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EDITORIAL

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Dear Colleagues,
In this issue, we feature two contributions relating to the complex but focused topic of vitamin D metabolism, which is in part yet to be explored, and to the necessary distinction between normal and optimal vitamin D levels.
The first article presents the fascinating interpretation of paleontology, according to which vitamin D, and in particular cholecalciferol, represents an extraordinary means which is finely regulated to respond above all to the necessity of improved intestinal absorption of calcium. This function became necessary when – during the course of evolution – vertebrates gradually moved from seas and oceans (where the availability of calcium was more than sufficient) onto land.
The second article of this issue is a contribution to the current discussion on the correct definition of vitamin D deficiency. It clarifies the fact that normal or optimal vitamin D values differ according to whether we are dealing with healthy populations or with patients with specific diseases who are particularly at risk of vitamin D deficiency or likely to suffer from its consequences.
Readers will note that a coherent definition of vitamin D deficiency is still lacking today on the part of the major international scientific associations that are concerned with this issue [1-4], as shown in Figure 1.
This lack of clarity indeed stems from the fact that different recommendations apply to different population types: these vary according to whether we are considering the healthy general population, for which 25OHD levels above 50 nmol/L seem to be sufficient to prevent known bone complications caused by deficiency, or if we are treating individual patients, such as those affected by osteoporosis – especially if elderly – for whom values above 75 nmol/L would appear to be optimal.
Clearly the correct definition of vitamin D deficiency influences prevention strategies, which will differ depending on the prevalence of this deficit: different definitions will apply to populations according to their various circumstances: phenotype, genetic make-up, age, race, geographical location, climate, nutrition, culture and lifestyle…
A recent position statement of the European Calcified Tissue Society [4] – which was rightly dedicated to the memory of Steven Boonen and Silvano Adami – acknowledged that vitamin D deficiency is a common condition in Europe, especially in southern countries, as well as in the Middle East. For this reason, it recommends general strategies of fortified foods for the general population as well as vitamin D supplementation for high-risk cate-

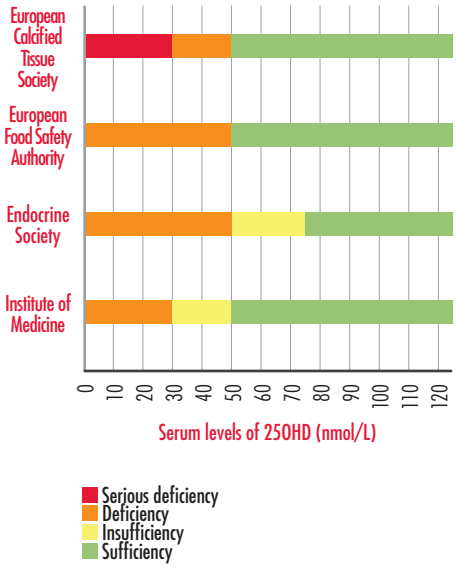

FIGURE 1.
Definition of vitamin D deficiency according to leading international scientific associations.

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VITAMIN D - UpDates
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gories: children until the age of 3, pregnant women, elderly persons in institutions or over the age of 70, immigrants and refugees.
What do you think?
I hope you enjoy reading this issue.

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CHOLECALCIFEROL: A PERFECT SYNTHESIS

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Vitamin D activation is realized by means of complex mechanisms included within the physiological regulation of mineral metabolism. This review focuses on metabolic systems that lead to vitamin D synthesis. Evolution has made it necessary to satisfy the needs of increasingly complex organisms, which are moreover located in areas with in areas with ever less calcium availability. Although the mechanism is not yet completely clear, the outline that has emerged – in spite of its complexity – helps us understand the key role that nature has always attributed to this particular vitamin.

VITAMIN D: ONE OR MANY?

Secosteroids are a subclass of tetracyclic steroids in which one of the rings has been "broken" (the prefix "seco-" derives from the Latin *secare*, to cut).

The prototype of these compounds is cholecalciferol (or vitamin D_3), although in reality several secosteroids show such markedly analogous structures that they are grouped under the name of vitamin D (Fig. 1).

In nature, there are essentially two main forms of vitamin D: ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Vitamin D_2 is found in plants and derives from irradiation with UVB ultraviolet rays (290-315 nm) of ergosterol or provitamin D_2 (Fig. 1A) [1].

Vitamin D_3, by contrast, is of animal origin and is produced on the skin, thanks to the action of the same kind of UVB irradiation on 7-dehydrocholesterol or provitamin D_3 present on the epidermis (Fig. 1A) [1].

If these are the two main forms of vitamin D, they are not the only ones. Thanks to UVB action, other tetracyclic steroids are converted into secosteroids which are structurally similar to cholecalciferol (Fig. 1B). The best known of these are vitamin D_4 and D_5 [2]. About others we still know little if anything at all, except that they are much less biologically active [2, 3].

Vitamin D_4 is structurally similar to D_3. By means of complex chromatographic techniques, it can be found in certain types of mushrooms (especially in the gills following exposure to sunlight) as well as in marine mollusks and yeasts, in which it is not distinguishable from D_3 in normal doses [2-5]. In mice, it has a much lower capacity (roughly half) than cholecalciferol in healing rickets [4], although its active metabolites have shown antiproliferative and differentiation effects at the cellular level (*in vitro*) similar to those produced by calcitriol [1-25(OH)2 vitamin D_3] [3].

Interesting data are also available for vitamin D_5. It is also of vegetable origin and has been identified (again through chromatographic studies) in some plants, in which, however, its physiological role is still completely unknown. The interest of researchers in vitamin D_5 is connected to the anti-neoplastic capacity of its hydroxylated metabolite in the 1 alpha position (1 alpha-OH vitamin D_5) [7]. *In vivo* and *in vitro* studies have shown its inhibitory effect against mammalian carcinogenesis in mice, with a toxicity framework that is completely negligible. Independently of the dose used, in mice there has in fact been no evidence of the typical toxic effect of hypercalcemia, which is rather seen with calcitriol at doses necessary to have a protective effect against carcinogenesis [7]. Beyond these interesting data on cellular life (proliferation and differentiation), vitamins D_4 and D_5 therefore present calciotropic hormonal activity which is either modest (vitamin D_4) or completely lacking (vitamin D_5); for this reason, they cannot be viewed in the same way as vitamin D_3 [3, 4].

As we know, cholecalciferol normally derives from the transformation of 7-dehydrocholesterol present in the epidermis. This precursor, though, can physiologically follow metabolic paths different from the classical one (Fig. 1C). Under the action of enzymes of the cytochrome P450 superfamily, it can undergo various transformations, even giving rise to the delta-7-steroid family. The latter, again by means of UVB action, can be transformed into secosteroids which are different from and alternative to cholecalciferol (Fig. 1C).

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VITAMIN D - UpDates
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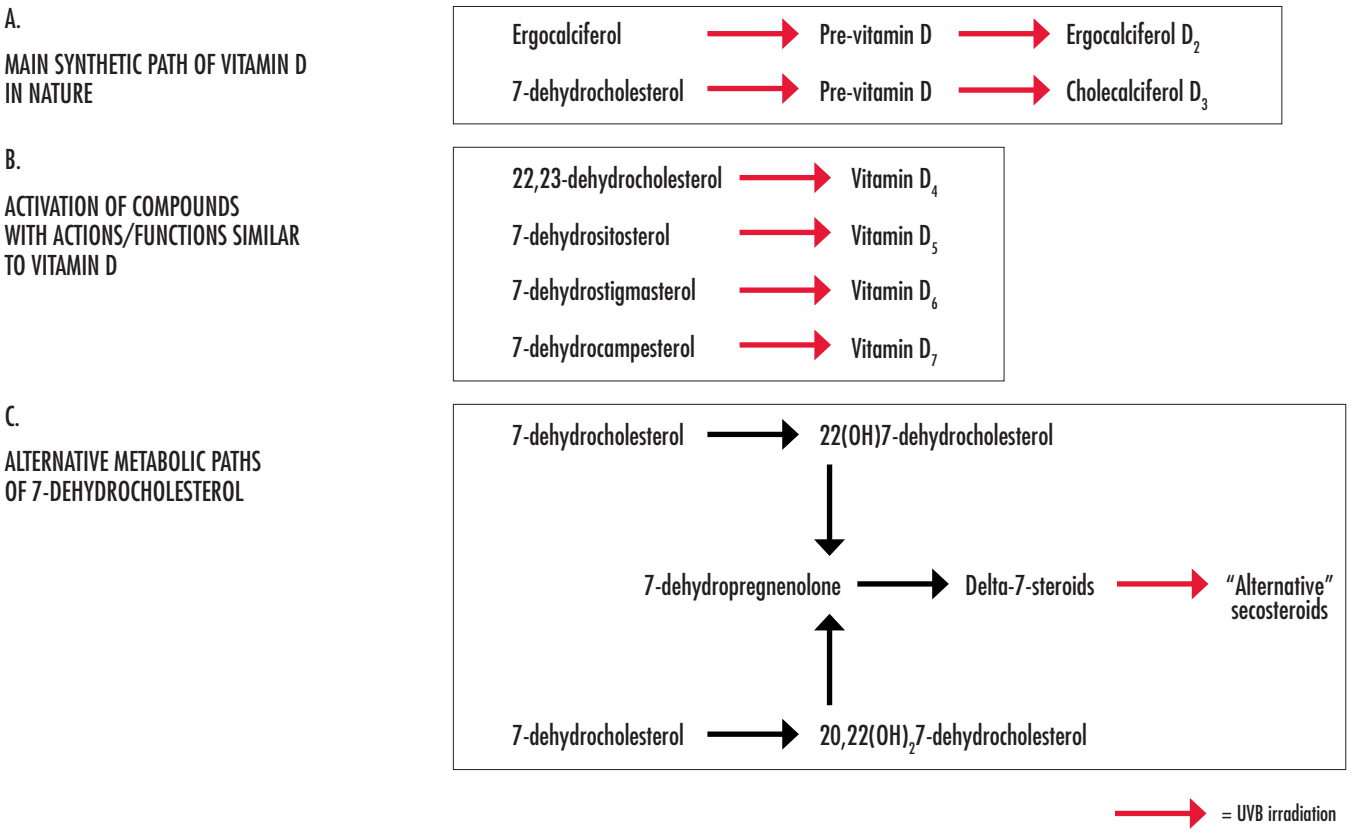

FIGURE 1.
Schematic summary of processes that lead to the synthesis of various secosteroids.

In a way similar to vitamins D_4 and D_5, these compounds can likewise produce metabolites which are biologically active at the cellular level (they can, for example, inhibit the proliferation of melanoma cells in a way similar to calcitriol) [8], without, however, producing any type of endocrine effect or of having consequences on bone metabolism [5].
In sum, ergocalciferol and above all cholecalciferol represent in humans the only efficient substrate – together with and thanks to PTH – to carry out important functions in regulating calcium and phosphorus. All other compounds, even if they often have great molecular similarities and sometimes even analogous autocrine activity, do not at all resemble vitamin D from either a functional or biological point of view in the way in which we normally consider it to be the center of skeletal metabolism.

THE IMPORTANCE OF SUNLIGHT AND HEAT

As is clearly seen in Figure 1, a key role in vitamin D synthesis is played by solar irradiation. Why? The reason is simple. Only energy from photons in the UV spectrum is able to effect the opening of the B ring in 7-dehydrocholesterol, which is essential for for the formation of the secosteroid prototype cholecalciferol – that is, of cholecalciferol [5]. The same thing obviously holds true for ergocalciferol. Nonetheless, UVB action turns out to be particularly efficient (both in quantitative terms and in the rapidity of transformation) when the precursor which it acts upon is located within the biological membrane. In the experiment shown in Figure 2, UVB irradiated both 7-dehydrocholesterol molecules inserted into a cellular membrane (lizard and human skin) and molecules in a biological solution. The rapidity and extent of the transformation process were much greater when the precursors were structured within a cellular membrane [9]. This explains why this reaction also occurs in biological materials which clearly possess little vitality, such as animal feces or hay [5].
The experiment proposed in Figure 2 also foregrounds the crucial role played by temperature. In fact, in the same structure (precursor in a membrane or in a solution), transformation turns out to be much more efficient at a higher temperature (25°C). Physiologically, 7-dehydrocholesterol, when placed at the cellular membrane level and subjected to UVB action, is transformed into previtamin D_3 (Fig. 1). The last stage of the cutaneous synthesis of vitamin D involves the conversion of previtamin D into vitamin D by means of a process of temperature-dependent isomerization. Previtamin D is not only an unstable molecule that must be "guided" in its transformation toward vitamin D; it is also and above all a biologically inactive compound for which this transformation becomes absolutely crucial.
In the course of the evolution of the species, nature has selected more and more efficient mechanisms. At first, precursors were diluted within cellular cytoplasm with a quite reduced transformation efficiency. Later, the isomerization yield was increased (by over 15 times) by the structure of the precursors within a membrane; finally it was made

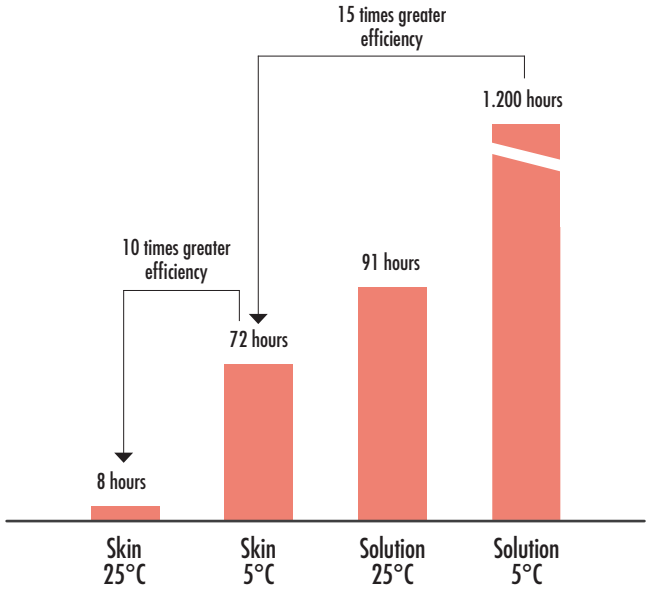

FIGURE 2.
Times of UVB irradiation necessary to transform 50% of 7-dehydrocholesterol into cholecalciferol (vitamin D_3). Comparison of transformation efficiency in different conditions: 1) precursor in biological solution or within cellular membrane (e.g., skin); 2) low and high temperatures. Maximum efficiency is obtained when precursor is within cellular membrane (e.g., skin) at high temperature: this condition is realized in warm-blooded animals (from Holick et al., 1995, modified) [9].

Evolution →

	MARINE VERTEBRATES	AMPHIBIOUS VERTEBRATES	TERRESTRIAL VERTEBRATES
ENVIRONMENT	Oceans	Coasts	Land
CALCIUM AVAILABILIT	High	Medium	Low
ORGANIZATION OF VITAMIN D PRECURSORS	Solution	Membrane	Membrane
TEMPERATURE CORPOREAL	Cold	Cold	Hot
PRE-VITAMIN D ISOMERIZATION	Reduced	**High**	**Very high**

FIGURE 3.
Hypothetical explanation of improvement of vitamin D synthesis mechanisms. In marine animals, the wide availability of calcium rendered improved absorption processes useless. With the gradual movement to sea and ocean layers closer to the surface and especially to land (where calcium is far less available), these mechanisms were perfected over time. At first, vitamin D precursors were arranged within the membrane (skin) such that solar energy could be harnessed. Then, with the evolution of warm-blooded animals, higher temperatures made possible the optimization of the final isomerization process of previtamin D into vitamin D.

even more productive and rapid (over 10 times so) by high temperatures (≥ 25°C) [5, 9]. This gradual optimization of processes of vitamin D synthesis connected to the action of light and temperature finds a possible explanation in the history of the evolution of vertebrates (Fig. 3) [5]. Large quantities of calcium were present in fertile oceans, more than enough to satisfy the needs of the first marine vertebrates. During evolution, however, animals began to move toward ocean layers closer to the surface and then onto land, an environment totally lacking in calcium. At first, solar energy was used, thanks to the precursors on the level of the skin (cold-blooded animals), with a clear improvement in synthetic efficiency. This was then further improved in warm-blooded animals thanks to the catalytic action of temperature (Fig. 3).

ENDOGENOUS FACTORS THAT CONDITION VITAMIN D SYNTHESIS

Melanin is an excellent solar filter able to block ultraviolet radiation, including UVB rays, which are necessary for vitamin D synthesis. This then explains why colored populations have a less efficient synthesis of vitamin D [10].
Age is a critical factor as well. Unfortunately, the concentration of 7-dehydrocholesterol in skin is progressively reduced as one ages [11]. For this reason, an elderly person has a decisively lower response in terms of cutaneous vitamin D synthesis with respect to a young person with the same exposure to sun [10]. Supplementation therefore represents the only way to satisfy vitamin D requirements in the elderly.

PROTECTION FROM EFFECTS OF EXCESSIVE SUN EXPOSURE

Solar irradiation is fundamental for the transformation of 7-dehydrocholesterol into pre-vitamin D. But what happens in the case of prolonged exposure to the sun? In reality, previtamin D also feels the effects of UVB radiation: in cases of overexposure, it undergoes further photolysis, with the formation of inactive compounds such as lumisterol and tocopherol (Fig. 4A, B). It should also be mentioned that cholecalciferol, once synthesized on the skin, must be rapidly captured by the cycle and removed. Otherwise, if it undergoes renewed exposure to the sun, it will also be subject to further photolysis, with the production of inactive final compounds (Fig. 4A) [10].

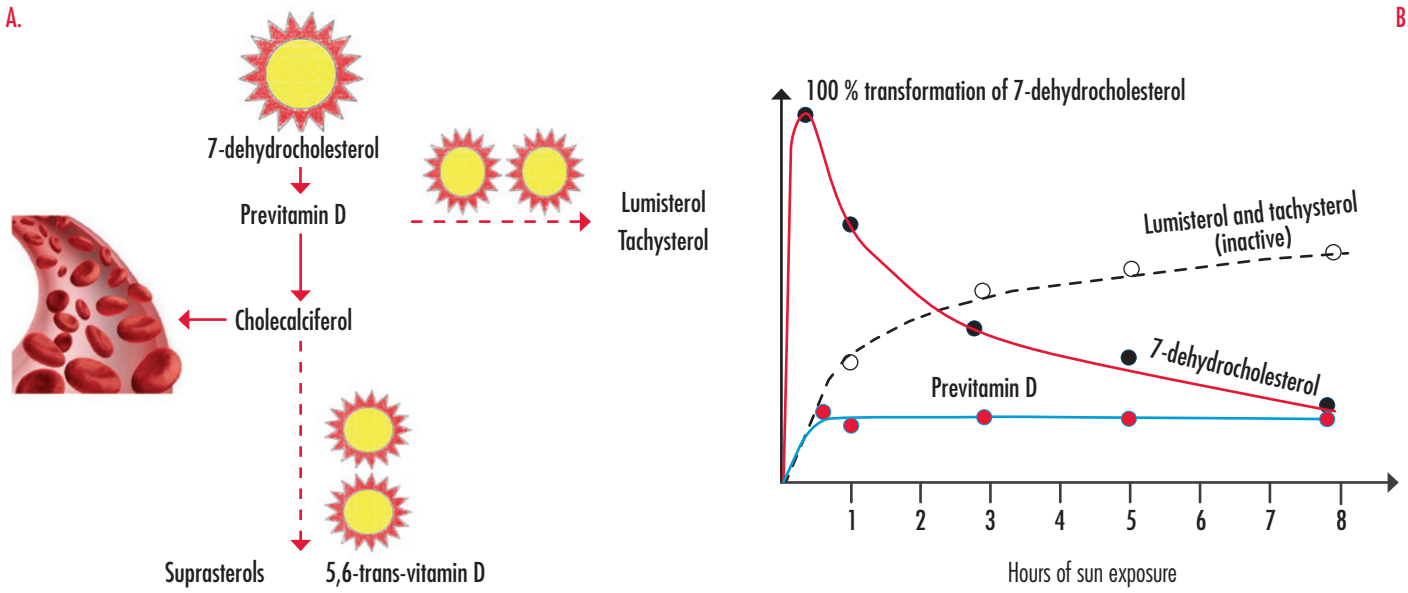

FIGURE 4.
Protection mechanisms against risk of excessive vitamin D synthesis in case of prolonged exposure to sun. A) Sun activates normal synthetic path of vitamin D (solid arrows). In case of prolonged exposure to sun (double suns), alternative paths are activated (dotted arrows). If exposed to UVB rays, previtamin D is transformed into inactive compounds (lumisterol and tachysterol). If cholecalciferol is not rapidly removed from the epidermis and enters the bloodstream, it is transformed under the action of UVB rays into inactive terminal compounds (suprasterol and 5,6 trans-vitamin D). B) Exposure to sun produces rapid (within 30 minutes) and complete transformation of 7-dehydrocholesterol, which is followed by a rapid increase of previtamin D. If exposure is prolonged, no further increase in previtamin D occurs but only an increased production of different, inactive metabolites (lumisterol and tachysterol) (from Holick, 1995, modified) [10].

CONCLUSIONS

The process leading to vitamin D synthesis appears to be particularly complex, as are, after all, the other features of its metabolism. There are many compounds with great structural similarities to cholecalciferol, yet none of these (with the exception of ergocalciferol of plant origin) is so biologically active to be considered vitamin D. This vitamin undoubtedly represents an extraordinary system to meet the needs of an improved intestinal absorption of calcium, a process that during the course of evolution became indispensable as vertebrates gradually moved from seas and oceans (where the availability of calcium was more than sufficient) to land.

For this reason, our organism created a perfect synthetic machine whose aim is to produce – according to need – the only truly efficient compound: cholecalciferol. Unfortunately, the synthesis does not always meet individual requirements, especially in elderly and fragile persons, for whom supplementation is therefore indispensable.

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RATIONALE FOR THE DEFINITION OF THE STATUS OF VITAMIN D: NORMAL AND OPTIMAL VALUES

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Vitamin D levels vary through different stages of life and according to season, latitude, degree of sun exposure, skin color and BMI. In addition, the analytical variability of vitamin D assay currently poses a significant difficulty, both in the field of research and in clinical practice.

Serum levels of 25(OH)D, which includes 25(OH)D2 and 25(OH)D3, are used today to determine vitamin D status, which is interpreted as the expression of the "vitamin D reserve." Serum 25(OH)D is relatively stable with a half-life of 2-3 weeks, while its activated form – 1,25(OH)2D – has a half-life of approximately 15 hours. Today, levels of 25(OH)D are normally determined by chemiluminescence immunoassay (CLIA), which has a variability – between different assays as well as inter-laboratory differences – of between 10 and 20%, such that the need for the standardization of doses is strongly felt, both for proper interpretation of clinical studies and for clinical practice [1].

The definition of normal and deficient vitamin D status is a much debated topic. While there is unanimous agreement that values of 25(OH)D < 10 ng represent a condition of severe deficiency, definitions of "normal" levels vary greatly. This factor has important repercussions both on epidemiological evaluations and on elements of clinical practice, as the question obviously influences prescriptions for vitamin D supplements.

The problem of the definition of a correct vitamin D level requires clarification of what is meant by "normal value" and by "optimal value." To identify a "normal level," reference is made to a statistic datum defined as ± 2 standard deviations (SDs) from the mean of detected values in a given population, a datum that has sparked the interest of researchers and institutions which study phenomena of the general population. In the case of vitamin D, there are different normal values for different geographical areas, age groups and seasons.

Distinct from "normal" values is the "optimal" or "desirable" level, which is defined as the value that has been demonstrated as effective in obtaining prevention of conditions related to vitamin D deficiency, such as fractures, on the basis of evidence provided by ad hoc observational and interventional studies. For this reason, Scientific Society provide a "recommended level" of vitamin D on the basis of the patient's profile and the outcome to be reached.

In 2011, the Institute of Medicine (IOM) defined values of deficiency, insufficiency and sufficiency at < 12 ng/mL, between 12 and 20 ng/mL, and between 20 and 30 ng/mL, respectively [2]. Other Scientific Society have suggested that levels of sufficiency could be represented by values ≥ 30 ng/mL (the Endocrine Society, the National Osteoporosis Foundation and the International Osteoporosis Foundation) [3]. In 2016, the Italian Society for Osteoporosis, Mineral Metabolism and Skeletal Diseases proposed a range of optimal levels at between 30 and 50 ng/mL [4].

There is solid evidence as well as unanimous agreement that 25(OH)D levels < 12 ng/mL (-30 nmol/L) are associated with rickets, osteomalacia and secondary hyperparathyroidism [5], to the extent that researchers are also in agreement that these values constitute a condition of deficiency [2-4].

More controversial, by contrast, is the definition of values of sufficiency. To determine the cutoff of 25(OH)D sufficiency, researchers have analyzed the associations between vitamin D levels and the correction of hyperpara-

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VITAMIN D - UpDates
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TABLE I.
Population at risk of hypovitaminosis.

- Institutionalized patients
- Conditions associated with inadequate sun exposure
- Pregnancy and breastfeeding
- Vegan diet
- Obesity
- Mineral metabolism and skeletal diseases
- Chronic renal insufficiency
- Tumors (in particular breast, prostate and colon)
- Anorexia nervosa
- Type 2 Diabetes mellitus
- Intestinal malabsorption and bariatric surgery
- Drugs which interfere with absorption or with hepatic metabolism (antiepileptic drugs, glucocorticoids, antiretrovirals AIDS, antifungals, cholestyramine)
- Cystic fibrosis
- Granulomatous diseases and some types of lymphoma

thyroidism, the intestinal absorption of calcium and several outcomes regarding skeletal health – fracture risk in particular. They have further analyzed other cutoff levels, such as those for mortality, tumors and falls.

In reality, data on optimal vitamin D levels with respect to outcomes concerning skeletal health are distributed over a range of values without a precise cutoff. Data relative to optimal 25(OH)D values on extra skeletal outcomes are even less consistent and not definable [6].

The attempt to associate the optimal 25(OH)D value to interaction with PTH does not appear convincing, because studies have found that 25(OH)D values that normalize PTH oscillate between 12 ng/mL (30 nmol/L) and 36 ng/mL (90 nmol/L) [7]. In addition, the interaction curve does not actually seem to have a real plateau point for PTH at 30 ng/mL of 25(OH)D as described; above all it varies considerably by age group and is strongly dependent on the calcium intake [8].

For the definition of an optimal level in the general population, we can take into account the association between vitamin D deficiency and fractures. There is significant consensus on the association between 25(OH)D values less than 20 ng/mL and increased fracture risk [9]. A recent meta-analysis has shown that for levels less than 20 ng/mL there is a 40% increase in femur fracture risk for each SD decrease of 25(OH)D [10]. Similarly, another meta-analysis on prospective cohort studies has reported that fracture risk is linearly reduced up to a 25(OH)D value of approximately 24 ng/mL (60 nmol/L). For values > 24 ng/mL, fracture risk ceases to decrease [11].

By contrast, there is no evidence that 25(OH)D values > 20 ng/mL are beneficial for skeletal health (BMD or fractures) in the general population. In a large randomized controlled study on healthy adults, elevated doses of cholecalciferol (equal to 100,000/IU per month) for approximately 4 years did not provide any benefit in terms of risk of fall and fracture with respect to the control group. Since 80% of the studied population had baseline values of > 25 ng/mL (60 nmol/L), these results indicate that this value is sufficient and adequate in the general population. Consequently, there is no reason for or advantage to supplementation in these subjects [12].

A recent meta-analysis on the musculoskeletal effects of vitamin D supplementation confirms these findings. Researchers have indeed concluded that there is no significant effect on BMD (bone mineral density) and fractures. Yet 55% of the studies included in the meta-analysis recruited patients with base values > 20 ng/mL (50 nmol/L), and only 6% looked at patients with levels < 10 ng/mL (25 nmol/L), again indicating that supplementation in subjects with values ≥ 20 ng/mL does not bring any benefit; this 25(OH)D level can therefore be considered adequate in the general population [13].

Another relevant aspect to be emphasized is that optimal 25(OH)D values ≥ 20 ng/mL (50 nmol/L) – that is, levels at which supplementation does not seem to produce benefits – refer to the normal population, in other words, to healthy subjects outside of institutional settings, persons who do not show the classic conditions of high risk of hypovitaminosis (Table I). These "healthy" persons often represent the majority of subjects included in prospective population studies and randomized trials in which supplementation with cholecalciferol did not produce clinically significant results. In a wide-ranging meta-analysis of 9 randomized controlled trials (RCTs) on healthy adult subjects – who were indeed selected because they did not have osteoporosis, fractures or a risk of fall and did not use osteopenia drugs – cholecalciferol supplementation with doses from 700 to 3,000 IU/day did not have any effect on fractures, mortality or morbidity [14].

The definition of a correct target of 25(OH)D values and of the categories of subjects in which supplementation is appropriate is therefore fundamental in order to prevent an excessive use of supplements in a broad sector of the population, which in particular will not receive any benefit from them [15]. The lack of such definitions has had the harmful result that vitamin D has been uncritically included among overused drugs and supplements and has attracted the attention of national regulatory agencies [16].

There is also general agreement as well as evidence that vitamin D supplementation is indispensable in subjects at risk of hypovitaminosis (Table I) and in those being treated with drugs able to reduce fracture risk (antiresorptive and anabolic).

In the RCT meta-analysis in which the overall effect of vitamin D supplements (with or without calcium) on fractures seems negative, researchers observed a significant benefit in terms of fracture risk reduction in the subgroup of patients who were either institutionalized or had previous fractures [13], a conclusion also supported by ESCEO and IOF [17].

In the RCT meta-analysis on vitamin D supplementation, those trials which showed an outcome of reduced femur and non-vertebral fracture risk saw significant reductions – 20% for non-vertebral and 18% for femur fractures – in subjects that reached 25(OH)D values > 30 ng/mL (75 nmol/L) [18, 19].

Today, we are paradoxically witnessing widespread vitamin D supplementation in population sectors that receive no advantage from it, while supplementation is not used by subjects who by contrast would benefit greatly, such as those at risk of fracture undergoing therapy with drugs for osteoporosis. Drugs for fracture risk reduction (which in Italy appear on the *Nota 79* list) were always associated with vitamin D supplements in the observational RCTs. A lack of vitamin D supplementation in association with these drugs significantly reduces the latter's anti-fracture effect, therefore worsening the cost-benefit relationship of the drugs themselves [20, 21]. Failure to combine anti-fracture drug therapies with vitamin D intake is the major cause of repeated fractures [22]. For this reason, it is crucial to assure that cholecalciferol supplementation accompanies any type of specific therapy for osteoporosis and also to guarantee that levels reach at least the optimal value of ≥ 30 ng/mL.

The upper limit of optimal values in the general population has been defined at 50 ng/mL (125 nmol/L) on the basis of some data that show a "U-shaped" tendency on several outcomes, such as falls and mortality,

TABLE II.
Definition of vitamin D status.

	Deficiency	Insufficiency	Optimal
General population	< 10 ng/mL	< 20 ng/mL	20-50 ng/mL
At-risk population*	< 10 ng/mL	< 30 ng/mL	30-50 ng/mL

*At-risk population for hypovitaminosis is shown in Table II. These values also apply to those subjects who are to begin or are undergoing anti-fracture therapy for osteoporosis. Multiply ng/mL by 2.5 to obtain values in nmol/L.

suggesting that beyond these values pathological events could reoccur. Thanks to the standardization of 25(OH)D doses, a recent study has shown that the curve between vitamin D levels and mortality is not U-shaped by rather flat ("J-shaped"). The plateau occurs at values of approximately 18-20 ng/mL (40-44 nmol/L) [23]. This finding indicates that in the general population reaching 25(OH) D levels far above 30 ng/mL is not particularly useful, even if it is relatively safe.

In conclusion, the definition of optimal 25(OH)D levels is fundamental, as it has repercussions not only on epidemiological estimates but also on daily clinical practice. In the general population, including in elderly subjects who are substantially healthy, a 25(OH)D value ≥ 20 ng/mL (50 nmol/L) should be considered adequate, while in patients with osteoporosis especially if they are undergoing therapy with a *Nota 79* drug, a value ≥ 30 ng/mL (75 nmol/L) should be considered optimal (Tab. II).

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