INTRODUCTION
In the last 20-30 years, increasing awareness of the role of vitamin D in the pathogenesis of some musculoskeletal and extra-skeletal diseases, together with substantial epidemiologic evidence on the prevalence of hypovitaminosis D in the general adult population and in the elderly, have given rise to an ever-growing debate concerning the appropriateness of the strategies for the prevention and treatment of vitamin D deficiency [1-9].

A number of scientific works have investigated the use of cholecalciferol and vitamin D metabolites (calcifediol in particular) for the prevention and treatment of vitamin D deficiency [1-9]. Undoubtedly, the great number of these randomized, controlled trials (RCTs) have produced a significant advance in our knowledge on this topic, highlighting extremely important clinical aspects [1, 3, 5, 6]. However, the quantity, quality (not always high) and heterogeneity of these published studies have also generated some doubts on the issue.

The aim of our narrative review is to describe the main attributes of vitamin D metabolites and to define their role in daily clinical practice, with the aim of aiding physicians in choosing adequate strategies to use in patients with proven vitamin D deficiency or with a risk of hypovitaminosis D.

VITAMIN D PRODUCTION AND METABOLISM
In general, the term vitamin D refers to both vitamin D3 (cholecalciferol), produced by animals and humans, and vitamin D2 (ergocalciferol), produced by plants [1]. Endogenous synthesis is the principal source of vitamin D for the organism; it derives from the conversion of 7-dehydrocholesterol after skin exposure to UV rays of a specific wavelength. This mechanism should produce most (approximately 80%) of the vitamin D requirements (vitamin D3), while lower quantities (approximately 20%) of vitamin D3 and vitamin D2 should come from dietary sources [1].

Vitamin D3 skin production is greatly influenced by seasonal change (lower during winter), latitude, the surface area and thickness of the skin exposed to sunlight (and perhaps also the use of sunscreens), and age (lower in the elderly) [1]. A lower dosage of vitamin D3 can be obtained from food, in particular from animal fats, while the amount of vitamin D3 in vegetable fats is totally negligible [1]. Dietary intake of vitamin D is significantly higher in those countries where vitamin D fortified foods with cholecalciferol are allowed [1].

Vitamin D is highly liposoluble, such that upon absorption it is stored in fatty tissues and released in small quantities. This explains why obese subjects are at higher risk of vitamin D deficiency, as it becomes “diluted” in the higher body mass [1]. As vitamin D does not stay in the body for very long, its concentration in the bloodstream is very low (1-2 ng/mL) [1]. In the liver, vitamin D is converted into 25-hydroxyvitamin D [25(OH)D] by the enzyme 25-hydroxylase. This transformation process can also take place in the presence of a significant reduction in the hepatic tissue function, although a high prevalence of hypovitaminosis D is evident in patients with correlated chronic hepatitis HCV [1].

Calcifediol, or 25(OH)D, has a high affinity for the vitamin D binding protein (VDBP) and represents the main vitamin D blood metabolite. Its concentrations are the most reliable representation of the vitamin D status of individuals [1]. Serum 25(OH)D concentrations constitute an accurate indicator of our storage of vitamin D. As a result, the question of vitamin status (deficiency, insufficiency and sufficiency) is exclusively based on serum 25(OH)D levels (Table I).
In part, 25(OH)D is a hydrophilic metabolite; it is stored only in the liver and muscles [1]. Its half-life is shorter than that of vitamin D, such that it is able to satisfy the organism’s requirements for no more than 12-18 days [1, 4]. 25(OH)D has a low affinity for the specific vitamin D receptor and thus needs to be transformed into calcitriol, or 1,25-dihydroxyvitamin D [1,25(OH)2D], to become metabolically active [1, 4].

The conversion of 25(OH)D into 1,25(OH)2D is achieved by the action of the 1-alpha-hydroxylase enzyme, which takes place predominantly in the kidneys, but it can also occur in other tissues [1]. Most of the production of 1,25(OH)2D, whose most important role is to control mineral metabolism, occurs in the renal proximal tubules. The production of 1,25(OH)2D by the action of the 1-alpha-hydroxylase enzyme involves the presence of the parathyroid hormone (PTH) and is in part modulated by serum calcium and phosphorus levels [1]. 1,25(OH)2D is not stored at tissue level and has a very short half-life [1, 4].

Renal insufficiency progressively reduces 1,25(OH)2D production [1]. Nevertheless, a significant decline in 1-alpha-hydroxylase enzyme activity, such that normal hormone levels are compromised, is only detected when associated to severe renal function impairment (generally 4-5/5D stage) [1, 10]. However, it should be emphasized that even when renal 1-alpha-hydroxylase enzyme activity is severely compromised, 25OHD levels must be kept within the normal range to ensure an adequate substrate for extrarenal 1-alpha-hydroxylase [1, 10].

By binding with a specific receptor (VDR, which is present both in the nucleus and in the cell membrane), 1,25(OH)2D (as an active metabolite) produces a biological response at the cellular level [1]. Such a response is produced both by triggering gene transcription (genomic mechanism) and through the action of cellular second messengers or the phosphorylation of some proteins (non-genomic mechanism) [1]. Vitamin D receptors are ubiquitous in the body.

CHOLECALCIFEROL, ERGOCALCIFEROL AND VITAMIN D METABOLITES

In addition to the two natural forms of vitamin D – vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol) – many other supplements/metabolites with the same vitamin D activity are available for daily clinical practice [1, 4]. Some of them, such as calcifiediol, were already clinically synthesized and utilized in the last century. Other forms, meanwhile, have been synthesized and primarily employed in nephrology (for example, paricalcitol) [4].

A comprehensive discussion of all vitamin D metabolites, particularly those which are mainly used in nephrology, is not the intent of our review. Our presentation will therefore focus on the most common types of vitamin D utilized in daily clinical practice; it will aim to describe their properties (Table 2) and to briefly summarize clinical data obtained from RCTs.

Cholecalciferol

Cholecalciferol (vitamin D3) is the natural form of vitamin D of animal and human production. Cholecalciferol (vitamin D3) is a prohormone, the precursor of the two vitamin D hydroxylated forms [25OHD and 1,25(OH)2D]; it therefore needs to undergo two processes of hydroxylation before being transformed into its metabolically active form [1, 4].

Cholecalciferol is normally stored in the adipose tissue, from where it is slowly released [1]. For this reason, its blood half-life is very short (estimated T1/2 = 19-25 hours), while its functional half-life (several weeks) is definitely longer (in correlation with its slow release from the adipose tissue) [4], making it an extremely flexible and adaptable substance to use in daily clinical practice and rendering possible intermittent administration regimes [1, 2].

Cholecalciferol is available on the market for oral and intramuscular use. With the exception of specific clinical conditions (malabsorption syndrome), oral administration is preferable to intramuscular injection because it is more effective in boosting serum 25OHD [11, 12].

Clinical studies have employed many administration regimes and different doses of cholecalciferol, ranging from 400 to 4000 IU/day and 25,000 to 50,000 IU per month [4, 6, 13-18]. Figure 1 illustrates the effect – in terms of mean increase of serum 25OHD (ng/mL) after three months – of different doses and therapeutic treatments with cholecalciferol. The lowest doses (e.g., 400-600 IU/day) have proved to be ineffective in achieving clinical endpoints (e.g., reduction of risk for fractures) [15]. Some RCTs have also investigated the effectiveness of massive doses (bolus) of cholecalciferol, with mixed results for clinical outcomes such as falls and fractures [1, 2, 6]. It is therefore recommended to avoid the bolus dosage of 100,000 IU and to distribute the administration of any higher therapeutic dosage (aiming to attain the optimal serum value of less than 30 ng/mL) over the course of two weeks [1, 2]. Recently, the Italian society SIOMMAS proposed a strategy for the prevention and treatment of vitamin D deficiency with cholecalciferol (Table 3), based on basal vitamin D status (25OHD) [2]. The cholecalciferol doses shown in Table 3 must be considered standard, although they are susceptible to variations in relation, for example, to the existence of risk factors (such as obesity) that could reduce the effect of cholecalciferol in increasing serum 25OHD values [16].

In the context of vitamin D metabolites correlated to vitamin D deficiency, cholecalciferol has by far been the one to attract most attention, both in clinical studies for the prevention and treatment of hypovitaminosis D and in RCTs evaluating its effectiveness on skeletal (falls and fractures) and extraskeletal

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**TABLE 1.**

Interpretation of blood levels of 25(OH)D (Adami et al. 2011, mod.; Rossini et al. 2016, mod.) [1, 2]

<table>
<thead>
<tr>
<th>DEFINITION</th>
<th>25OHD Units</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>nmol/l</td>
</tr>
<tr>
<td>Severe deficiency</td>
<td>&lt; 25</td>
</tr>
<tr>
<td>Deficiency</td>
<td>25-50</td>
</tr>
<tr>
<td>Insufficiency</td>
<td>50-75</td>
</tr>
<tr>
<td>Optimal Range</td>
<td>75-125</td>
</tr>
<tr>
<td>Excess</td>
<td>&gt; 250</td>
</tr>
<tr>
<td>Intoxication</td>
<td>&gt; 375</td>
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(e.g., pneumonia and neoplasia) endpoints [1-3, 6]. A systematic discussion of RCTs on cholecalciferol falls outside the aims of our review, which is limited to describing the most important findings in the field of osteo metabolic disorders.

Numerous RCTs have evaluated the efficacy of cholecalciferol in normalizing and maintaining the optimal level of serum 25OHD (> 30 ng/mL) [1, 2, 11-18]. These studies have shown that when used at appropriate doses and in suitable therapeutic regimes cholecalciferol was able to efficiently normalize 25OHD and keep it within the desired range (30-50 ng/mL) [1, 2, 13, 14, 16-18]. The definition of an appropriate dose must take into account both the basal serum 25OHD value and other clinical factors that may influence treatment response (e.g., body mass index, age, pathologies and pharmacological therapies) [1, 2, 16]. The prevention and treatment strategies described in Table III summarize a part of the evidence from these RCTs [2].

Cholecalciferol has noticeably been the focus of the greatest number of RCTs that aim to evaluate the efficiency of this metabolite in reducing the risk of fracture [6]. These RCTs and their meta-analyses have shown that cholecalciferol, when administered in appropriate doses and therapeutic regimes and when associated with adequate calcium supplementation – either through foods (only dietary calcium) or supplements – is able to produce a significant reduction in the risk of femur and non-vertebral fractures in at-risk populations [such as the elderly and adults with low 25OHD levels] [1, 2, 5, 6]. Reduced fracture risk was in part attributed to a significant reduction in the risk of falls. Even if the findings of these RCTs are not completely consistent, reports of higher scientific quality allow us to estimate a risk reduction in subjects treated with cholecalciferol of 16-30% for femur fracture and of ca. 14% for non-vertebral fracture [5, 6]. It should be emphasized that these findings derive from trials in which cholecalciferol was administered to appropriate patients (with, that is, vitamin D deficiency), and above all in suitable doses (between 800 IU and 2,000 IU/day) [5, 6, 15]. In the clinical studies, the administration of boli less than 100,000 IU turned out to be safe and free of side effects, including hypercalcemia and hypercalciuria [1, 2, 4, 6].

Finally, to provide a comprehensible framework for the use of cholecalciferol in clinical practice, we should highlight several points of undeniable importance:

• cholecalciferol has proven to be effective in reducing the risk of femur and non-vertebral fracture when it is used in doses that allow an appropriate level of 25OHD to be reached (> 30 ng/mL). In patients with osteoporosis at risk for fracture, treatment with cholecalciferol only is not sufficient to produce a significant reduction of this risk; it must be associated with anti-fracture pharmaco-
logical therapy, such as antiresorptive or osteoanabolic therapy;

- in all phase III pivotal RCTs, the active substance (bisphosphonate, denosumab or teriparatide) proved to be effective in reducing the risk of osteoporotic fracture in association with cholecalciferol;

- vitamin D deficiency (defined as a lack of cholecalciferol intake or reduced serum 25OHD) is probably the main cause of a lack of clinical response to pharmacological therapy for osteoporosis (particularly in the case of antiresorptive).

### Ergocalciferol

Ergocalciferol is natural vitamin D2 of plant origin. It is a prohormone that requires double hydroxylation to be transformed into its active form [1,25(OH)2D] [1].

It has been calculated that the T1/2 of circulating ergocalciferol is ca. 48 hours, while its functional half-life may be less than two months [4]. Ergocalciferol is available in the market in oral and intramuscular form. For years it was believed that ergocalciferol and cholecalciferol were equally effective and were therefore interchangeable [4].

Recently, however, several studies have shown that ergocalciferol is less effective in increasing serum 25OHD levels than cholecalciferol, with an estimated ratio of 3 to 1 (ergo- vs. cholecalciferol) [4,11,12].

Few RCTs have been conducted to evaluate the anti-fracture effectiveness of ergocalciferol, either in the general population or in at-risk groups, such as elderly persons in institutions. On the basis of the results of these RCTs, we can affirm that ergocalciferol – in the experimented doses – has proved to be substantially ineffective in reducing the risk of vertebral, non-vertebral and femur fracture [4,6,15].

Based on what has been described above, the use of ergocalciferol in daily clinical practice does seem justifiable.

### Calcifediol

Calcifediol (25OHD) is the hepatic metabolite of vitamin D. Compared to calcitriol (a biologically active metabolite), calcifediol has a higher affinity for VDBP, but a lower affinity for VDR [4]. For this reason, calcifediol must hydroxylate into its active form (calcitriol) in order to become biologically effective. Calcifediol is partially hydrophilic and is stored only in the liver and muscles [1]. It is thus unable to cause repletion of vitamin D storage (unlike cholecalciferol). The half-life of 25OHD is shorter than that of vitamin D3 and has been calculated to be ca. 10-22 days [4]. This shorter half-life (with respect to that of cholecalciferol, which is believed to be of many weeks) certainly makes calcifediol a less flexible and adaptable product in clinical practice. Its administration/intake must in fact follow more rigid therapeutic regimes, as its shorter half-life reduces the margins between one administration and another. It is indeed believed that a single dose is able to supply the body’s requirements for no more than 12-18 days (depending on the quantity of the dose) [1,4]. For this reason, daily or weekly protocols are usually followed, even if it has been proposed that monthly administration regimes [of high doses] are also effective [4,6-9,19,20].

In this context, treatment which is not regularly followed over long periods of time may make patients more susceptible to the risk of hypovitaminosis D or to a lesser response to serum 25OHD.

Recent pharmacokinetic studies have shown that calcifediol produces a more rapid increase in serum 25OHD than cholecalciferol in subjects with vitamin D deficiency [7-9,19,20]. In these studies, calcifediol was typically administered in doses of 20 μg/day [19,20]. Even if higher doses were also used, in daily clinical practice calcifediol is normally prescribed at doses between 5 and 20 μg/day [4,6-9,19,20]. This suggests that in the pharmacokinetic studies cited above calcifediol was used in medium to high doses, while cholecalciferol was administered in relatively low ones (800 IU/day, which, as we have seen, was described in the RCTs as the minimum effective dose for fracture risk reduction) [5-9,15,20]. This critical difference obviously complicates the interpretation of the findings of these pharmacokinetic studies on calcifediol (vs. cholecalciferol) and reduces their value on a clinical level.

Compared to the significant number of RCTs conducted on cholecalciferol to evaluate its effectiveness in reducing the risk of fracture, there have been decidedly fewer RCTs on calcifediol [4,6]. A recent meta-analysis by Cochrane reviewed therapeutic RCTs (investigating fracture risk reduction) conducted with vitamin D and its metabolites: the analysis identified only two studies on calcifediol which were deemed acceptable based on the quality of their experimental design [6].

It should be pointed out that in both of these studies the risk of bias was not measurable [6]. On the basis of the findings of these two studies, we can affirm that at present there is not sufficient scientific evidence to warrant the anti-fracture effectiveness of calcifediol [6,21].

In a more recent RCT published by Peacock et al., for example, the incidence of new vertebral and non-vertebral fracture turned out to be similar in subjects treated over four years with calcium (750 mg/day), calcifediol (15 μg/day) or a placebo [21].

On the whole, if we wish to summarize the available evidence, we can state that in clinical practice calcifediol has a single advantage compared to cholecalciferol: the greater rapidity at which the serum 25OHD level increases. In which situations this different pharmacokinetics is able to provide greater benefits on a clinical level (e.g., reduction of the risk of fracture) has not, however, been clearly defined, in part given the lack of clinical data from RCTs which have persuasively demonstrated its effectiveness in achieving primary endpoints.

As has been recently emphasized in a liter-
ature review, we must, finally, keep in mind the potential risk of toxicity with higher doses of calcifediol [Table I] [4], even if pharmacokinetic studies [20 μg/day] have not shown significant adverse events [20]. As indicated in the guidelines, it therefore does not seem appropriate to consider calcifediol a preferred drug for the prevention and treatment of hypovitaminosis D or in the prevention of fragility fractures in patients with osteoporosis, in association with antiresorptive or osteoanabolic medication. [1, 2]. It is, by contrast, necessary to emphasize that calcifediol represents the chosen vitamin D metabolite in treating patients with chronic liver disease and severe reduction of the hepatic function.

Calcitriol
Calcitriol [1,25(OH)2D] is the active metabolite of vitamin D and the natural ligand of VDR. It has a short half-life, calculated at ca. 5-8 hours [4]. For this reason it must be administered daily (in some studies it is also used with intermittent regimes) and sometimes in lower doses distributed over a 24-hour period [4, 6, 20]. Administered doses usually range from 0.25 to 1 μg/day [4, 6].

Since its discovery in the 1970’s, calcitriol has been successfully used in treating secondary hyperparathyroidism in patients affected with chronic renal insufficiency, or in the prevention of hypocalcemia in patients suffering from hyperparathyroidism [4, 20]. More recently, calcitriol has been used and studied in RCTs aimed at evaluating its effectiveness in reducing fracture risk [6, 20]. In some – though not all – of these RCTs, calcitriol has been shown to reduce the risk of fracture [4, 6, 20]. Nonetheless, these same RCTs have also reported a greater and more significant incidence of adverse events, such as hypercalcemia, hypercalciuria and nephrolithiasis in subjects treated with calcitriol [4, 6, 20].

Because of the lesser degree of safety and clinical practicality of calcitriol, the international scientific community agrees that its use should be limited to patients suffering from chronic renal insufficiency or to patients affected by hypoparathyroidism [2, 4, 20].

In the context of treating patients suffering from chronic renal insufficiency, two other aspects of the use of calcitriol should be mentioned (4, 10): numerious writers and opinion leaders propose the contemporary administration of cholecalciferol in patients affected by chronic renal insufficiency who are being treated with calcitriol: this recommendation is motivated by the activity of extra-renal 1-α-hydroxylase, which is not linked to feedback mechanisms and is not compromised by the reduced renal function; it has recently been suggested that the use of calcitriol be limited to patients suffering from chronic renal insufficiency with low cardiovascular risk profiles.

Alfacalcidol
Alfacalcidol, or 1-α-hydroxy-vitamin D, is a prodrug which requires 25-hydroxylation in the liver to become metabolically active [1,25(OH)2D]. It was first synthesized in the early 1970’s and used clinically beginning in 1973, with the aim of administering a prohormone that was able to bypass renal 1-α-hydroxylation and that would thus be usable even in the presence of reduced renal function [4, 20]. Alfacalcidol therefore represents an alternative to calcitriol.

For a certain period, the use of alfacalcidol in clinical practice was strongly encouraged. It was indeed believed that because alfacalcidol needs to be activated (25-hydroxylation) its pharmacokinetics was preferable to that of calcitriol, as its action is more enduring (because of its longer half-life) and it creates less exposure to the risk of hypercalcemia [4, 20]. This theoretical advantage, however, has not been realized in clinical practice. Although slightly longer than that of calcitriol, the half-life of alfacalcidol is ca. 12 hours [the time necessary for its total metabolic conversion] [4]. For this reason, alfacalcidol must also be administered daily. It has been calculated that a daily dose of 1 μg of alfacalcidol is the bioequivalent of 0.5 μg of calcitriol [20]. Administered doses typically range from 1 to 5 μg/day [4, 6].

As in the case of calcitriol, alfacalcidol is generally recommended for use in patients affected by chronic renal insufficiency [4, 20].

In some RCTs (and meta-analyses), alfacalcidol has been shown to significantly reduce the incidence of new fractures [4, 6, 20]. As with calcitriol, though, prolonged treatment with alfacalcidol can expose patients to a heightened risk of adverse events linked to its hypercalcemicizing action. For this reason, the use of alfacalcidol in clinical contexts should be subject to the same guidelines and limitations as those for calcitriol [4].

CONCLUSIONS
In daily clinical practice, cholecalciferol should be considered the preferred supplement for preventing and treating vitamin D deficiency and for the primary and secondary prevention of fragility fractures in patients with osteoporosis, in association with antiresorptive or osteoanabolic therapy. The use of other vitamin D metabolites – calcifediol, alfacalcidol and calcitriol in particular – should be limited to specific situations, such as conditions of chronic renal insufficiency or hypoparathyroidism (alfacalcidol and calcitriol), malabsorption syndrome, severe obesity or hepatic insufficiency (calcifediol). These recommended restrictions concerning the use of vitamin D metabolites are mainly due to the limited evidence proving their effectiveness in reducing the risk of fracture, the lack of appropriate studies that directly “pit” them against cholecalciferol, and the potential risk of adverse events linked to their hypercalcemicizing action (especially for 1-α-hydroxylated metabolites).

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