

Original Article

Use of [¹⁴C]-sodium bicarbonate/urea to measure physical activity induced increases in total energy expenditure in free-living healthy males

Darren M Roffey BAppSc, BHSc(Hons)^{1,2}, Natalie D Luscombe BSc(Hons) PhD¹,
Nuala M Byrne BHMS, MAppSc, PhD², Andrew P Hills PhD, FSMA², Max Bellon PhD³,
Chris Tsopelas PhD³, Ian D Kirkwood MBBS³ and Gary A Wittert MBBCh, MD, FRACP¹

¹ Department of Medicine, University of Adelaide, Royal Adelaide Hospital, Adelaide SA 5000

² School of Human Movement Studies, Faculty of Health, Queensland University of Technology, Brisbane QLD 4052

³ Department of Nuclear Medicine, Royal Adelaide Hospital, Adelaide SA 5000

The aim of this study was to evaluate the utility of the [¹⁴C]-sodium bicarbonate/urea technique to detect physical activity-induced increases in total energy expenditure in free-living healthy men. Thirteen healthy males aged 34.1 ± 11.7 yrs with body mass index 24.1 ± 3.1 kg/m² were studied on three separate occasions, during which [¹⁴C]-bicarbonate was infused over 48-hours and urine was collected during the second 24-hours. On three separate occasions and in random order, subjects either remained sedentary, or performed a bout of physical activity on an electro-magnetically braked cycle ergometer sufficient to increase energy expenditure by 7% or 11% above predicted sedentary total energy expenditure. Urine samples were analyzed to evaluate the amount of [¹⁴C]-bicarbonate incorporated into urinary urea, thereby reflecting the amount of CO₂ produced per day, and upon conversion, the number of kilojoules of energy expended in 24-hours. All 13 subjects successfully completed the two physical activity treatments and there were no adverse events. As measured by the [¹⁴C]-urea assay, mean total energy expenditure values were not significantly different between sedentary activity (17902 ± 905 kJ/day), the physical activity treatment designed to increase TEE by 7% (17701 ± 594 kJ/day) and the physical activity treatment designed to increase TEE by 11% (18538 ± 485 kJ/day) ($P=0.668$). In conclusion, although the [¹⁴C]-sodium bicarbonate/urea technique was well tolerated and did not interfere with normal daily activities, it was not able to accurately measure physical activity-induced increases in EE in the range of 7-11% above predicted sedentary total energy expenditure.

Key Words: energy expenditure, carbon dioxide production, exercise prescription, sodium bicarbonate, urea.

Introduction

Obesity is recognised as a major public health problem as excess body fat, or adiposity, influences morbidity, mortality, and the efficacy of medical treatment.¹ While obesity is not a new phenomenon, it has never been as prevalent in both adults and children worldwide as it is today.^{1,2} Summary results from the National Health Survey of Australia indicate that in 2001, 58% of adult males and 42% of adult females were classified as either overweight or obese according to body mass index (BMI) ratings above 25 kg/m.^{2,3}

It is commonly accepted that if energy intake (EI) exceeds energy expenditure (EE) for a sustained period of time, the excess energy will be stored as fat.^{4,5} Obesity has been linked with low voluntary physical activity⁶⁻⁸ and low rates of non-exercise activity thermogenesis.^{9,10} Accordingly, it is important to be able to study EE in reasonably large numbers of free-living individuals, not only to assess associations between modern habits of living and the obesity epidemic, but also to monitor the efficacy of

interventions.^{11,12} This may be particularly important given the recidivism post weight loss. While weight-loss induced through dietary restriction has poor long term success,¹² increased levels of physical activity facilitates the matching of daily EI and EE and consequently contributes to the maintenance of weight loss.¹³ A variety of methods have been applied to the measurement of total energy expenditure (TEE) and physical activity. Continuous indirect whole-body calorimetry is regarded as the reference method for measuring TEE.¹⁴ Subjects are, however, constrained within the controlled metabolic environment that does not reflect everyday living conditions, and the facilities required are expensive to build and maintain.¹¹

Correspondence address: Associate Professor Gary Wittert, Department of Medicine, University of Adelaide, Royal Adelaide Hospital, Adelaide SA 5000 Australia.

Tel: +61-08-8-8222 5502; Fax: +61-08-8-8223 3870

Email: gary.wittert@adelaide.edu.au

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Doubly labelled water (DLW) is considered the 'gold standard' method for measuring TEE in free-living unrestrained subjects.¹⁵ Nonetheless, widespread use of the DLW technique in large numbers of subjects is restricted by high costs, limited availability of isotopes, the need for specialised equipment and the inability to measure TEE over short durations, such as multiples of 24-hour periods.¹⁶ Heart rate monitoring may be useful in short-term studies,¹⁷ but the method may lack precision as the relationship between heart rate (HR) and oxygen uptake (VO_2) used to measure TEE becomes more variant during low-level physical activity, and is affected by stress, anxiety and posture.¹⁸ Pedometers and accelerometers can also be used to measure activity levels during certain movement patterns, but the tracking of activity is limited as neither device can detect static work such as isometric contractions during lifting and carrying.¹⁹ In addition, pedometers are unable to measure cycling movements and are unreliable during fast running and very slow walking. Accelerometers do not detect movements distal to the device.⁴ Physical activity questionnaires and diaries are often the chosen technique to gauge overall activity trends of subject populations, and to estimate and ensure the maintenance of daily physical activity levels.²⁰ However, they are less objective, are highly dependent on subject compliance, and if used to estimate TEE, result in substantial error.²¹ In summary, the available TEE measurement techniques cannot differentiate between voluntary and involuntary forms of physical activity, nor accurately ascertain the intensity, duration and frequency with which the physical activity is performed, especially within a free-living environment.²²

The [^{14}C]-sodium bicarbonate/urea technique is based on the principle of isotopic dilution of infused [^{14}C]-bicarbonate, and the measured specific activity of [^{14}C]- CO_2 incorporated into urinary urea. The assay for urinary [^{14}C]-urea allows indirect calculation of CO_2 production and ultimately an estimation of TEE.^{23,24} [^{14}C]-bicarbonate is inexpensive compared to DLW,²⁵ and in comparison to indirect calorimetry, the technique allows individuals to continue their normal activities of daily living.²⁵ Furthermore, it can measure TEE for 24-hours (or multiples thereof), potentially allowing for influences of dietary restriction and other aspects of clinical intervention such as physical activity to be analysed over on a day-by-day basis.²³ In a controlled environment, the [^{14}C]-sodium bicarbonate/urea technique has been shown to measure TEE with an accuracy of between 2-6% as compared to whole body calorimetry.²⁴⁻²⁶ In free-living conditions, the measurement of TEE using [^{14}C]-bicarbonate was found to be comparable to that obtained using DLW in obese women.²⁷

The utility of the [^{14}C]-sodium bicarbonate/urea technique to detect increases in voluntary physical activity in free-living subjects has not yet been determined. Accordingly, the aims of this study were (i) to evaluate the ability of the [^{14}C]-sodium bicarbonate/urea technique to detect physical activity-induced increases in TEE in healthy men and; (ii) to determine the acceptability of the [^{14}C]-sodium bicarbonate/urea technique to measure free-living TEE under sedentary conditions and during physical activity in active men.

Materials and Methods

Subjects

Fifteen male adults were recruited at the Queensland University of Technology. Subjects were included if they were aged 18-55 years, had a BMI between 18-33 kg/m^2 , and were weight stable (± 2 kg) for at least six months before recruitment. Respondents were excluded if they smoked, were taking any regular medication, and reported any cardiovascular, respiratory or gastrointestinal disease, diabetes mellitus, had elevated blood pressure or abnormal renal or liver function. The Human Ethics Re-search Committees of the Royal Adelaide Hospital, the University of Adelaide and the Queensland University of Technology approved the experimental protocol. Each subject gave written informed consent to participate in the study.

Experimental design

The study was conducted on an outpatient basis over an 8-week period. All clinical data was collected at the School of Human Movement Studies, Queensland University of Technology. Chemical and statistical analyses were conducted at the Department of Nuclear Medicine, Royal Adelaide Hospital and the Department of Medicine, University of Adelaide. Body weight and height were recorded at screening. All subjects underwent cardiorespiratory testing during a sub-maximal cycling trial either on the initial screening day or at least one week prior to the first randomised activity protocol. On three occasions, in random order, separated by a minimum of 11 days, measurements of resting metabolic rate (RMR), TEE, and level of physical activity were made in response to two 24-hour physical activity protocols and a 24-hour protocol containing no physical activity (i.e. control day). Physical activity was increased by performing a single bout of cycling on a stationary electromagnetically braked ergometer sufficient to increase EE by either 7% or 11% above predicted sedentary TEE. The duration and energy cost of the prescribed physical activity was determined for each subject from the individualised VO_2 (ml/kg/min) - Work (Watts) relationship calculated during a sub-maximal cycle test. During their first 3-day activity protocol, subjects completed three 24-hour diet diaries designed to give an indication of typical daily food intake. Throughout the study subjects were asked to maintain their usual dietary habits, in particular following as closely as possible for the day before and for the 2-days of the infusion the dietary intake recorded in their baseline diet diaries. They were also asked to maintain normal daily activity patterns and to not undertake any type of formal exercise outside of everyday physical activity routines on the day prior to the study measurements being made and on the day of testing. Following each TEE measurement, subjects completed a questionnaire evaluating the suitability and intrusiveness of the [^{14}C]-bicarbonate/urea technique on typical daily activities.

Exercise prescription

Measurements of VO_2 and VCO_2 during exercise at specific mechanical workloads on a cycle ergometer were

used to define the rate of energy expenditure.^{28,29} The time and intensity of cycle ergometer exercise that each subject was required to perform to induce an increase in EE 7% or 11% above predicted sedentary TEE, were calculated from the individualized mechanical to physiological work relationship. As an example for a 67kg subject, five calculation steps were used to derive the required ergometer exercise: (1) RMR = 7402 kJ \approx 1769 kcal, sedentary TEE = 1769 x 1.4 \approx 2477 kcal; (2) 10% RMR = 176.9 kcal \approx 7% above sedentary TEE; (3) Mean VO_2 at 100 Watts during sub-max test = 25.86 ml/kg/min \equiv 1.73-0.23 L/min = 1.50 L/min; (4) Energy equivalence = 1.50 x 5 kcal = 7.49 kcal/min; (5) 176.90 / 7.49 = 23.62 minutes at 100 Watts. Therefore the subject would have to cycle at 100 Watts for 23.37 minutes to induce a 7% increase in EE above predicted sedentary TEE. To calculate an increase in TEE of 11%, the 10% RMR value in the aforementioned equation was substituted with 15% RMR. The constant 1.4 is the index rating provided by the International Obesity Task Force (IOTF), and the International Dietary Energy Consultative Group (IDECG) that is used to predict TEE for sedentary adults.⁶ The number of kcal required to increase EE by 7% above predicted sedentary TEE was calculated by multiplying RMR by 0.10, which represented approximately 7% of predicted sedentary TEE. The amount of energy required during seated rest, referred to as 1 MET (3.5 ml/kg/min), was subtracted from the absolute VO_2 value (L/min) to ensure that the physical activity completed during the exercise bout was above that for the sedentary day.³⁰ The net kcal expended per minute during the 100 Watts workload of the sub-maximal cycling trial was calculated from mean VO_2 multiplied by 5 (a constant representing the relationship between oxygen consumption and energy expenditure, where 1 L of O_2 utilized = 5 kcal expended). It is recognised that the energy cost per litre of O_2 can range from 4.686 to 5.047 kcal as a function of substrate utilisation.²⁹ However, given the variable respiratory exchange ratio at 100 Watts across subjects, the standard conversion of 5 kcal/L O_2 was employed. To calculate the necessary duration of physical activity, the kcal required to increase EE by 7% above predicted sedentary TEE was divided by the net kcal of energy expended per minute at 100 Watts. An assumption made regarding the exercise prescription was that subjects would expend energy at the same rate as reflected by the same respiratory exchange whilst performing the prescribed physical activity bout as they did during sub-maximal cardio-respiratory cycling test.

Measurements

Height and body weight: Body height was measured without shoes to the nearest tenth of a centimetre using a Harpenden stadiometer. Body weight was measured in light clothing and bare feet to the nearest ten grams recorded on a digital scale. Body Mass Index (BMI = weight (kg) / height (m)²) was calculated for each subject using their measured height and weight.

Cardio-respiratory testing: Cardio-respiratory testing was performed following a minimum 3-hour fast. Subjects reported to the testing laboratory wearing lightweight,

comfortable clothing and were asked to abstain from strenuous exercise and consumption of caffeine, alcohol or salty foods in the 24-hour period preceding the test. Prior to the sub-maximal test, each subject was fitted with a nose clip and a Hans-Rudolf headset consisting of a two-way breathing valve and pneumotach. In addition, subjects were fitted with a Polar Coded Transmitter (Polar Electro, Kempele, Finland) and heart rate was recorded every 15 seconds throughout the test. To calculate oxygen consumption, and subsequently predict $\text{VO}_{2\text{max}}$, expired respiratory gases were collected continuously and analyzed using a Q-PLEX Gas Analysis System (Quinton Instrument Company, Seattle, USA). The O_2 and CO_2 analyzers were calibrated prior to each test against known high and low gas concentrations (3.0% CO_2 , 18.1% O_2 and 6.0% CO_2 , 15.0% O_2) reflecting the physiological range of the measurement. The sub-maximal cardio-respiratory test protocol required subjects to pedal continuously on an electro-magnetically braked cycle ergometer (Excalibur, Lode, Gronigen, The Netherlands) at a self-selected cadence of 60 ± 10 rpm for 5 minutes at incremental work rates of 50, 100 and 150 Watts (15 minutes total). The coefficient of variation (CV) of the Q-PLEX Gas Analysis System was found to be $7.1 \pm 0.0\%$ ($N = 13$ subjects) using the mean VO_2 values for the last 2 minutes at the 100 Watt workload during the sub-maximal cardio-respiratory cycling test.

Heart rate: Heart rate was measured during the two physical activity treatments designed to increase EE by 7% and 11% above predicted sedentary TEE. The receiver was placed either on the wrist of the subject or around the handlebars of the ergometer for ease of viewing. The Polar Coded Transmitter (Polar Electro, Kempele, Finland) was worn around the chest of the subject, via a narrow plastic band attached to an adjustable elastic strap. Average heart rate was recorded in beats per minute (BPM) every 15 seconds for the duration of the prescribed cycling bout.

Resting metabolic rate: Resting metabolic rate was measured for 30 minutes by indirect calorimetry using a ventilated hood and Deltatrac metabolic monitor (Datex, Helsinki, Finland). The Deltatrac ventilated hood system comprises an oxygen sensor, a carbon dioxide analyser, and a mass flow meter that allows variable airflow through the system. A calibration procedure was performed before each RMR test using a standard gas mixture (5% CO_2 , 95% O_2). Previous studies conducted by our group have found the CV of the metabolic monitoring procedure to be $1.7 \pm 0.4\%$ and $3.1 \pm 0.8\%$ for fasting REE and respiratory quotient respectively. On the day of testing, each subject was instructed to minimise physical activity prior to arrival and to fast for a minimum of 3 hours before the test. Subjects lay supine on a bed in a thermoneutral environment with a transparent hood placed over the head and shoulders, which was connected to the metabolic monitor. Following an initial equilibration phase of 20 minutes, RMR was continuously measured based on oxygen consumption and carbon dioxide production. Data obtained from the last 10 minutes of the measurement period was used for analyses.

Total energy expenditure (TEE): Total energy expenditure was measured using the [^{14}C]-bicarbonate/urea method.^{23,25} An area of skin (5 cm x 5 cm) adjacent to the navel was shaved and sterilized with alcohol wipes, and then a flexible infusion cannula bearing a 60 cm tube (MMT-316, MiniMed Technologies, NSW, Australia) was localized into the subcutaneous layer of abdominal fat of subjects laying in the supine position. Lignocaine injection (1% w/v; Delta West, WA, Australia) was diluted in saline (0.9% w/v; 5 mL) and administered as a bolus through the cannula. A priming dose of [^{14}C]-urea (9.25 kBq or ≈ 0.25 $\mu\text{Ci}/\text{mL}$; Amersham Pharmacia Biotechnology, NSW, Australia) diluted in water for injection (1 mL) was administered, and then the infusion cannula was connected to a 20 mL syringe (Terumo Syringe with Leur Lock tip, Terumo Corporation, Springfield, SA, Australia) containing 14.7 ± 0.2 mL of a sterile and pyrogen-free [^{14}C]-bicarbonate solution (95 kBq or ≈ 2.57 $\mu\text{Ci}/\text{mL}$; Amersham Pharmacia Biotechnology, NSW, Australia). The tracer dose was infused over 48 hours at a constant rate using a mini-pump syringe driver (Graseby MS16A, SIMS Australasia Pty Ltd, QLD, Australia). Total activity infused over the 48-hour infusion period was 1.4 MBq (or ≈ 37.8 μCi). At no stage was there any indication of leakiness at the site of skin contact or with any of the connections in all 15 subjects. On the morning after the [^{14}C]-bicarbonate infusion commenced (day 2 of the 3-day protocol), subjects were required to record the time their first urine sample was passed. Thereafter, all urine passed over the next 24 hours was collected in a 4L container that contained no preservative and was stored at $\approx 4^\circ\text{C}$ in the dark. On the day of the 24-hour urine collection, the subject performed the prescribed exercise bout. The total volume of urine collected was measured, and a small aliquot (500 mL) was stored in an airtight bottle (preservative-free) at -20°C prior to assays for measurement of urea, [^{14}C]-urea and creatinine.

The specific activity of urea was analysed using a method based on that of Elia *et al.*²³ Added to a 500mL round-bottomed flask was: urine containing 12mmol urinary [^{14}C]-urea, sodium citrate (1M; 100 mL; pH 5.2) and variable amounts of distilled water to achieve a final volume of 300 mL. The pH of the urine reaction solution prepared for each subject assay was always below 5.5. A silicone stopper (24/29) containing a nitrogen inlet and an outlet tube was inserted into the ground joint to isolate the solution. With the outlet tube exposed to the atmosphere, nitrogen gas (>99.999%) was bubbled through the solution at 4 L.min⁻¹ for 3 minutes and at 0.5 L/min for 2 minutes to remove traces of air. The gas outlet tube was then immersed in a 50 mL volumetric flask that contained a carbon dioxide trapping solution (0.305M; 8.2 mL) of ethanolic potassium hydroxide with an indicator (pH 9.3). This trapping solution was designed to react with 2.5 mmol of CO₂ gas and form an unreactive precipitate of potassium bicarbonate. The addition of Jackbean urease (Type III; 1000 unit.mL⁻¹; 4 mL; Sigma Aldrich, NSW, Australia) initiated the conversion of urinary [^{14}C]-urea to [^{14}C]-CO₂, where the gas evolved was delivered to the trapping solution via carrier gas at a flow rate of 0.5 L/min.

The reaction was complete when the trapping solution changed from purple to colourless (indicating the consumption of 2.5 mmol CO₂). The trapping solution was diluted to 50 mL with ethanol, and samples ($N = 3$, 5 mL) were dispensed under nitrogen atmosphere into scintillation vials containing scintillant cocktail (10mL). Samples were counted in duplicate using a liquid scintillation counter (Beckman Instruments, NSW, Australia) and the background corrected mean value was divided by the moles of carbon dioxide to give specific activity of [^{14}C]-urea (moles of CO₂ \equiv moles of urea). The specific activity of [^{14}C]-urea was used in the following equation to predict total CO₂ production per day.²⁵

Total CO₂ production (mol/day) = $[0.95 \times 0.85 \times \text{infused } [^{14}\text{C}]\text{-bicarbonate (dpm/day)}] / [\text{specific activity of urea (dpm/mol of CO}_2)]$. From the total CO₂ produced, TEE was calculated based on the assumption that CO₂ has an energy equivalent of 535 kJ/mol. This value approximates with that obtained in subjects close to nutrient balance whilst consuming a dietary intake with food quotient equal to 0.85.³¹ Preliminary research by our group determined that the [^{14}C]-sodium bicarbonate/urea assay had an intra-assay CV of $3.9 \pm 0.3\%$ in lean subjects (8 men) and $3.6 \pm 0.3\%$ in obese subjects (6 men/9 women), and that the CV between study days was $4.8 \pm 1\%$ in lean subjects and $9.7 \pm 1.3\%$ in obese subjects.

Assessment of physical activity level: An activity diary was maintained by each subject as a record that voluntary physical activity remained stable over the 3-day protocol (TEE measurements). All subjects were instructed how to complete diaries and were provided with written guidelines. The first diary entry commenced on the day the [^{14}C]-bicarbonate/urea infusion commenced (morning of day 1) and continued until the 24-hour urine collection and infusion was completed (morning of day 3). Each diary was reviewed with the subject at the completion of a measurement, and subjectively graded from a table specifying physical activity indexes (multiples of RMR) of eight different activity levels, in order to establish a 24-hour physical activity level.

Evaluation of the [^{14}C]-sodium bicarbonate/urea technique: The practicality and intrusiveness of the [^{14}C]-bicarbonate/urea technique on typical daily activities was evaluated using a questionnaire given to subjects at the completion of the 3-day study. The evaluation rating scale comprised the following four questions: (1) any pain associated with the insertion of the cannula; (2) any pain associated with the administration of [^{14}C]-urea; (3) the suitability and comfort of wearing the syringe infusion pump; and (4) the intrusiveness of the 48-hour subcutaneous infusion of [^{14}C]-bicarbonate. Subjects answered each question by rating from 1 (painless, uncomfortable, limits activity) to 10 (painful, extremely comfortable, does not limit activity) corresponding with the level of comfort, pain and suitability experienced. Each subject was also asked to comment on whether this technique interfered with normal routine.

Statistical analysis

All data are expressed as mean \pm SE, unless otherwise specified. Statistical analysis was performed using SPSS for Windows 10.0 software (SPSS Incorporated, Chicago, USA). The difference between TEE values, as well as total CO₂ production measured by the [¹⁴C]-bicarbonate/urea technique, was assessed using ANOVA with the three physical activity levels (sedentary activity, physical activity treatments designed to increase EE by 7% and 11% above sedentary TEE) as fixed factors. When a significance of effect between the sedentary activity and the physical activity treatments was found, paired sample T-test was used to distinguish between levels of physical activity. Statistical significance was considered as $P < 0.05$.

Results

Subject characteristics

Fifteen male subjects were recruited, thirteen completed the study and two were incomplete (only two TEE measurements were obtained). The two reasons provided by subjects for not completing the study were a lack of available time to participate, and the inconvenience associated with the requirements of the study. Only complete data ($N = 13$) was included in the statistical analysis. The mean physical characteristics (\pm SD) of the included subjects are shown in Table 1.

Physical activity features

The mean predicted increase in EE during the two physical activity treatments, the duration of cycling necessary to increase EE by 7% and 11% above calculated sedentary TEE, and the mean heart rates are shown in Table 2. The calculated 7% and 11% increases in EE above predicted sedentary TEE were significantly different (769 ± 57 kJ/day and 1154 ± 86 kJ/day respectively; $P < 0.0001$). The cycling times required to increase EE by 7% and 11% above predicted sedentary TEE were 22.1 ± 0.6 min and 33.4 ± 1.0 min respectively, and were also significantly different ($P < 0.0001$).

Carbon dioxide production and TEE measured using the [¹⁴C]-sodium bicarbonate/urea technique

The average 24-hour urinary creatinine values were similar, being 15.2 ± 0.8 mmol/L, 15.4 ± 0.9 mmol/L and 15.0 ± 0.7 mmol/L for 7%, 11% and the sedentary protocols respectively, suggesting the 24-hour urine collections were likely to be complete.

Table 3 shows the mean TEE and CO₂ production for the subject population. TEE measured using the [¹⁴C]-sodium bicarbonate/urea technique was not significantly different between the two physical activity treatments designed to increase EE above predicted sedentary TEE by 7% (17701 ± 594 kJ/day) and 11% (18538 ± 485 kJ/day) and the sedentary activity protocol (17902 ± 905 kJ/day) ($P = 0.668$). The [¹⁴C]-sodium bicarbonate/urea technique was shown to have an average intra-assay CV of $3.3 \pm 1.3\%$ over the three TEE measurement occasions.

Table 1. Summary of subject characteristics

Parameter	Mean \pm SD	Range
Age (yr)	34.1 \pm 11.7	19-54
Weight (kg)	76.7 \pm 9.4	63.1-91.6
Height (m)	1.79 \pm 0.07	1.66-1.89
BMI (kg.m ⁻²)	24.1 \pm 3.1	18.6-29.8
RMR (kJ)	7692.3 \pm 571.9	6728.2-8815.7
Predicted sedentary TEE (kJ)	10769.2 \pm 800.7	9419.5-12342.0
Predicted VO _{2max} (ml/kg/min)	46.6 \pm 7.5	34.8-60.0

Table 2. Description of the physical activity (PA) treatments designed to influence energy expenditure above TEE

Parameter	Mean \pm SE	Range
PA (kJ) [†]	769.2 \pm 15.9	672.8 - 881.6
Cycling time (min) [†]	22.1 \pm 0.6	18.1 - 26.0
Heart Rate (BPM) [†]	138.3 \pm 5.5	105.6 - 166.9
PA (kJ) [‡]	1153.8 \pm 23.8	1009.2 - 1322.4
Cycling time (min) [‡]	33.4 \pm 1.0	27.2 - 39.1
Heart Rate (BPM) [‡]	142.1 \pm 4.9	112.4 - 167.6

[†]7% and [‡]11% above predicted sedentary TEE

Table 3. Total carbon dioxide production and TEE measured using the [¹⁴C]-sodium bicarbonate/urea technique

Parameter	Mean \pm SE	Range
Sedentary CO ₂ (mol/day)	33.5 \pm 1.7	21.1-44.4
PA CO ₂ (mol/day) [†]	33.1 \pm 1.1	26.0-38.5
PA CO ₂ (mol/day) [‡]	34.6 \pm 0.9	29.0-40.0
Sedentary TEE (kJ/day)	17901.6 \pm 904.6	11290.2-3772.3
PA TEE (kJ/day) [†]	17701.0 \pm 593.5	13918.1-20589.0
PA TEE (kJ/day) [‡]	18537.9 \pm 484.6	15500.4-21429.3

[†]7% and [‡]11% above predicted sedentary TEE

Physical activity levels assessed from 3-day activity diaries

The average daily physical activity index values of 1.45 ± 0.01 , 1.44 ± 0.01 and 1.43 ± 0.01 , derived from the 3-day physical activity diaries for sedentary activity, and days when activity was increased by 7% or 11% respectively, were similar.

Suitability and comfort of the [¹⁴C]-bicarbonate/urea technique

None of the subjects reported any adverse reaction to the infusion of the [¹⁴C]-bicarbonate during the three activity treatments. On one occasion, some discomfort was experienced by a subject after subcutaneous insertion of the infusion needle into the abdomen, contributing to an

overall mean rating of 3.1 ± 0.3 (range 1-7) on a scale of 1 (painless) to 10 (very painful). The discomfort associated with the 48-hour infusion of [^{14}C]-bicarbonate solution was rated at a mean of 2.5 ± 0.2 (range 1-6) on the same scale. The comfort of wearing the syringe infusion pump over 48-hours in the free-living environment resulted in a mean rating of 4.1 ± 0.3 (range 2-9) on a scale of 1 (uncomfortable) to 10 (extremely comfortable). The suitability and practicality of wearing the syringe infusion pump in a free-living environment was rated 5.0 ± 0.3 (range 2-9) on a scale of 1 (limits activity/not practical) to 10 (does not limit activity/very practical). Fifty-nine percent of subjects stated that the infusions did not interfere with normal daily lifestyle activities. Activities that subjects cited as difficult to perform whilst wearing the infusion pump included showering and dressing, sitting for long periods and bending over. None of the subjects complained of any discomfort during either of the physical activity bouts performed on the cycle ergometer, although a small number (3) did note some skin irritation due to the adhesive tape fastening the cannula in place after the completion of each cycling bout.

Discussion

The [^{14}C]-sodium bicarbonate/urea technique was easily applied, acceptable to, and tolerated by all subjects, but it did not have sufficient sensitivity to detect physical activity-induced increases in EE in the range of 7-11% above predicted sedentary TEE. In 13 overweight men and women (BMI >25kg/m), our group previously established that the between-day coefficient of variation of the [^{14}C]-sodium bicarbonate/urea technique was $9.7\% \pm 1.3\%$. Accordingly, in the present study, the levels of physical activity were selected to produce an increase in EE well below and just above the limit of sensitivity of the technique (7% and 11%). The hypothesis proposed that the practical application of this technique in larger populations would depend on it being able to detect increases in EE due to voluntary physical activity of this magnitude.

In choosing these physical activity levels, it was also necessary to be reminded that cycling at too high an intensity or for extended periods might affect the distribution of the [^{14}C]-bicarbonate in vivo.^{23,25} Even so, a variance of 9.7% corresponds to an error of 1736 kJ/day for a mean measured sedentary activity TEE of 17902 kJ/day. As such, the increases in EE induced by the physical activity in the current study (769 ± 57 kJ/day and 1153 ± 86 kJ/day at 7% and 11% above predicted sedentary TEE respectively) were probably within the error of the technique.

It may be possible that the assumptions made in the calculation of TEE using the [^{14}C]-sodium bicarbonate/urea technique do not apply during physical activity. The calculation of CO_2 production, from which TEE is estimated, is dependent upon the assumption that recovery of [^{14}C]-bicarbonate as breath [^{14}C]- CO_2 is 95% of the total amount infused.^{25,32} The amount of infused label recovered in gaseous [^{14}C]- CO_2 increases following exercise as compared to sedentary activity.^{23,25} Specifically, the recovery of label as gaseous [^{14}C]- CO_2 was

140% following a bout of exercise at 75 Watts, and 139% following a bout 100 Watts.^{23,25} In the hours immediately after an exercise bout however, compensatory low recovery rates of 70% and 67% occurred that restored the overall balance to end-study levels of $95.6 \pm 1.3\%$ and $95.6 \pm 1.1\%$ over 24 hours.^{23,25}

Coinciding with the increased recovery of infused label as gaseous [^{14}C]- CO_2 in these studies was the assessment that the lowest specific activities in random breath samples were also taken during the periods of physical activity. An elevated local [^{14}C]- CO_2 production rate during exercise would most likely increase isotopic dilution, explaining why the specific activity of breath CO_2 was found to be at its lowest during exercise while the amount of infused label recovered as gaseous CO_2 was at its highest.²³ Accordingly, the use of a constant recovery factor in the calculation of mean TEE over small time periods might be incorrect where physical activity is expected to be variable.

The assumption that recovery of [^{14}C]-bicarbonate as breath [^{14}C]- CO_2 is 95% of the total amount infused may also be compromised due to increased losses of urea, and hence labelled CO_2 , via sweating. Whilst this possible source of error has received little attention in previous tracer studies, a number of other authors have identified sweat losses as a potentially important route for urea excretion during physical activity.³³⁻³⁵ In one particular study of eight healthy subjects cycling at approximately 45% of VO_2max for 90 minutes, it was found that 30% of total urea excretion (urine + sweat losses) during exercise was in the form of sweat losses.³⁴ However, given that the intensity and duration of the physical activity undertaken in this current study was designed to avoid the profuse sweating rates seen in these other studies, loss of urea via this route is not expected to be a significant source of error.

An additional parameter that may influence the precision of the [^{14}C]-sodium bicarbonate/urea technique is the assigned energy equivalent value of CO_2 . The energy equivalent of 1 mole CO_2 was assumed to be 535 kJ, a value suitable for use in subjects who are close to nutrient and energy balance, and consuming a 'Western' style diet.²⁵ It was previously shown however, that the energy equivalent of CO_2 may vary according to the relative proportions of fat, carbohydrate, protein and alcohol oxidised over the day.³² Although subjects were asked to maintain their normal dietary habits throughout the study, neither the nutrient nor energy balance were assessed for the subject population during the study period, making it unclear as to what the level of nitrogen intake was, what proportions of macronutrients were oxidised and whether the food quotient for the diet was indeed 0.85. For individuals with a food quotient of ≤ 0.8 or ≥ 0.9 , the error in the measurement of TEE could be around 600 kJ/day (based on production of 20 moles of CO_2 per day).

The mean sedentary TEE estimated using the prediction equation $\text{RMR} \times 1.4$ prescribed by the IOTF and IDECG was 10769 ± 801 kJ/day. In contrast, TEE measured under sedentary conditions on the control day using the [^{14}C]-sodium bicarbonate/urea technique was 17902 ± 905 kJ/day; a discrepancy between the two of

over 7000 kJ/day. As the exercise prescription method to achieve 7% and 11% increases in EE was based upon the prediction equation sedentary TEE value, and the discrepancy between measured and predicted TEE was well above the acknowledged error of the [¹⁴C]-sodium bicarbonate/urea technique, the subsequent increases in EE could have been too small to be measured. Since exhaled gas was not sampled during the cycling bouts, there was no precise measurement of energy expended per activity treatment. The average heart rate values between the two physical activity treatments varied by only 4 BPM; however one would not expect this to differ much more since the interventions were designed to generate a greater accumulative energy cost rather than increase cardiovascular demand.

Over the course of the study period, subjects were asked to maintain normal daily activity patterns and to abstain from additional physical activity in order to reduce the variation across the three physical activity treatments, thereby reducing daily variation in the measurement of TEE. The 3-day activity diaries provided by the subjects indicated that the level of voluntary physical activity remained constant throughout the study, and accordingly were unlikely to influence the TEE measurements.

The 48-hour infusion of [¹⁴C]-sodium bicarbonate/urea did not cause any local inflammation, was generally well tolerated and with no adverse side effects, in agreement with previous reports.^{23,24,26,27} In particular, the infusion did not prevent subjects from participating in the majority of normal daily activities.

In conclusion, results from the current study suggest the [¹⁴C]-sodium bicarbonate/urea technique cannot accurately measure modest increases in EE in free-living populations. Further research is required to optimise the technique using a larger sample size as well as by identifying potential sources of error. Approaches include exercising in a whole-body indirect calorimeter as well as determining the utility of the technique to detect physical activity-induced increases in EE up to 15-20%. However, if the *in vivo* distribution of [¹⁴C]-bicarbonate is significantly altered by cycling for ≤ 60 minutes at 100 Watts, a single bout of activity will need to be compared with two shorter bouts designed to induce the same overall increase in TEE.^{23,25} Moreover, because carbon dioxide production varies in response to physical activity, monitoring exhaled [¹⁴C]-CO₂ must be incorporated into future protocols.

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