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Review

Measuring energy expenditure in birds using bolus injections of ^{13}C-labelled Na-bicarbonate ☆

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ABSTRACT

The ^{13}C-labelled Na-bicarbonate technique uses stable isotopes to measure energy expenditure in birds. After administration, the isotopes reach equilibrium within the body's bicarbonate pools at a fast rate due to the small size of the bicarbonate pool in relation to CO_2 flux. This technique is therefore ideal for measuring energy expenditure over short-term activities. The major advantage of this technique is that it can be applied without the animal having to wear a respirometry mask or being enclosed in a respirometry chamber. Despite the technique's suitability for use in birds and other animals, there have been few studies that have used it to date and so its potential is not fully understood. Here we discuss the methodology and review previous applications.

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1. Introduction

Various methods are available to physiologists to measure the energy expenditure of animals (reviewed in this issue). By using indirect calorimetry, for example, one can quantify the energy expenditure of birds when CO_2 production rate is measured in the animal's breath. Animals are usually required to wear respirometry masks or are kept in respirometry chambers (see Welch this issue, Bech this issue). This, however, may impede or preclude some activities an animal would usually perform. The doubly labelled water (DLW) technique allows the measurement of energetics without the requirement of a mask or respirometry chamber (Speakman, 1997; Shaffer this issue). The DLW method is based on the relative washout rates of ^{18}O and ^{2}H-labelled water, which corresponds to the water flux and CO_2 production rate. But since an animal's body water pool is large in relation to its daily water flux and CO_2 production rate, it is not possible to measure the energetics of short-term activities.

This methodological gap is filled by the labelled Na-bicarbonate technique, modified for bolus injections according to Speakman and Thomson (1997). This technique allows quantification of an animal's CO_2 production rate while performing short-term activities, i.e. of several minutes duration, without restricting the animal's movements. The Na-bicarbonate method involves measuring the washout rate of ^{14}C- or ^{13}C-labelled Na-bicarbonate that is administered to the animal. Two major variations of this technique are currently in use; continuous infusion and bolus injection of Na-bicarbonate solutions labelled with heavy carbon. While we will briefly review general

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aspects of the technique and specific aspects of the continuous infusion technique, we will focus on the bolus injection of stable ^{13}C-labelled Na-bicarbonate during the remainder of the paper, since we believe that the bolus injection approach has more potential in birds.

The labelled Na-bicarbonate technique was developed originally using the radioactive isotope in the late 1960s to measure the energy expenditure of sheep (White and Leng, 1969; Young and Corbett, 1969; Corbett et al., 1971). In these first studies the isotope was administered by constant infusion to measure the CO_2 entry rate into the body bicarbonate pools. The stable isotope ^{13}C (or the radioactive isotope ^{14}C) was then incorporated into the body bicarbonate pool until equilibrium was reached. Then the heavy isotope was eliminated predominantly in expired CO_2 (Fig. 1). In a steady state the CO_2 incorporation rate should be the same as the sum of all the losses of CO_2 from the body bicarbonate pool, which are mainly from expiration (98%), urinary losses (less than 2%) and incorporation into other areas of the body (negligible).

There is more than one bicarbonate pool and therefore there is a degree of recycling between them. Irving et al. (1983) found three different pools in humans by analysing the elimination rate of ^{13}C in breath using multi-compartmental analysis. The first central circulatory pool is the pool most likely to dominate elimination kinetics, while the two additional pools were suggested to be the rapidly perfused (liver and brain), and the slowly perfused (bone) tissue pools due to their size and half-life of the isotope washout. There was no evidence however to confirm that the morphological pools actually map onto the three components identified from the compartmental analysis. The fact that there are multiple pools involved means that the labelled carbon elimination may not occur at a constant rate over the elimination period. However, Pallikarakis et al. (1991) found that multiple pools merge into one in exercising animals, because isotopic exchange among pools is enhanced when energy turnover is elevated.

The majority of the studies using the continuous infusion variation of the technique have involved either humans (Irving et al., 1983; Armon et al., 1990; Elia et al., 1995) or large agricultural animals, such as sheep, *Ovis aeries* (Young and Corbett, 1969; Corbett et al., 1971), pigs, *Sus scrofa* (Benevenga et al., 1992) or goats, *Capra hircus* (Junghans et al., 1997). Breath or urine is often the chosen type of sample for recovery of the labelled CO_2 in mammals. These probably cause less stress for the subject than blood samples, and in addition multiple samples can be taken over the sampling period. The breath sampling methodology is more indicative of simultaneous energy expenditure than the urine sampling methodology because breath is expired instantaneously while urine accumulates in the bladder over time, before being expelled. The potential for errors is high with urine sampling, because with an increased length of time that urine remains in the bladder, there is a higher possibility of mixing between different dilutions of the label, or exchange of the label with blood CO_2, both of which will affect the final rate of elimination of the isotope. Elia et al. (1988) found that when humans had labelled bicarbonate directly instilled into the bladder, 30%–40% of label was transferred across the bladder wall into the circulatory system every 30 min. They state, however, that this is a two-way process.

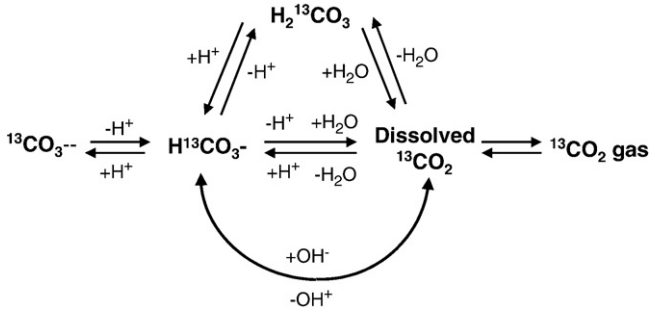

Fig. 1. Equilibrium of isotopic exchange between labelled dissolved CO_2 in blood and water based on Chinard et al. (1958). The catalyst for the main reaction is carbonic anhydrase.

Breath sampling seems to be the easiest and most precise method of isotope recovery. The method assumes that all of the isotope is eliminated through this source and can be recovered. In humans the recovery of the isotope in breath has been measured to be as high as 95.6% (Elia et al., 1992). In addition, the error calculated by using breath sampling to measure energy expenditure and ignoring urinary CO_2 was 1%–2% in sheep (Corbett et al., 1971). Lower recovery rates, particularly in the bolus injection approach of the Na-bicarbonate technique have been mostly associated with analytical errors (Leijssen and Elia, 1996).

Until 1997 the labelled Na-bicarbonate technique had only been used to measure energy expenditure in large animals. Speakman and Thomson (1997) adjusted the method for use in laboratory mice *Mus musculus*. Constant infusion techniques were difficult due to limitations in the size of mini-osmotic pumps available at this time, and so a single injection was the chosen method for isotope administration. The single injection technique has advantages over the constant infusion technique in that it is much easier to administer the isotope and the animal is free to move around and conduct normal activities throughout the measurement period. The isotope also reaches equilibrium within the pools at a fast rate due to the small size of the bicarbonate pool in relation to CO_2 flux, allowing measurements to be made soon after injection. The isotope is then eliminated with a biological half-life of typically around 10–20 min in small animals, highlighting the potential of this technique for measuring energy expenditure over short-term activities. The initial studies with single bolus injections of labelled Na-bicarbonate in agricultural animals have found that the elimination of labelled CO_2 is complex and conforms to a multi-exponential decline as the pools interact (White and Leng, 1969; Young and Corbett, 1969). In the few studies performed in birds however, a single exponential curve provides the best fit for the elimination of ^{13}C from the body, indicating that only a single pool may dominate the kinetics of isotopic washout during the first hour after injection (e.g. Hambly et al., 2002), however this needs to be investigated further in additional species.

The potential for the use of this technique in small animals and birds is still largely unknown. It is important for there to be more research to expand the capabilities of this approach for measuring short-term energetics. Several studies have now used the bolus injection technique to measure the energy expended during short flights in birds. The methodology and application of this technique will be discussed here.

2. Technical issues for the bolus injection technique

Thus far, the labelled Na-bicarbonate method has been used in 4 species of birds (Hambly et al., 2002, 2004a,b,c). The technique involves injecting a measured volume of ^{13}C-labelled Na-bicarbonate intraperitoneally (IP) into the bird. After a period of time to allow the ^{13}C to become mixed with the body bicarbonate pools, breath samples are collected prior to and after a period of activity. The difference between the amount of ^{13}C present in the breath before and after the activity, is directly related to CO_2 production and hence energy expenditure. There are several calibration steps that are required to enable the energy cost to be estimated.

2.1. Estimating the size of the bicarbonate pool

This initial step in this technique has been required for studies in medium to large sized bird species only (e.g. starlings (*Sturnus vulgaris*) and cockatiels (*Nyphicus hollandicus*) Hambly et al., 2004b,c) and is used to obtain a standard isotope dilution curve for each new solution of labelled Na-bicarbonate so that the size of the bicarbonate