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Stable heavy isotopes in human health

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Abstract

Applications of heavy stable isotopes in the field of human health are rather limited compared with applications employing radioactive isotope tracers. The elements reviewed in this paper encompass zinc, copper, iron, calcium, selenium and lead. Most research for the first five metals has addressed metabolic aspects, specifically fractional absorption ("bioavailability"). Lead isotopes, although employed for pioneering pharmacokinetic modelling research in the early 1970s, have not enjoyed widespread acceptance until more recent times. An example is given of the use of lead isotopes to detect changes to blood lead from dietary sources and the contribution of skeletal lead to blood lead.

Introduction

Most emphasis on isotopic methods in human health has been directed towards the use of light stable isotopes of carbon, nitrogen, oxygen and hydrogen for body composition, energy metabolism and macronutrient metabolism (Jones 1990). So-called "mineral" (?heavy) stable isotopes have been commonly employed as short-lived radioactive tracers. Limited use of thermal ionisation mass spectrometry (TIMS) and inductively coupled plasma mass spectrometry (ICP-MS) as well as fast atom bombardment mass spectrometry (FAB-MS) has also largely been directed towards metabolic studies, specifically bioavailability and, in the case of lead, evaluation of sources and pathways. Part of the reason for the limited use of these methods may be a narrowing of research into highly specialised fields, poor communication between disciplines, and arrogance of certain groups. Another reason for the limited use of isotopic methods in the health field is because of the perceived high cost of the measurements as a result of the high cost of the enriched isotopes. This problem is exacerbated by the use of low precision mass spectrometry (ICP-MS), which makes it necessary to use large doses of enriched isotopes.

In this paper, I will briefly review the use of stable heavy isotopes in human health, although the classification of the elements discussed may be disputed by some as not "heavy" and perhaps the term "mineral" should be employed. Elements reviewed are calcium, copper, iron, selenium and zinc with respect to; bodily functions, isotopes, methods of analysis, isotopic research and main references.

Information on bodily functions is mainly taken from Florence & Setright (1994).

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Calcium (Ca)

Bodily Functions: An adult body contains ≈ 1 kg Ca, 99% of which is located in bones and teeth in the form of apatite. The other ~1% is found in intra- and extracellular fluids, where it plays a vital role in directing cell functions and nerve impulses. The Ca concentration in blood serum is critical and lies between narrow limits of 90-100 mg/L^{-1}. Parathyroid hormone and Vitamin D regulate Ca intake. If the intake is too low, Ca is "resorbed" from bone stores. If bone depletion continues for extended periods, this condition can give rise to osteoporosis. Maintenance of Ca homeostasis during pregnancy and lactation is critically important for females.

Isotope*	40	***42***	43	***44***	46	49
% Abundance	96.9	0.65	0.14	2.08	0.03	0.19

* Isotopes in ***bold italic*** are those primarily used in investigations

Methods: TIMS/ Fast Atom Bombardment-MS.

Isotopic Research: Fractional Absorption (FA) especially in the study of bone turnover status/ bone loss and osteoporosis. In metabolic investigations of bioavailability (fractional absorption, FA; the amount of the substance taken up by the body compared with the total amount introduced to the body) employing dual isotopic methods, one of the isotopes is introduced orally and the other by intravenous injection. For example, ^{44}Ca may be introduced orally in milk and, a short time later, the ^{42}Ca isotope is injected. Blood and urine samples are collected serially, commonly over 24 hours. For example, in a study of Chinese children on a low Ca diet of 359 mg d^{-1}, the FA was 63%; with a high Ca diet of 862 mg d^{-1}, the FA was 55% (Lee *et al.* 1994). In Caucasian children, the FA was ≈40% (Lee *et al.* 1994). In a study of one male and one female subject, Price *et al.* (1990) measured a FA of 70%. Abrams (1993) employed TIMS to determine the rate of Ca deposition in bone and the size of the exchangeable Ca pool in bone in girls at puberty, the time of maximum growth associated with the peak of bone mass accumulation and Ca retention.

Main References: Abrams *et al.* (1991, 1993, 1994), TIMS; Eastel *et al.* (1989), TIMS; Lee *et al.* (1994), TIMS; Miller *et al.* (1989), FAB-MS; Moore *et al.* (1985), TIMS; Price *et al.* (1990), TIMS; Smith *et al.* (1985), FAB-MS; Yergey *et al.* (1987,1990,1994), TIMS.

Copper (Cu)

Bodily Functions: A 70-kg human contains about 80 mg Cu, mostly located in muscle and liver. Copper (and Fe) are the primary oxygen carriers in cells. A Fe-Cu-Zn enzyme, cytochrome oxidase, is present in mitochondria and is responsible for the catalysis of oxygen to water, an impotant step in cellular metabolism. Other Zn enzymes in mitochondria act as antioxidants to remove free radicals, which could otherwise promote cellular damage. Deficiency in Cu can result in anaemia, osteoporosis, reproductive failure, and heart failure.

Isotope	63	***65***
% Abundance	69.1	30.9

Method of analysis: TIMS.

Isotopic Research: Bioavailability (FA) for an adequate dietary intake of 1.68 mg d^{-1}, was 36%; on a low intake of 0.79 mg d^{-1}, the FA was 56%; and on a high intake of 7.5 mg d^{-1}, the FA was 12%. There is a Cu balance (homeostasis) with 0.8 mg Cu d^{-1}, and the regulation of Cu absorption and endogenous loss gives protection from Cu deficiency on the one hand, and toxicity on the other.

Main Player: Turnlund *et al.* (1989).

Iron (Fe)

Bodily Functions: In an adult male, ≈4 g Fe is distributed among the protein, haemoglobin (73%), ferritin and hemosiderin (12%), and myoglobin (14%). Haemoglobin is the main constituent of red blood cells. Its main function is to transport oxygen from the lungs in arterial blood to various organs and tissues, and return in venous blood carrying some carbon dioxide.

Isotope	***54***	56	***57***	***58***
% Abundance	5.8	91.7	2.14	0.28

Methods of Analysis: TIMS / ICP-MS / FAB-MS.

Isotopic Research: When added to the diet, there was a FA of ≈9% in the elderly, using faecal composites as the sampling medium (Turnlund 1983). Iron bioavailability in blood from Fe tablets was measured by Hansen *et al.* (1992). Iron distribution among the protein species transferrin, ferritin, and other haemoproteins was measured in liver and heart by Stuhne-Sekalec *et al.* (1992).

Main References: Turnlund (1983), TIMS; Stuhne-Sekalec *et al.* (1992), ICP-MS; Hansen *et al.* (1992), FAB-MS.

Lead (Pb)

Bodily Function: No natural physiological use.

Isotope	***204***	***206***	***207***	***208***
% Abundance	1.4	24.1	22.1	52.4

Methods of Analysis: TIMS / ICP-MS.

Main References: Pharmacokinetic Modelling, Rabinowitz *et al.* (1976), Heusler-Bitschy *et al.* (1988); pregnancy / personal monitoring, Manton (1985,1992); efficacy of chelating agents, Smith *et al.* (1994); source of Pb in humans, Yaffee *et al.* (1983), Tera *et al.* (1985), Faccheti (1989), Campbell & Delves (1989); Biokinetics of lead in pregnancy, Gulson *et al.* (1995 a,c, 1996c), Gulson & Calder (1995).

Example of the Use of Lead Isotopes: In a project on the "biokinetics of lead in human pregnancy", the primary objective was to establish if lead is released from the maternal skeleton during pregnancy and lactation (Gulson *et al.* 1995c). The main cohort were subjects from countries outside of Australia because the lead isotopic ratios in their skeleton are different from those in the "long-term" Australian population. The Australian population has $^{206}Pb/^{204}Pb$ ratios generally less than 17.0, whereas people from other countries (and especially Eastern Europe), have ratios >18.0. If a subject conceives and Eastern European lead, for example, is observed in the blood, then this is an indication that the lead is derived from maternal skeletal stores and not from Australian environmental sources such as diet , soil or dust. The subjects in this study who conceive had a matched non-pregnant control. During pregnancy, the subjects and controls were monitored monthly for blood and urine samples, and quarterly for environmental samples of house dust, drinking water, and a 6-day duplicate diet.

In one control subject, blood samples exhibited a sudden change in isotopic composition and increased lead concentration (Figure 1), in the direction which was predicted for a pregnant subject. Upon detailed questioning about dietary changes, the only plausible change was daily consumption of several glasses of beverages made with water from a newly-acquired Russian samovar. Testing of the samovar water showed it to have lead concentrations at least 20 times above fully-flushed kitchen tap water and isotope ratios consistent with Russian values.

Besides negating her role as a control, the other disconcerting aspect of these results was the rapidity of change in both isotopic composition and blood lead concentration. The implication of these rapid changes is that the bioavailability of the lead in the water is very high.

The other features of the data shown in Figure 1 are the changes in lead in blood when moving from one country to another. The blood lead concentrations in this subject are low when compared with a value of <10 µg Pb dl^{-1}, the Australian National Health and Medical Research Council National Goal for all Australians. There was little change in Pb until the samovar incident. In contrast, the isotopic composition showed a rapid decrease for all subjects from Eastern Europe when they

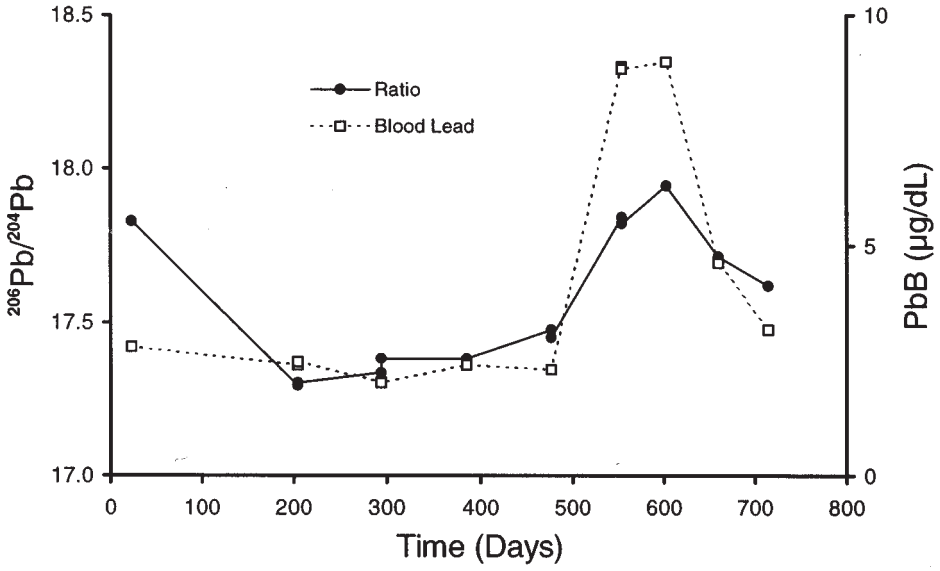

Figure 1. Time-series variation of isotope ratio, expressed as the abundance of the ^{206}Pb to ^{204}Pb, and blood lead (PbB) of a subject from Eastern Europe. The marked increase in $^{206}Pb/^{204}Pb$ ratio and PbB was traced to consumption of water from a Russian samovar.

arrive in Australia, arising from the exchange of European lead with that in the Australian environment (Gulson *et al.* 1995c). The results of this study demonstrated, for the first time, the quantitative contribution of skeletal lead to blood lead. In the case of the subject in Figure 1, skeletal lead is estimated to have a $^{206}Pb/^{204}Pb$ ratio of 18.0 and this is exchanging with Australian lead with a $^{206}Pb/^{204}Pb$ of 17.0. At 200-300 days, the $^{206}Pb/^{204}Pb$ in the blood of this subject was ≈17.4, which means that approximately 40% of the lead in her blood was deriving from skeletal sources.

Selenium

Bodily Functions: Selenium is an important antioxidant. For example, glutathione peroxidase converts H_20_2 to water, reducing cell destruction and hence combating degenerative diseases. There are 13 Se-containing proteins involved in the correct functioning of the thyroid gland. Selenium is also a strong inhibitor of platelet aggregation, a process involved in strokes, heart disease and cancer metastases.

Isotope	***74***	76	77	***78***	80	82
% Abundance	0.9	9.0	7.5	23.5	50	9.0

Methods of Analysis: ICP-MS.

Isotopic Research: FA is up to 70% when present as selenomethionine (organic form in food) compared with 40% in inorganic form. Selenium acts synergistically with Vitamin C as an antioxidant.

Main References: Kasper *et al.* (1984), Martin *et al.* (1988, 1989a,b), Sirichakwal *et al.* (1985), Solomons *et al.* (1986).

Zinc

Bodily Functions: In an adult, there is ≈2g Zn in muscle, bone and tissues. It is essential for bone formation, wound healing, immune system fuction, ageing, sexual function, anexoria nervosa, and reduction of cadmium toxicity. Over 200 enzyme systems require Zn for structural integrity or catalysis, including synthesis of DNA and RNA.

Isotope	***64***	66	***67***	68	70
% Abundance	48.8	27.8	4.1	18.6	0.62

Methods of Analysis: 'I'IMS / ICP-MS / FAB-MS.

Isotopic Research: A study by August *et al.* (1999) indicated that for a diet with Cu addition, FA was ≈40% in the young and ≈21% in the elderly; for a diet with no addition of Cu, FA was ≈31% in the young and ≈17% in the elderly (Turnlund *et al.* 1986). Later work, however, by Couzy *et al.* (1993) using radioisotope methods indicated that age differences were not significant for Zn FA. In another study, Fairweather-Tait *et al.* (1992) showed that there was no difference in the FA (≈30%) of Zn for white compared with wholemeal bread. Jackson *et al.* (1984) and Lowe *et al.* (1993) investigated Zn biokinetic modelling (definition of Zn compartments in the body).

Main Players: Turnlund *et al.* (1984, 1986, 1991), TIMS; Jackson *et al.* (1984), TIMS; Couzy *et al.* (1993), radioisotopes; Knudsen *et al.* (1995), ICP-MS; Eagles *et al.* (1989); Janghorbani *et al.* (1984, 1990), ICP-MS.

Concluding comments

This brief review illustrates that there is considerable scope for investigations in human health using isotopic techniques with stable isotopes. One concern that I have is whether or not many of the metabolic experiments for bioavailability are really applicable to "real life". The elements are commonly introduced as pure compounds and these species may not be in the appropriate bioavailable form. For example, the FA of Fe was measured in one experiment as ≈9% in elderly subjects. It is fairly well established that the bioavailability of Haeme

Fe varies according to the food item: in meat it is ≈20%, in fish ≈6%, in cereals and vegetables ≈2-5%, and in breast milk the bioavailability is ≈50% versus <5% for formula. Furthermore, drinking alcohol with meals enhances bioavailability of Fe (Florence & Setright, 1994).

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