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# UTILIZATION OF BLOOD-BORNE AND INTRAMUSCULAR SUBSTRATES DURING CONTINUOUS AND INTERMITTENT EXERCISE IN MAN

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SUMMARY

1. Substrate utilization in the legs during bicycle exercise was studied in five subjects when performing intermittent intense exercise (15 sec work – 15 sec rest) as well as continuous exercise during 60 min, with an almost identical average power output and oxygen uptake in both situations.

2. Muscle biopsies were obtained from vastus lateralis at rest, during, and after exercise in order to determine intramuscular lipid and carbohydrate utilization. The contribution from blood-borne substrates to total oxidative metabolism was determined by arterial-femoral venous (a-fv) differences for oxygen, FFA, glucose, and lactate and leg blood flow.

3. Intermittent and continuous exercise revealed a similar glycogen depletion and the intramuscular lactate accumulation was rather small. A similar uptake of blood-borne substrate (FFA, glucose) was found in both situations whereas a release of lactate only was observed in intermittent exercise.

4. ATP and CP levels oscillated between work and rest periods in intermittent exercise but were not resynthesized to resting levels at the end of the rest periods. The mainly aerobic energy release during each work period in intermittent exercise is partly caused by myoglobin functioning as an oxygen store; this factor was calculated to be more important than ATP and CP or lactate level oscillations.

5. The metabolic response to intermittent exercise was found to be similar to that found in continuous exercise with approximately the same average power output and oxygen uptake. This indicates that some factor in the intermediary metabolism, for instance citrate, functions as a regulator retarding glycolysis and favouring lipid utilization and an aerobic energy release in intermittent exercise.

## INTRODUCTION

Several studies have been undertaken to evaluate substrate utilization in the human skeletal muscle during different types of continuous exercise. During work of moderate intensity lipids contribute a great proportion of the substrate utilization, at least if the work duration is longer than 30–40 min. At heavier work intensities carbohydrates become of greater importance as a substrate source. Concomitant with a high rate of glycolysis there is a partly anaerobic metabolism. In daily life the most common situation is not continuous exercise but work performed as intermittent exercise with bursts of more intense activity interspersed with repeated periods of lighter activity or rest. In this situation lipids contribute relatively more to substrate utilization than during continuous exercise of the same intensity as indicated by the lower repiratory exchange ratio, and the fraction of energy covered by anaerobic metabolism is smaller as indicated by a lower blood lactate concentration (Åstrand, Åstrand, Christensen & Hedman, 1960; Christensen, Hedman & Saltin, 1960). However, there is no study where an evalution has been made by analyses of both the intra- and extramuscular lipid and carbohydrate sources.

The purpose of this study was to quantify substrate utilization from both intra- and extramuscular sources of lipids and carbohydrates during a prolonged period of intermittent intense exercise (15 sec work – 15 sec rest) and during continuous exercise, with the same average oxygen uptake ($\approx$ the same average power output). The net metabolic response seems to be similar in these two situations (Christensen, Hedman & Holmdahl, 1960; Edgerton, Essén, Saltin & Simpson, 1975), which indicates that some regulatory factors are brought into play retarding glycolysis and enhancing fat oxidation during intermittent exercise.

## METHODS

### *Subjects*

Five healthy male students participated in the study. They averaged 23 yr (21–26) in age, 181 cm (175–193) in height, 70 kg (62–75) in weight and 4·20 l./min (3·59–4·50) in maximal oxygen uptake ($\dot{V}_{O_2}$ max). All subjects were informed of the nature and purpose of the experimental procedure and of the possible risks involved before giving their voluntary consent to participate.

### *Procedure*

A few weeks before the first experiment maximal oxygen uptake was determined during bicycle exercise. On the day of the experiment the subjects came to the laboratory in the morning after an overnight fast. Teflon (Du Pont) catheters were inserted percutaneously into both femoral veins and one femoral artery for blood sampling and leg blood flow determination. The tips of the femoral catheters were introduced approximately 8 cm in the proximal direction. Another catheter

was introduced into one antecubital vein for the infusion of [$^{14}$C]oleic acid to enable the estimation of oleic acid turnover and free fatty acid (FFA) uptake.

Exercise was performed on an electrodynamically loaded bicycle ergometer (Siemens-Elema, Stockholm, Sweden). The work load chosen for the intermittent exercise was that demanding the subjects $\dot{V}_{O_2}$ max during continuous exercise and was on an average 299 Watt (270–343).

All subjects repeated the experimental procedure ½–1 yr later with continuous exercise. The physical activity of the subjects was then unchanged between the two experimental procedures. The work load was 157 Watt (138–180), chosen to correspond to the average oxygen uptake found during the intermittent exercise which was 55% $\dot{V}_{O_2}$ max. The duration was again 60 min and the total work output was approximately the same in the two situations. Oxygen uptake, heart rate, blood samples, leg blood flow, [$^{14}$C]oleic acid infusion and muscle biopsies were obtained at the same intervals in both intermittent and continuous exercise. A schematic illustration of the experimental procedure is given in Fig. 1.

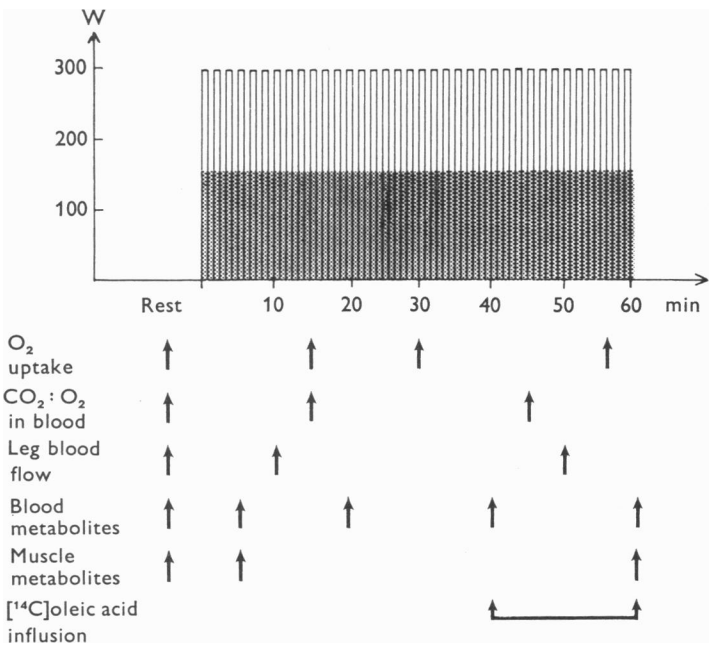


Fig. 1. A schematic illustration of the experimental procedure during 60 min of intermittent (15 sec work–15 sec rest, open bars) and continuous exercise with the same average power output. The arrows indicate at what time during the experiment the different measurements were made.

*Analyses*

*Oxygen uptake* was measured at rest, after 15 and 30 min and at the end of exercise. At 30 min of intermittent exercise oxygen uptake was measured both during work and rest periods by collecting the expired air during the rest periods in one Douglas bag and the expired air during the work periods in a second Douglas bag.

The volume of expired air was measured by a spirometer and air samples were analysed for oxygen and carbon dioxide by the Haldane technique.

*Heart rate* was recorded every 5 min and both during work and rest periods in intermittent exercise. Heart rate was counted over 15 sec from an e.c.g.-recording.

*Leg blood flow* was determined at rest and after 10 and 50 min of exercise by constant rate indocyanine green (Cardiogreen$^{R}$) infusion into the femoral artery (Jorfeldt & Wahren, 1971). The time for infusion of dye was 180 sec at rest and 90 sec during continuous and intermittent exercise before the blood sampling started. Four a-v differences were drawn during continuous exercise and three each during both rest periods and work periods in intermittent exercise. The dye concentration was determined spectrophotometrically at 805 nm. Hematocrit was obtained using a microcapillary haematocrit centrifuge (Garby & Vuille, 1961).

*Leg respiratory quotient* (R.Q.$_{L}$) was estimated at rest and after 15 and 50 min of exercise by analysing the oxygen and carbon dioxide content in arterial and venous blood by the Van Slyke technique.

*Metabolites in blood* were analysed in samples drawn from the femoral artery and vein simultaneously at rest and after 5, 20, 40 and 60 min of exercise. During intermittent exercise samples were collected during both a work and a rest period. For calculation purposes the average of a work and a rest period was used. Blood glucose was analysed by the glucose oxidase reaction (Hugett & Nixon, 1957). Blood, lactate, pyruvate, glycerol and citrate were determined by deproteinizing 0·2 ml. whole blood in 1 ml. ice-chilled 0·6 M perchloric acid and then neutralizing with $KHPO_4$. From the supernatant an aliquot was taken and analysed for the blood metabolites by enzymic fluorimetric methods (Lowry & Passonneau, 1973). Free fatty acids (FFA) in plasma were analysed by gas chromatography (Hagenfeldt, 1966).

*[$^{14}$C]oleic acid* (0·4 $\mu$Ci/min) was infused continuously at constant rate starting after 40 min of work and three samples from the femoral artery and vein were collected between 55 and 60 min for the estimation of [$^{14}$C]oleic acid specific activity (Hagenfeldt & Wahren, 1968). Calculations of fractional uptake and turnover rate of oleic acid as well as uptake and release of total FFA have been described previously (Ahlborg, Felig, Hagenfeldt, Hendler & Wahren, 1974).

*Muscle biopsies* were obtained from the vastus lateralis using the percutaneous needle biopsy technique (Bergström, 1962) at rest, after 5 min of work and immediately at the end of exercise. In addition, during intermittent exercise in four of the subjects muscle biopsies were obtained immediately after both a work and a rest period at 3, 8 and 15 min. The samples were immediately frozen in liquid nitrogen and stored at $-80°$ C until analysed. Glycogen, lactate, citrate, ATP and CP were analysed with fluorometric enzymic methods (Karlsson, Diamant & Saltin, 1970; Lowry & Passonneau, 1973). Triglycerides were analysed by the extraction of the neutral fats from the biopsy specimen with a Folch extract and determining its glycerol content after evaporation and hydrolysis of the chloroform phase (Chernick, 1969).

### *Statistics*

Results in the text, tables and figures are given as mean $\pm$ S.E. of mean or mean and range. Paired $t$ test and standard statistical methods have been used in the analysis of data.

## RESULTS

*Oxygen uptake, pulmonary ventilation, respiratory exchange ratio and heart rate* (Table 1). Exercise produced a nine- to tenfold increase in oxygen uptake. Mean oxygen uptake for the 60 min period of continuous exercise at 157 Watt (138–180) was 2·35 $\pm$ 0·08 l./min and was similar to 2·24 $\pm$ 0·09

during intermittent exercise at 299 W (270–343). During the first half of the experiment, however, oxygen uptake during intermittent exercise was $0{\cdot}21 \pm 0{\cdot}06$ l./min higher than during continuous exercise ($P < 0{\cdot}01$). During the second half of the experiment oxygen uptake was almost identical in the two situations. Comparing rest and work periods during intermittent exercise, oxygen uptake was found to oscillate only slightly around that calculated for the average power output and a $0{\cdot}38 \pm 0{\cdot}07$ l./min higher oxygen uptake was observed during the work periods as

TABLE 1. Oxygen uptake, respiratory exchange ratio, pulmonary ventilation and heart rate at rest and during continuous and intermittent exercise (each figure represents the mean ± S.E. of mean)

| | | Rest | 15 min | 30 min | 50 min |
|---|---|---|---|---|---|
| Oxygen uptake (l./min) | C | $0{\cdot}26 \pm 0{\cdot}01$ | $2{\cdot}14 \pm 0{\cdot}12$ | $2{\cdot}19 \pm 0{\cdot}16$ | $2{\cdot}29 \pm 0{\cdot}20$ |
| | I | $0{\cdot}29 \pm 0{\cdot}01$ | $2{\cdot}38 \pm 0{\cdot}18$ | $2{\cdot}56 \pm 0{\cdot}09$ (work)<br>$2{\cdot}18 \pm 0{\cdot}13$ (rest) | $2{\cdot}28 \pm 0{\cdot}17$ |
| Respiratory exchange ratio | C | $0{\cdot}83 \pm 0{\cdot}01$ | $0{\cdot}89 \pm 0{\cdot}01$ | $0{\cdot}91 \pm 0{\cdot}01$ | $0{\cdot}90 \pm 0{\cdot}01$ |
| | I | $0{\cdot}80 \pm 0{\cdot}04$ | $0{\cdot}88 \pm 0{\cdot}02$ | $0{\cdot}86 \pm 0{\cdot}02$ (work)<br>$0{\cdot}88 \pm 0{\cdot}03$ (rest) | $0{\cdot}87 \pm 0{\cdot}01$ |
| Pulmonary ventilation (l./min) | C | $8{\cdot}3 \pm 0{\cdot}9$ | $52{\cdot}2 \pm 1{\cdot}8$ | $56{\cdot}5 \pm 2{\cdot}6$ | $59{\cdot}3 \pm 4{\cdot}1$ |
| | I | $8{\cdot}1 \pm 0{\cdot}5$ | $61{\cdot}9 \pm 3{\cdot}3$ | $67{\cdot}0 \pm 3{\cdot}0$ (work)<br>$62{\cdot}5 \pm 4{\cdot}2$ (rest) | $65{\cdot}1 \pm 3{\cdot}7$ |
| | | 5 min | 20 min | 40 min | 60 min |
| Heart rate (beats/min) | C | $127 \pm 7$ | $144 \pm 7$ | $147 \pm 8$ | $154 \pm 10$ |
| | I | $141 \pm 5$ | $155 \pm 5$ | $158 \pm 5$ (work)<br>$158 \pm 5$ (rest) | $161 \pm 4$ |

compared with the rest periods ($P < 0{\cdot}01$). Pulmonary ventilation followed oxygen uptake closely and was significantly greater ($61{\cdot}9 \pm 3{\cdot}3$ l./min) after 15 min of intermittent exercise than pulmonary ventilation during continuous exercise ($52{\cdot}2 \pm 1{\cdot}8$ l./min) ($P < 0{\cdot}01$). During intermittent exercise the pulmonary ventilation was higher during work ($67{\cdot}0 \pm 3{\cdot}0$ l./min) than rest periods ($62{\cdot}5 \pm 4{\cdot}2$ l./min) ($P < 0{\cdot}05$). As a result the ventilatory coefficient $\dot{V}_E/\dot{V}_{O_2}$ was of a similar magnitude in intermittent ($27{\cdot}1 \pm 0{\cdot}7$) and continuous exercise ($25{\cdot}6 \pm 0{\cdot}7$). A rise in respiratory exchange ratio from a resting value of $0{\cdot}80 \pm 0{\cdot}04$ to a mean of $0{\cdot}88 \pm 0{\cdot}01$ was observed in intermittent exercise and a rise from $0{\cdot}83 \pm 0{\cdot}01$ to $0{\cdot}90 \pm 0{\cdot}01$ was found during continuous exercise. No significant difference was observed between the two situations. The mean heart rate during continuous exercise was $143 \pm 8$ beats/min, which was $11 \pm 2$ beat/min lower than during intermittent exercise ($P < 0{\cdot}001$). A progressive small increase in heart rate from the 5th to the 60th min was noted in both situations with a mean of 20 beats in intermittent and 27 beats in continuous exercise. The results for $\dot{V}_{O_2}$, ventilation, R.Q. and heart rate obtained in

the present study during continuous and intermittent exercise agree well with previous reports (Åstrand *et al.* 1960; Edwards, Ekelund, Harris, Hesser, Hultman, Melcher & Wigertz, 1973; Edgerton *et al.* 1975).

TABLE 2. Leg blood flow, respiratory quotient, a-fv oxygen difference, haematocrit and leg $\dot{V}_{O_2}$ at rest and during continuous and intermittent exercise (each figure represents the mean ± S.E. of mean)

| | | Rest | 15 min | 50 min |
|---|---|---|---|---|
| Leg blood flow (l./min) | C | 0·43 ± 0·03 | 4·93 ± 0·37 | 4·89 ± 0·42 |
| | I | 0·59 ± 0·07 | 4·36 ± 0·30 | 4·38 ± 0·25 |
| | | | 4·60 ± 0·39 (rest) | 4·53 ± 0·28 (rest) |
| | | | 4·12 ± 0·24 (work) | 4·20 ± 0·26 (work) |
| R.Q. | C | 0·81 ± 0·03 | 0·90 ± 0·03 | 0·86 ± 0·03 |
| | I | 0·80 ± 0·03 | 0·88 ± 0·02 | 0·91 ± 0·02 |
| a-fv oxygen differences (ml./l.) | C | 58·2 ± 13 | 144·8 ± 4·5 | 151·2 ± 5·3 |
| | I | 44·4 ± 3·4 | 159·0 ± 3·3 | 163·3 ± 5·5 |
| Haematocrit | C | 43·2 ± 0·72 | 48·0 ± 1·19 | 47·4 ± 0·82 |
| | I | 41·7 ± 0·99 | 47·1 ± 0·98 | 46·2 ± 0·66 |
| Leg $\dot{V}_{O_2}$ | C | 0·03 ± 0·0 | 0·71 ± 0·06 | 0·73 ± 0·04 |
| | I | 0·02 ± 0·0 | 0·69 ± 0·03 | 0·70 ± 0·04 |

*Leg blood flow, arterial-femoral venous oxygen difference,* R.Q.$_L$ *and haematocrit* (Table 2). Leg blood flow increased approximately eight- to tenfold with exercise over the resting values. No significant difference was seen between the values obtained at 15 and 50 min of exercise. Continuous exercise produced 0·56 ± 0·16 l./min greater flow ($P < 0·01$) than intermittent exercise where the mean value was 4·37 ± 0·28 l./min. The value given is for one leg. Calculations of flow in intermittent exercise made separately for blood samples taken only during work periods and during rest periods revealed a tendency towards a greater flow during rest periods 4·56 ± 0·34 l./min than during work periods 4·18 ± 0·25 l./min ($P < 0·01$).

The arterial-femoral venous (a-fv) oxygen difference increased three- to fourfold with exercise and was almost unchanged throughout the exercise period. It was significantly greater during intermittent exercise at both 15 and 50 min of exercise with a mean value of 161 ± 4 ml./l. as compared with 148 ± 5 ml./l. in continuous exercise ($P < 0·01$).

The leg oxygen uptake calculated from a-fv oxygen difference and leg blood flow was similar in the two situations. The mean value for one leg during intermittent exercise was 0·70 ± 0·03 l./min and 0·72 ± 0·05 l./min during continuous exercise.

There was good agreement between R.Q.$_L$ and respiratory exchange ratio both at rest and during exercise. The mean R.Q.$_L$ was not significantly different between intermittent and continuous exercise.

The haematocrit increased with exercise to a mean value of $46{\cdot}7 \pm 0{\cdot}8$ during intermittent and similarly to $47{\cdot}7 \pm 1{\cdot}0$ during continuous exercise.

*Arterial substrate concentrations* (Figs. 2 and 3). Arterial glucose concentration at rest was $4{\cdot}55 \pm 0{\cdot}11$ m-mole/l. and at end of continuous exercise $4{\cdot}89 \pm 0{\cdot}18$ m-mole/l. In intermittent exercise the resting value was $3{\cdot}96 \pm 0{\cdot}06$ m-mole/l. and $4{\cdot}79 \pm 0{\cdot}31$ m-mole/l. at 60 min. The resting values and the values at 5 min in intermittent exercise were slightly lower than in continuous exercise ($P < 0{\cdot}05$). Glucose concentration in both intermittent and continuous exercise did not deviate significantly from the resting values during the exercise period.

Arterial lactate concentration in the continuous exercise experiment was $0{\cdot}95 \pm 0{\cdot}08$ m-mole/l. at rest and rose to $3{\cdot}96 \pm 0{\cdot}32$ m-mole/l. ($P < 0{\cdot}001$) after 5 min of exercise but decreased progressively thereafter during the remaining exercise period ($P < 0{\cdot}01$). In intermittent exercise there was a rise from $0{\cdot}75 \pm 0{\cdot}15$ m-mole/l. at rest to $3{\cdot}18 \pm 0{\cdot}63$ m-mole/l. ($P < 0{\cdot}05$) after 5 min and the arterial concentration did not change significantly during the remaining intermittent exercise.

Arterial pyruvate concentration in intermittent and continuous exercise was similar, and during exercise followed the same pattern as for lactate. In continuous exercise there was an initial rise to $0{\cdot}130 \pm 0{\cdot}011$ m-mole/l. at 5 min ($P < 0{\cdot}05$) followed by a tendency towards a gradual decrease. In intermittent exercise the concentration rose to $0{\cdot}095 \pm 0{\cdot}015$ m-mole/l. at 5 min ($P < 0{\cdot}05$) and if any there was a slight gradual increase during the following period, to $0{\cdot}115 \pm 0{\cdot}021$ m-mole/l. at 60 min ($P < 0{\cdot}05$).

Arterial FFA concentration showed a similar pattern both in continuous and intermittent exercise, with an initial fall at the onset of exercise after which the concentration tended to increase progressively.

A gradual and similar increase of arterial glycerol was seen in both situations and at the end of exercise there was a fourfold increase over the resting values ($P < 0{\cdot}04$).

Arterial and femoral venous citrate concentration revealed a tendency to increase slightly in both situations with exercise. Citrate was the only metabolite in the blood that showed a significant difference in arterial and venous concentration between work and rest periods in intermittent exercise (Fig. 7). The mean arterial value during work periods was $0{\cdot}19 \pm 0{\cdot}03$ m-mole/l. as compared with $0{\cdot}14 \pm 0{\cdot}02$ m-mole/l. found during rest periods ($P < 0{\cdot}05$).

*Leg exchange of substrates* (Table 3, Figs. 2 and 3). Significant positive a-fv differences of glucose were observed in both intermittent and continuous exercise during the entire exercise period ($P < 0{\cdot}001$). A significant uptake of glucose was attained with a mean of $1{\cdot}12 \pm 0{\cdot}33$ m-mole/min at

TABLE 3. Arterial concentration, turnover, fractional extraction and leg exchange of oleic acid at 50–60 min of continuous and intermittent exercise (each figure represents the mean ± S.E. of mean)

| | | |
|---|---|---|
| Arterial concentration of oleic acid (μmole/l.) | C | 148 ± 52 |
| | I | 181 ± 35 |
| Turnover of oleic acid (μmole/min) | C | 343 ± 120 |
| | I | 398 ± 73 |
| Leg exchange of oleic acid | | |
| Fractional extraction | I | 0·19 ± 0·02 |
| | C | 0·21 ± 0·03 |
| Uptake (μmole/min) | C | 168 ± 24 |
| | I | 144 ± 48 |
| Release (μmole/min) | C | 72 ± 38 |
| | I | 46 ± 20 |

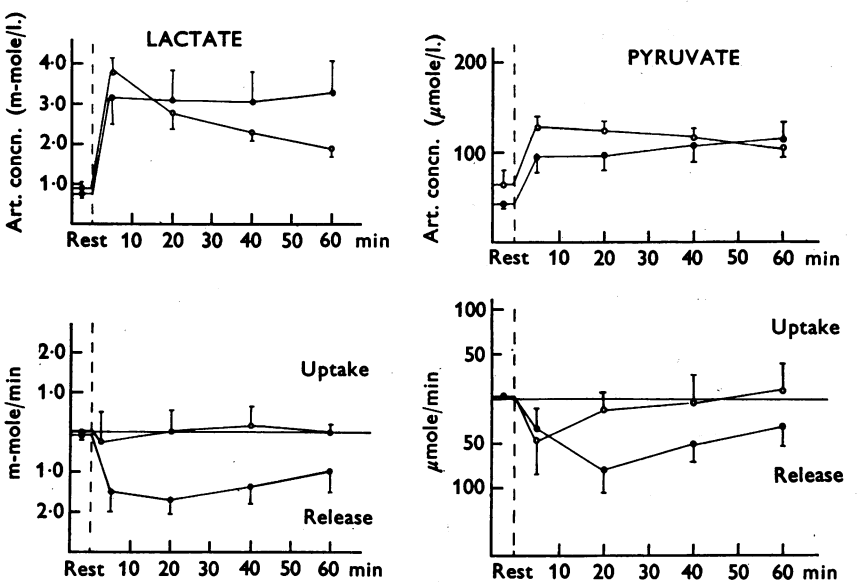


Fig. 2. Arterial lactate and pyruvate concentration, and muscle uptake and release of lactate and pyruvate at rest and during intermittent (●) and continuous (○) exercise (mean ± S.E. of mean).

the end of intermittent exercise ($P < 0·01$) which did not differ statistically from 0·87 ± 0·05 m-mole/min at the end of continuous exercise.

Significant negative a-fv differences of lactate ($P < 0·001$) and pyruvate ($P < 0·01$) were found only during intermittent exercise. A net production of lactate with a mean rate of 1·5 ± 0·4 m-mole/min and of pyruvate with a mean rate of 0·05 ± 0·03 m-mole/min for the whole exercise period were found. During continuous exercise a tendency towards release was observed

only at 5 min of exercise while during the remainder of the exercise period the a-fv differences did not differ significantly from zero at any time.

At the end of exercise the leg fractional uptake of oleic acid calculated from the extraction of [$^{14}$C]oleic acid was $0{\cdot}19 \pm 0{\cdot}02$ in continuous exercise which was similar to $0{\cdot}21 \pm 0{\cdot}03$ in intermittent exercise. Uptake and release of FFA calculated from fractional extraction of [$^{14}$C]oleic acid and a-fv difference of FFA did not differ significantly between the two situations. The net exchange of glycerol in the leg was insignificant and similar in intermittent and continuous exercise.

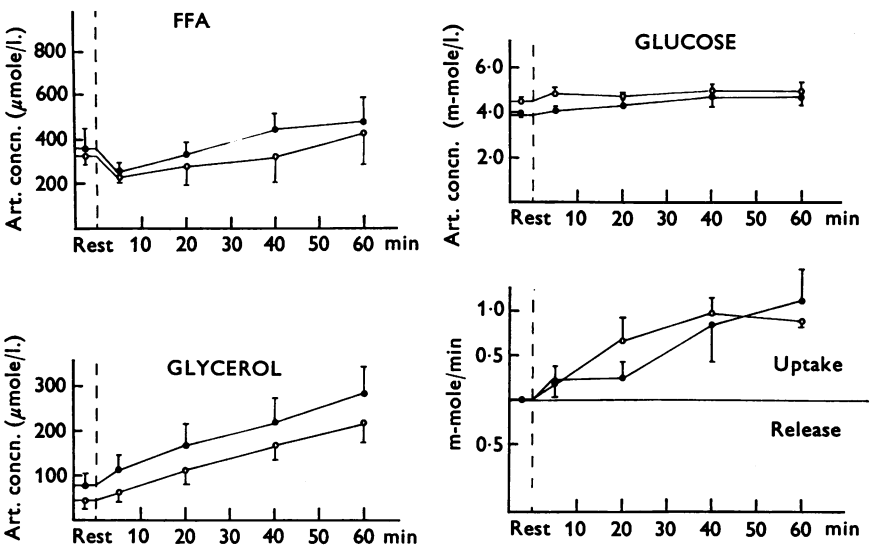


Fig. 3. Arterial FFA, glycerol and glucose concentration and muscle uptake of glucose at rest and during intermittent (●) and continuous (○) exercise (mean ± S.E. of mean).

*Intramuscular substrates* (Fig. 4). The glycogen concentration at rest was $76 \pm 4$ m-mole/kg w.w. before intermittent exercise and $79 \pm 4$ m-mole/kg w.w. before continuous exercise. Already after 5 min of both intermittent and continuous exercise there was a 20% decrease ($P < 0{\cdot}01$) in glycogen concentration and a 60% decrease at the end of exercise ($P < 0{\cdot}01$). There were no significant differences in glycogen depletion between continuous and intermittent exercise.

The triglyceride level revealed a tendency towards an initial decrease after 5 min in both situations. The mean triglyceride utilization after 60 min was $2{\cdot}26 \pm 0{\cdot}22$ m-mole/kg w.w. in intermittent exercise ($P < 0{\cdot}01$) and $3{\cdot}46 \pm 0{\cdot}84$ m-mole/kg w.w. ($P < 0{\cdot}01$) in continuous exercise. The difference between the two forms of exercise was significant ($P < 0{\cdot}05$).

*Intramuscular adenosine triphosphate (ATP), creatine phosphate (CP), lactate and citrate* (Fig. 5). At rest the values obtained for ATP and CP were not different in the two situations. A 20% lower total ATP + CP content was observed both at 5 and 60 min in intermittent exercise compared with continuous exercise ($P < 0.05$). In intermittent exercise, however, the ATP and CP content oscillated with rest and work periods. CP showed a significantly lower mean value at the end of a work period, $8.05 \pm 0.87$ m-mole/kg w.w., compared with $10.17 \pm 1.09$ m-mole/kg w.w. after a rest period ($P < 0.05$). The mean value of ATP at the end of a work period was $3.53 \pm 0.28$ m-mole/kg w.w. compared to $3.92 \pm 0.34$ m-mole/kg w.w. at end of a rest period, but the difference was not significant.

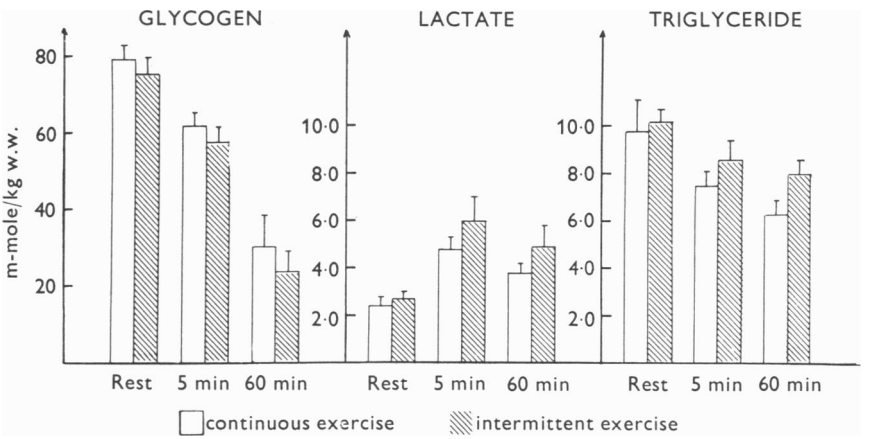


Fig. 4. Intramuscular glycogen, lactate and triglyceride concentration at rest and after 5 and 60 min of intermittent and continuous exercise (mean ± S.E. of mean).

The intramuscular lactate concentration at rest was similar and showed a 2–3 m-mole/kg w.w. increase in both situations at 5 min and 60 min of exercise ($P < 0.01$) (Fig. 4). In intermittent exercise the mean lactate concentration $4.40 \pm 0.44$ m-mole/kg w.w. was significantly greater after a work period compared with $3.33 \pm 0.41$ m-mole/kg w.w. after a rest period ($P < 0.05$).

The citrate concentration increased with continuous exercise from a resting value of $0.11 \pm 0.01$ m-mole/kg w.w. to $0.26 \pm 0.02$ m-mole/kg w.w. after 5 min and $0.20 \pm 0.02$ m-mole/kg w.w. after 60 min ($P < 0.01$). A similar resting value of $0.10 \pm 0.03$ m-mole/kg w.w. was observed before intermittent exercise. Mean citrate concentration was significantly higher at the end of a rest period ($0.31 \pm 0.05$ m-mole/kg w.w.) than at the end of a work period ($0.23 \pm 0.05$ m-mole/kg w.w.) ($P < 0.01$).

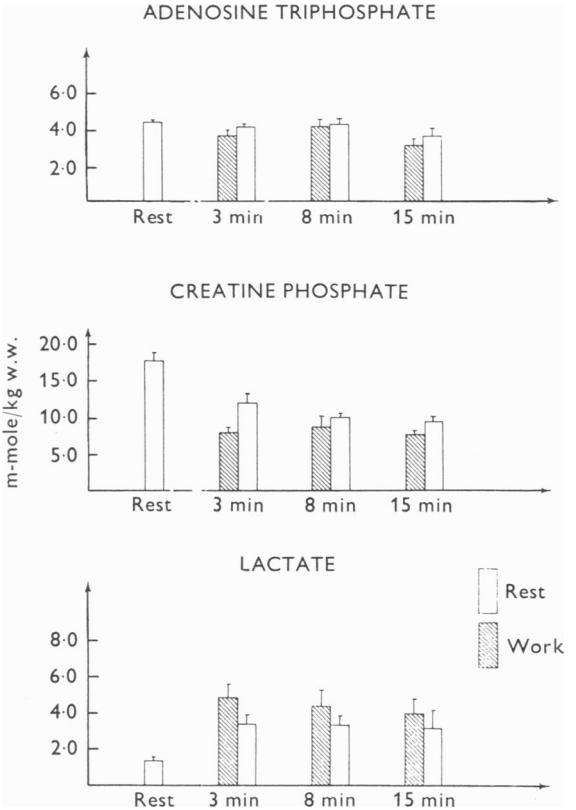


Fig. 5. Intramuscular ATP, CP and lactate concentration at rest and at end of a work and rest period at 3, 8 and 15 min in intermittent exercise (mean ± S.E. of mean).

## DISCUSSION

In this study a comparison has been made of the utilization of both blood-borne and intramuscular fat and carbohydrate substrates in intermittent intense exercise and continuous submaximal exercise with the same average oxygen uptake. In most respects, substrate metabolism was similar in the two forms of exercise performed by the same five subjects. This was the case despite the fact that the work intensity was twice as high in intermittent as in continuous exercise. Had work of the higher intensity been performed continuously for some min this would have produced a markedly different metabolic response (Åstrand *et al.* 1960; Edgerton *et al.* 1975).

The current findings demonstrate that the proportion of energy derived from fat and carbohydrate is similar in intermittent and in continuous

exercise with the same average oxygen uptake. This indicates that in intermittent exercise regulatory mechanisms prevent metabolism from attaining the pattern typical for the heavier work intensity in that situation.

*Substrate utilization*

*Continuous exercise.* Both glycogen (Fig. 4) and triglycerides were utilized in the exercising muscles during the continuous exercise. In an earlier study (Carlson, Ekelund & Fröberg, 1971) triglyceride concentration was lowered by 25% compared with 37% in the present study after continuous exercise at a similar work load. The depletion of glycogen in the present study was 64% and similar to that previously observed at 55% of $\dot{V}_{O_2}$ max (Saltin & Karlsson, 1971). The decrease in glycogen proceeded at a higher rate during the first five min than during the remainder of the exercise. The ATP and CP depletion and the small lactate accumulation was also of the magnitude expected (Karlsson *et al.* 1970).

The uptake of blood-borne substrates was slightly lower in the present study than that which certain authors have previously found during continuous exercise at similar absolute and relative work intensity (Wahren Felig, Ahlborg & Jorfeldt, 1971; Wahren, Hagenfelt & Felig, 1975; Juhlin-Dannfelt, Jorfeldt, Hagenfeldt & Hultén, 1977). Thus, the glucose uptake shown in their studies was 20–30% higher and the uptake of FFA 15–30% higher than observed in the present study. The difference was not due to a smaller calculated leg blood flow in the present study. One explanation for different results for FFA uptake may be that the subjects in this study had relatively low arterial FFA concentrations at the start of work since it has been shown that FFA uptake is related to arterial FFA concentration (Hagenfeldt & Wahren, 1971). A slight release of lactate which has been observed during the whole exercise period by some authors (Wahren *et al.* 1975; Juhlin-Dannfelt *et al.* 1977) was only observed at the beginning of exercise in the present study. This may be explained by the fact that subjects in this study were in a good state of physical training.

*Intermittent exercise.* Most of the methods used in this study are well established, although usually based on steady-state conditions such as in continuous exercise. With intermittent exercise, however, it is questionable whether the same methods are applicable because of oscillation between work and rest periods.

Respiratory gas exchange and leg blood flow fluctuations are seen between work and rest periods but the time lag which probably differs for the different variables must also be taken into account, and it is therefore not possible to draw specific conclusions regarding the observed differences

between 15 sec work and 15 sec rest periods. In the present study the results for substrate exchange, gas exchange, blood flow and arterial concentration were therefore calculated from the mean values for work and rest periods.

With the muscle biopsy technique, however, it is possible more accurately to follow the quick changes occuring in muscle phosphagen and metabolite concentrations after a work and a rest period (Saltin & Essén, 1971).

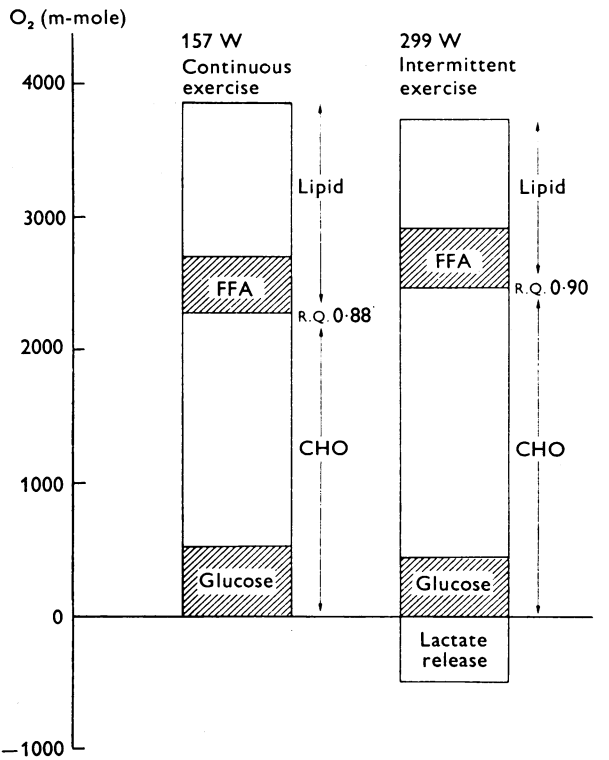


Fig. 6. Leg uptake of oxygen and blood-borne substrates during 60 min of intermittent and continuous exercise. The contribution from lipid and carbohydrate sources is shown from the R.Q.$_L$ values. The cross-hatched areas indicate FFA and glucose uptake and lactate release over the whole exercise period with the same fractional uptake as was measured at 50–60 min of exercise.

### *Intermittent exercise versus continuous exercise*

#### *Total leg metabolism*

The relative contribution of fat and carbohydrate as estimated from R.Q.$_L$ and respiratory exchange ratio seems to be similar in intermittent and continuous exercise although minor differences exist within the contribution from the intra- and extramuscular sources of these substrates.

The simultaneous measurement of leg oxygen uptake, leg uptake of blood-borne substrates and concentrations of intramuscular substrates can allow a direct estimation of the contribution made by each fuel to total oxidative metabolism over 60 min (Fig. 6). The contribution to total oxidative metabolism from blood-borne substrates was 13 % from FFA and 11 % from glucose in intermittent exercise and was the same in continuous exercise where 11 % was derived from FFA and 13 % from glucose. However, $R.Q._L$ suggests that FFA contributed 40 % of the total fat utilization in intermittent exercise and only 28 % during continuous exercise. This is in good agreement with the slightly greater intramuscular triglyceride utilization of 3·5 m-mole/kg w.w. found in continuous exercise than the 2·2 m-mole/kg w.w. in intermittent exercise.

Blood glucose covers approximately 17 % of the carbohydrate utilization in intermittent exercise and 23 % in continuous exercise. The remaining proportion of the carbohydrate utilized derives from intramuscular glycogen. While a direct comparison is possible between oxygen consumption and the fraction of oxidative metabolism covered by the blood-borne substrates, only an approximate estimation can be made of the intramuscular contribution to total oxidative and anaerobic metabolism assuming the biopsy sample to be representative for the mean glycogen utilization in the active muscle mass. Discounting the first 5 min of exercise, the period with the greatest anaerobic energy release, the carbohydrate utilization from intramuscular sources (as indicated from leg oxygen uptake, glucose uptake and lactate release) amounts to an equivalent of 2200 m-mole oxygen or 336 m-mole glucose in intermittent exercise and 1700 m-mole oxygen or 283 m-mole glucose in continuous exercise. The mean glycogen utilization observed from the biopsy samples was 34 m-mole/kg w.w. in intermittent exercise and 32 m-mole/kg w.w. in continuous exercise. This indicates that a greater muscle mass is likely to be involved in intermittent exercise (10–11 kg) compared with continuous exercise (8–9 kg).

*Muscle fibre involvement*

Previous studies have shown that during continuous exercise at 50–60 % of $\dot{V}_{O_2}$ max mainly type I fibres are recruited while during the higher intensity both type I and II fibres are involved (Gollnick, Piehl & Saltin, 1974). The greater decrease in intramuscular triglycerides in continuous exercise may be related to the greater triglyceride concentration found in type I than in type II fibres (Essén, Jansson, Henriksson, Taylor & Saltin, 1975). The release of lactate observed in intermittent exercise may likewise reflect a larger recruitment of type II fibres and anaerobic metabolism. Furthermore, it has been shown that both type I and II fibres are recruited in intermittent exercise (Edgerton *et al.* 1975).

*Role of myoglobin*

In intermittent exercise the greatest part of the energy is derived from aerobic metabolism which is dependent on the availability of oxygen in addition to substrates. It has been suggested that myoglobin in intermittent exercise plays an important role in the muscle as an oxygen store which can be used during the work periods and then replenished during the rest periods (Åstrand *et al.* 1960). Even if the low lactate values in intermittent exercise have suggested only a slight anaerobic metabolism the

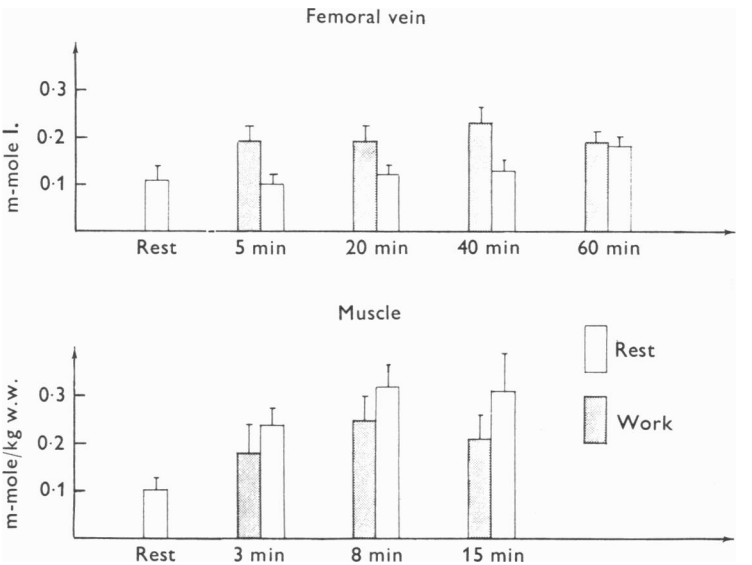


Fig. 7. Citrate concentration in femoral vein at rest and during work and rest periods in intermittent exercise at 5, 20, 40 and 60 min and intramuscular citrate concentration at rest and at end of work and rest periods at 3, 8 and 15 min (mean ± S.E. of mean).

splitting of ATP and CP and their resynthesis during each rest period is another factor to consider as an important energy source (Wittenberg, 1970). Since the oxygen uptake during work periods is smaller than the simultaneous oxygen demand, mechanisms must be operative which compensate this oxygen deficit during the rest periods.

The present data permit a calculation of the contribution from aerobic and anaerobic metabolism in the legs during each work period. The measured pulmonary oxygen uptake during a work period was 0·66 l. whereas an oxygen supply of 1·07 l. is assumed to be adequate for the work intensity used in this situation. The measured leg oxygen uptake was 60 % of the pulmonary oxygen uptake and the estimated demand during the

work periods is then 0·63 l. whereas the actual leg uptake was found to be 0·35 l. The deficit of 0·28 l. can then be covered by (1) ATP and CP depletion of 2·5 m-mole/kg w.w. equivalent to 8·7 ml. oxygen/kg w.w., (2) the lactate accumulation of 1·1 m-mole/kg w.w. equivalent to 5·5 ml. oxygen/kg w.w., (3) the oxygen bound to myoglobin which in the human muscle is estimated to be 0·5 m-mole/kg w.w. or 11·2 ml. oxygen/kg w.w. (Harris, Hultman, Kaijser & Nordesjö, 1975). In order to cover the observed oxygen deficit from these energy sources, a calculated muscle mass of 11 kg is involved and then ATP + CP contribute 34 %, lactate 22 % and myoglobin 44 % to the oxygen deficit (Fig. 8). The oxygen uptake during the rest periods is thus able to recharge the myoglobin store.

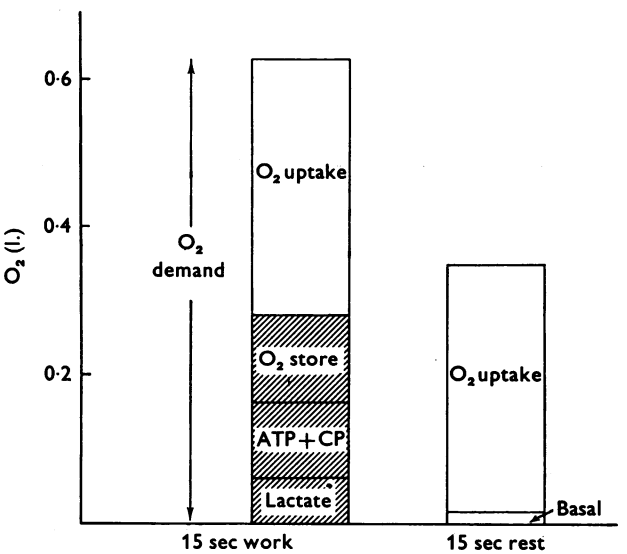


Fig. 8. The oxygen demand during 15 sec work and measured mean oxygen uptake in work and rest periods in the exercising legs in intermittent exercise. The contribution to the oxygen deficit during the work periods from lactate accumulation, phosphagen depletion and myoglobin is indicated by the cross-hatched areas.

*Metabolic regulation in intermittent exercise*

The present findings demonstrate that the contribution from fat and carbohydrate to the energy need is rather similar in intermittent and continuous exercise when the total amount of work performed is almost identical. One has to bear in mind here that the work intensity in intermittent exercise is nearly twice that used in continuous exercise. A very high rate of glycolysis would have occurred if the same work load as used in the intermittent exercise were performed continuously. However, when

performed intermittently in 15 sec periods an aerobic energy release predominates with a substantial contribution of lipid utilization, thus suggesting that the rate of glycolysis is lower than during continuous exercise at the same intensity. Possible mechanisms for such a retardation will be briefly discussed.

During each work period ATP is hydrolysed and the concentration of ADP and AMP increases, enhancing glycolysis and the rate of the citrate cycle. At the end of a work period or at the very beginning of a rest period, the ADP-AMP concentration must be high but during the rest period ATP level is restored. Citrate then accumulates and passes across the mitochondrial membrane into the cytoplasm. *In vitro* studies have shown that a raised cytoplasmic citrate concentration inhibits glycolysis (Randle, Newsholme & Garland, 1964). This may tend to slow down the rate of glycolysis during the ensuing work period, facilitating the utilization of lipid for aerobic energy release.

The observed fluctuations in blood citrate concentration between work and rest periods may reflect oscillations in muscular citrate concentration with the effect suggested above. The time relationship between citrate increase and phase of work cannot be determined from blood citrate concentrations because of the time lag. However, intramuscular citrate levels were found to be significantly higher at the end of a rest period than at the end of a work period, thus indicating that citrate could be another factor regulating the rate of glycolysis in human muscle metabolism during exercise (Fig. 7).

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## REFERENCES

AHLBORG, G., FELIG, P., HAGENFELDT, L., HENDLER, R. & WAHREN, J. (1974). Substrate turnover during prolonged exercise in man: splanchnic and leg metabolism of glucose, free fatty acids and amino acids. *J. clin. Invest.* **53**, 1080–1090.

ÅSTRAND, I., ÅSTRAND, P.-O., CHRISTENSEN, E. H. & HEDMAN, R. (1960). Myohemoglobin as an oxygen-store in man. *Acta physiol. scand.* **48**, 454–460.

BERGSTRÖM, J. (1962). Muscle electrolytes in man. *Scand. J. clin. Lab. Invest.* suppl. 68.

CARLSON, L. A., EKELUND, L. G. & FRÖBERG, S. (1971). Concentration of triglycerides, phospholipids and glycogen in skeletal muscle and of free fatty acids and β-hydroxybutyric acid in blood in man in response to exercise. *Eur. J. clin. Invest.* **1**, 248–254.

CHERNICK, S. S. (1969). Determination of glycerol in acyl glycerols. *Meth. Enzymology* **14**, 627–630.

CHRISTENSEN, E. H., HEDMAN, R. & HOLMDAHL, I. (1960). The influence of rest pauses on mechanical efficiency. *Acta physiol. scand.* **48**, 443–447.

CHRISTENSEN, E. H., HEDMAN, R. & SALTIN, B. (1960). Intermittent and continuous running. *Acta physiol. scand.* **50**, 269–286.

EDGERTON, V. R., ESSÉN, B., SALTIN, B. & SIMPSON, D. R. (1975). Glycogen depletion in specific types of human skeletal muscle fibres in intermittent and continuous exercise. In *Metabolic Adaptation to Prolonged Physical Exercise*, ed. HOWALD, H. & POORTMANS, J. R., pp. 402–415. Basel: Birkhäuser Verlag.

EDWARDS, R. H. T., EKELUND, L. G., HARRIS, R. C., HESSER, C. M., HULTMAN, E., MELCHER, A. & WIGERTZ, O. (1973). Cardiorespiratory and metabolic costs of continuous and intermittent exercise in man. *J. Physiol.* **234**, 481–497.

ESSÉN, B., JANSSON, E., HENRIKSSON, J., TAYLOR, A. W. & SALTIN, B. (1975). Metabolic characteristics of fibre types in human skeletal muscle. *Acta physiol. scand.* **95**, 153–165.

GARBY, L. & VUILLE, J. C. (1961). The amount of trapped plasma in a high speed microcapillary hematocrite centrifuge. *Scand. J. clin. Lab. Invest.* **13**, 642–645.

GOLLNICK, P. D., PIEHL, K. & SALTIN, B. (1974). Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates. *J. Physiol.* **241**, 45–57.

HAGENFELDT, L. (1966). A gas chromatographic method for the determination of individual free fatty acids in plasma. *Clinica chim. Acta* **13**, 266–268.

HAGENFELDT, L. & WAHREN, J. (1968). Human forearm muscle metabolism during exercise. II. Uptake, release and oxidation of individual FFA and glycerol. *Scand. J. clin. Lab. Invest.* **21**, 263–276.

HAGENFELDT, L. & WARHEN, J. (1971). Metabolism of free fatty acids and ketone bodies in skeletal muscle. In *Muscle Metabolism during Exercise*, ed. PERNOW, B. & SALTIN, B., pp. 153–163. New York: Plenum.

HARRIS, R. C., HULTMAN, E., KAIJSER, L. & NORDESJÖ, L-O. (1975). The effect of circulatory occlusion on isometric exercise capacity and energy metabolism of the quadriceps muscle in man. *Scand. J clin. Lab. Invest.* **35**, 87–95.

HUGETT, A. S. G. & NIXON, D. A. (1957). Use of glucose oxidase, peroxidase, and *o*-dianisidine in determination of blood and urinary glucose. *Lancet* **ii**, 368–370.

JORFELDT, L. & WAHREN, J. (1971). Leg blood flow during exercise in man. *Clin. Sci.* **41**, 459–473.

JUHLIN-DANNFELT, A., JORFELDT, L., HAGENFELDT, L. & HULTÉN, B. (1977). The influence of ethanol on free fatty acid and carbohydrate metabolism during exercise in man. *Clin. Sci.* (in the Press).

KARLSSON, J., DIAMANT, B. & SALTIN, B. (1970). Muscle metabolites during submaximal and maximal exercise in man. *Scand. J. clin. Lab. Invest.* **26**, 385–394.

LOWRY, O. H. & PASSONNEAU, J. V. (1973). *A Flexible System of Enzymatic Analysis*. New York: Academic.

RANDLE, P. J., NEWSHOLME, E. A. & GARLAND, P. B. (1964). Regulation of glucose uptake by muscle. *Biochem. J.* **93**, 652–665.

SALTIN, B. & ESSÉN, B. (1971). Muscle glycogen lactate ATP and CP in intermittent exercise. In *Muscle Metobolism during Exercise*, ed. PERNOW, B. & SALTIN, B., pp. 419–424. New York: Plenum.

SALTIN, B. & KARLSSON, J. (1971). Muscle glycogen utilization during work of different intensities. In *Muscle Metabolism during Exercise*, ed. PERNOW, B. & SALTIN, B., pp. 289–299. New York: Plenum.

WAHREN, J., FELIG, P., AHLBORG, G. & JORFELDT, L. (1971). Glucose metabolism during leg exercise in man. *J. clin. Invest.* **50**, 2715–2725.

WAHREN, J., HAGENFELDT, L. & FELIG, P. (1975). Splanchnic and leg exchange of glucose amino acids and free fatty acids during exercise in diabetes mellitus *J. clin. Invest.* **55**, 1303–1314.

WITTENBERG, J. B. (1970). Myoglobin-facilitated oxygen diffusion: role of myoglobin in oxygen entry into muscle. *Physiol. Rev.* **50**, 559–636.