Indirect calorimetry is the standard paradigm for determining the energy transfer rate and oxidative fuel utilization during exercise in humans, but only under steady-state conditions where CO2 output (V\text{CO}_2) is unaffected by acid-base disturbances. The requirement for steady state is a serious limitation, because most conditions of exercise are non-steady state. Consequently, there is an urgent need to extend the indirect calorimetric paradigm to determine the kinetics of muscle and whole body energetics and oxidation of fat and carbohydrate (CHO). The kinetic paradigm would provide opportunities to study 1) the contribution of fat and CHO to oxidative metabolism during the early transition to steady state, 2) the cellular mechanisms controlling oxidative metabolism, 3) the systemic controls involving alveolar gas exchange as well as convective O2 transport and conductive O2 diffusion, and 4) the interrelationship between oxidative V\text{CO}_2 and acid-base effects on measured V\text{CO}_2.

Molé, Paul A., and James J. Hoffmann. VO2 kinetics of mild exercise are altered by RER. J. Appl. Physiol. 87(6): 2097–2106, 1999.—We propose that variations in fat and carbohydrate (CHO) oxidation by working muscle alter O2 uptake (VO2) kinetics. This hypothesis provides two predictions: 1) the kinetics should comprise two exponential components, one fast and the other slow, and 2) their contribution should change with variations in fat and CHO oxidation, as predicted by steady-state respiratory exchange ratio (RER). The purpose of this study was to test these predictions by evaluating the VO2 kinetic model: \( VO2(t) = a_F + a_C[1 - \exp(\frac{t - TD}{-\tau_F})] + a_C[1 - \exp(\frac{t - TD}{-\tau_C})] \) for short-term, mild leg cycling in 38 women and 44 men, where VO2(t) describes the time course, \( a_F \) is resting VO2, t is time after onset of exercise, TD is time delay, \( a_F \) and \( a_C \) are asymptote and time constant, respectively, for the fast (fat) oxidative term, and \( a_C \) and \( \tau_C \) are the corresponding parameters for the slow (CHO) oxidative term. We found that 1) this biexponential model accurately described the VO2 kinetics over a wide range of RERs, 2) the contribution of the fast (fat) component was inversely related to RER, whereas the slow (CHO) component was positively related to RER, and 3) this assignment of the fast and slow terms accurately predicted steady-state respiratory quotient and CO2 output. Therefore, the kinetic model can quantify the dynamics of fat and CHO oxidation over the first 5–10 min of mild exercise in young adult men and women.

The complexity of physiological and metabolic processes makes developing a kinetic paradigm a daunting task. However, there are several principles of modeling that can help develop, as a first step, a simplified dynamic indirect calorimetric paradigm, which is based on O2 uptake (VO2) kinetics. First, the shape of the dynamic VO2 response provides information on pulmonary, circulatory, and metabolic processes. Second, mathematically decomposing the response into its component parts potentially can represent a specific process or some combination of processes. Critical to this task is the selection of a mathematical model, which contains parameters that reflect the dynamic characteristics of the process under study. At that point, each process can be identified by performing experiments that test the parameters of the model against theory.

Previous authors (4–6, 43, 45) have initiated decomposition of VO2 kinetics. Whipp and colleagues (45) introduced the concept that VO2 kinetics during light to moderate exercise below the performer’s lactate threshold involves three phases. Phase 1 is the rapid rise in pulmonary VO2 after the onset of exercise, which reaches a transient plateau or inflection point in 15–20 s. They proposed that this phase involves the rapid delivery of O2-poor blood for alveolar exchange and called it the “cardiodynamic phase.” After phase 1, the VO2 rises exponentially in phase 2 and reaches an apparent steady state (phase 3), reflecting an increase in VO2 of metabolically active tissues involved in exercise, primarily working muscles. Accordingly, phases 2 and 3 represent oxidative dynamics of working muscles. Results from simulation experiments by Barstow and coworkers (4, 5) are consistent with this assignment of circulatory (phase 1) and metabolic components (phases 2 and 3) to the dynamics of pulmonary VO2 for light to moderately intense exercise. It is important to notice that the shape of the VO2 kinetic response has information on the time course of cardiorespiratory (phase 1) and oxidative (phases 2 and 3) processes. Therefore, an accurate mathematical model of phase 2 and 3 VO2 kinetics may provide analytic information on the dynamics of oxidative metabolism of working muscles.

That the exercise-induced increase in pulmonary VO2 primarily represents the oxidation of working muscles is well established experimentally (1–3, 21, 23, 30, 33, 36, 39), thereby confirming the theoretical work (4, 5). For example, recently Odland et al. (30) showed that leg VO2 represents ~80% of whole body VO2 with virtually the same substrate oxidation [leg respiratory quotient (RQ) = 0.91 vs. respiratory exchange ratio (RER) = 0.92] during minutes 4–7 of cycling at 40% of maximal VO2 (VO2\text{max}). Therefore, it is reasonable to propose that the oxidation of fat and CHO in working muscles contributes to VO2 in phases 2 and 3. Furthermore, as developed below, we proposed that the initial
changes in the flux of these fuels are reflected in the dynamics of phase 2 \( \dot{V}O_2 \). Briefly, we propose that the flux of these fuels is initiated as a feedforward process driven by contraction-induced increases in calcium, cAMP, and epinephrine that simultaneously activate glycogenolysis, triglyceride (TG) lipolysis, and \( \beta \)-oxidation. Subsequently, feedback controls modulate their fluxes as dictated by alterations in the redox and phosphorylation potentials and intermediates, including pyruvate, citrate, and the ratio of acetyl CoA to CoA. Therefore, we propose that the contraction-induced changes in key enzymes controlling glycogen, intracellular TG, and fatty acid fluxes initially dictate the dynamic of redox and phosphorylation states, which in turn control and drive the dynamic of oxidative phosphorylation and phase 2 \( \dot{V}O_2 \) of working muscle.

As recently reviewed by Tschakovky and Hughson (40), if muscle \( O_2 \) utilization, particularly for light exercise, is limited by intrinsic metabolic inertia and not by \( O_2 \) transport inertia, then the primary determinants of phase 2 \( \dot{V}O_2 \) kinetics will include enzyme activation associated with phosphorylation and redox states in healthy individuals under normoxic conditions. Research from our laboratory supports this view (28). Our findings on myoglobin desaturation during plantar flexion exercise at various intensities suggest that intracellular \( P_{O_2} \) is not limiting, and therefore neither convective nor conductive \( O_2 \) transport is typically controlling oxidative dynamics in working skeletal muscle. How, then, is phase 2 \( \dot{V}O_2 \) controlled by metabolic inertia at the early unsteady state of exercise? Briefly, muscle contraction will rapidly increase cellular \( Ca^{2+} \) with activation of various ATPases and dehydrogenases. Also, the activity of various AMP protein kinases increases, which in turn activates glycogenolysis, intracellular TG lipolysis, and \( \beta \)-oxidation (38). Therefore, glycogen and intramuscular TG should be the primary fuels utilized during the early transient, non-steady state (10, 12, 15–17, 20, 31, 42, 48). Carbon flux from these sources is linked to \( \dot{V}O_2 \) by the formation of reducing equivalents, FADH\(_2\) and NADH, and their utilization by the electron transport chain (ETC). When fat oxidation dominates, such as when persons perform light to moderate exercise on a fat diet, we propose that TG lipolysis and \( \beta \)-oxidation are rapidly activated at exercise onset. Consequently, \( \beta \)-oxidation will be the dominant supplier of FADH\(_2\) and NADH to ETC and the formation of acetyl CoA. In this case, acetyl CoA from fat oxidation will dominate the early rapid increase in tricarboxylic acid (TCA) cycle carbon flux and the rise of \( \dot{V}O_2 \) in phase 2. Furthermore, TG oxidation would be expected to have feedback control over glycogenolysis (via citrate) and would attenuate activation of the pyruvate dehydrogenase (PDH) complex via acetyl CoA and NADH (7). More specifically, increases in the NADH-to-NAD and acetyl CoA-to-CoA ratios from TG oxidation could feed back to attenuate the activation of the PDH complex. Studies (9, 15) have shown that lactate can be transported into the mitochondria and converted to pyruvate and NADH by matrix lactate dehydrogenase. We propose that this would rapidly enhance the mitochondrial NADH-to-NAD ratio and downregulate PDH, particularly when fat oxidation is dominant. Under these conditions in which fat (intracellular TG) is the dominant oxidative substrate, PDH complex activity must be insufficient initially in supplying acetyl CoA to the TCA cycle. Thus pyruvate oxidation would increase more slowly than intramuscular TG lipolysis and \( \beta \)-oxidation at the onset of exercise. We think this slow CHO oxidation is not part of the so-called “\( \dot{V}O_2 \) drift,” as described by Molé and Coulson (29), since it appears as an exponential dynamic as opposed to a linear drift, which often develops during prolonged exercise at moderate to high intensities.

If \( \dot{V}O_2 \) distinctly tracks the dynamics of fat and CHO oxidation as proposed, then \( \dot{V}O_2 \) in phase 2 would have at least two components that change at different speeds. Moreover, the relative contribution of these substrates could affect the \( \dot{V}O_2 \) kinetics, since their ATP-to-O\(_2\) ratios differ because of differences in NADH and FADH\(_2\) production. This can be expressed by Eq. 1

\[
\frac{A}{P_{O_2}} = \frac{6.5f_{CHO} + 5.64f_{Fat}}{2} \quad (1)
\]

where \( f_{CHO} \) and \( f_{Fat} \) are the time-dependent fractions for CHO and fat oxidation, respectively. The coefficients in Eq. 1 were derived by assuming that glycogen and mixed intramuscular TG are oxidized during the first minutes of exercise up to the initial attainment of steady state. If the rate of ATP utilization is constant during constant-load muscular work, then the rate of \( O_2 \) utilization would vary according to the fractional oxidation of CHO and fat.

**MODEL OF \( O_2 \) UPTAKE KINETICS**

We propose that breath-by-breath \( \dot{V}O_2 \) in phase 2 increases as a biexponential and quantitatively represents the dynamics of intramuscular TG and glycogen oxidation during the transition from rest to the initial steady state of exercise. Furthermore, the dynamics of \( \dot{V}O_2 \) and the oxidation of these substrates are tightly coupled, and local controls are the dominant factors determining their kinetics. The controls could operate, as reviewed above, such that intramuscular TG oxidation increases more quickly than CHO oxidation with a step increase in work rate. In this case, \( \dot{V}O_2 \) kinetics during phase 2 should be expressed by two components, a fast component representing fat oxidation and a slow component reflecting the time course for CHO oxidation, as given by Eq. 2, which is modified from that of Barstow and Molé (6)

\[
\dot{V}O_2(t) = a_{R} + a_{F}(1 - \exp((t - TD)/\tau_{F})) + a_{C}(1 - \exp((t - TD)/\tau_{C})) \quad (2)
\]

where \( \dot{V}O_2 \) describes the time course of muscle oxidation during phases 2 and 3, \( \tau_{R} \) is the initial resting \( \dot{V}O_2 \), \( \tau_{F} \) is the time starting from the onset of exercise, TD is...
the time delay where phase 2 $V_{O2}$ equals $\alpha_F$, $\alpha_F$ and $\alpha_C$ are the fast (fat) and slow (CHO) asymptotes representing the steady-state increase in $V_{O2}$ at phase 3 due to fat and CHO oxidation for exercise, and $\alpha_F$ and $\alpha_C$ are the time constants defining the speed of fat and CHO oxidation, respectively. Given this assignment, steady-state RER can be estimated from the asymptotic parameters of Eq. 2 by calculating the fractional contribution of CHO ($f_{CHO}$, Eq. 3) or fat ($f_{Fat}$, Eq. 4)

$$RQ = 0.707 + 0.293f_{CHO}$$

where $f_{CHO} = \alpha_C/(\alpha_F + \alpha_C)$ and $f_{Fat} = 1.00 - f_{CHO}$. With RQ, oxidative $V_{CO2}$ can be calculated by using the definition of RQ as the ratio of oxidative $V_{CO2}$ to $V_{O2}$.

METHODS AND PROCEDURES

In the present study we tested these predictions by evaluating the parameters of the $V_{O2}$ kinetic model (Eq. 2) obtained for mild leg-cycling exercise in 82 subjects (38 women and 44 men) who had widely different steady-state RERs.

The following hypotheses were tested.

Hypothesis 1. Equation 2 will accurately describe phase 2 and $V_{O2}$ kinetics during mild exercise, as evidenced by the following equality: predicted $V_{CO2} = \text{measured steady-state } V_{CO2}$.

Hypothesis 2. The asymptote of the fast term ($\alpha_F$) and its fractional contribution [$\alpha_F/(\alpha_F + \alpha_C)$] will be inversely related to steady-state RER, since we propose that $\alpha_F$ is the oxidation of fat.

Hypothesis 3. Conversely, the asymptote of the slow term ($\alpha_C$) and its fractional contribution [$\alpha_C/(\alpha_F + \alpha_C)$] will be positively related to steady-state RER, since we propose that $\alpha_C$ is the oxidation of CHO.

Hypothesis 4. The fast time constant ($\tau_F$) will be negatively, whereas the slow time constant ($\tau_C$) will be positively, related to RER.

Hypothesis 5. The TD will not be related to RER.

Subsequently, subjects participated in three to eight sessions at 2- to 7-day intervals to characterize their $V_{O2}$ kinetics for mild cycling exercise. The intensity of these mild exercise bouts varied between subjects depending on their aerobic capacity and involved exercising for 5–10 min at 20–65 W (28–40% $V_{O2max}$). Because a wide range of RERs was desired, the subjects were studied 2–4 h postabsorptively or 10–14 h postabsorptively. Each subject was instructed to maintain his/her habitual dietary pattern, particularly on the day before and the day of testing. To assess compliance, each subject measured food intake by weight and volume on the day of testing and also over a 3-day period including 2 week days immediately before testing and 1 weekend day.

Characterization of subjects. On arrival at the laboratory, the subject's body composition was determined by underwater weighing or by air plethysmography by use of the BOD POD (Life Measurements, Concord, CA), as previously described (26). Residual lung volume was determined by the method of Wilmore (46). Percent fat mass was determined from body density with the Siri equation (28).

In the present study we tested these predictions by evaluating the parameters of the $V_{O2}$ kinetic model (Eq. 2) obtained for mild leg-cycling exercise in 82 subjects (38 women and 44 men) who had widely different steady-state RERs.

Subjects.

Eighty-two young adults (44 men and 38 women) volunteered to participate in the study after being informed of the requirements, procedures, and risks. Written consent was given in accordance with the requirements of the University’s Institutional Review Board for Human Subjects Experimentation. All subjects were physically active, but none were involved in intense training for athletic competition.

Experimental protocol. The first session involved characterizing each subject with respect to body composition, $V_{TH}$, $V_{O2max}$, and steady-state $V_{O2}$ as a function of cycle power output.

Fig. 1. $O_2$ uptake ($V_{O2}$) as related to leg-cycling power: comparison of women and men. Regression for women: $V_{O2} = (451\pm 26.4) + (9.36\pm 0.259)power$, standard error of estimate (SEE) = 162 ml/min, $R^2 = 0.90$; regression for men: $V_{O2} = (485\pm 33.1) + (11.20\pm 0.194)power$, SEE = 309 ml/min, $R^2 = 0.92$. These coefficients are different from those reported in RESULTS, because they were derived by regression of each group’s data.
The subject rested for ~10 min while the metabolic cart was recalibrated. Then the following test of VO\textsubscript{2max} was undertaken. Continuous breath-by-breath measurements were made throughout exercise to volitional exhaustion by using a ramp protocol. Exercise started with a power of 25 W increasing 25 W/min for the women, or with a power of 30 W increasing 30 W/min for the men. VO\textsubscript{2max} was estimated using 15-s averages of VCO\textsubscript{2} and VO\textsubscript{2} and the V-slope method as provided by the SensorMedics software. VO\textsubscript{2max} was defined as the highest VO\textsubscript{2} that did not change >110 ml/min or 1.6 ml·min\textsuperscript{-1}·kg\textsuperscript{-1} (standard error for repeated measurements, unpublished observations) with increments in power output and was accompanied by a RER ≥ 1.10. All tests satisfied these criteria.

VO\textsubscript{2} kinetics for mild cycling exercise. One week after characterization, the subject began a series of three to eight sessions at 2- to 7-day intervals. Data used for analyses came from an initial, single bout of submaximal cycle ergometer exercise performed each session. Breath-by-breath determinations of gas exchange were made at rest on the bicycle ergometer for 5 min and during the mild "warm-up" bout for 5–10 min at 20–65 W (28–40% VO\textsubscript{2max}).

Nonlinear regression analysis of VO\textsubscript{2} kinetics. The breath-by-breath VO\textsubscript{2} data for each bout were smoothed with a four-breath rolling average, interpolated to one value per second, and time aligned to the start of exercise. Next, the responses of three to eight experiments for the subject were averaged. Analysis of VO\textsubscript{2} kinetics excluded phase 1. The end of phase 1 or the start of phase 2 for VO\textsubscript{2} was determined as the breath before to the sudden, progressive fall in RER. This coincides with the inflection point or the end of the initial plateau of VO\textsubscript{2}. The data after phase 1 (e.g., phases 2 and 3) were then fit by a double-exponential model (Eq. 2) with increments in power output.

\[ \text{V} \dot{O}_2(t) = C_a e^{-t/a} + C_b e^{-t/b} + C_c \]

Nonlinear regression analysis of V\textsuperscript{2} kinetics. The breath-by-breath V\textsuperscript{2} data for each bout were smoothed with a four-breath rolling average, interpolated to one value per second, and time aligned to the start of exercise. Next, the responses of three to eight experiments for the subject were averaged. Analysis of V\textsuperscript{2} kinetics excluded phase 1. The end of phase 1 or the start of phase 2 for V\textsuperscript{2} was determined as the breath before to the sudden, progressive fall in RER. This coincides with the inflection point or the end of the initial plateau of V\textsuperscript{2}. The data after phase 1 (e.g., phases 2 and 3) were then fit by a double-exponential model (Eq. 2) with increments in power output.

\[ \text{V} \dot{O}_2(t) = C_a e^{-t/a} + C_b e^{-t/b} + C_c \]

Figure 1 shows the relationship between the V\textsuperscript{2} and leg-cycling power output for the men and women. Comparison of the intercepts, derived by linear regression analysis of each individual’s relationship, showed no significant difference between the men and women [500 ± 226 vs. 427 ± 164 (SD) ml/min, respectively]. In contrast, the women responded with a significantly lower slope than the men (9.98 ± 1.74 vs. 10.97 ± 1.70 ml O\textsubscript{2}·min\textsuperscript{-1}·W\textsuperscript{-1}, P = 0.02). These slopes are equivalent to 3.37 and 3.71 J metabolic energy/J mechanical work when the mean RER values of Table 2 are employed. The inverses of these values are estimates of the efficiency and indicate that the efficiency of leg cycling was 29.7 and 26.9% for the women and men, respectively.

Table 1 presents several characteristics of the volunteers. These results indicate that the participants’ relative fatness, VO\textsubscript{2max}, and V\textsubscript{TH} were in the expected range for nonobese, physically active young adults.

Table 2. Steady-state metabolic response to mild leg exercise

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Power, W</th>
<th>V\textsuperscript{2}O\textsubscript{2}, ml/min</th>
<th>V\textsuperscript{2}CO\textsubscript{2}, ml/min</th>
<th>RER\textsuperscript{a}, V\textsuperscript{2}O\textsubscript{2}/V\textsuperscript{2}CO\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>38</td>
<td>38.7 ± 9.5</td>
<td>890 ± 305</td>
<td>772 ± 258</td>
<td>0.87 ± 0.06</td>
</tr>
<tr>
<td>Men</td>
<td>44</td>
<td>44.3 ± 17.2</td>
<td>1,030 ± 261</td>
<td>892 ± 224</td>
<td>0.87 ± 0.06</td>
</tr>
<tr>
<td>Combined</td>
<td>82</td>
<td>41.3 ± 13.8</td>
<td>955 ± 292</td>
<td>828 ± 249</td>
<td>0.87 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SD. VO\textsubscript{2max}, maximal O\textsubscript{2} uptake; V\textsubscript{TH}, ventilatory threshold determined by V-slope method.

RER, respiratory exchange ratio. This level of exercise represents a VO\textsubscript{2} that is 40% and 28% of VO\textsubscript{2max} for women and men, respectively.}

Table 1. Characteristics of women and men

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Body Mass, kg</th>
<th>Fat Mass, %</th>
<th>VO\textsubscript{2max}, ml·min\textsuperscript{-1}·kg\textsuperscript{-1}</th>
<th>V\textsubscript{TH}, %VO\textsubscript{2max}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>38</td>
<td>22.8 ± 3.0</td>
<td>164.2 ± 6.1</td>
<td>56.8 ± 8.0</td>
<td>21.5 ± 6.1</td>
<td>39.0 ± 8.1</td>
<td>55.7 ± 9.4</td>
</tr>
<tr>
<td>Men</td>
<td>44</td>
<td>23.9 ± 5.6</td>
<td>179.8 ± 7.2</td>
<td>78.3 ± 12.5</td>
<td>14.3 ± 5.6</td>
<td>47.3 ± 8.3</td>
<td>51.8 ± 11.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. VO\textsubscript{2max}, maximal O\textsubscript{2} uptake; V\textsubscript{TH}, ventilatory threshold determined by V-slope method.
Table 3. Parameters for the VO2 kinetic model

<table>
<thead>
<tr>
<th>Group</th>
<th>(\alpha_F), ml/min</th>
<th>(\alpha_C), ml/min</th>
<th>(\tau_F), s</th>
<th>(\tau_C), s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>255 ± 68</td>
<td>330 ± 178</td>
<td>13 ± 7.0</td>
<td>14 ± 9.2</td>
</tr>
<tr>
<td>Men</td>
<td>319 ± 92</td>
<td>359 ± 178</td>
<td>12 ± 5.9</td>
<td>16 ± 10.4</td>
</tr>
<tr>
<td>Combined</td>
<td>284 ± 86</td>
<td>343 ± 178</td>
<td>12 ± 6.5</td>
<td>15 ± 9.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. TD, time delay; \(\alpha_F\), resting VO2; \(\alpha_C\) and \(\tau_F\), asymptote and time constant for fast (fat) oxidative term; \(\alpha_C\) and \(\tau_C\), asymptote and time constant for slow (carbohydrate) oxidative term. \(\tau_F\) vs. \(\tau_C\) are significantly different, \(P < 0.0001\).

presented in Table 3. No significant differences \(P > 0.05\) were found for the women and men. Comparison of asymptotes \((\alpha_F\) and \(\alpha_C\)) and time constants \((\tau_F\) and \(\tau_C\)) by dependent (paired) t-test for the combined group indicated that only the latter was significant \(P < 0.0001\).

The parameters of the model were grouped by RER in 0.05-unit increments (except for values <0.8, which were lumped together) and analyzed by one-way ANOVA. As shown in Table 4, the TD was unchanged over the range of RER groupings. Similarly, \(\tau_F\) was invariant with respect to RER = 0.78–0.97. In contrast, \(\tau_C\) was inversely related to RER \((r = -0.97)\). That is, for RER = 0.78 the slow time constant was 163 ± 59 s, but at RER = 0.97 the time constant of 14 ± 7.9 s was significantly faster (Table 4). At RER < 0.92, \(\tau_F\) and \(\tau_C\) were different \(P < 0.05\). These findings are illustrated in Fig. 2 by the relationships between the time constants and RER. Figure 2 shows that \(\tau_C\) converges toward \(\tau_F\) as RER approaches unity, making it impossible to discern a second-order dynamic response at RER ≈ 0.92.

The fast \((\alpha_F)\) and the slow \((\alpha_C)\) steady-state parameters of the kinetic model were significantly related to RER (Table 4, Fig. 3; \(P < 0.0001\)). More specifically, the absolute rate of the fast component was inversely related to RER and approached zero at RER = 1.00. The slow component showed the opposite (positive) relationship with RER. Because RER approximates RQ at steady state for mild exercise, as studied here, these relationships with RER imply that the fast and slow components represent fat and CHO oxidation, respectively. An initial test of this assignment is provided by the expected relationship between RQ and the fractional oxidation of fat \((f_{fat})\) at steady state (Eq. 4): RQ = 1.00 - 0.293f_{fat}, where \(f_{fat} = [\alpha_F/\alpha_F + \alpha_C]\). This prediction is illustrated by the theoretical dashed line of Fig. 4. Also shown are values we obtained for \(f_{fat}\) for the RER groupings. Notice how closely they approximate the theoretical \(f_{fat}\) at RER = 0.78, 0.82, and 0.88.

At RER ≥ 0.92, there was a clear discrepancy between the estimated and theoretical \(f_{fat}\). This occurred where the time constants approached each other (Fig. 2), making it technically difficult to discern two unique components for VO2 kinetics for mild cycling exercise.

Equation 4 provides a method for calculating RQ and oxidative \(\text{VO}_2\) from \(f_{fat}\). The estimated oxidative and measured \(\text{VO}_2\) at steady state as well as the differences between them for the RER groupings are presented in Fig. 5, A and B, respectively. The discrepancies for the mean RERS of 0.92 and 0.97 were small \((-25 \pm 5.8\) and \(-41 \pm 4.7\) ml/min, respectively) but statistically significant \(P < 0.05\). However, the predicted and measured \(\text{VO}_2\) were strongly related (Fig. 6A; \(r = 0.99\)). The mean difference was \(-11 \pm 3.0\) (SE) ml/min (Fig. 6B) over the domain of \(\text{VO}_2\) evaluated.

DISCUSSION

The present study showed that 1) our double-exponential model adequately described the VO2 kinetics for the subjects with RERs < 0.92, 2) \(\alpha_F\) was inversely correlated, whereas \(\alpha_C\) was positively correlated with steady-state RER, and 3) TD and \(\tau_F\) were independent of RER, whereas \(\tau_C\) was inversely related to RER. These findings suggest that the relative contributions of fat and CHO to oxidative metabolism determine the relative contributions of the fast and slow components of VO2 kinetics and the speed of the slow component. The possibility that these relationships reflect cause and effect is supported by the accurate estimates of steady-state RQ and \(\text{VC}_{O_2}\) obtained with our kinetic model and indirect calorimetric paradigm. Therefore, it would appear that the fast component of \(\text{VO}_2\) represents fat oxidation while the slow component of \(\text{VO}_2\) is due to CHO oxidation.

Our findings show that steady-state \(\text{VO}_2\) increased in proportion to leg-cycling power output, indicating that aerobic metabolism behaves as a linear system, as has been consistently reported for exercise intensities be-

Table 4. Grouping by steady-state RER for the parameters of the \(\dot{VO}_2\) kinetic model

<table>
<thead>
<tr>
<th>Grouping by RER</th>
<th>n</th>
<th>Power, W</th>
<th>(\alpha_F), ml/min</th>
<th>(\alpha_C), ml/min</th>
<th>TD, s</th>
<th>(\tau_F), s</th>
<th>(\tau_C), s</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.78 ± 0.016</td>
<td>9</td>
<td>39</td>
<td>306 ± 88</td>
<td>557 ± 159</td>
<td>206 ± 104</td>
<td>12 ± 4.0</td>
<td>21 ± 7.1</td>
</tr>
<tr>
<td>0.82 ± 0.015</td>
<td>23</td>
<td>44</td>
<td>289 ± 83</td>
<td>445 ± 203</td>
<td>297 ± 160</td>
<td>12 ± 7.5</td>
<td>14 ± 9.2</td>
</tr>
<tr>
<td>0.88 ± 0.014</td>
<td>23</td>
<td>37</td>
<td>264 ± 95</td>
<td>255 ± 73</td>
<td>336 ± 135</td>
<td>12 ± 5.4</td>
<td>17 ± 9.7</td>
</tr>
<tr>
<td>0.92 ± 0.014</td>
<td>22</td>
<td>44</td>
<td>297 ± 89</td>
<td>272 ± 119</td>
<td>393 ± 124</td>
<td>13 ± 7.5</td>
<td>13 ± 9.7</td>
</tr>
<tr>
<td>0.97 ± 0.014</td>
<td>5</td>
<td>39</td>
<td>260 ± 44</td>
<td>211 ± 49</td>
<td>397 ± 94</td>
<td>15 ± 6.0</td>
<td>10 ± 9.0</td>
</tr>
</tbody>
</table>

Values are means ± SD. TD and \(\tau_F\) were not different, but \(\tau_C\) was significantly lower \(P < 0.05\), when respiratory exchange ratio (RER) changed from 0.78 to 0.97. Below RER of 0.92, \(\tau_F\) and \(\tau_C\) were different \(P < 0.05\).
Furthermore, VO₂ kinetics of mild exercise below VO₂TH were strongly associated with RER, as seen in Table 4. For RER < 0.92, VO₂ kinetics in phases 2 and 3 responded as a second-order linear system, inasmuch as respiration could be described by a fast and a slower exponential term. At RER ≥ 0.92, the system was statistically discerned only as a first-order linear system, i.e., with only a fast exponential term. This apparent change in the system’s order came about because the slow time constant was inversely related to RER. That is, as RER increased, the slow time constant became faster, thereby converging toward the invariant fast time constant. This effect of
RER probably is an important reason why we (6) and others (14, 44) could only describe \( \dot{V}O_2 \) kinetics with a monoexponential model for exercise intensities below \( V_{\text{TH}} \). Our experimental evidence helps explain this apparent transition in the system’s order over the domain of RER.

It is thought that the rapid rise in muscle \( \dot{V}O_2 \) at the onset of exercise represents the oxidation of CHO. However, our finding of an inverse relationship between the fast \( \dot{V}O_2 \) component and RER suggests that this component reflects fat oxidation, not CHO oxidation. This is supported by the finding that our model accurately predicts steady-state RQ and oxidative \( \dot{V}CO_2 \) when the fast term is assigned to fat oxidation. Therefore, the transition from a double to a single exponential should occur as RER approaches unity, because the contribution of fat would progressively decrease and disappear at an RER of unity, leaving only CHO as the sole oxidative substrate. Unfortunately, it was difficult to identify the two components at RER \( \approx 0.92 \). This practical limitation of the model probably is due to the inherent breath-by-breath noise and to the variability in the time constants in individuals with different muscle fiber type composition and recruitment patterns, which may affect \( \dot{V}O_2 \) kinetics independent of RER (24). These factors, combined with the acceleration of the slow time constant, make it technically difficult to discern two dynamic components at high RERs. Nevertheless, it is expected that detection will improve at exercise intensities higher than that for very mild exercise employed in this study because of greater signal-to-noise ratio for breath-by-breath \( \dot{V}O_2 \).

We have presented only correlational evidence in support of our model. Preliminary results from research in progress show that dietary-induced metabolic adaptations alter \( \dot{V}O_2 \) kinetics as predicted by our model. That is, metabolic adaptations to a fat diet increase the contribution of the fast (fat oxidation) component and reduce the contribution of the slow (CHO oxidation) term. The converse occurs when adaptations are induced by a CHO diet. Figure 7 is an example of these preliminary results in one subject. Notice the slow rise in \( \dot{V}O_2 \) for the subject on the fat diet (Fig. 7A) that appears to disappear on the CHO diet (Fig. 7B). In both cases, there is excellent agreement between the predicted RQ and measured steady-state RER. These results are encouraging, but we must reserve judgment until a more complete assessment of

![Fig. 6. Comparison of measured and predicted \( \dot{V}CO_2 \): individual responses (A) and differences (B). Coefficient of determination (R²) was 0.99 (A), and difference (mean ± SE) was \(-11 ± 3.0 \text{ ml/min} \) (B); 95% confidence interval is shown relative to mean difference (B).](http://jap.physiology.org/)

![Fig. 7. Representative effect of fat (A) and CHO (B) diets on \( \dot{V}O_2 \) kinetics during mild leg-cycling exercise at 39 W in a man. For fat diet, solution to model Eq. 2 was as follows: \( \dot{V}O_2 = 481 + 607 \text{exp}(t - 15.6/17.5) + 234 \text{exp}(t - 15.6/197.8) \), R² = 0.92, measured RER = 0.79 vs. predicted respiratory quotient = 0.79. For CHO diet, solution to model Eq. 2 was as follows: \( \dot{V}O_2 = 239 + 456 \text{exp}(t - 4.6/32.5) + 479 \text{exp}(t - 4.6/42.4) \), R² = 0.98, measured RER = 0.87 vs. predicted respiratory quotient = 0.86.](http://jap.physiology.org/)
dietary effects can be made and other experimental tests of the model are undertaken and reported.

Other considerations suggest that the dynamics of muscle fat oxidation are fast and represent oxidation of intramuscular TGs, whereas the slow component represents primarily glycogen oxidation during the transition to the initial steady state (5–10 min). At the initiation of all intensities of exercise, glycogenolysis is rapidly activated. Wahren et al. (41) showed that, during the first 2 min of forearm exercise, net glucose is released, indicating that glycogen is the primary CHO substrate utilized during this early period. Connett et al. (10) found in contracting dog gracilis muscle that the initial burst in the glycolytic rate is independent of muscle Po2 and Vo2. Lactate rapidly accumulated, indicating a mismatch between pyruvate production and oxidation. They suggested that aerobic glycolysis functions to reduce the cytosolic redox state via lactate accumulation to support mitochondrial fat oxidation. Consistent with this suggestion are the results of computer simulation studies on work-work transitions in the pyruvate-perfused heart. Garfinkel et al. (15) showed that pyruvate is mainly removed by lactate accumulation in the first 30 s of a step increase in work. Thus their findings are consistent with the view that intramuscular TG oxidation rapidly increases to support aerobic production of ATP with a step increase in work.

However, studies on net utilization of muscle TG have reported conflicting results (34). Some studies (22, 38) have shown muscle TG concentration to be unaltered by exercise, whereas others (12, 18, 19, 35) have found exercise to decrease intracellular TG concentration. Intracellular TGs do turn over (35). Therefore, these discrepancies suggest that the balance between TG synthesis and utilization can be variable under various conditions of exercise. Differences between total fat oxidation by indirect calorimetry and plasma free fatty acid oxidation suggest that intracellular TG can account for ~50% of the fat oxidized at steady state during prolonged exercise (13, 16, 17, 20, 32).

It is not known what contribution intramuscular TG makes to oxidative metabolism during the transient period of exercise. However, skeletal muscle has the enzymatic pathway for quickly increasing lipolysis of intramuscular TG to support the rapid increase in Vo2 at the onset of exercise. Oscai and co-workers (31) studied an intramuscular TG lipase and showed that it is rapidly activated by protein phosphorylation mediated by cAMP-dependent protein kinase. Thus it is reasonable to assume that the activity of this lipase rapidly increases with the activation of phosphorylase at the onset of exercise. Presumably, β-oxidation also increases in concert with activation of lipolysis, inasmuch as the expected rise in AMP protein kinase would phosphorylate and inactivate acetyl CoA carboxylase, which is responsible for synthesis of malonyl CoA (47). Consequently, a rapid fall in malonyl CoA would diminish carnitine acyl transferase I, which is the rate-limiting enzyme in β-oxidation. Although more research on the dynamics of intracellular TG lipolysis is needed, these speculations suggest that the machinery is present and could activate intracellular TG oxidation rapidly to support the fast increase in muscle oxidation.

Consistent with this idea is indirect evidence which shows that the RER decreases at the start of phase 2 (24). Estimated “apparent” CO2 retention from the RER decline is markedly greater than can be accounted for by the alkalinization produced from net H+ removal and phosphocreatine utilization by the creatine kinase reaction (unpublished observations). Therefore, this suggests that fat oxidation likely is increasing faster than CHO oxidation during the early rise in Vo2. We have reviewed evidence elsewhere (26) which suggests that intramuscular TG is the immediate source of fatty acid oxidation and is rapidly oxidized. For example, Zierler (48) found that production of 14CO2 lags the uptake of 14C-labeled plasma fatty acids in humans at rest when the arteriovenous RQ across the muscle was low, indicating dominance of intracellular fat oxidation. Paul and Issekutz (32) observed a similar delay in plasma fatty acid oxidation at the onset of exercise in the dog, even though the specific activity of 14C-labeled plasma fatty acids was at steady state. This probably occurred because the intramuscular TG pool was not labeled adequately. Consequently, this resulted in a rapid increase in VCO2 from unlabeled TG. Also, Issekutz and Paul (20) showed that, after prolonged infusion of 14C-palmitate for 190 min to label muscle TGs, 14CO2 production rapidly increased during the onset of exercise, even though the specific activity of plasma 14C-palmitate had fallen to a nominal level 5 min into exercise after infusion was stopped. All these findings suggest that intramuscular TG is the immediate fuel for fatty acid oxidation at the onset of exercise.

Although it is unclear what specific factors influence the time constants, it is reasonable to assume that the fast time constant provides information related to the speed of fat oxidation. A comparison of our fast time constant with those reported for plasma 14C-palmitate oxidation demonstrates an important discrepancy between our data and the dynamics of plasma fatty acid oxidation. In this regard, plasma fatty acid oxidation probably does not contribute directly to the fast component during the initial phase of exercise, because its dynamic is very slow. Our fast time constant averaged 15 s (Table 3) and varied between 21 and 10 s for RERs of 0.78 and 0.97, respectively. In contrast, Havel et al. (16) reported that the time constant for plasma 14C-palmitate oxidation during walking at 3–4 miles/h in the fed state was 93–106 s and increased to 125–172 s in the fasted condition in six men. Notice that fasting slowed the kinetics, which is consistent with our finding that Vo2 kinetics are slow when RER is low. For more vigorous exercise, Friedberg et al. (13) reported a time constant of 126 s for plasma 14C-palmitate oxidation. Clearly, the time constant for the fast Vo2 component in our study is an order of magnitude faster than those reported for plasma fatty acid oxidation. Therefore, either our model is wrong or the fast Vo2 component reflects the oxidation of intramuscular TGs.
In summary, the dynamics of \( \dot{V}O_2 \) during the rest-work transition are influenced by RER. Statistically modeling \( \dot{V}O_2 \) kinetics as a double exponential and assigning the fast and slow terms to fat and CHO oxidation, respectively, were shown to accurately predict steady-state RQ and oxidative V\( \dot{C}O_2 \) over a wide range of RERs for mild exercise. These results are consistent with available data and our theory, which proposes that the rapid rise in muscle V\( \dot{O}_2 \) involves a concerted interaction of modulators of the redox and phosphorylation states that feed back to modify the contraction-induced activation of intramuscular TG lipolysis, \( \beta \)-oxidation, and glycogen utilization. If future experiments confirm the validation of this model and paradigm, then we will have a new quantitative method for assessing the gross dynamics of energy transfer and fat and CHO oxidation, which can be used to investigate various aspects of exercise metabolism and respiratory control in vivo.

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