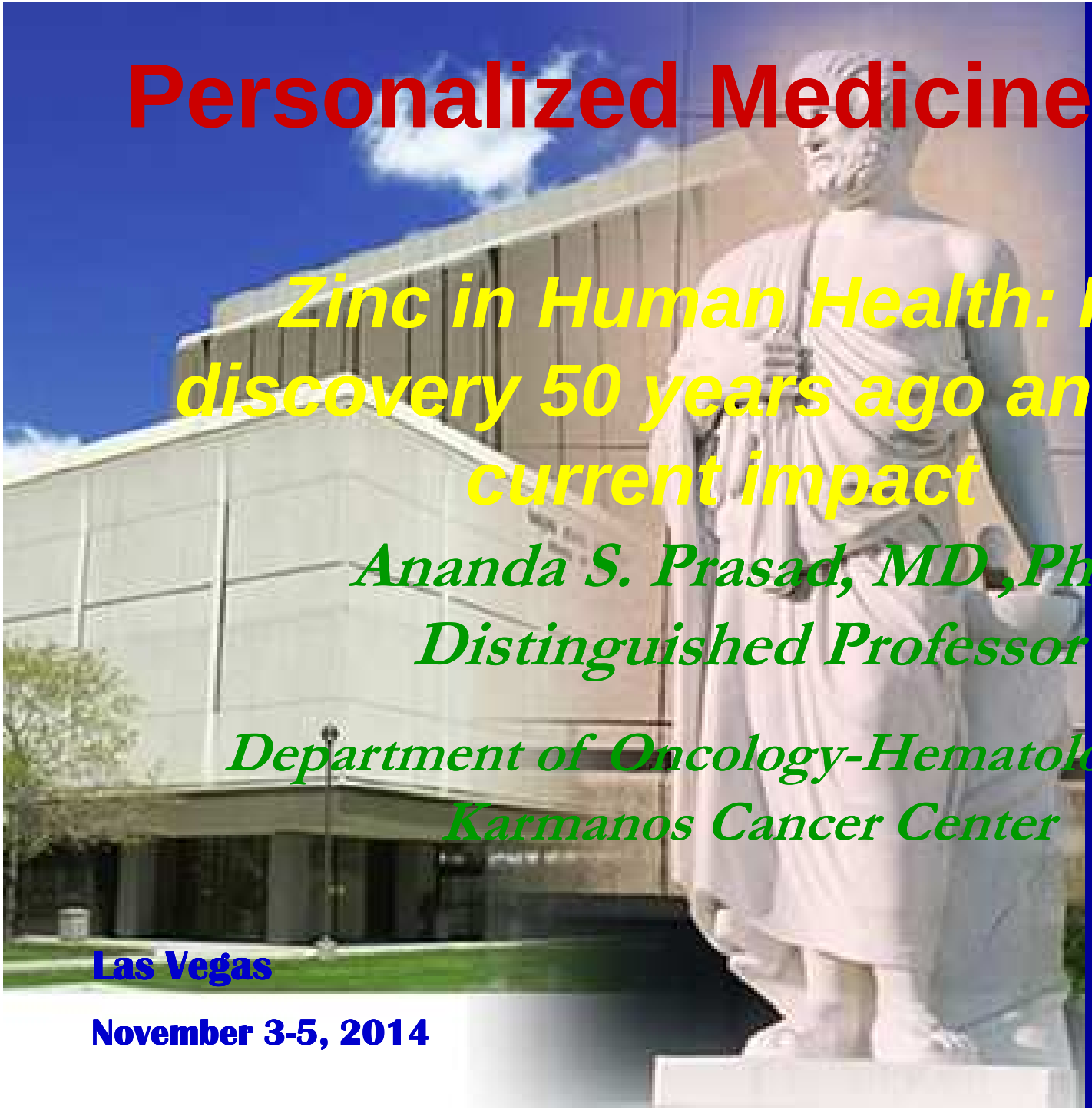
Personalized Medicine 2014



Zinc in Human Health: Its discovery 50 years ago and the current impact

Ananda S. Prasad, MD ,PhD

Distinguished Professor

Department of Oncology-Hematology and Karmanos Cancer Center

WAYNE STATE UNIVERSITY SCHOOL OF MEDICINE

Las Vegas

November 3-5, 2014

Zinc Deficiency in Microorganisms, Plants and Animals

1. RAULIN in 1869 - Aspergillus niger
2. SOMMER and LIPMAN in 1926 - Higher Plant Life
3. TODD, ELVEHJEM & HART in 1934 - Rats
4. TUCKER and SALMON in 1955 - Pigs
5. O'DELL and SAVAGE in 1958 - Poultry
6. Prasad et al in 1963 - Man


Prasad, A. S., Zinc Metabolism,
C. C. Thomas, Springfield, Ill.,1966

Cited in Nutrition Classics, Nutrition Review, July 1983

NUTRITION CLASSICS

THE
AMERICAN JOURNAL
OF MEDICINE

Volume 31, 1961
Pages 532-546

Syndrome of Iron Deficiency Anemia, Hepatosplenomegaly, Hypogonadism, Dwarfism and Geophagia

ANANDA S. PRASAD, M.D., JAMES A. HALSTED, M.D. and MANUCHER NADIMI, M.D.
Shiraz, Iran



Zinc-Deficient Farm Boy



LANDMARK ARTICLE

The Journal of
LABORATORY and CLINICAL MEDICINE

Volume 61 Number 4 APRIL 1963

Clinical and experimental

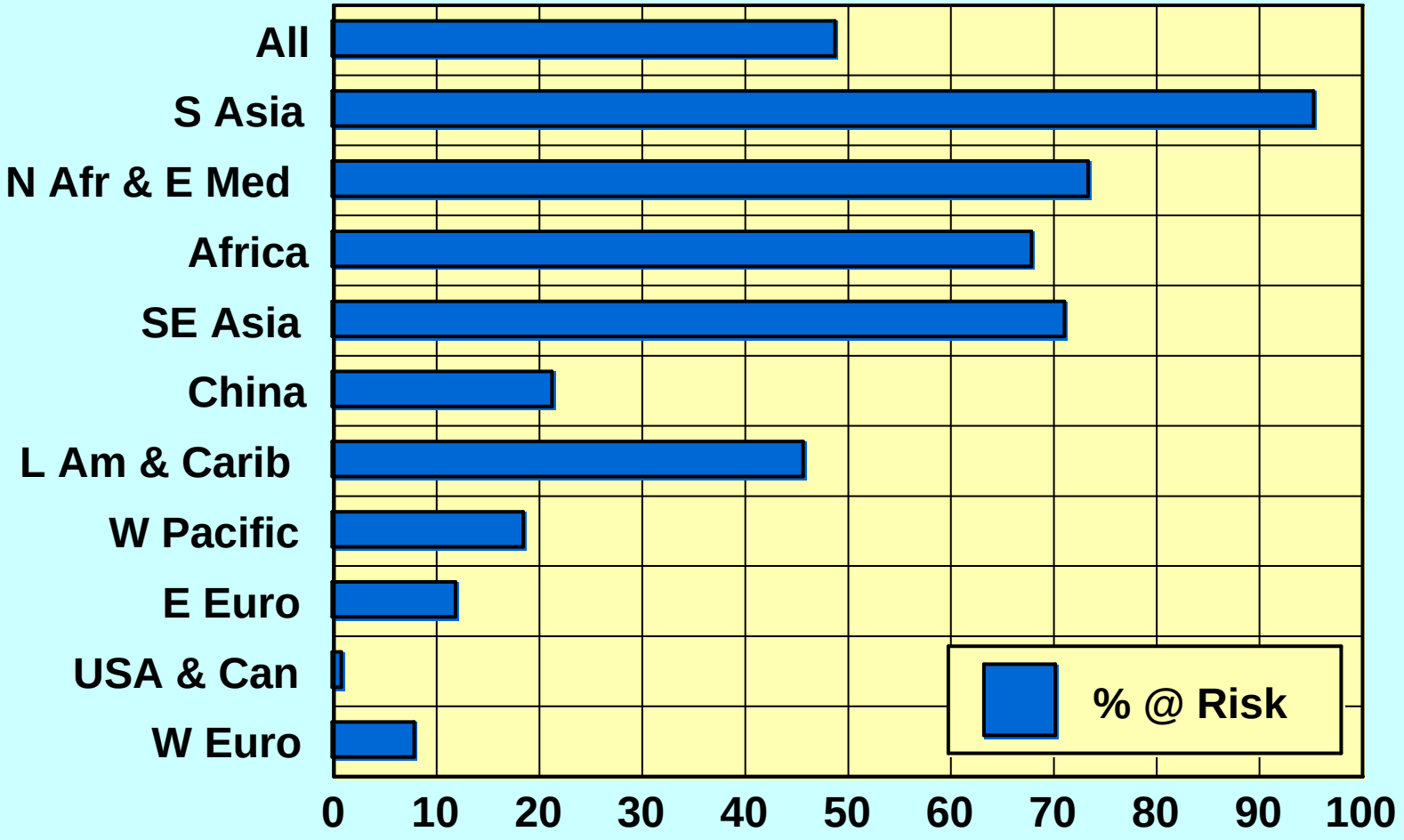
Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypogonadism

ANANDA S. PRASAD, AUGUST MIALE, JR., Z. FARID, H. H. SANDSTEAD, and ARTHUR R. SCHULERT *Cairo, U. A. R.*

CLINICAL MANIFESTATIONS IN ZINC DEFICIENT DWARFS

1. Growth Retardation
2. Hypogonadism in Males
3. Rough Skin
4. Poor Appetite
5. Mental Lethargy
6. Intercurrent Infections

Estimated Percentage of Population at Risk of Zinc Deficiency



Brown, et al Food Nutr Bull 2001;22:113-25

Prevalence of ZINC DEFICIENCY

Nearly two billion subjects in the developing world have nutritional deficiency of zinc. In the developing world the diet consists of mainly cereal proteins with high phytate content which complexes zinc and decreases its availability.

Conditioned deficiency of zinc is also widely prevalent throughout the world. Zinc deficiency has been reported in patients with liver disease, chronic alcoholism, malabsorption syndrome, chronic renal disease, and other chronic diseases including malignancy.

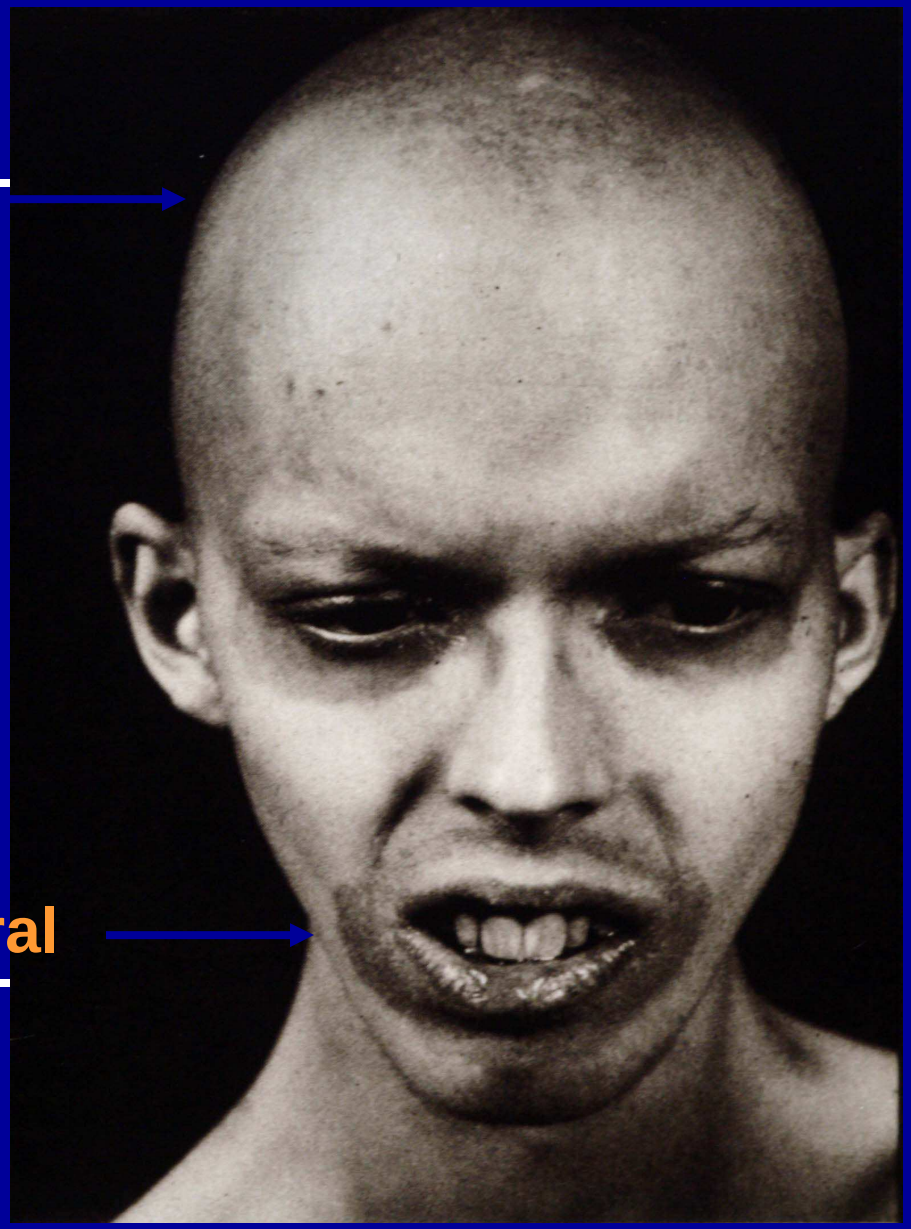
ZINC: Advances in Basic Sciences during the past 50 years

Fifty years ago, we knew of only three enzymes which required zinc. Today we know that nearly 10% of human proteins have zinc binding motifs, including nearly 1000 enzymes and over 1000 transcription factors.

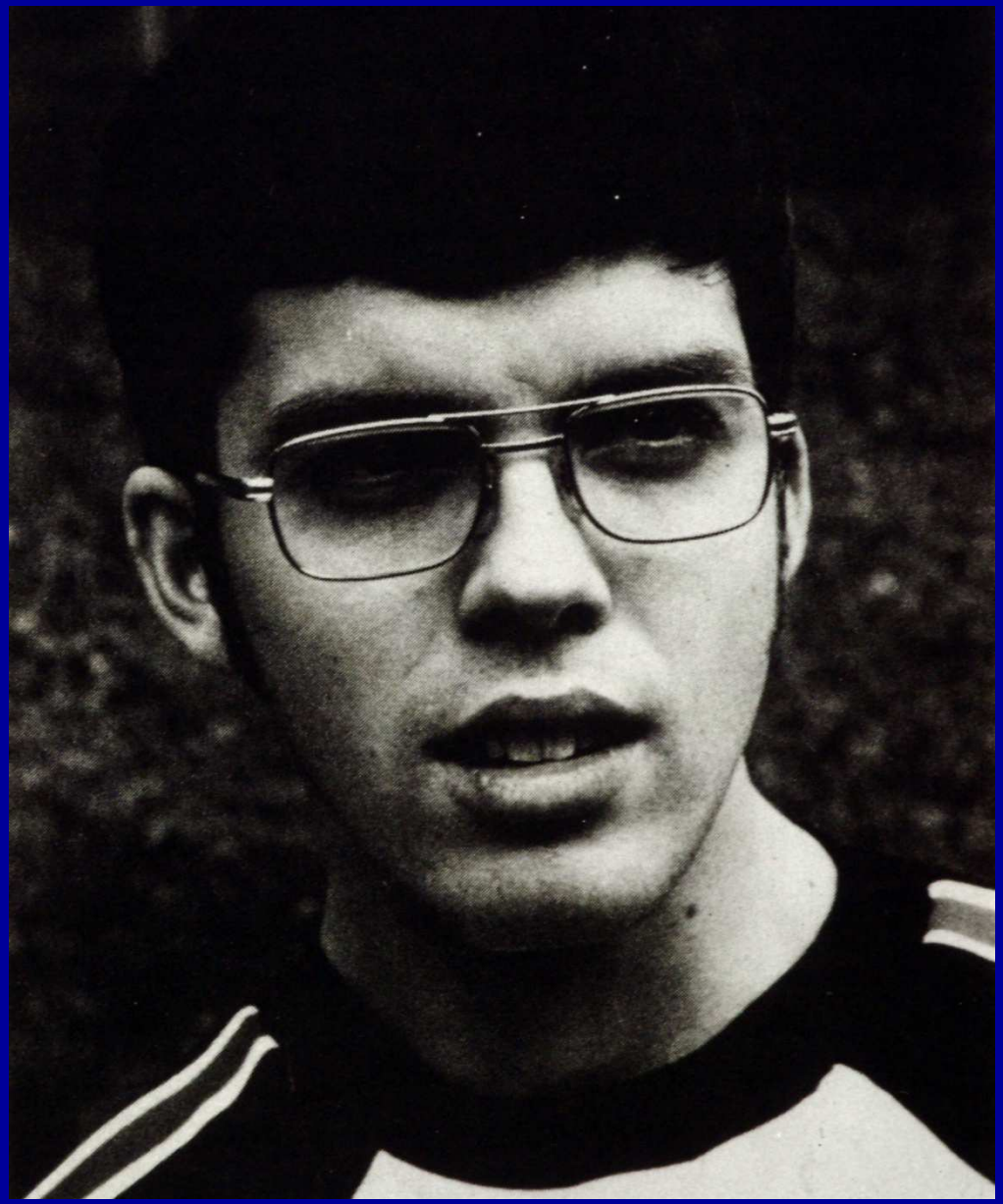
Intra cellular Zn homeostasis is maintained tightly by 14 ZIP and 10 ZNT transporters.

Most importantly we now know that Zn functions as a molecular signal.

Patient With Severe Zinc Deficiency



Photograph of Patient After Zinc Therapy



“ Mild” Deficiency of Zinc

Manifestations:

- Neuro-sensory changes
- Decreased serum testosterone
- Oligospermia
- Hyperammonemia
- Anergy
- Decreased NK activity
- Decreased production of IL-2
- Decreased thymulin activity
- Impaired cognitive functions

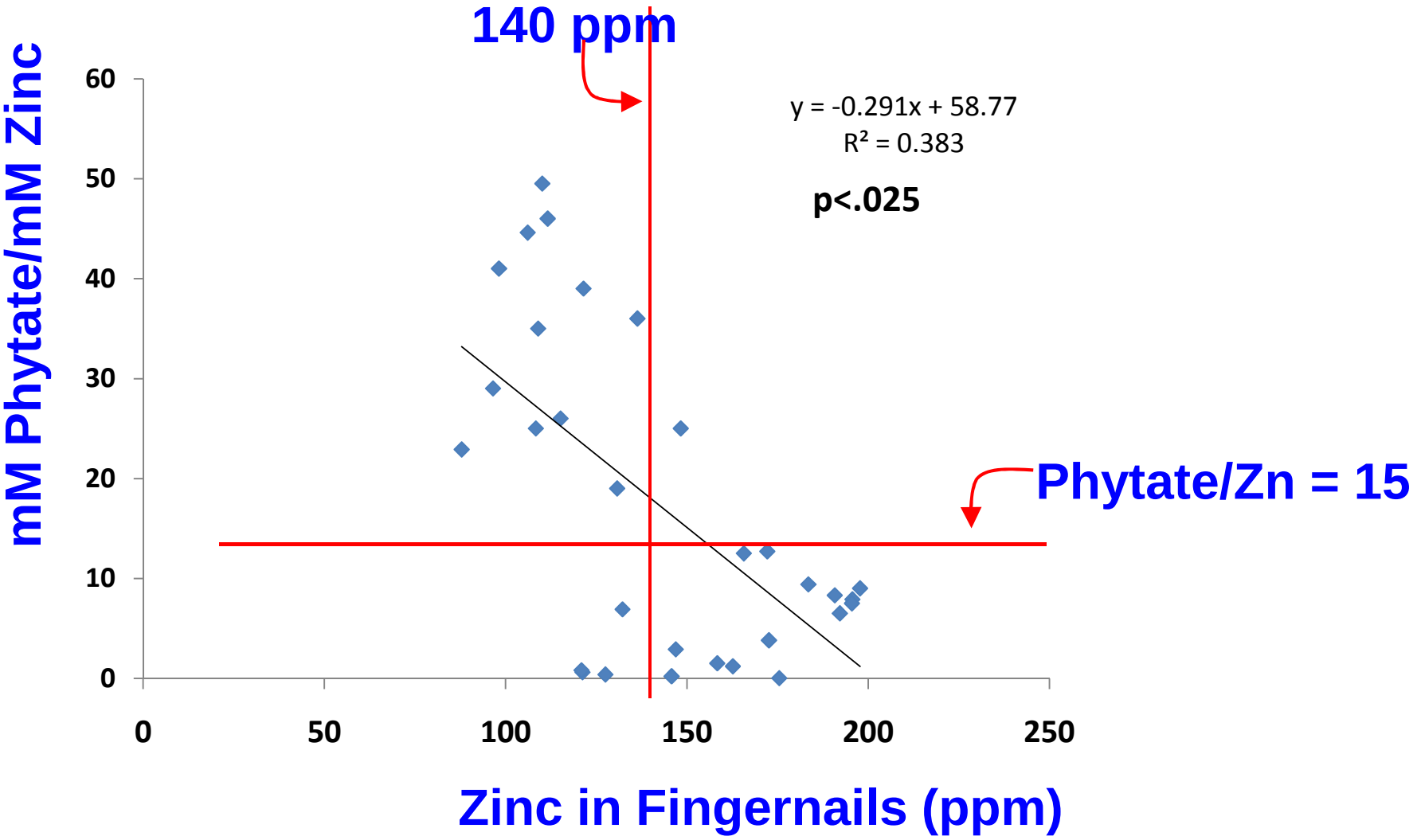
Observed in:

- Experimental human model studies.
- Elderly
- Pre-menopausal women

Bio-markers of Zinc Deficiency

1. Serum thymulin activity
2. Generated IL-2 mRNA and IL-2 cytokine from mononuclear cells
3. Lymphocte ecto 5’ Nucleotidase
4. Zinc in Lymphocytes and Granulocytes
5. Alkaline phosphatase in granulocytes
6. Intestinal Absorption of zinc
7. For chronic zinc status, assay of nail zinc by LIBS technique

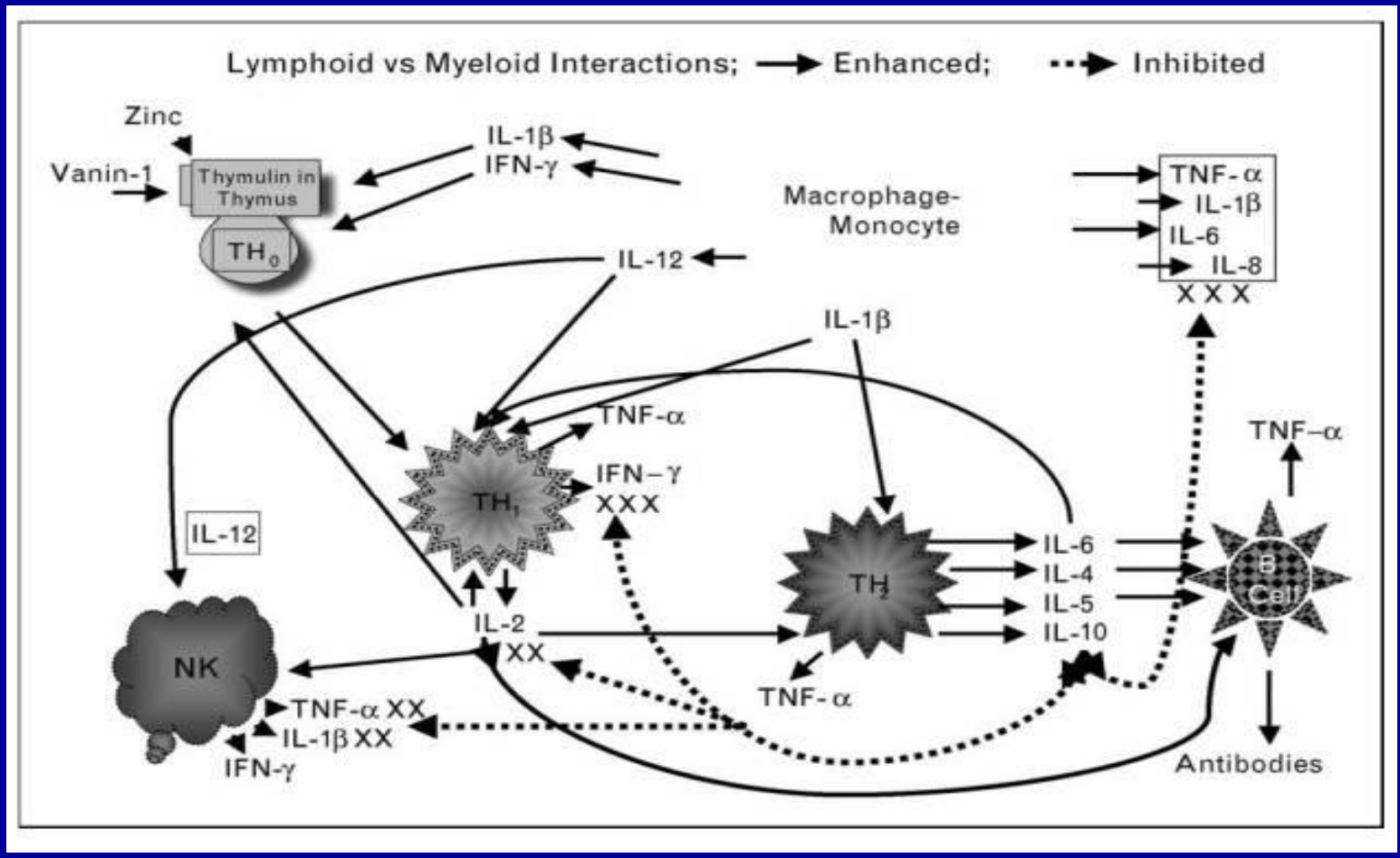
Nail Zn and dietary Phytate/Zn in healthy elderly subjects from Detroit



Nail Zinc phytate/zinc ratio	<140 PPM	>140 PPM
> 15	12	1
<15	3	12

N= 28
P < .001

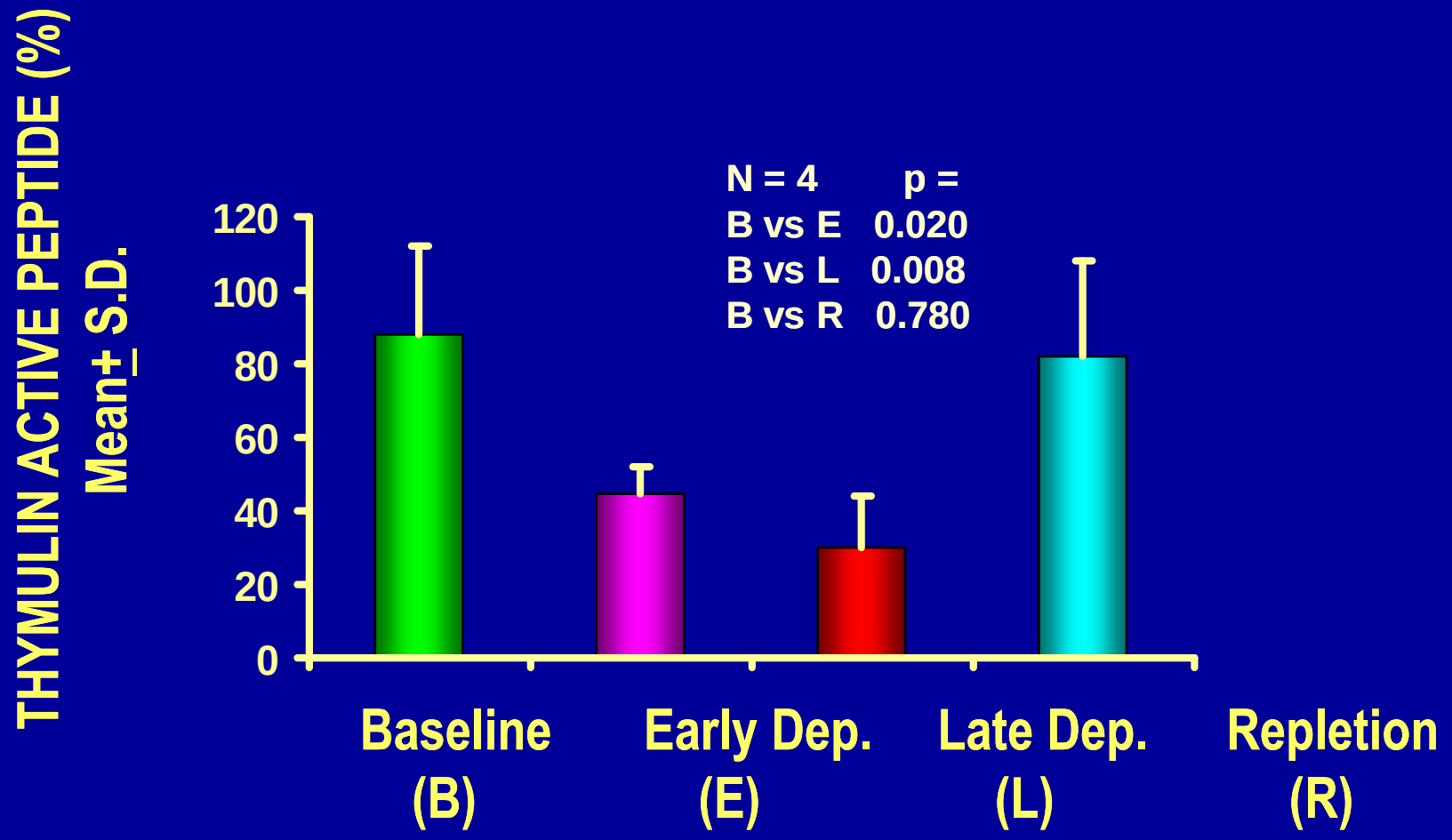
Effect of Zinc on Cell Mediated Immunity



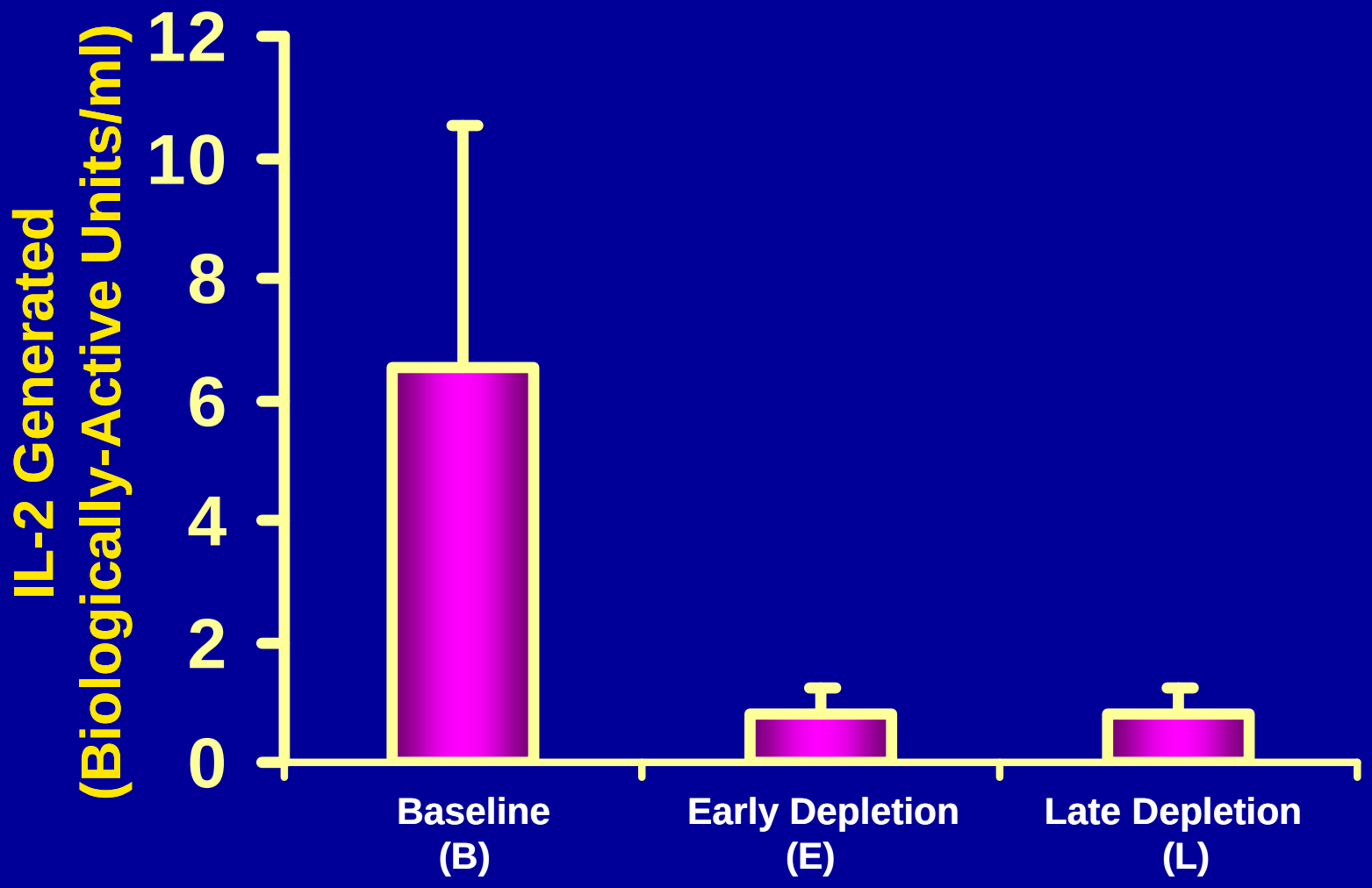
This figure shows the landscape of zinc effect on immune cells. Solid lines indicate pathways leading to generation of selected cytokines and dotted lines represent pathways, which lead to inhibition of cytokine generation. NK represents natural killer cells; Th1 represent activated Th1 type T-cells and secreted cytokines (small triangles). Th2 represent activated Th2 type T-cells and secreted cytokines (small rounds); B-cell represents B-cell lineages and associated immunoglobulin (triangles). The major adverse effects of zinc deficiency were on Thymulin, and Th1 functions. Zinc deficiency increased oxidative stress resulting in activation of monocytes-macrophages and generation of inflammatory cytokines. Th2 functions were not affected due to zinc deficiency.

Prasad AS. 2009. Zinc: role in immunity,oxidative stress and chronic inflammation. Current Opinion in Clin Nutr and Metab Care. 12:646-652.

Thymulin Active Peptide in Human Volunteers During Baseline (B), Early Zinc Depletion (E), Late Zinc Depletion (L) and Zinc Repletion (R)



Reduction in IL-2 Generated *Ex Vivo* due to Experimentally Induced Zinc Deficiency In Healthy Young Human Volunteers



Mean ± S.D. ; n= 5 Subjects

Therapeutic Impact of the Discovery of Essentiality of Zinc in Human

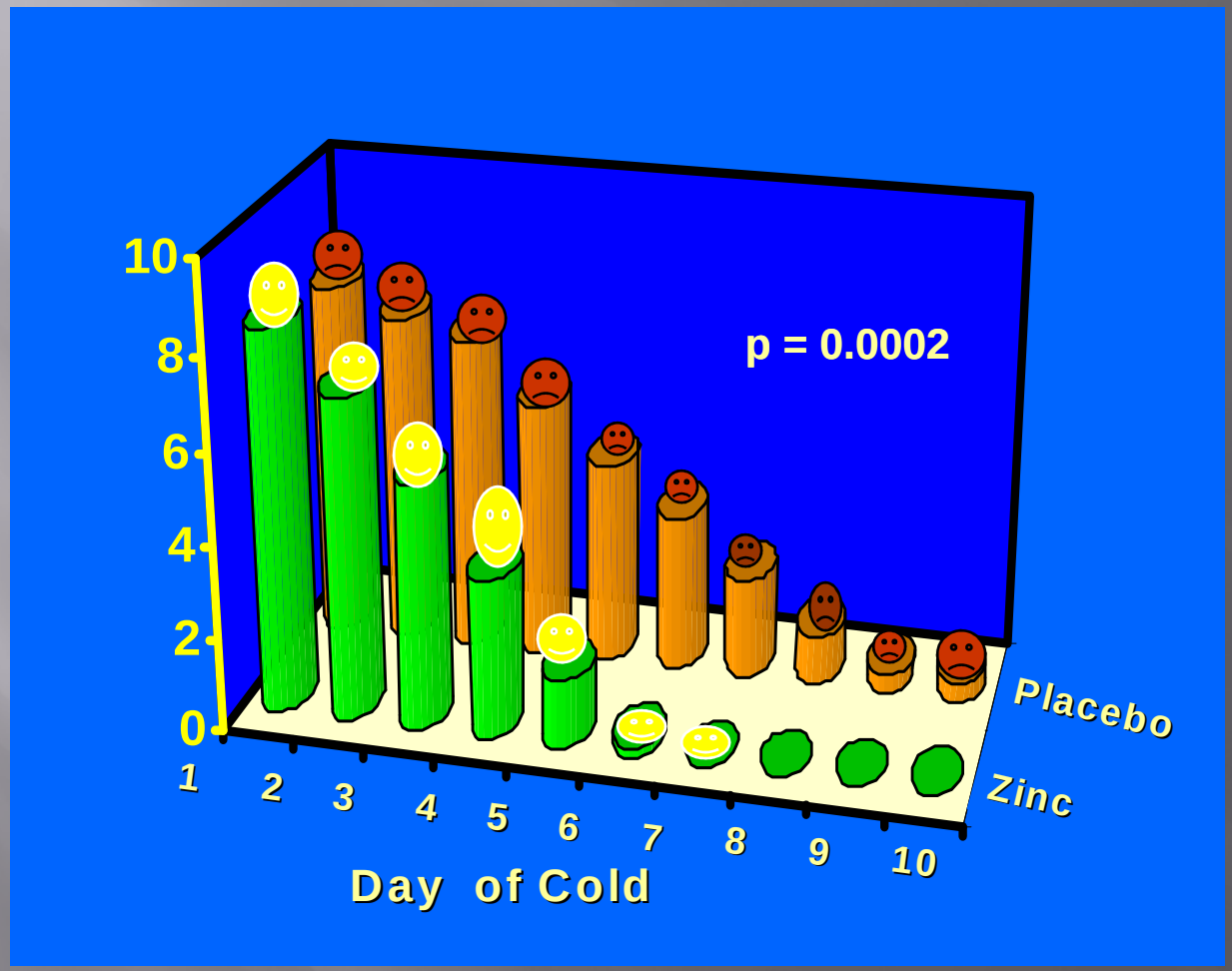
1. In infants and children in developing countries with acute diarrhea and respiratory tract infections
2. Wilson's Disease
3. Common Cold
4. Sickle Cell Disease
5. Prevention of blindness due to age related macular degeneration (AMD)
6. Acyzol for Co poisoning (*Russian Study*)
7. Decreased incidence of infections in the elderly

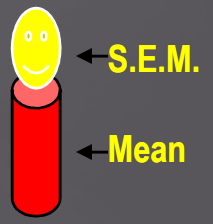
Evidence that Zinc Acetate Lozenges are Effective for Treatment of Cold Duration and Cold Symptoms

Duration (Days) of Symptoms of the Common Cold

Variable	Zinc Supplemented Group (n=25)	Placebo Supplemented Group (n=25)	p value
Overall Symptoms	4.00±1.04[a] (3.57 to 4.42)[b]	7.12±1.26 (6.59 to 7.64)	<0.0001
Specific Symptoms:			
Sore Throat	1.96±1.83 (1.2 to 2.71)	3.24±2.93 (2.02 to 4.45)	0.07
Nasal Discharge	3.00±1.63 (2.32 to 3.67)	4.56±3.01 (3.31 to 5.80)	0.02
Cough	2.16±1.70 (1.45 to 2.86)	5.08±2.97 (3.85 to 6.30)	<0.0001
Hoarseness	1.00±1.44 (0.40 to 1.59)	2.20±2.90 (1.00 to 3.39)	0.07
Muscle Ache	0.80±1.22 (0.29 to 1.30)	2.00±2.25 (1.06 to 2.93)	0.02
Fever	0.52±1.35 (0.04 to 1.05)	1.12±2.00 (0.29 to 1.94)	0.22

Group Mean Symptom Scores Logged During Treatment





Zinc treatment was significantly effective in reducing plasma levels of sICAM-1

Plasma sICAM-1 and ICAM-1 on the cell surfaces function as receptors for the HRV14 rhinovirus. Decreased presence of this “rhinovirus docking” receptor lessens the opportunity for rhinovirus attachment to cells.

Cochrane Review: Sickle Cell Disease (SCD) and Zinc

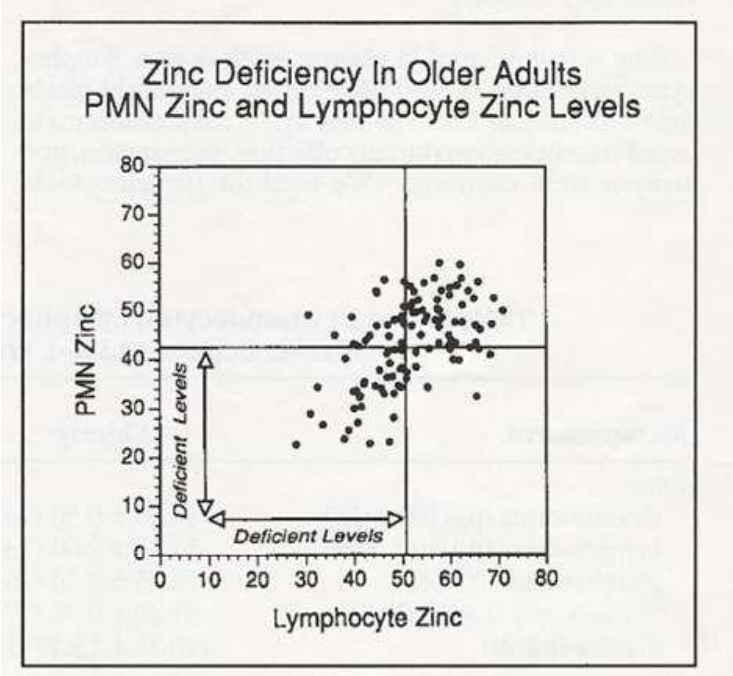
Evidence of benefit is seen with the reduction in the number of sickle cell pain crises among sickle cell patients who received one year of zinc supplementation and with the reduction in the total number of clinical infections among sickle cell patients who received zinc supplementation for both three months and for one year.

Currently there are limited treatment options for patients with SCD to prevent infection and painful crises, the two most difficult complications of this disease; zinc seems to be the only therapeutic option available at present.

Zinc in plasma and cells in the elderly

Measurement	Elderly	Younger Healthy Control	*P*
Zinc			
Granulocytes (µg/10^{10}cells)	44.20 ± 9.50 (149)	47.54 ± 5.20 (62)	<0.01
Lymphocytes (µp/10^{10} cells)	52.33 ± 9.60 (135)	56.57 ± 6.42 (65)	<0.01
Platelets (µg/10^{10}cells)	2.75 ± 0.56 (161)	2.71 ± 0.48 (69)	NS
Erythrocytes (µp/g Hb)	35.26 ± 6.35 (174)	36.32 ± 4.38 (61)	NS
Plasma (µg/dl)	110.57 ± 13.35 (180)	108.28 ± 12.14 (72)	NS
Plasma copper (µg/dl)	156.18 ± 39.71 (179)	116.09 ± 16.20 (63)	<0.01

Values are means ± SD with η in parentheses



Based on decreased levels of zinc in both cell lines, 36 of 118 subjects were zinc deficient. Correlation coefficient between granulocyte and lymphocyte zinc was r = 0.52, p = 0.001.

Prasad AS, et al. 1993. Zinc deficiency in the elderly patients. Nutrition 9, 218-224

Comparison of plasma zinc, oxidative stress markers and generation of inflammatory cytokines in young adults and elderly subjects

Variables[1]	Young Adults	Older Subjects	p value[3]
Plasma zinc (µg/dl)	101.4±10.0[1] (31)[2]	94.3±11.4 (49)	0.046
Plasma ICAM-1 (ng/ml)	538±112.7 (25)	652.6±169.8 (47)	0.001
Plasma VCAM-1 (ng/ml)	1766±480.4 (25)	2209±890.5 (46)	0.008
Plasma E- Selectin (ng/ml)	32.2±13.1 (19)	84.6±47.6 (69)	<0.001
Plasma NO (µM)	42.7±10.9 (24)	55.6±14.7 (36)	<0.001
Plasma MDA (µM)	0.36±0.10 (16)	0.49±0.15 (34)	<0.001
IL-1 β (% cells)	8.5±9.2 (28)	17.4±23.5 (48)	0.023
IL-1 β generated (pg/ml)	679.5±110.9 (31)	938.3±423.3 (28)	0.004
TNF-α(% cells)	10.18±10.86 (22)	18.25±20.5 (48)	0.035
TNF-α generated (pg/ml)	1522±390 (26)	1882±722.6 (24)	0.036

[1]Values represent mean ± SD; [2]Number of subjects; [3]t-Test

Young adults (20-50 y) and elderly subjects (55-85 y)

Prasad AS, et al. 2007. Zinc supplementation decreases incidence of infections in the elderly: Effect of zinc on generation of cytokines and oxidative stress. Am J Clin 85, 837-844

Effect of zinc and placebo supplementation on incidence of infections in the elderly

Variables	Percentage of subjects affected in one year		
	Zinc group (n=24)	Placebo group (n=25)	Chi square Fishers exact test p
Infection	29	88	<0.001
Upper Respiratory Tract Infection	12	24	0.136
Tonsillitis	0	8	0.255
Common cold	16	40	0.067
Cold sores	0	12	0.124
Flu	0	12	0.124
Fever	0	20	0.027
One infection each/yr.	29%	52%	
Two infections each/yr.	0	24%	
Three infections each/yr.	0	8%	
Four infections each/yr.	0	4%	
Received antibiotics	8%	48%	

Each person could appear in more than one sub-category of infection

Prasad AS, et al. 2007. Zinc supplementation decreases incidence of infections in the elderly: Effect of zinc on generation of cytokines and oxidative stress. Am J Clin 85, 837-844

Effect of Zinc and Placebo Supplementation on Plasma Zinc and Copper in the Elderly

	Baseline	12 months	p = (Time*Group[1])
Plasma Zinc (µg/dl)			
Zinc Suppl.[2]	92.9±9.45[3]	104±16.69	0.0002
Placebo Suppl.[2]	95.7±13.09	88.5±9.66	
Plasma Copper (µg/dl)			
Zinc Suppl.	182.2±50.5	210.7±60.7	0.750
Placebo Suppl.	193.4±61.6	215.4±58.7	

[1] p value for change in groups over time. Multivariate repeated measures analyses were used to examine measures over time. [2]Zinc (n=24) or Placebo (n=25) supplemented subjects. [3] Values represent Mean ± SD. There were no significant differences in plasma zinc or plasma copper between the two groups at baseline.

Prasad AS, et al. 2007. Zinc supplementation decreases incidence of infections in the elderly: Effect of zinc on generation of cytokines and oxidative stress. Am J Clin 85, 837-844

Effect of zinc and placebo supplementation on the generation of cytokines[1]

	Baseline	At 6 mo	At 12 mo	*P*[2]
Percentage of cells positive for selected cytokine[3]				
TNF-α				
Zinc group	18 ± 18[4]	19 ± 19	18 ± 18	0.060
Placebo group	17 ± 21	24 ± 24	38 ± 34	
IL-1β				
Zinc group	20 ± 24	23 ± 24	23 ± 26	0.240
Placebo group	14 ± 23	10 ± 11	24 ± 28	
IL-10				
Zinc group	10 ± 11	7 ± 7	8 ± 8	0.170
Placebo group	6 ± 6	11 ± 15	12 ± 18	
Cytokines generated ex vivo (pg/mL)[5]				
TNF-α				
Zinc group	1897 ± 1004	1344 ± 544	1411 ± 786	0.018
Placebo group	1728 ± 498	1923 ± 782	2698 ± 785	
IL-1β				
Zinc group	892 ± 372	984 ± 373	766 ± 295	0.137
Placebo group	878 ± 188	881 ± 206	955 ± 223	
IL-10				
Zinc group	1916 ± 1277	952 ± 785	934 ± 873	0.056
Placebo group	917 ± 608	858 ± 483	1018±834	

[1] TNF-α, tumor necrosis factor α; IL, interleukin. No significant differences (*t* test) were found in the percentage of cells positive for TNF-α, IL-1β, or IL-10 or in generated TNF-α and IL-1β between the 2 groups at baseline. The generated IL-10 concentration was, however, higher in the zinc group than in the placebo group ($P = 0.015$) at baseline, the explanation for which is unclear. [2] *P* value for change in groups over time (time x group interaction) (multivariate repeated-measures analyses). [3] $n = 24$ in both the zinc and placebo groups. [4] $\bar{x}$ ± SD (all such values). [5] $n = 12$ and 14 in the zinc and placebo groups, respectively. Means within group are repeated measures. The repeated measures by group (zinc and placebo) for TNF-α showed a significant change ($P = 0.012$ and 0.042, respectively), which means that, over time, TNF-α changed from baseline in both groups.

Effect of zinc and placebo supplementation on oxidative stress markers in the elderly

	Baseline	6 Months	p = (Time*Group[1])
MDA+HAE (µmol /l)			
Zinc Suppl.[2]	**1.66±0.34[3]**	**1.35±0.18**	**0.0002**
Placebo Suppl.[3]	**1.70±0.30**	**1.71±0.35**	
8-OHdG (ng/ml)			
Zinc Suppl.	**0.63±0.16**	**0.50±0.14**	**0.030**
Placebo Suppl.	**0.66±0.13**	**0.68±0.13**	
Nitric oxide (µmol /l)			
Zinc Suppl.	**87.34±8.08**	**79.01±10.96**	**0.180**
Placebo Suppl.	**89.43±11.72**	**86.74±9.28**	

[1] p value for change in groups over time. Multivariate repeated measures analyses were used to examine measures over time. [2] Zinc (n=13) or placebo (n=11) supplemented subjects. [3]Values represent Mean ± SD. There were no significant differences (t-Test) in oxidative stress markers between the two groups at baseline.

Prasad AS, et al. 2007. Zinc supplementation decreases incidence of infections in the elderly: Effect of zinc on generation of cytokines and oxidative stress. Am J Clin 85, 837-844

Effect of Zinc Supplementation on hs CRP (µg/L) in the Elderly

	Pre suppl.	Post suppl.	Paired t Test between Pre and Post	Change	ΔP
Zinc (20)	2.46±1.91	1.90±1.51	0.015	- 0.55	0.0298
Placebo (20)	2.14±1.71	2.49±1.71	0.149	0.36	

Δ P for differences (Pre compared with Post) between placebo and zinc group t Test

Bao B, et al. 2010. Zinc decreases C-Reactive protein, lipid peroxidation, and implication of zinc as an atheroprotective agent. Am J Clin Nutr 91, 1634-1641

Effect of Zinc and Placebo Supplementation IL-2 mRNA in the Elderly

	Baseline	6 months	p = (Time*Group[1])
IL-2 mRNA[2]			
Zinc Suppl.[3]	0.38±0.07[4]	0.63±0.03	<0.001
Placebo Suppl.[4]	0.40±0.05	0.39±0.04	
Plasma Zn[5]			
Zinc Suppl.	84.0±3.03	97.6±5.98	<0.008
Placebo Suppl.	86.8±2.04	89.2±3.06	

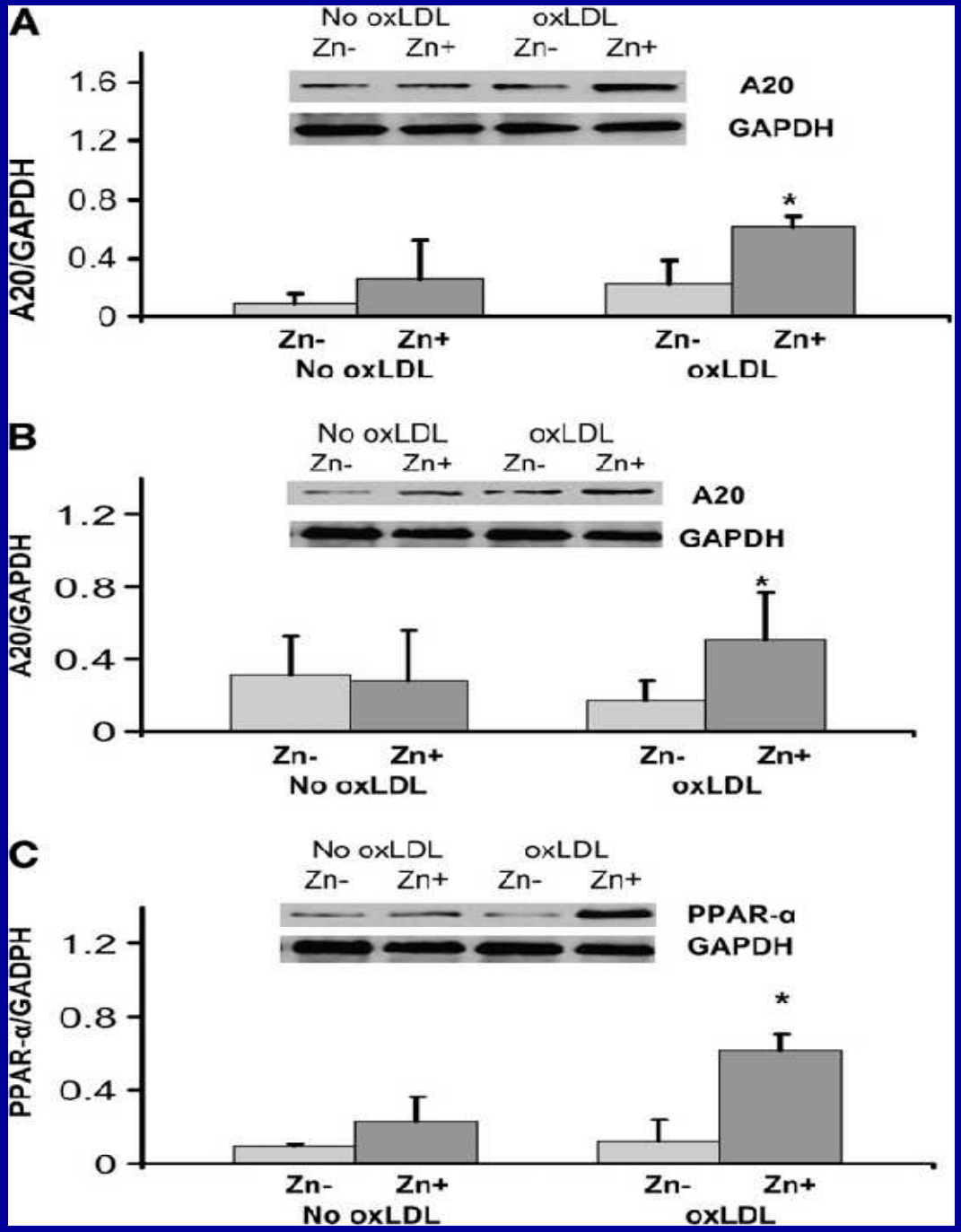
[1] p value for change in groups over time. Multivariate repeated measures analyses were used to examine measures over time. [2] Relative expression of IL-2 mRNA/18S. [3] Zinc supplemented (n=6) or placebo supplemented (n=6) zinc deficient elderly subjects. [4] Values represent Mean ± SD. There was no significant difference (t-Test) in IL-2 mRNA between the two groups at baseline. In spite of the random assignment of zinc deficient subjects into zinc and placebo group, the plasma zinc level was lower in the zinc group in comparison to the placebo group (p=0.016). [5] Plasma zinc levels (µg/dl).

. Prasad AS, et al. 2007. Zinc supplementation decreases incidence of infections in the elderly: Effect of zinc on generation of cytokines and oxidative stress. Am J Clin 85, 837-844

Effect of zinc on A20 and (PPAR-α) in THP-1 Cells (A) and HAEC cells (B & C)

The cells were incubated either in zinc-deficient (Zn^{++}, 1 µM) or zinc-sufficient (Zn^{++}, 15 µM) medium for 8 d (for THP-1) and for 6 d (for HAECs), followed by 24 H of stimulation with 50 µg oxLDL/ml. A20 and PPAR-α proteins were measured by Western blot analysis. *p <0.05 for Zn- compared with Zn+ (n = 3). GADPH, glyceraldehydes 3-phosphate dehydrogenase.

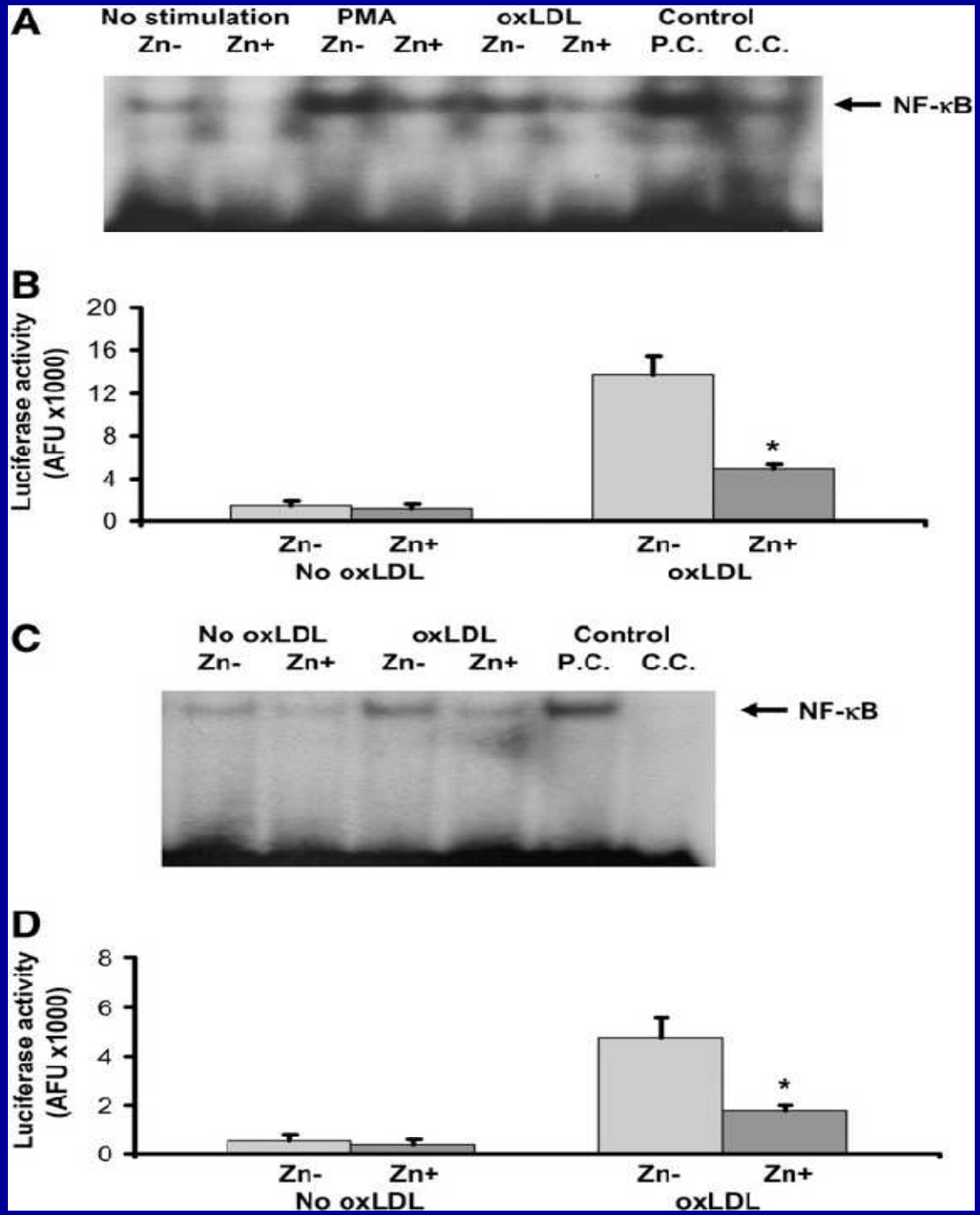
Bao B, et al. 2010. Zinc decreases C-Reactive protein, lipid peroxidation, and implication of zinc as an atheroprotective agent. Am J Clin Nutr 91, 1634-1641



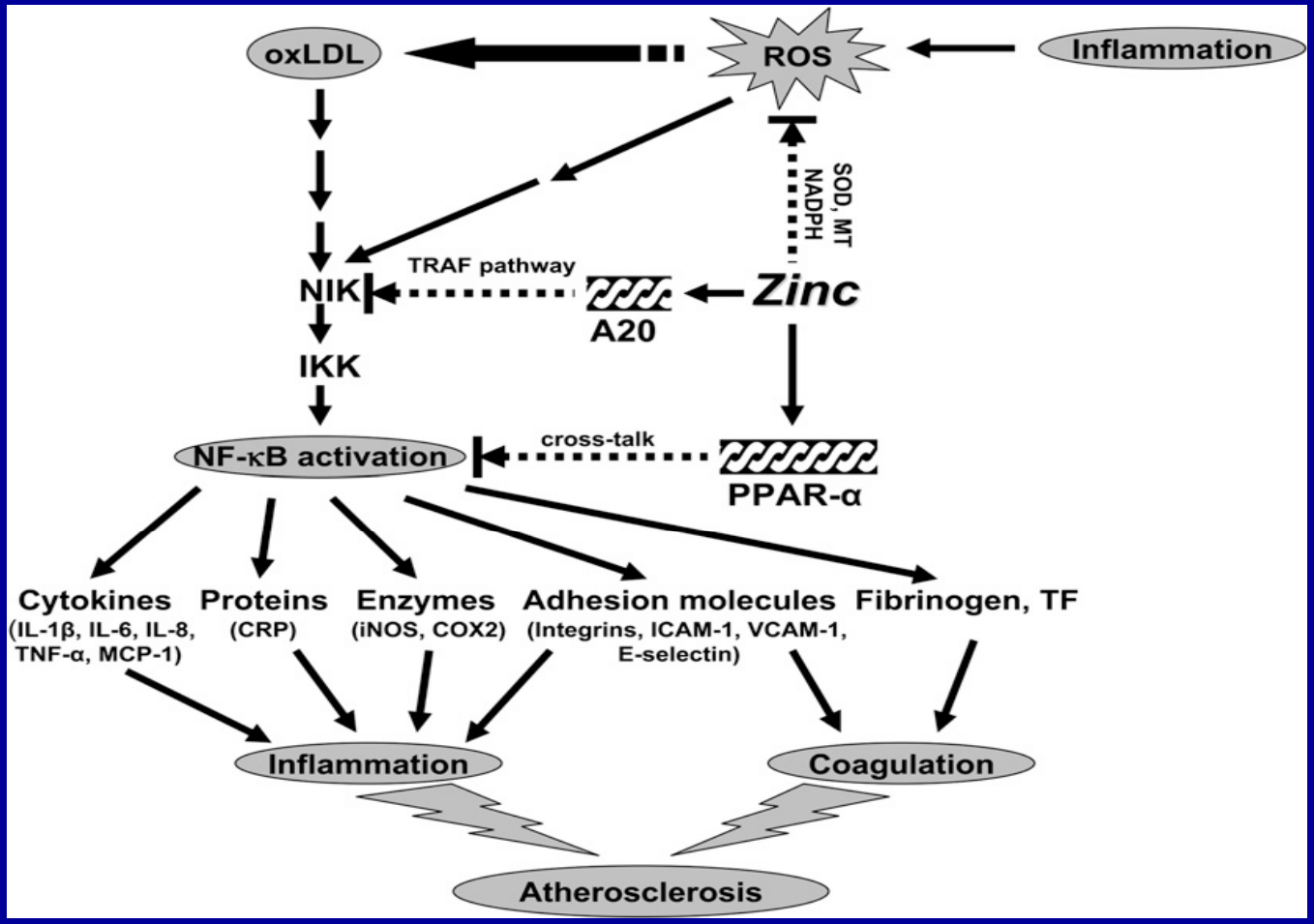
Effect of Zinc on NF-κB activation in THP-1 cells

A and B: Effect of zinc on nuclear transcription factor κB (NF-κB) activation in THP-1 cells after oxidized LDL (ox-LDL) or phorbol myristate acetate (PMA) stimulation. Zinc-deficient (Zn-) THP-1 cells and zinc-sufficient (Zn+) THP-1 cells were used for the measurement of NF-κB activation by electrophoretic mobility shift assay (EMSA; A) and luciferase reporter gene assay (B). C and D: Effect of zinc on NF-κB activation in human aortic endothelial cells (HAECs) after ox-LDL stimulation. Zn- HAECs and Zn+ HAECs were used for the measurement of NF-κB activation by EMSA (C) and luciferase reporter gene assay (D). *P <0.05 for zinc- compared with Zn+ (η = 3). AFU, arbitrary fluorescent unit/β-galactosidase U/100 μg protein; P.C., positive control; C.C., competition control.

Bao B, et al. 2010. Zinc decreases C-Reactive protein, lipid peroxidation, and implication of zinc as an atheroprotective agent. Am J Clin Nutr 91, 1634-1641



Signaling pathways of zinc action on oxidative stress markers and inflammatory cytokines



Signaling pathway for zinc prevention of atherosclerosis in monocytes/macrophages and vascular endothelial cells: a proposed hypothesis. Reactive oxygen species (ROS) induced by many stimuli modifies LDL into oxidized LDL (oxLDL) in macrophages and vascular endothelial cells. oxLDL or ROS can activate the nuclear transcription factor κB (NF-κB) pathway via NF-κB inducible kinase (NIK) activation, which eventually results in inflammation and progression of atherosclerosis.

Prasad AS. 2009. Zinc: role in immunity,oxidative stress and chronic inflammation. Current Opinion in Clin Nutr and Metab Care. 12:646-652.


Thank You for Your Attention