Total energy expenditure and physical activity measured with the bicarbonate-urea method in patients with human immunodeficiency virus infection


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1. Our objectives were to measure total energy expenditure, the daily variation in total energy expenditure and the physical activity level in a group of HIV-positive subjects using the bicarbonate-urea method. The study also aimed to assess the practicalities of using the bicarbonate-urea technique in free-living conditions.

2. Total energy expenditure was measured with the bicarbonate-urea method over 2 consecutive days (1 day in one subject) in 10 male patients with HIV infection (median CD4 count = 30). Resting energy expenditure was measured by indirect calorimetry. Physical activity level (total energy expenditure/resting energy expenditure) was calculated from these measurements and from activity diaries.

3. Resting energy expenditure was found to be 7.46 ± 0.87 MJ/day, 5% higher than predicted values. Total energy expenditure was 10.69 ± 1.95 MJ/day with an intra-individual day-to-day variation of 6 ± 6%. The measured physical activity level was 1.42 ± 0.14, higher than the diary estimate of 1.34 ± 0.16 (P = 0.029), and there were large inter-method differences in individual values. The subcutaneous infusion of bicarbonate was well tolerated and did not seem to restrict normal activities.

4. Total energy expenditure was not elevated in the group of HIV-positive subjects when compared with reference values for normal subjects. The physical activity level of the patients in this study was lower than that measured using other techniques in healthy young men, but was compatible with that expected for people leading a sedentary lifestyle. Reductions in physical activity in patients with HIV are likely to contribute to the wasting process and physical activity level may thus be a clinically useful measure. This study has also provided the first tracer estimate of the day-to-day variation in total energy expenditure. The bicarbonate-urea method represents an important new investigative tool for measuring total energy expenditure which has previously only been possible within the confines of a whole-body calorimeter or using the expensive doubly labelled water method.

INTRODUCTION

Infection with the human immunodeficiency virus (HIV) is often associated with profound wasting, particularly in the later stages of disease [1–3]. There has consequently been considerable interest in measuring the components of energy balance in patients with HIV infection to determine the causes of the weight loss. Several investigators have measured resting energy expenditure (REE) using indirect calorimetry and have generally found this to be raised at all clinical stages of disease, but particularly at the time of opportunistic infections [4–7]. It has been suggested that this elevation of REE might contribute to the pathogenesis of wasting [6, 7]. However, increased REE may not necessarily be associated with increased total energy expenditure (TEE) because the latter includes the energy cost of physical activity, which is variable and tends to be reduced in patients with disease [8]. There is surprisingly little information about how components of energy expenditure other than REE change with progression of HIV infection. In the only published study of TEE in patients with HIV, weight loss was associated with a reduction in TEE [9]. That study was undertaken using doubly labelled water which is expensive and requires considerable time and specialized equipment for analysis. In addition, the technique gives average values of TEE over an extended period of time (e.g. about 2 weeks in adults) and therefore cannot be used to assess day-to-day variation in TEE.

The bicarbonate-urea method is a new technique for measuring TEE which has been validated against whole-body calorimetry over 1 to 4 days and found to have an accuracy of 2–5% [10]. The method involves subcutaneous infusion of a solution...

Key words: bicarbonate-urea method, energy expenditure, human immunodeficiency virus infection, physical activity.

Abbreviations: BMI, body mass index; LBM, lean body mass; PAL, physical activity level; REE, resting energy expenditure; TEE, total energy expenditure.

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of $^{14}$C-bicarbonate and collection of urine. It is an isotopic dilution technique in which the specific activity of CO$_2$ incorporated into urinary urea is measured, from which energy expenditure can be indirectly calculated. The advantages of this method are that it is relatively cheap (approximately 10-fold cheaper than doubly labelled water), it involves simple laboratory equipment and techniques and can yield results very rapidly (within a few hours of completion of the urine collection). The method can also be used to analyse short-term changes in TEE. To date, however, there has been no evaluation of the feasibility of using this method in free-living individuals.

The aims of this study were to use the bicarbonate-urea technique in patients with HIV infection to measure TEE, the daily variation in TEE and the physical activity level (PAL) in comparison to that estimated by an activity diary, and to assess the practicalities of using the technique in free-living conditions.

METHODS

Subjects

Ten male HIV-positive subjects who were clinically stable and free of active untreated opportunistic infection were recruited from St. George's Hospital, London. Eight men had stage IV disease (AIDS) and two had stage II disease (asymptomatic infection) as defined in the criteria of the Centres for Disease Control and Prevention [11]. All patients were afebrile at the time of investigation and did not have oedema. Two patients were convalescent in hospital after treatment for cytomegalovirus infection but both were well enough to make visits outside the hospital during the period of the study. The remaining eight men participated in the study as outpatients.

The study was conducted according to the Declaration of Helsinki (1989) and was approved by the hospital research ethics committee. All subjects gave written informed consent.

Methods

On the first morning of the study, body weight was measured to the nearest 0.1 kg on digital electronic scales and height was measured to the nearest millimetre. Four-site skinfold thickness was determined following the method of Durnin and Womersley [12] to give an estimate of percentage fat from which lean body mass (LBM) was calculated. In two patients in whom skinfold thickness was not performed, percentage fat (and hence LBM) was estimated from the body mass index (BMI; weight in kilograms divided by square of height in metres) using the equation of Black [13]: 

$$\%\text{fat} = 1.281 \times \text{BMI} - 10.13.$$ 

Serum urea and bicarbonate were measured by standard automated assays at the start of the study and the CD4 count was recorded from the clinical notes.

Patients were asked to keep a record of activity over the 2 days of energy expenditure measurement. The investigator reviewed this record with the patient on completion of the study and graded each activity from tables of physical activity ratios [14]. A mean PAL was calculated for each 24 h period.

REE was measured after an overnight fast using a ventilated hood and metabolic monitor (Deltatrac; Datex Instrumentarium, Helsinki, Finland). The measurements were carried out at an ambient temperature of 23°C with the subjects resting in the recumbent position. REE was measured over 20 min, after a 5 min equilibration period. The mean of two measurements was used in seven subjects.

TEE was measured by the bicarbonate-urea method which has been described in detail elsewhere [10]. In brief, approximately 6–7 ml of a solution of $^{14}$C-sodium bicarbonate (5 μCi/ml) was administered by constant subcutaneous infusion using a mini-pump syringe driver (Graseby MS26 Syringe driver; Graseby Medical Ltd, Watford, U.K.) over a 3-day period. The exact dose of bicarbonate infused was determined by weighing the syringe, extension tube (100 cm Lectro-spiral extension tube; Vygon U.K. Ltd, Cirencester, Gloucester, U.K.) and syringe driver on an electronic balance sensitive to 0.001 g immediately before connection to the patient and again after completing the study. Allowance was made for the dead space in the cannula. The effective whole-body dose of radiation during the entire study was estimated to be equivalent to ≤1 day’s natural background radiation.

After cleaning an area of skin over the abdomen, a 22G cannula was inserted subcutaneously and a priming dose of a solution of $^{14}$C-urea (0.22 μCi/ml) was injected slowly through the cannula (see [10] for basis of calculations for estimating priming dose). The extension tube was then connected to the cannula and the whole secured to the skin with transparent plastic adhesive dressing (Opsite Flexi-grid, 10 × 12 cm; Smith And Nephew Medical Ltd, Hull, U.K.). The infusion was commenced and exact starting time was noted. The pump was placed in a cloth pouch which could be worn on a belt, hung around the neck or supported by straps in a holster position.

Patients were given two 51 plastic bottles, one for each day of the urine collection, and also several smaller (250 ml) bottles which could be carried around more easily and transferred to the larger bottles at a later stage. They were instructed to begin urine collection after the first morning specimen of the following day. A complete urine collection was continued for 48 h (day 2 and 3 of the bicarbonate infusion), ending with the first morning specimen of the third day. Aliquots of urine were taken from each collection period and stored at −20°C until analysed.

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Measuring energy in patients infected with HIV

The method of analysis has been previously described [10]. After removal of acid-labile CO₂ dissolved in the urine, urease was added and the CO₂ released from urea was sequestered by hyamine hydroxide. The specific activity of the trapped CO₂ was determined by scintillation counting.

Calculations

REE was calculated from CO₂ production and O₂ consumption using the equation of Elia and Livesey [15]:

\[ \text{Energy expenditure (kJ)} = 15.818 \times \text{O}_2 + 5.176 \times \text{CO}_2 \]

where O₂ and CO₂ are in litres at standard temperature and pressure. Results were compared with values predicted from Schofield’s equations for normal subjects [16]. Total CO₂ production was calculated from the tracer results using the following equation [10]:

\[ \text{CO}_2 \text{ production (mol/day)} = 0.95 \times 0.85 \times \frac{\text{infused bicarbonate (d.p.m./day)}}{\text{specific activity (d.p.m./mol)}} \]

TEE was calculated from net CO₂ production assuming an energy equivalent of 535 kJ/mol CO₂ [17]. Energy expenditure due to physical activity was calculated as the difference between 90% of TEE (assuming 10% of TEE was due to diet-induced thermogenesis) and REE.

RESULTS

The protocol was acceptable to all the patients who participated and several offered to repeat the study at a future date. The mini-pump was well tolerated and there was no local inflammatory reaction at the site of the infusion. Nine patients reported that urine collection was complete for days 2 and 3; one patient collected urine on day 2 only. All patients were able to continue with normal daily activities without hindrance. None of the patients were engaged in full-time work and most were leading sedentary lives although several did undertake short periods of exercise, cycling and weight training for example, during the study. All subjects continued to consume their habitual diet during the study period and none were known to be changing weight rapidly.

Clinical and anthropometrical characteristics of the subjects are shown in Table 1. The mean values of serum urea and bicarbonate were in the normal range for all subjects (mean 4.8 mmol/l and 23.5 mmol/l, respectively, at the start of the study) and these parameters remained stable around the study period. Changes in the size and specific activity of the urea pool are likely to make a negligible difference to the measurement of TEE [10].

The mean REE (7.46 ± 0.87 MJ/day) was 5 ± 6% higher than predicted by Schofield’s equations (P=0.018 by paired Student’s t-test). TEE was measured over 2 days in nine of the patients and over one day in the remaining patient. The mean value of TEE for the group of subjects was 10.69 ± 1.95 MJ/day. The mean energy expenditure due to physical activity was 2.17 ± 1.01 MJ/day. All three energy expenditure parameters were closely related to total body weight (r=0.88, P=0.0007; r=0.8, P=0.0057; r=0.63, P=0.052 for REE, TEE and physical activity energy expenditure respectively) and to LBM (r=0.90, P=0.0005; r=0.82, P=0.0036; r=0.66, P=0.038 for REE, TEE and physical activity energy expenditure respectively). The relationship between LBM and mean TEE and REE for each subject is shown in Fig. 1. It can be appreciated from the figure that the difference between TEE and REE, which is mainly due to physical activity energy expenditure, becomes larger as LBM increases. Mean values of the energy expenditure parameters expressed in terms of LBM and total body weight are given in Table 2.

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<td>LBM (kg)</td>
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<td>Peripheral blood CD4 count (cells/mm³)</td>
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<td>Energy expenditure parameters in HIV patients</td>
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<td>Absolute (MJ/day)</td>
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*Resting energy expenditure predicted from Schofield’s equations.
†Physical activity energy expenditure.

For the nine patients measured over 2 days, the mean day-to-day variation in TEE was 0.6 MJ/day, 6 ± 6% of mean total TEE or 8 ± 8% of mean REE.

The mean PAL, the ratio of TEE to REE, was 1.42 ± 0.14. PAL was significantly related to REE (r = 0.69, P = 0.025) and non-significantly associated with LBM (r = 0.61, P = 0.062), BMI (r = 0.47, P = 0.17) and stage of disease as indicated by the CD4 count (r = 0.33, P = 0.35). The mean PAL estimated from the activity diary was 1.34 ± 0.16. Individual daily estimates of PAL from the activity diary were correlated with the measured values (r = 0.53, two-tailed P-value = 0.018). However, comparison of the estimated and measured PAL by the technique of Bland and Altman [18] indicated that the activity diary significantly underestimated the measured PAL by a mean of 0.09 (P = 0.029) and the individual differences between methods were large (1 SD = 0.16).

**DISCUSSION**

This work, which represents the first use of the bicarbonate-urea method in free-living subjects, has shown that the method is well tolerated by subjects and apparently does not restrict normal daily activities. This provides encouragement for further use of the technique in free-living conditions.

This study has provided new information about some of the components of energy balance in patients with HIV infection. It confirms that REE is significantly elevated in HIV patients in the absence of opportunistic infections when compared with normal values predicted from Schofield’s equations (which are based on weight and height for appropriate age categories) [16]. Previous studies have shown that REE is elevated in HIV patients when compared with control subjects [5, 7] and prediction equations for normal subjects [7]. It is not possible to assess from the present data whether the increase in REE is related to a change in the composition of LBM or a change in the tissuespecific metabolic rates. In contrast to the elevated REE, TEE was not high in comparison with reference values for normal subjects (see below). This is partly because some subjects had a low body mass (and low BMI) and partly because the energy expended in physical activity was not high. The mean PAL ratio (1.42 ± 0.14) is entirely compatible with the sedentary lifestyle of the majority of our subjects (a PAL of 1.4 to 1.5 would be typical for unemployed men with light to moderate non-occupational activity) [14]. However, it is significantly lower than that measured in normal healthy male adults using the doubly labelled water method. In a compilation study of the results of individual doubly labelled water studies, Black et al. [19] produced the following reference ranges: TEE 13.8 ± 3.0 MJ/day and PAL 1.85 ± 0.33 for subjects aged 18–29 years, mean weight 75.6 kg, BMI 24 ± 5.3, n = 56; TEE 14.3 ± 3.1 MJ/day and PAL 1.77 ± 0.31 for subjects aged 30–39 years, mean weight 86.1 ± 31.4 kg, BMI 26.8 ± 8.8, n = 36. The low PAL values in our subjects persist even if the 5% elevation in REE (which effectively reduces the PAL ratio) is taken into account. Although none of the patients in the current study were acutely unwell, many had reached the advanced stages of HIV disease. Lethargy and fatigue are common symptoms in such patients and reduced levels of physical activity would be expected. The results of this study fall within the range of values previously reported in HIV patients using the technique of doubly labelled water (TEE 5.6 to 16.9 MJ/day) [9].

We also found that estimates of PAL from activity diaries were significantly lower than those calculated from measured TEE and REE. There were substantial differences between methods, although the two sets of estimates were significantly correlated. More detailed lifestyle questionnaires have also been shown to underestimate PAL and TEE in comparison with doubly labelled water estimates in HIV-positive subjects [20].

This study has also provided the first tracer estimates of the intra-individual day-to-day variation in TEE. Although this variation (6 ± 6%) is less than that of a group of young male adults (PAL 2.0) who were assessed using indirect calorimetry and an activity diary (12 ± 3%), the difference is not statistically significant [21]. It is not surprising that the day-to-day variation in TEE, which is mainly due to daily variation in physical activity, was small in our group of patients with comparatively low overall levels of physical activity.

The PAL may be a clinically useful measure in HIV patients since it may affect protein turnover in skeletal muscle and size of the LBM; reduced physical activity may thus play a role in the wasting process. PAL may also act as a marker of performance status and quality of life. The ability to measure the components of energy expenditure using tracer techniques provides a useful investigative tool.

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