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_2 consumption, CO_2 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_2 consumption ($\dot{V}O_2$) and CO_2 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

$$\text{glucose } (C_6H_{12}O_6) + 6\ O_2 \rightarrow 6\ H_2O + 6\ CO_2 \quad (1)$$

6 mol of O_2 are consumed and 6 mol of CO_2 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, $C_{55}H_{104}O_6$, chosen for reasons given in the APPENDIX; abbreviated throughout to PSOG) according to the equation

$$\text{PSOG } (C_{55}H_{104}O_6) + 78\ O_2 \rightarrow 55\ CO_2 + 52\ H_2O \quad (2)$$

consumes 78 mol of O_2 and produces 55 mol of CO_2 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_2 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_2 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_2 consumption and CO_2 production, respectively, in liters per minute.

These formulas are very similar to those given by other

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TABLE 1. *Volumes of O_2 consumed and CO_2 produced in oxidation of various fuels*

Fuel	O_2, l/g fuel	CO_2, 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_2 or H_2O, 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_2 and H_2O 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, $C_{18}H_{36}O_2$) is sometimes written in a balanced equation (10) as

$$3\ C_6H_{12}O_6 \rightarrow C_{18}H_{36}O_2 + 8\ O_2 \qquad (7)$$

However, evolution of O_2 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[1]; when combined, the overall process can be represented as

$$27\ \text{glucose} + 24\ \text{ATP} + 6\ O_2 \rightarrow 2\ \text{PSOG} + 24\ \text{ADP} + 52CO_2 \qquad (8)$$

(Inorganic phosphate and H_2O will be omitted in all equations.) In contrast to *Eq. 7*, this shows that there is a net consumption of oxidizing equivalents. The CO_2 is evolved "obligatorily" in the formation of acetyl-CoA from pyruvate (2 mol of CO_2 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

$$83\ \text{glucose} + 30\ O_2 \rightarrow 6\ \text{PSOG} + 168\ CO_2 \qquad (9)$$

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_2 are used and 52 mol (1,165 liters) of CO_2 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_2 and CO_2 equivalents of these processes from Tables 1 and 2

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

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

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

[1] For supplemental material order NAPS document 04098 from Microfiche Publications, PO Box 3513, Grand Central Station, New York, NY 10017.

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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

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

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

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

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 cyle. The individual steps are listed in the supplementary publication; when combined, the following overall equation is arrived at

$$2 \text{ alanine} + CO_2 + 10 \text{ XTP} \rightarrow \text{glucose} + \text{urea} + 10 \text{ XDP} \qquad (12)$$

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

$$\text{palmitate} + 26 \text{ alanine} + 23\ O_2 \rightarrow 13 \text{ glucose} + 3\ CO_2 + 13 \text{ urea} \qquad (13)$$

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

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

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

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

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

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

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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 β-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

$$2 \text{ palmitate} + 14\ O_2 + 70\ ADP \rightarrow 8\ AcAc^- + 8\ H^+ + 68\ ATP + 2\ AMP + 2PP_i \quad (15)$$

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*

$$AcAc\ (C_4H_5O_3^-) + H^+ + 4\ O_2 \rightarrow 4\ CO_2 + 3\ H_2O \quad (16)$$

$$2\ 3\text{-OHB}\ (C_4H_7O_3^-) + 2\ H^+ + 9\ O_2 \rightarrow 8\ CO_2 + 8\ H_2O \quad (17)$$

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-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.

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

Compound	Correction per Mole Stored/Excreted or Utilized		Additional Max Correction for Each Mole Stored or Utilized
	O_2, mol	CO_2, mol	CO_2, mol
Storage/excretion			
Acetoacetate	−1.75	0	−1
3-Hydroxybutyrate	−1.25	0	−1
Acetone	−1.75	−1	0
Utilization			
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).

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

$$\text{acetone}\ (C_3H_6O) + 3\ O_2 \rightarrow 3\ CO_2 + 3\ H_2O \quad (18)$$

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

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subject, the maximum rate of displacement of CO_2 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

$$\text{lactate } (C_3H_5O_3^-) + H^+ + 3\ O_2 \rightarrow 3\ CO_2 + 3\ H_2O \quad (19)$$

consumes 3 mol of O_2 and produces 3 mol of CO_2 per mole of lactate, and again up to 1 mol of CO_2 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_2 in the pyruvate dehydrogenase step in lipogenesis, the CO_2 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 possible, 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

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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 $(6Vc/G + 78Vf/F + P_on)$ l/min O_2 and produces $(6Vc/G + 55Vf/2F + P_cn)$ 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/2 G 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 $2Fc_f/27G$ g fat/min; and urinary nitrogen excretion, n g/min. Then

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

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

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

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

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

where $2Fc_f/27G$ 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

$$CH_mO_n + dO_2 \rightarrow e\ CH_pO_q + fCO_2 + gH_2O$$

(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 Gn_g/U g glucose/min, using Vn_g/U liters CO_2/min. Then

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

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

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

Since the rate of glucose formation, $c_s = Gn_g/28$, 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

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

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

$$f \simeq f_o - 0.09\ c_s$$

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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