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Research Article

Study of Isotope D/H Composition in Women Liquid Media during Lactation

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Abstract

Carried studies of isotope (D/H) composition in liquid media in women during physiological lactation revealed different deuterium concentrations presence, which significantly differ in saliva, blood plasma and woman breast milk. Thus the highest deuterium concentrations observed in the saliva, which surpassed deuterium content in the blood plasma (by 99 ‰) and breast milk (by 117 ‰). Inclusion in drinking diet deuterium depleted water (-614 ‰, volume 1.5 liters per day, for 30 days) led to significant decrease of deuterium concentration in all liquid media on average by 102-160 ‰, on background it was noted some smoothing of differences between biological media with preservation of deuterium concentration decrease gradient in the same sequence as in physiological conditions.

Keywords

Saliva; Blood plasma; Woman breast milk; DDW; NMR spectroscopy

Introduction

Study of various chemical element isotopes abundance in bio systems is actual question in modern biology and medicine [1], which is associated with heavier atoms ability to significantly affect living organisms' adaptive capabilities and their survival in changing environmental conditions. At present time scientists from different countries are conducting research aimed to studying of number of biogenic elements light and heavy non-radioactive isotopes possible participation (e.g., oxygen, hydrogen, carbon, nitrogen and sulfur) in physiological and some pathological processes regulation in living organisms [2-4], including its role in metabolism regulation [5], that may be due to isotopes distribution peculiarities in humans and animals tissues, as well as food and fluid intake [6-9].

Special place among biogenic isotopes elements belongs to deuterium, which, in comparison with other stable isotopes, can exert more pronounced effect on organism, due to sufficiently pronounced differences in ^{1}H and ^{2}H masses. Mainly there are scientific research works devoted to isotopic D/H ratio in blood plasma studying [10], also there are attempts to assess liquid medium with low deuterium content influence on physiological and morphological processes in different organization level biological objects [11,12].

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One of the key mechanisms leading to heavy isotopes concentration increase in organism as result of isotopic exchange reactions amplification is their admission into food and drinking rations composition, which is associated with a heavy atoms accumulation different degree into food products depending on their chemical composition [13]. This also explains the possibility of carrying out adjustments to change the heavy isotopes percentage *in vivo* by products with modified isotopic composition inclusion in food ration. For this reason biomedical scientific community growing interest towards hydrogen isotopes ratio caused by possibility to change organism adaptive potential and immune bodies structural alterations in experimental animals, to improve radio protective resistance, to influence on pro oxidant-antioxidant system and the other physiological processes using deuterium depleted water [14-18].

However, up to the present time deuterium/protium isotope exchange reactions in woman breast milk and saliva have not been studied, which is important for non-invasive diagnostic methods development, including environmental safety monitoring in the population. The latter fact is particularly important; because of saliva application in laboratory diagnosis will implement the non-invasive monitoring of heavy non-radioactive isotopes content in organism. Especially important D/H isotope composition in breast milk during lactation, it is actually the main (practically unique) food product that may include heavy isotopes able to provide negative effect on children growth and development.

NMR spectroscopy is one of the main precision methods for deuterium concentration analyzing in biological fluids [19], which is due to sample preparation and analytical process procedures simplicity can be used as express method in experimental and clinical practice, for example, while monitoring the isotopic (D/H) exchange processes of person using diet products with a modified isotopic composition with preventive or therapeutic purposes [20,21].

The main aim of the research is to study via isotope ratios (D/H) NMR spectroscopy in various biological fluids for non-invasive methods development of heavy hydrogen isotopes monitoring content in organism, including application of deuterium depleted water in drinking diet.

Materials and Methods

In this study the materials used was JNM-ECA-series 400 MHz FT NMR spectrometer (model NM-40TH5AT/FG2) having the following specifications: constant magnetic field induction of 9.389766 Tl, frequency range from 10 to 400 MHz with a step of 0.01 MHz, resonant D nucleus frequency of 61.37 MHz [22].

The main calibrated NMR tubes with a diameter of (4.97 ± 0.013) mm and a length of 178 mm were used. The substance for isotopic composition determination was placed into these tubes. Calibrated internal NMR tube (coaxial external standard) with a 40 μL end capillary 32 mm long was inserted into the main NMR tube. The reference material with the same molecular structure as of the examined substance but with the known isotopic composition was placed in the internal tubes and contained water with a known isotopic composition. The internal NMR tubes contained the

addition of dissolved europium trifluoromethanesulfonate (III), whereby ^2H water NMR signal of internal ampoule was displaced relative to determined substance signal from main tubes. This allowed integrating separate spectrum signals relative to each other. Calibration samples of pure water with a known deuterium content were used (impurity content less than 0.01 % by mass), in accordance with international standard introduced by the IAEA VSMOW and SLAP.

For $^2H/^1H$ ratio determination water sample with the $(CF_3SO_3)_3Eu$ content equal to (0.045 ± 0.005) mol/L was placed into internal NMR tube; in this case, deuterium content in this solution corresponded to examined deuterium concentrations range in the sample to be measured (typical obtained spectrum is shown in Figure 1). Other impurities content did not exceed 0.01 % by mass. For solutions preparation it was used 1 ml dispenser micropipette with a step by 0.010 ml with interchangeable tips and laboratory analytical balance with permissible error for single weighing by ± 0.0005 g. All experiments were carried out on samples series, including calibration ones, were carried out under the same conditions of NMR measurements and with the same device settings.

The following optimal parameters were selected for measuring the ^2H nuclei: gain was 60; shear was 5 ppm; sweep was 10 ppm; free induction decay observation time was 6 ms; scans number was 256; relaxion delay time was $10 \cdot T_1(^2D_2O) \geq 7$ s; temperature inside of resonator was 25 °C. Experiment results were expressed in ppm (here and below, the deuterium content is indicated).

^2H content was measured in biological substrates: saliva, blood plasma, and woman breast milk in two groups of the puerperas. In group A (n=14) were included women, consumed standard diet, in group B (n=8) were included women, consumed deuterium depleted (– 614 ‰) water in a volume of 1,5 liters per day for 30 days prior to examination beginning in addition to standard diet. It was carried out preliminary sample preparation before biological fluids isotopic composition analysis, including blood centrifugation (Eppendorf minispin centrifuge) at 1500 revolutions per minute (rpm) for 15 minutes; woman breast milk and saliva were centrifuged at 3000 rpm for 15 minutes. Due to specified centrifuge parameters it is able to save remaining main proteins, lipids and carbohydrates in biological fluids. Differences were considered significant at $p< 0.05$. It was used the Spearman's coefficient (r) to assess the correlation relationship.

Results and Discussion

It is known that deuterium concentrations under natural conditions in water, proteins, lipids and carbohydrates differ significantly due to different isotope (D/H) exchange reactions rate in different chemical bonds in biomolecules. Isotopic (D/H) metabolism in natural conditions is mainly expressed in bonds -OH, -SH, $-NH_2$ (=N-H), but practically absent in bonds $-R_2C-H$. According to the scientific literature, protein content in blood plasma is about 6.5 % to 8.5 %, in lipids – about 0.5 to 1.0 %, in carbohydrates – about 0.1 %, while in oral fluid and woman breast milk these indicators are differ in several times. Most lipids among studied objects were found in woman breast milk (about 4 %), while in oral fluid they were practically absent (0.006 % – 0.007 %) [23-25]. Protein content in blood plasma was significantly higher than in woman breast milk (about 1.0 %) and oral fluid (about 0.1 %). Thus, carbohydrates content in woman breast milk was ten times higher than that in plasma and reached by 6.9 %, while in the oral fluid those indexes were two orders of magnitude lower (about 0.03 %). As a result of carried studies it was established deuterium content gradient under physiological conditions (oral fluid >> blood plasma>female breast milk) in the group A, all these parameters in biological fluids are significantly different (Table 1). Therefore, in order to clarify reason for isotope D/H gradient described above appearance it was carried out correlation analysis of relationship between deuterium content in biological fluids and blood plasma, oral fluid and woman breast milk biochemical composition.

As a conducted correlation analysis result it was established that there is direct correlation relationship between content in biological fluids of water and deuterium content ($r=0.86$, $p<0.05$). Whereas between organic components content in biological fluids and deuterium content was revealed inverse relationship: for proteins r was from –0.55 ($p<0.05$), for carbohydrates r was –0.81 ($p<0.05$), for lipids r was –0.83 ($p<0.05$), which point to deuterium concentration dependence, primarily, on drinking ration, and to a lesser extent on the organic molecules content, with lipid molecules exception, which are characterized by high r. It can be explained by isotopic (D/H) exchange lowest intensity in hydrophobic (nonpolar) lipid radicals, which even under conditions of water with different isotopic composition consumption contribute a stable contribution to final deuterium concentration. In addition, such interrelationships between biomolecules and deuterium content must be taken into account while developing algorithms for noninvasive assessment of heavy non-radioactive isotopes content in organism, including deuterium, because biological fluids biochemical composition can be quite variable depending on person lifestyle (nutrition nature, physical activity and other factors). However, it is not possible to completely explain differences in isotopic composition with only these bio fluids biochemical composition peculiarities and, apparently, there are additional mechanisms for isotope exchange regulation in living system.

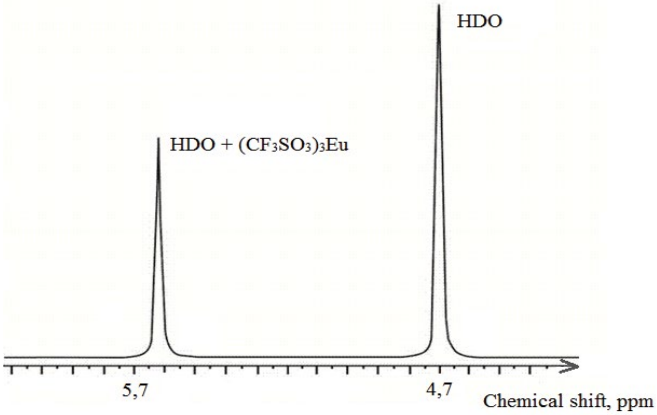

Figure 1: NMR spectrum, deuterium signal in the sample of biological fluid (right peak) in comparison to standard with shift reagent (left peak).

Table 1: Deuterium content in women biological fluids in lactation physiological period and during consume. deuterium depleted water

Parameter	Group A (M ± σ)	Group B (M ± σ)
Women breast milk, ‰	−91 ± 11*	−181 ± 20 ^
Blood plasma, ‰	−73 ± 15	−175 ± 18 ^
Saliva, ‰	26 ± 10*	−134 ± 29 *, ^

Note: *p<0,05 in comparison with blood plasma parameters,

^ - p<0,05 in comparison with parameters of group A.

Also, difference in blood plasma, saliva and woman breast milk isotope composition may be due to changes in metabolic processes rate in organism, when, under increased energy production conditions, there is increased water formation inside cells from hydrogen isotopes that are biological oxidation substrates part (i.e. various organic compounds classes). At the same time, water, formed during oxidative processes inside the cell, can significantly differ by deuterium content from extracellular water, replenished in organism mainly from diet ration. Another probable reason for this physiological isotope gradient appearance is possible selective mechanisms presence for heavy isotopes entry through histohematetic barriers, for example, hematosalivar and hemato-lactational ones. Its main function is permeability regulation for physiologically necessary substances in breast milk formation, in addition, it is one of key mechanisms for protecting infant in case of toxic substances entering in mother's blood.

In group B woman, consumed deuterium depleted water, significant decrease in deuterium concentration was noted (Table 1): in blood plasma – by 2.4 times ($p<0.05$), in saliva – by 160 ‰ ($p<0,05$), in woman breast milk – by 1,9 times ($p<0,05$), in comparison with group A results. It should be noted that although in group B there is an isotope (D/H) gradient (oral fluid>blood plasma ≥ woman breast milk) was preserved, absolute differences between deuterium content in biological fluids decreased more than in 2 times. Wherein, parameters in blood and woman breast milk did not have significant differences. Such deuterium concentration indexes dynamics can indicate deuterium content decrease in water comprised in studied bio fluids, with its practically unchangeable content in the organic substrates mentioned above, as well as it can be an indicator of possible smaller fluctuations in intracellular deuterium concentrations, especially in cells that synthesize breast milk, which can be explained by predominant formation in organic substrates lactocytes, not by its intake from blood plasma [26-29]. Wherein, intracellular water percentage formed directly in the cell, for example, as oxygen reduction in mitochondria to water processes result, can differ substantially both in different tissues (which is explained by their different metabolic activity) and in the same tissue while changing of metabolic reactions intensity in its vital activity process.

In addition, due to new methodological approaches application with lanthanide shifting reagents, it was succeed significantly shorten the time of the experiment.

Conclusion

Thus, on conducted studies basis it was established that in human biological fluids under physiological conditions there is isotope D/H gradient (oral fluid >> blood plasma>woman breast milk), its presence caused, in particular, by water content (r=0,86) and bio fluids biochemical composition features. Wherein, largest inverse correlation among organic components (r=– 0.83) was noted between deuterium concentration and lipids content in corresponding biological fluids.

While using deuterium depleted water in drinking diet, maximal alterations in deuterium concentration were observed in oral fluid and blood plasma, whereas fluctuations in deuterium concentration in breast milk were significantly less. Thus, it was noted the decrease of absolute D/H gradient values: oral fluid>plasma ≥ woman breast milk.

This study also demonstrated that developed methods for isotope ratios measurement by NMR spectroscopy with use of probes, containing shift reagent, can be aimed at solving environmental problems and human condition screening, specifically while natural origin fluids isotopic composition express control, as well as for deuterium concentration monitoring in organism while consuming products with modified isotopic composition. As a standard (etalon), it was chosen substance with the same molecular structure as the test material, with a lanthanide shifting reagent: europium (III) trifluoromethanesulfonate added in it.

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