Mechanisms of Ageing and Development 151 (2015) 93–100

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Review

Serum copper to zinc ratio: Relationship with aging and health status

Marco Malavolta*, Francesco Piacenza, Andrea Basso, Robertina Giacconi, Laura Costarelli, Eugenio Mocchegiani

National Institute of Health and Science on Aging (INRCA), Scientific and Technological Research Area, Nutrition and Ageing Centre, via Birarelli 8, 60100 Ancona, Italy

ARTICLE INFO

Article history:
Available online 7 February 2015

Keywords:
Zinc
Copper
Aging
Inflammation
Homeostasis

ABSTRACT

The serum concentrations of copper (Cu) and zinc (Zn) are strictly regulated by compensatory mechanisms that act to stabilize them within certain ranges of nutritional intake. However, there are mechanisms that are built to decrease serum concentration of Zn and to increase serum concentration of Cu in the presence of inflammatory conditions, so that a common feature of several age-related chronic diseases is an increase of the Cu to Zn ratio (CZr). Although the clinical potential of CZr has been extensively investigated, few authors addressed the mechanisms that mainly contribute to the increase of CZr in serum during aging, which signals drive this change and how cells respond to these changes. This review focuses on this topic and discusses how an increase of CZr during aging could reflect the homeostatic shade from a general systemic "growth and reproduction" status typical of juvenile age to a "repair and maintenance" status that evolved to preserve health status during old age.

Contents

1. Introduction 93
2. Factors driving the changes of serum CZr in aging 94
   2.1. Nutritional intake 94
   2.2. Oxidative stress 94
   2.3. Pro-inflammatory stimuli and cellular senescence 95
   2.4. Hormonal signals 95
3. Significance of increased serum CZr for the organism during aging 95
   3.1. Immune defense 95
   3.2. Metabolic activity and cell proliferation 96
   3.3. Stress Response 96
4. Conclusions 98
   Acknowledgements 98
   References 98

1. Introduction

The growing number of elderly people in developed countries has compelled the scientific community to increase focus on biomarkers of aging, physical function decline, frailty, age-related diseases, and mortality (Engelfriet et al., 2013; Ruiz et al., 2012). These biomarkers might be relevant in clinical practice to help clinicians in the early detection of disability or incipient diseases, as they could be used to unmask homeostatic failure even in the absence of the most prevalent pathological conditions. Most studied biomarkers such as soluble inflammatory mediators, hormones, free radicals, antioxidants, macro-, and micronutrients can be measured by a minimally invasive peripheral blood withdrawal. Among serum micronutrients, essential trace elements, in particular Cu and Zn seem particularly important to predict the development and course of disability as they appear to be mostly related to inflammatory parameters than nutritional ones (Malavolta et al., 2010). Actually, the serum concentration of these trace elements is strictly regulated by compensatory mechanisms that act to stabilize their concentrations within certain ranges of nutritional intake. This reg-

* Corresponding author. Tel.: +39 071 8004116; fax: +39 071 206791.
E-mail address: m.malavolta@inrca.it (M. Malavolta).

http://dx.doi.org/10.1016/j.mad.2015.01.004

ulation, which involves mainly the transfer of Cu or Zn from the intestinal lumen to the portal circulation through the absorptive cells has been extensively reviewed elsewhere (Cousins, 1985). The serum concentration of Zn appears to be slightly affected by nutritional changes unless during severe deficiency or supplementation periods (Lowe et al., 2012), and even less sensible than serum Zn is the serum concentration of Cu to nutritional changes (Araya et al., 2003). Conversely, there are other mechanisms that act to decrease serum concentration of Zn and to increase serum concentration of Cu in the presence of inflammatory conditions, such as the acute phase of various disease (Sullivan et al., 1979). A common feature of these conditions is an increase of the Cu to Zn ratio (CZr) due to the decrement of serum Zn and/or increment of serum Cu. Hence, in addition to the measurement of Zn and Cu alone, a particular consideration should be drawn to the ratio between Cu and Zn. In fact, the serum (or plasma) CZr is among those parameters that may be associated with reduced ability to maintain or regain homeostasis after a destabilizing event. Increment of this ratio above 2.0 in the elderly usually reflects an inflammatory response or a decreased nutritional Zn status (Malavolta et al., 2010). High serum CZr is also found in people with debilitating conditions, such as hospitalized elderly subjects (Belbraouet et al., 2007). Moreover, it has been associated with the risk of CVD death (Leone et al., 2006; Reunanen et al., 1996), malignancy (Cunzhi et al., 2003; Dìez et al., 1989) and all-cause mortality in very old subjects (Malavolta et al., 2010; Malavolta et al., 2010). These data suggest that this parameter may be a useful tool in clinical research as prognostic and predictive factor for a multitude of pathological and pre-pathological conditions and that its relevance might be comparable or even superior to the inflammatory biomarkers (i.e., CRP, ESR) that are measured in routine clinical laboratories. However, although the clinical potential of CZr has been extensively investigated, few authors addressed the mechanisms that mainly contributes to the increase of CZr in serum during aging. Less investigated is also how cells respond to this change and what does this change mean for the aged organism. Last, but not least, it is important to focus on which signals drive this change and from where these signals arise. Within this review we would like to address these questions and the related scientific challenges. In particular, we will attempt to provide evidence that an increase of CZr during aging reflects the homeostatic shade from a general systemic "growth and reproduction" status typical of juvenile age to a "repair and maintenance" status that evolved to preserve health status during old age.

2. Factors driving the changes of serum CZr in aging

2.1. Nutritional intake

Some studies based on national surveys (Ma and Betts, 2000; Pennington and Young, 1991; Martínez Tomé et al., 2011), suggest that daily intakes of Cu and Zn in the elderly could be frequently lower than current recommendations. However, human requirements of Cu and Zn in old age are currently undergoing scrutiny, and there is sufficient research to suggest that the prevalence of Zn and Cu deficiency in healthy, free-living, late middle-aged and older Europeans is below 11% and 20%, respectively, for Zn and Cu (Roman Viñas et al., 2011; Andriollo-Sanchez et al., 2005). From these data, it can be concluded that the prevalence of Cu deficiency in elderly is apparently in contrast with frequently increased levels of Cu in aging (Mocchegiani et al., 2012b) while the low prevalence of Zn deficiency in elderly is not enough to explain the observed decrease of plasma Zn with advancing aging (Mocchegiani et al., 2012a,b). Zn and Cu are also strictly related to their respective major carriers in plasma, albumin and ceruloplasmin (Cp). Synthesis of these proteins, as well as regulation of serum Cu and Zn levels occurs mainly at hepatic levels. Hence, among the mechanisms that drive the general increase of CZr with aging, it is important to consider which factors can promote a reduced synthesis and secretion of Zn–Alb as well as increased synthesis and secretion of Cu–Cp. Although it is possible that inadequate nutrition may also contribute to hypoalbuminemia in elderly, it has been reported that albumin levels remain virtually unchanged even in the presence of severe protein calorie malnutrition in those individuals near terminal starvation (Rigaud et al., 2000). Albumin is also known as a negative acute phase protein because inflammation, more than low caloric intake, is known to be a cause of decreased albumin synthesis (Fuhrman, 2002; Don and Kaysen, 2004). Similarly, it would be rather unrealistic to talk about increased nutritional intake of Cu to explain the raised levels of Cu–Cp during the inflammatory conditions that usually accompany the aging process and, in particular, age-associated diseases and disorders. Under normal circumstances the hepatic Cu pool is not rate-limiting for Cp synthesis, as the serum Cp concentration increases rapidly during infection, trauma, and pregnancy while the ratio of apo- to holo-Cp is maintained (Matsuda et al., 1974). Also cases of potential Cu deficiency that would results into a CZr ratio well below normal values in aged individuals seems to be restricted to rare clinical cases (Williams, 1983). On the basis of these studies, it is likely that the major contribution to the increased CZr in aging arises not from nutritional habits but from other systemic changes that occur during aging. Hence, a more complex etiology other than a simple reduced dietary intake may contribute to reduced levels of Zn and increased levels of Cu in aging.

2.2. Oxidative stress

Increased oxidative stress in aging and age-related disorders could play a major role in raising the levels of CZr by both decreasing plasma Zn and increasing plasma Cu. It has been shown that the albumin bound fraction of Zn decreases in the plasma while labile Zn is loaded to peripheral tissues during some forms of oxidative stress (Kelly et al., 2011; van Rij et al., 1981). Indeed, physiological and pathological changes in the redox state of human serum albumin critically influence its binding properties (Oettl and Stauber, 2007). The Zn ions displaced from albumin by increased oxidative stress are subsequently delivered into cells and tissues by specific transporters (Sekler et al., 2007). Therefore, oxidative stress can contribute to decrease levels of plasma Zn without a necessary concomitant decrease of albumin levels. The labile Zn loaded into peripheral tissues displays several functions among which emerges the induction of the potent antioxidant protein metallothioneins (MTs) (Malavolta et al., 2008). A strong response to increasing oxidative stress is also a feature of serum Cu and Cu–Cp. Indeed, infections and inflammation as well as other conditions that induce oxidative stress, including aging and age-related disorders, promote serum Cu–Cp levels to raise. Studies on changes of Cu and Cp with age in humans and mice have been conducted since several decades ago. A comparative study of mice and humans reported one fold and two fold increase of serum Cu and Cp, respectively, from 3 months to 30 months of age in C57BL/6J mice while no changes was found in humans from 17 to 98 years (Massie et al., 1979). Further studies observed that Cu and Cp could increase with age in disabled or sick elderly (Mezzetti et al., 1998; Malavolta et al., 2010) suggesting that Cu–Cp is an essential protein to face-off the increasingly stressing conditions occurring with typical disorders of advanced age. A slight increase of the ratio between Cu and Cp was also observed in both humans and mice (Massie et al., 1979) with advancing aging, suggesting a possible displacement of free Cu by oxidative stress. However, the role of serum free Cu (Squitti et al., 2011) is still elusive, especially in aging research. Anyway, taking into account that Cp bound 80–95% of total serum Cu with high affinity, it is likely that the increased levels of Cu in serum are a consequence of increased transcription of Cp in liver tissues.

By the way, transcription of Cp in liver tissues was found to be strongly affected by inflammation but not by hyperoxic conditions (Fleming et al., 1991). Therefore, although Cp is produced by stress response mechanisms, inflammatory mediators that act at hepatic level could be the major factors that determine an increment of Cp and Cu–Cp in serum during age-related disorders.

2.3. Pro-inflammatory stimuli and cellular senescence

One of the major features associated with human aging, is the presence of a low-grade chronic pro-inflammatory status (Franceschi et al., 2000). Studies in humans and animal models have clearly demonstrated that inflammatory stimuli modify serum concentration of Cu and Zn by increasing the former and decreasing the latter through a hepatic organized mechanism (Milanino et al., 1993; Garduño Espinosa et al., 1991). Some of the major cytokines relevant to the acute phase response are interleukin (IL)-6, IL-1 beta, tumor necrosis factor-alpha (TNF-alpha), and interferon gamma (IFN-gamma). These inflammatory biomarkers are known to suppress the synthesis of albumin (Moshage et al., 1987) and to upregulate the synthesis of Cp (Barber and Cousins, 1988; Mazumder et al., 1997; Sidhu et al., 2011). While the levels of IFN-gamma have been frequently shown to be reduced in elderly (Scheffer et al., 2000), an increase of this cytokine can be detected in aged lymphocytes subsets (CD8+) (Zanni et al., 2003) and the levels of IL-1, IL-6 and TNF-alpha are commonly found to be increased with aging (Franceschi et al., 2000). A transcription factor, FOXO1, which is involved in the promotion of the anti-oxidant response in presence of pro-inflammatory cytokines (i.e., IL-6), seems to be specifically involved in the synthesis of Cp (Sidhu et al., 2011). Pro-inflammatory cytokines can also upregulate the Zn importer Zip-14 in the liver contributing to decrease serum Zn during the inflammatory response (Beker et al., 2012). These data support the concept that the increased levels of CZr in aging are part of a complex mechanisms built to improve resistance to oxidative stress and infection. Consequently, the low grade chronic inflammatory status described in advanced age could be a major determinant of this phenomena. The source of this age-related systemic chronic inflammation, named inflammaging (Franceschi et al., 2000), was mainly attributed to the progressive activation of immune cells over time. However, recent studies have shown that epigenetic effects eventually triggered by both nutritional deficiencies (Wessels et al., 2013) and the process of cellular senescence can be important additional contributors to chronic inflammation. Indeed, senescent cells acquire a phenotype named "senescence-associated secretory phenotype" (SASP), characterized by the enhanced secretion of many modulators of inflammation (Campisi et al., 2011). Hence, the low-level pro-inflammatory status, likely sustained by the cell senescence secretome and by progressive activation of immune cells over time, might be a determinant of the raised CZr levels in aging.

2.4. Hormonal signals

Other important players that could affect Cu and Zn levels in plasma are circulating hormones, especially insulin. The albumin synthesis rate is stimulated by insulin, which in turn diminishes its action with advancing aging (Boirie et al., 2001). Although higher insulin levels compensate for a reduced insulin action in elderly, conditions where beta-cell function and insulin production can be partly or mostly compromised, such as pre-diabetes and diabetes, are common in the elderly. Taking into account the role of albumin as major Zn carrier in plasma, it is not surprising the finding that elderly with pre-diabetes have lower plasma Zn levels than controls (Islam et al., 2013) and that high CZr was measured in serum of patients with diabetes mellitus (Viktorínová et al., 2009). At the same time, insulin can attenuate the FOXO1 mediated Cp expression, as demonstrated in rat liver cells (Leyendecker et al., 2011). Therefore, an impaired insulin action in aging might be among the basic factors that contribute to raise CZr plasma levels. Well known hormonal modulators of plasma Cp include also ACTH, thyroxine, estrogens and glucocorticoids (Cousins, 1985). This last class of hormonal modulators could play a key role in regulating Cu and Zn plasma levels in aging as glucocorticoid responses to stress in aging individuals are likely to be prolonged due to blunted and delayed inhibition of ACTH secretion (Wilkinson et al., 2001). It has been shown that administration of a short-acting (methylprednisolone) and a long-acting (dexamethasone) synthetic glucocorticoid can lead to raised levels of CZr after 12 h, due to increased Cu levels and depressed plasma Zn (Yunice et al., 1981). Being prolonged the glucocorticoid responses in elderly individuals, the increased CZr levels can persist for a long time in aged blood following stressing conditions. An additional contributing factor to the raising of human CZr plasma levels with aging could be the loss of nocturnal surges of growth hormone (GH) and the parallel decline in plasma insulin-like growth factor-1 (IGF-1) (Corpas et al., 1993). The decline in high-amplitude growth hormone secretion and plasma IGF-1 concentrations are some of the most robust and well-characterized endocrine alterations that occur with age. Although, most studies have been focused on the effect of Zn on GH/IGF-1 activity, Zn itself is necessary for the expected growth promoting and proliferative activity of GH/IGF-1 as replacement of growth promoting stimuli in children with idiopathic GH deficiency can give rise to plasma Zn levels (Mocchegiani et al., 1991). Therefore, reduced growth promoting stimuli might also contribute to the declined levels of plasma Zn and to the concomitant raise of plasma CZr.

3. Significance of increased serum CZr for the organism during aging

3.1. Immune defense

The immunomodulatory and antimicrobial properties of Zn and Cu have been extensively studied. Both trace elements are claimed to provide protection against infectious diseases in vivo, and to regulate innate immune response (Stafford et al., 2013). However, the teleologic basis for the depression of plasma Zn and the increased Cu concentrations associated with pro-inflammatory conditions, especially during aging, has not been firmly established. However, it is known that the redistribution of Zn in the organism following an inflammatory stimulus follow a specific strategy; while plasma Zn decreases, liver, thymus, and bone marrow accumulate Zn (Cousins et al., 1988). This kind of hypozincemic response to inflammatory mediators has been suggested as a mechanism to restrict Zn from the acquisition systems of potential invading pathogens (Kehl-Fie and Skaar, 2010), which in turn require Zn for growth and virulence (Citiulo et al., 2012; Corbett et al., 2012). By the way, imaging of metal distribution of tissue abscesses caused by *Staphylococcus aureus* found that the abscess was virtually devoid of detectable Zn, while high levels of Zn were present in the surrounding healthy tissue (Corbin et al., 2008). Dislocation of Zn from serum and infected tissues into the liver and other compartments is also useful to support the production of acute phase proteins and to protect liver and tissues from oxidative stress mainly via induction of MTs (Parsons and DiSilvestro, 1994). Moreover, the involvement of pro-infammatory cytokines in initiating a sequence of proliferative steps in primitive hematopoietic cells lends functional significance to the enhanced Zn uptake in hematopietic tissues and in the thymus (Cousins et al., 1988). In other words, this redistribution of zinc may be used by the organism to provide support for hematopoietic cell production in bone marrow and pro-

liferative response of lymphocytes as well as to reduce growth of eventual invading pathogens. A major mediator that is responsible of the hypozincemic response following inflammatory stimuli is the Zn trasporter Zip14 (Beker et al., 2012). Up-regulation of Zip14 in the liver can occur by macrophage-produced (or systemic) IL-6 and by LPS via toll like receptor 4 (TLR4) and to the subsequent activation of the NFKB pathway. This process is usually accompanied by reduced hepatic synthesis of albumin (Moshage et al., 1987) as well as increased synthesis and release of acute phase proteins including Cp. Some data also provided evidence that experimental prevention of this hypozincemic response is detrimental for the organism (Braunschweig et al., 1997). Regarding the increased levels of Cu in plasma, it is possible that this phenomenon could be related to an improvement of macrophage antimicrobial function. In vitro studies also show that Cu regulates macrophage antimicrobial pathways. Some of the first evidence for this phenomenon came again from elemental analysis of macrophage phagosomes. Indeed, the macrophage activating cytokines IFN-gamma and TNF-alpha promoted the accumulation of Cu within the phagosomes of *Mycobacterium avium*-infected macrophages (Wagner et al., 2005). It has been suggested that Cu, imported in the macrophages by the IFN-gamma inducible transporter CTR1, may contribute to ROS-dependent killing in macrophages by catalyzing the generation of hydroxyl radical from H2O2 (White et al., 2009). Although the secreted Cu-containing protein, Cp, is mostly responsible for this rise in serum Cu during acute and chronic inflammation (Fox et al., 1995), the non-protein-bound fraction of Cu in the serum is also increased during inflammation (Auer et al., 1989). Moreover, Cu accumulates at sites of inflammation and within the exudates of wounds and burns where there exists an abundance of macrophages (Stafford et al., 2013). Aged monocytes or macrophages showed impaired capabilities to phagocyte both pathogenic bacteria and charcoal particles suggesting that phagocyte functional senescence contributes to the diminished immune response against pathogens in aged individuals (Li, 2013; Malavolta et al., 2013). Moreover, signaling and activation of phagocytes appears to be impaired with aging (Fulop et al., 2014). In this context, the findings shown above underscore the possibility that age-related changes in systemic Cu concentrations may provide localized reserves of Cu to improve macrophage-mediated functional activity as an attempt to counteract phagocyte dysfunction, tissue aging and degeneration. Macrophage dysfunctions are only a part of the changes of the immune system observed during aging. This kind of erosion of the immune system that affect both innate and adaptive immunity is termed immunosenescence (Müller et al., 2013). Taking into account exposure to persistent antigens (such as antigens from cytomegalovirus) is a common feature in elderly individuals, it has been proposed to consider immunosenescence in some respect as an infectious state (Vasto et al., 2007). The final result of this antigenic challenge associated with the accumulation of dysfunctional immune cells is predicted to be a kind of immunodeficiency for novel antigenic challenges as well as loss of responsiveness to persistent antigen themselves. As a consequence, the raised levels of CZr in aging might represent the downstream effects of mechanisms developed in the futile attempt to modulate the compromised immune response.

3.2. Metabolic activity and cell proliferation

The most prominent form of Zn and Cu in serum are Cu–Cp and Zn–albumin (Zn–Alb) which account for 75–95% of serum Cu and 60–70% of serum Zn, respectively (Malavolta et al., 2012). Changes in serum Cu and Zn can in certain cases reflect changes in Cu–Cp and Zn–Alb which, in addition to their carrier task, display several physiological functions, including modulation of metabolic activity and cell proliferation (Francis, 2010). These functions are exploited following endocytosis of albumin, which in turn is subsequently transferred to lysosomes for degradation to amino acids, to be utilized for energy metabolism or protein synthesis. However, energy metabolism and protein synthesis are process that require also Zn. Hence, it is not surprising that albumin plays also a role in facilitating the physiological delivery of Zn to endothelial cells (Rowe and Bobilya, 2000). Albumin receptors that preferentially recognize albumin molecules carrying Zn were demonstrated on the endothelial cell surface. By the way, it is also known that physiological Zn uptake is critical for cell proliferation. Tumors are positively-imaged with 65Zn and cellular Zn uptake is generally suppressed during growth arrest (Takeda et al., 2004). Indeed, late passages senescent T-cell clones in vitro display reduced Zn-related cellular biomarkers compared to their early proliferating passages (Malavolta et al., 2008). In agreement with this statement and with the statements reported in the chapter above, the findings of longitudinal studies reporting a gradual decline of albumin with aging (especially after the 70s decade) (Miyake et al., 2011; Gom et al., 2007) are not unexpected. Indeed, in addition to possible changes in nutritional habits (Iuliano et al., 2013) and inflammatory status (see chapter above), the reduced levels of albumin and Zn usually observed during aging might be consistent with a diminished demand of the organism for growth and proliferation. This kind of response could be important during aging or disease status to invest more resource in maintenance and repair mechanisms. Many of the studies investigating this question of whether albumin administration is salutary in age-related diseases have generally not shown any significant benefit. A large meta-analysis of 30 randomized controlled trials studying the effects of albumin infusions demonstrated a higher mortality in critically ill patients treated with albumin infusions than in control groups (Cochrane Injuries Group Albumin Reviewers, 1998). Thus, it is likely that the cause of hypoalbuminemia and hypozinchemia is associated with morbidity and mortality in elderly rather than a direct effect of low albumin or low Zn levels per se. Also serum Cu might be required for cellular proliferation (Turski and Thiele, 2009), but the most prominent metabolic effect in aging might be related to the influence of Cu–Cp on iron metabolism (Jeong and David, 2006). Indeed, Cu influences intestinal absorption of iron and iron release from body storage sites by being required for cellular Fe utilization during erythropoiesis. Moreover, Cp converts toxic ferrous iron to its nontoxic ferric form and is required for iron efflux from cells (e.g., hepatocytes and macrophages). Absence of Cp leads to iron accumulation and neurodegeneration (Harris et al., 1998). Interestingly, younger-onset Parkinson's disease (PD) patients have significantly lower levels of serum Cp compared to those with older-onset PD (Bharucha et al., 2008). These data suggest that increased levels of Cu and Cp might modulate metabolic changes that are necessary to regulate iron homeostasis during stressing conditions. Heme-iron and heme biosynthesis are known to decline with age (Cook and Yu, 1998), but total iron accumulation is widely accepted as a feature of the aging process particularly in post-mitotic tissues by emerging research in the last years (Seo et al., 2008; Bulvik et al., 2012). Iron dyshomeostasis is further exacerbated in age-associated disorders (Xu et al., 2012) where serum Cu and Cp levels are usually raised above normal levels. In spite of these relevant data, the effect on organismal metabolism of the raised Cu–Cp levels in aging and age-associated disorders have been poorly investigated. A possible attempt of Cu and Cu–Cp to normalize iron dyshomeostasis in age-related disorders appears foreseeable but further research is still needed to clarify this point.

3.3. Stress Response

Among the well-known functions of Zn and Cu, their role in the defense against free radical has been extensively studied. It has

Table 1
Potential significance of increased CZr for the organism during aging.

Area of impact	Phenomenon associated	Effect (potential)	References
Immune defence	Redistribution of Zn in the organism (while plasma zinc decreases, liver, thymus, and bone marrow accumulate zinc)	Restrict Zn from the acquisition systems of potential invading pathogens	Kehl-Fie and Skaar (2010)
		Support the production of acute phase proteins and protect liver and tissues from oxidative stress	Parsons and DiSilvestro (1994) and Liuzzi et al. (2005)
		Stimulate proliferation of hematopoietic cells	Cousins and Leinart (1988)
	Dislocation of Zn from serum albumin and delivery in the free form to other compartments	Improve immune function	Haase and Rink (2013)
	Increased levels of Cu in plasma	Improvement of macrophage antimicrobial function	Wagner et al. (2005) and White et al. (2009)
	Increased circulating Cu–ceruloplasmin	Restrict utilization of iron by pathogenic bacteria	White et al. (2012)
Metabolic activity and cell proliferation	Reduced circulating Zn–albumin	Reduce non-specific growth and proliferation	Takeda et al. (2004)
	Increased circulating Cu–ceruloplasmin	Conversion of toxic ferrous iron to its nontoxic ferric form and promote iron efflux from cells	Jeong and David (2006) and Harris et al. (1998)
Stress response	Dislocation of Zn from serum albumin and delivery in the free form into other compartments.	Oxidative stress response	Maret (2006), Putics et al. (2008) and Liuzzi et al. (2005)
		Resistance to apoptosis	Thambiayya et al. (2012)
	Increased circulating Cu–ceruloplasmin	Oxidizes ferrous ions to the ferric state for further incorporation into apo-transferrins	Osaki et al. (1996)
		Catalyzes Cu(I) oxidation	Stoj and Kosman (2003)
		Provide protection against oxidant production by myeloperoxidase	Chapman et al. (2013)
		Can display NO-oxidase, NO_2-synthase, glutathione-linked peroxidase and superoxide dismutase activity	Shiva et al. (2006), Park et al. (1999) and Vasil'ev et al. (1998)

been shown above that reduced serum levels of Zn with advancing aging might also occur, independently of a decrease in serum albumin, by dislocation of Zn from the serum albumin pool and its subsequent delivery in the free or labile form into other compartments. Interestingly, a different role for cellular function of Zn in the free or labile form than in the Zn–Alb ones has been observed. Indeed, the quota of free or labile Zn seems to be mostly involved in the oxidative stress response (Maret, 2006; Putics et al., 2008) by modulating the mechanisms of resistance to apoptosis (Thambiayya et al., 2012), immune function (Haase and Rink, 2014), as well as in the production of specific antioxidant proteins including MTs and acute phase response proteins (Liuzzi et al., 2005). Similarly, the reason for an increase of serum Cu and Cu–Cp during stressing conditions are easily attributed to the multi-functional role and enzymatic activity that make serum Cp an effective antioxidant, able to prevent oxidative damage to proteins and lipids. Indeed, serum Cp oxidizes highly toxic ferrous ions to the ferric state for further incorporation into apo-transferrins (Osaki et al., 1996), catalyzes Cu(I) oxidation (Stoj and Kosman, 2003) and provide a protective shield against inadvertent oxidant production by myeloperoxidase during inflammation (Chapman et al., 2013). Moreover, Cp is the only plasma protein demonstrat-

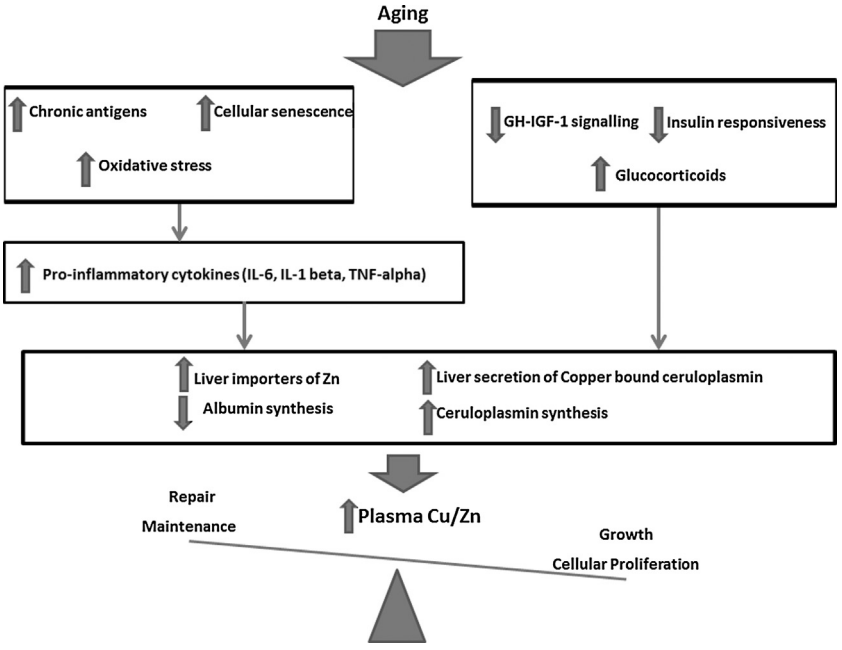

Fig. 1. A schematic representations of signals that during aging can synergically act to increase the copper to zinc ratio. On the basis of this scheme, the increased copper to zinc ratio is the downstream result of an overall attempt of the organism to move resources from growth and proliferation to maintenance and repair.

ing the activity of NO-oxidase, NO_2^- syntase (Shiva et al., 2006), glutathione-linked peroxidase (Park et al., 1999) and superoxide dismutase (Vasil'ev et al., 1998). In addition, in vitro binding studies and molecular modeling indicate that lactoferrin can bind to Cp such that a direct transfer of ferric iron between the two proteins is possible. This transfer of ferric iron would prevent both the formation of potentially toxic hydroxyl radicals and the utilization of iron by pathogenic bacteria (White et al., 2012). Although Cp is a known risk factor for cardiovascular diseases and was shown to be capable of oxidising LDL (Ehrenwald et al., 1994), recent observations suggests that the functional role of this protein "in vivo" is essential to protect cells and tissues during stressing conditions, such as it may occur during aging related diseases and disorders. By the other way, experimental models of infection with influenza virus (Samuel et al., 1980) and recent models of inflammatory bowel disease in Cp −/− mice confirm the strong protective role of Cp for the organism (Bakhautdin et al., 2013).

In conclusion, an increase with aging of serum CZr might reflect: (1) a decrease of serum Zn as a consequence of limited requirement of Zn–Alb for proliferation and growth or reduced nutritional intake, (2) a dislocation of Zn from the serum albumin pool to other tissue and compartments to sustain stress response, (3) an increase of Cu–Cp to face-off the increasingly stressing conditions occurring with advancing age and in the presence of age-related diseases. The overall putative effects that could be driven by an increase of Cu to Zn ratio in aging have been resumed in Table 1. Most, if not all, these effects appear to be a kind of defensive response against aging. Hence, the association of CZr with mortality in elderly could be the consequence of the factors that drive an increase of CZr or, alternatively and in analogy with the deregulated nutrient sensing (López-Otín et al., 2013), could be the result of this chronic response that may eventually become deleterious and aggravate aging.

4. Conclusions

The majority of studies on determinants of CZr in plasma have been focused on nutritional status, and the raised levels of this parameter have been described as a negative player for health status of elderly mainly due to inadequate nutritional intake of Zn. However, current knowledge on aging mechanisms suggests a more complex etiology of this phenomenon. Systemic signals promoting cellular proliferation and growth seem to be able to increase levels of plasma Zn in order to sustain the cellular requirement of this trace element whereas, other signals promoting "maintenance and repair" seem to induce a decrease of plasma Zn and an increase of Cu levels as a part of a complex mechanisms built to improve intracellular and extracellular anti-oxidant defense. According to recent studies, endocrine and metabolic changes with aging and accelerated aging diseases might be indicative of "survival" responses to genotoxic stress that could promote tumorigenesis (Schumacher et al., 2008). The suppression of the somatotropic axis in aging could represent an example of pathways that are modulated in order to make an attempt to survive through growth cessation and increased tissue maintenance despite existing genomic instability. Therefore, it emerges the presence of an evolutionarily conserved response that shifts the organism's resources from growth to maintenance as an adaptation to stresses in an attempt to protect the organism from cancer and to promote healthy aging. Hence, the aging process could be represented as a progressive switch from signals driving to growth and reproduction during young/adult age to signals driving to maintenance and repair in old age. These signals, including hormonal and inflammatory mediators can strongly affect plasma levels of Cu and Zn with a synergistic effect to increase CZr levels even independently of the nutritional status. It is not surprising that high CZr appears as a risk factor for disability and death in elderly as it might reflect the organismal response to the accumulation of genotoxic stress. In this context, plasma CZr might represent the downstream results of a series of complex mechanisms that gradually change with aging but that overall converge at increasing this parameter. A schematic picture of these mechanisms is reported in Fig. 1. For this reason, although the variability of CZr in humans is relatively large (Malavolta et al., 2010) a significant increase of this parameter with aging has been documented by several studies. However, it is still lacking a large study that might analytically describe the limits and potential of CZr as biomarker of aging. It should be also of interest to compare the levels of CZr between normal aging populations and centenarian's offspring. It has been reported that centenarian's offspring display several differences in systemic signals compared to offspring matched-controls including lower circulating IGF-I bioactivity (Vitale et al., 2012), that might predispose to an health and long lifespan. The question remains open as to whether an higher CZr plasma levels in response to infection and/or genotoxic stress could result into a slower or more rapid deterioration of various organ function contributing to delay or to accelerate the onset of age-related diseases. As first step in order to answer this question, it would be of interest to know whether CZr is affected by the particular changes occurring in systemic signals of centenarian's offspring. The results of the largest European Project built to establish biomarkers of aging (http://www.mark-age.eu/) will help to answer some of these questions.

Acknowledgements

This work was supported by The Italian National Institute of Health and Science on Aging (INRCA) and by a Grant from European Union within the FP7 framework: Project MARK-AGE (Co.N. HEALTH-F4-2008-200880).

References

Andriollo-Sanchez, M., Hininger-Favier, I., Meunier, N., Toti, E., Zaccaria, M., Brandolini-Bunlon, M., Polito, A., O'Connor, J.M., Ferry, M., Coudray, C., Roussel, A.M., 2005. Zinc intake and status in middle-aged and older European subjects: the ZENITH study. Eur. J. Clin. Nutr. 59 (Suppl. 2), S37–41.

Araya, M., Olivares, M., Pizarro, F., González, M., Speisky, H., Uauy, R., 2003. Copper exposure and potential biomarkers of copper metabolism. Biometals 16, 199–204.

Auer, D.E., Ng, J.C., Thompson, H.L., Inglis, S., Seawright, A.A., 1989. Acute phase response in horses: changes in plasma cation concentrations after localized tissue injury. Vet. Rec. 124, 235–239.

Bakhautdin, B., Febbraio, M., Goksoy, E., de la Motte, C.A., Gulen, M.F., Childers, E.P., Hazen, S.L., Li, X., Fox, P.L., 2013. Protective role of macrophage-derived ceruloplasmin in inflammatory bowel disease. Gut 62, 209–219.

Barber, E.F., Cousins, R.J., 1988. Interleukin-1 – stimulated induction of ceruloplasmin synthesis in normal and copper-deficient rats. J. Nutr. 118, 375–381.

Beker, A.T., Chang, S.M., Guthrie, G.J., Maki, A.B., Ryu, M.S., Karabiyik, A., Cousins, R.J., 2012. Zinc transporter ZIP14 functions in hepatic zinc, iron and glucose homeostasis during the innate immune response (endotoxemia). PLoS One 7, e48679.

Belbraouet, S., Biaudet, H., Tébi, A., Chau, N., Gray-Donald, K., Debry, G., 2007. Serum zinc and copper status in hospitalized vs: healthy elderly subjects. J. Am. Coll. Nutr. 26, 650–654.

Bharucha, K.J., Friedman, J.K., Vincent, A.S., Ross, E.D., 2008. Lower serum ceruloplasmin levels correlate with younger age of onset in Parkinson's disease. J. Neurol. 255, 1957–1962.

Boirie, Y., Gachon, P., Cordat, N., Ritz, P., Beaufrère, B., 2001. Differential insulin sensitivities of glucose, amino acid, and albumin metabolism in elderly men and women. J. Clin. Endocrinol. Metab. 86, 638–644.

Braunschweig, C.L., Sowers, M., Kovacevich, D.S., Hill, G.M., August, D.A., 1997. Parenteral zinc supplementation in adult humans during the acute phase response increases the febrile response. J. Nutr. 127, 70–74.

Bulvik, B.E., Berenshtein, E., Konijn, A.M., Grinberg, L., Vinokur, V., Eliashar, R., Chevion, M.M., 2012. Aging is an organ-specific process: changes in homeostasis of iron and redox proteins in the rat. Age (Dordr.) 34, 693–704.

Campisi, J., Andersen, J.K., Kapahi, P., Melov, S., 2011. Cellular senescence: a link between cancer and age-related degenerative disease. Semin. Cancer Biol. 21, 354–359.

Chapman, A.L., Mocatta, T.J., Shiva, S., Seidel, A., Chen, B., Khalilova, I., Paumann-Page, M.E., Jameson, G.N., Winterbourn, C.C., Kettle, A.J., 2013. Ceruloplasmin is an endogenous inhibitor of myeloperoxidase. J. Biol. Chem. 288, 6465–6477.
Citiulo, F., Jacobsen, I.D., Miramón, P., Schild, L., Brunke, S., Zipfel, P., Brock, M., Hube, B., Wilson, D., 2012. Candida albicans scavenges host zinc via Pra1 during endothelial invasion. PLoS Pathog. 8, e1002777.
Cochrane Injuries Group Albumin Reviewers, 1998. Human albumin administration in critically ill patients. Systematic review of randomized controlled trial. BMJ 317, 235–240.
Cook, C.I., Yu, B.P., 1998. Iron accumulation in aging: modulation by dietary restriction. Mech. Ageing Dev. 102, 1–13.
Corbin, B.D., Seeley, E.H., Raab, A., Feldmann, J., Miller, M.R., Torres, V.J., Anderson, K.L., Dattilo, B.M., Dunman, P.M., Gerads, R., Caprioli, R.M., Nacken, W., Chazin, W.J., Skaar, E.P., 2008. Metal chelation and inhibition of bacterial growth in tissue abscesses. Science 319, 962–965.
Corbett, D., Wang, J., Schuler, S., Lopez-Castejon, G., Glenn, S., Brough, D., Andrew, P.W., Cavet, J.S., Roberts, I.S., 2012. Two zinc uptake systems contribute to the full virulence of *Listeria* monocytogenes during growth in vitro and in vivo. Infect. Immun. 80, 14–21.
Corpas, E., Harman, S.M., Blackman, M.R., 1993. Human growth hormone and human aging. Endocr. Rev. 14, 20–39.
Cousins, R.J., 1985. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol. Rev. 65, 238–309.
Cousins, R.J., Leinart, A.S., 1988. Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin 1. FASEB J. 2, 2884–2890.
Cunzhi, H., Jiexian, J., Xianwen, Z., Jingang, G., Shumin, Z., Lili, D., 2003. Serum and tissue levels of six trace elements and copper/zinc ratio in patients with cervical cancer and uterine myoma. Biol. Trace Elem. Res. 94, 113–122.
Dìez, M., Cerdàn, F.J., Arroyo, M., Balibrea, J.L., 1989. Use of the copper/zinc ratio in the diagnosis of lung cancer. Cancer 63 (February (4)), 726–730.
Don, B.R., Kaysen, G., 2004. Serum albumin: relationship to inflammation and nutrition. Semin. Dial. 17, 432–437.
Ehrenwald, E., Chisolm, G.M., Fox, P.L., 1994. Intact human ceruloplasmin oxidatively modifies low density lipoprotein. J. Clin. Invest. 93, 1493–1501.
Engelfriet, P.M., Jansen, E.H., Picavet, H.S., Dollé, M.E., 2013. Biochemical markers of aging for longitudinal studies in humans. Epidemiol. Rev. (February (4)) (Epub ahead of print).
Fleming, R.E., Whitman, I.P., Gitlin, J.D., 1991. Induction of ceruloplasmin gene expression in rat lung during inflammation and hyperoxia. Am. J. Physiol. 260, L68–74.
Fox, P.L., Mukhopadhyay, C., Ehrenwald, E., 1995. Structure, oxidant activity, and cardiovascular mechanisms of human ceruloplasmin. Life Sci. 56, 1749–1758.
Franceschi, C., Bonafe, M., Valensin, S., 2000. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann. N. Y. Acad. Sci. 908, 244–254.
Francis, G.L., 2010. Albumin and mammalian cell culture: implications for biotechnology applications. Cytotechnology 62, 1–16.
Fulop, T., Page, A.L., Fortin, C., Witkowski, J.M., Dupuis, G., Larbi, A., 2014. Cellular signaling in the aging immune system. Curr. Opin. Immunol. 29, 105–111.
Fuhrman, M.P., 2002. The albumin-nutrition connection: separating myth from fact. Nutrition 18, 199–200.
Garduño Espinosa, J., Paniagua Sierra, R., Díaz Bensussen, S., Lifshitz, A., 1991. Response of serum zinc and copper to inflammatory stimulation in the rat with chronic liver damage. Arch. Invest. Med. (Mex.) 22, 289–294.
Gom, I., Fukushima, H., Shiraki, M., Miwa, Y., Ando, T., Takai, K., Moriwaki, H., 2007. Relationship between serum albumin level and aging in community-dwelling self-supported elderly population. J. Nutr. Sci. Vitaminol. (Tokyo) 53, 37–42.
Haase, H., Rink, L., 2014. Zinc signals and immune function. Biofactors 40, 27–40.
Harris, Z.L., Klomp, L.W., Gitlin, J.D., 1998. Aceruloplasminemia an inherited neurodegenerative disease with impairment of iron homeostasis. Am. J. Clin. Nutr. 67, 972S–977S.
Islam, M.R., Arslan, I., Attia, J., McEvoy, M., McElduff, P., Basher, A., Rahman, W., Peel, R., Akhter, A., Akter, S., Vashum, K.P., Milton, A.H., 2013. Is serum zinc level associated with prediabetes and diabetes?: a cross-sectional study from Bangladesh. PLoS One 8, e61776.
Iuliano, S., Olden, A., Woods, J., 2013. Meeting the nutritional needs of elderly residents in aged-care: are we doing enough. J. Nutr. Health Aging 17, 503–508.
Jeong, S.Y., David, S., 2006. Age-related changes in iron homeostasis and cell death in the cerebellum of ceruloplasmin-deficient mice. J. Neurosci. 26, 9810–9819.
Kehl-Fie, T.E., Skaar, E.P., 2010. Nutritional immunity beyond iron: a role for manganese and zinc. Curr. Opin. Chem. Biol. 14, 218–224.
Kelly, E., Mathew, J., Kohler, J.E., Blass, A.L., Soybel, D.I., 2011. Redistribution of labile plasma zinc during mild surgical stress in the rat. Transl. Res. 157, 139–149.
Leone, N., Courbon, D., Ducimetiere, P., Zureik, M., 2006. Zinc, copper, and magnesium and risks for all-cause, cancer, and cardiovascular mortality. Epidemiology 17, 308–314.
Leyendecker, M., Korsten, P., Reinehr, R., Speckmann, B., Schmoll, D., Scherbaum, W.A., Bornstein, S.R., Barthel, A., Klotz, L.O., 2011. Ceruloplasmin expression in rat liver cells is attenuated by insulin: role of FoxO transcription factors. Horm. Metab. Res. 43, 268–274.
Li, W., 2013. Phagocyte dysfunction, tissue aging and degeneration. Ageing Res. Rev. 12, 1005–1012.
Liuzzi, J.P., Lichten, L.A., Rivera, S., Blanchard, R.K., Aydemir, T.B., Knutson, M.D., Ganz, T., Cousins, R.J., 2005. Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response. Proc. Natl. Acad. Sci. U. S. A. 102, 6843–6848.
López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging. Cell 153, 1194–1217.
Lowe, N.M., Medina, M.W., Stammers, A.L., Patel, S., Souverein, O.W., Dullemeijer, C., Serra-Majem, L., Nissensohn, M., Moran, V.H., 2012. The relationship between zinc intake and serum/plasma zinc concentration in adults: a systematic review and dose-response meta-analysis by the EURRECA network. Br. J. Nutr. 108, 1962–1971.
Ma, J., Betts, N.M., 2000. Zinc and copper intakes and their major food sources for older adults in the 1994–1996 continuing survey of food intakes by individuals (CSFII). J. Nutr. 130, 2838–2843.
Malavolta, M., Basso, A., Piacenza, F., Costarelli, L., Giacconi, R., Mocchegiani, E., 2013. Peripheral mononuclear cell rejuvenation for senescence surveillance in Alzheimer disease. Curr. Pharm. Des. 19, 1720–1726.
Malavolta, M., Piacenza, F., Basso, A., Giacconi, R., Costarelli, L., Pierpaoli, S., Mocchegiani, E., 2012. Speciation of trace elements in human serum by micro anion exchange chromatography coupled with inductively coupled plasma mass spectrometry. Anal Biochem. 421, 16–25.
Malavolta, M., Giacconi, R., Piacenza, F., Santarelli, L., Cipriano, C., Costarelli, L., Tesei, S., Pierpaoli, S., Basso, A., Galeazzi, R., Lattanzio, F., Mocchegiani, E., 2010. Plasma copper/zinc ratio: an inflammatory/nutritional biomarker as predictor of all-cause mortality in elderly population. Biogerontology 11, 309–319.
Malavolta, M., Cipriano, C., Costarelli, L., Giacconi, R., Tesei, S., Muti, E., Piacenza, F., Pierpaoli, S., Larbi, A., Pawelec, G., Dedoussis, G., Herbein, G., Monti, D., Jajte, J., Rink, L., Mocchegiani, E., 2008. Metallothionein downregulation in very old age: a phenomenon associated with cellular senescence? Rejuvenation Res. 11, 455–459.
Martínez Tomé, M.J., Rodríguez, A., Jiménez, A.M., Mariscal, M., Murcia, M.A., García-Diz, L., 2011. Food habits and nutritional status of elderly people living in a Spanish Mediterranean city. Nutr. Hosp. 26, 1175–1182.
Maret, W., 2006. Zinc coordination environments in proteins as redox sensors and signal transducers. Antioxid. Redox Signal. 8, 1419–1441.
Massie, H.R., Colacicco, J.R., Aiello, V.R., 1979. Changes with age in copper and ceruloplasmin in serum from humans and C57BL/6J mice. Age 2, 97–101.
Matsuda, I., Pearson, T., Holtzman, N.A., 1974. Determination of apoceruloplasmin by radioimmunoassay in nutritional copper deficiency, Menkes' kinky hair syndrome, Wilson's disease, and umbilical cord blood. Pediatr. Res. 8, 821–824.
Mazumder, B., Mukhopadhyay, C.K., Prok, A., Cathcart, M.K., Fox, P.L., 1997. Induction of ceruloplasmin synthesis by IFN-gamma in human monocytic cells. J. Immunol. 159, 1938–1944.
Mezzetti, A., Pierdomenico, S.D., Costantini, F., Romano, F., De Cesare, D., Cuccurullo, F., Imbastaro, T., Riario-Sforza, G., Di Giacomo, F., Zuliani, G., Fellin, R., 1998. Copper/zinc ratio and systemic oxidant load: effect of aging and aging-related degenerative diseases. Free Radic. Biol. Med. 25, 676–681.
Milanino, R., Marrella, M., Gasperini, R., Pasqualicchio, M., Velo, G., 1993. Copper and zinc body levels in inflammation: an overview of the data obtained from animal and human studies. Agents Actions 39, 195–209.
Miyake, M., Ogawa, Y., Yoshida, Y., Imaki, M., 2011. Seven-year large cohort study for the association of serum albumin level and aging among community dwelling elderly. J. Anal. Bio.-Sci. 34, 284–286.
Mocchegiani, E., Fabris, N., Travaglini, P., Sartorio, A., De Min, C., Paolucci, P., 1991. Thymic endocrine activity in children with idiopathic growth-hormone deficiency. Int. J. Neurosci. 59, 151–157.
Mocchegiani, E., Costarelli, L., Giacconi, R., Piacenza, F., Basso, A., Malavolta, M., 2012a. Micronutrient (Zn, Cu, Fe)-gene interactions in ageing and inflammatory age-related diseases: implications for treatments. Ageing Res. Rev. 11, 297–319.
Mocchegiani, E., Malavolta, M., Lattanzio, F., Piacenza, F., Basso, A., Abbatecola, A.M., Russo, A., Giovannini, S., Capoluongo, E., Bustacchini, S., Guffanti, E.E., Bernabei, R., Landi, F., 2012b. Cu to Zn ratio, physical function, disability, and mortality risk in older elderly (ilSIRENTE study). Age (Dordr.) 34, 539–552.
Moshage, H.J., Janssen, J.A., Franssen, J.H., Hafkenscheid, J.C., Yap, S.H., 1987. Study of the molecular mechanism of decreased liver synthesis of albumin in inflammation. J. Clin. Invest. 79, 1635–1641.
Müller, L., Fülöp, T., Pawelec, G., 2013. Immunosenescence in vertebrates and invertebrates. Immun. Ageing 10, 12.
Oettl, K., Stauber, R.E., 2007. Physiological and pathological changes in the redox state of human serum albumin critically influence its binding properties. Br. J. Pharmacol. 151, 580–589.
Osaki, S., Johnson, D.A., Frieden, E., 1996. The possible significance of the ferrous oxidase activity of ceruloplasmin in normal human serum. J. Biol. Chem. 241, 2746–2751.
Park, Y.S., Suzuki, K., Taniguchi, N., Gutteridge, J.M., 1999. Glutathione peroxidase-like activity of caeruloplasmin as an important lung antioxidant. FEBS Lett. 458, 133–136.
Parsons, S.E., DiSilvestro, R.A., 1994. Effects of mild zinc deficiency, plus or minus an acute-phase response, on galactosamine-induced hepatitis in rats. Br. J. Nutr. 72, 611–618.
Pennington, J.A., Young, B.E., 1991. Total diet study nutritional elements, 1982–1989. J. Am. Diet. Assoc. 91, 179–183.
Putics, A., Vödrös, D., Malavolta, M., Mocchegiani, E., Csermely, P., Soti, C., 2008. Zinc supplementation boosts the stress response in the elderly: Hsp70 status is

linked to zinc availability in peripheral lymphocytes. Exp. Gerontol. 43, 452–461.
Reunanen, A., Knekt, P., Marniemi, J., Mäki, J., Maatela, J., Aromaa, A., 1996. Serum calcium, magnesium, copper and zinc and risk of cardiovascular death. Eur. J. Clin. Nutr. 50, 431–437.
Rigaud, D., Hassid, J., Meulemans, A., Poupard, A.T., Boulier, A., 2000. A paradoxical increase in resting energy expenditure in malnourished patients near death: the king penguin syndrome. Am. J. Clin. Nutr. 72, 355–360.
Roman Viñas, B., Ribas Barba, L., Ngo, J., Gurinovic, M., Novakovic, R., Cavelaars, A., de Groot, L.C., van't Veer, P., Matthys, C., Serra Majem, L., 2011. Projected prevalence of inadequate nutrient intakes in Europe. Ann. Nutr. Metab. 59, 84–95.
Rowe, D.J., Bobilya, D.J., 2000. Albumin facilitates zinc acquisition by endothelial cells. Proc. Soc. Exp. Biol. Med. 224, 178–186.
Ruiz, M., Cefalu, C., Reske, T., 2012. Frailty syndrome in geriatric medicine. Am. J. Med. Sci. 344 (November (5)), 395–398.
Samuel, I., Tomas, E., Istodor, A., Bârnaure, F., Cajal, N., 1980. The effect of ceruloplasmin on experimental infection with influenza virus APR 8/34 (H0N1) in the mouse. Virologie 31, 221–225.
Scheffer, C., Zawatzky, R., Rink, L., 2000. Interferon-gamma (IFN-gamma) levels finally become stable with increasing age as revealed by using an ELISA corresponding to the bioactivity. Mech. Ageing Dev. 121, 47–58.
Schumacher, B., Garinis, G.A., Hoeijmakers, J.H., 2008. Age to survive: DNA damage and aging. Trends Genet. 24, 77–85.
Sekler, I., Sensi, S.L., Hershfinkel, M., Silverman, W.F., 2007. Mechanism and regulation of cellular zinc transport. Mol. Med. 13 (July–August (7–8)), 337–343.
Seo, A.Y., Xu, J., Servais, S., Hofer, T., Marzetti, E., Wohlgemuth, S.E., Knutson, M.D., Chung, H.Y., Leeuwenburgh, C., 2008. Mitochondrial iron accumulation with age and functional consequences. Aging Cell 7, 706–716.
Shiva, S., Wang, X., Ringwood, L.A., Xu, X., Yuditskaya, S., et al., 2006. Ceruloplasmin is a NO oxidase and nitrite synthase that determines endocrine NO homeostasis. Nat. Chem. Biol. 2, 486–493.
Sidhu, A., Miller, P.J., Hollenbach, A.D., 2011. FOXO1 stimulates ceruloplasmin promoter activity in human hepatoma cells treated with IL-6. Biochem. Biophys. Res. Commun. 404, 963–967.
Squitti, R., Ghidoni, R., Scrascia, F., Benussi, L., Panetta, V., Pasqualetti, P., Moffa, F., Bernardini, S., Ventriglia, M., Binetti, G., Rossini, P.M., 2011. Free copper distinguishes mild cognitive impairment subjects from healthy elderly individuals. J. Alzheimers Dis. 23, 239–248.
Stafford, S.L., Bokil, N.J., Achard, M.E., Kapetanovic, R., Schembri, M.A., McEwan, A.G., Sweet, M.J., 2013. Metal ions in macrophage antimicrobial pathways: emerging roles for zinc and copper. Biosci. Rep. 33 (4), pii: e00049.
Stoj, C., Kosman, D.J., 2003. Cuprous oxidase activity of yeast Fet3p and human ceruloplasmin: implication for function. FEBS Lett. 554, 422–426.
Sullivan, J.F., Blotcky, A.J., Jetton, M.M., Hahn, H.K., Burch, R.E., 1979. Serum levels of selenium, calcium, copper magnesium, manganese and zinc in various human diseases. J. Nutr. 109, 1432–1437.
Thambiayya, K., Wasserloos, K., Kagan, V.E., Stoyanovsky, D., Pitt, B.R., 2012. A critical role for increased labile zinc in reducing sensitivity of cultured sheep pulmonary artery endothelial cells to LPS-induced apoptosis. Am. J. Physiol. Lung Cell. Mol. Physiol. 302, L1287–1295.
Takeda, A., Tamano, H., Ibuki, Y., 2004. Change of zinc uptake under growth arrest and apoptosis. Anticancer Res. 24, 3869–3874.
Turski, M.L., Thiele, D.J., 2009. New roles for copper metabolism in cell proliferation, signaling, and disease. J. Biol. Chem. 284 (January (2)), 717–721.
van Rij, A.M., Hall, M.T., Bray, J.T., Pories, W.J., 1981. Zinc as an integral component of the metabolic response to trauma. Surg. Gynecol. Obstet. 153, 677–682.
Vasil'ev, V.B., Kachurin, A.M., Soroka, N.V., 1998. Dismutation of superoxide radicals by ceruloplasmin – details of the mechanism. Biokhimiia 53, 2051–2058.
Vasto, S., Colonna-Romano, G., Larbi, A., Wikby, A., Caruso, C., Pawelec, G., 2007. Role of persistent CMV infection in configuring T cell immunity in the elderly. Immun. Ageing 4 (March (21)), 2.
Viktorínová, A., Toserová, E., Krizko, M., Duracková, Z., 2009. Altered metabolism of copper, zinc, and magnesium is associated with increased levels of glycated hemoglobin in patients with diabetes mellitus. Metabolism 58, 1477–1482.
Vitale, G., Brugts, M.P., Ogliari, G., Castaldi, D., Fatti, L.M., Varewijck, A.J., Lamberts, S.W., Monti, D., Bucci, L., Cevenini, E., Cavagnini, F., Franceschi, C., Hofland, L.J., Mari, D., Janssen, J., 2012. Low circulating IGF-I bioactivity is associated with human longevity: findings in centenarians' offspring. Aging (Albany NY) 4, 580–589.
Wagner, D., Maser, J., Lai, B., Cai, Z., Barry 3rd, C.E., Höner Zu Bentrup, K., Russell, D.G., Bermudez, L.E., 2005. Elemental analysis of *Mycobacterium avium*-, *Mycobacterium tuberculosis*-, and *Mycobacterium smegmatis*-containing phagosomes indicates pathogen-induced microenvironments within the host cell's endosomal system. J. Immunol. 174, 1491–1500.
Wessels, I., Haase, H., Engelhardt, G., Rink, L., Uciechowski, P., 2013. Zinc deficiency induces production of the proinflammatory cytokines IL-1β and TNFα in promyeloid cells via epigenetic and redox-dependent mechanisms. J. Nutr. Biochem. 24, 289–297.
White, K.N., Conesa, C., Sánchez, L., Amini, M., Farnaud, S., Lorvoralak, C., Evans, R.W., 2012. The transfer of iron between ceruloplasmin and transferrins. Biochim. Biophys. Acta. 1820, 411–416.
White, C., Lee, J., Kambe, T., Fritsche, K., Petris, M.J., 2009. A role for the ATP7A copper-transporting ATPase in macrophage bactericidal activity. J. Biol. Chem. 284, 33949–33956.
Wilkinson, C.W., Petrie, E.C., Murray, S.R., Colasurdo, E.A., Raskind, M.A., Peskind, E.R., 2001. Human glucocorticoid feedback inhibition is reduced in older individuals: evening study. J. Clin. Endocrinol. Metab. 86, 545–550.
Williams, D.M., 1983. Copper deficiency in humans. Semin. Hematol. 20, 118–128.
Xu, J., Jia, Z., Knutson, M.D., Leeuwenburgh, C., 2012. Impaired iron status in aging research. Int. J. Mol. Sci. 13, 2368–2386.
Yunice, A.A., Czerwinski, A.W., Lindeman, R.D., 1981. Influence of synthetic corticosteroids on plasma zinc and copper levels in humans. Am. J. Med. Sci. 282, 68–74.
Zanni, F., Vescovini, R., Biasini, C., Fagnoni, F., Zanlari, L., Telera, A., Di Pede, P., Passeri, G., Pedrazzoni, M., Passeri, M., Franceschi, C., Sansoni, P., 2003. Marked increase with age of type 1 cytokines within memory and effector/cytotoxic CD8+ T cells in humans: a contribution to understand the relationship between inflammation and immunosenescence. Exp. Gerontol. 38, 981–987.