The Protection Conferred by Chelation Therapy in Post-MI Diabetics Might be Replicated by High-Dose Zinc Supplementation

Mark F. McCarty, Catalytic Longevity, markfmccarty@gmail.com

Abstract

The recent Trial to Assess Chelation Therapy (TACT) study, enrolling subjects who had previously experienced a myocardial infarction, has provided strong evidence that intravenous chelation therapy can markedly reduce risk for mortality and vascular events in diabetics, whereas no discernible benefit was observed in non-diabetics. It has plausibly been suggested that this reflects a role for transition metal ions – iron or copper – in the genesis of advanced glycation end products, key mediators of diabetic complications that can destabilize plaque. Since phlebotomy therapy fails to prevent vascular events in diabetics, labile copper may be the chief culprit whose removal by chelation mediated the benefit observed in TACT. Indeed, a number of recent studies report that the copper-specific orally-active chelator trientine can reduce risk for range of diabetic complications in rodents; a clinical trial with this agent demonstrated some decrease in left ventricular mass in diabetics with ventricular hypertrophy. However, in light of the current exorbitant expense of this agent, supplementation with high-dose zinc may represent a more feasible alternative. Zinc opposes the absorption and redox activity of copper via induction of the antioxidant protein metallothionein, which binds copper tightly. A great many studies demonstrate that increased expression of metallothionine decreases risk for tissue damage in diabetic rodents, and in some of these studies metallothionine expression was boosted by supplemental zinc. Zinc supplementation also modestly improves glycemic control in type 2 diabetics, and might reduce risk for diabetes by protecting pancreatic beta cells from oxidative stress. A long term study assessing the impact of supplementing diabetics with high-dose zinc, assessing risk for mortality, vascular events, and diabetic complications, may be warranted. Histidine, which readily forms complexes with copper that possess superoxide dismutase activity, also has potential for alleviating the contribution of loosely bound copper to AGE formation; moreover, in a recent clinical study, supplemental histidine improved insulin sensitivity and exerted anti-inflammatory and antioxidant effects in women with metabolic syndrome.

Chelation Therapy Decreases Vascular Events in Diabetics – A Role for AGE Reduction?

IN the Trial to Assess Chelation Therapy (TACT), subjects who had previously experienced a myocardial infarction were randomized to receive intravenous EDTA or placebo solutions 40 times over approximately one year, and were followed for five years; primary endpoints assessed were death, re-infarction, stroke, coronary revascularization, or hospitalization for angina.[1] Somewhat surprisingly, a major reduction in the primary endpoints was observed in the 633 diabetic subjects (hazard ratio 0.59, 95% CI 0.44-0.79; $p<0.001$), whereas no hint of benefit was observed in the non-diabetics. (Separate analysis of the diabetics had been pre-specified in the study's protocol.) Some commentators have quite plausibly suggested that diabetics achieved selective benefit because transition metals (iron and copper) play an obligate role in the generation of advanced glycation end products (AGEs), which are thought to be key mediators of the micro- and macrovascular complications of diabetes.[1-3] The redox activity of these metals, interacting with the hydrogen peroxide that is typically elevated in diabetic tissues,

engenders hydroxyl radicals, which in turn convert reversible protein-bound Amadori products to irreversible AGEs.[4] It is notable that AGEs, the production of which is notably enhanced by the hyperglycemia and oxidative stress associated with diabetes, can act to destabilize plaque.[5] A reasonable interpretation of the TACT results would therefore be that chelation therapy, by decreasing tissue levels of labile transition metals and thereby lessening formation of AGEs, helped to stabilize the atheromatous plaque of diabetics, decreasing risk for vascular events.

Copper, Rather Than Iron, May be the Key Target of Chelation Therapy

If this formulation is essentially correct, is either iron or copper the primary culprit whose removal was protective in the TACT study? A previous study may cast some light on this issue. The Iron and Atherosclerosis Study (FeAST) randomized 1277 patients with stable symptomatic peripheral arterial disease to a control group or a group receiving repeated phlebotomies.[6] In treated patients who were reasonably compliant, baseline ferritin levels were reduced by approximately half, whereas these did not change in the controls. The secondary endpoints assessed during about 6 years of follow up were death plus non-fatal myocardial infarction or stroke. 473 diabetics were included in this study; among these, the secondary endpoints were observed in 34.5 % of the controls, and 33.2% of those who received phlebotomies. In other words, a substantial reduction in tissue iron stores did not influence subsequent risk for vascular events in high-risk diabetics. This result renders doubtful the possibility that removal of tissue iron was largely responsible for the protection afforded diabetics in the TACT study.

What then about copper? Over the last decade, a number of studies from several groups have demonstrated that treatment of diabetic rats or humans with the copper-selective chelating agent trientine - long used in the treatment of Wilson's disease – can exert a range of favorable effects: improving endothelium-dependent vasodilation, alleviating diabetes-associated heart failure and hypertrophy, preventing renal fibrosis and albuminuria, and decreasing AGE formation in the lens.[7-16] Clinically, in diabetic patients with left ventricular hypertrophy at baseline, a one-year course of oral trientine (600 mg twice daily) was associated with a gradual reduction in left ventricular mass, whereas no change was observed in the patients receiving placebo.[11] Cooper notes that the chelatable copper stores in diabetic rats are higher than those in non-diabetic rats; he suggests that this reflects the fact that certain AGE-modified proteins can bind copper ions in such a way that they have strong redox activity.[14, 17-19] Other researchers have reached a similar conclusion, and have shown that the tail tendons of diabetic rats, as compared to those of non-diabetic rats, have about twice the copper content .[20] Hence, the formation of AGEs may increase the level of redox-active labile copper in tissues, potentially catalyzing the formation of additional AGEs in a vicious cycle.

These findings are consistent with the proposition that copper removal was largely responsible for the benefits conferred on diabetics by chelation therapy in the TACT study, and that trientine or other copper-selective chelators may have important utility for decreasing risk for vascular events in diabetics, and likely decreasing risk for the range of diabetic complications as well. Unfortunately, though, trientine, as an orphan drug employed in the treatment of Wilson's disease, is currently very expensive – currently over $3,000 for 30 capsules (250 mg) in the U.S.

Supplemental Zinc, via Metallothionein Induction, Prevents Diabetic Complications

What alternative strategies might be feasible? It is well known that high intakes of supplemental zinc can be employed in the treatment of Wilson's disease (often as maintenance therapy after an initial course of trientine), owing to the fact that high-dose zinc promotes induction of the protein metallothionein.[21-24] This protein, an antioxidant rich in cysteine groups, binds not only to zinc, but also copper and cadmium; in this bound form, copper and cadmium are innocuous. Zinc-mediated metallothionein induction in gastrointestinal epithelium tends to inhibit absorption of dietary copper, as metallothionein-bound copper is trapped in these cells, which subsequently are sloughed off into the intestinal lumen.[25, 26] Hence, it is reasonable to propose that high-dose zinc supplementation might lessen the complications of diabetes by opposing the impact of tissue copper on AGE formation. The dose schedules of zinc that have been employed effectively in Wilson's disease, with good tolerance – 50 mg zinc 2 or 3 times daily, or 25 mg 3 times daily[27] – can be expected to achieve a notable induction of metallothionein.

There is indeed a substantial literature demonstrating that increased expression of metallothionein can be quite protective in diabetic rodents – whereas metallothionein knock out exacerbates the complications of diabetes.[28, 29] In diabetic rodents, a general or tissue-specific increase in metallothionein is reported to provide protection from diabetic cardiomyopathy, nephropathy, and neuropathy – and also protects pancreatic beta cells from oxidative damage, decreasing onset of diabetes in some models.[29-48] In many of these studies, transgenic strategies were used to increase metallothionein expression – but in some of them, zinc administration was employed. Zinc supplementation *per se* has been found to decrease pathogenic changes in the aorta, and inhibit the progression of diabetic cardiomyopathy and nephropathy.[29, 32, 36, 38, 45, 46, 48-50] These studies do not clarify whether copper sequestration contributed importantly to the observed benefits – cysteine-rich metallothionein can function as a radical scavenger - but, in light of the foregoing discussion, this is a highly credible possibility.

The ability of metallothionein to protect beta cells casts an interesting light on epidemiology suggesting that relatively high dietary intakes or serum levels of zinc may confer some protection from type 2 diabetes.[51, 52] Meta-analyses of controlled clinical trials examining the impact of supplemental zinc on glycemic control in diabetics concludes that such supplementation is modestly beneficial in this regard.[53, 54] Unfortunately, these were relatively short term studies not designed to test the impact of supplemental zinc on risk for diabetic complications. A long term trial of high-dose zinc supplementation, in amounts sufficient to achieve a notable induction of tissue metallothionein, examining as end points mortality, vascular events, and progression of diabetic complications (nephropathy, cardiomyopathy, retinopathy, neuropathy), may be warranted in light of the growing evidence that labile copper plays a key malign role in promoting AGE formation and the many AGE-driven complications of diabetes.

It should be noted that zinc supplementation, via metallothionein induction, can also oppose the toxicity of cadmium.[55-58] Increased body stores of cadmium, reflected in an increase in urinary cadmium, have been linked to increased risk for a number of pathologies, including nephropathy and atherosclerosis, key complications of diabetes.[59-64] These apparent adverse effects of cadmium are observed in the general population, not just in subgroups with high occupational exposure. It therefore seems likely that high-dose zinc supplementation might also protect diabetics by lessening the impact of cadmium exposure. Unfortunately, chelation therapy is not an effective strategy for removing cadmium from the body, which

persists with a half-life of 10-30 years.[60] Hence, the benefits observed in the TACT study were not likely mediated by cadmium reduction.

With respect to the safety and appropriateness of high-dose zinc supplementation, attention should be drawn to the AREDS1 study, which examined the impact of supplemental antioxidants or zinc (80 mg daily, accompanied by 2 mg of copper to prevent induction of copper deficiency) on progression of age-related macular degeneration.[65] During a median follow-up of 6.5 years, total mortality in the zinc-supplemented subjects, relative to those not receiving zinc, was found to be 27% lower (RR, 0.73; 95% CI, 0.61-0.89). This intriguing finding, though robust, appears to have attracted little attention; it may imply that metallothionein has greater protective health potential than is commonly appreciated. However, zinc supplementation may also decrease mortality among the elderly by supporting efficient immune function and decreasing risk for infection.[66, 67]

In the management of Wilson's disease, zinc supplementation is usually employed as maintenance therapy after a course of trientine therapy has decreased body copper stores.[21] If in the future trientine is approved for use in diabetes, and becomes available at a more reasonable cost that is insurance-reimbursable, a similar regimen – intermittent trientine, with zinc maintenance – might be the most effective strategy for diabetics. Although trientine may be inherently quicker and more thorough for decreasing extracellular copper levels than is zinc, metallothionein induction by zinc should provide some advantages – intracellular oxidant scavenging, cadmium detoxification – not achievable with trientine alone. Presumably, zinc could also be employed in conjunction with a course of EDTA chelation therapy, which, despite its cost and inconvenience, now has documented efficacy in diabetes.

Alternative Copper Chelators – Focus on Histidine

A number of agents – drugs and natural metabolites – have moderate affinity for copper, and their copper chelates have limited redox activity. Although these agents do not boost urinary loss of copper as trientine does, they have the potential to compete for the loosely bound copper affiliated with AGE-modified proteins, and hence may render this copper less pathogenic.[2, 68, 69] Baynes and colleagues have noted that a number of commonly used drugs can suppress copper-catalyzed oxidation of ascorbate – a standard assay for copper's redox activity – in low micromolar concentrations that may be clinically relevant.[2, 68, 69] Of particular interest is the fact that the amino acid histidine accomplishes this with an IC_{50} of 12 μM; its derivative carnosine is even more potent in this regard (IC_{50} = 4μM).[68] Remarkably, copper-histidine complexes have been reported to have superoxide dismutase activity with a rate constant similar to that of the enzyme.[70, 71] And, inasmuch as histidine is an oxidant scavenger, it has been suggested that any hydroxyl radical generated by interaction of hydrogen peroxide with a Cu-II-histidine complex would likely be quenched immediately by the histidine itself.[70] The fact that copper histidinate is well tolerated when administered parenterally to patients with the copper deficiency disorder Menkes' disease tends to confirm the innocuous nature of this complex.[72] It would be of interest to determine whether supplemental histidine might limit the contribution of labile copper to AGE production in diabetics.

This possibility is made all the more attractive by a reports that dietary histidine exerts anti-inflammatory and antioxidant effects in diabetic mice and in women with metabolic syndrome.[73, 74] In the clinical study, a randomized double-blind protocol, 2 g of histidine were administered twice daily; as compared to placebo-treated subjects, those receiving histidine achieved improved insulin sensitivity, a reduction in fat

mass, decreases in markers of oxidative stress, serum inflammatory cytokines (IL-6 and TNF-alpha), and non-esterified fatty acids, and an increase in adiponectin. In observational studies, plasma histidine correlates inversely with insulin resistance, oxidative stress, and inflammatory markers; in patients with chronic kidney disease, low plasma histidine also predicts increased mortality.[75-77] The many beneficial effects of orally administered carnosine documented in animal studies[78, 79] may be largely attributable to derived histidine, as carnosinase rapidly degrades carnosine in plasma.[80] Indeed, the antioxidant and acid-buffering merits of carnosine stem largely from its histidine moiety, and the purpose of carnosine synthesis in muscle fibers and neurons may be to trap a high concentration of non-protein histidine in these cells.[79] How histidine mediates anti-inflammatory effects is unclear; copper chelation, oxidant scavenging activity, a boost in carnosine synthesis, and increased generation of histamine conceivably might play a role.[81] Further clinical investigation of supplemental histidine is clearly warranted. Zinc histidinate may be a preferable source of zinc for supplementation in diabetics.

References

(1) Escolar E, Lamas GA, Mark DB et al. The Effect of an EDTA-based Chelation Regimen on Patients With Diabetes Mellitus and Prior Myocardial Infarction in the Trial to Assess Chelation Therapy (TACT). *Circ Cardiovasc Qual Outcomes* 2013 November 19.

(2) Frizzell N, Baynes JW. Chelation therapy: overlooked in the treatment and prevention of diabetes complications? *Future Med Chem* 2013 June;5(10):1075-8.

(3) Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation* 2006 August 8;114(6):597-605.

(4) Nagai R, Ikeda K, Higashi T et al. Hydroxyl radical mediates N epsilon-(carboxymethyl)lysine formation from Amadori product. *Biochem Biophys Res Commun* 1997 May 8;234(1):167-72.

(5) Paneni F, Cosentino F. Advanced glycation endproducts and plaque instability: a link beyond diabetes. *Eur Heart J* 2013 October 31.

(6) Zacharski LR, Chow BK, Howes PS et al. Reduction of iron stores and cardiovascular outcomes in patients with peripheral arterial disease: a randomized controlled trial. *JAMA* 2007 February 14;297(6):603-10.

(7) Inkster ME, Cotter MA, Cameron NE. Effects of trientine, a metal chelator, on defective endothelium-dependent relaxation in the mesenteric vasculature of diabetic rats. *Free Radic Res* 2002 October;36(10):1091-9.

(8) Cooper GJ, Phillips AR, Choong SY et al. Regeneration of the heart in diabetes by selective copper chelation. *Diabetes* 2004 September;53(9):2501-8.

(9) Hamada Y, Nakashima E, Naruse K et al. A copper chelating agent suppresses carbonyl stress in diabetic rat lenses. *J Diabetes Complications* 2005 November;19(6):328-34.

(10) Gong D, Lu J, Chen X et al. A copper(II)-selective chelator ameliorates diabetes-evoked renal fibrosis and albuminuria, and suppresses pathogenic TGF-beta activation in the kidneys of rats used as a model of diabetes. *Diabetologia* 2008 September;51(9):1741-51.

(11) Cooper GJ, Young AA, Gamble GD et al. A copper(II)-selective chelator ameliorates left-ventricular hypertrophy in type 2 diabetic patients: a randomised placebo-controlled study. *Diabetologia* 2009 April;52(4):715-22.

(12) Baynes JW, Murray DB. The metal chelators, trientine and citrate, inhibit the development of cardiac pathology in the Zucker diabetic rat. *Exp Diabetes Res* 2009;2009:696378.

(13) Lu J, Gong D, Choong SY et al. Copper(II)-selective chelation improves function and antioxidant defences in cardiovascular tissues of rats as a model of diabetes: comparisons between triethylenetetramine and three less copper-selective transition-metal-targeted treatments. *Diabetologia* 2010 June;53(6):1217-26.

(14) Cooper GJ. Therapeutic potential of copper chelation with triethylenetetramine in managing diabetes mellitus and Alzheimer's disease. *Drugs* 2011 July 9;71(10):1281-320.

(15) Lu J, Pontre B, Pickup S et al. Treatment with a copper-selective chelator causes substantive improvement in cardiac function of diabetic rats with left-ventricular impairment. *Cardiovasc Diabetol* 2013;12:28.

(16) Moya-Olano L, Milne HM, Robinson JM et al. Trientine and renin-angiotensin system blockade ameliorate progression of glomerular morphology in hypertensive experimental diabetic nephropathy. *Pathol Int* 2011 November;61(11):652-61.

(17) Saxena AK, Saxena P, Wu X, Obrenovich M, Weiss MF, Monnier VM. Protein aging by carboxymethylation of lysines generates sites for divalent metal and redox active copper binding: relevance to diseases of glycoxidative stress. *Biochem Biophys Res Commun* 1999 July 5;260(2):332-8.

(18) Monnier VM. Transition metals redox: reviving an old plot for diabetic vascular disease. *J Clin Invest* 2001 April;107(7):799-801.

(19) Saxena P, Saxena AK, Cui XL, Obrenovich M, Gudipaty K, Monnier VM. Transition metal-catalyzed oxidation of ascorbate in human cataract extracts: possible role of advanced glycation end products. *Invest Ophthalmol Vis Sci* 2000 May;41(6):1473-81.

(20) Eaton JW, Qian M. Interactions of copper with glycated proteins: possible involvement in the etiology of diabetic neuropathy. *Mol Cell Biochem* 2002 May;234-235(1-2):135-42.

(21) Schilsky ML. Treatment of Wilson's disease: what are the relative roles of penicillamine, trientine, and zinc supplementation? *Curr Gastroenterol Rep* 2001 February;3(1):54-9.

(22) Sullivan VK, Burnett FR, Cousins RJ. Metallothionein expression is increased in monocytes and erythrocytes of young men during zinc supplementation. *J Nutr* 1998 April;128(4):707-13.

(23) Cao J, Cousins RJ. Metallothionein mRNA in monocytes and peripheral blood mononuclear cells and in cells from dried blood spots increases after zinc supplementation of men. *J Nutr* 2000 September;130(9):2180-7.

(24) Aydemir TB, Blanchard RK, Cousins RJ. Zinc supplementation of young men alters metallothionein, zinc transporter, and cytokine gene expression in leukocyte populations. *Proc Natl Acad Sci U S A* 2006 February 7;103(6):1699-704.

(25) Fischer PW, Giroux A, L'Abbe MR. Effects of zinc on mucosal copper binding and on the kinetics of copper absorption. *J Nutr* 1983 February;113(2):462-9.

(26) Yuzbasiyan-Gurkan V, Grider A, Nostrant T, Cousins RJ, Brewer GJ. Treatment of Wilson's disease with zinc: X. Intestinal metallothionein induction. *J Lab Clin Med* 1992 September;120(3):380-6.

(27) Brewer GJ, Dick RD, Johnson VD, Brunberg JA, Kluin KJ, Fink JK. Treatment of Wilson's disease with zinc: XV long-term follow-up studies. *J Lab Clin Med* 1998 October;132(4):264-78.

(28) Tachibana H, Ogawa D, Sogawa N et al. Metallothionein deficiency exacerbates diabetic nephropathy in streptozotocin-induced diabetic mice. *Am J Physiol Renal Physiol* 2013 October 23.

(29) Islam MS, Loots dT. Diabetes, metallothionein, and zinc interactions: a review. *Biofactors* 2007;29(4):203-12.

(30) Chen H, Carlson EC, Pellet L, Moritz JT, Epstein PN. Overexpression of metallothionein in pancreatic beta-cells reduces streptozotocin-induced DNA damage and diabetes. *Diabetes* 2001 September;50(9):2040-6.

(31) Liang Q, Carlson EC, Donthi RV, Kralik PM, Shen X, Epstein PN. Overexpression of metallothionein reduces diabetic cardiomyopathy. *Diabetes* 2002 January;51(1):174-81.

(32) Wang J, Song Y, Elsherif L et al. Cardiac metallothionein induction plays the major role in the prevention of diabetic cardiomyopathy by zinc supplementation. *Circulation* 2006 January 31;113(4):544-54.

(33) Maret W. A role for metallothionein in the pathogenesis of diabetes and its cardiovascular complications. *Mol Genet Metab* 2008 May;94(1):1-3.

(34) Zheng S, Carlson EC, Yang L, Kralik PM, Huang Y, Epstein PN. Podocyte-specific overexpression of the antioxidant metallothionein reduces diabetic nephropathy. *J Am Soc Nephrol* 2008 November;19(11):2077-85.

(35) Zhou G, Li X, Hein DW et al. Metallothionein suppresses angiotensin II-induced nicotinamide adenine dinucleotide phosphate oxidase activation, nitrosative stress, apoptosis, and pathological remodeling in the diabetic heart. *J Am Coll Cardiol* 2008 August 19;52(8):655-66.

(36) Ohly P, Wang Z, Abel J, Gleichmann H. Zincsulphate induced metallothionein in pancreatic islets and protected against the diabetogenic toxin streptozotocin. *Talanta* 1998 June;46(2):355-9.

(37) Wang Y, Feng W, Xue W et al. Inactivation of GSK-3beta by metallothionein prevents diabetes-related changes in cardiac energy metabolism, inflammation, nitrosative damage, and remodeling. *Diabetes* 2009 June;58(6):1391-402.

(38) Tang Y, Yang Q, Lu J et al. Zinc supplementation partially prevents renal pathological changes in diabetic rats. *J Nutr Biochem* 2010 March;21(3):237-46.

(39) Bellomo EA, Meur G, Rutter GA. Glucose regulates free cytosolic Zn(2)(+) concentration, Slc39 (ZiP), and metallothionein gene expression in primary pancreatic islet beta-cells. *J Biol Chem* 2011 July 22;286(29):25778-89.

(40) Park L, Min D, Kim H et al. Tat-enhanced delivery of metallothionein can partially prevent the development of diabetes. *Free Radic Biol Med* 2011 November 1;51(9):1666-74.

(41) Park L, Min D, Kim H, Park J, Choi S, Park Y. The combination of metallothionein and superoxide dismutase protects pancreatic beta cells from oxidative damage. *Diabetes Metab Res Rev* 2011 November;27(8):802-8.

(42) Min D, Kim H, Park L et al. Amelioration of diabetic neuropathy by TAT-mediated enhanced delivery of metallothionein and SOD. *Endocrinology* 2012 January;153(1):81-91.

(43) Xue W, Liu Y, Zhao J, Cai L, Li X, Feng W. Activation of HIF-1 by metallothionein contributes to cardiac protection in the diabetic heart. *Am J Physiol Heart Circ Physiol* 2012 June 15;302(12):H2528-H2535.

(44) Carlson EC, Chhoun JM, Laturnus DI et al. Podocyte-specific overexpression of metallothionein mitigates diabetic complications in the glomerular filtration barrier and glomerular histoarchitecture: a transmission electron microscopy stereometric analysis. *Diabetes Metab Res Rev* 2013 February;29(2):113-24.

(45) Li B, Tan Y, Sun W, Fu Y, Miao L, Cai L. The role of zinc in the prevention of diabetic cardiomyopathy and nephropathy. *Toxicol Mech Methods* 2013 January;23(1):27-33.

(46) Ozcelik D, Naziroglu M, Tuncdemir M, Celik O, Ozturk M, Flores-Arce MF. Zinc supplementation attenuates metallothionein and oxidative stress changes in kidney of streptozotocin-induced diabetic rats. *Biol Trace Elem Res* 2012 December;150(1-3):342-9.

(47) Cong W, Ma W, Zhao T et al. Metallothionein prevents diabetes-induced cardiac pathological changes, likely via the inhibition of succinyl-CoA:3-ketoacid coenzyme A transferase-1 nitration at Trp(374). *Am J Physiol Endocrinol Metab* 2013 April 15;304(8):E826-E835.

(48) Miao X, Wang Y, Sun J et al. Zinc protects against diabetes-induced pathogenic changes in the aorta: roles of metallothionein and nuclear factor (erythroid-derived 2)-like 2. *Cardiovasc Diabetol* 2013;12:54.

(49) Jansen J, Karges W, Rink L. Zinc and diabetes--clinical links and molecular mechanisms. *J Nutr Biochem* 2009 June;20(6):399-417.

(50) Wang X, Li H, Fan Z, Liu Y. Effect of zinc supplementation on type 2 diabetes parameters and liver metallothionein expressions in Wistar rats. *J Physiol Biochem* 2012 December;68(4):563-72.

(51) Sun Q, van Dam RM, Willett WC, Hu FB. Prospective study of zinc intake and risk of type 2 diabetes in women. *Diabetes Care* 2009 April;32(4):629-34.

(52) Vashum KP, McEvoy M, Shi Z et al. Is dietary zinc protective for type 2 diabetes? Results from the Australian longitudinal study on women's health. *BMC Endocr Disord* 2013;13(1):40.

(53) Jayawardena R, Ranasinghe P, Galappatthy P, Malkanthi R, Constantine G, Katulanda P. Effects of zinc supplementation on diabetes mellitus: a systematic review and meta-analysis. *Diabetol Metab Syndr* 2012;4(1):13.

(54) Capdor J, Foster M, Petocz P, Samman S. Zinc and glycemic control: a meta-analysis of randomised placebo controlled supplementation trials in humans. *J Trace Elem Med Biol* 2013 April;27(2):137-42.

(55) Brzoska MM, Moniuszko-Jakoniuk J. Interactions between cadmium and zinc in the organism. *Food Chem Toxicol* 2001 October;39(10):967-80.

(56) Bulat ZP, Djukic-Cosic D, Malicevic Z, Bulat P, Matovic V. Zinc or magnesium supplementation modulates cd intoxication in blood, kidney, spleen, and bone of rabbits. *Biol Trace Elem Res* 2008 August;124(2):110-7.

(57) Rogalska J, Pilat-Marcinkiewicz B, Brzoska MM. Protective effect of zinc against cadmium hepatotoxicity depends on this bioelement intake and level of cadmium exposure: a study in a rat model. *Chem Biol Interact* 2011 September 30;193(3):191-203.

(58) Brzoska MM, Roszczenko A, Galazyn-Sidorczuk M, Majewska K. Zinc supplementation can protect from enhanced risk of femoral neck fracture in male rats chronically exposed to cadmium. *Exp Toxicol Pathol* 2011 July;63(5):491-8.

(59) Jarup L, Akesson A. Current status of cadmium as an environmental health problem. *Toxicol Appl Pharmacol* 2009 August 1;238(3):201-8.

(60) Nawrot TS, Staessen JA, Roels HA et al. Cadmium exposure in the population: from health risks to strategies of prevention. *Biometals* 2010 October;23(5):769-82.

(61) Everett CJ, Frithsen IL. Association of urinary cadmium and myocardial infarction. *Environ Res* 2008 February;106(2):284-6.

(62) Agarwal S, Zaman T, Tuzcu EM, Kapadia SR. Heavy metals and cardiovascular disease: results from the National Health and Nutrition Examination Survey (NHANES) 1999-2006. *Angiology* 2011 July;62(5):422-9.

(63) Lee MS, Park SK, Hu H, Lee S. Cadmium exposure and cardiovascular disease in the 2005 Korea National Health and Nutrition Examination Survey. *Environ Res* 2011 January;111(1):171-6.

(64) Suwazono Y, Sand S, Vahter M et al. Benchmark dose for cadmium-induced renal effects in humans. *Environ Health Perspect* 2006 July;114(7):1072-6.

(65) Clemons TE, Kurinij N, Sperduto RD. Associations of mortality with ocular disorders and an intervention of high-dose antioxidants and zinc in the Age-Related Eye Disease Study: AREDS Report No. 13. *Arch Ophthalmol* 2004 May;122(5):716-26.

(66) Prasad AS, Beck FW, Bao B et al. Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. *Am J Clin Nutr* 2007 March;85(3):837-44.

(67) Prasad AS. Zinc in human health: effect of zinc on immune cells. *Mol Med* 2008 May;14(5-6):353-7.

(68) Price DL, Rhett PM, Thorpe SR, Baynes JW. Chelating activity of advanced glycation end-product inhibitors. *J Biol Chem* 2001 December 28;276(52):48967-72.

(69) Nagai R, Murray DB, Metz TO, Baynes JW. Chelation: a fundamental mechanism of action of AGE inhibitors, AGE breakers, and other inhibitors of diabetes complications. *Diabetes* 2012 March;61(3):549-59.

(70) Naughton DP, Knappitt J, Fairburn K, Gaffney K, Blake DR, Grootveld M. Detection and investigation of the molecular nature of low-molecular-mass copper ions in isolated rheumatoid knee-joint synovial fluid. *FEBS Lett* 1995 March 20;361(2-3):167-72.

(71) Brigelius R, Spottl R, Bors W, Lengfelder E, Saran M, Weser U. Superoxide dismutase activity of low molecular weight Cu2 plus-chelates studied by pulse radiolysis. *FEBS Lett* 1974 October 1;47(1):72-5.

(72) Waslen TA, Houston CS, Tchang S. Menkes' kinky-hair disease: radiologic findings in a patient treated with copper histidinate. *Can Assoc Radiol J* 1995 April;46(2):114-7.

(73) Lee YT, Hsu CC, Lin MH, Liu KS, Yin MC. Histidine and carnosine delay diabetic deterioration in mice and protect human low density lipoprotein against oxidation and glycation. *Eur J Pharmacol* 2005 April 18;513(1-2):145-50.

(74) Feng RN, Niu YC, Sun XW et al. Histidine supplementation improves insulin resistance through suppressed inflammation in obese women with the metabolic syndrome: a randomised controlled trial. *Diabetologia* 2013 May;56(5):985-94.

(75) Niu YC, Feng RN, Hou Y et al. Histidine and arginine are associated with inflammation and oxidative stress in obese women. *Br J Nutr* 2012 July 14;108(1):57-61.

(76) Mihalik SJ, Michaliszyn SF, de las HJ et al. Metabolomic profiling of fatty acid and amino acid metabolism in youth with obesity and type 2 diabetes: evidence for enhanced mitochondrial oxidation. *Diabetes Care* 2012 March;35(3):605-11.

(77) Watanabe M, Suliman ME, Qureshi AR et al. Consequences of low plasma histidine in chronic kidney disease patients: associations with inflammation, oxidative stress, and mortality. *Am J Clin Nutr* 2008 June;87(6):1860-6.

(78) Hipkiss AR. Would carnosine or a carnivorous diet help suppress aging and associated pathologies? *Ann N Y Acad Sci* 2006 May;1067:369-74.

(79) Boldyrev AA, Aldini G, Derave W. Physiology and pathophysiology of carnosine. *Physiol Rev* 2013 October;93(4):1803-45.

(80) Everaert I, Taes Y, De HE et al. Low plasma carnosinase activity promotes carnosinemia after carnosine ingestion in humans. *Am J Physiol Renal Physiol* 2012 June 15;302(12):F1537-F1544.

(81) Kimura K, Nakamura Y, Inaba Y et al. Histidine augments the suppression of hepatic glucose production by central insulin action. *Diabetes* 2013 July;62(7):2266-77.