Horizons in Nutritional Science

Is zinc deficiency a risk factor for atherosclerosis?

John H. Beattie[1]* and In-Sook Kwun[2]

[1]*Division of Cellular Integrity, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, Scotland, UK*
[2]*Department of Food Science and Nutrition, Andong National University, Andong, Kyungpook, South Korea*

The development of atherosclerosis is influenced by genetic, lifestyle and nutritional risk factors. Zn and metallothionein deficiency can enhance oxidative-stress-related signalling processes in endothelial cells, and since changes in available plasma Zn may affect the Zn status of the endothelium, Zn deficiency could be a risk factor for IHD. Although the association of Zn with many proteins is essential for their function, three key signalling processes are highlighted as being principal targets for the effect of Zn deficiency: the activation of NF-κB, the activation of caspase enzymes and the signalling of NO. The need to develop a reliable indicator of Zn status is critical to any epidemiological approach for studying the relationship between Zn status and disease incidence. Studies using appropriate animal models and investigating how the plasma Zn pool influences endothelial intracellular labile Zn would be helpful in appreciating the importance of Zn deficiency in atherogenesis.

Zinc: Atherosclerosis: Metallothionein

Atherogenesis is a complex process involving mechanical, chemical and biological factors (Bannon *et al.* 2003). Damage to endothelial cells and activation, attachment and transmigration of monocytes through the endothelium are early events in atherosclerosis. It follows that factors modulating these processes could influence the development of the disease. The formation of atherosclerotic plaque and development of IHD is greatly influenced by lifestyle and nutritional factors, many of which have been clearly identified (Tunstall-Pedoe, 2003; Yancy *et al.* 2003). The impact of trace metal deficiencies on this process is less well defined and studied, although the influences of Se and Cu on atherogenesis and heart disease have attracted much attention (Strain, 1998; Kohrl *et al.* 2000; Alissa *et al.* 2003). Although Zn is regarded as an antioxidant (Powell, 2000), its deficiency has not been firmly associated with disease progression (Strain, 1998), probably due to the lack of a good indicator of Zn status (Hambidge, 2003). Zn binds to metallothionein (MT), which has been ascribed an antioxidant function (Miles *et al.* 2000), and seems to play an influential role on Zn metabolism during periods of stress (Carey *et al.* 2000). The possibility that Zn can help protect the vascular endothelium against oxidative stress has therefore been raised (Hennig *et al.* 1996) and we propose that MT may

influence Zn protection. In the present review, we examine whether current evidence supports the proposition that Zn deficiency is a risk factor for atherosclerosis and whether MT may influence atherogenesis.

Zinc requirements and deficiency

It is estimated that Zn deficiency affects one third of the World's population and is the major factor contributing to 1·4 % of all deaths worldwide (WHO, 2002). Although acute deficiency in developed countries is rare, marginal deficiency is thought to be relatively common (Hambidge, 2000). Major impacts of Zn deficiency of importance to health include reduced growth and suppression of immune function (Hambidge, 2000). Zn is required for structural and functional integrity of more than 2000 transcription factors (Coleman, 1992) and 300 enzymes (Vallee & Falchuk, 1993); therefore, almost every signalling and metabolic pathway is in some way dependent on at least one, and often several, Zn-requiring proteins. Many Zn-finger proteins bind Zn relatively tightly and have not been regarded as sensitive to changes in cellular total Zn concentrations, which are in the upper μM to lower mm concentration range. However, recent evidence suggests that 'free' Zn, which is most likely to influence activation

---

of Zn finger proteins and enzymes, is present at fM concentrations, at least in bacterial cells (Outten & O'Halloran, 2001). Hence, small, localised increases in 'free' or labile Zn within cells may have disproportionate influences on Zn-dependent protein activation in relation to the overall cellular Zn concentration. In addition, localised intracellular oxidative stress may enhance the lability of Zn within Zn fingers (Webster *et al.* 2001). With this in mind, it is quite credible that small changes in Zn homeostasis that result in a large changes in the cellular labile Zn may have quite profound effects on intracellular signalling. This raises the possibility that Zn status could influence the progression of chronic diseases such as heart disease, because some transcription factors involved in this process are sensitive to Zn. Due to the lack of a reliable indicator of Zn status, epidemiological study of an association between Zn status and disease incidence is rare (Hambidge, 2003). However, a protective effect of high Zn status on the risk of human CVD is compatible with some published data (Kok *et al.* 1988). Unfortunately little attempt has been made to study this association in appropriate animal models.

Cellular zinc homeostasis

As regards initiation of atherogenesis, a crucial question to be addressed is whether under physiological conditions, endothelial or mononuclear cells would ever become deficient in the pool of labile Zn that regulates transcription factor and enzyme activation. Although plasma contains $< 0.1\%$ whole-body Zn, it is the only medium for Zn transport, and so plasma Zn is turned over very rapidly (about 150 times per d in human subjects; King *et al.* 2000). The most labile Zn in human plasma is that bound to albumin (about 70 % plasma total Zn), with the remainder being more firmly associated with higher molecular mass proteins, predominantly α-2-macroglobulin (Chesters, 1997). Tissues such as liver also contain mobile reserves of Zn, but the kinetics of Zn turnover are slower than those in plasma and this tissue Zn is regarded as a separate pool in compartmental models of Zn metabolism (Lowe *et al.* 1993). Zn shows a rapid uptake into endothelial cells and may involve endocytosis of albumin-bound Zn (Rowe & Bobilya, 2000). The lability of albumin-bound Zn and the ease with which it is transported into endothelial cells suggests that the vascular endothelium may be particularly influenced by changes in plasma Zn levels, which are affected both by whole-body Zn homeostasis (Hambidge & Krebs, 2001) and by changes in the partitioning of Zn into different compartments (Philcox *et al.* 1995). Normal physiological levels of plasma Zn in developed countries are approximately $13-15 \mu M$ (Lentner, 1984) and Zn homeostasis is normally tightly regulated over a wide range of Zn intake (Hambidge & Krebs, 2001). However, increasing age > 25 years (Hotz *et al.* 2003), for example, is associated with lower plasma Zn levels. Tissue MT, induced by a wide variety of factors including bacterial endotoxin and ethanol, draws and binds Zn from the plasma and has a profound, albeit transient effect to suppress plasma Zn (Philcox *et al.* 1995; Carey *et al.* 2000). How endothelial cell Zn homeostasis is affected by plasma Zn levels will depend on issues relating to the regulation of Zn influx and efflux from the endothelial cells and the intracellular binding and compartmentalisation of absorbed Zn.

Monocyte activation

Once activated, peripheral blood monocytes form macrophages, which then infiltrate tissues and form part of the immune response against bacterial, viral, fungal and neoplastic pathogens. During activation, they release inflammatory cytokines such as IL-1 and -6, and TNF-α. These cytokines stimulate vascular endothelial cell production of adhesion factors, which encourage monocyte adhesion and diapedesis through the endothelium. Recent studies using the monocyte–macrophage cell line HL-60 show that Zn deficiency increases phytohemagglutinin-stimulated IL-1 and TNF-α gene expression, which if translated into secretion of these proteins, may enhance localised inflammatory effects of monocyte activation (Bao *et al.* 2003). However, the same group have previously reported that plasma IL-1 levels in elderly subjects of marginal Zn status are significantly lower than in younger subjects, and can be corrected by supplementation with Zn (Prasad *et al.* 1993). The major contribution to plasma IL-1 may come from lymphocytes, the predominant mononuclear cell type in blood, and so changes in plasma IL-1 may not reflect expression of this cytokine by monocytes–macrophages. Attachment of monocytes to damaged endothelium is a normal part of the repair process, but macrophage ingestion of oxidised LDL can result in the intimal formation of foam cells. Over time, this results in the formation of fatty streaks in the vasculature, and ultimately in the formation of atherosclerotic plaque. It has been suggested that physiological levels of Zn can prevent oxidation of LDL and its uptake into macrophages (Wilkins & Leake, 1994), although this is controversial and has yet to be confirmed.

Endothelial cell integrity

The vascular endothelium has a barrier function and must resist a range of chemical and mechanical stresses from the flow of blood containing potentially pro-oxidant and toxic compounds. Atherogenesis is associated with endothelial cell apoptosis, which may be initiated directly by oxidant stress from oxidised LDL or PUFA such as linoleic acid. It may also be initiated by inflammatory cytokines from activated monocytes–macrophages. Recent evidence suggests that Zn may have a protective effect on the vascular endothelium at a number of different levels. Apart from its direct proposed role as an antioxidant and stabiliser of cell membranes, Zn participates in the activity of numerous endothelial signalling processes, several of which are important to the maintenance of cell integrity.

NF-κB

NF-κB is a key transcription factor that regulates the expression of many genes and signalling pathways related to apoptosis and the immune and inflammatory responses

(May & Ghosh, 1998). Its role in vascular cell function has been well characterised and it is activated by several factors including IL-1, TNF-α, lipopolysaccharide, homocysteine and direct oxidative stress (De Martin *et al.* 2000). NF-κB activity is regulated by Zn, but while the effect of Zn is inhibitory in endothelial cells (Hennig *et al.* 1999*a*), it is stimulatory in a Th(0) cell line (Prasad *et al.* 2002). The reason for this cell-specific influence of Zn is unclear, but the marked inhibition of NF-κB by Zn pyrithione, a Zn ionophore, in endothelial cells (Kim *et al.* 1999) is striking evidence for the efficacy of this interaction. The binding of NF-κB to DNA is Zn-dependent (Kudrin, 2000), but the precise nature of the protein–Zn interaction is not known. A similarity between the DNA-binding domain of the p53 transcription factor, which binds Zn and is Zn dependent, and that of NF-κB has been noted; unlike p53, however, NF-κB does not contain structural Zn (Hainaut & Mann, 2001). Endothelial cells are activated to express the adhesion molecules intercellular adhesion molecule-1 and vascular adhesion molecule-1 and E-selectin by macrophage secreted TNF-α, encouraging further recruitment to the serosal endothelial surface and transmigration of monocytes through the endothelium. NF-κB is a key signalling process in adhesion molecule gene upregulation (De Martin *et al.* 2000), but the influence of endothelial Zn status on adhesion molecule expression and other NF-κB-dependent gene expression has not been studied.

Caspase-mediated apoptosis

Many, but not all, cells in culture show characteristic signs of apoptotic cell death when deprived of Zn (Truong-Tran *et al.* 2001). This was initially attributed to release of the inhibitory action of Zn on endonuclease activity (Duvall & Wyllie, 1986). However, DNA fragmentation and condensation of intact nuclei by cytosol from cells primed to undergo apoptosis was inhibited by Zn at lower levels than required to inhibit endonuclease activity, suggesting that Zn-mediated suppression of apoptosis may occur by independent mechanisms upstream of endonucleases. In addition, Zn inhibition of apoptosis can occur even in the absence of DNA fragmentation (Truong-Tran *et al.* 2000). Using purified substrate and recombinant enzyme, caspase-3, a pivotal enzyme for several apoptotic pathways, was found to be sensitively inhibited by Zn (Perry *et al.* 1997). Inhibition may occur by binding of Zn to a sulphydryl essential for enzyme activity (Maret *et al.* 1999). Zn is also a potent inhibitor of other caspases such as caspase-6 and -9 (Truong-Tran *et al.* 2001).

Atherogenesis involves apoptosis of endothelial cells; activation of caspase enzymes, particularly in response to oxidative stress, plays a major role in that process (Geng, 2001). Porcine endothelial cells made Zn-deficient by treatment with the Zn-chelator N′,N′,N′,N′-tetrakis (2-pyridylmethyl)ethylenediamine show a considerably higher level of apoptotic cell death and caspase-3 activity when challenged with oxidative stress-inducing conditions such as treatment with linoleic acid and TNF-α (Meerarani *et al.* 2000). Likewise, caspase-3 activity in human aortic endothelial cells showed an inverse relationship with media Zn

concentrations (Fanzo *et al.* 2002). There is therefore merit in the idea that Zn deficiency may enhance atherogenesis-related apoptosis of endothelial cells (Hennig *et al.* 1999*b*), but evidence supporting this *in vivo* is currently lacking.

Nitric oxide signalling

Changes in NO production have been linked to the development of atherosclerosis (Anderson, 2003). Recent studies have shown that Zn plays a crucial role in endothelial NO synthase function and in NO signalling. NO synthases are catalytically active only as a dimer of two subunits, the association of which is stabilised by the tetrahedral binding co-ordination of Zn with thiol ligands at the dimer interface (Zou *et al.* 2002). The activity of NO synthase is strongly inhibited by the formation of peroxynitrite, a product of superoxide radical reaction with NO, and is directly related to peroxynitrite-induced release of NO synthase-bound Zn. Since NO synthase expression is dependent on NF-κB activation, it is also possible that Zn deficiency could influence NO synthase by this mechanism.

NO also stimulates release of Zn from proteins by thiol nitrosylation. Treatment of endothelial cells with NO increases intracellular labile Zn, which can be detected using the Zn-binding probe Zinquin (Berendji *et al.* 1997). In particular, thiol nitrosylation of MT was found to release MT-bound Zn (Kroncke *et al.* 1994). A critical observation is that mesenteric arteries of mice with a knock-out mutation of MT-1 and -2 genes showed no myogenic reflex to increased luminal pressure. Treatment with the NO synthase inhibitor nitro-L-arginine methyl ester abolished the difference in myogenic response between arteries from MT-deficient and MT-normal mice, indicating that NO interaction with MT was an important factor in this reflex. It is hypothesised that an enhanced relaxing effect of NO during the myogenic reflex in MT-deficient vessels may be due to a lack of releasable metals from MT that normally would produce contraction (Pearce et al. 2000*a*).

Perspective

With so many Zn-binding ligands in endothelial cells, it is not an easy task to predict which proteins from which signalling or metabolic pathways might be most sensitive to changes in intracellular labile Zn under physiological conditions. Modulation of processes highlighted in the present review, namely NF-κB, caspase and NO activity, by physiological changes in Zn levels has been observed in isolated cell systems, but the relative effect of Zn at any particular level on all three processes simultaneously is unknown. However, Zn deficiency may influence localised pools of Zn differentially, which is clearly critical to effects on spatially localised or compartmentalised Zn-sensitive proteins. In some types of cell, labile intracellular Zn is rapidly compartmentalised within Zn containing vesicles (Haase & Beyersmann, 2002), and the presence of these vesicles has been demonstrated in endothelial cells (Pearce *et al.* 2000*b*).

Regulation of Zn homeostasis, both at a systemic and cellular level, is very efficient and so large changes in Zn

balance tend to be transitory. For example, an endotoxin-stimulated decrease in plasma Zn may persist for only a matter of hours. However, this may be sufficient to deplete vascular endothelial cells of labile Zn and stress factors such as endotoxin may then cause a more profound response when Zn sensitive processes involved in athero-genesis are more active. As discussed in the present review, MT may regulate labile Zn pools either through oxidative mechanisms, such as *S*-nitrosylation by NO (St Croix *et al.* 2002; Gow & Ischiropoulos, 2002), or by sequestering excess Zn released from other cellular ligands or derived from cellular import. In this regard, metal tran-scription factor-1, which is a cellular sensor of labile Zn, directly regulates MT transcription (Lichtlen & Schaffner, 2001).

As yet, there is no direct evidence linking Zn deficiency and IHD. However, there is now considerable evidence that Zn deficiency renders endothelial cells more susceptible to the effects of oxidative stress. The age-old problem of developing a reliable diagnostic indicator of Zn status needs to be re-addressed and resolved in order to critically evaluate the relationship between Zn status and human heart disease. In addition, considerably more effort should be devoted to animal studies of Zn deficiency, using appropriate models of heart disease, such as ApoE-null or LDL-receptor-null mice. Study of the dynamic relationship between available plasma Zn and endothelial labile Zn, vesicular or otherwise, would clarify whether Zn-sensitive signalling was likely to be influenced by sys-temic changes in Zn balance and status. Stress-induced endothelial dysfunction mediated through the signalling processes identified in the present review may predispose individuals to CHD, which we propose could be modulated by dietary Zn.

Acknowledgements

References

Alissa EM, Bahijri SM & Ferns GA (2003) The controversy sur-rounding selenium and cardiovascular disease: a review of the evidence. *Med Sci Monit* **9**, RA9–RA18.

Anderson TJ (2003) Nitric oxide, atherosclerosis and the clinical relevance of endothelial dysfunction. *Heart Fail Rev* **8**, 71–86.

Ball MJ & Ackland ML (2000) Zinc intake and status in Austra-lian vegetarians. *Br J Nutr* **83**, 27–33.

Bannon P, James N & Jessup W (2003) The endothelial cell in atherosclerosis. In *Atherosclerosis: Gene Expression, Cell Interactions and Oxidation*, pp. 137–158 [RT Dean and DT Kelly, editors]. Oxford: Oxford University Press.

Bao B, Prasad AS, Beck FWJ & Godmere M (2003) Zinc modu-lates mRNA levels of cytokines. *Am J Physiol Endocrinol Metab* **285**, E1095–E1102.

Berendji D, Kolb-Bachofen V, Meyer KL, Grapenthin O, Weber H, Wahn V & Kroncke KD (1997) Nitric oxide mediates intracytoplasmic and intranuclear zinc release. *FEBS Lett* **405**, 37–41.

Carey LC, Coyle P, Philcox JC & Rofe AM (2000) Ethanol decreases zinc transfer to the fetus in normal but not metallothionein-null mice. *Alcohol Clin Exp Res* **24**, 1236–1240.

Chesters JK (1997) Zinc. In *Handbook of Nutritionally Essential Mineral Elements*, pp. 185–230 [BL O'Dell and RA Sunde, editors]. New York: Marcel Dekker Inc.

Coleman JE (1992) Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. *Annu Rev Bio-chem* **61**, 897–946, 897–946.

De Martin R, Hoeth M, Hofer-Warbinek R & Schmid JA (2000) The transcription factor NF-kappa B and the regulation of vascular cell function. *Arterioscler Thromb Vasc Biol* **20**, E83–E88.

Duvall E & Wyllie AH (1986) Death and the cell. *Immunol Today* **7**, 115–119.

Fanzo JC, Reaves SK, Cui L, Zhu L & Lei KY (2002) p53 Protein and p21 mRNA levels and caspase-3 activity are altered by zinc status in aortic endothelial cells. *Am J Physiol* **283**, C631–C638.

Geng YJ (2001) Molecular signal transduction in vascular cell apoptosis. *Cell Res* **11**, 253–264.

Gow A & Ischiropoulos H (2002) NO running on MT: regulation of zinc homeostasis by interaction of nitric oxide with metal-lothionein. *Am J Physiol Lung Cell Mol Physiol* **282**, L183–L184.

Haase H & Beyersmann D (2002) Intracellular zinc distribution and transport in C6 rat glioma cells. *Biochem Biophys Res Commun* **296**, 923–928.

Hainaut P & Mann K (2001) Zinc binding and redox control of p53 structure and function. *Antioxid Redox Signal* **3**, 611–623.

Hambidge M (2000) Human zinc deficiency. *J Nutr* **130**, 1344S–1349S.

Hambidge M (2003) Biomarkers of trace mineral intake and status. *J Nutr* **133**, Suppl. 3, 948S–955S.

Hambidge M & Krebs NF (2001) Interrelationships of key vari-ables of human zinc homeostasis: relevance to dietary zinc requirements. *Annu Rev Nutr* **21**, 429–452.

Hennig B, Meerarani P, Ramadass P, Toborek M, Malecki A, Slim R & McClain CJ (1999b) Zinc nutrition and apoptosis of vascular endothelial cells: implications in atherosclerosis. *Nutrition* **15**, 744–748.

Hennig B, Meerarani P, Toborek M & McClain CJ (1999a) Anti-oxidant-like properties of zinc in activated endothelial cells. *Am J Coll Nutr* **18**, 152–158.

Hennig B, Toborek M & McClain CJ (1996) Antiatherogenic properties of zinc: implications in endothelial cell metabolism. *Nutrition* **12**, 711–717.

Hotz C, Peerson JM & Brown KH (2003) Suggested lower cutoffs of serum zinc concentrations for assessing zinc status: reanaly-sis of the second National Health and Nutrition Examination Survey data (1976–1980). *Am J Clin Nutr* **78**, 756–764.

Kim CH, Kim JH, Moon SJ, Chung KC, Hsu CY, Seo JT & Ahn YS (1999) Pyrithione, a zinc ionophore, inhibits NF-kappaB activation. *Biochem Biophys Res Commun* **259**, 505–509.

King JC, Shames DM & Woodhouse LR (2000) Zinc homeostasis in humans. *J Nutr* **130**, 1360S–1366S.

Kohrl J, Brigelius-Flohe R, Bock A, Gartner R, Meyer O & Flohe L (2000) Selenium in biology: facts and medical perspec-tives. *Biol Chem* **381**, 849–864.

Kok FJ, Van Duijn CM, Hofman A, Van der Voet GB, De Wolff FA, Paays CH & Valkenburg HA (1988) Serum copper and zinc and the risk of death from cancer and cardio-vascular disease. *Am J Epidemiol* **128**, 352–359.

Kroncke KD, Fehsel K, Schmidt T, Zenke FT, Dasting I, Wesener JR, Bettermann H, Breunig KD & Kolb-Bachofen V (1994) Nitric oxide destroys zinc-sulfur clusters inducing zinc release from metallothionein and inhibition of the zinc finger-type yeast transcription activator LAC9. *Biochem Biophys Res Commun* **200**, 1105–1110.

Kudrin AV (2000) Trace elements in regulation of NF-kappaB activity. *J Trace Elem Med Biol* **14**, 129–142.

Lentner C (editor) (1984) *Geigy Scientific Tables*, vol. 3. Basle: Ciba-Geigy Ltd.

Lichtlen P & Schaffner W (2001) The "metal transcription factor" MTF-1: biological facts and medical implications. *Swiss Med Wkly* **131**, 647–652.

Lowe NM, Green A, Rhodes JM, Lombard M, Jalan R & Jackson MJ (1993) Studies of human zinc kinetics using the stable isotope ^{70}Zn. *Clin Sci (Lond)* **84**, 113–117.

Maret W, Jacob C, Vallee BL & Fischer EH (1999) Inhibitory sites in enzymes: zinc removal and reactivation by thionein. *Proc Natl Acad Sci USA* **96**, 1936–1940.

May MJ & Ghosh S (1998) Signal transduction through NF-kappa B. *Immunol Today* **19**, 80–88.

Meerarani P, Ramadass P, Toborek M, Bauer HC, Bauer H & Hennig B (2000) Zinc protects against apoptosis of endothelial cells induced by linoleic acid and tumor necrosis factor alpha. *Am J Clin Nutr* **71**, 81–87.

Miles AT, Hawksworth GM, Beattie JH & Rodilla V (2000) Induction, regulation, degradation, and biological significance of mammalian metallothioneins. *Crit Rev Biochem Mol Biol* **35**, 35–70.

Outten CE & O'Halloran TV (2001) Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. *Science* **292**, 2488–2492.

Pearce LL, Gandley RE, Han W, Wasserloos K, Stitt M, Kanai AJ, McLaughlin MK, Pitt BR & Levitan ES (2000a) Role of metallothionein in nitric oxide signaling as revealed by a green fluorescent fusion protein. *Proc Natl Acad Sci USA* **97**, 477–482.

Pearce LL, Wasserloos K, St Croix M, Gandley R, Levitan ES & Pitt BR (2000b) Metallothionein, nitric oxide and zinc homeostasis in vascular endothelial cells. *J Nutr* **130**, 1467S–1470S.

Perry DK, Smyth MJ, Stennicke HR, Salvesen GS, Duriez P, Poirier GG & Hannun YA (1997) Zinc is a potent inhibitor of the apoptotic protease, caspase-3. A novel target for zinc in the inhibition of apoptosis. *J Biol Chem* **272**, 18530–18533.

Philcox JC, Coyle P, Michalska A, Choo KH & Rofe AM (1995) Endotoxin-induced inflammation does not cause hepatic zinc accumulation in mice lacking metallothionein gene expression. *Biochem J* **308**, 543–546.

Powell SR (2000) The antioxidant properties of zinc. *J Nutr* **130**, 1447S–1454S.

Prasad AS, Bao B, Beck FW & Sarkar FH (2002) Zinc enhances the expression of interleukin-2 and interleukin-2 receptors in HUT-78 cells by way of NF-kappaB activation. *J Lab Clin Med* **140**, 272–289.

Prasad AS, Fitzgerald JT, Hess JW, Kaplan J, Pelen F & Dardenne M (1993) Zinc deficiency in elderly patients. *Nutrition* **9**, 218–224.

Rowe DJ & Bobilya DJ (2000) Albumin facilitates zinc acquisition by endothelial cells. *Proc Soc Exp Biol Med* **224**, 178–186.

St Croix CM, Wasserloos KJ, Dineley KE, Reynolds JJ & Pitt BR (2002) Nitric oxide-inducing changes in intracellular zinc homeostasis are mediated by metallothionein/thionein. *Am J Physiol Lung Cell Mol Physiol* **282**, L185–L192.

Strain JJ (1998) Trace elements and cardiovascular disease. *Bibl Nutr Dieta* 127–140.

Truong-Tran AQ, Carter J, Ruffin RE & Zalewski PD (2001) The role of zinc in caspase activation and apoptotic cell death. *Biometals* **14**, 315–330.

Truong-Tran AQ, Ho LH, Chai F & Zalewski PD (2000) Cellular zinc fluxes and the regulation of apoptosis/gene-directed cell death. *J Nutr* **130**, 1459S–1466S.

Tunstall-Pedoe H (editor) (2003) *Monica Monograph and Multi-media Sourcebook: World's Largest Study of Heart Disease, Stroke, Risk Factors and Population Trends 1979–2002*. Geneva: WHO.

Vallee BL & Falchuk KH (1993) The biochemical basis of zinc physiology. *Physiol Rev* **73**, 79–118.

Webster KA, Prentice H & Bishopric NH (2001) Oxidation of zinc finger transcription factors: physiological consequences. *Antioxid Redox Signal* **3**, 535–548.

World Health Organisation (2002) Quantifying selected major risks to health. In *The World Health Report 2002*. Geneva: WHO.

Wilkins GM & Leake DS (1994) The oxidation of low density lipoprotein by cells or iron is inhibited by zinc. *FEBS Lett* **341**, 259–262.

Yancy WS, Westman EC, French PA & Califf RM (2003) Diets and clinical coronary events: the truth is out there. *Circulation* **107**, 10–16.

Zou MH, Shi C & Cohen RA (2002) Oxidation of the zinc–thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. *J Clin Invest* **109**, 817–826.