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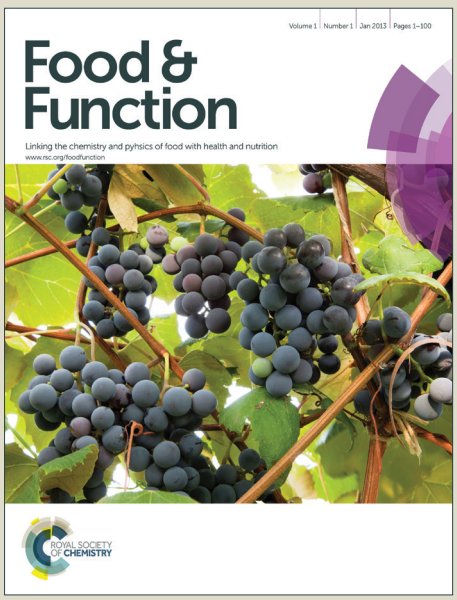

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ARTICLE

Zinc as a micronutrient and its preventive role of oxidative damage in cells

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Zinc is an essential trace element with special importance in the immune system. Deficiencies of zinc are seen in the course of ageing and in various diseases, such as diabetes mellitus or rheumathoid arthritis. The trace element is essential for a variety of basic cellular functions and especially important for various enzymes participating in the production and neutralization of reactive oxygen species (ROS) which are normally produced by the cell. Under normal conditions ROS are neutralized and are not able to harm the cell, but in case of ROS elevation oxidative damage within the cell is the result. Interestingly, zinc deficiency is directly associated with oxidative stress. Thus, control and regulation of the intracellular zinc content is essential with participation of various transporter and zinc-binding proteins, such as metallothionein. Oxidative stress is mainly caused by elevated ROS production and a decrease of antioxidant mechanisms. Zinc partly functions as an antioxidant although it is redox inert. Zinc supplementation is associated with decreased ROS formation exhibiting beneficial effects especially in ageing and diabetes mellitus. This review summarizes current findings concerning zinc as a micronutrient and its actions as a pro-antioxidant, and the association between zinc and oxidative stress under various conditions is highlighted.

Characteristics of zinc

Zinc is an essential trace element, being important for the growth and development of all organisms. It is essential for the function of more than 300 enzymes covering all enzyme classes and it is indispensable for various basic cellular functions, as for example proliferation, DNA and RNA synthesis, and apoptosis.[1] Moreover, zinc seems to be an essential cofactor for the function of approx. 10% of the proteins encoded by the human genome, including among each other DNA binding proteins, such as p53 and zinc finger proteins, and additionally enzymes, such as Copper/Zinc superoxide dismutase (Cu/Zn-SOD).[2]

Zinc deficiency has negative impact on cell status and is accompanied by reduced cell differentiation and proliferation. Thereby its effects are readily seen in the immune system by impaired immune functions, since this represents the system with highest proliferation.[3] Symptoms accompanying zinc deficiency comprise dermatitis, dysfunction of the immune system, hypogonadism, mental and growth retardation, and others.[4, 5]

The total amount of zinc within the body comprises 2-4g with a plasma concentration of only 12–16µM, emphasizing its classification as a trace element.[3] The existing plasma zinc pool is small but mobile and thus important for the distribution of zinc within the body. Since there is no existing storage system for zinc as for iron, a steady state of zinc intake and excretion is obligatory.[3] Zinc distribution within the body is widespread, with highest amounts found in the bones, liver, skeletal muscles, and skin, corresponding to the percentage of whole body zinc.[6, 7]

The zinc ion is a charged divalent cation and therefore not able to cross cell membranes by passive diffusion. To enable crossing of the membrane transport mechanisms are of high importance. Apart from this, it is essential to control zinc homeostasis and to prevent overaccumulation of zinc accompanied by toxic effects within the cell.[8] This is mainly ensured by two families of zinc transporters, namely by the Zip- (Zrt-, Irt-like protein)/SLC39A- (solute carrier family 39) family and the ZnT- (zinc transporter)/SLC30A-family in mammals, which are conserved at all phylogenetic levels.[9, 10] While ZnT-transporters conduct the transport of zinc out of the cytoplasm into the lumen of intracellular organelles or into the extracellular space, Zip-transporters manage the transport of zinc *vice versa*, *i.e.* out from organelles and the extracellular space into the cytosol.[8] So far, 10 ZnT- and 14 Zip- encoded transporters were identified within the human genome.[11]

Among foods zinc concentrations vary considerably (Table 1). Apparently the most important dietary source of readily bioavailable zinc is meat. Due to the presence of phytates in legumes and cereals, zinc absorption is inhibited by the small intestine and thus especially vegetarians might have problems with inadequate zinc intake.[12, 13] Moreover, people living in the developing world will also have problems caused by zinc

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See DOI: 10.1039/x0xx00000x

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deficiency since their major food source comprises cereal grains.[14, 15]

Recommendations of daily zinc intake vary between different countries and gender. In detail, zinc intake recommended by the German Society of Nutrition comprises 7 mg/d for female and 10 mg/d for male adults.[16] The recommended zinc intake advised by the US Food and Nutrition Board comprises 8 mg/d for female and 11 mg/d for male adults.[17] However, the molar ratio of phytate to zinc has to be considered, being optimal for adult men at a value of 10.[18] An inadequate molar ratio of phytate to zinc results in zinc deficiency even if the diet follows official recommendations concerning the amount of supplemented zinc.[19] For example, a phytate-rich diet with a molar ratio of 15 was shown to decrease zinc absorption to 50 % in young men compared to the basal diet. Thus, the phytate to zinc molar ratio might represent a useful index of zinc bioavailability.[20] The World Health Organization (WHO) classifies three categories of zinc bioavailability which are based on the phytate to zinc molar ratio. According to this, a molar ratio lower than 5 is associated with high zinc bioavailability, a molar ratio of 5 to 15 shows moderate zinc bioavailability, and a molar ratio greater than 15 shows low zinc bioavailability.[21] It seems likely that vegetarians can suffer zinc deficiency due to low intake of zinc combined with the intake of high amounts of phytate, accompanied by reduced zinc bioavailabilty. Apart from that, the administered form of zinc is important and varies considerably between different zinc supplements. Highest absorption is obtained by amino acid bound zinc complexes, in particular zinc bound to aspartate, cysteine, histidine, or methionine.[22]

Zinc deficiency is a worldwide problem not restricted to the developing world but also present in the industrialized world, primarily in the population of the elderly.[23] Apart from vegetarians, the elderly, and people living in the developing world, individuals in the growth phase, namely infants, children, adolescents, as well as pregnant and lactating women are especially prone to zinc deficiencies.[24] However, not all causes of zinc deficiency are due to malnutrition, but some rare forms are based on inherited disorders, as for example in *acrodermatitis enteropathica*.[25]

Reactive oxygen species and oxidative stress

Under normal conditions, the production of reactive oxygen species (ROS) is an elemental process of cellular respiration. If the metabolism of the cell is in balance, there are no difficulties resulting from ROS and severe cell damage is excluded.[26] However, if ROS are accumulating within the cell, the antioxidant defence mechanism, which is responsible for detoxifying ROS, is not able to neutralize all radicals anymore.[27] Consequently, excess ROS production causes a pathological response leading to cell and tissue damage. Oxidative stress is in particular caused by an imbalance between the increased production of ROS and the decreased protective action of antioxidants which are responsible for the neutralization and removal of ROS.

Apart from oxidative stress, chronic inflammation is important in the development of various chronic diseases, such as atherosclerosis, cancer, neurodegeneration, immunologic disorders, and ageing. ROS is comprised of H_2O_2, $^{\bullet}O_2^-$, and $^{\bullet}OH$, being continuously produced *in vivo* due to aerobic conditions. Although those molecules cause damage to the cell, they are necessary for the function of normal cell regulation and signalling.[26] The main ROS sources in eukaryotic cells comprise the flavoprotein oxidases, mitochondrial respiratory chain, microsomal cytochrome P450 enzymes, and peroxisomal fatty acid metabolism. The nicotinamide adenine dinucleotide phosphate oxidases (NADPHs) are plasma membrane-associated enzymes (Fig. 1), serving as electron donors and catalyzing the production of $^{\bullet}O_2^-$ from O_2 (reaction 1).

$$NADPHH^+ + 2O_2 \rightarrow NADP^+ + 2O_2^- + 2H^+ \qquad \text{(reaction 1)}$$

NADPH oxidase is inhibited by zinc, resulting in decreased ROS formation. Additionally, zinc works as a cofactor for the enzyme superoxide dismutase (SOD), which catalyzes dismutation of $^{\bullet}O_2^-$ to H_2O_2 (reaction 2), which is subsequently detoxified by catalase (CAT) and glutathione peroxidase (GPx).[28]

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \qquad \text{(reaction 2)}$$

In general, zinc is able to bind to sulfhydryl groups of various molecules, hence protecting them from oxidation. In the presence of zinc the activation of antioxidant proteins, such as glutathione (GSH), and enzymes, such as CAT, and SOD is increased, thereby reducing the activity of oxidant-promoting enzymes, such as inducible nitric acid synthase (iNOS) and NADPH oxidase.[28]

The antioxidant properties of zinc are supposed to be subdivided into acute and chronic effects. Long-term exposure to zinc results in chronic effects with subsequent induction of other molecules, primarily of ultimate antioxidants, such as metallothioneins (MTs). In contrast, long-term zinc deficiency results in increased sensitivity to oxidative stress. The acute effects comprise firstly the protection of sulfhydryl groups of proteins, and secondly the reduced $^{\bullet}OH$ formation from H_2O_2 and $^{\bullet}O_2^-$. This is done by antagonizing the redox-active transition metals copper and iron, which normally catalyze $^{\bullet}OH$ formation by the Fenton reaction (reaction 4), reaction 3 see below.[27]

Iron and copper are rather insoluble under physiological conditions. However, they remain in solution by getting complexed to low or high molecular components, respectively. Thereby, they serve as catalytic centers for free radical production. It is likely that intracellular free iron associates with low molecular structures, such as citrate, nucleotides, and glucose. In contrast, copper is more likely to bind to macromolecular structures, such as enzymes, carbohydrates, peptides, and DNA.[27] After complex formation the metal ion is trapped and serves as a site of free radical formation by repeated redox cycling of the metal. Due to this fixed position the formed reactive $^{\bullet}OH$ is able to attack adjacent structure causing severe damage, which is also described as a site

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specific mechanism.[27, 29] Minimal concentrations of soluble copper or iron are able to catalyze the conversion of $^{\bullet}O_2^-$ into the highly reactive $^{\bullet}OH$ by the metal catalyzed Haber-Weiss reaction (reaction 3) and the already mentioned Fenton reaction (reaction 4). Zinc effectively competes for Cu^{2+} and Fe^{2+} binding sites, thereby distributing protection against extensive radical formation.[29]

$$^{\bullet}O_2^- + Fe^{3+}/Cu^{2+} \rightarrow O_2 + Fe^{2+}/Cu^{+} \qquad \text{(reaction 3)}$$

$$Fe^{2+}/Cu^{+} + H_2O_2 \rightarrow Fe^{3+}/Cu^{2+} + {}^{-}OH + {}^{\bullet}OH \qquad \text{(reaction 4)}$$

Another biological structure which is affected by oxidative stress is the lipid bilayer. There is evidence that copper and especially iron are critical in the initiation process of lipid peroxidation, triggering a free radical chain reaction.[30] The metal-catalyzed $^{\bullet}OH$ can cause withdrawal of hydrogen from an unsaturated fatty acid within the lipid bilayer with subsequent lipid radical formation. This is followed by a cascade of cyclic reactions, leading to repetitive formation of short-chain alkanes and lipid acid aldehydes with final destruction of the bilayer. Apart from lipid peroxidation, proteins are as well targeted by radicals. Amino groups get hydrolyzed, resulting in oxidative modifications. By this, the protein is labelled for degradation and protein oxidation contributes to catalytically inactive enzymes, which are accumulated during oxidative stress and ageing.[31]

The first report describing the antagonism of a zinc-mediated reaction was the property of zinc to antagonize the iron-mediated xanthine/xanthine oxidase-induced peroxidation of erythrocyte membranes.[32] Moreover, zinc was shown to have protective effects in cardiac oxidative injury, possibly due to decreased $^{\bullet}OH$ formation as a consequence of decreased content of copper in the tissue after zinc supplementation.[27]

Zinc homeostasis and its pro-antioxidant effects

Zinc homeostasis seems to be important in the relationship between inflammation and oxidative stress.[33] Zinc is an essential element in the redox metabolism and elevated oxidative stress seems to be the result of zinc deficiency accompanied by oxidative damage to DNA, lipids, and proteins. Since zinc itself is redox inert, it is likely that it plays an indirect antioxidant role. If sufficient zinc supplementation is not guaranteed, the deficiency of zinc might lead to human diseases such as cancer and autoimmune diseases, e.g. rheumatoid arthritis and asthma.[2, 34, 35] Indeed, zinc supplementation *in vivo* was shown to decrease markers of oxidative stress, apart from inflammation and infection which will be discussed below in more detail.[36]

Different mechanisms exist to bind zinc and thereby to prevent overaccumulation of intracellular zinc in the cell. The transcription factor "metal regulatory transcription factor-1" (MTF-1) is a conserved zinc finger protein.[37] An increase of intracellular zinc can be sensed by MTF-1 which is followed by zinc-dependent gene induction, e.g. transcription of the gene encoding for MT. The probably most characteristic function of MT is its ability to act as a zinc buffer.[38] MTs are low molecular weight molecules of 6-7kDa and involved in a variety of cellular processes, such as metal detoxification and metal homeostasis, primarily with the ability to complex metal ions such as zinc. Up to seven zinc ions can be bound by the cysteine sulfhydryl groups of one MT protein, which are presented in the form of two clusters (Fig. 2).[1] Interestingly, the MT binding constants vary among the bound zinc ions. Moreover, the presence of reactive compounds or oxidative stress is responsible for oxidizing or at least for modifying the cysteine sulfur ligands of MTs thereby releasing zinc ions, which in turn leads to increased intracellular free zinc concentrations. Consequently, zinc is able to modify intracellular signalling in particular at picomolar concentrations.[39] MTs are redox-active proteins, whereas zinc itself is redox inert. To summarize the mechanism of zinc release, zinc ions are released of MTs during the reaction of oxidants with MT with subsequent formation of the oxidized MT protein (Fig. 3).

The metal-free protein thionein is supposed to be the biologically active form of MT. *In vivo*, various signals induce the synthesis of thionein, such as cytokines and phorbol esters next to metal ions. The MT bound metals are not trapped in an inflexible conformation rather metal transfer exists due to metal exchange within each MT cluster. Additionally, the cluster bound metals are able to exchange with metals in solution or with metals in different MT proteins.[1] Therefore, MT proteins are able to bind or release zinc ions into the cytoplasm depending on the redox status (Fig. 3).

As already mentioned zinc is no proper antioxidant, since an antioxidant is defined as a substance which impedes a free radical reaction and under physiological conditions zinc is not redox active.[40] Thus, it is obvious, that zinc is not directly involved in donating electrons to or accepting electrons from oxidant molecules. However, zinc acts as a Lewis acid accelerating the electron transfer, which characterizes its catalytic function within enzymes. Zinc binds to a water molecule or thiolate which get activated and produce nucleophiles. Consequently, the formal charge of zinc is affected and modulation of its Lewis acidity follows.[41] Hence, zinc does not function as an antioxidant *per se* and the antioxidant properties result from indirect mechanisms.[2] Therefore, the term "pro-antioxidant" was defined to describe the functions of zinc at least in a small range of concentrations. In contrast, pro-oxidant effects of zinc become predominant if the small range of zinc concentrations is below a limit or exceeded, since oxidative stress is accompanied by zinc deficiency or zinc overaccumulation.[42] Using the term "pro-antioxidant" gives a better description of the antioxidant effects of zinc without claiming a direct participation in redox biochemistry.[33] Protection of sulfhydryl groups of proteins is insufficient under conditions of zinc deficiency (Fig. 4), whereas zinc overload inhibits mitochondrial respiration and antioxidant enzymes, such as glutathione reductase (GSR), thereby resulting as well in the induction of oxidative stress. In particular, within MT the redox-active donor atoms of the cysteine sulfhydryl groups coordinate zinc association and

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dissociation. Under physiological conditions, zinc acts as a pro-antioxidant by binding to the sulfhydryl groups and thereby protecting the cell against inflammation induced by ROS (Fig. 4).[41]

All in all, the actions of zinc involve the induction of MT expression, which in turn acts as a radical scavenging protein. Importantly, zinc is able to cause inhibition of the superoxide-producing enzyme NADPH oxidase and displaces redox active metal ions from critical binding sites, such as copper and iron, hence preventing the manifestation of oxidative damage within the cell.

Antioxidant effects of zinc within immune cells

Phagocytes which comprise monocytes/macrophages and neutrophil granulocytes represent cells of the innate immune system. They are able to produce ROS by NADPH oxidase, whereby O_2 is transformed into $^{\bullet}O_2^-$ with subsequent H_2O_2 conversion and formation of other ROS molecules (Fig. 1).

ROS production by NADPH oxidase upon phagocytosis of pathogens causes effective pathogen killing.[43] The rapid release of ROS within phagocytes is called oxidative or respiratory burst accompanied by subsequent pathogen killing. The intracellular distribution of zinc might lead additionally to effective clearance of the intracellular pathogen.[44]

Although zinc deficiency is associated with impaired cell growth, it has obviously no suppressing effect on the amount of monocytes and neutrophils. In particular, impaired lymphopoiesis was seen in zinc-deficient mice, whereas the numbers of monocytes and neutrophils increased. Thus, it seems likely that the precursors of phagocytes are protected, whereas the precursors of lymphocytes are down-regulated.[45] Moreover, induced monocytic differentiation of the promyelocytic cell line HL-60 was accompanied by decreased intracellular zinc levels and induced expression of monocytic surface markers. Apart from that, monocyte functions, such as the oxidative burst and phagocytosis, were in fact increased during differentiation under conditions of zinc deficiency. It is known that monocyte differentiation is promoted by the second messenger "cyclic adenosine monophosphate" (cAMP) and zinc causes inhibition of the cAMP-producing enzyme adenylate cyclase (AC). In contrast, zinc deficiency causes increased cAMP production after adequate AC stimulation thereby leading once again to the assumption that intracellular free zinc levels limit the activity of AC and a decrease of zinc levels promote monocyte differentiation.[46]

The influence of zinc deficiency on monocytes of healthy human donors was studied and revealed elevated elimination of bacterial pathogens by phagocytosis and oxidative burst, since NADPH oxidase was probably not influenced due to low zinc levels.[47] This is in accordance with the fact that the presence of zinc is able to inhibit NADPH oxidase with subsequent inhibition of ROS production. Consistently, inflammatory cytokine production of interleukin (IL)-6 and tumour necrosis factor (TNF)-α was reduced, leading to the assumption of a shift from intercellular communication to basic innate defensive functions under conditions of zinc deficiency.[47] The consequent cytokine reduction might be the cause of short-term zinc deficiency, whereas under conditions of long-term zinc deficiency cytokine expression is increased upon stimulation, in particular regulated by redox-mediated and epigenetic mechanisms.[48]

In healthy normal individuals zinc probably functions as an anti-inflammatory and antioxidant agent. In fact, zinc-supplemented healthy individuals showed decreased plasma levels of lipid peroxidation products and DNA adducts compared to a control group. Moreover, lipopolysaccharide (LPS)-stimulated mononuclear cells (MNCs) from zinc-supplemented individuals showed reduction of IL-1β- and TNF-α-mRNA.[28] However, constitutive cytokine expression is increased after long-term zinc deficiency. Thus, inflammatory cytokine production and oxidative stress during inflammation could be limited by zinc supplementation.

Additionally, *ex vivo* zinc supplementation caused protection of MNC from TNF-α-induced "nuclear factor kappa-light-chain enhancer of activated B cells" (NF-κB) activation, thus keeping the normal following expression of pro-inflammatory cytokines low. Besides, zinc supplementation of the HL-60 cells was shown to enhance the upregulation of the zinc finger protein A20 which is a zinc-finger transactivating factor, being important in the down-regulation of IL-1β- and TNF-α-induced NF-κB activation.[28] In particular, A20 inhibits the activation of NF-κB via the TNF receptor associated factor (TRAF) pathway and hence, zinc supplementation might cause downregulation of inflammatory cytokines by upregulation of A20 acting as a negative feedback inhibitor.[49]

Moreover, N,N,N',N'-Tetrakis-(2-pyridyl-methyl)-ethylenediamine (TPEN), which is a zinc chelator causing zinc deficiency, was able to inhibit *E.coli* induced secretion of TNF-α and IL-1β by monocytes. However, no significant differences were seen in this study with regard to changes in oxidative burst and phagocytosis neither in monocytes nor in granulocytes under conditions of zinc deficiency.[50] Apart from that, macrophages are able to take up cellular debris or pathogens by phagocytosis and induce killing of the pathogens by oxidative burst. Here it was shown that phagocytosis as well as oxidative burst were affected by the intracellular zinc status. Additionally, macrophages produce various pro-inflammatory cytokines, e.g. IL-1β, IL-6, and TNF-α, requiring intracellular zinc signals. Cytokine production in macrophages can even be induced by stimulation with high extracellular zinc concentrations.[33]

Apart from this, macrophages are able to sequestrate essential micronutrients, such as zinc. In detail, infected macrophages which were activated by granulocyte-macrophage colony stimulating factor (GM-CSF) were shown to sequester free zinc by inducing its binding to MTs in a signal transducer and activator of transcription (STAT)3 and STAT5 transcription-factor-dependent manner. This zinc sequestration causes elevated function of phagosomal H^+ channels and triggers ROS generation by NADPH oxidase.[51] This mechanism represents a very effective mechanism in the control of intracellular pathogens by actively reducing the free zinc level within the cell.

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Besides phagocytosis and oxidative burst, neutrophil granulocytes possess another mechanism in the control of pathogens, namely the formation of neutrophil extracellular traps (NETs), also called NETosis. This is a special defence mechanism against fungi and bacteria by which a matrix of DNA is released by neutrophil granulocytes to serve as a scaffold for histones and antimicrobial proteins, thereby capturing extracellular pathogens.[52] Stimulation with phorbol-12-myristate-13-acetate (PMA) was shown to activate NETosis via protein kinase C (PKC) activation. In detail, treatment with PMA leads to a zinc signal in neutrophil granulocytes, finally resulting in NET formation and hence control of extracellular pathogens. In contrast, zinc chelation causes inhibition of NETosis and thus reduced capability to combat pathogens. Moreover, PKC-mediated NET formation seems to be dependent on ROS production, namely by the superoxide producing enzyme NADPH oxidase. Inhibition of NADPH oxidase results in suppressed ROS formation and thus suppressed NETosis. However, zinc seems not to be directly involved in the activation of PKC and production of ROS. In this case zinc seems likely to be essential for signals downstream of ROS production and influencing NETosis.[53]

Effects of zinc supplementation on oxidative stress in the elderly

The process of ageing is accompanied by accumulation of cellular damage, causing cellular dysfunction and apoptosis. In particular, characteristics of the ageing process comprise increased oxidative stress, accelerated cellular senescence as well as reduced tissue homeostasis and regeneration. Therefore, increased risk of the development of various chronic diseases is associated with ageing, such as vascular diseases, diabetes, and neurodegenerative disorders as a consequence of elevated oxidative stress.[28, 54] Since malnutrition among each other is responsible for symptoms of zinc deficiency, inadequate food intake in the elderly might as well be problematic, promoting the development of the before mentioned different diseases. In fact, reduced dietary zinc intake in the elderly seems to be quite prevalent as documented in the Zincage project.[55] A survey conducted in the Unites States of America revealed that only 42.5% of the elderly showed adequate zinc intake whereas consequently almost 60% were zinc deficient.[56] Uncontrolled production of free radicals is observed in ageing, possibly as a result of increased ROS production and decreased antioxidant defence mechanisms.[57]

Under physiological conditions different antioxidant enzymes, such as CAT, GPx, and SOD are able to neutralize the oxidative action of ROS thereby avoiding oxidative damage, especially in the development of ageing. Two of the three SOD isoforms are able to bind copper and zinc at its catalytic site, assuming a beneficial effect of zinc supplementation in the antioxidant defence.[54]

In a studied population of healthy elderly the activity of the SOD1 isoform in erythrocytes (eSOD) was seen to be significantly increased with age. In contrast, no age-related differences were observed in the activities of the SOD3 isoform in the plasma (pSOD), CAT, and GPx. Moreover, eSOD activity was found to be even higher in women than in men.[54] The increase of eSOD might be a response to elevated ROS in the ageing individual. Normal ageing is associated with a consistent loss of plasma zinc levels, probably reflecting a progressive decrease of zinc content in the whole body, thereby being consistent with the reduced finding of pSOD.[58] Short-term zinc supplementation of previously zinc-deficient elderly showed an increase in plasma zinc levels. Apart from that, significant increase in the activity of eSOD and pSOD was seen. In contrast, the enzymatic activities of CAT and GPx, responsible for detoxifying H_2O_2, were decreased after zinc supplementation.[54]

Different mechanisms of zinc action affecting the enzyme and thereby causing protection from destruction were assumed in former times. Direct binding of zinc to cysteine residues within the enzymatic catalytical site could be shown by our working group.[59] It is likely that zinc is able to protect sulfhydryl groups from oxidative damage by binding those groups and thereby suppressing their reactivity and preventing intramolecular disulfide formation. In particular, this was shown for the phosphatase "phosphatase and tensin homolog deleted on chromosome 10" (PTEN) which seems to be inactivated by zinc binding, leading to enhanced Akt activation, which in turn causes cell survival. It is likely that zinc inhibits PTEN by binding to its catalytic cysteine residue at position 124 (Cys124), which in turn is easily oxidized by H_2O_2 under zinc-deficient conditions (Fig. 4), thereby causing enzyme damage. During the oxidation process it is likely that Cys124 forms a disulfide bond with Cys71.[60] Thus, in case of elevated H_2O_2 formation due to elevated ROS production, zinc deficiency might lead to uncontrolled cell proliferation.

A zinc supplementation study conducted in an elderly population was shown to increase plasma zinc levels, whereas significantly decreasing inflammatory markers, such as plasma high-sensitivity C-reactive protein (hsCRP), IL-6, macrophage chemoattractant protein (MCP-1), secretory phospholipase A (sPLA), sE-selectin (soluble E-selectin), vascular cell adhesion molecule 1 (VCAM-1), and malondialdehyde and hydroxyalkenals (MDA+HAE) compared to the placebo group, emphasizing once again the antioxidant and anti-inflammatory properties of zinc.[61]

The most common form of heart disease is the ischaemic or coronary heart disease, mostly being the cause of atherosclerosis. In detail, during the development of atherosclerosis lipids infiltrate into the arterial intima which are digested by macrophages. In the course so-called foam cells are formed which lead to the formation of fibrous plaques. Those plaques in turn are likely to rupture and thereby enable blood influx and disruption of the arterial wall. Clinical manifestations of atherosclerosis include myocardial infarction, heart failure, angina, and sudden death. Zinc status seems to be important in the atherosclerotic development, on the one hand indicating a contribution of zinc deficiency to disease development and on the other hand a protective role

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of zinc supplementation.[62] Thereby, the association between lipoprotein metabolism and zinc seems to be an essential aspect. Increased levels of oxidized lipids in the vessel wall are characteristic. Lipid peroxidation can cause the formation of oxidized low-density-lipoproteins (oxLDL). OxLDL in turn activates the NF-κB inducible kinase (NIK)/IκB-kinase (IκBK)/NF-κB signalling pathway and upregulates various target genes, such as inflammatory cytokines and adhesion molecules. However, zinc supplementation was shown to increase antioxidant mechanisms, decrease the presence of inflammatory cytokines within the plasma, and decrease oxidative stress biomarkers in the elderly.[28]

In oxLDL stimulated THP-1 cells, which is a human monocytic leukemia cell line, and HAEC cells, which is a human aortic endothelial cell line, zinc increased the expression of A20 and peroxisome proliferator-activated receptor (PPAR)-α compared to zinc deficient cells. The nuclear receptor PPAR-α which mediates lipoprotein metabolism, inflammation, and glucose homeostasis is shown to play a protective role in the development and progression of atherosclerosis. Moreover, the zinc sufficient oxLDL stimulated cells showed decreased NF-κB activation compared to zinc deficient cells, shown for the first time in T helper cells.[28, 63]

In vitro experiments showed suppression of LDL oxidation by iron ions, endothelial cells, and macrophages, caused by zinc supplementation.[64] This is interesting in the consideration of zinc as an endogenous protective agent against the development of atherosclerosis.

Apart from this, current studies on the beneficial use of zinc in a South Australian population of elderly in the age of 65-85 years, revealed a positive outcome on genome stability events compared to the placebo group.[65]

The protective role of MT in ageing is questionable due to an increase of inflammatory and stress-like conditions potentially causing irreversible modifications of cysteine residues. This in turn might change the properties of MT from protective to harmful features. Therefore, the balance of zinc and MT might be important to prevent age-related chronic diseases. Comparison of the zinc status and MT expression of peripheral blood mononuclear cells (PBMCs) isolated from healthy and zinc-deficient atherosclerotic patients showed altered intracellular zinc distribution and increased MT expression during atherosclerosis.[66]

Besides oxygen involving radicals, other radicals seem to be important in cell damage. Under normal conditions NO protects endothelial cells against H_2O_2-induced toxicity and release by the endothelium is essential in vascular homeostasis. Release of intracellular zinc from zinc-sulphur containing proteins stimulated by NO was shown to activate GSH redox cycle in endothelial cells, ultimately causing protection against oxidative damage. However, zinc deficiency interrupts the NO-mediated increase of the catalytic subunit of glutamate cysteine ligase and thus of cellular GSH levels, showing again a protective role of zinc in oxidative stress.[67]

The zinc-dependent enzymes Cu/Zn-SOD and extracellular-SOD control the amount of superoxides and thus function to protect the cellular availability of NO. The ability of zinc to protect the redox-signalling functions of NO is likely to be ameliorated by a zinc deficient state or perturbed zinc utilisation in atherosclerosis.[62]

To summarize, zinc participates in various cellular redox and inflammatory processes, including NF-κB, NO, and A20-PPAR-α signalling pathways, leading to reduced generation of inflammatory cytokines. Impaired zinc homeostasis, primarily zinc deficiency, is associated with increased levels of oxidative stress and the induction of widespread genomic and proteomic changes, inducing cardiovascular diseases, in particular atherosclerosis in the elderly.

Zinc and its antioxidant effects in diabetes mellitus

Two main forms of diabetes mellitus exist, comprising diabetes mellitus type I and diabetes mellitus type II. The first is primarily characterized by autoimmune destruction of the insulin producing β-cells in the pancreatic islets of Langerhans, causing insulin deficiency.[68] Diabetes mellitus type II is characterized by the presence of glucose intolerance and hyperglycemia. The main pathophysiological effect is the induction of peripheral resistance to insulin action associated with a relative deficiency of insulin secretion in response to glucose. Chronic hyperglycemia in diabetes favors the manifestation of oxidative stress due to high ROS production and decreased antioxidant defence mechanisms. Manifestation of oxidative stress in patients affected by diabetes mellitus type II might be associated with changes in zinc metabolism.[69] Insulin resistance is followed by hyperlipidemia and hyperglycemia, leading to increased metabolism and mitochondrial oxidation in β-cells. This in turn leads to elevated mitochondrial membrane potential and superoxide production, thereby increasing exposure of the cell to ROS. The cells harbour a special protein, *i.e.* uncoupling protein 2 (UCP 2), to prevent the mitochondrial membrane potential, hence reducing production of ROS. However, the defence mechanisms of β-cells against ROS overaccumulation are limited since those cells possess only low levels of ROS-detoxifying enzymes and ROS is suggested to be a key contributor to β-cell failure during insulin resistance.[70]

Under normal conditions a rapid increase of blood glucose stimulates insulin secretion, resulting in a temporary increase of blood insulin levels. However, hyperglycemic condition might result in increased NADPH formation, consequently, leading to increased superoxide production as an important source for excessive ROS formation.[71]

Insulin-secreting β-cells of the pancreas are especially rich in zinc, predominantly found in secretory vesicles. Characteristic for diabetes is the increased urinary loss of zinc with subsequent decrease of total body zinc, causing hypozincemia.[72] Oxidative stress generated by ROS is known to significantly contributing to the pathogenesis of diabetes and since zinc is known to be involved in ROS formation, zinc might be an interesting pharmacological molecule. In fact pharmacologic protection against oxidative stress was able to

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ameliorate the severity of diabetes progression in an animal model of diabetes mellitus type II.[73] Interestingly, reduced plasma zinc levels were found to be associated with this type of diabetes. In association with this finding, zinc supplementation could be effective in promoting insulin signalling in the therapy of type II diabetes.[74] The effects of zinc on oxidative stress in diabetes mellitus affected patients have been studied by various working groups. Thereby, it was shown that zinc supplementation of diabetic patients positively influenced parameters of lipid peroxidation in diabetes mellitus type I as well as in type II. In mice, SOD overexpression was shown to increase the tolerance of β-cells of the pancreas to oxidative stress-induced diabetogenesis, thus contributing to the protection from the onset of diabetes mellitus type II. In contrast, high amounts of SOD in humans were not shown to protect diabetic patients from ROS damage, only plasma lipid peroxidation was shown to be significantly decreased.[75]

The effects of zinc in diabetes mellitus are versatile but might lead at least in some parts to a beneficial use of zinc in the treatment of ROS-induced cell damage.

Summary

Increased oxidative stress due to ROS elevation is associated with cell damage, in particular damage to lipids, proteins, and DNA, thereby causing diseases, such as cardiovascular diseases in the elderly or diabetes mellitus manifestation. Evidence for the relationship between zinc deficiency and increased production of ROS produced by NADPH oxidase is already stated for some years. Moreover, zinc-dependent SOD was shown to be involved in elevated oxidative stress under conditions of zinc deficiency, obviously not being able to neutralize ROS within the cell anymore. The use of zinc as an antioxidant in the prevention or therapy of ROS-associated diseases might be a promising approach.

To sum up, zinc positively influences ROS production and lipid peroxidation. Moreover, assessment of the current zinc status in human cells might be useful to assess the status of oxidative stress and this in turn might serve as a potential predictor in the development of different ROS-dependent diseases.

Acknowledgements

L. R. is a member of Zinc-Net.

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Table 1: Zinc Content of Selected Foods

Animal Products (mg/100 g)		Plant Products (mg/100 g)	
Beef fillet	3.6	Carrot	0.64
Calf fillet	4.3	Coconut	0.5
Cheese	1-5	Potato	0.2-0.3
Fish	1-2	Red cabbage	0.22
Liver	4-6	Rice	1.3
Oysters	20-150	Salad	0.22
Pork cutlet	1.3	Sweetcorn	1.2
Poultry	2-3	Vegetable oil	0.1-0.2
Roast beef	2.5	Wheat (white) flour	0.9
		Wholemeal flour	3.0

Modified from Ibs and Rink.[1]

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Fig. 1: ROS production initiated by NADPH oxidase. The membrane localized NADPH oxidase is found predominantly in the membrane of pathogen engulfing vesicles within phagocytes. NADPH oxidase is able to generate superoxide radicals ($^{\cdot}O_2^-$) by the use of oxygen (O_2) and cytosolic NADPH and can be suppressed by zinc. Afterwards $^{\cdot}O_2^-$ can be dismutated by superoxide dismutase (SOD), which uses zinc as a cofactor, resulting in the formation of hydrogen peroxide (H_2O_2) which in turn can be either transformed into the hydroxyl radical ($^{\cdot}OH$) under iron participation, into hypochlorous acid (HOCl) by the myeloperoxidase (MPO), into water and oxygen molecules by catalase activity (CAT), or into glutathione (GSH) via water and glutathione disulfide (GSSG) formation by glutathione peroxidase (GPx) with subsequent transformation into the final GSH with the help of glutathione reductase (GSR). $^{\cdot}O_2^-$, $^{\cdot}OH$, and H_2O_2 represent ROS and are indicated with red arrows. Zinc might be responsible for decreased GPx and CAT activity resulting in decreased detoxification of H_2O_2.

Fig. 2: Relationship between zinc and metallothionein (MT). Increased zinc levels are detected by the metal regulatory transcription factor-1 (MTF-1) which induces expression of the apo-metalloprotein thionein. Thionein is activated and binds intracellular zinc forming the active MT protein. Up to seven zinc ions can be bound by the 20 sulfhydryl groups of the cysteine residues which are presented in two clusters. One cluster is able to bind three zinc ions and the other cluster is able to bind four zinc ions. Zinc is released by MT as a consequence of ROS action (Fig. 3) which is subsequently provided for intracellular signalling.

Fig. 3: Schematic pro-antioxidant functions of zinc. Zinc is able to act as an antioxidant thereby preventing damage and subsequent inactivation of the corresponding protein or enzyme (left side). Oxidation of this binding releases the zinc ion and causes formation of a disulfide bond which is prone to ROS (right side).

Fig. 4: Cysteine residues presented in the catalytic site of PTEN. (A) Increase of intracellular zinc enables zinc binding to sulfhydryl groups of Cys124 and Cys71 causing enzymatic inactivity in case of PTEN. Decrease of zinc concentration recovers enzyme activity by reconversion of the enzyme. (B) Increased ROS formation promotes the formation of a disulfide bond within the catalytic site, resulting in enzyme inactivation. This mechanism is irreversible and the enzyme remains inactive after reduction of H_2O_2. (C) However, after zinc supplementation and increases in H_2O_2, the enzyme gets protected due to zinc binding within the catalytic site and H_2O_2 has no damaging properties. This mechanism is reversible due to the protection of the sulfhydryl groups by zinc. Thus, the enzyme can change again from its inactive state into an active state after H_2O_2 is reduced.

Fig. 5: Intracellular oxidative damage. ROS production can be inhibited in the presence of zinc. Otherwise ROS is able to attack fatty acids causing lipid peroxidation. One example is the formation of oxidized LDL (oxLDL) which activates the NF-κB inducible kinase (NIK)/IκB-kinase (IκBK)/NF-κB signalling pathway. This results in the translocation of NF-κB (p50/p65) into the nucleus with subsequent induction of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, causing

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inflammation with subsequent development of chronic inflammatory diseases, such as advanced ageing and cardiovascular disease. The kinases NIK and IκBK can be suppressed by zinc-bound A20 or PPAR-α, respectively.

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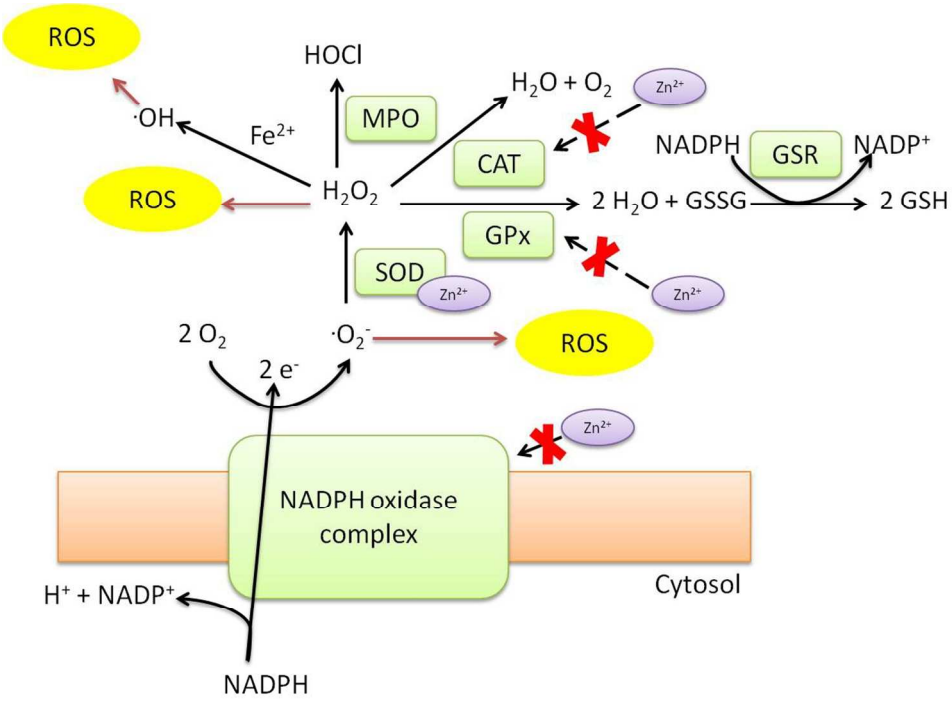

ROS production initiated by NADPH oxidase. The membrane localized NADPH oxidase is found predominantly in the membrane of pathogen engulfing vesicles within phagocytes. NADPH oxidase is able to generate superoxide radicals (.O2-) by the use of oxygen (O2) and cytosolic NADPH and can be suppressed by zinc. Afterwards .O2- can be dismutated by superoxide dismutase (SOD), which uses zinc as a cofactor, resulting in the formation of hydrogen peroxide (H2O2) which in turn can be either transformed into the hydroxyl radical (.OH) under iron participation, into hypochlorous acid (HOCl) by the myeloperoxidase (MPO), into water and oxygen molecules by catalase activity (CAT), or into glutathione (GSH) via water and glutathione disulfide (GSSG) formation by glutathione peroxidase (GPx) with subsequent transformation into the final GSH with the help of glutathione reductase (GSR). .O2-, .OH, and H2O2 represent ROS and are indicated with red arrows. Zinc might be responsible for decreased GPx and CAT activity resulting in decreased detoxification of H2O2.
222x156mm (150 x 150 DPI)

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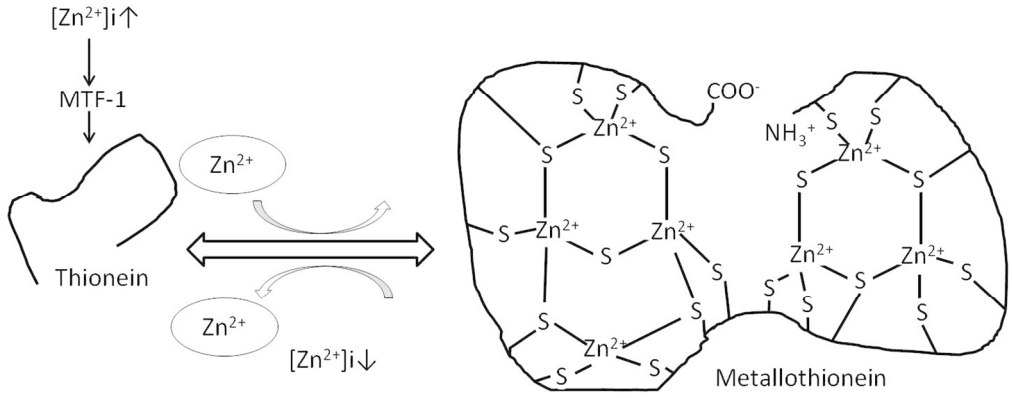

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244x99mm (150 x 150 DPI)

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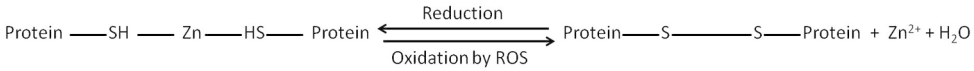

Schematic pro-antioxidant functions of zinc. Zinc is able to act as an antioxidant thereby preventing damage and subsequent inactivation of the corresponding protein or enzyme (left side). Oxidation of this binding releases the zinc ion and causes formation of a disulfide bond which is prone to ROS (right side).
325x25mm (150 x 150 DPI)

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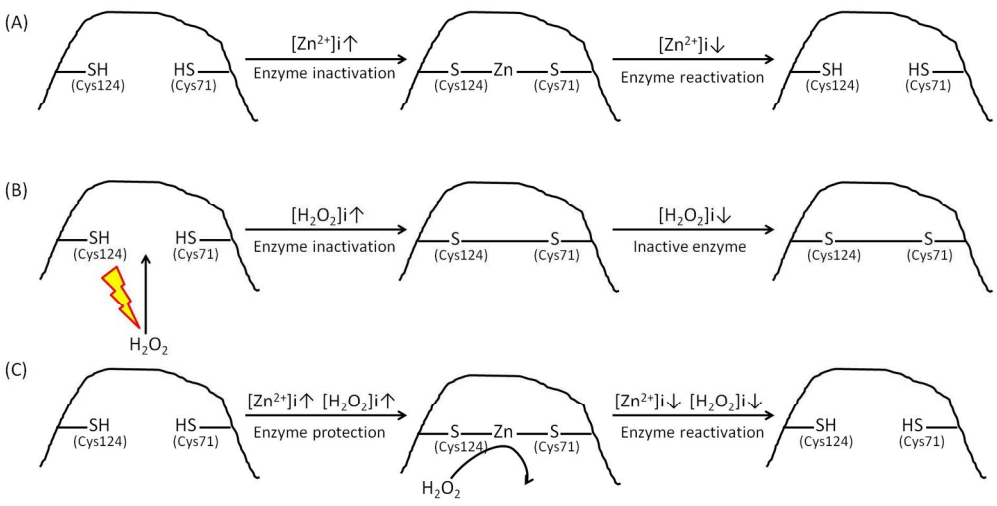

Cysteine residues presented in the catalytic site of PTEN. (A) Increase of intracellular zinc enables zinc binding to sulfhydryl groups of Cys124 and Cys71 causing enzymatic inactivity in case of PTEN. Decrease of zinc concentration recovers enzyme activity by reconversion of the enzyme. (B) Increased ROS formation promotes the formation of a disulfide bond within the catalytic site, resulting in enzyme inactivation. This mechanism is irreversible and the enzyme remains inactive after reduction of H2O2. (C) However, after zinc supplementation and increases in H2O2, the enzyme gets protected due to zinc binding within the catalytic site and H2O2 has no damaging properties. This mechanism is reversible due to the protection of the sulfhydryl groups by zinc. Thus, the enzyme can change again from its inactive state into an active state after H2O2 is reduced.
321x161mm (150 x 150 DPI)

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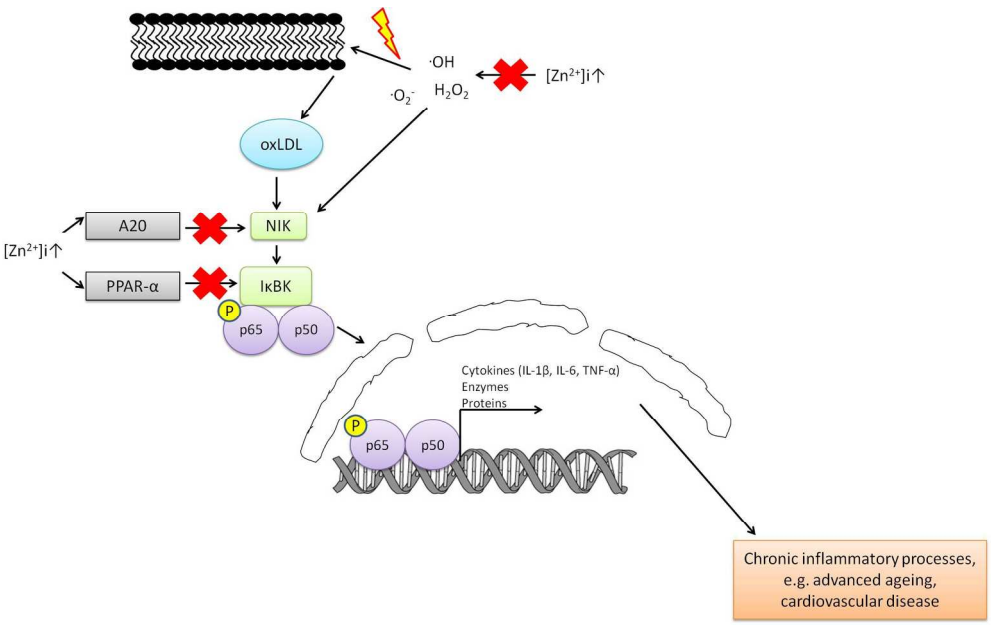

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364x224mm (150 x 150 DPI)

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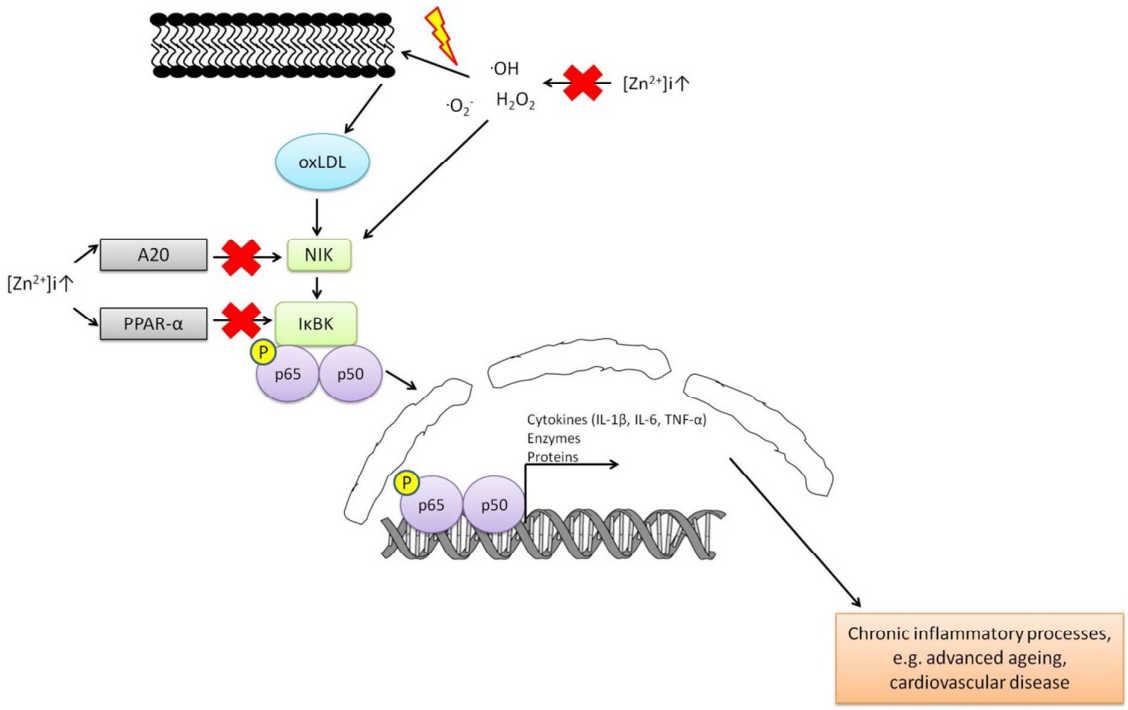
Zinc as a micronutrient and its preventive role of oxidative damage in cells

V. Kloubert[a] and L. Rink[a,*]

Zinc deficiency leads to increased ROS production, thereby causing lipid peroxidation. Subsequently, signalling via the NF-κB pathway is increased, resulting in the expression of pro-inflammatory cytokines which in turn cause chronic inflammatory diseases.



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