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FACTORS AFFECTING THE COMPONENTS OF THE ALVEOLAR CO_2 OUTPUT–O_2 UPTAKE RELATIONSHIP DURING INCREMENTAL EXERCISE IN MAN

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SUMMARY

The $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ (alveolar CO_2 output–alveolar O_2 uptake) relationship (V-slope) during increasing work rate (ramp) cycle ergometer exercise has two approximately linear components: a lower component slope (S_1) with a value of about 0·95 and a steeper, upper component (S_2). We examined the effect of muscle glycogen depletion (protocol 1) and the rate of increase in work rate (ramp rate) without muscle glycogen depletion (protocol 2) on S_1 and S_2. In protocol 1, ten healthy men with a mean age of 31·4 years (S.D. 6·2) were studied on each of 3 days (days 1 and 3 were control days). They performed a ramp exercise test to maximum tolerance and steady-state tests at rest, during unloaded pedalling and at two constant work rates below their anaerobic threshold (AT). To deplete muscle glycogen before the test on day 2, the subjects performed 2 h of very heavy cycle exercise on the preceding day and fasted overnight. S_1 was reduced on day 2 (0·79 compared with 0·95, $P < 0·001$), as was the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ slope derived from steady-state measurements (0·81 compared with 0·99, $P < 0·001$), but AT and the slope difference ($S_2 - S_1$) were unchanged. In protocol 2, seven healthy men with a mean age of 20·6 years (S.D. 2·4) performed ramp tests at three different rates of increasing work rate (15, 30 and 60 W min^{-1}), each ramp rate being performed twice in random sequence. The ramp rate did not affect S_1 but S_2 was steeper with the faster rates of work rate increase (1·27, 1·43 and 1·63, respectively, $P < 0·01$). Our findings support the concept that the lower component of the $\dot{V}$-slope plot (below AT) represents muscle substrate respiratory quotient (RQ) while the difference between S_1 and S_2 reflects 'excess CO_2' derived from bicarbonate buffering of lactic acid.

INTRODUCTION

Beaver, Wasserman & Whipp (1986) described the use of a continuous plot (V-slope) of alveolar carbon dioxide output ($\dot{V}_{CO_2}$) as a function of alveolar oxygen uptake ($\dot{V}_{O_2}$) to determine the anaerobic threshold (AT) during a progressively increasing work rate (ramp) exercise test. They found that this plot had two linear components: a lower component (S_1) assumed to reflect aerobic metabolism only, and an upper component (S_2) thought to reflect aerobic metabolism with additional CO_2 produced from bicarbonate buffering of lactic acid. The objective of this study was to challenge the physiological mechanisms hypothesized to underlie the two linear components of the V-slope plot.

In order to test the hypothesis that the slope of the lower component was primarily dependent on the substrate being used for exercise, we contrasted the lower and upper component slopes for increasing work rate on a bicycle ergometer with and without prior glycogen depletion of the muscles involved in cycle exercise. A shift towards fatty acid metabolism should reduce S_1 but not affect the difference between the lower and upper components ($S_2 - S_1$).

In order to test the hypothesis that the upper linear component was steeper due to 'excess

CO_2' evolved from bicarbonate buffering of lactic acid, in addition to CO_2 generated from aerobic metabolism, we studied the upper and lower component slopes at three different rates of increasing work rate (ramp rates). Faster accumulation of lactic acid with increasing ramp rates should effect a steepening of S_2 without changing the respiratory quotient (RQ) of the substrate (S_1) and the break-point reflecting the AT.

These two protocols were complementary in that they were designed to affect differently the lower and upper linear components of the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ relationship during ramp exercise.

METHODS

Seventeen healthy male volunteers were used in these experiments. The study was approved by the Harbor–UCLA Human Subjects in Research Review Committee and the subjects gave fully informed consent.

Protocol 1: effect of glycogen depletion

Ten subjects with a mean age of 31·4 years (S.D. 6·2) were studied on each of 3 days. The characteristics of each subject are shown in Table 1. The studies were performed at the same time of day with the same relationship to meals on the control days. The exercise tests were the same for each subject on each study day. Each subject performed three steady-state tests and a ramp test to exhaustion as described by Whipp, Davis, Torres & Wasserman (1981). The ramp rate was 20 W min^{-1} for all but one subject whose level of fitness exceeded that of the others. In the latter subject, it was 40 W min^{-1}. Two minutes into the recovery period of each ramp test, venous blood was drawn from an antecubital vein for the measurement of lactate, pyruvate and catecholamines. The steady-state tests were performed, each for 4 min, at unloaded pedalling and at two other constant work rates (see Table 1 for work rates). The work rates were selected according to the level of fitness of each individual subject with the intention that the highest constant work rate test would be just below the AT and the intermediate work rate be midway between this work rate and unloaded cycling.

During the afternoon following the first assessment, the subjects performed 2 h of outdoor, hill cycling exercise (estimated $\dot{V}_{O_2}$ being 2–3 l min^{-1}) and refrained from eating until after the study on the second day. The metabolic rate demanded by this procedure is known, from previous studies, to deplete muscle glycogen in the working muscles to less than 30% (Gollnick, Piehl & Saltin, 1974). The overnight fast was intended to minimize muscle glycogen replacement by the liver and to allow fatigued muscles to repair. The subjects resumed a normal diet after the test on the second day. The final (third) assessment was made 48 h later, under identical conditions to the first day. This test was regarded as a second control study, the subjects having returned to normal diet and activity for 2 days.

Protocol 2: effect of increasing work rate increment

Seven subjects with a mean age of 20·6 years (S.D. 2·4) were studied. The characteristics of each subject are shown in Table 2. Each subject performed ramp exercise tests on the cycle ergometer at three different ramp rates (15, 30 and 60 W min^{-1}). Each rate was performed twice yielding six studies for analysis for each subject except in subjects 5 and 7 in whom 60 W min^{-1} was performed only once because of technical problems. The order of the tests was randomized and the subjects were not aware of the ramp rate during the tests. The order of testing is shown in Table 2 for each subject.

Measurements

The exercise tests were performed using an electrically braked cycle ergometer (Gould-Godart BV, The Netherlands). The subjects breathed through a low-impedance turbine transducer (Alpha Technologies Inc., USA) for the measurement of inspired and expired volumes. The dead space of the mouthpiece and turbine device was 90 ml. Gas was withdrawn at 1 ml s^{-1} from the mouthpiece by a mass spectrometer (MG 1100; Perkin-Elmer Inc., USA) for the measurement of fractional concentrations of O_2, CO_2 and N_2. A computer (HP1000; Hewlett–Packard Inc., USA) aligned the gas concentrations and volume signals for the sampling transit delay and response time of the mass spectrometer. The signals then underwent analog-to-digital conversion and the computer calculated cardiopulmonary variables breath by breath using previously described algorithms (Beaver, Lamarra & Wasserman, 1981).

Table 1. *Age, height, weight and work rates of subjects studied with and without glycogen depletion (protocol 1)*

Subject no.	Age (years)	Height (m)	Weight (kg)	Rate of increase in work rate (W min^{-1})	Steady-state work rate (W)
1	35	1·90	84·0	20	0, 40, 80
2	32	1·80	80·0	20	0, 30, 60
3	45	1·81	82·7	20	0, 40, 80
4	29	1·83	83·6	20	0, 30, 60
5	35	1·75	68·2	20	0, 40, 80*
6	31	1·78	67·5	40	0, 80, 160
7	31	1·80	74·0	20	0, 30, 60
8	23	1·76	90·5	20	0, 50, 100
9	25	1·73	58·2	20	0, 30, 60
10	28	1·80	70·0	20	0, 30, 60*

* These work rates were above AT on glycogen-depleted days.

Table 2. *Age, height, weight and work rates of subjects studied with different rates of work rate increase (protocol 2)*

Subject no.	Age (years)	Height (m)	Weight (kg)	Order of incremental tests (W min^{-1})
1	21	1·69	60·0	60, 30, 30, 15, 60, 15
2	19	1·83	82·1	60, 30, 15, 30, 15, 60
3	23	1·79	82·7	30, 60, 15, 30, 15, 60
4	21	1·66	74·1	15, 30, 60, 15, 60, 30
5	24	1·87	100·0	15, 60, 30, 30, 15
6	18	1·89	82·7	60, 30, 15, 60, 15, 30
7	18	1·75	75·9	30, 15, 15, 30, 60

Venous blood lactate was determined using the enzymatic technique (Behring Diagnostics, USA) which forces the complete conversion of lactate to pyruvate by lactic dehydrogenase, the concentration of lactate being proportional to the change in photometric absorbance. Pyruvate was determined by an enzymatic fluorometric method (Olsen, 1971) and catecholamines were determined by radioenzymatic method and rapid thin-layer chromatography (Peuler & Johnson, 1977).

Data analysis

The relationship between $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ during the progressive non-steady-state tests was plotted from the breath-by-breath data files deriving rolling averages over nine consecutive breaths. The relationship was then analysed by the method of Beaver *et al.* (1986) which eliminates data from the first minute of exercise, when transient changes in CO_2 stores occur, and also disregards the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ relationship above the respiratory compensation point when acute hyperventilation (increase in $\dot{V}_E/\dot{V}_{CO_2}$) may occur. Between these limits two straight lines are fitted to the data by linear regression analysis and the anaerobic threshold (AT) is defined as the point of intersection between the lower (S_1) and upper (S_2) slopes.

The difference between test days for each group of subjects was examined using repeated measures analysis of variance and paired *t* tests with Bonferroni corrections where appropriate. Also, for the 3 days of protocol 1, a slope describing the steady-state $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ relationship during aerobic exercise was derived by regression analysis of the steady-state data of the constant work rate tests. Thus, for each subject in protocol 1, the slope of the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ relationship during aerobic exercise was derived by two separate methods: (i) from steady-state values of constant work rate tests and (ii) as the lower slope (S_1) of the *V*-slope analysis. These results were compared using Pearson product moment correlation analysis.

Table 3. *Effect of glycogen depletion on slope of* $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ *relationship*

Subject no.	Day	*R* rest	$\dot{V}_{O_2}$ at AT (l min^{-1})	Steady-state slope	Ramp lower slope	Ramp upper slope	ΔSlope†	$\dot{V}_{O_2,max}$ (l min^{-1})
1	1	0·88	1·79	0·99	1·01	1·56	0·55	3·14
	2	0·75	1·51	0·74	0·71	1·26	0·55	2·72
	3	0·83	1·66	0·94	0·97	1·40	0·43	3·54
2	1	0·83	1·34	0·98	0·96	1·67	0·71	2·85
	2	0·74	1·33	0·81	0·72	1·27	0·55	2·64
	3	0·85	1·30	1·01	0·91	1·60	0·69	2·71
3	1	0·81	1·62	0·87	0·96	1·12	0·16	3·80
	2	0·66	1·83	0·78	0·75	1·12	0·37	3·52
	3	0·78	1·75	0·81	0·84	1·20	0·36	4·00
4	1	0·93	2·00‡	1·08	1·06	1·30	0·24	3·11
	2	0·71	1·62	0·83	1·00	1·40	0·40	3·05
	3	0·87	1·67	1·02	1·09	1·53	0·44	3·38
5	1	0·88	1·47	1·09	0·93	1·48	0·55	3·21
	2	0·71	1·63	0·82	0·88	1·15	0·27	3·05
	3	0·87	1·45	1·06	1·01	1·36	0·35	3·21
6	1	0·98	2·57	0·94	0·92	1·29	0·37	4·57
	2	0·74	2·04	0·83	0·74	1·24	0·50	4·10
	3	0·95	2·80‡	0·97	0·89	1·11	0·22	4·21
7	1	0·92	1·28	0·98	0·93	1·52	0·59	3·05
	2	0·79	1·22	0·82	0·79	1·36	0·57	2·84
	3	0·82	1·36	0·97	1·05	1·42	0·37	3·16
8	1	0·95	1·75	0·96	0·84	1·40	0·56	4·01
	2	0·78	1·83	0·66	0·71	1·36	0·65	3·59
	3	1·02	1·95	0·89	0·76	1·91	1·15	3·95
9	1	0·92	1·22	1·14	0·91	2·01	1·10	2·73
	2	0·78	1·32	0·86	0·86	1·82	0·96	2·63
	3	1·00	1·05	1·02	1·03	2·10	1·07	2·55
10	1	0·88	1·25	0·97	0·95	2·01	1·06	2·30
	2	0·70	1·10	0·93	0·74	1·42	0·68	1·87
	3	0·94	1·27	0·98	0·95	2·14	1·19	2·17
Mean	1	0·90	1·63	1·00	0·95	1·54	0·59	3·28
	2	0·74**	1·54	0·81**	0·79**	1·34*	0·55	3·00**
	3	0·89	1·63	0·97	0·95	1·58	0·63	3·29
S.E.M.	1	0·02	0·13	0·03	0·02	0·09	0·10	0·21
	2	0·01	0·10	0·02	0·03	0·06	0·06	0·20
	3	0·03	0·16	0·02	0·03	0·11	0·12	0·21

* $P < 0·01$, ** $P < 0·001$ by repeated measures analysis of variance.
† ΔSlope is the difference between ramp lower and upper slopes.
‡ Because of the small difference between lower and upper slopes, AT was selected by visual inspection, i.e. when $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ slope broke from lower to upper slope, the latter being > 1·1.

RESULTS

Effect of glycogen depletion on exercise gas exchange

Table 3 shows the resting values of the gas exchange ratio (*R*) for the three study days. The mean values for the two control days were 0·90 (S.E.M. 0·02) and 0·89 (S.E.M. 0·03). Resting *R* was reduced, on average, to 0·74 (S.E.M. 0·01, $P < 0·001$) on the glycogen-depleted day, indicating that the metabolic substrate had changed from predominantly carbohydrate to fatty acids.

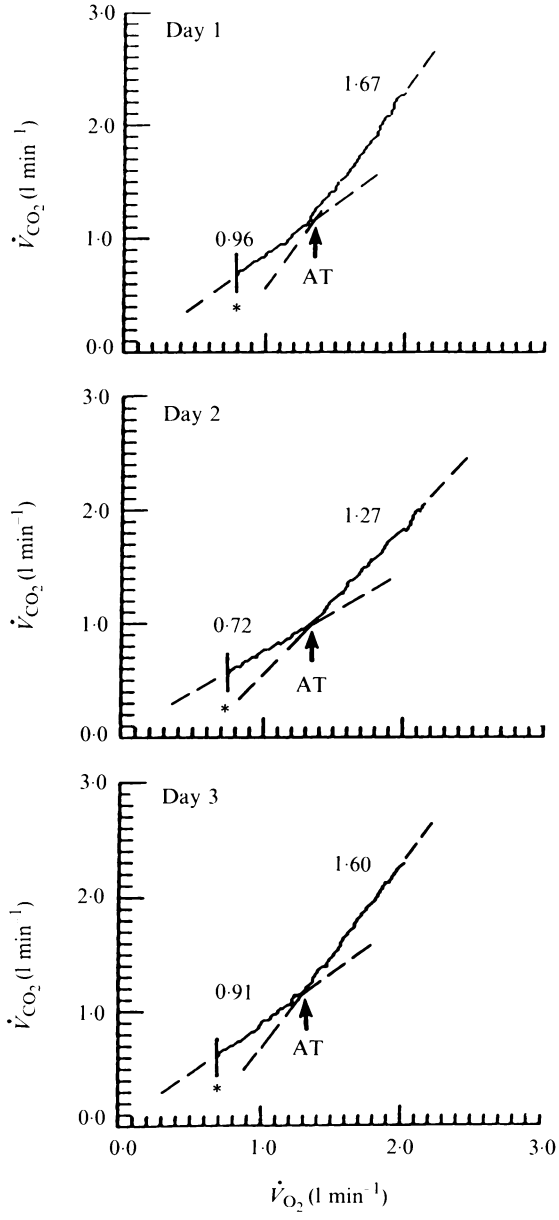

Fig. 1. $\dot{V}_{CO_2}$ $\dot{V}_{O_2}$ relationship after glycogen depletion (day 2) contrasted with the relationships observed in the normally nourished state (day 1 and day 3) for subject 2. See Table 3 for summary of results for all subjects. The lower component slope was reduced on the glycogen-depletion day whereas the anaerobic threshold (AT) was unaffected. *Start of *V*-slope analysis.

Figure 1 shows the relationship between $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ for progressively increasing work rate tests performed by one subject (no. 2) on each of the three study days. Inspection of Fig. 1 reveals a similarity in the responses on the two control days (days 1 and 3) whereas, on the glycogen-depleted day (day 2), the lower slope is reduced.

Values for the lower and upper slopes, together with the $\dot{V}_{O_2}$ corresponding to the AT and

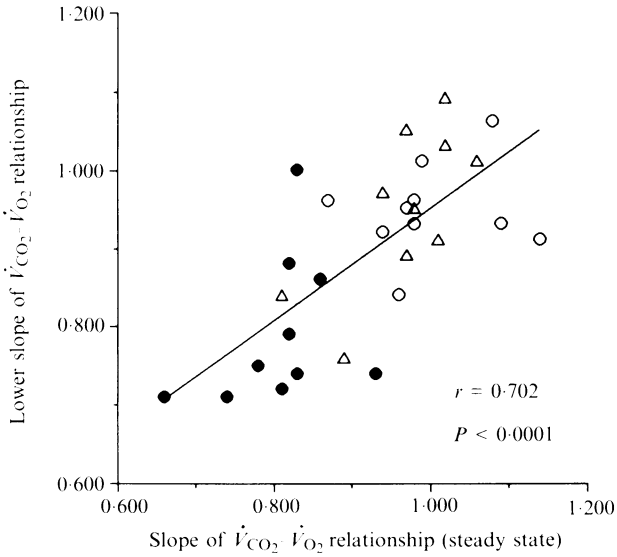

Fig. 2. Correlation between the lower slope of the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ relationship during the ramp pattern incremental exercise test and the slope of the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ relationship determined from steady-state measurements of six constant work rate tests (three levels in duplicate) for each subject. ○, ● and △ represent the results of studies on ten subjects on day 1, day 2 and day 3, respectively. The day 2 study followed a glycogen-depleting manoeuvre.

the peak $\dot{V}_{O_2}$ on each of the 3 days, are shown in Table 3 for each subject. There is agreement in each of these measurements for the two control days. However, the lower slope (S_1) of the increasing work rate test was reduced to 0·79 (S.E.M. 0·03, $P < 0·001$) on the glycogen-depleted day (day 2) compared with 0·95 (S.E.M. 0·02) and 0·95 (S.E.M. 0·03) on days 1 and 3 (control days), respectively.

The upper slope (S_2) of the increasing work rate exercise test was also reduced; 1·34 (S.E.M. 0·06, $P < 0·01$) compared with 1·54 (S.E.M. 0·09) and 1·58 (S.E.M. 0·11) on control days. However, the difference between S_1 and S_2 was not significantly affected by glycogen depletion (Table 3). A change in the $\dot{V}_{O_2}$ at AT was not detected but the peak $\dot{V}_{O_2}$ was reduced on the glycogen-depleted day, being 3·00 l min^{-1} (S.E.M. 0·20, $P < 0·001$) compared with 3·28 l min^{-1} (S.E.M. 0·21) on day 1 and 3·29 l min^{-1} (S.E.M. 0·21) on day 3.

The constant work rate tests yielded steady-state values of $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ for each subject at three different work rate tests below the anaerobic threshold. In two cases (noted in Table 1), the highest constant work rate was above the subject's anaerobic threshold on the glycogen-depletion day, as assessed by slowed $\dot{V}_{O_2}$ kinetics (Whipp & Wasserman, 1972) on these days. Therefore the results of the highest work rate study on these two subjects were disregarded for the purpose of comparing the steady-state $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ relationships, with the lower slope (S_1) of the V-slope analysis. The slope of the steady-state $\dot{V}_{CO_2}$ plotted as a function of steady-state $\dot{V}_{O_2}$ for the constant work rate tests was also reduced for all subjects on the glycogen-depleted day; being 0·81 (S.E.M. 0·02, $P < 0·001$) compared to 1·00 (S.E.M. 0·03) on day 1 and 0·97 (S.E.M. 0·02) on day 3. These slopes are similar to the lower slope (S_1) of the non-steady-state ramp tests for their respective days (Table 3). Figure 2 shows the highly significant correlation between S_1 and the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ slope determined from the steady-state values of the constant work rate tests ($r = 0·702$, $P < 0·0001$). The cluster of data points (●) in the lower left-hand corner of the plot represents the slopes on glycogen-depleted days.

Table 4. *Effects of rate of work rate increase on* $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ *relationship*

Subject no.	Ramp rate (W min^{-1})	$\dot{V}_{O_2}$ at AT (l min^{-1})	Lower slope	Upper slope	ΔSlope	
1	15	1·50	1·02	1·28	0·26	
	15	1·40	1·07	1·36	0·29	
	30	1·63	0·93	1·36	0·43	
	30	1·55	1·04	1·64	0·60	
	60	1·79	1·08	1·75	0·67	
	60	1·58	1·03	1·48	0·45	
2	15	1·50	0·96	1·18	0·22	
	15	1·58	0·93	1·25	0·32	
	30	1·83	0·89	1·55	0·66	
	30	1·87	1·03	1·38	0·35	
	60	2·08	1·07	1·64	0·57	
	60	2·15	1·05	1·71	0·66	
3	15	1·47	0·89	1·22	0·33	
	15	1·40	0·79	1·07	0·28	
	30	1·40	0·88	1·15	0·27	
	30	1·70	0·91	1·25	0·34	
	60	1·95	0·74	1·55	0·81	
	60	2·36	0·93	1·48	0·55	
4	15	1·94	0·93	1·30	0·37	
	15	1·84	0·96	1·30	0·34	
	30	1·89	0·84	1·46	0·62	
	30	1·81	0·91	1·42	0·51	
	60	1·91	0·81	1·87	1·08	
	60	1·66	0·81	1·42	0·61	
5	15	1·46	1·00	1·41	0·41	
	15	1·30	0·84	1·22	0·38	
	30	1·61	0·88	1·50	0·62	
	30	1·54	0·99	1·35	0·36	
	60	1·66	0·94	1·82	0·88	
6	15	1·69	0·98	1·33	0·35	
	15	1·70	0·96	1·22	0·26	
	30	1·55	1·00	1·43	0·43	
	30	1·91	0·99	1·50	0·51	
	60	2·08	0·98	1·97	0·99	
	60	2·26	0·86	1·53	0·67	
7	15	1·48	1·08	1·33	0·25	
	15	1·28	0·97	1·32	0·35	
	30	1·39	0·98	1·73	0·75	
	30	1·25	1·02	1·22	0·20	
	60	1·65	1·01	1·58	0·57	
Mean	15	1·54	0·96	1·27 } **	0·32	
	30	1·64 } **	0·95	1·42 } **	0·48	**
	60	1·93 }	0·94	1·65 }	0·71	**
S.E.M.	15	0·05	0·02	0·02	0·02	
	30	0·05	0·02	0·04	0·04	
	60	0·08	0·03	0·05	0·06	

* $P < 0·05$, ** $P < 0·01$ by paired t tests.

Effect of glycogen depletion on lactate, pyruvate and catecholamines

Lactate, pyruvate and catecholamine levels were determined from the venous blood drawn after 2 min of recovery at the end of each increasing work rate test. The mean lactate levels were 8·72 mmol l^{-1} (S.E.M. 0·72) and 8·89 mmol l^{-1} (S.E.M. 0·70) on the two control

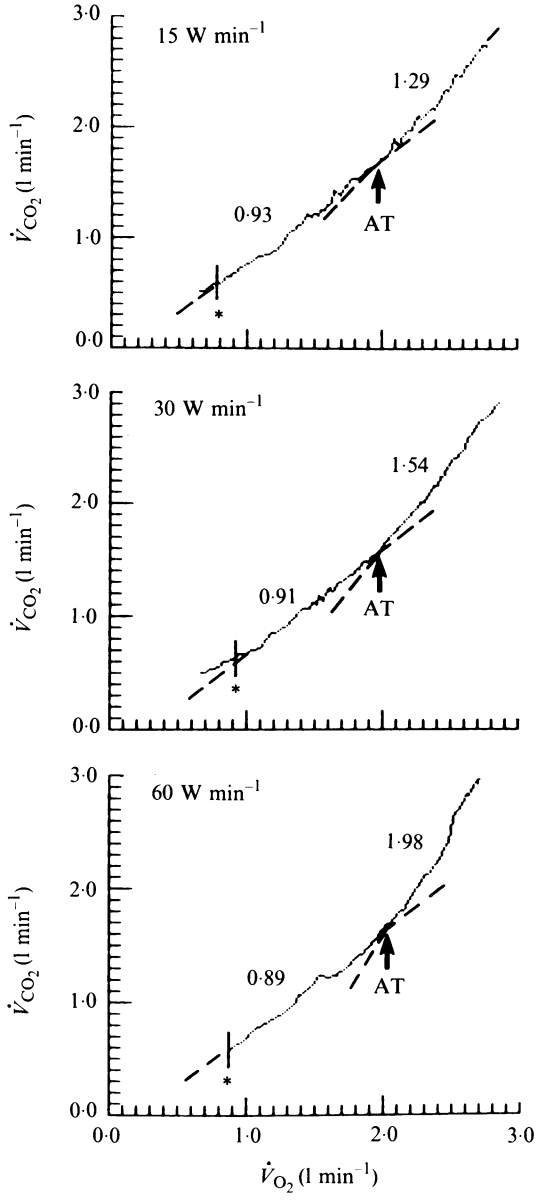

Fig. 3. $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ relationships at different rates of work rate increase (15, 30 and 60 W min^{-1}) for subject 4. See Table 4 for summary of results for all subjects. The upper slope was increased as the rate of work rate was increased. *Start of *V*-slope analysis.

days whilst the level was reduced to 4·52 mmol l^{-1} (S.E.M. 0·71, $P < 0·001$) on the glycogen-depleted day.

Pyruvate was 0·200 mmol l^{-1} (S.E.M. 0·020) on the glycogen-depleted day compared with 0·240 mmol l^{-1} (S.E.M. 0·026) and 0·235 mmol l^{-1} (S.E.M. 0·016) on control days, but the reduction was not significant.

Catecholamine levels were obtained in eight subjects and were not significantly different on the glycogen-depleted day (adrenaline = 148 mmol l^{-1} (S.E.M. 47), noradrenaline =

1363 mmol l^{-1} (S.E.M. 360)) as compared to the control days (adrenaline = 123 mmol l^{-1} (S.E.M. 24) and 126 mmol l^{-1} (S.E.M. 41), noradrenaline = 1602 mmol l^{-1} (S.E.M. 355) and 1147 mmol l^{-1} (S.E.M. 348)).

Effect of increasing work rate increment on the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ *relationship*

The lower slope of the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ relationship was not systematically affected by the ramp rate in protocol 2 (Table 4). Figure 3 shows the pattern of change in the *V*-slope plot as affected by the ramp rate for one representative subject (no. 4). The mean values for the lower slopes were respectively 0·96 (S.E.M. 0·02), 0·95 (S.E.M. 0·02) and 0·94 (S.E.M. 0·03) for ramp rates of 15, 30 and 60 W min^{-1} (Table 4). These values are not significantly different from the control days (S_1 = 0·95) of the ten subjects used in protocol 1 (Table 3). In contrast, the upper slope was steeper for a faster rate of work rate increase ($P < 0·01$), the mean upper slope being 1·27 (S.E.M. 0·02), 1·43 (S.E.M. 0·04) and 1·63 (S.E.M. 0·05) for the 15, 30 and 60 W min^{-1} studies, respectively (Table 4). The differences between the corresponding lower and upper slopes (ΔSlope) were, on average, 0·32 (S.E.M. 0·02), 0·48 (S.E.M. 0·04) and 0·71 (S.E.M. 0·06). These differences are significant ($P < 0·01$), in contrast to the findings from protocol 1 (glycogen depletion). The rate of increase in work rate appeared to have an influence on the AT as determined by the *V*-slope method in the case of the 60 W min^{-1} ramp rate.

DISCUSSION

The two protocols studied allowed us to demonstrate that the lower component slope is influenced by the respiratory quotient of the substrate of exercising muscle and is not affected by the rate of work rate increase. In contrast the upper component slope relative to the lower component was not affected by the energy substrate but was systematically increased with the rate at which work rate was increased. Analysis of the linear components of the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ relationship during ramp exercise tests has the advantage of being independent of the sensitivity of the ventilatory control mechanism (Beaver *et al.* 1986). The anaerobic threshold (AT) determined using this method correlates closely with the development of metabolic acidosis due to lactate increase. Beaver *et al.* (1986) also found that the *V*-slope method for determining the AT was more widely applicable and gave a smaller coefficient of variation than other gas exchange methods.

Carbon dioxide output increases approximately linearly as a function of oxygen uptake during a progressively increasing exercise test below the anaerobic threshold, with a slope (S_1) that is usually between 0·9 and 1·0 (Beaver & Wasserman, 1991). It would appear that CO_2 stores were not increasing during this component of the incremental period, since CO_2 storage should cause S_1 to be lower than the slope which purely reflects substrate metabolism. Within the first minute of increasing work rate from unloaded cycling, the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ slope is low and non-linear, and *R* decreases before it increases, suggesting CO_2 loading into stores during this early period of exercise. If CO_2 stores were continuing to increase significantly after 1 min, the slope would be artificially low, in contrast to the high value actually observed. In addition, the relationship of the steady-state values of $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ from the constant work rate tests had a similar slope to S_1 of the ramp test (Table 3). Since the stores are already saturated in the steady-state tests, the slope of the *increase* in $\dot{V}_{CO_2}$ must reflect the respiratory quotient (RQ) of exercising muscle. Hence these findings support the interpretation that S_1 is a measure of muscle metabolic substrate.

The high muscle RQ calculated from S_1 or the constant work rate steady-state values is consistent with the concept that glycogen is the primary substrate for exercise in the

normally nourished subject. Use of the *V*-slope method to determine muscle RQ from a ramp exercise test is a new approach to understanding the metabolism of contracting muscle. The concept relies upon the assumptions that body CO_2 stores are loaded early after the onset of exercise and thereafter the increases of alveolar $\dot{V}_{CO_2}$ and alveolar $\dot{V}_{O_2}$ truly reflect the metabolic events in exercising muscle. Fundamental to the understanding of the muscle RQ concept is an appreciation that since S_1 does not intercept with the origin (Beaver *et al.* 1986), it is not the total body respiratory gas exchange ratio (*R*) or total body RQ in the steady state (steady-state *R*). The total body *R* or total body RQ is lower than the RQ of exercising muscle as reflected by S_1. Furthermore, due to the negative *y*-intercept of the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ plot, the slope S_1 (or muscle RQ) can remain constant whilst the instantaneous value of total body *R* ($\dot{V}_{CO_2}/\dot{V}_{O_2}$) is changing.

Previous studies have demonstrated that during incremental exercise the total body *R* increases with increasing work rate (Naimark, Wasserman & McIlroy, 1964) and this has led some investigators to the conclusion that muscle RQ increases with incremental exercise independently of anaerobiosis (Clode & Campbell, 1969). However, if this were the case, $\dot{V}_{CO_2}$ should not increase linearly with respect to $\dot{V}_{O_2}$ for increasing work rate tests and constant work rate tests below the anaerobic threshold. The results of the present study (Fig. 1) and those of Beaver *et al.* (1986) contradict this suggestion demonstrating that a change in total body *R per se* cannot be assumed to indicate a change in the substrate utilization of exercising muscle. More likely, total body *R* increases as work rate increases, below levels causing a metabolic acidosis, because muscle utilizes a large proportion of high RQ substrate (glycogen) relative to other tissues. Different organs metabolize different proportions of glycogen and free fatty acids (i.e. they have different RQ values). When the metabolic rate of an organ with high RQ increases, total body RQ will increase but the RQ of individual organs need not change. As muscle contributes a proportionately larger fraction of the total body metabolism the total body *R* and total body RQ will approach the muscle RQ. Thus, the increase in total body *R* observed as work rate increases can be attributed to a greater contribution of muscle respiration to that of the total, rather than increasing muscle RQ with increasing work rate, a concept for which there is no real direct evidence.

Dietary manipulation is well known to influence the extraction of metabolic substrates by the exercising leg as measured by arteriovenous differences in glucose and free fatty acids (Jansson & Kaijser, 1982). Also muscle biopsies have confirmed that glycogen depletion is associated with less lactate accumulation during exercise (Grisdale, Jacobs & Cafarelli, 1990). However, there is little evidence to prove that fatty acid utilization is a significant component of metabolism in muscle with normal glycogen content exercising at work rates below the AT. Recently Hargreaves, Kiens & Richter (1991) reported an RQ of 0·87 across the whole leg during exercise using a one-legged knee extension model. Doubling plasma free fatty acid concentrations by intravenous infusion of Intralipid (Kabivitrum) reduced glucose uptake in the leg but did not affect glycogen degradation or RQ. The findings of this study support the idea of reliance on glycogen as the primary metabolic substrate and furthermore the RQ measured across the whole leg might not purely reflect the metabolism of the contracting knee extensors. We believe that it is necessary to study changes in $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ in order to obtain a true indication of the RQ of the actively contracting muscle.

Muscle glycogen content varies among normal, healthy subjects being about 10–20 g (kg wet muscle)$^{-1}$ (Bergstrom & Hultman, 1966; Hultman, 1967). This would be equivalent to about 400 g in a 70 kg man. Muscle glycogen is rapidly depleted by bouts of very heavy

exercise (Bergstrom & Hultman, 1967; Hermansen, Hultman & Saltin, 1967; Jacobs, 1981*b*). Gollnick *et al.* (1974) showed that 2 h of cycling at the level performed by our subjects reduced muscle glycogen to approximately 30% of baseline. In the study of Heigenhauser, Sutton & Jones (1983), three subjects had vastus lateralis muscle biopsies during a similar protocol of carbohydrate restriction and intensive exercise. At 24 h, muscle glycogen content remained less than 30% of the control value.

An RQ of 0·95 equates to 85% of the energy coming from carbohydrate. Two hours of exercise at a $\dot{V}_{O_2}$ of approximately 2 l min^{-1} would reduce the glycogen level in muscle by 2·5 g min^{-1} or 300 g for the 2 h period. Since total muscle glycogen is estimated to be about 400 g in a 70 kg man, this suggests that our subjects, exercising at a level of about 2–3 litres of O_2 consumption per minute for 2 h, must have markedly depleted their glycogen stores. Skeletal muscle glycogen repletion is extremely slow in fasted subjects (Hultman, 1967; Piehl, 1974). Therefore our subjects fasted overnight before returning to the laboratory for the second study. The purpose of this approximately 18 h delay in testing after glycogen depletion was to allow the muscles to rest and repair before re-study in the glycogen-depleted state. Also, it provided a period to further deplete liver glycogen stores. In the glycogen-depleted state, aerobic cellular metabolism becomes more dependent on the oxidation of fat (Newsholme, 1977). Our assumption of a reduced amount of glycogen available for metabolism was borne out by the reduced resting (from 0·90 to 0·74) and exercise R of our subjects when studied on the glycogen-depleted day in contrast to the studies on the two control days.

Both the lower slope of the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ relationship of the ramp test (0·79) and the slope of the steady-state $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ values of the constant work rate exercise tests (0·81) were reduced on the glycogen-depleted day compared to the control day (0·95) supporting the hypothesis that S_1 reflects muscle RQ. An alternative explanation for the determinant of S_1 is difficult to rationalize, given that it is measured over the work rate range during which CO_2 stores are stable.

While S_2 also decreased on the glycogen-depleted day, the difference between S_1 and S_2 was independent of the state of glycogen depletion (Table 3). These results demonstrate that S_1 of the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ relationship can be selectively manipulated by altering energy substrate, without altering the difference between the lower and upper components of the V-slope plot (see Table 3).

Dietary manipulation and muscle glycogen content are also known to affect lactate accumulation during exercise (Kelman, Maughan & Williams, 1975; Segal & Brooks, 1979; Jacobs, 1981*a*). In the present study, the anaerobic threshold as measured by gas exchange did not change (Table 3), consistent with the finding that during exercise in the glycogen-depleted state, lactate increases with a threshold pattern similar to that seen when subjects exercised while on a carbohydrate diet (Wilson & Cureton, 1984; Yoshida, 1984; McLellan & Gass, 1989). In the glycogen-depleted state, end-exercise lactate concentrations are known to be reduced (Wasserman, 1967; Jacobs, 1981*a*). In our study, the venous blood lactate levels after increasing work rate tests in the glycogen-depleted state were, on average, about 50% of the levels observed on control days. This can be partly explained by the fact that subjects achieved a lower maximum work rate after glycogen depletion of 231 W (S.E.M. 21) compared with 260 W (S.E.M. 20) on day 1 and 264 W (S.E.M. 20) on day 3 ($P < 0·001$). It is possible that the reduced ability to produce lactate, in the glycogen-depleted state, resulted in the reduced peak $\dot{V}_{O_2}$ and work rate. The mild metabolic acidosis which develops during fasting may also inhibit lactate production (Jones, Sutton, Taylor & Toews, 1977; Sutton, Jones & Toews, 1981; Kowalchuck, Heigenhauser & Jones, 1984).

Alternatively, lactate utilization may be increased in the glycogen-depleted state (Saltin & Hermansen, 1967) while lactate production is relatively unchanged for any given work rate.

If the increase in lactate beyond the AT on the glycogen-depletion day has the same functional relationship to $\dot{V}_{O_2}$ as found for subjects with normal glycogen stores, the reduction in lactate concentration caused by the reduced peak $\dot{V}_{O_2}$ in the glycogen-depleted state could be estimated from the equation

$$[La]' = [La]\left(\frac{\dot{V}_{O_2'}}{\dot{V}_{O_2}}\right)^{2.9},$$

derived by Beaver, Wasserman & Whipp (1985), where [La] and [La]', $\dot{V}_{O_2}$ and $\dot{V}_{O_2'}$ are the highest values of lactate and oxygen uptake on the non-depleted and glycogen-depleted days, respectively. Thus, the observed reduction in peak $\dot{V}_{O_2}$ on day 2 to 91 % of days 1 and 3 would result in a predicted decrease in peak lactate to 76 % of the day 1 and 3 values (i.e. 6·7 mmol l^{-1}). Since the peak lactate value on day 2 was found to be 4·5 mmol l^{-1} or 51 % of that on days 1 and 3, about half of the reduction in lactate (2·2 mmol l^{-1}) must have been due to factors other than the reduced peak work rate.

Given that glycogen is the source of lactate, reduced glycolysis is likely to be one of these factors. An important distinction must be drawn between aerobic and anaerobic glycolysis. Aerobic glycolysis (below the AT) is well known to be influenced by the availability of substrate and the reduction of S_1 by glycogen depletion is evidence of this effect. By contrast, anaerobic glycolysis (above the AT) is an obligatory source of energy due to the interruption of other metabolic pathways and relates to work intensity rather than oxygen uptake. A reduction in lactate accumulation at a given $\dot{V}_{O_2}$ above the AT would predictably reduce the difference $S_2 - S_1$ according to our hypothesis that excess CO_2 arises from bicarbonate buffering of lactic acid. Table 3 shows that $S_2 - S_1$ was 0·55 on day 2 compared with an average of 0·61 on control days. Although this difference was not statistically significant, a small decrease in excess CO_2 might have occurred.

In both protocols of this study and in the study of Beaver & Wasserman (1991), S_1 averaged approximately 0·95 (total of twenty-seven normal subjects in the three studies). S_1 is quite uniform in normally nourished subjects and is uninfluenced by the rate of change in work rate (Table 4). By contrast, the S_2 slope of the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ relationship (above the break-point) is affected by the rate of increase in work rate (Table 4). Hence, we observed a systematic increase in the difference $S_2 - S_1$ with faster ramp rates. Based on our hypothesis that the difference $S_2 - S_1$ reflects 'excess CO_2' from bicarbonate buffering of lactate, we conclude that the greater $\dot{V}_{CO_2}$ for any given $\dot{V}_{O_2}$ above the AT reflects a faster rate of accumulation of lactate as expected with faster rates of increasing work rate. This may be due in part to the lag in circulatory delivery of O_2 and therefore a slowed rate of $\dot{V}_{O_2}$ increase as observed by Hansen, Casaburi, Cooper & Wasserman (1988).

An alternative mechanism worth considering is the influence of arterial plasma potassium. Substantial quantities of potassium are released during volitional exercise and hyperkalaemia increases the sensitivity of the peripheral chemoreceptors (Linton & Band, 1985). Furthermore, the increase in arterial plasma potassium bears a close temporal relationship to increased ventilation during exercise (Paterson, Robbins & Conway, 1989). We believe that altered chemosensitivity is unlikely to influence gas exchange during exercise before the stage at which subjects develop respiratory compensation for metabolic acidosis. Hence, the region of analysis of the linear components S_1 and S_2 would not be affected and would remain insensitive to changes in the ventilatory control mechanism

(Beaver *et al.* 1986). Potassium might influence the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ relationship above this respiratory compensation point. However, an effect on $\dot{V}_{CO_2}$ would be relatively small because of the high rate of aerobic CO_2 production and $\dot{V}_{O_2}$ kinetics would not be affected by changes in ventilation (Casaburi, Weissman, Huntsman, Whipp & Wasserman, 1979).

The studies reported here contribute to the understanding of the factors affecting the upper and lower slopes of the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ relationship during a progressive exercise test. The computer-assisted method of Beaver *et al.* (1986) (*V*-slope analysis) for determining the anaerobic threshold and the upper and lower slope values appears to be reproducible in subjects performing the same protocol on different days (Table 3). The lower slope (S_1) was found to depend upon the availability of glycogen but not upon the rate of increase of work rate whereas the upper slope (S_2) was found to depend upon the lactic acidosis as well as the availability of glycogen. The difference between S_2 and S_1 was independent of the availability of glycogen but dependent on the rate of increase in work rate. These findings are consistent with the concept that the lower linear component of the *V*-slope analysis of the $\dot{V}_{CO_2}$–$\dot{V}_{O_2}$ relationship during a progressively increasing work rate test reflects muscle substrate RQ and the upper linear component reflects increasing CO_2 output from aerobic metabolism plus CO_2 produced from bicarbonate buffering of lactic acid.

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