Immediate biofeedback for energy balance via expired breath $\delta^{13}CO_2$*

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***Abstract*—Expired breath $\delta^{13}CO_2$ measured in real time serves as a useful biomarker of altered macronutrient metabolism in response to changes in energy balance. Altered breath $\delta^{13}CO_2$ is believed to be a result of changes in macronutrient oxidation and the kinetic isotope effect where enzymatic processes discriminate against metabolites naturally enriched with ^{13}C. Use of breath $\delta^{13}CO_2$ as a rapid biofeedback of energy balance status will enhance an individual's ability to modify behavior during weight loss efforts. Herein we describe a novel approach for immediate biofeedback for energy deficit using a moderate exercise challenge. Our new mid-infrared isotope ratio-meter for $\delta^{13}CO_2$ is a step toward miniaturization of a personal device for instant biofeedback for people attempting to lose weight.**

I. INTRODUCTION

Obesity has reached epidemic proportions worldwide [1]. The prevalence of overweight (Body Mass Index [BMI] ≥ 25 kg/m^2) and obese (BMI ≥ 30 kg/m^2) individuals has continued to rise in the U.S. The NHANES data from 2003-2004 indicate that 66.3% of adults age 20 and older are overweight or obese [2]. Both overweight and obesity status have been associated with numerous additional health problems including diabetes, hypertension, high cholesterol levels, arthritis, asthma, and generally poor health status [3]. Although almost 75% of Americans are attempting to lose or maintain weight [4], weight-loss and weight-maintenance strategies are clearly not working since the prevalence of obesity continues to increase.

Successful weight loss programs require patient compliance with an energy-restricted diet, and compliance is best achieved when the patient is positively reinforced through outcomes. Current methods of monitoring compliance to prescribed guidelines for weight loss include monitoring changes in weight and body composition and recording dietary intake. Tracking of weight and body composition allows for feedback on a weekly basis but is not sensitive enough for daily feedback. Monitoring food intake is a useful treatment technique for behavioral change, but maintaining this technique long-term is time-consuming and challenging for many individuals. In addition, while dietary record keeping provides increased awareness of eating, it is notoriously inaccurate [5] and does not give any direct positive reinforcement of successful efforts. Personalized daily feedback would allow for such immediate positive reinforcement or, conversely, counseling efforts to change intake patterns for unsuccessful attempts. Weinstein (1988) suggests that personalized feedback is an important component to awareness and motivation to change behaviors [6].

Naturally occurring stable isotope ratios in body proteins, fats and carbohydrates will reflect the isotope ratios of the diet consumed [7, 8]. Measurements of the isotopic abundance of ^{13}C are typically expressed as a relative change in the ratio of $^{13}C/^{12}C$ in a sample compared to a standard (Pee Dee Belemnite, PDB) using the delta per mil (‰) notation:

$$\delta^{13}C = \frac{(^{13}C/^{12}C\ \text{sample} - {}^{13}C/^{12}C\ \text{PDB})}{^{13}C/^{12}C\ \text{PDB}} \times 1000$$

As macronutrients are metabolized for energy, the carbon structures from the diet are converted to CO_2. Typically the body is oxidizing a mixture of carbohydrate, protein and lipid. During the conversion of glucose to lipids, enzymatic processes discriminate against ^{13}C [9]. This results in stored lipids being about 3.5‰ lighter than carbohydrates in the body [10]. Therefore, respired carbon stable isotope ratios

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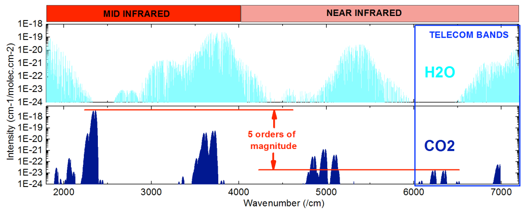

Fig. 1: Molecular absorption intensities of water (top) and CO_2 (bottom): Mid-Infrared Vs. Near-infrared.

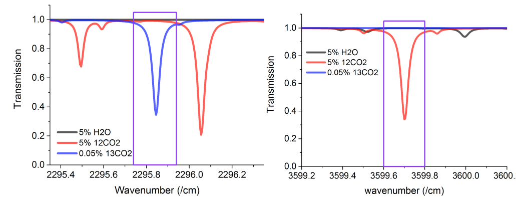

Fig. 2: A simulated spectrum of Breath at 200 mbar showing the selected $^{12}CO_2$ and the $^{13}CO_2$ molecular transitions.

may be useful for tracking the reliance on lipid oxidation [11]. Thus a decrease in ^{13}C in breath may indicate compliance with an energy-restricted diet [12, 13]. The concept of using stable isotopes of carbon to track the source of metabolic fuel has been applied successfully to animals and humans [14-16].

We have developed a non-invasive breath monitoring system based on naturally occurring breath stable isotopes that will enable immediate biofeedback on energy balance for individuals. Our approach uses a novel mid-infrared dual beam technique to optimally derive concentrations of $^{13}CO_2$ and $^{12}CO_2$ in expired breath. We report here the development details of our new isotope ratio-meter designed for determining the $\delta^{13}CO_2$ in breath, and its application as an immediate biofeedback tool for energy balance during weight maintenance and energy deficit. Our approach may provide an individualized immediate biofeedback system for use during weight management efforts.

II. Methods and Results

A. Novel Isotope Ratio-meter Development

Commercial cavity ring-down spectrometer (CRDS)-based carbon isotope analyzers operate in the near-infrared (IR) spectral region. Here molecules absorb radiation through the excitation of ro-vibrational overtones. Fundamental ro-vibrational absorption bands are to be found in the mid-IR spectral region and are much more intense than ro-vibrational overtones found in the near-IR; five orders of magnitude in measurement sensitivity can be gained from operation in the mid-IR compared to near-IR spectroscopy while still obviating water interference (**Fig. 1**). Until recently the convenience and economy of off-the-shelf telecoms diode lasers (DLs) and the lack of reliable and compact mid-IR laser sources have outweighed the sensitivity advantage of probing the fundamental absorption bands. However, more recently, solid-state continuous wave lasers such as Interband Cascade Lasers (ICLs) and Quantum Cascade Lasers (QCLs) have reached technical maturity, enabling commercially viable manufacturing, proven by the entrance of several manufacturers on the market (Thor Labs, AdTech, Alpes Lasers, Daylight Solutions, Hamamatsu). QCLs are compact uncooled, single mode, robust, tunable infrequency and reliable laser sources emitting in the mid-IR (3-20 µm).

Tunable laser-based isotope analysis has been an active area of research and development over the last few years in various research institutions [17]. Tunable Laser Absorption Spectroscopy (TLAS) techniques, using advanced infrared laser sources, offer the unique opportunity to overcome the drawbacks of mass spectroscopy and to enable real-time *in situ* monitoring of stable isotopes with high sensitivity, which is of particular relevance to breath analysis.

We have developed a portable dual laser system operating at room temperature using an antimonide distributed feedback (DFB) Diode Laser and a DFB QCL [18]. The dual wavelength approach stems from an exhaustive spectroscopic survey to select the optimum pair of $^{12}CO_2$ and $^{13}CO_2$ absorption lines [19] yielding $\delta^{13}CO_2$ measurements immune from artifacts due to sample temperature variation. This relaxes the temperature stability constraints usually associated with TLAS and CRDS isotope ratio systems.

As shown in **Fig. 2**, the $^{12}CO_2$ and $^{13}CO_2$ absorption intensities of the selected lines are similar for an exhaled breath sample. The selected lines are among the most intense to ensure high sensitivity for natural abundance of both $^{12}CO_2$ and $^{13}CO_2$, and are free from spectral interference from other gaseous species.

In addition to the unique line selection, a patented noise and baseline cancellation scheme underpins the instrument's capabilities [20]. As shown in **Fig. 3**, the radiation from the two lasers is combined, and then directed along both paths of a dual-channel absorption cell, where the laser radiation interacts with the sample. The instrument incorporates an on-board real-time referencing scheme, which ensures accurate and permanent calibration of the measurements relative to a known isotopic gas mixture. The use of two well-separated wavelengths allows spectral multiplexing in a single beam, ensuring that the radiation from the two lasers probe the sample in exactly the same way. Most importantly, a fast optical switch is employed for baseline noise cancellation. The optical beams are then de-multiplexed and

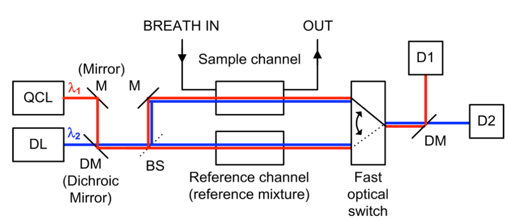

Fig. 3: Schematic of the isotope ratio-meter. λ_1 and λ_2 probe $^{13}CO_2$ and $^{12}CO_2$ respectively. Acronyms stand for: DL, Diode Laser; BS, Beam Splitter; D1 and D2, Detectors.

directed to detectors.

Using this method the ratio-meter is only sensitive to real changes in sample concentration with respect to the reference mixture. Any changes in the baseline or detector non-linearity are ideally fully compensated, as well as some of the technical noise.

The device is designed specifically to measure the $\delta^{13}CO_2$ in human breath. The top panel of **Fig. 4** shows a record of delta values over about three minutes, during which different samples were flown through the instrument. Using a high temporal resolution and long record, we demonstrated the device is precise to 0.1‰ within 100s of measurement time. The instrument precision is assessed using the concept of Allan deviation that indicates how the statistical standard deviation of measured values evolves as the integration time is increased (bottom panel of **Fig. 4).** The dependence is shown to be as the square root of time as expected from a measurement dominated by random noise.

B. Immediate Biofeedback for Energy Balance After Exercise

The University of North Dakota Institutional Review Board provided oversight of the following study. Volunteers resided in a metabolic research unit. Details of the diet and exercise protocol are described in detail elsewhere [21]. Briefly, on days 1-10, human volunteers consumed a weight maintenance (WM) diet and participated in individually prescribed, supervised, daily exercise episodes at 40-60% VO_{2peak}. On days 11-31, the volunteers were in 40% energy deficit (ED) and continued with daily exercise. The study diet was based on a 3-day menu that was individually adjusted to caloric needs for WM or ED for each study volunteer. Exercise episodes were conducted either during the morning (following breakfast and prior to lunch) or in the afternoon (following lunch and prior to dinner). Breath samples were collected prior to, immediately after, and 30, 60 and 90 minutes post exercise during weight maintenance and energy deficit. For the purpose of this study the $\delta^{13}CO_2$ was determined using an isotopic CO_2 CRDS (Picarro, CA, USA). The results are shown in **Fig. 5**. The breath $\delta^{13}CO_2$ values increase in response to exercise under WM conditions ($p \leq 0.05$), but do not significantly change following exercise under ED conditions.

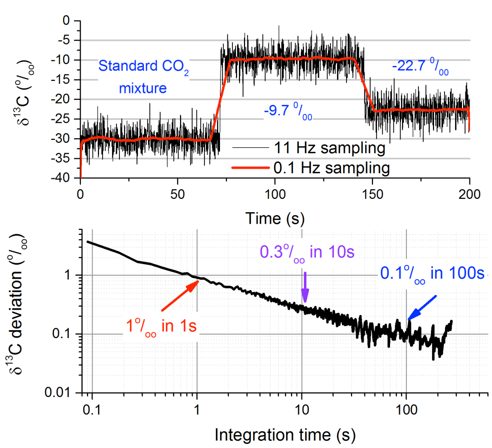

Fig. 4: Top: Calibrated data from the prototype during a 6-min data record with 5 measurements/s. **Bottom:** Corresponding Allan deviation analysis.

III. DISCUSSION

This study demonstrates a novel use of expired breath $\delta^{13}CO_2$ as a possible biofeedback tool for individuals attempting to lose weight by consuming an energy-restricted diet. Consumption of an energy-restricted diet resulted in a diminished change in the $\delta^{13}CO_2$ after a moderate exercise bout. In energy-restricted individuals, the breath $\delta^{13}CO_2$ values remained relatively depleted irrespective of the diet composition (**Fig. 5**), potentially indicating that breath was more representative of the substrate being utilized (i.e. stored fat) than contemporary diet consumption during energy restriction.

The ability to monitor compliance with an energy-restricted diet has value for investigators interested in dietary compliance and for individuals seeking motivation for weight loss. Personalized daily feedback, provided by breath $\delta^{13}CO_2$ values, could allow for immediate positive reinforcement or timely counseling efforts to change intake patterns for unsuccessful attempts. Behavioral psychology models suggest that personalized feedback is an important component for awareness and motivation to change behavior [6]. Timely biofeedback is important for individual

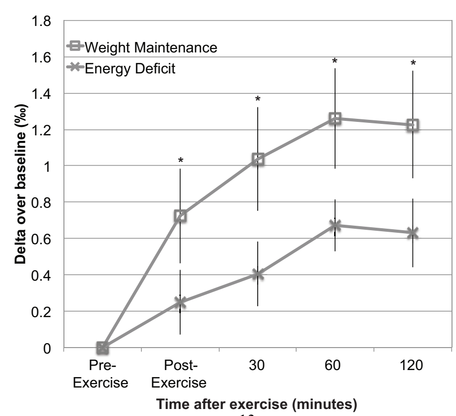

Fig. 5: Mean expired breath $\delta^{13}CO_2$ relative to baseline for weight maintenance or energy deficit conditions. Data are presented as mean with error bars representing the standard error of the mean. Asterisks (*) denote significant differences with $p \leq 0.05$. Data were analyzed with SAS version 9.4 using the Mixed procedure accounting for autocorrelation of repeated measures.

improvements in compliance to weight loss diets.

To enable this methodology as a weight loss aid for individuals we developed a new isotope ratio-meter designed for use in a health care or fitness center setting. This new device will enable individuals to use breath $\delta^{13}CO_2$ to non-invasively and rapidly monitor their energy balance to aid in weight management efforts, and allow positive or negative feedback far earlier than is currently possible.

Our device uses a novel dual beam approach to derive concentrations of $^{13}CO_2$ and $^{12}CO_2$. It is currently a lab bench setup, designed specifically for breath diagnosis. The wavelengths (2.7 μm and 4.3 μm) for determining concentrations of $^{12}CO_2$ and $^{13}CO_2$ were selected to absorb at equal strength and minimize $\delta^{13}CO_2$ artifacts induced by temperature variations. In contrast, alternate approaches for $\delta^{13}CO_2$ determination, such as CRDS, require the measurement cell to have exquisite control over temperature and pressure. For example, commercial CRDS analyzers control the temperature of the optical bench to 1/1000 of a degree Celsius [22]. Furthermore, to achieve a high precision of ~0.1‰ in 100 seconds of measurement, our technology does not need high reflectivity optics required by CRDS, which makes it more relevant to real-world unprocessed samples and less demanding in terms of maintenance. Typically a loss of ~10 ppm of CRDS mirror reflectivity incurs a sensitivity drop of two orders of magnitude.

Currently our system is 40x20x10 cm^3 without any efforts at reduction in form factor. The graphical user interface is also designed for an unskilled user with placement in healthcare or fitness club settings in mind. However, further reductions in size and complexity of the system are being developed to enable a hand-held device capable of breath carbon isotope ratio analysis. In its current stage our technology, nevertheless, offers options for center-based programs.

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