

# Calculation of substrate oxidation rates in vivo from gaseous exchange

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FRAYN, K. N. *Calculation of substrate oxidation rates in vivo from gaseous exchange.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 55(2): 628-634, 1983.—This paper reviews the assumptions involved in calculating rates of carbohydrate and fat oxidation from measurements of O<sub>2</sub> consumption, CO<sub>2</sub> production, and urinary nitrogen excretion. It is shown that erroneous results are obtained in the presence of metabolic processes such as lipogenesis and gluconeogenesis. The apparent rates calculated under these conditions can, however, be interpreted as net rates of "utilization." Thus the apparent rate of carbohydrate oxidation is the sum of the rates of utilization for oxidation and for lipogenesis minus the rate at which carbohydrate is formed from amino acids. The apparent rate of fat oxidation is the difference between the rates of oxidation and synthesis from carbohydrate, so that the apparently negative rates encountered in patients infused with glucose do quantitatively represent net rates of synthesis. Other processes such as synthesis of ketone bodies or lactate at rates greater than their utilization can also disturb the calculations, but the magnitude of the effect can be estimated from appropriate measurements. Methods of correcting the observed gaseous exchange in these circumstances are given.

carbohydrate oxidation; fat oxidation; gluconeogenesis; ketogenesis; lactate formation; lipogenesis

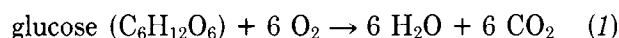
ALTHOUGH THE TECHNIQUE of indirect calorimetry has been used mainly for the measurement of metabolic rate, it has been recognized for nearly two centuries that measurements of O<sub>2</sub> consumption ( $\dot{V}O_2$ ) and CO<sub>2</sub> production ( $\dot{V}CO_2$ ) can also give information on the type and rate of fuel oxidation within the body (15).

The calculation of rates of glucose, fat, and protein oxidation, based on measurements of gaseous exchange together with urinary nitrogen excretion, has seen increasing use recently both in the study of acutely ill patients (13, 17) and in connection with parenteral nutrition (1, 23). However, these calculations are based on a number of assumptions that may not hold in such patients.

The purpose of this paper is to review the assumptions on which these calculations are based and to consider specifically two metabolic processes that may disturb such calculations, namely lipogenesis and gluconeogenesis. The influence of ketogenesis and of lactate formation will also be considered.

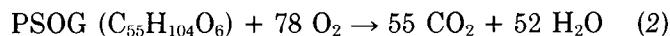
## CALCULATION OF SUBSTRATE OXIDATION UNDER NORMAL CIRCUMSTANCES

When glucose is oxidized according to the equation



6 mol of O<sub>2</sub> are consumed and 6 mol of CO<sub>2</sub> produced for each mole (180 g) oxidized (Table 1); the respiratory quotient (RQ =  $\dot{V}CO_2/\dot{V}O_2$ ) is thus 1.00. These values differ only slightly if carbohydrates other than simple hexoses are considered.

Oxidation of a typical fat (palmitoyl-stearoyl-oleoyl-glycerol, C<sub>55</sub>H<sub>104</sub>O<sub>6</sub>, chosen for reasons given in the APPENDIX; abbreviated throughout to PSOG) according to the equation



consumes 78 mol of O<sub>2</sub> and produces 55 mol of CO<sub>2</sub> for each mole (861 g) oxidized (Table 1); again slightly different values apply to different fats.

For protein, empirical values must be used; those in Table 1 are taken from Magnus-Levy (16). The amount of protein oxidized may be estimated from the urinary nitrogen excretion, since most urinary nitrogen (>80%) is in the form of urea (24, 25), 1 g urinary nitrogen arising from approximately 6.25 g protein (although the value used does not influence the following considerations).

In a subject oxidizing *c* grams of carbohydrate (as glucose) and *f* grams of fat per minute and excreting *n* grams of urinary nitrogen per minute, the total O<sub>2</sub> consumption, using values from Table 1, is thus given by

$$\dot{V}O_2 \text{ (l/min)} = 0.746 c + 2.03 f + 6.04 n \quad (3)$$

and the total CO<sub>2</sub> production by

$$\dot{V}CO_2 \text{ (l/min)} = 0.746 c + 1.43 f + 4.89 n \quad (4)$$

Equations 3 and 4 may be solved in terms of the unknown variables *c* and *f* (gaseous exchange and urinary nitrogen excretion being measurable) as follows

$$c = 4.55 \dot{V}CO_2 - 3.21 \dot{V}O_2 - 2.87 n \quad (5)$$

$$f = 1.67 \dot{V}O_2 - 1.67 \dot{V}CO_2 - 1.92 n \quad (6)$$

where  $\dot{V}O_2$  and  $\dot{V}CO_2$  represent O<sub>2</sub> consumption and CO<sub>2</sub> production, respectively, in liters per minute.

These formulas are very similar to those given by other

TABLE 1. Volumes of O<sub>2</sub> consumed and CO<sub>2</sub> produced in oxidation of various fuels

Fuel	O <sub>2</sub> , l/g fuel	CO <sub>2</sub> , l/g fuel
Glucose	0.746	0.746
Fat	2.03	1.43
Protein	0.966	0.782
	6.04*	4.89*

Values are calculated as described in the text for glucose and fat, those for protein are from the work of Magnus-Levy (16). One gram of urinary nitrogen is assumed to arise from 6.25 g protein; 1 mol of gas occupies 22.4 liters. \* Volumes expressed per g urinary nitrogen.

authors (3, 4); the slight differences are due to the choice of particular values in Table 1, but the values shown will be used throughout this paper for consistency.

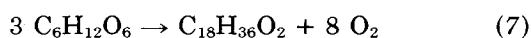
#### INFLUENCE OF OTHER METABOLIC PROCESSES

A general principle in these considerations is that intermediate metabolic processes do not influence overall conclusions. For instance, if glucose is converted to lactate in one tissue and that lactate is oxidized elsewhere, the overall RQ (and the effect on the calculations described above) is exactly as if the glucose had been oxidized directly. Thus, when considering gluconeogenesis, for instance, its influence is only relevant if glucose is formed and then not oxidized (i.e., either stored as glucose or as glycogen or excreted). It is under these circumstances, i.e., when there is accumulation within, or excretion from, the body of an intermediate or end product other than CO<sub>2</sub> or H<sub>2</sub>O, that the assumptions involved in deriving Eqs. 5 and 6 do not hold. The usefulness of Eqs. 5 and 6 under some such conditions will be considered in the following sections. The quantitative effects of lipogenesis and gluconeogenesis will be illustrated by numerical examples. More generalized treatments are given in the APPENDIX and show that the conclusions reached are generally applicable.

#### Lipogenesis

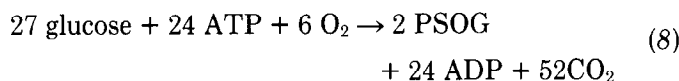
It is well known that conversion of glucose into fat elevates the RQ, but its quantitative effect on calculations of substrate oxidation has apparently not been investigated. It can be seen from the values in Table 1 that if the nonprotein RQ (i.e., the ratio of  $\dot{V}CO_2$  to  $\dot{V}O_2$  excluding their components from protein oxidation) is greater than 1.0, then net fat oxidation cannot be occurring; such RQ are often observed in control subjects and in some surgical patients receiving glucose infusions (1, 23). In this case rates of fat oxidation calculated by the standard formula (Eq. 6) are negative. Because Eqs. 5 and 6 are derived by considering the complete oxidation of substances to CO<sub>2</sub> and H<sub>2</sub>O and since fat synthesis, as shown below, is by no means a reversal of this process, it is not obvious how these negative values should be interpreted in a quantitative sense.

The conversion of glucose to fat (e.g., stearic acid, C<sub>18</sub>H<sub>36</sub>O<sub>2</sub>) is sometimes written in a balanced equation (10) as

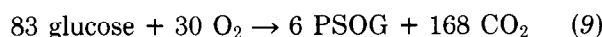


However, evolution of O<sub>2</sub> obviously does not occur in vivo; in fact, as shown below, the overall process of lipogenesis requires oxidizing equivalents.

Glucose is in fact converted to fat via pyruvate and acetyl-CoA, the glycerol 3-phosphate required for esterification arising through dihydroxyacetone phosphate. The equations for the individual steps in mammalian tissues are listed in the supplementary publication<sup>1</sup>; when combined, the overall process can be represented as



(Inorganic phosphate and H<sub>2</sub>O will be omitted in all equations.) In contrast to Eq. 7, this shows that there is a net consumption of oxidizing equivalents. The CO<sub>2</sub> is evolved "obligatorily" in the formation of acetyl-CoA from pyruvate (2 mol of CO<sub>2</sub> for each mole of glucose). The RQ for the process of lipogenesis by Eq. 8 is 52/6 = 8.7, in agreement with Elwyn and Kinney (5). However, this process obviously cannot function in isolation, since it requires synthesis of ATP, for instance by oxidation of glucose. As shown in the supplementary publication, inclusion of this leads to



The RQ for this overall process is thus 168/30 = 5.6. Equation 8 shows that for each 2 mol (1,723 g) of fat produced, 27 mol (4,865 g) of glucose and 6 mol (134 liters) of O<sub>2</sub> are used and 52 mol (1,165 liters) of CO<sub>2</sub> produced, leading to the values in Table 2.

The effect of lipogenesis on calculations of substrate oxidation using Eqs. 5 and 6 can now be examined by a numerical example. Consider a subject in whom the following metabolic processes are occurring at the rates shown: glucose oxidation, 0.12 g/min; fat oxidation, 0.02 g/min; glucose conversion to fat, 0.12 g glucose/min, thus forming 0.12 × 0.35 = 0.042 g fat/min; protein oxidation leading to urinary nitrogen excretion of 14.4 g/day or 0.01 g/min.

Combining the O<sub>2</sub> and CO<sub>2</sub> equivalents of these processes from Tables 1 and 2

$$\begin{aligned} \text{total } \dot{V}O_2 &= 0.12 \times 0.746 + 0.02 \times 2.03 \\ &\quad + 0.12 \times 0.028 + 0.01 \times 6.04 \\ &= 0.194 \text{ l/min} \end{aligned}$$

$$\begin{aligned} \text{total } \dot{V}CO_2 &= 0.12 \times 0.746 + 0.02 \times 1.43 \\ &\quad + 0.12 \times 0.239 + 0.01 \times 4.89 \\ &= 0.196 \text{ l/min} \end{aligned}$$

giving an RQ of 1.01. Substituting these values into Eqs. 5 and 6 gives  $c = 0.24$  g/min and  $f = -0.023$  g/min.

These results suggest that the rate of carbohydrate oxidation calculated under these circumstances is close

<sup>1</sup> For supplemental material order NAPS document 04098 from Microfiche Publications, PO Box 3513, Grand Central Station, New York, NY 10017.

TABLE 2. Volumes of  $O_2$  consumed and  $CO_2$  produced in lipogenesis and gluconeogenesis

	Unit	$O_2$ , l used/unit	$CO_2$ , l produced/unit	Other Products
Lipogenesis	1 g glucose	0.0276	0.239	0.354 g fat
Gluconeogenesis	1 g urinary N	0	-0.800	6.43 g glucose

Lipogenesis refers to synthesis of palmitoyl-stearoyl-oleoyl-glycerol from glucose, gluconeogenesis to synthesis of glucose and urea from alanine. Values are derived from *Eqs. 8 and 12* in the text.  $CO_2$  is used in gluconeogenesis.

to the sum of the rates of true oxidation and conversion to fat (i.e.,  $0.12 + 0.12$  g/min) and that the calculated rate of fat oxidation is the net rate of fat disappearance (oxidation-synthesis; i.e.,  $0.020-0.042$  g/min). It is shown in the APPENDIX that this is generally true; i.e., representing the rate of carbohydrate oxidation by  $c_o$ , the rate of conversion of carbohydrate to fat by  $c_f$ , the true rate of fat oxidation by  $f_o$ , and the rate of fat synthesis by  $f_s$ , then the apparent rate of carbohydrate oxidation,  $c$ , calculated from *Eq. 5* is given by

$$c = c_o + c_f \quad (10)$$

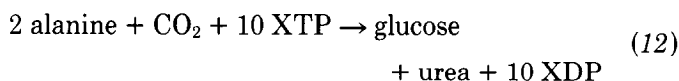
and the apparent rate of fat oxidation,  $f$ , calculated from *Eq. 6* is given by

$$f = f_o - f_s \quad (11)$$

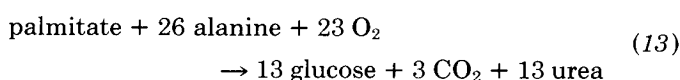
Apparently negative rates of fat oxidation, as seen for instance in patients on parenteral nutrition, thus quantitatively represent net rates of fat synthesis.

#### Gluconeogenesis

There are several substrates for gluconeogenesis, but in this context lactate and pyruvate arising from glucose breakdown are not relevant (since the Cori cycle, although consuming energy, does not involve gaseous exchange). Alanine is the major noncarbohydrate gluconeogenic precursor (6) and is converted to glucose via pyruvate; the amino group is transferred to form glutamate and eventually ammonia, which is used to form urea in the urea cycle. The individual steps are listed in the supplementary publication; when combined, the following overall equation is arrived at



where X represents adenosine or guanosine. The RQ for the process of gluconeogenesis itself is therefore  $-\infty$ , since  $CO_2$  is used and no  $O_2$  is consumed. The use of  $CO_2$  is for synthesis of carbamoyl phosphate on the pathway to urea production. As in the case of lipogenesis, this is an energy-requiring process, and assuming that the energy required comes from the oxidation of fat (e.g., palmitic acid), then the overall process could be balanced as follows



with an RQ of  $3/23 = 0.13$ .

From *Eq. 12* it can be seen that excretion of 1 mol of urea (28 g N) is equivalent to synthesis of 1 mol of glucose and use of 1 mol of  $CO_2$ , leading to the values in Table 2.

To illustrate the effect of gluconeogenesis on calculations of substrate oxidation, consider a subject with the following rates of metabolic processes: glucose oxidation, 0.10 g/min; fat oxidation, 0.12 g/min; urinary nitrogen excretion, 28.8 g/day = 0.02 g/min, of which 0.01 g/min arises from protein oxidation and 0.01 g/min arises from gluconeogenesis, equivalent to 0.064 g glucose produced/min.

Combining the  $O_2$  and  $CO_2$  equivalents of these processes from Tables 1 and 2

$$\begin{aligned} \text{total } \dot{V}O_2 &= 0.10 \times 0.746 + 0.12 \times 2.01 \\ &\quad + 0.01 \times 6.04 \\ &= 0.376 \text{ l/min;} \end{aligned}$$

$$\begin{aligned} \text{total } \dot{V}CO_2 &= 0.10 \times 0.746 + 0.12 \times 1.42 \\ &\quad + 0.01 \times 4.89 - 0.01 \times 0.80 \\ &= 0.286 \text{ l/min,} \end{aligned}$$

giving an RQ of 0.76. Substituting these values into *Eqs. 5 and 6* gives  $c = 0.038$  g/min and  $f = 0.113$  g/min.

The calculated rate of carbohydrate oxidation is therefore close to the net rate of oxidation (oxidation-synthesis; i.e.,  $0.10-0.064$  g/min), and again it is shown in the APPENDIX that this is generally true. Representing the rate of carbohydrate synthesis as  $c_s$ , with other symbols as above, the apparent rate of carbohydrate oxidation,  $c$ , calculated under these circumstances is thus

$$c = c_o - c_s \quad (14)$$

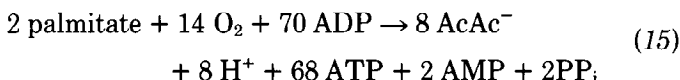
In the case of the rate of fat oxidation there is a small discrepancy (0.113 g/min calculated, 0.120 g/min actual). It has not been possible to put any physiological meaning on this error, although it is shown in the APPENDIX that, in units of mass, the rate of fat oxidation is underestimated by approximately one-tenth the rate at which glucose is being synthesized.

#### Other Metabolic Processes

Other metabolic processes commonly encountered in acutely ill patients that may involve excretion or storage of an intermediate compound include ketogenesis and lactate formation. If the intermediates are later removed by oxidation this will also involve gaseous exchange. These processes differ from those of lipogenesis and gluconeogenesis in that the compounds are not stored in the sense of being "laid down"; if they are synthesized faster than they are utilized (utilization in this context including excretion) then their concentration rises in some compartment of body water, and assuming a value for the distribution volume, the total amount stored or consumed within the body can thus be estimated from the change in plasma concentration. For this reason it is possible to calculate directly the amounts of  $O_2$  and  $CO_2$  involved in their synthesis or utilization and to correct

the observed gaseous exchange by these amounts so that calculations of fat and carbohydrate oxidation can still be made. There is, however, an addition problem with both ketone bodies and lactate in that they are produced along with hydrogen ions. The effect of these hydrogen ions on bicarbonate stores within the body is uncertain, as discussed below, and so accurate correction of  $\dot{V}CO_2$  is still difficult, although in most circumstances the effect of this will be very small.

**Ketone body formation and utilization.** Acetoacetate (AcAc) is produced by condensation of acetyl-CoA units liberated during  $\beta$ -oxidation of fatty acids. The individual steps are listed in the supplementary publication, which shows that the overall process of acetoacetate formation from a typical fatty acid, palmitate, can be represented by



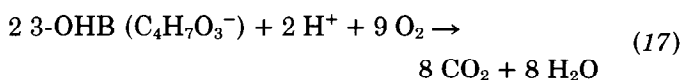
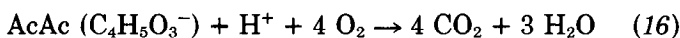
For each mole of AcAc produced and not oxidized, 14/8 or 1.75 mol of  $O_2$  are thus consumed.

Production of 3-hydroxybutyrate (3-OHB) involves a reduction step with oxidation of NADH, resulting effectively in a lessening of the  $O_2$  consumption by 0.5 mol (see supplementary publication); each mole of 3-OHB produced and not oxidized thus requires consumption of 1.25 mol  $O_2$ .

Acetone is formed by spontaneous decarboxylation of acetoacetate, 1 mol of  $CO_2$  being liberated for each mole of acetone formed.

The ketone bodies once formed may, if not oxidized, either accumulate in extracellular fluid or be excreted in either breath (acetone) or urine. Because both AcAc and 3-OHB are almost entirely dissociated at physiological pH an equivalent amount of hydrogen ion will be produced. This raises a difficulty in that it is not clear whether these hydrogen ions quantitatively displace an equivalent amount of  $CO_2$  from bicarbonate (discussed in Ref. 15), so that the correction to the gaseous exchange can only be placed between two extremes. Published values for the AcAc and 3-OHB distribution spaces are usually around 0.2 l/kg body wt (11), although a value of around 0.5 l/kg has recently been claimed (2). The distribution space for acetone is around 0.6–0.8 l/kg body wt (20). Once the total amounts stored and excreted have been calculated then the observed gaseous exchange can be corrected as shown in Table 3.

When the ketone body concentration is falling, i.e., ketone bodies previously stored are being utilized, then the effect will be to raise the RQ, the RQ for oxidation of AcAc being 1.00 and 3-OHB being 0.89 as shown in Eqs. 16 and 17



For each mole of AcAc oxidized, 4 mol of  $CO_2$  are thus produced and 4 mol of  $O_2$  consumed; in the case of 3-

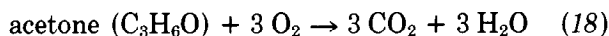
TABLE 3. Corrections to gaseous exchange for ketone body storage, excretion, and utilization

Compound	Correction per Mole Stored/ Excreted or Utilized		Additional Max Cor- rection for Each Mole Stored or Utilized
	$O_2$ , mol	$CO_2$ , mol	$CO_2$ , mol
<i>Storage/excretion</i>			
Acetoacetate	-1.75	0	-1
3-Hydroxybutyrate	-1.25	0	-1
Acetone	-1.75	-1	0
<i>Utilization</i>			
Acetoacetate	-4.0	-4.0	+1
3-Hydroxybutyrate	-4.5	-4.0	+1
Acetone	-3.0	-3.0	0

Storage is calculated from the increase in plasma concentration multiplied by the distribution volume (see text). To this must be added the amounts excreted in urine and (for acetone) in expired air. Additional correction to  $CO_2$  excretion allows for displacement of  $CO_2$  from bicarbonate by the associated hydrogen ion production. Utilization is calculated similarly from the fall in plasma concentration. Corrections are added to the observed  $\dot{V}O_2$  and  $\dot{V}CO_2$  (using 22.4 liters = 1 mol).

OHB 4 mol of  $CO_2$  are produced and 4.5 mol  $O_2$  consumed. Again there is an additional somewhat uncertain correction as hydrogen ions are consumed during the oxidation, and this could result in compensatory  $CO_2$  retention as bicarbonate.

Some plasma acetone may be removed by oxidation in humans (20). Although it is not clear whether or not this occurs after conversion to other compounds, the overall reaction must be

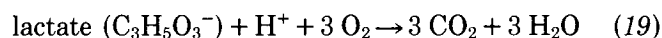


showing that 3 mol of  $O_2$  are consumed and 3 mol of  $CO_2$  produced for each mole of acetone disposed of in this way. Any correction for acetone utilization, however, must be somewhat speculative as it may also be removed by incorporation into other compounds and not necessarily oxidized (20).

**Lactate formation and utilization.** When lactate is produced and not oxidized there is no direct involvement of either  $O_2$  or  $CO_2$ , but, as in the case of ketone bodies, the associated hydrogen ion production may displace  $CO_2$  from bicarbonate stores. Again the stoichiometry of this is uncertain, and measurement of blood bicarbonate does not necessarily help (18), although Jones et al. (9) have recently shown that during heavy exercise the fall in blood bicarbonate concentration matches the rise in blood lactate. When exercise continues, however, there is additional  $CO_2$  evolution from an unidentified source (9), so that corrections made during exercise are uncertain. In other states it may be reasonable to assume that 1 mol of  $CO_2$  is evolved for each mole of lactate stored in a distribution volume of about 0.2 l/kg body wt (19). It is worth noting, however, that for all but very rapid changes in blood lactate concentrations this will be a very small correction. If the blood lactate concentration rises by 10 mmol/l in 10 h, for instance, in a 60-kg

subject, the maximum rate of displacement of CO<sub>2</sub> is about 4.5 ml/min.

The main pathways for lactate removal are oxidation and gluconeogenesis. If lactate is converted to glucose, which is then oxidized, then the effect on gaseous exchange will be the same as if the lactate were oxidized directly. After exercise, however, when there is resynthesis of muscle glycogen, lactate may be used without oxidation so that corrections for lactate utilization after exercise would be uncertain. Otherwise, however, lactate oxidation, represented by



consumes 3 mol of O<sub>2</sub> and produces 3 mol of CO<sub>2</sub> per mole of lactate, and again up to 1 mol of CO<sub>2</sub> may be retained as bicarbonate because of the consumption of hydrogen ions.

## DISCUSSION

With the increasing study of patients in states of disturbed metabolism, and particularly those patients receiving infusions of large amounts of nutrients, it is not obvious that the standard methods of calculating rates of carbohydrate and fat oxidation will still be valid. Whereas several authors have described the effects of processes such as gluconeogenesis and lipogenesis in qualitative or semiquantitative terms (e.g., 12, 21), there have been few attempts at quantitative treatment.

The approach used in this paper has been based on listing the individual metabolic steps involved in these processes. Approaches used in the past have usually been based in principle on the simple stoichiometry of the overall reactions (e.g., in *Eq. 7*). It is apparent, however, that there are cases in which this can be misleading. For instance, the loss of CO<sub>2</sub> in the pyruvate dehydrogenase step in lipogenesis, the CO<sub>2</sub> fixation involved in urea synthesis, and the difference in redox state between AcAc and 3-OHB might be missed in simpler approaches (e.g., *Ref. 17*).

The results given above (and in more general form in the APPENDIX) show that indeed the values of carbohydrate and fat oxidation calculated from the standard formulas no longer give "true" oxidation rates in the presence of other metabolic processes such as gluconeogenesis or lipogenesis. The rates calculated in fact represent, in general, net rates of "utilization" according to the following equations.

Calculated rate of carbohydrate oxidation

$$c = c_o + c_f - c_s$$

where, as above,  $c_o$  represents the true rate of oxidation,  $c_f$  represents the rate of conversion to fat, and  $c_s$  represents the rate of synthesis from amino acids.

Calculated rate of fat oxidation

$$f = f_o - f_s$$

where  $f_o$  represents the true rate of fat oxidation and  $f_s$  represents the rate of synthesis from carbohydrate, with the proviso that, in the presence of gluconeogenesis, there is a small error in the calculated rate of fat oxidation.

These relationships mean that it is not strictly possi-

ble, from indirect calorimetry alone, to calculate true rates of oxidation of either carbohydrate or fat. In the normal individual it may be reasonable to assume that rates of gluconeogenesis and lipogenesis are low, in which case the standard formulas give almost the true oxidation rates, but in other circumstances the calculated rates must be interpreted with the above in mind. Without other information, e.g., from isotopic studies, it is not possible to put any more detailed interpretation on the calculated rates.

These considerations may well be relevant in many of the patients studied nowadays by indirect calorimetry. For instance, in subjects infused with glucose the RQ may rise above 1.0 (1, 23), and in such subjects calculated rates of fat oxidation are negative. In such patients the calculated rate of glucose oxidation will overestimate the true glucose oxidation rate by the rate at which glucose is converted to fat.

It is more difficult to envisage situations in which gluconeogenesis might occur without oxidation of the glucose formed. One example is the patient in diabetic ketoacidosis as studied by Owen et al. (17) in whom there is a high rate of urinary glucose excretion. It is possible that gluconeogenesis might also occur in other types of acutely ill patient. In patients suffering from sepsis after injury or surgery, there is evidence from isotopic and other studies that glucose production continues despite hyperglycemia and despite glucose infusion (7, 14). If the glucose produced is stored as glycogen, or excreted, then the RQ will be lowered and the calculated rate of carbohydrate oxidation will be falsely low. A rough calculation of the likely magnitude of this effect in patients with abdominal sepsis studied by the Trauma Unit (23) shows that up to about one-fourth of the apparent impairment of carbohydrate oxidation in the most severely ill might be due to the operation of simultaneous gluconeogenesis. We have also observed low RQ in patients in accidental hypothermia (22), and again there is independent evidence that in such patients gluconeogenesis may continue despite marked hyperglycemia (26).

The importance of the inability to calculate absolute oxidation rates will depend on the purpose of the study. The results in this paper may be useful for the research worker attempting to understand the mechanisms underlying metabolic changes in acutely ill patients or other subjects. In clinical practice indirect calorimetry may be used to indicate the type of fuel preferentially used by a patient, and in this case the net rates of oxidation calculated from gaseous exchange may be more useful than true rates as determined, for instance, in isotopic studies. The other major clinical use of indirect calorimetry is in the determination of energy expenditure as a guide to nutritional requirements. This paper has not been concerned with assessment of energy expenditure, since this is covered by other authors (e.g., 3, 5, 25). In this case also, net, rather than true, rates of oxidation are required, since the free energy liberated, for instance, when glucose is converted to fat and then oxidized must be the same as for its direct oxidation. Caloric factors for net lipogenesis have been calculated by Elwyn and Kinney (5).

In conclusion, it is hoped that the information summarized in this paper shows that the calculation of rates

of substrate oxidation from gaseous exchange in all but the normal individual is liable to error; but that the errors can in fact be interpreted in a physiologically meaningful way.

## APPENDIX

### Choice of a Representative Triacylglycerol

Hirsch (8) gives the fatty acid composition of normal human adipose tissue. Taking a weighted mean gives the average fatty acid as being  $C_{17.4}H_{33.1}O_2$ ; the triacylglycerol formed from three such molecules would have the approximate formula  $C_{55}H_{104}O_6$ . Palmitoyl-stearoyl-oleoyl-glycerol (PSOG) has this formula and so has been taken as a representative molecule. The same choice was arrived at by Elwyn and Kinney (5).

### Effects of Lipogenesis and Gluconeogenesis on Calculations of Substrate Oxidation

The following symbols are used: molecular weights of glucose  $G$ , fat (PSOG)  $F$ , and nitrogen in urea (= 28)  $U$ ; gram molecular volume (l/mol),  $V$ ; protein oxidation, let 1 g urinary nitrogen be equivalent to  $O_2$  consumption (liters) of  $P_o$  and  $CO_2$  production (liters) of  $P_c$ .

Consider oxidation of a mixture of  $c$  g/min carbohydrate (as glucose),  $f$  g/min fat (as PSOG), and protein equivalent to  $n$  g/min urinary nitrogen. From *Eqs. 1* and *2* in the main text, this uses  $(6Vc/G + 78Vf/F + P_o n)$  l/min  $O_2$  and produces  $(6Vc/G + 55Vf/2F + P_c n)$  l/min  $CO_2$ . Eliminating the terms in  $f$  gives

$$c = G[78\dot{V}CO_2 - 55\dot{V}O_2 - n(78P_c - 55P_o)]/6V(78 - 55) \quad (A1)$$

Eliminating the terms in  $c$  gives

$$f = F[\dot{V}O_2 - \dot{V}CO_2 - n(P_o - P_c)]/V(78 - 55) \quad (A2)$$

*Equations A1* and *A2* are equivalent to *Eqs. 5* and *6* in the main text, i.e., the formulas for calculation of substrate oxidation.

*Lipogenesis.* Based on *Eq. 8* in the main text, conversion of 1 g glucose to fat uses  $V/2G$  liters  $O_2$  and produces  $2F/2G$  g fat and  $V/2G$  liters  $CO_2$ . Consider a subject with the following rates of metabolic processes: glucose oxidation,  $c_o$  g/min; fat oxidation,  $f_o$  g/min; glucose conversion to fat,  $c_f$  g/min, forming  $2F c_f/27G$  g fat/min; and urinary nitrogen excretion,  $n$  g/min. Then

$$\dot{V}O_2 = 6Vc_o/G + 78Vf_o/F + P_o n + 6Vc_f/27G$$

$$\dot{V}CO_2 = 6Vc_o/G + 55Vf_o/F + P_c n + 52Vc_f/27G$$

When these expressions are substituted in *Eqs. A1* and *A2* to calculate "apparent" rates of carbohydrate and fat oxidation, it can be seen by inspection that terms in  $n$  cancel out; rearranging and simplifying the remaining terms leads to

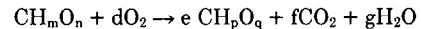
$$c = c_o + c_f \quad (A3)$$

$$f = f_o - 2F c_f/27G \quad (A4)$$

## REFERENCES

1. ASKANAZI, J., Y. A. CARPENTIER, D. H. ELWYN, J. NORDENSTRÖM, M. JEEVANANDAM, S. H. ROSENBAUM, F. E. GUMP, AND J. M. KINNEY. Influence of total parenteral nutrition on fuel utilization in injury and sepsis. *Ann. Surg.* 191: 40-46, 1980.
2. COBELLI, C., R. NOSADINI, G. TOFFOLO, A. MCCULLOCH, A. AVOGARO, A. TIENGO, AND K. G. M. M. ALBERTI. Model of the kinetics of ketone bodies in humans. *Am. J. Physiol.* 243 (*Regulatory Integrative Comp. Physiol.* 12): R7-R17, 1982.
3. CONSOLAZIO, C. F., R. E. JOHNSON, AND L. J. PECORA. *Physiological Measurements of Metabolic Functions in Man*. New York: McGraw-Hill, 1963, p. 316-317.
4. DURNIN, J. V. G. A., AND R. PASSMORE. *Energy, Work and Leisure*. London: Heinemann, 1967, p. 16-18.
5. ELWYN, D. H., AND J. M. KINNEY. A unique approach to measuring total energy expenditure by indirect calorimetry. In: *Assessment of Energy Metabolism in Health and Disease. Report of the First Ross*

where  $2F c_f/27G$  is, from above, the rate of synthesis of fat. It is shown in the supplementary publication that this is not dependent on the form chosen for *Eq. 8* in the text (i.e., does not depend on what assumptions are made regarding biochemical pathways). In general, for the interconversion of two substances  $A$  (empirical formula  $CH_mO_n$ ) and  $B$  (empirical formula  $CH_pO_q$ ) according to the equation



(where the coefficients  $d$ ,  $f$ , and  $g$  may be positive or negative; i.e.,  $O_2$  and  $CO_2$  may be consumed or produced in the reaction), then if apparent rates of oxidation of each are calculated from respiratory exchange ignoring interconversion, these apparent rates are given by  $A$ , apparent rate = oxidation rate + rate of conversion to  $B$ , and  $B$ , apparent rate = oxidation rate - rate of formation from  $A$ .

*Gluconeogenesis.* The effect of gluconeogenesis is more complex, since the urinary nitrogen excretion has to be partitioned between that arising from gluconeogenesis and that arising from protein oxidation.

According to *Eq. 12* in the main text, 1 g urea nitrogen arising from gluconeogenesis is equivalent to  $G/U$  g glucose formed and  $V/U$  liters  $CO_2$  used. Consider a subject with the following rates: glucose and fat oxidation,  $c_o$  and  $f_o$  g/min as before; urinary nitrogen excretion,  $(n_p + n_g)$  g/min, where  $n_p$  g/min arises from protein oxidation and  $n_g$  g/min from gluconeogenesis, thus forming  $G n_g/U$  g glucose/min, using  $V n_g/U$  liters  $CO_2$ /min. Then

$$\dot{V}O_2 = (6Vc_o/G + 145Vf_o/2F + P_o n_p) \text{ l/min}$$

$$\dot{V}CO_2 = (6Vc_o/G + 102Vf_o/2F + P_c n_p - V n_g/U) \text{ l/min}$$

Substituting in *Eq. A1* from above to calculate  $c$ , terms in  $n_p$  and  $f_o$  cancel out, leaving after simplification:  $c = c_o + G n_g (102P_o - 145P_c - 145V/U)/6V(145 - 102)$ . Substituting numerical values for  $P_o$ ,  $P_c$  (from Table 1),  $V$  and  $U$ , this becomes  $c = c_o + G n_g/27.7$ .

Since the rate of glucose formation,  $c_s = G n_g/28$ , then

$$c = c_o + c_s \quad (A5)$$

with an error of around 1% in  $c_s$ . (It has not been found possible to formulate this in any more general way to avoid introducing numerical values.)

Substituting  $\dot{V}O_2$  and  $\dot{V}CO_2$  in *equation A2* to calculate  $f$ , terms in  $c_o$  and  $n_p$  cancel out, leaving after simplification:  $f = f_o + 2F (V/U - P_o + P_c) n_g / (145 - 102)V$ , giving, after substitution of numerical values

$$f = f_o - 0.59 n_g \quad (A6)$$

Since the rate of glucose synthesis ( $c_s$ ) is given by  $c_s = 180 n_g \cdot 28 \text{ g}^{-1} \cdot \text{min}^{-1}$ , then *equation (A6)* becomes

$$f = f_o - 0.09 c_s$$

i.e., the calculated rate of fat oxidation underestimates the true rate by an amount approximately equal, in mass terms, to 0.1 the rate of glucose formation. It has not been possible to ascribe any physiological meaning to this small discrepancy.

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*Conference on Medical Research*, edited by J. M. Kinney. Columbus, OH: Ross Laboratories, 1980, p. 54-61.

6. FELIG, P., T. POZEFKY, E. MARLISS, AND G. F. CAHILL. Alanine: key role in gluconeogenesis. *Science* 167: 1003-1004, 1970.
7. GUMP, F. E., C. LONG, P. KILLIAN, AND J. M. KINNEY. Studies of glucose intolerance in septic injured patients. *J. Trauma* 14: 378-388, 1974.
8. HIRSCH, J. Fatty acid patterns in human adipose tissue. In: *Handbook of Physiology. Adipose Tissue*. Washington, DC: Am. Physiol. Soc., 1965, sect. 5, chapt. 17, p. 181-189.
9. JONES, N. L., G. J. F. HEIGENHAUSER, A. KUKSIS, C. G. MATSOS, J. R. SUTTON, AND C. J. TOEWS. Fat metabolism in heavy exercise. *Clin. Sci.* 59: 469-478, 1980.
10. KEELE, C. A., AND E. NEIL. *Samson Wright's Applied Physiology* (11th ed.). London: Oxford Univ. Press, 1965, p. 187.
11. KELLER, U., G. E. SONNENBERG, AND W. STAUFFACHER. *Valida-*

- tion of a tracer technique to determine nonsteady-state ketone body turnover rates in man. *Am. J. Physiol.* 240 (*Endocrinol. Metab.* 3): E253-E262, 1981.
12. KLEIBER, M. *The Fire of Life. An Introduction to Animal Energetics.* New York: Wiley, 1961, p. 89-93.
  13. LITTLE, R. A., H. B. STONER, AND K. N. FRAYN. Substrate oxidation shortly after accidental injury in man. *Clin. Sci.* 61: 789-791, 1981.
  14. LONG, C. L., J. L. SPENCER, J. M. KINNEY, AND J. W. GEIGER. Carbohydrate metabolism in man: effect of elective operations and major injury. *J. Appl. Physiol.* 31: 110-116, 1971.
  15. LUSK, G. *The Elements of the Science of Nutrition* (3rd ed.). Philadelphia, PA: Saunders, 1923, p. 18-25, 468-472.
  16. MAGNUS-LEVY, A. *The Physiology of Metabolism.* In: *Metabolism and Practical Medicine*, edited by C. von Noorden. London: Heinemann, 1907, vol. 1, p. 190.
  17. OWEN, O. E., V. E. TRAPP, G. A. REICHARD, M. A. MOZZOLI, R. SMITH, AND G. BODEN. Effect of therapy on the nature and quantity of fuels oxidized during diabetic ketoacidosis. *Diabetes* 29: 365-372, 1980.
  18. PETERS, J. P., AND D. D. VAN SLYKE. *Quantitative Clinical Chemistry. Interpretations* (2nd ed.). London: Baillière, Tindall & Cox, 1946, vol. 1, p. 25.
  19. RECORD, C. O., R. A. CHASE, R. WILLIAMS, AND D. APPLETON. Disturbances in lactate metabolism in patients with liver damage due to paracetamol overdose. *Metabolism* 30: 638-643, 1981.
  20. REICHARD, G. A., A. C. HAFF, C. L. SKUTCHES, P. PAUL, C. P. HOLROYDE, AND O. E. OWEN. Plasma acetone metabolism in the fasting human. *J. Clin. Invest.* 63: 619-626, 1979.
  21. SCHUTZ, Y., AND E. RAVUSSIN. Respiratory quotients lower than 0.70 in ketogenic diets. *Am. J. Clin. Nutr.* 33: 1317-1319, 1980.
  22. STONER, H. B., R. A. LITTLE, E. A. ELEBUTE, E. GROSS, J. TRESADERN, AND K. N. FRAYN. Some metabolic problems in patients with injuries or sepsis. In: *Clinical Nutrition 1981*, edited by R. I. C. Wesdorp and P. B. Soeters. Edinburgh: Churchill Livingstone, 1982, p. 297-300.
  23. STONER, H. B., R. A. LITTLE, K. N. FRAYN, E. A. ELEBUTE, J. TRESADERN, AND E. GROSS. The effect of sepsis on the oxidation of carbohydrate and fat. *Br. J. Surg.* 70: 32-35, 1983.
  24. VARLEY, H. *Practical Clinical Biochemistry* (4th ed.). London: Heinemann, 1969, p. 167.
  25. WILMORE, D. W. *The Metabolic Management of the Critically Ill.* New York: Plenum, 1977.
  26. WYNN, V. The metabolism of fructose during hypothermia in man. *Clin. Sci.* 15: 297-304, 1956.

