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# The Role of Zinc in Renal Pathological Changes in Diabetic Status

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**Abstract**

Diabetes mellitus (DM) was the 8th leading cause of death in 2011, resulting in 1.4 million deaths worldwide. One of the complications of DM is chronic kidney disease, which accounts for nearly 44% of all new cases of kidney failure in the US in 2011. Zinc (Zn), an essential trace element, plays an important role in regulating carbohydrate metabolism. Several studies have shown the beneficial effects of Zn on renal pathological changes in Type 1 and Type 2 diabetic subjects, by reducing levels of oxidative stress, glomerular damage, and urinary albumin excretion after Zn supplementation. In contrast, other studies have shown little effects of Zn supplementation on renal damage. This paper reviewed recent research developments and found promising results of Zn in reducing, and in some cases, completely preventing renal damage. Nevertheless, the use of Zn as a potential treatment and especially its long-term impact against renal pathological symptoms in DM patients needs to be further studied.

**Keywords:** Zinc; Diabetes mellitus; Oxidative stress; Chronic hyperglycaemia

## Introduction

Currently, there are 382 million people with diabetes mellitus (DM). Almost 600 million people worldwide will suffer from this condition by 2035 [1].

Hyperglycaemia, the main underlying symptom of DM, is caused by either a defect in insulin secretion and/or insulin action. Chronic hyperglycaemia and its defects in other related metabolic regulation may be associated with secondary damage in organ systems including the kidneys, eyes, nerves, and blood vessels [2].

There are two main types of diabetes, type 1 and 2 diabetes (T1D and T2D). Renal failure is second to myocardial infarction as a cause of death from DM. Three lesions are typically encountered in DM patients with renal disease, including glomerular lesions, renal vascular lesions, and necrotizing papillitis [3]. The most important glomerular lesions are capillary basement membrane thickening. Excessive glomerulosclerosis can produce adequate levels of ischemia to the kidneys that can develop into end-stage renal disease, requiring dialysis or renal transplantation [3].

Increased DM prevalence rates, predominantly in developing countries, follow the trend of lifestyle changes and certain diets. One particular area in research is zinc (Zn). The sufficient availability of this transition metal has shown to be essential for biological processes [4] including optimal nucleic acid and protein metabolism as well as cell growth, division, and function [5].

Interestingly, some studies have shown DM patients had increased levels of Zn excretion in urine compared to non-DM controls [6] and that DM patients are usually Zn deficient.

This has encouraged further research into the possible positive effects of Zn supplementation on DM patients. Several studies have shown its beneficial effects on renal pathological changes in T1D and T2D subjects by reducing oxidative stress, glomerular damage, lymphocytic infiltration, and urinary albumin excretion.

As DM incident rates increase rapidly worldwide, and the UK health service is predicted to spend 16.9 billion pounds on DM-related complications over the next 25 years, there is pressure to find treatment for this condition. Therefore, this particular study includes analysis of published findings and have found promising results that show Zn reducing, and in some cases completely preventing renal damage.

The intention of this paper is to highlight the significance of Zn and encourage further research in this area, and its potential use as a treatment against renal pathological symptoms in DM patients.

## Diagnosis of DM

Blood glucose level tests are the common methods of diagnosis for DM, which include fasting glucose concentration test, post-standard carbohydrate load, and oral glucose tolerance test (OGTT). Patients with abnormally high levels of glucose in any of these tests are considered to have impaired glucose tolerance and recognised as pre-diabetics or diabetics.

Another diagnostic test is to measure levels of glycated haemoglobin (HbA1c), which identifies the average plasma glucose concentration over a long period of time, for a period of two to three months. High levels of HbA1c are usually seen in DM patients and individuals with renal failure. HbA1c is formed when haemoglobin binds to plasma glucose. Due to high numbers of glucose molecules binding to haemoglobin in DM patients, high levels of HbA1c is typically expected. Together with the fasting plasma glucose test, the HbA1c test is mainly used to diagnose T2D.

## Main Types of DM and its Mechanisms

There are two main types of diabetes, T1D and T2D. Most patients depend on exogenous insulin for survival. Without insulin they develop severe metabolic complications such as ketoacidosis and coma. Although the clinical onset of T1D is abrupt, this disease results from chronic autoimmune attack on β-cells that usually begins several years before the disease becomes evident. The common symptoms of the disease such as hyperglycaemia and ketosis appear late in its course, after more than 90% of β-cells have been destroyed.

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T2D is caused by a combination of peripheral resistance to insulin activity and inadequate insulin secretion by pancreatic β-cells that precedes hyperglycaemia and is typically accompanied by β-cell hyperfunction and hyperinsulinemia in early stages of DM [3].

T2D is a complex multifactorial disease where both genetic [7-9] and environmental factors [10-12] play a role. Environmental factors such as lifestyle and dietary habits, and also genetic factors are involved in the pathogenesis as evidenced by the greater concordance rate of the disease in monozygotic twins (35% to 60%) compared to dizygotic twins [13,14].

Other types of DM fall into monogenic and secondary causes. Although the major types of DM arise by different pathogenic mechanisms, the long-term complications in kidneys, eyes, nerves, and blood vessels are the same and are the principal causes of morbidity and death. DM includes a group of metabolic disorders caused by the disruption of glucose reabsorption in kidney tubules.

### Glucose homeostasis and the process of filtration and reabsorption of glucose in the kidney

In diabetic circumstances, hyperglycaemia causes filtration of large numbers of glucose molecules from blood plasma to the Bowman's capsule. High concentration of glucose in urine leads to saturation of transport proteins in the epithelium, restricting glucose molecules from leaving the kidney tubules. Urine containing high glucose solute (glycosuria) causes osmotic diuresis, increasing the volume of urine excreted out of the body (polyuria).

Normal glucose homeostasis is regulated by three interrelated processes including the production of glucose in the liver, glucose uptake and utilization by peripheral tissues, and actions of insulin and other regulatory hormones such as glucagon. Insufficient levels of insulin in the body, which occurs in DM, can cause accumulation of glucose in cells and lead to cell damage, including in renal cells.

As glomerulosclerosis progresses in DM patients with nephrotic syndrome, symptoms such as proteinuria, hypoalbuminemia, and oedema become evident. Excessive glomerulosclerosis can produce adequate levels of ischemia to the kidneys that can be seen as granular lesions on its surface [15].

## Zinc in the Human Body

Zn is a trace element found in microorganisms, plants, and animals. Although trace quantities are contained within organisms, the presence of this transition metal has shown to be essential for biological processes [4]. Approximately 300 enzymes and more proteins contain Zn components, which emphasizes its essential role for human health. Optimal nucleic acid and protein metabolism, as well as cell growth, division, and function require sufficient availability of Zn [5].

In the human body, most Zn is found in the muscle and bone and smaller concentrations are found in some organs including the liver, gastrointestinal tract, and kidney. Oral ingestion of Zn leads to its absorption through the small intestine and then its release into blood serum where it can be absorbed into different areas of the body and ultimately excreted by binding to proteins such as albumin and transferrin [16].

Cellular Zn involves efficient homeostatic control that avoids accumulation of excess Zn [17] and redistributes Zn to avoid its deficiency in specific organs e.g. brain [18]. Cellular Zn homeostasis is mediated by two protein families; the Zn-importer (Zip, Zrt, Irt-like proteins) family containing 14 proteins that transport Zn into the cytosol, and the Zn transporter (ZnT) family, comprising 10 proteins transporting Zn out of the cytosol [19]. Regulation of homeostasis is crucial to maintain cellular viability and prevent deregulation, which can lead to damage of the cell and ultimately cell death.

Cellular Zn homeostasis is mediated by three main mechanisms (Figure 1). One is the transportation of Zn into the cytosol through the plasma membrane by importers from the Zip-family, and it is exported out of the cytosol by proteins from the ZnT-family. Another is due to Zn-binding proteins such as metallothionein (MT) that maintain Zn homeostasis. To enable Zn re-distribution, it needs to be mobilized from its tight binding sites by coordinating Zn to sulphur donors. This allows direct Zn transfer and redox reactions to occur to the redox-inert Zn ion. The final main mechanism is transporter-mediated sequestration of Zn into intracellular organelles, including endoplasmic reticulum, Golgi, and lysosomes.

Intracellular Zn homeostasis is maintained by the MT/thionein-system, where free Zn ions bind to apo-protein thionein (Tred) to form MT (Figure 1). Elevated levels of free Zn ions can bind to Zn finger structures of the metal-regulatory transcription factor (MTF)-1, inducing the expression of Tred. Oxidation of thiols by reactive oxygen (ROS) or nitrogen (RNS) species triggers the formation of the oxidized protein thionin (Tox), which generates Zn signals and reduces oxidant signals, thereby reducing oxidative stress [20] (Figure 1).

MTs have been widely recognised in their involvement with Zn

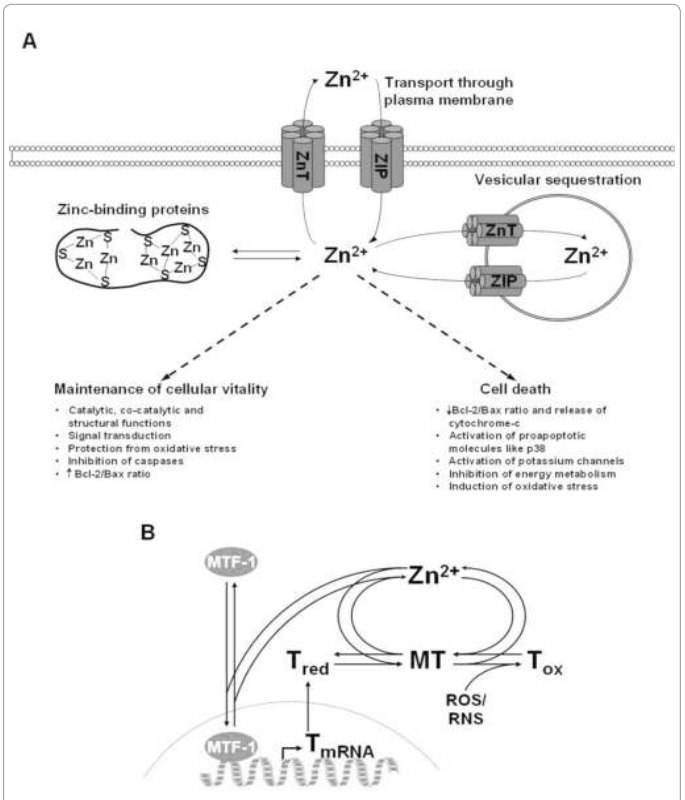


**Figure 1:** Cellular Zn homeostasis and its impact on cytotoxicity and during Zn deficiency. (A) Cellular Zn homeostasis. Zn is transported into the cytosol by Zip-family and exported out by ZnT-family. Zn-binding proteins (e.g. MT) maintain Zn homeostasis. It coordinates Zn to sulphur donors, allowing Zn transfer and redox reactions to occur. (B) Intracellular Zn homeostasis by the MT/thionein-system [20].

homeostasis. Overall, MT has a variety of functions including the regulation of apoptosis and more relevantly, the maintenance of Zn homeostasis. Due to the unique structural characteristics of MT, it can bind up to seven Zn ions. A study on the effects of MT on tubular epithelial cells in the kidney showed the up-regulation of MT stimulated the hypoxia-inducible factor and hypoxia-responsive elements (HIF-HRE) pathway, which stabilizes protein expression in cells and reduces renal injury caused by hypoxia [21].

Decreased levels of Zn in the body can cause damaging effects towards growth, neuronal development, and immunity. There are several causes of Zn deficiency including insufficient dietary intake e.g. diet with lower Zn content, malabsorption e.g. acrodermatitis enteropathica, increased excretion e.g. chronic renal disease [22,23], and increased Zn loss e.g. sickle cell disease [24].

## Zinc and Diabetes

In 1934, Zn was discovered as a component of insulin crystals therefore suggested the importance of Zn to maintain normal biological processes in the body. The deregulation of Zn homeostasis can lead to Zn deficiency or excess Zn in the body, which can affect the formation of insulin crystals that may lead to insufficient levels of insulin and result in DM.

Possible components that may be involved in Zn deregulation include defects in MT as this protein is predominantly involved in Zn homeostasis. Alternatively it could be caused by defects in the Zn transporter protein ZnT-8, which delivers Zn ion from the cytoplasm into insulin granules [25-27].

As well as being a significant component of insulin, Zn also relates to DM in the sense that studies on animal DM models and human have shown increase levels of Zn excretion in urine compared to non-DM controls [6]. This indicates that DM patients are usually Zn deficient. The definite cause for this is yet unknown, however a number of studies have indicated a positive correlation between increased zincuria with polyuria, since osmotic diuresis is one of the symptoms of DM [28-30]. Therefore high levels of Zn are excreted in urine, resulting in Zn deficiency in the body. Also hyperzincuria has been linked with blood glucose concentration, glycosuria and proteinuria [29,31,32].

### Improvements in control diabetic glycaemic level after zinc supplementation

The relationship between Zn deficiency and DM has been the subject of many studies, some using animal models [33-35] and some human subjects [36,37]. Diabetic subjects are commonly tested for blood glucose levels due to inadequate levels of insulin produced by the pancreas. A study on T1D rats (by STZ administration) showed lower blood glucose levels and 24-hour urinary protein levels after 3 months of Zn supplication in DM subjects (Blood glucose level 15.84 ± 1.38 mmol/L, 24 h urine proteins 42.28 ± 15.15 mg) compared to DM rats without Zn supplementation (Blood glucose level 18.03 ± 1.72 mmol/L, 24h urine proteins 59.25 ± 11.31 mg) [38]. This suggested that Zn might be involved in preventing hyperglycaemia and proteinuria, which are common symptoms of DM-induced renal damage.

Studies have also shown the reduction of HbA1c in T2D patients after Zn supplementation [39-42]. A study on T2D patients suffering from microalbuminuria showed that HbA1c was significantly reduced from 8.0 ± 1.4% before treatment, to 7.2 ± 1.4% after 3 months of Zn supplementation [41]. Another paper studied the effects of Zn supplementation on 101 T2D patients and the level of glycaemic control. Group 1, which included 50 patients, were given Zn, and 51 patients in Group 2 were given a placebo. Results showed a positive correlation between serum Zn levels and Zn intake. Also after 3 months of Zn supplementation, HbA1c% concentration decreased significantly while no significant changes were found in the control group. This indicates the beneficial effects of Zn supplementation in T2D as it elevated patients' serum Zn levels and improved their glycaemic control; shown by the reduction in HbA1c% concentration [40]. Results from another study showed patients given Zn supplementation in addition to multivitamins improved glycaemic control by significantly reducing HbA1C% levels and fasting and postprandial glucose compared to patients receiving multivitamin only and placebo [42].

### Improvements in diabetic glomerular function after zinc supplementation

The effects of a mixture of vitamins and minerals, including Zn have also shown improvements in glomerular function in T2D patients. Factors that significantly reduced are urinary albumin excretion (UAE) levels, fasting serum glucose and malondialdehyde concentrations. Malondialdehyde is a common marker for oxidative stress [43].

Several studies have investigated the effects of Zn supplementation on microalbuminuria in DM patients [41,44-46]. Results from studies show a significant decrease in UAE in subjects given Zn, whereas subjects not given Zn exhibited consistent high UAE levels [41]. This study has shown the renal-protective effects of Zn by significantly reducing UAE after Zn supplementation, which suggests reduced permeability of renal glomerulus to albumin in patients supplied with Zn. This also demonstrates the effectiveness of Zn as these improvements were seen within only 3 months, therefore shows the potential of Zn to possibly prevent renal damage at a relatively fast rate. However, the limitations of this study include only measuring fasting plasma glucose and not postprandial plasma glucose concentration. Hence these levels may change after Zn supplementation and alter the results.

Further, another study on the effects of Zn on T2D patients with micro-albuminuria showed a significant decrease in homocysteine levels in the group supplemented with 30 mg/d of Zn for 3 months, whilst the group receiving the placebo showed no significant changes. Also high homocysteine concentrations in plasma are associated with glomerular filtration impairment [45]. Hence a decrease in this amino acid after Zn supplementation shows promising results.

In addition to research on damaged renal tubules, other substances involved in renal damage have been studied. For example, renal tissues from Zn-deficient DM rats that were given Zn supplementation showed a reduction in levels of connective tissue growth factor (CTGF). This particular matricellular protein is critically involved in fibrosis and renal damage as it acts as a downstream mediator of transforming growth factor-beta (TGF-β1)-induced fibrosis [47-49]. A number of studies have shown a positive correlation between the level of renal CTGF mRNA expression and glomerulosclerosis in diabetic mice [47,48,50,51]. The effects of Zn supplementation on reducing CTGF levels were seen in a study that examined CTGF levels in human renal proximal tubular (HK-11) cells, which showed inhibited CTGF expression in pre-treated HK-11 cells with Zn compared to cells not treated with Zn [38]. This suggests that Zn supplementation had a positive effect on these renal cells by reducing the levels of this protein, which may prevent TGF-β1 induced fibrosis and therefore reduce pathological changes in renal tissues.

## Inhibition of diabetic oxidative stress after zinc supplementation

A study on the antioxidant effects of Zn on T2D showed initial increases in oxidative stress, which was measured according to the presence of plasma thiobarbituric acid reactive substances (TBARS), in T2D patients compared to healthy patients (3.32 +/- 0.05 micro mol/L vs. 2.08 +/- 0.04 micro mol/L). After six months of Zn supplementation, T2D patients showed a significant reduction in plasma TBARS, in contrast to the placebo group where no significant changes were seen [52]. Another similar study also showed the potential antioxidant effects of Zn, although when combined with chromium. Plasma TBARS reduced more significantly in patients receiving Zn, Cr or a mixture of Zn and Cr supplementation [Cr (13.6%), Zn (13.6%) and Zn/Cr (18.2%)] [53]. However a study on T2D male patients showed Zn supplementation does not decrease oxidative stress in patients with normal Zn levels [54].

Hyperglycaemia has been found to be a risk factor of causing oxidative stress in DM [55]. Therefore, the relationship between hyperglycaemia and its possible effects on MT is an interesting area to focus on, since MT is involved in the counteraction of oxidative stress. As Zn is related to the expression of MT, the relationship between Zn supplementation and levels of MT expression in cells have been studied in HK-11 cells using RT-PCR methods [38]. Results showed that pre-treatment of HK-11 cells with Zn induced MT expression. However, the study only used a single concentration of Zn (dissolved $ZnSO_4$ in tap water) and did not examine the effects of different doses of Zn supplementation, which may alter MT expression. Also, the study focused on tubular cells, so the effects on other types of renal tissue may differ.

However, another study on 2 months old MT-KO (MT-I and MT-II Knock Out) mice showed no significant differences in the molecular biomarkers, protein kinase B (Akt1 and Akt2), hexokinase II (HK2), and glycogen synthase kinase- 3beta (GSK-3β) expression between MT-KO mice with and without Zn supplementation [56]. Results also showed that Akt1, Akt2, HK2, and GSK-3β expression were increased in mice with MT expression after Zn supplementation. These results show that MT is required for Zn treatment to be therapeutically effective for renal protection, and also MT stimulates the phosphorylation of Akt and GSK-3 and increases HK2 expression. The reason for this is unknown however researches of this study assumed that without MT there would be limited Zn chaperones to release Zn to target molecules, which is required to stimulate Akt and GSK-3 phosphorylation and HK2 expression [57].

## Improvements in diabetic glucose metabolism after zinc supplementation

Akt1 and Akt2 genes are recognised for their role in the regulation of glucose uptake by mediating insulin-induced translocation of the SLC2A4/GLUT4 glucose transporter to the cell surface [58]. It also regulates cell survival by inactivating components of apoptosis machinery. GSK-3β is an important molecule in renal glucose metabolism as it is involved in the conversion of glucose to glycogen, and provides a preventative effect on pathogenic fibrosis of the kidney [59].

HK2 is a glycolytic enzyme that phosphorylates glucose to produce glucose-6-phosphate, the first step in most glucose metabolism pathways. Expression of the HK2 gene is insulin-responsive, and studies have shown its involvement in the increased rate of glycolysis [60,61]. It is also known to regulate cytoprotection and apoptosis based on the metabolic state of the cell [62].

Studies have shown that Zn may play a similar role to that of insulin, causing it to stimulate Akt phosphorylation and activate glucose metabolism [63-65] and a recent study on podocytes showed the protective effects of Akt2 against damage induced by chronic kidney disease [66]. Therefore the importance of Akt2 for glucose metabolism led to the assumption that the protective effect of Zn was dependent on this enzyme. However, the study on 2-month old Akt2-KO diabetic mice showed increases in Akt, p-GSK-3β, and HK2 expression, even when the Akt2 gene was absent. Therefore implying that the deletion of Akt2 gene does not affect basal Akt phosphorylation, basal and contraction-stimulated GSK-3β phosphorylation, glycogen synthase phosphorylation or activity [67].

Further studies on Akt-mediated molecules have shown a link between renal pathological damage at the last stages of DM with decreased levels of Akt function in the kidneys [68-70]. A study on 3-month old T1D OVE26 mice showed abnormalities such as significantly decreased phosphorylation of Akt and GSK-3β, decreased expression of HK2, and increased expression of the Akt negative regulators phosphatase and tensin homolog (PTEN), protein-tyrosine phosphatase 1B (PTP1B), and tribbles homolog 3 (TRB3) [56]. All of these were significantly prevented after Zn supplementation.

Inhibiting the negative regulators of Akt suggests that cells may be able to regulate glucose levels and improve intracellular conditions due to Akt availability. This may also lead to the reduction in hyperglycaemia and excess glucose in renal tissues.

## Improvement of diabetic renal pathology after zinc supplementation

Most studies investigating the effects of a substance in renal tissues usually carry out histological examinations to visually detect any changes in comparison to controls [71-73]. A particular study on 3-month old T1D OVE26 mice showed after Zn supplementation, segmental glomerulosclerosis and extracellular matrix significantly decreased [56]. DM mice given saline showed excessive accumulation of extracellular matrix, which caused glomerular enlargement (Figure 2A). In addition, Sirius red staining showed excess collagen accumulation in DM mice but not in DM mice given Zn (Figure 2B). Therefore these results reveal an improvement in pathological changes after DM mice were treated with Zn (Figure 2).

Along with histological examinations, semi-quantitative analysis can provide information on the state of the renal tissues. For example, the level of fibrosis in renal tissues can be measured by semi-quantitative analysis of mesangial index, tubulointerstitial damage, and GBM thickness. A study on renal cells in T1D rats showed fewer pathological changes of the glomeruli, renal tubules, and interstitium in DM subjects given Zn supplements compared to DM subjects given a placebo [38]. Levels of mesangial index, tubulointerstitial damage, and GBM thickness were lower in DM/Zn subjects, therefore displayed fewer renal damage (Figure 3). This suggests that Zn may play a protective role against cellular injury in renal tissues in addition to renal fibrosis, which is one of the pathological changes seen in DM.

## Protective effects of zinc supplementation on diabetic aorta oxidative damage

As well as investigating the beneficial effects of Zn supplementation

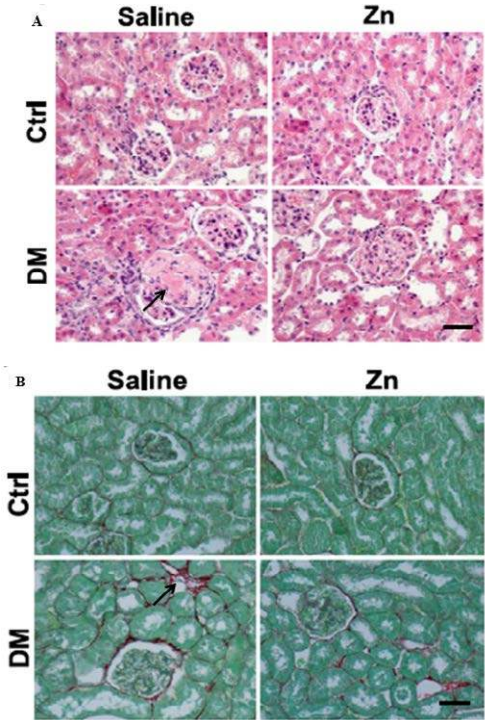


**Figure 2:** Histological examination of kidney tissues from 3-month old T1D OVE26 mice given saline or Zn supplementation. (A) Haematoxylin and eosin stain. Arrow showing accumulation of extracellular matrix, causing glomerular enlargement. (B) Sirius red stain. Arrow showing excess collagen accumulation [56].

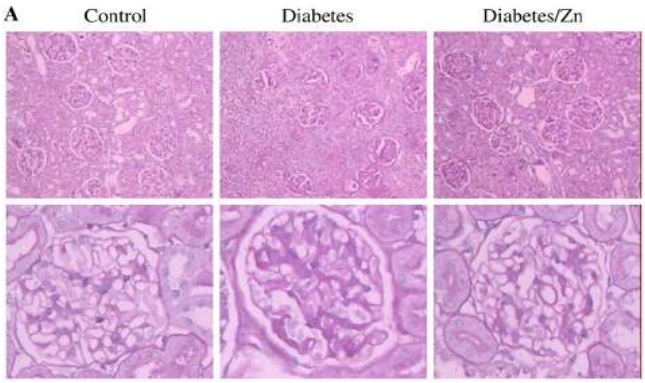


**Figure 3:** Semi-quantitative analysis of mesangial index, tubulointerstitial damage, and GBM thickness in T1D rats. Tissue examinations show damage in DM as thick GBM is clearly visible compared to DM/Zn tissue [38].

on renal organs, a study investigated the pathological effects of Zn supplementation in the aorta [74]. Results showed decreases in 4-hydroxynonenal (4-HNE) and neurotrophin-3 (3-NT) expression in tissues of T1D OVE26 mice with Zn supplementation compared to DM subjects without Zn supplementation. This provides important information on the state of the tissues, as these factors are involved in oxidant-induced cell signalling and apoptosis. Therefore higher quantities are usually found during oxidative stress.

The reduction in the expression of these factors as well as others (e.g. nuclear factor-like 2 transcription factor [74]) in DM subjects given Zn suggests that Zn supplementation decreases oxidative stress, and therefore reduces DM-induced oxidative damage in aortic tissue.

This provides valuable information that could be applied to renal blood vessels since renal atherosclerosis and arteriolosclerosis constitute part of the macrovascular disease seen in DM. Therefore this result may indicate that besides having beneficial effects on kidney cells, Zn supplementation could also have protective effects on renal blood vessels.

## Discussion

In summary, a large number of studies have shown the beneficial effects of Zn supplementation in a range of renal components including glycaemic control, glomerular function, and oxidative stress.

When levels of insulin secretion are abnormal or sensitivity to insulin is reduced, glycaemic levels (either fasting or postprandial glucose levels) are not controlled, which develops into DM. Prolonged DM causes Zn homeostasis disorders, which is shown by the reduction of serum Zn [41]. This could be due to a restricted diet, decreased intestinal Zn absorption, or increased zincuria. The mechanism of renal disease complications involves the development of glycaemic disorder, oxidative stress, and pathological changes of the glomerulus (e.g. glomerulosclerosis).

This paper showed the beneficial effects of Zn on various mechanisms of renal disease, including glucose transportation. This could be because increased amounts of Zn are transferred into pancreatic cells via increased Zn-T8 activities that also improves glucose transporter activity (e.g. SLC2 A4/GUT4) or Akt2 that increase glucose uptake. Hence this may improve insulin activity. Also Zn supplementation showed improvements to glycolysis by increased activity of HK2 (in the presence of MT), a key enzyme for the conversion of glucose into glucose-6-phosphate.

A number of papers also presented reductions in oxidative stress after Zn supplementation, which could be caused by improvements in MT and Copper-Zn-Superoxide dismutase (SOD) activities that reduce oxidative stress biomarkers, TBARS and malondialdehyde.

Subjects that presented high CTGF levels (involved in fibrosis and renal damage) showed formation of glomerulosclerosis. However after Zn supplementation, increased GSK-3β activity was seen, which may have inhibited that process and reduce fibrosis.

Histological examinations displayed the decrease in collagen accumulation after Zn supplementation (Figure 2B) and this also reduced excessive accumulation of extracellular matrix.

Further, Zn supplementation improved 4-HNE and 3-NT factors as well as others (e.g. nuclear factor-like 2 transcription factor) in DM subjects. These factors are involved in oxidant-induced cell signalling and apoptosis. This suggests that Zn supplementation decreases oxidative stress, and therefore reduces DM-induced oxidative damage.

The extent to which these have improved is significant, especially when taking into account the time period of the studies. Some of the studies have shown considerable visible improvements to renal damage within three months. This could have long-term improvements for DM patients and possibly prevent end-stage renal disease, as well as reducing the number of patients needing renal dialysis and transplantation.

This study has outlined substances that have only recently been associated with DM and/or Zn, such as MT and Akt2. Further research on the effects of Zn on MT in renal disease may lead to future treatments

relating MT and Zn supplementation. Likewise, additional knowledge on Akt2 and the effects of Zn on other Akt isoforms such as Akt1 and Akt3 would be relevant to finding treatments for DM. Therefore this needs to be studied in the future. In addition, the improvements in micro-albuminuria and levels of protein excreted in urine after Zn supplementation described in this study could lead to further research on the mechanism of Zn on reducing permeability of renal glomerulus to albumin and other proteins.

The majority of studies analysed in this paper, included research studies on animal models than human subjects. However in particular instances where human models are used, several studies showed similar beneficial effects of Zn as in the animal studies. Specifically, a study investigating the effects of Zn on micro-albuminuria in T2D patients showed a decrease in UAE as serum Zn increases, which is similar to that of many animal studies [41].

More research is required on Zn supplementation and its pathological effects on animal models to increase knowledge about suitable cellular environments needed for Zn to have its beneficial effects in renal tissues. This would provide vital information about the possible side effects of Zn supplementation and whether it is safe to use as a long-term treatment for DM patients. Further research on human subjects is crucial for this.

Studies on animal models were performed around the age of two to three months, and most of these studies show similar results to studies on human subjects over the age of 20 years, indicating that age may not be a contributing factor to the effects of Zn on renal pathological changes.

The effects of Zn supplementation on aortic damage were briefly discussed in this paper to see whether there are similarities between vascular tissues in the heart and in renal organs. Many studies showed positive effects after Zn supplementation. However there were also a small number not showing significant changes. For example, a study on forty males with T2D showed no significant differences with the baseline in the biomarkers F2- Isoprostanes (IsoPs) and allantoin in patients receiving Zn or the placebo [54]. IsoPs are involved in free radical peroxidation and have been used in a number of studies as a marker of oxidant injury in vivo [75-77]. Allantoin is a product of oxidation of uric acid by purine catabolism and has been used in clinical laboratories as a determination of oxidative stress in humans. Therefore unchanged levels of allantoin and IsoPs suggest Zn supplementation had no beneficial effects on oxidative stress.

Many previous studies have shown the positive effects of Zn, so this study provides important information that Zn supplementation may not have beneficial effects in certain situations. However, this study did not measure levels of Zn co-transporters in addition to Zn levels, and these are important factors to consider, as they are involved in the regulation of β-cell insulin processing and excretion. Therefore, variations in levels of co-transporters may have prevented Zn from having any effects. In addition, DM patients with low Zn levels were not used in this study and only males were included. Zn supplementation may have different effects on diabetic females with suboptimal or optimal Zn levels, which is frequently seen in DM patients. Also, the duration of this study was only 4 months so increasing the length of this study might produce different results.

## Conclusion

To conclude, all research articles mentioned in this study provides vital information about the relationship between Zn and DM because Zn deficiency is commonly seen in DM patients as a result of elevated urinary Zn excretion, decreased intestinal Zn absorption, and restricted diet. Therefore these findings emphasize the importance of regularly monitoring Zn levels in diabetic patients. The majority of studies aforementioned have demonstrated the beneficial effects of Zn supplementation on renal pathology of both T1D and T2D subjects. Despite this, there are some studies that have had limited impact on renal tissues after the administration of Zn. Therefore, the aim of this paper is to encourage further research in this area to consider Zn as a potential treatment against long-term renal damage in diabetic patients, which would decrease DM mortality rates due to renal damage, and reduce costs by national health services.

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