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Trace metals and the hemostatic system

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ABSTRACT

In this narrative review we report the main relationships between trace metals and the hemostatic system since this aspect has seldom attracted the attention of the scientific community.

A basic aspect to be considered is the importance of maintaining the fine control of all trace metals' levels since they have an important impact on the pathophysiology of the hemostatic system.

It is worth noting that poor diet habits are responsible for most trace metal deficiencies, while pollution is responsible for dangerous exposure to them with a consequent negative impact on the general population. This appears of paramount importance in planning the implementation of food and nutrient support to ameliorate the hidden hunger and the quality of life of people especially in developing countries and limiting poisons both in the air and food. As it often happens, when damage to certain mechanisms takes a very long time to appear, no attention is paid to the importance of a systematic prevention to avoid late negative outcomes.

1. Introduction

Platelets, blood coagulation and fibrinolysis are the three integrated steps that compose the hemostatic system [1,2,3,4]. The pathophysiological mechanisms of the hemostatic system are complex, and a deep understanding of this system is not easy to acquire. Further elucidation of this topic is important in daily practice because the hemostatic system is not only involved in several mechanisms whose abnormalities lead to either bleeding or thrombosis, but is also involved in an integrated pathway with the immune system [5]. In other words, both of these systems provide a physiological defensive barrier against foreign invaders, such as viruses, bacteria and neoplastic cells [6]. However, an aspect of the hemostatic system that is often neglected is its relationship with several trace metals, which are important aspects of human life. The aim of this chapter is to highlight the interplay among several trace metals and platelets, blood coagulation and fibrinolysis. To search for articles that address this review topic, we used an electronic-based strategy (Medline). A combined keyword search strategy was used to narrow the results. We identified and chose the following keywords for the search strategy: "zinc (Zn^{2+}), iron, cadmium, calcium (Ca^{2+}), magnesium (Mg^{2+}), selenium, copper and platelets, blood coagulation and fibrinolysis". Articles were selected based on the following criteria: a) direct relation to this review topic, b) good quality and easy to understand for a nonspecialized reader, c) publication in peer review journals and d) written in English. Since significant results in the literature were not found for all trace metals, we included only those articles in which a connection with the hemostatic system was evident. For these reasons, selenium and copper were excluded in the final assessment of this review.

1.1. Calcium

Calcium (Ca) (atomic number 20, atomic weight 40.078 g/mol) is a silvery metal ion in oxidation states of 0 and 2^{+}. The main part of Ca (99%) is in the bones as hydroxyapatite. Ca is essential for blood coagulation since it is a cofactor in the activation of several clotting factors [7]. In particular, Ca is essential for the functional state of vitamin K-dependent factors and natural anticoagulants [8]. Vitamin K (*Koagulation*) is a fat-soluble vitamin discovered by Henrik Dam in 1934 [9]. Vegetables such as spinach, cabbage, and broccoli and fruits such as

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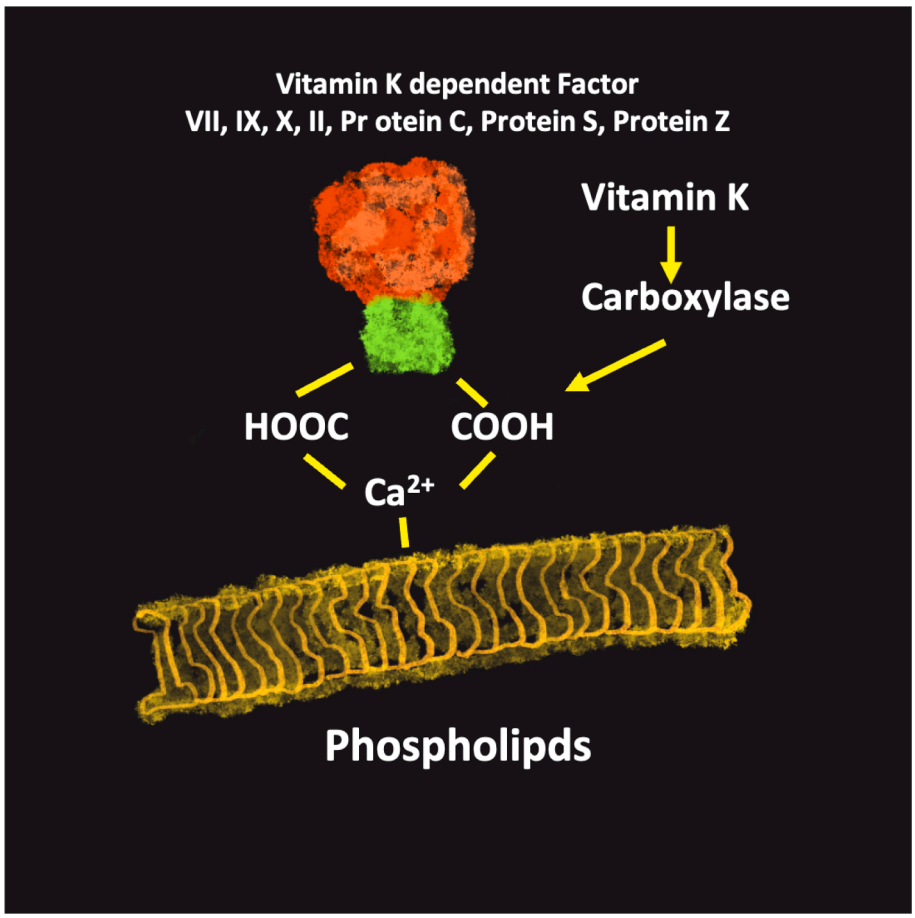

Fig. 1. The role of the vitamin K carboxylation of Gla residues for both an optimal adsorption and function of vitamin K dependent factors on phospholipids surfaces. The role of Ca^{2+} is essential for the clotting factors adsorption.

kiwi and bananas contain large amounts of vitamin K1 (phylloquinone). A controlled vitamin K diet has been found to avoid anticoagulation fluctuation during vitamin K-antagonist consumption [10]. Vitamin K2 (menaquinone) is produced by intestinal bacteria, but its role in contributing to the active role of this vitamin is uncertain [4]. Vitamin K is an anti-hemorrhagic factor; it drives a carboxylase that introduces carboxyl groups (–COOH) in the glutamate residues of proteins (Gla residues) to form gamma-carboxyglutamate. The carboxylated Gla residues can bind Ca ions, which are essential for the coagulability of four factors of blood coagulation (II, VII, IX, and X) on the phospholipid membranes [11] [Fig. 1]. However, this pathway is also essential for the function of other proteins involved in the control of blood coagulation activity, such as Protein C, Protein S, and Protein Z, the so-called natural anticoagulants [12]. Tight regulation of the coagulation system is therefore achieved. In healthy subjects, this fine control allows the blood to coagulate to repair tissue damage without excess thrombin formation, thus avoiding vascular thrombosis. Vitamin K is also important for the function of proteins involved in bone metabolism, such as osteocalcin, periostin, and the matrix Gla protein [13]. The discovery of vitamin K was later crucial for understanding the mechanism of action of coumarin anticoagulants, the use of which as antithrombotic drugs is still widespread worldwide [14]. Ionized hypocalcemia is frequently found in critically ill adults and is associated with increased mortality [15]. The hazard ratio is up to 5.1 when severe hypocalcemia is present (3.6 mg/dl), but decreases to 1.8 for mild hypocalcemia (3.6–4.6 mg/dl) when we consider the admission of patients to an intensive care unit [16]. In 2004, James et al. [17] published a study aiming to determine the threshold calcium value needed for the start of coagulation and the range over which changes in ionized calcium could influence coagulation activity. The study was performed in one hundred samples using thromboelastography for assessing coagulation. No sample with a $Ca^{2+} < 1.3$ mg/dl showed any clot formation. Normal coagulation measures were obtained in almost all samples for $Ca^{2+} > 2.2$ mg/dl. Therefore, Ca^{2+} levels between 2.2 and 2.4 mg/dl are required to avoid severe abnormalities in blood coagulation activity. However, citrate anticoagulant is present in infused blood components, resulting in worsening hypocalcemia [18]. Excess amounts of citrate anticoagulant may be present in fresh frozen plasma administration [19], thus further affecting blood coagulation in trauma. It is important to keep in mind that if the infusion is too rapid, a more pronounced fall off Ca^{2+} may occur [20]. Optimal management of hypocalcemia is therefore warranted in critically ill patients [21]. As it regularly happens in the pathophysiology, an opposite dangerous effect of an excess of Ca^{2+} may occur. Three cases of patients who suffered from clinical thrombotic events (stroke, pulmonary embolism and deep venous thrombosis) during the course of a hypercalcemia condition have been described by Koufakis et al. [22]. In all three cases, the final diagnosis of primary hyperthyroidism due to a parathyroid adenoma was established. The authors proposed several pathophysiological mechanisms that may link hypercalcemia to both venous and arterial thrombosis, and they determined that hypercalcemia may activate both platelet aggregation and blood clotting factors. However, other conditions may also be involved, such as dehydration, vasoconstriction and cell toxicity. In our opinion, these findings are of importance in clinical practice because serum Ca^{2+} levels should always be considered in a patient with a thrombotic event, while patients with hypercalcemia would benefit from anti-thrombotic prophylaxis with either low molecular weight heparins or fondaparinux.

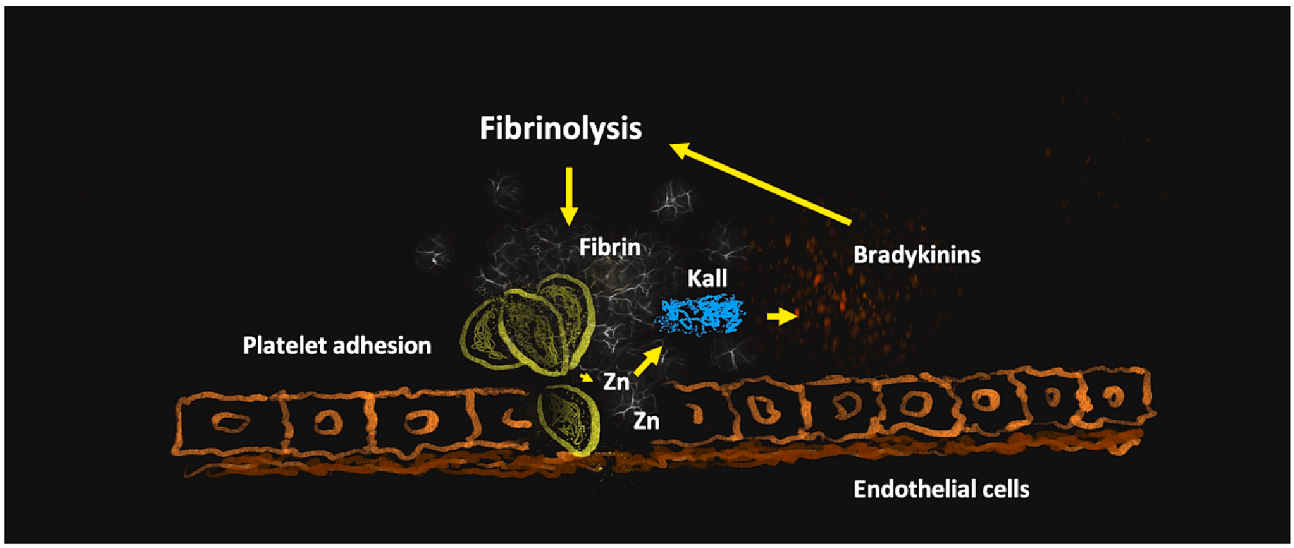

Fig. 2. Platelets release stored Zn^{2+} at the sites of vascular damage. This phenomenon contributes to the activation of the plasma kallikrein system on the endothelial cells. The kallikrein system generates bradykinins which in turn activate fibrinolysis so maintaining the anti-thrombotic function of the endothelial cells.

1.2. Zinc

Zinc (Zn^{2+}) is a blue-silver abundant metal in the body (atomic number 30, atomic weight 65.38 g/mol, density 7.14 g/mL). Foods that contain abundant Zn^{2+} are red meat, poultry, fish, seafood, legumes, nuts, whole grains, and dairy products [23]. Zinc is an important element present in many enzymes, where it works with its catalytic, cocatalytic, and/or structural functions. Zn^{2+} is also involved in cellular metabolism and protein stability [24]. The total concentration of Zn^{2+} in human plasma is between 10 and 20 μM, mostly bound to albumin and α_2 macroglobulin. Only 0.1–2 μM Zn^{2+} is free [25]. Zn^{2+} plays an important role since it has a favorable impact on hemostasis by some mechanisms related to platelet function, blood coagulation and fibrinolysis. First, platelets release stored Zn^{2+} at the sites of vascular damage [26]. This function contributes to the activation of the plasma kallikrein system on endothelial cells. The kallikrein system is important since it allows the generation of bradykinins, which in turn can activate fibrinolysis to maintain the antithrombotic function of endothelial cells [27] [Fig. 2]. Moreover, Zn^{2+} platelet transporter transmembrane protein 163 has been recently discovered to play a role in dense granule biogenesis [28]. Again, extracellular Zn^{2+} can enter the platelet cytosol, thus inducing full platelet activation and potentiating their activation by agonists. In other words, Zn^{2+} seems to be essential in granting normal efficient platelet function [29]. Second, Zn^{2+} facilitates the binding of FXI to activated platelets. In vitro experiments showed that FXI binds to glycocalicin in a zinc-dependent manner. Glycocalicin is a part of the GPIb-IX-V receptor, essential for optimal binding between FXI and activated platelets [30]. The binding of FXI to that receptor in activated platelets is crucial to support optimal blood clotting, allowing proper thrombin generation in sites where platelets aggregate [31]. Third, Zn^{2+} modulates fibrin structure because its fibers formed with Zn^{2+} are thicker than those without [32], thus contributing to clot stability. Recently, a new role for Zn^{2+} has been shown in the binding and activation of FXII at the platelet surface. This property seems to be crucial for FXII-dependent thrombin generation but unnecessary for hemostasis [33]. Fourth, Zn^{2+} binds tissue-type plasminogen activator (t-PA) and plasmin, limiting their activities and thus reducing fibrin dissolution. When Zn^{2+} is released from platelets, fibrinolysis becomes attenuated, so clot stability is preserved. All these findings clearly indicate that Zn^{2+} deficiency may significantly affect all hemostatic mechanisms. To counteract a poor dietary habit, especially in developing countries, food grains such as wheat, rice, and maize could be biofortified with zinc, thus allowing an improvement in micronutrient consumption [34].

1.3. Iron

Iron (Fe) is a metal with an atomic number of 26 and an atomic weight of 55.845 g/mol. Iron is a metal essential for life since it is a cofactor for mitochondrial enzymes and in DNA synthesis. In addition, iron plays an essential role in binding and transporting oxygen by hemoglobin and myoglobin [35]. Tight control of iron homeostasis is essential because, on the one hand, iron deficiency is a common cause of anemia, while on the other hand, iron overload may lead to toxic radical generation and tissue damage. Surprisingly, both iron deficiency and overload are risk factors for thromboembolic events [36]. Although not common in clinical practice, some case reports on the occurrence of both venous and arterial thrombosis in an iron deficiency setting have been published [37–41]. Since mild thrombocytosis is a frequent finding during iron deficiency anemia [42], this condition has been claimed to be the trigger of thrombotic events on the occurrence of both venous and arterial thrombosis in an iron deficiency setting [43]. A negative, although not close, correlation was found among serum iron, transferrin saturation and platelet count [44]. In other words, a relationship seems to be present among iron deficiency, anemia and thrombocytosis. Iron is important in the control of not only erythropoiesis but also the megakaryocyte pathway; this may be because an amino acid homology exists between erythropoietin and thrombopoietin, the growth factors of the two pathways [45]. However, this has not been proven to be the key point behind why iron deficiency is associated with thrombocytosis [46,47]. In our opinion, more simply, an ancestral defensive mechanism may provide a thrombocytosis reaction against an underlying bleeding condition. An opposite condition, i.e., iron overload, has been associated with cardiovascular diseases [48]. On the other hand, iron has prooxidant activity that leads to an increased production of hydroxyl radicals, which in turn induces the progression of atherosclerosis in an animal model [49]. Moreover, an association between ferritin, a marker of iron deposits, and atherosclerosis has also been proven in humans [50,51]. In particular, patients with myocardial infarction and premature coronary stenosis (<50 years) showed increased ferritin levels compared to controls. This association was not found in women [52]. In another study, higher iron overload was found in males with early-onset cardiovascular disease. Interestingly, this association was not found with C282Y or H63D mutations in human homeostatic iron regulator protein (HFE) [53]. These findings were confirmed by Claeys et al. some

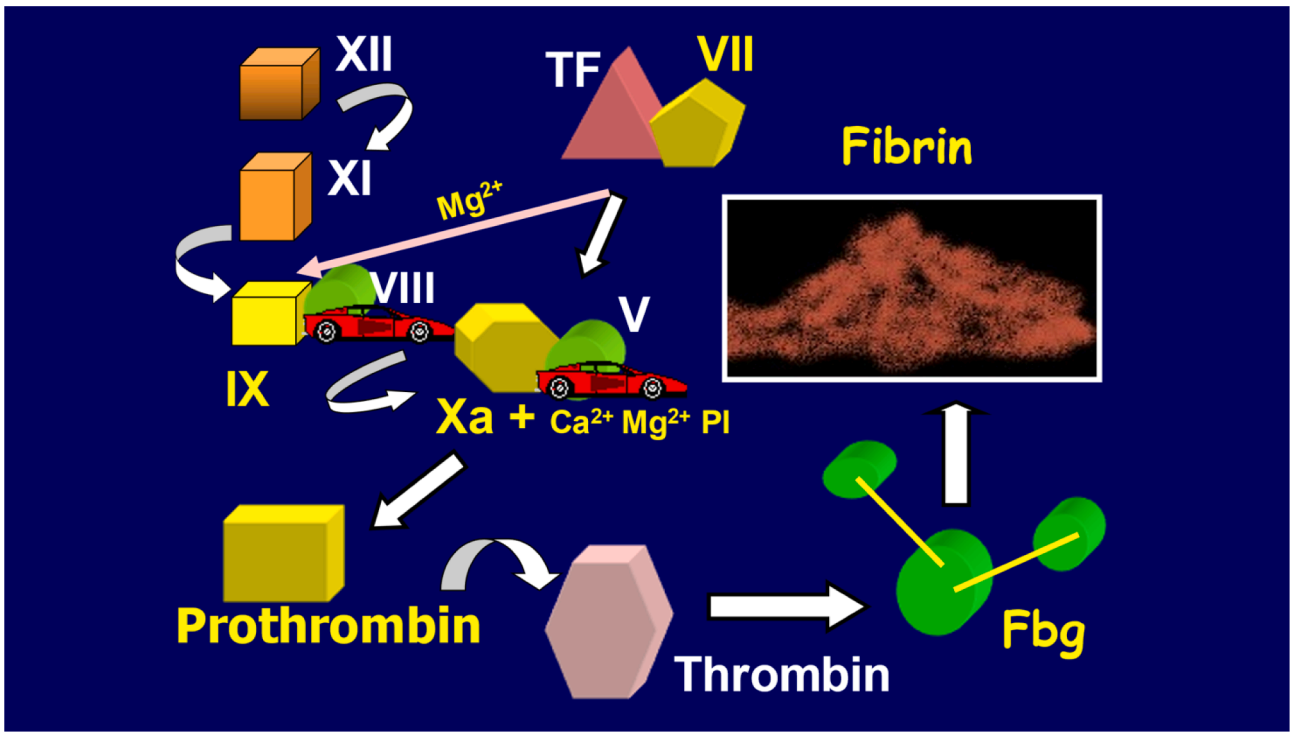

Fig. 3. Mg^{2+} accelerates the activation of factor IX by the complex Factor VII-Tissue Factor, the trigger of blood coagulation in vivo as wells as the activation of prothrombin to thrombin by factor Xa. TF: Tissue Factor, Pl: Phospholipids, Fbg: Fibrinogen.

years later [54]. The behavior of ferritin has recently been a subject of interest during the COVID-19 pandemic. High levels of ferritin have been found overall in groups of patients with severe COVID-19 [55]. Ferritin along with D-dimer and fibrinogen well reflect the association among coagulopathy, inflammation and COVID-19 [56].

1.4. Magnesium

Magnesium is an essential macronutrient, and in its ionic form (Mg2þ) [57], it is crucial for hemostasis and coagulation, although this property has often been overlooked. In 1996, Sekiya et al. [58] highlighted the role of Mg^{2+} in the coagulation enzymatic cascade. They showed that not only Ca^{2+} ions but also Mg^{2+} ions play a significant role in both stabilizing the conformation and enhancing the activity of Factor IX. Again, Mg^{2+} ions accelerate the activation of Factor X by factor IX in the presence of factor VIII, phospholipids and Ca^{2+}. Another important function of Mg^{2+} was the acceleration of the activation of factor IX by the complex Factor VII-Tissue Factor, the trigger of blood coagulation in vivo [Fig. 3]. In 2019, Sobczak et al. [59] found that 47 patients affected by type-1 and type-2 diabetes had lower Mg^{2+} levels than a control group. Since it is known that Mg^{2+} deficiency can induce plasma hypercoagulability [60], the authors examined fibrin clot formation and lysis parameters in the same population of diabetic patients by means of a turbidimetric method. The magnesium concentration was negatively correlated with the clot maximum absorbance ($p = 0.0215$) and lysis time ($p = 0.046$). Afterward, lysis time was shortened by adding Mg^{2+} in a purified system, thus demonstrating a further role of this macronutrient in the hemostatic system [61]. These findings confirm the role of Mg^{2+} in fibrinolysis control. As previously reported, increasing concentrations of Mg^{2+} added to whole blood were able to shorten the fibrin lysis time, probably by inhibiting plasminogen activator inhibitor-1 (PAI-1), thus enhancing t-PA activity [62]. Taken together, these findings underline an important, although well-known, concept regarding the fine regulation and modulation of a balance of many physiologic pathways not only referred to as the hemostatic system. From a practical point of view, it would be interesting to conduct a case-control study on long-term Mg^{2+} supplementation in both type-1 and type-2 diabetes to reduce cardiovascular events. This statement is supported by the findings of a meta-analysis on the beneficial effect of magnesium supplementation on fasting glucose, LDL levels, triglycerides and HDL in type-2 diabetes [63].

1.5. Cadmium

Cadmium is a heavy metal with important occupational and environmental poisonous properties. Smoking, air pollution and consumption of fruits and vegetables cultivated with an excessive use of fertilizers induce chronic cadmium toxicity. Cadmium is used by industry for coating steel, glass and plastics (including polyvinyl chloride) and for nickel cadmium battery production [64]. Cadmium exposure plays a negative role in the development of cardiovascular diseases, hypertension and atherosclerosis. Increased oxidative stress, inflammation and endothelial damage are the results of chronic cadmium toxicity [65,66]. In a Swedish population of 5,627 individuals with low to moderate cadmium exposure, high blood cadmium values have been found to be associated with coronary artery calcium scores, indicating that atherosclerosis is the main outcome of chronic cadmium toxicity [67]. The hemostatic system is also involved in the pathophysiology of the dangerous effects of this metal [68]. Animal studies indicate that cadmium provokes both platelet activation and the formation of fibrin characterized by increased thickness. The latter has been found to lead to dense matter deposits, as shown by scanning electron microscopy (SEM) analysis [69]. Again, analysis obtained in an ex vivo study [70] with the use of SEM along with confocal laser scanning microscopy revealed that blood exposed to cadmium presents ultrastructured changes. Activated platelets and significantly thicker fibrin fibers were found. Moreover, phosphatidylserine was detected on the membrane of spherocytic erythrocytes exposed to cadmium. This is an important finding because phosphatidylserine is an optimal substrate to allow clotting of the coagulation factors on the erythrocyte membrane [71]. In other words, this is a prothrombotic mechanism that may contribute over time to the development of atherosclerosis. Finally, Hara et al. [72]

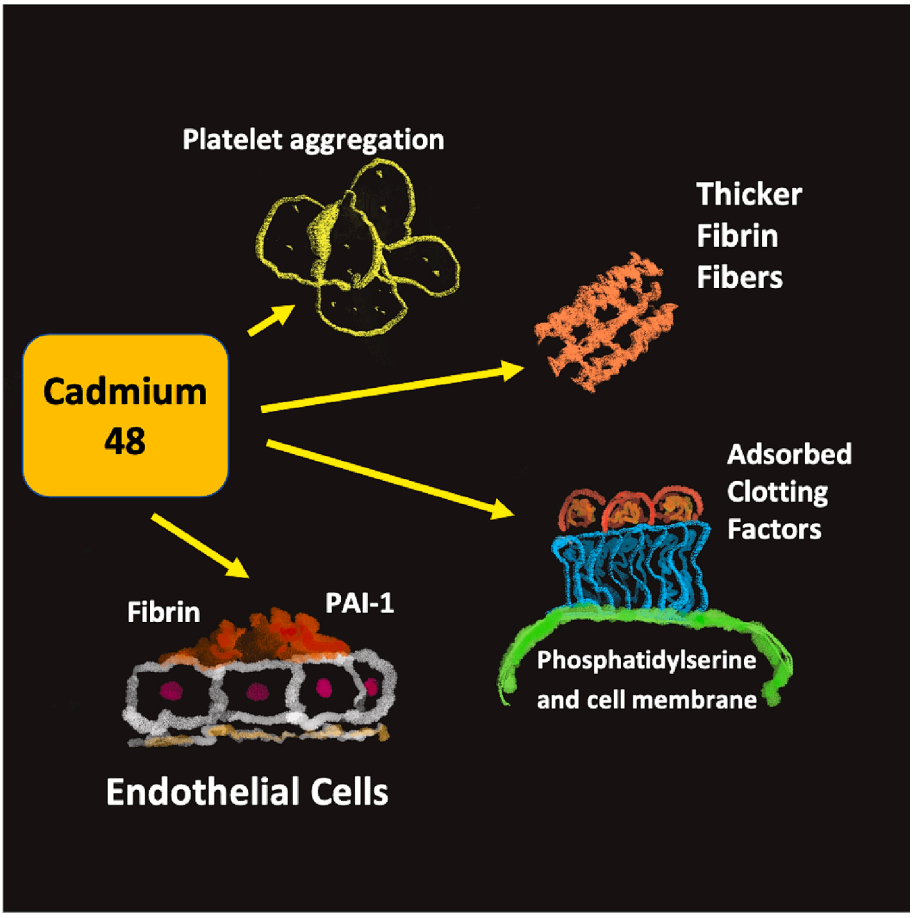

Fig. 4. The adverse and dangerous activities of Cadmium on the haemostatic system: activation of platelets, thicker fibrin fibers, exposition of phosphatidylserine which allows clotting factor adsorption and fibrin deposition on endothelial cells because of an increase in PAI1 levels.

demonstrated that cadmium induces the synthesis of PAI-1 in human endothelial cells; this is another prothrombotic mechanism since high PAI-1 levels can shift the balance between t-PA and its inhibitor PAI-1 in favor of the latter [73]. The final result may be harmful because a fibrinolysis shutdown may occur, thus limiting intravascular fibrin removal [Fig. 4].

2. Conclusions

In this brief review, we report the main relationship between trace metals and the hemostatic system to highlight the importance of this kind of connection.

Several trace metals have an impact on the hemostatic system, but this has seldom attracted the attention of researchers, students, and teachers. It is worth noting that poor dietary habits are responsible for most trace metal deficiencies, while pollution is responsible for dangerous exposure to them, with a consequent negative impact on the general population. This conclusion appears to be of paramount importance in planning the implementation of food and nutrient support to ameliorate the hidden hunger and quality of life of people, especially in developing countries, and limiting poisons both in the air and food.

Another aspect that we wished to illustrate in this review was the importance of maintaining the fine control of all trace metal levels, since they have an important impact on the pathophysiology of the hemostatic system. Recently, Fanni et al. described how trace metals could have a profound unfavorable impact on the development of atherosclerosis [74]. As it often happens, when damage to certain mechanisms takes a very long time to appear, no attention is given to the importance of systematic prevention to avoid late negative outcomes.

CRediT authorship contribution statement

F. Marongiu: Conceptualization. **S. Marongiu:** Methodology, Data curation. **M.F. Ruberto:** Supervision. **G. Faa:** Conceptualization. **D. Barcellona:** Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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