Excess Copper & Lead as Factors in the Aging Process

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Introduction

It has been postulated that trace metals may play an important role in the aging process. This concept is based upon the fact that trace metals often catalyze biochemical reactions, resulting in the formation of free radicals which may play an integral part of aging.

To illustrate this idea, the reaction of oxygen with serum fatty acids present in atherosclerotic plaques could be initiated by metals, e.g. Cu and Fe. These oxidative reactions are a probable continuous source of peroxide radicals and other potent tissue irritants.

Copper is an essential trace metal required for the function of eleven enzymes. Anemia, steely hair, central nervous system disorders, achromotrichia, cardiovascular, and skeletal defects are among the known disorders resulting from copper deficiency. Conversely, copper, when elevated, is known to be highly toxic, especially to aquatic organisms. Levels of less than 0.1 ppm, are lethal to certain species of fish and in mammals; 0.009 ppm have been observed to virtually eliminate immune response. Thus, the elevated copper levels in the elderly may produce the immune deficiency found with age. It has been postulated that free radicals catalyzed by trace metals may react with the receptors on immune cells, rendering them ineffective. Likewise, the depression of the immune system frequently noted in cancer patients could in part be attributed to the increasing levels of free radical reactions reflected by elevated serum copper levels in cancer.

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Trace metals may come into play with respect to aging via other modalities as well. For example, zinc is necessary for axonal and synaptic transmission, especially in the hippocampus, where it is thought to play an important role in the storage of histamine, which acts as a neurotransmitter, in the terminal vesicles of the mossy fibers.[3] The hippocampus is involved with cognition and memory. It follows that a decline in Zn with age could be an important factor in the decrement in cognition and memory seen with age.

There have been many studies concerning the effect of elevated aluminum on the CNS in relation to the production of epilepsy and other neurogenic disorders, but special interest has been devoted to its role in Alzheimer's disease and related types of senile dementia.

The toxicity of lead as a heavy metal has been extensively studied. Lead poisoning has been linked with a gamut of nervous and mental disorders including hyperactivity and schizophrenia, as it has been found that the presence of lead produces a decrement in neuronal transmission. This could have potentially serious effects especially in the amygdala and hippocampus of the limbic system, its major site of accumulation in the brain.

Another site of the accumulation of this most ubiquitous element is in the bone, where it may be metabolically inert. In fact, bone storage of lead seems to be regulated by the same hormonal factors which regulate calcium. Lead may be released into the blood, and thereby become metabolically activated, just as is calcium, under physiological conditions of bone demineralization with general aging, pregnancy, lactation, and the post-meno-

pausal osteoporosis often seen with elderly women.

Both increased levels and decreased levels in tissues of certain trace minerals have been reported in the elderly. Some of the increases reported may be due to the adventitious accumulation of environmental contaminants. For example, the accumulation of lead may come about from such environmental sources as automobile exhaust from leaded gasoline, the atmosphere of large cities, soft drinking water obtained via lead plumbing, lead-based paints, pica, plaster, and lead-soldered canned foods. Toxic lead levels may even be experienced in utero as lead ingested by the expectant mother is able to cross the placenta to the fetus, as well as the yet not fully developed hematoencephalic (blood-brain) barrier in the fetus.

In addition, excessive intake of copper may occur from drinking water obtained via copper plumbing and through the use of oral contraceptives.

Requirements for trace minerals differ and may be abnormal either due to the incidence of disease or the accumulated effects of a lifetime of nutritional excesses or deficiencies. The accumulation of heavy metals could contribute to the aging process and may necessitate changes in what may be considered the optimum intake of a particular metal. Several metals have been reported to exhibit changes which may correlate with age. Increases in serum copper levels with increasing age have been reported by Harman" and Bunker. Aluminum has been reported to accumulate in the brain as one gets older by Markesbery.[8,9] The present study examines blood trace mineral levels and other blood biochemical parameters with respect to age in male and female subjects.

Materials and Methods

Trace metal determinations were carried out on blood samples from 260 males and 321 females. The trace metal data were analyzed with respect to age and sex, both groups being divided into ten-year intervals from 11-20 years to 71-80 years. The subject pool consisted of patients coming to the Princeton Brain Bio Center for their first visit. The patient population was comprised primarily of those suffering from psychiatric problems, allergies, and metabolic disorders.

Zn, Cu and Fe concentrations in serum were determined utilizing the air acetylene flame of a Perkin Elmer 3030 atomic absorption spectrophotometer. Mn and Al concentrations were determined on whole blood using a Perkin Elmer HGA 400 graphite furnace in conjunction with the 3030 spectrophotometer, while Pb concentrations in whole blood were determined with a sample cup cuvette on a Hitach 170-70 atomic absorption spectrophotometer.

A biochemical profile was carried out on all patients by sending serum to MetPath for their Chem Screen Profile.

Results and Discussion

Tables 1 and 2 list the mean and standard deviations of trace metals for decennial age groups of males and females, while Table 3 lists the correlation coefficients of some sixteen variables which show a significant correlation with age. Increases in blood urea nitrogen (BUN), cholesterol, triglycerides, and glucose correlated well with age in female subjects and to a lesser extent in male subjects. These increases agree to a great extent with other studies done. For example, Werner et al[10] found that concentrations of urea nitrogen were similar up to the fifth decade; thereafter both the 50 percentile value and scatter of results increased. By the same token, cholesterol was found to rise sharply in the third decade. Thereafter, concentrations continued to rise markedly in males up to the fifth decade, flattening off later. In females, concentrations remained constant until menopause, increasing later to significantly higher values than those of males.

Our findings are also very similar to those reported in the Lipid Research Clinic Study 11 for 11 free living North American populations for cholesterol and triglyceride. High levels of cholesterol and triglycerides may well come into play in respect to the oxidation of serum fatty acids and the formation of the free radicals implicated in so many of the diseases of the aged.

Our results on glucose also agree well with other findings. Reed et al[12] reported an increase in the normal upper limits for both sexes. In the 50 and over age group, this glucose rise is statistically significant. A possible explanation for this occurrence is the presence of undiagnosed, prediabetic conditions in the elderly.

BUN levels are influenced by such factors

as the dietary intake of protein and the state of hydration. Glucosteroids and increases in thyroid hormone tend to raise BUN levels while androgens and growth hormone tend to decrease BUN levels. BUN is regarded as a rough guide to renal function; thus a decrease in this function, as indicated by increased BUN in the elderly, may contribute to the accumulation of metals such as Al and Pb in tissues of these people.

Albumin has been reported to decrease with age. We found a non-significant correlation in females, while in males a significant negative correlation at the .001 level was observed.

The fact that serum uric acid is higher in males than females is well recognized. Increased levels of uric acid occur with age in both sexes but this is only statistically significant for women.

These aforementioned biochemical parameters both serve to establish the external validity of our trace mineral study and to shed some light on diseases of aging. Since the Brain Bio Center results are in very good agreement with other studies, it seems that our patient pool is representative of the general population. It follows we may be relatively confident that our subsequent trace metal results are representative also, especially in the case seen with cholesterol and BUN. These biochemical factors may be closely linked to trace metals in their adverse effects upon the aged.

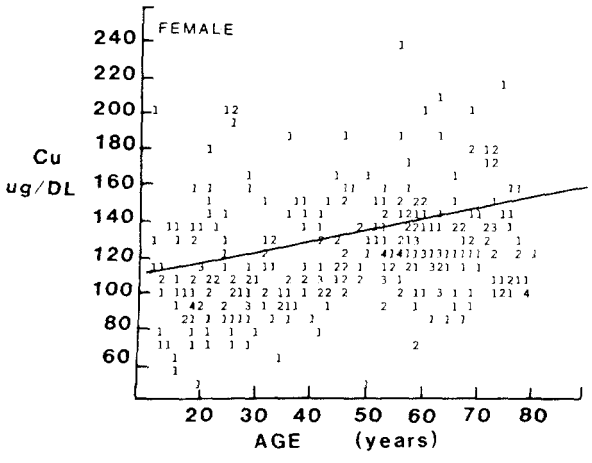

FIGURE 1
Regression Curve for CU Levels Versus Age for Female Subjects

Copper showed a significant correlation with age. Figure 1 is a regression curve of Cu versus age in female subjects. Higher serum copper levels in females is well established and may be explained in the younger age groups, either by the use of oral contraceptives, which alter circulating plasma levels, or by pregnancy due to the influence of estrogenic hormones. But this does not account for the higher levels found in older age groups. Chronic disease is known to raise serum copper levels and may play a role in the observed increase, although there is usually a concomitant decrease in serum zinc.

That excessive intake of copper may have a profound effect upon senescence and longevity was supported by work done with mice and varying concentrations of copper gluconate in their drinking water.[13]

Reports of zinc levels and aging in humans have been conflicting. Steideman and Harrill[14] report a decline in Zn with increasing age.

It has also been suggested that low serum albumin levels in the elderly may influence Zn levels. Other findings, though, including the present data showed no significant age related changes in Zn levels, although there is a trend in this direction.

Blood aluminum showed a positive correlation with age, but it did not reach statistical significance. The areas of greatest accumulation of aluminum in the body are in the lungs, liver, thyroid and brain. This being the case, high serum levels should correlate somewhat with the increased levels in the brain demonstrated by the aged.

There have been many studies concerning the effect of elevated Al on the CNS in relation to the production of epilepsy and other neurogenic disorders, but special interest has been devoted to its role in Alzheimer's disease and related types of senile dementia. Intracerebral injection of aluminum phosphate in rabbits produced a severe convulsive state accompanied by neurofibrillary degeneration (NFD) throughout the CNS, similar to that in human brain cells with Alzheimer's.[15] Cells of rabbits with NFD were noted to lack lysosomal and Golgi enzyme activities.

In cats, subdural spinal injections of aluminum hydroxide produced massive NFD degeneration of the neuronal parikarya (cell bodies of neurons) and cell process, in addition

to marked tumor growth in neuroglia and fibrillar aggregation inside the nuclei of glial cells.

In 1973, Crapper, Kirshner and Dalton[16] reported that concentrations of aluminum similar to those required to induce experimental epilepsy have been found in regions of the brains of patients diagnosed as having Alzheimer's. Pathological examination of these regions revealed brain atrophy, extensive NFD, senile plaques, and hippo-campal pyramidal cell granulovascular degeneration.

Significant increases in blood lead levels with age (regression plot of Pb vs Age) were found. Figure 2 is a regression plot of Pb vs. Age in females. As previously mentioned, both the accumulation and subsequent mobilization of lead could be important for the older individual since the biochemical effects of lead are far reaching.

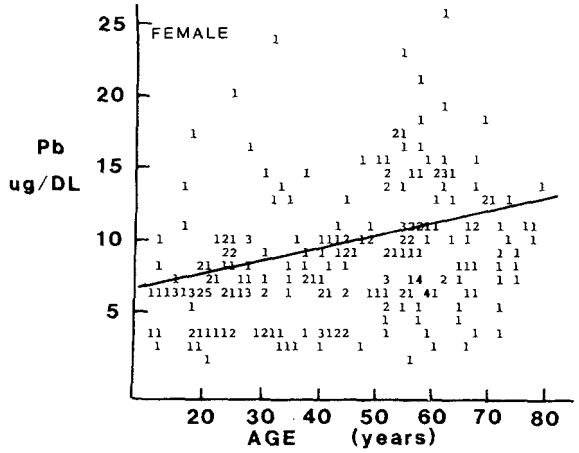

FIGURE 2
Regression Curve for Pb Versus Age for Female Subjects

Calcium showed a negative correlation with age in the males but no significant correlation in females. The relationship between lead and calcium has been documented. Sufficient amounts of dietary calcium have been found effective in preventing accumulation of lead in bone and soft tissue. This would seem to be substantiated by the finding, obtained via urinary lead excretion data, that the calcium salt CaNa(2) EDTA can be used to release significant amounts of the body's burden of chelatable lead.[17]

Phosphorus also showed a significant negative correlation in males but not in females. This is verified by studies done on brain trace elements and aging by Markesbery et al.[9], which showed a significant decline in P in the aging brain. Since P is primarily found in the form of phospholipids, it was postulated that the decline in P with age may reflect a loss of myelin phospholipids over time.

It is also interesting to point out that in this study Al showed a significant negative correlation with phosphorus. It has also been shown that high Al intake decreases P absorption and increases P excretion, suggesting that an antagonistic relationship exists related to the observed decreases in P. This point may be dubious, however, owing to the fact that Al and P concentrations differ drastically.

Table 4 shows that the percentage of individuals having abnormal Cu and Al levels increases with respect to age. What are the significance and possible ramifications with respect to the elderly of trace elements which vary out of the accepted normal range? By looking at the biochemical/physiological actions of these trace elements, some valid conclusions may be drawn. First, let us consider copper, which showed a significant increase with age.

In humans, high serum copper levels were found in many disease states including leukemia and Hodgkin's disease, as well as the age-associated diseases of rheumatoid arthritis, myocardial infarction, atherosclerosis, and cancer. With the case of the latter two, it is interesting to note that if oxidation of lipids producing tissue irritants such as peroxides plays a significant role in the development of these diseases, then the serum concentration of copper, an oxidation catalyst might be elevated in these individuals. This contention is supported by the fact that a statistically significant positive correlation has been shown between serum copper levels and myocardial infarction. Furthermore, a close relationship has been found between lipid peroxides and increasing age.[18] For example, antioxidants such as glutathione peroxidase showed increase with age (and greater levels in post-menopausal women than in men). Induction of glutathione peroxidase activity was demonstrated to be due to a higher rate of lipid peroxidation.

Interestingly enough, copper also exists in several protein-bound complex forms, two of

which are caeruloplasmin, a major antioxidant in serum, and a high molecular weight superoxide dismutase, an enzyme insuring the complete breakdown of superoxide radicals. In these cases copper is a necessary co-factor for the anti-oxidant effect of these proteins. Caeruloplasmin has shown age-related increases.

Thus, copper is a double-edged sword. Nonenzymatic copper *in vivo* would appear to have a pro-oxidant effect, whereas protein bound or enzymatic copper has an antioxidant effect. This suggestion also is supported by other studies.[19] In retrospect, then, to determine the exact ramifications of elevated copper levels reported in the elderly, one must determine the exact nature of its form. It may well be that its nonenzymatic free, pro-oxidant form is directly related to its increase in the enzymatic, antioxidant form.

Although the Brain Bio Center data shows no significant decrease in zinc with age, possible deficiency due to decreased absorption as reported by other sources may have several detrimental effects.

With the case of aluminum a positive cor-relation which did not reach statistical significance was demonstrated and while it may be true that Al does have a neurotoxic role in Alzheimer's, there is need to establish the exact pathogenic role of Al in this disease of the elderly. One cannot fall into the trap of assuming causation is necessarily implicated in correlation. Al is involved with the most severe diagnostic signs of Alzheimer's. It is not known to cause all cases of the disease.

Lead showed a significant increase with age, as did copper. Women who had been pregnant showed a smaller increase in plasma lead after menopause, which indicates that lead was mobilized from sites in the bone during pregnancy, depleting these stores for further mobilization after the menopause. The ramifications of such mobilization, in either the fetus or the older person, depend on the degree to which the lead reaches susceptible organs.

A major biochemical factor involved in lead toxicity involves its characteristic tendency to react with sulfhydryl or thiol groups. Thus levels or the activity of certain compounds vulnerable to sulfhydryl group interference might be affected by the elevated
lead levels in the elderly. One illustration of this concerns glutathione (GSH), the most abundant cellular nonprotein thiol compound. GSH makes up more than 80-90% of the total nonprotein sulfhydryl (NPSH) concentrations and plays a major detoxification role in the liver. Lead added to the diet of chicks was found to significantly increase liver and kidney concentrations of NPSH as well as hepatic concentrations of GSH.[20] Thus, it seems that GSH is intimately involved in compensating for the toxic effects of lead. One must consider that the heightened demand for glutathione occurs with respect to two age-related parameters. One, to compensate for the observed increase in lead and two, for the increase in free radicals and damaging peroxides, as glutathione peroxidase is a major antioxidant. It follows from this that a paralleled, increased need for cysteine is also the case here, for the availability of cysteine is a rate-limiting factor for the biosynthesis of GSH.

Another vulnerable target for increased lead mobilization is specifically the rod photoreceptors of the eye, thus offering a feasible explanation for possible deficits in low illumination visual acuity with older people. Studies done by Fox and Sillman[21] have shown that divalent lead ions caused a reversible concentration dependent decrease in the amplitude of rod response. Concentrations of 5.0 micromolar and 12.5 micromolar produced a 9 and 20% decrease respectively. The mechanism by which this is thought to occur again involves the vulnerability of sulfhydryl groups to lead. By binding thiol groups on the Na+ - K+ adenosine-phosphatase pump, lead may well interfere with the enzymatic reactions necessary for the phosphorylation of sodium channels in the outer membrane of the rod. This would, in effect, reduce sodium current and would therefore hyperpolarize the membrane of the rod, making it more difficult for the production and propagation of the action potential in response to a stimulus.

In summary, it can be said that the biochemical effects of lead are both far reaching and sinister in its subtlety, especially for the aged. Stores of metabolically inert lead in the bone, built up over time, released and activated in the older individual can be deleterious. This is true especially with respect to the brain and the eye (if not other

TABLE 1

MINERALS SHOWING POSSIBLE CORRELATION WITH AGING

AGE	21 — 30		31 — 40		41 — 50	
	M	F	M	F	M	F
n	41	41	41	23	41	30
Pb	8.2 ± 3.3	8.2 ± 3.3	10.7 ± 5.2	8.9 ± 5.3	11.6 ± 3.9	8.1 ± 3.5
Mg	1.8 ± 0.1	1.8 ± 0.2	1.9 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
Cu	105.2 ± 16.9	123.5 ± 36.3	111.1 ± 21.1	119.3 ± 24.3	103.5 ± 18.3	125.6 ± 22.8
Al	7.9 ± 6.2	11.4 ± 8.4	11.4 ± 6.9	9.6 ± 7.1	9.7 ± 5.0	9.6 ± 10.2
Ca	9.7 ± 0.3	9.6 ± 0.4	9.7 ± 0.3	9.4 ± 0.3	9.7 ± 0.3	9.4 ± 0.4
P	3.4 ± 0.5	3.4 ± 0.6	3.3 ± 0.4	3.1 ± 0.4	3.1 ± 0.5	3.1 ± 0.7
Zn	97.8 ± 34.9	97.0 ± 18.3	98.8 ± 13.0	91.6 ± 21.3	88.7 ± 14.9	90.8 ± 13.3

TABLE II

BIOCHEMICAL PARAMETERS SHOWING POSSIBLE CORRELATION WITH AGING

AGE	21 — 30		31 — 40		41 — 50	
	M	F	M	F	M	F
n	41	41	41	23	41	30
Bun	16.1 ± 5.5	11.6 ± 4.3	14.5 ± 4.3	11.6 ± 1.9	17.1 ± 5.4	13.1 ± 3.6
Cholesterol	175.0 ± 34.1	187.5 ± 30.8	190.6 ± 25.6	205.6 ± 37.0	209.8 ± 48.2	210.2 ± 38.8
Triglyceride	122.3 ± 76.1	86.2 ± 42.6	141.3 ± 78.0	100.1 ± 42.2	150.5 ± 74.0	101.5 ± 48.7
Glucose	89.1 ± 9.7	87.0 ± 12.0	92.4 ± 9.7	88.5 ± 10.0	93.4 ± 9.1	89.2 ± 10.4
Uric Acid	5.1 ± 1.0	3.9 ± 1.2	5.2 ± 1.2	3.5 ± 0.9	5.2 ± 1.2	3.9 ± 0.7
Total Protein	7.2 ± 0.3	7.1 ± 0.4	7.4 ± 0.3	6.9 ± 0.3	7.2 ± 0.4	7.2 ± 0.4
Albumen	4.6 ± 0.2	4.4 ± 0.3	4.5 ± 0.3	4.2 ± 0.2	4.4 ± 0.4	4.3 ± 0.3

	51 - -60		61- -70		71- -80	
	M	F	M	F	M	F
	46	55	44	38	30	31
	14.9	11.1	11.1	11.6	13.8	10.6
± 4.9		± 4.7	± 4.7	± 5.0	± 4.5	± 2.7
	1.9	1.8	1.9	1.9	1.9	2.0
± 0.2		± 0.1	± 0.1	± 0.2	± 0.1	± 0.2
	114.7	136.4	113.7	130.5	124.3	134.4
± 24.4		± 26.2	± 12.9	± 25.2	± 19.3	± 24.5
	12.8	9.4	13.0	12.4	12.0	15.6
± 8.2		± 5.4	± 12.2	± 13.1	± 5.8	± 10.4
	9.6	9.7	9.5	9.5	9.2	9.6
± 0.2		± 0.5	± 0.4	± 0.3	± 1.4	± 0.4
	3.1	3.4	3.1	3.4	3.0	3.1
± 0.6		± 0.5	± 0.5	± 0.5	± 0.5	± 0.5
	103.5	90.8	92.7	98.4	85.5	92.4
± 35.1		± 13.3	± 16.5	± 24.7	± 16.8	± 23.7

	51 — 60		61 — 70		71 — 80	
	M	F	M	F	M	F
	46	55	44	38	30	31
	16.6	15.9	17.4	17.8	21.0	19.4
	± 3.8	± 4.0	± 3.5	± 4.1	± 7.4	± 6.5
	214.2	230.9	216.0	246.0	220.0	235.3
	± 42.9	± 33.0	± 37.7	± 44.5	± 52.1	± 41.5
	168.4	134.0	172.1	172.6	164.4	158.0
	± 113.9	± 70.2	± 132.5	± 96.0	± 95.4	± 80.5
	92.0	97.5	100.4	104.4	106.1	98.8
	± 12.2	± 18.1	± 22.1	± 40.5	± 34.1	± 15.8
	5.3	4.3	5.2	4.6	5.7	4.6
	± 1.0	± 1.1	± 1.6	± 1.4	± 1.7	± 1.3
	7.2	7.1	7.0	7.0	7.0	6.9
	± 0.4	+ 0.4	± 0.4	± 0.4	± 0.5	± 0.4
	4.4	4.3	4.3		4.2	4.2
	± 0.3	± 0.3	± 0.3		± 0.3	± 0.3

TABLE HI

BIOCHEMICAL PARAMETERS SHOWING SIGNIFICANT CORRELATION WITH AGE

	FEMALES			MALES		
VARIABLES	**r**	**N**	**P**	**r**	**N**	**P**
1. BUN	.550	225	.001	.270	185	.001
2. CHOLESTEROL	.443	225	.001	.185	184	.050
3. TRIGLYCERIDES	.400	225	.001	.197	182	.010
4. PB	.308	185	.001	**.373**	117	.001
5. GLUCOSE	.278	225	.001	.279	185	.001
6. URIC ACID	.241	225	.001	.075	184	**NS**
7. MG	.229	217	.001	.177	175	.050
8. CU	.188	225	.010	.253	185	.001
9. TOTAL PROTEIN	.177	225	.050	-.275	184	.001
10. AL	.091	198	**NS**	.190	121	**NS**
11. ALBUMEN	.067	225	**NS**	-.387	184	.001
12. CA	.060	225	**NS**	-.242	184	.010
13. P	-.054	225	**NS**	-.253	184	.001
14. ZN	-.010	225	**NS**	-.158	185	.050

TABLE IV

PERCENTAGE OF INDIVIDUALS IN EACH AGE GROUP HAVING ABNORMAL TRACE METAL LEVELS

Age		**21-30**	**31-40**	**41-50**	**51-60**	**61-70**	**71-80**
Cu> 120	F	**32**	**35**	**50**	**73**	**63**	**61**
ug/DL	M	**14**	**24**	**21**	**31**	**39**	**62**
Zn < 80	F	**15**	**26**	**17**	**25**	**11**	**26**
ug/DL	M	**19**	**12**	**42**	**31**	**39**	**58**
Al > ,10	F	**38**	**35**	**32**	**27**	**43**	**50**
ppb	M	**21**	**39**	**23**	**43**	**43**	**53**

sensory organs also). In the hippocampus and the amygdala lead may indeed be involved with producing deficits in memory via a similar biochemical mechanism by which it causes a decrement in the response of the retinol rods, i.e., reaction with thiol groups on the ATPase pumps. It is certainly conceivable that lead may augment the effects of aluminum in Alzheimer's Disease in this manner. It would also seem logical that dietary supplementation of both calcium and cysteine (for glutathione) would be useful to the older individual, to help the body rid itself of its lead burden.

Conclusion

The increase in toxic heavy metals such as Pb, Cu and Al, could play an important role in the aging process. Deranged trace metal homeostasis may come about as a result of inadequate or excess oral intake, compounded by inborn errors in transport or metabolism. Toxic trace metals may be poorly handled due to a decline in efficiency of such physiological parameters as dysfunction of the intestinal tract or renal system. The accumulation of toxic trace elements may interfere with normal metabolism, neural transmission, or lead to the formation of free radicals by metal catalysis. These radicals would cause further damage characteristic of aging.

Acknowledgements

The authors wish to thank Vida Chu, Glen von Slooten, and Eva Nagy for trace metal analysis and Scott Lamola for the statistical analysis. We also wish to acknowledge the assistance of Brian Kurtz in the preparation of the manuscript.

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