

OPINION

Serum 25-hydroxyvitamin D and parathyroid hormone exhibit threshold behavior

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Most reported studies of PTH and serum 25-hydroxyvitamin D (25OHD) show a curvilinear relationship between the two variables, with PTH rising steeply at low 25OHD values, and tending to flatten out, or plateau, at high 25OHD values (1, 2). Is this low plateau real? Is there a threshold 25OHD value below which PTH rises? If so, is it important? I believe it is real, but of doubtful importance.

Virtually all physiological regulatory systems operate by means of negative feedback loops – they are what engineers call “error-driven systems”. Whether it be blood sugar regulation or extracellular fluid calcium concentration, organisms have evolved to function optimally around certain values which are canonized as notional setpoints (the analog of the setting of a thermostat in a heating system). When environmental or other factors lead to a departure of the regulated quantity from the ideal level (the “error”), physiological responses are called into play that act to reduce the departure and to restore the regulated quantity to its setpoint level. Thus, as blood sugar rises, insulin secretion rises as well, with the result being that the blood sugar returns toward a “normal” value. So too, as extracellular fluid calcium ion concentration [ECF (Ca^{2+})] falls, PTH rises to counteract the fall. Various refinements on these control loops have been discovered over many years of careful research, but the fundamental engineering analogy remains apt.

One of the features of most or all such systems is that the evoked response is proportional to the size of the error. For example, a 5 mg/dl rise in blood sugar evokes less of an insulin response than does a 50 mg/dl rise. The statement that the response is

proportional to the perturbation, when expressed symbolically in mathematical terms, is precisely an exponential relationship. Thus, to fit observed data derived from such a system to a decreasing exponential is not so much to impose an arbitrary model on reality as to take our verbal description of the system and encode it mathematically. In this case, if we accept the fact that calcium absorption is proportional to serum 25OHD, as many studies have suggested (3-5), then low 25OHD means low calcium absorption, evoking a correspondingly increased secretion of PTH. In other words, an exponential relationship not only fits the data statistically, but conceptually as well. However in all such systems, when the regulated quantity [ECF (Ca^{2+})] is at the setpoint level, the regulatory factor (PTH) falls to its practicable minimum, *i.e.*, it reaches a low plateau.

Inspection of the very large series of cases assembled by Chapuy et al. (2) shows very clearly that, at serum 25OHD values below ~80 nmol/l, there is a tendency for PTH and 25OHD to be inversely related, whereas above 80 nmol/l no such relationship exists. Indeed, separating the data into two subsets (Fig. 1), one above and one below 80 nmol/l, and subjecting the subsets to ordinary Pearsonian regression, shows that there is a highly significant inverse relationship for the values below 80, and none whatsoever for the higher values. PTH above 80 nmol/l has reached a plateau when expressed as a function of 25OHD. The most logical way to express the relationship for both sets recombined is to use a decreasing exponential, with the plateau being the steady state to which the exponential tends. This is what Chapuy et al. did. That model is logical because, as just described, that is how essentially all regulated quantities behave with respect to their control factors.

Moreover, plotting the data as log transforms does not alter the fact that there is still no relationship between PTH and 25OHD for the set of values above 80 nmol/l (while the fit for the transformed data below 80 nmol/l is even better than for the untransformed). And, even if there were a mathematically demonstrable relationship of the log-transformed values

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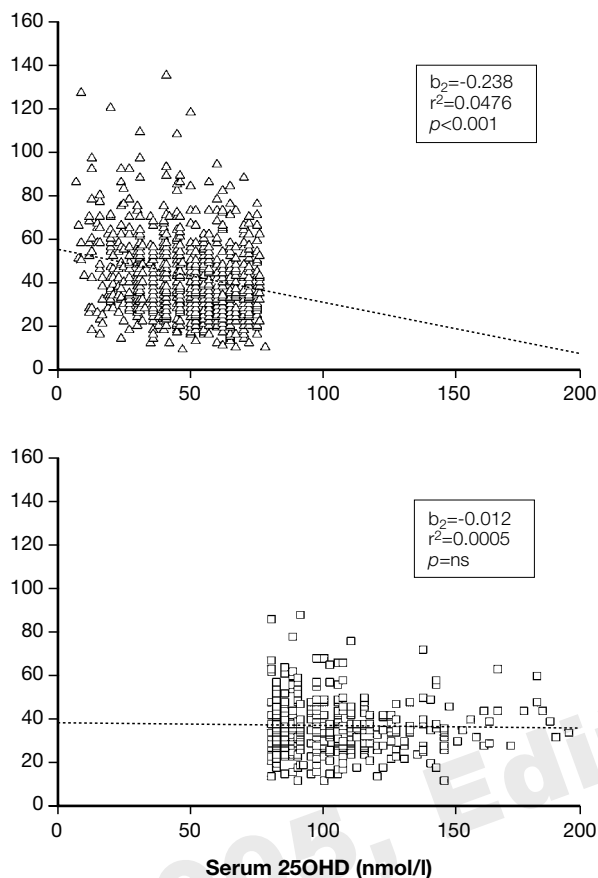


Fig. 1 - Redrawing of the data of Chapuy et al. (2), splitting the data at 25-hydroxyvitamin D (25OHD) values of 80 nmol/l. Panel A is for values below 80 and panel B for values above 80. For both panels, "b" is the slope of the linear regression equation. Copyright, Robert P. Heaney, 2005. Used with permission.

for the higher 25OHD values, the verbal translation of any log-log relationship is simply: "PTH falls by ever smaller amounts as 25OHD rises by ever larger amounts". In other words, the rate of change in PTH becomes so small as to constitute a virtual plateau. Thus, as a first approximation, a plateau seems the most parsimonious description of the PTH data above 25OHD values of ~80 nmol/l.

Why do we care? Only because the lower end of the plateau might be taken as an indicator of the lower end of the normal range for serum 25OHD. But, the more important point is the inverse relationship below ~80 nmol/l. Its recognition functioned usefully as a first indication that something might not be optimal about vitamin D status whenever 25OHD levels were associated with elevated PTH concentration (that concern remains apt whether or not the plateau exists or is important).

It must be said that the issue is somewhat more complicated than the glucose-insulin counterpart because, strictly speaking, serum PTH is not the regulated quantity in this feedback system. Rather PTH is a surrogate for that quantity, which may be taken as ECF (Ca^{2+}). Serum PTH is a useful, if not a perfect surrogate. It is useful because it amplifies perturbations in ECF (Ca^{2+}) by a factor of approximately 10- to 50-fold, thus allowing us to "see" perturbations undetectable by ordinary chemical measurement of (Ca^{2+}) itself. It is not perfect because its response saturates quickly and hence the relation of changes in ECF (Ca^{2+}) to changes in PTH is non-linear.

However, there is more at stake than just ECF (Ca^{2+}). Serum PTH is also the principal regulator of the quantity of bone remodeling in the body (remodeling is not actually regulated as a part of the control loop, but it is a collateral effect of such regulation). Bone remodeling is now recognized to be an important skeletal fragility factor (6). Hence high serum PTH heralds the presence of both inadequate calcium input to the system (intake and/or absorption) and increased bony remodeling activity – each leading in its own way to increased skeletal fragility.

While the earliest concerns with respect to adequacy of vitamin D status were raised because of the inverse relationship observed with PTH, other, more salient endpoints should be used to determine the actual vitamin D requirement. It is for this reason that the issue of a PTH plateau is today of uncertain importance. For example, our group has shown that calcium absorption efficiency from standard meals improves as serum 25OHD rises from 50 to approximately 80 nmol/l, but does not change appreciably above that level (7), i.e., absorption itself plateaus at 25OHD levels above 80 nmol/l. This is certainly a more direct indicator of vitamin D functioning, since it demonstrates that, below approximately 80 nmol/l, serum 25OHD level is rate-limiting for its classical physiological effect (absorption) but that, above 80 nmol/l, circulating 25OHD is adequate to allow the system to regulate itself through other controls (thereby avoiding hyperabsorption of calcium despite high levels of 25OHD). Thus, the PTH plateau seems congruent with vitamin D function in the calcium economy.

Fortuitously, parallel studies have shown that there is also significant fracture reduction when serum 25OHD is raised through approximately the same range (i.e., 53 to 75 nmol/l) (8). This important benefit cannot be unambiguously attributed solely to improved calcium absorption or reduced PTH secretion, since vitamin D is known to reduce fall frequency (9) and to improve lower extremity muscle function (10). Whatever the precise mechanism

of the fracture risk reduction, this improvement reinforces the conclusion that serum 25OHD concentrations below 75-80 nmol/l are suboptimal for health. Possibly higher values might be even more beneficial. We do not know. As we acquire new information with respect to yet other health status indicators influenced by vitamin D, the question about a PTH plateau (which would apply only to the calcium economy) becomes less and less pertinent. We may find that truly optimal levels are above 90 or 100 nmol/l (or even higher). In any case, health, however defined, itself represents, a kind of plateau state, one that is dynamically maintained. Where it is reached for all body systems along the continuum of serum 25OHD concentrations remains one of the most important open questions in nutritional science today.

REFERENCES

1. Thomas MK, Lloyd-Jones DM, Thadhani RI, et al. Hypovitaminosis D in medical inpatients. *N Engl J Med* 1998, 338: 777-83.
2. Chapuy M-C, Preziosi P, Maamer M, et al. Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int* 1997, 7: 439-43.
3. Barger-Lux MJ, Heaney RP. Determinants of calcium absorption. In: Burckhardt P, Heaney RP eds. *Nutritional Aspects of Osteoporosis '94*. Proceedings of 2nd International Symposium on Osteoporosis, Lausanne, May 1994. Ares-Serono Symposia, Rome, 1995, pp 243-51.
4. Devine A, Wilson SG, Dick IM, Prince RL. Effects of vitamin D metabolites on intestinal calcium absorption and bone turnover in elderly women. *Am J Clin Nutr* 2002, 75: 283-8.
5. Heaney RP, Dowell MS, Hale CA, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. *J Am Coll Nutr* 2003, 22: 142-6.
6. Heaney RP. Is the paradigm shifting? *Bone* 2003, 33: 457-65.
7. Heaney RP. Functional indices of vitamin D status and ramifications of vitamin D deficiency. *Am J Clin Nutr* 2004, 80 (6 Suppl): 1706S-9S.
8. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. *Br Med J* 2003, 326: 469-74.
9. Bischoff HA, Stähelin HB, Dick W, et al. Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial. *J Bone Miner Res* 2003, 18: 343-51.
10. Bischoff-Ferrari HA, Dietrich T, Orav EJ et al. Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or = 60 y. *Am J Clin Nutr* 2004, 80: 752-8.

There is no lower threshold level for parathyroid hormone as 25-hydroxyvitamin D concentrations increase

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The concept that there is a vitamin D-related plateau in PTH has been given much attention because of the desire to define some sort of objective target for an optimal plasma 25-hydroxyvitamin D [25(OH)D] level. The hope is to use the point where a threshold or plateau is attained as a way to define the optimal vitamin D intake (1, 2). The present debate is important, because official nutrition recommendations carry with them medico-legal implications for the doses of vitamin D that can be used in clinical trials. If we accept the concept of a plateau in PTH just for the sake of expediency, this will very likely put a constraint on the clinical research necessary to address the question of whether higher doses of vitamin D have therapeutic or health effects. Vitamin D [with this we refer only to cholecalciferol (3)] is a powerful, yet inexpensive orphan drug that has been very much underestimated and overlooked by modern clinical medicine.

It may come as a surprise to some, that for healthy people, PTH correlates far better with plasma 25(OH)D than with any other biochemical test including calcium or 1,25-dihydroxyvitamin D [1,25(OH)₂D]. While we are not aware that this has been emphasized before, we know it well from our own data. We saw this for adolescents, where the PTH vs 25(OH)D correlation r-value was -0.2 ($p < 0.001$) but for PTH vs plasma calcium the r-value was -0.09 ($p = 0.1$) (4). We saw this in the elderly, where for PTH vs 25(OH)D the r-value was -0.2 ($p < 0.001$) but for PTH vs plasma calcium the r-value was -0.05 ($p = 0.3$)

(5). Likewise, for 1741 endocrine outpatients there was better correlation for PTH vs 25(OH)D, r-value = -0.2 ($p < 0.001$), than for PTH vs plasma calcium, r-value = -0.08 ($p = 0.001$) (6). The relationship between parathyroid hormone (PTH) vs 25(OH)D has usually been presented in a style of graph that makes it look as if this reaches a plateau (7-11). This concept of a plateau suits the conventional thinking that levels of a hormone like PTH are determined according to the "set-point" of the thing that the hormone regulates, like plasma calcium (12). This traditional notion of a set-point makes it seem reasonable to conclude that because PTH levels do respond to 25(OH)D, then this should also behave according to some sort of vitamin D-related set-point.

One problem with this is that most of the feedback between PTH and vitamin D nutrition is poorly understood. In part, vitamin D nutrition affects PTH by promoting calcium absorption (13). However, if this were the major mechanism, the correlation between PTH and calcium should be far stronger than the one between PTH and 25(OH)D. This stronger mechanism involves metabolism of 25(OH)D within parathyroid tissue to generate 1,25(OH)₂D in a local, paracrine or autocrine manner (14). This local 1,25(OH)₂D suppresses PTH gene transcription, PTH synthesis and secretion (15). Consequently, the real question is: "Can the vitamin D-related mechanisms that suppress PTH become saturated (*i.e.* do they reach a plateau), or not?"

Chapuy et al. (7) have done fine work to establish the concept of a plateau by fitting an exponential decay function to data on 1569 French study subjects. They reported that a PTH asymptote of 36 pg/ml was reached at a 25(OH)D concentration of 78 nmol/l. Visual inspection of all such data does suggest a similar conclusion. Indeed, our own analysis of 1741 endocrine outpatients produced similar plateau results when we applied the mathematical strategy of Chapuy et al. (6, 7).

However, the mathematical approach just described is not objective, because it starts with the unfounded

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assumption that a plateau does exist. Any evaluation of the shape of a relationship cannot be objective if it guarantees an outcome because of the parameters or a cut-point selected by the investigator.

It should not come as a surprise that the concept of a PTH plateau vs 25(OH)D has resulted in many different estimates for the optimal 25(OH)D concentration – from 20 to 110 nmol/l (7, 8, 16-19). Differences in assays account for some of the variability (20), but most of the variability is because the decision about when a plateau is reached is quite arbitrary. It is affected by the range of 25(OH)D levels being looked at, the nature of the study population (6), and by the mind of whoever is looking at the data. We invite the reader to try to select a plateau for the PTH vs 25(OH)D data based on graphs of Sahota et al. (21) or Need et al. (22). This exercise will highlight just how subjective and arbitrary it is to decide upon a plateau for a relationship that is more appropriate for log-log scales.

Cleveland (23) recognized the problem of a researcher-driven bias in defining relationships between two variables, and he developed the locally weighted scatterplot smoother line (LOWESS) to provide a way to allow the data to speak for themselves. This method is implemented on popular statistical graphing software programs (for example, SPSS, Chicago, IL). We found that regression lines obtained using log PTH vs log 25(OH)D concentrations were a close match to LOWESS plots of the data, and this worked for patients of every age group (6). What we have now done is to expand this analysis to additional large cohorts from Lebanon, and spanning different age groups (Fig. 1) (4, 5, 24-26). Except for one group [the Bekaa cohort, in which there were only 15 out of 477 subjects with 25(OH)D >60 nmol/l (25)] all groups of subjects – children, adolescents, young adults and the elderly – produced LOWESS lines that sloped steadily downwards and definitely no plateau when graphed as log PTH vs log 25(OH)D. The LOWESS line for the combined data stands as strong evidence that this relationship is best linearized with log vs log axes, and without a plateau (Fig. 1).

We have shown elsewhere that the PTH vs 25(OH)D relationship varies with age, and that it does not reach a plateau at any age (6). Yan et al. compared population samples from China and the United Kingdom. They concluded that for both populations, PTH vs 25(OH)D does not reach a plateau, and they used log-log scales as the best match for the data. Anyone who insists on using PTH to establish a target level for 25(OH)D must bear in mind that PTH can always be suppressed further by increasing 25(OH)D, and that this happens in a log-dose-response manner (27, 28).

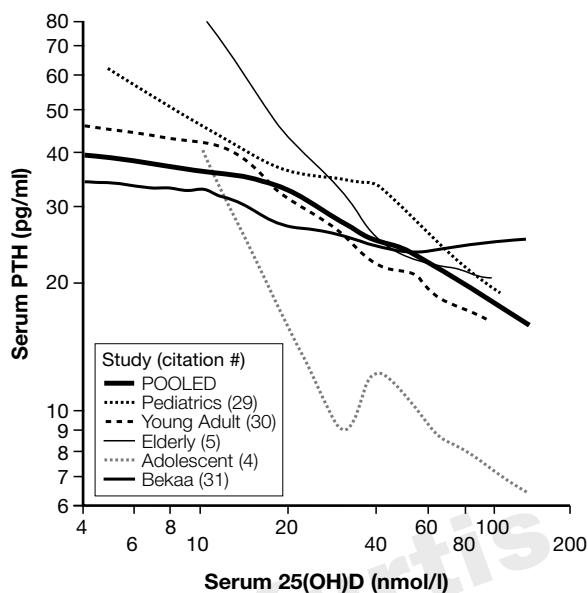


Fig. 1 - Relationship between PTH and 25-hydroxyvitamin D [25(OH)D] concentrations in several studies conducted in Lebanon. Each line represents the nonparametric regression of data for 346 children/adolescents (24), 362 children/adolescents (4), 213 young adults (26), 477 adults (25), 460 elderly (5). The heaviest, solid line represents the combined LOWESS plot of all 1858 data points represented here.

Barger-Lux et al. (27) conducted an elegant study comparing the efficacy of vitamin D₃, 25(OH)D and 1,25(OH)₂D at suppressing PTH. They showed that for each agent, the suppression of PTH was highly significant and inversely correlated to the dose; furthermore, serum calcium was increased only in those who took 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], but not the nutrient form of vitamin D. This highlights a rarely appreciated aspect of the vitamin D system: vitamin D and 25(OH)D are physiologic, non-calcemic suppressors of PTH secretion.

An expert panel recently agreed that 20-25 µg/day (800-1000 IU/day) of vitamin D is "optimal" based largely on PTH levels (2). However, the log-log relationship of Figure 1 can be translated as meaning that the percentage suppression of PTH that results from an increase in vitamin D intake from 5 to 20 µg/day (200 to 800 IU/day) is the same as the percentage suppression that will happen if we go from 20 to 80 µg/day (800 to 3200 IU/day) – all while remaining well within the physiologic range for vitamin D supply (29, 30). Therefore, there may still be a lot of room for more vitamin D to show greater efficacy for the prevention or treatment of osteoporosis, but it will be difficult to carry out clinical trials if we suggest that low intakes are "optimal".

It is helpful to point out that vitamin D nutrition relates to endocrine functions beyond just mineral metabolism. Insulin levels, insulin sensitivity, and glucose levels after a glucose challenge all relate favorably to higher 25(OH)D levels (31). A log-log graph describes the insulin vs 25(OH)D relationship just as well as it describes the PTH vs 25(OH)D relationship (31).

This debate about an optimal level of 25(OH)D is not a simple issue of semantics. Acceptance of a plateau is acceptance of an endpoint target that exists only for the sake of expedience. Acceptance of a plateau is a concession to a side issue whose purpose is to provide a "criterion for adequacy" to satisfy a need of those who must make official nutrition recommendations (1). As a basis for a vitamin D intake recommendation, regulators want an objective criterion like a PTH plateau that they can aim at. By itself, this is an admirable objective. But unfortunately, the resulting official nutrition recommendation creates medico-legal restrictions that affect the more important question of whether higher doses of vitamin D are relevant to health. Official recommendations define the limits for the vitamin D doses that ethics review panels and lawyers will easily allow for clinical research.

If we concede to the concept of a vitamin D-related threshold for PTH, then we will make it more difficult for researchers to explore vitamin D supplementation at a level beyond that imaginary plateau point. In contrast, if we accept the reality that there is no plateau, this means that future researchers will be less constrained in their exploration of the potential benefits of what may turn out to be a powerful orphan drug, vitamin D.

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REFERENCES

1. Yates AA, Schlicker SA, Sutor CW. Dietary reference intakes: the new basis for recommendations for calcium and related nutrients, B vitamins, and choline. *J Am Diet Assoc* 1998, 98: 699-706.
2. Dawson-Hughes B, Heaney R, Lips P, Meunier P, Vieth R. Vitamin D Round Table. In: Dawson-Hughes B, Heaney R, Burckhardt P eds. *Nutritional aspects of osteoporosis*. New York: Academic Press 2004.
3. Vieth R. Why "Vitamin D" is not a hormone, and not a synonym for 1,25-dihydroxy-vitamin D, its analogs or deltanoids. *J Steroid Biochem Mol Biol* 2004, 89-90: 571-3.
4. Arabi A, Nabulsi M, Maalouf J, et al. Bone mineral density by age, gender, pubertal stages, and socioeconomic status in healthy Lebanese children and adolescents. *Bone* 2004, 35: 1169-79.
5. Arabi A, El-Hajj Fuleihan G, Baddoura R, Awada H, Salamoun M. Hypovitaminosis D in a sunny country and its relation to musculoskeletal health in the elderly. *American Society for Bone and Mineral Research, Meeting Presentation, Seattle USA, 2004* (abstract).
6. Vieth R, Ladak Y, Walfish PG. Age-related changes in the 25-hydroxyvitamin D versus parathyroid hormone relationship suggest a different reason why older adults require more vitamin D. *J Clin Endocrinol. Metab* 2003, 88: 185-91.
7. Chapuy MC, Preziosi P, Maamer M, et al. Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int* 1997, 7: 439-43.
8. Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. *Lancet* 1998, 351: 805-6.
9. Meunier P. Vitamin D insufficiency: reappraisal of its definition threshold and bone consequences. In: Burckhardt P, Dawson-Hughes B, Heaney R eds. *Nutritional aspects of osteoporosis*. San Diego: Academic Press. 2001, 152-72.
10. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *N Engl J Med* 1997, 337: 670-6.
11. Gallagher JC, Kinyamu HK, Fowler SE, Dawson-Hughes B, Dalsky GP, Sherman SS. Calcitropic hormones and bone markers in the elderly. *J Bone Miner Res* 1998, 13: 475-82.
12. Brown EM. Four parameter model of the sigmoidal relationship between parathyroid hormone release and extracellular calcium in normal and abnormal parathyroid hormone tissue. *J Clin Endocrinol Metabol* 1983, 56: 572-81.
13. Heaney RP, Barger-Lux MJ, Dowell MS, Chen TC, Holick MF. Calcium absorptive effects of vitamin D and its major metabolites. *J Clin Endocrinol Metab* 1997, 82: 4111-6.
14. Segersten U, Correa P, Hewison M, et al. 25-hydroxyvitamin D(3)-1alpha-hydroxylase expression in normal and pathological parathyroid glands. *J Clin Endocrinol Metab* 2002, 87: 2967-72.
15. Beckerman P, Silver J. Vitamin D and the parathyroid. *Am J Med Sci* 1999, 317: 363-9.
16. Lips P, Wiersinga A, Van GF, et al. The effect of vitamin D supplementation on vitamin D status and parathyroid function in elderly subjects. *J Clin Endocrinol Metab* 1988, 67: 644-50.
17. Peacock M. Effects of calcium and vitamin D insufficiency on the skeleton. *Osteoporos Int* 1998, 8 (Suppl 2): S45-51.
18. Dawson-Hughes B, Harris SS, Dallal GE. Plasma calcidiol, season, and serum parathyroid hormone concentrations in healthy elderly men and women. *Am J Clin Nutr* 1997, 65: 67-71.
19. Krall EA, Sahyoun N, Tannenbaum S, Dallal GE, Dawson-Hughes B. Effect of vitamin D intake on seasonal variations in parathyroid hormone secretion in postmenopausal women. *N Engl J Med* 1989, 321: 1777-83.

20. Lips P, Dawson-Hughes B, Pols HA, Holick M. An international comparison of serum 25-hydroxyvitamin D measurements. *Osteoporos Int* 1999, 9: 394-7.
21. Sahota O, Mundy MK, San P, Godber IM, Lawson N, Hosking DJ. The relationship between vitamin D and parathyroid hormone: calcium homeostasis, bone turnover, and bone mineral density in postmenopausal women with established osteoporosis. *Bone* 2004, 35: 312-9.
22. Need AG, O'Loughlin PD, Morris HA, Horowitz M, Nordin BE. The effects of age and other variables on serum parathyroid hormone in postmenopausal women attending an osteoporosis center. *J Clin Endocrinol Metab* 2004, 89: 1646-9.
23. Cleveland WS. Robust locally weighted regression and smoothing scatterplots. *Journal of the American Statistical Association* 1979, 74: 829-36.
24. El-Hajj, Fuleihan GE, Nabulsi M, Choucair M, et al. Hypovitaminosis d in healthy schoolchildren. *Pediatrics* 2001, 107: E53.
25. Fuleihan GE, Deeb M. Hypovitaminosis D in a sunny country. *N Engl J Med* 1999, 340: 1840-1.
26. El Hajj Fuleihan G, Baddoura R, Awada H, Salam N, Salamoun M, Rizk P. Low peak bone mineral density in healthy Lebanese subjects. *Bone* 2002, 31: 520-8.
27. Barger-Lux MJ, Heaney RP, Dowell S, Chen TC, Holick MF. Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. *Osteoporos Int* 1998, 8: 222-30.
28. Vieth R, Pinto T, Reen BS, Wong MM. Vitamin D poisoning by table sugar. *Lancet* 2002, 359: 672.
29. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am J Clin Nutr* 1999, 69: 842-56.
30. Barger-Lux MJ, Heaney RP. Effects of above average summer sun exposure on serum 25-hydroxyvitamin d and calcium absorption. *J Clin Endocrinol. Metab* 2002, 87: 4952-6.
31. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr* 2004, 79: 820-5.

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