

Medicinal and Nutritional Aspects of Various Trace Metals Determined in *Ajuga Bracteosa*

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Abstract: *Ajuga bracteosa* (Lamiaceae) is known for its ethnomedicinal importance. In order to rationalize its medicinal applications and establish biogeochemical link, the mineral elements (Na, K, Ca, Mg, Zn, Mn, Cu, Fe, Cr) of leaves and roots of *Ajuga bracteosa* and the nearby soil were studied. The herb contains comparatively larger amounts of chromium (leaves 25 mg and roots 20 mg per 100 g) which may be correlated to its use as remedy for diabetes. The considerably larger amounts of potassium (leaves 139 mg, roots 159 mg per 100 g) than sodium (leaves 21 mg, roots 29 mg per 100 g) may have some correlation with the use of the herb in hypertension.

Key words: *Ajuga bracteosa*, trace elements, chromium, diabetes, hypertension

INTRODUCTION

Ajuga bracteosa Wall belongs to family Lamiaceae (Labiatae), which is a large family comprising about 170 genera.^[1,2] The genus *Ajuga* consists of about 40-50 species. *A. bracteosa* is distributed in subtropical and temperate regions from Kashmir to Bhutan, Pakistan, Afghanistan, China and Malaysia.^[3] In Pakistan it is found in northern hilly areas, where in local Hindko/Punjabi language it is called *kori booti* (lit., bitter herb) owing to its bitter taste. A decoction of the leaves of the herb is used in the traditional medicine for a number of diseases including diabetes, hypertension, fever, malaria and stomach pain. The plant is astringent and used as tonic and in the treatment of agues.^[4] The local people also use its leaves extract for blood purification.^[5,6] The juice of the root is also used in the treatment of diarrhea and dysentery.⁷ According to Asia Pacific Medicinal Plant Database the leaves are regarded as stimulant, diuretic and tonic.^[8] The extract is also used to cure swollen wounds, bites of insects, eye troubles, diseases of bladder, as well as tumors.^[9,10]

The chemical investigations^[11,12] have shown that *A. bracteosa* contains compounds of the class diterpenoids and withasteroids, which are a group of naturally occurring C₂₈ steroidal lactones built on an intact or rearranged ergostane framework. Withasteroids exhibit a number of biological activities such as antimicrobial, antitumor, anti-inflammatory, hepatoprotective, immunomodulatory, cytotoxic, antiseptic and insect-antifeedant properties.^[13-15]

The literature survey showed that no work on mineral elements of this herb has yet been reported. In order to develop biogeochemical link, establish

ethnomedicinal significance and investigate its possible pharmacological role, the mineral elements present in the leaves and roots of *Ajuga bracteosa* and in the soil samples collected from the area in which this herb grows in Abbottabad (NWFP, Pakistan) were studied and are being reported in this paper.

RESULTS AND DISCUSSION

The study of mineral constituents of the leaves and roots of *Ajuga bracteosa* indicated the presence of macronutrients (Na, K, Ca, and Mg) and micronutrients (Fe, Zn, Cr, Cu, and Mn) in variant amounts both in the herb as well as in the soil samples collected from its vicinity. The results are shown in Table-1 and Table-2 for the former and latter samples respectively. The latter values for trace elements in non-agricultural soil reflect quite divergence than the corresponding mineral contents recently reported in the agricultural soil of Peshawar, Swat and Kohat (NWFP, Pakistan)^[16]

The uptake pattern of various elements by the herbs/vegetables in the concerned areas of their soil depends upon various factors. These include synergetic/antagonistic behaviour of various elements, nature of aquatic organisms affecting the ecological conditions, pH related changes, etc.^[17-20]

In order to evaluate the pharmacological /medical /nutritional significance of *Ajuga bracteosa*, we need to investigate the physiological and other functional roles of various minerals found in this herb.

Sodium and Potassium: Sodium is the common mineral determined in *Ajuga bracteosa* for which Estimated Safe and Adequate Dietary Intake varies from 1100-3300 mg which is 1875-5625 mg for

potassium. The ions of these metals are electrolytes which maintain “body water balance” and carry out nerve functions. The requirement of K^+ for daily intake is obviously quite higher in concentration within body cells, while Na^+ (along with Cl^-) ions are found in excess especially in extra cellular fluids such as blood plasma.^[21] The role of Potassium in controlling blood pressure is well known. In this context, the higher quantity of potassium as compared to sodium makes this herb significant (Table-1). In case of K-deficient individuals, the intake of a decoction of this herb will certainly assist to overcome its deficiency and thus is likely to enhance its functional role.^[22]

Calcium and Phosphorus: Calcium and phosphorus together are important elements which remarkably contribute to body structure especially in the formation of bones and teeth. The bones have living tissues and secrete collagen, which forms the framework of connected tissues and bones. Calcium, with other elements, is involved in nerves transmission and blood clotting. The Recommended Dietary Allowance (RDA) necessitates 800 mg of Ca^{2+} as daily dose for adult males and females, while 1200 mg for teenagers and pregnant women. Overdose of Ca supplement may suppress utilization of other minerals. The intake of Ca reduces absorption of Na^+ and assists in reducing blood pressure. Its deficiency leads to stunted growth due to softening/ weakening of bones and causes diseases like rickets, *osteomalacia* and *osteoporosis*^[23,24]. The intake of aqueous extracts provides about 200 mg of calcium from 100 grams of this herb (Table-1). This will certainly mitigate the deficiency of calcium content in the needy individuals.

Magnesium: Magnesium^[25-34] is an important mineral element which is a component of bones and many enzymes. It plays important role in the regulation of blood sugar levels and blood pressure. It is necessary for the transmission of nerve impulses, which affects contraction and relaxation of muscles.

Generally speaking spontaneous deficiency of Mg^{2+} in humans is unlikely. However, its prolonged deficiency causes neurological disturbances. When Mg^{2+} deficiency falls to 1.0 meq/L, a syndrome resulting delirium tremens may be precipitated resulting in semi coma, tremor, carpopedal spasm and general neuromuscular irritability, with marked susceptibility to auditory, mechanical and visual stimuli. The administration of Mg^{2+} brings about prompt improvement.

We must bear in mind that Daily Requirement Allowance (RDA) of Mg^{2+} has not been fully established, but its Estimated Safe Intake Requirement falls between 200-300 mg/day. [Table-1] Luckily we

can get about 300 mg of Mg^{2+} from 100 g of *Ajuga bracteosa*, which can be utilized to play its pharmacological function to sustain a healthy body.

Zinc: Zinc^[35-43] is another important mineral which makes contribution to human health as a component of 70 enzymes with a variety of functions. It promotes proper growth along with sexual maturity. The adult RDA requirement for Zn^{2+} is about 15 mg. Its deficiency leads to the loss of appetite, retards growth, causes reproductive failure, impairs immune function, delays healing of wounds and decreases taste sharpness. Animals fed on peanut meal, having a supplement of Zn^{2+} ions, develop a syndrome called *parakeratosis* with nausea and vomiting.

The aqueous decoction of *Ajuga bracteosa*, which contains very small amount of Zn^{2+} 1.2-2.0 mg/100 g of the herb (Table-1), may not alleviate symptoms of this type of disease. However, the administration of .02 % $ZnCO_3$ in the diet can cure this disease. Its excess causes nausea and vomiting along with diarrhea. It may adversely affect Cu metabolism by depressing its absorption.

Iron: Like many other minerals, iron^[44,45] is a component of many enzymes and is an essential part of hemoglobin (blood protein) and myoglobin (muscle protein). The RDA requirements for adult males and females are 10 mg and 18 mg respectively. Thus, women require more dietary iron since they lose blood during menstruation and childbirth. They also need 30-60 mg of supplementary iron during lactation at least for 2-3 months after delivery. Deficiency of Fe may lead to anemic conditions and an individual feels “run down” and his work efficiency is adversely affected. Its prolonged deficiency impairs immune functions. The use of an aqueous decoction of *Ajuga bracteosa*, which can provide only 2-3 mg of Fe per 100 g of the herb, cannot yield appreciable pharmacological benefit in the absence of dietary iron.

Copper: Copper^[46-50] is involved in many biological functions. It is a component of various enzymes and plays its role in the synthesis of collagen, regulation of normal cardiovascular and immune functions. However, its severe and prolonged deficiency may promote anemic conditions, bring about cardiovascular changes and cause infirmity of bones (due to depression of iron metabolism). However, its depletion and enrichment both are damaging. Excess deposition of Cu in tissues is seen in man in Wilson’s disease, with pertinent associated effects. The Estimated Safe Adequate and Dietary Intakes for Cu^{2+} is only 0.9 mg. But on the average we get only 0.3mg/100g of *Ajuga bracteosa*, which is small but may still be physiologically useful.

Manganese: Manganese is an essential trace element.^[51,52] Definite requirement of Mn^{2+} for individuals has not been rationally established. However, Estimated Safe and Adequate Dietary Intake indicates 2.5-5.0 mg as its sufficient amount. Mn salts are poorly absorbed from intestine and get concentrated in liver and kidney, particularly in mitochondria. The aqueous decoction obtained from 100 g of *Ajuga bracteosa* (Table-1) contains about 2 mg of this trace element. This may partially be helpful in compensating its deficiency, if excessive amount of mineral is ingested, it may interfere with absorption of iron. Under such conditions resulting anemia may be prevented by increasing dietary intake of iron.

Chromium: Chromium^[53-64] is an other essential mineral that human require in trace amounts. It is an important component of many body building strategies to and in the development of lean muscle mass, as well as in the treatment of diabetes and for weight loss. Chromium increases the metabolism of proteins, fats and carbohydrates. Significantly, it enhances the efficiency of insulin to regulate blood sugar levels. A person deficient in chromium can lead to fatigue, high cholesterol levels, anxiety, atherosclerosis and slow healing. Its deficiency also diminishes the body's ability to convert glucose to energy and therefore leads to high blood glucose levels and increased insulin production. Within the body, chromium is also used as Glucose Tolerance Factor (GTF), which consists of chromium, niacin (Vitamin B3) and a number of amino acids. The GTF improves insulin efficiency and activity of insulin receptors in cells. Poor insulin production leads to hypoglycemia and adult diabetes. Thus it is essential to add chromium to patients fed insulin intravenously, to avoid the onset of diabetes. However, keeping in view of this toxic effect, it has been recommended that 200-400 $\mu g/day$ of Cr-picolenate may be used safely to avoid full blown diabetes. It has also been found that this compound affects a number of functions of neurotransmitter of people suffering from depression or other emotional disorders. Most of the vegetables/fruits provide very little chromium. Its absorption in intestinal tract is low. The absorbed chromium is stored in the liver, spleen, soft tissues and bones. The body's chromium content may be reduced under several conditions, e.g., infection, acute exercise, pregnancy, lactation, stressful states etc. To meet the nutritional requirement of Cr on daily basis, Recommended Dietary Allowance (RDA) has been developed along with Estimated Safe and Adequate Intake. Individuals suffering from diabetes when given a dose of 150-250 $\mu g/day$ were found to correct their diabetes symptoms. The older people are more vulnerable to chromium depletion than younger ones. There is considerable

possibility that supplemental chromium may help to treat impaired glucose tolerance and type-2 diabetes. In this connection, research activities are still going on. It is worthwhile to mention that 20-25 mg of Cr obtainable from 100 g of *Ajuga bracteosa* (Table-1), may be quite sufficient for the relief of diabetic patients. Hence, this herb may be taken in an appropriate manner to relieve diabetic patients and help in various other functions affected by chromium deficiency.

Conclusion: We may conclude that human body needs major minerals (Ca, P, S, K, Na and Mg), and trace elements (Fe, Zn, Cu, I₂, Mn, Cr and others), which are essential for growth, health and wellbeing, and reproduction. We must bear in mind that the consequences of essential trace mineral deficiency may be just as severe as those of a deficiency of a major essential mineral. Many elements are associated with one another in maintaining our normal growth and health. *Ajuga bracteosa* is characterized by its comparatively larger potassium to sodium ratio, and higher quantities of magnesium and chromium. As these elements have a role in regulating blood sugar level and blood pressure, the herb may prove to be a useful remedy for diabetes and hypertension.

MATERIALS AND METHODS

The chemicals and reagents used in the present work were of analytical grade (E. Merck, Germany). The solutions of the samples and the standard were prepared in double deionised water. The atomic absorption spectrophotometer used for trace elements analysis was Varian Model AA240.

The leaves and roots of the perennial herb *Ajuga bracteosa* (Lamiaceae) were collected in October 2007 from hills near Abbottabad, Pakistan, and were identified by the taxonomist Dr Syed Muqarrab Shah, Department of Botany, Hazara University. The soil samples were also collected from the vicinity of the herb.

Digestion of Leaves and Roots Samples: The samples of leaves and roots were cleaned visually to remove any dust particles and were then washed with the deionised water. The dried samples were then ground to fine powder.

Weighed and ground samples of leaves and roots were separately placed in a pre-cleaned silica crucible and heated on flame for about 10 minutes to remove moisture and volatile matter. Then in each case the crucible was heated strongly in a muffle furnace at 600°C for about 4 hours which converted the samples into ash. The ash was dissolved in conc.

Table 1: Quantity of Trace Elements in mg/100 g

	Elements	Leaves	Roots	Soil
1	Sodium, Na	21.4	28.6	40.6
2	Potassium, K	139.2	159.4	163.2
3	Calcium, Ca	201.3	211.3	220.4
4	Magnesium, Mg	271.6	312.7	138.6
5	Zinc, Zn	2.0	1.2	1.4
6	Chromium, Cr	25.0	20.2	0.48
7	Manganese, Mn	2.0	1.3	1.41
8	Iron, Fe	3.0	2.1	8.21
9	Copper, Cu	0.37	0.23	1.82

Table 2: Trace Elements in Various Soil Samples (Microgram/g)

	Elements	Abbottabad	Peshawar*	Swat*	Kohat*
1	Zinc, Zn	14.00	5.36	6.31	5.18
2	Chromium, Cr	4.80	2.26	1.98	2.18
3	Manganese, Mn	14.1	28.76	20.75	19.63
4	Iron, Fe	82.1	197.00	205.00	218.00
5	Copper, Cu	18.2	0.80	0.76	0.89

* Data collected by Agriculture University Peshawar¹⁶

HNO₃ (12 mL) by adding the acid gradually. A clear solution was obtained. Then the total volume was made 100 mL by adding twice distilled water to the dissolved material. The content was then filtered, and the filtrate was used for the analysis. The experiments were done in triplicate and the results were averaged (Table-1).

Digestion of Soil: The soil samples were taken from the depth of about 10 cm in vicinity of the herb. After drying at 105°C, the soil was screened to obtain fine dust. A weighed, ground sample of the soil was placed in a conical flask and treated with conc. HNO₃ (10mL) by adding the acid gradually. The flask was then heated on a hot plate for about an hour. Then, after adding 4 mL conc. HNO₃ and 2 mL H₂O₂ (30%), the flask was further heated for one hour. The contents of the flask were cooled and diluted with deionised water to 100 mL final volume. The solution was used for the analysis of trace elements (Table-1 & 2)

REFERENCES

- Hassan, M., G.A. Hazimi and G.A. Miana, 1994. *J. Chem. Soc. Pak*, 16: 1.
- Ali, S.I. and Y.J. Nasir, 1990. *Flora of Pakistan*, BCC & T Press, University of Karachi, 192: 14.
- Arfan, M., G.A. Khan and N. Ahmed, 1996. *J. Chem. Soc. Pak*, 18: 2.
- Chopra, R.N., S.L. Nayar and I.C. Chopra, 1986. *Glossary of Indian Medicinal Plants (Including the Supplement)*. Council of Scientific and Industrial Research, New Delhi.
- <http://www.siu.edu/~ebl/leaflets/zafeer.htm>.
- <http://hujra.org/gfx/usr/File/Marghuzar.pdf>.
- Manandhar, N.P., 2002. *Plants and People of Nepal* Timber Press, Oregon. ISBN 0-88192-527-6.
- <http://219.93.41.233/wapi/mctweb.dll/getObject?MID=MEDICINALPLANT&ObjID=1561>.
- Perry, L.M. and Metzger, 1980. *Medicinal Plants of East and Southeast Asia*, The MIT Press Cambridge, London, pp: 184.
- Chang, B.S., H.K. Lee and J. Woong, 1980. *Saengyak Hakhoe Chi.*, 11: 15.
- Bhakuni, R.S., Y.N. Shukla, R.S. Thakur, 1987. *Indian Journal of Pharmaceutical Sciences*, 49(6): 225-6.
- Naheed Riaz, Sarfraz A. Nawaz, Naveen Mukhtar, Abdul Malik, Nighat Afza, Samar Ali, Shafi Ullah, Pir Muhammad, 2007. *M. Iqbal Choudhary, Chemistry & Biodiversity*, 4: 72.
- Glatter, E., 1991. *Nat. Prod. Reports*, 8: 415.
- Budhiraga, R.D. and S. Sudhir, 1987. *J. Scient. Indian Res.*, 46: 488.
- Ray, A.B. and M. Gupta, 1994. *Prog. Chem. Org. Nat. Prod.*, 63: 1.

16. Sohail Noor, Abdul Wajid, Saeed Akhtar and Atif Latif, 2008. *J. Chem..Soc. Pak*, 30(3): 357.
17. Cornelis, R., 2002. *Anal. Bioanalytical Chemistry*, 373: 123.
18. Alia Bano Munshi, Tanzal Haider Usmani, Farooq A. Khan, 2008. *J. Chem..Soc. Pak*, 30(3): 352.
19. Aslam, S., M.S. Wahid, A. Ali, I. Ahmad and F.K. Bangash, 2007. *J. Chem..Soc. Pak*, 29(4): 332 and references therein.
20. Makhdoom, M.I., M. Ashraf and H. Pervez, 2008. *J. Chem. Soc. Pak*, 29(4): 275.
21. National Research Council, National Academy of Sciences (NAS), 1980. *Recommended Dietary Allowances*, 9th ed. Washington, DC. NAS.
22. Scrimshaw, N.J. and V.R. Young, 1976. *The Requirements of Human Nutrition*, *Scientific American*, 235: 50-64.
23. Underwood, E.J., 1977. *Trace Elements in Human and Animal Nutrition*, New York: Academic Press.
24. Abraham White, Philip Handler, Emil L. Smith and I. Robert Lehman, 1978. *Principles of Biochemistry*, International Students 6th Edition, McGraw Hill Kogakusha Ltd., pp: 1159.
25. Shils, M.E., 1999. Magnesium. In *Modern Nutrition in Health and Disease*, 9th Edition. (edited by Shils, ME, Olson, JA, Shike, M, and Ross, AC.) New York: Lippincott Williams and Wilkins, pp: 169-92.
26. Rude, R.K., 1998. Magnesium deficiency: A cause of heterogeneous disease in humans. *J Bone Miner Res.*, 13: 749-58.
27. Wester, P.O. Magnesium, 1987. *Am J Clin Nutr.*, 45: 1305-12.
28. Saris, N.E., E. Mervaala, H. Karppanen, J.A. Khawaja, A. Lewenstam, 2000. Magnesium: an update on physiological, clinical, and analytical aspects. *Clinica Chimica Acta*, 294: 1-26.
29. Institute of Medicine, 1999. *Food and Nutrition Board. Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride*. National Academy Press. Washington, DC.
30. Elisaf, M., H. Milionis, K. Siamopoulos, 1997. Hypomagnesemic hypokalemia and hypocalcemia: Clinical and laboratory characteristics. *Mineral Electrolyte Metab.*, 23: 105-12.
31. Appel, L.J., 1999. Nonpharmacologic therapies that reduce blood pressure: A fresh perspective. *Clin Cardiol*, 22: 1111-5.
32. Simopoulos, A.P., 1999. The nutritional aspects of hypertension. *Compr Ther*, 25: 95-100.
33. Appel, L.J., T.J. Moore, E. Obarzanek, W.M. Vollmer, L.P. Svetkey, F.M. Sacks, G.A. Bray, T.M. Vogt, J.A. Cutler, M.M. Windhauser, P.H. Lin, N. Karanja, 1997. A clinical trial of the effects of dietary patterns on blood pressure. *N Engl J Med.*, 336: 1117-24.
34. Abraham White, Philip Handler, Emil L. Smith and I. Robert Lehman, 1978. *Principles of Biochemistry*, International Students 6th Edition, McGraw Hill Kogakusha Ltd., pp: 1329.
35. Sandstead, H.H., 1994. Understanding zinc: Recent observations and interpretations. *J Lab Clin Med.*, 124: 322-327.
36. Institute of Medicine, 2001. *Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. National Academy Press. Washington, DC.
37. Solomons, N.W., 1998. Mild human zinc deficiency produces an imbalance between cell-mediated and humoral immunity. *Nutr Rev.*, 56: 27-28.
38. Prasad, A.S., 1995. Zinc: An overview. *Nutrition*, 11: 93-99.
39. Heyneman, C.A., 1996. Zinc deficiency and taste disorders. *Ann Pharmacother*, 30: 186-187.
40. Prasad, A.S., F.W. Beck, S.M. Grabowski, J. Kaplan, R.H. Mathog, 1997. Zinc deficiency: Changes in cytokine production and T-cell subpopulations in patients with head and neck cancer and in noncancer subjects. *Proc Assoc Am Physicians*, 109: 68-77.
41. Simmer, K. and R.P. Thompson, 1985. Zinc in the fetus and newborn. *Acta Paediatr Scand Suppl*, 319: 158-163.
42. Fabris, N. and E. Mocchegiani, 1995. Zinc, human diseases and aging. *Aging (Milano)*, 7: 77-93.
43. Institute of Medicine, 2001. *Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. National Academy Press. Washington, DC.
44. Abraham White, Philip Handler, Emil L. Smith and I. Robert Lehman, 1978. *Principles of Biochemistry*, International Students 6th Edition, McGraw Hill Kogakusha Ltd., pp: 1004-5.
45. Janet, L. Christian and Janet L. Greger, 1988. *Nutrition For Living*, 2nd Edition, The Benjamin/Cummings Publishing Company, Menlo Park, California, 321: 333-6.
46. Food and Nutrition Board, 2001. Institute of Medicine. *Copper. Dietary reference intakes for vitamin A, vitamin K, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc*. Washington, D.C. National Academy Press, pp: 224-257.
47. Turnlund, J.R., 2006. Copper. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ, eds. *Modern Nutrition in Health and Disease*. 10th ed. Philadelphia: Lippincott Williams & Wilkins; pp: 289-299.

48. Uauy, R., M. Olivares, M. Gonzalez, 1998. Essentiality of copper in humans. *Am J Clin Nutr*, 67(5 Suppl):952S-959S.
49. Linder, M.C., M. Hazegh-Azam 1996. Copper biochemistry and molecular biology. *Am J Clin Nutr.*, 63(5): 797S-811S.
50. Harris, E.D., 1997. Copper. In: O'Dell BL, Sunde RA, eds. *Handbook of nutritionally essential minerals*. New York: Marcel Dekker, Inc., 231-273.
51. Abraham White, 1978. Philip Handler, Emil L. Smith and I. Robert Lehman, *Principles of Biochemistry*, International Students 6th Edition, McGraw Hill Kogakusha Ltd., pp: 1330.
52. Janet, L. Christian and Janet L. Greger, 1988. *Nutrition For Living*, 2nd Edition, The Benjamin/Cummings Publishing Company, Menlo Park, California, pp: 321.
53. Doisy, R.J., D.H.P Streeten, M.L. Souma, M.E. Kalafer, S.L. Rekant, T.G. Dalakos, 1971. Metabolism of ⁵¹Chromium in Human Subjects. In: *Newer Trace Elements in Nutrition* (edited by Mertz W, Cornatzer WE). Dekker, New York, pp: 155-68.
54. Anderson, R.A., M.M. Polansky, N.A. Bryden, K.Y. Patterson, C. Veillon, W.H. Glinsmann, 1983. Effects of chromium supplementation on urinary Cr excretion of human subjects and correlation of Cr excretion with selected clinical parameters. *J Nutr*, 113: 276-81.
55. Bunker, V.W., M.S. Lawson, H.T. Delves, B.E. Clayton, 1985. The uptake and excretion of chromium by the elderly. *Am J Clin Nutr*, 39: 797-802.
56. Anderson, R.A., A.S. Kolovsky, 1985. Chromium intake, absorption and excretion of subjects consuming self-selected diets. *Am J Clin Nutr.*, 41: 1177-83.
57. Offenbacher, E.G., H. Spencer, H.J. Dowling, F.X. Pi-Sunyer, 1986. Metabolic chromium balances in men. *Am J Clin Nutr.*, 44: 77-82.
58. Anderson, R.A., M.M. Polansky, N.A. Bryden, J.J. Canary, 1991. Supplemental-chromium effects on glucose, insulin, glucagon, and urinary chromium losses in subjects consuming controlled low-chromium diets. *Am J Clin Nutr.*, 54: 909-16.
59. Anderson, R.A., N.A. Bryden, K.Y. Patterson, C. Veillon, M.B. Andon, P.B. Moser-Veillon, 1993. Breast milk chromium and its association with chromium intake, chromium excretion, and serum chromium. *Am J Clin Nutr.*, 57: 419-23.
60. Lim, T.H., T. Sargent, N. Kusubov, 1983. Kinetics of trace element chromium(III) in the human body. *Am J Physiol*, 244: R445-54.
61. Institute of Medicine, 20001. *Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. National Academy Press, Washington, DC.
62. Jeejeebhoy, K.N., R.C. Chu, E.B. Marliss, G.R. Greenberg, A. Bruce-Robertson, 1977. Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation in a patient receiving long-term total parenteral nutrition. *Am J Clin Nutr.*, 30: 531-8.
63. Freund, H., S. Atamian, J.E. Fischer, 1979. Chromium deficiency during total parenteral nutrition. *JAMA*, 241: 496-8.
64. Brown, R.O., S. Forloines-Lynn, R.E. Cross, W.D. Heizer, 1986. Chromium deficiency after long-term total parenteral nutrition. *Dig. Dis. Sci.*, 31: 661-4.