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Validation of the labeled bicarbonate technique for measurement of short-term energy expenditure in the mouse

Validierung der ^{13}C-Bicarbonat-Methode zur Messung des Kurzzeit-Energieumsatzes bei Mäusen

Summary The energy expenditure of free-living animals has been studied extensively by the doubly-labeled water (DLW) technique. This method provides a reasonably accurate estimate of daily energy needs. However, there is considerable interest in the energy demands of animals over much shorter timescales, for which the DLW technique is less useful. We examined the possibility of measuring the expenditure of small animals over these shorter timescales from the washout kinetics of a bolus dose of ^{13}C labeled bicarbonate. The study involved 19 laboratory mice which were injected either IP or SC with 0.2 ml of ^{13}C labeled bicarbonate in water. Mice were placed in a standard respirometry system, maintained at different temperatures to precipitate a 3 fold variation in metabolism. Samples of breath were collected from the chamber into vacutainers at one minute intervals for approximately 40 minutes to an hour. Samples were analyzed by admission to a mass spectrometer (VG Optima) via a GC interface which identified and admitted the CO_2 peak. The log converted isotope elimination was linear ($r^2 > 98\%$ in all cases) indicating a single pool was involved. We evaluated the pool size from a dilution series of the injectate in equilibrium with CO_2 gas. Conventional compartmental analysis produced an estimate which on average across the 19 individuals provided a reasonable estimate of the CO_2 production. Individual estimates were however imprecise and the overall correlation between isotope and calorimeter estimates had an r^2 of only 15%. Reasons for this discrepancy are unclear. Nevertheless an empirical model, using the elimination gradient, pool size and route of isotope administration as predictors explained 86% of the variation in CO_2 production. Elimination of a bolus dose of ^{13}C labeled bicarbonate provides a useful tool for estimating the energy metabolism of mice over intervals between 15 and 40 minutes.

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Zusammenfassung Der Energieumsatz von frei lebenden Tieren wurde in zahlreichen Untersuchungen mit der doppeltmarkierten Wassermethode (DLWM) bestimmt. Diese Methode liefert ausreichend genaue Ergebnisse für den täglichen Energiebedarf. Es besteht jedoch ein beachtliches Interesse für Messungen über kürzere Zeiträume, die mit Hilfe der DLWM nicht erfaßt werden können. Wir prüften die Möglichkeit zur Messung des Energieumsatzes von kleinen Tieren über kürzere Zeiträume anhand der Auswaschkinetik nach einem Bolus von ^{13}C-markiertem Bicarbonat. ^{13}C-markiertes Bicarbonat (gelöst in Wasser) wurde 19 Labormäusen i. p. oder s. c. injiziert (0,2 ml). Die Mäuse befanden sich in einem Standardrespiration-System. Die Messung fand bei 3 verschiedenen Temperaturen statt. Die Atemproben wurden minütlich über 40 - 60 min aus der Respirationkammer gewonnen und in Vacutainer abgefüllt. Die Proben wurden über ein GC-Interface in das Massenspektrometer (VG Optima) eingelassen und gemessen. Die halblogarithmische ^{13}C-Ausscheidung war linear ($r^2 > 98\%$ in allen Fällen), was auf einen einzelnen Pool hinweist. Wir bestimmten die Poolgröße aus der ^{13}C-Verdünnungskurve. Die Kompartimentanalyse lieferte für die 19 Tiere einen vernünftigen Mittelwert der CO_2-Produktion. Die individuellen Werte waren jedoch ungenau. Die Korrelation zwischen den Werten aus der Isotopenmethode und der indirekten Kalorimetrie war schlecht ($r^2 = 15\%$). Die Gründe für diese Diskrepanz sind unklar. Ein empiri-

sches Modell, welches den Ausscheidungsgradienten, die Poolgröße und die Applikationsart der Tracers berücksichtigte, erklärte 86% der Variation der CO_2-Produktion. Die ^{13}C-Methode ist ein brauchbares Hilfsmittel, um den Energieumsatz von Mäusen über einen Zeitraum von 15 - 40 min zu bestimmen.

Key words ^{13}C Labeled bicarbonate technique - short-term measurement - energy expenditure - mouse

Schlüsselwörter ^{13}C-markierte Bicarbonatmethode - Kurzzeitmessung - Energieumsatz - Maus

Introduction

The standard methodology for the measurement of energy demands is indirect calorimetry. The nature of the calorimetry apparatus however means that it can never fully reflect the subtleties of interaction in factors which promote variation in energy expenditure in the real world. For some time it has been recognised that there is a need therefore for a method which allows less precise but more realistic measures of free-living energy demands (3). The doubly-labeled water technique developed by Lifson and colleagues in the 1950's (4,5) provides one such method. This technique works on the principle that the oxygen of respiratory carbon dioxide comes to rapid and complete isotope exchange equilibrium with the oxygen of body water (8). Consequently an isotopic label of oxygen in body water is eliminated more rapidly than a label of hydrogen, because the former is eliminated predominantly across two routes and the latter along only one. An estimate of CO_2 production is thus feasible from the different elimination characteristics of the two isotopes (8).

The flux of carbon dioxide however is small relative to the size of the body water pool. Inevitably therefore it is possible to make measurements with this technique only over relatively long periods - a day in small mammals and birds (6), and one to two weeks in humans (7) and larger animals (8). This duration limits the technique to quantification of daily energy expenditure. It is of extreme interest, however, not only to quantify daily energy demands, but also the components of energy expenditure which contribute to the total expenditure over these more protracted periods. There is a need therefore for a method which provides the freedom of the doubly-labeled water protocol combined with the short duration possible in calorimetry measurements of, for example, basal metabolic rate.

In theory such a technique might be possible by labelling the body bicarbonate pool with heavy carbon (^{13}C or ^{14}C). This pool is in rapid equilibration with the dissolved CO_2 in blood and because the flux of CO_2 is large relative to the size of the bicarbonate pool the turnover should be sufficiently rapid to permit measurements over considerably shorter periods than are possible with doubly-labeled water. Recently a version of this approach has been validated in humans (1). The protocol in this latter instance was to continuously infuse the label subcutaneously and to make complete collections of urea from urine to trace the equilibrium level of body enrichment of ^{14}C. Both the method of adminstration and sample collection would thus compromise the utilisation of this method in free-living animals, hence negating its principle advantages over calorimetry.

Utilisation of labeled bicarbonate however need not rely on continuous infusion and a single dose/ elimination protocol similar to that employed in applications of doubly-labeled water should at least in theory be viable. Indeed some early studies involved such a protocol but ran into difficulties because of the complex multi-compartmental kinetics which appeared to be involved (9,10). In the present study we aimed to investigate the possibility of using a single dose/elimination protocol for measurement of energy demands of small mice using ^{13}C labeled bicarbonate.

Methods

We used a total of 19 individual MF1 mice. Measurements were made once on each individual. The mice were injected either subcutaneously or intraperitoneally with a weighed quantity (4 decimal places) of approximately 0.2 ml of ^{13}C labeled sodium bicarbonate in water. Within 2 minutes of injection the animals were placed into a small respirometry chamber, in a standard indirect calorimetry set-up, which has been described elsewhere (2). The chamber was modified so that air samples from the chamber could be periodically collected via a small needle (26 gauge) directly into 10 ml vacutainers. The chamber was maintained at different temperatures between 30 and 10 °C to precipitate variation in energy expenditure over a three-fold range. We measured oxygen consumption of the animals. This was converted to CO_2 production using the measured RQ of a subset of the animals.

Samples of the gas in the chamber were collected at intervals of 1 minute for a period of 40-60 minutes. The gas samples were admitted into a mass spectrometer (VG Optima) via a GC interface (VG Isogas) which identified and admitted the CO_2 peak in a stream of CP grade Helium. Isotope abundance of ^{13}C was measured as the

ratio of the major to minor beam ratio of the samples and a reference (delta) using a working standard gas as the reference material. We collected samples of respiratory CO_2 from the mice prior to injection and analyzed these for background levels of ^{13}C as well.

To evaluate the dilution spaces of the isotope we made a series of injections of varying mass into vacutainers, and combined these with a small quantity of the reference gas. The mixtures were then left for 3 days at 60 °C to equilibrate and allowed to cool to room temperature before being analyzed.

Results

The log converted enrichment of ^{13}C above the background isotope enrichment was linearly related to the time of the experiment (Fig. 1). In all cases the r^2 of this relationship was > 98% indicating the kinetics of the isotope elimination over this very early phase of the elimination probably involved only a single pool. If we took the enrichment of isotope at 4 minutes and 40 minutes post injection and reconstructed the elimination track from these two points alone we obtained virtually identical answers to those derived from regression analysis of the 40-60 data over the entire period.

The injections of the injectate into vacutainers containing varying quantities of CO_2 also produced a liner relation when it was log converted (Fig. 2). We interpolated the plateau enrichment of ^{13}C and also the back extrapolated enrichment at the time of injection (intercept enrichment) on this relationship to evaluate the size of the pool in which the ^{13}C was turning over. We then used conventional single compartment kinetics to evaluate the flux rate of CO_2 and compared this with the measured oxygen consumption over the period that the animals was in the respirometer (converting the former to oxygen consumption using the established RQ for a subset of these animals which averaged 0.91, sd = 0.03).

Across all 19 mice the plateau estimate of pool size when combined with the elimination constant provided an overestimate for oxygen consumption. However the intercept estimate on average resulted in a much closer comparison (Table 1). This close comparison however masked a great deal of individual variability. Indeed when individuals estimates for oxygen consumption using the two methods were plotted against each other the resultant relationship had an r^2 of only 15.1% (Fig. 3). Nevertheless we noted that residuals to this regression were strongly correlated with the components of the isotope elimination parameters (e.g., k_c : Fig. 4). Inevitably then an empirical model using the elimination constant (k_c), Pool size (N_c) and a coded variable for the injection route (1 for SC and 2 for IP) as predictors, resulted in a much better predictive equation. In fact using the empirically derived model explained 86% of the variation in the oxygen consumption (Fig. 5).

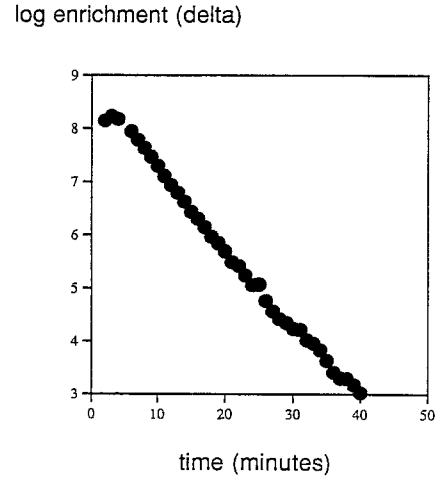

Fig. 1 Typical pattern of log converted enrichment of ^{13}C above background in the body of a mouse following injection at time 0. The incorporation of the label was extremely rapid (8 minutes) and the elimination over the first 40 minutes or so followed a mono-exponential decline. The r^2 value in this instance between 4 and 40 minutes post injection was 99.2%.

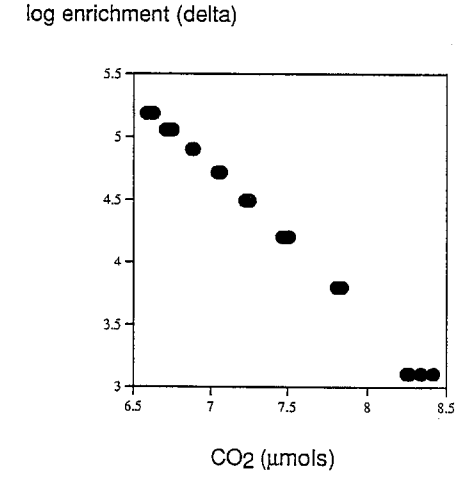

Fig. 2 Relationship between the equilibrium enrichment of ^{13}C resulting from mixtures of a standard amount of the injectate solution containing $NaH^{13}CO_3$ and varying amounts of CO_2. The log-log converted values were linearly related with an r_2 of 100%. This curve provided an estimate of the body water carbon pool size by interpolation of the plateau or intercept enrichments of ^{13}C enrichment derived from the washout curves of each experimental animal.

Discussion

Independent of the route of injection, the bolus dose of ^{13}C enriched bicarbonate appeared to have distributed itself within the body within 8 minutes because in no

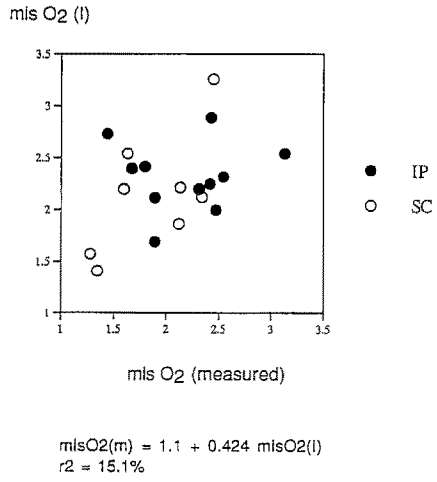

Fig. 3 Relationhsip between the estimated CO_2 production estimated from the measured oxygen consumption and an RQ of 0.91 (measured in a sub-set of the animals) and the estimated CO_2 production from the labeled bicarbonate washout kinetics using a standard one compartment washout model and the intercept estimate of the body bicarbonate pool. Subjects were 19 MF1 mice. Each point represents a different individual. Open symbols are subcutaneously injected and closed symbols are intraperitoneal injected animals. Although the levels of CO_2 production estimated by the two methods matched on average (Table one) the individual estimates were only poorly correlated (r^2 = 15.1%).

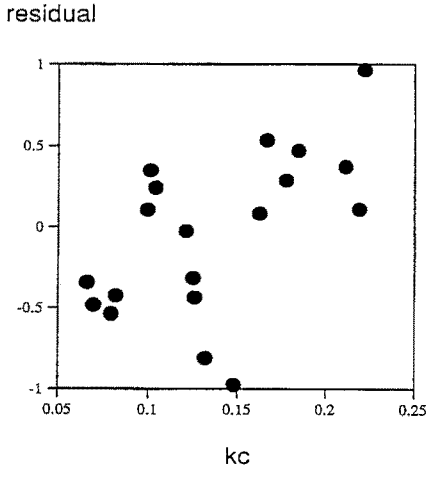

Fig. 4 Relationhsip between the residual error from the relationship illustrated in Fig. 3 and the turnover of labeled bicarbonate (k_c).

cases did we observe rising enrichments after this time. This rapid incorporation of the label was surprising but fortuitous given the very rapid elimination rate that was observed, with a biological half life of 10-20 minutes, depending on the metabolism. As the label was incorporated so fast an appreciable amount must have been

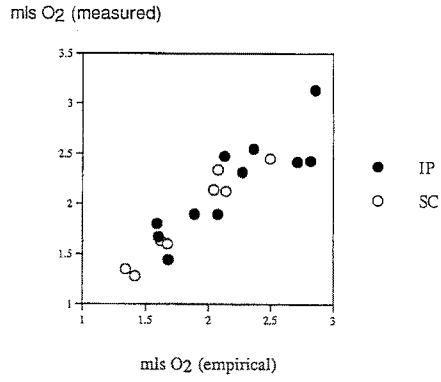

Fig. 5 Relationhsip between the estimated CO_2 production estimated from the measured oxygen consumption and an RQ of 0.91 (measured in a sub-set of the animals) and the estimated CO_2 production from the labeled bicarbonate washout kinetics using an empirical model with turnover, pool size and route of injection as predictors. Subjects were 19 MF1 mice. Each point represents a different individual. Open symbols are subcutaneously injected and closed symbols are intraperitoneal injected animals. The fit using the empirical model was much improved over the theoretical single pool kinetic model (Fig. 3) and the r^2 was 86%.

Table 1 Average levels of oxygen consumption across 19 individual MF1 mice, measured directly and inferred from the estimated CO_2 production from the labeled bicarbonate technique using either the plateau isotope enrichment or the intercept enrichment to evaluate the body carbon pool in which the label was turning over. Estimated oxygen consumption using bicarbonate was estimated from the measured CO_2 production using an RQ of 0.91 (measured in a sub-set of the animals). Estimates of O_2 consumption using the plateau method to estimate pool size were on average over twice the direct estimate. In contrast the estimates derived using the intercept estimate of carbon pool size were only 9.6% too large.

Technique	Mean Oxygen consumption
Respirometry	2.05 ml/min
Bicarbonate technique	
Plateau	4.43 ml/min
Intercept	2.24 ml/min

eliminated before the first sample was taken at around 4 minutes post injection. It was not surprising then that pool size estimates based on the back extrapolated intercept at time = 0 provided a much better estimate of oxygen consumption when compared with the plateau estimates.

The linearity of the elimination was unexpected, given previous studies which have clearly indicated multiple compartment kinetics. However, the time course of our collections was relatively short, and in some animals which we followed for slightly longer periods there was an indication of a second exponential beyond 60 minutes.

Strong linearity in the system over the initial phase was however extremely important because it meant only an initial and final sample were necessary to estimate the elimination gradient accurately. This clearly indicates the potential of the method for measuring free-living energy expenditures.

We are uncertain why the standard kinetic approach did not provide a good estimate for the oxygen consumption of individual animals but a good overall estimate. The empirical model for individuals was dominated by the elimination gradients k_e which points to variability in the estimated pool sizes as the cause of the variation in the kinetically derived estimates. The source of this variability was however unclear since our dilution experiment to derive the pool sizes from interpolation had an r^2 of 100%. Although the standard kinetic approach did not provide a useful method for estimating the energy demands of individuals we were greatly encouraged by the results of the empirical model. These data clearly indicate that the elimination of a bolus dose of ^{13}C will probably be a useful tool for the evaluation of short term energy demands of mice over periods of 15 to 40 minutes. The external validity of the empirical model however still needs to be checked in a control group of animals. Moreover, whether the technique will work in other species needs further validation.

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