Investigation into the Bioavailability of Some Essential Health Based Elements in Ripe and Unripe Noni Fruits (*Morinda citrifolia*)

S A Shettima[1]*, B A Madu[1] , A A Baffa[1] , A K Akinlabi[2], A S Abdulkadir[3]

[1]Chemistry Department, Federal University Gashua, Yobe State, Nigeria.
[2]Chemistry Department, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
[3]Environmental Toxicology Department, Southern University and A&M College, Baton Rouge, Louisiana, USA
*Corresponding Author's email: saidusa1961@gmail.com, Tel: +2348035054474

ABSTRACT

Noni (Morinda citrifolia) fruit has recently been gaining attention in the area of nutraceutical research due to its potential health benefits. The Fruit of the morinda citrifolia plant, also known as Indian mulberry, has been used for medicinal purposes for centuries in Polynesia and Southeast Asia and recently in Africa. The fruit has gained significant attention for its profound therapeutic and nutritional benefits in treating various ailments. This study aims to contribute to the existing literature by analyzing the presence and quantity of selected elements relevant to human health, namely Calcium, Manganese, Potassium, Phosphorus, Sodium, Nickel, Mercury, Lead, Arsenic, Chromium, and Cadmium, in both ripe and unripe Noni fruits. The highly sensitive atomic absorption spectroscopic (AAS) technique was employed for this purpose. The results revealed that ripe Noni fruit contained higher concentrations of Calcium, Potassium, Chromium, and Phosphorus, while unripe Noni fruit had higher levels of Manganese, Sodium, and Nickel.

Keywords: Nutraceutical, AAS, Morinda citrifolia, Noni, Macrominerals, Microminerals Therapeutics**.**

INTRODUCTION

Noni fruit has gained significant attention in the current era of nutraceutical research due to its profound therapeutic and nutritional health benefits. 'Noni' is the Hawaiian name for the fruit of *Morinda citrifolia L.* (Rubiaceae). The Fruit of the *Morinda citrifolia* plant, also known as Indian mulberry, has been used for medicinal purposes for centuries in Polynesia and Southeast Asia [1], and recently gained acceptance in America and Africa. This Fruit is a tropical plant belonging to the coffee family *Rubiacea*. It exists as a small evergreen tree or shrub growing to a height of

3 to 6m [2]. The Fruits are multiple, oblong, 5 to 7cm long, soft, and watery with a cheesy aroma. Since 1996, Noni products, mainly fruit juice, are available as health food worldwide. Fruit juice and leaf tea were approved by EU law as a novel food in 2003 [3]. Concurrently, Metals are essential constituents of all cellular structures, but they exhibit a narrow margin between their required intake and toxic levels [4].

This Fruit and its extracts are the subject of extensive research to elucidate their anti-cancer potential [5,6]. The fruits provide a rich source of essential minerals and trace elements that are vital for human health [7]. Numerous publications have demonstrated that Noni possesses immense potential to relieve various ailments, with its registered uses spanning across the Pacific, Asia, and Africa. In fact, two clinical studies have reported the alleviation of arthritis and diabetes upon Noni-consumption [8,9]. Such beneficial effects can be attributed to various elements and compounds such as scopoletin, nitric oxide, alkaloids, sterols, and many essential metals and non-metallic elements, as well as the potent antioxidant potential of Noni. Notably, the Fruit's reputation has led to its increased consumption in producing countries and the United States, Japan, Europe and Africa.

Our team of researchers had earlier reported from this laboratory on the investigation into the content and potential uses of Noni leaves and stembark [10].

In this work, we are investigating the presence of some essential elements in both ripe and unripe Noni fruits to explore their impact on longevity. This study employs Atomic Absorption Spectroscopy (AAS), a sensitive analytical technique to quantify the trace levels of metallic elements [11] in Noni fruit at different stages of ripeness. The elements of interest are Sodium (Na), Potassium (K), Calcium (Ca), Phosphorus (P), Cadmium (Cd), Nickel (Ni), Mercury (Hg), Lead (Pb), Arsenic (As), Manganese (Mn) and Chromium (Cr), which are widely distributed in fruits and have distinct roles in human health.

MATERIALS AND METHODS

The equipment and instruments used in this study were all calibrated to check their status in the middle of the experiments. Apparatus such as volumetric flask and measuring cylinder were thoroughly washed with detergents and tap water and then rinsed with deionized water. All glassware were cleaned with about 10% concentrated Nitric acid (HNO_3) in order to clear out any heavy metal on their surfaces and rinsed with distilled water.

Reagents and chemicals

The reagents and chemicals used for the laboratory works were all analytical grade; deionized water (chemically pure with conductivity of 1.5μs/cm and below was prepared in the Chemistry Research Laboratory of the Federal Univeristy, Gashua, Nigeria) was used for dilution of the sample, and intermediate metal standard solutions prior to analysis and rinsing glass wares and sample bottles.

Sample collection

The ripe and unripe Noni fruit (*Morinda citrifolia L.*) was collected from Nyanya area of Nasarawa State, near Abuja, Nigeria.

Sample preservation and storage

The Fresh fruits of Noni, ripe and unripe fruit flesh and seed were washed with running water, cut with a knife into small pieces, shade dried at room temperature, and then grounded. 20g of ripe and 22g of unripe dried Noni fruit powder were subjected to elemental analysis using a calibrated AAS machine (Buck scientific model 210GP). The preservation was done such that the sample contained 1% HNO_3 by adding 0.75ml of HNO_3 to the sample of Noni fruit and making the entire volume to 750ml using deionized water. Plastic containers were used to keep the sample to avoid contamination, and the samples were stored at 4^{o}C in a refrigerator prior to the analysis.

Preparation of standard and working solutions

The metal concentration for each of the metals was determined in the experimental solution based on its respective calibration curve. In plotting the calibration curves for Pb, Zn, Cd, and other elements, a stock solution for each metal ion supplied by the manufacturer's company was used, from which a standard working solution of 100 ppm was prepared.

The 100ppm working solution was prepared from the earlier 1000ppm stock solution. A simple dilution formula ($C_1V_1 = C_2V_2$) was used to calculate the volume of the stock solution to be diluted to the new desired concentration. 1mL of concentrated HNO_3

was added to each working standard and finally diluted to the desired volume with deionized water.

Determination of Elements Concentration Using AAS Method

Preparation of Calibrated Curve

Calibration curves were prepared to determine the concentration of the metals in the sample solution. The instrument was calibrated using a series of working standards. The working standards of solutions of each metal were prepared from standard solutions of their respective metals, and their absorbance was taken using the AAS. A calibration curve for each metal ion to be analyzed was prepared by plotting the absorbance as a function of metal ion standard concentration.

Determination of elements in the samples

The concentration of the metal ions in the samples was determined by reading their absorbance using AAS (Buck scientific model 210GP) and comparing it to the respective standard calibration curves. Three replicate determinations were carried out for each sample. The metals were determined by absorption/concentration mode, and the instrument readout was recorded for each solution manually. The same analytical procedure was employed for the determination of elements in digested blank solutions and for the picked samples.

RESULTS AND DISCUSSION

Results

The elemental contents obtained for both ripe and unripe Noni fruit samples obtained from Nyanya, Nasarawa-Abuja, Nigeria, are presented in the Table below. WHO standards/ maximum permissible levels for the selected elements are also presented for ease of comparison and benchmarking.

Table 1 Elemental Composition of Ripe and Unripe Noni Fruit

Detected Elements	Ripe (mg/kg)	UNRIPE (mg/kg)	WHO standard
Calcium	373.09	354.93	800
Manganese	4.76	5.46	6.06
Potassium	1836.56	1648.07	2000
Phosphorus	538.35	511.48	700
Sodium	281.43	525.10	1000
Nickel	0.629	0.880	10
Mercury	0.00	0.000	0.001
Lead	0.023	0.012	0.1
Arsenic	0.000	0.000	0.2
Chromium	0.270	0.149	1.3
Cadmium	0.000	0.000	0.02

The Table showed the mineral content of ripe Noni fruit as Calcium (373.09), Sodium (525.10), Manganese (4.76), Potassium (1836.55), Phosphorus (538.35), Nickel (0.629), Lead (0.023) and Chromium (0.270).

Similarly, for the unripe Noni fruits, the mineral content was Calcium (354.93), Manganese (5.46), Potassium (1643.07), Phosphorus (511.48), Sodium (281.43), Nickel (0.880), Lead (0.012) and Chromium (0.419).

On the other hand, the elements Cadmium, Mercury, and Arsenic were not detected in either ripe or unripe Noni fruit.

Discussion

Minerals are inorganic substances that are required for various biological processes in the body. The minerals can be classified into macrominerals (Na, K, Ca, Mg, P, S) that are needed in large quantities by the body and microminerals (Fe, Zn, Cu, Mn, I, Se, Mo, Cr, Ni) that are needed in trace quantities by the same body. All of these minerals have diverse roles in different cells and tissues. Some of them function as enzyme cofactors or structural components of biomolecules. Others regulate the membrane potential or the osmotic balance of cells. Still, others modulate the activity of hormones or neurotransmitters.

A comparative analysis of the results obtained for both the ripe and unripe Noni fruits using the AAS technique revealed that ripe Noni had higher concentrations of Calcium (373.09), Potassium (1836.56), Phosphorus (538.35), Chromium (0.270), while unripe Noni had higher concentrations of Manganese (5.46), Sodium (525.10) and Nickel (0.880).

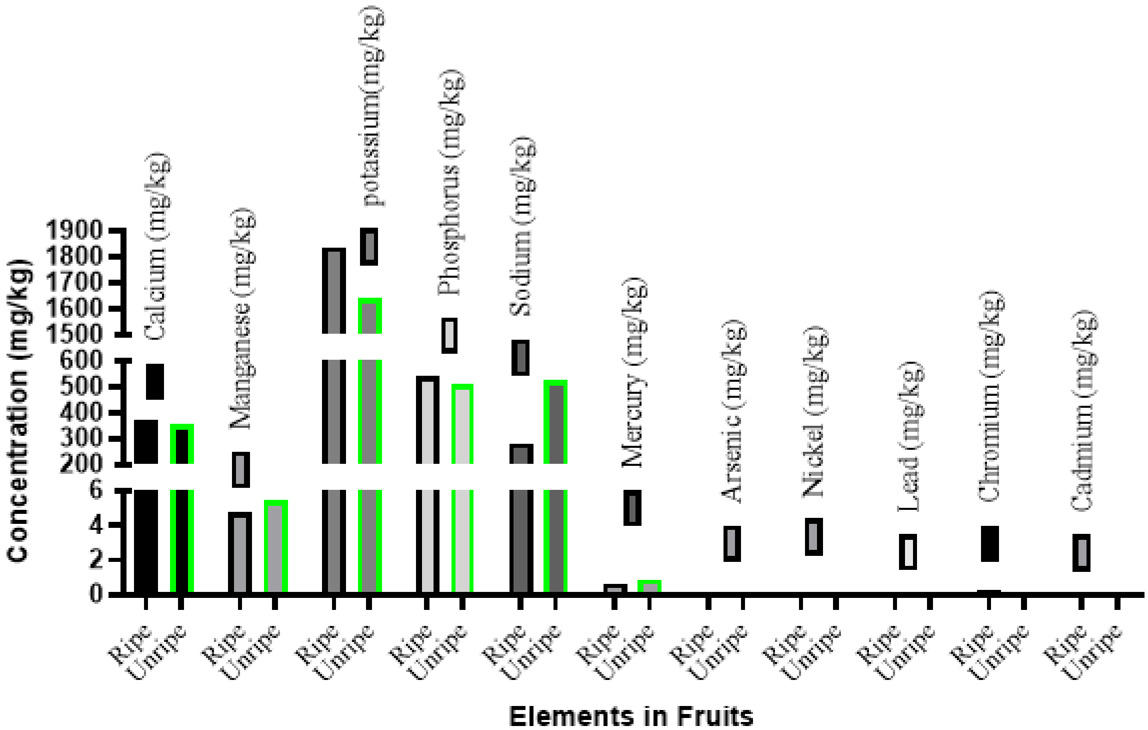

Figure 1: Bar chart comparing the concentration of elements in ripe and unripe Noni Fruits.

The results demonstrated that most of the elements tested in this work had similar concentrations in ripe and unripe Noni fruit, except for Sodium, which exhibited a significant difference. The ripe Noni fruit had a higher sodium content (525.10 mg/kg) than the unripe Noni fruit (281.43 mg/kg). This discrepancy may be attributed to the difference in the pH levels of the fruits, as unripe Noni fruit tends to have a higher alkalinity than ripe Noni fruit, which may influence their sodium content. Sodium is an essential mineral that regulates the body's fluid balance, nerve function, and muscle contraction. Although most fruits have low sodium levels, some fruits, such as avocados and tomatoes, contain small amounts of Sodium.

The results also indicated the calcium and potassium content of ripe and unripe Noni fruit and their implications for human health. Calcium is an essential mineral that supports the growth and maintenance of bones and teeth and the function of muscles, blood clotting, and nerves [12]. Several fruits are rich in Calcium, such as oranges, kiwis, and figs

[13]. Likewise, Potassium is another vital mineral that regulates blood pressure and fluid balance, as well as the function of muscles, nerves, and heart rhythm [13]. Some fruits that contain high amounts of Potassium are bananas, cantaloupes, and apricots. This study shows Potassium as having the highest concentration of all the tested minerals in the Noni fruit, with the ripe and unripe fruits having 1836.56 and 1643.07 mg/kg, respectively. These results suggest that potassium concentration in ripe Noni is closer to the WHO recommended amount of 2000 mg/L. While both ripe and unripe Noni have significant levels of Potassium, ripe Noni has a higher concentration, which may have more significant health implications. This may suggest that Noni fruit has beneficial effects on blood pressure and fluid balance, but excessive intake may also cause adverse effects such as hyperkalemia or kidney damage [14,15.]

Chromium is a trace element that is required for the metabolism of carbohydrates, proteins, and fats. It also plays a role in insulin sensitivity and may benefit individuals with type 2 diabetes [16]. Other fruits that are good sources of chromium include apples and grapes.

Phosphorus is a critical element that is necessary for the growth and maintenance of healthy bones and teeth. It also plays a role in energy metabolism and is involved in the function of nerves and muscles, including the heart [17]. Many fruits, such as grapes and pineapples, are rich sources of phosphorus. The high phosphorus concentrations of both ripe and unripe Noni fruit indicate that noni fruit may benefit bone health, energy production, and nerve and muscle activity.

Manganese is an essential trace element for the metabolism of carbohydrates, amino acids, and cholesterol. It is also involved in bone formation and wound healing [13]. Fruits that are rich in Manganese include raspberries and pineapples. In Noni fruit juice, Rybak and Ruzik (2013) reported that Manganese is complexed from flavonoids rutin, dyes like anthraquinone (alizarin), and glycosides asperulosidic acid [18]. Furthermore, this study detected Mn concentrations of 5.406 and 4. mg/l in ripe and unripe Fruit, which was more than a 20-fold increase compared to the 0.3 mg/l reported by Rybak and Ruzik (2013) study [18]. maintain healthy fluid balance in the body and blood pressure [19]. It also plays a role in nerve and muscle function [19]. While many fruits are low in Sodium, some, such as avocados and tomatoes, contain small amounts. Therefore, its concentration in food has been a topic of interest for its potential health implications due to excessive intake of Sodium in the Western diet, which can lead to health problems such as high blood pressure, heart disease, and stroke. The recommended daily intake of Sodium for adults is 1000 mg per day, with an ideal limit of no more than 1,500 mg per day [19]. The sodium concentration in unripe Noni, as reported, is 281.56 mg/L, while the sodium concentration in ripe Noni is reported to be 525.07 mg/L. The implications of consuming Noni fruit with varying Sodium levels on human health remain unclear and require further investigation; however, the risk factor is significantly reduced.

Nickel, a trace element required for the metabolism of certain enzymes in the body, is not considered an essential nutrient, although some research work suggested [21-25] that it may play a role in the immune system, metabolism, blood formation, and bone health. Nickel is a trace element that is required for the metabolism of certain enzymes in the body. It is important to note that the aforementioned mineral concentrations may vary depending on various factors, such as the region in which the Noni fruit is grown, the maturity of the Fruit at the time of testing, and the methods used for testing. The higher concentrations of Calcium, Potassium, and Phosphorus in ripe noni fruit are significant, as these elements are essential for maintaining healthy bone density, preventing osteoporosis nerve transmission, muscle contraction, and energy metabolism. Additionally, the higher concentration of chromium in ripe Noni fruit may have implications for its potential use as a natural supplement for individuals with type 2 diabetes, as chromium has been shown to improve insulin sensitivity [1,26–28].

Even though the concentrations of all elements in both ripe and unripe noni fruit are below the WHO maximum permissible levels, suggesting both ripe and unripe Noni fruit are safe for human consumption, the concentrations of mercury, nickel, lead, and arsenic are higher in unripe Noni fruit than in ripe noni fruit (Table 1). These elements are toxic to human health, as they can cause various adverse effects such as neurological damage, kidney failure, anemia, and cancer [4,29].

This study's findings are consistent with previous studies showing that Noni-fruit compounds have antioxidant and antiinflammatory properties[30]. In addition, the high Sodium, Potassium, Calcium, and Phosphorus levels in Noni-fruit compounds make them essential in maintaining proper body function.

CONCLUSION

This study denotes that noni fruit is a potential source of minerals, nutrition, and essential elements for human health. Our analyses revealed that ripe noni fruit had higher concentrations of Calcium, Potassium, Phosphorus, and Chromium, while unripe Noni fruit had higher concentrations of Manganese, Sodium, and Nickel. These findings have significant implications for the potential use of noni fruit as a natural supplement for maintaining healthy blood pressure, fluid balance, bone density, and improving insulin sensitivity and other health benefits. Furthermore, locational variation on where the Noni plant is grown, be it Africa, America. Asia or Europe does not seem to show significant variation in the nutritional and nutraceutical contents of the plant. Despite these findings, further research needs to be done to have deeper understanding on the health implications of these findings, including the bioavailability of other essential elements not investigated in this work present in the Noni fruit grown in Nigeria.

REFERENCE

1. Ali, A., Ma, Y., Reynolds, J., Wise, J. P., Inzucchi, S. E., & Katz, D. L. (2011). Chromium Picolinate for the Prevention of Type 2 Diabetes. *Treatment Strategies. Diabetes*, *3*(1), 34. /pmc/articles/PMC4169208/

2. Palu, A. K., Kim, A. H., West, B. J., Deng, S., Jensen, J., & White, L. (2008). The effects of Morinda citrifolia L. (Noni) on the immune system: its molecular mechanisms of action. *Journal of Ethnopharmacology*, *115*(3), 502–506. https://doi.org/10.1016/J.JEP.2007.10.023

3. David, B. (2003). Commission Decision of 5 June (2003)/426/EC: authorising the placing on the market of 'noni juice' (juice of the Fruit of Morinda citrifolia L.) as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliam ent and of the Council. *(Commission of the European communities*

4. Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R., & Sadeghi, M. (2021). Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. *Frontiers in Pharmacology*, *12*, 643972. https://doi.org/10.3389/FPHAR.2021.643972/BIBTEX

5. Chan-Blanco, Y., Vaillant, F., Mercedes Perez, A., Reynes, M., Brillouet, J. M., & Brat, P. (2006). The noni fruit (Morinda

citrifolia L.): A review of agricultural research, nutritional and therapeutic properties. *Journal of Food Composition and Analysis*, *19*(6–7), 645–654. https://doi.org/10.1016/J.JFCA.2005.10.001

6. Kaur, H., Gurjar, N., & Gill, R. (2018). The noni fruit (Morinda citrifolia L.): A systematic review on anti-cancer potential and other health beneficial pharmacological activities. *Journal of Medicinal Plants Studies*.

7. Basar, S., & Westendorf, J. (2012). Mineral and Trace Element Concentrations in Morinda citrifolia L. (Noni) Leaf, Fruit and Fruit Juice. *Food and Nutrition Sciences*, *3*, 1176–1188. https://doi.org/10.4236/fns.2012.38155

8. Solomon, N. & Udall, C. (1999). The noni phenomenon. 296.

9. Elkins, R. (1998). *Hawaiian Noni (Morinda Citrifolia): Prize Herb of Hawaii and the South Pacific*. 30. https://books.google.com/books/about/Hawaiian_Noni_Morinda_Citrifolia.html?id=EFeCm4KG7IsC

10. Shettima, S. A., Baffa, A. A., Uzoma, O. B., Akinlabi, A. K., & Shettima, A. A. (2023). Investigation into the Potential Uses of Noni (Morinda citrifolia) Leaves and Stem Bark. *Journal of Chemical Society of Nigeria*, *48*(2), 326–332. https://doi.org/10.46602/JCSN.V48I2.874

11. *Atomic Absorption Spectroscopy, How Does AAS Work, AAS FAQs | Agilent*. (n.d.). Retrieved 4 May 2023, from https://www.agilent.com/en/support/atomic-spectroscopy/atomic-absorption/flame-atomic-absorption-instruments/how-does-aas-work-aas-faqs

12. Harvart Chan. (2023). *Calcium | The Nutrition Source | Harvard T.H. Chan School of Public Health*. https://www.hsph.harvard.edu/nutritionsource/calcium/

13. Pal, M., & Molnár, J. (2021). Growing Importance of Fruits and Vegetables in Human Health. *International Journal of Food Science and Agriculture*, *5*(4), 567–569. https://doi.org/10.26855/IJFSA.2021.12.001

14. *A Primer on Potassium | American Heart Association*. (n.d.). Retrieved 3 August 2023, from https://www.heart.org/en/healthy-living/healthy-eating/eat-smart/sodium/potassium

15. David Rossiaky. (2023). 10 Effects of Hyperkalemia on the body. *Healthline*. https://www.healthline.com/health/high-potassium/effects-on-the-body

16. Kilner, J. A., Druce, J., & Ishihara, T. (2022). Electrolytes. *High-Temperature Solid Oxide Fuel Cells for the 21st Century: Fundamentals, Design and Applications*, 85–132. https://doi.org/10.1016/B978-0-12-410453-2.00004-X

17. Chan, H. (2023). *Phosphorus | The Nutrition Source | Harvard T.H. Chan School of Public Health*. 2023. https://www.hsph.harvard.edu/nutritionsource/phosphorus/

18. Rybak, J., & Ruzik, L. (2013). Application of chromatography and mass spectrometry to the characterization of cobalt, copper, Manganese and molybdenum in Morinda Citrifolia. *Journal of Chromatography A*, *1281*, 19–25. https://doi.org/10.1016/J.CHROMA.2013.01.040

19. American Heart Association. (n.d.). How much Sodium should I eat per day? | Retrieved 5 May 2023, from https://www.heart.org/en/healthy-living/healthy-eating/eat-smart/sodium/how-much-sodium-should-i-eat-per-day

20. Lewis, J. L. (2022). *Overview of Sodium's Role in the Body - Hormonal and Metabolic Disorders - Merck Manuals Consumer Version*. https://www.merckmanuals.com/home/hormonal-and-metabolic-disorders/electrolyte-balance/overview-of-sodiums-role-in-the-body

21. RxList. (n.d.). *Nickel: Health Benefits, Side Effects, Uses, Dose & Precautions*. Retrieved 4 August 2023, from https://www.rxlist.com/hiv_aids_myths_and_facts_slideshow_pictures/article.htm

21. Humanitas Research Hospital. (n.d.). *Nickel - Humanitas.net*. Retrieved 5 May 2023, from https://www.humanitas.net/wiki/mineral-salts/nickel/

22. Smialowicz, R. J., Rogers, R. R., Riddle, M. M., & Stott, G. A. (1984). Immunologic effects of nickel: I. Suppression of cellular and humoral immunity. *Environmental Research*, *33*(2), 413–427. https://doi.org/10.1016/0013-9351(84)90039-2

23. Di Gioacchino, M., Boscolo, P., Cavallucci, E., Verna, N., Di Stefano, F., Di Sciascio, M., Masci, S., Andreassi, M., Sabbioni, E., Angelucci, D., & Conti, P. (2000). Lymphocyte subset changes in blood and gastrointestinal mucosa after oral nickel challenge in nickel-sensitized women. *Contact Dermatitis*, *43*(4), 206–211. https://doi.org/10.1034/J.1600-0536.2000.043004206.X

24. Salsano, F., Francia, C., Roumpedaki, I., Proietti, M., Pisarri, S., Verna, N., Gabriele, E., Di Gioacchino, G., & Di Gioacchino, M. (2004). Immune Effects of Nickel. *Http://Dx.Doi.Org/10.1177/03946320040170S211*, *17*(2 Suppl), 63–69. https://doi.org/10.1177/03946320040170S211

25. Büdinger, L., & Hertl, M. (2000). Immunologic mechanisms in hypersensitivity reactions to metal ions: An overview. *Allergy: European Journal of Allergy and Clinical Immunology*, *55*(2), 108–115. https://doi.org/10.1034/J.1398-9995.2000.00107.X

26. Wang, Z. Q., Yu, Y., Zhang, X. H., & Komorowski, J. (2014). Chromium-Insulin Reduces Insulin Clearance and Enhances Insulin Signaling by Suppressing Hepatic Insulin-Degrading Enzyme and Proteasome Protein Expression in KKAy Mice. *Frontiers in Endocrinology*, *5*(JUL), 99. https://doi.org/10.3389/fendo.2014.00099

27. Feiner, J. J., Mcnurlan, M. A., Ferris, R. E., Mynarcik, D. C., & Gelato, M. C. (2008). Chromium picolinate for insulin resistance in subjects with HIV disease: A pilot study. *Diabetes, Obesity and Metabolism*, *10*(2), 151–158. https://doi.org/10.1111/j.1463-1326.2006.00681.x

28. Cersosimo, E., & DeFronzo, R. A. (2006). Insulin resistance and endothelial dysfunction: The road map to cardiovascular diseases. *Diabetes/Metabolism Research and Reviews*, *22*(6), 423–436. https://doi.org/10.1002/dmrr.634

29. Cleveland Clinic. (2022). *Heavy Metal Poisoning (Heavy Metal Toxicity): Symptoms, Causes & Treatment*. https://my.clevelandclinic.org/health/diseases/23424-heavy-metal-poisoning-toxicity