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# Trace elements and the carotid plaque: the GOOD (Mg, Zn, Se), the UGLY (Fe, Cu), and the BAD (P, Ca)?

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**Abstract. – Multiple epidemiological studies have suggested that industrialization and progressive urbanization should be considered one of the main factors responsible for the rising of atherosclerosis in the developing world. In this scenario, the role of trace metals in the insurgence and progression of atherosclerosis has not been clarified yet. In this paper, the specific role of selected trace elements (magnesium, zinc, selenium, iron, copper, phosphorus, and calcium) is described by focusing on the atherosclerotic prevention and pathogenesis plaque. For each element, the following data are reported: daily intake, serum levels, intra/extracellular distribution, major roles in physiology, main effects of high and low levels, specific roles in atherosclerosis, possible interactions with other trace elements, and possible influences on plaque development. For each trace element, the correlations between its levels and clinical severity and outcome of COVID-19 are discussed. Moreover, the role of matrix metalloproteinases, a family of zinc-dependent endopeptidases, as a new medical therapeutical approach to atherosclerosis is discussed.**

**Data suggest that trace element status may influence both atherosclerosis insurgence and plaque evolution toward a stable or an unstable status. However, significant variability in the action of these traces is evident: some – including magnesium, zinc, and selenium – may have a protective role, whereas others, including iron and copper, probably have a multi-faceted and more complex role in the pathogenesis of the atherosclerotic plaque. Finally, calcium and phosphorus are implicated in the calcification of atherosclerotic plaques and in the progression of the plaque toward rupture and severe clinical complications. In particular, the role of calcium is debated. Focusing on the COVID-19 pandemia, optimized magnesium and zinc levels are indicated as important protective tools against a**



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**severe clinical course of the disease, often related to the ability of SARS-CoV-2 to cause a systemic inflammatory response, able to transform a stable plaque into an unstable one, with severe clinical complications.**

*Key Words:*
Trace elements, Metals, COVID-19, SARS-CoV-2, Atherosclerosis, Plaque.

## Introduction

In recent years, multiple epidemiological studies have suggested that metal exposure may promote the insurgence and progression of atherosclerosis in humans. Industrialization followed by progressive urbanization has been indicated as the main factors responsible for the rising of atherosclerosis in the developing world[1]. The relevance of the trace metals' status in the progression of atherosclerosis has not been clarified yet. Epidemiologic and experimental evidence supports the role of cadmium and lead in the insurgence of atherosclerotic plaques and development of cardiovascular disease[2]. Higher concentrations of cadmium, chromium, copper, iron, and manganese have been reported in the blood of atherosclerosis patients, as compared with control healthy subjects[3]. Urine cadmium and tungsten have been associated with femoral and carotid intima-media thickness, respectively[4]. Moreover, changes in zinc status have been found in carriers of atherosclerotic plaques[5].

The aim of this review is to report the available data on the role of selected trace metals in atherosclerosis patients, with respect to the healthy subjects, to shed light on the possible role of these metals in the initiation and progression of atherosclerosis in humans. Daily intake, normal serum levels, role in physiology, main effects of increased and decreased stores, and specific roles of each metal on atherosclerosis will be reported, with the aim to give the readers a simple guide on the relationship between trace metal status and atherosclerosis.

## Metal Analysis

### Magnesium and Atherosclerosis

**Definition.** Magnesium is one of the ten essential metals in humans, and it is the second richest intracellular cation after $K^+$. Magnesium is a grey-white alkaline earth metal (atomic number 12, atomic weight 24.305 g/mol, density 1.738 g/mL); in its inorganic compounds, it presents the two oxidation states 0 and +2.

**Daily intake.** $Mg^{2+}$ is abundant in all green leafy vegetables, cereals, nuts, and legumes. The Recommended Daily Allowance (RDA) for magnesium is variable with the gender and age: 80 mg/day for children aged 1-3 years, 240 mg/day for children aged 9-13 years, and 320-400 mg/day, or 6 mg/kg/body weight for adults.

**$Mg^{2+}$ serum levels**. Even though only 1% of the total body magnesium is present in the blood, the serum magnesium concentration is the predominant test used in clinical practice to assess magnesium status. Normal values: 1.3-2.1 mEq/L or 0.65-1.05 mmol/L or 1.5 to 2.5 mg/dL.

**Intracellular $Mg^{2+}$**. Intracellular free magnesium may be measured in platelets using Mag Green fluorescent dye and flow cytometry. The mean platelet concentration of intracellular free magnesium is 450 mM with a range from 203 mM to 673 mM.

**Roles of magnesium in physiology.** Magnesium is a cofactor of over 300 different enzymes. It regulates collagen and elastin turnover in the vascular wall and matrix metalloproteinase activity. Magnesium protects the elastic fibers from calcium deposition and maintains the elasticity of the arterial vessels. It is a critical factor in immunological competence. $Mg^{2+}$ is vital for the formation of bone, favoring $Ca^{2+}$ assimilation.

**$Mg^{2+}$ and metalloenzymes**. Several metalloproteins require magnesium for their function, being involved in glycolysis and nucleic acid biochemistry. Moreover, magnesium regulates matrix metalloproteinase activity.

**Hypomagnesemia: main effects**. Serum magnesium levels <1.5 mg/dL determine hypomagnesemia. Magnesium deficiency, prevalent in older people and in carriers of celiac disease, is associated with clinical manifestations including hypertension, atherosclerosis, cardiac arrhythmias, stroke, changes in lipid metabolism, insulin resistance, metabolic syndrome, type 2 diabetes, osteoporosis, depression, and other neuropsychiatric disorders[6].

**Hypermagnesemia: main effects**. Hypermagnesemia is a serum magnesium concentration >2.6 mg/dL. The major cause is renal failure. Clinical consequences include hypotension, respiratory depression, and cardiac arrest.

**Specific roles of magnesium in atherosclerosis**. Hypomagnesemia has a role in the pathogenesis of atherosclerosis. Low serum magnesium levels are associated with an increased mean carotid wall thickness, higher incidence of cardiovascular disease, and hypertension. Patients with coronary artery disease submitted to magnesium supplementation show a more favorable clinical outcome.

**Mg and arterial calcification and inflammation**. An inverse relationship between serum magnesium concentration and vascular calcification has been reported[7]. There are two leading hypotheses for this protective activity of magnesium: i) magnesium may bind phosphate and delay calcium phosphate crystal growth; ii) magnesium may halt vascular smooth muscle cell transdifferentiation toward an osteogenic phenotype[8]. In short, $Mg^{2+}$ has a protective effect on the calcification of the plaque.

**Interactions with other trace metals**. $Mg^{2+}$ is involved in the modulation of intracellular $Ca^{2+}$ homeostasis. Decreased extracellular or intracellular $Mg^{2+}$ is associated with an increase in $Ca^{2+}$ levels. In vascular smooth muscle cells (VSMCs), cytosolic $Ca^{2+}$ concentration is one of the principal factors determining their contractile properties and their ability to migrate into the intima. $Mg^{2+}$ counteracts $Ca^{2+}$ and functions as a physiological $Ca^{2+}$ blocker[9].

**Magnesium supplementation: possible influence on plaque development**. Magnesium, acting as a calcium antagonist, potentiates the production of vasodilator mediators, such as prostacyclin and nitric oxide, and modulates vascular responses to a variety of vasoactive substances, including endothelin-1, angiotensin II, and catecholamine. $Mg^{2+}$ balances the effects of catecholamines in acute and chronic stress. Magnesium deficiency is associated with the development of insulin resistance, hyperglycemia, and changes in lipid metabolism, which favor the insurgence of the atherosclerotic plaque[10] (Figure 1).

**Magnesium and COVID-19: possible influences on the carotid plaque**. Elderly people, obese, hypertensive, and diabetic patients are among the high-risk category groups more prone to SARS-CoV-2 infection, which is often severe or fatal in these subjects. Magnesium status might explain why these categories share an increased risk of severe COVID-19, hypomagnesemia being strongly associated with old age, type 2 diabetes, and obesity. Regarding hypertension, hydrochlorothiazide often leads to magnesium deficiency.

## *Zinc and Atherosclerosis*

**Definition.** Zinc is a blue-silver, slightly brittle metal (atomic number 30, atomic weight 65.38 g/mol, density 7.14 g/mL); in its inorganic compounds it presents in different oxidation states: -1, 0, +1 and +2. It is the first element in group 12 of the periodic table. The most common zinc ore is sphalerite (zinc blende), a zinc sulfide mineral.

**Daily intake.** The Recommended Dietary Allowance (RDA) for adults is 8 mg/day for women and 11 mg/day for men. RDA shows relevant changes according to different age: from 7 months to 3 years of age is 3 mg/day; from 3 to 8 years of age RDA, is 8 mg/day; during pregnancy, RDA increases up to 13 mg/day; during lactation, women require 12-14 mg of zinc daily[11]. Elderly aged (<69 years) are apparently at increased risk of zinc deficiency. Zinc is widely distributed in foods. Some important dietary sources of zinc include red meat, poultry, fish, seafood, legumes, nuts, whole grains, and dairy products. The vast majority of zinc is absorbed by the small intestine through a transcellular process. Paracellular transport may occur at high zinc intakes. In malabsorption syndromes, including celiac disease, zinc absorption is reduced. Transfer from the intestine is via the portal system with most newly absorbed zinc bound to albumin.

**Zinc Serum levels.** Plasma or serum zinc is the most frequently used index for evaluating zinc status. Values vary diurnally, decrease after meals, and appear related to gender and age. The lower limit of normal (morning) fasting plasma

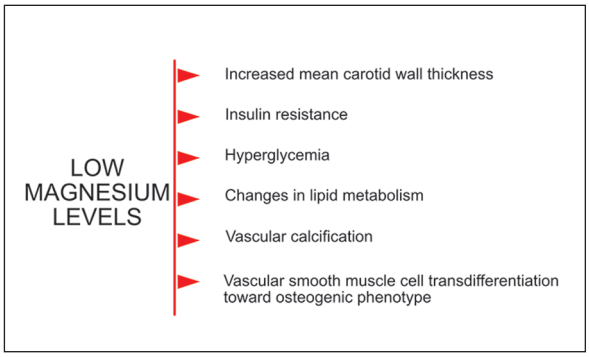


**Figure 1.** Magnesium deficiency consequences and associations with human pathology conditions.

zinc has been set at 10.7 mmol/L (700 mg/L). A cutoff value of 11.5 mmol/L (750 mg/L) might be a more reliable index of zinc status.

**Intracellular $Zn^{2+}$ status.** In cells, zinc is distributed in the cytoplasm (50%), nucleus (30–40%), and cell membrane (10%). While most of $Zn^{2+}$ is bound to proteins and inaccessible, a pool of labile $Zn^{2+}$ is non-protein bound and complexed to a variety of low molecular weight ligands. There is growing evidence of a labile $Zn^{2+}$ pool in cytoplasm organelles. Small molecule probes and sensors[12] can be used to quantify the subcellular distribution of labile $Zn^{2+}$, whereas mass-spectrometry and X-ray fluorescence-based techniques can be used to map the distribution of total $Zn^{2+}$.

**Roles of $Zn^{2+}$ in physiology.** Zinc is an essential trace element with important biological functions. It plays a critical role in cellular integrity, protein synthesis, and nucleic acid metabolism, contributing to cell growth, proliferation, differentiation, and death. Zinc also regulates the expression and activation of biological molecules such as transcription factors, enzymes, adapters, channels, and growth factors, along with their receptors. $Zn^{2+}$ participates in gene expression regulation, the maintenance of chromatin and biomembranes, immunity, and protection against free radicals[13].

**$Zn^{2+}$ and metalloenzymes.** A zinc metalloenzyme is an enzyme that specifically requires zinc as a cofactor or as an integral firmly complexed moiety within the structure of the protein. In the latter circumstances, $Zn^{2+}$ ions may participate at the active site of the enzyme in its catalytic activity in either the 4- or 5-coordination configurations in association with a water molecule. Alternatively, complexed zinc plays a role in stabilizing the conformation of the protein. Zinc is required for the activity of >300 enzymes that participate in a wide variety of metabolic processes, including carbohydrate, lipid, protein and nucleic acid synthesis, regulation and degradation.

**Low zinc levels.** The central role of zinc in cell growth and differentiation may easily explain its deficiency in tissues with rapid cell turnover, especially skin, gastrointestinal tract mucosa, and immune system. The relevance of zinc for humans was recognized in patients with growth retardation, hepatomegaly, splenomegaly, hypogonadism, and severe iron deficiency anemia. Zinc deficiency causes dysfunction of cell-mediated immune responses and increases susceptibility to infections. There have also been reports of benefits of an optimal zinc status with regard to other illnesses, such as Wilson's disease and chronic hepatitis C, and in many chronic diseases, such as rheumatoid arthritis, diabetes, and cancer, which are associated with chronic inflammation and oxidative stress.

**Specific roles of $Zn^{2+}$ in atherosclerosis.** $Zn^{2+}$ deficiency can enhance oxidative-stress-related signaling processes in endothelial cells, and plasma $Zn^{2+}$ levels may affect the $Zn^{2+}$ status of the endothelium. Atherogenesis is associated with endothelial cell apoptosis, which may be initiated directly by oxidant stress from oxidized low-density lipoprotein (LDL) or polyunsaturated fatty acids (PUFA) such as linoleic acid. $Zn^{2+}$ may have a protective effect on the vascular endothelium at a number of different levels (Figure 2). Apart from its direct proposed role as an antioxidant and stabilizer of cell membranes, $Zn^{2+}$ participates to the activity of numerous endothelial signaling processes, several of which are important to the maintenance of endothelial cell integrity[14].

**$Zn^{2+}$ and arterial calcification and inflammation.** $Zn^{2+}$ deficiency has been related to the development of a cardiovascular disease, which involves aberrant arterial wall calcification. Zinc is a cofactor of many enzymes and has anti-inflammatory and anti-proliferative properties. Zinc has specific anti-atherogenic properties by inhibiting oxidative stress-responsive transcription factors that are activated during the inflammatory response in atherosclero-

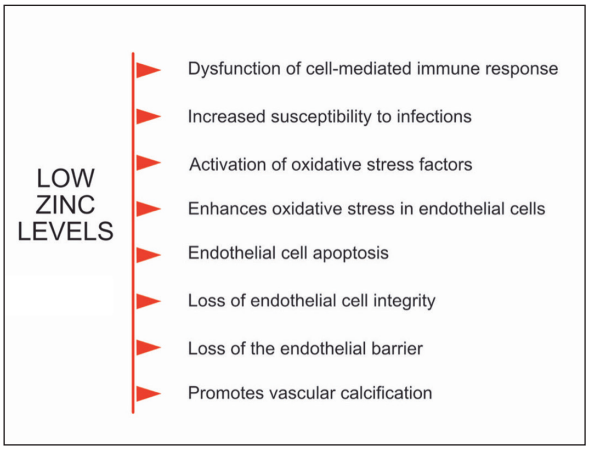


**Figure 2.** Zinc deficiency related human pathology damage.

sis. Zinc supplementation ameliorates phosphate-induced osteogenic/chondrogenic transdifferentiation of vascular smooth muscle cells and vascular calcification through an active cellular mechanism resulting from GPR39-dependent induction of TNFAIP3 and subsequent suppression of the NF-kB pathway. Zinc supplementation may be a simple treatment to reduce the burden of vascular calcification in chronic kidney disease[15].

**Possible interactions of $Zn^{2+}$ with other trace metals.** Elevated concentrations of iron, often present in early atherosclerotic lesions, may provide an accelerated process of atherogenesis in specific regions of the artery wall. Zinc concentrations are inversely correlated with the depth of the lesion in the arteries. The lower the local $Fe^{3+}$ level and the higher the local $Zn^{2+}$ level, the less developed is the atherosclerotic lesion. Iron may be a promoter of atherosclerosis, and zinc may have an anti-atherosclerotic effect[16].

**Zinc supplementation: possible influence on the plaque development.** It is well established that highly fibrotic calcified plaque is less prone to rupture than lipid-rich, matrix-poor, lesions. High zinc levels may promote plaque stability by binding to matrix components and stabilizing lesion structures. The decreased extent of cardiovascular events in people with high zinc levels may therefore merely be an indicator of calcium accumulation and fibrosis and hence decreased propensity to lesion rupture[17].

**Zinc and COVID-19: possible influences on carotid plaque.** Cardiovascular disease, hypertension, diabetes are common comorbidities in patients with COVID-19. Zinc deficiency is frequent in the elderly and leads to changes similar to those that occur in oxidative inflammatory aging (oxi-inflamm-aging) and immune-senescence. Zinc deficiency causes a significant decline in the innate and adaptive immune responses and promotes systemic inflammation. Inflammation is a basic pathogenetic element in the development of atherosclerotic disease and its manifestation, especially the instability of the atheromatous plaque. Zinc supplementation may reduce plaque instability and its vascular complications.

### *Selenium and Atherosclerosis*

**Definition**. Selenium (Se) (atomic number 34, atomic weight 78.97 g/mol, density 4.81 g/mL in gray selenium, the most stable among its allotropic forms) is a trace element crucial for many biological functions and for human health; in its inorganic compounds it presents in different oxidation states: −2, 0, +2, +4, and +6. Its deficit may cause severe disorders, but its overload may have severe consequences. Se mediates its effects through incorporation in several selenoproteins. Selenoprotein P is responsible for Se transport and storage.

**Daily intake of selenium**. 55 μg is the recommended daily intake of selenium.

**Selenium serum levels**. 70 ng/L are considered the minimum normal selenium serum level in adults. 0-2 months: 45-90 ng/L; 3-6 months: 50-120 ng/L; 7-9 months: 60-120 ng/L; 10-12 months: 70-130 ng/L >1 year; 70-150 ng/L adults.

**Roles of selenium in physiology**. Selenium is involved in antioxidant defense systems and in the immune response. It has important antioxidant properties, being a cofactor of many antioxidant enzymes[18]. Regarding the immune system, selenium stimulates the activity of T-helper lymphocytes, cytotoxic T cells, and Natural Killer (NK) cells[19].

**Selenium and metalloenzymes.** The finding of a significantly elevated selenium plasma concentration in patients with Wilson disease treated on a long-term basis with zinc indicates the possible mutual interaction between zinc and selenium[20]. More than hundred selenoproteins are detectable in human cells, including glutathione-peroxidase and thioredoxin reductase. Selenium does not only exist in the cell as selenocysteine (as in GPx) but also as selenomethionine, in place of methionine

**Low selenium levels: main effects**. Low selenium levels are associated with the insurgence of cardiovascular disease[21]. Selenium deficiency is associated with impaired humoral and cell-mediated immunity, increased viral virulence, decreased immunoglobulin titers, diminished NK-cell cytotoxicity[22]. Moreover, selenium deficiency during gestation has been associated with changes in fetal programming of the fetal immune system, with consequences later in life[23]. Men with serum selenium ≤1 μmol/L have a significantly increased risk of ischemic heart disease. Recently, low selenium levels have been associated with the insurgence of autoimmune diseases (Figure 3)[24].

**High selenium levels: main effects**. 400 micrograms/day is the threshold of selenium that

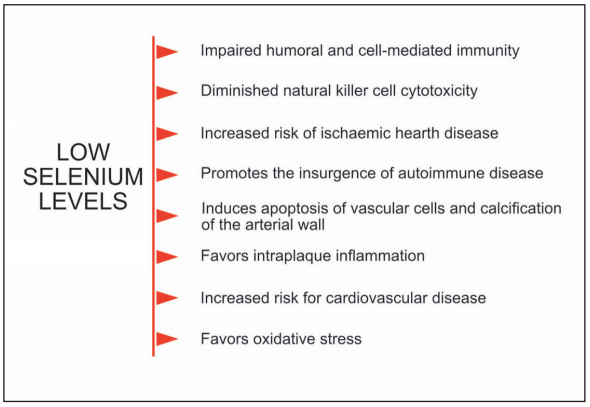


**Figure 3.** Main effects of selenium deficiency.

should not be exceeded. 700 micrograms are surely a toxic dose. The range between therapeutic and toxic doses is very narrow. Fatigue, cardiovascular, respiratory and gastrointestinal symptoms are described in patients undergoing excessive dietary supplement of Se[25].

**Specific roles of selenium in atherosclerosis**. Selenium intake can prevent atherosclerosis. Its underlying mechanisms include inhibiting oxidative stress, modulating inflammation, suppressing endothelial dysfunction, and protecting vascular cells against apoptosis and calcification[26]. Evidence from animal studies shows that selenium and selenoproteins might prevent experimental atherosclerosis. Selenoproteins of particular relevance to atherosclerosis are glutathione peroxidases, thioredoxin reductase 1, selenoprotein P, selenoprotein S. Supplementation with selenomethionine, an organic form of selenium, in ApoE-/- mice, an animal model of atherosclerosis, prevents atherosclerosis progression, modulates the intraplaque inflammation and has the ability to regress already established plaques[27]. The positive activity of selenium supplementation in atherosclerosis is probably due to its antioxidant and anti-inflammatory properties that might halt neutrophil and macrophage activation and infiltration of the plaque. Selenium's underlying mechanisms include inhibiting oxidative stress, modulating inflammation, suppressing endothelial dysfunction, and protecting vascular cells against apoptosis and calcification[28].

**Selenium and arterial calcification and inflammation**. Selenium has beneficial effects in counteracting oxidative stress, inflammation, and vascular endothelial dysfunction[29].

**Selenium supplementation: possible influence on plaque development.** A significant inverse association between selenium or selenoprotein status and risk for cardiovascular disease has been reported. With regard to the effects of selenium supplementation on the prevention of atherosclerosis, further investigations are needed to confirm the roles of selenoproteins in atherosclerosis prevention and clarify the underlying mechanisms[26].

**Selenium and COVID-19: possible influences on the carotid plaque**. Given selenium's role in the immune response, through the activation of B lymphocytes, T lymphocytes, and natural killer cells, all involved in the immune response against SARS-CoV-2, subjects with low selenium stores might be included in the high-risk group for developing a severe form of COVID-19.

## *Iron and Atherosclerosis*

**Definition.** Iron (Fe) is a silver gray metal (atomic number 26, atomic weight 55.845 g/mol, density 7.874 g/mL); in its inorganic compounds it presents a variety of oxidation states -1, -2, 0, +2, +3, +4 and +6, being +2 and +3 the most common ones. It is a trace metal essential for life. However, when present in higher doses, iron may participate in diverse pathological processes by catalyzing the formation of reactive oxygen free radicals.

**Daily intake.** The Recommended Dietary Allowance (RDA) for all age groups of men and women is 8 mg/day; the RDA for premenopausal women is 18 mg/day. The median dietary intake of iron is approximately 16 to 18 mg/day for men and 12 mg/day for women. The Tolerable Upper Intake Level (UL) for adults is 45 mg/day of iron.

**Serum levels**. Normal values of total serum iron: 26-170 mg/dL in women and 76-198 mg/dL in men.

**Main roles of iron in physiology**. Iron is essential for life, but many remains to be learned about iron physiology and pathophysiology despite major recent advances.

**Low iron levels.** Iron serum levels below 26 mg/dL for women and below 76 mg/dL for men allow a diagnosis of hyposideremia.

**Hypersideremia and atherosclerosis**. In the early eighties, Dr. Jerome Sullivan formulated the 'Iron Hypothesis', suggesting that high iron stores might promote cardiovascular diseases, whereas iron deficiency may have a protective

effect against atherosclerosis. However, during the years, multiple studies failed to confirm this hypothesis. Moreover, the reports that patients with hemochromatosis and carriers of beta-thalassemia do not show an increased risk of atherosclerosis appeared incongruous with Sullivan's hypothesis. Recent reports have elucidated the complex signaling pathways regulating iron, with a particular focus on hepcidin, the master regulator of body iron homeostasis[30]. Bone morphogenetic protein signaling is the major pathway required for induction of hepcidin expression in response to increasing iron levels. According to this hypothesis, a new role for iron homeostasis and hepcidin should be assigned in the development of the atherosclerotic plaque[31].

**Specific roles of iron in atherosclerosis.** High iron levels may contribute to atherosclerosis and its complications as factors in a multifactorial disease[32]. "Normal" serum levels of iron may contribute to various diseases of aging, including atherosclerosis[33]. An iron deposition is prominent in atherosclerotic plaques, providing histological evidence to support the detrimental role of iron in vascular damage and progression of atherosclerosis[34] (Figure 4). Differences in Fe content in carotid plaques have been reported between symptomatic and asymptomatic patients[35]. High iron serum levels have been reported in atherosclerotic patients and increased with the severity of the coronary atherosclerotic disease, suggesting a basic relationship between the serum iron level and severity of atherosclerosis[36].

High iron levels have been frequently associated with an increasing risk for complicated atherosclerosis, although low iron levels might play a role in atherosclerosis. Iron deficiency is frequently seen in patients with end-stage renal disease, particularly in those under dialysis. These patients are prone to undergo calcification of the atherosclerotic plaques, leading to cardiovascular disease and the increased mortality associated herewith[37].

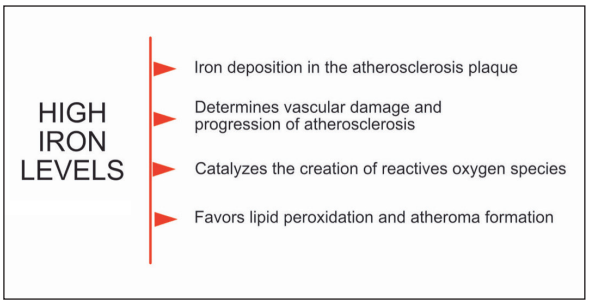


**Figure 4.** Progression's phases of iron overload leading atherosclerosis.

**Iron and arterial calcification and inflammation.** Recently, new insights on the role of iron in the development of the atherosclerotic plaque have been published. According to these studies, the prevailing view of iron being capable of accelerating coronary atherosclerotic disease progression, mainly through lipid peroxidation, may not fully take into account the multi-faceted role of iron in the pathogenesis of the atherosclerotic plaque[38].

**Possible interactions of iron with other trace metals.** Inverse correlations between iron status and blood cadmium, cobalt, and copper have been reported. On the contrary, iron status is positively correlated with mercury and selenium concentrations.

**Iron supplementation/depletion**: **possible influence on plaque development.** Given that, in atherosclerosis, iron catalyzes the creation of reactive oxygen free radicals that contribute to lipid modification, an essential factor toward atheroma formation, no iron supplementation is suggested.

**Iron and COVID-19:** COVID-19 has been recently proposed as a new character in the hyperferritinemia syndrome spectrum, being characterized by high serum ferritin levels and a life-threatening hyper-inflammation sustained by a cytokines storm which eventually leads to multi-organ failure[39].

## *Copper and Atherosclerosis*

**Definition.** Copper (Cu) is a red-orange metal (atomic number 29, atomic weight 63.546 g/mol, density 8.96 g/mL); in its inorganic compounds it presents in different oxidation states: 0, +1 and +2. It is a trace element essential for life, being involved in collagen biosynthesis, wound healing, angiogenesis, and cell growth. On the other hand, copper is a potentially toxic metal, unbound copper ions being involved in the production of hydroxyl radicals that are responsible for oxidative stress and cell death[40].

**Daily intake of copper**. Copper-rich food includes shellfish, nuts, and chocolate[41]. The recommended dietary allowance of copper in adults is 340 micrograms/day, whereas, in pregnant women, RDA goes up to 1 mg/day per day. In the human body, copper is relatively high. A healthy adult contains about 110 mg of

copper, the highest part in skeleton and bone marrow (46 mg), skeletal muscles (26 mg), liver (10 mg), brain (8.8 mg), and blood (6 mg)[42].

**Serum levels.** The normal serum value of healthy people[43] is between 639 and 1495 mg/L.

**Intracellular copper: how to evaluate it**? The method available to measure the changes in intracellular copper concentration is based on inductively coupled plasma mass spectrometry (ICP-MS)[44].

**Roles of copper in physiology**. Copper function in physiology is mainly linked to its redox ability as a cofactor of multiple enzymes, including mitochondrial cytochrome c oxidase (CcO) and superoxide dismutase 1 (SOD1)[45]. Moreover, copper status plays a relevant role in cardiomyocyte function[46].

**Copper and metalloenzymes**. More than 300 enzymes need a copper atom for their specific function. Copper atom propensity to cycle between the two oxidation states $Cu^{+}$ and $Cu^{2+}$ accounts for its frequent presence as a cofactor in many physiological processes through Cu-containing enzymes, including mitochondrial energy production (via cytochrome c-oxidase), protection against oxidative stress (via superoxide dismutase), and extracellular matrix stability (via lysyl oxidase)[42]. Copper is an essential component of lysyl oxidase, an enzyme involved in the biosynthesis of collagen, which is a major constituent of the extracellular matrix of the atherosclerotic plaque [47].

**Hypocupremia**. Serum copper is a mix of copper loosely bound to various proteins, including albumin, and amino acids and copper incorporated within proteins, essentially ceruloplasmin. The dietary copper deficit results in severe derangement in growth and impaired erythropoiesis. Low serum copper levels may also be observed in Wilson disease due to a decrease in the synthesis of ceruloplasmin[42].

**High copper levels**. Increasing excessive intracellular copper levels may severely affect mitochondria, leading to mitochondrial destruction and cell death[48]. Wilson disease, a genetic metabolic disorder, is the pathological example of what copper overload can lead[49,50]. In this disease, the evolution of tissue copper storage is responsible for liver fibrosis, cirrhosis, and neuropsychological deterioration. The therapy with penicillamine, trientine, and zinc is essential to slow down or improve Wilson disease's progression[51].

**Specific roles of copper in atherosclerosis**. Copper transport systems and their regulators have also been linked to various cardiovascular pathology, including hypertension and atherosclerosis. A greater appreciation of the central importance of copper transporters and copper chaperones in cell signaling and gene expression in cardiovascular biology offers the possibility of identifying new therapeutic targets for cardiovascular disease[52]. It has been proposed that the oxidative modification of low-density lipoprotein (LDL) is a key event in human atherogenesis. Copper ions can catalyze the oxidative modification of LDL *in vitro*, and there is some evidence that they may also participate in the oxidation of LDL within the atherosclerotic plaque. Moreover, copper ions form an intrinsic constituent of superoxide dismutase and ceruloplasmin, enzymes that may be involved in preventing oxidative injury (Figure 5)[47].

**Copper and arterial calcification and inflammation.** Atherosclerosis occurs when damaged endothelial cells allow low-density lipoprotein cholesterol (LDLc) to leak into the subintimal tissue. As the endothelial damage is repaired, the subintimal LDLc is trapped inside the subintimal space, where it induces an inflammatory response in the overlying endothelium, which expresses chemotactic peptides. Chemotactic peptides attract circulating monocytes, which enter into the arterial wall, where they become tissue macrophages that phagocytize the irritating LDLc in the atheroma[53]. Oxidized LDLc is toxic to macrophages, and it may cause their death, contributing to inflammation in the atherosclerotic plaque. In this process, excess copper might play a role, inducing peroxidation of LDLc and triggering the sequence of pathological events leading to atherosclerosis.

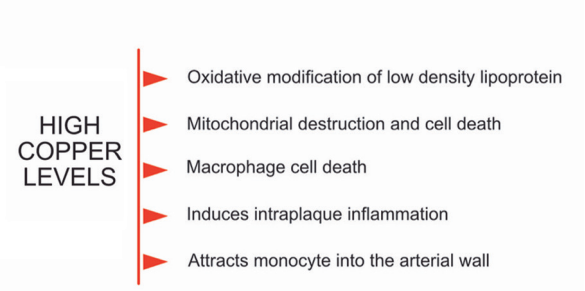


**Figure 5.** Tissue and cellular injury leaded by copper overburden.

**Possible interactions of copper with other trace metals.** Copper plays a role in iron homeostasis, particularly in iron transport in the blood and in liver uptake[54]. Copper can interact with other transition metals, for example, iron and aluminum, enhancing the production of reactive oxygen species, leading to the exacerbation of cellular oxidative stress and neuronal cell death. The interaction between copper and aluminum could begin and spread the inflammatory response in the elderly brain and in Alzheimer's disease patients[55]. Copper with other metal ions (aluminum, zinc, and iron) might differentially influence the formation of the Amyloid aggregation process in Alzheimer's disease[56].

**Copper supplementation/depletion: possible influence on plaque development.** High copper levels may contribute to atherosclerosis and its complications. The direct detection and quantification of transition metal ions evidenced elevated levels of copper in human atherosclerotic plaques[57]. A correlation for low-density lipoprotein cholesterol and copper in the serum was observed. Serum copper concentration seems to be higher in the patients with hemorrhagic in comparison to those with calcified plaque[52]. The copper value was lower in human ulcered atherosclerotic plaque in comparison with the normal control group[58].

**Copper and COVID-19.** The combination of copper with candidate antiviral drugs has been proposed as a treatment for patients positive for SARS-CoV-2[59]. Copper is involved in the functions of immune cells involved in the response against pathogens. Therefore, Cu-deficient is related to susceptibility to infections in humans. In particular, copper is able to kill several viruses, including SARS-CoV-2[60]. Adequate intake and status of many nutrients, including copper, are suggested to lead to an increase in resistance to infections and, consequently, a decrease in COVID-19 burden[61].

### *Phosphorus (Phosphates) and Atherosclerosis*

**Definition**. Phosphorus (P) (atomic number 15, atomic weight 30.973 g/mol, density 1.823 in its white form) is a highly reactive element, so it does not exist in nature as a free element, but it is usually found as phosphates. It constitutes the second most abundant mineral of the human body and represents almost 1% of body weight. A complex organ network, including bone, kidney, and intestine, is involved in its homeostatic control.

**Daily intake**. Phosphorus rich-foods are milk and dairy products followed by meat and poultry. The organic forms are less efficiently absorbed (40-60%) in contrast to inorganic added phosphate salts, which are briskly and efficiently absorbed (80-100%). Currently, there is no Recommended Dietary Allowance (RDA) yet, but the Adequate Intake (AI) has been established in 160 mg/day for infants between 7 and 11 months, 250-640 mg/day for children, and 550 mg/day for adults[62].

**Serum levels** (normal values). Phosphorus may be measured both in plasma and serum, even if it does not exactly reflect the total-body phosphorous content. The reference interval varies according to age, and it is between 2.5 to 4.5 mg/dL (0.81-1.45 mmol/L) in adults.

**Intracellular P**: how to evaluate it? For every molecule of phosphate found in the extracellular space, there are about 100 molecules in the intracellular space. There are still no methods to evaluate intracellular phosphate in clinical practice.

**Roles of phosphorus in physiology**. Phosphorous is considered essential to life molecules. Phosphates are implied in DNA, RNA, phospholipids, and energetic molecules, e.g., ATP. In the human body it is distributed between bone (85%), soft tissues (14%), teeth (0.4%), blood (0.3%) and extravascular fluids (0.3%). Calcium and phosphates are involved in bone homeostasis. Another fundamental role is phosphate regulation of PTH mRNA stability, leading to post-transcriptional parathyroid function control.

**Low P levels:** main effects. Hypophosphatemia becomes symptomatic when phosphate plasma levels are <0.32 mmol/l. It presents with proximal myopathy, weakness, and bone pain with osteopenia, osteomalacia, and rhabdomyolysis. Hypophosphatemia has also been related to myocardial dysfunction due to ATP depletion and cardiac dysrhythmias. Regarding the respiratory system, the main effects are respiratory failure, with depression of diaphragmatic contraction, and failure of ventilator weaning. Patients with hypophosphatemia may also present with coma, seizures, encephalopathy, paresthesia, metabolic acidosis, hemolysis, and leucocyte dysfunction.

**High Phosphorus levels: main effects** (Figure 6). Hyperphosphatemia is characterized by a serum phosphate concentration >1.46 mmol/L. High serum phosphorus is independently related to the risk of cardiovascular and all-cause mortality[63]. Gastrointestinal symptoms, e.g., nausea, vomiting, and osmotic diarrhea, have been reported in healthy subjects taking more than 750 mg/day phosphorus supplements. In patients with end-stage kidney disease, hyperphosphatemia has been related to secondary hyperparathyroidism, bone loss or deformations, and ectopic calcification[62].

**Specific roles of phosphates in atherosclerosis**. In young adults, even if within the normal range, higher values of serum phosphates are associated with coronary atherosclerosis development 15 years after serum phosphate evaluation[64]. In males with no renal or cardiovascular disease, serum phosphorus concentration adjusted for eGFR and atherosclerotic risk factors is directly associated with the mean carotid intima-media thickness. The early phase of atherosclerosis is characterized by endothelial dysfunction, which is associated with high serum phosphorus levels in patients affected by hypertension[65]. Furthermore, hyperphosphatemia induces the transformation from the vascular phenotype of smooth muscle cells to an osteogenic one inside the plaques[66].

**Phosphorus and arterial calcification and inflammation** Hyperphosphatemia in *in vitro* models showed an important role in the early transformation of the vascular-phenotype of smooth muscle cells to an osteogenic phenotype through upregulation of chondrocyte and osteoblast-like gene expression and downregulation of the smooth muscle-specific ones. This transformation may put the basis for the subsequent calcification[67]. In support of this hypothesis, a positive association between high serum phosphate levels and coronary calcification and obstruction has been demonstrated in patients with preserved renal function and coronary artery disease[68].

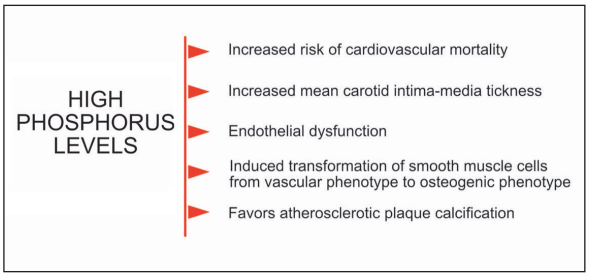


**Figure 6.** Pathophysiology of Phosphorus load.

**Possible interactions of phosphorus with other trace metals.** Calcium and phosphorus metabolisms[62] are strictly related, and the whole-body calcium to phosphorus molar ratio ranges between 1.4:1 to 1.9:1.

**Phosphorus supplementation/depletion: possible influence on plaque development.** Most studies on the influence of phosphorus on plaque development are related to hyperphosphatemia induced by chronic kidney disease. Limited results in lowering phosphorous levels have been described to be associated with dietary phosphate depletion and dialysis removal, but there are numerous phosphate binders able to reduce phosphate serum levels. Their usage might be essential to halt the atherosclerotic process. In this regard, it has been reported that an early control of hyperphosphatemia might reduce coronary calcification, even more in dialyzed patients[66].

**Phosphorus and COVID-19: possible influences on the carotid plaque.** Serum phosphorus may interfere with Fibroblast Growth Factor 23 (FGF-23) production by osteocytes since hypophosphatemia has been associated with a decrease in FGF-23 serum levels[69]. On the other hand, FGF-23 inhibits the Angiotensin-Converting Enzyme 2 (ACE2) gene expression in the kidney, increasing Angiotensin II, which plays an important role in inflammation and remodeling of the atherosclerotic plaque[70]. Some authors[71] reported that ACE2, the cellular attachment site of spike proteins of SARS-CoV-2, which is inhibited by its binding, might have an atheroprotective behavior. There is the possibility that the same atherogenic effect related to ACE2 inhibition by FGF-23 might be induced through SARS-CoV-2 binding to ACE2.

## *Calcium and Atherosclerosis*

**Definition.** Calcium (Ca) (atomic number 20, atomic weight 40.078 g/mol, density 1.55 g/mL) is a silvery metal ion; in its inorganic compounds, it presents the two oxidation states 0, and +2. It is the principal extracellular divalent cation in the human body. About 99% of body calcium is stored in the bone as hydroxyapatite. The remainder is distributed in the intravascular, interstitial, and intracellular fluids.

**Daily intake.** $Ca^{2+}$ needs vary at different times of life. In adults, the $Ca^{2+}$ requirements are approximately 320 mg per day, provided by an intake of 1000 mg daily. The intestinal absorption of the calcium intake is 200-400 mg, while the rest is excreted in urine, feces, and sweat. The main sources of calcium in diet are milk and dairy products, especially cheese and yogurt.

**Calcium serum levels.** The normal serum concentration of $Ca^{2+}$ is maintained constant throughout life at about 8.8-10.3 mg/dL (2.20-2.58 mmol/L) by the efficient homeostatic mechanism. Three forms of calcium are in equilibrium in serum[72]: diffusible ionized calcium, which is the physiologically active form and it represents approximately 50% of the total calcium[73]; non-diffusible calcium (around 45% of the total) bound to proteins, primarily albumin[74]; diffusible nonionic calcium (5%) combined with lactate, bicarbonate, phosphate, sulfate, citrate, and other anions.

**Intracellular calcium.** The intracellular calcium concentration is approximately 0.1 μM, about 10,000 times lower than that in the extracellular fluid (ECF). The entrance of $Ca^{2+}$ into the cell occurs by the electrochemical gradient involving several $Ca^{2+}$ channel types, and the extrusion from the cells is mainly favored by $Ca^{2+}$-ATPase. Several methods have been developed to analyze and measure intracellular $Ca^{2+}$ concentration; fluorescent indicators are particularly useful[72].

**Roles of calcium in physiology.** Calcium is an essential element for the biochemical events in human physiology. In addition to being the main component of bone and teeth, calcium is involved in many molecular pathways, including nerve excitability, muscle contraction, hormone actions, enzyme secretion, cell motility, and blood coagulation. Calcium is strongly involved in the signal transduction pathway as a second messenger in a variety of cells.

**Calcium and metalloenzymes[73,74].** The potential role of matrix metalloproteases (MMPs) in the modulation of inflammation and vascular calcification has been recently discussed. Some findings suggest that MMPs play a critical role in arterial calcification. Moreover, MMPs expression is upregulated within human atherosclerotic lesions. The mechanism by which MMPs may modulate the development of calcified lesions in atherothrombosis has not been demonstrated yet. However, MMPs may act in the osteogenic differentiation of vascular smooth muscle cells (SMCs). Additionally, MMPs degrade extracellular matrix components such as elastin and collagen. Once degraded, they are prone to calcium binding; thus, they may serve as initiation sites for calcification. The role of MMPs in modulating calcium deposit has been observed for MMP-3 and MMP-9 in carotid lesions. In coronary arteries, there is a significant correlation between calcified plaque and MMP-2 activation. MMP-10 has been associated with calcified hemorrhagic plaques in the progression and complications of atherosclerosis.

**Hypocalcemia: main effects** – Decreased levels of serum calcium concentration (<8.8 mg/dL) may be due to inadequate $Ca^{2+}$ absorption or excessive $Ca^{2+}$ loss. Causes include hypoparathyroidism, vitamin D deficiency, and renal disease. Symptoms of hypocalcemia most commonly include paresthesia, muscle spasms, cramps and can lead to tetany, characterized by sustained muscle contraction. Hypocalcemia can also present with numbness and tingling, bronchospasm and wheezing, laryngospasm and dysphagia, neuromuscular irritability, cognitive impairment, personality disturbances, electrocardiographic changes that mimic myocardial infarction, or heart failure.

**Hypercalcemia: main effects** – Hypercalcemia is a total serum calcium concentration >10.4 mg/dL. Principal causes include hyperparathyroidism, vitamin D toxicity, and malignancy. Elevations of calcium above 11.5 mg/dL can lead to symptoms including nausea, vomiting, altered mental status, headache, confusion, abdominal pain, constipation, depression, weakness, myalgia, arthralgia, polyuria, and polydipsia. High $Ca^{2+}$ concentration in the body affects the neuromuscular system. It has inotropic and chronotropic effect in the heart, with changes in the ECG, and it could lead to cardiac arrest. The excess of $Ca^{2+}$ can deposit in different soft tissues, especially in kidneys.

**Specific roles of calcium in atherosclerosis** – The role of calcium in atherosclerosis is controversial, and the relationship between the amount of vascular calcification and plaque vulnerability has not been evaluated yet. Although calcifications are present in approximately 50% to 60% of carotid plaques, their association with ischemic cerebrovascular events remains unclear[75]. In most data, carotid plaque calcification is associated with plaque stability,

and it may be considered as a protective plaque feature. These studies support the concept that calcified atherosclerotic plaques may be more biomechanically stable, less prone to disruption, and less likely associated with symptoms than non-calcified carotid plaques[75-78]. Conversely, other reports stated that a high level of calcification of the carotid plaque may not be necessarily associated with lower vulnerability[79-83]. Thus, the level of carotid calcification cannot be considered an element of plaque stability. The calcification process within the plaque can be an evolution of an intraplaque hemorrhage determining a vulnerable plaque; eventually, it may predict cerebral ischemic lesions, independent of stenosis grade[84]. These discrepancies may be explained by the recent demonstration that not only the quantity, but also the types and chemical composition of calcification in the atheromatous plaques, could affect the plaque stability[85].

**Calcium and arterial calcification and inflammation**[84-87] – The exact mechanism of arterial calcification remains to be elucidated. Vascular calcium deposition may be classified by the location of calcium in medial and intimal calcification. Whereas the intimal calcification is affected by lipid deposition and inflammation within the atherosclerotic lesion progression, the medial calcification is more correlated with advanced tissue degeneration. There are several types of arterial calcification by histology: micro-calcification, punctate, fragmented, sheet-like, and nodular calcification. Recent pathologic studies have shown that they have different key roles in atherosclerotic progress: sheet calcification is highly prevalent in stable plaques, while micro-calcifications, punctate, and fragmented calcifications are more frequent in unstable lesions (Figure 7). Therefore, the presence of small calcifications may be a better predictor of unstable plaque; large calcification may be a better predictor of stable plaque.

**Possible interactions of $Ca^{2+}$ with other trace metals**[35,17] – Different amounts of co-localized calcium and zinc have been detected in areas of mineralized atherosclerotic plaques. No association between the vulnerability of plaque and the amount of calcium, zinc, potassium has been established. Few studies have investigated the levels of metal ions (zinc, iron, calcium, and copper) in atherosclerotic carotid lesions. Highly significant associations between zinc and calcium were detected in all lesions, maybe due to their binding to similar ligands *in vitro*. High zinc levels may be an indicator of calcium accumulation and fibrosis. However, it is still unknown whether high zinc levels are a consequence or the promotion of calcification. It has not been established whether calcium accumulation occurs concurrently with zinc or whether calcium, zinc, iron, and copper ions accumulate independently.

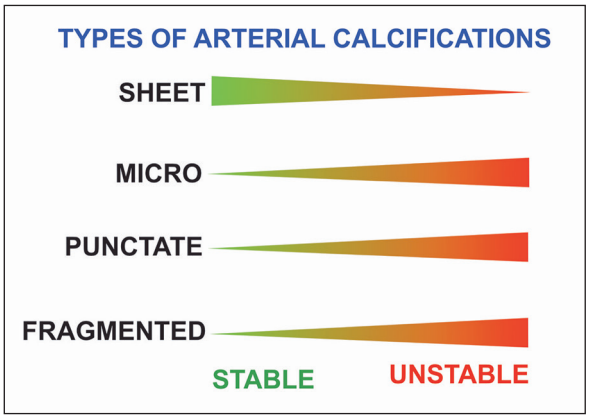


**Figure 7.** Classification of calcification and inflammation.

**Calcium supplementation: possible influence on the plaque development** – Calcium is a widely used dietary supplement in elderly individual's prevention and treatment of osteoporosis. However, recent evidence on the adverse effect of calcium supplementation on the cardiovascular system has been reported, even though high dietary calcium intake was shown to have a protective effect[88]. Calcium supplements may be associated with adverse cardiovascular events and atherosclerosis, as measured by increase the risk for coronary artery calcification. There are several possible pathophysiological mechanisms for these effects, including effects on vascular calcification, on the function of vascular cells, and on blood coagulation[89,90].

**Calcium and COVID-19: possible influences on carotid plaque** – Some scholars[91,92,26] have investigated the correlations between serum calcium and clinical severity and outcomes in patients with COVID-19. Hypocalcemia is highly incident in COVID-19 patients. The cause of hypocalcemia in COVID-19 patients with severe status is not established; several mechanisms may be suggested for this. Serum calcium modifications may also be due

to a direct effect caused by SARS-CoV-2. Experiments *in vitro* and in animal models infected with SARS-CoV-2 demonstrated that alterations of intracellular calcium homeostasis could promote the activation of inflammatory pathways. These mechanisms seen in SARS-CoV-infected patients could be the same for SARS-CoV-2[93]. Consequently, hypocalcemia may have a negative impact on cardiac outcomes and may be even lethal when severe and acute[94]. The association of hypocalcemia and COVID-19 on carotid plaques has not been investigated yet.

### Matrix Metalloproteinases (MMPs): a New Medical Therapeutic Approach to Atherosclerosis?

**Definition** – Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases, which mediate tissue remodeling and degradation of extracellular matrix (ECM). These proteolytic enzymes have high homology in sequence and structural domain. They consist of a zinc-catalytic domain, a linker peptide, and a hemopexin domain. Human 23 MMPs are classified based on their specific substrates into collagenases (MMP-1, MMP-8, MMP-13), stromelysins (MMP-3, MMP-10, MMP-11), gelatinases (MMP-2, MMP-9), matrilysin (MMP-7), membrane-type (MT)-MMPs (MMP-14, MMP-15, MMP-16, MMP-17), and other MMPs[95].

**Serum levels** (Normal levels) – The serum levels of MMPs can be determined using a commercially available enzyme-linked immunosorbent assay (ELISA). Normal reference values vary according to the different types of MMPs (ng/ml).

**Intracellular MMPs** – MMPs are synthesized as inactive zymogens (pro-MMPs), which are cleaved to the active form by various proteinases, including other MMPs. MMPs are either released from the cells into the intercellular space in extracellular vesicles (EVs), or anchored to the plasma membrane by special transmembrane domains[96]. To quantify MMP enzymatic activity, the analysis of MMPs may be based on the zymography technique.

**Role of MMPs in physiology** – MMPs are produced by multiple cell types, and their activity is essential in several physiological processes. MMPs are expressed in vascular tissues such as endothelial cells, vascular smooth muscle cells (VSMCs), fibroblasts, and leukocytes. MMPs play a role in vascular tissue remodeling during various biological events (angiogenesis, embryogenesis, morphogenesis, and wound repair). The substrate specificity of MMPs includes many extracellular matrix (ECM) and surface proteins such as collagen and elastin, which are essential for the structural integrity of the vascular wall.

Because of their role in promoting the turnover of various ECM proteins, MMPs are highly distributed in most connective tissues. Important sources of MMPs are proinflammatory cells, osteoblasts, macrophages, neutrophils, lymphocytes, and platelets. Although MMPs are traditionally recognized as extracellular proteases, more recent research has shown that they may also regulate other substrates, including chemokines and other cytokines. This suggests that intracellular MMPs have both proteolytic and non-proteolytic functions, including a signaling role in maintaining homeostasis[97].

**Specific role of MMPs in pathological conditions** – MMPs can be regulated in the human body by four endogenous tissue inhibitors of metalloproteinases (TIMPs), namely TIMP-1, TIMP-2, TIMP-3, and TIMP-4. The MMP/TIMP ratio often determines the extent of ECM protein degradation and tissue remodeling. The overexpression of MMPs or the insufficient control by TIMPs is a condition that may lead to the MMP/TIMP imbalance. These alterations in MMPs expression may be associated with leukocyte infiltration, inflammation, and dysregulation of tissue remodeling, causing various pathological conditions. Therefore, MMPs play key roles in cardiovascular diseases (atherosclerosis, aneurysms formation, hypertension, heart failure, ischemic cardiomyopathy, venous disorders), musculoskeletal disorders (osteoarthritis, bone resorption), remodeling of the respiratory airways, and cancer invasiveness.

**Specific role of MMPs in atherosclerosis**[98] – Accumulating evidence in human and animal models[99] suggests that MMPs expression and activity are strongly associated with all the stages of atherosclerotic plaque development and rupture. MMPs are involved in the plaque initiation with the recruitment of immune cells and vascular inflammation. MMPs are secreted constitutively or after activation by inflammatory response not only by monocytes and macrophages, but also by foam cells. MMPs participate in the induction of endothelial dys-

function (MMP2) and in the degradation of ECM by migration and proliferation of VSMCs into the intima. This contributes to increase the intimal thickness and progression of plaque. Furthermore, the activity of MMPs in vessel wall remodeling and ECM proteolysis results in the release of vascular endothelial growth factor (VEGF) from ECM that promotes capillary structure formation and plaque neovascularization (MMP-9 and MMP-2). MMPs determine vascular calcification in the advanced atherosclerotic plaque, and their activity is involved in platelet aggregation resulting in prothrombotic plaques (MMP-2) (Figure 8). Several MMPs promote plaque instability by weakening the fibrous cap (MMP-9, MMP-2, MMP-10) and facilitating penetration, proliferation, and apoptosis of inflammatory cells, including macrophages. Accordingly, all these mechanisms could lead to plaque rupture. MMPs may be important biomarkers to predict future atherothrombotic major cardiovascular events.

**High MMPs levels: main effect** – The imbalance between MMPs and TIMPs in unstable carotid plaques is reflected in the plasma levels of these markers[100]. High levels of MMPs (MMP-2, MMP-8, and MMP-9) have been correlated with an unstable carotid plaque composition, significant carotid stenosis with spontaneous embolization, active process of plaque rupture, and future risk of cardiovascular events.

**MMP and COVID-19: possible influences on carotid plaque**[101] – 2019-nCov has the ability to target cells by binding to angiotensin-converting enzyme 2 (ACE2). This enzyme is expressed in the cardiovascular system, and it may have a possible role in vascular remodeling, atherogenesis, and other pathological vessel conditions. Interestingly, ACE2 was also found in carotid atherosclerosis, and its functions and effects are mediated by MMPs (MMP-2, MMP-3, MMP-9), which are also related to cytokine recruitment and inflammation. Besides, elevated plasma ACE2 activity is an independent predictor of major cardiac events (Figure 9).

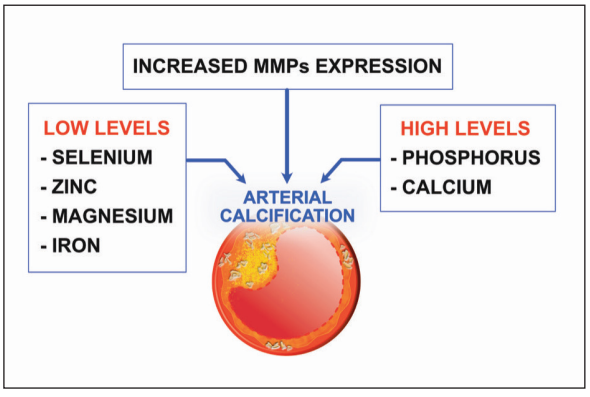


**Figure 8.** Schematic representation of the role of MMPs in the development of atherosclerosis.

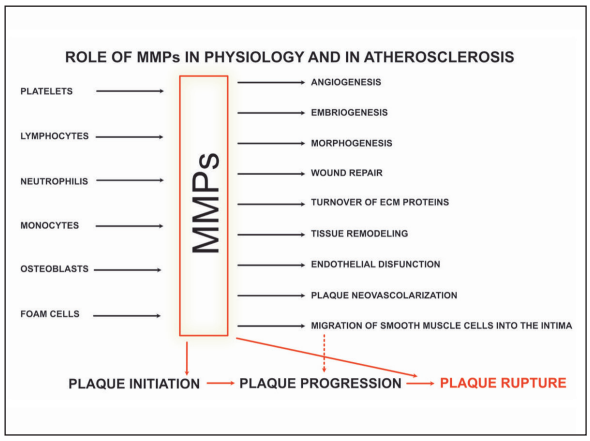


**Figure 9.** Role of MMPs in physiology and disease pathogenesis.

**Potential therapeutic targets**[102] – MMPs suggest a possible role as biomarkers in stratifying patients with cardiovascular risk, and they may represent a valuable diagnostic and potential therapeutic target for cardiovascular disease therapy. The involvement of MMPs in atherosclerosis has been driving the development of MMP specific inhibitors to retard plaque progression and rupture. Four generations of synthesized MMP inhibitors (MMPIs) have been designed in the last decades. The progress expanded from small molecules or peptides containing zinc and no-zinc catalytic domain to selective allosteric inhibitors. Most of them have failed in clinical trials due to several reasons. To date, among several biological inhibitors, only doxycycline has been approved by Food and Drug Administration (FDA) for clinical use because of its role in plaque stabilization and reduction of inflammation. Moreover, statins may inhibit MMPs activity in atherosclerotic plaques due to the reduction of cardiovascular inflammation in animal models. In clinical trials, statins have shown the effect of decreasing plasma levels of MMPs (MMP-9), increasing fibrous cap thickness, and plaque stability[103].

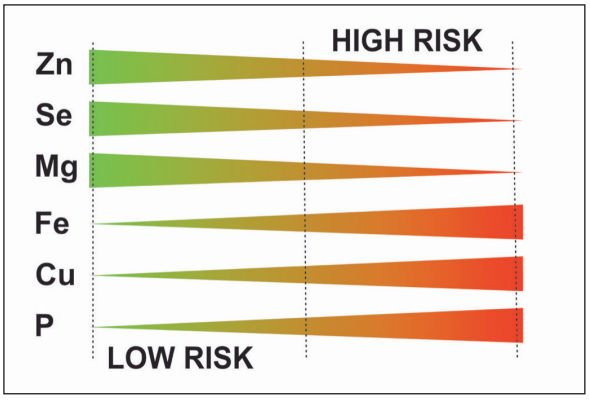


**Figure 10.** Schematic representation showing the balance of the different trace elements in order to establish a protective role (left) versus the circumstance favoring the plaque evolution (right).

With the recent technological innovation, new promising structurally selective MMPs inhibitors, namely antibody-based inhibitors and protein-engineering inhibitors have been developed, and their effectiveness in cardiovascular disease needs to be examined. Despite some positive results, further studies *in vivo* are needed. Designing highly specific MMP inhibitors is challenging because the proteolytic activity of MMPs is a dynamic process, with variability in tissue regions and stages of diseases. In the future, highly selective MMPIs may be synthesized via the combined inhibition of both the active site and MMP-specific sites to be targeted individually.

## Conclusions

Nutritional and toxic trace metals present emerging health and environmental hazard[104]. In recent years, the majority of studies on trace metal toxicity have been focused on their role in carcinogenesis, including the role of dietary iron on the insurgence of urinary bladder cancer[105].

Data reported indicate that trace element status may influence both atherosclerosis insurgence and plaque evolution toward a stable or an unstable status. The role of trace elements here analyzed appears to be different: some trace elements may have a protective role, whereas others probably have a role in the progression of the plaque toward rupture of the plaque and severe clinical complications (Figure 10). The role of calcium, in particular, appears debated even though the finding of sheet calcifications inside the plaque appears as a positive sign. Further investigations are warranted to better understand the role and, more importantly, the interactions among the different trace elements to better understand the atherosclerotic routes and ideally find new therapeutical approaches for targeting atherosclerosis regression in the carotid arteries.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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