Nutrition and Diet Research Progress

Rebecca Morton
Editor

Zinc Deficiency

Etiology, Screening Methods and Health Implications

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ZINC DEFICIENCY

ETIOLOGY, SCREENING METHODS AND HEALTH IMPLICATIONS

REBECCA MORTON
EDITOR

New York

Library of Congress Cataloging-in-Publication Data

Library of Congress Control Number: 2015959368

ISBN: 978-1-63484-429-1 (eBook)

Published by Nova Science Publishers, Inc. † New York

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PREFACE

Zinc is the second most prevalent trace found in the human body after iron. It is essential for normal cell function and metabolism, playing a central role in over 300 enzymatic reactions, and protects cells from free radical damage. The central role of zinc in cell growth and differentiation explains the dramatic effect of zinc deficiency in tissues with a rapid cell turnover such as hair growth. In recent years, much interest has been generated by the possibility that subclinical zinc deficiency may significantly increase the incidence of and morbidity and mortality from diarrhea and upper respiratory tract infections. Clinical manifestations of zinc deficiency include delayed sexual maturation, impotence, hypogonadism, oligospermia, alopecia, dysgeusia, immune dysfunction, night blindness, impaired wound healing, and various skin lesions. After bariatric surgery, zinc deficiency is often associated with other micronutrients deficiencies, mainly iron. Chapter One in this book provides a review of the etiology, health implications and management of zinc deficiency after bariatric surgery. Currently, recommendations regarding routine screening for zinc deficiency or the use of daily oral zinc supplementation in individuals after bariatric surgery are not based on clinical trials. Gaps that exist in the understanding of zinc deficiency are summarized in Chapter Two. Chapter Three brings attention to the important implications of zinc in carcinogenesis. It provides a comprehensive review of the current literature on the role of zinc in carcinogenesis, as well as highlights the significance of zinc homeostasis mechanisms, which are essential for understanding the key roles of this trace element in carcinogenesis.

Chapter 1 – Bariatric surgery leads to a significant body weight reduction, and improvement of obesity-related comorbidities. However, it is associated with a higher risk of presenting some nutritional deficiencies. These

deficiencies are especially relevant after mixed or malabsorptive procedures. Deficiencies in micronutrients after bariatric procedures are a known threat if not corrected appropriately. Though zinc deficiency is not considered among the most frequent deficiencies after bariatric surgery, several studies have shown that its frequency might overcome 10%, even after restrictive procedures and in patients with multivitamin supplements intake. Zinc is the second most prevalent trace found in the human body after iron. It is essential for normal cell function and metabolism, playing a central role in over 300 enzymatic reactions, and protects cells from free radical damage. The central role of zinc in cell growth and differentiation explains the dramatic effect of zinc deficiency in tissues with a rapid cell turnover such as hair growth. In recent years much interest has been generated by the possibility that subclinical zinc deficiency may significantly increase the incidence of and morbidity and mortality from diarrhea and upper respiratory tract infections. Clinical manifestations of zinc deficiency include delayed sexual maturation, impotence, hypogonadism, oligospermia, alopecia, dysgeusia, immune dysfunction, night blindness, impaired wound healing, and various skin lesions. After bariatric surgery, zinc deficiency is often associated with other micronutrients deficiencies, mainly iron. It has been demonstrated that zinc and iron levels, both within the normal range, but close to the minimum level of the range, can be associated with hair loss, mainly between the 6^{th} and 9^{th} postoperative month. For the evaluation of zinc status, plasma levels are generally a good index of zinc status in healthy individuals. Zinc supplements are usually indicated for patients with low zinc levels, depending upon the clinical context. In obese patients after bariatric surgery, zinc supplementation can be considered even in patients with serum levels within the normal range, when iron levels are also close to the minimum value of normality and the patient complain of alopecia.

Chapter 2 – The rising prevalence of obesity (body mass index of >/= 30 kg/m^2) in more than 100 countries has been described as a global pandemic. Studies have found that surgery results in more significant and sustained weight loss, when compared to dietary and lifestyle interventions, in individuals with medically-complicated obesity. Zinc is an essential component of the catalytic site of hundreds of different metalloenzymes where it functions as a Lewis acid. The small intestine is the main site of zinc absorption via transporter expression which is regulated in part by dietary zinc intake. Zinc deficiency is a clinical concern since it may result in multiple clinical symptoms. After bariatric surgery, the prevalence of biochemical zinc deficiency has been reported to range from 5% to 83%. It has been suggested

that bariatric procedures that result in restriction may have lower rates of zinc deficiency compared to bariatric procedures that produce restriction as well as malabsorption. The mechanisms for zinc deficiency after bariatric surgery have not been fully delineated but may include decreased intake, poor food tolerability, decreased surface area for absorption, rapid oral-cecal transit, altered compensatory mechanisms, and/or small intestinal bacterial overgrowth. Elevated serum folic acid levels, a marker for small intestinal bacterial overgrowth, is common after gastric bypass surgery, and small bowel bacterial overgrowth is associated with decreased zinc absorption. The authors evaluated 230 patients after gastric bypass surgery and 145 patients had elevated serum folate levels, supporting 63% prevalence for small intestinal bacterial overgrowth. In 54 patients with normal serum folate levels, 43 had normal zinc levels, while among 118 patients with elevated serum folate, 78 had low serum zinc (Chi-squared 2X2: $P < 0.001$), supporting upper gut bacterial overgrowth as a mechanism for biochemical zinc deficiency. They have evaluated 43 patients who underwent upper endoscopy after gastric bypass surgery. In 17 out of 43 (40%) of these patients, there was an abnormal finding at upper endoscopy: gastrojejunal anastomotic stricture (obstruction) or gastrojejunal anastomotic ulcer. In 8 patients with gastrojejunal ulceration, 66% of the patients had low serum zinc levels. In patients who had elevated serum folate levels, there was no increased risk of gastrojejunal ulceration (Chi-squared 2X2: $P = 0.53$). Currently, recommendations regarding routine screening for zinc deficiency or the use of daily oral zinc supplementation in individuals after bariatric surgery are not based on clinical trials. Gaps that exist in the authors' understanding of this clinical disorder are summarized in this chapter. Since present physiological evidence supports zinc absorption primarily in the jejunum by a transcellular route involving a zinc-specific transporter, Zip4, proposed studies should examine potential post-operative changes in zinc transport and the potential for reversibility with antibiotic treatment of small intestinal bacterial overgrowth.

Chapter 3 – Zinc is the second most abundant trace element in humans and is essential for the activity of more than 300 enzymes. It affects the conformation of many transcription factors associated with control of cell proliferation, apoptosis, and signaling. Therefore, immune function also depends heavily upon its regulation. Studies of this mineral suggest that alterations in zinc homeostasis are linked to the development of numerous diseases, including cancer. Several epidemiologic and experimental studies have investigated the relationship between zinc intake and the risk for development of many cancer types. These include mammary and prostate

tumors, and especially cancer of digestive tract. However, experimental outcomes have remained inconsistent. For example, some studies suggest that increased zinc intake is negatively associated with colorectal and esophageal cancer, while other studies show a positive or null association. Zinc concentration can affect the body system by many means. In general, zinc effects are based on intermediary metabolism and bioenergetics effects, proliferative/apoptotic effects and motility and invasive effects. Zinc deficiency adversely affects the immune system, increases oxidative stress, and increases the production of inflammatory cytokines. Mitochondrial accumulation of zinc inhibits aconitase activity, halting citrate oxidation. This results in decreasing the cellular energy production requirement of malignant cells. The physiological zinc levels can also affects intracellular signaling pathways. For example, the high intracellular zinc level inhibits the activity of transcription factor NF-κB resulting in promestatastic and proangiogenic molecules reduction. By contrast, zinc deficiency increases perphosphorylation of protein kinase B (Akt), E3 ubiquitin-protein ligase (Mdm2), and reduces nuclear p53 accumulation. Overall, more research into the mechanisms of zinc homeostasis are required to better understand its impact on carcinogenesis. Furthermore, studies that can provide the tissue's zinc concentration profile in tumorigenesis, will improve our understanding of its role in cancer development.

In: Zinc Deficiency
Editor: Rebecca Morton
ISBN: 978-1-63484-414-7

Chapter 1

ZINC DEFICIENCY AFTER BARIATRIC SURGERY: ETIOLOGY, HEALTH IMPLICATIONS AND MANAGEMENT

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ABSTRACT

Bariatric surgery leads to a significant body weight reduction, and improvement of obesity-related comorbidities. However, it is associated with a higher risk of presenting some nutritional deficiencies. These deficiencies are especially relevant after mixed or malabsorptive procedures.

Deficiencies in micronutrients after bariatric procedures are a known threat if not corrected appropriately.

Though zinc deficiency is not considered among the most frequent deficiencies after bariatric surgery, several studies have shown that its frequency might overcome 10%, even after restrictive procedures and in patients with multivitamin supplements intake.

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Zinc is the second most prevalent trace found in the human body after iron. It is essential for normal cell function and metabolism, playing a central role in over 300 enzymatic reactions, and protects cells from free radical damage. The central role of zinc in cell growth and differentiation explains the dramatic effect of zinc deficiency in tissues with a rapid cell turnover such as hair growth.

In recent years much interest has been generated by the possibility that subclinical zinc deficiency may significantly increase the incidence of and morbidity and mortality from diarrhea and upper respiratory tract infections. Clinical manifestations of zinc deficiency include delayed sexual maturation, impotence, hypogonadism, oligospermia, alopecia, dysgeusia, immune dysfunction, night blindness, impaired wound healing, and various skin lesions.

After bariatric surgery, zinc deficiency is often associated with other micronutrients deficiencies, mainly iron. It has been demonstrated that zinc and iron levels, both within the normal range, but close to the minimum level of the range, can be associated with hair loss, mainly between the 6^{th} and 9^{th} postoperative month.

For the evaluation of zinc status, plasma levels are generally a good index of zinc status in healthy individuals.

Zinc supplements are usually indicated for patients with low zinc levels, depending upon the clinical context. In obese patients after bariatric surgery, zinc supplementation can be considered even in patients with serum levels within the normal range, when iron levels are also close to the minimum value of normality and the patient complain of alopecia.

INTRODUCTION

Zinc is the second most prevalent trace found in the human body after iron. Along with iron, iodine, and vitamin A, zinc deficiency is one of the most important micronutrient deficiencies globally. Serum zinc deficiency can determine the appearance of several diseases. Most of these deficiencies are caused by inadequate nutrition. Meats, nuts and lentils are excellent sources of zinc. However, it has been estimated that nearly 45% of adults may have inadequate zinc intakes. Mild zinc deficiency appears to be common, especially in developing countries. Individuals in developing countries are at risk of zinc deficiency because the diet is relatively low in zinc and contain significant amounts of phytates (which reduce zinc absorption) [1, 2]. The Food and Nutrition Board of the Institute of Medicine and the World Health Organization (WHO) have published standard recommendations for daily zinc intake. Requirements vary by age and gender, rising from 3 mg daily in early

childhood to 8 mg daily for adult women, and 11 mg daily for adult men. Requirements are slightly higher during pregnancy and lactation [3].

ZINC METABOLISM

Around 60% of total body zinc is in bone and muscle pools with slow turnover. Zinc is absorbed mainly in the duodenum and jejunum, and to a lesser extent in the ileum and colon. Typically, zinc absorption is 20 to 40% efficient, and may be related to zinc status [4].

During digestion, dietary zinc is released and forms complexes with different ligands, namely amino acids, phosphates, organic acids, and histidines. Zinc-ligand complexes are then absorbed through the intestinal mucosa. Pancreatic enzymes are necessary for release of dietary zinc, and pancreatic juices may contain zinc-complexing ligands. Once absorbed, the portal circulation carries zinc to the liver. There is an intricate homeostatic control of zinc absorption, regulated by metallothionein, a metalloprotein that binds copper and other divalent cations [5].

The major route of zinc excretion is via the gastrointestinal tract. Up to 10% of the circulating zinc is also excreted through urine. Zinc homeostasis is probably maintained by a combination of changes in fractional absorption and endogenous fecal zinc excretion [1].

BIOLOGICAL ROLE OF ZINC

The biological role of zinc mainly depends on the ability to form tight bonds with certain amino acids, especially histidine and cysteine. When zinc binds four amino acids (tetradentate configuration), it serves a structural role maintaining protein structure (such as the beta-pleated sheet), and maintains nuclear stability and histone structure. When zinc binds three amino acids, the fourth site is temporarily taken by a water molecule; in this form, zinc can play a role in the metabolic activity of many proteins. Approximately 250 proteins contain zinc. These include enzymes such as angiotensin converting enzyme, alkaline phosphatase, carbonic anhydrase, DNA and RNA polymerases, copper-zinc superoxide dismutase, and metallothionein, as well as a large family of zinc proteins involved in gene transcription (such as the zinc finger proteins). Zinc has important roles both in cell division as well as apoptosis

(programmed cell death) [1]. Zinc plays a central role in over 300 enzymatic reactions, and protects cells from free radical damage [6, 7].

ZINC DEFICIENCY

Mild dietary zinc deficiency impairs growth velocity while severe depletion of zinc leads to growth retardation. Other clinical manifestations of zinc deficiency include delayed sexual maturation, impotence, hypogonadism, oligospermia, alopecia, dysgeusia, immune dysfunction, nictalopia, impaired wound healing, and various skin lesions. Other signs and symptoms of zinc deficiency include loss of taste, change in hair color, impaired appetite and decreased sperm counts [8].

Findings such as these, suggesting zinc deficiency, have been described in chronic diseases such as malnutrition, malabsorption syndromes (such as chronic inflammatory bowel disease), prolonged breast feeding, cirrhotic patients and elderly individuals with poor diet quality. In the latter cases, the dietary zinc deficiency may have been exacerbated by medications that increase urinary losses of zinc, including thiazides, loop diuretics, and angiotensin receptor blockers [9].

In recent years much interest has been generated by the possibility that subclinical zinc deficiency may significantly increase the incidence of and morbidity and mortality from diarrhea and upper respiratory tract infections [8].

ZINC DEFICIENCY AFTER BARIATRIC SURGERY

In the recent decades, reduced zinc absorption and stores have been demonstrated in patients who have undergone bariatric surgery, mainly malabsorptive procedures [6]. Though zinc deficiency is not considered among the most frequent deficiencies after bariatric surgery, several studies have shown that its frequency might overcome 10%, even after restrictive procedures and in patients with multivitamin supplements intake. In malabsorptive procedures, as it has been previously explained, zinc is absorbed in duodenum and jejunum, which are the main parts of small bowel that are bypassed in malabsorptive techniques. Similar to zinc, other minerals are also absorbed in these locations of the small bowel (iron, calcium,…).

Therefore, deficiencies in micronutrients after bariatric procedures are a known threat if not corrected appropriately [9-11]. Another factor that might contribute to an impairment of zinc levels, is a reduced stomach production of hydrochloric acid required for zinc bioavailability and absorption [12, 13]. The reduction of gastric acid segregation is probably the main explanation for zinc deficiencies after restrictive procedures, such as sleeve gastrectomy, as there is no malabsorption associated with this procedure. Absorption is also related to zinc concentration in food and red meat is the richest common source of readily available zinc, providing about 50% of dietary intake [14]. Some studies which evaluated food records found that zinc intake was not enough in almost all patients in the first months after bariatric surgery, based on a limited quantity of red meat intake. In patients undergoing any type of bariatric surgery, solid food intake begins 1 month after surgery, but chicken is the main meat included in this first solid diet. Red meat is progressively introduced, but most patients complained of difficult digestions with this red meat and they decide to reduce the ingestion. It is around the 3rd postoperative month, when they can ingest red meat without problems.

EVALUATION OF ZINC STATUS

Serum levels of zinc are often the only method performed to evaluate zinc status. After bariatric surgery, serum levels of zinc are included in the routinary blood analysis performed for monitoring the nutritional status in the postoperative course. However, it has been demonstrated that serum levels do not necessarily correlate with tissue levels and do not reliably identify individuals with zinc deficiency. Although plasma levels can be a good index of zinc status in healthy individuals, these levels are depressed during inflammatory disease states. It is important to remember that obesity is considered as a pro-inflammatory entity, and the rapid weight loss after surgery is identified by the organism as an aggression, associating an inflammatory response, that ends once a weight stability is achieved [15]. Thus, the accuracy of serum zinc levels for the diagnosis of zinc deficiency is low.

Erythrocyte concentrations of zinc may provide a more useful measure of zinc status during acute or chronic inflammation. Serum superoxide dismutase and erythrocyte alkaline phosphatase activities are functional indices that can also be used to indirectly assess zinc status [16]. The main limit of these tests is that they are not widely available.

ALOPECIA AND MILD ZINC DEFICIENCY AFTER BARIATRIC SURGERY

The central role of zinc in cell growth and differentiation explains the dramatic effect of zinc deficiency in tissues with a rapid cell turnover, such as the hair growth [12]. The phenomenon of postoperative hair loss is multifactorial. Though hair loss is mainly due to a rapid weight loss, micronutrient deficiencies must be discarded. Zinc is the most frequently suspected deficiency with rapid clinical responses reported from therapy [6]. A recent study of our group showed that zinc levels within the normal range, but close to the minimum value, can be associated with hair loss in females between the 3rd and the 9th postoperative months, especially when iron levels are also close to the minimum limit. We proposed to study a new variable, called Addition (serum zinc + serum iron). When Addition levels are below 115, these values are associated with alopecia, that disappeared with zinc supplementation [17]. These findings support the fact that serum levels of zinc within the normal range in the postoperative course of bariatric surgery, are not a good marker for zinc status.

MANAGEMENT OF ZINC DEFICIENCY

The fact that after zinc supplement intake hair loss stopped in most patients of our study, even in those with zinc levels within normal range, reveals that there is a mild zinc deficiency after sleeve gastrectomy, that probably alone is not enough to produce hair loss, but associated with iron mild deficiency (also with levels within normal range) it can appear. There are currently no guidelines for zinc supplementation and optimal dose and rules of prescription after bariatric surgery are still to be determined. In our series, just a small dose of 12.5mg/day (125% RDA) during 2 months was enough to slightly increase the serum zinc levels and to stop hair loss. As prophylactic measure against hair loss after bariatric surgery, it could be hypothesized a routinely supplementation with low doses of zinc [17].

During zinc supplementation, it is recommended to monitor periodically the serum concentrations in order to avoid the appearance of adverse effects associated with overload. Anyway, humans are very tolerant of high zinc intakes up to 100 mg/day (1000% RDA). Mega-dose supplementation or high zinc intake from contaminated food has been associated with nonspecific

gastrointestinal symptoms, including abdominal pain, diarrhea, nausea, and vomiting. Zinc overload may interfere with copper absorption, and high zinc intakes (> 150 mg/day) can lead to copper deficiency. Treatment for zinc toxicity is primarily supportive, although chelation with calcium disodium ethylenediaminetetraacetate (CaNa2EDTA) has been used in some cases of severe toxicity [18].

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In: Zinc Deficiency
Editor: Rebecca Morton
ISBN: 978-1-63484-414-7

Chapter 2

MECHANISMS OF ZINC DEFICIENCY AND ITS CLINICAL SIGNIFICANCE AFTER BARIATRIC SURGERY

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ABSTRACT

The rising prevalence of obesity (body mass index of >/= 30 kg/m^2) in more than 100 countries has been described as a global pandemic. Studies have found that surgery results in more significant and sustained

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weight loss, when compared to dietary and lifestyle interventions, in individuals with medically-complicated obesity.

Zinc is an essential component of the catalytic site of hundreds of different metalloenzymes where it functions as a Lewis acid.

The small intestine is the main site of zinc absorption via transporter expression which is regulated in part by dietary zinc intake. Zinc deficiency is a clinical concern since it may result in multiple clinical symptoms.

After bariatric surgery, the prevalence of biochemical zinc deficiency has been reported to range from 5% to 83%. It has been suggested that bariatric procedures that result in restriction may have lower rates of zinc deficiency compared to bariatric procedures that produce restriction as well as malabsorption.

The mechanisms for zinc deficiency after bariatric surgery have not been fully delineated but may include decreased intake, poor food tolerability, decreased surface area for absorption, rapid oral-cecal transit, altered compensatory mechanisms, and/or small intestinal bacterial overgrowth.

Elevated serum folic acid levels, a marker for small intestinal bacterial overgrowth, is common after gastric bypass surgery, and small bowel bacterial overgrowth is associated with decreased zinc absorption. We evaluated 230 patients after gastric bypass surgery and 145 patients had elevated serum folate levels, supporting 63% prevalence for small intestinal bacterial overgrowth. In 54 patients with normal serum folate levels, 43 had normal zinc levels, while among 118 patients with elevated serum folate, 78 had low serum zinc (Chi-squared 2X2: $P < 0.001$), supporting upper gut bacterial overgrowth as a mechanism for biochemical zinc deficiency.

We have evaluated 43 patients who underwent upper endoscopy after gastric bypass surgery. In 17 out of 43 (40%) of these patients, there was an abnormal finding at upper endoscopy: gastrojejunal anastomotic stricture (obstruction) or gastrojejunal anastomotic ulcer. In 8 patients with gastrojejunal ulceration, 66% of the patients had low serum zinc levels. In patients who had elevated serum folate levels, there was no increased risk of gastrojejunal ulceration (Chi-squared 2X2: $P = 0.53$).

Currently, recommendations regarding routine screening for zinc deficiency or the use of daily oral zinc supplementation in individuals after bariatric surgery are not based on clinical trials. Gaps that exist in our understanding of this clinical disorder are summarized in this chapter. Since present physiological evidence supports zinc absorption primarily in the jejunum by a transcellular route involving a zinc-specific transporter, Zip4, proposed studies should examine potential post-operative changes in zinc transport and the potential for reversibility with antibiotic treatment of small intestinal bacterial overgrowth.

INTRODUCTION

The Pandemic of Obesity

As shown in Table 1, obesity is consistent with a Body Mass Index (BMI) >/= 30 kg/m^2 while overweight people have a BMI >/= 25 kg/m^2 [1]. Over the past decades, the rising prevalence of overweight and obesity in more than 100 countries has been described as a global pandemic [2-4].

Table 1. Obesity can be defined by body mass index or BMI

BMI (kg/m^2)	Definition	Obesity Class
18.5-24.9	Normal	
25-29.9	Overweight	
30-34.9	Obesity	I
35-39.9	Obesity	II
40.0-	Obesity	III

There is a universal concern about health risks associated with increased obesity.

According to a recent study, the conditions overweight and obesity were estimated to cause 3.4 million deaths, 4% of life years lost and 4% of disability adjusted life years across the globe in 2010 [5]. Worldwide, the combined prevalence of overweight and obesity rose by 27.5% for adults and 47.1% for children between 1980 and 2013 (see Figure 1). The worldwide number of overweight and obese adults increased from 857 million in 1980 to 2.1 billion in 2013.

Data from studies in the United States have suggested that during the past 20 years, there has been a dramatic increase in obesity and therefore rates remain high (see Figure 2). More than one-third of U.S. adults (34.9%) and approximately 17% (or 12.7 million) of children and adolescents aged 2 to 19 years-old are obese [6, 7].

The connection between the rising rates of obesity and rising medical spending is also clear. In 1998 the medical costs of obesity were estimated to be as high as $78.5 billion and these costs increased to $147 billion by 2008 (see Figure 3). In 2006, obese individuals had medical spending that was $1,429 greater than spending for normal-weight individuals [8].

Figure 1. There was a rise in the worldwide prevalence (in percentages) of men and women who were overweight or obesity in 1980 and in 2013.

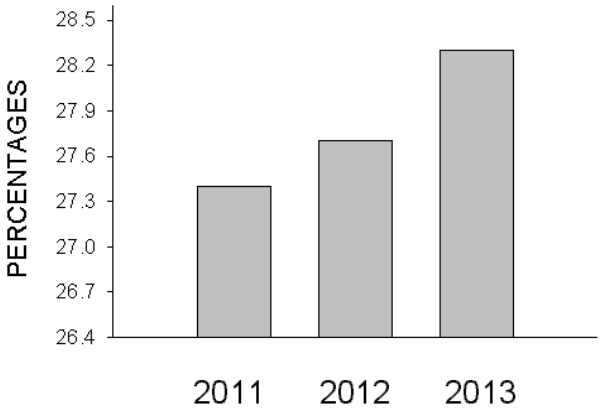


Figure 2. There was a rise in percentages of adults aged 18 years and older who were obese in the United States in 2011, 2012, and 2013.

Surgical Management of Obesity

Morbid obesity has been directly associated with an extensive list of significant co-morbid conditions, including the metabolic syndrome, cardiovascular diseases, obstructive sleep apnea, certain carcinomas (i.e., endometrial, breast, colon), osteoarthritis and non-alcoholic steatohepatitis.

Childhood obesity is associated with a higher chance of obesity and disability when reaching adulthood.

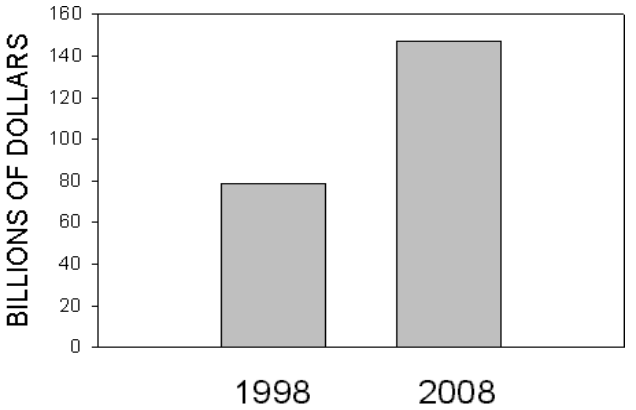


Figure 3. There were increased estimates of the medical costs (in billions of dollars) related to obesity in the United States in 1998 and in 2008.

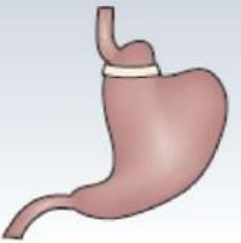
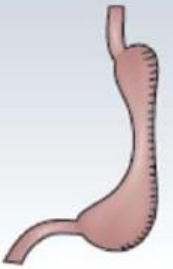
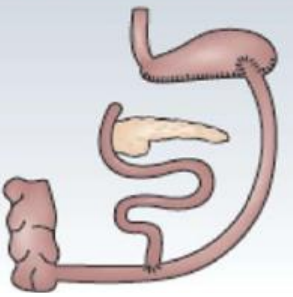
In addition to increased future risks, obese children experience breathing difficulties, increased risk of fractures, hypertension, insulin resistance, and psychological disorders.

Management options for obesity include dietary and activity program, pharmacological therapy, endoscopic interventions, and surgical procedures. The initial management of obesity includes dietary and lifestyle interventions. Recent studies have showed that more focus on healthy eating and physical activity have led to a potential decline in obesity rates in school children in a school based program [9].

However dietary and activity program, and pharmacological therapy, have resulted in poor weight loss and/or poor maintenance of weight loss especially in individuals with severe obesity [10, 11]. Controlled studies and cohort studies have found that bariatric surgery results in more dramatic and sustained weight loss as compared to nonsurgical interventions [12-14].

Bariatric surgical procedures (see Figure 4) are considered for individuals with a body mass index >39.9 kg/m^2 or a body mass index of >34.9 kg/m^2 with an associated significant co-morbidity, e.g., diabetes mellitus, obstructive sleep apnea, Pickwickian syndrome, obesity-related cardiomyopathy, or disabling osteoarthritis. Currently, only about 1% of the morbidly obese population is being evaluated for this surgical strategy. Clinical outcome studies have shown significant reductions in long term mortality after gastric

bypass surgery, including mortality caused by cardiovascular events, diabetes, and cancer [15-18]. These data support the current indications of bariatric surgery for the treatment of medically-complicated obesity.

Procedure	Anatomy
Adjustable gastric banding	
Sleeve gastrectomy	
Roux-en-Y gastric bypass	
Billopancreatic diversion	

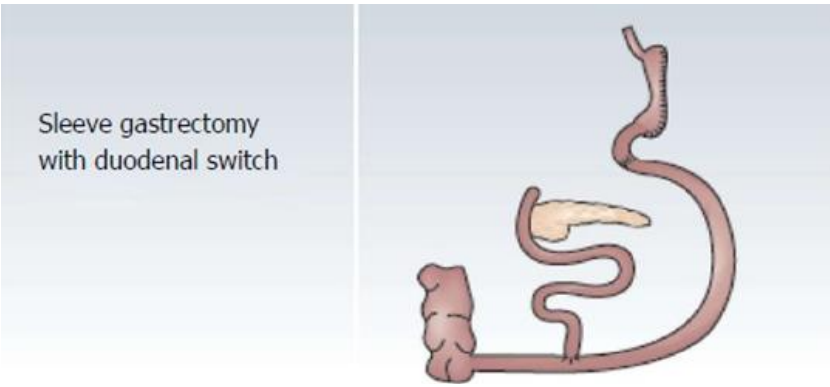


Figure 4. Representations of major bariatric surgical procedures. Restrictive bariatric surgical procedures include the adjustable gastric band and the vertical sleeve gastrectomy. Bariatric surgical procedures that result in both restriction as well as in some malabsorption include the Roux-en-Y gastric bypass, the biliopancreatic diversion, and the duodenal switch. In gastric bypass surgery and in biliopancreatic diversion, the length of the biliopancreatic limb (e.g., bypassed small intestine) is related to the distance from the pylorus to the jejuno-jejunal anastomosis. In gastric bypass surgery and in biliopancreatic diversion, small intestine distal to the jejuno-jejunal anastomosis (e.g., the common channel) is required to allow mixing of micronutrients with bile and pancreatic secretions (Reproduced with permission from Bal, BS; et al. Nature Rev Endocrinol 2012; 8: 544-556).

Data from a global survey shows that the total number of bariatric procedures performed worldwide in 2013 was 468,609, in which 95.7% were completed laparoscopically. The highest number (n = 154,276) were performed in the USA/Canada region. The most commonly performed procedure in the world was Roux-en-Y gastric bypass (RYGB), at 45%, followed by the vertical sleeve gastrectomy (SG), at 37%, and then the adjustable gastric banding (AGB), at 10%. The most significant changes were the worldwide rise in utilization of SG from 0% in 2003 to 37% of bariatric surgical procedures in 2013, with a related decline in the performance of AGB after its peak in 2008 [19]. The number of procedures performed in the USA/Canada has increased over time, with the number being 179000 in 2013 [23], which results in approximately 40% of the global total.

Bariatric surgeries involving the stomach are restrictive in general, while surgery involving the small intestine can also result in malabsorption. Vertical sleeve gastrectomy and adjustable gastric band are the commonly performed restrictive procedures, while Roux-en-Y gastric bypass and biliopancreatic diversion (more rarely performed due to the risks of malnutrition) result in malabsorption in addition to restriction. Procedures designed to include a

malabsorptive component produce greater nutritional deficiencies than restrictive surgeries.

In Roux-en-Y gastric bypass, a small gastric pouch is created by complete division of the stomach, and then the proximal gastric pouch is directly connected to jejunum that was divided commonly 40 to 50 cm distal to the ligament of Treitz. The location of the jejuno-jejunal anastomosis is important because a long proximal alimentary limb (which is distal to the proximal gastric pouch) results in a short common channel (e.g., the small intestine distal to the jejuno-jejunostomy). Patients with a short common channel are more at risk for developing malabsorption and malnutrition, compared to patients with longer common channels, due to decreased surface area of small intestine available for the absorption of micronutrients and possible alteration of bacterial flora [24].

The vertical sleeve gastrectomy is gaining popularity worldwide and is now commonly performed in the USA. The gastric fundus and body are surgically excised, leaving a narrow, tubular stomach along the lesser curvature of the stomach. It allows for an immediate restriction of caloric intake without an intestinal anastomosis, placement of an extrinsic foreign body, or the need for adjustments [24].

The adjustable gastric band is primarily a restrictive strategy that does not bypass any part of the gastrointestinal tract. A plastic latch is used to hold a soft, silicone ring circumferentially around the upper stomach just distal to the gastroesophageal junction, leaving a very small pouch available for food. A subcutaneous access port is connected via tubing to the silicone ring or band. Sterile fluid added to the port induces extrinsic compression of the proximal gastric lumen by inflating the balloon positioned around the proximal stomach [24]. Biliopancreatic diversion involves a distal gastrectomy with closure of the duodenal stump. The jejunum is divided and the distal limb is anastomosed to the proximal stomach, while the proximal limb (termed the biliopancreatic limb) is anastomosed to the ileum. A variation of this procedure, termed biliopancreatic diversion with duodenal switch, was developed to decrease the adverse effects of dumping syndrome. In this procedure restrictive component is maintained by a partial gastrectomy of 70% to 80% of the greater curvature of the stomach in a gastric sleeve configuration [25].

Vertical-banded gastroplasty is another bariatric procedure that was developed to be restrictive in nature and was designed to decrease morbidity. Due to an unacceptably high failure to maintain long term weight loss, this procedure is now rarely performed [26].

PHYSIOLOGY OF ZINC

Zinc is the second most abundant trace element in the body after iron. The average adult stores a total of 2 to 3 grams of zinc, mainly present in muscle and bone. In clinical research studies of zinc metabolism, an exchangeable zinc pool mathematically estimates compartments in the body in which zinc can rapidly exchange with serum zinc. The exchangeable zinc pool in adults has been correlated with dietary zinc intake. Therefore, this concept may provide a useful measure in studies of zinc metabolism. Zinc is required as a cofactor for more than 100 metalloenzymes where it functions as a Lewis acid. These enzymes are involved in synthesis of DNA and RNA, release of vitamin A from liver, metabolism of carbohydrates, disposal of free radicals, biosynthesis of heme and essential fatty acids, and transport of carbon dioxide. In addition, zinc appears to stabilize cell membranes, assists in immune function, and is involved in the synthesis, storage and release of insulin from the pancreas. Zinc is physiologically important for normal taste perception, wound healing, production of sperm, and fetal development.

Zinc is principally absorbed in the small intestine. The rate of the zinc absorption varies from 15-40% depending on the zinc status in the body: if more zinc is needed, more is absorbed. Dietary factors also influence its absorption, and zinc is better absorbed when the stomach is empty. Meat, shellfish, and nuts are good sources of bioavailable zinc, whereas zinc in grains and legumes is less bioavailable. Absorption of dietary zinc may be inhibited by dietary phytate, fiber, oxalate, iron, and copper.

Zinc is absorbed into intestinal mucosal cells by passive carrier-mediated diffusion. A portion of absorbed zinc is utilized in the metabolic functions of the cell itself. Alternatively, zinc may be retained within mucosal cells by interaction with metallothionein, a binding protein. By this mechanism, metallothionein in intestinal cells may be involved in the regulation of zinc absorption. This step is mediated by an energy dependent process, is regulated by body stores of zinc, and is potentially altered by the presence of iron cations [28, 29].

The level of zinc supplementation does not have a significant differential effect on the magnitude of its absorption. Recent advances have shown that zinc absorption is a complex process involving the participation of several transporters from both the ZIP (solute linked carrier SLC39) and the ZnT (solute-linked carrier SLC30) families. ZIP4 is considered the primary regulator of zinc uptake in the intestinal cells, and ZnT1 is pivotal for the zinc efflux from the enterocyte into the blood [30-33]. The expression of these zinc

transporters is regulated by cytokines, hormones, and zinc levels in the body [30]. It is believed that surgery on the small intestine can cause changes in the expression of zinc transporters, resulting in important effects on zinc homeostasis [31].

Zinc's main transport vehicle in the blood is albumin. Some zinc also binds to transferrin. In conditions of iron overload, transferrin is saturated hence leaving too few sites available for zinc binding. The converse is also true: large doses of zinc inhibit iron absorption. A proportion of the circulating zinc is taken up by the pancreas, where it is incorporated into digestive enzymes that are released at mealtime. This recycling of zinc in the body from small intestine to the pancreas and back to the small intestine is known as enteropancreatic circulation. As zinc circulates through the small intestine, it may not be absorbed by intestinal mucosal cells. Zinc is also excreted in intestinal cells shed into the intestinal lumen. The primary excretion of zinc from the body is within feces. The body also loses small amounts of zinc in urine, hair, skin cells that are sloughed, sweat, menstrual fluid, and semen.

PREOPERATIVE AND POSTOPERATIVE ZINC METABOLISM

Zinc and Obesity

An estimated 17.3% of the world's population is at risk of inadequate zinc intake [34]. Preoperative determination of zinc levels in obese individuals considering bariatric surgery suggests that biochemical zinc deficiency (e.g., low serum levels of zinc) is common in this population. Multiple studies evaluating morbidly obese individuals preoperatively have found that the prevalence of zinc deficiency ranges from 15% to 73.9% [35-39]. Such wide differences in results may be attributable to poor dietary habits in the studied population, socioeconomic factors, gastrointestinal pathologies, and/or the use of different cutoff points for defining biochemical zinc deficiency.

Sanchez et al. evaluated 103 morbidly obese women for micronutrient deficiencies before proceeding to gastric bypass surgery. The mean BMI of these individuals was 43.1 ± 5.3 kg/m^2 and the mean age was 36.0 ± 9.6 years-old in the study group. Low hair zinc concentrations were detected in about 15% of the patients [35]. In a second study by Gobato at al, biochemical zinc

deficiency was found in 20 (55.5%) of 36 morbidly obese patients presenting for preoperative evaluation for gastric bypass surgery [36].

Although the exact mechanism to explain zinc deficiency in the obese patient population is not clear, several mechanisms have been hypothesized. Dietary intake may be both an origin for obesity as well as an origin for the development of micronutrient deficiencies. The obese patient usually has a high intake of calorie dense food rich in large amounts of carbohydrate and fat, but poor in vitamins and minerals [40-44]. A substantial proportion of these patients also have problematic eating behaviors such as loss of control eating, night eating syndrome, binge eating disorder, and bulimia nervosa [45]. Additionally, these individuals have altered bioavailability of micronutrients, due to "high calorie malnutrition," which is a state of excess intake of calories with concurrent nutrient deficiencies resulting in inadequate ability to utilize these calories efficiently; the toxic by-products of incomplete biochemical reactions create a vicious cycle resulting in further weight gain, eating disorders, metabolic syndrome, and fatigue [41]. Another potential explanation for biochemical zinc deficiency could be dilution effects of extracellular concentrations due to elevated quantities of total body water in individuals with obesity, since the extracellular compartment is relatively larger than the intracellular compartment, causing imbalances in the vitamin status [46]. It has also been postulated that there is decreased levels of bioavailable zinc in the obese patients due to sequestration of a majority of zinc in increased body fat [47, 48]. Small intestinal bacterial overgrowth (SIBO), especially in diabetic patients with concurrent malabsorption is another potential explanation for the development of zinc deficiency.

Zinc and Bariatric Surgery

Although bariatric surgical procedures have shown promising results in sustained extensive weight loss, chronic complications including macronutrient and micronutrient deficiencies occur. Micronutrient disorders including biochemical zinc deficiency are commonly found in individuals before bariatric surgery and have been shown to persist or worsen after bariatric surgery.

Clinical zinc deficiency is a major clinical concern in bariatric surgery patients because of the important function of this mineral and the co-morbidities related to its deficiency, such as growth retardation, hypogonadism, dermatitis, alopecia, poor immunity, acrodermatitis, poor

healing, and impaired neuropsychological performance [49-51]. The potential role of zinc in development of diarrhea is not as clear since many individuals with diarrhea can develop biochemical zinc deficiency. Many bariatric surgery patients are women in child bearing age, and zinc deficiency has been associated with adverse effects on maternal health and pregnancy outcomes, including hypertension [52, 53].

Biochemical zinc deficiency after bariatric surgery has been supported by multiple studies (see Table 2). There are wide variations reported in the post-operative prevalence of reduced serum zinc levels possibly due to the type of hospital involved in bariatric surgery, the length of postoperative follow-up, patients' socioeconomic factors, and the use of different cutoff levels in defining a biochemical nutritional deficiency.

In a study from University Hospital in Memphis, TN [54], 100 individuals had serum zinc levels examined at 1 year after surgery. In this study the prevalence of zinc deficiency was 36%. In a more recent study [36], 36 individuals had serum zinc levels measured six months after Roux-en-Y gastric bypass. The prevalence of zinc deficiency in this long term study was 61.11%. In an additional study from Switzerland, Gehrer et al. found that 34.9% of patients were zinc deficient after Roux-en-Y gastric bypass, compared to 30% after vertical sleeve gastrectomy, in postoperative follow up at 1 year [55].

Table 2. Prevalence of zinc deficiency after specific bariatric procedures

Surgery*	Post-op period	No. of patients	Zinc deficiency	Reference
RYGB	6 months	36	61.11%	[55]
RYGB	12 months	100	36%	[54]
RYGB	12 months	86	34.88%	[56]
SG	12 months	50	30%	[56]
SG	12 months	200	5%	[57]
SG	12 months	28	10.7%	[58]
BPD/DS	6 months	24	83%	[66]
BPD/DS	18 months	180	38.3%	[65]

*RYGB: Roux-en-Y Gastric Bypass; VSG: Vertical Sleeve Gastrectomy; BPD/DS: Biliopancreatic Diversion/Duodenal Switch.

Due to its restrictive nature, vertical sleeve gastrectomy is thought to result in less frequent and less severe postoperative nutritional deficiencies compared to gastric bypass surgery. Despite the absence of surgically induced malabsorption in individuals undergoing vertical sleeve gastrectomy, there is emerging literature suggesting that zinc deficiency is common after sleeve gastrectomy. This is important due to the worldwide rise in the utilization of vertical sleeve gastrectomy, as mentioned above. There have been short term as well as long term studies of the prevalence of zinc deficiency after vertical sleeve gastrectomy. In a one year follow up of 200 individuals after vertical sleeve gastrectomy, van Rutte et al. reported a 5% prevalence of zinc deficiency [40]. In a similar study, Saif et al. reported a 10.7% prevalence of zinc deficiency at one year follow up of 28 individuals after vertical sleeve gastrectomy. These results were confirmed by an additional 4 year follow up that showed a 14.3% prevalence of zinc deficiency in these patients [56].

In contrast to vertical sleeve gastrectomy, the worldwide performance of adjustable gastric banding has rapidly declined in recent years. Studies evaluating zinc deficiency after this bariatric procedure are scarce. In 30 patients who underwent laparoscopic adjustable gastric banding, at 6 months after the surgery Boyuk et al. found that the postoperative zinc levels (500 ± 130 ng/ml) were statistically significantly lower than the preoperative zinc levels (740 ± 230 ng/ml) [59], but this decline did not result in clinical zinc deficiency.

Biliopancreatic diversion with duodenal switch may be superior to other bariatric procedures in terms of weight loss, as supported by recent meta-analyses [58-60]. However, biliopancreatic diversion is also associated with more frequent postoperative nutritional disorders due to increased malabsorption, and so this bariatric procedure is generally restricted to super-morbidly obese patients (body mass index of $\geq$ 50 kg/m^2) for whom greater weight loss is required [61, 62]. An increased risk of zinc deficiency has been reported in individuals after BPD/DS by Botella and associates [63]. The authors report results from 180 patients with BMI decreasing from a preoperative value of 52.9 ± 7.7kg/m^2 to 30.8 ± 5.2kg/m^2 after surgery. In this group, 38.3% of the patients were found to have low serum zinc levels at 18 months follow up. In a similar study, when evaluating 24 patients after BPD/DS, Salle et al. reported that 83% and 91.7% of the patients had biochemical zinc deficiency at 6 months and at 12 months postoperatively [64].

MECHANISMS OF ZINC DEFICIENCY

Postoperative Bariatric Surgery

Despite multiple studies describing low zinc levels and its outcome, the exact underlying mechanisms involved in the development of postoperative zinc deficiency are not well understood (see Table 3). Three major factors can influence zinc status in humans: reduced zinc intake, reduced zinc absorption, and increased losses.

Table 3. Proposed mechanisms for development of zinc deficiency after bariatric surgery

• Reduction in total food intake due to decreased gastric capacity
• Poor tolerability of food with high zinc contents
• Post-operative vomiting
• Substantial reduction in intestinal absorption surface
• Dumping syndrome with rapid oral-cecal transit
• Altered bioavailability of zinc in obesity
• Small intestinal bacterial overgrowth
• Concomitant administration of iron and copper
• Altered compensatory mechanisms post-operatively

An important factor associated with zinc deficiency early after bariatric surgery is reduction in dietary nutrient intake due to decreased gastric capacity. Studies that evaluated food records found that zinc intake was inadequate in almost all patients presenting for follow up after bariatric surgery [65-67]. In addition to this decrease in total zinc intake, zinc ingestion related to use of meat products (beef, lamb, and oysters) can also be decreased due to low tolerance [68-70] or the use of a low fat, high protein sources in the postoperative diet.

Zinc absorption takes place mainly in the duodenum and initial portions of the jejunum. These portions of the intestine are bypassed during Roux-en-Y gastric bypass which may result in a substantial reduction in intestinal absorption surface and, in turn, less absorption capacity. In a recent study evaluating the patients who underwent Roux-en-Y gastric bypass surgery, percentage zinc absorption from a standard diet decreased significantly from

32.3% to 13.6% at 6 months and to 21% at 18 months after surgery [71]. Although vertical sleeve gastrectomy restricts the volume of the stomach without bypass of the small intestine, it also leads to accelerated gastric emptying. Subsequently, rapid gastric emptying might promote nutrient deficiencies [72], as observed in a recent study describing increased fecal excretion of fatty acids which induced moderate malabsorption [73]. Due to the high frequency of postoperative iron deficiency, frequent administration of iron supplements may also increase a patient's risk of zinc deficiency due to competitive interactions for uptake of these minerals [74].

Post-surgery zinc deficiency could also be due to a defect in compensatory mechanisms. Zinc is subject to strong homeostatic regulation via the gut and liver. Zinc absorption is affected by zinc status and absorption efficiency is inversely correlated with the zinc level in the body [75]. Zinc is mainly excreted by the intestine through intestinal and pancreatic secretions. It is believed that zinc homeostasis can be altered by malabsorptive surgery. This result may be partly due to excessive loss of zinc arising from secondary diarrhea after these surgeries [76]. A major compensatory mechanism in the presence of biochemical zinc deficiency (e.g., greater intestinal absorption and reduced excretion of zinc) can therefore be reduced [77].

Zinc and Small Intestinal Bacterial Overgrowth

Small intestinal bacterial overgrowth after gastrointestinal surgery has long been known to lead to cyanocobalamin or vitamin B12 deficiency. Our group has further examined the role of small intestinal bacterial overgrowth in development of thiamine or vitamin B1 deficiency. The results of our studies support small intestinal bacterial overgrowth as a potential mechanism to explain postoperative micronutrient deficiencies [78]. These studies have also expanded upon prior reports of increased serum levels of folate (which is produced by upper intestinal bacteria and then absorbed into the serum) in individuals with small intestinal bacterial overgrowth [79] by comparing elevated serum folate levels with abnormal breath hydrogen levels in individuals examined by glucose-hydrogen breath testing after Roux-en-Y gastric bypass surgery.

Small intestinal bacterial overgrowth can result in malabsorption as a result of the effect of gut flora on intestinal absorptive functions. In normal individuals, colonization of bacteria in the proximal gastrointestinal tract is in low concentrations. However, in individuals with small intestinal bacterial

overgrowth, the flora of the proximal gut can be changed toward higher concentrations of colonic-type anaerobic bacteria that can induce fermentation.

Small intestinal motility and the migrating motor complex are important defense mechanisms against upper gut bacterial overgrowth, likely due to passage of bacteria into the colon [80]. Bariatric surgery may disrupt these defense mechanisms. The creation of a shorter small intestine may result in less time and less intestinal surface area for absorption of bile acids, gastric acid, and pancreatic enzymes.

These post-procedural disruptions may alter small intestinal motility and could permit bacteria to have prolonged access to intestinal chyme further promoting bacterial overgrowth [81].

A clinical diagnosis of upper gut bacterial overgrowth can be difficult since patients can present after bariatric surgery with non-specific abdominal symptoms including cramping abdominal pain, abdominal bloating or distension, increased flatulence, or diarrhea. These abdominal symptoms can overlap with symptoms of malabsorption caused by lactose intolerance, fructose intolerance, or surgical bypass of small intestine.

Based upon prior reports of B vitamin deficiencies induced by small intestinal bacterial overgrowth, our group examined the association between zinc absorption and small intestinal bacterial overgrowth [82].

This study was a retrospective review of 452 patients who underwent Roux-en-Y gastric bypass surgery from 1999 to 2005. As a marker of small intestinal bacterial overgrowth, an elevated serum folate level (a serum level above the upper limit of normal for the serum folate assay) has a reported specificity of 79%.

All patients were identified who had measurement of serum folate levels. Levels of serum folate were available in 230 patients, while 172 patients had determination of both serum folate and serum zinc levels. This study involved 199 females and 31 male patients with an average age of 46 years-old (range was 21 to 68 years old). The mean body mass index was 53 kg/m^2 with a range of 40 to 100 kg/m^2.

For results involving the total 230 patients, 145 had elevated serum folate levels supporting 63% prevalence for upper gut bacterial overgrowth. As shown in Table 4, among the 172 patients who had measurement of both serum folate and serum zinc levels, in 54 patients with normal serum folate levels, 43 had normal serum zinc; among 118 patients with elevated serum folate, 78 had low serum zinc (Chi-squared 2x2: $P < .001$), supporting upper gut bacterial overgrowth as a mechanism for biochemical zinc deficiency.

While these results support upper gut bacterial overgrowth as a mechanism for the development of zinc deficiency after bariatric surgery, in previous studies we showed that treatment of Vitamin B1 deficiency with oral Vitamin B1 supplements requires concomitant antibiotic therapy for small intestinal bacterial overgrowth [78].

This finding suggests that oral zinc supplements may not be fully effective for the treatment of zinc deficiency in individuals who have upper gut bacterial overgrowth after bariatric surgery. Further studies need to be performed to evaluate treatment of small intestinal bacterial overgrowth with antibiotics and the subsequent absorption of dietary zinc.

Table 4. Relationship between serum folate levels and serum zinc levels after gastric bypass surgery*

		SERUM FOLATE LEVELS		
		NORMAL	ELEVATED	TOTALS
SERUM ZINC LEVELS	NORMAL	43	40	83
	LOW	11	78	89
TOTALS		54	118	172

*Chi-squared 2X2: P < .001.

The high prevalence (52%) of biochemical zinc deficiency in our above study of patients after gastric bypass surgery raised the question of the clinical importance of this finding.

We evaluated 43 patients who underwent upper endoscopy for vomiting, abdominal pain, or bleeding after gastric bypass surgery from May 2007 to May 2008 [83].

There were 40 female and 3 male patients with an average age of 46 years-old (range was 21 to 63 years). In 17 out of 43 (40%) of these patients, there was an abnormal finding at upper endoscopy: gastrojejunal anastomotic stricture (obstruction) or gastrojejunal anastomotic ulcer. In 8 patients with gastrojejunal ulceration, 66% of the patients had low serum zinc levels. In patients who had elevated serum folate levels, there was no increased risk of

gastrojejunal ulceration (Chi-squared 2X2: P = 0.53), but there was a strong trend toward decreased serum zinc levels (P = .16) supporting a role of upper gut bacterial overgrowth in developing zinc deficiency. Based on these results, in this subset of patients after Roux-en-Y gastric bypass surgery, zinc deficiency was associated with formation of marginal gastrojejunal ulceration. Since zinc in an important component in the biosynthesis of Cu-Zinc superoxide dismutase, these findings support the importance of bioavailable zinc in tissue homeostasis. There was no evidence to support the notion that marginal gastrojejunal ulceration was caused by small intestinal bacterial overgrowth.

CLINICAL MANIFESTATIONS AND PREVENTION OF ZINC DEFICIENCY

Potential Clinical Screening Methods

Due to its high prevalence after bariatric surgery, clinicians should be aware of the risk of zinc deficiency and the need to monitor bariatric patients closely. Regular follow-up and clinical recognition could be important for preventing the development of zinc deficiency in this susceptible postoperative group.

The pivotal role of zinc in cell growth and differentiation can explain the substantial effect of zinc deficiency in tissues with a rapid cell turnover, particularly the skin, gastrointestinal tract mucosa, gonads and immune system. As summarized in Table 5, numerous signs and symptoms have been associated with zinc deficiency. Mild zinc deficiency is associated with depressed immunity, dysgeusia (loss of taste), anosmia (loss of smell), impaired wound healing and decreased spermatogenesis. Severe zinc deficiency may be characterized by growth retardation, alopecia, acrodermatitis, severe infections due to altered immune function, and cognitive decline [51, 84-91].

Ongoing clinical and biochemical assessment of zinc status poses many challenges because, despite the large number of methods proposed, all of the available methods have problems that alter their validity. The search for a reliable indicator of zinc deficiency has been problematic because the effective regulation of zinc homeostasis buffers the functional response to dietary deficiency and excess. According to a recent systematic analysis by Lowe et

al. plasma zinc concentration is the only biomarker that can be used to measure zinc status in individuals and reflects zinc intake [92, 93]. However, there are various confounders of plasma zinc concentrations and clinical interpretation should be made while keeping in mind these limitations and constraints. Measurement of 24-hour urinary zinc excretion may be useful in monitoring response to zinc supplements, but their role in detecting low zinc levels is not clearly validated. Lowe and associates concluded that zinc concentration in erythrocytes, polymorphonuclear cells, and mononuclear cells and platelets, as well as plasma alkaline phosphatase levels, do not appear to be useful biomarkers of zinc status according to the current evidence. Other bioactive measures such as the exchangeable zinc pool [94] and endogenous zinc measurement with the use of stable isotopes [73] are potential biomarkers. Clearly, there is a clinical need to develop reliable biomarker of zinc status, and more high quality studies are required to assess the effects of the current indices in a variety of populations included postoperative bariatric patients.

Table 5. Common clinical manifestations of zinc deficiency

• Alopecia	• Stomatitis
• Delayed wound healing	• Dysarthria
• Acrodermatitis	• Nystagmus
• Growth retardation	• Hypogonadism
• Anorexia	• Dysgeusia
• Anosmia	• Immune dysfunction
• Impaired concentration	

Zinc Deficiency after Bariatric Surgery

As described above, there is a high prevalence of micronutrient deficiencies after gastric bypass surgeries. The extent of and severity of micronutrient undernutrition is related to the extent and severity of disruption of normal gastrointestinal anatomy and physiology [95]. Post-operatively, it is presently recommended that all individuals who have undergone Roux-en-Y gastric bypass or vertical sleeve gastrectomy receive daily micronutrient supplementation consisting of two multivitamin supplements (to include iron, folic acid, thiamine, and Vitamin B12), 1500 mg of elemental calcium, and at least 3000 international units or 75 mcg of daily vitamin D for minimally the

first 3 to 6 months after surgery [96]. Currently, there are no recommendations with regards to oral zinc supplementation in this susceptible postoperative population due to insufficient data, although multivitamins containing mineral supplements routinely include zinc. There is some evidence to support routine screening for zinc deficiency after malabsorptive bariatric surgical procedures and to support the use of routine supplementation following biliopancreatic diversion with duodenal switch [65, 97]. Again, the dosage of zinc in such patients needs to be further studied.

The high rates of zinc deficiency are concerning and this diagnosis should be considered in all post-operative patients who present with the signs and symptoms summarized in Table 4. The recommended "optimal" dose of oral zinc supplementation for individuals with symptomatic zinc deficiency has varied in the available literature and there is no present consensus. The concentration of zinc in oral multivitamins used in supplementation programs after bariatric surgery may not be sufficient as described above. While treating with oral supplements, potential risks of interactions with other trace minerals such as iron must be considered. The timing of the zinc in relation to the meals is also important as its absorption is 60% during fasting but decreases to 20 to 30% when taken with a meal [98]; indeed many patients take their multivitamin supplements with a meal after bariatric surgery. The Recommended Dietary Allowances for zinc is 11 mg/day for male and 8 mg/day for female in healthy, non-operative adults who are 19 years or older according to the Institute of Medicine of the National Academy [99]. However, the doses of zinc needed to replete zinc in patients with zinc deficiency, when zinc intake and absorption are reduced by significant alterations in the anatomy and physiology of the gastrointestinal tract, are unknown.

Ruz et al. studied the effects of zinc supplement in 56 morbidly obese patients undergoing bariatric surgery [73]. These patients were divided into two groups of 27 and 19 patients respectively. One group received standard supplement with daily dose of 7.5 mg/day while the other group received double the dose, e.g., 15 mg/day. Despite supplementation, the mean zinc levels decreased from 87.2 µg/dL to 80.3 µg/dL in the group receiving standard zinc supplements and from 87.2 µg/dL to 80.5 µg/dL in the group receiving double zinc supplements. Based on their data, the investigators suggested routine supplementation of the zinc, but at a higher dose of 40 to 60 mg/day. Zinc absorption can plateau at a dose of about 20 mg/day [100], but in situations of zinc malabsorption, doses as high as 60 mg/day of zinc are advised. There is a need for additional studies to determine the appropriate

dose and monitoring of the patients with varied levels of biochemical zinc deficiency after different types of bariatric surgery.

CONCLUSION

The prevalence of obesity is rising worldwide. Bariatric surgical procedures remain a major option for treatment of medically-complicated obesity. Currently, only about 1% of the morbidly obese population is being evaluated for this surgical strategy. With an expected future rise in the prevalence of obesity and the popularity of bariatric surgeries, the worldwide total number of bariatric surgeries will likely rise. These bariatric surgical procedures are not without complications and nutritional deficiencies including zinc deficiency remain significant clinical concerns. Bariatric patients who have zinc deficiency are at risk for developing a myriad of symptoms.

The most common symptoms of zinc deficiency include delayed wound healing, dermatitis, cognitive impairment, hypogonadism and recurrent infections. This susceptible postoperative population has a high prevalence of biochemical zinc deficiency, even prior to bariatric surgery. These risks further increases after bariatric surgical procedures especially if there has been insufficient screening for and treatment of zinc deficiency, both pre-operatively and long-term post-operatively. Bariatric surgical procedures that include a restrictive component with less malabsorptive component may develop lower rates of zinc deficiency in the short term period.

However, long term follow up of postoperative bariatric patients show that other mechanisms may be important for the persistence of zinc deficiency, sometimes overwhelming the use of oral zinc supplements. Our studies support the concern that small intestinal bacterial overgrowth may induce malabsorption of oral zinc. This is a potential mechanism for persistent zinc deficiency in patients after bariatric surgery receiving oral zinc supplements. Clinicians should maintain increased suspicion for the presence of small intestinal bacterial overgrowth in those patients with persistent zinc deficiency following bariatric surgery.

To date, there are no recommendations based on high quality clinical studies either for postoperative screening of serum zinc or for the routine daily supplementation of zinc after bariatric surgery.

Doses of oral zinc supplements higher than the RDAs are required to supplement those individuals who develop zinc deficiency after bariatric

surgery. Currently, there are many gaps in our understanding including the exact mechanisms involved in pre-operative and post-operative zinc deficiency, the potential need for screening and routine supplementation after different bariatric procedures, and to what extent low serum zinc levels can be normalized in postoperative patients. It will be important in this field of research to find the answers to these questions by completing high quality translational studies.

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In: Zinc Deficiency
Editor: Rebecca Morton
ISBN: 978-1-63484-414-7

Chapter 3

THE ROLE OF ZINC IN CARCINOGENESIS

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ABSTRACT

Zinc is the second most abundant trace element in humans and is essential for the activity of more than 300 enzymes. It affects the conformation of many transcription factors associated with control of cell proliferation, apoptosis, and signaling. Therefore, immune function also depends heavily upon its regulation. Studies of this mineral suggest that alterations in zinc homeostasis are linked to the development of numerous diseases, including cancer.

Several epidemiologic and experimental studies have investigated the relationship between zinc intake and the risk for development of many cancer types. These include mammary and prostate tumors, and especially cancer of digestive tract. However, experimental outcomes have remained inconsistent. For example, some studies suggest that

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increased zinc intake is negatively associated with colorectal and esophageal cancer, while other studies show a positive or null association.

Zinc concentration can affect the body system by many means. In general, zinc effects are based on intermediary metabolism and bioenergetics effects, proliferative/apoptotic effects and motility and invasive effects. Zinc deficiency adversely affects the immune system, increases oxidative stress, and increases the production of inflammatory cytokines. Mitochondrial accumulation of zinc inhibits aconitase activity, halting citrate oxidation. This results in decreasing the cellular energy production requirement of malignant cells.

The physiological zinc levels can also affects intracellular signaling pathways. For example, the high intracellular zinc level inhibits the activity of transcription factor NF-κB resulting in promestatastic and proangiogenic molecules reduction. By contrast, zinc deficiency increases perphosphorylation of protein kinase B (Akt), E3 ubiquitin-protein ligase (Mdm2), and reduces nuclear p53 accumulation. Overall, more research into the mechanisms of zinc homeostasis are required to better understand its impact on carcinogenesis. Furthermore, studies that can provide the tissue's zinc concentration profile in tumorigenesis, will improve our understanding of its role in cancer development.

INTRODUCTION

The intent of this chapter is to bring attention to the important implications of zinc in carcinogenesis. This chapter will provide a comprehensive review of the current literature on the role of zinc in carcinogenesis, as well as highlight the significance of zinc homeostasis mechanisms, which are essential for understanding the key roles of this trace element in carcinogenesis. Increasing awareness, amplifying interest, and accelerating research into the role of zinc in carcinogenesis is the aim of the following chapter.

Zinc is the most prolific trace element in cells and the second most abundant trace element in human body. It is found in all body tissues and secretions, contributing to approximately 2–4 g of the adult body (Jansen et al. 2009; Chasapis et al. 2012). Approximately 60% of zinc is stored in skeletal muscle, 30% is in bones, and 5% is in the liver and skin. In tissues, zinc concentration is highest in the prostate (approximately 200 μg/g), while there is approximately 14–16 μM of total zinc in the plasma, available to cells (200–300 μM) (Jansen et al. 2009; Kambe et al. 2014; Maret, 2015).

Zinc is a component of almost 10% of all the proteins produced in humans. Out of these nearly 3,000 proteins, more than 300 are enzymes that

require zinc as a cofactor. These proteins are involved in a number of different physiological processes, such as, intracellular signaling enzymes, defense against oxidative stress, DNA damage and repair, transcription factors (Zn-finger proteins) that require Zn for their structural stability and binding to DNA, and matrix metalloproteinases (MMPs), a family of Zn-dependent endopeptidases that regulate tissue remodeling. Furthermore, zinc is critical for cell proliferation, cell cycle regulation, differentiation and apoptosis (Prasad, 1995, 2003; Rink & Gabriel, 2000; Beyersmann & Haase, 2001; Theocharis et al. 2004; Song et al. 2009a, b; Prasad et al. 2009; Chasapis et al. 2011; Lin et al. 2011; Sharif et al. 2011; Alam & Kelleher, 2012; Song & Songming, 2013).

The zinc homeostasis is controlled by the coordinated regulation of the uptake, efflux, distribution, and storage of zinc (Vallee & Falchuk, 1993; Krebs, 2000; Kambe, 2004; Liuzzi et al. 2004; Soybel & Kohler, 2011).

Intestinal epithelial cells take up dietary zinc. The absorptive efficiency increases in the small intestine when zinc is limited in the diet. Deficiency in total-body zinc is mediated via reduced pancreatic and intestinal release, while excess stores are depleted through gastrointestinal secretions and the sloughing of mucosal and integument cells (King et al. 2000; Krebs, 2000; Hambidge & Krebs, 2001).

Some important dietary sources of zinc include red meat, poultry, fish, other seafood, legumes, nuts, whole grains, and dairy products. However, the zinc concentration in food depends on soil, water and fodder concentrations of the element. The absorption of zinc may be affected by quantity ingested, presence of phytate in foods—which inhibits absorption—and by some physiological factors such as age and genotype (Hambidge et al. 2010; Stathopoulou et al. 2012; Lowe et al. 2013).

The large number of enzymes that require zinc, and the diversity of cellular zinc transporters, indicate that controlling both serum and intracellular concentrations of the element is a significant process. However, the processes of cellular signaling are complex, and the role of zinc and zinc transporters in cellular signaling is less defined.

Similar to many hormones, growth factors, and cytokines, zinc impacts intracellular signaling. For example, zinc inhibits protein tyrosine phosphatases (PTPs), an important class of enzymes implicated in dephosphorylation of many proteins. By inhibiting PTPs, zinc facilitates the net phosphorylation of the insulin receptor, thereby promoting the activation of its signaling cascade (Haase & Moret, 2003, 2005a, b; Yamasaki et al. 2007; Wilson et al. 2012).

Thus, insulinergic effects of zinc include stimulating lipogenesis in adipocytes and glucose uptake from the plasma (Tang & Shay, 2001; Tang et al. 2001). Further downstream, tyrosine phosphorylation of the insulin receptor substrate-1 and the insulin/IGF-1 receptor occur as a result of zinc signaling (Haase & Moret, 2003, 2005a, b). Zinc has also been implicated in the activation of the epidermal growth factor receptor (EGFR) (Taylor, 2008; Taylor et al. 2008), mitogen-activated protein kinases (MAPKs), and transcription factor FOXO1a as well as two important regulators of gluconeogenesis: glucose 6-phosphatase (G6Pase) and phosphoenol pyruvate carboxykinase (PEPCK) (Cameron et al. 2010; Hogstrand et al. 2009).

Owing to the importance of this trace element, alterations in zinc concentration have shown to impart a profound effect on health, beginning at the cellular level. Supplementation of zinc promotes DNA synthesis, while depletion of this mineral has an inhibitory effect. Zinc deficiency in humans was initially discovered by Prasad et al. (1961). Since then, many zinc-deficiency symptoms such as growth retardation, diarrhea, skin lesions, alopecia, hypogonadism, immune system dysfunction, and neurological disorders have been described in the literature (Prasad et al. 1985, Prasad, 1991; Hambidge, 2000; Fraker and King, 2004).

In the United States, the first case of zinc deficiency was reported in 1969 in a Puerto Rican subject presenting with dwarfism, hypogonadism, hypogammaglobulinemia, giardiasis, strongyloidosis, and schistosomiasis. Zinc supplementation proved to have a positive effect on restoring growth and development (Caggiano, et al. 1969).

In developing countries, zinc deficiency contributes to 4% of the global morbidity and mortality of young children, and for 176,000 diarrhea-related and 406,000 pneumonia-related deaths of children less than 5 years of age (Penny, 2013). As a result, both the reports recommend the inclusion of zinc in oral rehydration solutions for infants and children to reduce diarrhea (Wardlaw, et al. 2010).

Zinc supplementation and zinc deficiency in human health has been extensively described in the literature. As a second messenger for immune cells, zinc provides an important antioxidant defense against free radicals. Studies have shown that zinc supplementation improves immune functions and decreases the incidence of infections in elderly people (Haase et al. 2006). Supplementation is also indicated for elderly patients with blindness or those with the risk of developing it due to age-related macular degeneration and vision loss (Kambe et al. 2014). On the other hand, zinc deficiency increases oxidative stress and the generation of inflammatory cytokines (Prasad et al.

2009). Studies also suggest that cancer may present disrupted zinc status, showing that zinc deficiency may lead to DNA damage and the initiation of cancer. Therefore, the antioxidant and anti-carcinogenic mechanisms associated with zinc homeostasis appear to have an inhibitory effect on neoplastic growth in people who are deficient. This chapter is intended to comprehensively review the literature considering the role of zinc in carcinogenesis.

ZINC AND CARCINOGENESIS

Carcinogenesis

Cancer is second among fatal diseases and it is considered a chronic degenerative disease. This disease originates in our own cells, however, both intrinsic and extrinsic factors can add to the risk of cancer development.

The development of cancer is a complex and multi-step process. Berenblum and Schubik first proposed the concept of multi-stage carcinogenesis, in 1948, in a murine skin carcinogenesis model. Each animal was treated topically with a single dose of a polycyclic aromatic hydrocarbon (ie, initiator), followed by repeated topical doses of croton oil (ie, promoter). This model was supported by later studies, and expanded to other rodent tissues. Other systems studied included the colon, esophagus, mammary glands, and trachea. Actually, the oncology recognizes three main phases: initiation, promotion and progression (Weston & Harris, 2003) (Figure 1).

Initiation

The initiation event results in irreversible DNA changes within somatic cells exposed to a causative agent. The initiation of carcinogenesis involves one or more stable genetic changes arising spontaneously or induced by exposure to a carcinogen. Stable genetic mutations are crucial for allowing a cell to have the potential for pre-neoplastic cellular development. For progression to neoplastic development, further genetic mutations and/or changes to the cellular environment must present (Trosko et al. 1992; Cox 1994). Thus, either the activation of a proto-oncogene or the inactivation of a tumor-suppressor gene can be categorized as a tumor-initiating event.

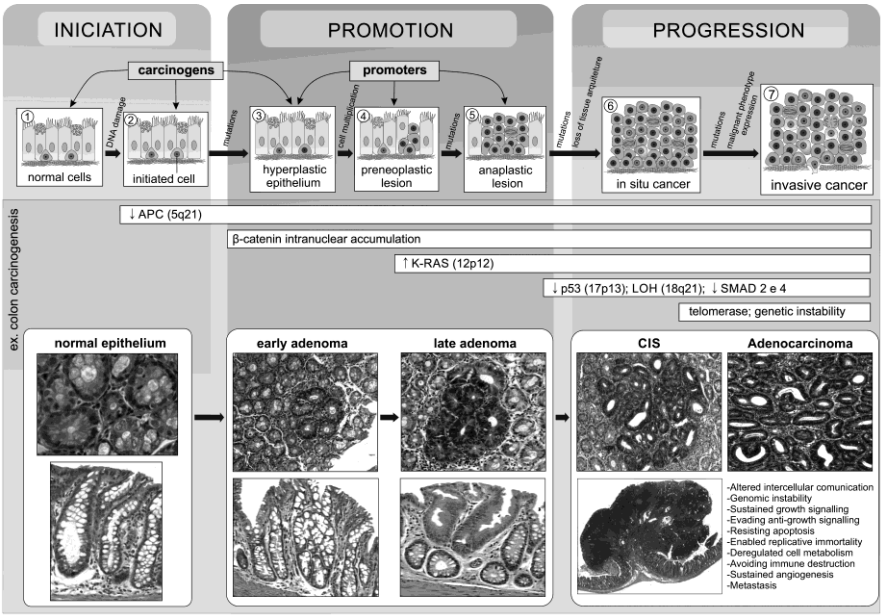

Figure 1. Illustration of stepwise tumor development. Initiated cells have irreversible DNA changes. In the presence of promoter, the initiated cells are stimulated to proliferate wich can lead a preneoplastic lesion formation or benign tumor. With successive genetic and epigenetic alteration, the expression of the malignant phenotype can emerge. The photomicrographs represent the stages of colon carcinogenesis.

Promotion

The initiated cells must be stimulated to undergo further proliferation, upset the cellular balance, and lead to neoplastic transformation. Expression of the initial mutation depends on interaction with other oncogenic mutations and on factors that can change the patterns of specific gene expression (eg. cytokines, lipid metabolites, and certain phorbol esters) (Trosko et al. 1992). The accumulative rate of mutations is proportional to the rate of cell division. Therefore, tumor promotion occurs when there is selective clonal expansion of initiated cells that will then be at risk for malignant conversion (Cairns, 1975; Verma & Boutwell, 1980; Weston & Harris, 2003).

Progression

In this phase, successive changes, such as mutations and chromosomal aberrations, continue to drive erratic cellular proliferation. Finally, these lead to the expression of the malignant phenotype, as the propensity for genomic instability and uncontrolled growth surpasses intrinsic safety nets. As the

tumor grows in size, the cells may undergo further mutations, leading to increasing cell population heterogeneity. The malignant cells can acquire more aggressive characteristics over time (Weston & Harris, 2003).

The Role of Zinc in Carcinogenesis

Zinc is an essential prerequisite for the execution of many signaling pathways in eukaryotes. Experimental and epidemiological studies have associated zinc deficiency with the potentiation for development of many tumors (Gurusamy, 2007; Al-Ebraheem et al. 2009; Franklin et al. 2011; Costello & Franklin, 2012). Conversely, zinc treatment could inhibit their development. Studies report that zinc can inhibit the proliferation of cancer cells and regulate proangiogenic and prometastatic molecules. Zinc deficiency, however, promotes cell proliferation and it is associated with an increase in size and stage of tumors (Fong et al. 2001, 2006; Jaiswal & Narayan, 2004; Tayler et al. 2012). Furthermore, high concentrations of zinc in human tissues are strongly associated with reduced risk of tumor development, demonstrating the protective effects of the mineral (Abnet et al. 2005).

However, there appears to be multiple mechanisms by which zinc exerts its effects. Many of these are somewhat cell-specific. Zinc can play opposite effects in different cancer types. This paradox is especially prevalent in mammary carcinogenesis. For this reason, the effects of zinc in mammary carcinogenesis will be discussed separately.

In general, however, studies explain the functional role of zinc in carcinogenesis based on intermediary metabolic/bioenergetic effects, proliferative/apoptotic effects, and motility/invasive effects in both zinc deficiency and supplemented conditions (Figure 2, Figure 3). Additionally, zinc's effects on carcinogenesis are well established in prostate cancer models. Evolving evidence indicates hepatocellular carcinoma, pancreatic adenocarcinoma, ovarian, colon, esophageal, and other head and neck cancers exhibit zinc relationships similar to prostate cancer (Tashiro et al. 2003; Gurusamy et al. 2007; Al-Ebraheem et al. 2009; Costello et al. 2011; Costello & Franklin, 2012). For other cancers, the role of zinc is not so clear.

In the subsections below, the metabolic and physiologic effects of zinc on carcinogenesis will be based largely on the research linking zinc to prostate cancer. Individual differences that are unique to specific cancers will be discussed briefly for completeness.

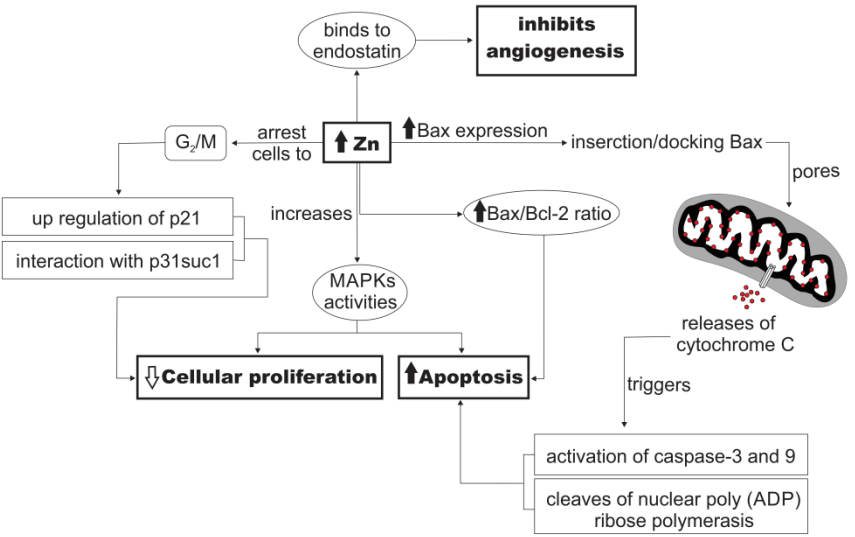

Figure 2. Illustration of zinc mechanisms which potentially link against carcinogenesis. High intramitochondrial zinc levels inhibit mitochondrial aconitase activity—inhibiting citrate oxidation. This inhibition decreases cellular energy production and, consequently, decreases proliferation. The high zinc levels also can inhibits the proliferation by arresting cells at the G2/M cell cycle check point that may involves the up-regulation of p21 gene and the interaction of zinc with the p13suc1 subunit of Cdc2 kinase. Zinc can inhibit angiogenesis by binding with endostatin. The cellular accumulation of zinc translocates the cytochrome-c from the mitochondria to the cytosol, which triggers the activation of the caspase-9 and caspase-3, resulting in apoptosis. Zinc facilitates the insertion/docking of Bax that are associated with the pore-forming process for release of cytochrome c. In addition, zinc causes an increase in Bax, resulting in an increase of Bax/BCL2 ratio wich is a classic initiating apoptotic signal in cells. The anti apoptogenic zinc effects is also attributed to MAPKs.

ZINC DISTRIBUTION IN THE SERUM AND MALIGNANT TISSUES

Several epidemiologic and experimental studies have investigated the relationship between tissue zinc level and the risk for development of many types of cancer, especially digestive tract cancers. These include esophageal, gastric, and colorectal cancer (Tashiro et al. 2003; Franklin et al. 2005; Gurusamy et al. 2007; Al-Ebraheem et al. 2009; Johnson et al. 2010; Costello & Franklin, 2012; Zapaterini et al. 2012; da Silva et al. 2013). However, the results are still not consistent.

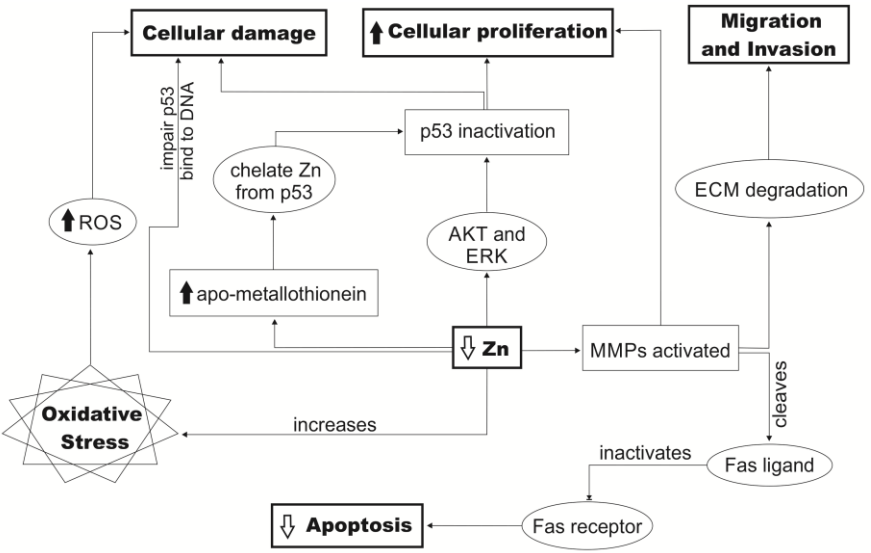

Figure 3. Illustration of zinc mechanisms that may contributes to carcinogenesis. The depletion of intracellular zinc results in hyperphosphorylation of proteins Akt and ERK and reduces the nuclear p53 accumulation, resulting in increase of cellular proliferation. Zinc deficiency also induces oxidative stress that lead to the formation of ROS which damages cellular macromolecules and impairs the ability of p53 to bind DNA. Additionally, under zinc deficient conditions, apo-metallothionein can chelate zinc from p53. It may promote increased proliferation and survival. Finally, the zinc deficiency activates MMPs which promote cell proliferation, invasion and migration, angiogenesis, and block apoptosis by degrading ECM and cleaving Fas ligand.

The level of zinc in all cells is carefully maintained and regulated to support their growth, proliferation, metabolism, and functional activities. However, the process and factors involved are complex and still largely unknown and speculative.

Plasma zinc concentration is homeostatically regulated, but under certain non-nutritional conditions such as infection and stress, plasma zinc concentration is lower than normal. Because cancer is characterized by uncontrolled growth, and zinc is required for cell growth, a reduced plasma zinc concentration and a higher zinc concentration in cancer tissues suggest a redistribution of zinc from normal tissues to the cancer tissues to supply the nutrients that are necessary for cancer development. Furthermore, in vitro and in vivo studies have shown that zinc depletion suppresses DNA synthesis and this inhibition can be reversed by zinc supplementation (Shirley & Xu, 2001; Woo & Xu, 2002).

Reduced caloric consumption and zinc intake are also contributors to zinc deficiency in plasma. Therefore, questions are raised whether this altered zinc distribution in serum and cancer tissue is a consequence of the tumor itself, chronic stress, altered zinc nutrition, or of a combination of all these effects.

Vallee and Falchuk (1993) described total cellular zinc in normal mammalian cells as approximately 200-800 μM. Under these conditions, cells possess mechanisms to protect themselves from the potential adverse effects of zinc. It is important to recognize, however, that zinc requirements for a malignant cell differ greatly from the requirements of a normal cell. It has been demonstrated in several cancer types that malignant tissue has a much lower concentration of zinc when compared to normal tissue. In fact, accumulated zinc has highly cytotoxic effects in malignant cells, which do not occur in healthy/normal cells (Franklin et al. 2005; Cortesi et al. 2008; Johnson et al. 2010; Costello & Franklin, 2012).

Many malignant cells are susceptible to the potential cytotoxic effects of physiological levels of zinc, suggesting that the protective mechanisms that exist in normal cells are lost in malignancy. Therefore, potentially malignant cells develop adaptations to balance the cytotoxic effects of zinc with the inherent need for the mineral to support survival, cellular activities, and proliferation.

There are many studies that suggest that decreased zinc is a result of malignancy rather than a contributing factor in the development of malignancy. However, as cited above, zinc deficiency occurs in cancerous tissue segments and in the non-cancer components surrounding the lesion—indicating that this depletion is an early step in cancer proliferation process, and precedes the transformation of cells from normal to cancerous. It is apparent that a decrease in zinc will not cause malignancy in the absence of the initial oncogenic transformation of the normal cell to a neoplastic cell (Costello et al. 2011).

The downregulation of zinc transporters that control the import, export, and zinc redistribution are all implicated in a cellular imbalance of zinc.

ZINC TRANSPORTERS

The intracellular concentration of zinc is tightly controlled to manage a number of biological processes, and excessive zinc is known to be cytotoxic (Choi & Koh, 1998; Sekler et al. 2007). The control of zinc homeostasis falls largely on zinc transporters, due to the selective permeability of the cell

membrane. Thus, diffusion to and from the cell is not allowed when there is an intact plasma membrane (Gaither et al. 2001; 2004).

Zinc is transported into the cell and between subcellular compartments by members of two metal-transporter families: the ZIP family (Zrt-like, Irt-like proteins), which imports zinc from extracellular fluid, and the ZnT family (zinc transporter), which exports or redistributes zinc intracellularly in mitochondria and the endosome/lysosome compartments (Kambe et al. 2004; Cousins et al. 2006).

After it enters the cell, approximately 35% of the total amount of zinc resides in the nucleus, and 65% in the cytoplasm. It is estimated that 50% of cytoplasmic zinc resides between the cytoplasmic organelles in the cytosol, and the ZnT (SLC30A) family of transporters mediates transference between them (Costello & Franklin, 2012).

The ZnT family has 10 members identified in humans, and ZnT-1 is the only zinc transport protein that is localized to the plasma membrane (Kambe et al. 2004; Liuzzi & Cousins, 2004; Foster et al. 2011). The induction of zinc transporter genes occurs through a variety of factors, and some of them are recognized as contributing to tumorigenesis. In LNCaP and PC-3 human prostate cancer cells, the expression of ZnT-1 is induced by high zinc concentrations, and the lack of expression of the ZnT7 gene—a zinc transporter localized on the Golgi membrane that has been implicated in prostatic carcinogenesis. Null-mutation of the Znt7 gene accelerated the prostate tumor formation in a transgenic adenocarcinoma of the mouse prostate model (TRAMP) (Hasumi et al. 2003; Tepaamorndech et al. 2011).

As already described, malignant cells adapt to prevent the cytotoxic effects of zinc via the reduction of cellular zinc levels. The ZIP transporter family has 14 mammalian ZIP members identified, however, only ZIP1, ZIP2, ZIP3, and ZIP4 are localized to the plasma membrane. It is assumed that these transporters are responsible for the import of zinc from the intercellular space (Kambe et al. 2004; Kim et al. 2004; Foster et al. 2011; Kolenco et al. 2013). The transporter, ZIP1 is vital for extracting zinc from circulation, while ZIP2 and ZIP3 maintain intracellular concentrations. Adenocarcinoma of the prostrate is a condition in which expression of all these transporters is significantly reduced compared to surrounding benign or healthy glandular tissue. ZIP1 is described as the primary transporter mediating zinc uptake in both transformed and normal prostatic cellsn (Franklin et al. 2005; Huang et al. 2006; Kolenco et al. 2013).

Therefore, since expression of zinc uptake transporters can increase intraprostatic zinc, the malignant prostate cell can silence the expression of zinc uptake transporters to escape the anti-tumor effects of zinc.

Transporters of zinc in hepatocytes contributing to hepatocellular carcinoma (HCC) provide further evidence to the adaptive nature of malignant cells to protect against zinc cytotoxicity. The ZIP14 transporters in these cells are absent, while expression of this protein is highly abundant and active in normal hepatocytes. Well-differentiated, early stage malignancy is characterized by low levels of cellular zinc, and persists in advancing malignancy. Thus, when viewed in the context of carcinogenesis, it is possible to propose that the silencing of ZIP14, and the resulting decrease in zinc, represent an early event in HCC carcinogenesis (Costello & Franklin, 2014).

Zinc transporters are subject to regulation by a variety of factors including zinc itself, hormones, growth factors and potentially cellular redox state. Therefore, it is unclear if zinc itself or zinc transporters are the actual mediators of malignancy-associated events (El-Tanani & Green, 1995, 1996; Liuzzi et al. 2001; Kagara et al. 2007; Maret & Krezel; 2007).

It is important to emphasize that these protective mechanism are influenced by the in vivo conditions in normal and malignant cells, where in vitro studies might not be representative for identification of the protective mechanisms (Costello & Franklin, 2012). Furthermore, the other zinc transporters and other protective mechanisms could be involved in zinc distribution, but there is not sufficient information regarding normal cell and malignant cell zinc cytotoxic protective mechanisms.

ZINC AND IMMUNE FUNCTION

Zinc is a second messenger for several immune functions. A decrease in intracellular free zinc is critical for lipopolysaccharide (LPS) mediated CD4 + T-cell activation by dendritic cells (Dcs). LPS binds to Toll-like receptor 4 on DCs and initiates Myd88 and TRIF-mediated signaling, which increases ZnT (a solute carrier)-5 mRNA and decreases ZIP-6 mRNA. This results in a decrease of intracellular free zinc in DCs that increases surface expression of major histocompatibility complex class II molecules—an essential effect for the activation of CD4 + T-cells (Kitamura et al. 2006; Hirano et al. 2008).

Zinc is also considered an important anti-inflammatory agent through its actions on macrophages. Zinc regulates phagocytosis and proinflammatory cytokine production. LPS stimulation of zinc-sufficient monocytes results in

down-regulation of inflammatory cytokines such as TNF-a, IL-1b, IL-6, and IL-8 (Kitamura et al. 2006; Haase & Rink, 2007; Hirano et al. 2008; Bao et al. 2010, 2011; Rosenkranz et al. 2011). Additionally, zinc inhibits the membrane phosphodiesterase, leading to the suppression of the nuclear factor NF-κB. This transcription factor is a pillar of the inflammatory reaction, and increases the expression of TNF-a, IL-1b, and other inflammatory cytokines. Furthermore, zinc deficiency can result in an imbalance of Th1 and Th2 function. It decreases of natural killer cell lytic activity and the percentage of cytotoxic T cells precursors (Guo et al. 2010; McCormick et al. 2014).

Therefore, a disruption of zinc level, can affect the immune system. This disruption has long been implicated in both the initiation and progression of cancer.

Zinc also provides an important antioxidant defense against free radicals, and attracts attention particularly with regard to cellular controls that regulate zinc dyshomeostasis in cancer. Oxidative stress causes severe damage to biological macromolecules, affecting normal metabolism and physiology. Oxidative processes occur most intensely in the wake of an imbalance of trace elements incorporated into the structure of enzymes responsible for antioxidant protection (Mao & Huang, 2013). Reactive oxygen species (ROS)-driven oxidative stress has been recognized as a critical inducer of cancer cell death in response to therapeutic agents. Zinc plays a role in antioxidant defense in different ways such as induction of metallothionein expression, copper and zinc superoxide dismutatase activity, protection against the oxidation of sulfhydryl groups of enzymes, and by regulation of apoptosis (Alam & Kelleher, 2012; Bobrowska-Korczak et al. 2012).

Metallothioneins are stress-inducible proteins with antioxidant properties that protect cells against free radicals and reactive oxygen species. Metallothioneins have a specific and high binding affinity for metals such as zinc and copper, maintaining negligible amounts of "free" cytosolic Zn^{+}. Sequestration of zinc via binding to redox-sensitive cysteine moieties allows metallothioneins to act as a Zn^{+}-donor or a Zn^{+}-acceptor (apo-metallothionein), maintaining the redox status of the cells (Theocharis et al. 2004; Maret, 2006; Alam & Kelleher, 2012).

Due to high cysteine content, metallothioneins are able to defend against oxidative stress by binding free radicals and protecting macromolecules against the oxidative damage (Zangger et al. 2001; Alam & Kelleher, 2012).

However, elevated metallothionein levels in cancer cells support uncontrolled growth. They protect transformed cells against free-radical damage, thereby inhibiting apoptosis and promoting cell proliferation. In

addition, the interaction of metallothionein with zinc ions regulates multiple transcription factors. These activities could explain the positive association of high metallothionein expression with histological grade in invasive ductal carcinoma of the breast, as well as explain the resistance to radiation and chemotherapeutics of these cells (Satoh et al. 1994; 2004; Yap et al. 2009).

METABOLIC/BIOENERGETIC EFFECTS OF ZINC

Regulation of mitochondrial zinc pools is critical for several cellular processes including bioenergetics and apoptosis (Alam & Kelleher, 2012).

The citrate cycle and beta-oxidation pathways are essential for the synthetic and energetic requirements for the malignant process. High intramitocondrial zinc levels inhibit mitochondrial aconitase activity—inhibiting citrate oxidation. This inhibition truncates the Krebs cycle and decreases cellular energy production. Thus, if the malignant cells can induce zinc depletion, they can maintain the citric acid cycle and the energy production needed by malignant cells (Costello & Franklin, 2000; Singh et al. 2006). Therefore, the inability to accumulate zinc could result in the loss of these inhibitory effects, leading to the development and progression of cancer.

In normal prostate gland cells, the decrease in oxidative production of ATP that accompanies the high intramitocondrial zinc levels is compensated for by an increase in aerobic glycolysis. These zinc-induced metabolic conditions do not exist in the malignant cells. Moreover, all clinical evidence shows that malignant glands consistently exhibit a marked decline of zinc and citrate levels and these metabolic decreases occur early in the development of malignancy (Costello et al. 2005; Costello & Franklin, 2006).

GROWTH AND PROLIFERATIVE EFFECTS OF ZINC

The main hallmark of cancer is uncontrolled cellular proliferation with alterations in the expression of proteins. The ability to evade apoptotic signals and survive in a growth-suppressing environment is another important feature of cancer cells.

The growth/proliferative effects of zinc are manifested by promotion of apoptosis and inhibition of cell cycle activity.

Zinc is an essential cofactor for cell proliferation, differentiation, apoptosis and cell cycle activity control. During normal cell cycle progression, zinc is requisite for G1/S transition and DNA synthesis. Zinc is also required for S-phase and subsequent G2/M transition phase. However, under normal conditions, zinc is associated with S-phase slow-down (Prasad & Beck, 1996; Chesters & Petrie, 1999; Beyersmann & Haase, 2001; Alam & Kelleher, 2012). In prostate cancer cells, the intracellular accumulation of zinc inhibits the proliferation by arresting cells at the G2/M cell cycle check point, suggesting that the inhibitory effect of high intracellular zinc on cell growth involves the up-regulation of p21 gene and the interaction of zinc with the p13suc1 subunit of Cdc2 kinase (Liang et al. 1999; Kolenko et al. 2013).

The mechanism for zinc regulation of signaling pathways is not well understood. Studies suggest that cytoplasmic zinc availability modulates kinase and phosphatase activities. For example, zinc modulates the mitogen-activated protein kinases (MAPKs) that are a diverse family of enzymes responsible for mediating the cellular response—especially those that are involved with growth, proliferation, and cell survival (Hirano et al. 2008). In experimental studies, zinc exposure increased MAPK activity in rat fibroblasts, while zinc chelation partially decreased MAPK stimulation, proving that zinc is a critical modulator of MAPK signaling (Lefebvre et al. 1999).

An important characteristic of cancer cells is their ability to evade apoptotic signals and survive in a growth-suppressing environment. The specific actions of zinc are complex, diverse, and cell-specific. In general, apoptosis is inversely correlated to the level of labile intracellular zinc (Zalewski et al. 1993).

Endogenous zinc inhibits the activity of caspases that initiate (caspase-8) and execute (caspase-3, −6, and −7) apoptosis (Stennicke & Salvesen, 1997). Recently, and perhaps a more physiologically relevant contributing factor to the anti-apoptotic effect of zinc, is its role in maintaining the functional configuration of inhibitor-of-apoptosis proteins (IAPs) (Roscioli et al. 2013).

The pro-survival proteins, BCL-2 and BCL-XL, and pro-apoptotic BCL-2 family of proteins, BAX and BAD are central to activation of mitochondrial outer membrane permeabilization and resultant loss of cytochrome c. The cellular accumulation of zinc translocates the cytochrome-c from the mitochondria to the cytosol, which triggers the activation of the caspase-9 and caspase-3, the cleavage of nuclear poly(ADP)-ribose polymerase (PARP), and apoptosis (Feng et al. 2000, 2002). Furthermore, the zinc-mediated release of cytochrome c can be attributed to zinc's effect on the mitochondria that

facilitates the insertion/docking of Bax and subsequent oligomerization that are associated with the pore-forming process for release of cytochrome c. In addition, zinc causes an increase in Bax, resulting in an increase of Bax/BCL2 ratio. This is a classic initiating apoptotic signal in cells.

The increase in cellular Bax is attenuated by zinc's induction of events that cause an increased expression of the Bax gene. Several response elements for transcription factors (TFs), reportedly activated by zinc, are present in the Bax promoter. For example, Egr-1 is an immediate-early gene and a variant of the hypoxia-inducible factor (HIF-1α) are both induced by zinc (Park & Koh, 1999; Chun et al. 2001).

Physiological levels of zinc result in activation of pro-survival and mitogenic kinases such as ERK and AKT serine-threonine kinases mediated by phosphorylation from RTKs. Phospho-AKT and ERK then phosphorylate BAD—a pro-apoptotic protein whose modulation results in sequestration in the cytosol. The net effect is survival promotion in response to normal levels of zinc (Beyersmann & Haase, 2001; Claerhout et al. 2007).

The tumor microenvironment, particularly the extracellular matrix, is considered as a key player in influencing cancer progression by promoting angiogenesis, tumor growth, and metastasis. Extracellular matrix metalloproteinases (MMPs) are calcium-dependent endopeptidases that require a zinc ion to mediate catalysis, and are produced by tumor cells, fibroblasts, macrophages, mast cells, polimorphonuclear neutrophiles (PMNs), and endothelial cells (ECs). MMPs can affect many stages of tumor development. They promote tumor cell proliferation, invasion and migration, angiogenesis, and they block apoptosis (Bhowmick et al. 2004; Kalluri & Zeisberg, 2006; Cathcart et al. 2015). Matrix metalloproteinases, especially MMP-7, confer anti-apoptotic signals to cancer cells. These MMPs cleave Fas ligand—a transmembrane stimulator of the death receptor, Fas. This inactivates the Fas receptor, and induces the resistance to apoptosis (Strand et al. 2004; Kirkin et al. 2007).

Apoptosis can also be induced by production of ROS. Zinc deficiency results in accumulation of iron in sites previously occupied by zinc. This induces oxidative stress that lead to the formation of ROS which damages cellular macromolecules. Additionally, zinc deficiency increases p53 expression in response to DNA damage. The ability of p53 to bind DNA, however, is impaired (Ho & Ames, 2002; Mackenzie et al. 2002). This is because this protein requires zinc for stable binding. Furthermore, under zinc deficient conditions, apo-metallothionein can chelate zinc from p53 and disrupt the architecture of the DNA binding domain. As a consequence, p53

adopts a conformation identical to mutant forms of p53, and is inactivated (Hainaut & Milner, 1993; Meplan et al. 2000a, b). If apo-metallothionein overexpression persists in tumor cells, it may promote increased proliferation and survival.

Among the DNA-binding domains, the zinc finger (ZF) motif is the most commonly found and represents 3% of the genes of the human genome (Tupler & Green, 2001; Klug, 2010). The zinc finger is a small protein domain that requires coordination of one or more zinc ions to stabilize its structure. The zinc ion serves to stabilize the integration of the protein itself, and is generally not involved in binding targets. The "finger" term refers to the secondary structures (α-helix and β-sheet) that are held together by the zinc ion (Lait et al. 2001). The zinc finger was first recognized in 1985 as a repeated zinc-binding motif, containing conserved cysteine (S) and histidine (N) ligands. Other common Zn^{2+} ligands found within proteins include aspartate (O) and glutamate (O) residues (Miller et al. 1985; Pace & Weerapana, 2014).

Zinc fingers are structurally diverse, and exhibit a wide range of functions. These include gene transcription, translation, mRNA trafficking, chromatin remodeling, zinc sensing, protein folding, cytoskeleton organization, epithelial development, and cell adhesion (Laity et al. 2001). One of the main questions about zinc finger proteins has been whether the linkers play an active or passive role in DNA binding. Some mutagenesis studies suggested that the linkers have a role in DNA binding, with single site mutations reducing the binding affinity by as much as 20-fold (Laity et al. 2001).

Some studies have demonstrated that ZNF family proteins are tumor suppressors and are epigenetically silenced by DNA methylation in multiple human cancer types (Cheng et al. 2010, 2012; Severson et al. 2013). The ZNF671 protein, which contains C2H2-type zinc fingers (ZFs) and a Krüppel associated box (KRAB) domain, is hypermethylated in bladder urothelial cancer samples from patients and cancer cell lines in renal cell and cervical cancers (Huang et al. 2007; Arai et al. 2012; Hansel et al. 2014). This supports the postulate that ZNF671 could inhibit tumor growth and invasion through down-regulation of determinants of dedifferentiation (methylomics). The expression of other ZNF members, such as ZNF382 and ZNF545 seems to be able to inhibit colony formation, proliferation, and induce apoptosis via repression of the NF-κB and AP-1 signaling pathways in multiple tumors (Cheng et al. 2010, 2012).

Studies also showed that the expression of ZNF23 can enhance the expression of p27KIP1 to inhibit cancer cell growth, while ZNF668 expression

can enhance the stability of the p53 tumor suppressor by preventing Mdm2-mediated p53 ubiquitination and its subsequent proteosomal degradation, in breast cancer (Huang et al. 2007; Hu et al. 2011). The ubiquitination process is involved in many physiological responses including cell growth, cell death and DNA damage repair, and it is a highly ordered. This process depends on three classes of enzymes: a ubiquitin-activating enzyme (E1) that utilizes adenosine triphosphate (ATP) to catalyze the formation of ubiquitin-conjugating enzyme (E2) and a ubiquitin-ligase (E3) that recognizes a substrate for ubiquitination. E3-ubiquitin-protein ligase is the most specific enzyme of the ubiquitination system, and participates in the turnover of many key regulatory proteins and in the development of cancer (Nakayama, 2010; Moretti & Brou, 2013). The depletion of intracellular zinc in prostate cancer cell lines results in hyperphosphorylation of protein kinase B (Akt) and E3 ubiquitin-protein ligase (MDM2), as well as reduction in nuclear p53 accumulation (Wade & Li, 2012).

In studies performed in animal models, a zinc-deficient diet seems to enhance the effects of esophageal carcinogens (e.g., N-nitrosomethyl benzylamine) by different mechanism. These mechanisms included the increase of cell proliferation, over expression of 7 cyclin D1, and p53 deficiency. Other mechanisms involved were cyclooxygenase-2 (COX-2) over expression, activating S100A8 inflammation, P450-dependent metabolism of nitrosamines, and reduced alkyl guanine DNA methyltransferase activity (Uzzo et al. 2002; Chang et al. 2004; Golovine et al. 2008). Furthermore, a chronically zinc-deficient diet in rats induced a pro-tumorigenic micro RNA signature (miR-31 and miR-21) that fosters squamous cell carcinoma development (Leitzmann et al. 2003). Meanwhile, zinc supplementation could affect tumor progression in rodents by inducing apoptosis in malignant cells, and reversing over expression of S100A8 (Key et al. 1997; Kristal et al. 1999; Gallus et al. 2007).

PROMETASTATIC AND PROANGIOGENIC ZINC EFFECTS

Metastasis is another hallmark of cancer cells and involves a number of sequential events by which cancer cells “escape” from the primary tumor, invade adjacent tissues, migrate to distant sites through the surrounding microvasculature, and invade secondary site to form new tumors (Friedl & Alexander, 2011; Hanahan & Weinberg, 2011). To acquire the migratory and invasive capacity the epithelial cells need to lose cell-cell adhesion and it is

coordinated by activation of epithelial-mesenchymal transition (EMT) (Heo et al. 2015). In the neoplastic process, angiogenesis is very important to support tumor growth. It is the supply for nutrients and oxygen for tumor cells (Fuents et al. 2015). Angiogenesis facilitates the invasion of malignant cells into the circulation, and is important for the establishment of these cells at the site of metastasis (Benelli et al. 2006; Singh et al. 2007; Rucci et al. 2011, Fuents et al. 2015).

The angiogenesis process involves the migration, proliferation, and differentiation of endothelial cells (EC) resulting in formation of new blood vessels. A blood supply is necessary for tumor growth and spread. The new blood provides nutrients and oxygen for tumor growth.

Therefore, four hallmarks of cancer including migration, invasion, metastasis, and angiogenesis are dependent on the surrounding microenvironment. It has been shown that MMPs can promote tumor development in several ways, including tumor invasion, metastasis and angiogenesis (Benaud et al. 1998; Hadler-Olsen et al. 2011).

MMPs mediate proteolysis of extracellular matrix (ECM) components and many other proteins, which facilitates movement of cells through ECM, and cleaves cell-ECM adhesion proteins and cell-cell junction proteins. MMP activities are highly dependent on zinc binding to the catalytic domain. The interaction of cysteine residue with zinc ion maintains the enzyme in its latent form. However, disruption in this interaction causes protein unfolding which activates the enzyme, exposing the active site to the ligand (Benaud et al. 1998; Klein & Bischoff, 2011). This contributes to invasion and metastasis during the carcinogenesis process. In an in vitro study, prostate cancer cell lines treated with physiological zinc levels showed a reduction of MMP-9 as well as a reduction of vascular endothelial growth factor (VEGF), IL-6, and IL-8 (Uzzo et al. 2002, 2006).

MMPs also support angiogenesis by releasing pro-angiogenic factors and degrading ECM to support EC migration (Czochara et al. 2014). In addition, the ability of zinc ion to bind with endostatin—a potent angiogenesis inhibitor in vitro and in vivo—is essential for its anti-angiogenic activity (Taylor et al. 2012). However, MMPs cleave cell surface growth factor receptors, which results in inhibition of tumor development (Czochara et al. 2014). Also, some ADAMTS display anti-angiogenic and antimetastatic properties. One possible explanation, especially for ADAMTS-1, is that this molecule undergoes auto-proteolytic cleavage or even proteolytic impairment of its catalytic site, which could account for these outcomes (Rocks et al. 2008; El-Hour et al. 2010).

THE ROLE OF ZINC ON BREAST CANCER

Breast cancer is a chronic disease with endogenous and environmental etiology, in which genetic susceptibility interacts with sociodemographic, reproductive, lifestyle related hormonal imbalances, nutritional factors and other risk factors related to lifestyle (AlQallaf et al. 2007).

Invasive breast carcinoma is the most common malignant tumor in women (Riesop et al. 2015). Mortality from breast cancer is not caused by the primary tumor, but rather, from tumour growth in secondary locations, leading to breast cancer metastasis (Weigelt et al. 2005). Then, to metastasize the cancer cells must undergo a phenotypic transition from epithelial cells to mesenchymal cells. The mesenchymal cells have greater motility and are capable of gaining access to the blood circulation or lymphatic system by penetrating through the basement membrane. Thus, cancer cells can migrate and to acquire ability to attach to the extra-cellular matrix (ECM). The integrins are the primary mediators of cell to ECM adhesion and are therefore important in cell migration during cancer metastasis. Many members of the integrin β1 family, as α5/β1 (fibronectin receptor) have been implicated in cancer metastasis. It has been shown the blocking of integrin α5/β1 reduces the adhesion of a highly metastatic breast cancer cell line to a model endothelium (Bliss et al. 1995). The role of zinc in integrin-mediated adhesion of breast cancer cells and its role in the process of breast cancer metastasis are not yet well unknown (Chavakis et al. 1999). However, a study showed the role of zinc as an inhibitor of MDA-MB-231 cell migration on fibronectin through inhibiting magnesium-dependent integrin-, likely integrin α5/β1-, mediated adhesion, suggesting that zinc could function as an inhibitor of integrin-mediated breast cancer metastases (Lymburner et al. 2013).

The zinc is an essential trace element and its functions as an antioxidant and its role in the maintenance of genomic stability have been widely described (Eide, 2011). The increased intracellular concentration of reactive oxygen species, as well as the damage DNA repair, allows nucleic acid susceptibly to oxidative impairment in, which contributes significantly to mammary carcinogenesis (Cai et al. 2004).

Inadequate nutrition of zinc could disturb the function of signaling molecules and proteins directly involved in DNA replication and repair. Limited availability of cellular zinc due to zinc deficiency could result in loss of activity of these zinc-dependent proteins involved in the maintenance of DNA integrity and may contribute to the development of cancer (Yan et al. 2008). In the breast cancer, part of these proteins are involved in the defense

against oxidative stress, including metallothionein (MT) (Theocharis et al. 2004), Cu/Zn superoxide dismutase (SOD) (Oteiza et al. 2001), and more than 2,000 transcription factors (Zn-finger proteins) that require zinc for their structural stability and binding to DNA (Brown et al. 2002). These also include the proteins controlling responses to DNA damage and repair (Prasad, 1998; 2003; Song et al. 2009), intracellular signaling enzymes (Vallee & Auld, 1993; Prasad, 1995) and p53 protein, which regulate functions related to DNA repair, cell cycle checkpoint regulation and induction of apoptosis (Harris, 1996; Gasco et al. 2002). Furthermore, the matrix metalloproteinases (MMPs), a family endopeptidases that regulate tissue remodeling, (Lin et al. 2011), as well plays an important role in invasion and metastasis of tumors when their functions are unregulated; also is zinc-dependent (Alam & Kelleher, 2012). Thus, the zinc function in antioxidant and anti-carcinogenesis mechanisms associated with its homeostasis appear to play an inhibitory role on neoplastic cell growth (Alam & Kelleher, 2012).

The major challenge in the management of breast cancer as well as in other types of cancers is the understanding biochemical pathways. In general, the relation to zinc and tumors can be regarded from the perspective of dysregulation of their intracellular and serum zinc levels and from the perspective of abundant or deficient dietary zinc income and thus resulting alteration of its biochemical roles (Gumulec et al. 2011). The disturbances in zinc homeostasis may induce biochemical changes characteristic of many diseases, including neoplastic diseases.

Normal mammary gland development and function is highly dependent on zinc homeostasis (Kelleher et al. 2009) which is necessary for the tight coupling of cell proliferation (MacDonald, 2000) and programmed cell death (Clegg et al. 2005). The zinc transporting network in the mammary gland is unique in that it plays a dual role in maintaining normal levels for basic cellular zinc requirements. This is coupled to its secretion during lactation, and is under the control of the lactogenic hormones such as prolactin (Kelleher et al. 2009). Since the signaling pathways that modulate cell growth in cancer cells also abrogate the controls for cell death, understanding zinc dysregulation in the context of breast cancer development could be important in prevention, diagnosis, targeted therapeutics design and management of the disease (Kelleher et al. 2009).

The complexity of zinc homeostasis requires its compartmentalization in intracellular organelles, which is tightly regulated through the integration of these metal transport mechanisms (Kelleher et al. 2011). The zinc ion is a charged divalent cation and therefore not able to cross cell membranes by

passive diffusion. The crossing of the membrane transport mechanisms are of high importance. Therefore, it is essential to control zinc homeostasis and to prevent the accumulation of zinc accompanied by toxic effects within the cell (Eide, 2006).

Zinc concentrations have been reported to be significantly higher in breast cancer tumors relative to healthy breast tissue, and lower in blood serum and erythrocytes of breast cancer patients when compared to healthy controls (Margalioth et al. 1983; Alam & Kelleher, 2012; Lopez et al. 2011; Tinoco-Veras et al. 2011). Epidemiological studies have established a relationship between high breast tissue zinc levels and development of breast cancer (Cui et al. 2007). During uncontrolled growth, cancer cells may be using more zinc than they normally use which generates a need to replenish zinc from plasma (Taylor et al. 2011). Moreover some studies also show a relationship between low plasma Zn levels and the risk for developing breast cancer (Kuo et al. 2002; Adzersen et al. 2003; Taylor et al. 2011), suggesting that this is a prognostic and therapeutic factor for this disease (Borges de Araújo et al. 2015).

Furthermore, since zinc is essential for growth and cancer is characterized by uncontrolled growth, zinc accumulation suggests an involvement of zinc in breast tumorigenesis through of proliferation increases, which can be confirmed by some studies (Sukumar et al. 1983; Lee et al. 2003).

Supplemental dietary intake of zinc may also influence IGF-1 signaling, which is related with systemic growth, and cancer risk (Giovannucci et al. 2003). Elevated IGF-1 is associated with an increased risk of developing several cancers including the breast cancer (Stoll, 1997). IGFs have shown to play role on mitogenic, transforming, and antiapoptopic mechanisms when coupled with growth factors (Dupont et al. 2000). IGF-1 also promotes angiogenesis and thus may be a key target in the prevention of cancer cell migration and metastasis (Tang et al. 2007; Shigematsu et al. 1999). Furthermore, IGF-1 appears to induce the expression of the ER, and estrogens which can enhance IGF-1R mediated signaling (Kahlert et al. 2000).

The excess of zinc increases superoxide generation (Donadelli et al. 2009) and intracellular acidosis and triggers both the caspase-dependent (Rudolf & Cervinka, 2004; Jayaraman & Jayaraman, 2011) such as independent apoptosis (Donadelli et al. 2009). In addition, overexpressed MT in breast carcinomas (EL Sharkawy & Farrag, 2008; Yap et al. 2009) can also be associated with the inhibition of p53 and resistance to apoptosis (Puca et al. 2009). Therefore, this supports the idea that abnormal Zn metabolism is a common link in cancer development (John et al. 2010).

The zinc accumulation in tumor tissues is also correlated with increased expression of cellular zinc importing proteins compared with normal tissues, suggesting that tumor cells selectively increase zinc uptake using common mechanisms and that zinc transporters can contribute to the severity of cancer (Taylor, 2000; Kagara et al. 2007; Taylor et al. 2008 (Chasapis et al. 2011). Zinc transporters (ZnTs) proteins facilitate cellular zinc homeostasis (Chasapis et al. 2011) and several proteins within these families appear disturbed in breast cancer cells. The antioxidant protein MT is also known to have a significant role in cellular Zn metabolism (Larner et al. 2015).

Investigation of breast cancer biopsies as well as in cultured tumor cells have shown high expression of some proteins that play a role in zinc homeostasis, such as ZIP6 (Taylor, 2000), ZIP7 (Taylor et al. 2008), ZIP10 (Kagara et al. 2007), and ZnT2 (Lopez et al. 2011) that participate of the network of zinc transporters and are associated with development of breast cancer. Thus, the presence of breast tumor leads to overexpression of genes encoding the Zip6, Zip7 and Zip10 proteins, which promote zinc inflow to the initiated cells and inhibit transmembrane transporters of this mineral to other cells. This predisposes the cells to malignant transformation and suggests that breast tumor cells selectively increase zinc absorption in the disease (Grattan & Freake, 2012). Furthermore, the ZIP 6 and ZIP 10 proteins are associated with histological grade of breast cancer and metastasis of lymph node (Kasper et al. 2005; Taylor et al. 2007; Grattan & Freake, 2012).

Breast cancer appears to be unique in its acquisition of zinc, suggesting the potential implication for this mineral in the breast malignancy involvement (Grattan & Freake, 2012). A recent study has shown that the marginal deficiency of zinc (15 mg Zn/kg diet) provided in the diet of female mice resulted in high zinc accumulate in the breast tissue and increased expression of zinc transporters ZIP 6 and ZIP 10. These established a toxic microenvironment (Bostanci et al. 2015). The effects include oxidative stress, ductal and stromal inflammation, fibrosis and expansion of the mammary gland, and increased estrogen receptor expression (ER). All of these carry potential risks leading to the onset of breast cancer (Bostanci et al. 2015), suggesting (paradoxically) zinc deficiency also induces zinc accumulation and oxidative stress (Bostanci et al. 2015).

On the other hand, in vitro and in vivo studies have revealed that zinc deficiency leads to increased oxidative stress and DNA damage wich contributed to breast cancer (Fenech & Ferguson, 2001; Ho et al. 2003; Milner, 2004; Finley, 2005; Yan et al. 2008). Zinc supplementation, however, has been shown to inhibit cancer development (Paski & Xu, 2001; Franklin &

Costello, 2007; Hashemi et al. 2007), and high levels of zinc supplementation have shown a positive effect on decreasing oxidative stress and improving immune responses in cancer patients (Federico et al. 2001).

The hyper-accumulation of zinc in the malignant breast tumor cells is correlated with ZnT2 overexpression and increase vesicular Zn pools (Lopez et al. 2011). Depletion of ZnT2 increases cytosolic zinc pools and induces autophagy, suggesting that abundant ZnT2 expression in malignant cells protects the breast tumor cells from zinc-induced cytotoxicity by redirecting it into vesicular compartments (Lopez et al. 2011). Since malignant breast cancer cells accumulate zinc (Geraki et al. 2002; Geraki et al. 2004), and exposure to high levels of zinc activates apoptosis (Truong-Tran et al. 2000), mechanisms have been evolved to protect cells against zinc modulated cell death. Then, The ZnT2 dysfunction is also related to breast disease (Seo et al. 2011).

It is important to relate that breast cancer is a heterogeneous disease and there are differences in zinc network dysregulation between breast cancer subtypes [i.e., luminal, basal, and human epidermal receptor 2 (HER2) tumors] (Hsiao et al. 2010; Bertos & Park, 2011). Also, measuring zinc in invasive ductal breast cancer has shown different results between estrogen positive (ER+) and estrogen negative (ER−) samples. Estrogen receptor positive tumor samples presented approximately 80% higher zinc concentrations than in ER negative samples, as described by Farquharson et al. (2009).

Zinc is also involved in epigenetic alterations, which heritable changes in gene expression occur without changes in DNA sequence (Jones & Laird, 1999; Yoo & Jones, 2006). Thus, zinc deficiency results in decreased DNA and histone methylation (Wallwork & Duerr, 1985). Some studies suggest that histone lysine methyltransferases and histone deacetylases are zinc dependent enzymes (Finnin et al. 2001; Fatemi et al. 2001; Somoza et al. 2004; Zhang et al. 2002). Histone deacetylase inhibitors have been shown to reactivate the estrogen receptor (ER) in estrogen receptor negative (ER−) breast cancer cells (Zhou et al. 2007). Furthermore, zinc deficiency also may result in greater concentrations of the ER (Om & Chung, 1996) through increased expression of aromatase enzyme (CYP19) (Om & Chung, 1996; James et al. 1987). This enzyme is needed for the conversion of testosterone and androstenedione (Buzdar & Robertson, 2006) to estrogens, allowing the provision of required needed for proliferation (James et al. 1987).

Therefore, there are some divergences about zinc level in mammary tumorigenesis, but, in general, the high levels of zinc in mammary tumor are positively correlated with tumor development and negatively in other tumor types, as discussed in the previous sessions.

CONCLUSION AND FUTURE PERSPECTIVES

Given our current understanding, several facts may explain the role of zinc in carcinogenesis. Zinc is an essential component of a number of biological processes. It is a cofactor for various enzymes crucial for DNA integrity, cell differentiation, and division. Furthermore, zinc is involved in a number of metabolic processes such as protein synthesis, immunological function, and growth. However, zinc accumulation has potentially toxic effects. Thus, it is reasonable that dysregulation of zinc might leads to unpredictable cytotoxic and pathological conditions, including cancer.

The functional role of zinc in carcinogenesis is based on intermediary metabolism and bioenergetics effects. Its effect on proliferation, apoptosis, motility, and invasive potential make it an intriguing mineral to study in carcinogenesis. In general, zinc levels are lower in malignant cells than in normal cells. Due to the susceptibility of tumor cells to the cytotoxic effects of accumulated zinc, this makes sense. Thus, to prevent the manifestation of zinc toxicity, malignant cells develop adaptive mechanisms to decrease zinc levels. This is primarily mediated via the downregulation of zinc transporters that control the import, export, and redistribution of zinc. It is not conclusive if zinc decreases as a result of malignancy, or if it is a contributing factor in the development of malignancy. However, many studies suggest that zinc depletion is an early step in the cancer proliferation process, but it will not cause malignancy in the absence of the initial oncogenetic transformation.

Moreover, different cancer types show differential sensitivity to zinc exposure and zinc dysregulation in cancer is cell-type specific, especially in mammary carcinogenesis.

Therefore, this is a promising and challenging field that must be explored in order to establish and clarify the effects of zinc in carcinogenesis, as well as to achieve new mechanisms that could be involved in the initiation and progression of cancer.

ACKNOWLEDGMENTS

The figures used in this chapter were created with support from Marcos Correa Dias, PhD, Federal University of Mato Grosso, Health Sciences Institute, Brazil.

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