VITAMIN D

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EDITORIAL

Maurizio Rossini

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Dear Readers,
As you can see, in this issue we are featuring the contribution of Professor Lello, whom we have asked to provide us with an update on the role of vitamin D in pregnancy.
It is known that during pregnancy vitamin D metabolism is modified. In particular, maternal calcitriol serum levels are doubled to meet the increased need for calcium, which is necessary for fetal skeletal mineralization and, probably, to increase immune system tolerance in this particular condition as well. This is achieved thanks to the contribution of the placenta, which acts as an extra-renal site for the conversion of 25(OH)D in calcitriol, and also to the reduction of the expression of the gene encoding the enzyme that catalyzes the active metabolite.
As you will read, vitamin D deficiency has been associated with an increased risk of preeclampsia, impaired tolerance to glucose and bacterial vaginosis in mothers, as well as with low birth weight, hypocalcemic convulsions and impaired skeletal development in newborns. Even if experiences with supplementation are still limited – as you will see – benefits have been observed in women taking cholecalciferol supplements from the beginning of pregnancy, with daily doses comparable to those used by the general population and with a good safety profile.
For this reason, the statement in the current package insert for cholecalciferol – that "in the first six months of pregnancy, vitamin D should be taken with caution…" – could excessively discourage its use, while according to the findings summarized in this issue it is often sustainable and appropriate. At the same, however, it is important to take caution with regard to dosages, given the noted risks of teratogenic effects in overdoses: only the daily dosage is recommended in pregnancy, while bolus administrations should be avoided.
As I myself am about to become a grandfather, I will confess that I have advised my daughter to take 750 IU (3 gtt) of cholecalciferol daily from the first months of pregnancy.
The other contribution that you will find in this issue is by Professor Fagiolini, who highlights a strong link between vitamin D deficiency and depression, psychotic disorders and cognitive dysfunction. As you will see, researchers admit that so far it is unclear whether vitamin D deficiency is a cause or effect of mental pathology. You will certainly appreciate to what degree the meaning changes if I affirm that persons with vitamin D deficiency are 3.5 times more likely to have hallucinations, delirium or symptoms of paranoia, or, conversely, that persons with these symptoms are 3.5 more likely to have vitamin D deficiency.
Furthermore, research has yet to determine whether adding vitamin D supplements can prevent and/or cure these pathological conditions in individuals with vitamin D deficiency. Indeed available studies on the role of vitamin D supplementation have thus far produced contrasting results, perhaps in part because studies in this complex field are not easy to conduct and are for the most part poorly designed.
What do you think?

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Vitamin D and Psychiatric Illnesses

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Abstract

Vitamin D is known not only for its essential role in calcium homeostasis and bone health but also for maintaining a healthy mind. A number of recent studies, in fact, have demonstrated a correlation between vitamin D deficiency and psychiatric illness. In addition to all its other functions, vitamin D acts as a potent neurosteroid hormone, critical to brain development and normal brain function; it is known for its anti-inflammatory properties, which are able to affect many aspects of human health.

The vitamin D receptor, which mediates many of its biological actions, has been found throughout the body, including in the central nervous system. Vitamin D deficiency is common in patients with serious mental illnesses, such as depression, schizophrenia and neurocognitive disorders.

Several risk factors, such as genetic and environmental factors, season of birth, latitude and migration, have been linked to vitamin D deficiency and can explain, at least in part, the association between hypovitaminosis D and mental illness.

The causal link between hypovitaminosis D and mental illness is probably bi-directional; mental illness increases the risk of hypovitaminosis D, and hypovitaminosis D increases the risk of developing mental illness.

The biological mechanism at the base of the relationship between hypovitaminosis D and mental illness is most likely related to vitamin D action on the regulation of inflammatory and immunological processes, which in turn can act as mediators or modulators for the development of clinical symptoms and/or treatment response.

Our review has found sound proof of a significant association between mental illness and vitamin D deficiency, yet it has also highlighted the need to further investigate, in future studies, the direction of the causal link of the relationship between vitamin D deficiency and other specific variables that are involved. This would be important in order to determine the best prevention and treatment strategies for hypovitaminosis D in patients with mental illnesses such as depression, psychosis and neurocognitive disorders.

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Since its discovery in 1921, vitamin D has been known for its role in calcium homeostasis and bone health. Low levels of vitamin D have been associated with bone disorders such as rickets, osteomalacia and osteoporosis [1]. However, these disorders can be considered as simply "the tip of the iceberg" in vitamin D deficiency. Recent studies have shown that most tissues and cells of the human body, including the brain, have vitamin D receptors, thus providing new information about its function [2].

Vitamin D plays an important role in the pathophysiology of psychiatric diseases, as has been shown by various studies on the presence of this vitamin, its receptors (Vitamin D Receptors, or VDRs) and its associated enzymes (CYP24A1 and CYP27B1) in many parts of the brain. The expression of vitamin D receptors (VDRs) in the prefrontal cortex, cingulate gyrus, thalamus, hypothalamus, amygdala, hippocampus and substantia nigra suggests a possible key role of vitamin D in the pathophysiology of psychiatric illnesses such as depression and psychosis [3-6].

It has been proved that vitamin D plays an important role in neurodevelopment, neuroprotection, neuroplasticity and neuromodulation, not only by exercising its biological action, but also by influencing gene expression at the cellular level [6-8].

In addition, there is new evidence regarding the neuroprotective mechanism of vitamin D action on inflammatory processes in the brain [9-19], such as the upregulation of pro-inflammatory cytokines associated with depression and with mental illness [11].

The discovery of vitamin D receptors in extraskeletal systems has caused increased interest in its function in these systems. Further studies have shown a relationship between vitamin D deficiency and cancer, chronic conditions such as diabetes, and metabolic, autoimmune, infective and cardiovascular diseases [2, 12-20].

Clinical observational studies and subsequent systematic reviews have demonstrated that a relationship between vitamin D deficiency and mental illnesses seems biologically possible, especially for those related to affectivity, sense perception and the elaboration of attention, concentration and memory, as well as to neuroendocrine aspects.

It is not clear whether this relationship is the result of serious mental illness with consequent social isolation, or if vitamin D has a regulatory role on those genes in the neuronal network that influence affectivity, cognition and sense perception [21-24].

It has further been shown that patients with mental disorders are at a higher risk for vitamin D deficiency than the general population. In particular, patients with schizophrenia have a greater risk of lacking vitamin D than those affected by other mental illnesses [21, 22, 25-30].

DEPRESSION

Several studies have shown a strong correlation between vitamin D deficiency and depression. A study entitled the *Third National Health and Nutrition Examination Survey* [31] assessed a sample of 7970 U.S. residents aged between 15 and 39 years, and found that people with serum vitamin D levels ≤ 50 nmol/L are at a significantly higher risk for having depression compared to those with serum vitamin D levels that are greater or equal to 75 nmol/L.

For example, a study conducted on 1282 adults of age between 65 and 95 years in the Netherlands [32] showed that persons suffering from depression have levels of 25 hydroxyvitamin D that are 14% lower compared to controls. Moreover, a relationship was found between the severity of the depression and low serum levels of 25 hydroxyvitamin D, which remained significant after adjusting for variables such as age, gender, smoking status, body mass index and number of comorbid chronic diseases.

Reduced serum levels of 25 hydroxyvitamin D and elevated serum levels of the parathyroid hormone (PTH) have been associated with depressive symptoms in various clinical settings. Of interest is an inverse association between 25 hydroxyvitamin D serum levels and depression, which was found even after taken into account several influencing factors such as lifestyle and health among European patients [33].

The relationship between depression and vitamin D was also investigated in older populations and/or in subjects with medical comorbidities [34, 35]. Many studies have shown a significant relationship between vitamin D deficiency and late-life depression as well as in people living at northern latitudes [36].

In a further assessment of an older cohort of a population living at northern latitudes [37], a moderate inverse relationship between vitamin D serum levels and depressive symptoms was observed among both genders. In addition, older men with low vitamin D levels (< 30 nmol/L) were twice as likely to show depression at the time of evaluation compared to men of similar ages whose vitamin D blood levels were adequate (≥ 50 nmol/L), even after correcting for factors such as hypertension and diabetes, which may also contribute to depression.

Interestingly, no significant relationship was found between vitamin D levels and current depression among women. Ultimately, high vitamin serum levels were found to be protective against the development of post-stroke depression (PSD) [38]. The study further found a relationship between low vitamin D serum levels and the development or presence of stroke, as well as an association between low levels of vitamin D and PSD development at one month post-stroke [38].

A recent large cohort study has demonstrated an association between low levels of vitamin D and both the presence and severity of depression, suggesting the possibility that hypovitaminosis D signals an underlying biological susceptibility to depression [39].

Similar results were obtained in the evaluation of a group of subjects affected by secondary hyperparathyroidism (n = 21), in whom low vitamin D serum levels were significantly related to higher scores on the Beck Depression Inventory (BDI), as compared to a control group [40].

In a six-year longitudinal study, Milaneschi, et al. (2010) [41] examined the association between levels of vitamin D at stratum basale and subsequent depression on a sample of 954 adults aged 65 and older. It was found that individuals with low 25 hydroxyvitamin D levels at stratum basale (that is, < 50 nmol/L or < 20 ng/mL) scored significantly higher on the depression rating scale in the two follow-up periods (3 and 6 years) compared to those individuals with elevated 25 hydroxyvitamin D levels at stratum basale, with a more pronounced association (between levels of vitamin D at stratum basale and subsequent depression) in women than in men.

Milaneschi, et al. (2013) [42] studied the association between 25 hydroxyvitamin D levels and depressive disorders in a large cohort aged between 18 and 65 from a Dutch study on depression and anxiety. In this study, lower levels of 25 hydroxyvitamin D were quantified in participants with current clinical depression, in particular in those with more serious symptomatology as compared to the controls.

A negative correlation between vitamin D

serum levels and clinically significant depressive symptoms measured in five weekly assessments was found among a group of young adult women [43]. These findings showed that young black women were more likely to have vitamin D insufficiency and to be depressed compared to other women: this result was in line with those obtained in previous studies [44-46].
Robinson et al. [47] reported that low vitamin D serum levels during pregnancy represent a risk factor for the development of postpartum depression symptoms. Similar results were found by Murphy et al. [48] in evaluating the relationship between vitamin D levels and depressive symptoms in a sample of 97 women, who were assessed monthly for the first seven months of the postpartum period. In this study, women with lower vitamin D levels constantly showed higher rates of depression as compared to women with higher vitamin D levels.
Two additional studies have shown a significant negative correlation between vitamin D serum levels in the first trimester of pregnancy and the presence of depressive symptoms in the second trimester [49, 50].
In addition, researchers have investigated the relationship between vitamin D serum levels in the second trimester of pregnancy and postpartum depression during the first six months after pregnancy [51]. This study showed that lower maternal 25 hydroxyvitamin D levels in the second trimester of pregnancy were associated with more severe depressive symptoms at one week, six weeks and six months into the postpartum period.
A systematic review with a meta-analysis conducted by Anglin et al. (2013) [52] assessed the relationship between depression and hypovitaminosis D, reporting an association between low levels of vitamin D and depression.
Even though most research confirms the hypothesis that a low concentration of vitamin D is associated with depression, some studies have failed to demonstrate this relationship. For example, a large epidemiologic study in China [53] did not find any relationship between vitamin D and depression in a sample of 3,262 men and women of between the ages of 50 and 70.
In another paper, Zhao et al. (2010) [54] carried out a large cross-sectional study among adults of all ages. They were not able to discover a significant correlation between vitamin D deficiency and depression after correcting for potential confounding factors (such as degree of sun exposure, level of physical activity, diet, age and body mass index).
In addition, Black et al. (2014) [55] carried out a cross-sectional study on young adults recruited from the *Western Australian Pregnancy Cohort Study* in order to investigate the relationship between 25 hydroxyvitamin D serum concentrations and symptoms of depression, anxiety and stress. After adjusting for confounding factors (that is, age, race, BMI and physical activity), an increase of serum 25 hydroxyvitamin D of 10 nmol/L was associated with a reduction of only 8% in depression rating scores in males (though not in females), though no significant associations with anxiety and stress symptoms were found.
Almeida et al. (2015) [56] conducted an observational study to assess retrospective, cross-sectional and prospective associations between vitamin D concentrations and depression in a sample of 3,105 elderly men. The authors of this study interpreted their results as not supporting a role of vitamin D in causing depression.

COGNITIVE DISORDERS

Low levels of vitamin D have also been associated with more severe general cognitive deficits [57] and dementia [58-60]. Low concentrations of vitamin D have been associated with cognitive impairments such as memory and orientation [61], executive function disability [62], and Alzheimer's disease [63].
Results from a large study conducted in Italy between 1998 and 2006 suggest that people with severe vitamin D deficiency (<25 nmol/L) have a greater risk of obtaining a substantial reduced score on the Mini-Mental State Examination as compared to people who have sufficient levels of vitamin D (≥ 75 nmol/L) [64]. Low levels of vitamin D in elderly women have been associated with an increased risk of Alzheimer's disease but not with other forms of dementia [65]. Polymorphisms of vitamin D receptors have been associated with depression and poor cognitive performance [66].

PSYCHOTIC DISORDERS

Vitamin D deficiency has been linked to a wide range of important psychiatric disorders and constitutes an emerging research area of interest. Low levels of vitamin D have been found in both inpatients and outpatients with psychosis and schizophrenia, with an inverse correlation between symptom severity and vitamin D serum levels having been observed.
Although the mechanism is not clear, recent studies suggest that the action of vitamin D on the regulation of inflammatory and immunological processes is likely to influence the manifestation of clinical symptoms and treatment response in schizophrenic patients [67].
Results from narrative and systematic reviews or meta-analyses agree in reporting an association between vitamin D deficiency and schizophrenia [68-74]. A significant inverse correlation between vitamin D levels and schizophrenia was also observed in the majority of case-control studies conducted on serum levels and schizophrenic subjects, as compared to healthy controls [75].
The correlation between vitamin D deficiency and development of schizophrenia has been researched among patients of all ages around the world. Recently, one meta-analysis reviewed 19 studies published between 1988 and 2013 and found a strong association between vitamin D deficiency and schizophrenia. Of the 2,804 participants from these studies, over 65% of participants with schizophrenia were vitamin D deficient. Vitamin D deficient participants were therefore 2.16 times more likely to have schizophrenia than participants with sufficient levels of vitamin D. In addition, lower levels of vitamin D were have also been found in cases of consolidated psychosis [76] and first episode psychosis [77].
The risk of schizophrenia and vitamin D levels vary with season of birth, migration status, latitude of residency and skin pigmentation [78-80]. The UV rays needed to produce vitamin D are reduced during the winter months, the same months that are most associated with an increase in births of individuals who later will develop schizophrenia. A review which screened a total of 437,710 individuals with schizophrenia revealed that most were born in January and February. These newborns were thus exposed to lower levels of UV rays during their prenatal and perinatal periods.
An increased rate of schizophrenia has also been observed at higher latitudes, especially among immigrants. This may be again related to the UV availability and the resulting vitamin D status. At higher altitudes, a dark-skinned individual will also have a more pronounced reduction of vitamin D as compared to a lighter-skinned individu-

al. The lighter-skinned individual will have less melatonin, allowing the skin to absorb UV rays more effectively. It is estimated that dark-skinned individuals who live at higher latitudes are more likely to develop schizophrenia than individuals in the general population [67].

Several epidemiologic studies have linked low levels of vitamin D to schizophrenia and psychotic disorders. Norwegian researchers using a structured clinical interview to identify psychosis found consistently low levels of 25 hydroxyvitamin D among immigrants and native Norwegians with psychotic symptoms [81].

Swedish researchers reviewed medical records at a psychiatric outpatient department to identify possible factors that could predict vitamin D deficiency. Over 85% of the 117 psychiatric patients had suboptimal levels of vitamin D. Those with schizophrenia and autism had the lowest levels. Being of Middle Eastern, Mediterranean, Southeast Asian or African ethnic origin was a strong predictor of low vitamin D. Patients receiving vitamin D supplements to correct their deficiencies achieved considerable improvement of psychosis and depression symptoms [82].

In Israel, vitamin D concentrations were measured in 50 patients with schizophrenia, aged 19–65: lower mean vitamin D concentrations were detected among patients with schizophrenia (15 ng/mL) compared to controls (20 ng/mL), after correcting for the impact of sun exposure and supplements [83]. Similarly, in New Zealand 92% of 102 psychiatric adult inpatients had suboptimal levels of vitamin D, more than double with respect to Europeans with serious levels of deficiency (lower than 10 ng/mL) [84].

An inadequate neurosteroid action of vitamin D on the brain, especially during development, is associated with changes such as inflammatory and immunologic disorders, which are also present in schizophrenia [85 86].

In more recent studies on humans, vitamin D deficiency has been linked to hippocampus dysfunction, a region thought to be involved in the pathogenesis of psychotic disorders. A positive correlation between vitamin D and gray matter volume has also been found [87]. Early deprivation of vitamin D or during prenatal life may increase the risk of developing late-onset schizophrenia [5].

A Finnish study of a neonatal cohort showed that vitamin D intake during the first year of life reduced the likelihood of schizophrenia [88]. A study among 8,411 Swedish women showed an association between low levels of vitamin D and psychotic symptoms [89].

In another pilot study, researchers measured serum levels of 25 hydroxyvitamin D in the third trimester of pregnancy and found that lower maternal vitamin D levels were associated with an increased risk of schizophrenia [90]. These findings suggest that low prenatal vitamin D levels may have a negative impact on brain development, thus increasing the risk of the onset of schizophrenia in adulthood.

McGrath et al. (2010) [91] investigated the relationship between neonatal vitamin D status and a later risk of schizophrenia. They identified 424 cases of schizophrenia from the Danish Psychiatric Central Research Register and analyzed neonatal dried blood spot samples. Not surprisingly, they found significant seasonal variation in vitamin D status as well as levels of vitamin D significantly lower in the offspring of mothers who immigrated to Denmark. They also found that those with lower neonatal concentrations of vitamin D had an increased risk of schizophrenia. Researchers estimated that if all these neonates had optimal vitamin D levels, more than 40% of schizophrenia cases could have been prevented.

Furthermore, a link has been hypothesized between vitamin D deficiency and psychotic symptoms. Adolescents [92] or children [93] with vitamin D deficiency suffered more often from psychotic symptoms compared to those with normal levels of vitamin D.

In a prospective study of 3,182 children in England, researchers measured vitamin D levels at age 9.8 years and assessed psychotic experiences at 12.8 years. Vitamin D concentrations during childhood were associated with psychotic experiences during early adolescence. In the case that psychotic experiences were correlated to the development of schizophrenia, this would support a possible protective association of higher vitamin D concentrations with schizophrenia [93].

In addition, cross-sectional analyses were carried out on adolescents aged between 12 and 18 years who requested either hospitalization or partial hospitalization. Of the 104 patients examined, 72% had insufficient vitamin D levels. Vitamin D status was correlated to the severity of the mental illness. Those with vitamin D deficiency were 3.5 times more likely to have hallucinations, delirium or paranoia (Gracious et al., 2012). A second study supports these findings. Vitamin D was analyzed in 20 patients with first-episode schizophrenia. A greater severity of negative symptoms (affective flattening, emotional withdrawal, poor socialization, social withdrawal, abstract thinking and implicit stereotypes) was strongly correlated with lower vitamin D levels [92].

CONCLUSIONS

Evidence suggests a possible association between vitamin D deficiency, depression, psychotic disorders and cognitive dysfunction. However, it remains unclear whether vitamin D deficiency is the cause or the effect of these mental pathologies. Subjects affected by such diseases are more likely to develop low levels of vitamin D, due to reduced outdoor activity and lesser intake of nutrients and pharmacological treatment.

Conversely, the causal link could work in the opposite direction. In fact, the presence of vitamin D receptors in those areas of the brain that have been associated with the development of depression, psychosis and neurocognitive disorders strengthens the plausibility [7] of a common pathogenic pathway between vitamin D and mental illness and of interactions that affect cellular mechanisms which lead to different clinically noted phenotypes.

Furthermore, it remains to be established whether adding supplements of vitamin D may prevent and/or treat such pathologic conditions in individuals with vitamin D deficiency. Indeed studies on the role of vitamin D supplementation have produced contradictory results. This may be attributable to several reasons, including the use of different dosages of vitamin D supplements for different time periods in several studies, the use of different parameters to define vitamin D deficiency, the use of numerous psychometric instruments to measure mental health, and the different frequencies at which vitamin D is administered (i.e., daily, weekly or monthly).

Because of variations in the methodology used in different studies, it is difficult to establish the exact role of vitamin D in preventing or treating mental illness. The literature already provides enough data relative to the screening and treatment of vitamin D deficiency in subjects with mental illness. These are easy, cost-effective practices and may improve the outcome of these diseases.

The non-specific relationship between vitamin D and psychiatric disease may reflect a hidden immune system dysfunction and oxi-

dative stress which – when combined with other genetic and environmental factors and with comorbidity – determine the different phenotypes observed among clinical populations.

Depression has been associated with a function of the immune system mediated by aberrant cells, alterations in the antioxidative blood levels, an increase of reactive oxygen species (free radicals) and oxidative and nitrosative stress leading to neurodegeneration [60, 94, 95].

It has also been hypothesized that immune system dysregulation, oxidative stress and later neurodegeneration may play roles in the pathogenesis of bipolar disorder and schizophrenia [96-100]. Low-content vitamin D cells produce high levels of inflammatory cytokines, while cells with an adequate content of vitamin D release them at significantly lower levels.

It is therefore possible that adequate levels of vitamin D might act as an anti-inflammatory mechanism [67]. Vitamin D modulates the transcription of most of the genes involved in the molecular pathway for the development of schizophrenia, including genes involved in synaptic plasticity, neural development and protection against oxidative stress [57]. Animal studies have shown that vitamin D deficiency during gestational periods affects dopamine metabolism, altering the dopaminergic system in the developing brain. It has been proved that dopamine is involved in the pathogenesis of schizophrenia. Vitamin D deficiency during pregnancy may also affect those cerebral structures associated with schizophrenia [25]. Finally, Alzheimer's disease and other forms of dementia have been linked to dysfunctions of the immune system and oxidative stress [101-104].

Most research utilizes cross-sectional studies that allow scientists to examine the role of vitamin D in mental illnesses at a specific moment, which is not conducive to making inferences about the direction of the relationship. Consequently, further longitudinal, randomized controlled studies are necessary to better understand the causal link. In addition, from the literature there emerges a great heterogeneity of variables used and limits imposed, in addition to multiple definitions of each variable. In particular, there are limited data on psychotic drugs, on vitamin D and on clinical results. Future studies may consider the use of variables compatible with those used in past research in order to reinforce known associations or to discover new ones, thereby standardizing conclusions.

Because of the possible role of vitamin D deficiency in the etiology of commonly treated psychiatric and non-psychiatric disorders, it would be important to identify and address the obstacles to adequate vitamin D intake for the general health of patients with mental illnesses.

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VITAMIN D AND PREGNANCY

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In pregnant women, especially during the first phases, the role of vitamin D appears to be mainly immunomodulatory, rather than – more traditionally – a calcium-regulating factor, although this function does retain its importance. Interestingly, vitamin D inadequacy in the early stages of pregnancy could be an instance of the "Barker Hypothesis" [1]. According to this theory, certain diseases in adults might have their roots in nutrient insults experienced in the perinatal period (either *in utero* and/or during the early months of life).
Still today there is little agreement on the optimal vitamin D dosages to be utilized as supplementation during pregnancy.

VITAMIN D: METABOLISM IN PREGNANT AND NONPREGNANT WOMEN

Marked differences exist in vitamin D metabolism in pregnant women, non-pregnant women and developing fetuses. Although scientists have long known about these differences, they have received little attention until recently [2, 3].
The rate of conversion of vitamin D to 25(OH)D appears unchanged during pregnancy, in line with zero-order enzyme kinetics. (We should point out here that zero-order kinetics is observed in an enzyme catalyzed reaction when, in response to high substrate concentrations, the velocity of the reaction reaches a maximum value which becomes constant following complete substrate saturation; in other words, no more enzyme is available) [4].
Conversely, the conversion of 25(OH)D to $1,25(OH)_2D$ has a unique profile during pregnancy. In fact, at no other time during life is 25(OH)D associated with the production of $1,25(OH)_2D$. Starting at 12 weeks of gestation, $1,25(OH)_2D$ concentration is double that of a nonpregnant woman, and continues to rise two to three times with respect to baseline values, reaching levels that would be toxic – because of hypercalcemia – to a nonpregnant woman, but which are essential during pregnancy [5]. The rise in $1,25(OH)_2D$ levels in mother and fetus has been thought to be a mechanism to regulate calcium levels and to preserve the maternal skeleton, in addition to maintaining fetal skeletal development. In reality, it seems that calcium homeostasis is not linked with $1,25(OH)_2D$, because from the twelfth week of gestation there is no demand for calcium increase in either mother or fetus. By contrast, the increase of $1,25(OH)_2D$ levels sustained during pregnancy is not preserved during breastfeeding, when the demand for maternal calcium is as high as during pregnancy [6].
Therefore, the increase in $1,25(OH)_2D$ in mother and fetus during pregnancy is dependent on substrate availability, that is, of 25(OH)D, but it is largely independent of calcium homeostasis [5].
Various hypotheses have been used to explain why calcium metabolism is uncoupled during pregnancy and not during breastfeeding. One theory is that $1,25(OH)_2D$ is an important modulator of the immune system, correlated to maternal tolerance to the fetus. For instance, epidemiologic studies involving pregnant women with preeclampsia, a condition characterized by inflammation and vasculitis, has demonstrated an association between this disorder and vitamin D deficiency [7]. Moreover, animal studies have shown that vitamin D deficiency could be potentially related to placental dysfunction (one of the pathogenic mechanisms of preeclampsia) [8].
We should also emphasize that the placenta represents the most active extra-renal site for the conversion of 25(OH)D to calcitriol. At such a level, the expression of the codifying gene for the enzyme catabolizing the active form of vitamin D (24-hydroxylase) is reduced. Additionally, $1,25(OH)_2D$ cannot cross the placental barrier, while it seems that 25(OH)D can [9].
However, as we have already mentioned, $1,25(OH)_2D$ maternal levels have the tendency to increase during the first trimester and to continue to rise during the entire gestational period, during which its concentration is double that of a nonpregnant woman or during

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puerperium. In this respect, the progressive increase of calcitriol levels during pregnancy plays a crucial role in the modulation of maternal and fetal phosphate and calcium homeostasis, with a possible increase of calcium absorption during pregnancy [10].

A number of randomized trials and placebo-controlled studies [5, 11-17] have dealt with the topic of safety in vitamin D supplementation during pregnancy, with dosages of 400 to 4,000 IU per day: these showed no safety profile abnormalities.

Indeed low levels of vitamin D during pregnancy have been linked to the following conditions:

- intrauterine growth restriction
- preterm birth
- small for gestational age (SGA)
- bacterial vaginosis
- gestational diabetes
- preeclampsia

In particular, a 2018 review [18] evaluated the association between vitamin D deficiency and adverse complications during pregnancy by means of a literature analysis of observational studies performed in developing countries. Included were thirteen studies showing a variation of vitamin D deficiency prevalence between 51.3 and 100%. Results from ten studies have shown at least one association between vitamin D deficiency and adverse maternal and/or neonatal complications, such as preeclampsia, gestational diabetes, postpartum depression, urgent cesarean section, low birth weight, small for gestational age and growth restriction.

The World Health Organization (WHO) does not currently recommend routine vitamin D screening or supplementation during pregnancy [19] because randomized trials and placebo-controlled studies (RCTs) have not provided high quality scientific evidence for which it would be desirable to activate these kind of procedures. Indeed, as we shall see, the relationship between maternal levels of vitamin D and various pathologic conditions encourages us to more carefully assess both vitamin status and the appropriateness of vitamin D supplementation during pregnancy.

We will now review several pregnancy related complications connected to vitamin D levels.

VITAMIN D AND PREECLAMPSIA

In regard to the physiopathology of preeclampsia [20], the following phases have been traditionally described: 1) abnormal placentation and trophoblast invasion; 2) placental ischemia; 3) endothelial damage; 4) vasoconstriction + platelet activation – hemostatic abnormalities; and 5) preeclampsia.

Low vitamin D levels appear to be related to an impaired mechanism of placentation. Observational studies also report that women who developed preeclampsia had lower vitamin D levels than those who did not. Furthermore, it has been found that the risk of developing severe eclampsia is 5 times greater in pregnant women with 25(OH)D levels < 20 ng/mL [20, 21].

Specifically, in a 2007 study Bodnar [21] evaluated the effects of low maternal 25(OH)D levels on the risk of developing preeclampsia, as well as the vitamin D status in newborns of preeclamptic mothers. This was a case-control study on pregnant women who were followed from before the 16th week of gestation until delivery. The patients were nulliparous pregnant women with singleton pregnancies, who either did or did not develop preeclampsia.

Preeclampsia was defined as a new episode of gestational hypertension associated with proteinuria and developing for the first time after 20 weeks of pregnancy. Concentrations of 25(OH)D at early pregnancy were lower in women who subsequently developed preeclampsia compared to controls. A dose-response relationship between 25(OH)D levels at less than 22 weeks of gestation and the risk of preeclampsia was evident. After adjustment for confounding factors and a reduction of 20 ng/mL, the threshold value implied a relative risk of preeclampsia equal to 2.4 (95% confidence interval [CI]: 1.1,5.4). In addition, newborns of preeclamptic mothers were more likely to have levels of 25(OH)D less than 15 ng/mL (adjusted odds ratio [OR]: 2.2; 95% CI: 1.2, 4.1).

This study concluded that maternal vitamin D deficiency was an independent risk factor for preeclampsia and that vitamin D supplementation should be explored for preventing preeclampsia and promoting neonatal well-being.

Baker's case-control study [20], published in 2010, investigated the association between midgestation vitamin D deficiency and development of severe preeclampsia. Midgestation maternal levels of 25(OH)D were lower in women who subsequently developed severe preeclampsia compared with controls. In addition, 25(OH)D maternal levels of less than 20 ng/mL were associated with an almost 4-fold increase of this risk (OR: 3.63; 95% CI, 1.52, 8.65) compared with levels of at least 30 ng/mL. The observed association become more evident after adjustment for confounders (adjusted OR: 5.41; 95% CI, 2.02, 14.52). Findings from this study showed that vitamin D deficiency during maternal midgestation was associated with an increased risk of severe preeclampsia and that vitamin D deficiency could be a modifiable risk factor.

Considering the effects of vitamin D on preeclampsia physiopathology (Table 1), it should be noticed that, at different levels, vitamin D is able to mitigate the principal mechanisms for the onset of this pathology.

Interestingly, Cochrane's review, published in 2012, suggested that pregnant women who received vitamin D and calcium supplementation were less likely to develop preeclampsia compared to pregnant women who received no supplementation and who had a risk ratio of 0.51 (95% CI, 0.32, 0.80).

A more recent study [23] assessed the effect of vitamin D supplementation (4400 vs. 400 IU/day) begun early in pregnancy (8-10 weeks) on the development of preeclampsia. The effects of vitamin D serum levels [25(OH)D] on the incidence of preeclampsia in pregnant women were also examined both at the beginning of the study and during the third trimester (28-32 weeks). Findings from this study revealed that a supplementation started at approximately 10-18 weeks of gestation does not reduce the incidence of preeclampsia. However, vitamin D levels of 30 ng/mL or greater detected at the beginning of the study and during late pregnancy were associated with a lower risk of preeclampsia.

A literature review [24] published in 2018 assessed the association between low maternal vitamin D levels and increased risk of hypertension. This review included all interventional, observational and nutritional studies, thereby providing a broad evaluation of data. The results of this analysis indicated that both vitamin D and calcium have a protective effect with regard to the development of preeclampsia. Conflicting data from observational studies in this area were attributed to several factors, such as high study design heterogeneity, a lack of consistency with the definitions of the obstetric outcomes, the varying quality of laboratory tests for measuring 25(OH)D and uncertainty about vitamin D status.

TABLE I.
Preeclampsia pathogenesis and vitamin D effects.

PATHOGENIC MECHANISMS	EFFECTS OF VITAMIN D
Abnormal placentation associated with inflammatory mechanisms	Reduction of immune response susceptibility
	Regulation of genes associated to placental invasion and implantation
Vascular endothelial dysfunction	Regulation of vascular structure, of elasticity and of intima-media thickness
	Reduced blood pressure (regulation of renin-angiotensin-aldosterone system)
Proteinuria mediated by Vascular Endothelial Growth Factor (VEGF) at renal level	Increase of proliferation of vascular smooth muscle cells through an increase of VEGF gene transcription

VITAMIN D AND LOW BIRTH WEIGHT

A positive correlation exists between maternal vitamin D levels and birth weight. Low birth weight (LBW) refers to both term and near-term newborns with a weight at birth of < 2500 g. These infants may be "small for gestational age" (SGA), or may have intrauterine growth restriction. The mortality rate of "small for gestational age" newborns has increased compared to those with normal weight [25]. Vitamin D plays an important role in fetal growth, both for its relation to parathormone and for its participation in the phosphate and calcium homeostasis. Several studies have in fact shown that insufficient levels of prenatal and postnatal vitamin D play a significant role in inadequate bone mineralization; such levels are also associated with SGA newborns, who are more frequent in winter-month pregnancies characterized by vitamin D deficiency [26, 27].
A Chinese study [28] evaluated the association between maternal vitamin D deficiency during pregnancy and the risk of SGA and LBW infants. A positive correlation was found between the maternal serum 25-hydroxyvitamin D level and offspring birth weight ($r = 0.477$; $p < 0.001$). Further analysis showed that 4.98% of neonates were LBW infants among subjects with vitamin D deficiency (RR = 12; 95% CI, 4.37, 33) and 1.32% among those with vitamin D insufficiency (RR = 3.18; 95% CI, 1.07, 9.48). After adjustment for confounders, the RR for LBW newborns was 12.31 (95% CI, 4.47, 33.89) among subjects with vitamin D deficiency and 3.15 (95% CI, 1.06, 9.39) among those with vitamin D insufficiency. Results from this study again confirmed the association between low maternal vitamin D levels and the risk of giving birth to LBW offspring.
Another Chinese study [29] examined the association between maternal vitamin D status at the first prenatal examination, on one hand, and the measurements of newborns and placental weight, on the other, in a cohort of women with singleton pregnancies (n = 747). In this group of women, 76.9% (95% CI, 74%, 78%) were defined as vitamin D deficient. The incidence of SGA was 13.3% (95% CI, 10.8%, 15.7%).
In addition, a nonlinear relation was found between 25(OH)D levels, birth weight and head circumference ($p < 0.01$). Interestingly, birth weight and head circumference increased by 69 g (95% CI, 38, 122) and 0.31cm (95% CI, 0.22, 0.40), respectively, per each ng/mL increase in 25(OH)D levels, before levelling off.
SGA distribution across the 25(OH)D quartiles ranged between 3.7% in the fourth to 24.1% in the first quartile. In addition, for each unit decrease of the plasma concentration of 25(OH)D, the unadjusted and adjusted risk of SGA increased by 19% (OR = 1.19 [95% CI, 1.13, 1.25], $p < 0.001$) and 9% (1.08 [1.03, 1.16], $p = 0.009$), respectively. By means of a multivariate analysis using vitamin D deficiency vs. other clinical variables, the adjusted risk of SGA increased by 205% (OR= 3.05 [95% CI, 2.24, 4.40], $p = 0.001$).
An analogous association has also been shown by European studies. In a study performed in the Netherlands [30] on a multiethnic cohort of 3,730 women with singleton pregnancies, researchers assessed the association between maternal vitamin D status, measured during the first stages of pregnancy, and neonate birth weight, as well as the prevalence of SGA infants and postnatal growth (weight and length). Vitamin D levels were measured during the first phases of pregnancy (13 weeks on average) for defining vitamin D deficiency, insufficiency or sufficiency.
Six ethnic groups were identified: Dutch, Surinamese, Turkish, Moroccan, other Western and other non-Western. Multilevel regression analysis was used to assess the association between neonatal data. Data showed that women with vitamin D deficiency had more LBW offspring (-114.4 g; 95% CI, -151.2, -77.6) and a higher risk of SGA (OR = 2.4, 95% CI, 1.9, 3.2) compared to women who had normal levels of vitamin D. Neonates born of women with vitamin D deficiency showed an accelerated growth rate for weight and height during the first years of life. Although vitamin D deficiency influenced birth weight, SGA risk and neonatal growth, it played no role in explaining ethnic differences.
Another study conducted in Australia [31] also showed an association between maternal vitamin D deficiency and frequency of LBW infants.

VITAMIN D AND PRETERM LABOR

Vitamin D levels may affect the physiopathological mechanisms of preterm labor through the modulation of inflammation and of some immune activities [32]. Vitamin D plays a role in the activation of the toll-like receptors that trigger innate immune response. Vitamin D deficiency therefore increases the risk of infection by causing a reduction in the production of catelicytines, a peptide with antifungal properties produced by macrophages [33]. At the same time, several observational studies have not found a significant association between maternal vitamin D levels and preterm labor [34, 35].
One study [37] compared vitamin D levels in 120 American women who delivered at term to those of 40 women who delivered between the 23rd and the 35th week of pregnancy. No differences in vitamin D levels between the groups were observed. Another study [32], which evaluated levels of vitamin D in a group of American women of mixed ethnicity with twin pregnancies, found significantly lower vitamin D levels in those who delivered before the 35th week com-

pared to those who delivered after the 35th week of pregnancy. Women with vitamin D levels lower than 30 ng/mL delivered prematurely in 49.4% of the cases, compared to 26.2% of preterm deliveries in women with vitamin D concentrations higher than 30 ng/mL.
It is necessary to point out that some of these studies included women with a previous history of preterm birth or twin pregnancies or who were at risk of preeclampsia. It is interesting to note that a meta-analysis [38] failed to show an association between maternal vitamin D levels and preterm birth. This conclusion is perhaps explained by the fact that the meta-analysis considered observational studies which were quite heterogeneous (in terms of dosage types, vitamin D levels measured at different gestational ages, etc.).
By contrast, an American research project [39] on supplementation in a mixed ethnicity population suggests an inverse relationship between maternal vitamin D levels and preterm labor. In this study, vitamin D concentrations were measured at the first examination, at which a supplementation of 5.000 IU capsules of vitamin D was offered. Additional measurements were made at the 24th and 28th weeks of gestation. The study found that women with vitamin D concentrations higher than 40 ng/mL at the time of delivery had a 62% lower risk of delivering prematurely compared to women with vitamin D concentrations lower than 20 ng/mL.
In addition, in women who had vitamin D levels below 40 ng/mL at their first examination, and who reached concentrations higher than 40 ng/mL at the follow-up visit, the risk of preterm birth was lower than 60%. Interestingly, the inverse relationship between vitamin D levels and risk of preterm birth was found to be true for all ethnic groups, suggesting that adequate vitamin D concentration may be beneficial for all women regardless of ethnicity. Similar outcomes were confirmed in another study performed in the United States [12].

VITAMIN D AND BACTERIAL VAGINOSIS

Bacterial vaginosis continues to be a problem during pregnancy: since vitamin D induces the expression of antibacterial proteins and enhances antibacterial activity in various tissues, various studies have focused on the relationship between this condition and vitamin D [40]. In a study in the U.S. on a group of 469 pregnant women, half Caucasian and half Black [41], vitamin D concentrations were measured before the 16th week of gestation, while a vaginal smear was performed simultaneously. It was found that vitamin D deficiency was associated with bacterial vaginosis in Black women, but not in Caucasians.
A meta-analysis on observational studies found an inverse relationship between maternal vitamin D concentrations and the risk of bacterial vaginosis [38]. Meanwhile, two randomized studies failed to show a positive effect of vitamin D supplementation on the occurrence or recurrence of bacterial vaginosis during pregnancy [5, 14].

VITAMIN D AND CAESAREAN SECTION

Regarding the relationship between vitamin D and the risk of caesarean section delivery in women with vitamin D deficiency, it has been proposed that this condition may reduce pelvic muscle strength, leading to prolonged labor [42]. A study conducted in the U.S. showed that in pregnant women of mixed ethnicity a significantly higher risk of caesarean section was associated with vitamin D concentrations below 15 ng/mL, after accounting for race, age and educational level [43].
Another American study examined a cohort of 1,153 low-income women and found a significantly higher risk of caesarean section in women with vitamin D concentrations lower than 12 ng/mL between the 8th and 18th weeks of gestation [42].
A British study analyzed the motivations behind elective and emergency C-sections [44]. After adjusting for such cofactors as BMI, ethnicity and age, no differences were found in vitamin D concentrations measured between the 11th and 13th weeks of gestation in women who would have vaginal deliveries, elective or emergency C-sections.

CONCLUSIONS

Low levels of vitamin D are frequent in the general population during pregnancy and lactation. Maternal vitamin D status during pregnancy may affect fetal and neonatal skeletal developmental mechanisms. This effect may continue after development, even reaching the stage of peak bone mass. (Such data represent an additional and important motivation for a prophylaxis of vitamin D during pregnancy).
On the evidence of results from epidemiologic observational studies and meta-analyses, low concentrations of vitamin D have been associated with a broad range of adverse complications regarding both the mother and the fetus and infant.
In women at risk of vitamin D deficiency who are pregnant or who are breastfeeding, vitamin D supplementation should be at least 600 IU/day, although vitamin D doses of 1500-2000 IU/day may be necessary to maintain 25(OH)D serum levels above 30 ng/mL. Indeed some studies in this review report doses of up to 4000 IU per day of vitamin D supplementation during pregnancy.
Currently, available studies seem to recommend starting prophylaxis with vitamin D at the beginning of the pregnancy, to be continued throughout the entire pregnancy and during lactation.

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