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METHOD DEVELOPMENT FOR CHARACTERIZATION OF NOVEL COPPER CHELATORS IN PATIENTS WITH DIABETES

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ABSTRACT
Defective copper regulation is implicated as a causative mechanism of organ damage in diabetes. Treatment with trientine, a divalent-copper-selective chelator, improves arterial and renal structure/function in diabetes, wherein it also ameliorates left-ventricular (LV) hypertrophy. Oxidative stress and mitochondrial dysfunction have been identified by many workers as key pathogenic mechanisms in ageing-related metabolic, cardiovascular and neurodegenerative diseases (for example diabetes mellitus, heart failure and Alzheimer's disease). Copper is a fundamental element for the homeostasis of the body. It is the third most abundant essential transition metal in humans. On the other hand, excessive exposure to copper can cause toxicity in many human organs, leading to various systemic alterations. In the kidney, increased copper concentration in the blood can cause deposition of this element in the kidneys, leading to nephrotoxicity. One of the most interesting aspects of copper balance is its influence on diabetes and the progression of its complications, such as Diabetic Kidney Disease (DKD). Several studies have shown a close relationship between copper serum levels and altered glycemic control. An imbalance of copper can lead to the progression of diabetes-related complications and impaired antioxidant homeostasis. The aim of this review is to evaluate the possible role of copper in DKD patients. TETA prevents tissue damage and causes organ regeneration by acting as a highly-selective CuII chelator which suppresses copper-mediated oxidative stress and restores anti-oxidant defenses. My group has employed TETA in a comprehensive programme.

KEYWORDS: Diabetic Kidney Disease; Copper; Diabetes; Zinc; Diabetic nephropathy; Chronic kidney disease.

INTRODUCTION
Copper (Cu) is an essential trace metal that is the third most abundant essential transition metal in humans. The mains sources of copper are foods such as vegetables, cereals, meat, fish, poultry, and legumes.[1] The average daily intake of copper is between 1 and 1.6 mg, with the recommended dose for adults being 900 mcg/day.[2] Copper is present in the body in concentrations of at least 50 mg to a maximum of 120 mg. This microelement is found in high concentrations in the liver, brain, and bones, and to a lesser extent in the heart, pancreas, and kidneys.[3,4] Copper is absorbed in the stomach and proximal small intestine, and is favored by the acidic environment that dissociates copper from dietary macromolecules.[5,6] The absorption process occurs by active transport processes when the daily copper intake is low, whereas it occurs by passive diffusion when the intake is high. The ingested copper binds to albumin and plasma amino acids and is transported to the liver where it is internalized. At this point, ceruloplasmin present in the liver binds the copper and transports it to peripheral tissues. The transporter binds to the cell surface and releases the transported copper. The liver also produces metallothionein, which can serve as a storage protein. Its concentration in the body depends on the balance between absorption in the small intestine and excretion via the liver with bile.[7] Copper plays an important role in the regulation of numerous enzymes and the synthesis of structural components and is involved in many physiological pathways and biological processes including angiogenesis, response to hypoxia, and neuromodulation.[8] A key role in preventing copper deficiency or toxicity is played by the P-type Wilson ATPase, which is responsible for transporting copper from the liver into the secretory pathway (about 50% of copper is excreted via bile, the rest via other gastrointestinal secretions).[9] Mutations of this gene lead to a lack of copper transport from the liver into the bile and to a deficient incorporation of copper into ceruloplasmin. Other copper-containing enzymes are: Zinc-Cu superoxide dismutase, which plays a fundamental role in oxidative processes; dopamine mono-oxygenase, which is involved in the synthesis of neurotransmitters; lysyl oxidase, which is involved in

bone formation; Leiden factor V, the deficiency of which leads to coagulation disorders; cytochrome C oxidase, the deficiency of which can manifest itself through several systemic symptoms.[10] Copper deficiency is characterized by hair and skin changes, muscle weakness, neurological disorders such as ataxia, neuropathy and cognitive impairment, edema, hepatosplenomegaly, and osteoporosis. It can also lead to anemia and neutropenia, the main hematologic features of copper deficiency.[5] In addition, copper is involved in processes that regulate oxidative stress (OS). Under physiological conditions, there is a balance between the products of metabolic processes that use oxygen (O2) as fuel for energy production, the so-called reactive oxygen species (ROS) and antioxidant agents. When this balance is disturbed, an increase in circulating ROS leads to the phenomenon of OS, which can cause damage to several cellular structures. If not adequately controlled, OS may be involved in the development of chronic and/or degenerative diseases such as cancer and cardiovascular disease.[11] In addition, minor copper deficiencies may contribute to the onset and progression of several pathologies, including diabetes. On the other hand, excessive copper concentration in the body can cause toxicity in many human organs, resulting in various diseases and, in rare cases, death.[6] Triethylenetetramine (TETA) has recently been identified as the first in a new class of anti-diabetic molecules through the original work reviewed here, thus providing a new use for this molecule, which was previously approved by the US FDA in 1985 as a second-line treatment for Wilson's disease. TETA acts as a highly selective divalent copper (CuII) chelator that prevents or reverses diabetic copper overload, thereby suppressing oxidative stress. TETA treatment of diabetic animals and patients has identified and quantified the interlinked defects in copper metabolism that characterize this systemic copper overload state. Copper overload in diabetes mellitus differs from that in Wilson's disease through differences in their respective causative molecular mechanisms, and resulting differences in tissue localization and behavior of the excess copper.[7] Elevated pathogenetic tissue binding of copper occurs in diabetes. It may well be mediated by advanced-glycation end product (AGE) modification of susceptible amino-acid residues in long-lived fibrous proteins, for example, connective tissue collagens in locations such as blood vessel walls. These AGE modifications can act as localized, fixed endogenous chelators that increase the chelatable-copper content of organs such as the heart and kidneys by binding excessive amounts of catalytically active CuII in specific vascular beds, thereby focusing the related copper-mediated oxidative stress in susceptible tissues. In this review, summarized evidence from our clinical studies in healthy volunteers and diabetic patients with left-ventricular hypertrophy, and from nonclinical models of diabetic cardiac, arterial, renal and neural disease is used to construct descriptions of the mechanisms by which TETA treatment prevents injury and regenerates damaged organs.[8] Our recent phase II proof-of-principle studies in patients with type 2 diabetes and in nonclinical models of diabetes have helped to define the pathogenetic defects in copper regulation, and have shown that they are reversible by TETA. The drug tightly binds and extracts excess systemic CuII into the urine whilst neutralizing its catalytic activity, but does not cause systemic copper deficiency, even after prolonged use. Its physicochemical properties, which are pivotal for its safety and efficacy, clearly differentiate it from all other clinically available transition metal chelators, including D-penicillamine, ammonium tetrathiomolybdate and clioquinol. The studies reviewed here show that TETA treatment is generally effective in preventing or reversing diabetic organ damage, and support its ongoing development as a new medicine for diabetes.[9] Trientine (TETA dihydrochloride) has been used since the mid-1980s as a second-line treatment for Wilson's disease, and our recent clinical studies have reinforced the impression that it is likely to be safe for long-term use in patients with diabetes and related metabolic disorders. There is substantive evidence to support the view that diabetes shares many pathogenetic mechanisms with Alzheimer's disease and vascular dementia. Indeed, the close epidemiological and molecular linkages between them point to Alzheimer's disease/vascular dementia as a further therapeutic target where experimental pharmacotherapy with TETA could well find further clinical application. Diabetes represents a real pandemic problem both for the public economy and for global health.[12] The development of novel therapies has helped to counteract this global phenomenon and ensure a more personalized approach, but dietary regulation and the adequate intake of essential elements are an indispensable aspect of treatment strategies.[13,14] In the kidney, a correct balance of copper seems to be essential: an increased blood concentration of this ion in the kidney may condition its renal deposition, leading to nephrotoxicity associated with interstitial damage that can lead to progressive renal function impairment.[15] Copper excretion in the urine may be related to dissociation from the albumin–copper complex of the serum as it passes through the kidney. In diabetics with progressive renal dysfunction, urinary excretion of this element may be due to dissociations of both albumin–copper and ceruloplasmin–copper complexes filtering through the damaged glomerulus. Urinary copper overload of the altered renal tubules may play a role in the progression of renal dysfunction in patients with advanced CKD.

Chelation therapy for diabetic complications

Advanced glycation end products (AGE) accumulate in long-lived tissue proteins with age and at an accelerated rate in diabetes and other chronic inflammatory diseases[1,2], including atherosclerosis and neurodegenerative diseases. The increase in AGEs develops in parallel with other chemical modifications of proteins, including advanced lipoxidation end products (ALE) and protein oxidation products (PrOP).[3,4] Tissue damage accrues not only because of protein chemical

modification and cross-linking reactions but also because of inflammatory processes initiated by the interaction of modified proteins with the receptor for AGE as well as scavenger and toll-like receptors.[5–7] Metal-catalyzed autoxidation reactions, i.e., oxidation by molecular oxygen, participate in the formation of ALEs, PrOPs, and most AGEs, supporting the close relationship between oxidative stress and age- and disease-related chemical modification of proteins. Protein carbonyls are intermediates in the formation of AGEs, ALEs, and PrOPs, and AGE inhibitors such as pyridoxamine actually inhibit the formation of all three classes of chemical modifications of protein.[8–10] However, in a recent Perspectives article in the journal Diabetes[11], we made the following observations. There is no evidence that AGE inhibitors actually trap carbonyl or dicarbonyl compounds in vivo or that dicarbonyl cross-links, the putative targets of AGE breakers, actually exist in vivo. Chelation is the common mechanism of action of both AGE inhibitors and breakers. Indeed, LR compounds, which have no nucleophilic functional groups with potential carbonyl-trapping activity, are both AGE inhibitors and AGE breakers and have strong chelating activity.[12,13]

Role of copper on DM

Diabetes is a multi-faceted metabolic disorder whose current definition is based on the demonstration of pathologically elevated blood glucose levels. It causes most of its morbidity and mortality through its propensity to cause damage in organs, including the heart, arteries, kidneys, retina and nerves. In many respects, diabetes can be considered as a microvascular disease, which is a leading cause of blindness, renal failure and nerve damage, and diabetes-accelerated atherosclerosis leads to increased risk of myocardial infarction, stroke and limb amputation.[11] However, blood vessel disease detectable before glucose levels become elevated is an early pathological finding before and at its onset, and hyperglycaemia may be neither the only nor indeed the earliest metabolic perturbation to occur in either of the major forms of diabetes. The question of whether elevated glucose is the cause of blood vessel disease in diabetes remains unresolved. Intensive blood glucose control may or may not prevent or reverse diabetic tissue damage, which is probably more responsive to anti-hypertensive therapy, and diabetic vascular disease remains largely refractory to all available treatments. The precise nature of oxidative stress in diabetes, and of the exact mechanism by which it might cause tissue damage, are questions that are yet to be answered.[3] One prominent suggestion has focused on four main molecular mechanisms that may reflect a single hyperglycaemia-induced process of superoxide overproduction by the mitochondrial electron-transport chain leading to progressive compromise of anti-oxidant defenses. Another less widely known suggestion implicates excessive transition metal-catalyzed oxidative stress.[1,3] Free iron and copper ions are highly redoxactive, and could contribute to tissue damage by generating ROS, in part through hydroxyl radical formation,[8] but the in vivo bioavailability of catalytically active iron and copper is usually very restricted. There is evidence, reviewed below, that diabetes itself may increase the bioavailability of catalytically active CuII, causing oxidative stress and tissue damage that is suppressible by highly selective copper chelation.

Regulation of cell copper uptake, distribution and export of copper regulation

The main biochemical role for copper is catalytic, and many copper enzymes act as oxidases that catalyze the reduction of dioxygen. There is a high degree of conservation of copper-regulating mechanisms between all eukaryotes. Copper is an essential but highly toxic nutrient, so its metabolism must be closely regulated in physiology.[12] Cellular copper homeostasis is coordinated by processes that ensure its delivery to specific sub cellular compartments and copper requiring proteins without releasing free copper ions that could otherwise damage cellular structures. These processes are mediated by proteins including integral transmembrane transporters such as high-affinity copper transporter 1 (Ctr1), metallo regulatory sensors and diffusible cytoplasmic metallo chaperone proteins that protect and carry metal ions to copper metalation targets such as cytochrome c oxidase and copper/zinc superoxide dismutase 1 (SOD1). Alongside these are parallel cellular mechanisms that can defend against any untoward release of ‘free’ copper ions, including sequestration by metallo thioneins and export via the copper-transporting adenosine triphosphatases (ATPases), ATP7A and ATP7B.

Role of copper in diabetic kidney disease

Renal failure and diabetes are also associated with disturbances in antioxidant homeostasis and chronic inflammation. The study by Stancic A et al. compared the activity of copper-zinc SOD in diabetic hypertensive patients with or without renal insufficiency and a control group. The results showed that SOD activity was significantly higher in diabetics with renal insufficiency, suggesting that disturbances in antioxidant homeostasis are associated with complications of diabetes such as hypertension and renal failure.[13]

The extent of copper excretion in urine was associated with the different stages of DKD. In studies by Ito S. et al. , 41 type 2 diabetic patients with different stages of nephropathy and 10 healthy controls were recruited and serum copper/albumin and copper/ceruloplasmin ratios were determined and tested whether they tended to dissociate in response to changes in urine pH. The results showed that urinary copper was significantly increased only in patients with macroalbuminuria. Urinary copper/ceruloplasmin and copper/albumin ratios were greater than in serum and equal between patients and healthy controls, except for the copper/albumin ratio in patients with macroalbuminuria. Reports in urine

decreased when nephropathy worsened. Copper tends to dissociate from its carrier protein under acidic pH conditions. A damaged glomerulus due to nephropathy may cause greater dissociation of the copper/albumin and copper/ceruloplasmin complexes, and urinary copper overload may, in turn, play an important role in the progression of nephropathy.

Copper Deficiency in Humans

Copper deficiency in humans is rare, but has been observed under a number of scenarios. These include long-term parenteral nutrition with insufficient copper intake, infants recovering from protein-energy malnutrition and feeding of cows' milk to premature infants. Documented signs of copper deficiency in humans include low plasma copper (hypocupraemia) with low serum caeruloplasmin values, oedema, anaemia and neutropenia, all of which respond to increased copper intake.[14] Genetic defects in copper metabolism that cause cellular copper deficiency through defective copper transport have also been identified: these include Menkes' disease, in which severe neurodegeneration is caused by mutations in the ATP7A gene that encodes the copper transport protein ATP7A (for review, see Lutsenko et al.), and defects in the SCO2 gene that encodes a copper chaperone that transports copper to/metalates cytochrome c oxidase and deficiency of which causes defective intracellular transport of copper to that enzyme, leading to its impaired function and a lethal cardiomyopath.[14,15]

Chronic copper toxicity in mammals

The generally low incidence of chronic copper toxicosis in mammals, despite considerable variation in copper intakes, probably reflects the efficiency of the homeostatic control mechanisms that operate at the levels of both intestinal absorption and biliary excretion to keep tissue copper concentrations within a tolerable range. Different mammals display quite different tolerances to toxic levels of copper exposure, due probably to variation in species-specific factors as well as genetics, age and diet.[15] For example, pigs are relatively resistant to high copper intakes, whereas sheep are much more susceptible, possibly because they are less able to excrete excess copper into the bile. Neonatal and milk-fed animals are more susceptible to copper poisoning than their adult counterparts, probably because of the high efficiency of copper absorption and the immaturity of biliary excretory mechanisms, an observation that may help to explain why chronic copper toxicosis in humans manifests mainly in childhood. Dietary antagonists of copper metabolism, such as sulfur and other trace metals, and of hepatotoxins or protective factors are also important. For example, dietary zinc supplements, which can restrict the uptake and accumulation and modify the distribution of copper in the liver, can be used to prevent copper toxicosis, both in sheep and in patients with Wilson's disease.

Molecular pathways that combat tissue damage by copper excess

To avoid copper-induced toxicity, most organisms use a redundant combination of copper regulated import inhibition, sequestration and enhanced export mechanisms.[16] Combinations of these mechanisms are used to provide a detoxification pathway controlled through copper binding proteins at the transcriptional, translational or enzymatic-function levels. In yeast, an important regulatory mechanism occurs via copper-mediated activation of the copper-binding transcription factor Ace1p, which regulates key aspects of copper metabolism through binding to the copper regulon, up-regulating anti copper defences such as those mediated by SOD1 and metallothionein, whilst concomitantly down regulating the high-affinity cell membrane copper transporter, Ctr1and its associated iron-dependent copper reductase, Fre1 In mammalian cells, copper is partially detoxified by sequestration in the metal-binding metallothioneins, or export via the copper-translocating ATPases; these pathways can be redundant in function and the molecular processes that coordinate them are incompletely understood at present. Trientine in Wilson's Disease: A Brief Summary Wilson's disease is a rare disorder of copper metabolism that causes progressive copper overload and consequent tissue damage particularly affecting the liver, CNS and eyes, although it can also damage other organs, including the heart It is caused by genetically transmitted defects in the ATP7B transporter gene, and, if untreated, is almost always fatal. Tissue distribution of ATP7B thus determines the location of copper excess in Wilson's disease, which in turn localizes the tendency to tissue damage.[17]

The mainstay of treatment in Wilson's disease is copper chelation with penicillamine, to which ammonium tetrathiomolybdate, a second copper chelator and/or zinc therapy may be added in certain cases. Trientine is a potent copper chelator that was first registered by the US FDA in 1985 for the second-line treatment of Wilson's disease. In brief, it is effective both in reducing excess body copper storage and in ameliorating symptoms in patients with Wilson's disease. The drug has generally demonstrated acceptable safety and efficacy profiles, although it has occasionally been linked to haematological abnormalities such as sideroblastic anaemia and thrombocytopenia, and has also been reported to cause low serum iron without anaemia. Based on its pharmacological and physicochemical properties, I chose to employ trientine over other known chelators such as penicillamine or tetrathiomolybdate, to probe systemic copper regulation in diabetes. I took this decision in part because of perceived downsides to the others, including the high rates of adverse reactions and other attendant risks of penicillamine, and the known propensity of molybdenum overload to induce/exacerbate diabetes, to cause neurodegeneration under some circumstances, and therefore, potentially to

aggravate diabetic neuropathy and to cause molybdenosis.

Clinical application of copper chelation therapy

A chelator is a chemical compound able to selectively bind, due to its structure, a particular atom/ion, with the formation of a stable complex ring-like structure.[18] Metal chelating agents are used as nutritional supplements, for designing radiopharmaceuticals, as additives for cleaning chemicals, cosmetics, plastics, fertilizers, growth supplements in aquaculture, and to remove toxic metals from soil and in the body (chelation therapy).[9] For a detailed biochemical description of several copper chelating agents, the reader is directed to a previously published review.[10] Copper overload toxicity as well as clinically significant copper deficiency are rare and mostly associated with genetic defects of copper transport such as Wilson's disease (copper overload) and Menkes disease (copper deficiency).

On the other hand, copper is an essential catalytic cofactor in redox biochemistry; consequently, copper dyshomeostasis leading to its unpaired distribution has been linked with several disorders including diabetes, neurological disorders and cancer.[11] Different chelating drugs have been shown to modulate copper levels by different mechanisms; in particular, penicillamine, trientine, and dimercaptosuccinic acid form complexes which are excreted in the urine, while tetrathiomolybdate promotes copper biliary excretion. In addition, administration of zinc salts has been suggested as maintenance treatment for Wilson's disease; zinc interferes with the gastrointestinal copper uptake by inducing metallothionein, which chelates copper, preventing absorption and allowing for its excretion in the feces. The use of copper chelating drugs such as trientine in Wilson's disease and in cancer patients has been considered safe[12,13]; nonetheless, the specific risk–benefit ratio for each therapeutic indication should be carefully evaluated by additional randomized clinical trials.

Copper chelation and radiotherapy

Increased efficacy of radiotherapy against primary tumors with reduced side effects can be achieved when combined with antiangiogenic agents. Along these lines, an additive effect of radiotherapy and copper chelation therapy has been observed in a Lewis lung high metastatic carcinoma mouse tumor model.[19]

Copper chelation and immunotherapy

Immunotherapy treatments have been designed to modulate patient's own immune system to fight against cancer. There are several immunotherapy strategies, including the use of monoclonal antibodies, immune cell activators, immune checkpoint inhibitors and oncolytic viral vectors. In the following subsections we review the main copper chelation and immunotherapy combination strategies.

Copper chelation and monoclonal antibodies immunotherapy

The monoclonal antibody Cetuximab, which binds specifically to the epidermal growth factor receptor (EGFR) thereby blocking transmission the relative proliferative signaling pathways, is an example of an immunotherapeutic agent.[20] Combination of TM and Cetuximab has been evaluated in a murine model of head and neck squamous cell carcinoma but no statistically significant differences were observed between single and combined treatments . Therefore, further investigations are needed to determine the clinical significance of combining copper chelation and monoclonal antibodies-mediated immunotherapy.

Copper chelation and immune activation

Copper chelation has been proposed in conjunction with immune activation for cancer immunotherapy. In particular, Zhou et al. recently developed a copper chelator used to prepare nanoparticles suitable for loading and delivery to the tumor the Toll-like receptor agonist R848, in order to stimulate antitumor immunity by dendritic cells activation.[21] This strategy of nanoparticle-based copper chelation and immune stimulation effectively inhibits breast tumor growth and metastasis in experimental models both in vitro and in vivo.

Copper chelation and immune checkpoint inhibitors

An important strategy for cancer immunotherapy targets the interactions between the immune checkpoints programmed cell death protein 1 (PD-1) and the programmed cell death ligand 1 (PD-L1) using specific antibodies. A positive correlation between the copper transport protein CTR1 and PD-L1 expression has been observed in neuroblastoma and glioblastoma tumor cells. Interestingly, copper chelation reduces PD-L1 expression, promoting a significant increase in tumor-infiltrating lymphocytes in a syngeneic mouse model of neuroblastoma. Therefore, copper chelation therapy may promote the efficacy of PD-1/PD-L1 based immunotherapy.

Copper chelation and oncolytic virotherapy

Oncolytic vectors selectively replicate and promote lysis of cancer cells triggering the patient's immune system against tumor antigens. Changes in the tumor microenvironment in response to induced oncolysis may limit the efficacy of oncolytic virotherapy.[21,22] Therefore, is has been hypothesized that combination of copper chelation therapy, which affects both tumor microenvironment and angiogenesis, may promote the efficacy of oncolytic virotherapy. In addition, serum copper levels have a detrimental effect on herpes virus infection. Based on these premises, it has been described that concomitant copper chelation therapy increases antitumor effect of herpes simplex virus–derived oncolytic viruses.[22]

CONCLUSIONS

Most of the studies reported in our review show an association between advanced age and higher copper levels in patients with diabetes, while in the younger population the risk of developing T1DM still increases 15-fold for one standard deviation of copper levels. Age-related chronic diseases lead to mechanisms that increase serum concentrations of copper and decrease serum concentrations of zinc, especially in the presence of inflammatory conditions, thus a common feature of several age-related chronic diseases is an increase in the copper/zinc ratio. The copper/zinc ratio is also associated with physical decline and mortality in the elderly population.

Accumulation of CuII-binding AGEs in the ECM of long-lived proteins, such as collagen, is thought to initiate the retention and targeting of catalytically active/chelatable CuII in the vasculature of organs such as the kidney and the heart, and to mediate localized oxidative stress via catalysis of ROS such as hydroxyl radicals. Treatment with highly selective copper chelation is a new experimental therapy for the treatment of diabetes that has so far been proven to be safe and efficacious in nonclinical models and in patients with T2DM, and merits further clinical investigation. The metabolic defect that initiates diabetic vasculopathy is unlikely to be triggered by hyperglycaemia, but probably begins with alterations in the homeostasis of lipoproteins and lipids perhaps driven by adiponectin deficiency. Glucose-mediated processes may well contribute to the generation of AGEs and consequent disturbance of copper regulation downstream in the development of the pathogenetic process. According to the available evidence, copper levels, diabetes, and its complications are not simply positively correlated, but have a complex relationship. Nevertheless, more specifically designed prospective articles should be conducted to understand the dynamic relationship of copper, copper/zinc balance, and the progression of diabetes. These data indicate the need for dietary and health behavior changes to better control the potential complications associated with T1DM and T2DM. Measurement of trace elements is a supportive tool in the management of this pandemic disease. People with DKD are more likely to have a disturbance in the homeostasis of essential metals. The imbalance of copper can lead to the progression of diabetes-related complications and impaired antioxidant homeostasis. A high Zn/Cu ratio seems to be associated with improved renal function and a lower risk of poor glycemic control in T2DM patients. In addition, the extent of urinary copper excretion appears to be related to the DKD progression, which plays a possible role in the opposing DM complications. Experimental models in vitro, as well as animal and human studies, suggest that maintaining adequate copper levels may play a role in the pathophysiology of diabetes and DKD. Unfortunately, there is a lack of large and interventional studies focusing on copper balance to confirm these findings. Evidence on the association between copper and DKD is still scarce and the results remain inconsistent. It should also be emphasized that the function of copper should be considered as part of a more complex system of factors that influence oxidative stress and condition its protective/damaging influence on DM and DKD. More studies are needed to adequately investigate the role of copper in DKD and the results available in the literature are still controversial.

REFERENCES

1. Wolff SP, Jiang ZY, Hunt JV. Protein glycation and oxidative stress in diabetes mellitus and ageing. Free Radic Biol Med., 1991; 10(5): 339-52.
2. Wolff SP. Diabetes mellitus and free radicals: free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. Br Med Bull, Jul, 1993; 49(3): 642-52.
3. Monnier VM. Transition metals redox: reviving an old plot for diabetic vascular disease. J Clin Invest, Apr, 2001; 107(7): 799-801.
4. Thomas MC, Baynes JW, Thorpe SR, et al. The role of AGEs and AGE inhibitors in diabetic cardiovascular disease. Curr Drug Targets, 2005; 6: 453-74.
5. Jain S, Purohit A, Nema P, Vishwakarma H, Jain PK. A brief review on nutraceuticals and its application. Asian Journal of Dental and Health Sciences, Mar 15, 2022; 2(1): 7-13.
6. Martinez A, Portero-Otin M, Pamplona R, et al. Protein targets of oxidative damage in human neurodegenerative diseases with abnormal protein aggregates. Brain Pathol, Mar, 2010; 20(2): 281-97.
7. Forbes JM, Coughlan MT, Cooper ME. Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes, Jun, 2008; 57(6): 1446-54.
8. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 4th ed. Oxford: Oxford University Press, 2007.
9. Gil JM, Rego AC. Mechanisms of neurodegeneration in Huntington's disease. Eur J Neurosci, Jun, 2008; 27(11): 2803-20.
10. Browne SE, Ferrante RJ, Beal MF. Oxidative stress in Huntington's disease. Brain Pathol, Jan, 1999; 9(1): 147-63.
11. Barber SC, Shaw PJ. Oxidative stress in ALS: key role in motor neuron injury and therapeutic target. Free Rad Biol Med., 2010; 48: 629-41.
12. Qureshi A, Jain PK, Shrivastava A, Jain S, Nema P, Jain H. Evaluation of antidepressant activity of aqueous and ethanolic extracts of glycyrrhiza glabra. Asian Journal of Pharmaceutical Education and Research, 2022; 11(2): 84-92.
13. Cooper GJS, Aitken JF, Zhang S. Is type 2 diabetes an amyloidosis and does it really matter (to patients)? Diabetologia, 2010; 53: 1011-6.
14. Zhang S, Liu H, Yu H, et al. Fas-associated death receptor signaling evoked by human amylin in islet beta-cells. Diabetes, Feb, 2008; 57(2): 348-56.
15. Aitken JF, Loomes KM, Scott DW, et al. Tetracycline treatment retards the onset and slows

the progression of diabetes in human amylin/islet amyloid polypeptide transgenic mice. Diabetes, Jan, 2010; 59(1): 161-71.

16. Nema P, Jain S, Vishwakarma H, Purohit A, Jain PK. A complete review on aromatherapy: a complementary alternative medication therapy with recent trend. International Journal of Medical Sciences and Pharma Research, 2021; 7(4): 1-7.
17. Yusuf S, Dagenais G, Pogue J, et al., The Heart Outcomes Prevention Evaluation Study Investigators. Vitamin E supplementation and cardiovascular events in high-risk patients. N Engl J Med., Jan 20, 2000; 342(3): 154-60.
18. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20536 high-risk individuals: a randomised placebo-controlled trial. Lancet, 2002; 360: 23-33.
19. Vishwakarma H, Thakur K, Purohit A, Jain S, Nema P, Jain PK. A herbal approach for the treatment of kidney stone. International Journal of Medical Sciences and Pharma Research, 2022; 8(1): 1-9.
20. Gong D, Lu J, Chen X, et al. Molecular changes evoked by triethylenetetramine treatment in the extracellular matrix of the heart and aorta in diabetic rats. Mol Pharmacol, Dec, 2006; 70(6): 2045-51.
21. Pohlers D, Brenmoehl J, Loffler I, et al. TGF-beta and fibrosis in different organs-molecular pathway imprints. Biochim Biophys Acta, Aug, 2009; 1792(8): 746-56.
22. Roth M. ‘‘Glycated hemoglobin,’’ not ‘‘glycosylated’’ or ‘‘glucosylated’’ [letter]. Clin Chem., Nov, 1983; 29(11): 1991.