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PRELIMINARY REPORT

# Determination of Doubly Labeled Water by Gas-Phase Fourier Transform Infrared Spectroscopy

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**Both $^{2}H$ (deuterium) and $^{18}O$ (oxygen 18) in isotopically enriched water have been detected by gas-phase Fourier transform infrared (FTIR) spectroscopy at 2,720 and 3,661.8 $cm^{-1}$, respectively. A linear relationship between varying concentrations of each of these isotopes and their absorbance at the above frequencies indicates that gas-phase FTIR may provide a rapid and potentially less expensive approach to measure doubly labeled water in biological fluids for the estimation of energy expenditure and total body water.**


IN 1988, A METHOD to determine total energy expenditure in free-living humans was reported.[1] The method is based on the determination of $CO_2$ production from the difference in elimination rates of deuterium ($^{2}H$) and oxygen 18 ($^{18}O$) from body water labeled by the prior administration of $^{2}H_2O$ and $H_2\,^{18}O$. In addition, either of these isotopes can be used to determine, by isotope dilution, total body water. Concentrations of these isotopes in samples of body water (eg, urine, saliva) are determined by isotope ratio mass spectrometry (IRMS). The determination of $^{18}O$ requires a 24-hour equilibration of the sample (eg, urine) with $CO_2$ to exchange and bring into isotopic equilibrium the oxygen 16 of the $CO_2$ and the $^{18}O$ of the water. The resulting $C^{18}O_2$ is injected into the IRMS for $^{18}O$ analysis. Deuterium is released by a Zn–heat-mediated decomposition of the doubly labeled water followed by injection of the deuterium gas into the IRMS. This method, although unique in its capacity to measure the energy expenditure of unrestricted individuals, is limited by the very high cost of the highly specialized IRMS instruments. In addition, the sample preparation is time-consuming, which adds to the overall costs of the assays. An alternative procedure for rapid quantitation of $^{2}H$ and $^{18}O$ using a less expensive instrument is therefore highly desirable. Gas-phase Fourier transform infrared (FTIR) spectroscopy, as described below, may provide such an approach.

## MATERIALS AND METHODS

The natural abundance of $^{2}H$ is 0.015 atom %, and that of $^{18}O$ is 0.20. When natural water is enriched with any one or both of these isotopes, their enrichment is expressed as atom percent excess (APE), which indicates the excess of a particular isotope above its natural abundance. $^{2}H_2O$ (99.9%) and $H_2\,^{18}O$ (8.54%) were purchased from Icon (Summit, NJ). Serial isotopic enrichment of drinking water obtained from a tap was made with these commercial isotopically enriched water samples. Based on the above-mentioned natural abundance of $^{2}H$ and $^{18}O$ in tap water, the APEs of both $^{2}H$ and $^{18}O$ were adjusted in such a manner that they were within the expected ranges for total body water and total energy expenditure measurements.

An infrared (IR) gas-phase sample cell, to be described in detail elsewhere, was constructed. The cell is made from a solid block of aluminum into which a chamber with two IR-transparent windows

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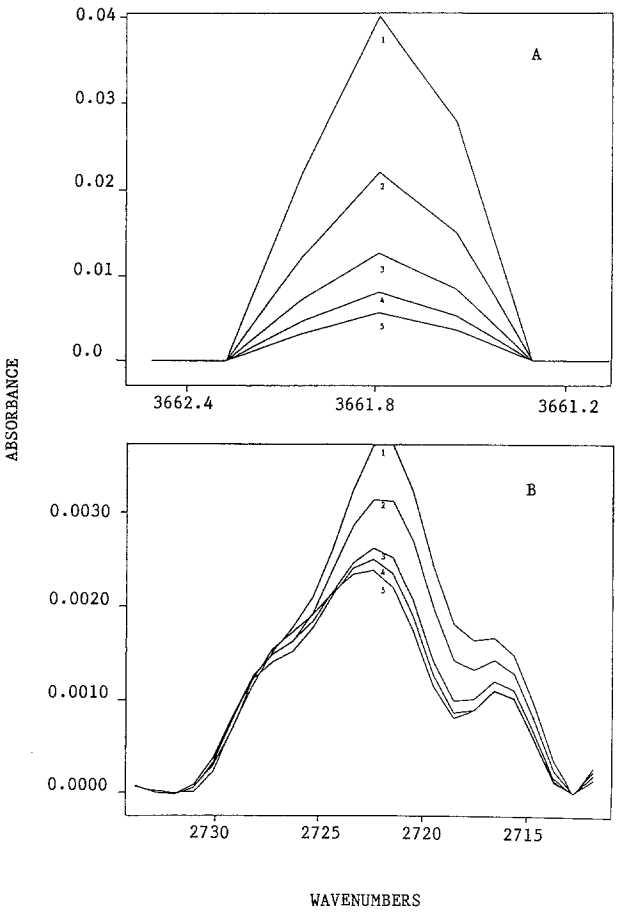


**Fig 1. (A) FTIR spectra for various $^{18}O$ concentrations (APE) as numbered: 1, 2.85; 2, 1.43; 3, 0.71; 4, 0.36; and 5, 0.18. (B) FTIR spectra for $^{2}H$ concentrations (APE): 1, 0.067; 2, 0.038; 3, 0.019; 4, 0.0095; and 5, 0.0050.**

is machined. A heating element maintains the cell at 120°C, and provisions are made to connect the cell to a vacuum source. After evacuation, a 40-μL volume of sample (ie, urine) is injected into the cell, where it instantaneously evaporates in a system that filters off the solid residue of unpurified biological fluids. This is necessary to keep the cell windows clean.

All FTIR measurements were made with a Mattson Research Series 1 FTIR spectrometer (Madison, WI) equipped with a wide-band mercury-cadmium-telluride detector. IR absorption bands at 3,661.8 and 2,720 $cm^{-1}$, corresponding respectively to $^{18}O$ and $^{2}H$, were identified and found appropriate for quantitative analysis. A resolution of 0.5 $cm^{-1}$ with 32 scans was required to obtain a quantitatively measurable $^{18}O$ IR spectrum, whereas a suitable spectrum for $^{2}H$ required 200 scans at a resolution of 2 $cm^{-1}$.

## RESULTS AND DISCUSSION

The IR spectral bands for $^{18}O$ and $^{2}H$ are shown in Fig 1A and B, respectively. The five tracings (no. 1 through 5) in each figure correspond to the different APE of each isotope as indicated in the figure legend. The minimum required line width for the $^{18}O$ bands is 0.5 $cm^{-1}$, and for $^{2}H$, 2 $cm^{-1}$. At this high resolution, the water signals are well separated and the peak due to $^{18}O$ becomes clearly observable in the gas-phase spectrum of doubly labeled water. The broadness of the IR bands of liquid water precludes observation of the $^{18}O$ peak. Previously, both $^{18}O$ and $^{2}H$ in water had been detected by gas-phase IR,[2] but only when present as pure $^{2}H_2{}^{16}O$, $^{2}H_2{}^{18}O$, or $^{1}H_2{}^{18}O$. For body composition and energy expenditure measurements, both these isotopes remain as isotopic mixtures, ie, $^{1}H^{2}H^{16}O_{1/2}{}^{18}O_{1/2}$, which have not been detected previously. The $^{2}H$ signal, on the other hand, has been detected in liquid phase and used successfully to measure total body water in humans.[3] The coefficient of variation for the solution-phase IR method has been reported to be 2.5%, whereas we found it to be 1.56% in the gas phase. This is indicative of the high stability of the gas-phase IR procedure.

Figure 2 shows the regression analysis of peak absorbances of five different concentrations (as APE) of $^{18}O$ (Fig 2A) and $^{2}H$ (Fig 2B). The straight lines were obtained with very high correlation coefficients, ie, .9987 for $^{18}O$ and .9948 for $^{2}H$. Such linearity implies that the observed $^{18}O$ and $^{2}H$ peaks can be used to determine the concentration of these isotopes in the water of any biological fluid. However, the sensitivity of the gas-phase FTIR method is considerably lower than that of the IRMS procedure. The quantitation by FTIR of low $^{18}O$ concentrations (ie, <0.25 APE) becomes unreliable. With the doses of doubly labeled water

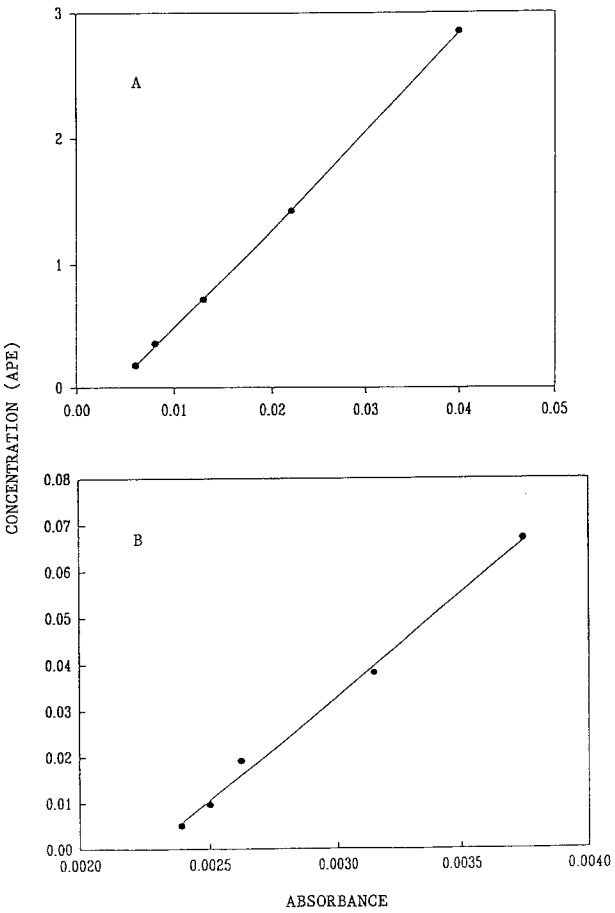


**Fig 2. Plots of IR absorbances *v* concentrations (APE). (A) $^{18}O$; (B) $^{2}H$.**

currently used for the determination of energy expenditure, the sensitivity of the gas-phase FTIR would therefore be inadequate and higher doses (three to four times higher) would be required. The use of these higher doses would obviously increase the cost of the procedure, making it impractical except for the determination of energy expenditure in small children and experimental animals. On the other hand, considering the major savings in instrumentation costs and the simplicity and rapidity of the FTIR procedure (which requires no sample preparation and only 5 to 7 minutes per determination), this method may ultimately prove economically advantageous over the IRMS procedure. It is also anticipated that the price of $^{18}O$-labeled water will decrease as the determination of total energy expenditure by doubly labeled water becomes more frequently and widely used.

## REFERENCES

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