# Serum levels of copper and zinc in diabetic retinopathy: Potential new therapeutic targets (Review)

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**Abstract.** Diabetic retinopathy (DR) is a microvascular disorder occurring due to the long-term effects of diabetes, leading to vision-threatening damage to the retina. The human body has an elaborate system for managing and regulating the number of key trace metals circulating in the blood and stored cells. Inadequate zinc (Zn) and concurrent excess of copper (Cu) levels are associated with an increased level of oxidative stress, which may aggravate the microvascular lesions in diabetes mellitus. Several studies have revealed a significantly lower serum Zn concentration and increased Cu levels in DR when compared with diabetic patients without retinopathy and normal controls. These abnormalities are correlated with the duration of diabetes and higher levels of HbA1C. Multiple pathological mechanisms are proposed to explain these changes including hyperzincuria associated with polyuria, glycosuria, and proteinuria in diabetic patients, as well as impaired absorption of Zn at the gastrointestinal level. Increased levels of free Cu ions may be attributed to glycation and the release of Cu ions from the Cu-binding sites of proteins. Zn supplements and selective Cu chelators may be useful to alleviate oxidative stress and prevent DR progression.

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**Contents**

## 1. Introduction

Diabetes mellitus (DM) is a global health problem that has seen an increasing incidence in the last three decades. According to a recent study by Liu *et al*, the overall incidence of DM increased by 102.9% between 1990 and 2017, with the majority (98.3%) of cases being type 2 DM (T2DM) (1). With a silent evolution for years, DM causes a tremendous burden on the health national systems, due to its microvascular and macrovascular complications (1,2).

The microvascular complications include neuropathy, nephropathy, and retinopathy, while the macrovascular complications are related to the acceleration of atherosclerosis, resulting in coronary artery disease, cerebrovascular events and peripheral arterial disease (3,4). Microvascular and macrovascular complications often occur concomitantly, sharing the same risk factors and involving similar pathological mechanisms. Duration of diabetes, poor glycemic control, increased blood pressure and altered lipid profile are independently associated with both microvascular and macrovascular damages (5).

Previous studies have revealed that T2DM is a chronic metabolic disorder associated with alterations in the status of trace elements, including zinc (Zn) and copper (Cu) (6-10). Homeostatic mechanisms maintain their concentration in the human body within a normal range (11). They play important catalytic, structural, and regulatory roles in proteins and enzymes of general cell metabolism, including gene expression, antioxidant defense systems, and mitochondrial processes (6,10,12). DM is associated with an increased level of free radicals and increased reactive oxygen species (ROS), as a consequence of the effect of chronic hyperglycemia in mitochondria and the endoplasmic reticulum (13).

DR may be the most common microvascular complication of diabetes and is considered the first cause of preventable blindness worldwide, with a profound effect on the quality of life (14,15). At a macroscopic level, obesity and a sedentary lifestyle are well-known risk factors for T2DM, but at a molecular level, oxidative stress is regarded as the primary contributor to the pathogenic processes leading to cellular and vascular damage (16). A high level of glycemia induces specific changes in the retinal microvasculature via the intracellular polyol pathway, increased oxidative stress, and accumulation of advanced glycation end-products (AGEs) (17). The retina is a tissue extremely metabolically active and is susceptible to variations in concentrations of these trace elements. As free ions, they participate in angiogenesis, nerve myelination, endorphin action, and synaptic transmission (18). The most abundant metal in the retina is Zn, followed by iron and Cu (19).

Clinically, DR can be classified as non-proliferative DR (NPDR) and proliferative DR (PDR). NPDR is characterized ophthalmoscopically by the presence of microaneurysm and dot-blot hemorrhages. Severe NPDR is characterized by cotton wool spots, venous beading, and loops, as well as intraretinal microvascular complications. If left untreated, PDR may occur with retinal neovascularization. Consequences of PDR are retinal and vitreous hemorrhage and tractional retinal detachment, which ultimately results in blindness. Despite the significant development of effective treatments, DR remains the leading cause of blindness (20).

Minerals play an important role in the progression of complications of DM, including DR. The metabolism of several minerals has been reported to be altered in DM. Among these minerals Zn and Cuare the most important (21).

The aim of the present review was to assess the data regarding the changes in serum Cu and Zn encountered in patients with DR, the mechanisms and pathological association, and the therapeutic strategies that may control the progression of DR associated with Cu-Zn imbalance. A comprehensive search was performed on PubMed and Google Scholar using the key words 'diabetic retinopathy' and 'Cu' or 'Zn'. All English articles for which full text was freely available were included in the review. After duplication removal a total of 70 articles were identified. Due to the limited available studies regarding the subject, all available studies on the topic were included in the present review.

## 2. Biological role of Zn in the retina

Zn is considered an integral component of Cu-Zn superoxide dismutase (SOD) enzyme which is a very important antioxidant defense system and protects the cells from the damages caused by free radicals (22). Zn ions are encountered in more than 300 enzymes and proteins, as well as in Zn finger transcription factors, immune function, and the metabolism of carbohydrates and proteins (5,6,9). It plays a fundamental role in conserving normal ocular function. It is present in high concentrations in the ocular tissue, particularly in the retina and the choroid (17). As demonstrated by PIXE and synchrotron XRF imaging, Zn is found at high levels in the retinal pigment epithelium (RPE)/choroid, photoreceptor inner segments (RIS)/outer limiting membrane (OLM) layer, outer plexiform layer (OPL), and inner nuclear layer (INL) (18,22). In the RPE, Zn is important in the antioxidant systems and the function of retinol dehydrogenase, an enzyme required in the visual cycle of retinol (10). In the plexiform layer, free Zn ions regulate the activity of the membrane receptors and channels at the level of bipolar and horizontal cells, while higher concentrations of Zn were also reported in the intracellular organelles of Muller cells involved in multiple metabolic functions (23-25). Morrison *et al* suggested that rods are more sensitive than cones to Zn deficiency, presenting 6 cases with Zn deficiency and nocturnal vision adaptation abnormalities, that responded favorably when treated with Zn supplements (26).

Low levels of Zn cause tissue and cellular damage, due to increased levels of free radicals. Prolonged Zn depletion induces retinal and EPR damages, and the involved pathological mechanisms include increased oxidative stress, lipofuscin accumulation in the RPE, and photoreceptor disruption (10,27).

In increased exposure to Zn, no retinal manifestations were described, probably due to the efficiency of homeostasis of retinal mechanisms. However, in experimental conditions, high Zn overload generated retinal toxicity, due to mitochondrial injury, apoptosis, and oxidative stress (18,28).

## 3. Zn metabolism disturbances in DM

Conversely, Zn is an essential trace element that is directly involved in the synthesis, storage, and release of insulin (29). It is present in secretory vesicles within β-cells of the pancreas

where it participates in the crystallization of insulin and is thus released alongside insulin into the plasma (30). Insulin homeostasis is disrupted by Zn deficiency, resulting in a decreased insulin secretion by β-cells (31). Zn supplementation has been revealed to improve insulin and glucose levels in diabetic subjects and decrease the risk of developing T2DM (32).

In a study conducted by Devi *et al*, Zn levels in diabetics, both with complications and without complications, were revealed to be lower than in the control group (33). Similar results were revealed in other studies (21,33-36).

Hyperglycemia and hyperinsulinemia increase the production of free radicals. Thus, the protection of Zn against free radicals generated in this chronic disease will be diminished (33).

A significant negative association between the duration of diabetes, fasting blood sugar, glycosylated hemoglobin levels of diabetic patients and their serum Zn levels has been revealed (34-36). Anderson *et al* reported that 30% of patients with DM were found to be Zn deficient (37). However, the exact mechanisms of Zn depletion in diabetes could not be established. Increased urinary excretion, decreased gastrointestinal absorption or a combination of both may explain Zn deficit in diabetics (38). Several studies indicated a significant correlation between increased zincuria and polyuria, due to increased urine volume, while others correlated Zn excretion with glycosuria and proteinuria (38-40).

## 4. Biological role of Cu in the retina and optic nerve

Cu has bidirectional actions as an antioxidant and prooxidant, depending on the concentrations and tissue sensitivity. On the one hand, Cu is an essential element for the existence of life, important in several cellular mechanisms. Cu is part of the COX1 and COX2 subunits of the respiratory enzyme, cytochrome c oxidase, monoamine oxidase, SOD, tyrosinase, and ascorbic oxidase (41). Cu is an important antioxidant participating, along with Zn in the Cu-Zn SOD.

At the retinal level, higher Cu concentration was identified at the RPE/choroid and RIS/OLM. Previously, Cu was revealed to also be involved in neurotransmission at the level of the plexiform layer (19).

Cu deficiency is reported to cause neuropathy in general, but also decreased vision and optic nerve involvement (42-44). The signs and symptoms reported were decreased visual acuity, visual field changes, disturbances in color vision, optic atrophy, and retinal nerve fiber thinning, but with normal electroretinogram (ERG), suggesting normal retinal function. The pathogenic mechanism involves demyelination, and Cu supplementation may terminate, but not cure the damages (45-50).

By contrast, chronic increased free Cu levels, not bound by ceruloplasmin which acts as a potent antioxidant, may cause increased oxidative stress and ROS accumulation, with macromolecule injuries (46). In addition, free Cu ions are potent inhibitors of enzymes, induce cellular membrane and mitochondrial damages, and may generate optic nerve fiber lesions via glutamatergic NMDA311 and AMPA222 receptors and activation of nitric oxide synthetase (19). In an experimental study on rats, Civelek *et al* revealed that free Cu ions may favor glycation and lipid peroxidation (51).

Thus, an adequate balance of serum Cu levels should be maintained.

## 5. Cu metabolism disturbances in diabetic patients

Numerous clinical studies have revealed that the high Cu levels are associated with several metabolic and inflammatory diseases (52-57). High Cu levels were revealed to be correlated with fatty liver, metabolic syndrome, and diabetes, possibly explained by the chronic low-grade inflammation and raised glycemic values. Insulin reduces Cu concentration in the liver through the regulation of at least one Cu-transporting ATPase, ATP7B (58). An accumulation of Cu in the liver may be due to decreased insulin levels (59).

Devi *et al* have revealed increased Cu levels in diabetic patients, both with complications and without complications when compared with the control group (33). A similar finding was observed by other previous studies (60-62). The increase in Cu ion levels in patients may be attributed to hyperglycemia that may stimulate glycation and release of Cu ions from Cu-binding sites of proteins (63). The release of Cu ions into the blood further accelerates oxidative stress. Cu in its free form is a potent cytotoxic element, due to its oxidative capacities (22). The main Cu-binding proteins in plasma are ceruloplasmin and serum albumin. Chronic hyperglycemia alters the Cu binding properties of both (64). Low Zn levels may also favor Cu toxicity, as Zn competes with Cu and iron in the cell membrane (65). Cu, bound to glycated proteins, may blunt normal endothelium-derived relaxing factor (EDRF)-dependent relaxation of diabetic arteries, and provide a rationale for the use of transition metal chelators in the therapy of diabetic vasculopathy and neuropathy (63,66).

## 6. Serum Cu and Zn levels in DR vs. controls in clinical studies

Oxidant-antioxidant imbalance is the major cause of complications in diabetes, including DR. It is estimated that serum vitamin A and Zn levels may be decreased due to hyperglycemia which induces oxidative stress pathways (13). Zn is an inducer of gene and protein expression of metallothionein (MT), which is an effective antioxidant (67). Zn deficiency is associated with the progression of chronic disease states such as metabolic syndrome, diabetes, diabetic microvascular complications, and DR (6,66).

Several studies have documented the comparative serum levels of patients with DR (Table I). Although the findings revealed wide variations, there are similar conclusions. Zn levels were significantly decreased in patients with DR vs. T2DM without retinopathy and normal subjects. Additionally, Zn levels were negatively correlated with Hba1C and diabetes duration (68). Conversely, serum Cu was increased in DR, when compared with non-retinopathy diabetics and controls.

Zn protection in DR may be related to stabilizing the membrane structure, activating MT, decreased lipid peroxidation, and protecting pericytes. Zn has an anti-inflammatory effect, reducing the expression of VEGF which is related to inflammation in the ischemic retina, thus decreasing exudation and neovascularization (9). Zn deficiency causes decreased antioxidant protection and is associated with cellular and

Table I. Serum copper and zinc levels in diabetic retinopathy, type 2 diabetic patients without retinopathy and controls in clinical studies.

| Study | Controls ($\mu$g/dl) | DM no retinopathy ($\mu$g/dl) | DM with retinopathy ($\mu$g/dl) |
|---|---|---|---|
| Mor *et al* (6) | Copper: 103±7.08<br>Zinc: 67.6±6.23 | No information | Copper: 129±14.3<br>Zinc: 54±6.09 |
| Luo *et al* (9) | No information | Zinc: 84.9 (41-137) | Zinc: 78.45 (52.3-183) |
| Ganiger *et al* (66) | Copper: 106.4±19.03<br>Zinc: 90.16±19.4 | Copper: 166.3±24.35<br>Zinc: 67.50±13.85 | Copper: 238.1±27.39<br>Zinc: 50.5±11.9 |
| Rostamkhani *et al* (70) | Zinc: 70.1±2.7 | No information | Zinc: 66.2±2.4 (NPDR)<br>60.9±2.5 (PDR) |

DM, diabetes mellitus; DR, diabetic retinopathy; NPDR, non-proliferative DR; PDR, proliferative DR.

tissue damage at the retinal level. As Zn and Cu homeostasis are closely linked, notably, through competition during intestinal absorption and through shared transporter proteins such as serum albumin, a deficiency in one can affect the other (69).

Impaired metabolism of Zn and Cu are associated with higher sensitivity to oxidative damage, as both Zn and Cu are required for the activity of the antioxidant enzyme SOD, whose activity is reduced. In lower serum levels of vitamin A and Zn, the risk of DR and its severity increases (70).

## 7. Future therapeutic implications

Although tight glycemic control and preventing the additional risk factors, such as increased blood pressure and dyslipidemia remain the main therapeutic targets in the prevention of initiation and progression of DR, antioxidants may be a suitable choice for inhibiting intrinsic changes within the retinal capillary that lead to the development of DR. Close interdisciplinary follow-up of diabetic patients is essential to prevent progression to life-threatening complications. Diabetic patients were a neglected vulnerable group during the early phase of the COVID-19 pandemic. Furthermore, epidemiological studies evidenced that diabetes and obesity are among the main risk factors for death in Sars-Cov-2 infections (71-74). Several studies have revealed the beneficial role of Zn supplements on oxidative pathways in diabetes, by multiple beneficial effects including antioxidative effects, particularly in protecting sulfhydryl (SH) groups, but also through the modulating effect of Zn on insulin sensitivity (7,69,75-77).

Zn supplementation is effective to control the progression of DR, according to Naghizadeh *et al* (77). Rostamkhani *et al* (70) have revealed that an increase in serum Zn and vitamin A levels reduced the risk of DR by 25.7 and 31.1%, respectively. The antioxidants may be a suitable choice for inhibiting intrinsic changes within the retinal capillary that could lead to the development of DR (70). Supplements with Zn at a dose of 30 mg/day were revealed to decrease lipid peroxidation and improve antioxidant mechanisms in DM (75).

Diabetes is a cause of delayed wound healing following surgery and abnormal inflammatory response, due to multiple altered metabolic pathways. Senapati *et al* found a significant correlation between low Zn levels and wound complications following surgery (78). Catabolism and surgical aggression are known to cause hyperzincuria in the following days after surgical intervention (79). Zn supplementation was revealed to be beneficial following surgical interventions even in non-diabetic patients (79,80). Correction of serum Zn levels may be considered preoperatively, to minimize complications in safe surgery management of diabetic patients (80). However, Zn over-supplementation can lead to Cu deficiency, as suggested by Duncan *et al*, due to competitive mechanisms of gastrointestinal absorption (80).

Selective divalent Cu chelation in increased Cu levels may be a possible therapeutic approach targeting these Cu-mediated pathogenic mechanisms, which suppresses Cu-mediated oxidative stress and restores antioxidant defenses (6,81).

## 8. Conclusions

Trace elements are recognized as important substances for human health due to their metabolic characteristics and functions. Lower Zn status accompanied by increased Cu levels were reported in patients with DR. Disturbances in serum levels of Cu and Zn may play a significant role in the onset and progression of DR, particularly due to increased oxidative stress. Zn supplementation and Cu chelation may help to reduce the complications of DM. However, further studies on larger study groups are required to confirm these findings.

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## Authors' contributions

AMD, AAD, DSta, LCT and DrS contributed to the conception and design of this study. AMD, DSer and AAD performed the

literature research. VAN, ACC, DOC, MST, LCT, AZ, MT, CT and LD were responsible for the data collection and analysis. AMD, AAD, SAB, VAN, ACC and DaS were in charge of drafting the manuscript. MST, SAB, LD, DOC, MT, CT, DSer and DT revised the manuscript critically for important intellectual content. The final version was read and approved by all authors. Data authentication is not applicable.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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