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RESEARCH ARTICLE

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Deuterium-depleted water: A new tracer to label pulmonary surfactant lipids in adult rabbits

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Abstract

Stable isotope tracing can be safely used for metabolic studies in animals and humans. The endogenous biosynthesis of lipids (lipogenesis) is a key process throughout the entire life but especially during brain and lung growth. Adequate synthesis of pulmonary surfactant lipids is indispensable for life. With this study, we report the use of deuterium-depleted water (DDW), suitable for human consumption, as metabolic precursor for lipogenesis. We studied 13 adult rabbits for 5 days. Four rabbits drank tap water (TW) and served as controls; in four animals, DDW was substituted to drinking water, whereas five drank deuterium-enriched water (DEW). After 5 days, a blood sample and a bronchoalveolar lavage (BAL) sample were collected. The $^{2}H/^{1}H$ ($\delta^{2}H$) of BAL palmitic acid (PA) desaturated phosphatidylcholine (DSPC), the major phospholipid of pulmonary surfactant, and of plasma water was determined by high-resolution mass spectrometry. We found that the $\delta^{2}H$ values of DDW, DEW and TW were −984 ± 2‰, +757 ± 2‰ and −58 ± 1‰, respectively. After 5 days, plasma water values were −467 ± 87‰, +377 ± 56‰ and −53 ± 6‰, and BAL DSPC-PA was −401 ± 27‰, −96 ± 38‰ and −249 ± 9‰ in the DDW, DEW and TW, respectively. With this preliminary study, we demonstrated the feasibility of using DDW to label pulmonary surfactant lipids. This novel approach can be used in animals and in humans, and we speculate that it could be associated with more favourable study compliance than DEW in human studies.

KEYWORDS
deuterium-depleted water, deuterium-enriched water, isotope ratio mass spectrometry, lipogenesis, pulmonary surfactant, tracer

1 | INTRODUCTION

The endogenous biosynthesis of lipids (lipogenesis) is a key process throughout the entire life but especially during fetal and neonatal growth, chiefly for the brain and the lungs, where it is essential to generate phospholipids for brain cell membranes[1] and for lung surfactant,[2] a lipoprotein complex essential for lung function. The lipogenesis is also an important anabolic process in the adipose tissue, where it plays an essential role in energy storage and for thermogenesis.[3]

Application of stable isotope tracers in humans and animals metabolic studies provides information on dynamics of selected metabolites like fractional synthesis and turnover rate; in addition, the use of stable isotopes can help in identifying the endogenous or exogenous components of several key metabolites/nutrients such as glucose, lipids and proteins.[4]

In the past years, we used stable isotope lipid precursors (deuterated water, ^{13}C-glucose and $^{2}H_3$-palmitate) to quantify the fractional synthesis of pulmonary desaturated phosphatidylcholine (DSPC), the major component of pulmonary surfactant, in adults and in preterm infants with respiratory distress syndrome.[2,5,6] Deuterated water is widely used for measuring de novo lipogenesis, because the deuterium atoms are incorporated into fatty acids via exchange between body water and the hydrogens of acetyl-CoA, malonyl-CoA and NADPH.[7]

Stable isotopes are non-toxic and safe, and studies can be safely undertaken in humans.[8,9] However, there are sometimes concerns with the use of stable isotopes as tracers in humans, especially during the most critical phase of life like pregnancy and the early postnatal period. Moreover, chemically synthesized stable isotope tracers are relatively expensive, and the cost varies depending on the number of labels and the site of labelling in the molecule. Common experiments in the literature use stable isotope enrichment levels from 1% to 20%. These high enrichment levels are required due to the imprecision and inability of the conventional gas chromatograph–mass spectrometer to perform stable isotope analysis of samples with enrichment levels below 1%. This necessitates larger isotope doses and increases study costs. Subtle variations in the abundance of stable isotopes enrichments or difference in the natural abundances of stable isotopes in organic and inorganic compound can be accurately measured by isotope ratio mass spectrometry (IRMS). IRMS instruments are designed to measure precisely small differences in the abundances of isotopes (between 0.01% and 0.001%), like $^{2}H/^{1}H$ and $^{13}C/^{12}C$.

The natural abundance of deuterium in water is approximately one deuterium atom in 6400 hydrogen atoms (156 ppm), or 0.0156%,[10] with small variations and the minimum level of deuterium observed in the ice of Antarctic (Standard Light Antarctic Precipitation [SLAP]), whose $\delta^{2}H$ is 89 ppm. A somewhat higher content of deuterium (125 ppm) is registered in Greenland ice and corresponds to the standard Greenland Ice Sheet Precipitation (GISP).[11] The deuterium abundance in water varies depending not only on the latitude but also with altitude, with values ranging between 156 ppm at sea level and 130 ppm at heights above 3000 m.

Given these premises, the search for better and safer methods for metabolic investigation is of a great importance for the scientific community. To this aim, we present a novel approach to label pulmonary surfactant lipids using a commercially available deuterium-depleted water (DDW) as metabolic precursor.

2 | MATERIALS AND METHODS

2.1 | Chemicals

The chemicals were obtained from Merck (Darmstadt, Germany). DDW, 25 ppm, was from Qlarivia™ (Bucharest, Romania), and the deuterated water (D_2O, 99.5% purity) was from Cambridge Isotope Laboratories (Andover, UK).

DDW was ready to use and contained 25 ppm of deuterium. Deuterium-enriched water (DEW) was prepared by adding a small amount of 99.9% D_2O (0.0116% v/v) to tap water.

2.2 | Animals and sample collection

The experimental procedure was approved by the intramural Animal Welfare Body and the Italian Ministry of Health (Prot. No. 1300-2015-PR) and complied with the European and Italian regulations for animal care.

A total of 13 adult rabbits, 7 to 8 weeks old, were studied. Four served as control and drank tap water (TW), four rabbits drank DDW and five rabbits DEW. Drinking waters were provided ad libitum for 5 days before the bronchoalveolar lavage (BAL) collection. After 5 days, all rabbits underwent BAL procedure using 20 ml/kg of prewarmed (37°C) 0.9% NaCl solution as previously reported.[12] At the same time, a blood sample was collected. BAL samples were immediately centrifuged at 100 × *g* for 10 min to sediment cells and cell debris. Blood samples were centrifuged at 1400 × *g* for 10 min to obtain the plasma sample. BAL supernatant and plasma were aliquoted and stored at −80°C.

The diet (dry chow) was the same for all animals and was kept constant throughout the study.

The deuterium enrichment of DDW, DEW and TW was analysed before the study design.

2.3 | Extraction and isolation of DSPC from BAL samples

BALs were thawed on ice and then homogenized by vortex for 10 s. Lipids were extracted by the method of Bligh and Dyer.[13] Extracted lipids were oxidized with osmium tetroxide, resuspended in chloroform and spotted on silica gel G thin-layer plates (Merck, Darmstadt, Germany). The plates were developed with chloroform: methanol:isopropanol:0.25% potassium chloride:triethylamine (40:12:33:8:24).

DSPC was visualized against a standard and scraped off from the silica gel plate. DSPC saturated fatty acids were derivatized as methyl ester by adding 2 ml of 3-M HCl methanol and heating at 100°C for 1 h. Methyl esters were then extracted with hexane.

2.4 | Plasma sample preparation

Plasma samples were thawed, and 100 µl was used for analysis. Plasma was deproteinized with sulphosalicylic acid (6% w/v), vortexed and spun at 11,000 × *g* to precipitate proteins. The supernatant was introduced into the upper part of a minicentrifuge tube of 10 kDa and centrifuged at 11,000 × *g* for 90 min at 4°C. The filtrated supernatant at the bottom of the tube was collected and transferred to a vial.

2.5 | Stable isotope analysis

2.5.1 | GC-P-IRMS

The online determination of the $^{2}H/^{1}H$ isotope ratios of DSPC fatty acids was performed with a Delta V Advantage IRMS (Thermo Fisher Scientific, Bremen, Germany) coupled by an 'open split' via a pyrolysis reactor (ceramic tube [Al_2O_3], reactor temperature 1400°C) to an HP 6890 gas chromatograph (GC). The GC was equipped with an Ultra-2 (25-m, 0.32-mm and 0.52-µm film thickness) column (Agilent). The following conditions were employed: a 1-µl on-column injection; a temperature program starting from 60°C (isothermal for 1 min), increased by 30°C/min to 195°C (isothermal for 2 min), and increased by 30°C/min to 240°C (isothermal for 7 min); and helium flow of 1 ml/min.

The separated fatty acids, represented mainly by palmitic acid (PA), eluting from the GC column were converted into H_2 in the pyrolysis interface. Analysis was performed in the mass spectrometer by simultaneous recording of Masses 2 ($^{1}H^{1}H$) and 3 ($^{1}H^{2}H$).[14]

Determinations were carried out five times, and the mean value of the last three injections was used. The isotope ratios are expressed as $\delta^{2}H‰$ (δ‰) against Vienna Standard Mean Ocean Water (V-SMOW):

$$\delta 2H\,(‰) = \frac{R\,sample - R\,SMOW}{R\,SMOW} * 1000,$$

where *R* is the isotope ratio of $^{2}H/^{1}H$. The results are expressed as the $\delta^{2}H‰$ of PA of alveolar surfactant DSPC.

2.5.2 | TC-EA/IRMS

The online determination of the $^{2}H/^{1}H$ of water samples and of plasma water pool was performed using a high-temperature conversion elemental analyser (TC-EA) coupled with a Delta^{Plus}XL IRMS via a ConFlo III Interface (Thermo Fisher Scientific, Bremen, Germany). The $^{2}H/^{1}H$ ratios were corrected for the $3H+$ effect. Each sample was injected five times, and the mean value of the last three injections was used to calculate the $^{2}H/^{1}H$ enrichment value against the reference gases injected in the same run. Reference water was interspersed every 20 injections to ensure that memory effects were not relevant. The isotope ratios $^{2}H/^{1}H$ are expressed as ‰ against V-SMOW.

Results are expressed as mean and standard deviation.

3 | RESULTS

DDW was administered for 5 days to four rabbits, with a body weight of 1.9 ± 0.2 kg (min 1.7 kg and max 2.1 kg), DEW to five rabbits with a body weight of 1.6 ± 0.1 kg (min 1.4 kg and max 1.7 kg) and TW to four rabbits with a body weight of 2.2 ± 0.1 kg (min 2.2 kg and max 2.3 kg). No adverse effects were observed during DDW and DEW administration.

The $\delta^{2}H$ value of administered DDW and of DEW was −984 ± 2‰ and +757 ± 2‰, respectively, whereas the $\delta^{2}H$ value of TW was −58 ± 1‰.

At the end of the experiment, the $\delta^{2}H$ value of plasma water pool was −467 ± 87‰, +377 ± 56‰ and −53 ± 6‰, and the $\delta^{2}H$ value of BAL DSPC was −401 ± 27‰, −96 ± 38‰ and −249 ± 9‰ in DDW, DEW and TW groups, respectively. The mean $\delta^{2}H$ value of drinking water, plasma water pool and BAL DSPC-PA is depicted in Figure 1.

After 5 days, the mean difference between the $\delta^{2}H$ value of the DSPC-PA found in the BAL of rabbits drinking DDW or DEW and those drinking TW was −153 ± 27 and −153 ± 38 δ‰, respectively.

4 | DISCUSSION

In this pilot study, we demonstrated that even with subtle variation in the deuterium content of the drinking water and the use of high-precision isotope ratio mass spectrometer, it is possible to obtain measurable changes of the deuterium background of both plasma water pool and lung phospholipid palmitate.

The incorporation of deuterium from water into lipids has been studied for decades, and it is an attractive method for determining the fractional synthesis rate of fatty acids and cholesterol especially in humans.[2,6,15,16] The use of deuterated water offers some advantages, as compared with other tracers: The label is administered orally, and the isotope is relatively inexpensive. On the other hand, there are several shortcomings associated with the use of deuterated water for kinetic studies. First, the level of deuterium incorporation depends both on the deuterium enrichment in the water pool and on the maximum number of deuterium atoms (N) incorporated into the newly synthesized molecules. Deuterium or hydrogen from body water can be incorporated in only 21 out of the 32 hydrogen atoms of PA and only 27 out of the 46 atoms of cholesterol; thus, the biochemistry analysis requires proper correction.[17] The information on the extent of the isotopic effect during the lipogenesis is not fully understood especially at high deuterium concentrations.[16,18]

In addition, there are concerns on the toxic effect of very high concentration of deuterium tested in animal studies.[19]

The effect of deuterium on living organisms has been investigated extensively[20] after its discovery by Urey et al.[21] Thomson investigated the physiological effects of deuterium on rats. In about 10 days when

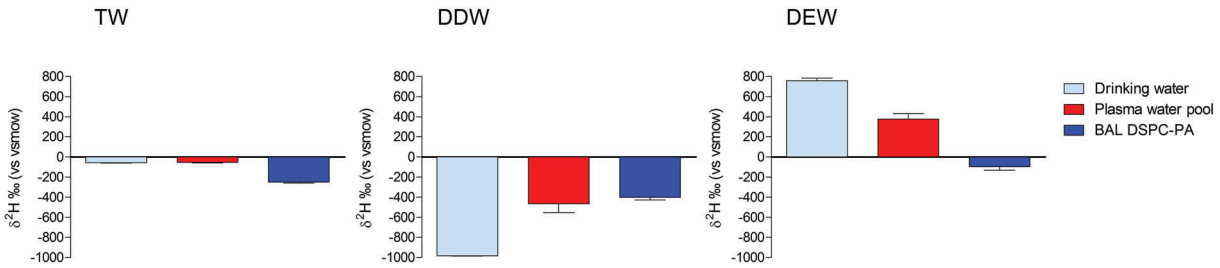

FIGURE 1 δ^2H values of drinking water, plasma water pool and desaturated phosphatidylcholine–palmitic acid (DSPC-PA). Data are expressed as mean values and standard deviation. δ^2H values of drinking water and of plasma water pool were determined by TC-EA/IRMS, whereas δ^2H value of DSPC-PA was determined by GC-P-IRMS. BAL, bronchoalveolar lavage; DDW, deuterium-depleted water; DEW, deuterium-enriched water; TW, tap water

15% of the body water was replaced by deuterium, the animal showed hyperactivity. When 20% to 25% of the body water was replaced by D_2O, the rats became hyperexcitable, and at 30% replacement, their body weight decreased, and the animal died from respiratory depression.[22] D_2O is known to be toxic in animals at high enrichment levels (>15%).[19] However, adverse effects become noticeable at 0.5% enrichment in bacterial cultures when bacterial growth is significantly reduced.[23] Although these adverse effects were reported at concentrations of at least 10 times higher than those employed in human studies,[2,6,16] still some concerns do exist.

Compared with the biological changes determined by the excess of deuterium in the water, the effects of reducing the concentration of deuterium in water are less studied. DDW can be found as a commercially available drinking water that has a sixfold to sevenfold less concentration of the naturally occurring deuterium than in the common TW (20–25 vs. 150 ppm).

In animal and cell-based studies, scientists have examined the potential positive effects of DDW on cell growth,[24] antioxidant protection,[25] energy production and metabolism,[26] and long-term memory in rats.[27] Cells exposed to DDW are reported to be more active, grew faster and have a lower level of inflammation. No side effects on human consuming DDW as drinking water have been reported so far.

The novelty of our pilot study resides on the use of DDW for the study of lipogenesis. To the best of our knowledge, DDW has never been used to change the enrichment of body water in living subjects with the aim of labelling the endogenous lipids pool. Moreover, the administered dose of D_2O was significantly lower than the dose that is usually used in animal and in human experiments to study lipid metabolism.[2,6,15]

In this pilot study, DDW appears to behave as DEW, and these preliminary results are noteworthy. After 5 days, the δ^2H of DSPC-PA in the BAL pool was −401 ± 27‰ and −96 ± 38‰ in DDW and DEW groups, respectively, with a mean difference of 153‰ from the δ^2H value of the DSPC-PA found in the BAL of the control group (−249 ± 9‰). Our analytical error for GC-P-IRMS analysis of BAL DSPC-PA processed in single and injected in quintuplicate is 3‰. Under this condition, the difference in the δ^2H of DSPC-PA was 51 times higher than the analytical error. Based on the obtained preliminary information, we can be confident that 5 days of DDW as drinking water leads to measurable changes in endogenous water and endogenous lipids (DSPC-PA).

We recognize that this study has several limitations. First, this method is not suited for short-term tracer studies as priming is not possible and steady state will be difficult to achieve given the slow turnover of body water. However, we foresee that, in clinical settings, and when enough time for depleting deuterium body water is possible, this method could be useful for the study of lipogenesis in very delicate situations, such as during human pregnancy or in preterm infants. During such studies, repeated samples for body water enrichment will be possible by measuring deuterium enrichment either in urine and plasma water or in saliva. A significant limitation is also the expensive instrumentation (GC-IRMS) required and the need for highly specialized technical people for its use.

5 | CONCLUSION

In conclusion, our pilot study demonstrates the feasibility of using DDW to label pulmonary surfactant lipids. This novel approach uses inexpensive commercially available DDW, and it can be used in animals as well as in humans. We speculate that the use of DDW as tracer could be associated with a more favourable study compliance in human studies, as it is not associated with the existing concerns for the use of DEW.

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CONFLICT OF INTERESTS

Francesca Ricci, Chiara Catozzi and Fabrizio Salomone are Chiesi Farmaceutici S.p.A. employees. Chiesi Farmaceutici S.p.A. sponsored the study but had no influence in the analysis and interpretation of data and in the manuscript drafting. Paola Cogo received a grant from Chiesi Farmaceutici S.p.A. Virgilio Carnielli received speaker fees from Chiesi Farmaceutici S.p.A. Apart from this, the authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Paola Cogo and Virgilio Carnielli conceptualized and designed the study. Fabrizio Salomone and Francesca Ricci contributed to the experimental plan design. Francesca Ricci, Chiara Catozzi and Matteo Storti carried out the in vivo experiments and collected the clinical data. Manuela Simonato and Sonia Giambelluca were in charge of sample analysis. Alessio Correani performed the data analysis. The first draft of the manuscript was written by Manuela Simonato, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Suzuki R, Ferris HA, Chee MJ, Maratos-Flier E, Kahn CR. Reduction of the cholesterol sensor SCAP in the brains of mice causes impaired synaptic transmission and altered cognitive function. *PLoS Biol*. 2013;11(4):e1001532
2. Cogo PE, Ori C, Simonato M, et al. Metabolic precursors of surfactant disaturated-phosphatidylcholine in preterms with respiratory distress. *J Lipid Res*. 2009;50(11):2324-2331.
3. Song Z, Xiaoli AM, Yang F. Regulation and metabolic significance of de novo lipogenesis in adipose tissues. *Nutrients*. 2018;10(10):1-22.
4. Kim IY, Suh SH, Lee IK, Wolfe RR. Applications of stable, non-radioactive isotope tracers in in vivo human metabolic research. *Exp Mol Med*. 2016;48(1):e203
5. Cogo PE, Simonato M, Danhaive O, et al. Impaired surfactant protein B synthesis in infants with congenital diaphragmatic hernia. *Eur Respir J*. 2013;41(3):677-682.
6. Simonato M, Baritussio A, Ori C, et al. Disaturated-phosphatidylcholine and surfactant protein-B turnover in human acute lung injury and in control patients. *Respir Res*. 2011;12(1):36
7. Biagetti C, Vedovelli L, Savini S, et al. Double blind exploratory study on de novo lipogenesis in preterm infants on parenteral nutrition with a lipid emulsion containing 10% fish oil. *Clin Nutr*. 2016;35(2):337-343.
8. Facco M, Nespeca M, Simonato M, et al. In vivo effect of pneumonia on surfactant disaturated-phosphatidylcholine kinetics in newborn infants. *PLoS ONE*. 2014;9(12):e93612
9. Gázquez A, Prieto-Sánchez MT, Blanco-Carnero JE, et al. In vivo kinetic study of materno-fetal fatty acid transfer in obese and normal weight pregnant women. *J Physiol*. 2019;597(19):4959-4973.
10. Hagemann R, Nief G, Roth E. Absolute isotopic scale for deuterium analysis of natural waters. Absolute D/H ratio for SMOW. *Tellus*. 1970;22:712-715.
11. de Wit JC, van der Staaten CM, Mook WG. Determination of the absolute hydrogen isotopic ratio of V-SMOW and SLAP. *Geostand Geoanal Res*. 1980;4(1):33-36.
12. Ricci F, Catozzi C, Murgia X, et al. Physiological, biochemical, and biophysical characterization of the lung-lavaged spontaneously-breathing rabbit as a model for respiratory distress syndrome. *PLoS ONE*. 2017;12(1):e0169190
13. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol*. 1959;37(8):911-917.
14. Hilkert AW, Douthitt CB, Schluter HJ, Brand WA. Isotope ratio monitoring gas chromatography/mass spectrometry of D/H by high temperature conversion isotope ratio mass spectrometry. *Rapid Commun Mass Spectrom*. 1999;13(13):1226-1230.
15. Previs SF, Herath K, Nawrocki AR, et al. Using [^{2}H]water to quantify the contribution of de novo palmitate synthesis in plasma: enabling back-to-back studies. *Am J Physiol Endocrinol Metab*. 2018;315(1):E63-E71.
16. Previs SF, Mahsut A, Kulick A, et al. Quantifying cholesterol synthesis in vivo using ^2H_2O: enabling back-to-back studies in the same subject. *J Lipid Res*. 2011;52(7):1420-1428.
17. Diraison F, Pachiaudi C, Beylot M. In vivo measurement of plasma cholesterol and fatty acid synthesis with deuterated water: determination of the average number of deuterium atoms incorporated. *Metabolism*. 1996;45(7):817-821.
18. Lakshmanan MR, Veech RL. Measurement of rate of rat liver sterol synthesis in vivo using tritiated water. *J Biol Chem*. 1977;252(13):4667-4673.
19. Jones PJ, Leatherdale ST. Stable isotopes in clinical research: safety reaffirmed. *Clin Sci (Lond)*. 1991;80(4):277-280.
20. Misra PM. The effects of deuterium on living organisms. *Curr Sci*. 1967;36(17):447-453447.
21. Urey HC, Brickwedde FC, Murphy GM. A hydrogen isotope of mass 2. *Phys Rev*. 1932;39(1):164-166.
22. Thomson JF. Physiological effects of D_2O in mammals. *Ann N Y Acad Sci*. 1960;84(16):736-744.
23. Xie X, Zubarev RA. Effects of low-level deuterium enrichment on bacterial growth. *PLoS ONE*. 2014;9(7):e102071
24. Syroeshkin AV, Antipova NV, Zlatska AV, et al. The effect of the deuterium depleted water on the biological activity of the eukaryotic cells. *J Trace Elem Med Biol*. 2018;50:629-633.
25. Zhang X, Gaetani M, Chernobrovkin A, Zubarev RA. Anticancer effect of deuterium depleted water-redox disbalance leads to oxidative stress. *Mol Cell Proteomics*. 2019;18(12):2373-2387.
26. Halenova T, Zlatskiy I, Syroeshkin A, Maximova T, Pleteneva T. Deuterium-depleted water as adjuvant therapeutic agent for treatment of diet-induced obesity in rats. *Molecules*. 2019;25(1):1-15.
27. Mladin C, Ciobica A, Lefter R, Popescu A, Bild W. Deuterium-depleted water has stimulating effects on long-term memory in rats. *Neurosci Lett*. 2014;583:154-158.

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