

# Vitamin D Deficiency Epidemic and Clinical Practice Styles

Months ago, one of my patients –with a long-standing, idiopathic, dilated cardiomyopathy, and functional limitations in his daily life– consulted a renowned clinician for a different opinion. He returned very happy as a result of that evaluation: low vitamin D levels in blood were detected, which –in the judgement of the professional– could explain the patient's problem. He added some comment about the lack of awareness in cardiologists about the importance of the issue; I had some difficulty to determine whether it came from the colleague or it had been added by the patient, disappointed by my inexperience. –Perhaps I had made a serious mistake for never having measured vitamin D in blood–, I said to myself. I encouraged him to take the supplementation prescribed by the colleague, and after several months of repeated metabolic studies, his levels had returned to normal, with no functional changes in his heart failure or any other visible benefit. Perhaps my mistake had not been that serious.

Instead of feeling surprised once again, I preferred to analyze –for the purpose of this letter– what information is available on the assessment of vitamin D levels and on treatments to increase them in patients with cardiovascular disease, or for its prevention. Similarly, I will try to discuss some of the modalities or styles to practise clinical medicine.

Measurement of calcium and vitamin D levels has grown significantly, not only for osteoporosis prevention but also for its increased relationship between vitamin D deficiency and other conditions.

It is worth recalling that even in postmenopausal women, we are far from knowing the efficacy of calcium plus vitamin D supplementation, except in very special circumstances. The largest clinical trial that assessed calcium and vitamin D supplementation in postmenopausal women was the WHI, which recruited 36,282 women between 50 and 79 years of age, and it did not show a significant clinical benefit. The only benefit was an improvement in bone density, with no reduction of hip fracture and significant increase of kidney stones. (1) This lack of effect was also observed in women with low vitamin D and calcium levels in blood. But let's leave this discussion aside and pass on to the abundant information available on the relationship between vitamin D and cardiovascular risk, trying to approach a current rational behavior.

As an advance, we have pathophysiological bases to explain the association between low vitamin D levels and the development of diabetes and cardiovascular disease, and epidemiological studies that show an association with increased cardiovascular and all-

cause mortality rates. (2) We lack large clinical trials about the role of vitamin D supplementation, and up to now, analyses are contradictory. In this situation, the reader will recognize some similarity with the issue of antioxidants and the saga of the homocysteine a decade ago, prior to the large trials that prospectively assessed the efficacy of folic acid and vitamin supplements. Outcomes could not have been worse. A meta-analysis that included 68 trials on 232,606 participants concluded that, in large trials, the use of antioxidants increased mortality: beta-carotene (vitamin B) with a relative risk (RR) of 1.07 (CI 95% 1.02-1.11), vitamin A, RR 1.16 (CI 85% 1.1-1.24), and vitamin E, RR 1.04 (CI 95% 1.01-1.07). (3) Neither benefit nor harm was observed with selenium or vitamin C supplements.

Will it be the final scenario of vitamin D or are we facing a panacea that prevents diabetes, cardiovascular disease, stroke, and cancer? In a few years, we will know it through the controlled trials now in progress, so the most important question is what we do in the meantime.

We will review the summarized information, and then discuss the thinking mechanisms and the action styles of the medical community faced with uncertainty.

## VITAMIN D AND CARDIOVASCULAR DISEASE

Vitamin D has a significant role in the metabolism of calcium and phosphorus, and in the bone synthesis, and its deficiency is associated with disorders such as rickets in children and osteomalacia in adults. However, its physiological action is much more complex.

### Sources of vitamin D and physiological effects

More than 90% of circulating vitamin D is synthesized at cutaneous level through the exposure to UV rays, and at hepatic level, it becomes its main circulating metabolite; the 25-hydroxyvitamin D, chemically 25(OH) vitamin D. It comes from two main sources: ergocalciferol or vitamin D<sub>2</sub>, which is provided by vegetables, and colecalciferol or vitamin D<sub>3</sub>, which is synthesized 98% at cutaneous level and is also found in some food such as fatty fish. At kidney level, it undergoes its transformation at 1.25(OH)<sub>2</sub>D, active metabolite that works as a true hormone in several tissues with receptors. It changes the functioning of 200 genes, and influences –in addition to the calcium-phosphorus metabolism and bone synthesis– in the production of cytokines and the regulation of inflammation, pancreatic insulin secretion, genesis

of kidney renin, role of macrophages on smooth muscle, and myocytes. The deficit of vitamin D has been associated with increased parathormone levels, stressed insulin resistance, and predisposition to systemic inflammation, hypertension, left ventricular hypertrophy, and diabetes. (4)

One of the postulated mechanisms is that low vitamin D levels are associated to an increased production of parathormone PTH, which would have harmful cardiovascular effects. This issue has been in part explained with a study on 2,312 individuals with no cardiovascular disease who were followed for 14 years, the Cardiovascular Health Study, which explored the relationship between vitamin D and PTH levels and the cardiovascular morbidity and mortality. The study observed a relationship between low vitamin D levels and a 9% increase of mortality rate per each difference of 10 ng/ml, and a 25% increased incidence of myocardial infarction. Subjects with levels < 15 ng/ml (17% of the population) had an increased mortality rate of 29%. High levels of PTH > 65 pg/ml (25% of the population) were associated with an increased heart failure of 30%, but not in other events. These outcomes suggest that the pathway of PTH does not explain the association between vitamin D and cardiovascular risk. (5)

### Epidemiological studies

In several follow-up cohorts, low vitamin D levels were associated with the future development of cardiovascular disease, stroke, hypertension, diabetes, and mortality rate.

The Framingham Offspring study recruited 1,739 participants with a mean age of 59 years, and no history of cardiovascular disease. Specified cut-off point was 15 ng/ml degree of 25-OH-D, and during a mean follow-up of 5.4 years, an increased risk of developing cardiovascular disease was observed when levels were low, RR 1.62 (CI 95% 1.11-2.36). The effect was evident in participants with hypertension, but was statistically insignificant in those without hypertension. (6)

The Health Professionals Follow-up Study assessed, with a nested case-control design, the association between vitamin D levels and the occurrence of a first myocardial infarction. Of the 18,225 participants, 454 men had myocardial infarction during follow-up, and they were compared with a control group of 900 participants who did not have it. Taking as reference of risk 1 the normal value  $\geq 30$  ng/ml, the relative risk of myocardial infarction with multivariate adjustment was 2.1 (CI 1.24-3.5) for those with levels < 15 ng/ml, 1.6 (CI 95% 1.1-2.3) for levels between 22.6 and 29.9, and 1.43 (CI 95% 0.96-2.1) for levels between 15 and 22.5 ng/ml. (7)

The third large cohort was the NHANES III, which assessed 13,331 individuals  $\geq 20$  years old with a mean long-term follow-up of 8.7 years, associating the circulating vitamin D levels with all-cause or

cardiovascular disease mortality. The first significant observation was the association between low vitamin D levels with low socio-economic status, the presence of diabetes, smoking habit, increased BMI and low physical activity. It was observed that the lowest quartile of vitamin D levels, < 17.8 ng/ml, had a 26% increase in total mortality (CI 95% from 8% to 46%) compared with the highest quartile. Cardiovascular disease mortality was higher in the group with < 17.8 ng/ml, and it resulted in the limit of significance when it was adjusted by multiple factors, such as hypertension and diabetes, RR of 1.22 (0.9-1.65) compared with the highest quartile, > 32 ng/ml. Surprisingly, relative risks in the two intermediate groups were lower than both extremes, 0.85 and 0.89 respectively, none of them with statistical significance. (8)

In the joint analysis of this information, the AHRQ review (9) commented that two of the studies suggested a complex approach, with higher risk for individuals with < 15 ng/ml or in the low quartile, coincidental among the three studies. However, the NHANES study followed a U-shaped curve, with lower risk in the groups with intermediate values, and increased risk in those with > 50 ng/ml. The confluence of multiple covariates associated with vitamin D levels weakens the conclusions.

A recent publication provided data about coronary patients with this problem. A subanalysis of the TNT study compared atorvastatin 80 mg vs 10 mg in 10,001 patients with stable CHD, followed for five years; a nested case-control analysis included 497 patients with CV events, compared with 1,012 without an event. There was no relationship between baseline vitamin D levels or its evolutive variation and CV events or mortality. (10)

### INTERVENTIONAL STUDIES ON VITAMIN D AND/OR CALCIUM SUPPLEMENTATION

#### Large trials in progress

There are currently three large trials in progress to assess the hypothesis of the vitamin D effectiveness: VIDAL, VITAL, and VIDA.

The VIDAL trial (11), Vitamin D and Longevity, is organized by the London School of Hygiene & Tropical Medicine in Great Britain, and is now in its first phase of feasibility, in which general practitioners will recruit 1,600 patients aged 65 or older. Patients will receive a dose equivalent to 3,200 IU/day of vitamin D3, very high and close to the recommended upper intake of 4,000 IU, with the aim of increasing the levels in blood over 30 ng/ml, based on the epidemiological fact that, in their community, 80% of the people at that age have levels lower than this value. If feasibility is appropriate, then it will progress to a trial on 20,000 patients with a 10-year follow-up.

The VITAL study (12), Vitamin D and Omega-3, is organized by the Brigham and Women's Hospital, and supported with public funds from different national

institutions of the United States. A total of 20,000 participants aged 50 or older, with no prior history of cancer or heart disease are already in the enrollment phase. They will receive, in a factorial design, 2,000 IU/day of vitamin D3 and a daily capsule of fish oil supplement. Endpoints will be the incidence of cancer, heart disease, or stroke at five years. They hope to have the outcomes by the year 2016.

The VIDA study (13) is organized by the University Hospital of Auckland, and supported with public funds from Australia and New Zealand. It is in its enrollment phase of 5,100 participants between 51 and 84 years old, who will receive treatment with 200,000 IU capsule at baseline, and then 100,000 IU capsule monthly, aside from supplementation in each June (winter) for four years. It will have a follow-up of 4 years and 6 months, and its main endpoint is the incidence of fatal and non-fatal heart disease.

The fact that authorities of public health in Great Britain, United States and Australia-New Zealand consider it necessary to develop large trials to provide a definitive answer to the recommendations of vitamin D supplement indicates that the information we have available is not enough to answer the question.

### Previous studies focusing on fracture prevention

There are several studies that have assessed the role of vitamin D and calcium to prevent fractures, and these studies have provided information about mortality rate and (in a somewhat less discriminatory way) about the incidence of cardiovascular diseases. Several meta-analyses have also been carried out about it.

As representative of this still unclear situation, I will summarize two large studies published recently, the RECORD trial and the branch of the Women's Health Initiative, which assessed the calcium and vitamin D supplementation.

The RECORD trial recruited 5,292 participants aged at least 70 years, 85% women, with previous low-trauma fractures. They were treated in four groups in a factorial design: daily vitamin D 800 IU, calcium 1000 mg, both, or placebo for 2-5 years, with a follow-up of 3 years after intervention. In the intention-to-treat analyses, there were neither benefits in reduction of all-cause mortality 31.6 % vs 33.3 %, vascular disease mortality 13.2 % vs 14.2 %, or neoplasms 5.7 % vs 6.7 %, nor in cancer incidence 12.8 % vs 11.9% with calcium, vitamin D or its association. In the analysis adjusting for compliance with treatment, trends for reduced mortality with vitamin D and increased mortality with calcium supplementation were accentuated, although both remained nonsignificant. (14)

The WHI CaD trial enrolled 36,282 postmenopausal women aged 51-82 years, who were assigned to 1,000 mg of calcium and 400 IU of vitamin D3 or placebo, with a follow-up of 7 years. The HR for reduced mortality 0.91 (CI 95% 0.83-1.01) was nonsignificant, and the same trend was observed in stroke and cancer mortality, though the trend was

clear for coronary artery disease or vascular disease mortality. (H) Interestingly, there was an increased risk of myocardial infarction or infarct event or stroke in patients who had not previously received calcium and vitamin D. For that reason, a meta-analysis of the studies on calcium supplementation was carried out, which confirmed an increased risk of myocardial infarction RR 1.24 (1.07-1.45) and of the combined endpoint of myocardial infarction or stroke 1.15 (1.03-1.27). (16)

### Meta-analyses of trials with vitamin D and calcium supplementation

Several meta-analyses or reviews with apparently conflicting conclusions have been published.

In 2007, Autier et al (17) carried out a meta-analysis of 18 trials that included 57,311 participants with a mean follow-up of 5.7 years. The mean daily vitamin D dose was 528 IU, varying from 300 to 2,000 IU in different trials. Relative risk for mortality was 0.93 (CI 0.87-0.99) in the limit of significance. There was no indication for heterogeneity among the trials. An aspect that reinforced the conclusions was that the effect was concentrated on the best designed trials. In the 9 trials with high statistical power, the RR was 0.92 (0.86-0.99), while in the 9 trials with low statistical power, the RR was 1.15 (0.79-2.73).

Over the past two years, two meta-analyses reviewed a larger number of trials and had a longer follow-up than other trials like the RECORD.

Elamin et al (18), based on a requirement by The Endocrine Society in the United States, carried out a review of 51 trials. For the analysis on mortality, information was obtained from 30 trials with 62,231 participants. It showed a trend toward reduction in mortality RR 0.96 (CI 0.93-1) p 0.08, with no effect on myocardial infarction RR 1.02 (CI 95% 0.93-1.13) and stroke RR 1.05 (CI 95% 0.88-1.25). The general impression is that the quality of the available studies was low to moderate at best, and with heterogeneity among trials. Changes in lipid and glycemia levels or in systolic and diastolic blood pressure were not verified either.

The Cochrane Collaboration (19) published a meta-analysis with 50 trials and a total of 94,148 participants, with data available for the analysis of mortality. They observed a significant –but of little magnitude– reduction of mortality RR 0.97 (CI 95% of 0.94-1), with no heterogeneity and 0% inconsistency. Only the studies on vitamin D3 supplementation showed that trend toward reduction of mortality RR 0.94 (0.91-0.98), with no effects associated with vitamin D2, alphacalcidol, or calcitriol. It also showed an increase in nephrolithiasis when vitamin D was associated with calcium supplementation. In the conclusions, and based on the analysis of subgroups, they affirm that mortality is predominantly reduced in older women, most of them inpatients in centers and under care due to their dependence. They agree with the previous meta-analysis in that quality of the trials

is moderate, and surprisingly, although RR is almost identical, in this case reaches a marginal statistical significance, perhaps because of the statistical methodology used by the Cochrane, which is the random method. This form of analysis provides higher standard to smaller trials, and many times leads to unsustainable conclusions, as in the case of the meta-analysis of magnesium in myocardial infarction.

As a conceptual exercise, I have carried out a new meta-analysis including only large trials (more than 1,000 patients enrolled), which is summarized in the figure below. Thus, taking into account 9 trials that included 57,645 participants, the resulting relative risk is 0.99 (0.95-1.03), with significant heterogeneity and I<sup>2</sup> inconsistency of 69.8%.

### RECOMMENDATIONS OF THE SCIENTIFIC SOCIETIES

The Institute of Medicine is an American institution that recommends about therapeutical and ethical practices. Based on the AHRQ 9 review, they released a very conservative proposal by recommending a dietary supplement of 600 IU daily for subjects younger than 70 years of age, and 800 IU daily for those older than 70, focusing on achieving a plasma level over 20 ng/ml. (20) In the guidelines, The Endocrine Society in the United States has suggested higher intake levels, between 1,000 and 2,000 IU; it agreed in not recommending vitamin D for cardiovascular disease prevention or quality of life improvement, but only in aiming at bone metabolism improvement. (21) The opinion of the IOM, reinforced by the JAMA publication of an analysis about their reasons (22), is that the epidemiological relationship between vitamin D levels and cardiovascular risk is not consolidated,

and that they are not convinced of the therapeutical effects of vitamin D supplementation for preventing diabetes or cardiovascular events. They recommend to wait for the outcomes of controlled trials before adopting a more active policy in this regard. "Despite biologic plausibility for a role of vitamin D in the prevention of cardiovascular disease and diabetes, the evidence from available research is inconsistent, inconclusive as to causality mortality and causality, and insufficient to set nutritional requirements".

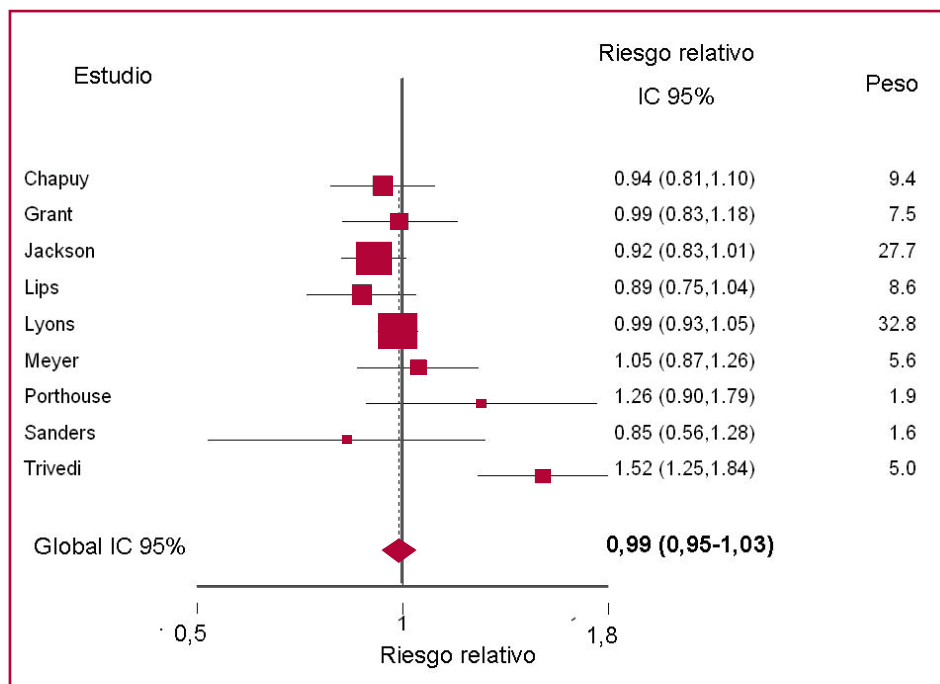
This position has been severely criticized by other authors, and it may be of interest to read the exchange of letters to the editor (23) about this issue between Shapses and Manson defending the position of IOM, and O'Keefe, Lavie and Holick, who suggest a more current and active policy. This position was reinforced in a publication a few years ago (24) and in a recent review published in the JACC by the same authors. (25) In that publication, they argue that the assessment of 25(OH)D levels in many adults is reasonable, especially in those with cardiovascular disease or at high risk to develop it, and that treatment not only is easy to implement but also gets results in plasma in a short time. Potential toxicity is very low, and no vitamin D adverse effects were observed in multiple series, in doses of up to 4,000 IU/day.

### TODAY, WHAT CAN WE DO WITH THIS INFORMATION?

#### Medicine styles and consequences for patients

Schematically, we have two alternatives:

- 1) Follow the general guidelines that recommend vitamin D intake and exposure to sunlight, independently from cardiovascular disease, diabetes or risk to have it. In this case, we will



**Fig. 1.** Meta-analysis of the randomized controlled trials that comparatively analyzed vitamin D effect compared with a control group or placebo on mortality. Only studies with more than 1,000 patients treated between both groups were included. Overall, no benefit was observed with vitamin D supplementation. None of the trials had statistically significant effects individually.



not measure 25 (OH) in blood in our patients with heart diseases, except under clinical indications (institutionalized cases, with nutritional deficiency or previous low-trauma fractures).

- 2) Find among our patients with heart disease those with levels below the desirable 25 (OH) D and try to change them with supplementation, controlling the effects. It is also possible that this approach leads us to check those patients with low levels for their bone metabolism, through densitometry and functional assessment of calcium and phosphorus metabolism.

It is interesting to speculate about which the results of the search will be in case of choosing the second option.

Which is the cut-off point to determine low vitamin D values?

Table 1 summarizes two proposed classifications to define normal or desirable levels of 25 OH vitamin D in plasma.

As shown, there are no coincidences in determining normal or pathologic values. There is agreement in that levels below 10-12 ng/ml would be low, but the IOM considers sufficient levels those above 20 ng/ml, while other classifications suggest > 30-40 ng/ml. Here are some data about Argentine surveys on vitamin D levels in blood to understand what these criteria imply, and estimate the incidence of low vitamin D in cardiovascular patients.

In Argentina, there are several epidemiological studies in populations with no cardiovascular disease, but none of them is large. As an example, a trial that enrolled 224 women older than 30 years who went to medical clinic offices, showed an incidence of levels < 20 ng/ml in 26.8% and an additional 29.9% between 20-30 ng/ml. The mean age was 58.3 years, and deficiency was significant in older, sedentary patients, in those with little exposure to sun, in obese patients, and in those with low calcium levels. (27) Levels vary between winter and summer, and a study on young subjects showed that 50% of the men and 42.6% of the women had levels < 20 ng/ml in winter. (28) In patients with a history of cardiovascular disease, it is likely that incidence be even higher. In some international series of elderly inpatients, 100% had levels < 15 ng/ml. Gathered together in a review article on the topic, the mean level in different Argentine groups in winter was not over 20 ng/ml. 4

It is clear that if we requested vitamin D dosage in cardiovascular patients, much more than the half would have levels considered low.

What message would be transmitted to the patient?

Patient should be informed that a low value has been found –although very common in the population– about which we have no certainties of its significance and utility of changing it. Thus, we can share doubts and make a joint decision.

The alternative is simply to inform about this low level that becomes a disorder, vitamin D deficiency,

**Table 1.** Two criteria to define the adequate levels of 25 OH vitamin D. (25-26)

Levels of 25 OH vitamin D in ng/ml	Vitamin D status
Modified classification of McKeena & Freaney <sup>26</sup>	
<= 10	Severe deficiency
10-20	Moderate deficiency
20-30	Mild to moderate deficiency
>= 30	Sufficient
40-50	Ideal
50-150	Undetermined state
> 150	Toxicity
Definition of the Institute of Medicine	
<12	Risk of deficiency
12-19	Risk of inadequacy
20-50	Sufficient
>50	Possibly harmful

and the “need” to complement with metabolic studies and treat it, which would lead us to a multiplicity of images, metabolic controls, and checking of results.

We are facing an uncertainty, approaching two medical styles that may underline different medical concepts: Table 2.

1) A leading, pathophysiologic vision, aimed at correcting metabolic disorders suspected of bringing about potential problems, though not yet confirmed. This way of practising medicine implies to define risk factors as pathological, whose utility to change them is unknown, as if they were diseases. It is close to the biological-statistical definition of disease. (29) In this bag we could put the management of high levels of CRP, HOMA, independent lipoprotein fractions, or of the strict controls of glycemia in type II diabetes. This strategy has a great advantage: if the utility of the adopted strategy is confirmed, we would benefit patients many years in advance. In this case, we will have no definitive information until the year 2017. Disadvantages are also obvious: we would be emphasizing the enforcement of controls whose utility is unknown, being aware that the level of compliance of indications is low and has several cultural obstacles. (30)

2) An initially conservative vision, which we may say is based on therapeutic evidences, and which prevents the patient from information about problems whose relevance is not known to us. What we must change is what invalidates or causes suffering, and our prevention must be adjusted to what we know for sure. It approaches to the functional, estimate definition of disease.<sup>29</sup> The central advantage of this approach is that it allows to concentrate on current problems of the patient and work hard on the efficacy

Medicine styles	Based on therapeutic evidences	Pathophysiological
Conduct about vitamin D dosage	Not to perform it.	To perform it, and add supplementation to increase it to desired levels.
Consequences		
Benefit	Avoids diagnosis of uncertain value and non-confirmed therapies. Omit an information that could be of therapeutic help.	If the hypothesis is true, definitive evidences are obtained many years earlier. Induce new diagnoses and medical interventions in patients with multiple strategies validated in their efficacy, but of difficult compliance.
Risk		
Definition of disease	Functionalist-valorative	Biological-statistical

**Table 2.** Medicine styles and the vitamin D problem

of the therapies, with the obvious limitations of the individual variability. Another advantage in this case is that it will be far from the possibility of adding calcium supplementation, which has been associated with increased cardiovascular risk in various research works. In another approach to the analysis, we should not forget that consultations are always brief, and that every aspect of the strategy for secondary cardiovascular prevention takes time. To convince a patient not to lower statin doses to experiment, or not to stop doing exercise, or to have a dialogue about the patient's moment and concerns, will have to compete with the explanation of periodical dosages of 25 OH D in blood and their modification, as well as calcium metabolism, which are temporarily unimportant issues. With how many conceptual priorities can we charge a person for the purpose of improving his/her health?

The reader may have noticed that I like the conservative view. I think we have the great responsibility to avoid making patients sick by prescribing dosages and interventions to change plasma levels of factors whose relevance is still unknown. There are a lot of negative historical examples with different interventions such as estrogen therapy, correction of homocysteine levels, vitamins and antioxidants in general, strict control of glycemia, and strict control to reach low levels of blood pressure in type II diabetic patients, and another long list of conducts based on fragile observational evidences or brilliant pathophysiological reasonings that have shown to increase mortality rate.

Of course, it does not preclude individual experimentation in patients who do not improve with usual treatments, where therapeutic creativity is always welcome. But this should not allow experimentation at the expense of the patient or the health care system, with massive evaluations and interventions of dubious efficacy.

**Dr. Carlos D. Tajer<sup>MTSAC</sup>**

Director of Revista Argentina de Cardiología

## BIBLIOGRAPHY

1. Jackson R, LaCroix A, Gass M, Wallace R, Robbins J, Lewis C, et al. Calcium plus Vitamin D Supplementation and the Risk of Fractures. *N Engl J Med* 2005;354: 669-83.
2. Botet J. VitaminaD: ¿un nuevo factor de riesgo cardiovascular? *Clin Invest Arterioscl*.2010;22:72-8 .
3. Goran Bjelakovic G, Nikolova D, Gluud L, Simonetti R, Gluud C. Mortality in Randomized Trials of Antioxidant Supplements for Primary and Secondary Prevention: Systematic Review and Meta-analysis. *JAMA*. 2007;297:842-57.
4. Costanzo P, Salerni H. Hipovitaminosis D: afectaciones no clásicas. *RAEM* 2009;46:2-23.
5. Kestenbaum B, Katz R, de Boer I, Hoofnagle A, Sarnak M, Shlipak M, et al. Vitamin D, Parathyroid Hormone, and Cardiovascular Events Among Older Adults *J Am Coll Cardiol* 2011;58:1433-41.
6. Wang T, Pencina M, Booth S, Jacques P, Ingelsson E, Lanier K, et al. Vitamin D Deficiency and Risk of Cardiovascular Disease. *Circulation*. 2008;117:503-11.
7. Giovannucci E, Liu Y, Hollis B, Rimm E. 25-Hydroxyvitamin D and Risk of Myocardial Infarction in Men. A Prospective Study. *Arch Intern Med*. 2008;168:1174-80.
8. Melamed M, Michos E, Post W, Astor B. 25-hydroxyl Vitamin D Levels and the Risk of Mortality in the General Population *Arch Intern Med*. 2008;168: 1629-37.
9. Chung M, Balk EM, Brendel M, Ip S, Lau J, Lee J, et al. Tufts Evidence-based Practice Center. Vitamin D and Calcium: A Systematic Review of Health Outcomes: Evidence Report No. 183. Rockville, MD: Agency for Healthcare Research and Quality; AHRQ 2009.
10. Bittner V, Wenger N, Waters D, DeMicco D, Messig M, LaRosa, J. Vitamin D levels do not predict cardiovascular events in statin-treated patients with stable coronary disease *J. Am. Coll. Cardiol*. 2010;55:A167.E1570.
11. Inscripto en el registro de ensayos con el código ISRCTN46328341 . Accesible en <http://www.controlled-trials.com/isrctn/pf/46328341>
12. Inscripto en el registro de ensayos con el código NCT01169259. Accesible en <http://clinicaltrials.gov/ct2/show/NCT01169259>
13. Inscripto en el registro de ensayos de Australia y Nueva Zelanda como VIDA Trial Vitamin D con el código ACTRN12611000402943. Accesible en [http://www.anzctr.org.au/trial\\_view.aspx?id=336777](http://www.anzctr.org.au/trial_view.aspx?id=336777).
14. Avenell A, MacLennan GS, Jenkinson DJ, et al. Long-term follow-up for mortality and cancer in a randomized placebo-controlled trial of vitamin D3 and/or calcium (RECORD trial). *J Clin Endocrinol Metab* 2011; DOI:10.1210/jc.2011-1309.
15. LaCroix A, Kotchen J, Anderson G, Brzyski R, Cauley J, Cummings S et al. Calcium plus vitamin D supplementation and mortality in postmenopausal women: the Women's Health Initiative calcium-vitamin D randomized controlled trial. *J Gerontol A Biol Sci Med Sci*. 2009 ;64:559-67
16. Bolland MJ, Avenell A, Baron JA, Grey A, MacLennan GS, Gamble GD, Reid IR. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. *BMJ* 2010 Jul 29; 341: c3691. doi: 10.1136/bmj.
17. Autier P, Gandini S. Vitamin D Supplementation and Total Mortality. A Meta-analysis of Randomized Controlled Trials. *Arch Intern Med*. 2007;167:1730-7.

18. Elamin M, Nisrin O, Elnour A, Elamin K, Fatourehchi M, Aziz A, et al. Vitamin D and Cardiovascular Outcomes: A Systematic Review and Meta-Analysis *J Clin Endocrinol Metab* 2011; 96: 1931-42
19. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti R et al. Vitamin D supplementation for prevention of mortality in adults. *Cochrane Database Syst Rev*. 2011;):CD007470.
20. Institute of Medicine. Dietary reference intakes for calcium and vitamin D. [http://books.nap.edu/openbook.php?record\\_id=13050](http://books.nap.edu/openbook.php?record_id=13050).
21. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96:1911-30.
22. Shapses S, Manson J. Vitamin D and Prevention of Cardiovascular Disease and Diabetes. Why the Evidence Falls Short. *JAMA*, 2011; 305:2565-6.
23. O'Keefe J, Lavie C, Holick M. Vitamin D Supplementation for Cardiovascular Disease Prevention. Y respuesta a continuación de Shapses S, Manson J *JAMA*: 2011; 306:1546-7.
24. Lee J, O'Keefe J, Bell D, Hensrud D, Holick M. Vitamin D deficiency an important, common, and easily treatable cardiovascular riskfactor? *J Am Coll Cardiol*. 2008;52:1949-56.
25. Lavie C, Lee J, Milani R. Vitamin D and Cardiovascular Disease: Will It Live Up to its Hype? *J. Am. Coll. Cardiol*. 2011;58:1547-56.
26. McKenna MJ, Freaney R: Secondary Hyperparathyroidism in the Elderly: means to Defining Hypovitaminosis D. *Osteoporos Int* 1998;8:S3-S6,.
27. Arevalo C, Nuñez M, Barcia R, Sarandria P, Miyazato M. Déficit de vitamina D en mujeres adultas de la Ciudad de Buenos Aires. *Medicina* 2009;69:635-9.
28. Costanzo P, Elías N, Kleiman J, Garcia Basavilbaso N, Piacentini R, Salerni H. Variaciones estacionales de 25 (OH) vitamina D en jóvenes sanos y su asociación con la radiación ultravioleta en Buenos Aires. *Medicina* 2011;71: 336-42.
29. Tajer, C. Una mirada de la cardiología argentina en la perspectiva del bicentenario: Segunda parte. *Rev Argent Cardiol*. 2010;78:98-102.
30. Yusuf S, Islam S, Chow CK, et al. Use of secondary prevention drugs for cardiovascular disease in the community in high-income, middle-income, and low-income countries (the PURE Study): a prospective epidemiological survey. *Lancet* 2011; DOI:10.1016/S0140-6736(11)61215-4.