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PFAS and correlation with vitamin D metabolism

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Summary

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a class of compounds widely used in industry and consumer products. They are resistant to degradation and tend to accumulate in the environment and in living beings with possible toxic effects. These contaminants are a public health problem, especially in some areas of the Veneto Region, but have recently been isolated in waters from other parts of Italy. Perfluorooctanoic acid (PFOA) is the predominant form in human samples and has been shown to induce serious health consequences, such as neonatal alterations, neurotoxicity and immunotoxicity. Toxicological studies indicate that PFAS accumulate in bone tissue and alter bone development. Epidemiological studies have reported an inverse relationship between PFAS blood levels and bone health, especially in terms of bone mineral density (BMD). Osteopenia and osteoporosis have been shown in several cohorts, ranging from post-menopausal women to young men. Since the interaction between this class of compounds and certain nuclear hormone receptors (such as the thyroid hormone receptor and the androgen receptor) has already been demonstrated, an interaction with the vitamin D receptor has also been hypothesised, which is essential for the proper regulation of phosphocalcic metabolism, the main determinant of bone density. This study summarises the experimental and clinical evidence supporting the interference of PFOA with the vitamin D signalling pathway.

INTRODUCTION

Perfluoroalkyl and polyfluoroalkyl substances are molecules that can interfere with the endocrine system, i.e., they belong to the category of EDs (*endocrine disruptors*). An endocrine disruptor is defined as any chemical entity or mixture of compounds that is capable of interfering with any aspect of hormonal action and that is therefore responsible for altering hormone homoeostasis [1]. EDs exert their toxicity by promoting tissue growth and development. The mechanism of interaction with the reproductive system through the binding of these substances to the androgen receptor (AR) and the oestrogen receptor (ER) is well known. Binding of the endocrine disruptor to the receptor will result in a receptor agonist or receptor antagonist response, manifested by an increase or decrease in the cellular response to the physiological hormone stimulus [2].

PERFLUOROALKYL SUBSTANCES

Perfluoroalkyl and substances (PFAS) are a very broad class of organic molecules that belong to the category of polyfluorinated compounds. They are artificially produced molecules that do not occur naturally. The structure of PFAS has a hydrocarbon skeleton where all hydrogen atoms are replaced by fluorine atoms. The presence of fluorine enables the molecules to acquire special physical and chemical characteristics, above all amphiphilicity: they have an apolar and a polar side, being at the same time both hydrocarbons and strong acids. The polar side contains the functional group, which may be a carboxyl, a sulphuric group, an alcohol group or several others. The polar functional group and the length of the fluorocarbon chain define the individual PFAS. For example, the two most frequently studied compounds, because they occur most frequently in polluted areas, are perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). The main sources of exposure can be ingestion of contaminated drinking water or food with high levels of these compounds (e.g., fish and seafood). Several perfluoroalkyl compounds have

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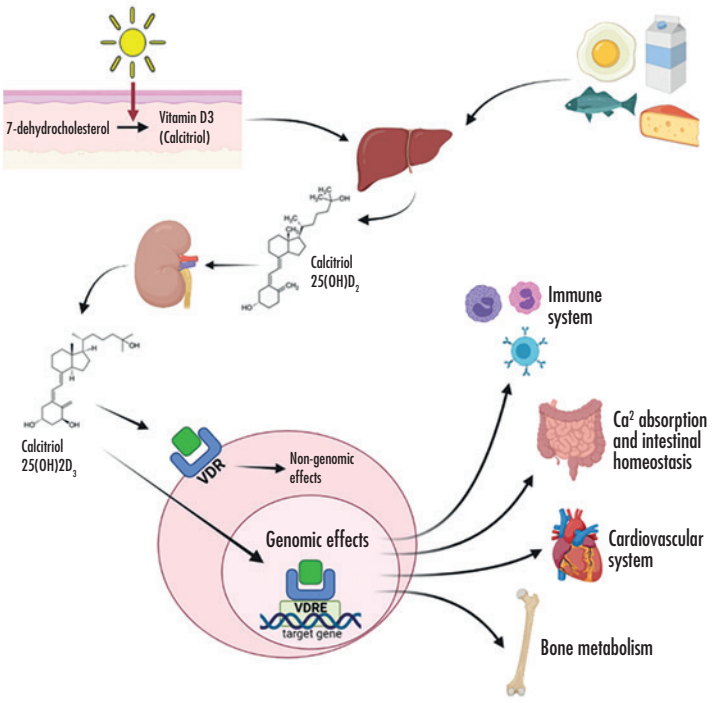

FIGURE 1.
Vitamin D metabolism: Vitamin D is produced endogenously by the skin following exposure to sunlight or exogenously through dietary ingestion. The vitamin D precursor (cholecalciferol) is then converted to 25-hydroxyvitamin D (25(OH)D, calcifediol) by the 25-hydroxylase enzyme in the liver. In its turn, 25(OH)D is hydroxylated in the kidney at the 1-α position in its biologically active metabolite, 1,25-hydroxyvitamin D (1,25(OH)D. Calcitriol performs its biological functions in different organs by acting on its nuclear receptor, which, following binding to the agonist, migrates into the nucleus and binds to specific recognition sequences (VDREs) at the level of target gene promoters.

been found in body fluids: serum, seminal fluid, breast milk and even in the umbilical cord, suggesting that exposure to these compounds is life-long from its inception. PFOA and PFOS induce serious human health consequences such as neonatal mortality, neurotoxicity and immunotoxicity. Health surveillance data in the USA have shown that PFAS are detectable in the serum of 95% of the population [3].
In humans, PFAS serum levels vary depending on the level of exposure. In the general population that has not been exposed, average concentrations of 5.5 ng/mL have been found for PFOA and 2.1 ng/mL for PFOS [4]. In the population living in the polluted areas of the Veneto region, PFOA concentrations range from 54 to 540 ng/mL [5].

SKELETAL TOXICITY

For about a decade, it has been known that the risk of osteoporosis and pathological fractures is associated with exposure to some environmental pollutants (lead, cadmium and mercury). PFAS are among the pollutants that interact with bone metabolism. Today, few studies are available on the interaction of these substances with bone metabolism. Foetal bone malformations have been reported in rodents with prenatal exposure to PFOS and, still in mice, environmental exposure to PFOS results in rapid accumulation in bone tissue. In humans, the presence of perfluoroalkyl compounds (predominantly PFOS) in bone has been demonstrated by analysis of skeletal findings from autopsies of subjects exposed to the contamination [6].
The most recent analyses concern those carried out by the two studies on the health of the American population, which highlighted the correlation between high serum levels of PFAS in contaminated areas and reduced bone mineral density, which varied based on the type of perfluoroalkyl substance considered. Looking more specifically at individual PFAS, there is a higher prevalence of osteoporosis and lower bone density in the tibia and femur and a high prevalence of osteoporosis among women associated with PFOA, PFNA, and PFHxS can be noted. More recently, these findings have been confirmed in other studies on adolescents or young adults [7,8].
These latter data were confirmed in a cohort of young men (aged 18 to 21) from polluted areas in the Veneto Region. In exposed subjects, the association between exposure to PFAS and the risk of fracture was demonstrated [9].

VITAMIN D METABOLISM

Most vitamin D3 is produced in the skin from provitamin 7-dehydrocholesterol through a photochemical reaction involving the UV component of solar radiation. Once synthesised in the skin, or absorbed from the intestine, vitamin D is found in the circulation bound to its serum *D binding protein* (DBP), an α-globulin synthesised by the liver. To be biologically active, vitamin D3 requires a double hydroxylation (Fig. 1).
The first hydroxylation, at position 25, is carried out by mitochondrial and microsomal enzymes that belong to the cytochrome P450 superfamily. The second hydroxylation is carried out in the kidney by an enzyme present in the proximal convoluted tubule, the 1α-hydroxylase, which also belongs to the cytochrome P450 family (CYP27B1). Unlike the previous one, this hydroxylase is strongly regulated: parathormone and hypophosphoremia activate it, while calcium, phosphate, FGF-23 and calcitriol (negative feedback mechanism) inhibit it. This results in 1,25-dihydroxy-cholecalciferol, or calcitriol, the true vitamin D derived hormone.
The vitamin D receptor (VDR) is expressed in numerous cell types and tissues. Calcitriol, the active form of vitamin D, directly or indirectly regulates more than 200 genes involved in cell proliferation, differentiation, apoptosis and neoangiogenesis. It is convenient to divide the biological effects of vitamin D into skeletal, i.e. those that affect phospho-calcium and extra-skeletal metabolism [10] (Fig. 1).

PFAS INTERFERENCE WITH VITAMIN D METABOLISM

Although the number of epidemiological studies confirming a negative effect of these substances on skeletal metabolism is increasing, the mechanisms that may induce this association have not yet been fully demonstrated. Vitamin D, a steroid hormone, which

acts by stimulating intestinal reabsorption of calcium in favour of an anabolic action on bone is a key hormone in skeletal development. Several exogenous factors, such as obesity, diet and pollution, are known to influence circulating vitamin D levels. Vitamin D homoeostasis may also be affected by endocrine disruptors, since the biologically active metabolite, 1,25-hydroxyvitamin D, is very similar in its structure to classic steroid hormones. Its nuclear receptor is also comparable to receptors for thyroid or steroid hormones. For example, an inverse association between bisphenol A and phthalates and vitamin D levels has been reported in two epidemiological studies [11]. Given the similarity between steroid hormones, in particular testosterone, and vitamin D, and between the respective steroid receptors, such as the androgen receptors, in particular the androgen receptor, and the vitamin D receptor, it can be assumed that the reported interference of PFAS with steroid hormone function can also be extended to vitamin D metabolism.

This mechanism could demonstrate the previously reported associations between PFAS exposure and impaired skeletal development and osteoporosis. On the basis of this evidence, a role for PFAS in altering vitamin D metabolism has been hypothesised. This mechanism could be one of the possible modes of skeletal alteration induced by these substances. Vitamin D homoeostasis could be affected by endocrine disruptors, as this hormone is steroidal in origin and endocrine interference of PFAS with steroid receptors, such as the androgen receptor, has already been demonstrated [12].

A very recent study by Prof. Foresta's Group [13] has shown that PFAS interfere with the vitamin D receptor, inducing a reduced response of skeletal cells to vitamin D, which manifests itself in reduced bone mineralisation (Fig. 2). Firstly, PFOA competes with calcitriol at the same vitamin D receptor (VDR) binding site, leading to an alteration in the receptor's structural flexibility. Secondly, this interference leads to an altered response of vitamin D-sensitive genes in two cell populations targeted by this hormone, osteoblasts and colorectal epithelial cells. Third, mineralisation in human osteoblasts is reduced when coincubated with PFOA and calcitriol. Finally, in a cohort of healthy young males, vitamin D was not decreased in the exposed group, but PTH levels were higher in association with

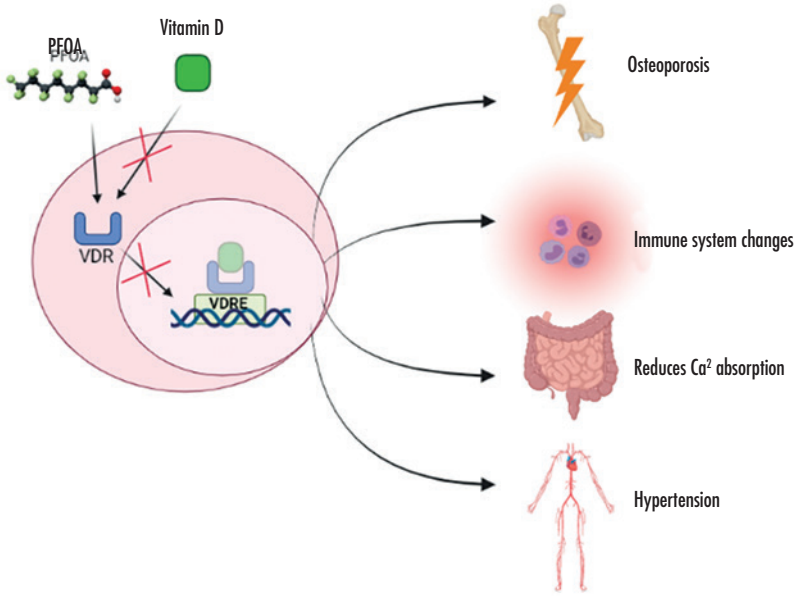

FIGURE 2.
PFAS' endocrine disruption mechanism on vitamin D: PFAS inhibit vitamin D from binding to its receptor (VDR) and preventing it from binding to the target gene promoters. This interference leads to a state of functional hypovitaminosis D in which, even with normal levels of vitamin D, it is unable to perform its biological function in the target cells. This mechanism may explain the several clinical manifestations observed in populations exposed to PFAS pollution and related to vitamin D activity, such as osteoporosis, reduced immune response, reduced calcium absorption and cardiovascular conditions.

PFAS exposure, suggesting a compensatory mechanism in response to functional D hypovitaminosis. Overall, this finding shows an important pathophysiological involvement in vitamin D deficiency associated with environmental exposure to endocrine disrupting chemicals and could explain the epidemiological observations of reduced bone mass in this context. These results, in addition to clarifying the mechanisms by which PFAS interfere with the activity of this important hormone, suggest a possible role for these pollutants in the pathogenesis of osteoporosis, the main pathology related to reduced vitamin D levels.

CONCLUSIONS

Epidemiological and experimental evidence shows that PFAS alter vitamin D homoeostasis and therefore represent a risk factor for bone tissue in all age groups, from developmental age (growth phase) to post-menopause, the high-risk phase for osteoporosis. Monitoring of vitamin D status and skeletal health is highly recommended in exposed populations. At the same time, subclinical vitamin D deficiency (a widespread problem in Western societies) is a susceptibility factor to the effects of PFAS exposure. Therefore, it is particularly important to develop non-pharmacological awareness and prevention campaigns in exposed populations, based on the promotion of physical activity, proper exposure to sunlight and nutrition.

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