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ASSESSMENT OF ENERGY EXPENDITURE AND FUEL UTILIZATION IN MAN

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DIRECT AND INDIRECT CALORIMETRY

Introduction

The assessment of energy expenditure in man has been carried out by direct and indirect calorimetry for nearly a century. Major advances in the study of energy metabolism were made by the outstanding work of many investigators during the first decades of this century, and the recent development of new technologies for measuring heat losses and gas exchanges has improved our ability to study energy turnover in man. This presentation deals with recent developments in the assessment of energy expenditure and fuel utilization in

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man. It is not intended to review the ancient literature, which has often been cited in many classical textbooks.

The method of direct calorimetry measures the heat dissipated by the body, whereas indirect calorimetry measures the heat released by the oxidative processes. Since for each liter of oxygen consumed there is a known amount of heat released, the measurement of the subject's oxygen consumption is the principle on which indirect calorimetry is based. The amount of heat released per liter of oxygen consumed is not constant but depends on the type of nutrient oxidized. By measuring carbon dioxide production and urinary nitrogen excretion in addition to oxygen consumption, it is possible to determine the proportion of the different nutrients that are oxidized, and the heat released can be precisely calculated.

It is important to emphasize that direct and indirect calorimetry do not measure the same energy. The human body constitutes a large heat capacity that undergoes changes in heat storage. Thus an increase in heat released by oxidative processes can be immediately assessed by indirect calorimetry, but is not accompanied by a similar increase in heat losses. Indirect calorimetry has the advantage of a small response time, whereas direct calorimetry gives a delayed response. The response time of indirect calorimetry is short because the body's oxygen stores are very small and the capacity of anaerobic synthesis of ATP is limited. With the exception of transient abrupt changes of energy expenditure occurring at the start of exercise (24), oxygen consumption follows ATP production on line and is directly related to the subject's energy expenditure. By contrast, the delayed response of direct calorimetry in regard to the changes in energy expenditure represents a limitation of this technique. Direct calorimetry cannot be used to assess the short-term effect of feeding or the energy expended during exercises of short duration because a large part of the heat that is generated is stored within the body. This results in a rise in body temperature; the assessment of the amount of heat stored in the body is very difficult since the changes of temperature are not uniformly distributed within the tissues (8).

Heat Balance Equation

For a subject at rest, the body heat balance equation is

$$\dot{M} = (\dot{R} + \dot{C} + \dot{K} + \dot{E}) + \dot{S}, \qquad 1.$$

where $\dot{M}$ is heat production from metabolism,
$\dot{R}$ is the radiant heat exchange,
$\dot{C}$ is the convective heat transfer,
$\dot{K}$ is the conductive heat transfer,
$\dot{E}$ is the evaporative heat transfer, and
$\dot{S}$ is the rate of storage of body heat.

The term $\dot{M}$ is measured by indirect calorimetry and represents the rate of heat production. The metabolic reactions involved in the release of free energy from substrates are coupled to the synthesis of ATP, and it is in this form that energy is made available to sustain metabolism. Approximately one third of the nutrient energy is lost as heat when ATP is synthesized, and two thirds of the energy is released through ATP hydrolysis (14, 24). However, all the energy released is eventually transformed into heat.

The terms $\dot{R}$, $\dot{C}$, $\dot{K}$, and $\dot{E}$ are measured by direct calorimetry. The rate of heat storage $\dot{S}$ can be either positive (i.e. heat gain, with a rise in body temperature) or negative (i.e. cooling of the body).

Direct Calorimetry

The direct measurement of the heat losses of an animal has been used since the time of Lavoisier at the end of the eighteenth century. Lavoisier built a calorimeter that was a small chamber with a double wall containing ice; the heat dissipated by the animal inside the chamber was assessed by the amount of melted ice. The development of more precise techniques has allowed accurate measurement of the heat losses by animals or man (13, 16, 22, 38). Today, three principles are used: (*a*) the isothermal principle established by Atwater & Benedict (4); (*b*) the gradient-layer system, which was greatly improved by Benzinger & Kitzinger (6); and (*c*) the water-cooled garment built by Webb et al (37).

ISOTHERMAL PRINCIPLE An isothermal calorimeter is a chamber with a double wall and a controlling system that maintains a mean temperature gradient of zero across the chamber walls (10). A heat exchanger allows one to extract the sensible heat produced by the subject inside the calorimeter. Since the two walls are maintained at the same temperature, there is no heat flux across the two walls and all the sensible heat of the subject is measured through the heat exchanger. The subject's evaporative heat loss is assessed by measuring the water pressure differences between the air entering and leaving the calorimeter and the flow rate of air passing through it.

The Vienna group (36) has developed a similar calorimeter, but the principle is slightly different. Air enters the chamber at a relatively low temperature and its temperature is raised by the heat output of the subject and a compensatory heater, the latter being used to maintain a constant temperature within the chamber.

Isothermal calorimeters measure the overall energy expended by a subject when the duration of the test is sufficiently long to minimize the effect of heat storage within the body. Dauncey (12) made simultaneous measurements of heat production by indirect calorimetry and of heat losses by an isothermal direct calorimeter in eight subjects; the mean ($\pm$ SEM) difference between the two measurements was 1.2 $\pm$ 0.14%. This shows that the measurement of

energy expenditure by direct calorimetry agrees with indirect calorimetry over a 24-h period. However, for periods less than 24 h it is not possible to obtain the subject's heat production by direct calorimetry because of circadian variations in body temperature and heat storage by the body.

Large direct calorimeters often have long response times. Garby's group (17) recently built a 24-m^3 direct heat-sink calorimeter; by extensive use of real-time processing to compensate for physical time constants and delays, the response times for both evaporative and sensible heat losses have been greatly reduced (95% of the response to a step change was obtained in 15 minutes).

GRADIENT-LAYER SYSTEM Direct calorimeters using the gradient-layer system have the advantage of a low thermal inertia and short response time. The principle of measurement of heat flow by a gradient layer has been described by Benzinger & Kitzinger (6). It consists of a precise measurement of the difference in temperature between the two surfaces of a layer made of insulating material that surrounds the calorimeter. In the calorimeter built in Lausanne (35), the insulating layer was made of an epoxy resin with a printed circuit of copper on both sides. The temperature difference between the inner and outer surfaces of the layer is obtained by measuring the electrical resistances of the two copper circuits. Evaporative heat losses are assessed by condensing the subject's water vapor losses in a heat exchanger and by measuring the heat released during this process.

WATER-COOLED GARMENT A third type of direct calorimeter is the water-cooled garment built by Webb et al (37, 38). This calorimeter is a water-cooling garment made of small vinyl plastic tubing held together by vinyl plastic clips. It is a flow calorimeter, in which a known mass of water flows over the subject whose heat loss is to be measured; by measuring the temperature change of the water and multiplying it by the mass flow, the rate of heat loss is derived.

Indirect Calorimetry

The heat released by the metabolic oxidative processes ($\dot{M}$) can be calculated from the measurement of oxygen consumption. The technique of indirect calorimetry has been used for nearly two centuries. With the technical advances in the construction of open-circuit ventilated hood systems and large respiration chambers for measuring energy expenditure over prolonged periods, there has been a renewed interest in studying energy output in man.

Metabolic rate is calculated by using equations, such as Weir's equation (39), based on the measurement of oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and urinary nitrogen excretion ($\dot{N}$). Table 1 shows the factors used by different authors to compute metabolic rate from

these three measurements. The differences between the values of the different factors (a, b, and c respectively) are due to the use of slightly different constants for the amounts of O_2 used and that of CO_2 produced during oxidation of the three classes of nutrients.

The nonprotein respiratory quotient (NPRQ) can be calculated, and an equation yielding the same results as Weir's equation can be used:

$$\dot{M} = [4.686 + 1.096\ (NPRQ - 0.707)] \times N\dot{P}\dot{V}O_2 + 4.60 \times P\dot{V}O_2, \quad 2.$$

where $\dot{M}$ is the metabolic rate in kcal/min; (to obtain $\dot{M}$ in kJ/min, multiply the above result by 4.184), NPRQ is the "nonprotein" respiratory quotient $= N\dot{P}\dot{V}CO_2/N\dot{P}\dot{V}O_2$,

$N\dot{P}\dot{V}O_2$ is the "nonprotein" oxygen consumption (liters/min),
$P\dot{V}O_2$ is the "protein" oxygen consumption (liters/min),
$N\dot{P}\dot{V}CO_2$ is the "nonprotein" CO_2 production (liters/min),
$P\dot{V}CO_2$ is the "protein" CO_2 production (liters/min),

$$P\dot{V}O_2 = \dot{N} \times 6.25 \times 0.966 \quad 3.$$

where $\dot{N}$ is the total nitrogen excreted in urine (g/min)

$$P\dot{V}CO_2 = \dot{N} \times 6.25 \times 0.774 \quad 4.$$

$$N\dot{P}\dot{V}O_2 = \dot{V}O_2 - P\dot{V}O_2 \quad 5.$$

$$N\dot{P}\dot{V}CO_2 = \dot{V}CO_2 - N\dot{P}\dot{V}CO_2. \quad 6.$$

When the NPRQ = 0.707, $N\dot{P}\dot{V}O_2$ is entirely due to lipid oxidation, and the caloric equivalent of one liter of oxygen consumed is 4.686 kcal (or 19.61 kJ).

Table 1 Effect of using different factors on the calculation of metabolic rate ($\dot{M}$) from the rate of oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and urinary N excretion ($\dot{N}$)

Authors	Ref.	$\dot{M} = a\ \dot{V}O_2 + b\ \dot{V}CO_2 - c\ \dot{N}$† a	b	c
Weir (1949)	39	3.941	1.106	2.17
Consolazio et al (1963)	9	3.78	1.16	2.98
Brouwer (1965)	7	3.866	1.20	1.43

†$\dot{M}$ is in kcal per unit of time, $\dot{V}O_2$ and $\dot{V}CO_2$ are in liters per unit of time, and $\dot{N}$ is in g per unit of time. As an example, if $\dot{V}O_2$ = 600 liter/day, $\dot{V}CO_2$ = 500 liter/day, and $\dot{N}$ = 25 g/day, the calculated $\dot{M}$ values are 2864, 2773, and 2884 kcal/day for the equations of Weir, Consolazio, and Brouwer respectively.

When the NPRQ = 1.0, the caloric equivalent of one liter of O_2 consumed is 5.007 kcal (or 20.95 kJ), and $NP\dot{V}O_2$ is entirely due to glucose oxidation. Note that carbohydrates are mainly oxidized as glucose; therefore the caloric equivalent of glucose oxidation should be used (i.e. 5.007 kcal/liter O_2) instead of the caloric equivalent of starch (5.047 kcal/liter O_2).

These equations to calculate $\dot{M}$, i.e. Weir's equation or the equation based on the nonprotein respiratory quotient, are valid even in the presence of lipogenesis from carbohydrates (15), or gluconeogenesis (5). This fact can be intuitively understood since the energy metabolism is determined by the reactants and the products of combustion, irrespective of the intermediary steps involved (18).

Indirect Calorimetry with a Respiration Chamber

A respiration chamber is an airtight room ventilated with fresh air, the air flow rate being continuously measured (usually at the outlet of the chamber) (19). Both the inlet-outlet difference in oxygen concentration and the difference in outlet-inlet carbon dioxide concentration are continuously measured. In steady-state conditions, air flow is set to maintain a concentration of CO_2 in the chamber of approximately 0.5%. The subject's oxygen consumption ($\dot{V}O_2$) depends on two terms:

$$\dot{V}O_2 = (\dot{V}O_{2in} - \dot{V}O_{2out}) + \Delta VO_2/\Delta t, \qquad 7.$$

where ($\dot{V}O_{2in} - \dot{V}O_{2out}$) represents the difference between the flow rates of O_2 at the inlet and outlet of the chamber, and $\Delta VO_2/\Delta t$ represents the change in the O_2 content of the chamber during the time Δt.

It is important to take into account in the calculation of $\dot{V}O_2$ that the flow rate at the inlet is different from that of the outlet; this is because the CO_2 production rate ($\dot{V}CO_2$) is usually smaller than the O_2 consumption ($\dot{V}O_2$).

Thus, using Haldane's correction, $\dot{V}O_2$ is obtained by Equation 8:

$$\dot{V}O_2 = \frac{1}{1 - F_{in}O_2}\left[\dot{V}_{out}(\Delta FO_2 - \Delta FCO_2 \times F_{in}O_2) + V\left(\frac{dF_{out}O_2}{dt} - \frac{dF_{out}CO_2 \times F_{in}O_2}{dt}\right)\right], \qquad 8.$$

where $F_{in}O_2$ = fraction of O_2 at the inlet,
$F_{out}O_2$ = fraction of O_2 at the outlet,
$F_{in}CO_2$ = fraction of CO_2 at the inlet,
$F_{out}CO_2$ = fraction of CO_2 at the outlet,
$\Delta FO_2 = F_{in}O_2 - F_{out}O_2$,

ΔFCO_2 = $F_{out}CO_2 - F_{in}CO_2$,
$\dot{V}_{out}$ = rate of air flow at the outlet,
V = volume of the chamber.

The production rate of carbon dioxide ($\dot{V}CO_2$) is

$$\dot{V}_{2} = {}_{out} \times \Delta FCO_2 + V \frac{dF_{out}\ CO_2}{dt}. \quad 9.$$

The Haldane correction is not necessary for $\dot{V}CO_2$. The metabolic rate of the subject within the chamber is calculated with Equation 2.

FUEL UTILIZATION IN MAN

The measurement of respiratory exchange is, under ideal conditions, the sum of all the oxidative processes occurring within the body. By measuring oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and urinary nitrogen excretion, it is possible to calculate overall substrate oxidation. However, these data give no information about the substrates being oxidized in individual organs and tissues.

An index of protein oxidation is obtained from the amount of nitrogen excreted in the urine during the test period. By subtracting the volumes of oxygen consumed and carbon dioxide produced per gram of protein oxidized (Table 2) from the total $\dot{V}O_2$ and $\dot{V}CO_2$ measured by indirect calorimetry, the respective nonprotein $\dot{V}O_2$ and $\dot{V}CO_2$ and nonprotein respiratory quotient (NPRQ) can be obtained. Figure 1 illustrates that there is not only a direct relationship between the NPRQ and the energy equivalent of a liter of oxygen, but that it also represents the proportion of the $NP\dot{V}O_2$ used for fat and carbohydrate oxidation. If we take the example of a NPRQ of 0.775 we can see that 75% and 25% of the $NP\dot{V}O_2$ (liter/min) were used for fat and carbohydrate oxidation respectively. Thus,

$$\text{Fat oxidation} = \frac{(0.75 \times NP\dot{V}O_2)}{2.019}\ \text{g/min} \quad 10.$$

$$\text{Carbohydrate oxidation} = \frac{(0.25 \times NP\dot{V}O_2)}{0.829}\ \text{g/min} \quad 11.$$

$$\text{Protein oxidation} = \frac{P\dot{V}O_2}{0.966}\ \text{g/min}. \quad 12.$$

The values 2.019, 0.829, and 0.966 liters of oxygen per gram substrate

Table 2 Oxygen consumption; carbon dioxide production per gram of protein, fat, and carbohydrate oxidized within the body; and the respective respiratory quotients

	Protein (liter/g)	Fat (liter/g)	Carbohydrate (liter/g)
Oxygen consumed (liters)	0.966	2.019	0.829[a]
CO_2 produced (liters)	0.774	1.427	0.829[a]
Respiratory quotient	0.80	0.71	1.00

$$\dot{P}\dot{V}CO_2 = \dot{N} \times 6.25 \times 0.774^{b}$$

$$\dot{P}\dot{V}O_2 = \dot{N} \times 6.25 \times 0.966^{b}$$

$$NPRQ = \frac{\dot{V}CO_2 - \dot{P}\dot{V}CO_2}{\dot{V}O_2 - \dot{P}\dot{V}O_2}$$

[a] This number (0.829) represents the oxygen consumed or the carbon dioxide produced (liters) per gram of starch or glycogen oxidized; when expressed as glucose oxidation, the value is 0.746 liter.

[b] $\dot{P}\dot{V}CO_2$ is the carbon dioxide production rate due to protein metabolism (liters/min); $\dot{P}\dot{V}O_2$ is the oxygen consumption rate due to protein metabolism (liters/min); and $\dot{N}$ is the urinary nitrogen excretion in g/min.

oxidized have been taken from Table 2; the value of 0.829 liter O_2 corresponds to the volume of oxygen consumed during the oxidation of 1 g starch or glycogen.

Another way of calculating substrate oxidation from respiratory exchange data is again to make the assumption that all of the oxygen consumed and all of the carbon dioxide produced are due to oxidation of the three major energetic substrates. Thus taking the values from Table 2, the following equations are obtained:

$$\text{Total } \dot{V}O_2 = 0.829 \text{ CHO} + 2.019 \text{ fat} + 0.966 \text{ prot.} \quad 13.$$

$$\text{Total } \dot{V}CO_2 = 0.829 \text{ CHO} + 1.427 \text{ fat} + 0.774 \text{ prot.}, \quad 14.$$

where carbohydrate, fat, and protein are expressed in grams per unit of time.

By solving these equations for carbohydrate and fat oxidation (g per unit of time) we obtain

$$\text{Carbohydrate oxidation} = 4.113\ \dot{V}CO_2 - 2.907\ \dot{V}O_2 - 0.375 \text{ prot.} \quad 15.$$

$$\text{Fat oxidation} = 1.689\ \dot{V}O_2 - 1.689\ \dot{V}CO_2 - 0.324 \text{ prot.} \quad 16.$$

Providing the respiratory exchange measurements have been made accurately, it is interesting to consider the limitations of these formulae, which fall into two basic categories: theoretical and physiological.

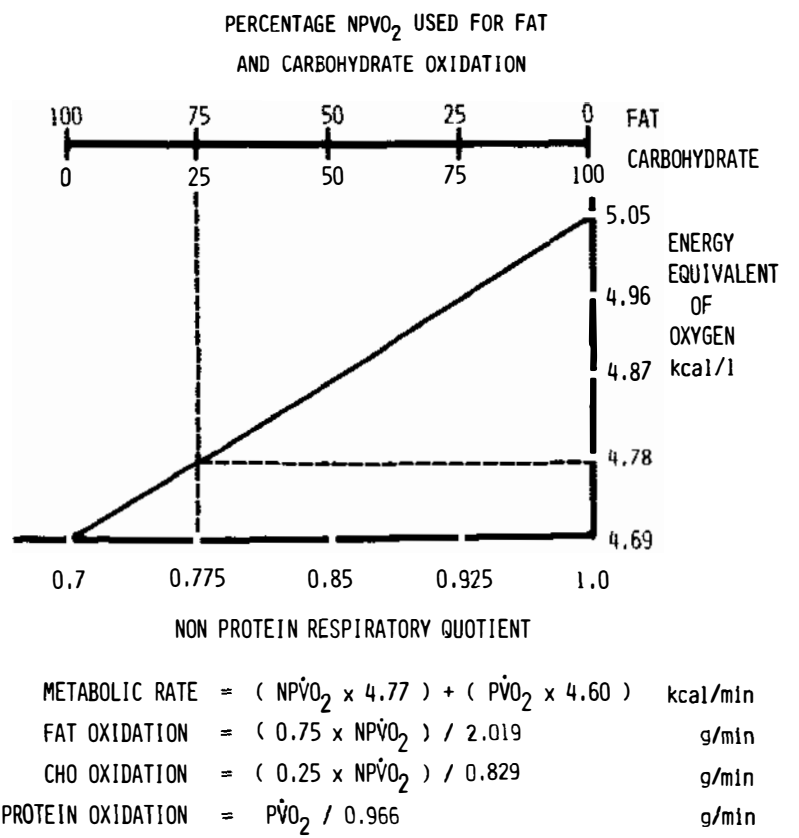


Figure 1 Relationships between the nonprotein respiratory quotient (NPRQ) and (*a*) the proportion of the $N\dot{P}V\dot{O}_2$ used for fat and carbohydrate oxidation and (*b*) the energy equivalent of a liter of oxygen. Also shown is an example of how metabolic rate and substrate utilization are calculated from a NPRQ of 0.775.

Theoretical Limitations

From Table 2 and Equations 10 to 12 it can be seen that we have taken a single value for the volumes of oxygen consumed and carbon dioxide produced during the complete oxidation of one gram of each of the substrates. However, Table 3 shows that these values depend very much upon the type of substrate being oxidized. The volume of oxygen consumed per gram of protein will vary depending upon whether the protein is principally of meat, milk, or cereal origin. With carbohydrates, oxygen consumption depends on whether the sugar is a mono-, di-, or polysaccharide. The carbohydrate energy source of the body in the postabsorptive state is glycogen, which will

consume 829 ml O_2 per gram of glycogen oxidized. If, however, one is studying a subject or patient who is receiving glucose, carbohydrate oxidation expressed as glucose will be underestimated by 11% if the starch (glycogen) value is used in the calculations.

A further complication occurs when one considers an acute study in which a subject is studied in the postabsorptive state and again after ingestion or infusion of glucose because the exogenous glucose load will not necessarily be oxidized immediately (2), i.e. there is a period of time during which glycogenolysis is decreasing and exogenous glucose oxidation is increasing. In such a situation the error in calculating glucose oxidation increases from 0 to 11% as more and more exogenous glucose is oxidized by the organism. We therefore recommend using glucose equivalents for assessing carbohydrate intake and oxidation, knowing that 1 g starch (or glycogen) is equivalent to 1.12 g glucose and 1 g disaccharide is equivalent to 1.05 g glucose. Unlike protein and carbohydrates, the amount of oxygen consumed per gram of fat oxidized varies little depending on whether it is of animal or vegetable origin.

Physiological Considerations

When performing respiratory exchange measurements, we make a further assumption that the body pools that might influence our measurements, e.g.

Table 3 Oxygen consumption for nutrient oxidation

	Oxygen consumed per gram of nutrient oxidized (liters)	Possible error compared with Lusk's values[a] (%)
Protein		
Beef muscle	1.008	4
Casein	1.008	4
Zein	1.052	9
Protein[a]	0.966	—
Carbohydrates		
Starch[a]	0.829	
Sucrose	0.786	6
Lactose	0.786	6
Glucose	0.746	11
Fat		
Corn oil	2.015	0.2
Pig fat	2.037	0.9
Fat[a]	2.019	—

[a] Values taken from Lusk, G. 1928. *The Elements of the Science of Nutrition*, pp. 64–67. Philadelphia: Saunders.

the urea pool and the bicarbonate pool, remain stable or in equilibrium. This is very important when calculating metabolic changes with respect to time. For example, if there is a decrease in the urea pool during an experiment, this implies that either urea excretion has increased or that its rate of appearance into the pool has decreased. In this situation, urinary nitrogen analysis will overestimate protein oxidation during the experiment. The converse is true with an increase in the urea pool. A possible correction for a decrease in the urea pool is presented in Table 4.

Figure 2 illustrates the influence of hyperventilation on the measured oxygen consumption, carbon dioxide production, and the respiratory quotient. During hyperventilation carbon dioxide is eliminated in excess of that produced by oxidative metabolism and there is a decrease in the bicarbonate pool. Carbon dioxide elimination increases rapidly and exceeds concomitant oxygen consumption; this results in a respiratory quotient greater than 1.00. The period of hyperventilation is followed by a compensatory period of hypoventilation during which metabolically produced carbon dioxide is stored to re-equilibrate the bicarbonate pool. Provided that the respiratory exchange measurements encompass these transient changes, the mean values for carbohydrate and fat oxidation will be correct. However, considerable errors are incurred if the measurements began, finished, or occurred during these transient periods. Since, $\dot{V}O_2$ changed very little during hyperventilation, the calculated energy expenditure is not greatly affected.

Not all respiratory quotients outside the usual range 0.7 to 1.00 should be attributed to ventilation changes or measurement errors. There are certain specific situations in which metabolic respiratory quotients (RQs) less than

Table 4 Example of calculation of protein oxidation rate from urinary nitrogen excretion in the presence of a decrease in the urea pool during a 4-h experiment

Urinary N	= 0.5 g/h		
Blood urea start	= 6.3 mmol/liter	Blood urea end	= 5.6 mmol/liter
Δ Blood urea	= −0.7 mmol/liter	Mol weight urea	= 60
Fraction of N in urea	= 0.47	Time between samples	= 4 h
Urea pool	= 0.60 body weight		
Body weight	= 70 kg		

$$\text{Correction} = \frac{\overbrace{-0.7 \times 60 \times 0.47}^{\Delta \text{ urea N g}} \times \overbrace{0.60 \times 70}^{\text{urea pool}}}{4 \times 1000} = 0.21 \text{ g/h}$$

Calculated protein oxidation rate = (0.5 − 0.21) × 6.25 = 1.81

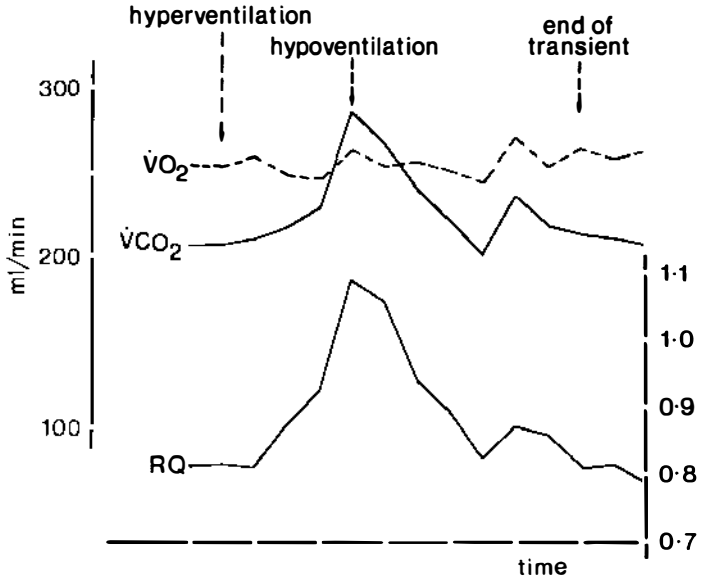


Figure 2 Influence of a period of hyperventilation and hypoventilation on the measured oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and the respiratory quotient (RQ). The arrows represent the beginning of hyperventilation and hypoventilation respectively, and the time intervals represent 5-minute periods.

0.7 or greater than 1.00 can be encountered. It is necessary to know under what conditions these extraneous respiratory quotients might occur and whether the basic formulae are valid or not.

Metabolic RQs of 0.7 or less are most likely to be encountered in starvation victims, individuals receiving high-fat, low-carbohydrate hypocaloric diets, alcoholics, and diabetics in the postabsorptive state. In some cases, an RQ less than 0.7 may be due to the oxidation of the fourth energetic substrate, alcohol, which has an RQ of 0.67, but it is more likely to be due to an increase in substrate flux through metabolic pathways that have low RQs, e.g. gluconeogenesis and ketogenesis.

The synthesis of glucose from amino acids has an RQ of approximately 0.4 but the influence of this low RQ only manifests itself on the overall RQ if the neoformed glucose is stored. This is an unlikely circumstance since gluconeogenesis becomes quantitatively important in fasting or starving conditions, where the glucose would be utilized almost immediately. If the glucose molecule that has been synthesized or another molecule of glucose in another tissue is subsequently oxidized to carbon dioxide and water, then the overall RQ will be that of complete protein oxidation: i.e. the overall RQ is independent of intermediate metabolic processes and of the different tissues in which they are occurring:

$$\text{Protein} \rightarrow \text{amino acids} \rightarrow \text{glucose} \rightarrow CO_2 + H_2O \qquad RQ = 0.80$$

It is difficult to refer to a respiratory quotient for ketogenesis, since the stoichiometric reaction indicates that oxygen is consumed but no carbon dioxide is produced. If the ketone bodies that are formed are either retained or excreted in the urine or via respiration, it is possible to observe RQs less than 0.7. Again if they are subsequently, completely oxidized, then the RQ for the whole reaction is the same as that for the complete oxidation of the fatty acid precursor. In this situation of ketoacidosis, ketone bodies that are excreted are difficult to quantitate and it is therefore difficult to correct the measured oxygen consumption. The measurements are also further perturbed by changes in the bicarbonate pool that occur in order to compensate for the metabolic acidosis.

Metabolic respiratory quotients greater than unity are associated with the net synthesis of fat from carbohydrate and have been observed in rodents prior to hibernation (27) and in fattening animals fed high-carbohydrate diets (28, 41).

From the stoichiometric examples given below it can be seen that the equations used for calculating substrate utilization and energy expenditure remain valid even in the presence of net lipid synthesis; however, it should be noted that the ATP balance or yield is different in each situation.

In the following examples, the numerical coefficients used are those for glucose and palmitate oxidation, i.e.

$$\text{Glucose oxidation (g/min)} = \text{NP}\dot{V}O_2 \times \frac{(\text{NPRQ} - 0.696)}{0.304 \times 0.746} \qquad 17.$$

$$\text{Palmitate oxidation (g/min)} = \text{NP}\dot{V}O_2 \times \frac{(1 - \text{NPRQ})}{0.304 \times 2.012} \qquad 18.$$

$$\text{Energy equivalent per liter } O_2 = 4.657 + \frac{(\text{NPRQ} - 0.696) \times 0.350}{0.304}, \qquad 19.$$

where 0.304 is the difference between the RQ for glucose oxidation and that of palmitate, i.e. 1.000 − 0.696; and 0.350 is the difference between the energy equivalent (kcal) of a liter of oxygen when glucose and palmitate are oxidized, i.e. 5.007 − 4.657.

Stoichiometry

Glucose oxidation alone

$$4.5C_6H_{12}O_6 + 27O_2 \rightarrow 27CO_2 + 27H_2O + 3029 \text{ kcal} \quad 20.$$

$$\text{NPRQ} = 27/27 = 1,$$

$$\text{Energy equivalent per liter} = \frac{3029}{(27 \times 22.39)} = 5.01 \text{ kcal/liter } O_2$$

$$\text{ATP balance} = 4.5 \times (38 - 2) = +162 \text{ ATP}$$

Palmitate oxidation alone

$$C_{16}H_{32}O_2 + 23O_2 \rightarrow 16CO_2 + 16H_2O + 2398 \text{ kcal} \quad 21.$$

$$\text{NPRQ} = 16/23 = 0.696$$

$$\text{Energy equivalent per liter } O_2 = \frac{2398}{(23 \times 22.39)} = 4.657 \text{ kcal/liter } O_2$$

$$\text{ATP balance} = 131 - 2 = +129 \text{ ATP}$$

Palmitate synthesis from glucose alone The following equation is a theoretical example since palmitate synthesis never occurs alone in the body; it has to be coupled with substrate oxidation:

$$4.5C_6H_{12}O_6 + 4O_2 \rightarrow C_{16}H_{32}O_2 + 11CO_2 + 11H_2O + 631 \text{ kcal} \quad 22.$$

$$\text{NPRQ} = 11/4 = 2.75$$

$$\text{Energy equivalent per liter } O_2 = \frac{631}{(4 \times 22.39} = 7.05 \text{ kcal/liter } O_2$$

$$\text{ATP balance} = 40 - 34 = +6 \text{ ATP}$$

Simultaneous glucose and palmitate oxidation If Equations 20 and 21 are combined, Equation 23 is obtained representing simultaneous glucose and palmitate oxidation:

$$9C_6H_{12}O_6 + 54O_2 \rightarrow 54CO_2 + 54H_2O + 6058 \text{ kcal} \quad 20.$$
$$+ \quad C_{16}H_{32}O_2 + 23O_2 \rightarrow 16CO_2 + 16H_2O + 2398 \text{ kcal} \quad 21.$$
$$9C_6H_{12}O_6 + C_{16}H_{32}O_2 + 77O_2 \rightarrow 70CO_2 + 70H_2O + 8456 \text{ kcal} \quad 23.$$

NPRQ = 70/77 = 0.909 ATP balance = 473 − 20 = +453 ATP
Glucose oxidation = 9 × 180 = 1620 g
Palmitate oxidation = 1 × 256 = 256 g

$$\text{Energy equivalent per liter } O_2 \frac{8456}{77 \times 22.39} = 4.905 \text{ kcal/liter } O_2$$

We can now use Equations 17, 18, and 19 to calculate glucose and palmitate oxidation, and the energy equivalent of a liter of oxygen:

From Equation 17 glucose oxidation is 1619 g,
Equation 18 palmitate oxidation is 256.6 g,
Equation 19 energy equivalent per liter O_2 is 4.902 kcal/liter O_2.

Simultaneous glucose and palmitate oxidation with concomitant palmitate synthesis where palmitate oxidation exceeds palmitate synthesis By combining Equations 20–22 it is again possible to obtain Equation 23, a situation in which glucose and palmitate oxidation are occurring at the same time as palmitate synthesis. In this example palmitate oxidation is greater than palmitate synthesis:

$$4.5C_6H_{12}O_6 + 27O_2 \rightarrow 27CO_2 + 27H_2O + 3029 \text{ kcal} \quad 20.$$
$$4.5C_6H_{12}O_6 + 4O_2 \rightarrow C_{16}H_{32}O_2 + 11CO_2 + 11H_2O + 631 \text{ kcal} \quad 22.$$
$$2C_{16}H_{32}O_2 + 46O_2 \rightarrow 32CO_2 + 32H_2O + 4796 \text{ kcal} \quad 21.$$
$$9C_6H_{12}O_6 + C_{16}H_{32}O_2 + 77O_2 \rightarrow 70CO_2 + 70H_2O + 8456 \text{ kcal} \quad 23.$$

NPRQ = 70/77 = 0.909 ATP balance = +473 − 47 = 426 ATP

Since oxygen consumption, carbon dioxide production, and heat production are the same with or without palmitate synthesis in the presence of glucose and palmitate oxidation, the energy equivalent of a liter of oxygen is the same as in the previous example, i.e. there are two different ways to obtain Equation 23.

Simultaneous glucose and palmitate oxidation with concomitant synthesis of palmitate, where palmitate synthesis exceeds palmitate oxidation The following example illustrates a situation of net palmitate synthesis:

$$4.5C_6H_{12}O_6 + 27O_2 \rightarrow 27CO_2 + 27H_2O + 3029 \text{ kcal} \quad 20.$$

$$9C_6H_{12}O_6 + 8O_2 \rightarrow 2C_{16}H_{32}O_2 + 22CO_2 + 22H_2O + 1262 \text{ kcal} \quad 22.$$

$$C_{16}H_{32}O_2 + 23O_2 \rightarrow 16CO_2 + 16H_2O + 2398 \text{ kcal} \quad 21.$$

$$13.5C_6H_{12}O_6 + 58O_2 \rightarrow C_{16}H_{32}O_2 + 65CO_2 + 65H_2O + 6689 \text{ kcal} \quad 24.$$

NPRQ = 65/58 = 1.12 ATP balance = 382 − 79 = +303 ATP

$$\text{Energy equivalent per liter } O_2 = \frac{6689}{58 \times 22.39} = 5.15 \text{ kcal/liter } O_2$$

Glucose utilization = 13.5 × 180 g and is the amount of glucose that is oxidized and converted to palmitate.

In this situation, indirect calorimetry measures glucose disappearance or disposal rather than glucose oxidation. From Equation 19 the energy equivalent per liter O_2 = 5.15 kcal/liter O_2. If Equations 17 and 18 are now used, we obtain

$$\text{Glucose disappearance} = NPVO_2 \times \frac{(1.12 - 0.696)}{0.304 \times 0.746}$$

$$= NPVO_2 \times 1.8696$$
$$= 1298.6 \times 1.8696$$
$$= 2428 \text{ g}$$

$$\text{Palmitate oxidation} = NPVO_2 \times \frac{(1 - 1.12)}{0.304 \times 2.012}$$

$$= NPVO_2 \times (-0.1962)$$
$$= 1298.6 \times (-0.1962)$$
$$= -255 \text{ g}$$

The negative value indicates an increase in the fat content of the system and is numerically equal to the 256 g of palmitate synthesized.

Since 810 g of glucose are necessary to synthesize 256 g of palmitate, glucose oxidation = 2428 − 810 = 1618 g. This value accounts for the glucose that was directly oxidized and that which was used for palmitate synthesis and was subsequently oxidized.

Metabolic RQs greater than 1.00 have been measured in the marmot, with an RQ of 1.39 (27), and in pigs, with RQs of 1.1 to 1.5 (28, 41), but the importance of net lipogenesis in human nutrition is less evident. In two experiments, Passmore et al (25, 26) demonstrated that very little net lipogenesis occurred in lean young men and obese women who ingested large quantities of carbohydrate. Similarly we did not observe net lipogenesis in lean healthy young men who consumed the equivalent of 500 g of carbohydrate (1). In a subsequent experiment (3) the subjects ingested 500 g of

dextrin maltose after having followed diets either high in fat, mixed, or high in carbohydrate during the days preceding the test. Net lipogenesis was more pronounced in those subjects whose antecedent diet was rich in carbohydrate. It should be noted that net lipogenesis amounted to 9 ± 1 g, which was surpassed by subsequent fat oxidation during the test. However, we have observed respiratory quotients continuously greater than 1.00 in three individuals during the last 5 days of a 7-day carbohydrate overfeeding experiment (34). At the end of the overfeeding period, net lipid synthesis could account for approximately 150 g of the daily positive fat balance, which was +218 g/day (unpublished results).

It should be stressed that such artificial conditions are rarely encountered. Even the acute ingestion of 500 g carbohydrate is very unlikely; thus the contribution of de novo lipogenesis in increasing the body fat stores after a balanced meal appears to be quite small or nonexistent in man.

NONCALORIMETRIC METHODS WITH REFERENCE TO THE $^2H_2{}^{18}O$ TECHNIQUE

Over the years, various noncalorimetric methods have been developed to assess total energy expenditure in man (33). They have been based either on (*a*) physiological measurements (pulmonary ventilation or heart rate); (*b*) human observations (activity diary); (*c*) kinematic recordings (mechanical activity meters); or (*d*) isotopic dilution of doubly labeled water.

About two decades ago, a general enthusiasm was manifested for the use of the heart rate method. The problems inherent in the prediction of energy expenditure from heart rate are not related to the heart rate measurement per se: today new heart rate monitors (of small size and light weight) are available, and both their accuracy and precision are excellent. The basic problem stems from the conversion of heart rate into a rate of energy expenditure using individually determined regression lines. Unfortunately, the relationship between these two variables is not linear within the usual range of measurements; this is primarily due to the confounding effect of variations in stroke volume. In the range where resting and working heart rates overlap (typically between 80 and 110 beats/min) the prediction of energy expenditure from heart rate is uncertain and this is precisely where the heart rate of most people engaged in light activity is encountered. Also a variety of factors (such as meals, change in posture, and cigarette smoking) affect heart rate proportionally more than energy expenditure so that a transient shift in the regression line occurs, with a resultant error of prediction. In a comparative study performed with a respiration chamber, Dauncey & James (11) found that the average error (± SD) of the heart rate method was 3 ± 10% at a light level of activity and 3 ± 7% at moderate levels of activity.

Human observation (activity diary) and kinematic recordings (mechanical

activity meters) are useful methods to obtain an index of physical activity, but as such they cannot be considered as methods to measure energy expenditure.

More recently, the doubly labeled water method using $^2H_2{}^{18}O$ has been used in humans to measure total energy expenditure for prolonged periods of time. This method is the most interesting development in the area of human energy metabolism over the last years and is briefly described below.

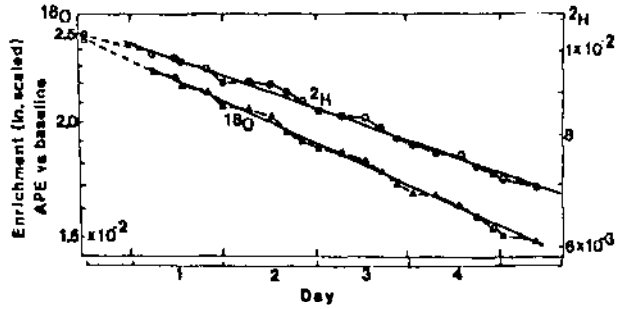
The method was first described by Lifson et al in 1955 (21) and has been the object of extensive studies in small animals. The principle is based on the fact that differences in the rates of ^2H_2O and $H_2{}^{18}O$ turnover in body water are used to measure CO_2 production rates, and hence the rate of energy expenditure. Briefly, when a subject is loaded with a single dose of $^2H_2{}^{18}O$, $^2H_2$ is assumed to leave the body water as ^2H_2O, and corresponds to the water output, while ^{18}O is assumed to leave the body as both $H_2{}^{18}O$ and $C^{18}O_2$, from which both water and CO_2 output can be assessed. Because of the action of carbonic anhydrase, there is a rapid exchange of oxygen between water and CO_2, leading to an isotopic equilibrium of ^{18}O in CO_2 and body water. The CO_2 production rate is calculated from the difference between these two fluxes. This involves several steps, described in Figure 3: (*a*) the measurement of $^2H_2$ and ^{18}O disappearance rates over 2–3 biological half-lives and the measurements of the ^2H_2O and $H_2{}^{18}O$ distribution spaces; (*b*) the transformation of these values into estimates of H_2O and CO_2 output, which requires a knowledge of the extent of isotope fractionation when H_2O and CO_2 leave the body; and (*c*) the conversion of CO_2 production rates into energy expenditure, which requires a knowledge of the respiratory quotient (RQ).

An estimate of CO_2 production can be obtained using the following equation (29):

$$rCO_2 = \frac{N}{2}(k_{18} - k_2), \qquad 25.$$

where rCO_2 is the CO_2 production rate (mol per day), N is the total body water pool size (mol), and k_2 and k_{18} are rate constants for ^2H and ^{18}O disappearances respectively (per day). The constant 2 represents a molar correction for O_2. Several underlying assumptions are required (23): (*a*) the body is in a steady state and acts as a single compartment with regard to the labeled water; (*b*) the labeled water is distributed only in the body water compartment; (*c*) the isotopes are lost from that water compartment only as water and CO_2; (*d*) the water and CO_2 leaving the body have the same isotopic enrichment as the body; (*e*) natural baseline enrichment values remain constant during the period of measurement; and (*f*) labeled or unlabeled H_2O or CO_2 in the environment does not enter the body via respiratory or skin surfaces.

An ideal situation for obtaining high accuracy with the $^2H_2{}^{18}O$ method



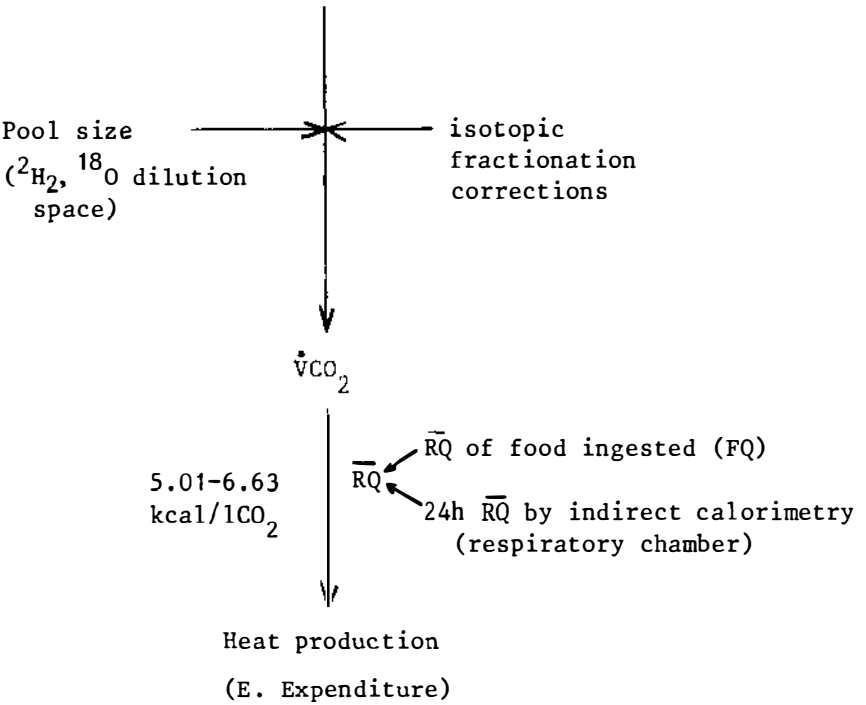


Figure 3 Principle of measurement used in the doubly labeled water ($^{2}H_2{}^{18}O$) technique. Following a loading dose of $^{2}H_2{}^{18}O$ urine (i.e. the difference between the two slopes), together with a knowledge of the total body water pool and a correction for isotopic effects, provides a measure of the CO_2 production rate ($\dot{V}CO_2$). Note that the greater the $\dot{V}CO_2$ when compared to the water turnover, the larger is the difference between the two slopes and hence the greater the accuracy. The calculated $\dot{V}CO_2$ is then converted into total heat production (or total energy expenditure) by using the energy equivalent of $\dot{V}CO_2$, which varies substantially (from 5.01 to 6.63 kcal/liter CO_2 at respiratory quotients (RQ) of 1.0 to 0.7 respectively. Since the RQ is not measured, it must be estimated from other parameters. The uncertainty due to the RQ could theoretically generate a maximum error of as much as 32%. However, if the dietary intake of the subject is quantitatively measured and if the subject is in energy equilibrium over the period of measurement (i.e. maintains body weight), the RQ of the diet can be used to predict the actual RQ of gas exchange and the error may be reduced to 5–10% (31).

occurs when the difference in the two rate constants is large, i.e. when the CO_2 turnover (energy expenditure) is high and the water turnover is low. This situation is not often encountered in life; for example, when compared with adults, babies have a high rate of energy expenditure per kilogram body weight but their water turnover rate is also elevated. Athletes who are in a training period show high rates of energy expenditure but the rate of water turnover is also increased.

In laboratory conditions, i.e. under controlled experimental conditions (physical activity, food, environment), recent studies have compared the energy expenditure obtained using the $^2H_2{}^{18}O$ method with that measured by indirect calorimetry in a respiration chamber (20, 31, 40) or by other independent methods (29, 30, 32). These investigations have demonstrated that the *mean* error involved in the use of the doubly labeled water method ranged between −4% (40) and +8% (30). In free-living conditions, additional assumptions are required since the RQ is unknown and the proportion of water fractionated by evaporation at epithelial surfaces, which varies according to environmental conditions, is also unknown. The RQ can be derived from the metabolizable RQ of the diet (sometimes referred to as the food quotient, FQ) since in individuals who maintain their body composition constant over a long period of time both the RQ and FQ must be equal. However, in order to obtain an FQ estimate, a quantitative knowledge of the food that has been eaten over the experimental period (over 2–3 weeks) is required. Furthermore, in individuals who are growing (i.e. babies) and retaining nutrients (i.e. pregnant

Table 5 Relative advantages and disadvantages in the use of the $^2H_2{}^{18}O$ method to assess total energy expenditure in humans

Advantages
1. Tracer is a nonradioactive isotope.
2. Long-term energy expenditure determination (2–3 weeks).
3. Simple and convenient technique.
4. Simultaneous assessment of body composition via total body water.
5. Can be used to study a large number of subjects simultaneously.
6. In lactating women, the method provides a simultaneous way of determining breast milk production and estimating the infant's energy expenditure.
Disadvantages
1. Expensive technique [cost of isotope (^{18}O) and cost of analysis].
2. Measures CO_2 production ($\dot{V}CO_2$) and not O_2 consumption ($\dot{V}O_2$), hence a five times greater potential error on the energy expenditure calculation by using the caloric equivalent of $\dot{V}CO_2$ than with the caloric equivalent of $\dot{V}O_2$.
3. Needs various correction factors and assumptions (e.g. isotope fractionation factors), which make the magnitude of the error in various situations uncertain.
4. Gives an overall mean value of energy expenditure for a period of 2–3 weeks; it is not possible to measure 24-hour energy expenditure and the day-to-day variability in 24-h energy expenditure.

women) or losing nutrients (i.e. obese under restrictive conditions) the method requires additional corrections since the FQ is no longer equal to the metabolic RQ. Therefore, in order to decrease the potential error involved when the RQ is unknown, the $^2H_2{}^{18}O$ method must be combined with other techniques such as a respiration chamber or dietary survey methods.

Additional difficulties involved in the use of the $^2H_2{}^{18}O$ method stem from the fact that various correction factors have to be applied in the model used to calculate energy expenditure (e.g. isotope fractionation factors); the magnitude of these corrections obviously influences the final calculation. For example in a recent study performed in our laboratory, Schoeller et al (31) clearly demonstrated that, depending upon the above corrections and the type of assumptions made in the model, the same set of data can generate average errors (±SD) ranging from $-4 \pm 10\%$ to $+11 \pm 7\%$. Therefore in future work in the field, it will be necessary to adjust fractionation factors and assumptions to specific environmental conditions and/or physiological status in order to eliminate the uncertainty of the method. The relative advantages and shortcomings of the $^2H_2{}^{18}O$ method are summarized in Table 5.

In conclusion, because of the effects of fractionation and compartmentation of isotopes, more research is needed to evaluate the $^2H_2{}^{18}O$ method further in free-living subjects under various conditions of climate, food intake, and physical activity.

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