Eur J Appl Physiol (1999) 80: 436–447

ORIGINAL ARTICLE

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Effect of work and recovery duration on skeletal muscle oxygenation and fuel use during sustained intermittent exercise

Accepted: 14 April 1999

Abstract The purpose of this study was to compare rates of substrate oxidation in two protocols of intermittent exercise, with identical treadmill speed and total work duration, to reduce the effect of differences in factors such as muscle fibre type activation, hormonal responses, muscle glucose uptake and non-esterified fatty acid (NEFA) availability on the comparison of substrate utilisation. Subjects ($n = 7$) completed 40 min of intermittent intense running requiring a work:recovery ratio of either 6 s:9 s (short-interval exercise, SE) or 24 s:36 s (long-interval exercise, LE), on separate days. Another experiment compared O_2 availability in the vastus lateralis muscle across SE (10 min) and LE (10 min) exercise using near-infrared spectroscopy (RunMan, NIM. Philadelphia, USA). Overall (i.e. work and recovery) O_2 consumption ($\dot{V}O_2$) and energy expenditure were lower during LE ($P < 0.01$, $P < 0.05$, respectively). Overall exercise intensity, represented as a proportion of peak aerobic power ($\dot{V}O_{2peak}$), was [mean (SEM)] 64.9 (2.7)% $\dot{V}O_{2peak}$ (LE) and 71.4 (2.4)% $\dot{V}O_{2peak}$ (SE). Fat oxidation was three times lower ($P < 0.01$) and carbohydrate oxidation 1.3 times higher ($P < 0.01$) during LE, despite the lower overall exercise intensity. Plasma lactate was constant and was higher throughout exercise in LE [mean (SEM) 5.33 (0.53) mM, LE; 3.28 (0.31) mM, SE; $P < 0.001$)]. Plasma pyruvate was higher and glycerol was lower in LE [215 (17) μM, 151 (13) μM, $P < 0.05$, pyruvate; 197 (19) μM, 246 (19) μM, $P < 0.05$, glycerol]. There was no difference between protocols for plasma NEFA concentration ($n = 4$) or plasma noradrenaline and adrenaline. Muscle oxygenation declined in both protocols ($P < 0.001$), but the nadir during LE was lower [52.04 (0.60)%] compared to SE [61.85 (0.51)%; $P < 0.001$]. The decline in muscle oxygenation during work was correlated with mean lactate concentration ($r = 0.68$; $P < 0.05$; $n = 12$). Lower levels of fat oxidation occurred concurrent with accelerated carbohydrate metabolism, increases in lactate and pyruvate and reduced muscle O_2 availability. These changes were associated with proportionately longer work and recovery periods, despite identical treadmill speed and total work duration. The proposal that a metabolic regulatory factor within the muscle fibre retards fat oxidation under these conditions is supported by the current findings.

Key words Intermittent exercise · Substrates · Near-infrared spectroscopy · Muscle O_2 availability

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Introduction

Intermittent exercise is composed of frequent periods of intense work followed by periods of less-intense recovery. This type of exercise reflects the nature of many physical occupations and is the basis of the games sports (e.g. soccer and tennis). Nevertheless, patterns of net substrate utilisation during sustained intermittent intense exercise are not clearly established. The early studies reported by Essen (1978a) remain the principal investigations of substrate utilisation in prolonged intermittent intense exercise under steady-state conditions.

Essen et al. (1977) showed that overall (i.e. work and recovery) carbohydrate and fat oxidation rates as well as glycogen depletion were similar during 60 min of intermittent intense and continuous submaximal exercise with the same overall (i.e. work and recovery) O_2 consumption ($\dot{V}O_2$). Overall exercise intensity, represented as a proportion of maximum aerobic power ($\dot{V}O_{2max}$), was approximately 55% $\dot{V}O_{2max}$ in both exercise protocols (Essen et al. 1977). In contrast, we found a lower overall fat oxidation and higher carbohydrate oxidation

rate during 90 min of intermittent intense compared to continuous submaximal exercise with similar overall $\dot{V}O_2$ and identical energy expenditure (Christmass et al. 1999). Overall exercise intensity, represented as a proportion of peak aerobic power ($\dot{V}O_{2peak}$), was approximately 70% $\dot{V}O_{2peak}$ in both exercise protocols (Christmass et al. 1999).

Recently, it has been suggested that accelerated carbohydrate metabolism may inhibit fat oxidation due to a decline in plasma non-esterified fatty acid (NEFA) availability as well as reduced transport (and oxidation) of long-chain fatty acids into skeletal muscle mitochondria (Coyle et al. 1997; Sidossis et al. 1997). Factors other than differences in plasma NEFA availability are likely to have been responsible for the difference in whole-body fat oxidation noted in our study (Christmass et al. 1999). One possibility is that carbohydrate metabolism inhibits the oxidation of fat in skeletal muscle during intermittent exercise (i.e. metabolic regulation). However, any effect of metabolic regulation per se on the difference in fuel oxidation between the two exercise protocols is confounded by the higher exercise intensity (corresponding to 120% $\dot{V}O_{2peak}$) required during the work periods of intermittent exercise (i.e. work-period intensity; for discussion see Christmass et al. 1999). This higher work-period intensity was attributable to the higher treadmill speed used during intermittent compared to continuous exercise.

The aim of the current study was to compare substrate oxidation rates between two protocols of sustained intermittent exercise with identical treadmill speed (i.e. work-period intensity), work:recovery ratio and total work duration in order to minimise the effect of differences in factors such as muscle fibre type activation, hormonal responses, muscle glucose uptake and NEFA availability on the comparison of substrate utilisation.

With identical work-period intensity and work:recovery ratio, a proportional increase in the duration of the work and the recovery period results in higher circulating lactate concentrations (Astrand et al. 1960a; Saltin and Essen 1971) and increased rates of glycogen depletion (i.e. accelerated carbohydrate metabolism) during intermittent exercise (Saltin and Essen 1971). On this basis, we hypothesised that a work:recovery ratio of 24 s:36 s (long-interval exercise, LE) compared to one of 6 s:9 s (short-interval exercise; SE) would result in higher carbohydrate oxidation and lower fat oxidation, despite identical treadmill speed and total work duration. Furthermore, during intermittent exercise a role for myoglobin (Mb) as an O_2 store, reducing glycolytic demand during short work periods (5–15 s), was originally proposed by Astrand et al. (1960a) and is supported by the findings of Saltin and Essen (1971). Since Mb stores may be depleted during long work periods in intermittent exercise, we also hypothesised that muscle O_2 availability (oxyhaemoglobin; HbO_2) would be lower during LE compared to SE. The study comprised two experiments: (1) a comparison of fuel oxidation and plasma metabolite levels and, (2) a comparison of muscle HbO_2 using near-infrared spectroscopy (NIRS), during two protocols of intermittent exercise with identical treadmill speed, work:recovery ratio and total work duration.

Methods

Subjects

The same group of healthy male volunteers who participate regularly in interval-type running exercise completed two experiments. Experiment 1 (intermittent exercise, expt. 1, $n = 7$) compared fuel oxidation and capillary plasma metabolite and blood gas concentrations during two protocols of sustained intermittent exercise. Experiment 2 (NIRS expt. 2, $n = 6$) compared muscle HbO_2, measured by NIRS, during two protocols of intermittent exercise (with identical work:recovery duration and treadmill speed compared to expt. 1) as well as 40 s of continuous intense exercise. The [mean (SEM)] age, height, body mass, $\dot{V}O_{2peak}$ and maximum heart rate (HR_{max}) for the subjects were: 24 (3) years, 179 (3) cm, 74.7 (4.1) kg, 57.6 (2.8) ml · kg^{-1} · min^{-1}, and 189 (5) beats · min^{-1}, respectively. All subjects provided their informed consent to participate in the studies, and the experimental procedures were approved by the Human Rights Committee of The University of Western Australia, in accordance with the Declaration of Helsinki.

During the first laboratory session the subjects' standing height, body mass and $\dot{V}O_{2peak}$ were measured according to methods previously described (Christmass et al. 1999).

Experimental protocols

Intermittent exercise (experiment 1)

Subjects completed intermittent running protocols (40 min) that required a cycle of SE and LE on a motorised treadmill. The experimental sessions involving the two exercise protocols were separated by a minimum of 5 days (range 5–18 days) and the order of completion of the two experimental sessions was randomised and balanced. Treadmill speed was determined individually for each subject at the time of the first experimental session. The determination of treadmill speed involved a 5-min warmup at 10 km · h^{-1} followed by 5–10 min of intermittent running (LE) during which treadmill speed was adjusted to elicit a respiratory exchange ratio (R) of 0.96–0.99. A computerised gas analysis system, used for preliminary $\dot{V}O_{2peak}$ tests, was used to determine R during this period.

Immediately following this preliminary procedure subjects performed 40 min of intermittent exercise (i.e. SE or LE). The alternate schedule of work and recovery duration was completed during the second experimental session (i.e. 5–18 days later). The treadmill speed for both protocols was identical (range 17.5–22.0 km · h^{-1}).

For metabolite assays and for measurement of blood gases and haematocrit, capillary blood was collected at rest and at approximately 10-min intervals (i.e. 10, 21, 32 and 43 min) during the experiment. Blood for measurement of plasma catecholamine levels was collected at rest and immediately following the experimental procedure. On the basis of previous results indicating the potential instability of the plasma bicarbonate (HCO_3^-) pool during the initial 10 min of intermittent intense exercise (Christmass et al. 1999), measurements for respiratory gas exchange (using open-circuit spirometry) commenced at 16 min and were performed at 10-min intervals, across 1-min collection periods (i.e. 16, 27 and 38 min). Subjects were trained in the use of the plastic mouthpiece that was connected to the spirometry system and breathed through this system for approximately 5 min during each collection period. Heart rate (HR) was monitored at 10-min intervals with the aid of a short-range radio telemetric HR monitor (Polar Electro Sports tester, Kempele, Finland).

Near-infrared spectroscopy (experiment 2)

Subjects completed three distinct exercise protocols, performed on a motorised treadmill during a single experimental session. Experimental sessions for expt. 1 and expt. 2 were separated by a minimum of 4 days. The protocols consisted of 10 min of intermittent intense running involving SE, followed by 10 min of intermittent intense running involving LE and 40 s of continuous intense running. Treadmill speed was unchanged for each exercise protocol during expt. 2 and, for each subject, was identical to the speed used during expt. 1. Subjects completed a warmup consisting of 8 min continuous submaximal running followed by 5 min intermittent intense running (SE). For the intermittent intense exercise warmup, treadmill speed commenced at 10 km · h^{-1} and was increased by 2 km · h^{-1} every 30 or 60 s until the required speed was attained. The LE was excluded from the warmup, and the order of completion of the experimental protocols was maintained for each subject to avoid the possible confounding effect of elevated lactate levels of HbO_2 (Stringer et al. 1994). The initial 5 min of both SE and LE were excluded from data analysis (see NIRS data collection and analysis) and were used in addition to the intense warmup, to minimise the influence of exercise-induced hyperaemia on the comparison of muscle HbO_2. Muscle HbO_2 was monitored continuously by NIRS throughout each experimental session in expt. 2.

Nutrient intake

For each experiment, subjects were requested to maintain a similar dietary and exercise regime in the 3 days prior to the experimental protocol. In expt. 1 subjects were studied in the post-absorptive state.

Respiratory gas exchange and indirect calorimetry

Open-circuit spirometry was used for the measurement of respiratory gas exchange. Expired air was separated from inspired air by means of a low-resistance valve (Hans-Rudolph) and was collected into a 350-1 Tissot spirometer (Collins, USA) for measurement of minute ventilation ($\dot{V}_E$). The spirometer was flushed twice and a gas sample removed for measurement of the fractional concentration of O_2 and CO_2, according to methods previously described (Christmass et al. 1999). Measurements of $\dot{V}O_2$ and CO_2 output ($\dot{V}CO_2$) were used to calculate energy expenditure using caloric equivalents of the nonprotein respiratory quotient (Lusk 1928) and to calculate rates of carbohydrate and fat oxidation using the stoichiometric equations and assumptions outlined by Frayn (1983).

Blood sample collection and treatment

For metabolite assays, capillary blood (≈60 μl) was collected and treated according to methods described previously (Christmass et al. 1999). For the determination of lactate, pyruvate and glycerol, plasma was immediately immersed in liquid N_2 prior to storage at −80°C. Plasma for NEFA determination was acid denatured immediately (i.e. 30 μ1 plasma and 30 μl of 0.2 M HCl for 1 min, neutralised with 30 μl 0.2 M NaOH/0.1M TES) according to a modification of the method of Kather and Weiland (1984) and then immersed in liquid N_2. For measurement of plasma catecholamine levels, blood (10 ml) was collected by venepuncture into a heparinised vacutainer (Becton Dickinson, Meylen, Cedex-France), immediately centrifuged with sodium metabisulphite (1 mg) at 4°C for 10 min at 8000 *g* (Jouan BR 3.11 refrigerated centrifuge, Jouan, St-Nazaire, France), and the plasma was stored with sodium metabisulphite (1 mg) at −80°C until analysis. For blood gas determination, capillary blood was collected and analysed according to methods described previously (Christmass et al. 1999). Haematocrit was measured in duplicate using a micro-haematocrit reader (Hawksley, Sussex, UK).

Plasma metabolite and catecholamine analysis

Plasma for catecholamine determination was extracted according to a modification of the method described by Henry et al. (1975), and quantitation was performed by high-performance liquid chromatography using electrochemical detection (Dept. Renal Medicine, Royal Prince Alfred Hospital, Sydney, Australia). Untreated plasma for lactate, pyruvate, and glycerol determination was deproteinised and analysed according to methods outlined previously (Christmass et al. 1999). Plasma NEFA concentrations were determined according to Christmass et al. (1998). All samples were measured in triplicate and were remeasured if two determinations in a triplicate differed by more than 10%. For each method, interassay variability was less than 10%. The recovery of known concentrations of appropriate standards added to plasma was: 98 (2)% (lactate), 103 (3)% (pyruvate), 99 (2)% (glycerol) and 100 (4.5)% (NEFA).

Near-infrared spectroscopy

RunMan NIRS unit

To monitor changes in tissue HbO_2 NIRS was performed using a commercially available unit (RunMan NIM, Philadelphia, Pa., USA). Validation, calibration and operation of the RunMan unit, as well as the representation and analysis of NIRS data from the experimental protocol, have been described previously (Christmass et al. 1999). Briefly, changes in the NIRS signal were represented relative to the minimum and maximum values recorded during cuff ischaemia. This in vivo calibration is necessary because the absolute pathlength of near-infrared (NIR) light in human tissue is unknown (Mancini et al. 1994), and therefore actual concentrations cannot be determined. At the end of the experiment a blood pressure cuff was inflated around the upper thigh. The lowest NIRS signal recorded during cuff ischaemia was assigned 0%, and the highest signal following cuff release (reactive hyperaemia) was assigned 100%. Data from the final 5 min of the two intermittent exercise protocols (SE, LE) were transferred from a MacLab (ADInstruments Castle Hill, Australia) chart computer display to a data analysis package (ClarisWorks, Claris, Santa Clare, USA) in consecutive 60-s periods (i.e. 1 and 4 work:recovery cycle/s for LE and SE, respectively). The results from these sections of the analysis period were integrated to produce a single (average) 60-s period of NIRS data representative of each intermittent exercise protocol. Data for the single 40-s period of continuous intense exercise were transferred to the data analysis package and an average NIRS signal response was calculated. The mean NIRS signals across 1-s periods were used to calculate the decline and range for HbO_2 in each protocol, with means across 3-s periods used for all other analyses.

Statistical analysis

Analysis of plasma metabolite, HR, *R*, blood gas and NIRS data was performed using a two-way analysis of variance (ANOVA) with repeated measures for comparisons across time for expts. 1 and 2. The Bonferroni post hoc test was used to compare means where significant interactions between time and exercise protocol occurred. Paired *t*-tests were used to determine differences between the decline and range for mean HbO_2 ($n = 6$) across the 5-min analysis period. Temporal changes for NIRS were modelled using non-linear curve fitting techniques (DATA DESK, Data Description, Ithaca, USA). Cubic polynomial equations were found to provide the most appropriate fit for the data, and means for data predicted from these equations were compared using paired *t*-tests. Data analysis and statistical calculations were performed using DATA DESK (ANOVA, non-linear curve fitting) or STATVIEW SE (paired *t*-test, correlation; Abacus Concepts, Berkley, Calif., USA). Results were considered to be significant at the $P < 0.05$ level and are presented as means (SEM) unless stated otherwise.

Results

Intermittent exercise (experiment 1)

Respiratory gas exchange and indirect calorimetry

From 16 min to 38 min of exercise, $\dot{V}O_2$, $\dot{V}CO_2$, R and energy expenditure remained constant, and from 27 min to 38 min $\dot{V}_E$ was constant (Fig. 1). Overall $\dot{V}O_2$ [36.8 (0.5) ml · kg^{-1} · min^{-1}, LE; 40.7 (0.8) ml · kg^{-1} · min^{-1} SE; $P < 0.01$] and energy expenditure [0.77 (0.01) kJ · min^{-1} · kg^{-1}, LE; 0.84 (0.02) kJ · min^{-1} kg^{-1}, SE; $P < 0.05$] were both lower during LE (Fig. 1). Overall exercise intensity was 64.9 (2.7)% $\dot{V}O_{2peak}$ during LE and 71.4 (2.4)% $\dot{V}O_{2peak}$ during SE.

The R was higher during LE [0.95 (0.01)] compared to SE [0.88 (0.09); $P < 0.01$], despite the lower overall exercise intensity in the former. There was no difference between the exercise protocols for $\dot{V}_E$ (Fig. 1). A protocol · time interaction effect ($P < 0.05$) indicated that the pattern of change over time for carbohydrate oxidation was different between LE and SE. Post hoc tests revealed that the carbohydrate oxidation rate was higher at all timepoints for LE (Fig. 1). There was no significant effect for time and carbohydrate oxidation rate. The fat oxidation rate was lower ($P < 0.01$) during LE [11 (2) μmol · kg^{-1} · min^{-1}] compared to SE [30 (2) μmol · kg^{-1} · min^{-1}]. There was no change in fat oxidation rate throughout exercise in either LE or SE.

Plasma metabolite, catecholamine and blood gas concentrations

Plasma lactate concentrations were similar at rest and increased significantly to the first measurement in both exercise protocols ($P \leq 0.0001$). From 10 min to the end of the experiment there was no change in lactate concentration during either exercise protocol. A protocol · time interaction effect ($P < 0.001$) indicated that the pattern of change for lactate concentration during the experiment was different in the two protocols. Plasma lactate levels were higher for all measurements during exercise in LE compared to SE ($P < 0.001$; Fig. 2). Pyruvate concentration followed a similar pattern to that observed for lactate (i.e. it exhibited a protocol · time interaction effect, $P < 0.05$). Pyruvate concentration was higher at 10, 32 and 43 min in LE compared to SE ($P < 0.01$), although there was no difference at 21 min. From 10 min to the end of the experiment pyruvate concentration remained constant in both exercise protocols (Fig. 2).

Plasma glycerol concentration increased from the resting level to 32 min in both protocols ($P \leq 0.0001$). However, glycerol concentration was lower ($P < 0.05$) in LE [197 (19) μM] compared to SE [246 (19) μM]. Due to difficulties with the treatment of plasma for NEFA determination, results from three subjects were excluded from statistical analysis. For the four remaining subjects, NEFA concentration was higher at the end of exercise relative to the value at 10 min ($P < 0.01$). Despite these increases and the apparently lower total body fat oxidation that occurred during LE, there was no difference between the exercise protocols for plasma NEFA concentration [537 (74) μM and 883 (148) μM for LE and SE, respectively].

There was no difference in noradrenaline concentration in venous plasma at rest in LE [3.6 (0.2) nM] and SE

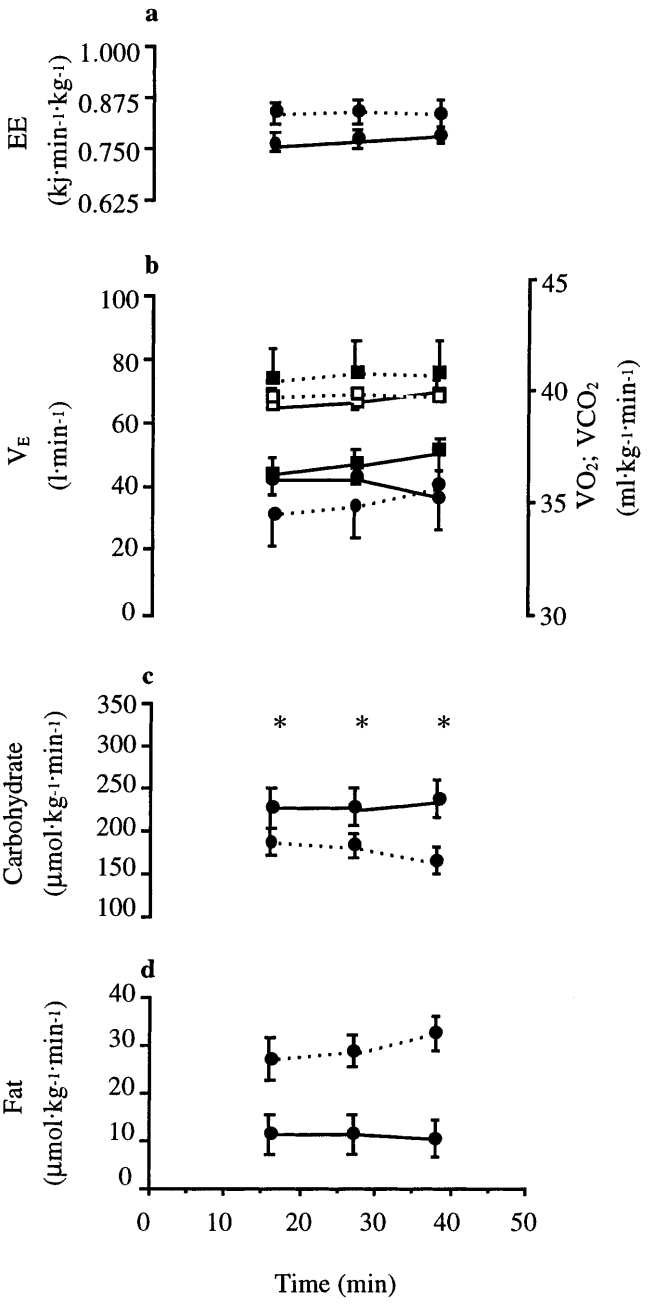

Fig. 1 **a** Energy expenditure (*EE*), **b** expired volume (V_E) (□), oxygen consumption (VO_2) (■), and carbon dioxide production (VCO_2) (●) **c** carbohydrate oxidation rate (*carbohydrate*) and **d** fat oxidation rate (*fat*) during intermittent exercise with a work:recovery ratio of either 24 s:36 s (*solid lines*) or 6 s:9 s (*dashed lines*) in experiment 1. *$P < 0.05$ Significant difference between exercise protocols

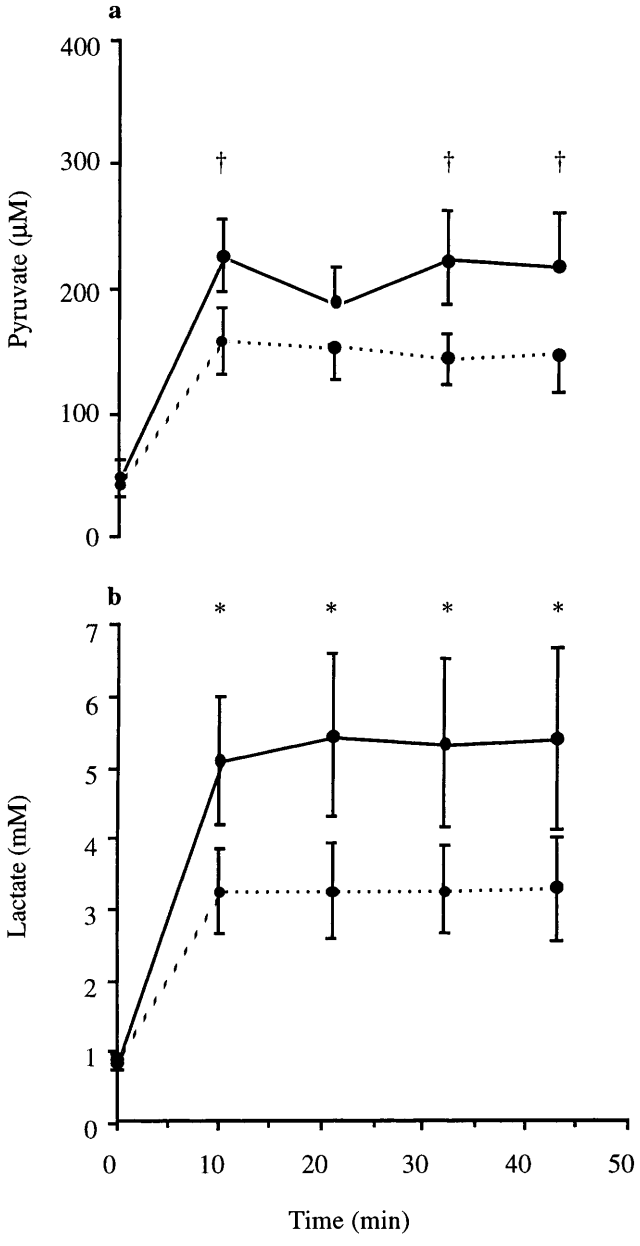

Fig. 2 a Capillary plasma pyruvate and **b** lactate during intermittent exercise with a work:recovery ratio of either 24 s:36 s (*solid lines*) or 6 s:9 s (*dashed lines*) in experiment 1. $^{\dagger}P < 0.01$, $^{*}P < 0.001$ Significant difference between exercise protocols

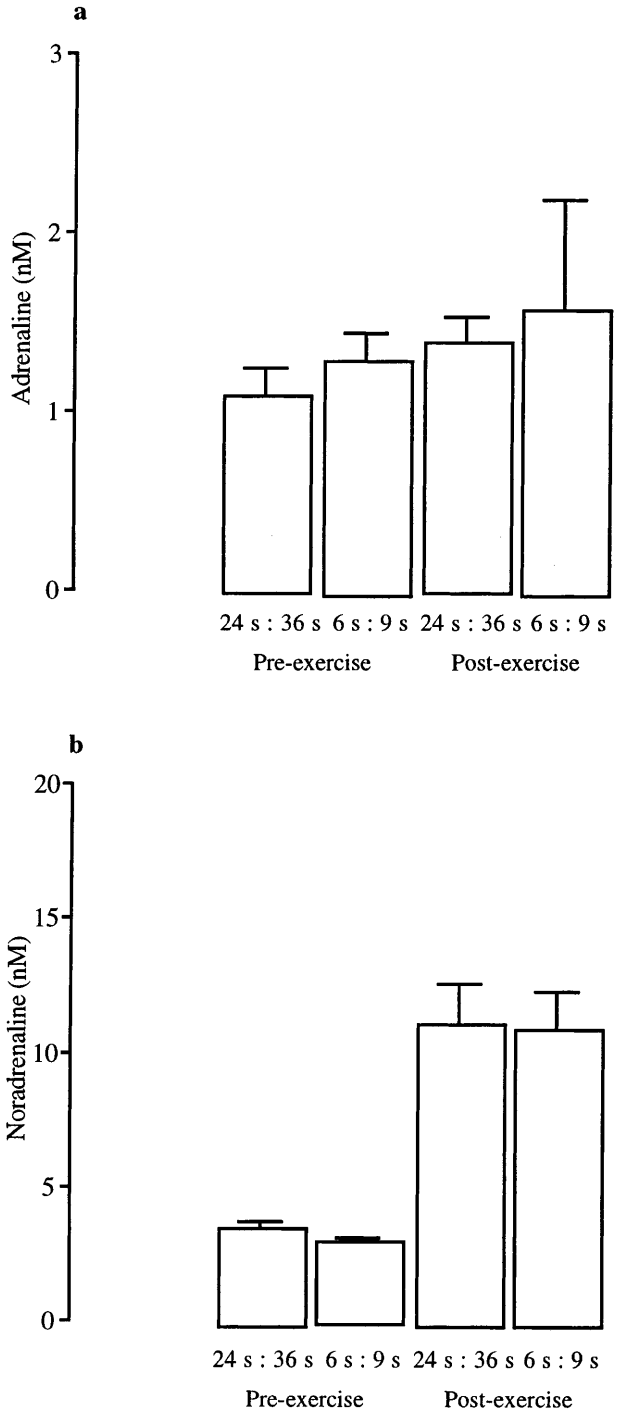

Fig. 3 a Venous plasma adrenaline and **b** noradrenaline pre and post-intermittent exercise with a work:recovery ratio of either 24 s:36 s or 6 s:9 s

[3.1 (0.3) nM]. Noradrenaline levels increased from rest in both protocols ($P < 0.01$, LE; $P < 0.05$, SE), although the response to exercise was not different [11.2 (1.5) nM, LE; 11.0 (1.5) nM, SE; Fig. 3]. Resting plasma adrenaline was not different in either protocol [1.1 (0.2) nM, LE; 1.3 (0.2) nM, SE]. There was no change in adrenaline from the resting level in either LE or SE and, accordingly, there was no difference between protocols for the adrenaline response to exercise (Fig. 3).

Capillary blood pH decreased from rest to 10 min ($P < 0.01$), returned to a level not significantly different from the resting value, and remained constant throughout exercise in both protocols (Fig. 4). During both protocols, blood partial pressure of CO_2 (PCO_2) and HCO_3^- concentration declined significantly from the resting value to 10 min of exercise ($P \leq 0.0001$). From 10 min to the end of exercise there was no further change in either PCO_2 or HCO_3^- for either exercise

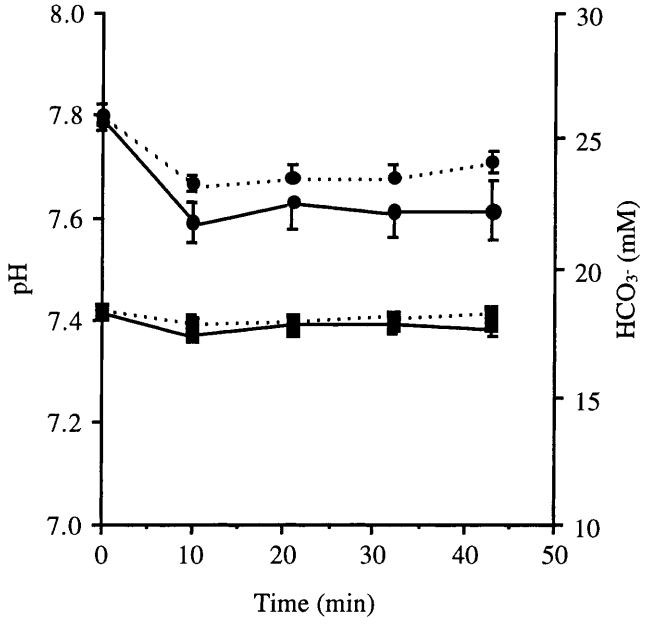

Fig. 4 Capillary plasma pH (■) and bicarbonate (HCO_3^-) (●) during intermittent exercise with a work:recovery ratio of either 24 s:36 s (*solid lines*) or 6 s:9 s (*dashed lines*) in experiment 1

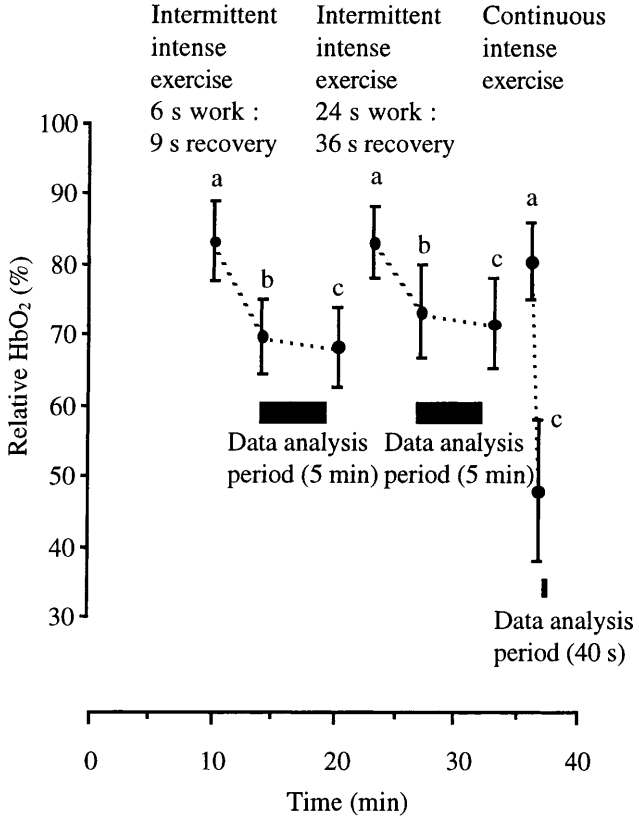

Fig. 5 Relative oxygenation (oxyhaemoglobin, HbO_2) of the quadriceps muscle (mean ± SEM for each subject) at baseline (**a**), commencement of the period of data analysis (**b**) and at the end of the period of data analysis (**c**; i.e. end of exercise) for the three exercise protocols during experiment 2

protocol. Blood HCO_3^- was lower ($P < 0.05$) during LE [23.0 (0.5) mM, LE; 24.7 (0.3) mM, SE; Fig. 4].

The haematocrit level was not different between LE and SE. Therefore, it is likely that observed differences between conditions for plasma metabolites are a consequence of differences in the metabolic response to exercise rather than differences in the degree of haemoconcentration.

Near-infrared spectroscopy (experiment 2)

The linearity of the signal output from the RunMan unit and the relationship to the O_2 content of human blood have been reported previously (Christmass et al. 1999). There was no difference in the baseline level of HbO_2 (subject mean) in the three protocols. Muscle HbO_2 declined between the start of exercise (i.e. baseline) and the commencement of the period of data analysis in both LE and SE (9.92%, $P < 0.01$; 13.55% $P < 0.01$, respectively). However, a dynamic steady state for muscle HbO_2 was apparent in LE and SE during the period of data analysis, as confirmed by the absence of an overall change in NIRS signal across this 5-min interval (Fig. 5). The onset of continuous intense exercise (Fig. 6a) was associated with NIRS values outside the maximum range assigned following release of cuff ischaemia (i.e. in vivo calibration). This outcome has been observed previously during continuous intense exercise (Christmass et al. 1999; 120% $\dot{V}O_{2peak}$, 40 s) and is likely to be the result of exercise-induced hyperaemia.

A protocol · time interaction effect indicated that during the analysis period, the pattern of change for muscle HbO_2 across the representative 60 s was different between the two protocols ($P < 0.001$). Based on the results for expt. 1, overall $\dot{V}O_2$, and hence exercise intensity, would be expected to be lower in LE compared to SE during expt. 2 (Fig. 1). Despite this, the nadir of relative muscle HbO_2 was lower during LE [52.04 (0.60)%] compared to SE [61.85 (0.51)%; $P < 0.001$; Fig. 6b]. The decline in muscle HbO_2 (i.e. first HbO_2 value − lowest HbO_2 value) during 24-s work periods was larger than the decline during 6-s work periods [22.82 (5.63)%; 9.58 (2.43)%, respectively; $P < 0.05$]. Accordingly, the range for HbO_2 across the 60-s analysis period (i.e. maximum HbO_2 − minimum HbO_2) was greater for LE [27.70 (4.50)%, LE; 11.68 (2.50)%, SE; $P < 0.01$]. The average muscle decline in HbO_2 (deoxygenation) was associated with the onset of a work-period and, conversely, an increase in HbO_2 (reoxygenation) was observed during recovery (Fig. 6b). The mean lactate concentration (measured during the corresponding protocol in expt. 1) was correlated with the decline in HbO_2 in intermittent exercise ($r = 0.68$; $P < 0.05$; $n = 12$). In contrast to the steady state observed across the final 5 min of both of the intermittent intense exercise protocols (Fig. 5), HbO_2 decreased progressively in continuous intense exercise, reaching a nadir at 40 s [49.76 (1.52)%; $P < 0.001$; Fig. 6a].

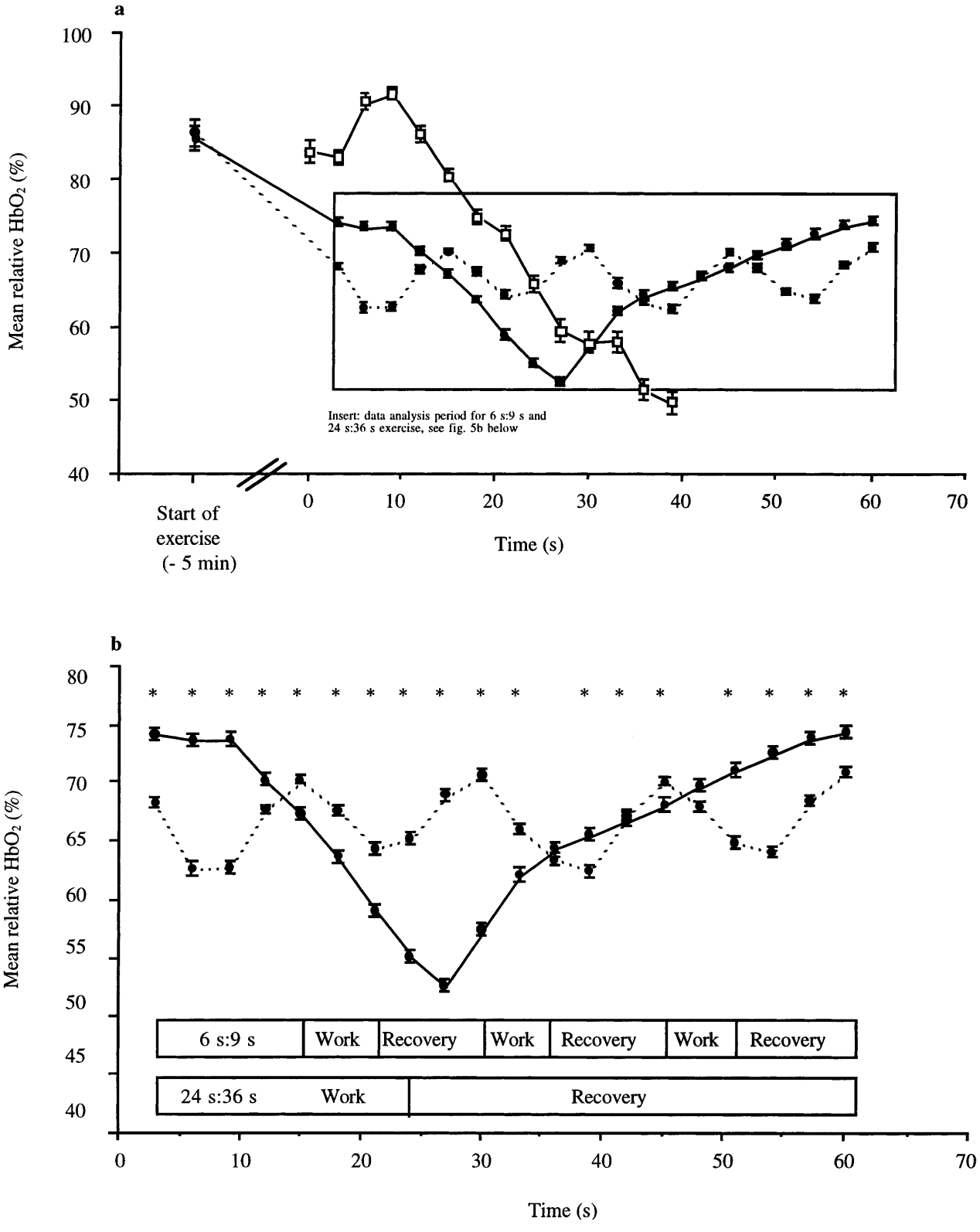

Fig. 6 a Relative oxygenation (oxyhaemoglobin, HbO_2) of the quadriceps muscle during the data analysis period in intermittent exercise with a work:recovery ratio of either 24 s:36 s (LE, *solid lines*) or 6 s:9 s (SE, *dashed lines*), and in continuous intense exercise (□). Results are means ± SEM, represented as a single average (60-s) period for intermittent intense exercise (i.e. one work:recovery cycle for LE, four work:recovery cycles for SE), and the 40-s period for continuous intense exercise. **b** Relative oxygenation of the quadriceps muscle during the data analysis period in intermittent exercise LE (*solid lines*) and SE (*dashed lines*) in experiment 2 (from *inset* in Fig. 6a above). $^*P < 0.001$ Significant difference between exercise protocols

Table 1 Results [mean (SEM)] for parameters predicted from raw near-infrared spectroscopy data using non-linear curve-fitting techniques. (HbO_2 oxyhaemoglobin)

Exercise protocol	Work		Recovery	
	6 s	24 s	9 s	36 s
Maximum rate of decrease in HbO_2 ($\% \cdot s^{-1}$)	−3.04 (0.79)	−2.22 (0.56)		
Maximum rate of increase in HbO_2 ($\% \cdot s^{-1}$)			1.63 (0.32)	1.91 (0.53)
Mean rate of decrease in HbO_2 ($\% \cdot s^{-1}$)	−1.42 (0.39)	−0.87 (0.29)		
Mean rate of increase in HbO_2 ($\% \cdot s^{-1}$)			0.83 (0.23)	0.75 (0.17)
Increase or decrease in HbO_2 (%)	−8.72 (2.30)	−26.24 (5.69)	9.08 (2.18)	26.63 (6.02)
Time to maximum HbO_2 (s)	0	4.67 (1.48)	7.88 (0.31)	36.0 (0.0)
Time to minimum HbO_2 (s)	5.75 (0.13)	25.0 (0.0)		

Results for the parameters that were predicted from raw NIRS data using non-linear curve fitting techniques are presented in Table 1. The maximum rate and the mean rate of deoxygenation during work and reoxygenation during recovery were not different between protocols (Table 1). The decrease in HbO_2 during work ($P < 0.01$) and the increase in HbO_2 during recovery ($P < 0.01$) were, in both cases, larger during LE, which supports the results from the raw data. The decline in HbO_2 during work was correlated with the increase in HbO_2 during recovery ($r = 0.94$, $P < 0.01$, LE; $r = 0.99$, $P < 0.001$, SE; $n = 6$), confirming the observation (from raw data) that muscle oxygenation attained a dynamic steady state across the analysis period in both protocols. During work the maximum HbO_2 level was attained after approximately 5 s in LE, whereas HbO_2 declined immediately at the onset of exercise in SE ($P < 0.05$; Table 1). Minimum muscle HbO_2 was attained after approximately 6 s during SE, and after approximately 25 s during LE.

Discussion

The main objective of the present study was to compare rates of substrate oxidation in two protocols of intermittent exercise, with identical treadmill speed and total work duration, in order to minimise the effect of differences in factors such as muscle fibre type activation, hormonal responses, muscle glucose uptake and NEFA availability on the comparison of substrate utilisation. These factors confound an explanation, based on metabolic regulation, of our previous results demonstrating lower fat oxidation and higher carbohydrate oxidation during intermittent intense compared to continuous submaximal exercise (Christmass et al. 1999). The current findings show that fat oxidation is approximately 3-fold lower and carbohydrate oxidation 1.3-fold higher during intermittent exercise with proportionately longer work and recovery period duration (i.e. LE compared to SE). This is despite the fact that treadmill speed [i.e. work-period intensity, equivalent to 109 (5)% $\dot{V}O_{2peak}$] and total work duration (i.e. 16 min) were identical in both exercise protocols. The differences in substrate oxidation closely resemble the 3-fold lower rate of fat oxidation and 1.2-fold higher rate of carbohydrate oxidation observed in the comparison of intermittent and continuous exercise (Christmass et al. 1999), and is supported by the observation that the respiratory quotient increases with longer work and recovery duration in intermittent intense exercise (Saltin and Essen 1971).

During continuous exercise, an increase in $\dot{V}O_2$, resulting in an increase in relative exercise intensity from 65 to 70% $\dot{V}O_{2max}$, would be expected to be associated with an increase in carbohydrate oxidation (Romijn et al. 1993). In contrast, during intermittent exercise a higher overall $\dot{V}O_2$, and hence higher relative exercise intensity (65 and 71% $\dot{V}O_{2peak}$ for LE and SE, respectively), was associated with lower rates of carbohydrate oxidation. A higher overall $\dot{V}O_2$ as well as higher energy expenditure at the same treadmill speed, suggests a lower efficiency of exercise during SE. This reduced efficiency may be due in part to the additional $\dot{V}O_2$ required to step on and off the moving treadmill belt as a consequence of the more frequent onset of work periods compared to LE. In addition, since the oxidation of fat compared to carbohydrate requires more O_2 for the same ATP production, the higher rates of fat oxidation in SE are likely to have contributed to this reduced efficiency.

Conditions for the appropriate interpretation of respiratory gas exchange measurements, and the reliable use of indirect calorimetry to estimate fat and carbohydrate oxidation rates in the current study, have been discussed previously (Christmass et al. 1999). Briefly, data for respiratory gas exchange were interpreted as representing an average of work and recovery combined (i.e. overall) across a measurement period. For indirect calorimetry, the reliable estimation of tissue $\dot{V}CO_2$ requires the presence of a stable HCO_3^- pool (Romijn et al. 1992).

In both the LE and SE trials, plasma lactate concentration increased in the initial 10 min, but subsequently remained stable throughout the experiment (Fig. 2). Wasserman (1986) concluded that changes in lactate levels, similar to the changes for lactate observed in our study, are associated with an initial decrease in HCO_3– concentration (corresponding to the initial increase in lactate) followed by no further change during

sustained exercise at the same intensity. Accordingly, capillary PCO_2 and HCO_3^- were constant from 10 min until the end of the experiment during both exercise protocols in the current study. During the same period (i.e. 10–43 min) capillary pH remained constant and was not different to the resting value in both protocols (Fig. 4). Taken together, these results suggest the presence of a stable plasma lactate and HCO_{3-} pool. The combination of constant values for $\dot{V}_E$ and $\dot{V}CO_2$ (Fig. 1) and a stable HCO_{3-} pool support the use of indirect calorimetry for estimating substrate oxidation rates across the final two measurement periods (i.e. 27–38 min). Information concerning the intermediate metabolic processes, gluconeogenesis, lipogenesis and ketogenesis during intermittent exercise is scarce, however the influence of these processes on the calculation of net substrate oxidation rates during high-intensity (85% $\dot{V}O_{2max}$) continuous exercise has been outlined previously (Romijn et al. 1992).

In the current study, differences in muscle fibre type recruitment between the two intermittent exercise protocols were not determined, but are unlikely to have influenced substantially the observed difference in substrate utilisation. Essen (1978a) showed that glycogen depletion occurred in similar proportion in type I and II fibres in both continuous intense exercise (100% $\dot{V}O_{2max}$) and intermittent intense exercise (15 s:15 s; work-period intensity 100% $\dot{V}O_{2max}$). In contrast, glycogen depletion was observed predominantly in type I fibres during continuous submaximal exercise at half this exercise intensity (55% $\dot{V}O_{2max}$). These results indicate that exercise intensity is the principal determinant of muscle fibre type activation, whereas the type of exercise (i.e. intermittent or continuous) affects the rate of glycogen depletion in all fibre types (Essen 1978b). The findings of Vollestad and Blom (1985) and Vollestad et al. (1984) support this conclusion and confirm the importance of both exercise intensity and duration in determining the type of muscle fibre recruited. Since treadmill speed and total work duration were identical in LE and SE, differences in muscle fibre type activation are unlikely to explain completely the observed differences in substrate utilisation.

The increase in plasma noradrenaline levels in response to exercise was similar in the two protocols (Fig. 3). In neither protocol was there a change in plasma adrenaline following exercise. The principal hormonal influences on muscle glycogenolysis are catecholamines, while peripheral (adipose tissue) lipolysis is regulated by changes in both insulin and catecholamines (Chastiosis 1983; Hales et al. 1978). Our finding indicates that the observed differences in fat and carbohydrate oxidation cannot be explained on the basis of differential effects of circulating catecholamines in LE and SE.

The decline in fat oxidation observed during LE exercise is not likely to be due to limited plasma NEFA availability (Romijn et al. 1995). The rate of lipolysis, as estimated from plasma glycerol, increased progressively in both exercise conditions and was approximately 26% higher during the SE trial. Despite this, plasma NEFA concentration was similar in the two exercise conditions and was considerably higher than the levels suggested to impair fat oxidation (Romijn et al. 1995). Furthermore, a progressive increase in NEFA levels occurred during LE with a trend towards a similar increase in SE. Combined with a relatively stable R, these results suggest that NEFA availability was not limiting for whole-body fat oxidation in either exercise condition.

The identical treadmill speed and total work duration in each exercise protocol, combined with a similar catecholamine response and plasma NEFA availability between SE and LE, support the concept that factors extrinsic to the muscle fibre (i.e. muscle fibre type activation and circulating hormone levels) are unlikely to be the basis for the current results. Instead, our findings suggest that a metabolic factor (or factors) within the oxidative muscle fibres is (are) responsible for the difference in substrate utilisation between the LE and SE.

The two exercise protocols differed principally on the basis of the duration of the work and recovery periods. At the same treadmill speed, the higher plasma lactate and pyruvate concentrations noted during LE exercise confirm the well-established effect of increases in work and recovery duration on the metabolic response to intermittent exercise (Astrand et al. 1960a). Astrand et al. (1960a) showed that increases in both work and recovery duration (i.e. 1:1 work:recovery ratio) from 30 s to 3 min resulted in increases in blood lactate accumulation and perceived exertion. A decline in the duration of recovery, whilst work remains constant, is associated with increased blood lactate concentration during exhaustive treadmill running (18 $km \cdot h^{-1}$, 15% grade; Margaria et al. 1969).

In the present study, stable, high lactate and pyruvate levels were associated with an increase in carbohydrate oxidation and a decline in fat oxidation during sustained steady-state intermittent intense exercise. These results are supported by the finding that in addition to lactate accumulation, the respiratory quotient measured across the exercising leg is elevated during intermittent cycling exercise at the same work-period intensity when the duration of work and recovery periods are increased (Saltin and Essen 1971).

With identical work-period intensity, the duration of the work period, rather than recovery or total work output, was originally suggested to be of primary importance in terms of the metabolic response to intermittent intense exercise (Astrand et al. 1960b). The connection between increased work duration and a higher rate of carbohydrate utilisation and lactate and pyruvate accumulation observed in the current study could be due to inadequate availability of O_2 secondary to depletion of Mb stores (Astrand et al. 1960b; Saltin and Essen 1971). In terms of metabolic regulation, lower

O_2 availability may be associated with higher rates of glycogenolysis (Ren et al. 1992) and glycolysis through increases in free ADP, AMP and inorganic phosphate (for review see Katz and Sahlin 1990).

Astrand et al. (1960a) originally proposed that Mb may act as an O_2 store that is repeatedly used and reloaded during work and recovery in intermittent exercise. According to this concept, as the duration of the work period increases Mb stores are reduced, resulting in a decline in O_2 availability relative to utilisation (Astrand et al. 1960a, b). In a similar approach to the current study, Saltin and Essen (1971) compared intermittent exercise protocols with proportionately different work and recovery durations (i.e. 1:2 work:recovery ratio). The higher muscle glycogen and phosphocreatine depletions observed with longer work duration provide quantitative support for the proposed role of Mb (Astrand et al. 1960a, b). The use of NIRS in the current study has enabled a comparison of relative O_2 availability in the quadriceps femoris between the two exercise protocols. Differences in the absorption characteristics of NIR light by the oxygenated and deoxygenated forms of haemoglobin and Mb enable NIRS to detect relative changes in tissue oxygenation (Jobsis 1977). The origin of the NIRS signals in muscle, validation of the technique and in vivo calibration during exercise in humans have been discussed previously (Chance et al. 1992; Mancini et al. 1994).

Following an initial period of overall decline across several cycles of work and recovery, relative muscle HbO_2 attained a dynamic steady state in both LE and SE (Fig. 5). During this steady-state period, average HbO_2 changed across 60 s in both intermittent exercise protocols and was characterised by deoxygenation in work periods and reoxygenation during recovery (Fig. 6b). The high correlation between the extent of deoxygenation in work and reoxygenation in recovery (from results for non-linear curve fitting techniques) further supports the occurrence of a dynamic steady state during both protocols. In contrast to intermittent exercise, muscle HbO_2 declined progressively during 40 s of continuous intense exercise (i.e. at the treadmill speed used for work periods of intermittent exercise), to reach a nadir in the final measurement (Fig. 6a). This finding demonstrates the distinct discrepancy between O_2 supply and demand at the treadmill speed (i.e. work-period intensity) required during intermittent exercise. In addition, the importance of the recovery period to enable a steady-state balance between deoxygenation and reoxygenation to be achieved in both intermittent protocols is emphasised by this result.

The lower nadir HbO_2 and the larger decline in HbO_2 that occurred during work periods in LE compared to SE indicates a comparably lower muscle HbO_2 (i.e. hypoxia) during LE. This relative hypoxia occurred despite a lower overall exercise intensity (i.e. lower overall $\dot{V}O_2$ based on results from expt. 1) in LE and an identical treadmill speed in both protocols. During LE and SE the time to reach the minimum HbO_2 level during work (from results for non-linear curve fitting techniques) was approximately 25 s and 6 s, respectively, suggesting that muscle deoxygenation occurs progressively throughout exercise in both protocols (Table 1). Furthermore, the mean and the maximum rates of this progressive deoxygenation were similar in both protocols. These results indicate that the larger decline in HbO_2 across 24 s of work is not a consequence of differences in O_2 metabolism between the protocols per se. Instead, the current findings confirm that the contrast in HbO_2 between the two intermittent exercise protocols is a consequence of the duration of the work period (Astrand et al. 1960b).

Reduced muscle O_2 availability (Sahlin 1990), resulting in an increase in carbohydrate utilisation, may have implications for inhibition of fat oxidation (Sidossis et al. 1997) in reverse of the classic glucose-fatty acid cycle (Randle et al. 1964) during LE exercise. The LE protocol was characterised by a 63% lower rate of whole-body fat oxidation compared to the SE protocol. During continuous exercise, whole-body fat oxidation attains maximal levels at a moderate exercise intensity (65% $\dot{V}O_{2max}$), but decreases in both absolute and relative terms at high exercise intensity (85% $\dot{V}O_{2max}$; Romijn et al. 1993). The reasons for the absolute decline when the intensity of exercise increases are unclear. Several studies have shown a decrease in the rate of appearance of plasma NEFA during high-intensity exercise (Jones et al. 1980; Romijn et al. 1993). However, plasma NEFA availability cannot account for the 3-fold lower fat oxidation rate observed for LE. Indeed, Romijn et al. (1995) have demonstrated that the availability of plasma NEFA can, at most, account for approximately 50% of the absolute decline in fat oxidation during high-intensity continuous exercise (85% $\dot{V}O_{2max}$).

The current results suggest that differences in the rate of fat oxidation within skeletal muscle provide an explanation for the difference in whole-body fat oxidation between SE and LE. Indeed, recent evidence suggests that decreased fat oxidation in the presence of accelerated carbohydrate metabolism results, at least in part, from reduced transport of long-chain fatty acids into skeletal muscle mitochondria (Coyle et al. 1997; Sidossis et al. 1997). Increases in the availability of pyruvate, as a consequence of rapid glycolysis, and lactate and pyruvate formation during high-intensity exercise, may result in a decline in fat oxidation by inhibiting the transport of long-chain NEFA into the mitochondria (Coyle et al. 1997; Sidossis et al. 1997). The lower fat oxidation observed in LE was associated with a higher rate of carbohydrate oxidation in the presence of elevated plasma lactate and pyruvate. Conversely, the 3-fold greater fat oxidation in SE was accompanied by lower carbohydrate oxidation and lower plasma lactate and pyruvate concentrations.

During continuous exercise the well-established decline in the contribution of fat to oxidative metabolism occurs when exercise intensity increases (Romijn et al.

1993). However, in the current study reduced rates of fat oxidation occurred in association with a lower overall exercise intensity and energy expenditure. In contrast to continuous exercise, the decline in fat oxidation noted during intermittent exercise was associated with an increase in the work and recovery duration at the same work-period intensity (i.e. treadmill speed). Furthermore, longer periods of work resulted in a comparatively lower muscle O_2 availability, which in turn was correlated with the mean lactate level during exercise. Sahlin (1990) speculated that a decline in fat oxidation as a consequence of a decrease in free carnitine (resulting in diminished transport of NEFA into the mitochondria) in the presence of elevated lactate and pyruvate may be beneficial in situations of O_2 deficiency, since carbohydrate oxidation requires less O_2 for a given ATP synthesis compared to fat. This is supported by the finding that carbohydrate metabolism increases in skeletal muscle when the O_2 supply is reduced (Zinker et al. 1994).

In summary, we have shown that lower rates of fat oxidation and higher rates of carbohydrate oxidation occur during sustained steady-state intermittent exercise involving LE compared to that involving SE. The treadmill speed and total work duration were identical in both exercise protocols. Although plasma glycerol was slightly higher in SE, there was no difference in NEFA concentration. The plasma catecholamine response to exercise was similar in both exercise protocols. Plasma lactate and pyruvate accumulation were higher during LE, and this was associated with a comparatively lower muscle O_2 availability (as determined using NIRS). We conclude that a metabolic factor (or factors) within the oxidative muscle fibres is (are) responsible for the difference in substrate utilisation observed between the LE and SE protocols.

Acknowledgements The authors thank Professor Peter Hartmann of the Department of Biochemistry, The University of Western Australia, for his valuable advice and support throughout the study. The authors also thank Vittoria Lazzaro and Narelle Willis from the Renal Laboratory, Department of Renal Medicine, Royal Prince Alfred Hospital, Sydney, Australia, for the determination of plasma catecholamine concentrations, and the staff of the Western Australian Institute of Sport for the use of their exercise physiology laboratory facilities during expt. 1.

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