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High-precision isotopic analysis of essential mineral elements in biomedicine: natural isotope ratio variations as potential diagnostic and/or prognostic markers

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ABSTRACT

High-precision isotopic analysis of essential mineral elements, mainly Ca, Cu, Fe and Zn, provides relevant biomedical information. For this application, multi-collector ICP-mass spectrometry is the preferred technique. Variation in the isotopic signature of these elements is governed by alterations in their uptake, metabolism and/or excretion. Therefore, diseases that affect mineral metal metabolism, such as hemochromatosis, cancer, liver cirrhosis and Wilson's disease, affect the isotopic composition of these elements in some body compartments. This review discusses how natural isotope ratio variations in biofluids can potentially be exploited as alternative approaches for the diagnosis of diseases that can otherwise only be established at a later stage or *via* a more invasive method and/or for prognostic purposes. This discussion also includes an evaluation of the isotopic variability in biofluids for apparently healthy individuals and in biofluids, soft tissues and bone of experimentally controlled animals. Physiological and lifestyle factors were also paid attention to.

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1. Introduction

Elements with two or more isotopes may show measurable natural variations in their isotopic composition, and thus isotope ratios, as a result of mass-dependent isotope fractionation accompanying physical processes and/or biochemical reactions, *i.e.* isotopes of the same element take part to a slightly different extent to these processes/reactions [1,2]. The lighter of two isotopes reacts slightly faster (kinetic effect) and the heavier one shows a slight preference for the strongest bond (thermodynamic or equilibrium effect). These effects are more pronounced in light elements, (e.g., H, C, N, O and S), which show larger *relative* mass differences between the

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isotopes, and therefore, their isotopic signatures have already been studied in various contexts. Within the last 50 years, the $^{13}C/^{12}C$ ratio determined *via* gas source isotope ratio mass spectrometry, for instance, provided an enhanced insight in human metabolism and nutritional status. Its potential for doping control in sports and for tracing down alterations caused by disease has been reported [3–7]. Advances in analytical instrumentation are relied on to develop new applications within this field; changes in the ($^{13}CO_2/^{12}CO_2$) ratio as measured *via* laser-based high-resolution cavity-enhanced absorption spectroscopy in exhaled breath CO_2 can be used for the accurate real-time monitoring of individuals with small intestinal bacterial overgrowth and inflammatory acute phase response (early detection of sepsis) [6,7].

Currently, several research groups are exploring the possibility of using high-precision isotopic analysis of 'heavier' essential elements, such as Ca, Cu, Fe, Zn, and to a lower extent, S and Sr, for biomedical applications. Multi-collector ICP-mass spectrometry (MC-ICP-MS) is nowadays the preferred technique for tracing down and quantifying such small isotope ratio variations caused by isotope fractionation effects. The low sample throughput of thermal ionization mass spectrometry (TIMS, typically <10 samples per day) and its limitation to elements with an ionization energy ≤7.5 eV (at least for the production of M^+ ions) reduces its applicability in this research context, which generally involves analysis of large sample collections. In addition, MC-ICP-MS provides an isotope ratio precision (down to 0.002% RSD under optimum conditions) that rivals with that attainable *via* TIMS (down to 0.001% RSD in the best of cases) [8].

The biomedical applications reported on so far focused on human biofluids, such as whole blood, serum and/or urine, and to a less extent on the body fluids, soft tissues and bone of experimentally controlled animals (e.g. [9–11],) and/or archaeological bone samples [12–15]. In general, high-precision isotope ratio measurements by MC-ICP-MS and TIMS are performed in solution, after acid digestion of the sample and isolation of the target element from the sample matrix. Two applications combined laser ablation (LA) with MC-ICP-MS for direct isotope ratio determination in solid samples, such as thin sections of animal tissue [16] or dried urine spots [17].

The overall goals of this type of research are (i) obtaining a more profound understanding of human metabolism and (ii) providing alternative approaches or potential biomarkers for the early diagnosis of diseases that can otherwise only be established at a later stage or *via* a more invasive method, such as a biopsy, and/or for prognosis. In this review, we illustrate the potential of high-precision isotopic analysis of essential elements, such as Ca, Cu, Fe and Zn, for such purposes. A *conditio sine qua non* for this type of application is a realistic view on the spread of the isotope ratio measured within healthy individuals (reference population or controls) and an understanding of the sources of variation, primarily related with physiological and lifestyle factors (e.g., gender, age, ethnicity, diet). The analytical protocols used to date and the isotope ratio data reported so far are also covered in this review.

2. High-precision isotopic analysis of essential mineral elements

2.1. Analytical protocol

Since most of the biomedical applications relying on high-precision isotopic analysis of essential mineral elements published today rely on MC-ICP-MS, this section is mainly focused on the use of this analytical technique. Isotope ratio data obtained *via* MC-ICP-MS can be compromised by instrumental mass discrimination – the bias thus caused is larger than that caused by isotope fractionation in TIMS. Mass discrimination is a result of the dependence of the ion transmission efficiency on the ion mass; the ion transmission efficiency is higher for the heavier of any two isotopes (by approximately 1% per atomic mass unit (amu) for most of the elements of interest discussed in the context of this paper and by approximately 5% per amu for S). The matrix composition and even the target element concentration itself affect the extent of instrumental mass discrimination. Therefore, an efficient separation of the target element(s) from the sample matrix, typically by ion-exchange chromatography, is mandatory to avoid such matrix effects and to reduce potential spectral interference to the largest possible extent. Quantitative element recoveries should be obtained to avoid on-column fractionation, caused by the slightly different affinities for the stationary phase resin and/or phase exchange rates displayed by the different isotopes of the same element [18], from affecting the final results. Table 1 compiles some procedures for the isolation of Ca, Cu, Fe and Zn from the matrix *prior* to their isotopic analysis *via* MC-ICP-MS. As can be seen, Cu, Fe and Zn can be isolated from the matrix and from one another during a single process by using anion exchange chromatography, whereas for Ca, the combination of cationic, anionic and element-specific resins is necessary for avoiding spectral interference from doubly charged Sr^{2+} ions, the isobars of Ti, and polyatomic ions containing K. These procedures are labor-intensive and time-consuming and therefore, the development of methodologies able to increase the sample throughput is highly desired, especially in the context of biomedical research. For this purpose, Fe isotopic analysis in whole blood after acid digestion followed by simple dilution of the sample [27] or Fe precipitation have been proposed [28].

The potential occurrence of spectral interference is also a critical issue in MC-ICP-MS. To overcome spectral overlap, medium or high mass resolution, potentially in combination with aerosol desolvation, are used in the isotopic analysis of the aforementioned essential mineral elements by MC-ICP-MS [19,29–31]. Only a few MC-ICP-MS instruments (one instrument type, the Nu Plasma 1700 from Nu Instruments) sold so far allow full resolution of the spectral peaks of analyte and interfering ion while preserving flat-topped peaks [8]. In most types of MC-ICP-MS instrumentation, the analyte signal is not fully resolved from that of the interfering polyatomic ion(s), but in a section of the resulting spectral peak, there is a plateau to which only the analyte signal contributes. This approach is sometimes referred to as pseudo-high resolution [31]. Ca and Fe ion beam intensities are measured at a mass-to-charge ratio located in the flat shoulder of the spectral peak [29,32] and Cu and Zn ion beam intensities left-of-center of the corresponding plateau [33]. As can be seen in Fig. 1, a measurement position approximately –0.038 u away from the shoulder centre allows one to avoid any effect of the Na-based spectral interference ($ArNa^+$) on the Cu isotope ratio measurements at medium resolution (ThermoScientific Neptune MC-ICP-MS unit). Under these conditions, the bias between the measured and the 'true' value is reproducible and therefore, it can be corrected for. Mass bias correction methods can be found in many reviews and books, e.g. [8,34–36]. The most widely used approach in biomedical applications is external correction in a sample-standard bracketing approach in combination with internal normalization using an admixed internal standard. The double spike approach, using an artificial standard containing ^{42}Ca and ^{48}Ca, has been used to correct for instrumental mass discrimination in Ca isotopic analysis in urine *via* TIMS [37]. This approach can only be applied to elements with at least four stable isotopes, and therefore, within the context discussed, its use would be limited to the isotopic analysis of Ca, Fe and Zn using either MC-ICP-MS or MC-TIMS. Gravimetric mixing of the sample with an isotopic spike in different proportions and applying the principles of isotope dilution has been proposed for isotopic analysis of Mg, an element with potential metabolism-induced isotopic effects awaiting to be explored, in human urine *via* MC-TIMS [38].