A model for measurement of lactate disappearance with isotopic tracers in the steady state

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1. The irreversible disappearance of lactate carbon from the body (RdL) is commonly calculated from data obtained with a continuous infusion of isotopically labelled lactate tracer. The tracer infusion rate divided by the steady-state lactate specific radioactivity in blood is taken to give the rate of lactate disappearance. 2. Measurement of lactate disappearance is complicated by the fact that it is reversibly converted into pyruvate as well as being irreversibly removed from the system. 3. We analysed a four-compartment model of lactate metabolism, representing blood lactate, tissue lactate and pyruvate carbon pools. 4. The standard method of calculating RdL from the lactate tracer infusion rate divided by the specific radioactivity of lactate was not validated. 5. We found that RdL can be calculated from the infusion rate and the pyruvate specific radioactivity, multiplied by the fraction of the total carbon flow out of pyruvate that goes to lactate. 6. Therefore, if almost all of the pyruvate carbon flows back to lactate, then RdL approaches the tracer infusion rate divided by the pyruvate specific radioactivity. On the other hand, if the rate of oxidation is large in relation to the rate of pyruvate conversion into lactate, then RdL is overestimated when calculated from the pyruvate specific radioactivity. 7. Calculation of RdL with the arterial lactate specific radioactivity results in an underestimate of the true RdL .

INTRODUCTION

Isotopically labelled tracers have been used extensively to measure the flux of various compounds in living organisms. Various models have been developed to describe how to calculate the flux of 'tracee' from the measurement of both tracee and tracer in the blood. The work of Mann & Gurpide [1] dealt in general terms with cases where not all the tracer and tracee flowed through the pool which was sampled. We recently addressed this same question with a model that also accounted for the circulation [2]. The model presented by Heath & Barton [3] considered the simultaneous formation and removal of tracee in the same pool, and present a method for correcting for this phenomenon.

Carbon-labelled lactate tracers have been used for over 30 years to assess the rates of the various pathways of lactate metabolism [4–12]. It is generally assumed that one can calculate the irreversible loss of lactate carbon from the body by dividing the lactate tracer infusion rate by the plateau lactate specific radioactivity in blood [7]. Sahlin [13] recently noted that measurement of lactate turnover might be complicated by the fact that lactate tracer is reversibly converted into pyruvate before being irreversibly removed from the system. He suggested that dilution of lactate tracer in the pyruvate pool would make the concentration of lactate tracer dependent upon the glycolytic rate, and that the rate of lactate turnover, as calculated from the lactate tracer infusion rate and the lactate specific radioactivity, would be equal to the rate of pyruvate production, and not a measure of irreversible lactate removal from the system. However, examination of the literature on lactate kinetics suggests that this

might not be the case [11], especially during muscular exercise, when the rates of pyruvate formation and oxidation are increased severalfold.

To our knowledge there is no theoretical model that adequately describes measurement of lactate kinetics with isotopic carbon tracers. We recently presented [2] a theoretical model for the selection of the appropriate site for sampling blood for specific radioactivity in experiments designed to measure the whole-body turnover rate of a metabolite. We did not consider that a special case might exist for compounds that pass through a metabolically active intermediate before entering an irreversible reaction. In the present paper we construct a theoretical model for tracer measurement of lactate kinetics that takes into account pyruvate metabolism.

A MODEL OF LACTATE METABOLISM

We analyse a four-compartment model (Fig. 1), representing the arterial blood lactate (AL), venous blood lactate (VL) and tissue lactate (TL) carbon pools and pyruvate (P) carbon pools. The tissue compartment would primarily be comprised of muscle and liver [8]. We consider the steady state, in which the mass of tracee (q) and isotopically labelled tracer (q^*) in each compartment, the lactate tracer infusion rate (F^*) and the appearance *de novo* of pyruvate in the tissue (I_T) are unchanged with time. Our model is of a system, not of data. However, the model and the restrictive steady-state assumption are, of course, dictated by experimental realities. Each fractional transfer coefficient $k_{j,i}$ from compartment i to compartment j is assumed constant

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Fig. 1. A four-compartment model of lactate metabolism

Pools of arterial lactate (AL), tissue lactate (TL), venous lactate (VL) and pyruvate (P) are connected by arrows labelled with fractional transfer coefficients ($k_{i,j}$). Substrate mass appears in the tissue compartment at rate I_T , and lactate tracer is infused into the arterial blood at rate F^* .

and independent of time and concentration in the compartment. The mass flow rate from one compartment to another will be denoted as the product of k and q ; for example the flow rate of lactate out of the tissue lactate compartment and into the venous blood lactate compartment is $k_{VL,TL} q_{TL}$. I_T denotes the mass rate of unlabelled pyruvate entry *de novo* into the tissue compartment (primarily from glycolysis), and $k_{O,P}$ is the transfer coefficient for the irreversible flow of tissue pyruvate out of the system (mainly through mitochondrial oxidation). Entry of carbon into the system from alanine is considered part of I_T , and irreversible removal of pyruvate carbon atoms through alanine (i.e. protein synthesis) is included in $k_{O,P} q_P$. We further assume a constant infusion of carbon-labelled lactate tracer into the arterial lactate pool (F^*). The usual tracer assumptions are made [14]: tracer concentrations are small compared with those of tracee, there is no isomeric selection (so fractional transfer coefficients are the same for tracer and tracee), and tracer is well-mixed in the compartments. In the steady state the rate of carbon flow *de novo* into pyruvate (I_T) will equal the rate of irreversible carbon loss from the system ($k_{O,P} q_P$), and the rate of lactate conversion into pyruvate ($k_{P,TL} q_{PT}$) will equal the rate of pyruvate conversion into lactate ($k_{TL,P} q_P$). It should be noted that the sources of labelled and unlabelled pyruvate are identical in our model, and thus q_P^*/q_P will be the same in either venous or arterial blood, or the tissue compartment.

Our purpose is to derive an equation for the rate of irreversible loss of carbon from the lactate pools. Tissue lactate is oxidized to form pyruvate at rate $k_{P,TL} q_{TL}$. Pyruvate then has two fates: irreversible oxidation in the mitochondria at rate $k_{O,P} q_P$, or reduction with NADH to form lactate at rate $k_{TL,P} q_P$. One is generally interested in the irreversible loss of lactate from the system, which can be defined as the rate of lactate conversion into

pyruvate ($k_{P,TL} q_{TL}$) times the fraction of pyruvate flow that is irreversibly lost from the system [$k_{O,P} q_P / (k_{O,P} q_P + k_{TL,P} q_P)$]. Thus the irreversible loss of lactate carbon can be expressed as:

$$RdL = (k_{P,TL} q_{TL}) [k_{O,P} q_P / (k_{O,P} q_P + k_{TL,P} q_P)] \quad (1)$$

Eqn. (1) can be rewritten in terms of the tracer. In the steady state, the rate of tracer carbon loss from the system ($k_{O,P} q_P^*$) is equal to the rate of tracer infusion (F^*). If both rates are multiplied by q_P/q_P^* , then $F^* q_P/q_P^*$ is equal to $k_{O,P} q_P$, so:

$$RdL = (k_{P,TL} q_{TL}) [F^* (q_P/q_P^*) / (k_{O,P} q_P + k_{TL,P} q_P)] \quad (2)$$

In the steady state, $k_{P,TL} q_{TL} = k_{TL,P} q_P$, so eqn. (2) can be rearranged to give:

$$RdL = [F^* / (q_P^*/q_P)] \{1 / [1 + (k_{O,P} q_P / k_{TL,P} q_P)]\} \quad (2a)$$

Thus calculation of RdL from the pyruvate specific radioactivity overestimates the true value by the factor $\{1 / [1 + (k_{O,P} q_P / k_{TL,P} q_P)]\}$. If the rate of pyruvate carbon flow to lactate is very large compared with the rate of irreversible pyruvate oxidation, then the correction factor in eqn. (2a) approaches 1. In other words, if almost all of the pyruvate carbon flows back to lactate, RdL will nearly equal $F^* / (q_P^*/q_P)$. On the other hand, if the rate of pyruvate production *de novo* [I_T , which is equal to pyruvate removal ($k_{O,P} q_P$)] is large in relation to the rate of pyruvate conversion into lactate ($k_{TL,P} q_P$), then RdL is overestimated when calculated from the pyruvate specific radioactivity.

The rate of lactate disappearance is commonly calculated as the rate of lactate tracer infusion divided by the arterial lactate specific radioactivity [4,5,7,12]. It would therefore be useful to express eqn. (2a) in terms of the arterial lactate specific radioactivity. For the system illustrated in Fig. 1 the mass-balance equations for the pyruvate pool give:

$$(k_{O,P} q_P + k_{TL,P} q_P) (q_P^*/q_P) = k_{P,TL} q_{TL} (q_{TL}^*/q_{TL}) \quad (3)$$

for tracer and:

$$k_{TL,P} q_P = k_{P,TL} q_{TL} \quad (4)$$

for tracee. Similar equations for the tissue lactate pool can also be derived:

$$k_{TL,AL} q_{AL} (q_{AL}^*/q_{AL}) + k_{TL,P} q_P (q_P^*/q_P) = (k_{VL,TL} q_{TL} + k_{P,TL} q_{TL}) (q_{TL}^*/q_{TL}) \quad (5)$$

for tracer and:

$$k_{TL,AL} q_{AL} = k_{VL,TL} q_{TL} \quad (6)$$

for tracee. Substituting eqn. (3) into eqn. (4), and eqn. (5) into eqn. (6), and eliminating the tissue lactate specific radioactivity, it can be seen that:

$$(q_{AL}^*/q_{AL}) = [1 + (k_{O,P} q_P / k_{TL,AL} q_{AL}) + (k_{O,P} q_P / k_{P,TL} q_{TL})] (q_P^*/q_P) \quad (7)$$

so:

$$(q_P^*/q_P) = (q_{AL}^*/q_{AL}) / [1 + (k_{O,P} q_P / k_{TL,AL} q_{AL}) + (k_{O,P} q_P / k_{P,TL} q_{TL})] \quad (7a)$$

Now we can substitute eqn. (7a) into eqn. (2a) to get RdL in terms of arterial lactate specific radioactivity:

$$RdL = [F^* (1 + k_{O,P} q_P / k_{TL,AL} q_{AL} + k_{O,P} q_P / k_{P,TL} q_{TL}) / (q_{AL}^*/q_{AL})] \{1 / [1 + (k_{O,P} q_P / k_{TL,P} q_P)]\} \quad (8)$$

This equation can be simplified further:

$$RdL = \left(\frac{F^*}{(q_{AL}^*/q_{AL})} \right) \left(1 + \frac{k_{O,P} q_P / k_{TL,AL} q_{AL}}{1 + k_{O,P} q_P / k_{TL,P} q_P} \right) \quad (9)$$

Thus RdL is the infusion rate divided by arterial lactate specific radioactivity as commonly measured, times a correction factor involving the rates of pyruvate oxidation, lactate transport into the tissue compartment and pyruvate conversion into lactate. If the rate of irreversible pyruvate removal from the system ($k_{O,P} q_P$) is much smaller than both the rates of arterial lactate flux into the tissue compartment ($k_{TL,AL} q_{AL}$) and pyruvate conversion into lactate ($k_{TL,P} q_P$), then the arterial lactate radioactivity will reflect the irreversible loss of lactate from the system.

DISCUSSION

Estimations

We have presented two equations [eqns. (2a) and (9)] for the calculation of the irreversible loss of carbon from the lactate pool from the lactate tracer infusion rate divided by the specific radioactivity of either pyruvate (eqn. 2a) or arterial lactate (eqn. 9) times a correction factor. Clearly it would be extremely useful to approximate the values of these two factors.

First, one can estimate the correction factor given the pyruvate specific radioactivity (eqn. 2a) by examining the enzymic control of the fate of pyruvate in various tissues *in vivo* [15]. The correction factor, $\{1/[1 + (k_{O,P} q_P / k_{TL,P} q_P)]\}$, is equal to the fraction of the total carbon flow out of pyruvate that goes to lactate [$k_{TL,P} q_P / (k_{O,P} q_P + k_{TL,P} q_P)$]. Skeletal muscle is the primary site of lactate formation [6,7,12,15], especially during exercise. The activity of lactate dehydrogenase in the gastrocnemius muscle of the rat (677 $\mu\text{mol}/\text{min per g}$ [16]) is 97% of the activity of the combined total of the activities of lactate dehydrogenase, mitochondrial pyruvate oxidase (0.55 $\mu\text{mol}/\text{min per g}$ [17]), alanine aminotransferase (22.7 $\mu\text{mol}/\text{min per g}$ [16]) and malic enzyme (0.69 $\mu\text{mol}/\text{min per g}$ [16]). This suggests that most of the apparent carbon flow out of pyruvate goes to lactate in this tissue, so $\{1/[1 + (k_{O,P} q_P / k_{TL,P} q_P)]\}$ may approach 1.0, and RdL might nearly equal $F^*/(q_P^*/q_P)$.

The correction factors can also be calculated from estimates of lactate conversion into pyruvate ($k_{P,TL} q_{TL}$) with infusion of [$2\text{-}^3\text{H}$]lactate [5,9], and of pyruvate loss from the system ($k_{O,P} q_P$) from the rate of carbohydrate oxidation as determined by indirect calorimetry. The carbon flow rates between pyruvate and lactate are equal at steady state. This method assumes that the tritium atoms on C-2 are transferred to NAD^+ when lactate is converted into pyruvate, and are not transferred back to lactate in the reverse reaction. Katz and co-workers [9] infused [$2\text{-}^3\text{H}$]lactate and [$\text{U-}^{14}\text{C}$]lactate into rats, and observed that the calculated turnover rate was twice as high with [$2\text{-}^3\text{H}$]lactate. Donovan & Brooks [5] observed a similar relationship between the two tracers at rest and during exercise. An estimate of $k_{O,P} q_P$ can be made with indirect calorimetry, assuming that all of the carbohydrate oxidized is reflected in $k_{O,P} q_P$. Assuming that $k_{O,P} q_P$ is roughly 70 $\mu\text{mol}/\text{min per kg}$ [5], $k_{P,TL} q_{TL}$ is 381 $\mu\text{mol}/\text{min per kg}$ [5] or 169 $\mu\text{mol}/\text{min per kg}$ [9], then our final term in eqn. (2a) is 0.84 [5] or 0.71 [9] in resting rats. These estimates indicate that the fraction of carbon

flow from lactate is large. However, during submaximal treadmill running the estimated value falls to 0.71 [5]. These estimates are based on the doubtful assumption that none of the secondary labelled NADH is recycled back on to newly formed lactate [2]. If reduction of pyruvate to form lactate is a primary route for cytosolic NADH oxidation, then there would be extensive recycling of ^3H from lactate to NADH and back to lactate, which would result in a large underestimation of $k_{P,TL} q_{TL}$, and of the correction factor in eqn. (2a). If the same estimations are made assuming $k_{P,TL} q_{TL}$ is twice the value obtained from the [^3H]lactate data, the correction factor in eqn. (2a) increases to 0.92 [5] or 0.83 [9]. If it is made using 10 times the measured value it increases further to 0.98 [5] or 0.96 [9]. Clearly the degree of error in the calculation of RdL from the pyruvate specific radioactivity is dependent on the rate of lactate carbon flow to pyruvate.

Similar estimations can be made for the correction factor in eqn. (9) involving the rates of pyruvate oxidation, lactate transport into the tissue compartment and pyruvate conversion into lactate. Values for lactate transport into the tissue compartment ($k_{TL,AL} q_{AL}$) can be estimated from the arterial lactate concentration and blood flow [2,3]. In resting rats the data of Donovan & Brooks [5] and Katz and co-workers [9] both give a correction factor 1.20, which suggests that the commonly used method of calculating RdL from the arterial specific radioactivity results in a 20% underestimation of the true value. Increasing $k_{TL,P} q_P$ 2-fold or 10-fold increases the correction factor to 1.23 or 1.25 respectively in resting rats. These estimates suggest that calculation of RdL from the arterial lactate specific radioactivity in resting rats underestimates the actual RdL by about 20–25%.

Wolfe *et al.* [18] recently assessed the equilibration of lactate tracer with pyruvate by infusing carbon-labelled lactate tracer and measuring the pyruvate and mixed venous lactate specific radioactivities. If $k_{P,TL} q_{TL}$ is much greater than $k_{O,P} q_P$, then $k_{TL,P} q_P^*$ will be almost as great as $k_{P,TL} q_{TL}^*$, and q_{TL}^*/q_{TL} will be nearly equal to q_P^*/q_P . Methodological problems prevent accurate measurements of q_P^*/q_P when [^{14}C]lactate is infused. Pyruvate spontaneously forms dimers, and also is decarboxylated to form acetate [19]. Since measurement of q_P^*/q_P with radiocarbon labels requires accurate measurement of the pyruvate concentration, it is not possible to obtain accurate measurement of q_P^*/q_P . Alternatively, one could measure q_P^*/q_P with an infusion of [^{13}C]lactate tracer. With this method q_P^*/q_P is measured directly by g.l.c.–m.s. analysis [18], and is given as the molar enrichment of pyruvate with [^{13}C]pyruvate. However, there is the practical problem of how to sample the tissue compartment. One can infuse the tracer into the left heart chambers, and sample the venous compartment from the pulmonary artery to obtain an estimate of the pyruvate enrichment in the tissue compartment [2]. Layman & Wolfe [20] demonstrated that this may be a valid assumption in anaesthetized dogs. Wolfe *et al.* [18] infused [$3\text{-}^{13}\text{C}$]lactate into the left ventricle of anaesthetized dogs using the primed continuous-infusion method, and sampled the venous pool from the right ventricle. The molar enrichment of pyruvate was 92% of the molar enrichment value for lactate. Thus the molar enrichment of pyruvate was very near that of lactate, which suggests that $k_{P,TL} q_{TL}/(I_T + k_{P,TL} q_{TL})$ is near 1.0, and that the lactate specific activity would accurately

reflect the pyruvate specific activity for the calculation of the *RdL*.

Limitations of the model

Our model is strongly dependent on the assumption that there is a steady state in both tracee and tracer concentrations, tracer infusion rate and the rate of pyruvate formation *de novo*. We also assume that the rate laws are the same for tracer as for tracee. These assumptions cannot be relaxed.

Conclusions

Tracer measurement of lactate kinetics is complicated by the reversible conversion of lactate into pyruvate before pyruvate is irreversibly removed from the system. The rate of irreversible lactate disappearance from the system is inversely proportional to the pyruvate specific radioactivity, and proportional to the fraction of the carbon flow out of the tissue pyruvate compartment that goes to lactate. If there is a rapid rate of lactate conversion into pyruvate relative to the rate of pyruvate carbon appearance *de novo* in the system, the pyruvate and lactate tracer specific radioactivities in the tissue become nearly equal and the rate of lactate removal from the system is inversely proportional to either specific radioactivity.

We thank Robert R. Wolfe for graciously supplying us with his data on the subject, and Richard A. Neese for his thoughtful review of our manuscript. W. C. S. was supported by a postdoctoral fellowship from the California Affiliate of the American Heart Association, with funds from the San Francisco Chapter.

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Received 23 May 1988/20 September 1988; accepted 13 October 1988