M. Riley · K. Wasserman · P.C. Fu · C.B. Cooper

Muscle substrate utilization from alveolar gas exchange in trained cyclists

Accepted: 15 April 1995

Abstract The respiratory exchange ratio (R) during steady-state exercise is equivalent to whole-body respiratory quotient (RQ), but does not represent muscle metabolism alone. If steady-state values of carbon dioxide production (\(V\)\(_{\text{CO}_2}\)) and oxygen uptake (\(V\)\(_{\text{O}_2}\)) are plotted for different work rates, the slope of the line fitting these points should estimate muscle RQ. Twelve cyclists randomly performed five 8-min, constant work rate tests of 40, 80, 120, 160 and 200 W. Whole-body R, averaged over the final 2 min of each exercise bout, increased with increasing work rate. When \(V\)\(_{\text{CO}_2}\) was plotted as a function of \(V\)\(_{\text{O}_2}\), the regression lines through the five points displayed excellent linearity, had negative y-intercepts, and a slope of 0.915 (0.043) [mean (SD)], which was greater than the whole-body R at any individual work rate [range 0.793 (0.027) at 40 W to 0.875 (0.037) at 200 W]. This slope was comparable to the lower slope of the \(V\)\(_{\text{CO}_2}\) versus \(V\)\(_{\text{O}_2}\) plot of an increasing work rate (ramp) protocol [0.908 (0.054)]. We conclude that, during mild and moderate exercise of relatively short duration, contracting muscle has a high and constant RQ, indicating that carbohydrate is the predominant metabolic substrate. Whole-body R does not accurately reflect muscle substrate utilization and probably underestimates muscle RQ at a given work rate.

Key words Cycle ergometer exercise · Indirect calorimetry

Introduction

Indirect calorimetry from alveolar gas exchange has been used as a means of assessing muscle metabolism during exercise for nearly a century (Chaveau 1896; Krogh and Lindhard 1920; Christensen and Hansen 1939). The respiratory exchange ratio (R), i.e. the ratio of \(CO_2\) production (\(V\)\(_{\text{CO}_2}\)) to \(O_2\) consumption (\(V\)\(_{\text{O}_2}\)), differs depending on the proportions of lipid and carbohydrate being oxidized, being 1.0 for exclusive carbohydrate utilization and approximately 0.7 for exclusive fat utilization (Jéquier and Felber 1987). R measured under steady state conditions is equivalent to the whole-body respiratory quotient (RQ). During conditions before steady state is established, changing body gas stores preclude the use of R as an accurate reflection of whole-body RQ.

Examination of R has been used to provide information on changes in muscle metabolism with dietary manipulation. With a high carbohydrate diet, R rises and with a high fat diet, it falls (Krogh and Lindhard 1920; Christensen and Hansen 1939). Resting R in the non-fasting state is generally about 0.8, indicating mixed fat and carbohydrate oxidation, with a small contribution from amino acid oxidation. R usually rises with exercise (Gollnick 1985) and increases progressively with increasing exercise work rate. This has been used to infer an increasing reliance on carbohydrate substrate by muscle (Christensen 1932; Edwards et al. 1934; Havel 1971; Gollnick 1985; Saltin 1990).

Muscle metabolism accounts for approximately 20% of resting \(V\)\(_{\text{O}_2}\) (Passmore and Draper 1964), and this may rise to approximately 90% during heavy exercise (Sullivan et al. 1987). We hypothesized that if muscle substrate RQ is greater than the average of other organs, whole-body R is likely to underestimate working muscle RQ, due to the dilution effect from other organs. Refinements in the techniques used for estimation of muscle metabolism have been developed. However,
these are invasive and include measurement of gas exchange directly across the exercising limb, arterial-venous differences in substrate concentrations, and percutaneous muscle biopsy (Gollnick et al. 1972; Essén et al. 1977). In addition estimations of the gas content of blood draining from exercising muscle may be subject to considerable error (Ungerer et al. 1990).

Measurement of alveolar gas exchange is both non-invasive and accurate (Huszczyk et al. 1990). If it were possible to identify the component of the total gas exchange due to exercising muscle, this methodology would be preferable to the invasive techniques described above. Increments in gas exchange between levels of constant work intensity should mostly represent changes in muscle metabolism, since muscle is the main tissue changing its metabolic rate. In a similar fashion, the initial slope of the relationship of $1/\text{CO}_2$ to $1/\text{O}_2$ during a single incremental exercise test [V-slope plot (Beaver et al. 1986)] may represent muscle metabolism (Cooper et al. 1992), assuming gas stores are not changing. In the latter study, exercise under conditions of muscle glycogen depletion resulted in a lower slope than during glycojen replete conditions. This was consistent with previous invasive work that demonstrated an increase in muscle lipid metabolism when glycogen is unavailable (Jansson and Kaijser 1982). Above the anerobic threshold (AT), the $V\text{CO}_2 - V\text{O}_2$ slope rises more steeply due to buffering of lactate by bicarbonate, releasing non-metabolic CO_2. The upper portion of the plot is not therefore a consequence of aerobic metabolism alone.

The aim of the current study was to estimate muscle metabolism accurately from alveolar gas exchange, utilizing five separate constant work rate exercise intensities. Gas exchange was measured during periods following achievement of steady-state conditions. The changes in $V\text{CO}_2$ and $V\text{O}_2$ resulting from changes in work rate were calculated. This analysis provided a means of excluding the metabolism of other tissues which may comprise a significant part of the total gas exchange measurement at any particular work rate. The increments in $V\text{CO}_2$ and $V\text{O}_2$ among the steady-state tests were then compared with the initial slope of the V-slope plot, to determine if the latter could provide a reliable estimate of muscle metabolism.

**Methods**

Subjects

Twelve competitive racing cyclists, all involved in training programs, acted as subjects. Their mean (SD) age was 35.3 (7.8) years, and their mean weight, 82.7 (8.6) kg. Each subject completed all tests apart from subject 6 who did not carry out the 80-W and 120-W constant work rate tests (see below).

Ethical approval

Ethical approval for the study was granted by the Harbor-UCLA Human Subjects in Research Review Committee. Informed consent was given by all subjects.

Experimental protocol

The subjects presented to the laboratory after an 8-h fast at 7 a.m. on 2 non-consecutive days. Tobacco and caffeine were prohibited during the period of fasting although water was permitted. A Teflon cannula was inserted into an antecubital vein, and, after familiarization with the equipment, three exercise tests separated by 15 min rest were performed on each day. The exercise comprised (1) a 40-W min$^{-1}$ continuous incremental ramp protocol which was always performed as the final test on day 1, and (2) five 8-min, constant-load exercise tests with work rates of 40, 80, 120, 160 and 200 W in random order, two on day 1 and three on day 2. All tests were preceded by 4 min rest, and in the case of the ramp, by a further 3 min of unloaded pedalling. Exercise tests were performed using an electrically braked cycle ergometer (Ergometrics 900; Ergoline, Bitz, Germany). Gas exchange was determined breath-by-breath using a metabolic cart (SensorMedics 2900; Yorba Linda, Ca, USA). Calibration of the cart was performed prior to each exercise bout with a precision syringe and gases of known concentration. During all five constant work rate tests blood samples were drawn for measurement of lactate, glucose, glycerol and free fatty acids (FFA). Sampling was performed at rest, after 4 min of exercise, immediately prior to the end of each exercise bout and after 2 min recovery. The venous cannula was flushed with 0.9% saline avoiding the use of heparin. Blood samples for glucose and lactate were drawn into heparinized syringes and held on ice for no more than 20 min prior to analysis using a rapid analyzer (Yellow Springs Instruments, Yellow Springs, Ohio). Samples for FFA and glycerol were allowed to clot at room temperature and the serum separated and stored at $-20^\circ\text{C}$. FFA were assayed by an enzymatic colorimetric method (Wako Laboratories, Va., USA) as was glycerol (Randox Laboratories, Northern Ireland). The coefficients of variation for the metabolite assays were: FFA 4.5%, glycerol 3.1%, glucose 1.7% and lactate 2.4%.

Data analysis

**Ramp test**

$V\text{CO}_2$ as a function of $V\text{O}_2$ was plotted from moving averages of nine consecutive breaths. Analysis was then performed using the method of Beaver et al. (1986), eliminating the data from rest and the 1st min of exercise, when transient changes in CO\textsubscript{2} stores are known to occur. Data were also eliminated during very heavy exercise above the respiratory compensation point, when acute hyperventilation occurs. Between these upper and lower limits two straight lines were fitted by linear regression analysis so as to minimize the total residual sum of squares. The AT was defined as the point of intersection of these lines (Cooper et al. 1992). The slope ($S_1$) of the lower line was compared with the slope derived from the constant work rate tests (Fig. 1).

**Constant work rate tests**

Breath-by-breath $F\text{O}_2$ and $F\text{CO}_2$ data were averaged for the final 2 min (minutes 6–8) of each exercise period.