Biol Trace Elem Res (2014) 162:64–71
DOI 10.1007/s12011-014-0109-2

Protein Energy-Malnutrition: Does the In Vitro Zinc Sulfate Supplementation Improve Chromosomal Damage Repair?

Gisel Padula · Horacio F. González · Ana Varea · Analía I. Seoane

Received: 22 July 2014 / Accepted: 13 August 2014 / Published online: 28 September 2014

Abstract Protein-energy malnutrition (PEM) is originated by a cellular imbalance between nutrient/energy supply and body's demand. Induction of genetic damage by PEM was reported. The purpose of this study was to determine the genetic effect of the in vitro zinc sulfate ($ZnSO_4$) supplementation of cultured peripheral blood lymphocytes from children with PEM. Twenty-four samples from 12 children were analyzed. Anthropometric and biochemical diagnosis was made. For the anthropometric assessment, height-for-age index, weight-for-age index, and weight-for-height index were calculated (WHO, 2005). Micronutrient status was evaluated. A survey for assessed previous exposure to potentially genotoxic agents was applied. Results were statistically evaluated using paired sample *t* test and χ^2 test. Each sample was fractionated and cultured in two separate flasks to performed two treatments. One was added with 180 μg/dl of $ZnSO_4$ (PEMs/$ZnSO_4$) and the other remains non-supplemented (PEMs). Cytotoxic effects and chromosomal damage were assessed using the cytokinesis-block micronucleus assay (CBMN). All participants have at least one type of malnutrition and none have anemia, nor iron, folate, vitamin A, and zinc deficiency. All PEMs/$ZnSO_4$ samples have a significant reduction in the micronucleus (MNi) frequency compared with PEMs (t=6.25685; p<0.001). Nuclear division index (NDI) increase in PEMs/$ZnSO_4$ (t=−17.4226; p<0.001). Nucleoplasmic bridge (NPBs) frequency was four times smaller in PEMs/$ZnSO_4$ (χ^2=40.82; p<0.001). No nuclear buds (NBuds) were observed. Cytotoxic effects and chromosomal damage observed in children suffering from PEM can be repaired in vitro with zinc sulfate supplementation.

Keywords Protein-energy malnutrition · Chromosomal damage · Zinc sulfate · Children

Introduction

Growth is defined as a continuous process resulting from the complex interaction between inheritance and environment [1]. In this context, Protein energy-malnutrition (PEM) results from food insufficiency as well as from poor social and economic conditions [2, 3]. Malnutrition is originated by a cellular imbalance between nutrient/energy supply and body's demand in order to ensure growth and maintenance [4]. The term protein-energy malnutrition applies to a group of related disorders that develop in children and adults whose consumption of protein and energy (measured by calories) is insufficient to satisfy the body's nutritional needs (primary PEM). This problem affects approximately one third of children worldwide [5], and approximately 60 % of deaths that occur in children under five are directly related to malnutrition and associated diseases. The total number of underweighted and stunted children has not dropped significantly since 1980 [6]. Malnutrition is a serious public health problem, and then the adequate nutritional support of severe PEM in infants represents a great challenge [7].

G. Padula
Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, Argentina

G. Padula (✉) · A. I. Seoane
Instituto de Genética Veterinaria (IGEVET) Ing. Fernando Noel Dulout, Facultad de Ciencias Veterinarias, UNLP-CONICET, 60 y 118 s/n, La Plata B-1900-AVW, Argentina
e-mail: giselpadula@conicet.com.ar

H. F. González · A. Varea
Instituto de Desarrollo e Investigaciones Pediátricas (IDIP) "Prof Dr Fernando Viteri" del Hospital de Niños de la Plata, Min de Salud/CIC-PBA, La Plata, Argentina

G. Padula
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), La Plata, Argentina

Many micronutrient deficiencies were described in PEM, for example, zinc, iron, and vitamin A [6, 8–10]. It has long been recognized that vitamins and minerals play a crucial role in health and disease [11–14]. Such essential micronutrients are required for the synthesis of DNA maintenance factors and repair mechanisms [14, 15]. In fact, not only nutritional deficiencies or excess can greatly alter physical function neither inadequate intake of micronutrients contributes significantly to DNA damage and cancer risk [16–21].

In these sense, in previous works, we studied the relationship between PEM and genetic damage [10, 22, 23]. We found that children aged 1–60 months with PEM exhibit increased frequencies of chromosomal aberrations (dicentrics chromosomes, gaps, isogaps, breaks, and telomeric association) in peripheral blood lymphocytes. Similar results in peripheral blood lymphocytes and bone marrow cell cultures have been found in another work, abnormalities persist even after the children have attained normal height and weight [24]. In addition, it was reported that low-protein diets induce chromosome breaks and deletions in bone marrow cells and lymphocytes of rats and female mice [25, 26].

Zinc (Zn) is an essential component for more than 1,000 proteins including copper/Zn super oxide dismutase (SOD) as well as a number of other Zn finger proteins [27, 28]. The function of Zn involves a wide range of biological processes including cell proliferation, immune function, and defense against free radicals [27–32]. In addition, Zn appears to regulate key physiological processes such as cellular response to oxidative stress, DNA repair, cell cycle regulation, and apoptosis [29, 30, 33–48].

There are various methods to measure genome stability but to date the cytokinesis blocked micronucleus assay (CBMN) appears to be the best validated tool for measuring DNA damage as a result of nutrient deficiency [13]. This method can provide data on cytotoxic effects as well as chromosomal damage. In the CBMN assay, a cytokinesis blocking agent (cytochalasin B) is used to produce once-divided binucleated cells that can express micronuclei (MNi) which are biomarkers of whole chromosome loss and/or breakage. In addition, it is also possible to measure nucleoplasmic bridges (NPBs), which arise from dicentric chromosomes and nuclear buds (NBuds), a biomarker of gene amplification, replication stress, or unresolved DNA repair-DNA protein complexes [13, 49–51]. The presence of these DNA damage endpoints are a strong indicator of chromosomal damage and instability within a cell [49].

Taking into consideration that in vivo and in vitro supplementation with vitamins and minerals reversed the damage caused by different genotoxic agents [13, 52–58], the purpose of this study was to determine the genetic effect of in vitro zinc sulfate ($ZnSO_4$) supplementation of cultured peripheral blood lymphocytes from children suffering from PEM without associated infections.

Materials and Methods

Experimental Procedure

Twenty-four samples obtained from twelve primary malnourished infants attending to Primary Health Care Rooms, La Plata, Argentina, were included in this analysis (Table 1). Children aged 1–60 months. Anthropometric were performed to assess nutritional condition. Biochemical evaluation was made to determined micronutrients status. Prior to blood collection, we interviewed each individual's parent to complete a semi-structural survey specifying age, dietary habits, viral or bacterial diseases, previous exposure to diagnostic X-rays, and use of therapeutic drugs. All children were disease-free, and had not been exposed to X-rays, drug therapy (including vitamins or minerals), or viral infections in 1 month prior to the study. Also, children with anemia or signs of vitamin deficiency were not included. Specific written information about the aims of the study was provided to all participants. Written informed consent was obtained from the participant's parents. The "Comité de Bioética del Hospital de Niños Sor María Ludovica" (La Plata, Argentina) provided the IRB approval.

Cytogenetic Analysis

Heparinized venous blood samples were used to obtain lymphocytes from the participants and set up two flasks, each one containing 0.5 ml of total blood in 5 ml RPMI 1640 (Gibco-Invitrogen Buenos Aires, Argentina) containing 1 % phytohemagglutinin (Gibco-Invitrogen), 50 IU penicillin, and 50 μg/ml streptomycin (Sigma, St. Louis, MO, USA). One culture was supplemented with $ZnSO_4$ (PEMs/ $ZnSO_4$) while the other remains non-supplemented (PEMs); by this way, each child was its own control. $ZnSO_4$ concentration was 180 μg/dl; it is in the middle of the normal physiological range established in children and was able to reduce the genetic damage induced by zinc deficiency in preliminary studies in our laboratory with in vitro cultured human lymphocytes (manuscript in preparation). Cytotoxic effects and chromosomal damage were assessed by CBMN assay according to the method of Fenech [49]. Briefly, after 6 days in culture, cells were resuspended in fresh culture medium in the presence of cytochalasin-B (3 μg/ml final concentration) (Sigma, St. Louis, MO, USA) for 24 h. At the seventh day, cultures were harvested. The cell suspension was centrifuged and the pellet was resuspended in 5 ml of fixative (methanol:acetic acid 3:1). The cells were washed three times with fresh fixative; they were later resuspended, dropped onto clean slides, and finally stained with 5 % of Giemsa for 10 min. The chromosome damage biomarkers scored are micronuclei (MNi), nucleoplasmic bridges (NPBs), and nuclear buds (NBuds). One thousand binucleated cells (BN)

Table 1 Sex, age, and anthropometric data of the malnourished children

Samples	Sex	Age (year)	Weight (kg)	Height (cm)	WAZ	HAZ	WHZ
1	M	1.631	9.45	78	−1.57	−2.08	−0.39
2	M	4.255	13.54	94.9	−1.72	−2.35	−0.2
3	M	3.764	13.12	88.1	−1.53	−3.33	1.14
4	M	0.906	8.86	71	−0.52	−1.46	0.46
5	M	3.847	14.28	95.6	−0.93	−1.62	0.21
6	M	1.42	9.86	70.5	−0.76	−4.06	2.41
7	M	2.039	12.2	83.9	−0.03	−1.18	1
8	M	2.983	12.12	95.5	−1.38	−0.12	−2.08
9	M	2.39	9.85	81.5	−2.42	−2.83	−0.86
10	F	1.428	9	75	−0.91	−1.68	0.15
11	F	2.736	12.2	87.5	−0.66	−1.45	0.35
12	F	1.225	10.47	73.5	0.75	−1.34	2.08

were analyzed per experimental point. Fenech's scoring criteria for MNi, NPBs, and NBuds determinations were used [49]. Cytostatic effects were analyzed through the nuclear division index (NDI), estimated by the ratio of mono-, bi-, and multinucleated cells in 500 viable cells, using the formula $=(M_1+2M_2+3M_3+4M_4)/N$; where $M_{1\text{-}4}$ represent the number of cells with 1–4 nuclei and N is the total number of viable cells scored [49].

Anthropometric Evaluation

Height (H) and weight (W) were measured. Height-for-age index (HA), weight-for-age index (WA), and weight-for-height index (WH) were calculated. *Z*-scores for height-for-age (HAZ), weight-for-age (WAZ), and weight-for-height (WHZ) were assessed with the WHO standards using the ANTHRO 2005 program [59]. To determine the prevalence of stunting (HA), underweight (WA), and wasting (WH), the cut-off was −1.1. Malnutrition degrees were established according to Torun and Chew [60].

Micronutrients Status

Part of the blood samples were taken for hemogram, ferritin, intra-erythrocyte folates, vitamin A, and zinc determinations. Samples were collected in aluminum foil-wrapped tubes with EDTA for hemogram determinations and with heparin to determine the other parameters. Samples were then transported to the Children's Hospital laboratory within 2 h after extraction. Hemogram samples were processed immediately. The separated plasma is being kept frozen at −70 °C and will be analyzed when all samples were collected to avoid inter- and intra-assay variations. Another aluminum foil-wrapped tube is being kept for vitamin A dosage. Hematocrit (Ht), hemoglobin (Hb), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were analyzed with an automated system. Ferritin and folate will be assessed by automated chemiluminescence immunoassay. Serum retinol concentration will be determined by HPLC and zinc by atomic absorption spectrophotometry at 213.9 nm (Shimadzu AA 6200).

Indicators and cut-off points. Anemia (Hb value <11 g/dl) [61]; iron deficiency (blood ferritin <12 ng/ml) [61]; folate deficiency (intra-erythrocyte folates <140 ng/dl) [62]; vitamin A deficiency (plasma retinol <20 μg/dl) [63]; and zinc deficiency (blood zinc levels <70 μg/dl [64].

Statistical Analysis

Data were statistically analyzed with the paired sample *t* test with the Statgraphics® 5.1 software and χ^2 test [65] with $p<0.05$ considered statistically significant.

Results

Table 1 summarizes anthropometric data of the sample. All participant have at least one type of malnutrition (stunting, underweight, and/or wasting). Stunting is the prevalent form of PEM (92 %). Figure 1 shows that most of the children have mild malnutrition (grade I).

Hematological and biochemical variables are summarized in Table 2. None of the children have anemia nor iron, folate, vitamin A, or zinc deficiency.

Table 3 shows the results of CBMN assay for each paired sample (MNi and NDI). All PEMs/$ZnSO_4$ samples have a significant reduction in the frequency of micronucleus when compared with PEMs ($t=6.25685$; $p<0.001$). Zn concentration also bring about impact on the nuclear division index, with significant increase for this variable in PEMs/$ZnSO_4$ ($t=-17.4226$; $p<0.001$). Figure 2 summarizes the DNA-damage

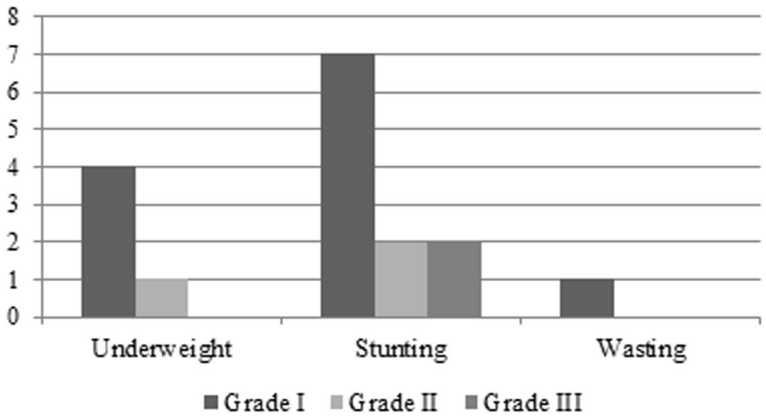

Fig. 1 Type and degree of PEM in the malnourished children

endpoints (MNi, NPBs, and NBuds) and NDI. The global MNi frequency was five times smaller in supplemented samples with regard to non-supplemented (χ^2=77.95; p<0.001). Similar trend was observed for NPBs (χ^2=40.82; p<0.001). No NBuds were observed in both groups.

Discussion

PEM is originated by a cellular imbalance between nutrient/energy supply and body's demand in order to ensure growth and maintenance [4]. It must be considered that energy and amino acids are required for protein synthesis and DNA repair [66–68]. In this sense, we recently carried out a research in order to study the occurrence of structural chromosomal aberrations in children suffering from PEM and we found that chromosomal damage could be attributed to several factors such as a severe deficiency of essential nutrients and energy required for the synthesis of DNA maintenance factors; deterioration of repair mechanisms; and/or the lack of specific factors that are needed to protect the cell against oxidative DNA damage.

Results presented here showed a significantly increased frequency of chromosomal damage and cytotoxic effects in lymphocytes of malnourished children. These results agree with those of other authors performed in children with severe PEM [10, 24, 69] and our own previous research conducted with lymphocytes of malnourished children in the same range of age (1–60 months) who exhibit increased frequencies of structural chromosomal aberration (dicentrics chromosomes, gaps, isogaps, breaks, and telomeric association). Likewise, Cervantes-Ríos and coworkers [70] reported the induction of micronuclei in reticulocytes of children with malnutrition and infections but Celik and coworkers [71] did not found DNA damage increase in marasmus cases. In addition, some results were reported in mice or rats fed with low-protein diets who exhibit high rates of chromosomal damage [24, 72–74] and citomolecular damage showed by the alkaline single cell electrophoresis assay [75].

In this work, the increased frequency of chromosomal damage and cytotoxic effects observed in lymphocytes of

Table 2 Hematological and biochemical variables

Samples	Ht (%)	Hb (g/dl)	MCV (μm^3)	MCH (g/dl)	Ferritin (ng/ml)	Folate (ng/dl)	Retinol (μg/dl)	Zinc (μg/dl)
1	40	12.6	79.8	25.5	26.7	359.0	29.4	129.4
2	41	14.0	77.0	26.2	13.0	376.0	30.7	118.7
3	34	11.1	76.9	25.8	29.1	299.0	26.3	101.5
4	38	12.8	83.0	27.5	48.9	364.7	27.9	102.1
5	39	13.4	83.0	28.3	14.0	554.6	32.3	84.0
6	34	11.3	72.0	23.5	15.8	298.5	21.9	83.0
7	33	11.1	74.0	24.6	27.4	467.3	31.6	80.25
8	34	11.5	74.0	25.1	52.7	306.4	27.1	85.75
9	35	11.7	78.0	26.4	63.7	345.6	25.7	113.4
10	34	11.4	67.0	22.3	25.3	372.9	22.8	97.2
11	37	12.3	81.0	27.0	12.1	345.2	28.1	102.6
12	36	11.7	72.0	23.3	19.1	355.8	24.7	105.7

Table 3 Results of CBMN assay for each paired sample (MNi and NDI)

	MNi (‰)		NDI (500)	
Samples	PEMs	PEMs/$ZnSO_4$	PEMs	PEMs/$ZnSO_4$
1	8	0	1.5	2.1
2	22	2	1.2	1.88
3	2	0	1.66	2.21
4	5	2	1.55	1.95
5	25	9	1.12	1.6
6	17	3	1.4	1.85
7	6	0	1.52	2.18
8	10	2	1.49	1.89
9	20	5	1.36	1.74
10	11	1	1.51	1.98
11	19	4	1.3	1.7
12	7	1	1.48	1.9

PEMs was significantly minimized with zinc sulfate supplementation. Zinc plays an important role in children growth. The association of zinc deficiency with growth retardation and hypogonadism was first described in 1963 from Iran [76] and is now well established from animal and human studies, demonstrating a critical role of this micronutrient in cellular growth, cellular differentiation, and metabolism [77]. Despite it is known that growth retardation, immune dysfunctions, and cognitive impairment are major effects of zinc deficiency [78] also is recognized that these effects are reversible by zinc supplementation [78–80]. The function of Zn involves a wide range of biological processes including cell proliferation, immune function, and defense against free radicals [27–32]. In addition, Zn appears to regulate key physiological processes such as cellular response to oxidative stress, DNA repair, cell cycle regulation, and apoptosis [29, 30, 33–48]. The role of zinc in modulating oxidative stress has been recognized [13]. Most of the beneficial effects displayed by zinc might be related to its action as an "hormetin", i.e., by the induction of a mild pro-oxidant activity that improve the anti-oxidative defensive responses leading ultimately to an improved capacity of the cell to respond to additional stressors or harmful agents [81]. In this sense, Sliwinski and coworkers conducted a study in normal human lymphocytes and human myelogenous leukemia K562 cancer cells treated with zinc sulfate and hydrogen peroxide; they analyzed the induction of cyto- and genotoxicity and reported that zinc may protect normal cells against DNA-damaging action of hydrogen peroxide and on the contrary amplify genetic damage induced by this agent in cancer cells [82].

The development or identification of nutrient-dense foods and ingredients that are rich in micronutrients required for DNA replication and repair and for the prevention of genome-damaging events is essential for individuals to achieve their daily nutrient requirements for genome health maintenance [15]. At present day, there is an international increasing interest to redefine recommended dietary intakes (RDIs) of minerals and vitamins to prevent developmental abnormalities and degenerative diseases of old age as well as optimizing cognition [14]. In this sense, prevention of DNA damage is an important parameter for the definition of new dietary reference values because increased loss of genome integrity has been repeatedly demonstrated to be prospectively associated with increasing risk of degenerative diseases [14, 15]. In this context, our results become important if we consider that all participants in the present study showed blood zinc levels within the normal physiological range established for children.

We conclude that, in our study, cytotoxic effects and chromosomal damage observed in children with PEM can be repaired in vitro with zinc sulfate supplementation. Moreover, the normal physiological range for this important micronutrient must be revised for DNA damage prevention. The strength of this work is, as far as the authors know, to be the first in which the role of the in vitro zinc sulfate supplementation of peripheral blood from children suffering from PEM is evaluated. Future investigations in this field should focus on

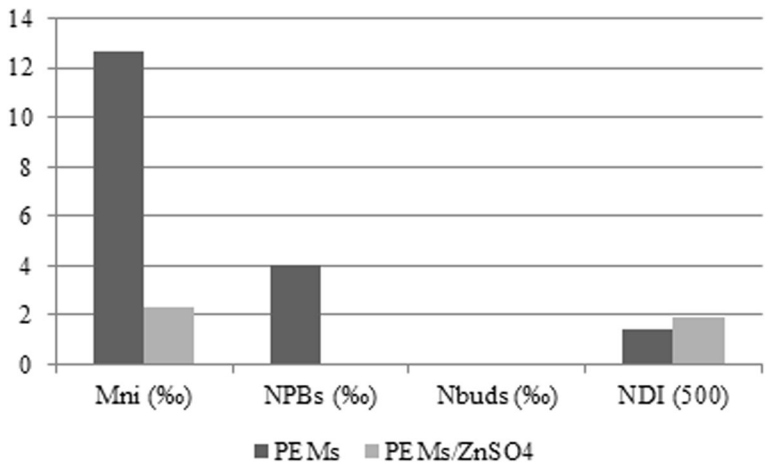

Fig. 2 Frequency of DNA-damage endpoints (MNi, NPBs, and NBuds) and NDI

elucidating the underlying mechanisms of the nutritional modulation of DNA repair, studying redox, as well as epigenetic regulation of DNA repair pathways and changes in gene expression. In addition, further studies should analyze the protective genotoxic effect of Zn in children with severe malnutrition and compare MNi, NPBs, and NDI frequencies, in healthy, well-nourished children.

Acknowledgments The authors are thankful to all participants and their parents. This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) [PIP Nro. 0187].

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Dulger H, Arik M, Sekeroglu MR, Tarakcioglu M, Noyan T, Cesur Y (2002) Pro-inflammatory cytokines in Turkish children with protein-energy malnutrition. Mediat Inflamm 11:363–365
2. Sereebutra P, Solomons N, Aliyu MH, Jolly PE (2006) Sociodemographic and environmental predictors of childhood stunting in rural Guatemala. Nutr Res 26:65–70
3. Shils ME, Olson JA, Moshe S (1999) Modern nutrition in health and disease. Lippincott Williams & Wilkins, Philadelphia
4. Gray VB, Cossman JS, Powers EL (2006) Stunted growth is associated with physical indicators of malnutrition but not food insecurity among rural school children in Honduras. Nutr Res 26(11):549–555
5. El-Hodhod MAA, Nassara MF, Zakib MM, Moustafaa A (2005) Apoptotic changes in lymphocytes of protein energy malnutrition patients. Nutr Res 25:21–29
6. Vásquez-Garibay E, Méndez-Estrada C, Romero-Velarde E, Vizmanos B, Campollo-Rivas O (2005) Impact of nucleotides and nutritional support on growth and body composition of severely malnourished infants. Nutr Res 25(8):727–736
7. Makonnen B, Venter A, Joubert GA (2003) Randomized controlled study of the impact of dietary zinc supplementation in the management of children with protein-energy malnutrition in Lesotho. I: mortality and morbidity. J Trop Pediatr 49(6):340–352
8. Amesty-Valbuena A, Pereira-Medero N, Núñez-González JR, García D, de Villaroel MV, Granadillo V (2006) Serum levels of Zn in children with different degrees of nutritional deficiency. Investig Clin 47(4):349–359
9. de Fátima Costa Caminha M, da Silva DA, Falbo AR, de Arruda IK, Serva VB, de Albuquerque LL (2008) Serum retinol concentrations in hospitalized severe protein-energy malnourished children. J Trop Pediatr 54(4):248–252
10. Padula G, Seoane AI (2008) Chromosomal effects of infections in malnourished and eutrophic children of Gran La Plata. J Basic Appl Genet 19(1):15–20
11. Ames BN (2001) DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutat Res 475:7–20
12. Fenech M, Ferguson LR (2001) Vitamins/minerals and genomic stability in humans. Mutat Res 475:1–6
13. Sharif R, Thomas P, Zalewski P, Fenech M (2012) The role of zinc in genomic stability. Mutat Res 733:111–121
14. Fenech MF (2014) Nutriomes and personalised nutrition for DNA damage prevention, telomere integrity maintenance and cancer growth control. Cancer Treat Res 159:427–441
15. Fenech MF (2010) Dietary reference values of individual micronutrients and nutriomes for genome damage prevention: current status and a road map to the future. Am J Clin Nutr 91:1438S
16. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB, Ames BN (1997) Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci 94:3290
17. Ames BN (1998) Micronutrients prevent cancer and delay aging. Toxicol Lett 5:102–103
18. Prise KM, Ahnstrom G, Belli M, Carlsson J, Frankenberg D, Kiefer J, Lobrich M, Michael BD, Nygren J, Simone G (1998) A review of DsB induction data for varying quality radiations. Int J Radiat Biol 74:173
19. Ames BN, Wakimoto P (2002) Are vitamin and mineral deficiencies a major cancer risk? Nat Rev Cancer 2:694
20. Fenech M, Crott JW (2002) Micronuclei, nucleoplasmic bridges and nuclear buds induced in folic acid deficient human lymphocytes-evidence for breakage-fusion-bridge cycles in the cytokinesis-block micronucleus assay. Mutat Res 504:131
21. Courtemanche C, Huang AC, Elson-Schwab I, Kerry N, Ng BY, Ames BN (2004) Folate deficiency and ionizing radiation cause DNA breaks in primary human lymphocytes: a comparison. FASEB J 18:209
22. Padula G, Salceda SA, Seoane AI (2009) Protein-energy malnutrition contributes to increase structural chromosomal alterations frequencies in Argentinean children: a case-control study. Nutr Res 29(1): 35–40
23. Padula G, Seoane AI (2011) Genotoxic effects of malnutrition and infections in Argentinean children. In: Malnutrition: risk factors, health effects and prevention. Nova Science Publishers Inc, New York, pp 91–100
24. Armendares S, Salamanca F, Frenk S (1971) Chromosome abnormalities in severe protein calorie malnutrition. Nature 232:271–273
25. Terreros MC, De Luca JC, Dulout FN (1993) The effect of hypoproteic diet and ethanol consumption on the yield of chromosomal damage detected in bone marrow cells of mice. J Vet Med Sci 55:191–194
26. Thorburn MJ, Hutchinson S, Alleyne GA (1972) Chromosome abnormalities in malnourished children. Lancet 1:591
27. Dreosti IE (2001) Zinc and the gene. Mutat Res 475:161–167
28. Ho E (2004) Zinc deficiency, DNA damage and cancer risk. J Nutr Biochem 15:572–578
29. Bray TM, Bettger WJ (1990) The physiological role of zinc as an antioxidant. Free Radic Biol Med 8:281–291
30. Falchuk KH (1998) The molecular basis for the role of zinc in developmental biology. Mol Cell Biochem 188:41–48
31. Powell SR (2000) The antioxidant properties of zinc. J Nutr 130: 1447S–1454S
32. Cousins RJ, Blanchard RK, Moore JB, Cui L, Green CL, Liuzzi JP, Cao JA, Bobo JA (2003) Regulation of zinc metabolism and genomic outcomes. J Nutr 133:1521S–1526S
33. Richard MJ, Guiraud MT, Leccia MT, Beani JC, Favier A (1993) Effect of zinc supplementation on resistance of cultured human skin fibroblasts towards oxidant stress. Biol Trace Elem Res 37:187–199
34. McGowan A, Fernandes R, Verhaegen S, Cotter T (1994) Zinc inhibits UV radiation-induced apoptosis but fails to prevent subsequent cell death. Int J Radiat Biol 66:343–349
35. Sunderman FW Jr (1995) The influence of zinc on apoptosis. Ann Clin Lab Sci 25:134–142
36. Bagchi D, Bagchi M, Stohs SJ (1997) Comparative *in vitro* oxygen radical scavenging ability of zinc methionine and selected zinc salts and antioxidants. Gen Pharmacol 28(1):85–91
37. Parat MO, Richard MJ, Pollet S (1997) Zinc and DNA fragmentation in keratinocyte apoptosis: its inhibitory effect in UVB irradiated cells. J Photochem Photobiol 37:101–106

38. Urrutia R (1997) Exploring the role of the homeobox and zinc finger proteins in pancreatic cell proliferation, differentiation and apoptosis. Int J Pancreatol 22:1–14
39. Favier A (1988) Is zinc a cellular mediator in the regulation of apoptosis. In: Bratter P, de Bratter VN, Khasanova L, Etienne JC (eds) Metal ions in biology and medicine. John Libbey Eurotext, Paris, pp 164–167
40. Lin YL, Shivji MK, Chen C (1998) The evolutionarily conserved zinc finger motif in the largest subunit of human replication protein A is required for DNA replication and mismatch repair but not for nucleotide excision repair. J Biol Chem 273:1453–1461
41. Rosado JL (1998) Deficiencia de zinc y sus implicaciones funcionales. Salud Publica Mex 40:181–188
42. Leccia MT, Richard MJ, Favier A (1999) Zinc protects against ultraviolet A1-induced DNA damage and apoptosis in culture human fibroblast. Biol Trace Elem Res 69(3):177–190
43. Szuster-Ciesielska A, Stachura A, Slotwińska M, Kamińska T, Śniezko R, Paduch R, Abramczyk D, Filar J, Kandefer-Szerszen M (2000) The inhibitory effect of zinc on cadmium-induced cell apoptosis and reactive oxygen species (ROS) production in cell cultures. Toxicology 145:159–171
44. Bae SN, Lee YS, Kim MY, Kim JD, Park LO (2006) Antiproliferative and apoptotic effects of zinc-citrate compound (CIZAR(R)) on human epithelial ovarian cancer cell line, OVCAR-3. Gynecol Oncol 103:127–136
45. Chimienti F, Jourdan E, Favier A, Seve M (2001) Zinc resistance impairs sensitivity to oxidative stress in HeLa cells: protection through metallothioneins expression. Free Radic Biol Med 31: 1179–1190
46. Chung MJ, Walker PA, Brown RWC, Hogstrand C (2005) ZINC-mediated gene expression offers protection against H_2O_2-induced cytotoxicity. Toxicol Appl Pharmacol 205:225–236
47. Clegg MS, Hanna LA, Niles BJ, Momma TY, Keen CL (2005) Zinc deficiency induced cell death. IUBMB Life 57:661–669
48. Chang KL, Hung TC, Hsieh BS, Chen YH, Chen TF, Cheng HL (2006) Zinc at pharmacologic concentrations affects cytokine expression and induces apoptosis of human peripheral blood mononuclear cells. Nutrition 22:465–474
49. Fenech M (2007) Cytokinesis-block micronucleus cytome assay. Nat Protoc 2(5):1084–1104
50. Fenech M, Kirsch-Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, Norppa H, Eastmond DA, Tucker JD, Thomas P (2011) Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis 26:125–132
51. Xu B, Sun Z, Liu Z, Guo H, Liu Q, Jiang H, Zou Y, Gong Y, Tischfield JA, Shao C (2011) Replication stress induces micronuclei comprising of aggregated DNA double-strand breaks. PLoS One 6: e18618
52. Galvano F, Piva A, Ritieni A, Galvano G (2001) Dietary strategies to counteract the effects of mycotoxins: a review. J Food Prot 64(1): 120–131
53. Astley SB, Elliott RM, Archer DB, Southon S (2002) Increased cellular carotenoid levels reduce the persistence of DNA single strand breaks after oxidative challenge. Nutr Cancer 43:202–213
54. Song Y, Chung CS, Bruno RS, Traber MG, Brown KH, King JC, Ho E (2009) Dietary zinc restriction and repletion affects DNA integrity in healthy men. Am J Clin Nutr 90:321–328
55. Ramos AA, Azqueta A, Pereira-Wilson C, Collins AR (2010) Polyphenolic compounds from *Salvia* species protect cellular DNA from oxidation and stimulate DNA repair in cultured human cells. J Agric Food Chem 58:7465–7471
56. Ramos AA, Pereira-Wilson C, Collins AR (2010) Protective effects of ursolic acid and luteolin against oxidative DNA damage include enhancement of DNA repair in Caco-2 cells. Mutat Res 692:6–11
57. Collins AR, Azqueta A, Sabine A, Langie S (2012) Effects of micronutrients on DNA repair. Eur J Nutr 51:261–279
58. Åsgård R, Hellman B (2013) Effect of β-carotene on catechol-induced genotoxicity in vitro: evidence of both enhanced and reduced DNA damage. Free Radic Res 47(9):692–698
59. WHO ANTHRO (2005) Software for assessing growth and development of the world's children. Geneva: WHO; available at http://www.who.int/childgrowth/software/en/
60. Torun B, Chew F (1994) Protein energy malnutrition. In: Shill ME, Olson JA, Shike M (eds) Modern nutrition in health and disease. Lea and Febiger
61. UNICEF/UNU/WHO (2001) Iron deficiency anaemia assessment. Prevention and control. A guide for programme managers
62. Food and Nutrition Board, Institute of Medicine: Dietary Reference Intakes (1998) Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. A report of the Standing Committee on the Scientific Evaluation of Dietary References Intakes and its Panel on Folate, Other B Vitamins and Choline and Subcommittee on Upper Reference Levels of Nutrients. National Academy Press, Washington DC
63. Sommer A, Davidson FR (2002) Annecy Accords. Assessment and control of vitamin A deficiency: the Annecy Accords. J Nutr 132(9): 2845S–2850S
64. International Zinc Nutrition Consultative Group (IZiNCG) (2007) Assessing population zinc status with serum zinc concentration. IZiNCG Technical Brief No. 2. Davis CA
65. Epi Info. CDC/OMS (2001) Procesador de texto, Base de datos y Estadística, para la Salud Pública. Versión 6.04d
66. Natarajan AT, Obe G, Hayashi M, Ikushima T (2002) Chromosomal aberrations: perspectives for the 21st century. Mutat Res 504:1–2
67. González C, Nájera O, Cortés E, Toledo G, López L, Betancourt M (2002) Susceptibility to DNA damage induced by antibiotics in lymphocytes from malnourished children. Teratog Carcinog Mutagen 22:147–158
68. Natarajan AT (2002) Chromosome aberrations: past, present and future. Mutat Res 504:3–16
69. Memduf A, Handan A, Ugur D, Srtkr O, Mevlirt I, Dursun Kaya M (1996) Chromosome abnormalities in protein-energy malnutrition. Nutr Res 16(9):1487–1494
70. Cervantes-Ríos E, Ortiz-Muñiz R, Martínez-Hernández AL, Cabrera-Rojo L, Graniel-Guerrero J, Rodríguez-Cruz L (2012) Malnutrition and infection influence the peripheral blood reticulocyte micronuclei frequency in children. Mutat Res 731(1–2):68–74
71. Celik M, Sermatov K, Abuhandan M, Zeyrek D, Kocyigit A, Iscan A (2012) Oxidative status and DNA damage in chidren with marasmic malnutrition. J Clin Lab Anal 26(3):161–166
72. Alu V, Murthy PB (1993) Chromosomal abnormalities in starved and marginally malnourished rats and in utero upon rehabilitation. Experientia 49:258–262
73. Gupta R, Gupta M, Ramdeo IN (1977) Chromosomal abnormalities in protein-calorie malnutrition. Am J Clin Nutr 30(12):1974–1978
74. Kinding DEF, Chrisman CL, Rogler JC (1987) Cytogenetic analysis of oocytes in mice with protein or energy malnutrition. Nutr Rep Int 35:1261–1268
75. Cortés E, González C, Betancourt M, Ortíz R (2001) Assessment of DNA damage in spleen, bone marrow, and peripheral blood from malnourished rats by single cell gel electrophoresis assay. Teratog Carcinog Mutagen 21:231–247
76. Moynahan EJ (1974) Letter: acrodermatitis enteropathica: a lethal inherited human zinc-deficiency disorder. Lancet 2(7877): 399–400
77. Prasad AS (1991) Discovery of human zinc deficiency and studies in an experimental human model. Am J Clin Nutr 53(2):403–412
78. Prasad AS, Bao B, Beck FWJ, Kucuk O, Sarkar FH (2004) Antioxidant effect of zinc in humans. Free Radic Biol Med 37(8): 1182–1190

79. Brown KH, Peerson JM, Baker SK, Hess SY (2009) Preventive zinc supplementation among infants, preschoolers, and older prepubertal children. Food Nutr Bull 30(1):S12–S40
80. Imdad A, Bhutta ZA (2011) Effect of preventive zinc supplementation on linear growth in children under 5 years of age in developing countries: a meta-analysis of studies for input to the lives saved tool. BMC Public Health 11(3):S22
81. Mocchegiani E, Costarelli L, Giacconi R, Piacenza F, Basso A, Malavolta M (2011) Zinc, metallothioneins and immunosenescence: effect of zinc supply as nutrigenomic approach. Biogerontology 12: 455–465
82. Sliwinski T, Czechowska A, Kolodziejczak M, Jajte J, Wisniewska-Jarosinska M, Blasiak J (2009) Zinc salts differentially modulate DNA damage in normal and cancer cells. Cell Biol Int 33(4):542–547