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Skeletal Muscle Phosphagen and Lactate Concentrations in Ischaemic Dynamic Exercise*

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Summary. Five young males performed dynamic, submaximal contractions to exhaustion with the quadriceps muscle under arterial occlusion. The work load was 14.7 Watt (W). After 10 min rest with intact arterial circulation, the subjects commenced another bout to exhaustion; this process was repeated until a total of 10–16 bouts had been performed. Muscle biopsies were obtained immediately after the second, fifth, eighth, and last bout as well as 30 min after the last bout. The concentrations of adenosine triphosphate (ATP), creatine phosphate (CP), lactate, and glycogen were measured in each sample and some material underwent histochemical analysis. Muscle lactate was highest following the second work bout [22.9 mmol/kg wet weight (ww)] and gradually declined to 7.0 mmol/kg ww by the end of the last bout. CP level was low in all postexercise samples with the exception of a remarkably high CP (11.7 mmol/kg ww) after the last bout. Glycogen utilization tended to parallel muscle lactate levels, the rate of depletion being most rapid initially. Histochemical staining for glycogen depletion revealed that both type I and II fibres were low in glycogen, although type I was depleted most uniformly. In the first work bouts the high lactate and low CP levels in the total muscle could be responsible for the fatigue; none of these factors seem adequate to explain the development of the fatigue experienced in the later work bouts. It is concluded that muscle fatigue in this type of exercise is not related to substrate depletion or accumulation of metabolites, further that the fibre recruitment pattern is determined by the type and relative severity of performed work rather than local metabolic factors.

Key words: Glycogen – Muscle fibre types – Muscular fatigue

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Muscular fatigue is a challenged concept in work physiology as its cause remains obscure [1]. Several factors have been taken into account, such as impairment in the CNS, in the neuromuscular transmission, and in the muscle tissue itself (see refs. in [22]). Several studies in man have investigated the muscle constituents after submaximal dynamic [17] and isometric [16] fatiguing exercise. These investigations indicate that the lack of phosphagen and the level of lactate seem to be unlikely explanations for the fatigue developed during these type of muscle contractions. Differences between dynamic and static exercise may be of importance in evaluating the cause of muscle fatigue: it has been demonstrated that muscle fibre recruitment differs as type I fibres are mainly used during dynamic exercise, whereas type II fibres play a major role during static exercise [6]. Muscle blood flow is also different as it is arrested in isometric work at levels above 25–50% of maximal voluntary contraction (MVC) [3], whereas it increases with increasing intensity in dynamic exercise [7]. An experimental model combining ischaemia and dynamic exercise may thus be informative in muscle fatigue studies. In an attempt to study possible local factors limiting skeletal muscle performance we followed the concentrations of adenosine triphosphate (ATP), creatine phosphate (CP), lactate, and glycogen in the quadriceps muscle during sessions of ischaemic dynamic exercise at submaximal levels. The results indicate that changes in these muscle metabolites do not seem to be directly related to muscular fatigue induced by this type of exercise.

Material and Methods

Five healthy male medical students participated in the study. Their mean age, height, and weight (range) were 25 years (24–27), 1.77 m (1.68–1.82), and 69 kg (62–82), respectively. They were fully informed of the experimental procedure and informed consent was given by all subjects before the study. Submaximal dynamic exercise was performed on a table with the subject in the prone position, allowing pure quadriceps contractions. A cable was attached to the right ankle and was passed over a pulley. A 10-kg weight was attached to the cable, and a second cable ensured that the movement was 0.45 m. The subject was placed so that the movement of the knee occurred equally around 90°, thereby minimizing the influence of the weight of the leg. The subjects were well acquainted with the experimental set up. The circulation to the leg was occluded by means of a 7 cm wide inflatable cuff, which was placed as proximally as possible on the thigh. The cuff was filled almost instantaneously by a Kidde automatic tourniquet to 300 mm Hg. The subjects started to lift the weight with a frequency of 20 per min, which equalled a work load of 14.7 Watt (W) (= 90 kpm/min = 10 kg × 0.45 m × 20 min^{-1}; work output = work load × work time). After some contractions all subjects experienced pain in the muscle, but they were able to ignore this sensation and to continue the performance 'through' the pain until the moment, when the muscle was completely unable to contract. All subjects described this inability as if a very heavy load was added to the 10-kg load. When the subject was unable to continue the exercise the occlusion was released. Following a 10 min rest with the cuff released the subject commenced another bout to exhaustion. After the second bout the rest interval was 30 min, and thereafter each rest interval lasted 10 min. The experiment was terminated when a plateau of endurance times for successive bouts was reached, denoted B_L. The work output in each bout has been related to the estimated weight of the quadriceps muscle, comprising 37% of the lean thigh volume (Halskov, unpubl. data). For description of thigh volumes see [14].

Muscle samples were obtained from the lateral portion of the quadriceps femoris muscle [2] after the second (B_2), fifth (B_5), eighth (B_8), and last bout (B_L) as well as 30 min after the last bout. After biopsy the occlusion was released. Additional biopsies were obtained in two subjects; before

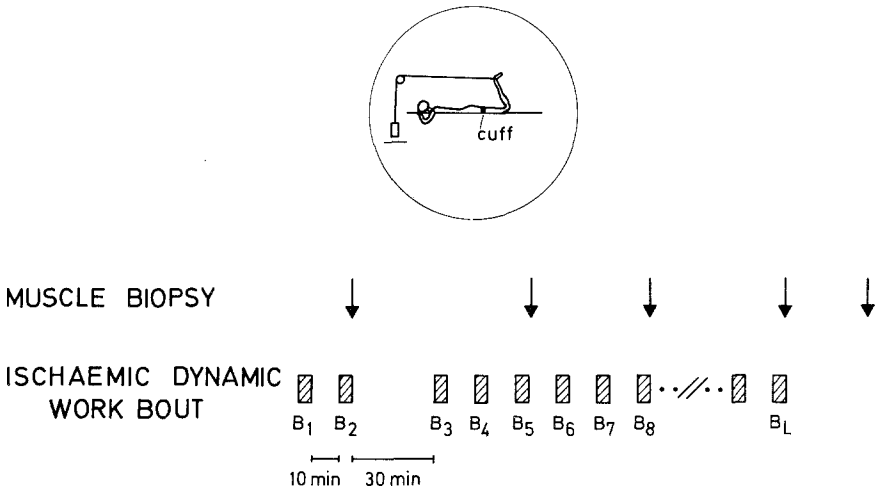

Fig. 1. The experimental protocol

and after the first bout and 10 min after the second. The experimental protocol is shown in Fig. 1.

Each muscle sample was divided into two: One portion was immediately frozen in liquid nitrogen for subsequent analysis of ATP, CP, glycogen, and lactate [15]; the methodological errors as estimated from duplicate samples ($n = 12$) were 6.5, 10.8, 6.1, and 9.8%, respectively. The results are given in mmol/kg ww. The remaining portion was embedded in Tissue-Tek II O.C.T. Compound and frozen in isopentane cooled in liquid nitrogen. Serial sections (4 μm) were cut at −20° C and stained for myofibrillar ATPase pH 9.4 [8, 19] after preincubation at pH 10.3, NADHTR (NADH-tetrazolium reductase) [18], α-GPD (α-glycerophosphate dehydrogenase) [24] and for glycogen using the PAS stain [11, 20]. NADHTR indicates the oxidative potential and α-GPD the glycolytic potential. Muscle fibres were identified as type I and II on the basis of the ATPase stain [5].

In one of the experimental subjects the staining intensity of the histochemically treated samples from two biopsies (rest and after the tenth bout) was quantified using microphotometry of single cells [8] and thickness measurements [10]. The same 90 fibres of each type were measured in serial sections (NADHTR, α-GPD, and PAS); the mean concentration in each fibre type is given by absorbance (A) divided by the section thickness in μm.

Conventional statistical methods were used. The significance of intra-individual differences were tested using the paired t-test.

Results

The endurance time averaged (SD) 2.76 (0.41) min for the first work bout (B_1) whereafter the time declined through the following bouts: 2.45 (0.24) B_2; 2.30 (0.31) B_3; 2.07 (0.13) B_4; 1.88 (0.25) B_5. There were more or less rapid decreases, the plateau being reached after 8–14 bouts; the endurance time for B_L averaged (SD) 1.41 (0.08) min; (B_1 vs. B_2: $p < 0.05$, B_2 vs. B_5: $p < 0.05$ and B_5 vs. B_L: $p < 0.001$).

Following the second work bout, muscle lactate averaged 22.9 mmol/kg ww and declined gradually to 7.0 mmol/kg ww by the end of the last bout. After

Table 1. Mean concentration (range) of muscle metabolites (mmol/kg ww) of five subjects during repeated periods of fatiguing ischaemic dynamic contractions. For explanation of B see text. Mean resting values (*R*) reported by Karlsson and Saltin [17] are included for comparison

	ATP[a]	CP[a]	Lactate[a]	Glycogen[b]
B_2	3.9 (2.8–5.0)	3.9 (1.8–7.4)	22.9 (13.9–31.3)	57 (41–77) $p < 0.05$
B_5	3.7 (3.0–4.1)	3.3 (1.2–4.5)	18.5 (14.1–21.5)	34 (23–49)
B_8	3.3 (3.1–3.5)	3.8 (2.8–5.0) $p < 0.01$	15.1 (11.2–17.7) $p < 0.01$	30 (17–38)
B_L	3.5 (3.0–3.9)	11.7 (4.9–18.8) $p < 0.05$	7.0 (2.9–12.1)	35 (28–48)
30 min after B_L	3.6 (2.5–4.1)	16.0 (7.0–23.8) $p < 0.05$	3.3 (1.8–6.0)	42 (21–62)
R	4.0	15.6	1.3	80

[a] mmol/kg ww; [b] mmol glucose/kg ww

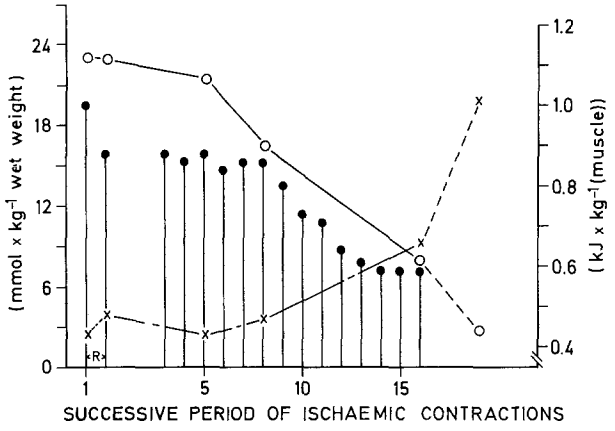

Fig. 2. Lactate (○) and creatine phosphate (×) concentrations (mmol/kg ww, left ordinate) in total muscle biopsies at exhaustion in one subject after the first, second, fifth, eighth, and sixteenth bouts, as well as 30 min after the last bout. ↑ represents the work output in each bout, expressed in relation to the estimated weight of the quadriceps muscle (J/kg, right ordinate), R denotes rest interval

30 min rest the mean concentration was 3.3 mmol/kg ww (Table 1). CP levels were low in all postexercise samples, with the exception of a remarkably high level (11.7 mmol/kg ww) after the last bout (Table 1). An example of the changes in lactate and CP is shown in Fig. 2, which also gives the work output in each bout.

The values of CP and lactate concentrations for the whole material are shown in Fig. 3. The CP concentration decreases curvilinearly with increasing

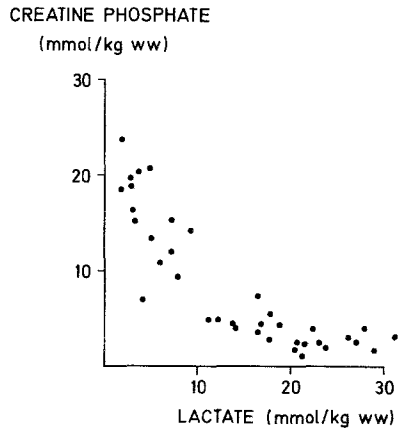

Fig. 3. Comparison between creatine phosphate and lactate content in muscle; all values from the present study are included

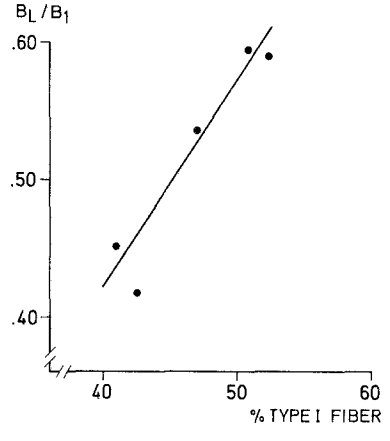

Fig. 4. Decline in ischaemic dynamic exercise, expressed as fractions of initial value versus relative distribution of type I fibres in M. vastus lateralis for five subjects ($r = 0.95$, $p < 0.05$)

lactate concentration. A very close exponential correlation was found between these two variables [$r = -0.885$, $n = 37$, $p < 0.001$, the equation for the regression line: ln CP = -0.085 (lactate) $+2.99$].

The ATP concentration showed no significant changes (Table 1).

Recovery values for lactate and CP obtained 10 min after the 2nd work bout in two subjects averaged 8.4 and 14.8 mmol/kg ww, respectively.

The relative number of type I muscle fibres in the lateral portion of quadriceps averaged 47% (41–52%). The individual level of ischaemic work output, expressed in relation to the initial value (B_L/B_1) was positively related ($r = 0.95$, $p < 0.05$) to the proportion of type I fibres in the contracting muscles (Fig. 4).

In resting muscle the histochemically determined mean glycogen concentration (PAS) in type I and type II fibres was 0.066 (SD 11%) and 0.068 (SD 15%) A/μm, respectively. No statistically significant difference was revealed between the two fibre types. The glycogen content in the sample taken after the tenth work bout was 0.035 (45%) and 0.041 (61%) A/μm in type I and type II,

respectively; $p < 0.001$. In the control situation there was no significant correlation between glycogen content and oxidative potential (NADHTR). In the sample taken after the tenth bout of ischaemic dynamic exercise a positive correlation was demonstrated between glycogen content and oxidative potential in the type II fibres ($r = 0.357$, $p < 0.001$), whereas no such correlation was found for type I fibres (Fig. 5). At no time was there a correlation between glycolytic potential (α-GPD) and glycogen content in either fibre type.

Discussion

The results of the present study demonstrate that the metabolic pattern in the total muscle biopsy changed at exhaustion during repeated bouts of ischaemic submaximal dynamic exercise. Muscle lactate concentration was highest at the beginning of the experiment and it thereafter gradually declined. The CP concentration showed an inverse pattern as demonstrated in Fig. 3. These changes in lactate and CP are similar to those found in isometric and dynamic exercise [21]. The general pattern of interplay between these anaerobic energy sources may thus support the hypothesis relating these metabolites to decreased intramuscular pH (lactate production), which in turn is known to affect the equilibrium constant of the creatine kinase reaction [21].

The present finding of a plateau for endurance times of successive ischaemic dynamic contractions (Fig. 2) concurs with the endurance time of successive isometric contractions sustained to fatigue [e.g., 4]. Thus, a general pattern for endurance times of repeated muscle contractions under ischaemic conditions seems to exist irrespective of the type of exercise.

The initial levels of muscle lactate and CP are in accordance with those levels observed at exhaustion in dynamic [17] and isometric exericse [16]. The changes in CP and lactate concentrations may be the fatiguing factors in the early work bouts of the present study. The postexercise CP and lactate concentrations at exhaustion changed during the experimental period. The decrease in lactate content was greater than expected from the decrease in performed ischaemic work (e.g., Fig. 2). This could be a consequence of the dual concept of the human skeletal muscle consisting of two main fibre types with different physiological, structural and chemical properties [5, 8]. The altered concentrations of CP and lactate at exhaustion in the total muscle biopsy during the experimental period may reflect changes in the number of recruited fibres, and the same metabolic event may occur in the recruited muscle fibre at exhaustion.

Muscle Fibre Recruitment

In the present study, after ten work bouts a significantly higher glycogen content was found in the type II fibres than in type I fibres (Fig. 5). This indicates that type I fibre motor units had been used to a greater extent than type II fibre units. This finding that some type II fibres had scarcely been recruited is in accordance

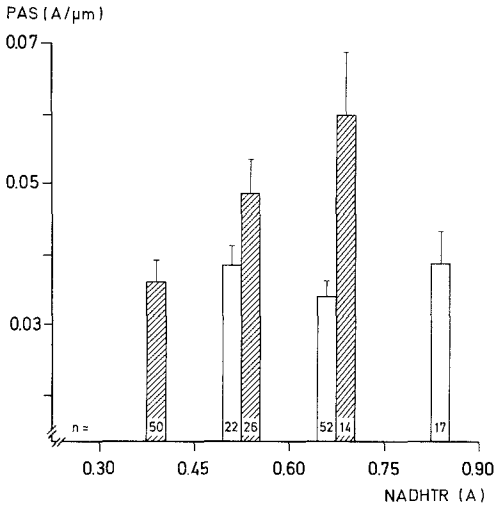

Fig. 5. Mean value (SEM) for glycogen concentration (PAS, A/µm) in single fibres in relation to the oxidative potential (NADHTR) after ten bouts of ischaemic dynamic exercise. The same cell was measured in both sections. □ type I fibres; ▨ type II fibres ($r = 0.357$, $p < 0.001$). N-values represent numbers of single fibres within the single oxidative interval. Mean difference (type II to type I) 0.010 A/µm ($t = 1.73$, $p > 0.05$) and 0.026 A/µm ($t = 4.22$, $p < 0.001$), for the oxidative intervals 0.45/0.60 (A) and 0.60/0.75 (A), respectively

with the conclusion of Stephens and Taylor [22]. They stressed the importance of neuromuscular function fatigue early in maximal voluntary contractions, a phenomenon which was most marked in high threshold motor units. Later a contractile element fatigue, particularly affecting the low threshold units appeared. The high threshold motor units (type II fibres) might have dropped out very early in the exercise experiment of the present study due to neuromuscular function fatigue, the changes in metabolites therefore being very small in these muscle fibres. The low threshold units (type I fibres) continue to work until 'fatiguing substances' have reached a level where they interfere with one of the excitation – contraction processes. One might also suggest that there may be a correlation between these high oxidative type II fibres and the subgrouping of type II based upon different ATPase stainings. As discussed elsewhere [9], no relation between the oxidative potential and these type II subgroups seems to be detectable. The present result of a positive correlation between the decline in capacity of ischaemic dynamic exercise and the muscle fibre composition (Fig. 4) concurs with our finding that some type II fibres had dropped out early in the experimental period. The type I muscle fibres, which usually are regarded as aerobic, have thus the potential to work during ischaemic conditions, and the type of contraction seems to be more important for the recruitment pattern than the intracellular biochemical state.

Metabolism and Energetic Relationships

The depletion of total muscle glycogen tended to parallel muscle lactate levels (Table 1), in that very little glycogen was utilized and very little lactate produced in the later work bouts. This finding raises the question: what is the fuel for muscular metabolism during the last working sessions in the present experiment? The energetic relations for the first and last ischaemic work bouts were therefore

Table 2. Estimated energetic relationships for the first and the last bout of ischaemic dynamic exercise to exhaustion in one subject (the same as illustrated in Fig. 2)

	Measured postexercise value (mmol/kg ww)	Estimated change (mmol/kg ww)	ATP equivalent (mmol/kg ww)	Energetic calculations (J/kg)
First bout				
Lactate[a]	23.1	22	33	Work output: 981
Creatine phosphate[b]	2.5	13	13	49 mmol ATP[d] equal 2,646 J
Oxygen store[c]		0.47	3	NE = 0.37
			Total 49	
Last bout				
Lactate[a]	7.9	7	11	Work output: 590
Creatine phosphate[b]	9.4	6	6	$17 + \times$ mmol ATP[d] equal $(17 + \times) \cdot 54$ J
Oxygen store[c]		? 2	$\times$	$0.37 = 590/(17 + \times) \cdot 54$
			Total $17 + \times$	$\times = 13$ mmol ATP

ATP equivalents for fuels:

[a] 1.5 mol ATP/mol lactate [1]

[b] 1 mol ATP/mol CP [1]

[c] 6 mol ATP/mol O_2 [1]

[d] 1 mol ATP equals 54 kJ [21]

The energetic output in each bout was calculated from the work output related to the estimated size of the quadriceps muscle. The energetic input from the lactate production and the CP depletion was estimated from the differences between the actual postexercise values and the normal resting levels. The oxygen store level in the resting muscle was obtained from Harris et al. [12]. The net efficiency (NE) of the work in the first bout was calculated from the estimated ATP production compared to the external work. Thereafter, the oxygen store in the last bout was calculated assuming the same NE (the result in italics)

determined from the values obtained in the subject illustrated in Fig. 2. Table 2 summarizes the results and the applied assumptions. Aerobic contributions to total energetic exchange were more important during the last bout of exercise than during the first bout. A lower net efficiency in the last bout as compared with the first bout (and this may be the case [4]) will not invalidate the conclusions, as the oxygen store would then be larger. The suggested four-fold increase in the oxygen store may be explained by increased concentration of haemoglobin in the muscle due to a greater number of open capillaries.

The increased aerobic metabolism indicates that working capacity in the later bouts is mainly dependent on the function of type I fibres. The interpretation of the estimated energetic relationships concurs with the present result, demonstrating that the level of ischaemic work is positively correlated with the number of type I fibres (Fig. 4). Our results are in accordance with Hultén et al. [13], who found a positive correlation between endurance time of isometric work (at 50% MVC) and the proportion of type I fibres. Thus, local metabolic relationships seem to be unimportant for the recruitment pattern of muscle fibre; the type and the severity of the work performed seems to be more critical.

From the present study it is difficult to point to a specific factor for muscle fatigue. Previous studies in animals have demonstrated the motor end-plate zone to be extremely sensitive to ischaemia [23]. Electromyographic studies in humans have, as previously mentioned, focused on impairment of neuromuscular function early in maximal voluntary contractions [22]. Thus, we would hypothesize that it is the neuromuscular functions which cease to work, and thereby limit muscle function during ischaemic dynamic exercise.

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