Original Article

Use Of [^{14}C]-sodium bicarbonate/urea to measure total energy expenditure in overweight men and women before and after low calorie diet induced weight loss

Natalie D Luscombe PhD[1,4], Christopher Tsopelas PhD[2], Max Bellon DipMedTech, ADNucMed [2], Peter M Clifton MBBS, PhD,FRACP,[3], Ian Kirkwood MBBS, FRACP[2] and Gary A Wittert MBBch, MD, FRACP[4]

[1] *University of Adelaide, Department of Physiology, South Australia, 5000*
[2] *Royal Adelaide Hospital, Department of Nuclear Medicine, South Australia, 5000*
[3] *CSIRO Health Science and Nutrition, Adelaide, South Australia, 5000*
[4] *University of Adelaide, Department of Medicine, South Australia, 5000*

The aim of this study was to evaluate the use of the [^{14}C]-sodium bicarbonate/urea technique to measure the change in total energy expenditure after weight loss and a period of weight maintenance. Eleven healthy subjects (6 men and 5 women aged 50 ± 3 yrs, BMI 34.1 ± 2.1 kg/m^2, body fat 38.7 ± 3%) underwent 8 weeks of energy restriction using a combination of 'Modifast™' formula and one small meal per day (≈3.3 MJ/day). For an additional 2-weeks, subjects resumed a solid food diet that contained enough energy to stabilize body weight at the newly reduced level. Body composition, total energy expenditure (TEE), resting energy expenditure (REE) and the thermic effect of a 2.7 MJ test meal (TEF) were measured at both weeks 0 and 10. At week 10 as compared to week 0, body weight decreased by 12.2 ± 1.6 kg (12.5%) ($P < 0.001$). Total fat and lean mass decreased by 8.4 ± 1.0 kg (20.4%) and 3.8 ± 0.7 kg (6.7%), respectively ($P < 0.001$). REE decreased by 500 ± 128 kJ/day (5.6 ± 1.3%) ($P < 0.002$). Decreases in the TEE (0.18 ± 3.7%) and TEF (1.4 ± 0.9%) were not significant. In conclusion, although [^{14}C]-sodium bicarbonate/urea was well tolerated and did not interfere with normal daily activities, it did not have sufficient sensitivity to accurately measure weight loss induced changes in TEE in the range of 0.1-10%.

Key Words: energy expenditure, thermic effect of feeding, weight loss, low calorie diet, clinical intervention.

Introduction

Most obesity treatments involve energy restriction to induce a negative energy balance and promote weight loss. Diet-induced weight loss is often,[1-5] but not always[6-8] accompanied by a decrease in total energy expenditure (TEE) that can predispose to weight regain over time if not compensated by a proportional reduction in habitual energy intake, or an increase in voluntary energy expenditure. TEE is comprised of three components: resting energy expenditure (REE) accounts for 60-75% of TEE,[9-11] the thermic effect of feeding (TEF) accounts for 6-15%,[12-13] and energy expenditure due to physical activity (includes voluntary and involuntary activity) accounts for the remaining proportion. Previous studies have suggested that the majority, if not all, of the reduction in TEE following weight loss is accounted for by the cumulative reduction in one or more of its components.[5,11] As REE is the major determinant of TEE in sedentary people, it is likely that a persistent decrease in REE following an energy-restricted diet, is largely responsible for the positive energy balance and weight regain that is often seen when energy intake returns to its' pre-restricted levels.[4,11,14,15] There is also evidence that the reduction in TEE involves decreases in the TEF,[5,14,15] and decreases in the energy cost of physical activity because of a smaller body size.[4,16] At present few studies have simultaneously assessed the impact of diet-induced weight loss on free-living TEE as well as REE, TEF and physical activity.[4,7]

A relatively new alternative to doubly labeled water and whole-body indirect calorimetry for the measurement of TEE is the [^{14}C]-sodium bicarbonate/urea method. It is an isotopic dilution technique that involves subcutaneous infusion of [^{14}C]-sodium bicarbonate/urea and collection of urine. The specific activity of CO_2 incorporated into urinary urea is measured, from which TEE can be indirectly calculated.

The method is unique because it allows the assessment of CO_2 turnover and TEE under free-living conditions over a period of 24 hours (or multiples thereof).[17] In addition, it is considerably cheaper (≈10-fold) than doubly labelled water, it uses simple laboratory equipment and results can be obtained within several hours of the urine collection. Moreover, [^{14}C]-bicarbonate/urea can provide information about

Correspondence address: Professor Gary Wittert, University of Adelaide, Department of Medicine, Royal Adelaide Hospital, Adelaide SA 5000
Tel: +61-08-8222 5502; Fax: +61-08-8223 3870
Email: gary.wittert@adelaide.edu.au
Accepted 10th October 2005

physical activity when used in conjunction with indirect calorimetry for measuring REE and TEF.[18-20] Two studies in healthy men[17,21] and a third in men with small-cell lung cancer,[19] have shown that the values of CO_2 production and TEE measured using [^{14}C]-bicarbonate/urea are within 2 to 5% of those obtained using whole-body indirect calorimetry over a 1-day or 4-day period. In addition, Gibney and colleagues[18] found in six grossly obese women that free-living TEE, measured using [^{14}C]-bicarbonate/urea as compared to doubly labeled water, was similar (mean difference of 3.6%). Accordingly, these previous studies have shown that [^{14}C]-bicarbonate/urea may be a reasonable alternative to whole body calorimetry and doubly labeled water, for measuring TEE.

The aim of this study was to examine the use of the [^{14}C]-sodium bicarbonate/urea technique for measuring a decrease in total energy expenditure after weight loss and a period of weight maintenance. We hypothesized that a reduction in TEE may be accounted for by a cumulative reduction in REE and TEF.

Research Design and Methods

Subjects

Fifteen subjects (6 men/ 9 women) with a BMI of greater than 27 kg/m^2 were recruited by public advertisement for a weight loss trial at the Royal Adelaide Hospital. Men were included if they were over 35 years of age and women had to be postmenopausal or sterile. Exclusion criteria included malignancy; type 1 or 2 diabetes mellitus; renal, liver or thyroid disease; unstable cardiovascular, respiratory or gastrointestinal disorders; and significant weight loss in the month prior to commencing the study. Subjects on anti-hypertensive, lipid lowering, respiratory or gastrointestinal medication were asked to maintain them at pre-study doses. A detailed information session attended by all subjects stressed the importance that they be motivated to lose approximately 10% of their initial body weight. All subjects gave informed written consent to participate in the study, which was approved by the Human Ethics Research Committee of the Royal Adelaide Hospital.

Experimental protocol

The study was conducted on an outpatient basis over 10 weeks. It consisted of 8 weeks of energy restriction (i.e. week 0 to week 8) and 2 weeks of energy balance (i.e. week 8 to week 10). Measurements of body weight and composition, TEE, REE, TEF and respiratory quotient (RQ) were made at week 0 (before energy restriction) and at week 10 (after 2 weeks of prescribed energy balance for maintenance of the reduced body weight as assessed at week 8). In addition, body weight was recorded every 2-weeks while subjects were at the research unit for dietary counselling. For each subject, all measurements were per-formed in the morning, on the same day of the week and at the same time of day. Following TEE measurements on the two separate occasions, subjects completed a questionnaire evaluating the intrusiveness of the [^{14}C]-sodium bicarbonate/urea method on their daily lifestyle. Throughout the study all subjects were asked to continue their usual physical activity routine.

Dietary prescription

Over the period from week 0 to week 8 all subjects followed a fixed energy restricted diet to induce a minimum weight loss of 10%. The daily energy provided was 3,300 kJ/day and it reflected a mean restriction of 60% of daily energy requirements. Energy was predominately derived from a liquid dietary formula (Modifast™, Novartis, Australia) that contained 50% of the total energy as protein, 40% as carbohydrate and 10% as fat; all essential micronutrients were incorporated in the formula. The majority of subjects had 5 sachets of the Modifast™ formula per day. Several subjects, however, replaced two sachets of Modifast™ with one low calorie meal (≈ 1470 kJ) at night. The low calorie meal consisted of 120g of lean meat, chicken or fish plus green vegetables or salad. Following the 8-week energy-restrictive phase, subjects resumed a solid food, low-fat diet for an additional two weeks. Subjects were instructed to consume enough energy to maintain body weight at the newly reduced level. The energy level of the weight maintenance period was calculated from each subject's resting metabolic rate multiplied by a physical activity index. Resting metabolic rate was not directly measured at the end of the energy-restricted period. Instead, it was calculated using the Schofield formula[22] that incorporates the measured value of body weight after weight loss. A suitable physical activity index was derived from physical activity information collected by the dietitian at week 8 and a table that specified indexes (multiples of REE) for eight different levels of activity.[23,24] Prior to commencing the study, subjects received detailed dietary guidelines and a meal plan from a dietitian experienced in the use of Modifast™. Subjects were asked to refrain from drinking alcohol throughout the 10-week study. Once a week for the first two weeks of the energy-restrictive phase, subjects visited the same dietitian to receive the formula and dietary counseling. Thereafter, they received fortnightly counseling until the commencement of the weight maintenance phase where they resumed counseling once a week. A three-day food diary (commenced the day prior to the energy expenditure measurements being made and continued until the end of the TEE measurement) was collected at weeks 0 and 10. For each dietary phase, energy intake (EI) and macronutrient composition was determined from food diaries using Diet 1 Nutritional software (Xyris Software, Highgate Hill, Queensland, Australia). This program is based on Australian food composition tables and food manufacturer's data. In addition, the ratio of RQ-to-FQ (FQ represents food quotient) was determined. RQ/FQ is an additional index to the equation of TEE-EI that is used to reflect the state of energy balance of an individual; a RQ/FQ of 1 indicates that energy intake matches energy expenditure. The FQ of the subjects' diet was calculated using the equation cited by Toubro and colleagues.[25] The calculation was based on the average macronutrient composition of the diet that the subjects consumed over the 3-days of the energy expenditure measurements. Fasting RQ, instead of mean 24-hour RQ was used in the calculation of the RQ/FQ ratio.

Measurements

Body weight and composition

Body weight (Seca™ digital scales, model 220; Wedderburn Scales, Hilton, South Australia) was recorded in the morning after emptying the bladder. Subjects wore light clothing and bare feet. Total fat mass, total body lean mass and abdominal fat mass were assessed using whole-body dual-energy X-ray absorptiometry (DEXA) (Norland densitometer XR36; Norland Medical Systems, Fort Atkinson, Wisconsin, USA; CV of 2.3% for total fat mass and 2.1% for total lean mass).

Total energy expenditure (TEE)

TEE was measured using the [^{14}C]-bicarbonate/urea method,[17] an isotopic dilution technique that indirectly calculates TEE from the measured specific activity of CO_2 incorporated into urinary urea. An area of skin on the abdomen was anaesthetized with 2% lignocaine hydrochloride (Delta West Pty Ltd; Bentley, Western Australia), and a small flexible infusion cannula (MMT-316 Sofset Infusion Set with a 60 cm tube); MiniMed Technologies; West Chatswood, NSW, Australia) was inserted subcutaneously while subjects were supine. A bolus priming dose of ^{14}C-urea (9.25 kBq or ≈0.25 µCi in 1 ml of water for irrigation) (Amersham Pharmacia Biotech; Castle Hill NSW, Australia) was administered, and thereafter the infusion cannula was connected to a 20 ml syringe (Terumo Syringe with Leur Lock tip; Terumo Corporation, Springfield SA, Australia) containing 14.2 ± 0.08 ml of a sterile and pyrogen-free [^{14}C]-bicarbonate solution (64 kBq or ≈ 1.74 ± 0.01 µCi/ml). The [^{14}C]-bicarbonate solution was administered via a constant infusion syringe driver (SIMS Graseby MS16A Syringe driver; SIMS Australasia PTY.Ltd; Bundall QLD, Australia), over two consecutive 24-hour periods (Fig. 1). no sign of leakiness at the infusion site or with any of the connections for any of the 11 subjects. On the second day of the infusion, subjects completed a 24-hour urine save after voiding the first specimen of that morning. Aliquots of the 24-hour urine collection were stored at -20ºC until analyzed for the specific activity of urinary urea.

The specific activity of urinary urea was measured using a method based on that of Elia and colleagues.[17] Added to a 500ml round-bottom flask was: urine containing 12 mmol 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 solution prepared for each assay was always below 5.5. A silicone stopper (24/29) containing a nitrogen inlet and 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 (0.305 M; 8.2 ml) of ethanolic potassium hydroxide with an indicator (pH 9.3). This trapping solution was designed to react with 2.5 mmol CO_2 gas and form an unreactive precipitate of potassium bicarbonate. The addition of Jackbean urease (Type III; 1000 unit.ml^{-1}; 4 ml; Sigma Aldrich, Castle Hill, Australia) initiated the conversion of urinary [^{14}C]-urea to [^{14}C]-CO_2, 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 CO_2). The trapping solution was diluted to 50 ml with ethanol and samples (N=3;5 ml each) were dispensed under nitrogen atmosphere into scintillation vials containing 10 ml of scintillant cocktail (Starscint; Packard BioScience Company, Meriden,USA).

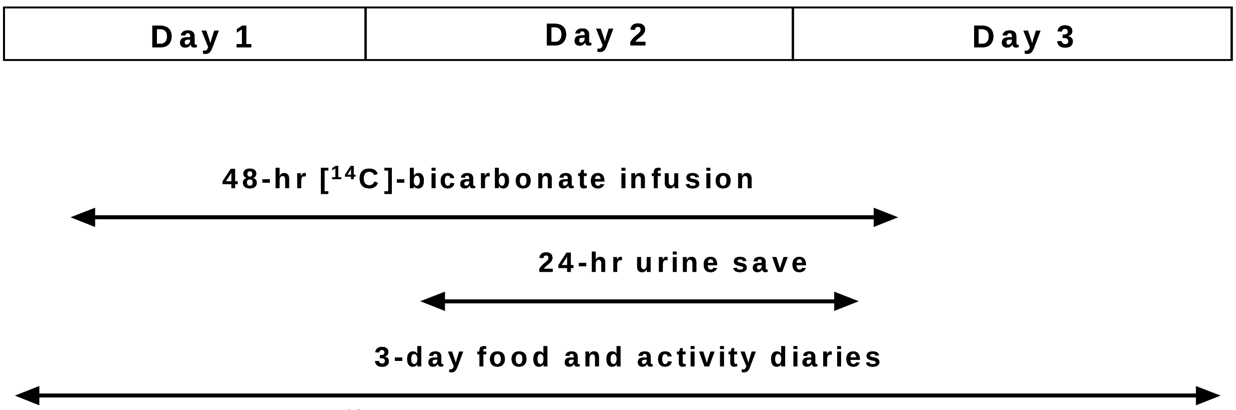

Figure 1. The 48-hour infusion of [^{14}C]-sodium bicarbonate/urea used to measure total energy expenditure.

On day 1, at approximately 9 am, a dose of [^{14}C]-urea (9.25 kBq or ≈0.25 µCi) was administered via a small flexible cannula that was inserted into the subcutaneous layer of fat on the abdomen. Thereafter, the infusion cannula was connected to a 20 ml syringe containing 91 kBq or ≈24.7 µCi of sterile and pyrogen free [^{14}C]-sodium bicarbonate. The [^{14}C]-sodium bicarbonate infusion continued from day 1 until the morning of day 3. After the first morning specimen of urine on day 2, all urine was collected over following 24-hour period. Aliquots of the 24-hour collection were frozen in order to measure the specific activity of urinary urea from which TEE was calculated.

The 48-hour infusion commenced immediately after the measurement of REE (on day 1). The first 24-hours of the [^{14}C]-bicarbonate/urea infusion is necessary to allow the solution to equilibrate with the body's bicarbonate pools. Total activity from the infused [^{14}C]-bicarbonate over the 48-hour period was 91 kBq (or ≈ 24.7 µCi/ml). There was Samples were counted in duplicate using a liquid scintillation counter (Beckman Instruments; Gladesville, NSW, Australia) and the background corrected mean value was divided by the moles of CO_2 to give the specific activity of [^{14}C]-urea (moles of CO_2 ≡ moles urea). The specific activity of [^{14}C]-urea was used in the following equation to predict total CO_2 production per day[17]:

CO_2 production (mol/day) = 0.95 x 0.85 x infused [^{14}C]-bicarbonate (dpm/day)

specific activity of urea (dpm/mol of CO_2)

The calculation of CO_2 production is based on two assumptions: that (i) the recovery of [^{14}C] exhaled as gaseous CO_2 is 95% of the total amount of [^{14}C] that was infused,[21] and (ii) the specific activity of urinary urea is equivalent to 85% that of CO_2 in arterialised blood or breath.[26] From the total CO_2 produced, TEE was calculated based on the assumption that CO_2 has an energy equivalent of 535 kJ/mol. This value approximates the value obtained in subjects close to nutrient balance while consuming a dietary intake with a food quotient equal to 0.85).[27]

Prior to using the [^{14}C]-bicarbonate/urea method to measure changes in TEE during dietary interventions, our group carried out preliminary research to determine its' reproducibility and reliability. Repeated measurements were made at least 7 days apart in free-living, weight stable, non-obese and obese subjects. We found that the method had an intra-assay CV of 3.9 ± 0.3% [range 1.69 to 5.89%] in non-obese subjects (8 men) and, 3.6 ± 0.3 [range 1.24 to 5.69%] in obese subjects (6 men/9 women). The CV between study days was 4.8 ± 1% [range 0.95 to 9.24%] in the non-obese subjects and 9.7 ± 1.3% [range 1.74 to 18.03%] in the obese subjects.

Resting energy expenditure (REE) and Respiratory quotient (RQ)

Fasting REE and RQ were measured over 30 minutes by indirect calorimetry using a ventilated canopy and Deltatrac™ metabolic monitor (Datex Division Instrumentarium Corp., Helsinki, Finland). The equation incorporated in the Deltatrac™ for the measurement of REE is:

REE (kJ/day) = 5.50 VO_2 (ml/min) + 1.76 VCO_2 (ml/min) – 1.99 U_N (g/24h)

Calibration of the Deltatrac™ was performed before each measurement. Subjects lay supine on a bed in a thermoneutral environment with a clear plastic hood over their head and shoulders, and the REE and RQ were recorded for 30 minutes. The first 10 minutes of data were discarded to ensure all subjects had reached equilibrium, and the remaining 20 minutes of data were averaged and represented the values for fasting REE and RQ. The intra-individual CV of the Deltatrac system was established to be 1.7 ± 0.41% for fasting REE, 3.10 ± 0.8% for fasting RQ, and 7.8 ± 1.5% for TEF.[14]

Thermic effect of feeding (TEF)

Immediately following the measurements of fasting REE and RQ, each subject consumed, within 20 minutes, a 2791 kJ test meal. The meal consisted of 4 slices of white bread, 10g of Flora Lite™ margarine, 50g of lean leg ham, 21g of Kraft Free™ cheese, 30g of lettuce, 350g of orange juice, and a 31g fruit museli bar, and the macronutrient composition was 18% of the total energy as protein, 62% as carbohydrate and 20% as fat. Thereafter, subjects returned to the hood for 180 minutes during which RQ and REE values were recorded continuously. A value for TEF was determined at 20-minute intervals from the measurement of postprandial REE (i.e. fasting REE was subtracted from the mean postprandial REE which was calculated every 20 minutes). The 20-minute TEF values were then averaged to determine the mean TEF over the entire 180 minute period. TEF was expressed as a percentage of the energy consumed during the test meal.

Assessment of physical activity level

To ascertain that voluntary physical activity remained stable over the 3 days of energy expenditure measurements, a three-day physical activity diary was maintained. Individuals were instructed on how to complete diaries and were provided with written guidelines. The diary commenced the day prior to the energy expenditure measurements being made and continued until the end of the TEE measurement. The investigator reviewed this record with the subject at the completion of the TEE measurement and subjectively graded each day's overall activity from a table that specified physical activity indexes (multiples of resting metabolic rate) for eight different levels of activity.[23,24] In addition, the level of physical activity derived from the diary was compared to a level of physical activity calculated from measured TEE divided by measured REE.[24]

24-hour urinary creatinine

Urinary creatinine was measured using the Jaffe reaction[28] to determine the completeness of the 24-hour urine save.

Questionnaire assessing the suitability and comfort of the [^{14}C]-bicarbonate/urea method

The intrusiveness of the [^{14}C]-bicarbonate/urea method and its' effect on usual daily activity was evaluated using a questionnaire that rated, on a scale from 1 to 10, the suitability and comfort of wearing the syringe infusion pump, and the intrusiveness of the 48-hour infusion. Subjects were also asked whether the method interfered with their normal lifestyle.

Statistical analysis

All data are represented as means ± SEM, unless otherwise specified. Statistical analysis was performed using SPSS for Windows 10.0 software (SPSS Inc, Chicago, USA). The effect of weight loss was assessed using repeated-measures ANOVA with variables measured at weeks 0 and 10 as the within-subject factor. The study was not specifically powered to look for effects of gender on energy expenditure, but there were approximately equal numbers of both genders and so we considered it reasonable to include gender as a between-subject factor. Simple linear regression was used to determine the relationship between energy expenditure and body composition and the method of Poehlman and Toth was used to normalize energy expenditure for body size and composition.[29] Significance was set at $P < 0.05$.

Results

Subject characteristics

Of the fifteen subjects who were recruited for the study, only eleven (6 men/5 women) completed it. Three women withdrew due to family and work commitments during the course of the study. Data from another subject was excluded from the analysis because she declined, for personal reasons, to have the final [^{14}C]-bicarbonate/urea measurement of TEE made. The physical characteristics of subjects at baseline are shown in Table 1.

Energy intake and urinary creatinine during the 3 days of energy expenditure measurements

The mean energy intake over the 3 days of the energy expenditure measurements was 8022 ± 682 kJ/day (range 5110 to 12619 kJ/day) at week 0 as compared to 5700 ± 317 kJ/day (range 3871 to 6992 kJ/day) at week 10 (P< 0.001); the mean change was 2326 ± 556 kJ/day (range –5627 to 790 kJ/day). The percentage of energy derived from carbohydrate (42 ± 2 vs 43 ± 2%, P = 0.8), fat (32 ± 2 vs 29 ± 1.2%, P = 0.1), and protein (20 ± 1.1 vs 22 ± 1.6%, P=0.2) were similar at week 0 and week 10. Although subjects were asked to refrain from alcohol several subjects had reported having a social drink and the mean energy derived from alcohol was 3.0 ± 1.4 at week 0 vs 3.0 ± 1.4%. At week 0 as compared to week 10, fasting RQ (0.80 ± 0.01 vs 0.78 ± 0.02), FQ (0.85 vs 0.84), and the RQ-to-FQ ratio of the diet (0.91 vs 0.95) were not different (P>0.1). Twenty-four hour urinary creatinine (Table 2) was similar at weeks 0 (P =0.23) indicating that the subjects' 24-hour urine collections were likely to be complete.

Body weight and composition

The change in body weight over the course of the 8-week period of energy restriction and the 2-week period of energy balance is shown in Figure 2. After 8 weeks of energy restriction, mean body weight decreased by 11.7 ± 1.8 kg (range -6 to -27 kg) (P<0.001). After a further 2 weeks at prescribed energy balance, body weight had decreased an additional 0.51 ± 0.3 kg (range -2.16 to +1.4 kg) (P = 0.04). There was, however, an effect of gender on weight loss from week 0 to week 8. From week 8 to the end of week 10, women lost an additional 1.2 ± 0.2 kg (P = 0.004) of weight, whereas body weight remained stable for the men. When female number 5 (Table 1), who lost a further 2.2 kg during the prescribed weight maintenance phase was removed from the analysis, the overall weight loss for the remaining 10 individuals was not significant (P = 0.08). Total fat mass and total lean mass at week 0 and week 10 are shown for all subjects in Table 1. Total fat mass decreased 8.4 ± 1.0 kg (20.4 ± 1.6%; range –13.2 to –27.2%) from week 0 to week 10 (P< 0.001) and this represented 68% of the total weight loss. Abdominal fat mass decreased 2.3 ± 0.4 kg (22.5 ± 2.3 %, range -12.2 to –34.8%) (P<0.001). After 10 weeks, total lean mass was reduced 3.8 ± 0.7 kg (6.7 ± 0.9%, range -0.8 to –8.5%) (P <0.001) which represented 31% of total weight loss. There was no affect of gender on the reduction in fat mass, but abdominal fat mass was reduced approximately 59% more in the men than in the women (-3.2 ± 0.3 vs -1.3 ± 0.2 kg, P = 0.003). There was a greater reduction in lean mass (by 51%) in the men than in the women (-4.9 ± 1.0 vs -2.4 ± 0.6 kg, P = 0.056).

Total and resting energy expenditure, and the thermic effect of feeding

Individual values and the means of the group for TEE, REE, and TEF at weeks 0 and 10 are shown in Table 2. After a mean weight loss of 11.8%, TEE expressed as an absolute value was not statistically reduced (mean decrease in TEE was 181 ± 454 kJ/day; or 0.18 ± 3.7%, range –24% to 22%). This finding remained consistent after the removal of subject number 5, who was not weight stable at week 10. TEE was associated with lean mass and fat mass at week 0 (r = 0.88 for lean mass and r = 0.62 for fat mass, P < 0.05) and at week 10 (r = 0.88 for lean mass and r = 0.62 for fat mass, P < 0.05).

From week 0 to week 10, the change in TEE normalized for lean mass, was 11 ± 1.2% (from 9912.3 ± 1020 at week 0 to 8784 ± 928 kJ/day at week 10, P = 0.07). TEE was greater (by ≈30%, P= 0.06) in the men than in the women, at both week 0 (13751 ± 1774 vs 9440 ± 460 kJ/day) and week 10 (13320 ± 1622 vs 9560 ± 651 kJ/day).

Resting energy expenditure (expressed as an absolute) decreased by 507 ± 125 kJ/day (or 5.6 ± 1.3%, range -12 to + 2.8%) after 10 weeks (P = 0.002). REE was

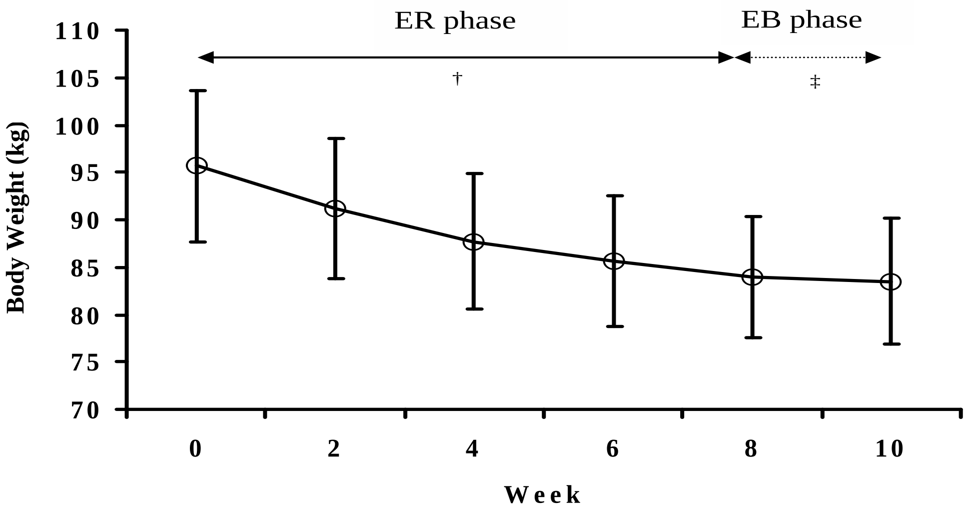

Figure 2. Change in body weight following 8 weeks of energy restriction (ER phase, weeks 0 to 8) and 2 weeks of weight maintenance at energy balance (EB phase, weeks 8 to 10) in the 11 subjects who completed the study. Data are expressed as means ± SEM. Weeks 0, 2, 4, 6, 8 and 10 were compared using repeated-measures ANOVA with gender as the between subject factor. †Significant reduction in weight from week 0 to week 8, P < 0.001. ‡Significant reduction in weight, P = 0.04.

Table 1. Age, BMI and body composition at week 0 and week 10 of the 11 subjects who completed the study

	Age (yrs)	BMI (kg/m^2)		Weight (kg)			Fat mass (kg)		Lean mass (kg)	
Subject/ Gender	Week 0	Week 0	Week 10	Week 0	Week 8	Week 10	Week 0	Week 10	Week 0	Week 10
1/F	54	30.9	28.1	82.1	77.0	75.5	39.8	34.0	43.2	41.5
2/F	44	32.0	28.0	83.0	72.5	72.5	40.7	33.4	42.3	39.2
3/F	61	33.8	27.9	79.5	69.5	68.0	40.6	33.3	39.0	34.7
4/F	60	28.1	24.0	66.0	58.0	57.0	34.2	27.4	31.7	29.6
5/F	60	40.1	36.7	100.1	93.0	91.5	58.0	50.3	42.0	41.2
6/M	51	28.4	25.9	91.0	83.5	83.0	29.1	24.9	61.9	58.1
7/M	41	37.2	32.1	105.0	90.5	90.5	37.8	29.0	67.2	61.5
8/M	49	29.6	26.2	83.5	73.0	74.0	29.2	20.7	54.3	53.3
9/M	34	52.1	49.4	168.6	141.7	142.7	75.9	58.5	92.7	84.2
10/M	48	28.7	25.1	92.0	81.5	80.5	30.4	23.4	61.6	57.2
11/M	45	34.9	28.9	101.0	84.0	83.5	42.4	30.9	58.6	52.6
Mean ± SEM	50 ± 3	34.0 ± 1.7	30.2 ± 2.2	95.7 ±8.0	84.0 ± 6.4	83.5 ± 6.6	41.7 ± 4.2	33.2 ± 3.5	54.0 ± 5.2	50.3 ± 4.6

BMI, body mass index. Week 0 measurements were made prior to dietary intervention, week 8 measurements were made immediately after 8-weeks of energy restriction and week 10 measurements were made after a further 2-weeks of energy balance. The physical characteristics of subjects at week 0 were assessed for differences using a one-way ANOVA with gender as the fixed factor

Table 2. Energy expenditure variables at week 0 and at week 10 following 8 weeks of energy restriction and 2 weeks of weight maintenance

	TEE (kJ/day)		REE (kJ/day)		PA index derived from 3-day diary		PA index derived from TEE/REE†		TEF (% EI)		24h urinary creatinine mmol/L	
Subject/Gender	Week 0	Week 10	Week 0	Week 10	Week 0	Week 10	Week 0	Week 10	Week 0	Week 10	Week 0	Week 10
1/F	10245	9618	6320	6149	1.5	1.6	1.6	1.6	9.2	8.8	9.8	9.8
2/F	10335	10041	6827	6290	1.5	1.5	1.5	1.6	9.0	4.3	10.7	9.5
3/F	8804	8578	6883	7074	1.5	1.5	1.3	1.2	5.1	2.9	8.7	8.2
4/F	7954	7888	5121	5028	1.5	1.5	1.6	1.6	8.1	6.7	7.5	7.2
5/F	9863	11675	7683	7040	1.5	1.5	1.3	1.7	6.0	4.2	19.8	13.2
6/M	11231	10562	7819	7062	1.5	1.5	1.4	1.5	9.5	10.5	16.2	12.2
7/M	15955	12081	9791	9077	1.5	1.5	1.6	1.3	7.6	8.0	14	9.4
8/M	13013	13397	8165	7912	1.7	1.7	1.6	1.7	4.6	9.0	13.8	15.1
9/M	21207	21137	14628	13505	1.7	1.7	1.5	1.6	9.1	2.0	17.8	18.7
10/M	8740	10628	8221	7894	1.5	1.5	1.1	1.4	9.7	6.3	8.8	9.6
11/M	12360	12114	9184	8071	1.7	1.7	1.4	1.5	10.3	10.1	16.7	17.9
Mean ± SEM	11792±1165	11612±1070	8244±49	7737±664	1.55±0.03	1.55± 0.03	1.47±0.04	1.51±0.05	8.0 ±	6.6±0.9	13.0±1.3	11.9±1.2
Change		-181 ± 454		-507±125†		0.0±0.012		0.05±0.06		-1.4±0.9		1.1±0.1

TEE, total energy expenditure as measured using the [^{14}C]-bicarbonate/urea method; REE, resting energy expenditure measured by indirect calorimetry and a ventilated hood; PA index, physical activity index derived from the physical activity diary kept over 3 days of the TEE measurement; TEF, the thermic response to a 2791 kJ test meal, expressed as the % increase in energy per kJ intake over 3 hours Week 0 and 10 data were compared using repeated-measures ANOVA with gender as the between subject factor. †Significant decrease in absolute REE from week 0 to week 10, $P = 0.002$

associated with lean mass at both weeks 0 (r=0.95, $P<0.001$) and 10 (r = 0.92, $P<0.001$). REE was associated with fat mass at week 0 only (r = 0.69, $P<0.02$). The change in REE normalized for lean mass was from 6623 ± 699 at week 0 to 5512 ± 603 kJ/day at week 10 (P = 0.001). There was an overall effect of gender (P = 0.04) on REE (expressed as an absolute) at both week 0 (9634 ± 1043 vs 6575 ± 423 kJ/day) and week 10 (8920 ± 954 vs 6316 ± 373 kJ/day) such that men had a higher REE than women; this effect remained significant after normalization for lean mass (P = 0.001). The mean TEF did not change significantly from week 0 to week 10 (mean decrease 1.4 ± 0.9%, range -7.1% to 4.4%) (Table 2). There was no affect of gender on TEF.

Physical activity

The average daily physical activity index derived from the 3-day physical activity diaries was not different at week 10 as compared to week 0 (1.56 ± 0.03 vs 1.56 ± 0.03) (Table 2). The level of physical activity calculated from measured TEE divided by REE was also not different from week 0 to week 10 (1.47 ± 0.04 vs 1.51 ± 0.05) (Table 2). The physical activity index derived from the diary as compared to that derived from TEE/REE, were similar at week 0 and at week 10 (P >0.1). Gender had no effect on the physical activity index derived from the activity diary.

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

None of the subjects reported any adverse reaction during the infusion of the [14C]-bicarbonate/urea solution. Discomfort associated with the 48-hour infusion of the solution was given a mean rating of 2.3 ± 0.4 (range 1 to 5.5) on a scale from 1 (painless) to 10 (painful). The comfort of wearing the syringe infusion pump over 48 hours in the free-living environment was rated 4.6 ± 0.44 [range 2 to 8.5] on a scale from 1 (uncomfortable) to 10 (comfortable). The suitability of wearing the syringe infusion pump in the subjects' free-living environment was rated 5.2 ± 0.53 [range 2 to 7.5] on the scale from 1 (not practical) to 10 (practical). When asked if the method interfered with their normal lifestyle 58% of the subjects replied 'No'. Activities that subjects cited as difficult to perform while wearing the infusion pump included showering and bending over (another subject who participated in a pilot study for the use of this method in our department reported that it prevented him from swimming but not from running or cycling).

Discussion

The [14C]-sodium bicarbonate/urea method was easily applied and well tolerated by all subjects, but it did not have sufficient sensitivity to measure weight loss induced decreases in TEE in the range of 0.1 to 10%.

Based on the literature that had shown a reduction in TEE after weight loss[4,5,13,16] we expected that the fall in TEE would be approximately equal or greater than a 0.8 to 1% per 1% decrease in body weight. For example, Leibel *et al.*,[4] using doubly labelled water to measured TEE in 7 men and 11 women, observed that the stabilization of body weight at a level 10% below the initial weight was associated with a 17% decrease in TEE normalized for lean mass. Using whole-body calorimetry for 7 obese women, Froidevaux and colleagues[16] found that TEE measured at 12 weeks post-weight loss (15.4 kg or 19%), remained 15% (or 1.5 MJ/day) lower than at baseline. In the present study the change in TEE, even when normalized for lean mass, was variable and not related to changes in either body weight or composition, which were similarly variable. One of the 11 subjects had a decrease in TEE of 24%, and 2 experienced an increase in TEE of approximately 20%. For the remaining 8 individuals, the decrease (7 subjects) or increase (1 subject) in TEE ranged from 0.33% to 6.9%. As a result of the variability, the study had only 65% power to detect a mean decrease in TEE of 10%. Upon resumption of a usual *ad libitum* energy intake, a persistent decrease in TEE of 1 to 7% could potentially lead to a weight regain of approximately 2 to 9 kg over 1 year and therefore it is important that future studies using the [14C]-bicarbonate method identify and resolve potential sources of error within specific subject populations as well as include a larger sample size to detect small changes in TEE in heterogeneous populations.

Recognised sources of error within the [14C]-bicarbonate/urea method include: i) the preparation and administration of the [14C]-bicarbonate solutions, ii) the assumptions that 95% of the infused labelled is recovered as breath CO_2, and that the specific activity of urinary urea was approximately 85% of gaseous CO_2, iii) the choice of energy equivalent for CO_2. Pilot work performed by our group (unpublished) found that the mean day-to-day variation in TEE for 8 non-obese men was 4.8%, of which ~3.9% was due to analytical error in the measurement of the specific activity of urea. This overall error was the same as reported by Elia and colleagues (range for day-to-day variation was 2.4 to 6%), and the TEE values they reported were similar to our observed values.[17-20] The present study was the first study to examine the use of [14C]-bicarbonate/urea to measure weight-loss induced changes in TEE in an obese population, and the mean day-to-day variation was 9.7% (of which ~3.6% was due to analytical error within the assay for the specific activity of urea). Because the day-to-day variation in the non-obese group as well as the analytical error was less than 5%, we are confident that the first source of error stated above was well controlled. Instead, we suspect that the second and third assumptions stated above may not be precise enough for an obese population who had recently lost a significant amount of weight. Furthermore, the choice of energy equivalent for CO_2 may not be valid for this population. In 6 weight-stable grossly obese (mean BMI 52 kg/m^2) women, Gibney *et al.*,[18] showed that these assumptions were valid and the TEE values obtained were the same as measured using gaseous exchange in the respiration chamber. However, no studies have yet used the [14C]-bicarbonate/urea in a mixed population of obese men and women who have recently undergone significant weight loss. Accordingly, validation of assumptions ii and iii still need to be performed in an obese but recently weight-reduced population, and this requires that exhaled [14C]-CO_2, as well as [14C]-urinary urea, are measured at specific periods throughout the measurement day(s). In this study, we used the value of 535 kJ as the energy equivalent of CO_2 because the macronutrient and energy intake collected from the subjects indicated that they had a food quotient of 0.82 to 0.88, and were therefore close to nutrient balance. It is well documented that obese individuals often under-report their energy and macronutrient intakes and we therefore calculated that if this did occur, the food quotient might equate to ≤ 0.8 or ≥ 0.9. In the present study, this may result in an error in TEE of ≥ 600 kJ/day or ≈6%. Further research is required to determine the exact energy equivalents of CO_2 when individuals are in various phases of energy and macronutrient balance.

Although we acknowledge that a lack of statistical power is probably the main reason that we observed no significant decrease in TEE, there are several other issues that may have contributed to the lack of effect observed in this study, and they might also help explain inconsistent findings within the literature. Firstly, placing all subjects on a fixed intake of 3300 kJ/day may have caused considerable differences in the energy deficit between subjects, which in turn may have contributed to the variability in the energy expenditure response. The aim of the study was to see whether energy expenditure was reduced after a weight loss of more than 10%, and accordingly the diet was designed to guarantee this magnitude of weight loss. We expected that the decrease in TEE would have been greater in those individuals who lost more weight. Ss discussed above, however, the change in TEE was variable and we did not observe an association between the reduction in TEE and body weight or composition. The timing of energy expenditure and body composition measurements may also in part explain disparate findings. Several studies have measured energy expenditure immediately after energy restriction[5,13] and as such the results may reflect a metabolic adaptation to an acute negative energy balance and/or the reduced cost of substrate oxidation due to the reduced energy intake. Studies that have assessed the changes in TEE during energy restriction and after a period of weight maintenance at the reduced level have demonstrated that TEE returns to baseline after 6 to 15 months.[8] DeGroot's study, and others[6-8,30], show that TEE is not always reduced following stabilisation at a reduced body weight. It could be argued that a number of the subjects in the present study were still in a state of dynamic weight loss at the week 10 measurement of energy expenditure. Removing subject number 5 (who lost a further 2.2 kg between weeks 8 to 10) from the data analysis showed that the remainder of the group was weight stable. Regardless of whether or not this one subject was removed from the data analysis, TEE was not significantly reduced at week 10 as compared to baseline. Poehlman and Toth[29] have also demonstrated that the statistical procedure used to normalize energy expenditure for body size and composition may also lead to different results. They concluded that dividing energy expenditure by weight or body composition is not appropriate and data should be normalized using a regression-based approach.

We hypothesized that a reduction in TEE following weight loss would be accounted for by cumulative reductions in REE and TEF, and possibly involuntary physical activity. Several studies have shown that a decrease in TEE of 9 to 18%, after body weight was stabilized at a reduced level, was due to a persistent and large reduction in REE (both 14%).[4,13] In the present study, REE expressed as an absolute value, was reduced by 5.6 ± 1.3% following weight loss, and when normalized for lean mass the decrease in REE was variable (ranged from 14 % to 26 %). Therefore another reason why this study may not have detected a decrease in TEE is because the decrease in REE itself was small and given that the study was designed to detect a 10% or greater fall in TEE, then statistical power becomes an issue. Nevertheless, using double-labelled water or whole-body calorimetry, other investigators[6,7,30] have observed that a coincident fall in TEE may not be occur if there is only a small reduction in REE. Furthermore, even if the decrease in REE is greater than 10%, a fall in TEE may not necessarily be observed. In 18 obese women, Amatruda *et al*.,[7] observed a 12% (748 kJ/day) decrease in REE, but no significant fall in TEE (measured using doubly labelled water) after body weight was reduced and stabilized 26% below the initial weight.

The thermic effect of food, expressed as a percentage of ingested energy, is generally 6-15%.[12-13] However, values as low as 2% have been observed in some individuals.[4,13,31] In this study, the average thermogenic response to a standard 2791 kJ test meal was 8% before and 6.6% after weight loss. There was, however, substantial variability between individuals in the change in TEF; 8 subjects had a decrease in TEF of 0.2 to 7.1% whereas in 3 subjects the TEF increased by 0.4 to 4.4%. Since the mean error in the measurement of TEF is 7.8%, changes smaller than 8% may not be accurately detected. Consequently, from this study, no conclusions can be made regarding the effect of diet-induced weight loss on TEF.

In order to minimize the impact of variations in physical activity from obscuring the effect of weight loss on TEE, subjects were asked to maintain a constant level of voluntary activity throughout the study. The diary method of assessing voluntary activity levels was used so that the subjects could account for the activity that they had engaged in during baseline measurements, and therefore maintain the same patterns throughout the study. The average daily level of voluntary physical activity remained similar before and after weight loss. There was, however, a trend for the activity index derived from TEE/REE to increase from week 0 to week 10, for 8 of the 10 individuals. This suggests that an increase in involuntary activity may have offset some of the reduction in REE and thereby masked a fall in TEE. Additionally, it must be acknowledged that the energy cost of physical activities changes as the body composition of individuals change. Therefore, even when subjects reportedly maintain similar activity patterns during a study, a difference in the assigned activity index of as little as 0.1 might cause an estimated error of ~16% in energy requirements (or 1.4 MJ/d) in individuals with identical REE values. Unfortunately the accurate measurement of physical activity remains problematic and all of the existing techniques have numerous limitations. Consequently, it is extremely difficult to precisely determine the magnitude of measurement error that arises from these inaccuracies.

This study confirms previous reports that the 48-hour infusion of [^{14}C]-sodium bicarbonate/urea is accepted and well tolerated by subjects and does not prevent normal daily activities.[16-20] Some individuals reported that bending over and showering were more difficult than usual, and in one case, running or cycling had to be substituted for swimming. This study used the [^{14}C]-sodium bicarbonate/urea method to measure TEE over a single 24-hour period, but others have shown that this method can be well-tolerated for at least 5 consecutive days.[17-20] Given the variance observed in the 24-hour measurements of TEE in the present study, future studies using [^{14}C]-sodium bicarbonate/urea may provide greater insight into the relationship between weight loss and changes in TEE, if the infusion is continued over 2 to 3 days.

In conclusion, the current study suggests that the [^{14}C]-sodium bicarbonate/urea method cannot yet accurately measure decreases in TEE after moderate weight loss in free-living obese populations. Due to the observed large degree of variability between subjects for the changes in TEE and body composition after weight loss, future research using the method must identify potential sources of error within the technique and optimize it using larger and more homogeneous populations. Approaches include measuring TEE at energy balance as well as at positive and negative energy balances in a whole-body indirect calorimeter. The measurement of exhaled [^{14}C]-CO_2 must be incorporated into all further research using this technique.

Acknowledgement

We gratefully acknowledge Kristy Robertson and the Department of Nuclear Medicine for the assistance in performing these studies. Dr Luscombe was supported by a PhD Dawes Scholarship from the Royal Adelaide Hospital.

References

1. Ravussin E, Bogardus C. Energy balance and weight regulation: genetics versus environment. Br J Nutr 2000; 83:S17-S20
2. Schoeller DA. Balancing energy expenditure and body weight. Am J Clin Nutr 1998; 68:956S-961S.
3. Bennett WI. Beyond overeating. N Engl J Med 1995; 332:673-674.
4. Leibel RL, Rosenbaum M, Hirsch J. Changes in energy expenditure resulting from altered body weight. N Engl J Med 1995; 332:621-628.
5. Ravussin E, Burnand B, Schutz Y, Jequier E. Energy expenditure before and during energy restriction in obese patients. Am J Clin Nutr 1985; 41:753-759.
6. Rumpler WV, Seale JL, Miles CW, Bodwell CE. Energy-intake restriction and diet-composition effects on energy expenditure in men. Am J Clin Nutr 1991; 53:430-436.
7. Amatruda JM, Statt MC, Welle SL. Total and resting energy expenditure in obese women reduced to ideal body weight. J Clin Invest 1993; 92:1236-1242.
8. DeGroot LC, van Es EA, van Es RJ, Vogt JE, Hautvast JG. Energy metabolism of overweight women 1 mo and 1 y after an 8-wk slimming period. Am J Clin Nutr 1990; 51:578-583.
9. Keesey RE, Hirvonen MD. Body weight set-points: determination and adjustment. J Nutr 1997; 127:1875S-1883S.
10. Weigle DS. Appetite and the regulation of body composition. FASEB J 1994; 8:302-310.
11. Ravussin E, Lillioja S, Knowler WC, Christin L, Freymond D, Abbott WG, Boyce V, Howard BV, Bogardus C. Reduced rate of energy expenditure as a risk factor for body-weight gain. N Engl J Med 1988; 318:467-472.
12. D'Alessio DA, Kavle EC, Mozzoli MA, Smalley KJ, Polansky M, Kendrick ZV, Owen LR, Bushman MC, Boden G, and Owen OE. Thermic effect of food in lean and obese men. J Clin Invest 1988; 81:1781-1789.
13. Bessard T, Schutz Y, Jequier E. Energy expenditure and postprandial thermogenesis in obese women before and after weight loss. Am J Clin Nutr 1983; 38:680-693.
14. Luscombe ND, Clifton PM, Noakes M, Parker B, Wittert G. Effects of energy-restricted diets containing increased protein on weight loss, resting energy expenditiure, and the thermic effect of feeding in Type 2 diabetes. Diabetes Care 2002; 24: 652-657.
15. Luscombe ND, Clifton PM, Noakes M, Farnsworth E, Wittert G. Effect of a high-protein, energy-restricted diet on weight loss and energy expenditure after weight stabilization in hyperinsulinemic subjects. Int J Obes Relat Metab Disord 2003 May; 27 (5): 582-90.
16. Froidevaux F, Schutz Y, Christin L, Jequier E. Energy expenditure in obese women before and during weight loss, after re-feeding, and in the weight-relapse period. Am J Clin Nutr 1993; 57:35-42.
17. Elia M, Jones MG, Jennings G, Poppitt SD, Fuller NJ, Murgatroyd PR, Jebb SA. Estimating energy expenditure from specific activity of urine urea during lengthy subcutaneous NaH14CO3 infusion. Am J Physiol 1995; 269:E172-E182.
18. Gibney, E.R., Murgatroyd, P., Wright, A., Jebb, S.A., and Elia, M. Measurement of total energy expenditure in grossly obese women: comparison of the bicarbonate/urea method with whole-body calorimetry and free-living doubly labeled water. Int J Obes Relat Metab Disord 2003, 27; 641-647.
19. Gibney E, Elia M, Jebb SA, Murgatroyd P, Jennings G. Total energy expenditure in patients with small-cell lung cancer - results of a validated study using the bicarbonate/urea method. Metabolism: Clinical & Experimental 1997; 46:1412-1417.
20. Paton NJ, Elia M, Jebb SA, Jennings G, Macallan DC, Griffin GE. Total energy expenditure and physical activity measured with the bicarbonate/urea method in patients with human immunodeficiency virus infection. Clinical Science 1996; 91:241-245.
21. Elia M, Fuller NJ, Murgatroyd PR. Measurement of bicarbonate turnover in humans: applicability to estimation of energy expenditure. Am J Physiol 1992; 263: E676-E687.
22. Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985; 39 Suppl 1:5-41.
23. Report of National Health and Medical Research Council. In: Recommended dietary intakes for use in Australia. Canberra, Australian Government Publishing Services, 1991.
24. FAO/WHO/UNU Expert Consultation. Energy and protein requirements. Report no. 724. Geneva, World Health Organisation. Technical Report Series, 1985.
25. Toubro S, Sorensen TI, Hindsberger C, Christensen NJ, Astrup A. Twenty-four-hour respiratory quotient: the role of diet and familial resemblance. J Clin Endocrinol Metab 1998; 83:2758-2764.

26. Fuller NJ and Elia M. Does mitochondrial compartmentation of CO_2 exist in man? Clin Physiol 1989 Aug; 9 (4): 345-52.
27. Elia M. Energy equivalents of CO_2 and their importance in assessing energy expenditure when using tracer techniques. Am J Physiol 1991; 260: E75-E88.
28. Narayanan S, Appleton HD. Creatinine: a review. Clin Chem 1980; 26: 1119-1126.
29. Poehlman ET, Toth MJ. Mathematical ratios lead to spurious conclusions regarding age- and sex-related differences in resting metabolic rate. Am J Clin Nutr 1995; 61:482-485.
30. Racette SB, Schoeller DA, Kushner RF, Neil KM, Herling-Iaffaldano K. Effects of aerobic exercise and dietary carbo-hydrate on energy expenditure and body composition during weight reduction in obese women. Am J Clin Nutr 1995; 61:486-494.
31. Weststrate JA. Resting metabolic rate and diet-induced thermogenesis: a methodological reappraisal. Am J Clin Nutr 1993; 58:592-601

Original Article

Use Of [^{14}C]-sodium bicarbonate/urea to measure total energy expenditure in overweight men and women before and after low calorie diet induced weight loss

Natalie D Luscombe PhD[1,4], Christopher Tsopelas PhD[2], Max Bellon DipMedTech, ADNucMed [2], Peter M Clifton MBBS, PhD,FRACP,[3], Ian Kirkwood MBBS, FRACP[2] and Gary A Wittert MBBch, MD, FRACP[4]

[1] *University of Adelaide, Department of Physiology, South Australia, 5000*
[2] *Royal Adelaide Hospital, Department of Nuclear Medicine, South Australia, 5000*
[3] *CSIRO Health Science and Nutrition, Adelaide, South Australia, 5000*
[4] *University of Adelaide, Department of Medicine, South Australia, 5000*

利用 C^{14} 重碳酸钠/尿素测低卡路里饮食导致男人和女人体重降低前后总能支出

目的：评价 ^{14}C 重碳酸钠/尿素技术在测定体重降低和一段时间的体重保持后总能支出的改变中的应用。方法：通过结合运用 Modifast™ 配方和每日少量一餐的方法，11 个健康人（6 男 5 女，50±3 岁，BMI 34.1 ± 2.1 kg/m^2，体脂含量 38.7 ± 3%）经历 8 周的能量摄入限制（≈3.3 MJ/day）后，所有研究对象摄入两周足够能量的固体食物，使体重保持在降低后的新水平。第 0 周和第 10 周分别测身体组成、总能支出（TEE）、静息状态能量支出（REE）和摄食测试餐（能量含量 2.7MJ）后的生热作用。结果：与第 0 周相比，第 10 周时体重降低了 12.2 ± 1.6 kg (12.5%) (P < 0.001)； 总脂肪和瘦体质分别降低了 8.4 ± 1.0 kg (20.4%)和 3.8 ± 0.7 kg (6.7%) (P < 0.001)； REE 降低了 500 ± 128 kJ/day (5.6 ± 1.3%) (P < 0.002)；；TEE 和 TEF 有所降低（0.18 ± 3.7%和 1.4 ± 0.9%），但差异不显著。结论：虽然 ^{14}C 重碳酸钠/尿素技术具有较好的耐受度且与每日正常活动互不干扰，但并不能灵敏地反映由体重减轻引起的 TEE 在 0.1-1.0％范围内的精确变化。

关键词：能量支出，摄食的生热作用，体重降低，低卡路里饮食，临床干扰