# Classical respirometry and the doubly-labelled-water ($^2H_2{}^{18}O$) method: appropriate applications of the individual or combined techniques

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Two techniques are widely used for measuring energy expenditure in humans: classical respirometry and more recently the doubly-labelled-water method. This presentation deals with a few applications of these techniques and the interest in combining both approaches.

Indirect calorimetry is an accurate method for short-term measurement of energy expenditure. With ventilated-hood open-circuit indirect calorimetry oxygen consumption and carbon dioxide production can be measured with an accuracy of ±2%. From these measurements, the respiratory quotient is obtained, and when urinary nitrogen excretion is measured, the overall substrate oxidation rate can be calculated. This method provides a non-invasive technique for studying the fate of nutrients in man (Jéquier, 1984*a*,*b*). A recent example of the use of this technique is illustrated by studies on glucose metabolism which have shown that impairment of non-oxidative glucose disposal in obese and non-insulin-dependent diabetic patients is a progressive defect characterizing the marked resistance to the action of insulin (DeFronzo *et al.* 1985). The thermogenic responses to single nutrients (Pittet *et al.* 1974; Schutz *et al.* 1983) or to a mixture of nutrients (Vernet *et al.* 1986) have been re-assessed by open-circuit indirect calorimetry. The results of these studies have improved our knowledge of the net energy available from nutrients given orally or parenterally to patients.

## *Respiration chamber*

*The components of energy expenditure.* The construction of respiration chambers (Dauncey *et al.* 1978; Jéquier & Schutz, 1983; Ravussin *et al.* 1986) has allowed more prolonged studies of energy expenditure in man. The respiration chamber offers the opportunity to study 24-h energy expenditure under standardized living conditions; it allows measurement of both $O_2$ consumption and $CO_2$ production with high accuracy, since the error of both measurements is within ±2% (Jéquier & Schutz, 1983). The response time of modern respiration chambers is about 2–4 min: this means that steady-state of $O_2$ consumption during exercise at constant intensity is obtained within 10 min. Thus, it is possible to measure the changes in energy expenditure which occur after meals and during physical activity; it also provides an opportunity to measure metabolic rate during sleep (Dauncey *et al.* 1978; Schutz *et al.* 1984; Ravussin *et al.* 1985).

The respiration chamber permits the partition of the three main components of energy expenditure, i.e. basal metabolic rate (BMR), thermogenesis, and the energy expended for physical activity (Fig. 1). BMR is measured when the subject is still in bed, in the morning, in the fasting state. Thermogenesis represents the energy expended above BMR in the resting state; it mainly includes the thermogenic response to meal ingestion. A method to assess the overall thermic effect of meals has been described by Schutz *et al.* (1982, 1984) (Fig.1). In brief, the movements of the subject in the chamber are detected by a radar system based on the Doppler effect. A linear regression line is obtained for energy expenditure *v.* physical activity measured by radar. The intercept of the regression line at zero activity determines the mean resting energy expenditure ($EE_{rest}$) from 07.00 to 23.00 hours, which includes the thermic effect of the meals. The difference

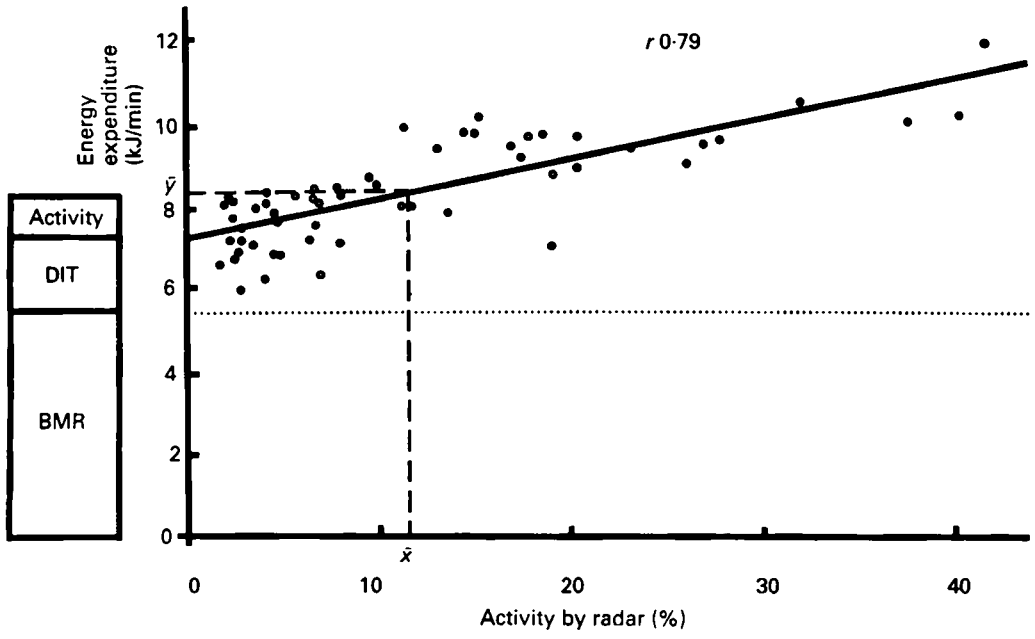


Fig. 1. Individual regression line between percentage physical activity measured by radar (Doppler effect) and energy expenditure (kJ/min). Percentage physical activity represented the percentage of time during which the subject was moving at a rate detected by the radar. Each point represents mean values for a 15-min period from 07.00 to 23.00 hours. The intercept of the line at zero activity is the value of the mean resting energy expenditure ($EE_{rest}$). Dietary induced thermogenesis (DIT) is $EE_{rest}$ minus basal metabolic rate (BMR),

$$\text{DIT (kJ)} = (7{\cdot}21 - 5{\cdot}40) \times 60 \times 16 = 1738 \quad (1)$$

$$\text{DIT (as \% of energy intake)} = 1738/16736 = 10{\cdot}4 \quad (2)$$

In equation 1, 60×16 represents the period (min) during which energy expenditure was measured.

between $EE_{rest}$ and BMR computed over 16 h represents an estimate of the thermic effect of the meals. The energy expended for physical activity is obtained from the difference between total energy expenditure and $EE_{rest}$, computed over 16 h; the period of 16 h represents the time-period from getting up at 07.00 hours until going to bed at 23.00 hours. The partition of total energy expenditure measured by the chamber in subjects with *ad lib.* activity was found to be 73% for BMR, 15% for thermogenesis and 12% for physical activity (Jéquier, 1984*a*,*b*).

An appropriate application of the respiration chamber technique is the assessment of total energy expenditure in lean and obese individuals. There is good agreement among various groups of investigators who have reported a greater total energy expenditure in obese than in lean subjects, either with *ad lib.* activity (Ravussin *et al.* 1981) or fixed activities (Prentice *et al.* 1986). When total energy expenditure was expressed on a per kg fat-free mass or per kg body-weight basis, there was no difference between groups of obese and lean subjects, but the individual variability was large (Ravussin *et al.* 1981, 1986). This means that obese individuals must consume more energy than lean sedentary controls in order to sustain their obesity. This simple conclusion from respiration chamber studies is, however, often overlooked, and claims of low energy requirements for obese patients are frequently made.

A second example of the use of a respiration chamber is the study of the change in energy expenditure due to overfeeding (Schutz *et al.* 1985). Ravussin *et al.* (1985) showed

that approximately one-quarter of the excess energy intake is dissipated through an increase in energy expenditure; after 9 d of overfeeding a mixed diet (1·6 times the maintenance requirements), one-third of the increase in 24-h energy expenditure was explained by a rise in BMR and the remainder was due to the thermic effect of food and the increased cost of physical activity related to body-weight gain. These findings do not support the concept of 'luxuskonsumption' (Neumann, 1902) in man during short-term overfeeding and show that the total increase in energy expenditure can be accounted for by known mechanisms.

*Nutrient balance and the relative contribution of carbohydrate and fat to energy expenditure.* The combined measurements of $O_2$ consumption, $CO_2$ production and urinary nitrogen excretion permit the calculation of the fuel mixture oxidized, and the nutrient balance if we know the amount of nutrients ingested. When a maintenance mixed diet is given to subjects in the respiration chamber, the fuel mixture oxidized over 24 h is closely related to the composition of the ingested nutrients (Hurni *et al.* 1982). If the proportion of carbohydrate intake is increased and that of lipid decreased in a maintenance diet (while maintaining constant energy intake) the fuel mixture oxidized matches the changes in intake: more carbohydrate and less lipid are oxidized than with the previous diet. This is in keeping with the limited capacity to store carbohydrate and the adjustment of carbohydrate oxidation to carbohydrate intake (Acheson *et al.* 1982).

Flatt (1985) has defined as the food quotient (FQ) the ratio, $CO_2$ produced:$O_2$ consumed when a representative sample of the diet consumed is oxidized in a bomb calorimeter. The studies of Hurni *et al.* (1982) described previously indicate that under

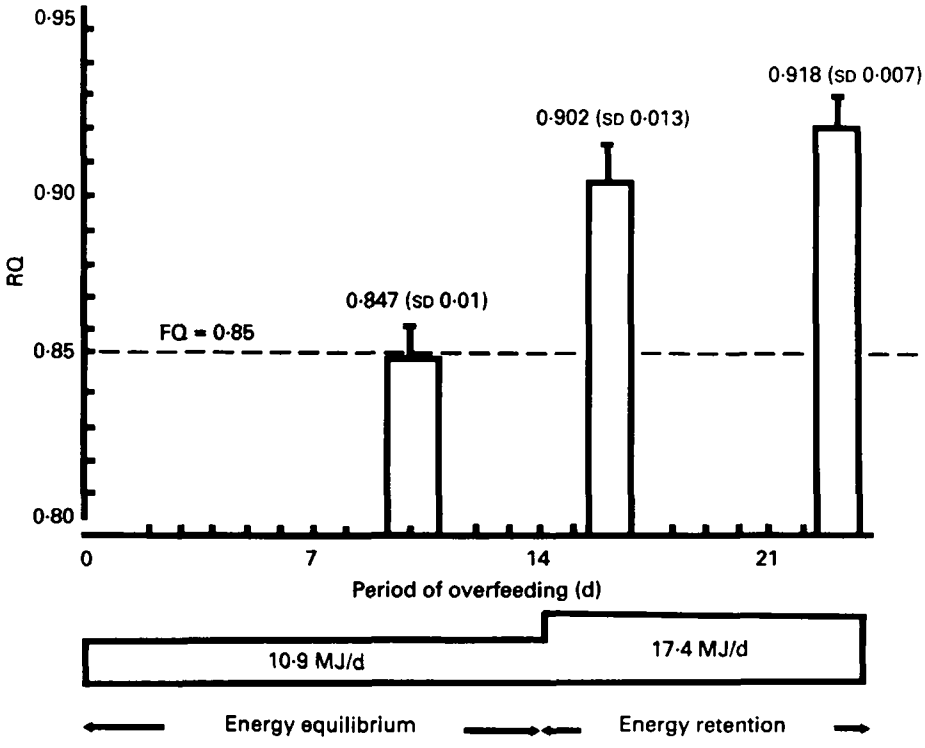


Fig. 2. Mean respiratory quotient during an overfeeding study with a mixed diet for five healthy subjects. FQ, food quotient of the diet.

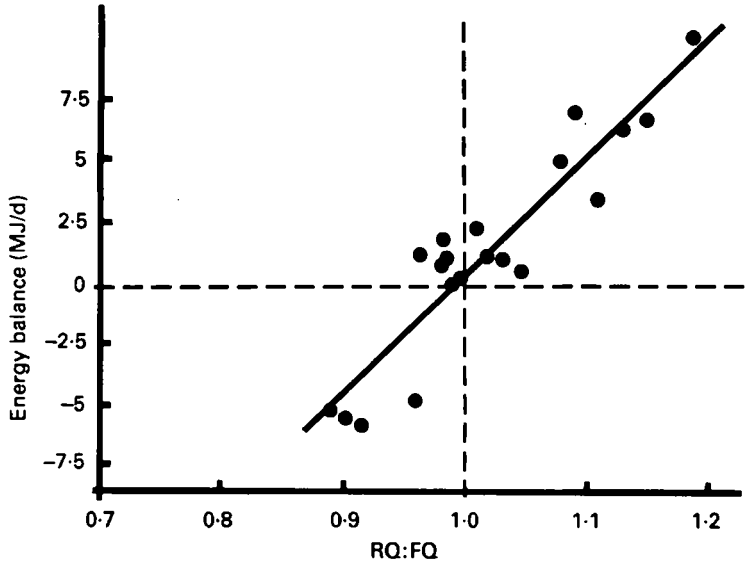


Fig. 3. Relationship between respiratory quotient (RQ):food quotient (FQ) ratio and the energy balance in man. Each point represents the values for a given subject measured over a 24-h period.

conditions of energy balance FQ is equal to the mean 24 h respiratory quotient (RQ) of the subject. By contrast, conditions of positive energy balance induce an RQ:FQ value greater than 1·0 (Fig. 2) indicating a preferential stimulation of carbohydrate oxidation, whereas the excess lipid intake is stored; this elevated RQ:FQ can also be explained by lipogenesis when carbohydrate intake is very large (Acheson *et al.* 1982). Conversely, when the energy balance is negative, RQ:FQ is less than 1·0, showing that the fuel mixture oxidized includes endogenous lipid, which lowers RQ.

Fig. 3 shows a linear relationship between RQ:FQ and energy balance. This implies that measurement of RQ:FQ over 24 h permits an indirect assessment of the energy balance state of the subject.

Short-term nutrient balance measured with a respiration chamber allows the assessment of changes in body composition. For example, the maximal glycogen storage capacity of young adult men was found to be approximately 500 g (K. J. Acheson, personal communication); after depletion of glycogen stores with a low-carbohydrate diet for 3 d, the glycogen stores were repleted during 7 d of carbohydrate overfeeding (Schutz *et al.* 1985). Measurement of nutrient balance showed that a cumulative gain of 500 g glycogen can be accommodated before net lipid synthesis contributes to increasing body fat mass. On the last day of the overfeeding period, the subjects ingested 981 (SE 43) g carbohydrate and the total rate of disappearance of carbohydrate (oxidation + conversion into lipid) was found to be 1010 (SE 37) g, which indicates that carbohydrate balance tends to be equilibrated. From the 1010 g carbohydrate utilized, approximately 540 g were oxidized and 470 g were converted into lipid, which corresponds to a gain of 150 g fat. The mean 24 h RQ was greater than 1·0, indicating the absence of net lipid oxidation. During the last day of the overfeeding period, the fat intake was 70 g, and the fat balance was therefore +220 g (70+150). There is no other technique which allows the assessment of such small changes in body composition in man.

*Advantages and disadvantages of respiration chambers.* In summary, the respiration chamber is an accurate method for the measurement of energy and nutrient balances in man. It permits the partition of energy expenditure into its main components; BMR, thermogenesis and the energy cost of physical activity. It is the only technique which allows the assessment of short-term changes in body composition.

The main disadvantage of the respiration chamber is the artificial conditions of living in a closed environment: spontaneous physical activity is somewhat limited while living in a chamber. The method is therefore not suitable for the assessment of energy requirements for every-day life. It gives, however, a value which corresponds to sedentary energy requirements. It is interesting to note that in obese individuals, 24-h energy expenditure measured with the chamber was found to be greater than the measured energy intake (James, 1985; Prentice *et al.* 1985). This shows that measurements of energy intake in obese individuals underestimate the true energy requirements of obese individuals.

## *Doubly-labelled-water technique*

The most interesting development in the area of human energy metabolism has been the use and validation of the doubly-labelled-water ($^2H_2{}^{18}O$) technique. The method has been described in the previous paper of this symposium by Coward (1988). This is an ideal non-invasive technique for measuring total daily energy expenditure in free-living people over extended periods, i.e. 8–14 d. The method gives an overall value of the energy expended over the total period; it cannot be used, however, to measure energy expenditure over a single day, or to assess energy expenditure during sleep.

The method involves several steps: (a) the measurement of $^2H_2$ and $^{18}O$ disappearance rate over two to three biological half-lives in body fluids and the measurement of the $^2H_2O$ and $H_2{}^{18}O$ distribution spaces; (b) the transformation of these values into the turnover of water and water plus $CO_2$, from which $CO_2$ production is calculated by difference; (c) the calculation of energy expenditure from $CO_2$ production rate which requires an assumption of the mean RQ.

*Combined techniques: respiration chamber and doubly-labelled water.* Validation of the doubly-labelled-water method has been carried out by comparing the isotopic determination of total energy expenditure with that of continuous measurements with the chamber. These investigations have shown that the mean error involved in the use of the doubly-labelled water ranged from –4% (Klein *et al.* 1984) to +8% (Schoeller & Webb, 1984). Under strictly controlled conditions, Coward *et al.* (1985) reported a total error of the method of ±5%.

The combined technique (respiration chamber plus $^2H_2{}^{18}O$ method) allows measurement of the mean RQ (with the chamber), a value needed to calculate energy expenditure from $CO_2$ turnover obtained with the doubly-labelled-water method. In addition, the chamber permits the partition of energy expenditure into its three components: BMR, thermogenesis and energy cost of physical activity under the restricted conditions of living in a chamber, whereas the doubly-labelled-water technique can be used to obtain total energy expenditure under free-living conditions. The difference between total energy expenditure measured using the doubly-labelled-water technique and that measured using the chamber mainly corresponds to the cost of extra-physical activity in free-living conditions compared with that in the chamber. It is surprising that in sedentary individuals, this difference is small: in a group of twelve healthy women (age 23–40 years) including a wide range of body-weights (50–120 kg), Prentice *et al.* (1985) reported that total energy expenditure measured with the doubly-labelled-water technique was only 4% higher than that measured with the

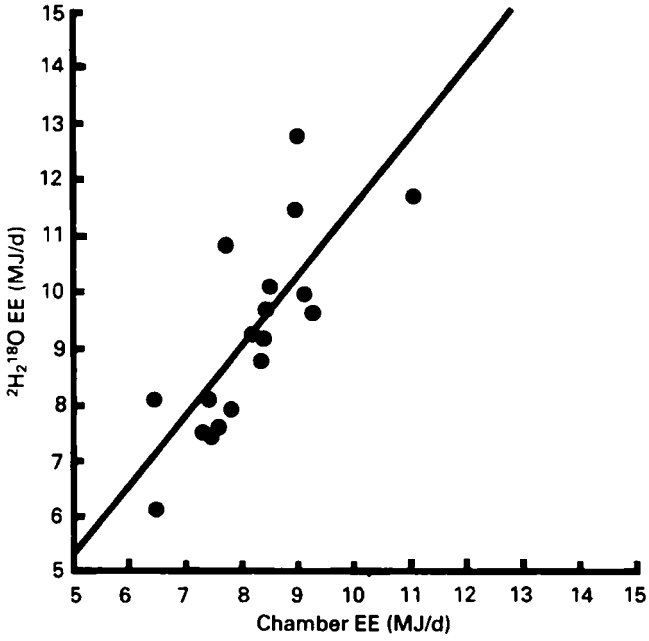


Fig. 4. Relationship between energy expenditure (EE) measured using the respiration chamber and EE measured under free-living conditions with the doubly-labelled-water ($^2H_2{}^{18}O$) method in eighteen subjects (from values of Prentice *et al.* 1986).

$$y = -0{\cdot}96 + 1{\cdot}24x \ (r\ 0{\cdot}78,\ P<0{\cdot}001)$$

respiration chamber under conditions of a rigidly-imposed activity protocol. In another study on thirteen lean and nine obese women (Prentice *et al.* 1986), total energy expenditure with the doubly-labelled-water technique was 8 and 14% higher than that measured in the chamber. This confirms the assumption that the respiration chamber gives a lower estimate of total energy expenditure than the doubly-labelled-water method, but the difference between the results of the two techniques is small for sedentary individuals. A much larger difference is obviously to be expected in subjects with high physical activity under free-living conditions. For sedentary subjects, a linear relationship was found between total energy expenditure measured with the chamber and that obtained from the doubly-labelled-water technique (Fig. 4). This shows that energy expenditure obtained in the calorimeter bears a relationship to that found in free-living conditions.

In summary, the two techniques for measurement of energy expenditure are complementary. The respiration chamber can be used to follow the time-course of energy expenditure over 24 h and to partition total energy expenditure between BMR, thermogenesis and physical activity. It also provides a method for assessing nutrient and energy balance in short-term studies, whereas the doubly-labelled-water technique is a method which can be used to assess the total energy expenditure of individuals under free-living conditions during periods of 1–2 weeks. The latter method is therefore appropriate for assessing the energy requirements of people with different activity levels, whereas the respiration chamber only assesses sedentary energy requirements.

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