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A prospective randomised study comparing oral ^{13}C -bicarbonate tracer technique versus indirect calorimetry for measurement of energy expenditure in adults

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Abstract

Background and Aims—Accurate assessment of energy expenditure (EE) is important in guiding nutritional therapy but current methods are unsatisfactory. This study compared the oral ^{13}C -bicarbonate tracer (BT) technique using the IRIS® system (Wagner, Germany) against indirect calorimetry (IC, ventilated-hood) to measure CO_2 output (VCO_2) and thus estimate EE.

Methods—Ten overnight-fasted healthy male volunteers were randomised to studies at rest or mild exercise in a crossover manner. During each study BT-IRIS® and IC were used simultaneously to measure VCO_2 and thus EE. Participants ingested a drink labelled with 50mg ^{13}C -bicarbonate and breath samples were collected every 5 min for 180 min and analysed using IRIS®. Bland-Altman plots were used to assess agreement between the two techniques in measurements of VCO_2 (L/day) and estimates of EE (kJ/day).

Results—Mean $\pm$ SE age and BMI of participants were 21.1 ± 1.1 yrs and 23.6 ± 0.6 kg/m². Both at rest and exercise, there was small bias but overall poor agreement between the two techniques as evident by the wide 95% limits of agreement in measurements of VCO_2 and EE: rest VCO_2 (bias 1.4, SD 93, 95% limits of agreement –180 to 183), rest EE (–8.3, 1830, –3595 to

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3578), exercise VCO_2 (49.3, 66.1, -80.4 to 178.9) and exercise EE (1083, 1944, -2727 to 4893). Furthermore, there was also evidence of systematic error in these measurements.

Conclusion—Prior to clinical application, further optimisation of the BT-IRIS® system should be undertaken, given the poor agreement with IC in measuring VCO_2 and estimating EE.

Keywords

energy expenditure; bicarbonate; tracer; calorimetry; exercise; rest; adult

Introduction

Accurate assessment of energy expenditure (EE) is of importance in guiding nutritional therapy in malnourished, critically ill and obese hospitalised patients^{1, 2}. Under-provision of energy compounds the catabolic state that is associated with critical illness, resulting in decreased muscle strength, ventilator dependence and increased infectious complications³⁻⁶. Overfeeding results in metabolic, hepatic and cardiopulmonary complications including hyperglycaemia, fatty liver and hypercapnea^{2, 7, 8}. Although the Harris-Benedict formula⁹ is widely utilised to estimate energy requirements in routine clinical practice, it does not allow for inter-individual variations in energy requirements associated with critical illness¹⁰⁻¹². Indeed previous studies have demonstrated both overestimates of up to 50% in calculated EE¹³ and underestimates¹⁴ when compared to indirect calorimetry (IC). Furthermore, EE calculated using predictive equations that apply an average weight adjustment, to allow for obese patients, correlates poorly with data from IC¹⁵. Finally, whilst IC remains the most utilised technique to measure EE, it is not readily applicable in the clinical setting due to cumbersome equipment, requirement for regular calibration and need for experienced operators¹⁶. The oral ^{13}C -bicarbonate tracer (BT) technique has been suggested as a more user-friendly alternative to IC in the clinical setting. This dilution technique involves oral ingestion of labeled bicarbonate with regular collection of expired breath samples into bags. The latter may be analysed using the infrared isotope analyser IRIS® system (Wagner Analysen Technik GmbH, Germany) which calculates endogenous CO_2 production (VCO_2) using previously described equations¹⁷. Data from human studies comparing the oral BT against IC are presently limited to studies of adolescents at various activity levels²⁰, and small numbers of adults at rest¹⁷ and exercise²¹. Furthermore, only one previous small ($N=4$ participants) human study has investigated the utility of BT-IRIS® system in measuring EE¹⁷. The aim of the present study was to compare the oral ^{13}C -bicarbonate tracer technique (utilising the IRIS® system) against IC (ventilated-hood technique) for measuring EE at rest (REE) and during mild exercise in healthy adults.

Methods and Materials

Ten healthy adult males, aged 18-30 yrs, body mass index (BMI) $20\text{--}25\text{ kg/m}^2$, were included in this randomised 2-way crossover study, which was approved by the University of Nottingham Medical School Ethics Committee. Informed written consent was obtained prior to enrolment. Subjects attended the temperature-controlled (23°C) metabolic laboratory following an overnight fast having avoided vigorous exercise, alcohol and caffeine for the 3 preceding days. On arrival, height and weight were measured and subjects

familiarised with study procedures. They were rested reclined in bed (30°) for 25 min following which they were randomised (computer generated randomisation codes concealed in sequentially numbered opaque envelopes in duplicate) to undergo study at rest or exercise, with the second visit performed following an interval period of 7 days. Calorimetry was undertaken using the ventilated-hood open circuit indirect calorimeter (GEM Nutrition Ltd, Cheshire, UK), calibrated with alcohol burns and span gases according to manufacturer's operating procedures. Indirect calorimetry derives EE values from measurements of VCO_2 production, O_2 utilisation and calculation (as opposed to estimates) of respiratory quotient (RQ). After taking baseline breath samples, volunteers ingested 125 ml of a sugar-free cordial labelled with 50 mg ^{13}C -sodium bicarbonate (Cambridge Isotope Laboratories Inc, Andover, MA, USA). Breath samples were subsequently collected into specialised bags with a mouth-piece attached (IRIS-breath bag, Wagner Analysen Technik GmbH, Germany) every 5 min for 180 min. The mouth-piece was passed to the volunteer under the ventilated-hood thereby permitting near-simultaneous measurements of EE using both techniques. During the rest study subjects rested reclined in bed (30°), under the ventilated hood, for the duration of the study. The exercise study was performed as per the study at rest with the exception of placement of 1-2 kg weights around volunteers' ankles. They were subsequently instructed to keep hips and knees flexed at 90°. The exercise involved alternate leg extension and flexion for 10 min periods (or less if the subject tired) with rest periods (10 min) interspersed between each exercise period. This exercise regimen was designed to increase REE by at least 50% whilst permitting simultaneous measurements of EE using BT-IRIS® and IC. At the end of each study day, breath samples were analysed using the IRIS® system. The latter uses measurements of VCO_2 together with an estimated respiratory quotient (RQ) value of 0.85 and an estimated ^{13}C fractional recovery value of 0.7 to calculate energy expenditure via the Weir equation as previously described^{18, 19}. In order to validate IRIS® measurements, a 10 ml sample from each breath bag was aspirated (using a 3-way tap and syringe) into 12 ml Exetainer® vials (Labco Ltd, Buckinghamshire, UK) which were measured using continuous flow isotope ratio mass spectrometry (IRMS, AP2003, Analytical Precision Ltd, Northwich, Cheshire, UK) against a calibrated reference gas, and the results expressed as % relative to Pee Dee Belemnite, an international standard of known ^{13}C composition. Statistical analyses were performed using IBM SPSS Statistics® for Windows™ v19 software (IBM SPSS Statistics, Feltham, UK). Data are presented as mean $\pm$ SE and differences between paired observations determined using the paired t-test (significance at $p < 0.05$). Bland-Altman plots²² were used to assess agreement and bias between BT-IRIS® and IC. Three comparisons were undertaken in this study: 1) VCO_2 and EE at rest derived using BT-IRIS® *versus* IC, 2) VCO_2 and EE during mild exercise derived using BT-IRIS® *versus* IC, and 3) IRIS®-derived ^{13}C delta over base (*dob*) measurements *versus* those derived from IRMS.

Results

The mean $\pm$ SE age and BMI of study participants were 21.1 ± 1.1 yrs and 23.6 ± 0.6 kg/m² respectively. All subjects completed the study without side effects. IC and BT-IRIS® demonstrated the exercise protocol to increase REE by 72% and 56% respectively (Table 1). Whilst there appeared to be no statistically significant differences between IC and BT-

IRIS® in measurements of mean EE at rest and exercise (Table 1), Bland-Altman analyses revealed poor agreement between the two techniques (Fig. 1). This was evident by a small bias but wide 95% limits of agreement and negatively sloping regression lines indicating systematic error in the measurements (Fig. 1). Similarly, there was poor agreement between measurements of VCO₂ at rest and exercise with significant negatively sloping regression lines indicating systematic error (Fig. 1). Finally, although there was reasonable agreement between IRIS® *dob* measurements of ¹³C and those derived from IRMS, there remained evidence of systematic error (Fig. 2).

Discussion

This study has demonstrated that in healthy volunteers undergoing measurement of EE at rest and mild exercise, the BT-IRIS® had poor agreement with the IC ventilated-hood technique. Furthermore, there was evidence of systematic error in BT-IRIS® measurements obtained.

The aim of the present study was to undertake near-simultaneous measurements of EE both at rest and during mild exercise. The exercise study was designed to test the oral BT during a 'hypermetabolic-state' simulating the situation of critically ill patients. Whilst there appeared to be no significant differences in mean values of EE acquired using the two techniques, there was poor agreement between the 2 techniques highlighting the limitations of tests of significance in assessing agreement²². Systematic error, which was greatest during the exercise study, in the BT-IRIS® technique may have resulted from pre-programmed estimates of RQ of 0.85. Indeed the mean $\pm$ SD RQ, determined using IC, during the present rest and exercise studies was 0.78 ± 0.07 and 0.80 ± 0.07 respectively. This study highlights the limitations of using estimates of RQ. Indeed were it sufficiently accurate to use an estimated RQ; then EE could be calculated in a simpler way by obtaining VCO₂ readings from the ventilators used on intensive care units. Systematic error may also have resulted from IRIS®-calculations using an estimated ¹³C fractional recovery value of 0.7. Although we did not measure fractional recovery of ¹³C, a previous study of similar design albeit utilising a smaller number of older subjects¹⁷, reported this to vary between 63.8% to 68.7%. Variability in the fractional recovery of orally ingested ¹³C-bicarbonate may occur secondary to differences in rates of gastric emptying, absorption, breakdown and trapping of ¹³C-bicarbonate into pools that turnover slowly in the body¹⁸. Although there was reasonable agreement between ¹³C *dob* values obtained via the IRIS® system and IRMS, there remained evidence of systematic error. However, the latter may have resulted from our sampling technique whereby 10 ml aliquots from each breath sample were aspirated into Exetainer® vials. Previous studies comparing the BT with calorimetry in exercising adolescents^{20, 21} and in resting adults¹⁷ have reported little difference between the 2 techniques. Horswill *et al*²⁰ compared the BT with IC in 8 adolescents at various activity levels and demonstrated good correlation between VCO₂ and rate of appearance of CO₂ (RaCO₂) and with no differences (multiple comparison Tukey tests) in measurements of EE during exercise between the 2 techniques, a finding similar to the present study. A subsequent study of 8 healthy exercising volunteers supported the use of the BT in measuring the energy costs of short duration physical activity in the field setting²¹.

However, both studies were performed in the postprandial state (2–3 h after the last meal), did not undertake simultaneous measurements of EE using the two techniques, and the study by Horswill *et al*²⁰ performed a test of significance between the two measurements, which is irrelevant to the question of agreement²². The final study compared the 2 techniques in only 4 participants¹⁷. In conclusion, the present study has demonstrated that despite there being no statistically significant differences between mean EE measured during rest and mild exercise, using BT-IRIS® and IC, there was poor agreement between the two methods. Further optimisation of the BT-IRIS® technique should therefore be undertaken prior to application in the clinical setting.

Acknowledgments

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Abbreviations

BMI	body mass index
BT	oral ¹³ C bicarbonate tracer
dob	delta over base
EE	energy expenditure
IC	indirect calorimetry
IRMS	isotope ratio mass spectrometry
RaCO₂	rate of appearance of CO ₂
REE	resting energy expenditure
RQ	respiratory quotient
VCO₂	endogenous CO ₂ production

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Fig. 1. Bland-Altman plots of simultaneous measurements of energy expenditure (EE, kJ/day) and CO_2 production (VCO_2 , L/day) made using indirect calorimetry (IC) and the oral ^{13}C -bicarbonate-IRIS (BT-IRIS®) system

1A EE during the rest study, bias -8 , SD of bias 1830, 95% limits of agreement -3595 to 3578 ; **1B** EE during the exercise study, bias 1083, SD of bias 1944, 95% limits of agreement -2727 to 4893 ; **1C** VCO_2 during the rest study, bias 1.4, SD of bias 93, 95% limits of agreement -180 to 183 ; **1D** VCO_2 during the exercise study, bias 49, SD of bias 66, 95% limits of agreement -80 to 179 .

Fig. 2. Bland-Altman plots of *delta over base (dob)* ^{13}C analysed using the infrared isotope spectrometer IRIS® system and isotope ratio mass spectrometry (IRMS) during the rest (A) and exercise (B) studies

Table 1
Energy expenditure (EE, kJ/day) at rest and mild exercise measured by indirect calorimetry technique and the oral ^{13}C -bicarbonate-IRIS® (BT-IRIS®) technique

	EE during rest study		EE during exercise study	
	IC	BT-IRIS®	IC	BT-IRIS®
Mean	7174	7183	12 321	11 238
95% CI	6358 - 7990	5825 - 8540	11086 - 13555	9315 - 13160
SE	361	600	546	850
p-value	0.781		0.161	

N=10 participants. BT was analysed using the IRIS infrared isotope analyser system (Wagner Analysen Technik GmbH, Germany)