Determinants of the variability in respiratory exchange ratio at rest and during exercise in trained athletes

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Goedecke, Julia H., Alan St Clair Gibson, Liesl Grobler, Malcolm Collins, Timothy D. Noakes, and Estelle V. Lambert. Determinants of the variability in respiratory exchange ratio at rest and during exercise in trained athletes. Am J Physiol Endocrinol Metab 279: E1325–E1334, 2000.—We examined the variability and determinants of the respiratory exchange ratio (RER) at rest and during exercise in 61 trained cyclists. Fasting (10–12 h) RER was measured at rest and during exercise at 25, 50, and 70% of peak power output (Wpeak), during which blood samples were drawn for [lactate] and [free fatty acid] ([FFA]). Before these measurements, training volume, dietary intake and muscle fiber composition, [substrate], and enzyme activities were determined. There was large interindividual variability in resting RER (0.718–0.927) that persisted during exercise of increasing intensity. The major determinants of resting RER included muscle glycogen content, training volume, proportion of type 1 fibers, [FFA] and [lactate], and %dietary fat intake (adjusted r2 = 0.59, P < 0.001). Except for muscle fiber composition, these variables also predicted RER at 25, 50, and 70% Wpeak to different extents. The key determinant at 25% Wpeak was blood-borne [substrate], at 50% was muscle [substrate] and glycolytic enzyme activities, and at 70% was [lactate]. Resting RER was also a significant determinant of RER at 25 (r = 0.60) and 50% (r = 0.44) Wpeak.

It has been recognized that both dietary fat and carbohydrate (CHO) serve as substrates for energy metabolism in exercising subjects, and that the relative contribution of these two substrates to power production can be influenced by factors such as the preexercise diet (3, 5, 38, 39), training status (3, 6, 8, 28), exercise intensity (3, 6, 37), and the relative hormonal milieu during exercise (17).

However, there also appears to be individual variability in substrate oxidation during exercise in both untrained (22, 43) and trained (5, 36) subjects. This variability in substrate utilization during exercise may be related to differences in skeletal muscle characteristics. Indeed, Wade et al. (43) found that the respiratory exchange ratio (RER) during mild exercise (100 W) in fed subjects was inversely associated with the proportion of type I (slow-twitch) muscle fibers. More recently, Geerling et al. (18) and Helge et al. (22) were unable to corroborate these findings in untrained subjects exercising at the same relative exercise intensity [55% relative exercise intensity (V˙O2 max)] in the fasted state. Moreover, Zurlo et al. (47) also failed to demonstrate a relationship between 24-h RER (24hRER) and muscle fiber composition in a small sample of non-obese, sedentary men and women. However, they did demonstrate an inverse correlation between 24hRER and muscle β-hydroxacyl-CoA dehydrogenase activity, even after adjusting for age, sex, and %body fat [r = −0.70, P = 0.005 (47)].

Further studies examining the variability and determinants of substrate utilization were undertaken in untrained, often obese, subjects fed a standardized diet in a metabolic chamber. In a recent study of 71 healthy siblings from 31 families, Toubro et al. (42) found that a substantial part of the interindividual variation in 24hRER could be explained by age, gender, energy balance, current dietary intake, and fasting plasma insulin and free fatty acid (FFA) concentrations. However, they also found that there was a strong familial resemblance in 24hRER, which had previously been shown by Zurlo et al. (46).

However, there are no studies, of which we are aware, that have comprehensively examined the variability and possible determinants of substrate utilization both at rest and during exercise in trained athletes eating their habitual diets. Variability in substrate utilization in trained athletes has only been examined indirectly, as demonstrated by two recent studies from this laboratory (5, 36). These studies demonstrated that not all trained cyclists could increase the contribution of fat to oxidative metabolism during prolonged exercise trials associated with muscle glycogen depletion (5, 36). The cyclists who did not increase their relative rates of fat oxidation during prolonged steady-state exercise had higher starting and overall rates of CHO oxidation and fatigued earlier than those cyclists.
who had higher relative rates of fat oxidation (and lower rates of CHO oxidation).

Therefore, the aim of the present study was, first, to examine the variability in fasting whole body substrate utilization (RER) at rest and during steady-state exercise at different intensities in a group of cyclists with above-average performance ability. Second, this study aimed to identify relationships between substrate utilization at rest and during exercise, as well as possible determinants of RER, including skeletal muscle characteristics, training, dietary intake, and body composition.

METHODS

Subject Selection and Sampling

Forty-five male and sixteen female endurance-trained cyclists participated in this investigation, which was approved by the Research and Ethics Committee of the Faculty of Health Science of the University of Cape Town. To participate in the study, subjects had to: 1) have completed a local 104-km cycle race in <3.5 h for men and in <4 h for women and 2) have no known metabolic conditions that may have adversely affected intermediary metabolism (e.g., diabetes, thyroid hormone disorder, hyperlipidemia). Subjects were not taking any medications for chronic conditions such as high blood pressure (e.g., β-adrenergic receptor antagonists) or stimulants for conditions such as asthma (e.g., β-adrenergic receptor agonists). The subjects were informed of the nature of the trial, and all potential risks and benefits were explained to them. Informed written consent was obtained before the start of the trial.

Females were used in the study to characterize any differences in substrate utilization at rest and during exercise between males and females. Because of time and logistical constraints, we were unable to control for the menstrual cycle in the female subjects.

Preliminary Testing

Anthropometry. Anthropometrical measurements, including the sum of seven skinfolds (biceps, triceps, subscapular, suprailiac, abdomen, thigh, and calf), waist and hip circumference, and muscle mass, were determined. Percentage body fat was estimated using the equations of Durnin and Womersley (13).

Peak power output and peak oxygen consumption. Peak oxygen consumption (V\text{O}_2\text{peak}) and sustained peak power output (W\text{peak}) were measured on an electronically braked cycle ergometer (Lode, Groningen, Holland) modified with toe clips and racing handle bars, as described previously by Hawley and Noakes (21). Work rates were started at 3.33 W/kg body mass for men and 150 W for women. After 150 s, the workload was increased by 50 W and then by 25 W every 150 s until the subjects were exhausted. Exhaustion was defined as a >10% reduction in pedaling frequency, or an RER of >1.10, or both. W\text{peak} was defined as the highest exercise intensity the subjects completed for 150 s, in W, plus the fraction of time spent in the final work rate multiplied by 25 W. The W\text{peak} values were used to determine the relative workloads of the subsequent experimental tests.

During the progressive exercise test, ventilation volume, oxygen uptake (V\text{O}_2), and CO\text{2} production (V\text{CO}_2) were measured over 15-s intervals by use of a breath-by-breath Oxycron Alpha Analyzer (Jaeger, Wuerzburg, Netherlands). Before each test, the gas meter was calibrated with a Hans Rudolph 3-liter syringe (Vacumed, Ventura, CA), and the analyzers were calibrated with room air and a 4% CO\text{2}-96% N\text{2} gas mixture.

Training history. A detailed retrospective training history was obtained from each subject. In addition, the subjects completed a training diary during the 2 wk preceding the experimental trial. Training was quantified according to intensity and duration in metabolic equivalents (METS, per week).

Dietary analysis. Three days before the experimental trial, the subjects completed a weighed dietary record. The dietary records were analyzed with the Food Finder program (Medtech, Medical Research Council, Tygerberg, South Africa) to determine the subjects’ energy intake and macronutrient consumption.

Experimental Trial

Muscle fiber type, substrate content, and enzyme activities. The day before the experimental trial, the subjects were instructed to train routinely for a duration not longer than 1 h before 1800. On the subsequent day, the subjects reported to the laboratory after a 10- to 12-h overnight fast. Resting muscle biopsies were taken from the vastus lateralis muscle of 56 of the subjects by means of the percutaneous needle biopsy technique. A portion of the muscle sample was frozen rapidly in liquid N\text{2} and stored at −80°C for subsequent analysis of glycogen and triglyceride content and enzyme activities, including the activities of carnitine acyltransferase (CAT), citrate synthase (CS), phosphofructokinase (PFK), hexokinase (HK), 3-hydroxyacyl-CoA dehydrogenase (3-HAD). The remaining sample was oriented and imbedded in Tissue-Tek (Miles Laboratories, Naperville, IL), frozen in liquid nitrogen-cooled n-pentane (Saarchem, Muldersdrift, South Africa), and stored at −20°C for subsequent fiber type determination by the myofibrillar ATPase (mATPase) method. Briefly, serial sections (10 μm for pH 9.4 and 20 μm for pH 4.3 and 4.6) were cut in a cryostat at −20°C. Adjacent muscle sections were assayed for mATPase at pH 9.4 after acidic (pH 4.3 or 4.6) and alkaline (pH 10.4) preincubation to identify the three major fiber types (12).

Before biochemical analysis of muscle glycogen and triglyceride content, a portion of the frozen muscle biopsy sample (~50 mg) was freeze-dried and dissected free of any visible fat or connective tissue. Muscle glycogen content was determined as glucose residues (glucose oxidase method; Glucose Analyzer 2, Beckman Instruments, Fullerton, CA) after hydrolysis of the muscle sample in 2 M HCl at 95°C for 3 h (35). Glycerol concentrations were measured using a commercial glycerol kit (Boehringer Mannheim, Mannheim, Germany) after the triglyceride was lipolyzed to glycerol and FFAs, as described by Kiens and Richter (30).

For the determination of muscle enzyme activities, 15–50 mg (wet wt) of muscle were homogenized on ice in a phosphate buffer (1:19 wt/vol) and sonicated on ice for 3 × 10 s (Virsonic 60, The Vitrus, New York, NY). CAT and PFK activities were measured using the method of Crabtree and Newsholme (10) and Ling et al. (32), respectively. HK and 3-HAD were measured using the techniques described by Bass et al. (2). CS activity was measured using the technique described by Srere (40). Enzyme activities are expressed relative to protein content and were assayed in duplicate by the Bio-Rad Protein assay (Bio-Rad Laboratories, Munich, Germany), with bovine serum albumin as a standard.

Steady-state RER. After the muscle biopsy, the subjects rested for 30 min and/or until their heart rate returned to prebiopsy levels. V\text{O}_2, V\text{CO}_2, and RER were then measured for...
15 min at rest and during a steady-state cycle ride at 25, 50, and 70% of \( W_{\text{peak}} \), respectively, in which a pedaling frequency of 90 rpm was maintained. These workloads corresponded to 41, 63, and 80% \( \dot{V}\text{O}2_{\text{peak}} \), respectively. Gas exchange measurements were recorded for 5 min at rest and at each exercise workload, after a 10-min “stabilization period,” as described above.

The reliability of the Oxycon Alpha Analyzer was tested on a weekly basis by burning absolute ethanol [99% analytical reagent, Associated Chemical Enterprises (Pty), Glenvista, South Africa] as a reference. The reliability of RER, at rest and during exercise at different exercise intensities, has been previously tested in our laboratory (unpublished observations). The coefficient of variation (CV) for RER at rest, tested previously in our laboratory (unpublished observations), was 2.1%. The CV for RER at 25, 50, and 70% of \( W_{\text{peak}} \), tested on nine endurance-trained male cyclists on three occasions, was 2.0, 1.5, and 1.4%, respectively. The intraclass correlation coefficients for RER at rest and at 25, 50, and 70% \( W_{\text{peak}} \) were 0.847, 0.658, 0.814, and 0.843, respectively.

**Blood sampling and analysis.** Blood samples (~6 ml) were drawn from a forearm vein at rest and during the last minute of each workload. One aliquot (3 ml) was placed in a tube containing potassium oxalate and sodium fluoride for subsequent analysis of plasma lactate concentrations. The remaining aliquot (3 ml) was placed in a tube containing gel and clot activator for determinations of serum (nonesterified) FFA concentrations. All samples were kept on ice until centrifuged at 3,000 rpm at 4°C for 10 min upon completion of the trial. The plasma and serum were then stored at −20°C for later analyses.

Plasma lactate concentrations (Lactate Pap, Bio Merieux, Marcy-L’Etiole, France) and serum FFA concentrations (FFA Half-micro test, Boehringer Mannheim) were determined via enzymatic spectrophotometric measurements with commercial kits.

### Statistical Analysis

Bivariate correlations were used to explore the relationships between steady-state RER, at rest and during exercise, and physiological characteristics, muscle characteristics, training, and diet. These variables were then used in multivariate analysis, by use of backward stepwise regression, to determine the model which best predicted RER at rest and during exercise at different intensities. A repeated-measures ANOVA was used to investigate differences in RER between males and females. All results are presented as means ± SD, and an α level of \( P < 0.05 \) was considered to be statistically significant.

### RESULTS

#### Subject Characteristics

The subject characteristics are presented in Table 1. Although the subjects performed at similar levels (completing a 104-km cycle race in the top 20% of the field), there was a large intersubject variation in body composition and physical work capacity (Table 1). The subjects’ reported energy and macronutrient intakes for the 3 days preceding the experimental trial are also presented in Table 1. As there were no differences between men and women for RER at rest and during exercise at 25, 50, and 70% of \( W_{\text{peak}} \) (Fig. 1), all data were combined for all analyses performed in this study.

#### Variability in RER

Resting RER ranged from 0.927 to 0.718, which corresponds to a relative rate of fat oxidation of 23 to 93% (Fig. 2A). As the exercise intensity increased, there was a corresponding increase in mean RER (Fig.
Mean RER increased from 0.817 ± 0.051 at rest to 0.862 ± 0.037, 0.900 ± 0.040, and 0.976 ± 0.043 at 25, 50, and 70% Wpeak, respectively. However, the variability in RER persisted during exercise at all intensities, with RER during submaximal exercise (50% Wpeak) ranging from 0.818 to 0.983 (Fig. 2B).

Correlations with RER at Rest and During Exercise

Bivariate analysis was used to describe the relationships between RER, at rest and during exercise, and different physiological and metabolic parameters (Table 2). Variables that were positively correlated with resting RER were the proportion of type I muscle fibers, resting muscle glycogen content, and plasma lactate concentrations. The proportion of type IIa muscle fibers, serum FFA concentrations, and dietary fat intake were negatively associated with resting RER. Resting RER was also significantly correlated with RER at both 25 and 50% Wpeak (r = 0.60, P < 0.0001, and r = 0.44, P < 0.0001, respectively) but not at 70% Wpeak (r = 0.17, not significant).

At 25% Wpeak, muscle fiber composition and glycogen content were not correlated with RER. However, serum FFA and plasma lactate concentrations, dietary fat intake, and training volume were significantly correlated to RER. At 50% Wpeak, serum FFA and plasma lactate concentrations, dietary fat intake, and training volume were significantly correlated with RER (Table 2). In addition, muscle triglyceride content and CS activity were negatively correlated with exercising RER, whereas the PFK-to-CS and HK-to-CS ratios (PFK/CS and HK/CS), both representing glycolytic flux, were positively correlated with RER at 50% Wpeak. At 70% Wpeak, only plasma lactate concentrations, HK/CS and PFK/CS, and training volume were significantly associated with RER (Table 2).

Multivariate Analysis for the Determination of RER at Rest

The variables, proportion of type I muscle fibers, muscle glycogen content, training volume, dietary fat intake, and resting serum FFA and plasma lactate concentrations were included in the model that accounted for 59% of the variance in resting RER (P < 0.0001, standard error of the estimate (SEE) = 0.035, Table 3). The proportion of type I muscle fibers was positively associated with resting RER and was the most important determinant of RER in this equation.

In addition to the muscle fiber composition, muscle glycogen content was also an important determinant of resting RER in this equation. Training volume, resting FFA concentration, and dietary fat intake were less well correlated, but still significant determinants of resting RER. Muscle enzyme activities, muscle triglyceride content, percent body fat, and VO2peak were not significant predictors of RER at rest and were therefore not included in the model predicting RER at rest.
Determinants of RER During Low- to Moderate-Intensity Exercise

The statistical model that best predicted RER at 25% W_peak still included muscle glycogen and training volume as important determinants but did not include muscle fiber type. Rather, muscle CS activity, serum FFA concentrations, and dietary fat intake contributed to the model significantly. When these variables were used in the equation, 45% of the variance in RER at 25% W_peak was accounted for (P < 0.0001, SEE = 0.029, Table 4). However, when resting RER was used as a variable in the equation, the adjusted R^2 increased to 0.575 (P < 0.0001, SEE = 0.029, Table 4). In this model, training volume and muscle glycogen content became less important determinants of RER at 25% W_peak, whereas the relative importance of the muscle enzymes, including CS and PFK activities, increased.

Although the intensity of the exercise bout doubled from 25 to 50% W_peak, the variables found to best predict RER were similar (Table 5). However, at this higher exercise intensity, muscle triglyceride content became a significant predictor of RER, whereas circulating FFA concentrations contributed less to the model than at 25% W_peak. This model accounted for 42% of the variance in RER at 50% W_peak (P < 0.0001, SEE = 0.031, Table 5).

When resting RER was used as a variable in the model to predict RER at 50% W_peak, the fit of the model improved (adjusted R^2 = 0.50, SEE = 0.029, P < 0.0001; Table 5). Other variables in the equation included the proportion of type I muscle fibers, muscle triglyceride content, and, less importantly, circulating plasma lactate concentrations. Interestingly, the proportion of type I muscle fibers was a better predictor of RER at 50% than the proportion of type IIa muscle fibers (b weighting = −0.47 and −0.16 for the proportions of type I and IIa fibers, respectively).

Determinants of RER During High-Intensity Exercise

During high-intensity exercise (70% W_peak), circulating plasma lactate concentration contributed significantly to the model predicting RER (Table 6). This model, which also included the variables of dietary fat intake, training volume, muscle glycogen content, and circulating FFA concentrations, accounted for 56% of the variance in RER (P < 0.0001, SEE = 0.031, Table 6). Dietary fat intake and training were both negatively associated with RER and contributed equally to the model, whereas muscle glycogen content was positively associated with RER but contributed less.

Possible Confounding Variables

There are a number of variables that were associated with, and may have confounded, the interpretation of the relationships with RER described above. Age was significantly correlated with percentage body fat and VO2 max (r = 0.34, P = 0.007 and r = −0.31, P = 0.015, respectively). Percent body fat correlated negatively with VO2 max (r = −0.75, P < 0.001) and positively with muscle triglyceride content (r = 0.30, P = 0.03). Training volume also correlated positively with VO2 max (r = 0.45, P = 0.001) and negatively with muscle glycogen content (r = −0.28, P = 0.01). Training volume also correlated positively with VO2 max (r = 0.45, P = 0.001) and negatively with muscle glycogen content (r = −0.28, P = 0.01).
Table 5. Multivariate analysis for RER at 50% $W_{peak}$

<table>
<thead>
<tr>
<th>Variable</th>
<th>$b$</th>
<th>$\beta$</th>
<th>$P$ level</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS activity</td>
<td>-0.408</td>
<td>-0.0003</td>
<td>0.002</td>
</tr>
<tr>
<td>Muscle glycogen content</td>
<td>0.376</td>
<td>0.0001</td>
<td>0.006</td>
</tr>
<tr>
<td>Training volume</td>
<td>-0.301</td>
<td>-0.002</td>
<td>0.019</td>
</tr>
<tr>
<td>Muscle triglyceride content</td>
<td>-0.254</td>
<td>-0.001</td>
<td>0.030</td>
</tr>
<tr>
<td>Dietary fat intake, %</td>
<td>-0.250</td>
<td>-0.001</td>
<td>0.033</td>
</tr>
<tr>
<td>[FFA] at 50% $W_{peak}$</td>
<td>-0.191</td>
<td>-0.038</td>
<td>0.114</td>
</tr>
</tbody>
</table>

$R = 0.704$, adjusted $R^2 = 0.421$, SEE = 0.031, $P < 0.0001$.

With Resting RER as a Variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>$b$</th>
<th>$\beta$</th>
<th>$P$ level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting RER</td>
<td>0.590</td>
<td>0.096</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Type I muscle fiber content</td>
<td>-0.341</td>
<td>-0.001</td>
<td>0.007</td>
</tr>
<tr>
<td>Muscle triglyceride content</td>
<td>-0.311</td>
<td>-0.0007</td>
<td>0.005</td>
</tr>
<tr>
<td>[Lactate] at 50% $W_{peak}$</td>
<td>0.270</td>
<td>0.012</td>
<td>0.016</td>
</tr>
</tbody>
</table>

$R = 0.739$, adjusted $R^2 = 0.503$, SEE = 0.029, $P < 0.0001$.

However, in these studies, the hormonal changes were not directly associated with changes in fasting plasma metabolite concentrations (22, 24). Furthermore, our measurements took place at a minimum of 40 min postbiopsy and were conducted in the entire group, thereby minimizing possible bias.

**Determinants of Fasting RER at Rest**

The determinants of resting RER included the proportion of type I muscle fibers, muscle glycogen content, dietary fat intake, training, and blood metabolites (plasma lactate and serum FFA concentrations), all of which accounted for ~56% of the variance in RER (Table 3). The positive association with type I (slow-twitch) muscle fibers and negative association with type IIa (fast-oxidative) muscle fibers and RER was unexpected. Type I muscle fibers more often use fatty acids as a fuel source, because they have a high oxidative capacity due to a high mitochondrial and capillary density (26). Moreover, Wade et al. (43) found that RER, measured during mild exercise at an absolute intensity of 100 W in 11 sedentary male subjects, was inversely related to the proportion of type I muscle fibers. However, Zurlo et al. (47), and more recently, Helge et al. (22) found no association between muscle fiber composition and RER in untrained subjects.

We cannot fully explain the reasons for the positive association between the proportion of type I muscle fibers and resting RER in this study. A possible explanation may be the coexistence of myosin heavy-chain isoforms for types I and IIa fibers found in endurance-trained subjects but not in sedentary individuals (31). If there is a large coexistence of type I and type IIa isoforms in the muscle samples, as expected in these trained cyclists, the conventional mATPase techniques for fiber typing may react histochemically as type I fibers, overestimating the proportion of type I fibers. However, we cannot exclude the possibility that the relationship between muscle fiber type and RER may be influenced by other factor/s not identified in this study.

**Association Between RER and Body Composition**

We found no association between fasting RER at rest and body composition as demonstrated by Zurlo et al. (46) and Weyer et al. (45) in untrained subjects measured using underwater weighing and total body dual-energy X-ray absorptiometry, respectively (Table 2). The lack of association between RER and body composition may be due to the fact that our subjects were

Table 6. Multivariate analysis of RER at 70% $W_{peak}$

<table>
<thead>
<tr>
<th>Variable</th>
<th>$b$</th>
<th>$\beta$</th>
<th>$P$ Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Lactate] at 70% $W_{peak}$</td>
<td>0.580</td>
<td>0.012</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dietary fat intake (%)</td>
<td>-0.324</td>
<td>-0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>Training volume</td>
<td>-0.300</td>
<td>-0.002</td>
<td>0.011</td>
</tr>
<tr>
<td>Muscle glycogen content</td>
<td>0.262</td>
<td>0.0001</td>
<td>0.02</td>
</tr>
<tr>
<td>[FFA] at 70% $W_{peak}$</td>
<td>-0.212</td>
<td>-0.068</td>
<td>0.04</td>
</tr>
</tbody>
</table>

$R = 0.782$, adjusted $R^2 = 0.562$, SEE = 0.031, $P < 0.0001$. |
leaner and had a smaller range of body fat (9–30%) compared with the subjects in the studies of Zulro et al. (46) (5–50%) and Weyer et al. (45) (3–53%). We also did not show any association between exercising RER and body composition, previously described by Wade et al. (43) \(r = 0.538, P < 0.001\) in 50 male subjects with a range in body fat similar to that in our study (4–28%). Their subjects were, however, untrained and were not fasted when RER was measured during mild exercise at the same absolute, but not relative, exercise intensity (100 W). In agreement with our findings, both Helge et al. (22) and Geerling et al. (18), who replicated the study of Wade et al. (43), failed to find a relationship between exercising RER and body fatness in untrained, healthy male subjects.

In our study and previous studies (1, 42) there is a consistent relationship between circulating FFA concentrations and RER (Tables 2 and 3). Havel et al. (20) demonstrated that most of the energy supplied to skeletal muscle at rest is derived from plasma FFAs. Furthermore, plasma FFA turnover is mainly controlled by substrate availability at rest (4) and is highly dependent on recent dietary intake (29). Therefore, preceding dietary intake can alter fuel utilization by modifying substrate availability (7). This relationship between dietary fat intake in the 3 days preceding the measurement of RER was demonstrated in the present study (Tables 2 and 3) and the study of Toubro et al. (42).

### Determinants of Fasting RER During Short-Term Steady-State Exercise

Another important finding of this study was that the major determinants of fasting RER at rest were different from those during exercise. Furthermore, as the intensity of the exercise increased, the variables used in the models to predict RER at the different exercise intensities changed, or their relative contribution to the model changed. At rest, muscle fiber composition was the most important determinant of fasting RER, but it did not contribute significantly to exercising RER. During low-intensity exercise (25% \(W_{peak}\)), blood-borne substrate concentrations were important determinants of RER, whereas during moderate-intensity exercise (50% \(W_{peak}\)), muscle substrate concentrations and enzyme activities became more important variables in predicting substrate utilization. At high-exercise intensities (70% \(W_{peak}\)), plasma lactate concentration was the most significant determinant of RER.

Muscle glycogen content was an important determinant of both resting and exercising substrate utilization in the fasted state. What may appear surprising is that this association was present at rest and during low-intensity exercise (25% \(W_{peak}\)) (Tables 3 and 4). Romijn et al. (37) found that, at 25% \(V_{O_2\ max}\) in trained subjects in the fasted state, the majority of energy was provided by plasma FFA and glucose, with little or no reliance on muscle glycogen. However, Friedlander and co-workers (15, 16) found that CHO accounted for >50% of total energy expenditure during exercise at 45% \(V_{O_2\ max}\) in untrained male and female subjects tested 2–3 h after they ingested a standardized meal (448 kcal) containing 80 g of CHO. It therefore appears that, irrespective of the exercise intensity, substrate oxidation may be regulated by substrate availability. Indeed, previous studies (5, 44) have shown that muscle glycogen utilization is determined, in part, by muscle glycogen content at the start of exercise.

Training volume was also a major determinant of RER, both at rest and during exercise (Tables 3–6). This is confirmed by numerous studies (3, 8, 28) that have demonstrated higher rates of fat oxidation (and lower rates of
CHO oxidation) in trained vs. untrained subjects, even during exercise at the same relative intensity.

As mentioned previously, blood-borne substrate concentrations, particularly serum FFA concentrations, were important determinants of RER during low-intensity exercise (25% \( \dot{W}_{\text{peak}} \)). However, as the exercise intensity increased to 50% \( \dot{W}_{\text{peak}} \), the relative weighting of serum FFA concentration decreased, and muscle triglyceride content became a more important determinant of RER (Tables 4 and 5). These findings are consistent with those of Romijn et al. (37) who found that peripheral lipolysis (and presumably oxidation) was higher during low-intensity exercise (25% \( \dot{V}_{\text{O}_2, \text{max}} \)) and declined progressively as the exercise intensity increased. Moreover, they showed that intramuscular triglyceride content, measured indirectly with RER and stable isotope tracers, did not contribute significantly to energy production at 25% \( \dot{V}_{\text{O}_2, \text{max}} \) (10% of total fat oxidized), whereas at 65% \( \dot{V}_{\text{O}_2, \text{max}} \), intramuscular triglyceride content accounted for 50% of the total fat oxidized.

Not only were intramuscular substrate concentrations important determinants of RER during exercise, but muscle enzyme activities contributed significantly to the model predicting RER, especially during moderate-intensity exercise (50% \( \dot{W}_{\text{peak}} \)). CS activity was inversely correlated with RER at 50% \( \dot{W}_{\text{peak}} \) (\( r = 0.30, P = 0.026 \), Table 2) and contributed significantly to the models that best predicted RER at 25 and 50% \( \dot{W}_{\text{peak}} \) (Tables 4 and 5). Both Zurlo et al. (47) and Helge et al. (22) failed to demonstrate this association, most likely because their subjects were untrained and had lower CS activities with a smaller range of values than our subjects. Rather, Zurlo et al. demonstrated an inverse relationship between 24hRER and 3-HAD activity (\( r = -0.75, P = 0.002 \), Ref. 38) that was not supported by our data (\( r = 0.008, P = 0.952 \)). This discrepancy in findings cannot be explained by differences in the range of values but may relate to methodological differences or to possible confounding factors outlined in Table 7.

We also demonstrated a positive relationship between RER and HK/CS and PFK/CS during moderate-intensity exercise (Table 2). These ratios provide an indication of glycolytic flux, which may attenuate fatty acid oxidation (39), possibly by controlling the rate of fatty acid transport into the mitochondria (38).

During high-intensity exercise (70% \( \dot{W}_{\text{peak}} \)), plasma lactate concentration was the most important determinant of RER in the regression equation (Table 6). During exercise of this intensity, CHO oxidation and muscle glycogen utilization are increased, with CHO providing more than two-thirds of the energy needed for exercise (for review, see Ref. 9). This results in accelerated rates of plasma lactate production, which may limit lipolysis (27). Moreover, during high-intensity exercise, FFA appearance in the plasma may also be decreased by entrapment of FFA within adipose tissue because of decreased blood flow (23).

Resting RER as a Determinant of Exercising RER

In this study we also found that resting RER, independent of all other factors, was a good predictor of RER during low- and moderate-intensity exercise \([r = 0.60 \text{ and } r = 0.44 \text{ at } 25 \text{ and } 50\% \dot{W}_{\text{peak}} \text{ (} P < 0.0001 \text{), respectively}]. Therefore, as exercise intensity increases from rest to exercise of increasing intensity, the short-term exercise RER of individuals with low resting RERs will increase but remain relatively low compared with those of individuals with high resting RERs, which will increase even further. When included in the regression equation to predict RER during both low- and moderate-exercise intensities, resting RER improved the fit of the models (Tables 4 and 5). This has important implications for exercise metabolism research because, first, it provides a useful tool to easily estimate relative substrate utilization in athletes without requiring that the athletes be studied during exercise. Second, in studies in which substrate utilization during exercise is under investigation, it may be important to consider the initial, individual fasting RER, because it may impact on the interpretation of these studies.

Gender Differences

In the present study, there was no difference in fasting RER between the male and female subjects at rest or during exercise (Fig. 1). When gender was forced into the model as a variable to predict RER at rest and during exercise, it did not contribute significantly to the model or alter its interpretation. These findings are contrary to the findings of Horton et al. (25), Friedlander and colleagues (14, 16), and Tarnopolsky et al. (41), who found that women tested in the midfollicular phase of the menstrual cycle tended to oxidize more fat during exercise at the same relative intensity than men. Although there was no significant difference in average training per day between the male and female subjects in the present study (13.5 vs. 14.6 METS/day, respectively), there was a significant difference in average training per day between the male and female subjects in the present study (13.5 vs. 14.6 METS/day, respectively), there was a significant difference in their dietary intake. Both energy intake and %dietary fat intake were significantly higher, and %CHO intake was significantly lower, in the male subjects than in the female subjects (12,642 ± 3,247 vs. 8,377 ± 2,654 kJ, 32.6 ± 6.5 vs. 27.0 ± 9.0% fat, and 45.6 ± 6.9 vs. 53.4 ± 9.2% CHO, for males and females, respectively, \( P < 0.01 \)). In contrast, in the studies of Tarnopolsky et al. (41) and Horton et al. (25), the dietary intake of the subjects was controlled for 2–3 days before testing. Although this discrepancy in CHO intake did not result in differences in muscle glycogen content of the male and female subjects (427 ± 126 vs. 436 ± 116 mmol/kg dry wt, respectively), it may have masked any potential gender differences in substrate utilization. Indeed, the female subjects had a significantly higher proportion of type I (slow-oxidative) muscle fibers than the male subjects (58.4 ± 10.0 vs. 49.6 ± 12.7%, respectively, \( P = 0.03 \), as previously shown (34). However, these data need to be interpreted with
caution because of the small number of females in this study.

Furthermore, the phase of the menstrual cycle was not controlled in the female subjects, which may have influenced the interpretation of the RER measurements. However, the results of existing research, examining whether the cyclic fluctuations of female sex hormones affect substrate utilization during exercise, are inconclusive (11, 19, 29). Moreover, Kanaley et al. (29) found that amenorrhea did not influence substrate utilization during prolonged exercise, compared with eumenorrheic athletes tested in the early and late follicular phases and midluteal phase of their menstrual cycle.

In the present study, we found a large variability in substrate utilization in a group of trained cyclists who have above-average performance ability. However, it is not known whether this variability in RER, measured during short-term steady-state exercise, will persist during more prolonged exercise, in the fed state, under conditions of low glycogen availability, or when CHO is ingested during exercise, for example.

The major determinants of this variability and/or their relative contribution to substrate utilization differed at rest and during exercise of different intensities. However, training, dietary intake, and the consequent muscle glycogen content and circulating substrates consistently predicted RER at rest and during exercise in this study. These findings suggest that, by manipulating training and/or dietary intake, an athlete’s ability to alter substrate utilization during steady-state exercise may be modified. Furthermore, resting RER was a significant independent determinant of RER at low and moderate intensities. This finding may have important implications for metabolic research, both as a tool for estimating intensities. This finding may have important implications for metabolic research, both as a tool for estimating

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