Applications of the doubly-labelled-water ($^2H_2{}^{18}O$) method in free-living adults

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The doubly-labelled-water (DLW) method is about to take off as a new investigative tool for human studies. This is well-illustrated by the fact that the amount of new DLW information presented at this meeting alone has far exceeded the total amount in the published literature. It is therefore an appropriate time to make a critical examination of the results obtained so far in order to test whether initial optimism and confidence in the new technique are really justified.

The present paper will use values from four DLW studies conducted by the Dunn Nutrition Unit, and will investigate whether any known or potential bias in the technique could invalidate the original conclusions drawn from the results. The studies assessed energy expenditure in women living modern, inactive lifestyles; in obesity; during pregnancy and in African women farmers. The major conclusions were based on comparisons of group means, and the distinction between errors and bias is therefore important. The emphasis in the present paper will be on searching for possible sources of bias, since random error would only increase the within-group variability and decrease the chances of demonstrating significant between-group differences. Although the potential errors discussed earlier in this symposium by Coward (1988) are crucial in some studies, it is only consistent, unidirectional bias which would confound the interpretation of the studies to be discussed here.

Study 1. Unexpectedly low levels of energy expenditure in normal women

This study reported the first field measurements of energy expenditure by the DLW method, and concluded that total free-living energy expenditure (TEE) in a group of normal, healthy women in Cambridge was only 1·38 times basal metabolic rate (BMR) (Prentice *et al.* 1985). This result was at variance with the widely-held assumption that an energy expenditure of 1·5 times BMR was the minimum level compatible with normal life, and also contrasted with energy intakes of 1·59 times BMR recommended by the Food and Agriculture Organization/World Health Organization/United Nations University (1985) for women at light activity levels and 1·71 times BMR recommended by the Department of Health and Social Security (1979) for women in most work categories. We were therefore claiming not only that it was possible for people to exist with expenditures below 1·5 times BMR, but that in practice many sedentary women (and presumably men) habitually did so in modern societies.

The first test which should be applied to any results such as these which challenge current tenets is that of biological plausibility, and in this study we used whole-body calorimetry to demonstrate that such low levels of expenditure were indeed possible. Each of the subjects spent 24 h in a whole-body indirect calorimeter before the isotope measurement period. They kept to a protocol which was representative of a sedentary day but included 1·5 h standing and 1 h of imposed exercise at a light work load. A typical trace from one of the subjects is illustrated in Fig. 1 in which energy expended in excess of BMR is indicated. In this case 24 h energy expenditure in the calorimeter (CAL EE) was 1·30 times BMR which is in the middle of the observed range of 1·25–1·35 times BMR. Other whole-body calorimetry studies have found similar results. Fig. 2 shows isotopically-derived TEE *v*. energy expenditure in the calorimeter for each subject. In

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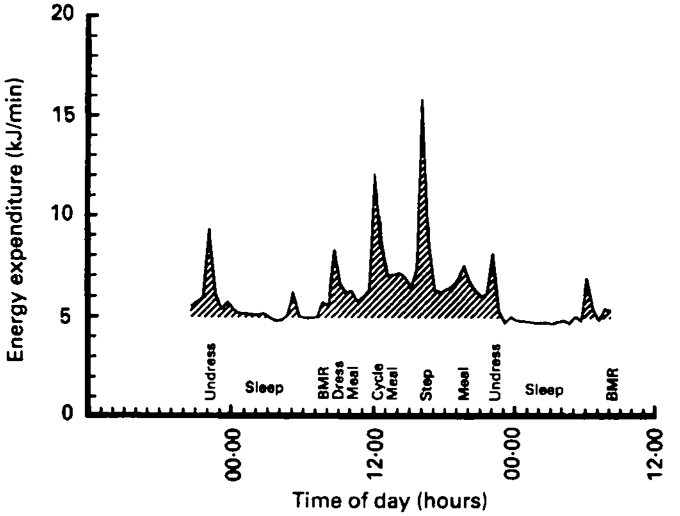

Fig. 1. Study 1. Whole-body calorimeter trace of energy expenditure (CAL EE) on a light activity protocol. (▨), Expenditure in excess of basal metabolic rate (BMR). CAL EE = 1·30 times BMR.

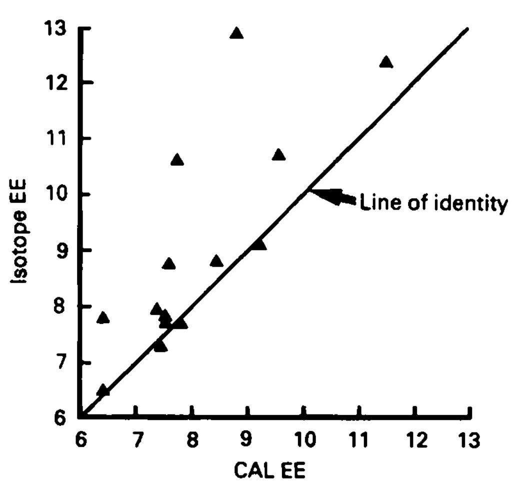

Fig. 2. Study 1. Relationship between energy expenditure in normal life (isotope EE) and in the calorimeter (CAL EE).

every case the values for free-living energy expenditure fall on or above the line of identity demonstrating that they are all physiologically plausible in spite of being so low.

The next stage is to check each step in the process of calculating TEE for separate or additive sources of bias. The calculation can be considered in two parts: derivation of

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carbon dioxide production rates from urinary isotope enrichment levels, and subsequent conversion of CO_2 to energy expenditure using the classical equations of indirect calorimetry. The first part of the process contains most of the potential sources of error including technical errors in sample handling and analysis, possible deviations from the stipulated conditions of the initial model set out by Lifson & McClintock (1966), the method of calculation and correction for physical fractionation effects as the isotopes pass from liquid to gaseous phases during evaporation across epithelial membranes. The second part of the calculation contains only a single source of error involving the need to estimate the mean respiratory quotient (RQ) over the entire measurement period in order to assign a correct energy value to the CO_2 production.

A number of cross-validation studies against whole-body calorimetry or intake–balance measurements of expenditure have now been performed in man in an attempt to check the errors in DLW. None of the studies raises serious cause for concern about the accuracy of DLW, but precision has been relatively poor in many of them. This could easily arise from the fact that the errors inherent in the 'reference' measurements are at least as great as, and usually greater than, those anticipated for DLW. In our own cross-validation study we compared simultaneous measurements of CO_2 production by DLW and by continuous whole-body calorimetry (precision and accuracy <0·5%) over 12-d periods in four adult men (Coward *et al.* 1985; Coward & Prentice, 1985). The protocols were designed to provide a wide between-subject range in energy expenditure (9·5–14·0 MJ/d) and in average RQ (0·79–0·90) by manipulation of the levels of exercise and dietary composition. The mean error in DLW was 1·9% with a coefficient of variation also of 1·9%. This indicated that our technical procedures, method of calculation and assumptions regarding fractionation were satisfactory under these conditions.

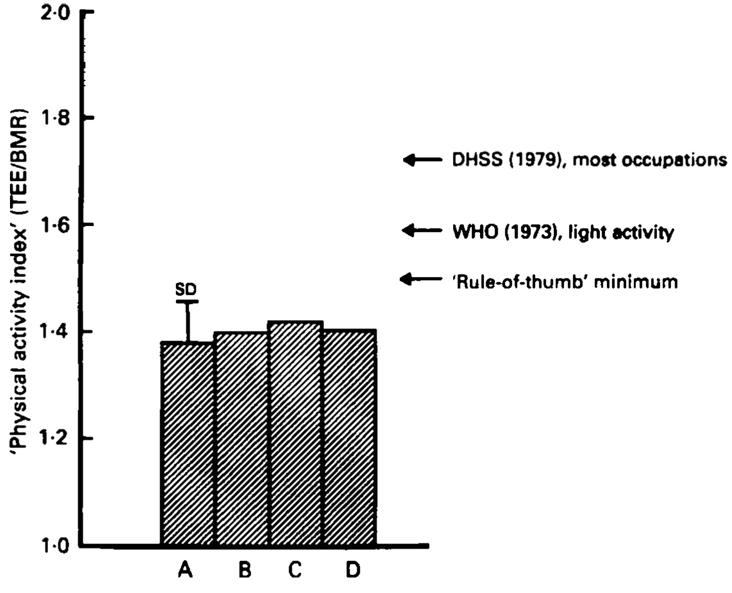

Fig. 3. Study 1. Effect of altering the method of calculation or fractionation and respiratory quotient (RQ) assumptions on energy expenditure. A, original assumptions (mean and standard deviation represented by vertical bar); B, two-point method of calculation used; C, individual fractionation assumptions (see p. 262); D, RQ changed by 2 SD; TEE, total free-living energy expenditure; BMR, basal metabolic rate; DHSS, Department of Health and Social Security; WHO, World Health Organization.

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Since there has been considerable discussion on the relative merits of Schoeller's two-point method *v.* the Cambridge multi-point method (Schoeller, 1984; Coward & Prentice, 1985; Schoeller & Taylor, 1987), we have recalculated TEE by applying the two-point method to our values from fifty free-living measurements in adult women. The two-point method differed from the multi-point method by $-1{\cdot}8 \pm 7{\cdot}4\%$, indicating that there was no appreciable bias. The precision term of $\pm 7{\cdot}4\%$ is compatible with errors in each individual method of approximately $\pm 5{\cdot}2\%$, or of $\pm 3{\cdot}5\%$ in the multi-point method (as predicted by propagation of error analysis) and $\pm 6{\cdot}5\%$ in the two-point method. In the remainder of the present paper it will be assumed that neither technical errors nor the method of calculation are likely to introduce appreciable bias in any of the data sets.

The only remaining sources of bias in the TEE values for the inactive Cambridge women relate to the fractionation and RQ assumptions. Fig. 3 shows the effects of recalculating the original values using different assumptions in order to test the possible limits of bias. In the initial publication we assumed that 0·5 of the total water turnover was fractionated and we now believe this to be an overestimate. The effect of changing this assumption to 0·4 would be to increase average TEE by 2·9%. Rather than applying a uniform guestimate of the fractionated proportion to all subjects, in common with Schoeller *et al.* (1986), we now favour estimating the proportion from the first crude (uncorrected) estimate of CO_2 production based on the fact that lung CO_2 and water losses are closely correlated. We therefore use individual estimates of the fractionated proportion calculated as: total water turnover $\div$ (non-sweating insensible water losses + lung losses) where the first term in the denominator is guessed to be 500 g/d for all adults and where lung losses (g/d) are calculated as $0{\cdot}8 \times$ crude CO_2 production expressed in litres per day. Recalculating the original values using individual estimates derived in this way increased average TEE by 3·1%.

In all our calculations of energy expenditure from CO_2 production we use an RQ value derived by adjusting individual dietary food quotients (obtained from 7-d weighed intakes) for changes in energy balance as described in full elsewhere (Black *et al.* 1986). When this procedure is used the errors arising from the RQ assumption are negligible. Nonetheless Fig. 3 contains an indication of the change in average TEE that would have been caused by changing the RQ assumptions by 2 SD from the true value (0·841 (2 SD 0·011)). This demonstrates that even when less rigorous approaches to the RQ problem are used, any bias is likely to be small.

We therefore conclude that the results as originally published contained a systematic underestimate of about 3%, resulting from the use of an inappropriate fractionation assumption, but that other sources of bias are likely to be negligible and that the original conclusion concerning the very low levels of energy expenditure in modern life still holds.

Study 2. High levels of energy expenditure in obese women

The second study examined the question of whether obese women have abnormally low energy requirements as a result of a metabolic or behavioural defect in energy expenditure. Nine women with established post-partum obesity (56% above ideal body-weight) were compared with thirteen lean controls using 7-d weighed dietary intakes, whole-body calorimetry and DLW (Prentice *et al.* 1986). The recorded energy intakes averaged 7·9 MJ/d in the lean subjects and only 6·7 MJ/d in the obese group; however, the obese group was losing weight over the measurement period and when corrected for the estimated mobilization of body fat the energy intakes were 8·2 and 8·3 MJ/d in the two groups respectively. This observation that obese subjects apparently eat

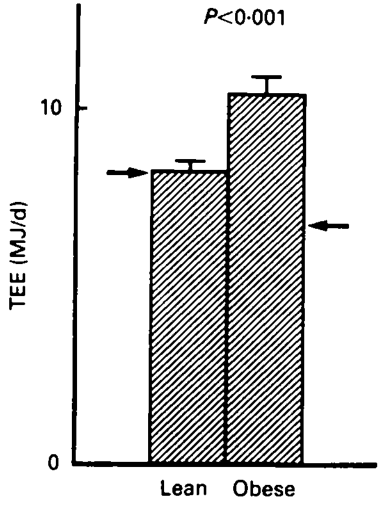

Fig. 4. Study 2. Total free-living energy expenditure (TEE) measured by the doubly-labelled-water method in lean and obese subjects. Arrows indicate recorded food intakes. Values are means and standard deviations represented by vertical bars.

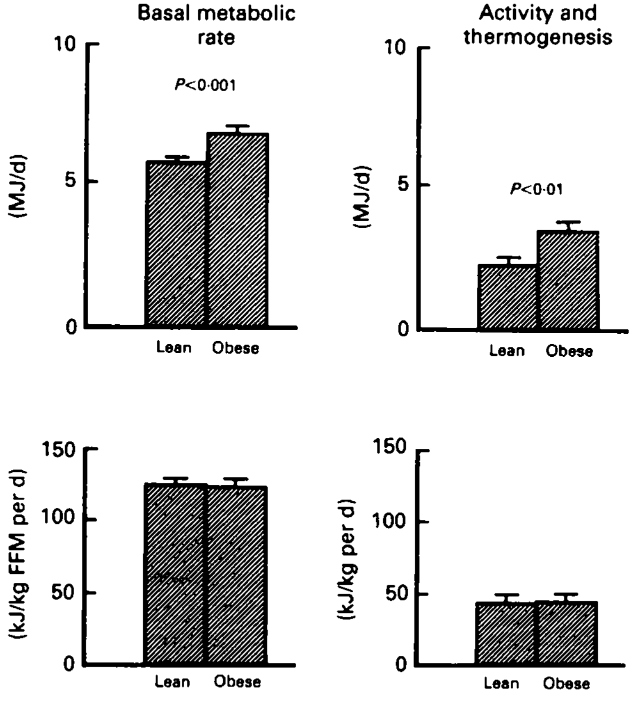

Fig. 5. Study 2. Basal metabolic rate (BMR) and activity plus thermogenesis in lean and obese subjects. FFM, fat-free mass. Values are means and standard deviations represented by vertical bars.

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no more than lean subjects in spite of their increased body mass replicates many previous studies and has been the main piece of evidence in establishing the hypothesis that obesity is both caused and perpetuated by low levels of energy expenditure.

The DLW measurements of TEE revealed a very different picture as illustrated in Fig. 4. TEE was 28% higher in the obese women who had apparently been under-recording their food intake by 3·5 MJ/d. Using the calorimetry measurements of BMR the total expenditure in each group was subdivided into maintenance and activity-plus-thermogenesis (derived as TEE−BMR). When these components of energy expenditure were expressed per kg fat-free mass (FFM) and per kg body-weight respectively, they were on average identical in the two groups (Fig. 5), refuting the possibility of energy-sparing defects in these particular obese women.

Once again the biological plausibility test can be applied using the whole-body calorimeter values. Fig. 6 shows 36-h traces from the two median weight subjects in each group. It is clear that under the fixed conditions of the calorimeter protocol the obese subject always expended considerably more energy than the lean subject. This result applied throughout, and the group mean CAL EE values were 7·4 and 9·0 MJ/d in the lean and obese subjects respectively. The isotope TEE results were perfectly consistent with this finding.

In searching for possible sources of bias we have assumed that technical problems and the method of calculation are not likely to be major contributors for the same reasons as outlined in the discussion of study 1. However, the fact that the obese women were in negative energy balance during the TEE measurement period and were deriving 18% of their daily energy from body fat stores was a potential concern. First, if we had merely guessed their RQ to be the same as the dietary food quotient we would have overestimated TEE by 2·5% in the obese group. This is mentioned merely as a caveat since in our study we did account for the fat loss and the error from the RQ assumption must have been negligible. Second, the weight loss would presumably have been associated with a slight contraction of the total body water pool thus breaking one of the stipulations of Lifson's model (Lifson & McClintock, 1966). Third, the mobilization and oxidation of body fat would have liberated hydrogen ions which were not in isotopic equilibrium with the total body water. We have computed the possible effects of the second and third sources of bias and conclude that they are most unlikely to exceed 1% of TEE.

The final factor to be considered is once again the fractionation assumption. In the original publication a constant assumption of 0·4 was used in both groups of subjects. In fact it is likely that the fractionated proportion was larger in the obese group than in the lean group since calculated surface area and TEE were 21 and 28% higher respectively, while water turnover was only 3% higher. However, recalculation of the results, using individually estimated fractionation assumptions as described previously, increased TEE in the lean group by 0·9% and in the obese group by 1·7%; a between-group difference of only 0·8% which would not affect our original conclusions.

Study 3. Variability in energy expenditure during pregnancy

This study made serial measurements of TEE at 6-weekly intervals during pregnancy following baseline measurements in the prepregnant state (Davies *et al.* 1988). The subjects were seven well-nourished women in Cambridge and the major finding was that the metabolic and behavioural responses to pregnancy are extremely variable. This is illustrated in Fig. 7(*a*) which shows the range in the energetic costs of pregnancy (exclusive of stored energy) calculated as the cumulative difference between pregnancy TEE values and the non-pregnant baseline. The estimates varied from −14 MJ to +568

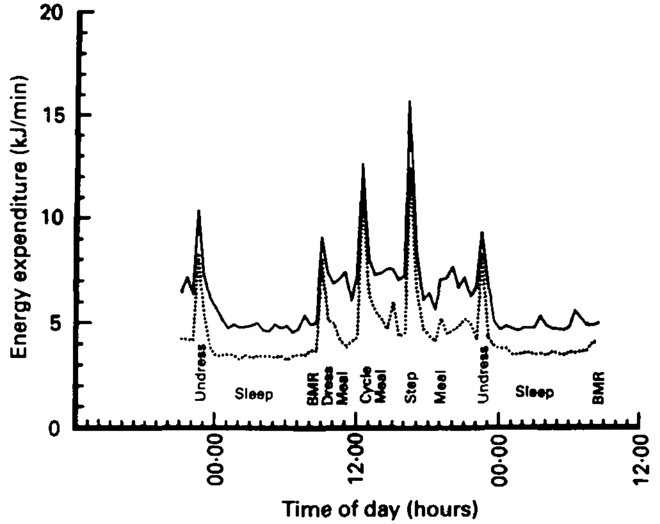

Fig. 6. Study 2. Whole-body calorimeter traces of energy expenditure in representative lean (. . . . .) and obese (————) subjects on identical activity protocols. BMR, basal metabolic rate.

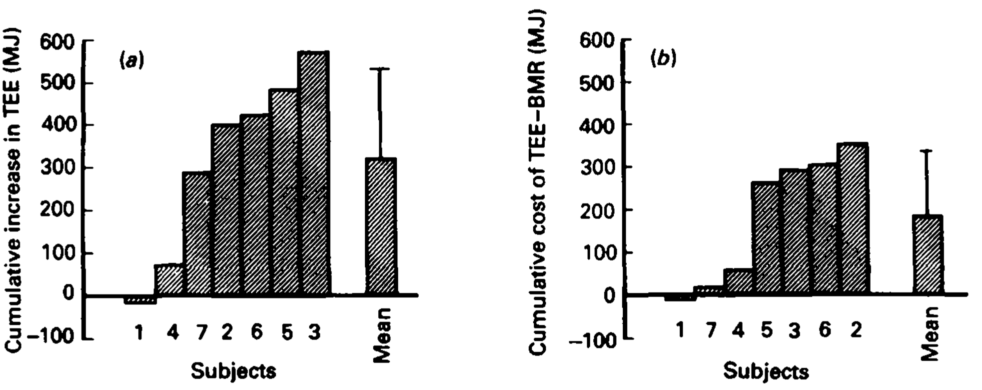

Fig. 7. Study 3. Ranges of (*a*) cumulative total energy expenditure (TEE) and (*b*) cumulative cost of physical activity (TEE−basal metabolic rate (BMR)) in seven well-nourished pregnant women. Vertical bars represent standard deviations.

MJ. Fig. 7(*b*) shows the cumulative changes in the amount of physical activity (plus thermogenesis) derived as TEE−BMR. Somewhat contrary to initial expectations none of the subjects showed any energy-sparing decreases in the cost of physical activity.

These results seem plausible in view of the fact that there were also very wide variations in the changes in BMR during pregnancy with some subjects showing a depression of BMR up until 24–30 weeks, while others showed progressive increases in BMR. The cumulative maintenance costs of pregnancy, measured with very high precision by whole-body calorimetry, had a coefficient of variation of 93% and this forms a large part of the variability in cumulative TEE. The increased costs of physical activity are also consistent with a constant pattern of activity which has a progressively rising cost due to the increase in body-weight.

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Since the previously mentioned conclusions were based on within-subject changes from a non-pregnant baseline, any constant, absolute bias would be immaterial and only progressive bias would matter. Three possible sources of progressive bias can be identified. First, the increase in body-weight during pregnancy will be associated with some binding of ^2H in newly synthesized protein and fat, and second, there will be a gradual increase in the body water pool. Each of these effects is computed to introduce less than a 1% bias even if it is unreasonably assumed that all fat deposited is synthesized *de novo*. The third potential source of progressive bias arises from the need to assume that each subject's true isotopic background levels stay the same as when initially measured in the prepregnant state. This is necessary because the closely repeated measurements prevented the re-establishment of baseline enrichments. If background enrichment levels (in local water supplies and foodstuffs) varied appreciably throughout a pregnancy, errors would occur in the estimation of TEE. We did not conduct the optimal check for such changes which would consist of making repeated measurements of background levels in comparable, but undosed, subjects. However, the constancy of isotopic enrichment levels in the local water was such that we can be confident that changes would not have introduced more than a 1% bias. A fourth possible source of progressive bias would occur if there were changes in the fractionation assumption which

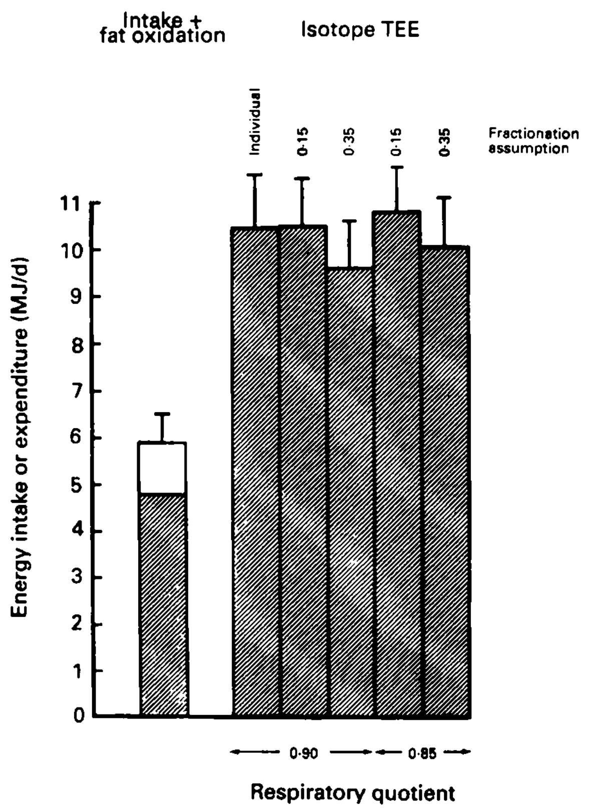

Fig. 8. Study 4. Energy intake and isotopically-measured total energy expenditure (TEE) assessed simultaneously in thirteen Gambian women at a time of peak agricultural activity. (□), Estimate of energy derived from body fat. Values are means and standard deviations represented by vertical bars.

had been ignored. In this study we applied individual estimates of the fractionated proportion and believe that potential changes have been adequately accounted for.

We therefore conclude that, as in the first two studies, our findings based on the DLW measurements are secure.

Study 4. Energy expenditure in Gambian farming women

The final study to be scrutinized involved DLW estimates of TEE in thirty Gambian women during the peak of the agricultural season (Singh *et al.* 1988). The farming season coincides with a 'hungry' season to produce a transient period of negative energy balance in all adults in the rural village studied. The purpose of this study was to try to discriminate between disparate estimates of energy expenditure based on activity diaries and on energy intake assessed by direct weighing.

Using individual fractionation assumptions and an RQ assumption of 0·90, the mean TEE in the whole group was 10·5 MJ/d or nearly 2·0 times BMR. This is consistent with the fact that the women spent 8 h/d involved in arduous farm work and closely matched activity diary estimates of 9·8 MJ/d derived in other women at the same time of year by Lawrence (1988). Fig. 8 shows the results from a subgroup of thirteen women in whom simultaneous measurements of food intake were also made. The discrepancy between TEE and measured food intake was 5·4 MJ/d of which only 1·1 MJ/d could be accounted for by our estimates of fat mobilization. Due to the imprecision inherent in short-term measurements of changes in body fat stores the extent of this discrepancy between the intake and expenditure measurements cannot be exactly defined. However, it is inconceivable that this can account for more than a small proportion of the residual 4·3 MJ/d discrepancy.

Fig. 8 also shows the DLW results when recalculated using extreme assumptions for fractionation and RQ. The low fractionation values in The Gambia are due to very high water turnovers caused by high levels of sweat loss which are assumed to be unfractionated. The chosen RQ of 0·90 was calculated from food quotients averaging 0·93 adjusted for the energy imbalance. Fig. 8 illustrates that even when quite unreasonable fractionation and RQ assumptions are substituted for the original ones it makes little difference to the estimates of TEE, and that the discrepancy between TEE and energy intake strongly suggests errors in the food intake measurements rather than in the DLW method.

Conclusion

A critical analysis of the potential sources of bias in the DLW method has failed to reveal any reason to modify the initial conclusions from each of the four studies scrutinized in the present paper. We therefore have considerable confidence in the technique and believe that it is fulfilling its initial promise as an exceptional methodological breakthrough.

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