_{ow}$ = 7,6

Vitamin D binding protein (DBP) binds vitamin d metabolites, but D3 has the lowest affinity

→ **More un-bound D3 can freely diffuse into adipose tissue given its higher lipo-solubility**

Meccanismo di rilascio del contenuto dei droplet lipidici in seguito a stimolo adrenergico



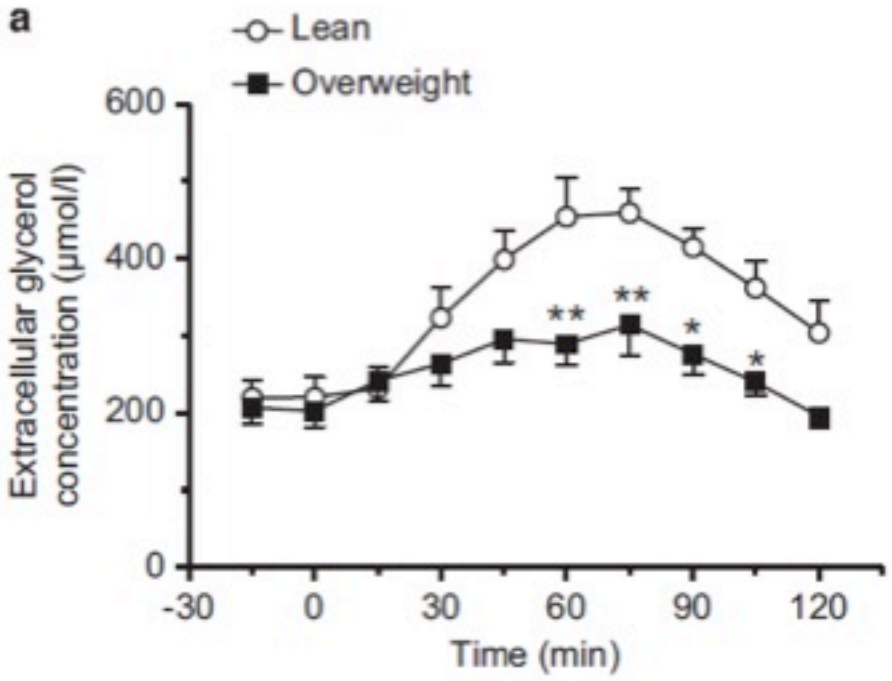
International Journal of Obesity (2015), 1–7

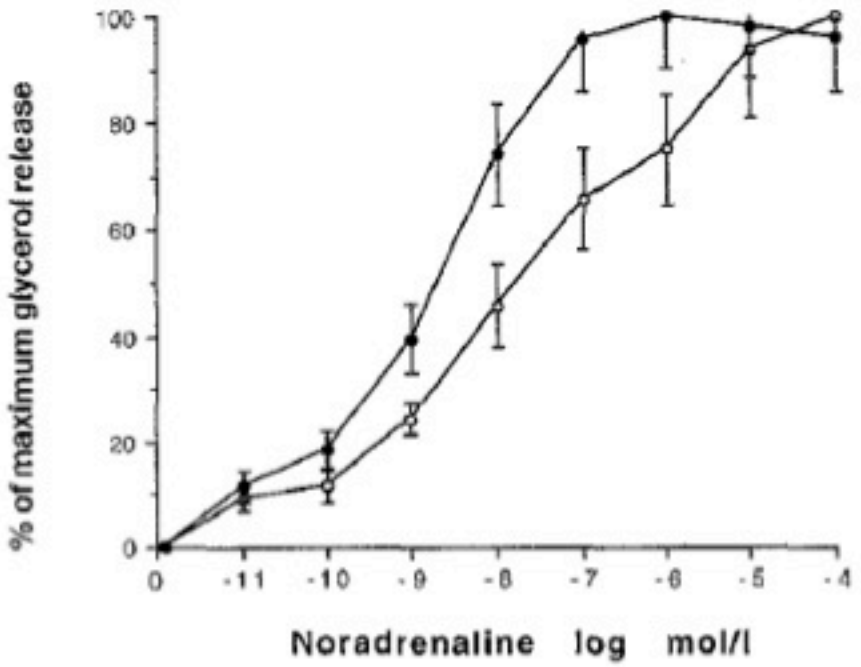
www.nature.com/ijo

ORIGINAL ARTICLE

Impaired atrial natriuretic peptide-mediated lipolysis in obesity

M Rydén[1], J Bäckdahl[1], P Petrus[1], A Thorell[2], H Gao[3], M Coue[4,5], D Langin[4,5,6], C Moro[4,5] and P Arner[1]



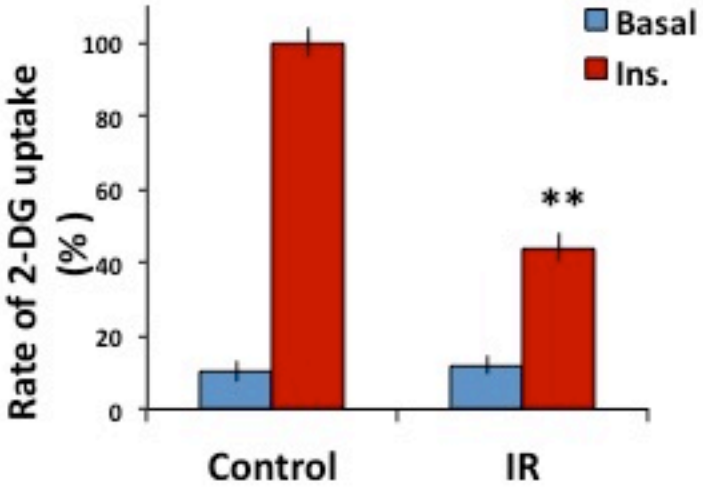


Catecholamine resistance in fat cells of women with upper-body obesity due to decreased expression of beta$_2$-adrenoceptors

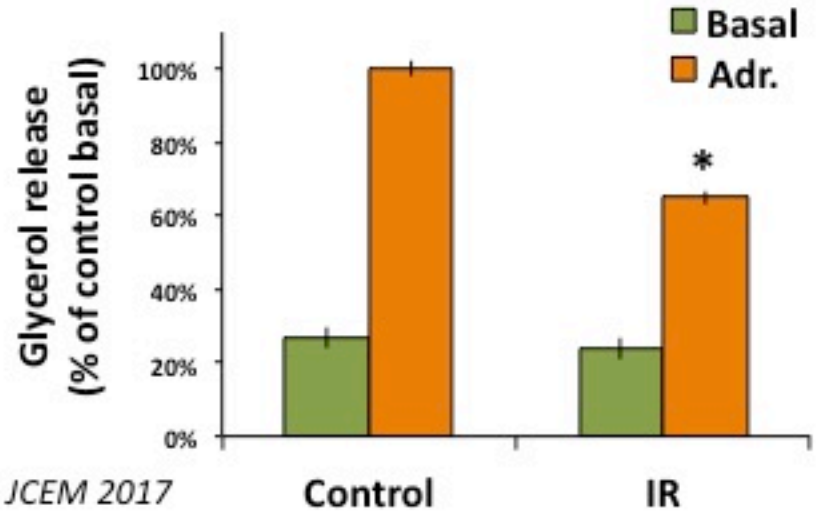
S. Reynisdottir[1], H. Wahrenberg[1], K. Carlström[1], S. Rössner[2], P. Arner[1]

Diabetologia (1994) 37: 428–435

1) Uptake di 2-DG in adipociti normali e insulino-resistenti in risposta a stimolo insulinico

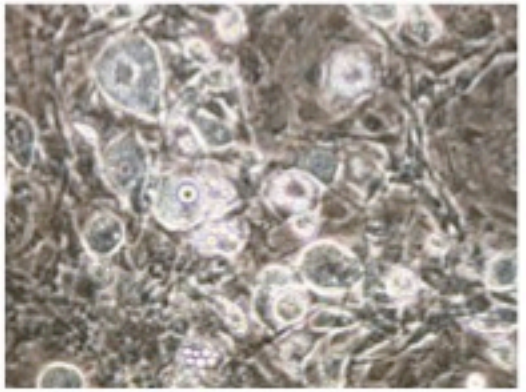


2) Rilascio di glicerolo in adipociti normali e insulino-resistenti in risposta a adrenergico

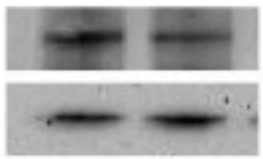


Foresta et al. JCEM 2017

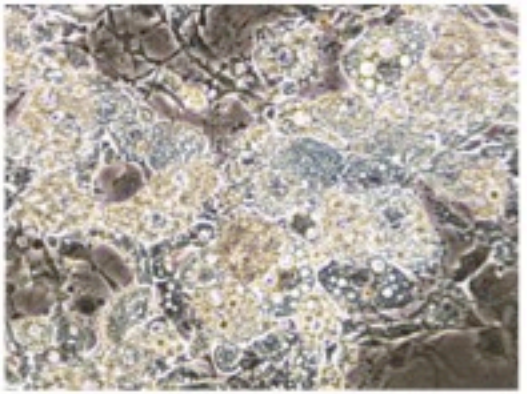
Standard Adipocyte culture From 3T3-L1



Espressione recettore β_2-adrenergico



Insulin Resistant Adipocytes



J Clin Endocrinol Metab, July 2017

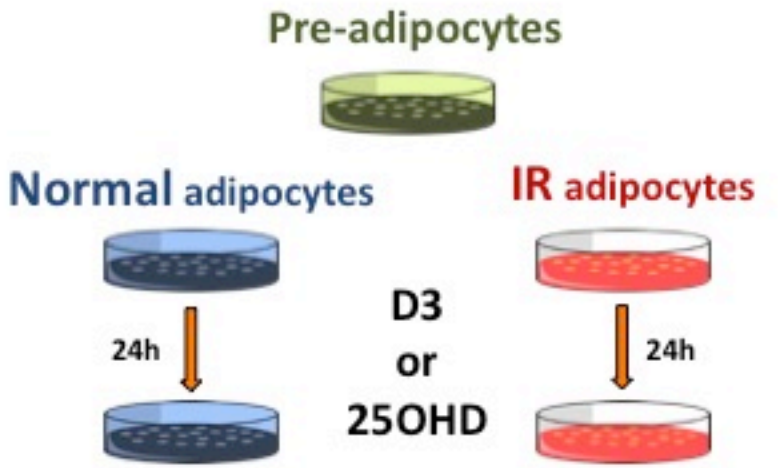
Impaired Release of Vitamin D in Dysfunctional Adipose Tissue: New Cues on Vitamin D Supplementation in Obesity

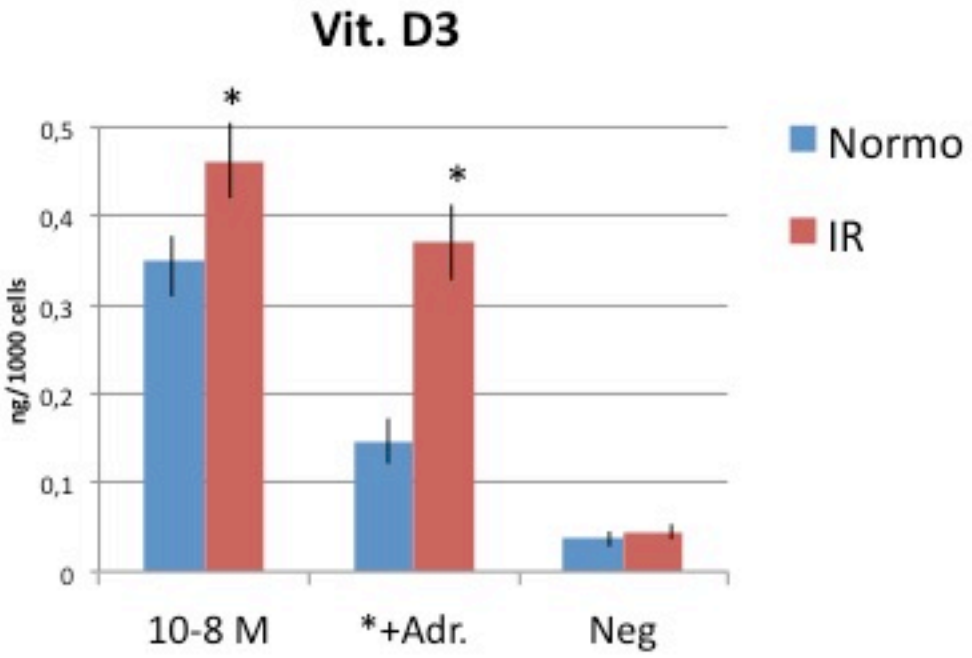
Andrea Di Nisio,[1] Luca De Toni,[1] Iva Sabovic,[1,5] Maria Santa Rocca,[1] Vincenzo De Filippis,[2] Giuseppe Opocher,[5] Bruno Azzena,[6] Roberto Vettor,[3] Mario Plebani,[4] and Carlo Foresta[1]

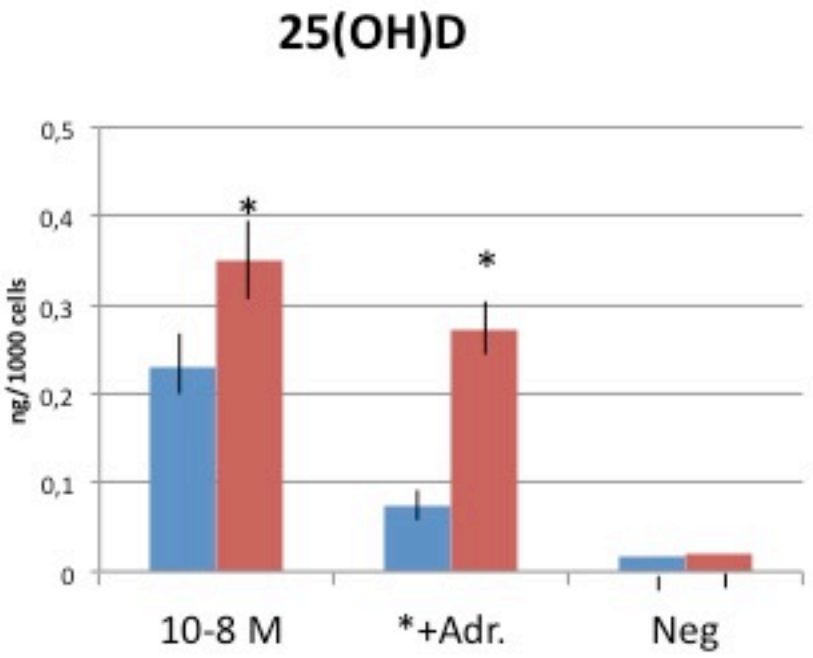
Results: In IR adipocytes, uptake of D3 and 25-hydroxyvitamin-D3 was higher, but, after adrenaline stimulation, the decrement in D3 and 25-hydroxyvitamin-D3 was stronger in control cells, which also showed increased expression of *Cyp27a1* and *Cyp27b1* and higher levels of 25-hydroxyvitamin-D3. In SAT from obese subjects, adrenaline-induced release of D3 and 25-hydroxyvitamin-D3 was blunted; in both IR cells and obese SAT, protein expression of β2-adrenergic receptor was reduced. Supplementation with 25-hydroxyvitamin-D3 was more effective in achieving vitamin D sufficiency in obese, but not in normal weight subjects.

Conclusion: Dysfunctional AT shows a reduced catecholamine-induced release of D3 and 25-hydroxyvitamin-D3 and altered activity of vitamin D–metabolizing enzymes; for these reasons supplementation with 25-hydroxyvitamin-D3 is more effective in obese individuals. (*J Clin Endocrinol Metab* 102: 2564–2574, 2017)

1) Adipociti incubati in presenza di vitamina D3 e 25(OH)D per 24h
2) Stimolo con adrenalina
3) Quantificazione D3 e 25(OH)D3 con LC-MS







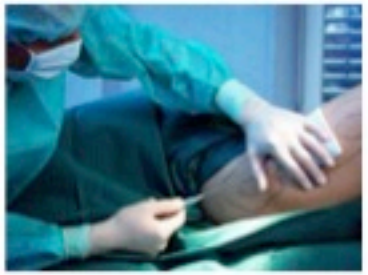
Normo IR

Foresta et al. JCEM 2017

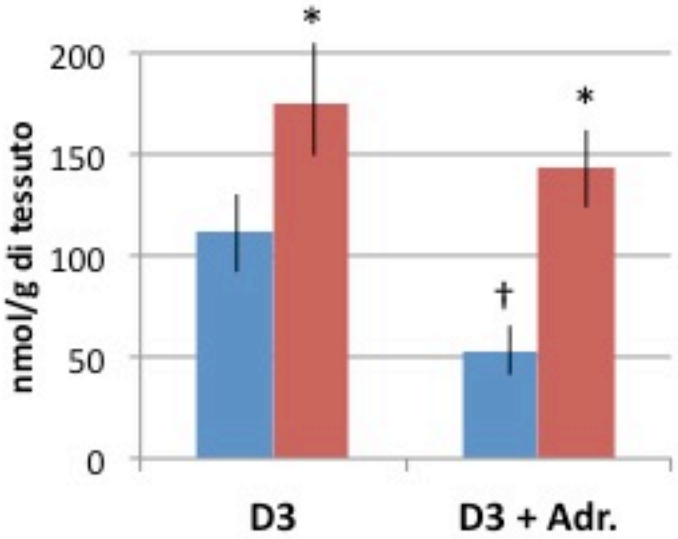
Foresta 2017 JCEM

Ex-vivo study

Tessuto adiposo sottocutaneo



Vit. D3



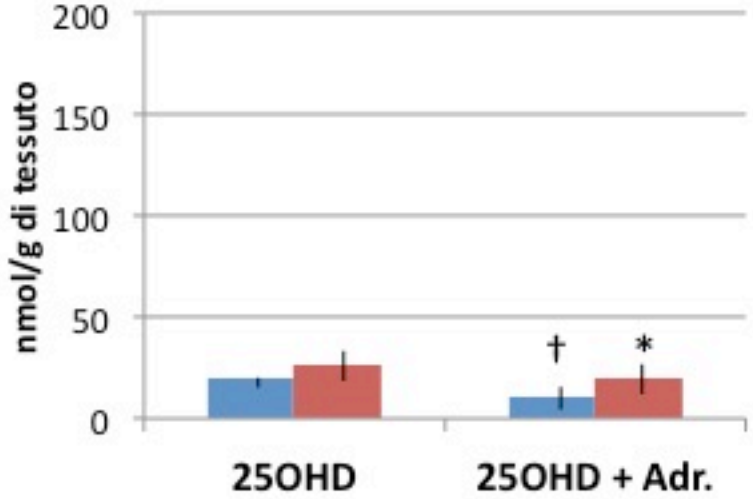
Normopeso

Obeso

*= P<0,05 vs Normo

† = p<0,05 vs D3

25(OH)D



- D3 e 25(OH)D si accumulano di più nell'adiposo disfunzionale
- E' un sequestro scarsamente reversibile

Foresta et al. 2009 Clinical Endocrinology

Table 1. Clinical characteristics and hormonal levels in controls and obese men

	Controls ($n = 64$)	Obese men ($n = 31$)
Age (year)	34·7 ± 6·4	35·6 ± 8·8
BMI (kg/m^2)	23·2 ± 1·5	34·9 ± 3·2*
T (nmol/l)	19·2 ± 3·4	16·4 ± 5·1†
SHBG (nmol/l)	30·4 ± 4·7	27·5 ± 5·0‡
FT (pmol/l)	432·3 ± 103·8	380·9 ± 120·5‡
E_2 (pmol/l)	41·4 ± 27·1	59·5 ± 27·2†
LH (IU/l)	3·1 ± 1·1	2·9 ± 2·9
FSH (IU/l)	2·9 ± 1·3	3·0 ± 2·5
INSL3 (pg/ml)	587·8 ± 170·7	422·9 ± 181·3*

*$P < 0·001$ *vs.* controls; †$P < 0·005$ *vs.* controls; ‡$P < 0·05$ *vs.* controls.

Impaired Release of Vitamin D in Dysfunctional Adipose Tissue: New Cues on Vitamin D Supplementation in Obesity

Andrea Di Nisio,[1] Luca De Toni,[1] Iva Sabovic,[1,5] Maria Santa Rocca,[1] Vincenzo De Filippis,[2] Giuseppe Opocher,[5] Bruno Azzena,[6] Roberto Vettor,[3] Mario Plebani,[4] and Carlo Foresta[1]

J Clin Endocrinol Metab, July 2017, 102(7):2564–2574

Table 1. Baseline Clinical, Metabolic, Hormonal Parameters, and Anthropometric Measures of Subjects

	Normal Weight (n = 38)	Obese (n = 59)
Age, y	42.28 ± 6.59	42.48 ± 6.19
Height, cm	170.34 ± 6.12	171.08 ± 8.02
Weight, kg	65.21 ± 8.26	98.09 ± 10.10[a]
BMI, kg/m^2	22.25 ± 2.07	33.47 ± 2.16[a]
Waist, cm	94.40 ± 4.60	124.32 ± 11.57[a]
Testosterone, nmol/L	16.32 ± 2.47	15.47 ± 2.40
LH, IU/L	4.29 ± 2.22	3.72 ± 2.09
Estradiol, pmol/L	94.59 ± 20.28	111.76 ± 37.42[a]
Fasting glucose, mg/dL	76.45 ± 7.76	94.19 ± 11.82[a]
Insulin, mU/L	4.34 ± 3.29	9.56 ± 5.58[a]
HOMA-IR	0.87 ± 0.43	2.03 ± 0.73[a]
25(OH)D, nmol/L	34.4 ± 5.5	33.0 ± 7.3
PTH, ng/L	83.20 ± 8.00	86.28 ± 7.04
Calcium, nmol/L	2.36 ± 0.10	2.38 ± 0.09
Posphorus, nmol/L	1.08 ± 0.19	1.06 ± 0.19

Endocr (2009) 35:252–261
DOI 10.1007/s12020-008-9134-2

ORIGINAL PAPER

Selective transcriptional regulation of aromatase gene by vitamin D, dexamethasone, and mifepristone in human glioma cells

Josune G. Yague · Luis M. García-Segura · Iñigo Azcoitia

Vitamin D upregulates aromatase expression in different cell lines

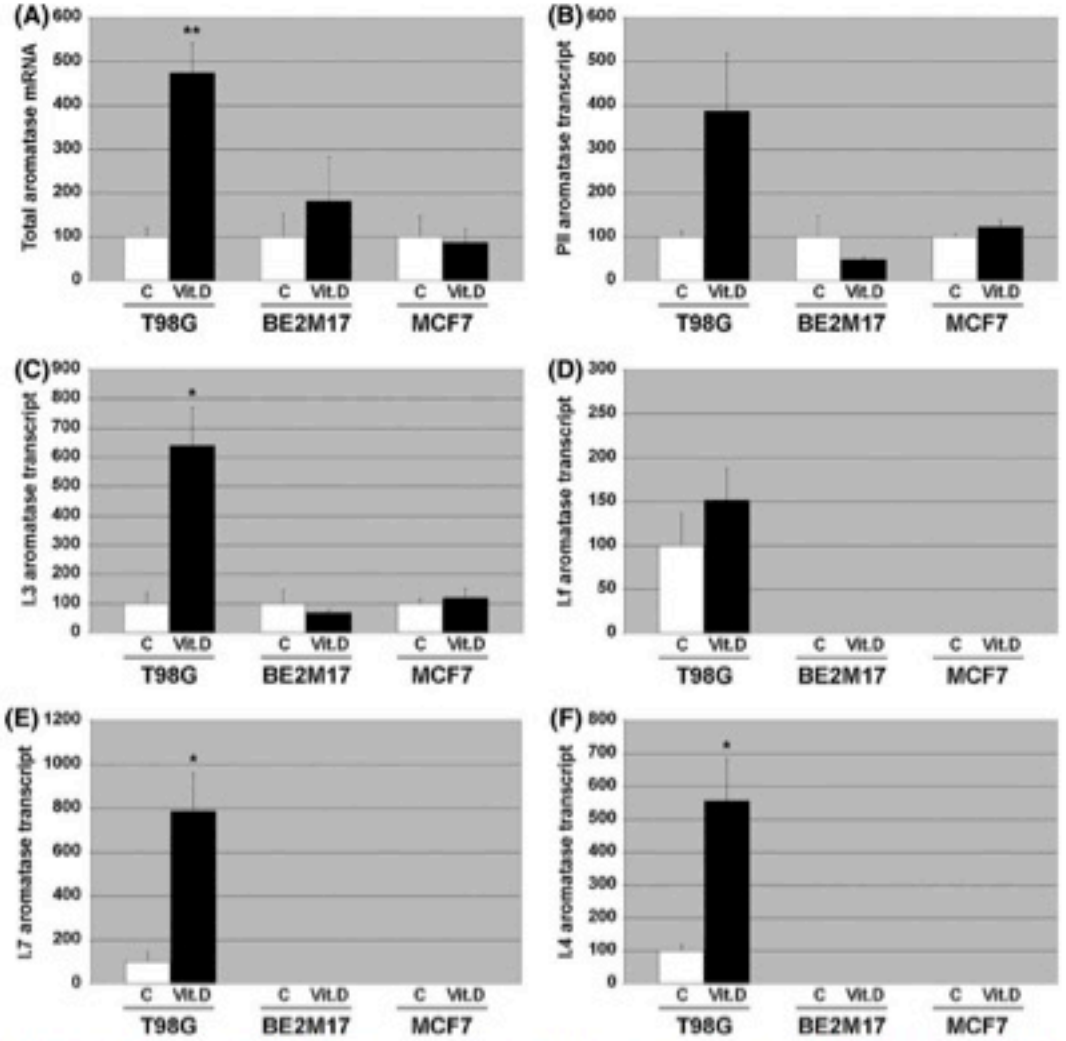
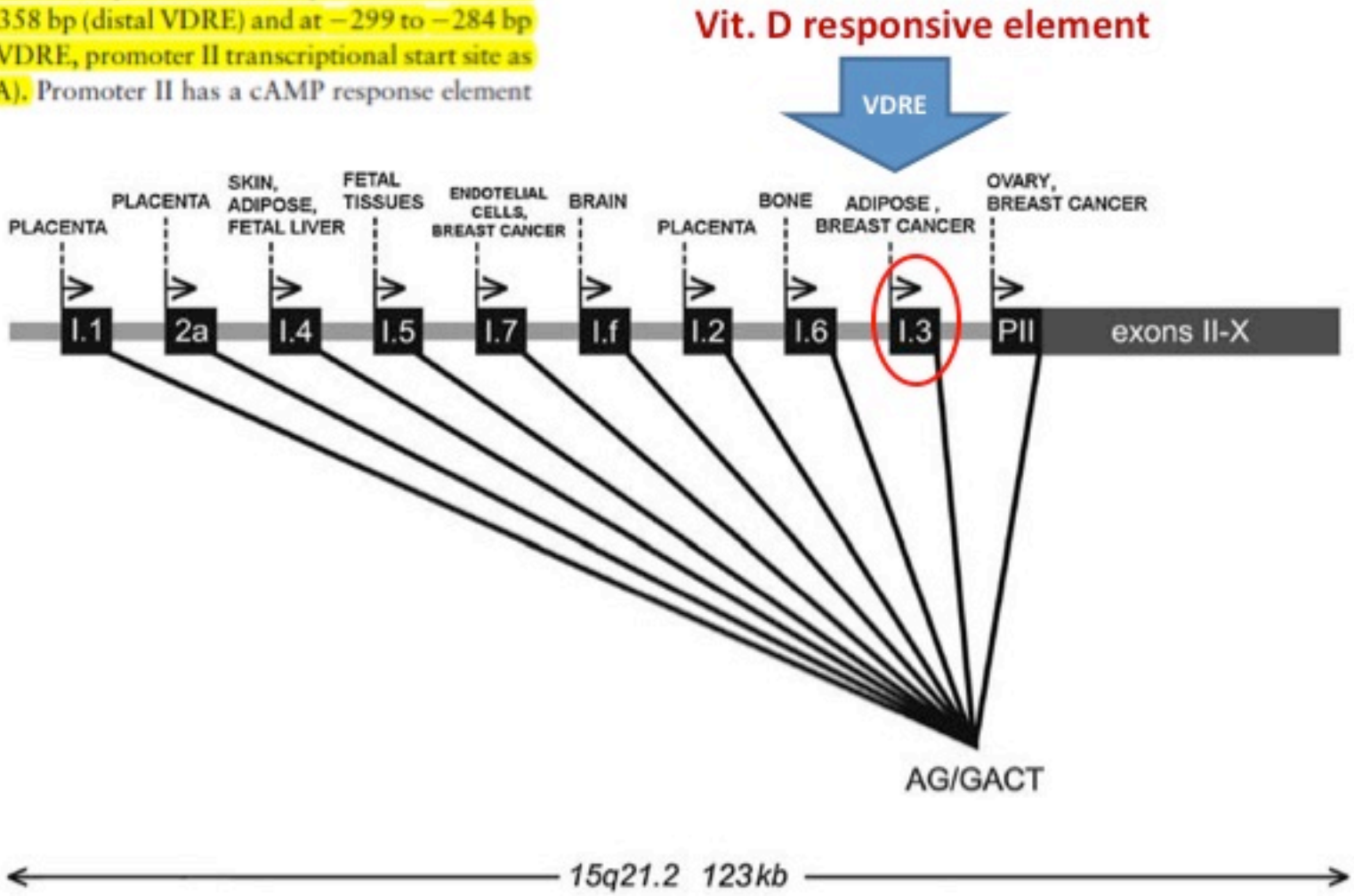


Fig. 3 Quantitative PCR analysis of total aromatase mRNA expression and aromatase transcript levels in glioma (T98G), neuroblastoma (BE2M17), and breast cancer (MCF7) cells treated with vitamin D (100 nM) or vehicle for 24 h. A, Total aromatase mRNA. B, PII transcript. C, L3 transcript. D, Lf transcript. E, L7 transcript. F, L4 transcript. mRNA values are expressed in arbitrary units. Data represent the mean ± standard error of the mean (SEM) from 3 to 5 independent experiments. Asterisks, statistical difference with respect to control values (*$P < 0.05$, **$P < 0.01$, Student's t-test). C, control. Vit.D, vitamin D

Tissue-Selective Regulation of Aromatase Expression by Calcitriol: Implications for Breast Cancer Therapy

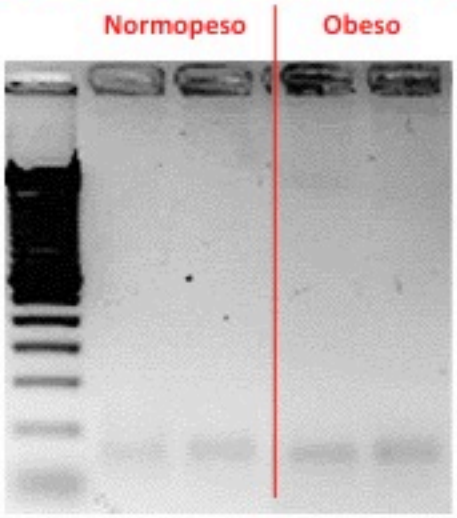
In silico analysis of the aromatase promoter I.3/II sequence revealed the presence of two putative VDREs at −373 to −358 bp (distal VDRE) and at −299 to −284 bp (proximal VDRE, promoter II transcriptional start site as +1, Fig. 5A). Promoter II has a cAMP response element



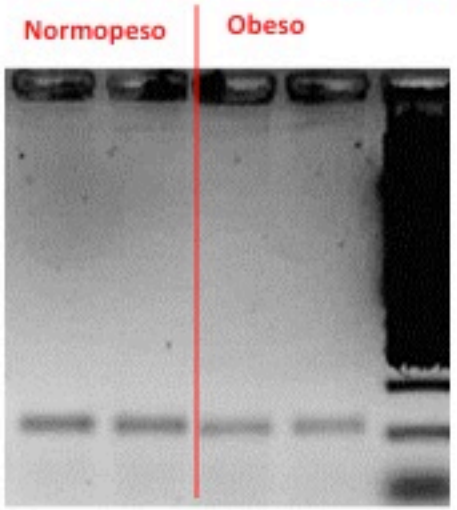
Risultati dello studio di espressione eseguito su tessuto adiposo di soggetto normopeso e obeso

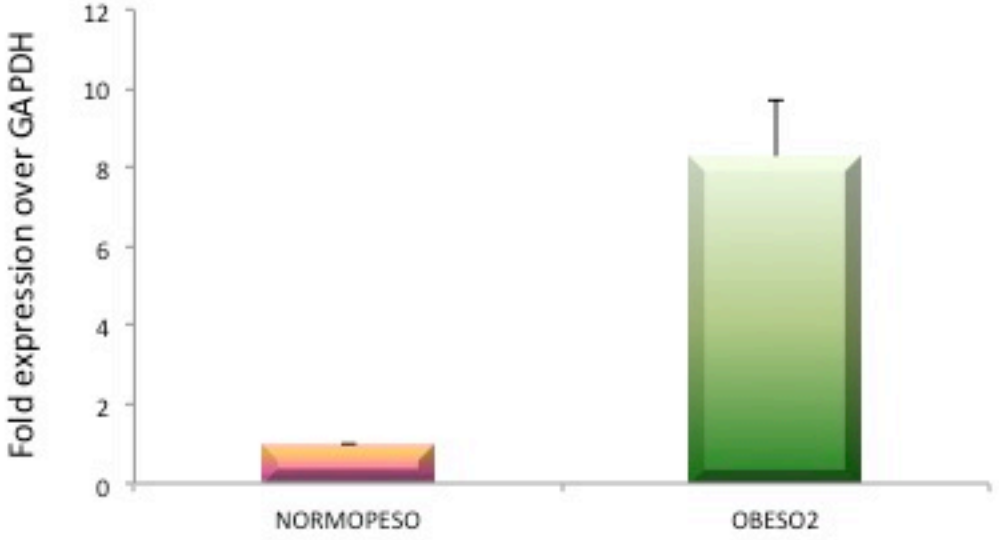
Risultati gel Real Time PCR

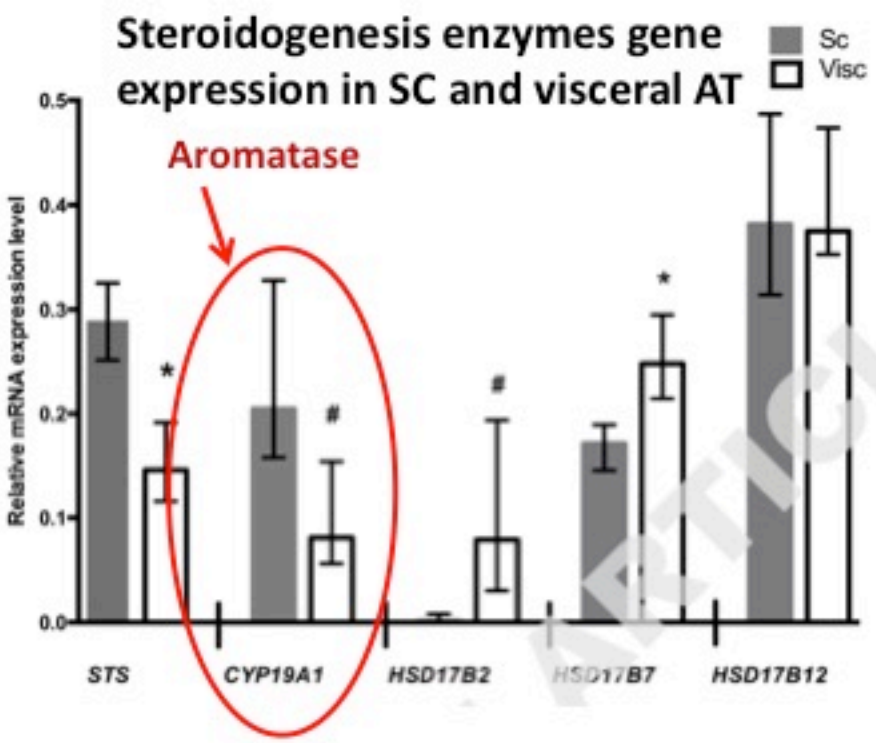
CYP19A1 (Aromatasi)



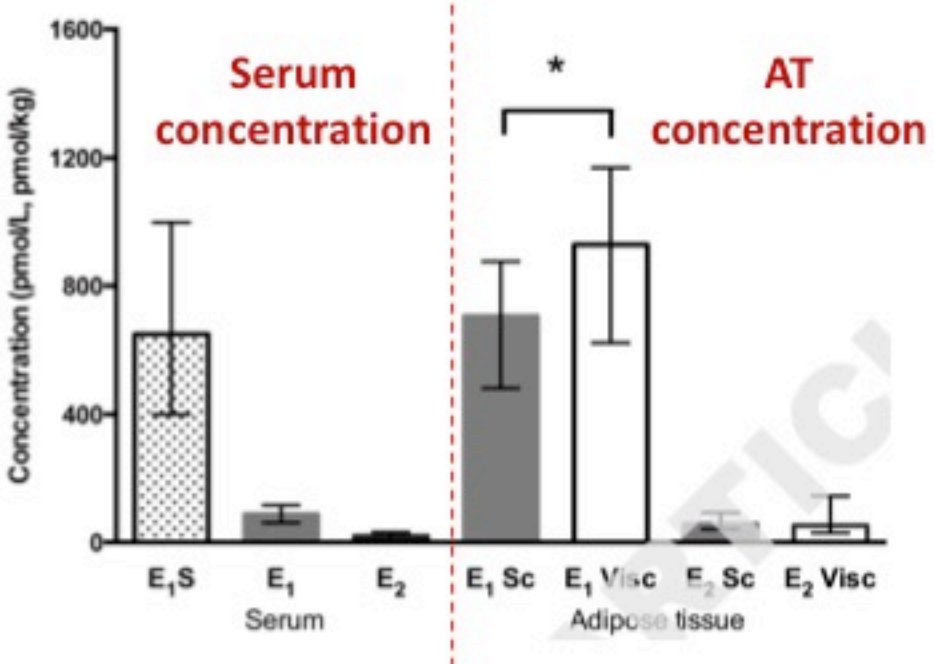
GAPDH (housekeeping)







1) Aromatase is expressed in adipose tissue, mainly in subcutaneous AT



2) Both Estrone and Estradiol increased with adiposity and BMI

Considerazioni

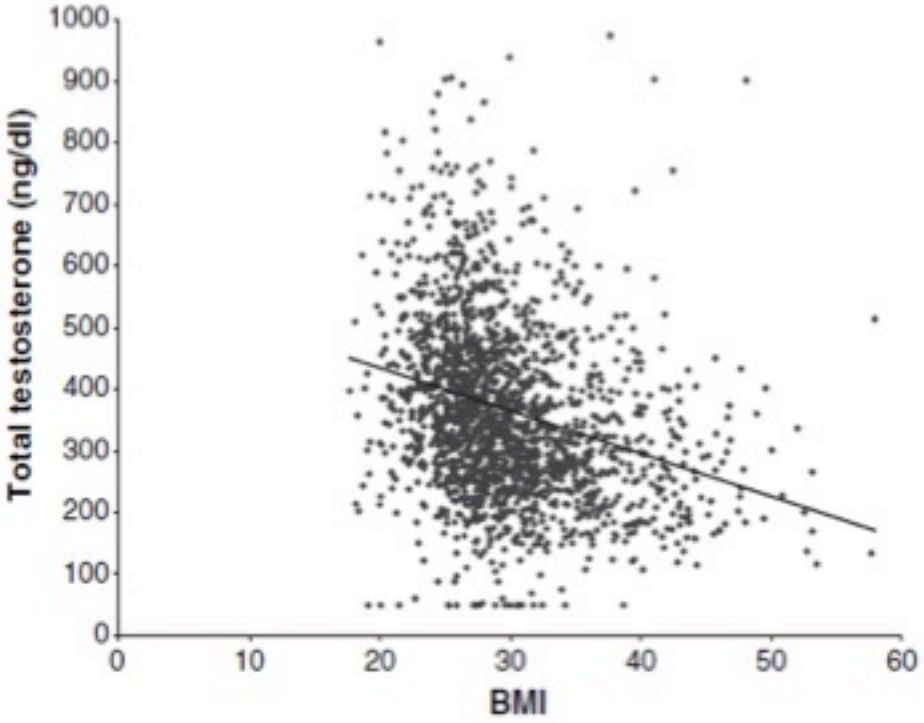
- Nel soggetto obeso è più frequente l'ipovitaminosi D rispetto al normopeso

- Le cause che inducono ipovitaminosi D possono essere ricondotte a:
  - Accumulo di 25(OH)D ma soprattutto D3 nel tessuto adiposo
  - Alterato rilascio di 25(OH)D e D3 dal tessuto adiposo insulino-resistente dell'obeso

Prevalence of hypogonadism in males aged at least 45 years: the HIM study

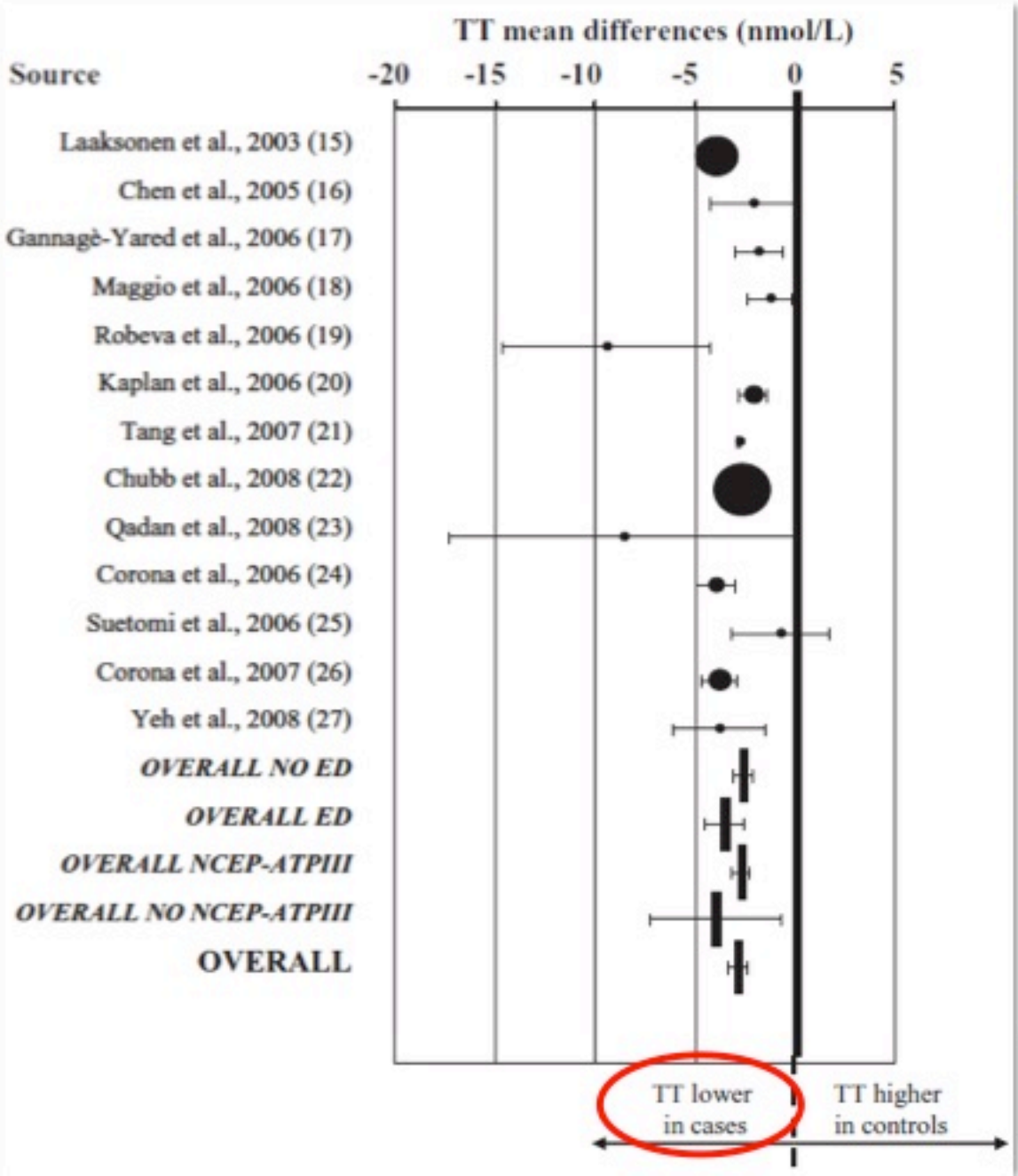
T. MULLIGAN,[1] M. F. FRICK,[2] Q. C. ZURAW,[2] A. STEMHAGEN,[2] C. MCWHIRTER[3]
[1]Division of Geriatrics, Malcom Randall VAMC GRECC and University of Florida, Gainesville, FL, [2]Global Clinical Practice and Strategic Development Services, Covance Periapproval Services, Inc., Radnor, PA, [3]Men's Health Clinical Development & Medical Affairs, Solvay Pharmaceuticals, Marietta, GA, USA

- Estimate the prevalence of hypogonadism in men ≥45 years in primary care
- 2,165 men (mean age 60.5±10.3 years)
- Hypogonadism defined as:
  - TT <300 ng/dL
  - FT <52 pg/mL
- Prevalence rate – 36.3% based on TT
  - 40% based on FT
  - 45% based on BAT



Mulligan T, et al. Int J Clin Pract 2006;60(7):762-769

MetS and testosterone



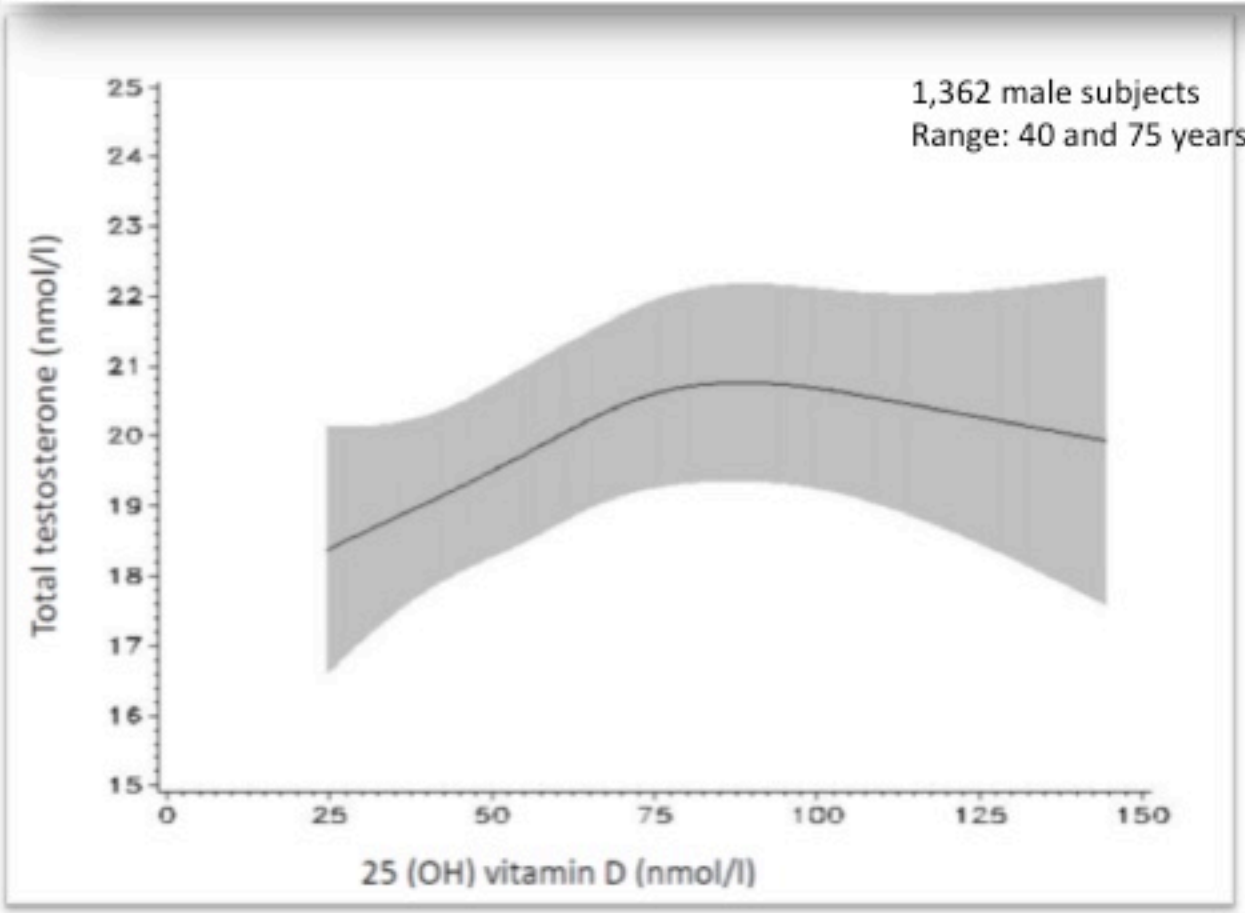
MetS patients have significantly lower T plasma levels

Corona G et al. J Sex Med 2011

ORIGINAL ARTICLE

Association between plasma 25-OH vitamin D and testosterone levels in men

Katharina Nimptsch*[,1], Elizabeth A. Platz[2], Walter C. Willett*[,§,¶] and Edward Giovannucci*[,§,¶]



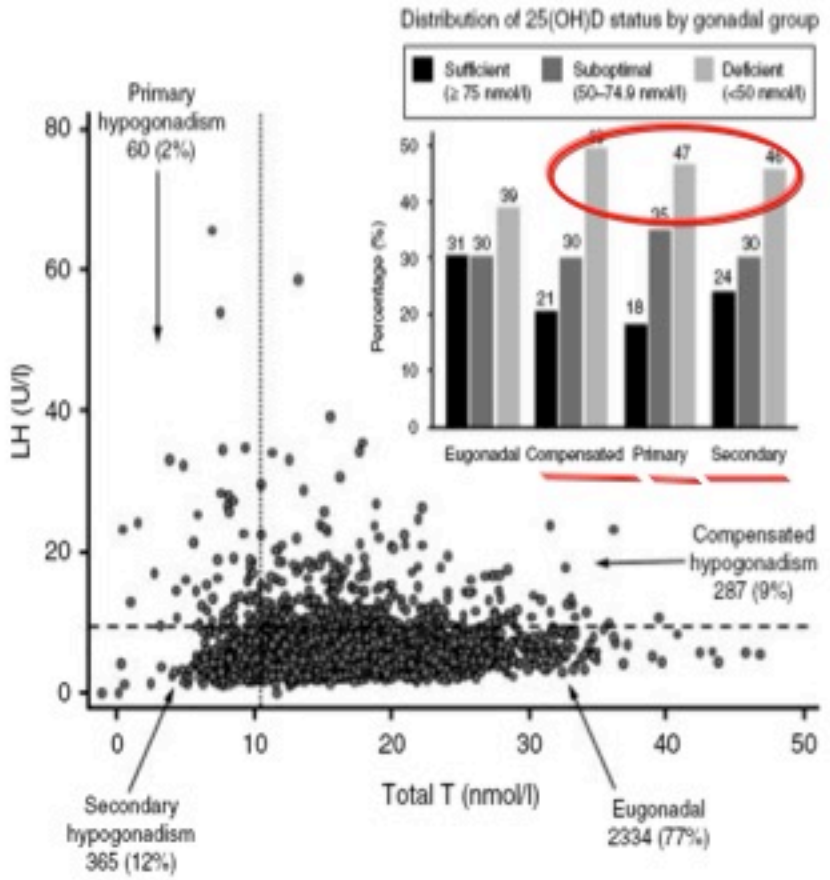
The association between 25(OH)-vitamin D and total and free testosterone is linear at lower levels of 25(OH)-vitamin D, reaching a plateau at higher levels

European Journal of Endocrinology (2012) 166 77–85 ISSN 0804-4643

CLINICAL STUDY

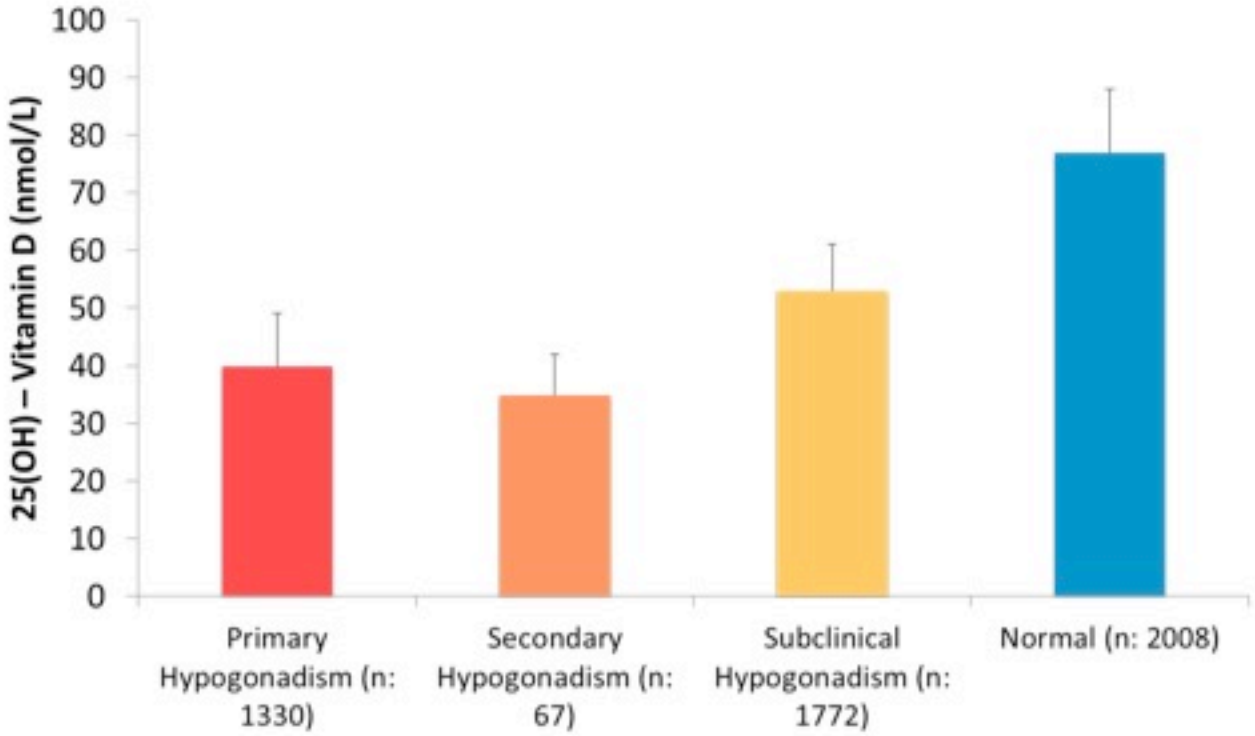
Association of hypogonadism with vitamin D status: the European Male Ageing Study

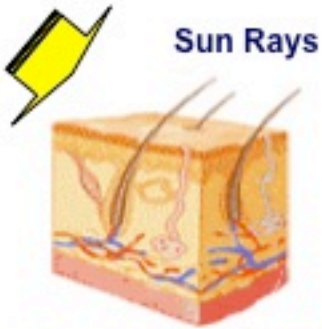
David M Lee, Abdelouahid Tajar, Stephen R Pye, Steven Boonen[1], Dirk Vanderschueren[2], Roger Bouillon[3], Terence W O'Neill, Gyorgy Bartfai[4], Felipe F Casanueva[5,6], Joseph D Finn[7], Gianni Forti[8], Aleksander Giwercman[9], Thang S Han[10], Ilpo T Huhtaniemi[11], Krzysztof Kula[12], Michael E J Lean[13], Neil Pendleton[14], Margus Punab[15] and Frederick C W Wu[7], the EMAS study group[‡]



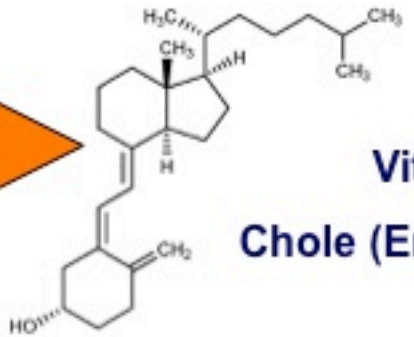
3,369 male subjects
Range: 40 and 79 years

25(OH)–Vitamin D
(n: 5,177)





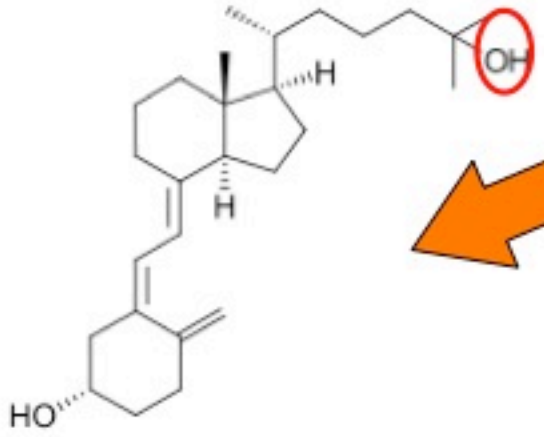
Sun Exposure (~90%)



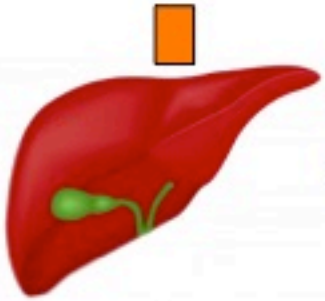
Vitamin D

Chole (Ergo)-calciferol

Nutritional (~10%)



25OH D

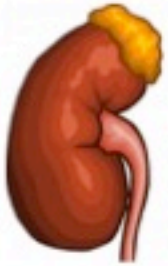


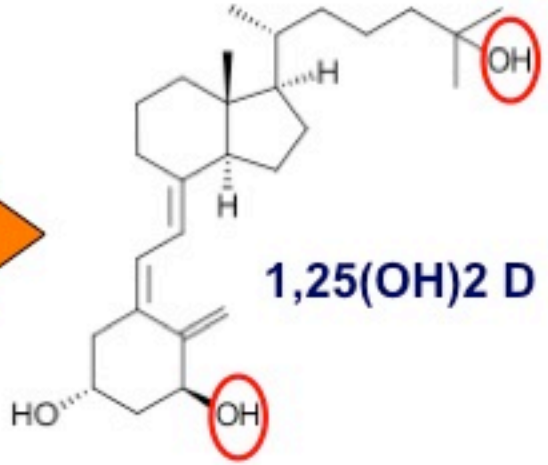
Mitochondrial CYP27A1

Highest expression in liver

Mitochondrial CYP27B1

Highest expression in kidney





1,25(OH)2 D

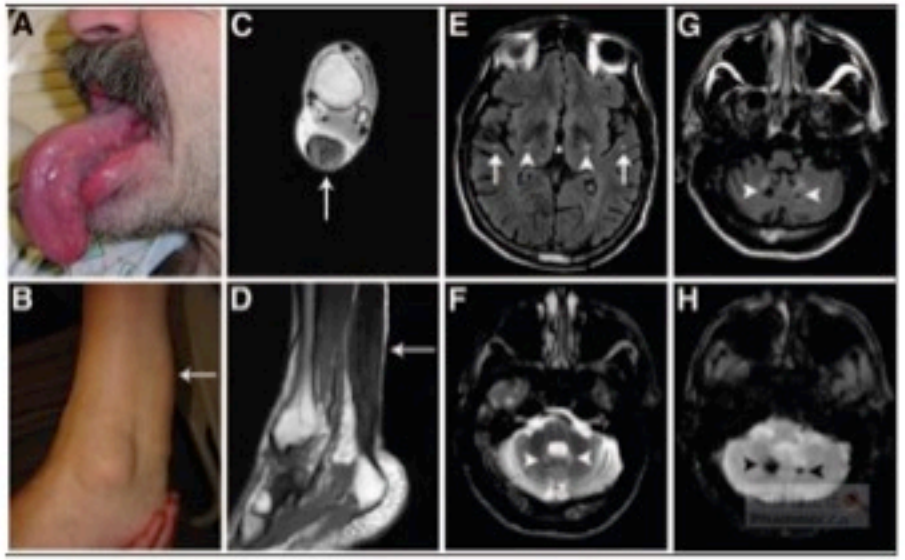
Inactivation of *CYP27A1*: Evidences from Human Models

Mutational analysis of CYP27A1: assessment of 27-hydroxylation of cholesterol and 25-hydroxylation of vitamin D

Ram P. Gupta[a], Kennerly Patrick[b], Norman H. Bell[a,*]

Metabolism Clinical and Experimental 56 (2007) 1248–1255

In humans, mutations of the *CYP27A1* gene cause cerebrotendinous xanthomatosis (CTX), an autosomal recessive disorder characterized by abnormal synthesis of bile acids with development of cataracts, tendon xanthomas, and progressive neurologic deterioration [12-14]. Some patients with CTX have a low or low normal serum 25-OHD and a low bone mass.



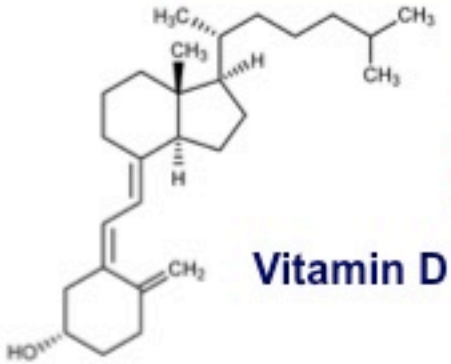
Role of *CYP2R1* in Vit D Metabolism

Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase

Jeffrey B. Cheng*, Michael A. Levine†, Norman H. Bell‡, David J. Mangelsdorf§¶, and David W. Russell*||

PNAS | May 18, 2004 | vol. 101 | no. 20 | 7711–7715

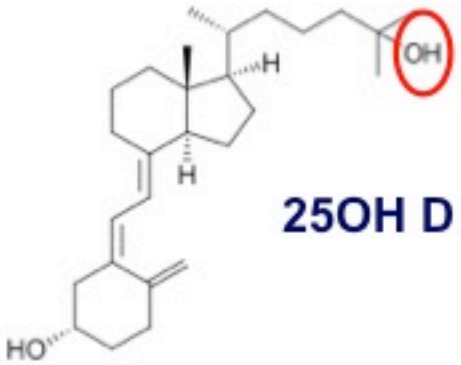
In the present study, we describe the molecular analysis of a patient with abnormally low plasma levels of 25-hydroxyvitamin D_3 and classic symptoms of vitamin D deficiency, including skeletal abnormalities, hypocalcemia, and hypophosphatemia (17, 18). This individual is homozygous for a substitution mutation in exon 2 of the CYP2R1 gene on chromosome 11p15.2. The CYP2R1 mutation changes a leucine residue at position 99 to a proline and eliminates the vitamin D_3 25-hydroxylase activity of CYP2R1.



Vitamin D

Microsomal CYP2R1

!



25OH D

Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase

Jeffrey B. Cheng*, Michael A. Levine†, Norman H. Bell‡, David J. Mangelsdorf§¶, and David W. Russell*||

PNAS | May 18, 2004 | vol. 101 | no. 20

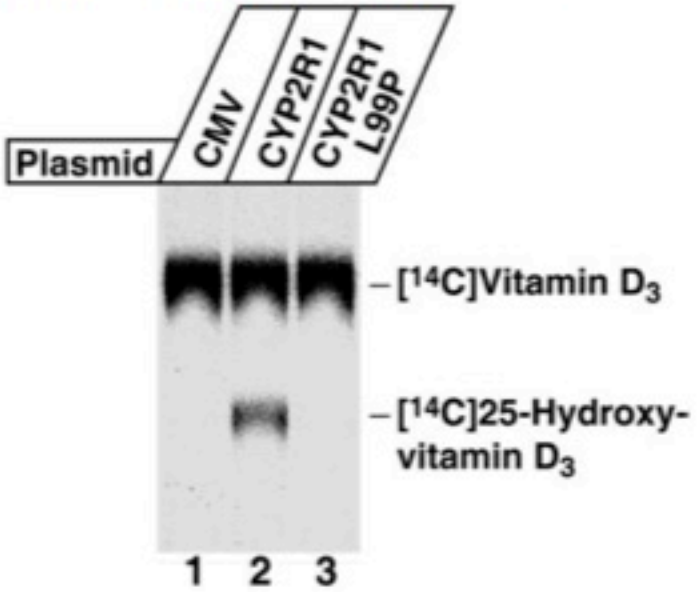


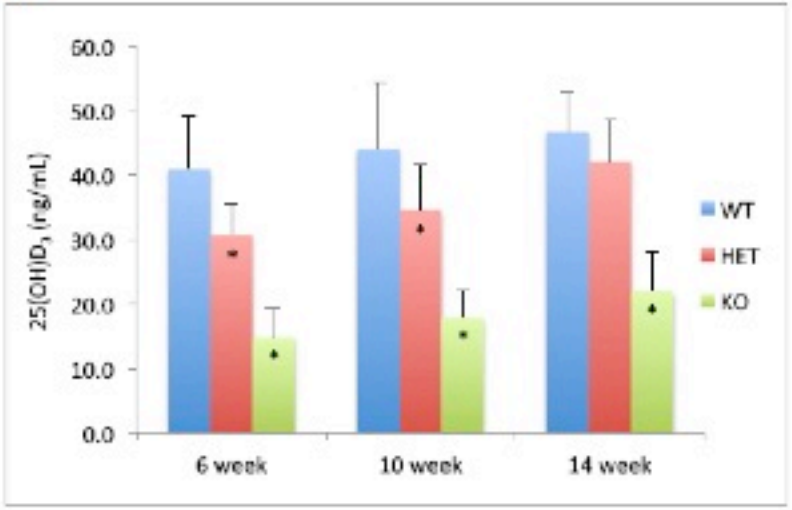
Fig. 3. Biochemical assay of CYP2R1 vitamin D 25-hydroxylase enzyme activity. HEK 293 cells were transfected with the indicated expression plasmids for a period of 18–20 h. Thereafter, the medium was made 4.6×10^{-7} M in [4-^{14}C]vitamin D_3 and the incubation continued for an additional 96 h. Lipids were extracted from cells and medium into chloroform:methanol (2:1, vol/vol), and vitamin D metabolites and standards were separated by TLC on 150-Å silica gel plates (Whatman, catalog no. 4855–821) in a solvent system containing cyclohexane:ethyl acetate (3:2, vol/vol). After development, radioactivity was detected by PhosphorImager analysis, and the positions to which authentic vitamin D_3 and 25-hydroxyvitamin D_3 migrated on the plate were determined by staining with iodine.

CYP2R1 is a major, but not exclusive, contributor to 25-hydroxyvitamin D production in vivo

Jinge G. Zhu*, Justin T. Ochalek*, Martin Kaufmann*, Glenville Jones†, and Hector F. DeLuca*‡

PNAS | September 24, 2013 | vol. 110 | no. 39

A

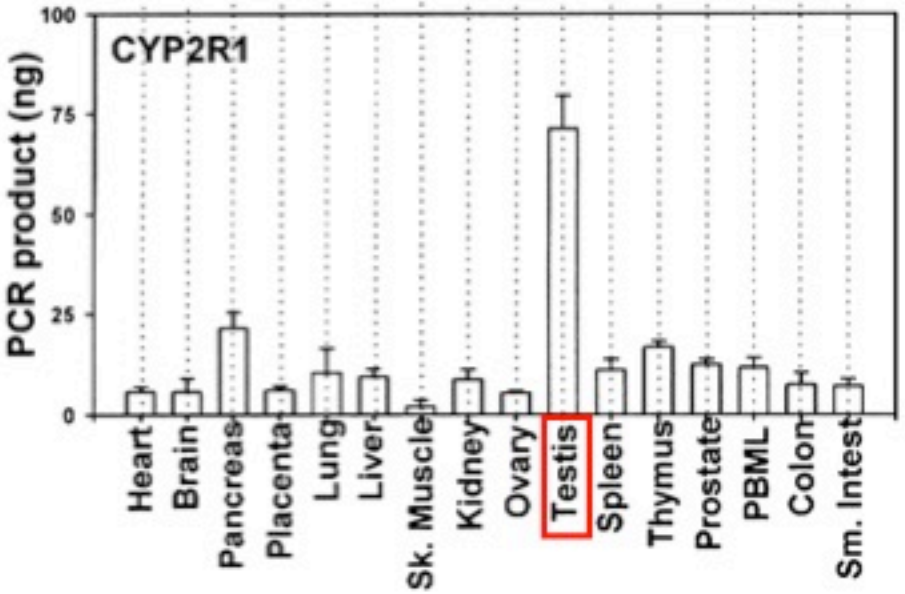


B

A known inactivating mutation (Leu99Pro) of CYP2R1 associates to

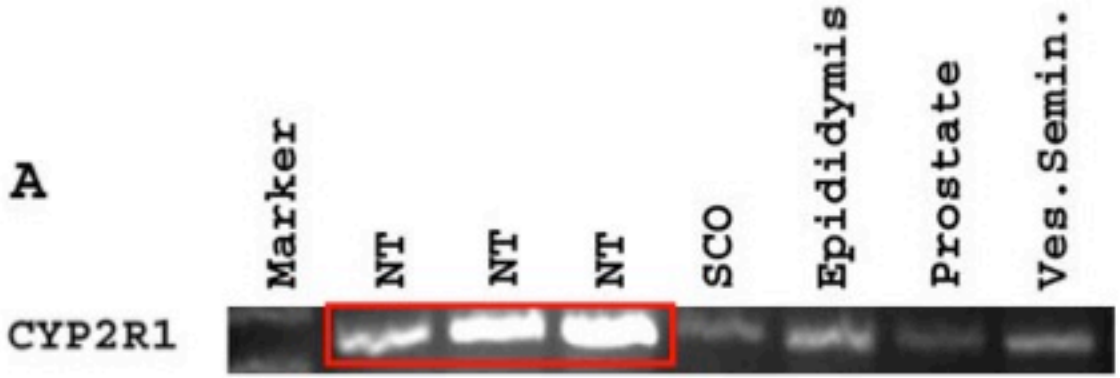
- **deficiency of vitamin D**
- **defective calcium homeostasis**
- **classical bone lesions referred to as rickets**

Gene Expression Pattern of *CYP2R1*



Expression patterns of mouse and human CYP orthologs during development and in diVerent adult tissues

(Choudhary D *2005 Archives of Biochemistry and Biophysics*)

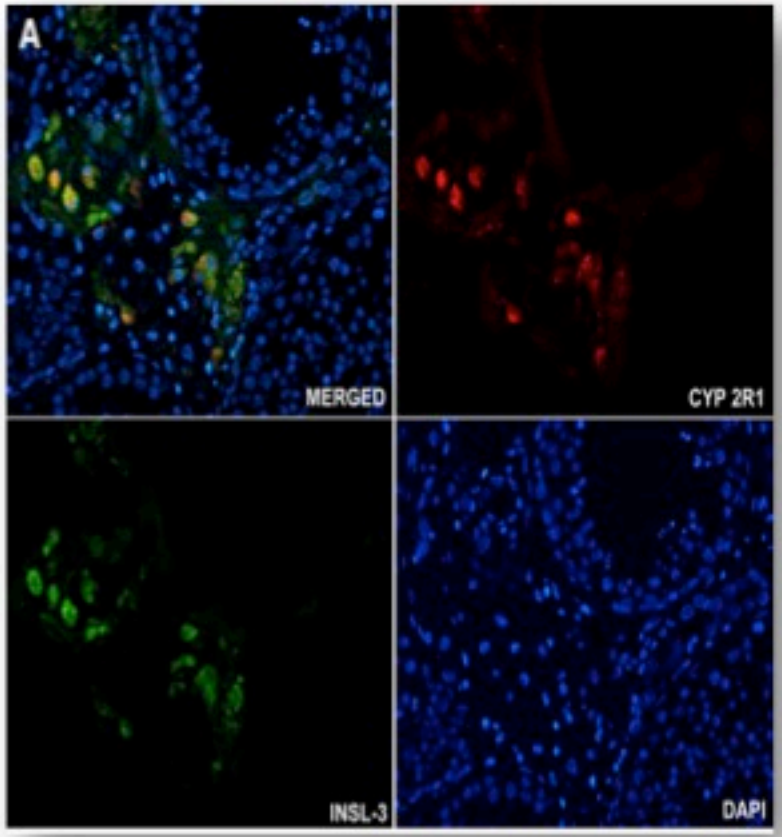


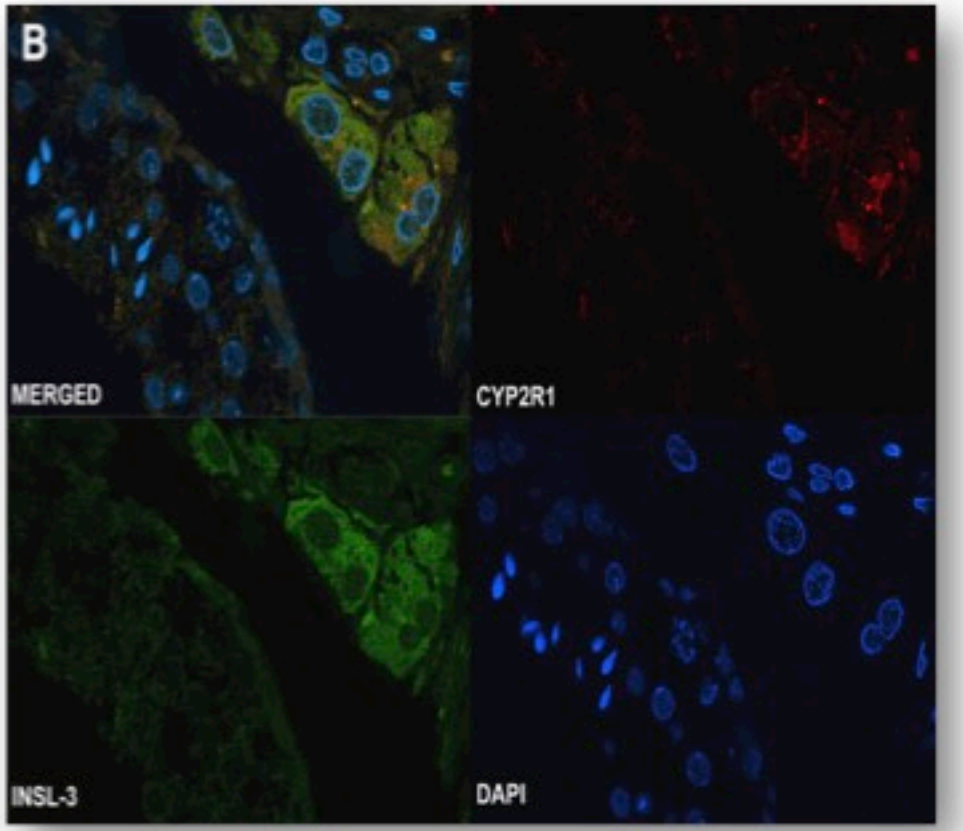
NT: Normal Testis

Bone Mineral Density and Testicular Failure: Evidence for a Role of Vitamin D 25-Hydroxylase in Human Testis

Carlo Foresta, Giacomo Strapazzon, Luca De Toni, Lisa Perilli,
Antonella Di Mambro, Barbara Muciaccia, Leonardo Sartori, and Riccardo Selice

J Clin Endocrinol Metab, April 2011, 96(4):E646–E652





Testiculopathy and vitamin D insufficiency

*Carlo Foresta, Riccardo Selice, Antonella Di Mambro, Giacomo Strapazzon

THE LANCET

Vol 376 October 16, 2010

	Controls (41)	Bilateral orchiectomized patients (15)
25OH D (nmol/L)	74.9 ±38.8	30.2 ±16.3 *

*P< 0.05 vs controls

Clinical features of patients:

- Age matched with controls (34.8±6.4 *vs* 35.8±6.2 years)
- Radical orchiectomy for bilateral testicular cancer without chemo- or radiotherapy
- No nutritional derangements
- Properly compensated with testosterone-replacement therapy

Bone Mineral Density and Testicular Failure: Evidence for a Role of Vitamin D 25-Hydroxylase in Human Testis

J Clin Endocrinol Metab, April 2011, 96(4):E646–E652

Carlo Foresta, Giacomo Strapazzon, Luca De Toni, Lisa Perilli, Antonella Di Mambro, Barbara Muciaccia, Leonardo Sartori, and Riccardo Selice

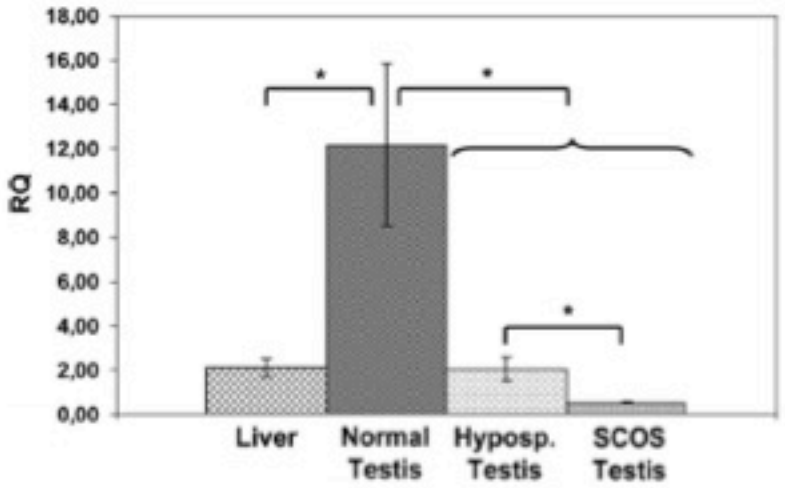
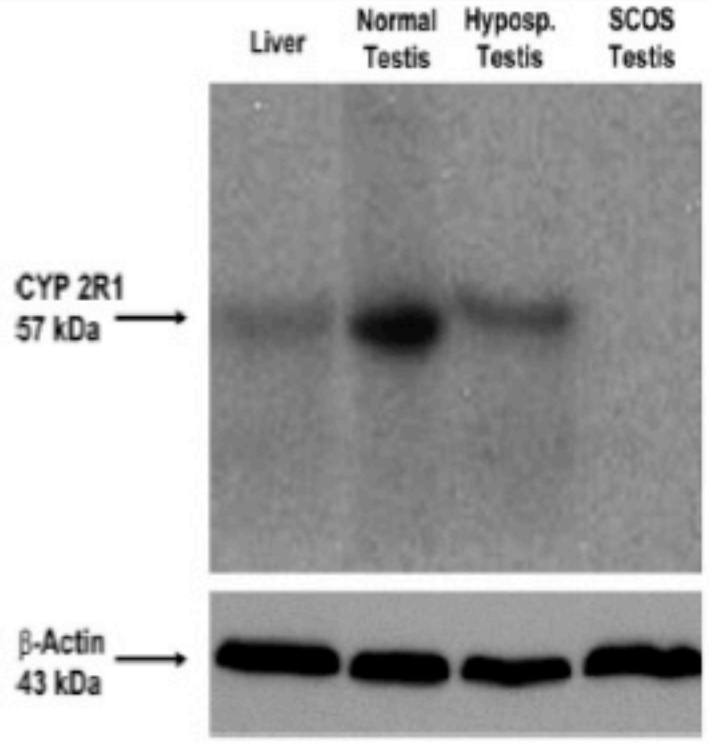


FIG. 1. Gene expression analysis. mRNA expression of *CYP2R1* in normal testis, testis affected by hypospermatogenesis (Hyposp. Testis), and testis affected by Sertoli cells only syndrome (SCOS Testis) compared with commercial liver cDNA library used as control for gene expression. Gene expression was normalized on *β-actin*. Significance (Student's *t* test): *, $P < 0.05$. Results are given mean ± SD. RQ, relative quantity.



→ Primary testiculopathy: reduced expression of CYP2R1

	Controls	Severe Hypospermatogenesis	SCOS
OsteoPENIA	0/41 (0%)	3/36 (22.2%)	8/21 (38.1%)
OsteoPOROSIS	0/41 (0%)	2/36 (5.6%)	4/21 (19.0%)
Bone disorders (Osteopenia + Osteoporosis)	0/41 (0%)	10/36 (27.8%)*	12/21 (57.1%)*

*P<0.0001 vs controls

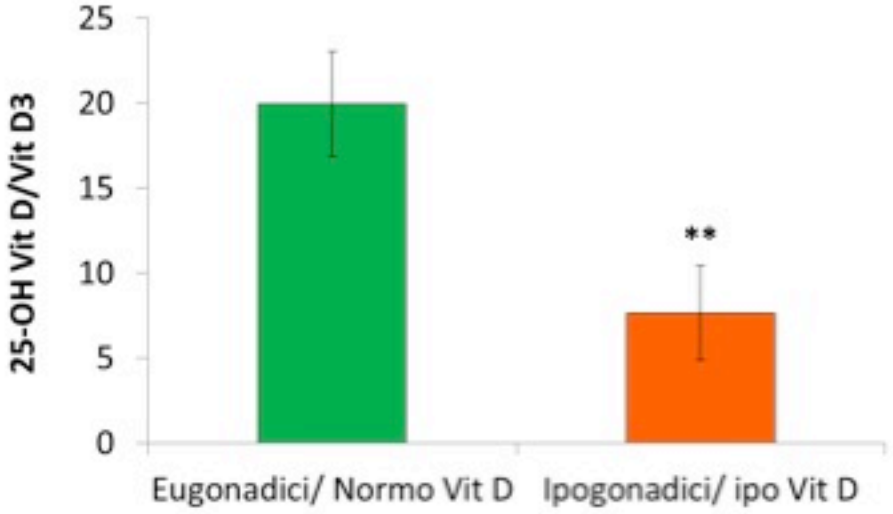
Testiculopathic men show low 25OH Vit D (due to low CYP2R1 activity) and high risk of osteoporosis/penia, despite normal T levels (compensated hypogonadism)

Foresta et al., JCEM 2011

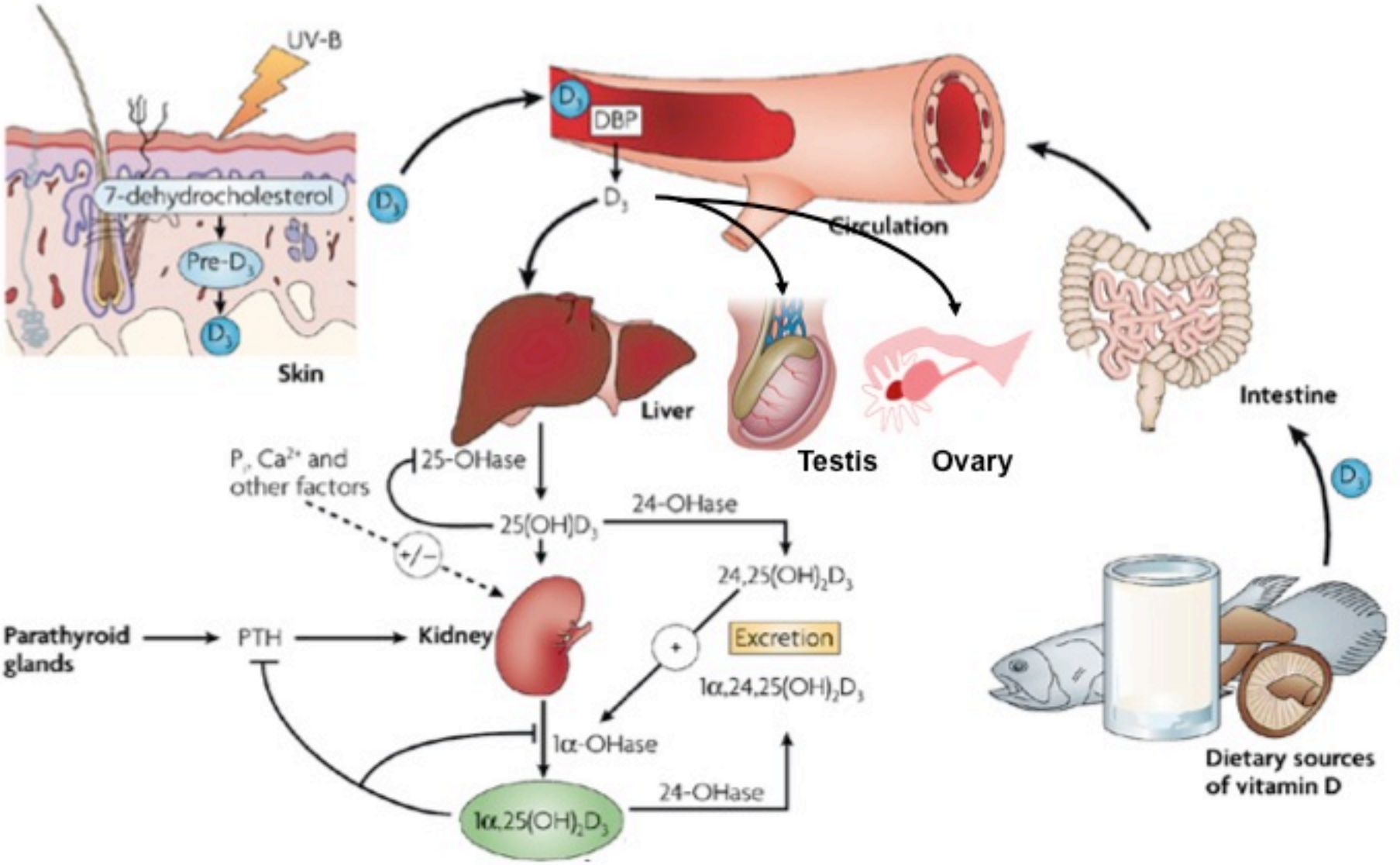
Rapporto 25(OH)D / vitamina D3 in soggetti eugonadici e ipogonadici

HPLC-MS

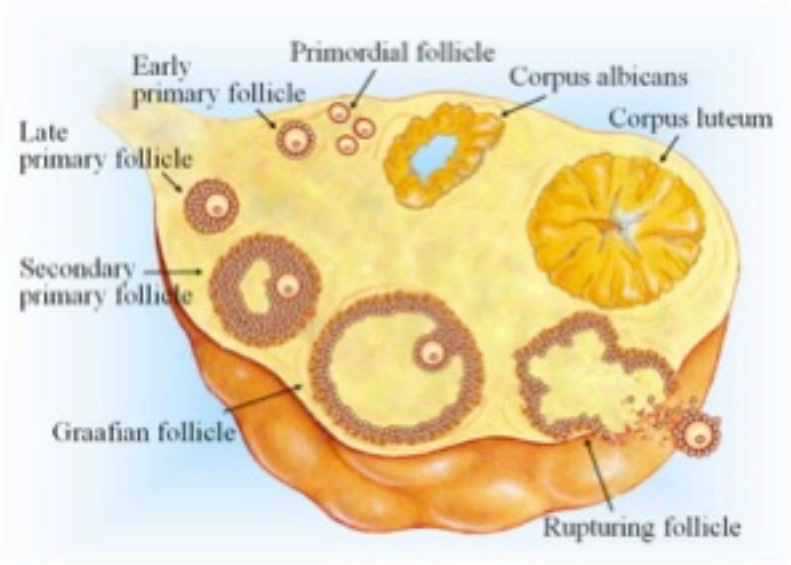
	N	D3 (nM)	25OH-Vit D (nM)	Ratio 25OH-D/ Vit D
Eugonadici	5	5,3	83	19,95
Ipogonadici	6	**7,1****	**46****	**7,65****

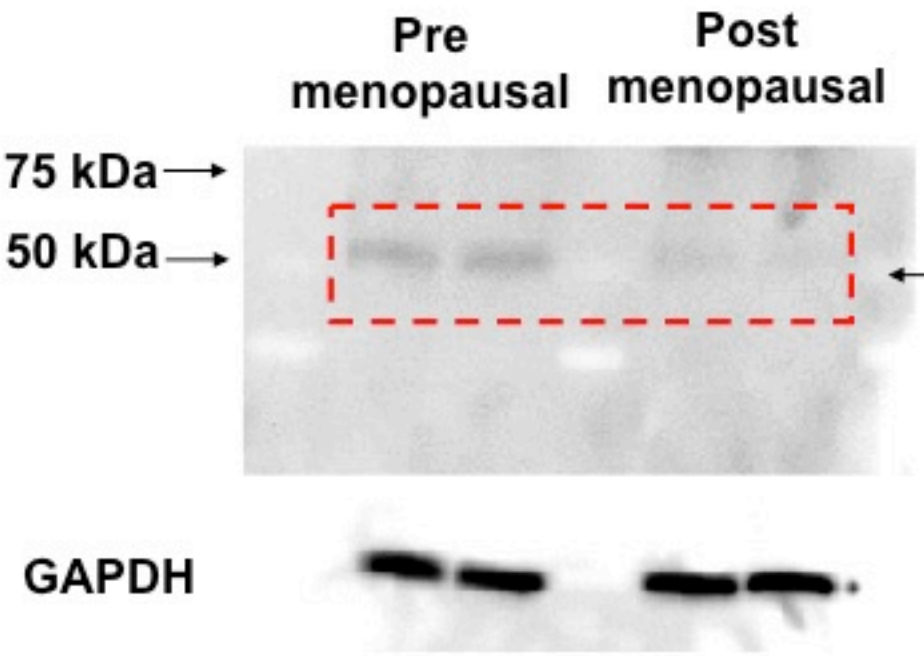


**= P<0.01


UV-B
7-dehydrocholesterol
Pre-D3
D3
Skin
D3
DBP
D3
Circulation
Liver
Testis
Ovary
Intestine
D3
P, Ca2+ and other factors
25-OHase
25(OH)D3
24-OHase
+/−
24,25(OH)2D3
Parathyroid glands
PTH
Kidney
+
Excretion
1α,24,25(OH)2D3
1α-OHase
1α,25(OH)2D3
24-OHase
Dietary sources of vitamin D

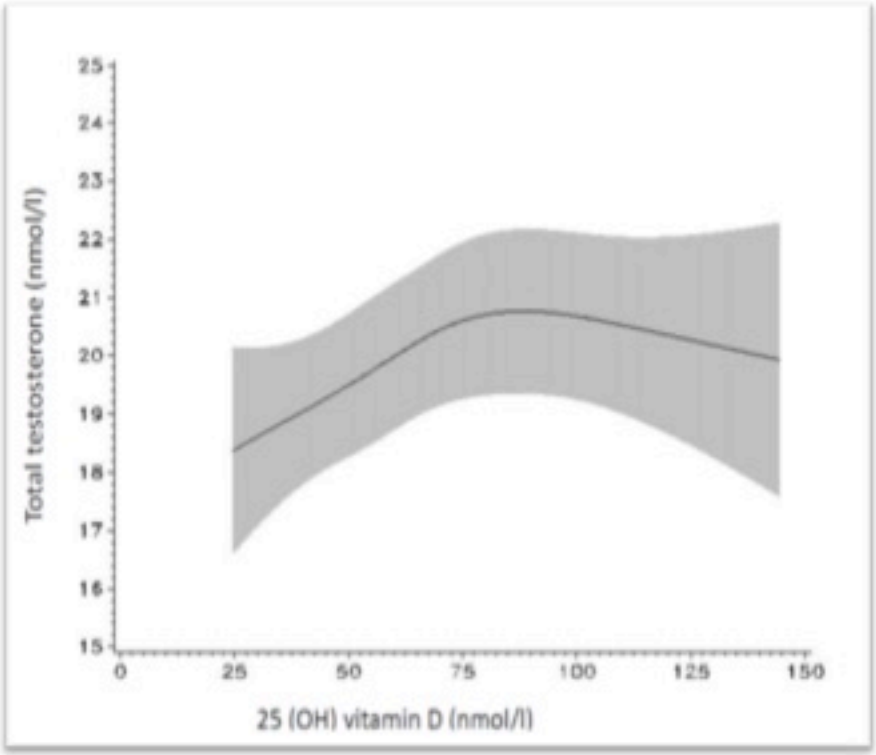
CYP2R1 protein expression in human ovary

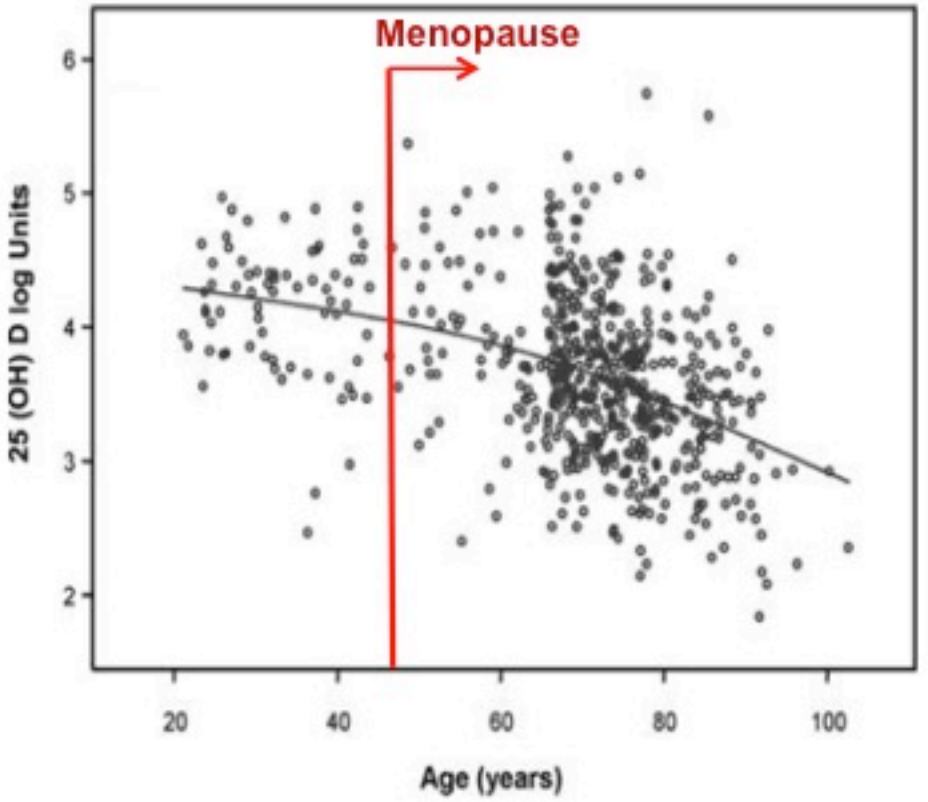




Specific signal for CYP2R1

Vitamin D and gonadal function in men and women



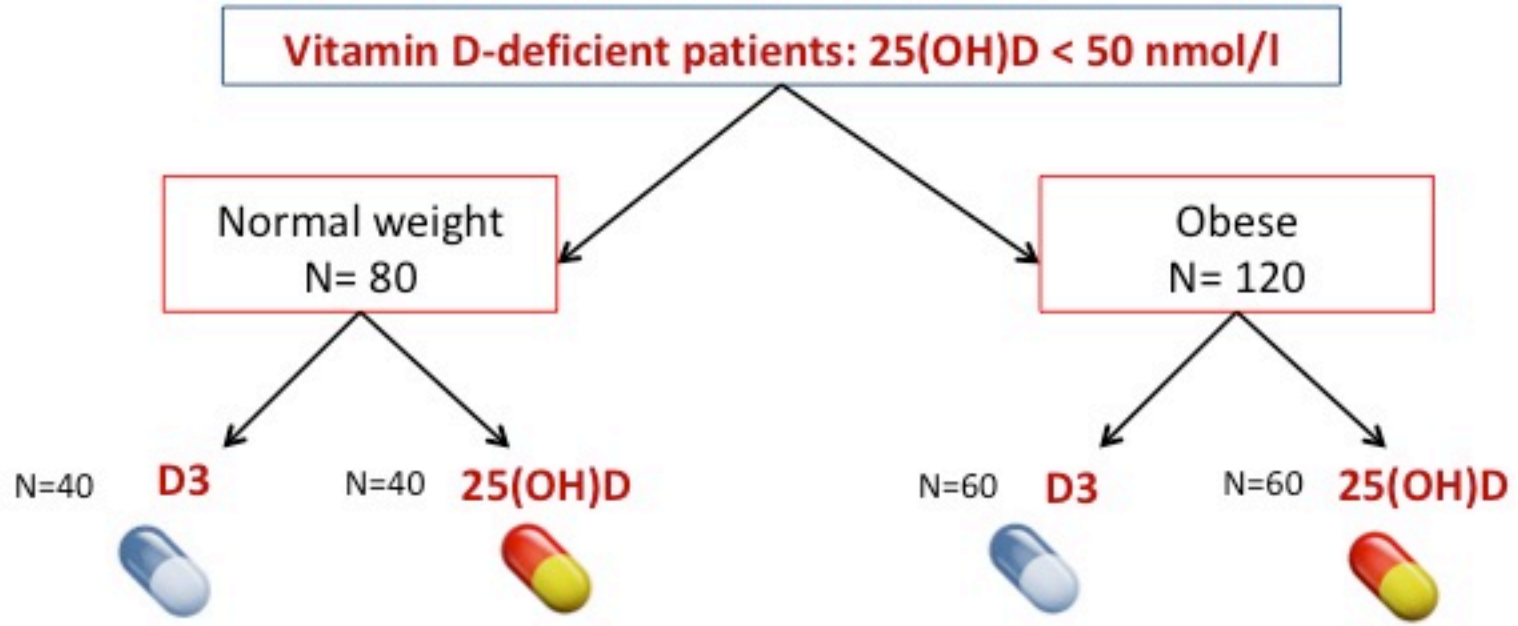


Maggio et al, J of Geront. 2005

Ipovitaminosi D:

- Ipo-calcifediolemia (↓ 25OHD)
- ipo-colecalciferolemia (↓ D3/D2)?

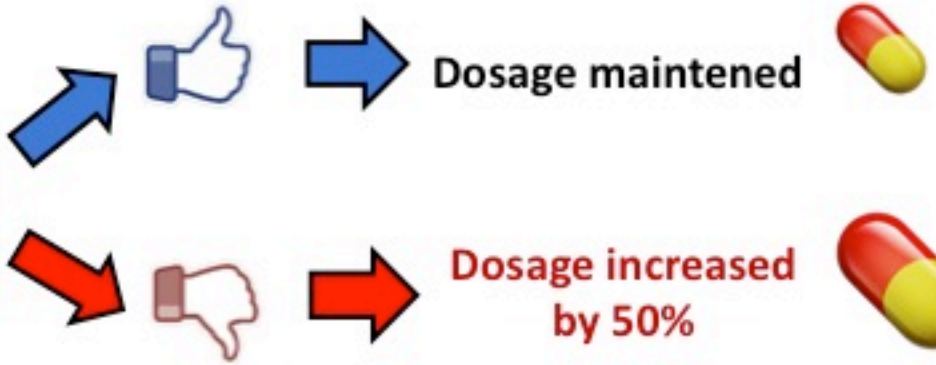
Efficacy of vitamin D supplementations (**D3 or 25OHD**) in the normalization of serum 25(OH)D levels **(>50 nmol/l)** in **normal weight and obese patients**



- D3 starting dose: 150 µg/week
- 25(OH)D starting dose: 50 µg/week

- Duration of the study: **2 years**

- **25(OH)D serum levels evaluated every 6 months: is sufficiency achieved?**



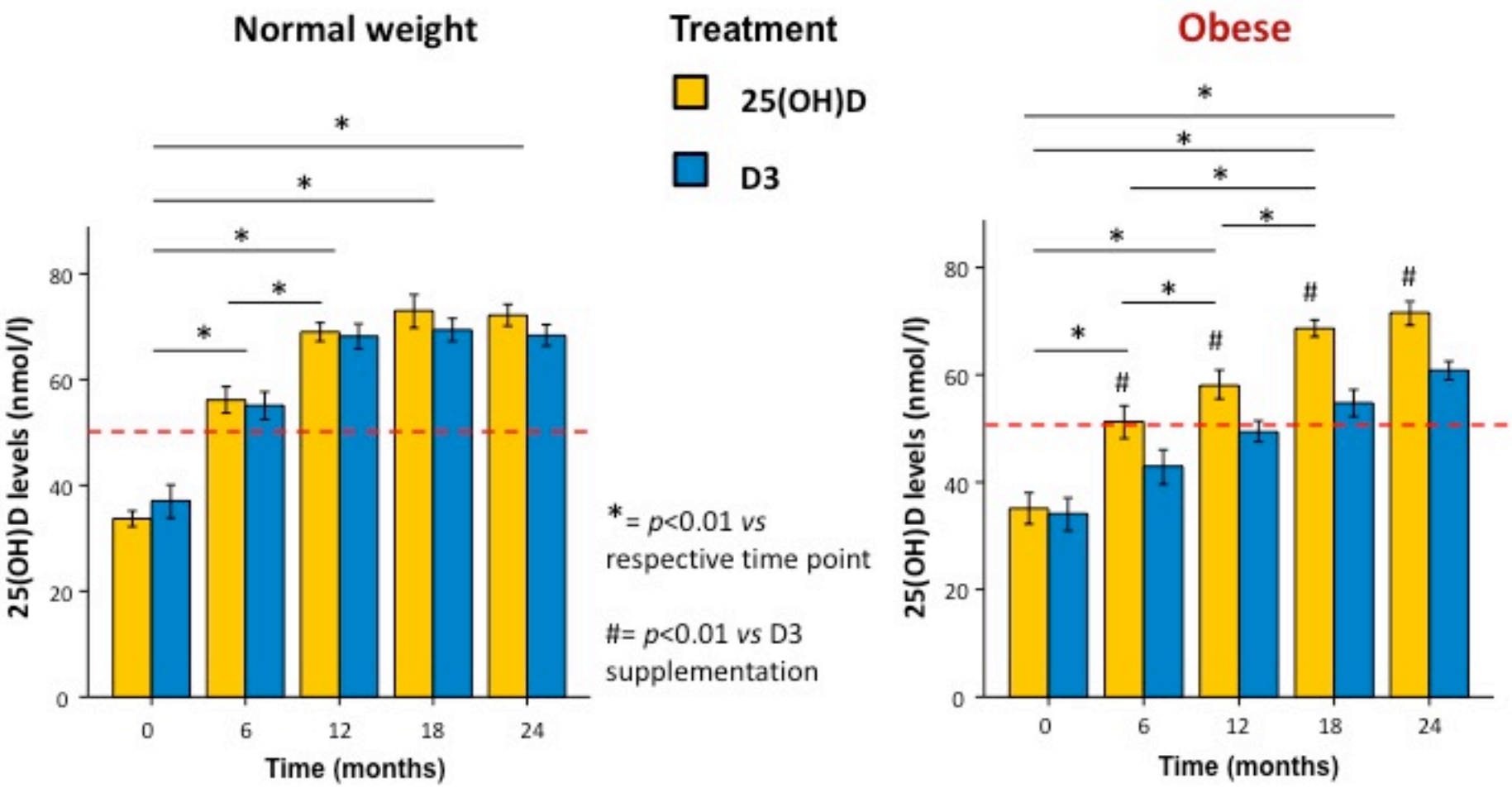
Foresta et al. JCEM 2017

Patients characteristics

	Normal weight	Obese
Age	42,13 ± 5,77	42,44 ± 4,33
BMI	22,25 ± 2,07	**33,47 ± 2,16 ***
Testosterone	16,70 ± 2,02	15,24 ± 2,70
LH	4,29 ± 2,22	3,72 ± 2,09
Estradiol	94,59 ± 20,28	**111,76 ± 37,42 ***
Fasting glucose	76,45 ± 7,76	**94,19 ± 11,83 ***
Insulin	4,34 ± 3,29	**9,56 ± 5,58 ***
HOMA	0,87 ± 0,44	**2,03 ± 0,73 ***
25(OH)	**35,36 ± 6,69**	**34,63 ± 8,54**
PTH	**83,45 ± 7,79**	**86,28 ± 7,65**
Calcium	2,36 ± 0,10	2,38 ± 0,09
Phosphorus	1,09 ± 0,24	1,01 ± 0,21

*p<0.05

Foresta et al. JCEM 2017

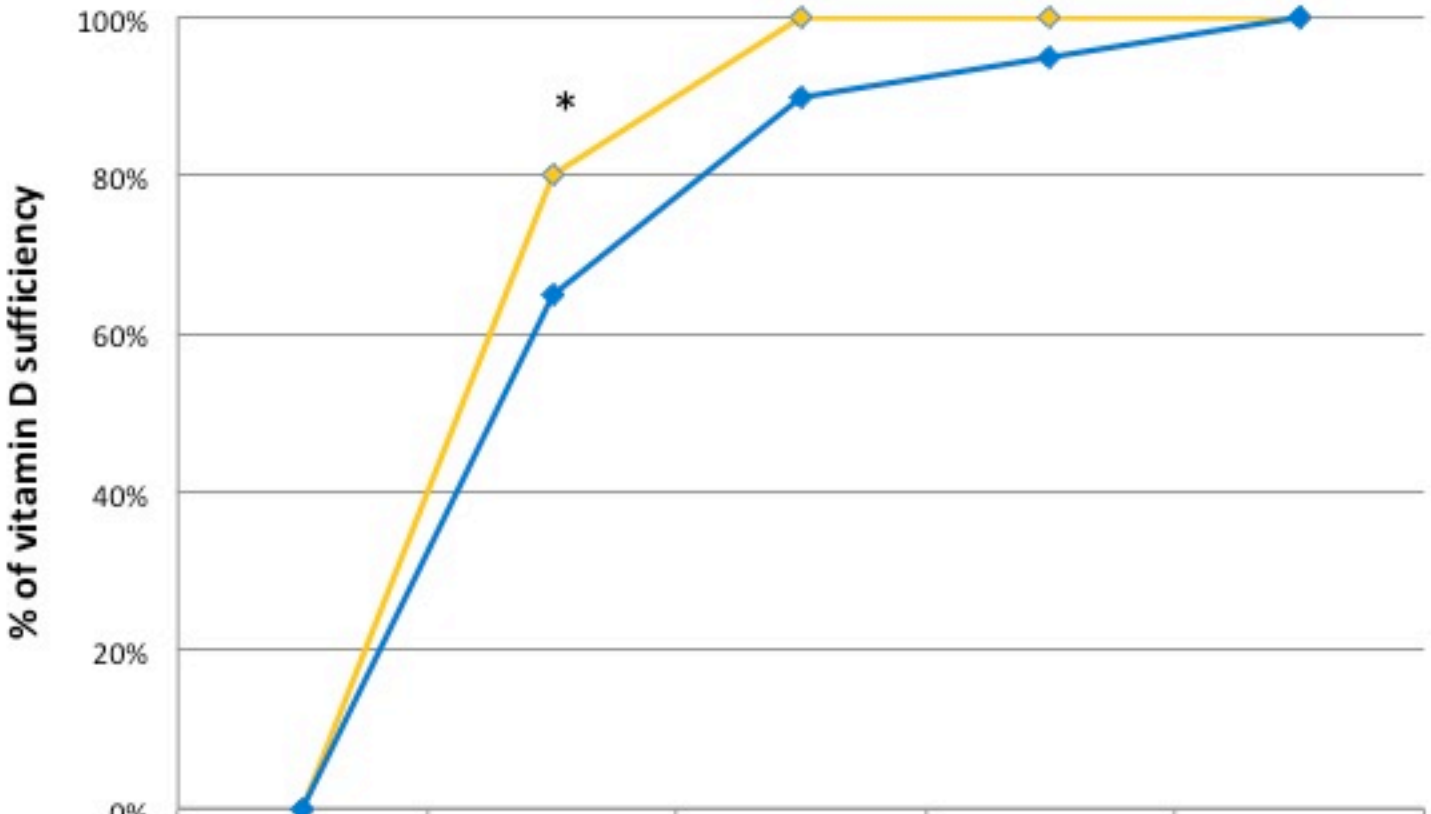


- No difference between 25(OH)D and D3 in incraesing serum 25(OH)D levels in normal weight subjects
- In obese patients, 25(OH)D is much more effective than D3 in achieving optimal 25(OH)D levels

Foresta et al. JCEM 2017

Normal weight

Prevalence of vitamin D sufficiency (25OHD > 50 nmol/l)



Treatment

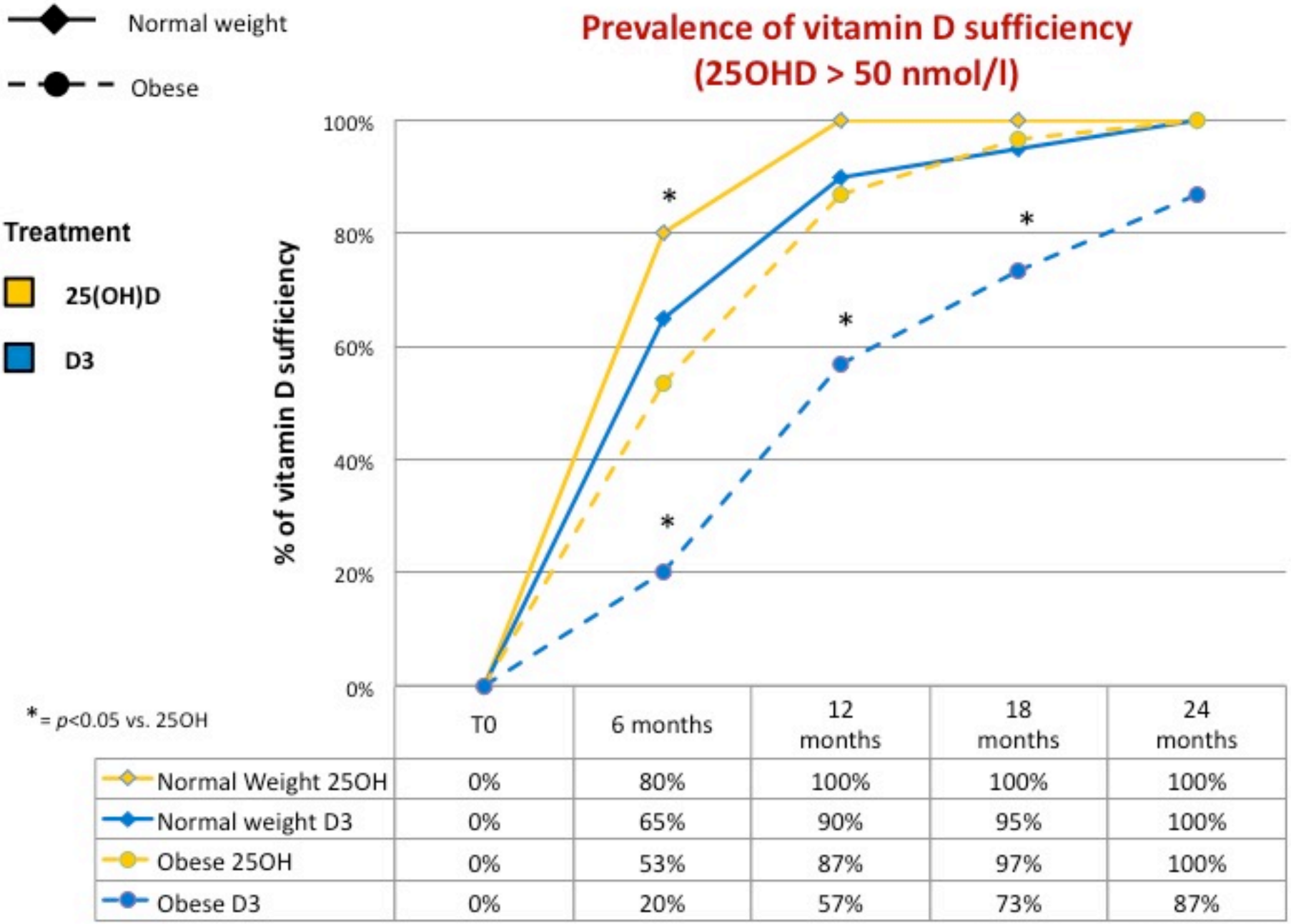
25(OH)D

D3

*= $p<0.05$ vs. 25OH

	T0	6 months	12 months	18 months	24 months
Normal Weight 25OH	0%	80%	100%	100%	100%
Normal weight D3	0%	65%	90%	95%	100%

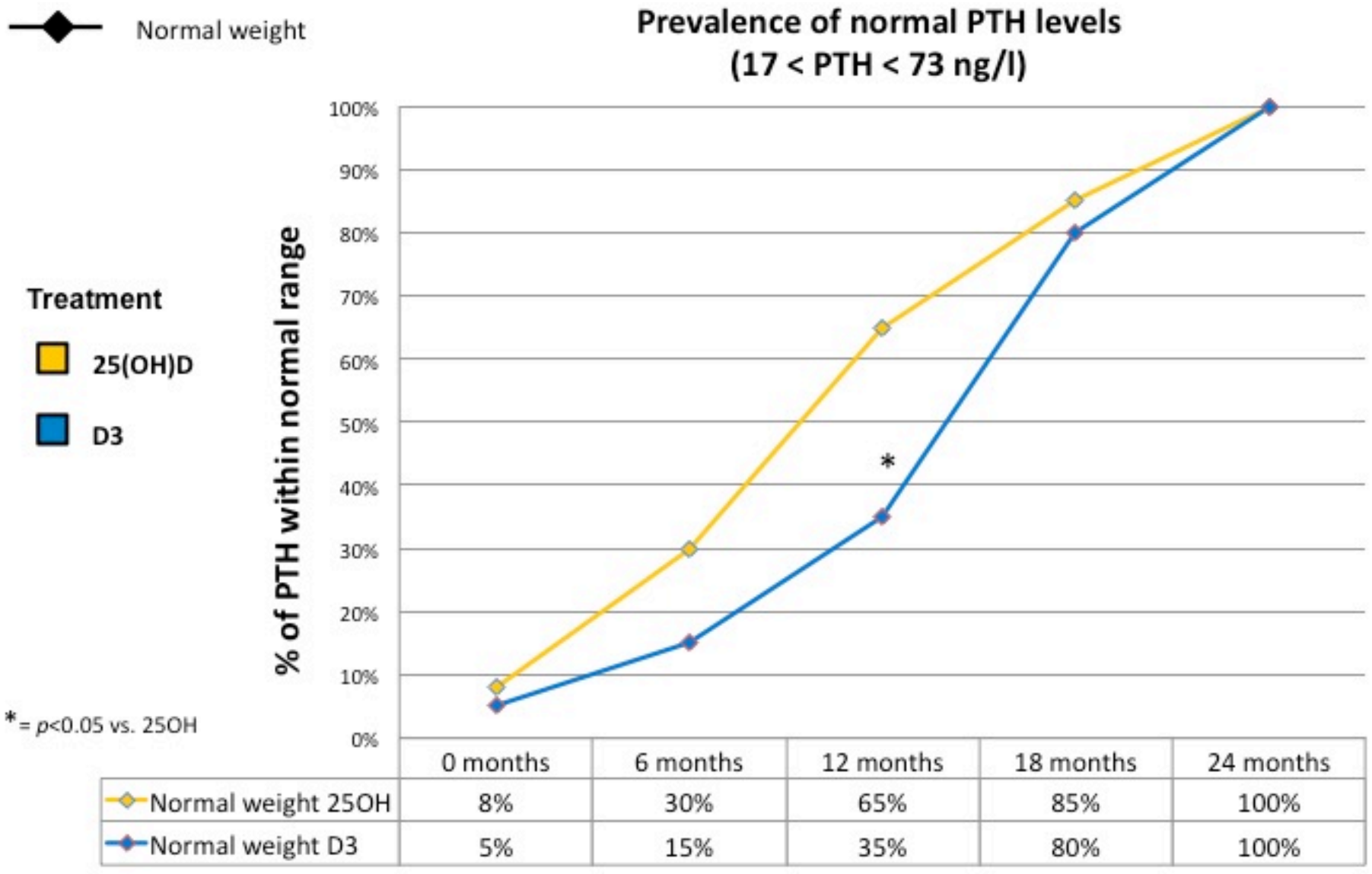
Foresta et al. JCEM 2017



*= $p<0.05$ vs. 25OH

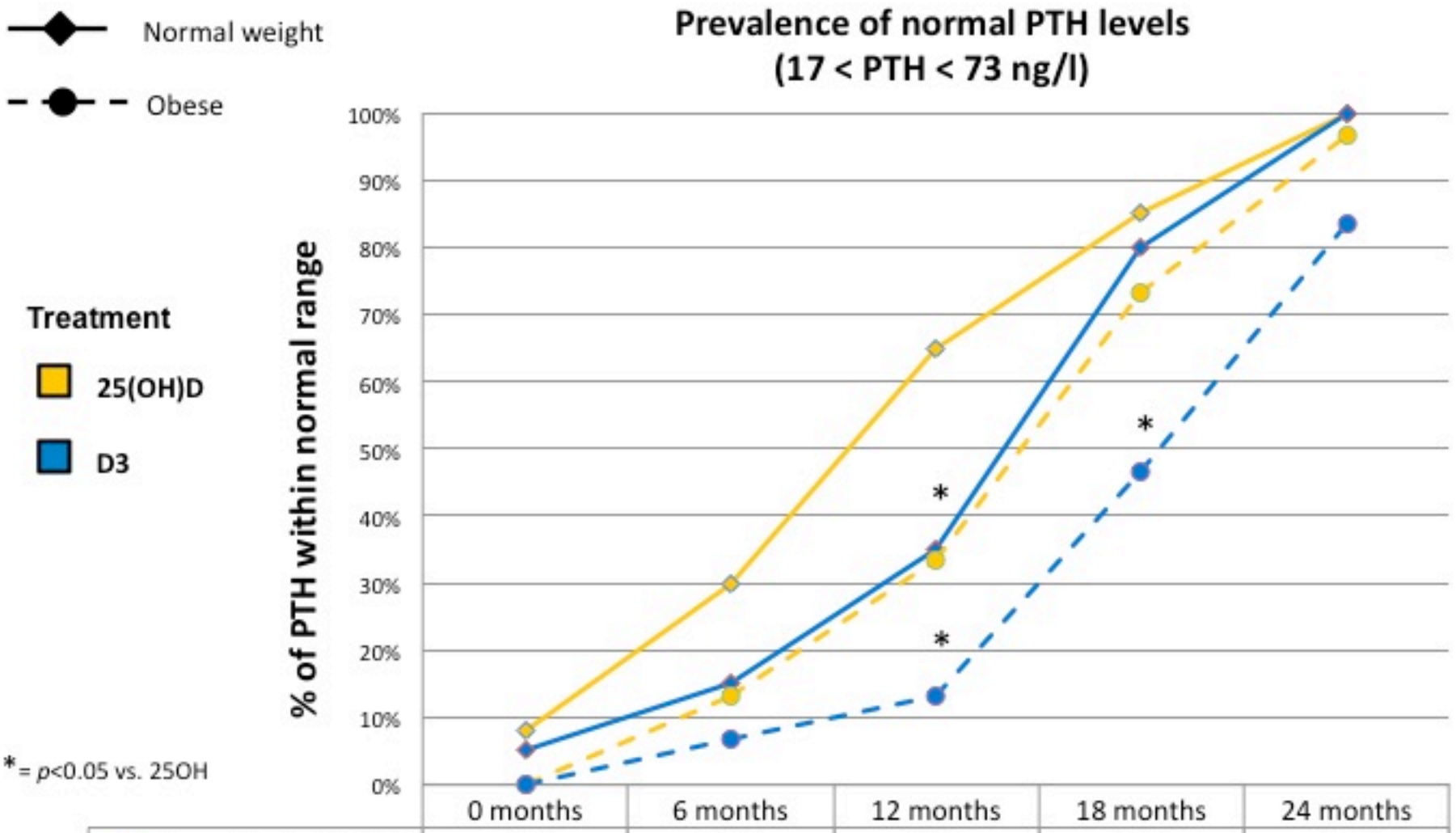
	T0	6 months	12 months	18 months	24 months
Normal Weight 25OH	0%	80%	100%	100%	100%
Normal weight D3	0%	65%	90%	95%	100%
Obese 25OH	0%	53%	87%	97%	100%
Obese D3	0%	20%	57%	73%	87%

Foresta et al. JCEM 2017



	0 months	6 months	12 months	18 months	24 months
Normal weight 25OH	8%	30%	65%	85%	100%
Normal weight D3	5%	15%	35%	80%	100%

Foresta et al. JCEM 2017



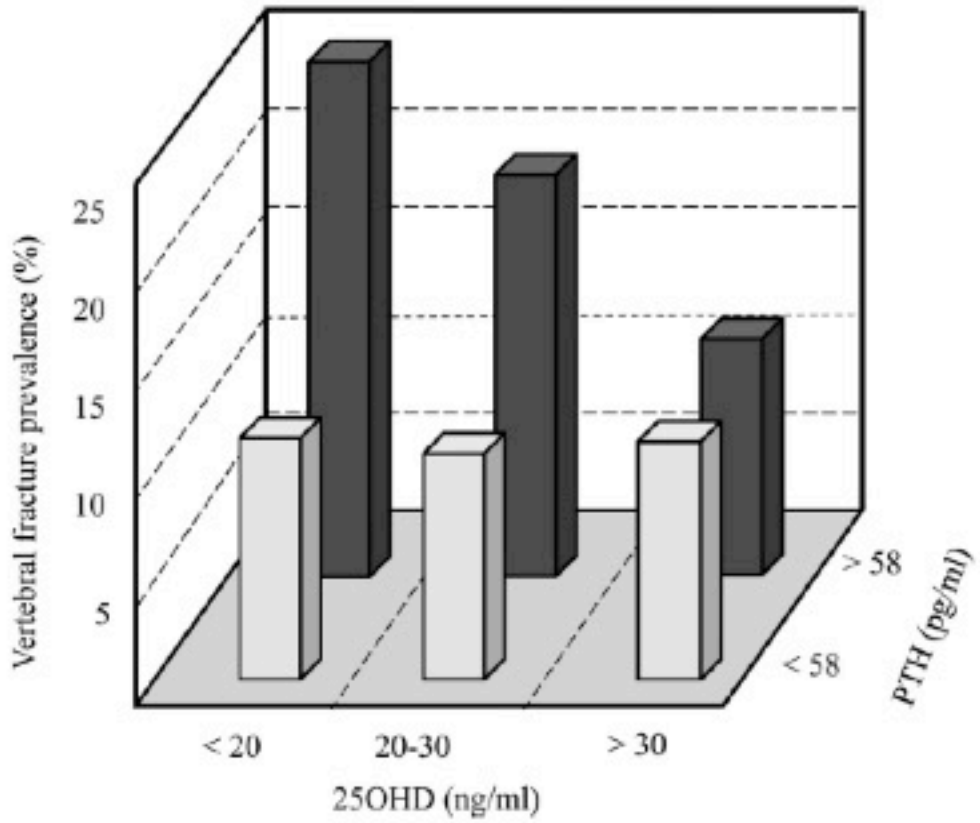
	0 months	6 months	12 months	18 months	24 months
Normal weight 25OH	8%	30%	65%	85%	100%
Normal weight D3	5%	15%	35%	80%	100%
Obese 25OH	0%	13%	33%	73%	97%
Obese D3	0%	7%	13%	47%	83%

Foresta et al. JCEM 2017

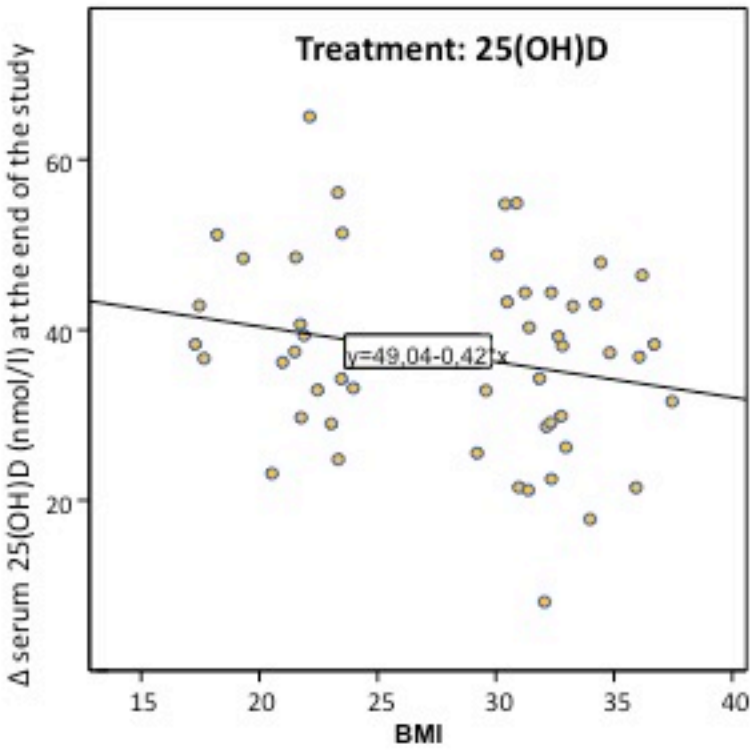
J Clin Endocrinol Metab, April 2013, 98(4):1711–1717

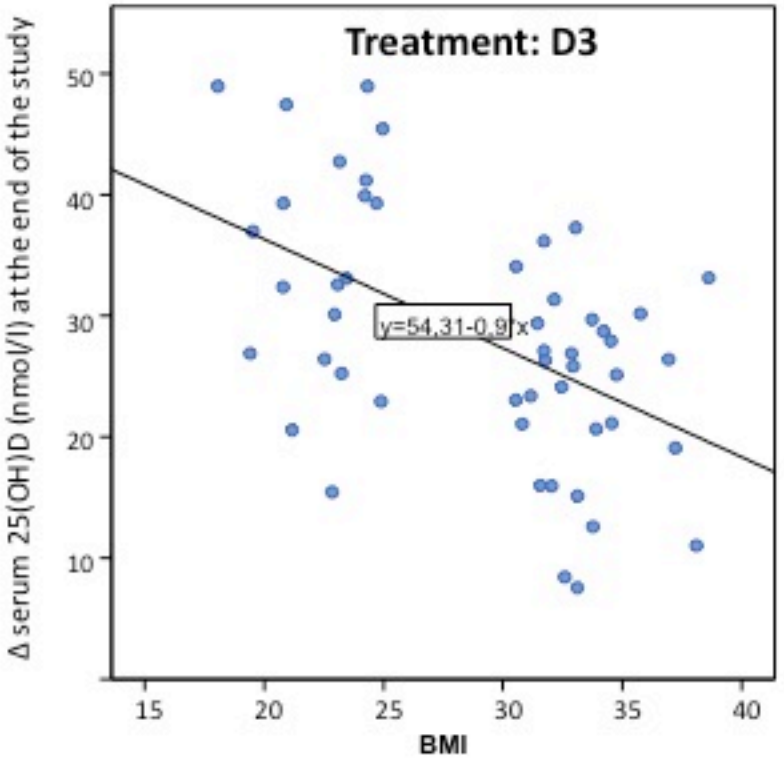
Influence of Vitamin D Status on Vertebral Fractures, Bone Mineral Density, and Bone Turnover Markers in Normocalcemic Postmenopausal Women With High Parathyroid Hormone Levels

Conclusion: Elevated PTH levels are associated with increased prevalence of vertebral fractures, low bone mass, or higher BTM only in the presence of hypovitaminosis D. An adequate nutritional status in the vitamin appears to protect the bone from the deleterious effect of a high PTH. (***J Clin Endocrinol Metab*** **98: 1711–1717, 2013)**



Correlazione tra BMI e incremento dei livelli serici di 25(OH)D a seconda della supplementazione utilizzata





Mean dosage to achieve vitamin D sufficiency at the end of the study (2 years)

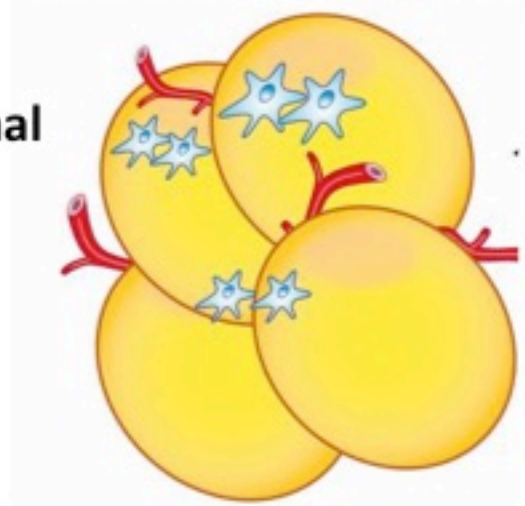
	µg/week
Normal weight 25(OH)D	**54,68**
Obese 25(OH)D	**61,72**
Normal weight D3	**175,31**
Obese D3	**252,19**

+ 12% dosaggio 25(OH)D negli obesi per raggiungere la sufficienza rispetto ai normopeso

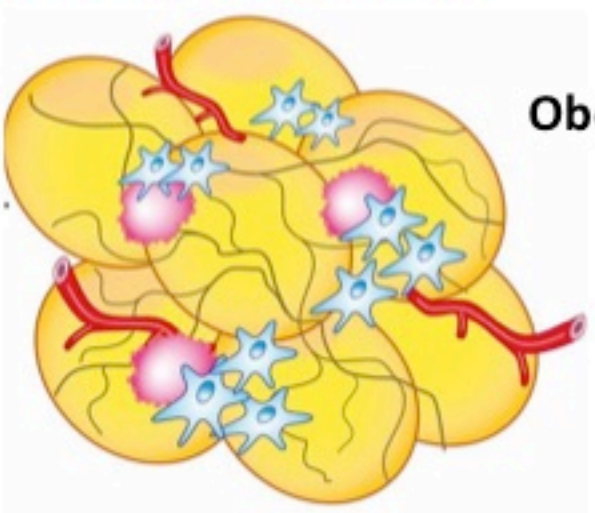
+ 43% dosaggio D3 negli obesi per raggiungere la sufficienza rispetto ai normopeso

Why is D3 prefentially accumulated in adipose tissue?

Normal



Differential accumulation of D3 and 25(OH)D in normal and obese adipose tissue?



Obese

DBP affinity:

- 25(OH)D: $7x10^8$ M^{-1}
- 1,25(OH)D: $5x10^7$ M^{-1}
- **Vitamin D3: $4x10^7$ M^{-1}** **-20X**

Liposolubilità (coefficiente di ripartizione acqua/n-ottanolo a 25°C)

- **D3: $logK_{ow}$ = 10,2** **150X**
- 25(OH)D: $logK_{ow}$ = 8,43
- 1,25(OH)D: $logK_{ow}$ = 7,6

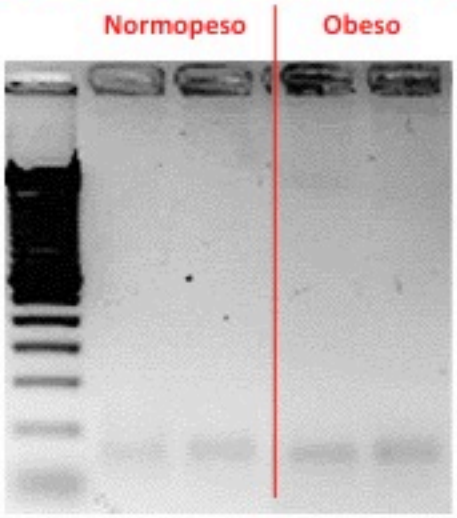
Vitamin D binding protein (DBP) binds vitamin d metabolites, but D3 has the lowest affinity

→ **More un-bound D3 can freely diffuse into adipose tissue given its higher lipo-solubility**

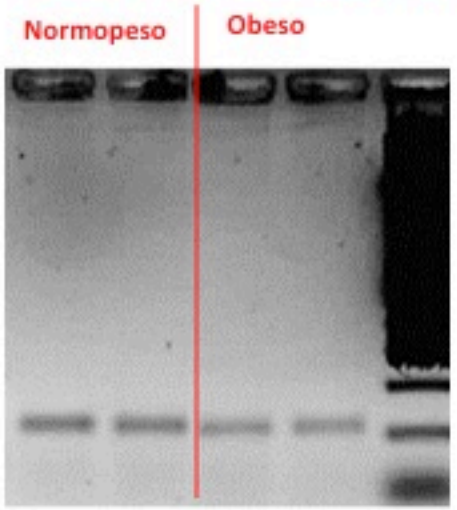
Risultati dello studio di espressione eseguito su tessuto adiposo di soggetto normopeso e obeso

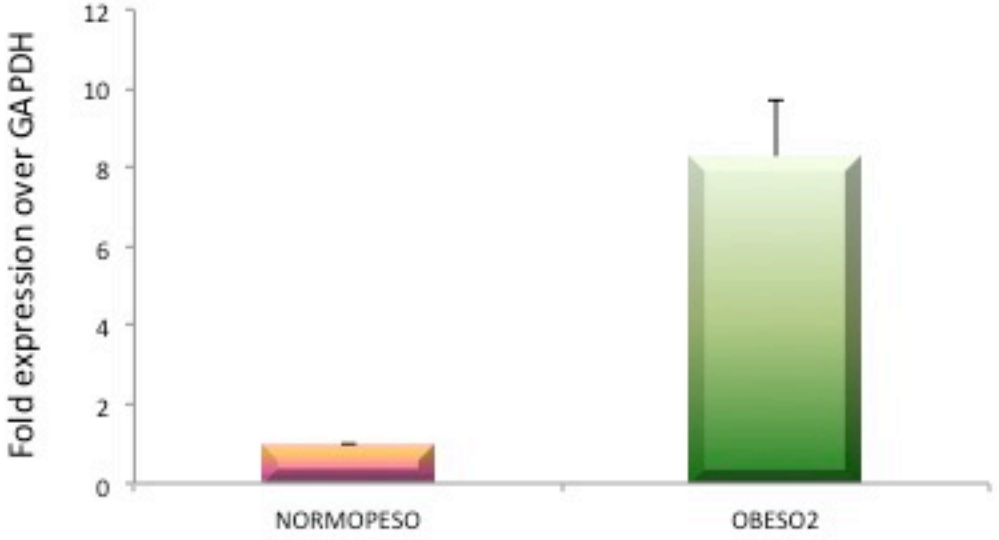
Risultati gel Real Time PCR

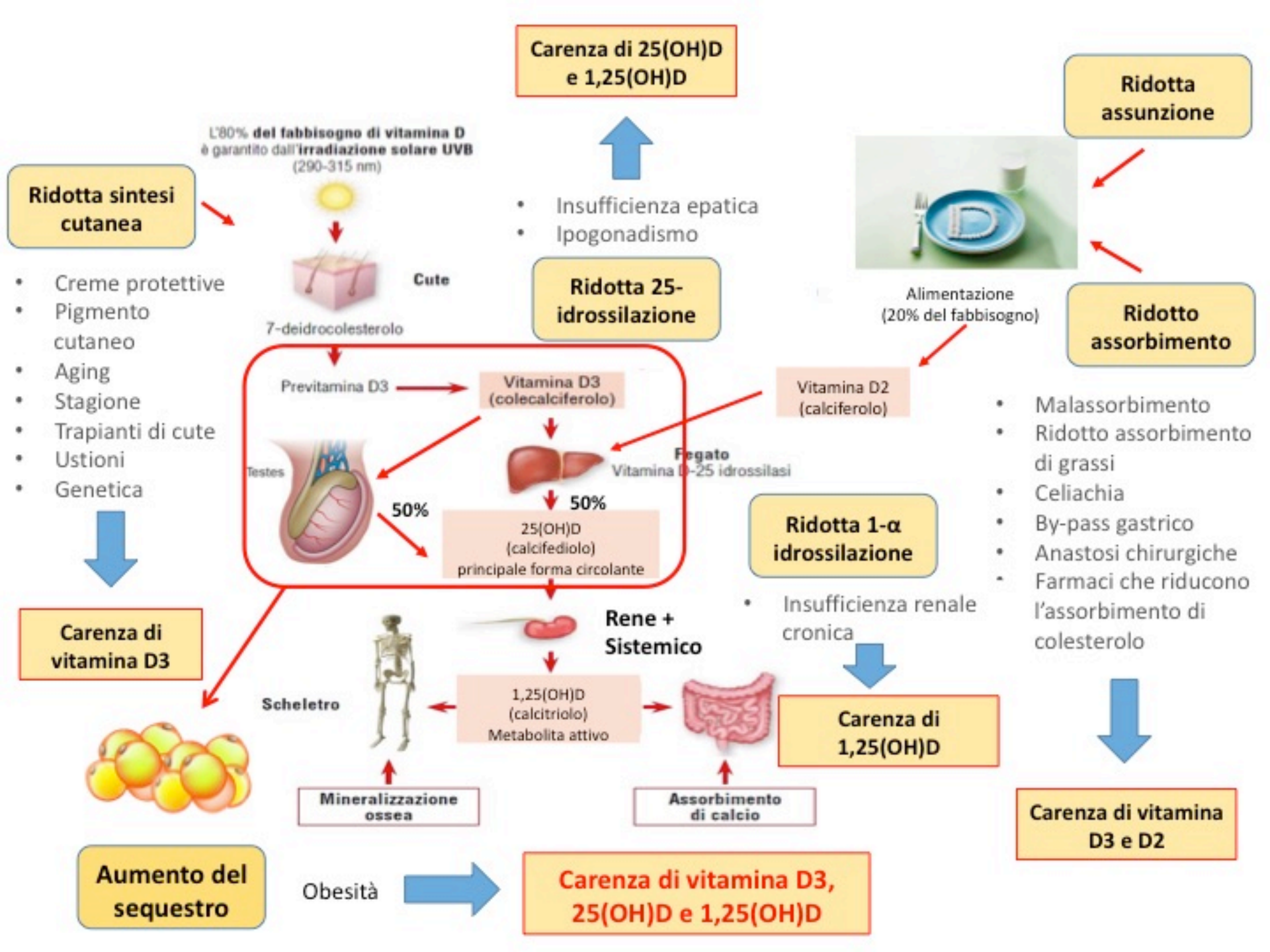
CYP19A1 (Aromatasi)



GAPDH (housekeeping)



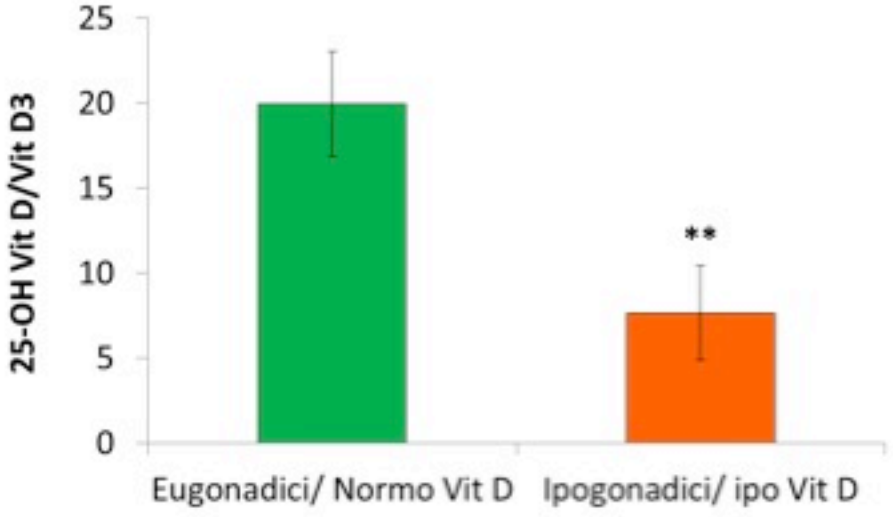



Carenza di 25(OH)D e 1,25(OH)D
Ridotta assunzione
L'80% del fabbisogno di vitamina D è garantito dall'irradiazione solare UVB (290-315 nm)
Ridotta sintesi cutanea
Insufficienza epatica
Ipogonadismo
Cute
Creme protettive
Pigmento cutaneo
Aging
Stagione
Trapianti di cute
Ustioni
Genetica
7-deidrocolesterolo
Ridotta 25-idrossilazione
Alimentazione (20% del fabbisogno)
Ridotto assorbimento
Previtamina D3
Vitamina D3 (colecalciferolo)
Vitamina D2 (calciferolo)
Testes
Fegato
Vitamina D-25 idrossilasi
50%
50%
25(OH)D (calcifediolo) principale forma circolante
Malassorbimento
Ridotto assorbimento di grassi
Celiachia
By-pass gastrico
Anastosi chirurgiche
Farmaci che riducono l'assorbimento di colesterolo
Ridotta 1-α idrossilazione
Carenza di vitamina D3
Rene + Sistemico
Insufficienza renale cronica
Scheletro
1,25(OH)D (calcitriolo) Metabolita attivo
Carenza di 1,25(OH)D
Mineralizzazione ossea
Assorbimento di calcio
Carenza di vitamina D3 e D2
Aumento del sequestro
Obesità
Carenza di vitamina D3, 25(OH)D e 1,25(OH)D

Rapporto 25(OH)D / vitamina D3 in soggetti eugonadici e ipogonadici

HPLC-MS

	D3 (nM)	25OH-Vit D (nM)	Ratio 25OH-D/ Vit D
Eugonadici	5,3	83	19,95
Ipogonadici	**7,1****	**46****	**7,65****



**= P<0.01

Osteoporos Int (2015) 26:2193–2202
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ORIGINAL ARTICLE

Role of vitamin D levels and vitamin D supplementation on bone mineral density in Klinefelter syndrome

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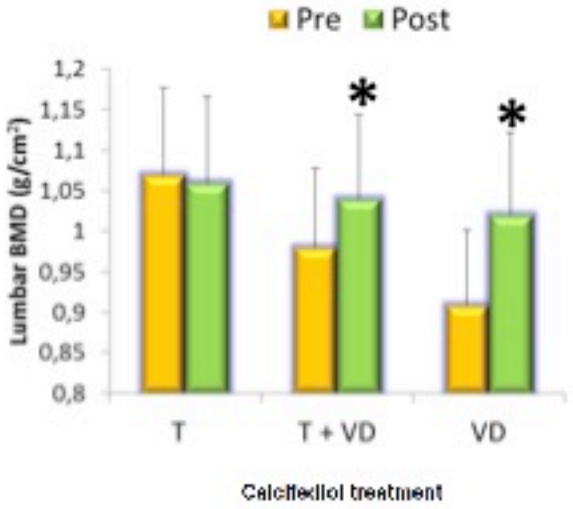
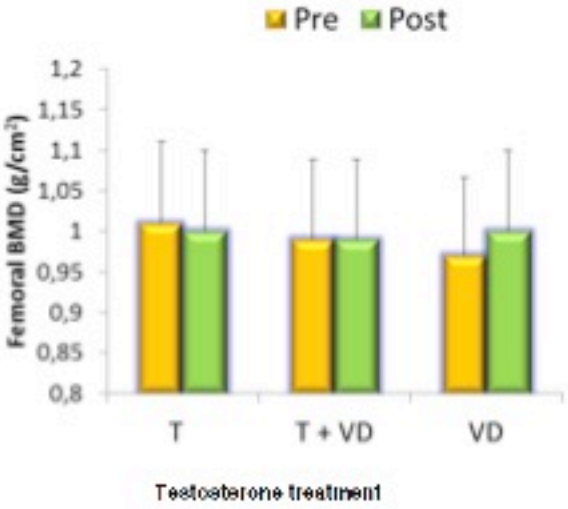
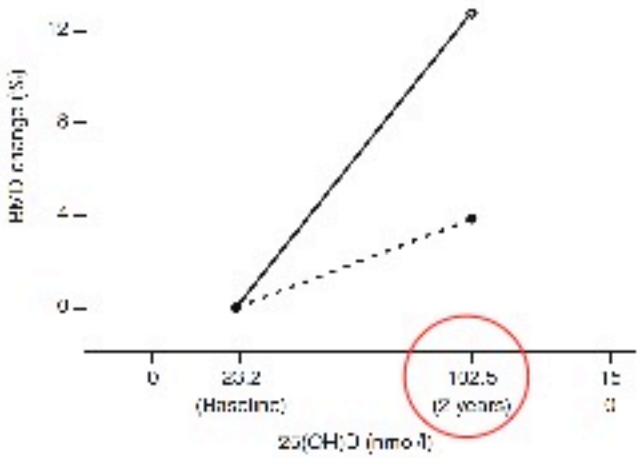
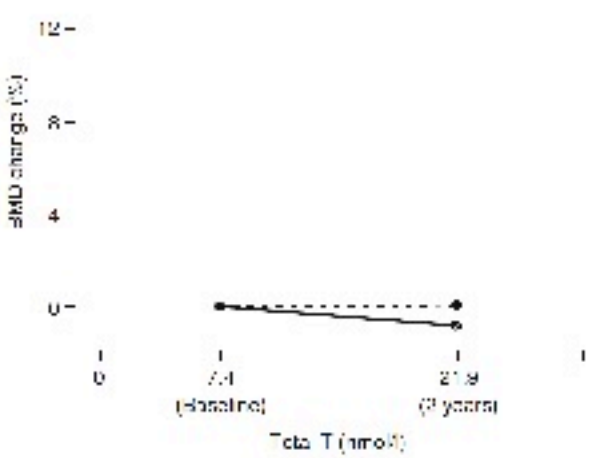








Conclusioni

- L'ipovitaminosi D è una sindrome complessa

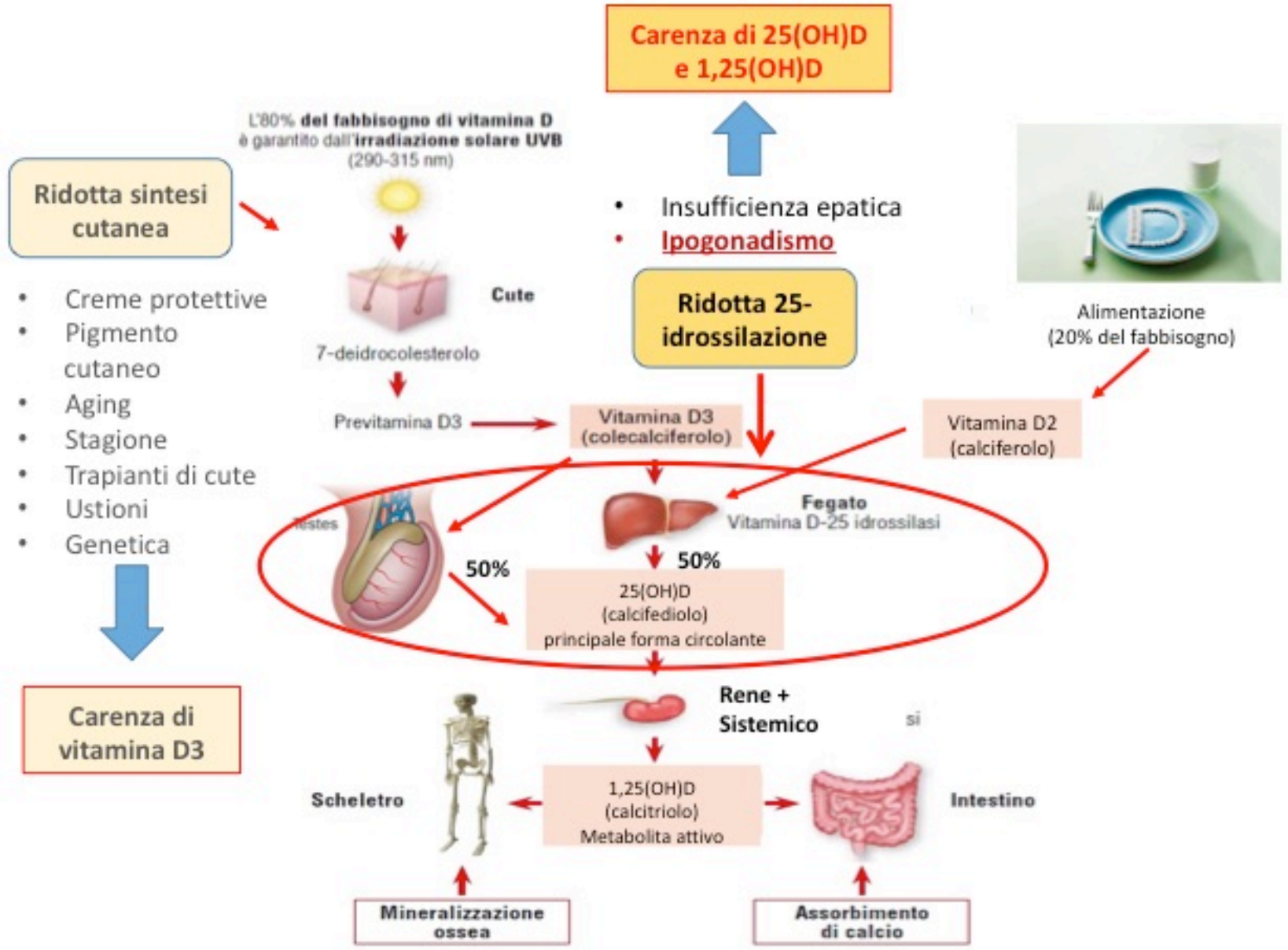
- La patogenesi dell'ipovitaminosi D racchiude diverse cause e diverse patologie di ipovitaminosi:

  A) ipo-calcifediolemia
  B) ipo-colecalciferolemia
  C) ipo-calcitriolemia

- Il trattamento delle ipovitaminosi D deve essere mirato e deve essere effettuato con formulazioni coerenti con la relativa patogenesi per:

  A) normalizzare precocemente i livelli plasmatici
  B) normalizzare precocemente i livelli di PTH
  C) evitare accumuli di substrati con conseguenti effetti collaterali

UOC Andrologia e Medicina della Riproduzione


Carenza di 25(OH)D e 1,25(OH)D
L'80% del fabbisogno di vitamina D è garantito dall'irradiazione solare UVB (290-315 nm)
Ridotta sintesi cutanea
Insufficienza epatica
Ipogonadismo
Ridotta 25-idrossilazione
Creme protettive
Pigmento cutaneo
Aging
Stagione
Trapianti di cute
Ustioni
Genetica
Cute
7-deidrocolesterolo
Previtamina D3
Vitamina D3 (colecalciferolo)
Alimentazione (20% del fabbisogno)
Vitamina D2 (calciferolo)
Testes
Fegato
Vitamina D-25 idrossilasi
50%
50%
25(OH)D (calcifediolo) principale forma circolante
Rene + Sistemico
Scheletro
1,25(OH)D (calcitriolo) Metabolita attivo
Intestino
Carenza di vitamina D3
Mineralizzazione ossea
Assorbimento di calcio