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Review

Zinc in gastrointestinal and liver disease

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Contents

Abstract

Zinc is an essential trace element with important biological functions, depending on the structural and/or catalytic role played by zinc ions in a large variety of enzymes. Zinc plays a critical role in cellular integrity, protein synthesis, nucleic acid metabolism, contributing to cell growth, proliferation, differentiation and death. The present review reports data on zinc homeostasis and metabolism, zinc absorption, intercellular trafficking, intracellular transport inside enterocytes and hepatocytes. Particular emphasis is given to data regarding the role of zinc carriers ZnTs and Zips, and to their expression in liver and gut in experimental and in human studies. The role of zinc in the gastrointestinal tract and in the liver as a powerful antioxidant and its relationship with apoptosis is discussed. Possible implications of zinc status in different disorders of the gastrointestinal tract are presented, focusing on its possible introduction in the therapy of inflammatory bowel diseases. Data on the role of zinc and zinc carriers in the evolution of liver fibrosis towards cirrhosis are also discussed. Finally, data on the ability of zinc therapy to obtain regression of liver cirrhosis in patients affected by Wilson's disease are reported, and the hypothesis that zinc could protect against liver fibrosis in chronic liver disease of different origin is presented.

Keywords: Zinc; Liver; Gut; Chronic liver disease; Inflammatory bowel disease; Apoptosis

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1. Introduction

Recent years have registered an explosion of interest by the scientific community regarding the role of zinc, the second most prevalent trace element in the human body, in human and animal diseases, together with the understanding of the large spectrum of functions of zinc at the subcellular level [1]. The relevance of zinc in metabolic processes of living cells has been underlined by the discovery of a large number of molecules involved in the uptake and in the excretion of zinc, which regulate the intracellular molecular pathways of this trace element in mammals [2].

The number of metalloproteins which need zinc ions to stabilize their structure and to allow their function is increasing: members of oxidoreductase, hydrolase–ligase, lyase family [3] and Cu/Zn superoxide dismutase [4] are among the several hundred zinc–proteins present in our body. Zinc is so ubiquitous in cell metabolism that even minor changes in its availability may have relevant biological and, eventually, clinical consequences.

The central role played by zinc in cell growth and differentiation may easily explain the dramatic effects of its deficiency in tissues with rapid cell turnover, especially the skin, the gastrointestinal tract mucosa and the immune system. With respect to fetal development, the consequences of zinc deficiency on the outcome of pregnancy can be severe, like fetal abnormalities, growth retardation, and eventually fetal death [5]. In order to protect fetal growth and development, and maintain fetal zinc homeostasis, a complex regulatory mechanism of zinc transporters has been developed in the human placenta. This mechanism mediates zinc transfer between mother and fetus, assuring a coordinated regulatory response to changes in dietary zinc intake [6].

The encouraging recent progress in the field of molecular biology, regarding absorption, tissue distribution, intracellular transport and excretion of zinc in mammals, may be identified as the future area of research, focusing on the role of this trace element in human health and disease.

Recent evidence of a putative efficacy of zinc supplementation in the cure of liver disease and, in particular, in halting liver fibrosis, could recommend the field of zinc and liver as an important future area of research, with a possible relevance of zinc towards a clinical application.

The antioxidant role of zinc, together with its ability to protect human cells from apoptosis, the mechanism of cell death involved in acute and chronic inflammatory diseases of the liver and of the gastrointestinal tract, may suggest the field of zinc and gut relationship as an interesting field of research. The aim of this paper is to give a general view on the current status of the scientific basis for the role of zinc in human health and disease, with particular attention towards the interaction of zinc status and liver function in health, in acute and in chronic liver disease of different aetiology and in inflammatory bowel disease. Data on other trace elements–zinc interactions from the level of enterocyte to other systems in the body are presented. Possible implications of zinc in apoptosis and in the pathogenesis of different liver diseases are also prospected. Finally, the putative role for zinc in prevention and therapy of liver fibrosis is discussed.

2. Role of zinc

2.1. Zinc homeostasis and functions

Zinc is an essential trace element for normal cell function and metabolism, i.e. for life [7]. It participates in all aspects of metabolism, in the regulation of gene expression, in the structural maintenance of chromatin and biomembranes, in immunity and in protection against free radicals [8]. Zinc ions are key structural components of a large number of proteins with highly specific functions: the matrix metalloproteinases, responsible for cartilage destruction seen in rheumatoid arthritis [9] and for removal of fibrosis in chronic liver disease [10], are a class of metalloenzymes requiring zinc at active site for catalytic activity [3]. Zinc is essential to the structure and function of over 300 enzymatic reactions [11]. It has both structural and catalytic roles in enzymes: in some proteins, zinc participates directly in chemical catalysis, in others it is important in maintaining protein structure and stability. The Zn-binding site is a conserved histidine–glutamine–X–glycine–histidine sequence, in which X is a hydrophobic residue [3].

Zinc-binding sites in proteins are made up of the sulfur of cysteine, the nitrogen of histidine or the oxygen of aspartate and glutamate, or a combination of the two [11]. In zinc-finger motifs, zinc provides a scaffold that organizes protein subdomains for the interaction with either DNA or other proteins [3]. Zinc is critical for the function of a large number of metalloproteins, including members of oxido-reductase, hydrolase and ligase families. In some enzymes such as alcohol dehydrogenase, zinc ions are an indispensable structural part of the molecule (structural zinc atoms); in other enzymes, such as carboxypeptidase, zinc participates directly in the catalytic function (catalytic zinc ions); in alkaline phosphatase, zinc has a co-activating function, enhancing or diminishing the catalytic function of the enzyme [12]. The zinc ion does not participate in redox reactions, which makes it a stable ion in a biological medium whose potential is in a constant flux [3]. Zinc homeostasis results from a coordinated regulation played by multiple proteins involved in uptake, intercellular trafficking, intracellular storage and excretion of the metal [13]. The principal role of these proteins is to provide zinc ions to newly synthesized proteins. The latter play an important role in the structural maintenance of chromatin and cell membranes, in maintaining cell homeostasis, and protecting cells against free radical damage [14]. Other zinc–proteins allow a proper function of multiple transcription factors which regulate gene expression [15], inhibit apoptosis [16], regulate cell proliferation, act in the modulation of the immune system [17], and eventually control the balance between cell life and death [7]. The role of zinc is extremely relevant in all aspects of cell metabolism, regulating the function of a large variety of metabolic processes. In fact, even minor changes in zinc concentration in the human body are likely to have multiple biological and clinical effects, particularly in older subjects [18].

Given its relevant biological functions, zinc is necessary for proper liver function and zinc deficiency has been related to the pathogenesis of multiple liver disease. In particular, different

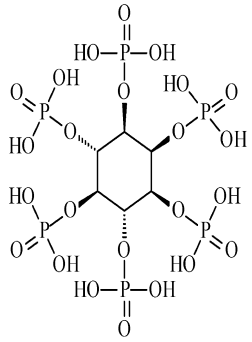
studies have shown that liver cirrhosis is associated with the reduction of zinc concentration in the liver [19] and in blood [20]. On the other hand, liver plays a key role in zinc homeostasis, regulating the incorporation of zinc ions in a large variety of enzymes which play a central role in the metabolism of the human body. The consequence of this interaction between zinc and liver [21] is that in every liver disease of different aetiology, the death of hepatocytes decreases the ability of liver cells to incorporate zinc ions into the enzymes which require zinc for their function. Since these enzymes express their function not only in the liver but in the whole organism, the disarrangement of hepatocytes in regulating zinc incorporation in macromolecules may severely affect zinc homeostasis, with relevant consequences in other organs. In addition, zinc deficiency may participate in the origin and the evolution of liver disease, lowering the efficacy of the antioxidant apparatus. This antistress action of zinc [22] is mainly due to a proper function of multiple zinc–proteins and it is probably at the basis of the ability of metalloproteinases to remove the excess of collagen fibers produced by hepatic stellate cells (HSC) as a consequence of liver cell death.

2.2. Zinc absorption

Zinc homeostasis is primarily regulated by the epithelial cells covering the mucosa of the gastrointestinal tract, by absorption of exogenous zinc contained in food, and by excretion of endogenous zinc. Adjustments in zinc absorption and excretion in the gastrointestinal tract are the primary means of maintaining zinc homeostasis in the human body [5].

Cellular uptake and intracellular distribution in the gut of the essential nutrient zinc is a precisely orchestrated process, coordinated by several membrane-associated proteins to ensure that zinc is removed from the intestinal lumen and is subsequently bound to small cytosolic zinc chaperones delivering zinc ions to specific subcellular compartments and zinc-requiring proteins. The recommended dietary allowance (RDA) for adults is 8 mg/day for women and 11 mg/day for men. RDA shows relevant changes according with different age: from 7 months to 3 years of age it is 3 mg/day; from 3 to 8 years of age RDA is 8 mg/day; during pregnancy, RDA increases up to 13 mg/day; during lactation, women require 12–14 mg of zinc daily [23]. The tolerable upper intake level (UL) for adults is 40 mg/day: prolonged high dietary intake of zinc may produce nausea or vomiting and can cause copper deficiency, but dietary zinc poisoning is virtually unknown [24].

The amount of zinc absorbed is related to different factors, which may significantly affect the level of absorption of the metal: zinc concentration in food, presence of dietary promoters and/or of dietary inhibitors, physiological states such as pregnancy, lactation and early infancy, all of which increase the demand for absorbed zinc [25]. Zinc content in food is very variable. Red meat is the richest common source of readily available zinc, providing about 50% of dietary zinc for most people [23]. Avoidance of red meat is, in fact, considered responsible for zinc and iron deficiency, particularly in childhood and in young women [26]. Fish usually contains small amounts of zinc, while some sea food, such as oysters, shows very high zinc concentrations, ranging from 2.3 up to 120 mg/100 g [27], with an average of 36 mg/100 g [28]. On the other extreme of the spectrum we can find vegetables and, in particular, tomatoes, which show a very low zinc concentration, below 1 mg/100 g [23]. This datum may clearly explain why zinc deficiency is frequently observed in people living in poor countries, as well as in vegans and vegetarians [1]. The zinc concentration in cow milk is analyzed in a small range of 310–445 μm/100 ml [29].



1,2,3,4,5,6 hexakis (di-hydrogen phosphate) *myo*-inositol

Phytic acid

Bioavailability of zinc entering the gastrointestinal tract is influenced not only by the amount of zinc present in foods, but even by other food constituents. Zinc absorption is inhibited by some non-digestible plant ligands, such as phytate (1,2,3,4,5,6 hexakis (di-hydrogen phosphate) *myo*-inositol), contained in corn, cereals, rice, legumes, which form insoluble complexes with zinc ions that are excreted in the stool [30]. The stoichiometries of these insoluble species can reach a Zn/phytate ratio up to 5:1, according to the value of the starting ratios [31–33]. Martin and Evans [34] also obtained a $pK_{sol} = 30.3$ for Zn/phytate 4:1 complexes. The literature about metal phytate complexes is thoroughly reviewed in the paper by Sammartano in this number of CCR [35]. A direct relationship has been discovered in children between dietary phytic acid intake and the risk of having a zinc hair content below 1.68 μmol/g, short height, watery stools and other symptoms related to zinc deficiency [36]. The amount and the quality of proteins in a meal may affect zinc absorption: in general, the amount of proteins is positively correlated with zinc bioavailability; some proteins, such as casein in milk and soy protein, have been reported to have an inhibitory effect on zinc absorption [37]. Promoters of zinc absorption are considered some amino acids, such as histidine and methionine, citrate and EDTA [30].

This may be due to two different factors: the formation of complexes prevents zinc–phytate precipitation and in addition it favours Zn^{2+} transport through intestinal membrane. To better understand the behavior of zinc complexes as pH function, the distribution plots of some representative amino acid and citrate complexes (Fig. 1), as natural occurring molecule are reported in Fig. 2.

The small bowel pH range, from 6.4 in proximal portion to 7.5 in distal portion [38], is emphasized in grey on the plots. The distribution plots were calculated using the literature formation constants reported in Table 1, at two different zinc concentrations

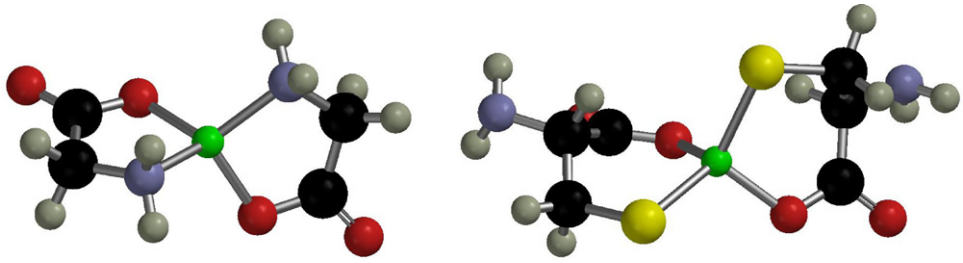


Fig. 1. Glycine (left) and cysteine (right) ZnL_2 lowest energy complex structures calculated with SPARTAN'06 program.

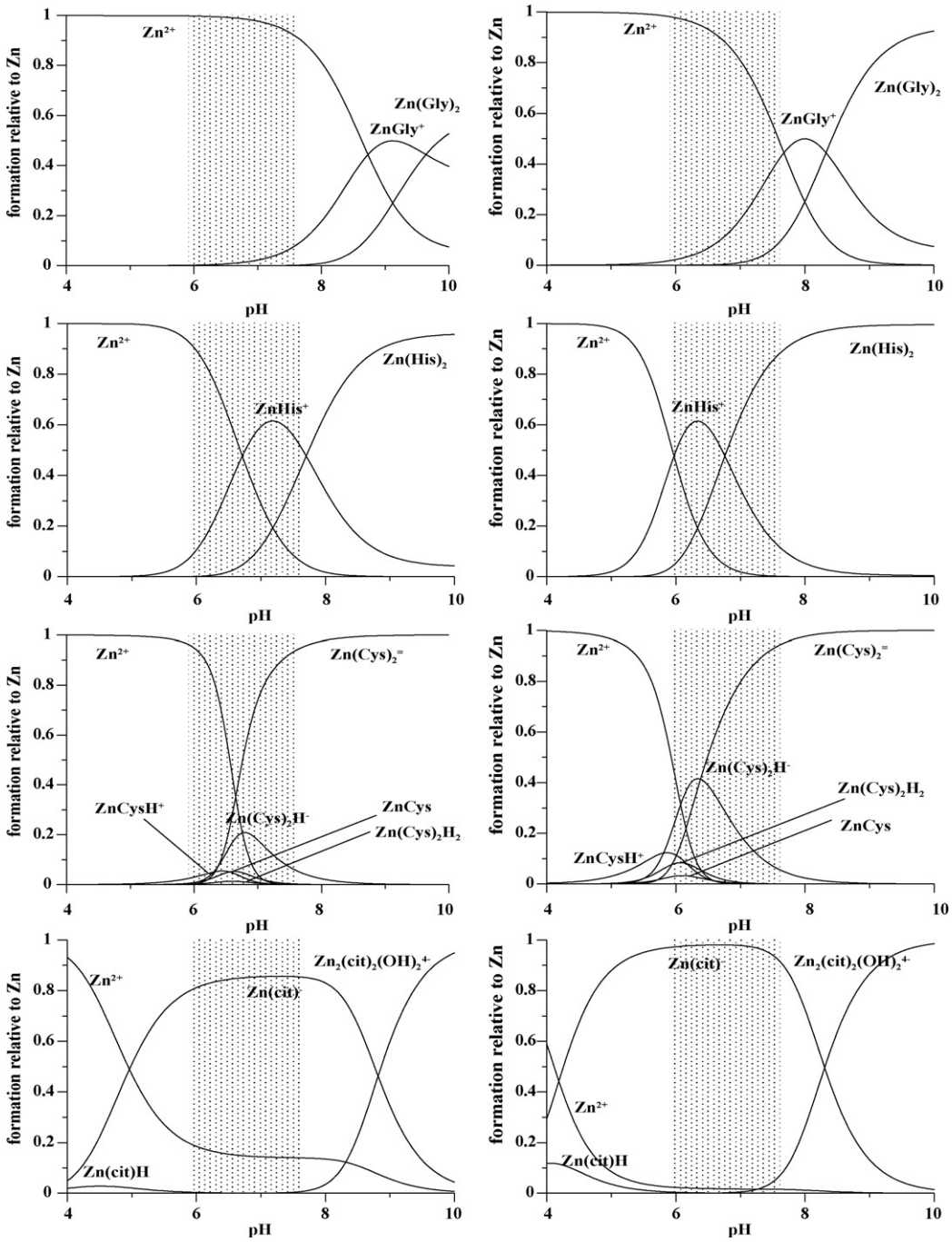


Fig. 2. Distribution curves of Zn complexes with glycine, histidine, cysteine and citrate (from top to bottom) at Zn^{2+} 10^{-5} M and 10^{-4} M and ligand 10^{-4} M and 10^{-3} M on the left and on the right, respectively. The pH range in gut is represented by grey area.

Table 1
The literature complex formation constants

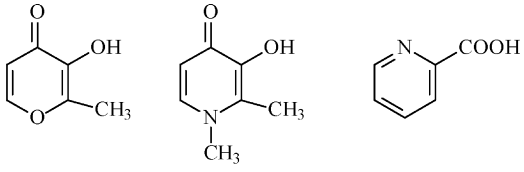
Species	Glycine [40,41]	Histidine [42]	Cysteine [43,44]	Citric acid [45,46]
LH	9.57	9.11	10.16	5.61
LH_2	11.89	15.16	18.26	9.91
LH_3	–	16.88	–	12.78
ZnLH	4.93	–	14.76	8.43
ZnL	–	6.51	8.20	4.83
ZnL_2H_2	–	–	29.93	–
ZnL_2H	–	–	24.43	–
ZnL_2	9.26	12.01	18.05	–
$Zn_2L_2H_{-2}$	–	–	–	−2.94
Zn_2L_3	–	–	29.2	–

(10 μM and 100 μM) according the estimate values of Sullivan before and after a meal, respectively [39]; 10-fold ligand concentrations were used.

These plots, in which the intestinal pH range is emphasized in grey, call for some considerations

- Glycine, which represents all amino acid chelating zinc only through carboxylic and amino groups, does not form significative amount of complexes in the pH range of interest with Zn^{2+} 10^{-5} M and ligand 10^{-4} M, while at higher concentration (Zn^{2+} 10^{-4} M and ligand 10^{-3} M) the 1:1 complex starts to appear.
- Cysteine and histidine, which in coordination use the –SH group and imidazol nitrogen, respectively, form zinc complexes almost quantitatively in the whole pH range at both concentration.
- Citrate forms at intestinal pH the 1:1 deprotonated negatively charged complex alone.

The formation of more stable mixed amino acid complexes with small ligands, as maltol, deferiprone, picolinic acid and its derivatives occasionally present in the gut, presented in a recent congress by Horvath et al. [47], can further enhance zinc absorption, changing the coordination geometry from tetrahedral to octahedral.



Maltol Deferiprone Picolinic acid

Zinc bioavailability in the gastrointestinal tract is also affected by the interaction among zinc and other trace elements present in foods. When iron is added to the diet, a significant reduction in zinc absorption is generally found [48]. The molecular basis for such an antagonism between iron and zinc has been identified in the intestinal divalent metal transporter 1 (DMT1) [49]. Recently, DMT1 has been shown to be an unlikely site for this competition, because zinc is not transported by the DMT1 [50]. Cadmium, contained in cereals, also inhibits zinc absorption [30]. Copper, at low doses, does not appear to affect zinc bioavailability in the gut [51], although high doses of zinc severely interfere with copper absorption. It remains to be elucidated whether high copper intake may affect zinc absorption. A low zinc intake may have negative effects on copper homeostasis [52], mainly in pregnancy and lactation of adolescent women [53]. Zinc toxicity due to acute or chronic ingestion of high doses of the metal leads to inhibition of iron and copper absorption and to hypocupremia [54]. Conflicting results have been reported on the effect of calcium on zinc absorption: the first studies showed an inhibitory effect of calcium on zinc absorption, probably due to the tendency of calcium to form insoluble complexes with phytate and zinc [55]. In other studies, calcium has been hypothesized to make more zinc available for absorption, competing with zinc cations for phytate [37]. At any rate all these interferences by various metal ions can be rationalized in terms of a direct involvement in the zinc complexation equilibria competing with the same ligands according to their stability constants and to their concentrations.

A major role in maintaining zinc balance is played by reabsorption of the considerable quantities of endogenous zinc secreted in the oral cavity by saliva, in the duodenum by pancreas and in all the other compartments of the gastrointestinal tract with each meal [5]. Dietary inhibitors of the normal reabsorption of endogenous zinc include phytate and unabsorbed fat [25].

The understanding of zinc transport at molecular level started with the cloning and functional characterization of ZnT1, the first mammalian zinc transporter discovered [56]. Mammalian transporters of zinc are within two gene families: the ZnT proteins, encoded by the solute-linked carrier 30 (SLC-30) family gene; the Zip (Zrt- and Irt-like proteins) family encoded by the SLC-39 gene family [2]. ZnTs and Zips appear to have opposite roles in zinc homeostasis: Zip proteins promote zinc uptake and vesicular zinc transport into the cytoplasm; ZnT carriers promote zinc efflux and/or zinc sequestration into vesicles. The roles of ZnT1 in regulating zinc efflux from cells, and of Zip transporters in zinc uptake have been recently confirmed in man [57]. In the gastrointestinal tract, zinc absorption is regulated by different Zip proteins, whose expression contributes to maintain zinc homeostasis. In humans, Zip4 (SLC39A4) has been localized at the apical pole of enterocytes and is considered the most important zinc transporter from the intestinal lumen into the enterocytes [58]. In biopsies from the small intestine of human subjects supplemented with zinc, it has been shown that Zip4 may change its expression, contributing to maintenance of zinc status in the body [58]. Intestinal Zip4 is markedly up-regulated in Zn-depletion conditions [59]. The prominent role of Zip4 in zinc metabolism has been evidenced in patients affected by acrodermatitis enteropathica, a autosomal recessive genetic disorder of zinc metabolism, caused by a mutation in the gene SLC39A4, which encodes for the zinc transporter Zip4 [60]. A mutation in the SLC39A4 gene, located on chromosome 15(q15–q21), is responsible for the block of zinc uptake, which causes zinc deficiency and a clinical picture characterized by poor growth, retarded development, suppression of cell-mediated immunity and severe dermatitis, mainly affecting the peri-oral, peri-anal and peri-ungueal areas [61]. Other members of the Zip family have been reported to be expressed in the enterocytes: Zip1 [62],

Zip3 [2], and Zip5 [63], but their role in zinc trafficking in the gastrointestinal tract remains to be elucidated. Recently, Zip1 and Zip3 were found to be down-regulated in zinc-supplemented human subjects [57]. Zip5 probably plays a central role in human zinc homeostasis, by antagonizing the action of Zip4 [64]. Different members of the ZnT family have been detected in the epithelium of intestinal mucosa. The majority of zinc transporters have been shown to be expressed in the small intestine, which plays a key role in Zn absorption in physiological conditions [65]. When the small intestine is impaired, also caecum and colon can participate in zinc uptake [66]. ZnT1, the first mammalian zinc transporter discovered [56], is expressed at the basolateral membrane of villus enterocytes of the small intestine, where it probably participates in zinc transfer to the circulation [67]. ZnT1 is probably also involved in cadmium transport [68]. ZnT2 is localized to vesicles (late endosomes) at the apical surface of enterocytes [69]; its putative function is to sequester zinc ions into vesicles and to down-regulate zinc absorption. ZnT4, whose mutation is responsible for the lethal milk mouse disorder in mice [70], is localized to enterocytes of the small intestine [69]. ZTL1, a zinc transporter which belongs to the cation diffusion facilitator family, is localized to the enterocyte apical membrane and putatively plays a role in the absorption of dietary zinc across the enterocyte membrane [71]. The divalent cation transporter 1 (DTC-1), also called Nramp2, is a zinc transporter which also shows iron transport capabilities [72]. DTC-1 is located at the basolateral membrane of enterocytes [49]. It has been suggested that a zinc–protein, cystein-rich intestinal protein (CRIP), which plays an important role in the regulation of the gut-associated lymphoid tissue, also may facilitate zinc transport across the enterocyte and its transfer across the basolateral membrane into the blood. CRIP could also compete with metallothionein, inhibiting zinc absorption [65].

While in physiological conditions the jejunum and ileum play a key role in zinc absorption, during zinc deficiency the colon and caecum can participate in zinc absorption [66]: ZnT4 and ZnT2 are the major Zn transporters subtypes expressed in the colon, which is considered a very sensitive organ for Zn homeostasis [73].

2.3. Zinc transport in blood

Albumin is the principal zinc carrier in plasma [74]. Zinc may also form complexes with cysteine or histidine [75]. Zinc concentration in plasma is about 15 μmol/l, 84% of which is bound to albumin, 15% is tightly bound to an alpha-2-macroglobulin and 1% to amino acids [3]. Albumin is also involved in zinc transport across the endothelial membrane, with a mechanism of vesicular cotransport [76]. Vesicular sequestration of zinc is facilitated by ZnT2 [77]. Since a great portion of plasma zinc is bound to alpha-2-macroglobulin [78], this protein has been suggested to be a plasmatic carrier of the metal. Recently, however, it has been shown that the macroglobulin fraction does not function in zinc transport and distribution [79]. It has been suggested that transferring may also play a role as a carrier of zinc ions in plasma [74]. Zinc plasma levels have been proved to be modulated by different hormones: growth hormone (GH) increases zinc plasma level, whereas prolactin decreases it [80]. Zinc might also be transported into erythrocytes as a zinc–histidine complex [81].

2.4. Zinc uptake and storage by hepatocytes

Zinc movement in the cell is tightly regulated and intracellular free zinc is very limited [82]. Trafficking of zinc in the hepatocyte is complex and involves several transport proteins. Two mechanisms have been suggested for Zn uptake in cultured liver cells: a high affinity saturable pathway and a low affinity linear pathway [83]. Within the hepatocytes, within the enterocytes as well as within other cells, zinc is mainly stored bound to metalloproteins, which include metalloenzymes, gene regulatory molecules, storage proteins and zinc carriers [84]. Within the hepatocytes zinc is mainly bound to metallothioneins. These are ubiquitous proteins characterized by low molecular weight and high cysteine content [3]. Human MTs are encoded by a multigene family located on chromosome 16 [85]; they are a family of at least 17 closely related gene products. Each MT contains 60–68 amino acids, 20 of which are cysteines, and binds seven zinc atoms. MTs bind to zinc, cadmium and copper [86]. The metallothionein/thionein system safeguards zinc cytoplasmic concentration, and controls the readily available zinc pool. The metallothioneins have two main functions: (i) to sequester and to store excess zinc and (ii) to rapidly release zinc by events which signal its requirement [87]. Metallothionein (MT) can also function as a chaperone for zinc transport from the cytoplasm into liver mitochondria [88]. The protective role played by MTs in human cells, and particularly in the enterocytes and in the hepatocytes has been suggested to be similar to that of glutathione. Other than storing zinc ions, metallothioneins also participate to intracellular zinc trafficking: it has been shown that the oxidation of MT by oxidized glutathione (GSSG) releases zinc to other specific ligands [89]. The rate of zinc release from MT depends on the amount of GSSG in the hepatocyte: in conditions of oxidative stress, the more oxidative the redox state becomes, the more efficiently zinc is released from MT [90]. Zinc transfer from MT probably needs a protein–protein interaction between MT and the zinc acceptor, which causes conformational changes, delivering zinc to specific proteins [91]. Zinc-binding MTs expression may affect a number of important cellular processes, such as gene expression, proliferation and differentiation [92]. One of the most important functions of MT is maintaining high levels of zinc in liver cells and in enterocytes. The coordination between zinc and MT is thus that MT retains zinc in the hepatocytes and in the enterocytes under physiological conditions, and releases zinc under oxidative stress, leading to potent antioxidant action [93]. The mechanism of action of MT, and in particular of zinc–MT, has been only recently clarified. MTs are rich in thiol groups, which have a strong affinity for zinc and for transition metals, such as iron and copper, which are bound to MT in a thermodynamically stable binding. Oxidation of the thiolate cluster causes formation of MT-disulfide followed by zinc release. The most critical advance in MT research is the demonstration of the redox regulation of the interaction between zinc and sulfur [94] MT redox cycle ends with the reduction of MT-disulfide to MT

by glutathione, restoring the capacity of the protein to bind zinc [95]. In pathology, MTs were found to be increased in the liver of patients affected by proinflammatory and cholestatic conditions and by systemic diseases, such as idiopathic bowel disease (IBD) [96]. MTs have been shown to maintain high levels of zinc in the liver, suggesting that the protective action of MTs in the liver is mediated by zinc [93]. In the same study, zinc has been shown to protect liver from acute alcohol-induced liver injury [93]. In ethanol-treated MT-knockout mice, zinc administration was able to protect intestinal mucosa cells of the small intestine from acute alcohol-induced damage [97]. In the cirrhotic rat liver, Zn–MT was induced by zinc treatment [98]. Since MT has been thought to be involved in cell defence against oxidative stress, the metallothionein induction exerted by zinc in the hepatocytes is considered to play a role in halting the progression of liver fibrosis [98].

2.5. Zinc excretion

The main excretory route for zinc in humans is gastrointestinal secretion, which is calculated as 2.5–5.5 mg/day [3]. It occurs via apoptosis of epithelial cells lining the intestinal mucosa or via salivary glands, pancreatic, biliary and intestinal cell secretion. Liver plays a pivotal role in the homeostasis of zinc, by extracting the trace metal from plasma, storing it, metabolizing zinc ions and inserting them in various proteins, and redistributing zinc in various forms into bile or back to the bloodstream [99]. Bidirectional transport across the sinusoidal pole of hepatocytes allows the liver to control plasma zinc concentration and, therefore, zinc availability to peripheral tissues. Zinc transport at the biliary pole of hepatocytes into bile canaliculi appears to be mainly unidirectional, and is considered the major excretory pathway for excess zinc in the human body [99].

Fecal loss may range from less than 1 mg/day with a zinc-poor diet, up to 5 mg/day with a zinc-rich diet. Renal zinc secretion, which accounts for about 0.3–0.7 mg/day, is considered of lower relevance [100]. Zinc loss from the body is also attributed to epithelial cell desquamation, sweat, semen, hair and to the menstrual cycle.

2.6. Zinc as an antioxidant

The generation of reactive oxygen species (ROS), which derive from electron transfer reactions in mitochondria and endoplasmic reticulum, is inherent in aerobic cell metabolism [79]. Zinc plays different roles in protecting biological structures from damage by free radicals: it maintains an adequate level of metallothioneins; it is an essential component of superoxidodismutase (SOD) [79]; it is a protective agent for thiol groups; it prevents the interaction between thiol groups and/or other chemical and disulfide formation, which may result in the loss of enzyme activity [101]; it prevents lipid peroxidation in mitochondria and microsome membranes; it stabilizes the cell membrane structure and prevents osmotic fragility of erythrocyte membranes [3].

Iron and copper participate in a catalytic cycle to produce dangerous ROS radicals. Their reduction to iron(II) and copper(I) is essential to catalyze Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet$$

The reduction potential of iron and copper is strongly affected by the ligands in the biological environment according the fact they stabilize oxidized or reduced form. One of the principal groups involved in iron and copper reduction is the thiol group, being –SS–/SH reduction potential −220 mV. The coordination of zinc exerts a protective action on thiol group oxidation: even if the stability of thiol–zinc complexes are lower than those with many other transition metal ions, the high zinc concentration in the biological environment allows a decrease of free –SH groups of 1/2 magnitude orders, corresponding to a decrease of 50–100 mV in the –SS–/SH reduction potential. This decrease can be enough in many instances to prevent iron and copper reduction.

Due to its antioxidant capabilities, zinc may inhibit lipid peroxidation [102], which can result in the loss of membrane fluidity, receptor alignment and cell necrosis [103]. It may also minimize the toxic effects of free radicals and of reactive oxygen species, producing SOD enzymes and reduced glutathione (GSH), which are pivotal in antioxidant defence in humans [80]. The liver contains high concentrations of Cu/Zn SOD, present in the cytosol, in the hepatocytic nucleus and in peroxisomes, where it catalyses superoxide anions to oxygen and hydrogen peroxide [104].

Multiple zinc enzymes contribute through GSH synthesis or the GSH redox cycle to regenerate GSH from its oxidized form GSSG. Catalase and GSHPx utilize GSH as a sacrificial reducing agent to reduce hydrogen peroxide and alkyl hydroperoxides into water and alcohol, respectively [104]. Reduced glutathione (GSH2), synthesized in hepatocytes, is considered the main intracellular defence against damage from free radicals, reactive oxygen species and xenobiotics. The role of GSH in protecting liver cells against xenobiotics has been demonstrated, in clinical practice, by the observation of fulminant hepatitis in patients assuming high doses of paracetamol, an antipyretic drug. Paracetamol is metabolized, in the hepatocytes, by the drug metabolizing system, with production of the unstable metabolite *N*-acetyl-*p*-benzoquinoneimine which can be rapidly inactivated by GSH and when a patient takes high quantities or suicidal doses of the drug, the amounts of metabolites deplete hepatic GSH, causing liver cell necrosis and diffuse apoptosis with dramatic clinical consequences [105]. GSH, the main component of the antioxidant liver defence, maintains the integrity of cell membranes and protects cells against ionizing radiations [106] and ultraviolet light [107]. The amount of reduced glutathione (GSH) present in liver cells is mainly related to zinc stores in the cell. It has been shown that zinc-rich metallothioneins, in the presence of GSSG, are oxidized, and lose their zinc atoms which are released to other proteins which need Zn for their function. As a result, oxidized glutathione is reduced to GSH [108]. Free radical activity may contribute to disease activity in inflammatory bowel disease mainly when associated with low glutathione values in the intestinal mucosa [109] Free radical production is

increased in patients affected by inflammatory bowel disease. Tissue lipid peroxide levels, including malondialdehyde, have been reported in colon mucosa from subjects with ulcerative colitis [110] and in children with Crohn disease [111].

2.7. Zinc and apoptosis

Cell death by apoptosis, a regulated biological mechanism required for the removal of superfluous or damaged cells, is one of the most important cellular processes influenced by the zinc status [112]. Apoptosis is a major mechanism of cell death in response to many infectious or toxic agents and, in particular, its dysregulation is central to the pathogenesis in diseases such as chronic liver disease [113] and inflammatory bowel disease. The input signalling pathways that commit cells to apoptosis converge, inside the cell, onto a central pathway masterminded by the 14 members of the caspase family; these are activated by cleavage by other caspases, resulting in a cascade of proteolytic events [3,114]. It has been shown that systemic changes in zinc homeostasis can influence cell susceptibility to apoptosis, which appears to be regulated by a specific labile pool of intracellular zinc [16]. Zinc has been shown to inactivate caspase-3, blocking the apoptotic process and protecting cells from death [115]. Increased apoptosis in vivo may occur as direct or indirect consequence of a decrease in intracellular zinc concentrations [115]. On the other hand, an increase of zinc concentration toward toxic levels inside the hepatocyte may trigger the apoptotic cascade, by allowing cytochrome *c* release from mitochondria [116], which is followed by the activation of caspases. Zn may also block caspase-6, which is considered the most sensitive apoptosis-related molecular target of zinc [117]. Low zinc levels may allow the activation of caspase-3 and -8, triggering the apoptotic process [118]. Zinc addition in cell culture has been shown to prevent apoptosis induced by tumor necrosis factor alpha (TNF alpha) [119]. Zinc may also act on the balance between the antiapoptotic protein Bcl-2 and the proapoptotic protein Bax, by increasing the Bcl-2/Bax ratio thereby increasing cell resistance to apoptosis [120].

2.8. Zinc in disease of the gastrointestinal tract

The single layer of epithelial cells in the mucosa of the gastrointestinal tract, held together by tight junctions, provides a barrier between the external environment and the body. Zinc is considered to be a key factor for the preservation of structural integrity of the intestinal barrier [97]. Several key enzymes in the epithelial cells of the intestinal mucosa, such as carbonic anhydrase, which regulate the secretion of mucosal protective bicarbonate ions, are metalloenzymes and require zinc for their action [121]. Every mechanism, every stress that breaches the integrity of the gastrointestinal barrier may modify the state of health of the gut mucosa, with biological and clinical consequences. Gastrointestinal disorders associated with physical stress include inflammatory bowel disease (IBD), gastroesophageal reflux, irritable bowel syndrome (IBS), nonsteroidal anti-inflammatory drugs and chemotherapeutic agents [22]. In this context, zinc may halt the progression of the gastrointestinal disease by its participation in free radical scavenging, halting the inflammatory process. During inflammation of the gut, zinc plays a relevant role as a powerful antiinflammatory agent. This antiinflammatory activity of zinc ions is probably due to the inhibition of nitric oxide, an ubiquitous signalling molecule that modulates inflammation in the gut [122]. On the other hand, the reduced absorption of zinc and the increased intestinal loss of the metal due to the malfunction of the stressed gut mucosa are at the basis of modifications in zinc homeostasis observed in patients with IBD. In intestinal biopsies from subjects affected by Crohn's disease, a decrease in Cu–Zn SOD activity was reported [123]. A study on 54 adult patients affected by CD showed marked micronutrient deficiencies: in particular more than 50% of patients had low plasma zinc concentrations [124]. A kinetic study on zinc clearance in CD gave evidence that the absorption of zinc seems to be intact and suggested that hypozincemia in these patients could be related to an accelerated turnover of the trace metal [125]. In intestinal biopsies from subjects with CD, a decrease in Cu–Zn SOD activity was reported [123]. Children with Crohn's disease showed lower serum zinc levels, probably as a consequence of the impaired zinc absorption by the inflamed intestinal mucosa [126]. Low zinc absorption and inappropriately high endogenous fecal zinc losses were reported to may cause a worse zinc balance [127]. Patients affected by ulcerative colitis, a disease mainly localized in the colon and rectum, did not show relevant changes in zinc status [126,128]. This observation underlines the relevance of the integrity of the small intestine, mainly affected in Crohn's disease, in zinc absorbance and homeostasis. The role of zinc deficiency in inflammatory bowel disease has been underlined by the observation of a marked decrease in the Cu/Zn-SOD expression within the intestinal mucosa of patients affected by ulcerative colitis and Crohn's disease [129]. In another study, a decrease in zinc content was observed in the mucosa of patients affected by ulcerative colitis, associated with the increase in reactive oxygen intermediates [123]. Very low serum concentrations of several nutrients, including zinc, have been reported in a study on 46 patients with ulcerative colitis [130]. These observations confirm the presence of disturbances in the zinc-related antioxidant cascade in the gut of patients affected by IBD. Zinc status appears to be of great value in the oxidant–antioxidant balance within the intestinal mucosa and in the clinical evolution of IBD since Cu/Zn-SOD plays a relevant anti-inflammatory role in the gut by triggering the clearance of neutrophils accumulated in the intestinal mucosa in ulcerative colitis and in Crohn's disease [129]. Moreover, the role played by zinc in IBD could be related to the influence of the metal on host defence. Zinc acts in T-lymphocyte activation [131], whereas zinc deficiency decreases chemotaxis by neutrophils and phagocytosis by macrophages [132].

The role of zinc in maintaining the integrity of the intestinal mucosal barrier is well evidenced in pathology. In trinitrobenzene sulfonic acid-induced colitis, an animal model of Crohn's disease, the administration of zinc-carnosine had a protective effect on the evolution of the disease [133]. Zinc-carnosine was also shown to prevent acetic acid-induced colitis in rats, through

induction of heat shock protein 72 and suppression of the transcription factor NF-KB [134]. Diarrhoea occurs in children with zinc deficiency and responds to oral zinc supplementation [132]. Increased losses of zinc can occur during diarrhoea; absorption of zinc is impaired by mucosal inflammation, anorexia, dietary restrictions, typically observed in patients with IBD. All these factors may well explain the development of zinc deficiency in association with intestinal diseases occurring in a variety of chronic intestinal disorders [135]. In the early cases of acrodermatitis enteropathica (AE) reported, diarrhoea was considered a prominent feature, underlying the role of the inherited zinc deficiency in the integrity of the intestinal mucosa [61]. In Crohn's disease, the combination of anorexia, malabsorption from the disease itself and intestinal losses of cells and plasma make it plausible that zinc deficiency could occur in this chronic gut disease [136]. In fact, in subjects affected by Crohn's disease plasma zinc levels were found to be very low [137]: the causes of this observation could be identified in inflammation of intestinal mucosa, with the consequent poor intake of protein and a reduction in plasma levels of albumin, the main plasma carrier of zinc atoms. Subnormal levels of plasma zinc were also reported in subjects affected by inflammatory bowel disease [3]. The role of zinc status in the evolution of IBD could also be related to the role played by zinc in wound healing, which should be mainly considered in Chron's disease patients with persistent fistulae [138].

Zinc homeostasis is also important for the integrity of gastric mucosa cells. Zinc deficiency of different origin may predispose the gastric mucosa to damage from xenobiotics and from exposure to stress [139]. Zinc ions have been shown to present anti-ulcer activity: zinc-carnosine is an anti-ulcer drug commonly used in the therapy of gastric ulcer in Japan [140]. The growth of Helicobacter pylori (Hp), a major aetiological agent in chronic gastritis and in peptic ulcer, is inhibited by zinc compounds [141]. Thus, the role of zinc supplementation in patients affected by Hp-related gastritis may not be restricted to the control of the growth of this pathogenic bacterium. It has been suggested that zinc could also enhance the protective mechanisms of the gastric mucosa cells.[142]. Zinc has been shown to influence alcohol metabolism in the stomach. Several studies have revealed that a significant fraction of ingested alcohol is oxidized in gastric mucosa cells by a zinc-enzyme, alcohol dehydrogenase. This metabolic step is known as the first-pass metabolism of ethanol and is considered a gastrointestinal barrier against the systemic toxicity of ethanol [143]. Chronic alcohol consumption and some drugs, such as H2 antagonists, may interact with metabolism of ethanol in gastric mucosa, diminishing alcohol dehydrogenase activity and increasing toxic systemic effects of ethanol [144].

In patients affected by Wilson's disease, an inherited disease of copper metabolism, zinc therapy increased duodenal concentrations of metallothioneins by 1500% [145]. Zinc homeostasis may even play a role even in maintaining the integrity of the oesophageal mucosa cells. Patients with endoscopic oesophagitis showed serum zinc concentrations significantly lower than control subjects [146].

2.9. Zinc in liver disease

Since liver plays an important role in the metabolism of zinc, it is not surprising that zinc metabolism is disturbed in the course of every acute or chronic liver disease. Patients with alcoholic cirrhosis often present acrodermatitis enteropathica-like symptoms: dry eczematous skin lesions, stomatitis, cheilitis, and alopecia, which respond to zinc supplementation [147]. On the other hand, nutritional zinc deficiencies also predispose to the pathogenesis and or perpetuation of liver damage, given the pivotal role of zinc ions as powerful antioxidants, in the imbalance between the formation of reactive oxygen species and antioxidant defence mechanisms.

Collagenase is a zinc-metalloenzyme [148] and zinc is the most effective inhibitor for prolyl hydroxylase, an enzyme which plays a key role in collagen synthesis [149]. These two assumptions could easily explain the role of zinc in collagen deposition and reabsorption in liver disease, the role played by zinc in liver fibrosis and in the evolution of chronic hepatitis toward cirrhosis [150].

Zinc deficiency has been well documented in several liver diseases [21]. Low serum zinc levels in cirrhotic patients affected by alcoholic liver disease (ALD) were first reported, in the fifties [151]. In recent years, reduced serum levels of zinc have been reported in several liver diseases of different aetiology [152]: acute and chronic viral hepatitis are associated with reduced serum zinc levels, without any difference among groups classified according with the aetiology or the clinical course of the liver disease [153]. The linkage between zinc homeostasis and viral infection could be identified with the assumption that viruses produce severe oxidative stress in the hepatocytes, leading to apoptosis or hepatocytic necrosis and causing a decrease in protein synthesis. In fact, low serum levels of zinc were found to correlate with low serum albumin concentrations in patients with viral hepatitis [154]. Metabolism of hepatitis C virus (HCV) is strictly related to zinc status in the hepatocyte. In fact, NS5A, a viral protein which is considered fundamental for viral replication, is a zinc-metalloprotein, which needs zinc coordination to function properly [155]. At the cellular level, Zn administration has been shown to inhibit apoptosis of liver cells induced by ethanol, through the suppression of the Fas/FasLigand-mediated pathway [156].

The role of zinc in the evolution of chronic liver disease of different aetiology is mainly related to its influence on the remodelling of liver fibrosis and on cirrhosis, the end stage consequence of liver fibrosis [150]. Liver cirrhosis has been correlated, in different human [157] and experimental studies [158], to reduced serum and hepatic zinc levels, compared with healthy individuals. In some studies, Zn concentration was inversely correlated with the degree of liver damage or with the evolution toward cirrhosis [159]. Liver fibrosis is the excessive deposition of extracellular matrix (ECM) proteins including collagen, resulting from chronic liver cell damage. Activated hepatic stellate cells, portal fibroblasts and myofibroblasts of bone marrow origin have been identified as major collagen-producing cells in the injured liver [113]. Accumulation of extracellular matrix in the liver results from both increased synthesis and decreased

degradation [160]. Activated hepatic stellate cells (HSC) are the main collagen-producing cells in the injured liver [161]: they secrete large amounts of extracellular matrix and regulate its degradation. A complex interplay among different liver cells takes place during liver fibrosis [113]: hepatocytes are targeted by hepatotoxic agents; damaged hepatocytes release reactive oxygen species; apoptosis of hepatocytes stimulates the fibrogenic action of myofibroblasts [162]. Kupffer cells, the resident macrophages of the liver, when activated by liver cell necrosis, produce different cytokines, such as tumor growth factor-beta 1 (TGF-β1), that participate in activation of hepatic stellate cells [163]. Platelet derived growth factor (PDGF), mainly produced in liver by Kupffer cells, is considered the predominant mitogen for activated hepatic stellate cells, and regulates collagen synthesis in HSC [164]. Oxidative stress may represent a relevant pro-inflammatory stimulus for HSC: H_2O_2 may act as intracellular signal mediator of the pro-fibrogenic action of TGF-β1 produced by Kupffer cells [165].

Recent developments in our understanding of hepatic fibrogenesis give a picture of a dynamic process, suggesting a previously unknown capacity for recovery even in cases of severe fibrosis and cirrhosis [166]. Zinc probably plays a key role in the remodelling fibrous septa, and in the degradation of multiple extracellular matrix components, among which collagen I is the predominant factor. The matrix metallo-proteinases (MMP), a family of zinc-metalloenzymes produced by Kupffer cells and by HSC, are also known as collagenases, and have the capability to degrade various ECM components [10]. MMP1 is the best characterized collagenase in man, and is widely expressed in the liver. MMPs are released by Kupffer cells as inactive pro-enzymes, and are activated by the protease plasmin which confers enzymatic activity to collagenases [167]. Activated hepatic stellate cells play a central role in the balance between pro-fibrotic and anti-fibrotic factors. Activation of HSC is regulated by several soluble factors: promoters of HSC proliferation, such as PDGF, basic fibroblast growth factor and insulin-like growth factor1; factors promoting fibrillar ECM components deposition, such as transforming growth factor beta1 [168]. HSC not only produces a fibrogenic environment within the liver through an overproduction of extracellular matrix components, but also may counterattack the collagenolytic machinery. In fact, activated HSC have been shown to produce different tissue inhibitors of metalloproteinases (TIMPs). In the human liver TIMP1 is able to block collagenases preserving the integrity of fibrous septa. Moreover, activated HSC also produce plasminogen activator inhibitor 1 (PAI1), which inhibits plasmin synthesis: as a consequence, MMPs pro-enzymes are not activated and cannot remove collagen fibers. Zinc interferes in this balance, favouring the activation of collagenases, and directly activating mast cells to produce nerve growth factor [169] that may induce apoptosis of hepatic stellate cells, i.e. the goal of every antifibrotic therapy [170].

2.10. Zinc and Wilson's disease

Wilson's disease, an inherited disorder of copper metabolism characterized by copper accumulation in different organs with chronic liver disease and evolution toward cirrhosis, may be considered a model for the study in human pathology of the effects of zinc as an antioxidant and as a pro-collanenolytic co-factor. In fact, Wilson's disease represents the unique chronic liver disease in which therapy with zinc salts is considered fully effective [171]. The mechanism of action of zinc is multifactorial and, at least in part, it is not completely understood. Zinc interferes with copper absorption in the gut, at least with two mechanisms:

- by inducing metallothionein synthesis in the enterocytes, blocking copper transport toward the basolateral membrane of the enterocyte [172];
- by competing for the same carrier in enterocytes [173].

Recently, it has been shown that zinc treatment may increase glutathione availability by increasing liver content of reduced glutathione and lowering the high concentrations of oxidized glutathione observed in patients with Wilson's disease under penicillamine treatment [174]. Excess copper ions in Wilson's disease appear to potentiate lipid peroxidation of liver cells' membranes, by reacting with thiol groups and with hydrogen peroxide, which causes the production of highly reactive hydroxyl radicals followed by lipid peroxidation [175]. Zinc atoms react with thiol groups, preventing the reaction of iron and copper ions with these groups and, eventually, protecting cell membranes from peroxidation-related damage. Experimental data on the ability of zinc ions to block toxic effects exerted on liver cells by copper overload have been recently confirmed in clinical practice [176]. Zinc sulfate was administered to 22 asymptomatic children affected by Wilson's disease for 10 years. A marked decrease in transaminases serum levels were detected 5 years after the start of zinc therapy in all but one child. All histological signs of liver damage (steatosis, inflammation, fibrosis and copper storage) were significantly decreased after treatment. The excellent clinical results in all patients induced the authors to indicate zinc as the treatment of choice in pediatric patients with Wilson's disease [176]. In a study carried out with sequential liver biopsies in patients affected by Wilson's disease under zinc therapy, a dramatic regression of liver fibrosis was observed in two patients with histologically proven cirrhosis (D. Fanni, unpublished data).

3. Conclusions

Zinc research may be considered still at an early stage of its evolution. There is a need for accelerate its progress, which could be relevant in human health and disease and, in particular, to better understand the pathogenesis and progression of liver and gastrointestinal disease. The correlation of zinc status in liver and gut in patients affected by chronic hepatitis or inflammatory bowel disease respectively could help to better analyze the role played by pro-inflammatory factors and by antiinflammatory components in the evolution of these diseases. An in deep study of the tissue expression of zinc carriers in the epithelial cells covering the various sections of the intestinal tract, both in physiology and in disease, will allow us to improve our knowledge of the molecular basis of the disarrangement

in zinc metabolism in human disease. Consequently, there may be promising possibilities for in the introduction of zinc in the treatment of acute and/or chronic gastrointestinal disorders. The study of zinc carrier expression in the liver of subjects affected by acute or chronic hepatitis could lead to the introduction of zinc, as a powerful antioxidant, even in the therapy of liver disease. The ability of zinc to halt hepatic fibrosis and to favour regression of liver fibrosis, well accepted in patients affected by Wilson's disease, could be the basis of its introduction in clinical practice with beneficial effects in cirrhosis of different aetiology.

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