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Exhaled Breath Vapor of Humans Reflects the Changes in Deuterium Concentration in the Body Water

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ABSTRACT

The concentration of the heavy isotope of hydrogen, deuterium (D), is not routinely measured in (human) medical laboratory tests, even though an increasing number of papers prove the pivotal role of D in tumor growth, cell cycle regulation, cell metabolism, and aging. Data from a prospective phase 2 clinical study and numerous retrospective clinical studies proved the anticancer effect of deuterium depletion achieved by replacing the regular water intake with deuterium-depleted water (DDW). In previous studies, the changes in serum D concentration of DDW-consuming patients were followed using blood samples and mass spectrometry, which was invasive, costly, and time-consuming. As future clinical trials will also require a follow-up of internal D level and the patient's compliance, a new sampling device and procedure was developed based on condensing the exhaled breath water vapor and measuring its D content using a liquid water isotope laser analyzer. Test results showed that the device provided accurate, reliable, and reproducible data. According to the data, the internal D level in a person consuming normal water was stable. In contrast, exclusive consumption of DDW for several days resulted in a gradual decrease of D concentration in exhaled breath condensate (EBC), which was proportional to the D concentration of DDW. These data confirm that orally applied DDW equilibrates with the person's water pool quickly, leading to a reduced internal D level reflected in the D content of EBC.

1. INTRODUCTION

Studies have shown that using deuterium-depleted water (DDW, 30 ppm) instead of water with a natural deuterium concentration (150 ppm) significantly reduces cell growth in vitro and demonstrates anticancer effects in mice studies [1, 2]. This anticancer effect of DDW is attributed to oxidative stress induced by redox imbalance [3]. Both prospective [4] and retrospective clinical studies [5-7] indicate that integrating deuterium depletion into existing therapies can substantially extend the median survival time of cancer patients. Subsequent studies on rats and clinical trials in humans have revealed that deuterium concentration strongly influences metabolic processes. Specifically, concentrations within the 125 - 140 ppm range optimize insulin signaling, reduce fasting glucose levels, and improve insulin resistance [8, 9]. Another study demonstrated the antiaging benefits of deuterium depletion [10]. These findings highlight that the deuterium/hydrogen (D/H) ratio is a key signal regulating cell growth, metabolism, and gene expression. Studies using DDW suggest that cellular D/H balance is influenced by the activity of the Na^+/H^+ exchanger in the cell membrane, which can increase the D/H ratio, along with mitochondrial metabolic water production, which produces DDW and helps set the cellular D/H ratio [11]. Further clinical trials are needed to realize DDW's therapeutic potential in cancer treatment fully. Additionally, implementing DDW in patient care requires a reliable method to monitor changes in deuterium concentration in patients' bodies, gather robust kinetic data, and ensure patient compliance.

The first such data were gained in a prospective clinical study investigating the clinical efficacy of DDW in metabolic diseases and published in 2020 [8]. Before starting DDW consumption, the subjects' blood D level was in a narrow range of 146 - 150 ppm but varied between 125 - 143 ppm by the end of the study; the high variation being due to differences in body weight, type, and quantity of diet, and the consumed regular water beside DDW. In this study, mass spectrometry of venous blood samples was used but proved too expensive in terms of money and time for routine usage [8]. Associated with the progression in this field of research, evaluation of the effect of deuterium depletion in healthy, tumorous, and diabetic patients requires a rapid, easy-to-use, and reliable measurement to determine the D concentration and its changes in the body. Such a method would optimize the deuterium depletion therapy based on treatment results.

To find a suitable solution, it was investigated whether exhaled breath condensate (EBC) was a usable sample to monitor D concentration in the body precisely. The latter is determined by the D concentration of the fluids and water content of solid foods consumed and can be purposely influenced by DDW.

The composition of EBC has been thoroughly studied and published [12-15]. Pulmonologists focused on respiratory gases, but recent studies investigated nonvolatile biomarkers such as cytokines, ions, and urea, suggesting that the water in the condensate can be a suitable analyte, too [13]. Nearly all water in the exhaled air evaporates from the inner surfaces of the lungs, originating from the liquid portion of the blood, the plasma, of which 92% is water. Typically, 45 μL liquid water can be extracted from one liter of saturated exhaled air [12]. Air temperature, humidity, and minute ventilation influence the amount of exhaled water; high temperature (35˚C) reduces while cool temperature (−10˚C) and high heart rate (140 bpm) increase the amount of water lost by exhaled air [14].

Based on the available information on the composition of exhaled breath vapor, a new medical device for collecting EBC samples was designed and constructed, and its applicability was tested by following the changes in the body's D concentration while the subjects consumed normal water or DDW.

2. MATERIALS AND METHODS

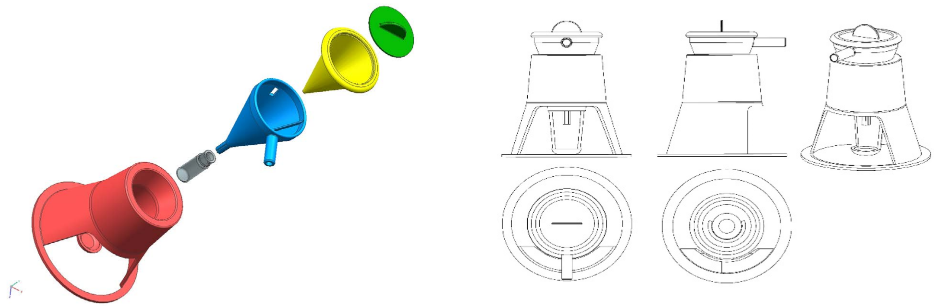
2.1. Structure and Operation of the EBC Sampling Device

The exhaled breath condensate sampling device, developed at HYD LLC, is designed to collect condensate from exhaled air efficiently. The EBC sampling device consists of four parts, as shown in **Figure 1(a)** and **Figure 1(b)**.

The device, which weighs approximately 30 grams and measures 110 × 90 × 80 mm, was constructed from biocompatible PA12 polyamide powder using an SLS 3D printer.

2.2. Operation Procedure

The way of use and measurement of D concentration:



(a) (b)

Figure 1. The EBC sampling device. Parts of the device (a): Red: Stand; Blue: Outer part of the condensation unit; Yellow: Inner part of the condensation unit; Green: Cap of the condensation unit; Grey: Condensate collecting vial; (b): Schematic diagram of the device.

1) Water is filled into the inner part of the condensation unit (**Figure 1(a)**).
2) The cap is placed back on the top of the inner part of the condensation unit.
3) Part Yellow is placed into the outer part of the condenser unit.
4) The complete condensation unit (Parts Yellow, Green, and Blue) is placed into the stand and put in a freezer set at −18˚C.
5) When the water in Part Yellow freezes (within 4 - 5 hours), the sample vial is decapped and placed under the condensation unit. Now, the sampling device is ready to use. The subject can start to exhale through the stub of Part Blue.
6) Exhaled air flows between the outer and inner parts of the condensation unit, precipitating water on the cold surface of Part Yellow and draining into the vial. The test found that the cooling capacity (volume and temperature) of the ice in Part Yellow is far greater than what is required to condense 1 mL of exhaled vapor.
7) When EBC volume reaches 1 mL in the vial (usually in 6 - 8 minutes), it can be removed and closed. Before the next use, the parts of the condensation unit (Part Yellow) are wiped dry; a standard surface disinfectant disinfects the stub, and the device, after refrigeration, is ready for subsequent use. These steps show that the novel EBC sampler is easy to use and allows in-home sampling.
8) The EBC sample, now collected and prepared, is ready for measurement by a liquid-water isotope analyzer.

Given the ice volume and temperature, its cooling capacity is far greater than required to condense 1 mL of exhaled vapor. This is evidenced by the fact that most of the water remains frozen inside the device by the end of the sample collection, and the temperature of the surface of the inner part of the condensation unit does not exceed 0˚C.

The instrument (Liquid-Water Isotope Analyzer-24d, manufactured by Los Gatos Research Inc., San Jose, CA, USA) uses off-axis integrated cavity output spectroscopy to measure the absolute abundances of D-containing water molecules via laser absorption (IAEA, 2009). The deuterium concentration is given in ppm (with ±1 ppm accuracy as stated by the manufacturer).

2.3. Excluding the Effect of Air Humidity

Air humidity can potentially influence the D concentration of the collected samples if there is time for an exchange reaction. To exclude this impact, the sampling conditions were varied. The extensive exhalation in 4 minutes produced 0.6 mL of condensed water, and the slow exhalation in 16 minutes produced 1.5 mL. Measuring the D concentration of the samples obtained in the two ways showed no difference (131.2 ppm and 130.3 ppm, respectively). The device was also tested alone, allowing the air humidity to precipitate [16].

Still, less than 100 μL was collected during an hour, suggesting that air humidity does not impact the D concentration of EBC. This is in line with the data that at room temperature, the water content of one liter of air is less than 1 μL.

3. RESULTS

3.1. D Concentration in the EBC of Persons Consuming Regular Water (Water with Natural D Concentration) Consistently Showed No Major Fluctuation, Reinforcing the Stability of the Results

The device was used as described above by subjects of both sexes aged 8 to 56, with body weights ranging from 26 to 110 kg. Table 1 summarizes the data collected.

Table 1. D concentration in the EBC of different persons consuming regular water.

Gender	Age (years)	Body weight (kg)	Number of samples	D (ppm) Mean ± SD
Female	8	26	7	145.9 ± 1.28
Female	8	27	8	144.5 ± 1.72
Female	13	50	9	144.7 ± 0.57
Female	46	58	6	144.8 ± 0.41
Female	48	72	6	144.2 ± 0.69
Male	46	110	9	143.3 ± 0.99
Male	63	80	3	145.5 ± 2.00
Male	50	77	11	138.3 ± 0.93

The samples were taken once a day (except for one subject; see below). Three persons provided new samples one year after giving the first ones. In two cases, there were no differences (144.82 - 144.45 ppm, 143.3 - 144.76 ppm); in one case, a difference of 5.2 ppm (138.3 - 143.5 ppm) was found. The difference between the minimal and maximal D levels in one volunteer's samples was 1.07 to 5.81 ppm. Interestingly, the highest differences (3.48 ppm and 5.81 ppm) were detected in the two volunteers of age 8. Among the adults, the differences were between 1.07 and 5.2 ppm. Considering that the difference, and hence the SD value, for each volunteer was low, we can conclude that in persons consuming normal water, the D concentration is stable and independent of age, gender, body weight, and sampling time. The data also indicate that the device provided reliable, reproducible samples for measurement.

One person (female, 56 years old, weighing 78 kg) gave two samples daily, at 10 a.m. and 10 p.m., for a week. The EBC D concentration results (145.1 - 144.8 ppm ± 0.64) further confirmed the stability of body water D levels.

Based on all 59 samples, the average D concentration was 143.61 ppm ± 2.64. This aligns with our earlier findings, where 30 volunteers were enrolled in a clinical study (mentioned above), and their average serum D concentration before consuming DDW was 147.57 ppm. (146 - 150 ppm) [8].

3.2. Drinking Regular and Deuterium-Depleted Water within One Day Causes the Body's D Concentration to Fluctuate

Consuming exclusively DDW is the basic rule to maximize the anticancer effect of deuterium depletion. The subsequent study represents and follows the changes when the patient's compliance is not adequate. The test person's daily water intake of 1.5 L consisted of 0.5 L of deuterium-depleted (105 ppm D) and 1.0 L of regular water (148 ppm D), or oppositely, over 33 days, as shown in Table 2. During this period, the overall decrease of D concentration in the EBC was 6.7 ppm. The changes of D concentration in the EBC corresponded to the varying proportions (2:1 to 1:2) of the two types of water, with a manifest decrease on the days of higher (cca. 2:1) DDW-to-normal water ratio but a temporary increase when the majority of the water consumed was normal (Table 2).

Table 2. The effect of consuming normal water and DDW together on the D concentration of the EBC.

Period	Ratio of DDW and regular water	D concentration—the beginning and end of the period (ppm)	Difference (ppm)
Day 1-Day 7	2:1	133.4 - 129.9	−3.5
Day 8-Day 18	1:2	129.9 - 131.8	+1.9
Day 19-Day 35	2:1	131.8 - 126.7	−5.1

3.3. D Concentration in EBC of Two DDW-Consuming Persons

The following data were obtained from a 64-year-old woman who consumed DDW with different D concentrations. The data show a correlation between the length of DDW consumption, the D concentration of the water consumed, and the measured D concentration in the exhaled air (Figure 2).

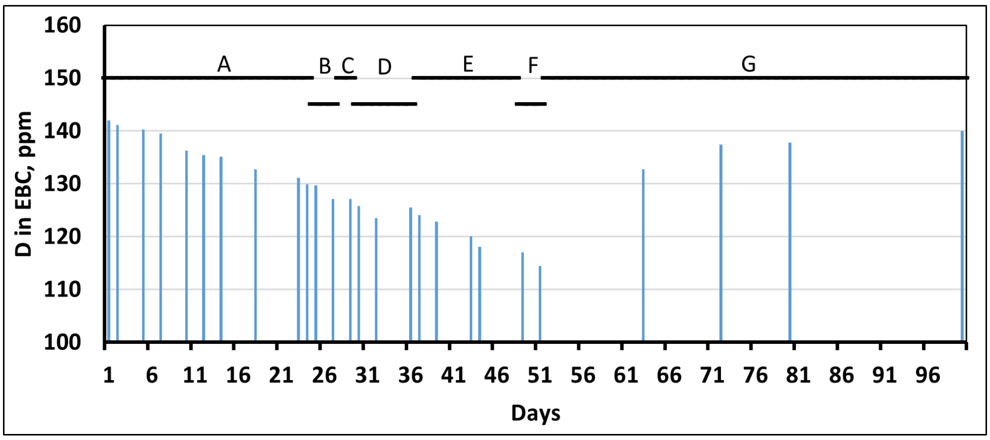


Figure 2. Time course of D level in EBC samples of a person consuming DDW for 51 days and regular water for the subsequent 49 days. The changes showed a strong correlation proportional to the D concentration of the consumed drinking water. For periods A to G, see text.

Over 51 days of consuming exclusively DDW (except for two days), a 27.54 ppm decrease was detected (0.69 ppm per day). During these 51 days, the person consumed DDW of five different D concentrations. In period A, DDW with 105 ppm D concentration resulted in a 10.81 ppm decrease within 22 days. In periods B and D, the deuterium concentration of the DDW was 85 ppm, but in period C, she consumed regular water. There was a 5.71 ppm decrease in D concentration of EBC in 13 days (periods B and D) but no additional decrease during regular water consumption in period C (127.09 - 127.09 ppm). In periods E and F, the person consumed DDW with 65 ppm and 45 ppm D concentration, resulting in a 5.74 ppm decrease within ten days and 2.62 ppm within two days. The average decrease of D concentration was 0.47 ppm/day in period A, 0.92 ppm in period B, 0.57 in period E, and 1.31 in period F. The person stopped drinking DDW on the 51st day. Starting to consume regular water (145 ppm) on the 52nd day caused an increase in D concentration (period G). The increase was faster (18.29 ppm) in the first 12 days, while it was only 7.66 ppm in the subsequent 37 days when D concentration almost reached the original level (141.99 ppm).

Another subject, a 68-year-old male consumed DDW of 105 ppm. Figure 3 shows a rapid decrease in D concentration in the first 4 - 5 days, with an additional decrease in the following days. However, the D concentration reached equilibrium within three weeks, and no additional decrease was observed.

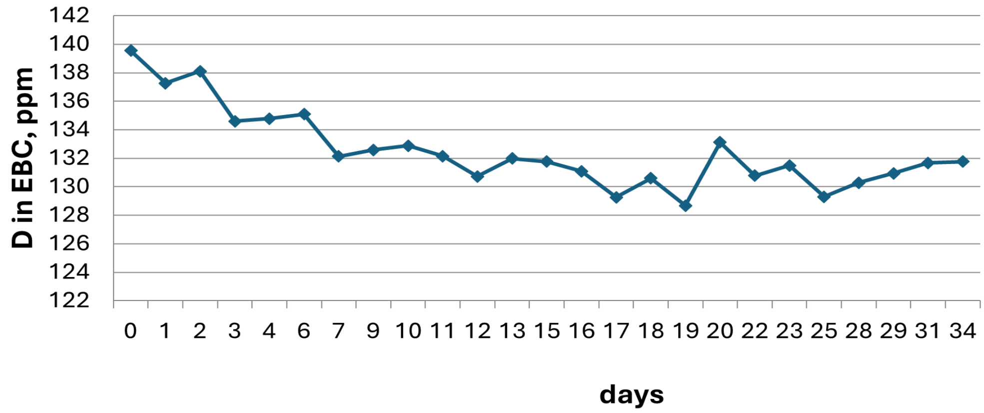


Figure 3. **D concentration decrease in the EBC samples of a person consuming DDW of 105 ppm D in the measured period.**

3.4. Rapid Change of D Concentrations in EBC of Persons Consuming DDW with 25 ppm D Concentration

The next question addressed was whether significant deuterium (D) concentration changes could be observed within half a day after ingesting deuterium-depleted water (DDW) of 25 ppm D concentration. This would also help determine how quickly D concentration equilibrates in the body. Two individuals (one female and one male, with body weights of 92 kg and 77 kg, respectively) consumed DDW containing 25 ppm D between 7:30 a.m. and 8:30 p.m. Over the 13 hours, a decrease of 4.64 ppm and 4.03 ppm in D concentration was measured after consuming 1.2 liters and 1 liter of DDW, respectively.

4. DISCUSSION

The antitumor efficacy of deuterium depletion, supported by *in vitro* tests and clinical applications, has gained increasing acceptance [3, 4, 9]. Ongoing research and planned clinical investigations require precise technology to monitor D concentration in patients. This is crucial to proving the correlation between decreased D concentration and treatment efficacy, as well as tracking patient compliance. The newly developed sampling device offers several advantages: it is simple and non-invasive, compact and portable, and requires only a household freezer.

Test results confirmed that the method for measuring D concentration in exhaled breath condensate (EBC) is both stable and reproducible, as demonstrated by the consistency of D levels in individuals consuming regular water. This makes it a suitable tool for monitoring changes in the body's D concentration induced by deuterium-depleted water (DDW) consumption. The data also showed that a consistent water consumption habit (whether normal water or DDW) leads to stable D concentrations in the body.

Furthermore, the results indicate that minor fluctuations in D concentration can occur even when consuming the same type of water, with differences of over five ppm observed in the same individual. Daily consumption of both regular and DDW in varying ratios led to fluctuations in D concentration, suggesting that equilibration occurs within a day.

Based on the DDW consumption patterns of participants in previous clinical studies, it can be inferred that their internal deuterium (D) levels likely reached the low values of around 120 - 130 ppm observed in the present study. Their prolonged survival further supports the efficacy of deuterium depletion [4-6]. Various *in vitro* tests [1, 2, 5] and animal experiments [1] have demonstrated that effective D levels are in the interval of 30 - 105 ppm. It is now evident that even a moderate reduction in D levels can have significant clinical benefits.

Consuming DDW leads to rapid and proportional changes in the body's D concentration. This study revealed that oral administration of DDW is reflected in the D concentration of EBC, indicating that equilibrium is achieved shortly after DDW intake. This finding is consistent with previous heavy water tests to determine total body water [17].

The deuterium (D) concentration in humans (12 - 14 mmol/L) is approximately six times higher than that of Ca^{2+} in the blood, yet it is not included in standard blood tests. Given findings from studies with deuterium-depleted water (DDW), which demonstrate D's critical role in regulating metabolism and cell growth, it is essential to conduct large-scale testing across diverse populations. Such studies could reveal potential correlations between individuals' D concentrations and the prevalence of diseases like cancer and diabetes.

Mitochondria metabolize carbohydrates to produce metabolic water with a D concentration of approximately 150 ppm. In comparison, fat metabolism results in metabolic water with a lower D concentration of about 118 ppm [18]. This suggests that modern dietary recommendations favoring high carbohydrate and low fat intake may contribute to a higher average D concentration in the body compared to populations from 50 - 60 years ago. The application of the new device may help to generate enough data to get answers to these questions.

The above-described application trials showed that the novel EBC collecting device is practical, easy to use, and provides accurate and reliable data. Given the possibly increasing involvement of deuterium depletion in cancer therapy and prevention, the device may gain substantial importance in the future.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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