Enzyme levels of the NADH shuttle systems: measurements in isolated muscle fibres from humans of differing physical activity

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The aim of the present study was to investigate enzyme levels of the malate-aspartate and alpha-glycerophosphate shuttles in type I (slow-twitch) and type II (fast-twitch) fibres of human skeletal muscle. The influence of endurance training on these levels was also elucidated. Biopsy specimens were obtained from the lateral part of the quadriceps femoris muscle of six untrained and six endurance-trained subjects. *Type I vs. type II.* In both groups the type I fibres exhibited higher levels of the TCA cycle marker enzyme citrate synthase (CS), as well as of the malate-aspartate shuttle enzymes (cytoplasmic and mitochondrial malate dehydrogenase (cMDH, mMDH), and aspartate aminotransferase (cASAT, mASAT)). A more pronounced difference between type I and type II fibres was noted for cMDH (58%) than for mMDH (16%), cASAT (20%), mASAT (18%) and CS (25%). In contrast to these enzymes, the levels of cytoplasmic glycerol-3-phosphate dehydrogenase (cGPDH), the enzyme representative of the alpha-glycerophosphate shuttle, were higher (25%) in the type II fibres. *Endurance-trained vs. untrained.* In the endurance-trained group, both fibre types were characterized by higher levels of CS (mean for both fibre types: 48%) as well as of mitochondrial malate-aspartate shuttle enzymes (mMDH: 47%, mASAT: 48%) than in the corresponding fibre types in the untrained group, while the differences in the levels of cytoplasmic malate-aspartate shuttle enzymes (cMDH: 13%, cASAT: 16%) were not statistically significant. Nor were the differences in cGPDH levels (8%) between the untrained and endurance-trained groups statistically significant. It is concluded that in human skeletal muscle, malate-aspartate shuttle enzymes are expressed to a higher degree in type I (slow) fibres than in type II (fast) fibres, with cMDH exhibiting the most marked difference. The single fibre analysis indicated that the muscle's activity level might exert a greater influence on the mitochondrial isoenzymes than on the cytoplasmic ones. In contrast to the malate-aspartate shuttle enzymes, the alpha-glycerophosphate shuttle is expressed to a higher degree in type II fibres and its capacity appears to not be influenced by endurance training. The present studies demanded considerable methodological investigations which also are presented in this paper.

Key words: alpha-glycerophosphate shuttle, enzyme activity, fibre types, human skeletal muscle, malate-aspartate shuttle, physical training.

The glycolytic pathway is of great importance for energy production in the working muscle. During the breakdown of glucose or glycogen to

pyruvate, the coenzyme NAD^+ is reduced to NADH in the reaction catalysed by glyceraldehyde-phosphate dehydrogenase. The muscle cytosolic NAD^+ content is, however, only of the order of 1/50-1/100 of that needed per minute of intense physical activity such as short-term bicycle exercise of high intensity or

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sprinting (Sahlin 1983, Saltin & Karlsson 1971, Newsholme & Leech 1983). Thus, rapid re-oxidation of NADH is necessary in order for glycolysis to proceed. This re-oxidation can be achieved in two different ways: anaerobically through the reduction of cytosolic pyruvate to lactate or aerobically through the mitochondrial respiratory chain. The transfer of NADH into mitochondria is hindered by the impermeability of the inner mitochondrial membrane to NADH (Lehninger 1951). Aerobic oxidation of cytosolic NADH can be accomplished, however, through the transfer of reducing equivalents into mitochondria via the malate-aspartate (MA) and alpha-glycerophosphate (GP) shuttles (Dawson 1979). It has previously been shown that endurance training leads to increased levels of the MA shuttle enzymes in human skeletal muscle while those of the GP shuttle do not change (Schantz *et al.* 1986). The aim of the present study was to obtain more detailed knowledge about these shuttle systems in skeletal muscle as well as the adaptation to training. For this purpose we have studied enzyme levels of the NADH shuttles in pools of type I (slow-twitch) and type II (fast-twitch) fibres dissected out from skeletal muscle biopsies obtained from untrained and endurance-trained individuals. Methodological considerations pertaining to this matter is presented in the section below.

SUBJECTS AND METHODS

Six untrained and six endurance-trained subjects were studied. The average age, height and weight were 24 ± 2 ($\pm$ SE) years, 1.71 ± 0.05 m and 62 ± 5 kg, respectively, for the untrained group and 36 ± 4 years, 1.79 ± 0.02 m, and 70 ± 3 kg for the endurance-trained group. Four subjects in the untrained group were females, the rest were males. It was not considered necessary to match the two groups exactly with regard to sex or age. The reason for this is that no primary sex-dependent (Haralambie 1979, Green *et al.* 1983, Howald *et al.* 1985, Simoneau *et al.* 1985, Blomstrand *et al.* 1986, J. Henriksson, unpublished results) or age-dependent (Örlander *et al.* 1978, Grimby *et al.* 1982) differences in oxidative enzyme levels, capillary supply and mitochondrial volume density have been reported. The same appears to be true for the adaptive response to endurance training in these variables (Bylund *et al.* 1977, Suominen *et al.* 1977, Nygaard 1981, Grimby *et al.* 1983). Large interindividual variations in these variables do exist, however. Thus, if minor differences between the sexes exist, despite the evidence to the contrary cited above, they would

be of virtually no practical importance. The endurance-trained group consisted of runners with an average training kilometrage of 82 km (range 20–170) per week. The subject with the lowest running kilometrage also bicycled 180 km per week. The subjects were informed about the procedures and risks involved in the experiments before they volunteered. The study was approved by the Committee on Ethics of the Karolinska Institute. Other aspects have been studied previously on the same individuals (Schantz *et al.* 1986).

Biopsy procedure. Muscle biopsies were taken from the middle portion of the quadriceps femoris muscle, vastus lateralis, of both the left and right leg with a Weil-Blakesley conchotome (AB Wisex, Sweden) (Henriksson 1979). Samples for enzyme assays were frozen immediately in liquid nitrogen and stored at -80°C until freeze-drying.

Preparation of single type I and type II fibres. The muscle samples were freeze-dried for 8 days at -37°C , followed by 1 h at room temperature. The samples were stored in vacuum at -80°C until dissection. The dissections were performed at 20°C and about 25% relative humidity. The samples were kept under these conditions for about 30 h. The freeze-dried material was placed under a dissection microscope, fragments of single muscle fibres were dissected out and the fibre type was identified by staining a small piece for myofibrillar ATPase at pH 9.4 after pre-incubation at pH 10.3 (Engel 1962). The remainder of the fibre fragments were after identification grouped into pools of type I and type II fibres and stored in vacuum at -80°C until homogenization. The procedure of preparing and typing fragments of single type I and type II fibres has been described in detail elsewhere (Essén *et al.* 1975).

Enzyme assays. The pooled fragments of single type I (121–293 fragments weighing 166–254 μg , dry wt) and type II (54–293 fragments weighing 166–264 μg , dry wt) fibres were weighed (Cahn electrobalance 4100, Cahn/Ventron Corporation USA) under dissection conditions (see above). Freeze-dried material absorbs O_2 , N_2 and moisture when removed from the vacuum but reaches equilibrium in a few seconds (Lowry & Passoneau 1972). Absorption in this manner can be assumed to amount to approximately 5% under the prevailing conditions. This was corrected for when recalculating enzyme activities in relation to wet weight, which was done in order to simplify comparisons with literature data. Enzyme activities based on dry weight were therefore divided by the factor 4.14 ($100 \cdot 1.05^{-1} \cdot 23^{-1}$, assuming a muscle water content of 77%). Results indicate that complete enzyme extraction from single fibres requires structural disintegration (Schantz 1986). The fibres were therefore homogenized (1:800, dry wt/v) by sonication (5 s) (Sonifier B-12, Branson Sonic Power Co., USA) in 0.3 M potassium phosphate buffer, pH 7.7,

with 0.05% (w/v) bovine serum albumin and 0.05% (v/v) Triton X-100 (Cooney *et al.* 1981) cooled by a mixture of ice and NaCl. The homogenate was divided into portions (one portion/two enzyme assays) and stored at -80°C until analysed. The following enzyme activities were determined spectrophotometrically: citrate synthase (CS, EC 4.1.3.7.; Alp *et al.* 1976, at 25°C), malate dehydrogenase (MDH, EC 1.1.1.37.; Bücher *et al.* 1964), aspartate aminotransferase (ASAT, EC 2.6.1.1.; Bergmeyer & Bernt 1974, at 25°C), 6-phosphofructokinase (PFK, EC 2.7.1.11.; Opie & Newsholme 1967, at 25°C) and the cytoplasmic as well as mitochondrial glycerol-3-phosphate dehydrogenases (cGPDH, EC 1.1.1.8.; Bergmeyer 1974) (mGPDH, EC 1.1.1.99.5.; Kubista *et al.* 1971). 3-Hydroxyacyl-CoA dehydrogenase (HAD, EC 1.1.1.35., at pH 7.0) was determined fluorometrically as described by Lowry *et al.* (1978). The HAD, PFK and mGPDH activities were assayed only in the methodological investigations. The assays were performed in duplicate at 30°C (unless stated otherwise) under such conditions that reaction rates were proportional to enzyme concentration. For the enzyme assays on fibre homogenates, the reaction volume was minimized to 125 μl by using 0.5 ml microcuvettes (Hellma GmbH & Co., FRG), thereby allowing small sample volumes. Through comparisons of enzyme activities with 1 ml and 125 μl reaction volumes it was shown that this scaling-down procedure did not affect the activities.

MDH isoenzyme assays. MDH isoenzyme activities were determined by also assaying, in addition to total MDH, the mitochondrial isoenzyme of MDH (mMDH) and calculating the cytoplasmic isoenzyme activity as the difference between the total MDH and mMDH activities. The mMDH activity was measured in a homogenate in which cMDH had been inactivated by pre-incubation in 50% (v/v) ethanol as described by Shonk & Boxer (1964) and modified by Schantz *et al.* (1986). To accommodate the high MDH activities in trained type I fibres the NADH concentration was increased from 75 μM , the concentration previously used (Schantz *et al.* 1986), to 220 μM (i.e. the same as that used by Bücher *et al.* 1964). Linearity between enzyme concentration and activity was tested and found to exist over a 10-fold range both for total MDH and mMDH. With 220 μM NADH, on the average 12% higher total MDH activity, 2% higher cMDH, and 26% higher mMDH were noted ($n = 3$). This reduced the cMDH/mMDH ratio by approximately 17%.

In line with the other assays, the cMDH inactivation was also scaled-down. Ten microlitres of homogenate was applied at the bottom of an Eppendorph micro test tube (0.7 ml, Eppendorph Gerätebau Netheler & Hinz GmbH, FRG), followed by addition of 10 μl of ethanol, whereafter it was mixed well. In these tubes, unlike others tested, the

fluid remained in the state of a droplet and thus in a controllable state. Inactivation of cMDH in this fashion was reproducible, and gave results identical to those obtained when using a three times greater volume.

Upon analysing the MDH isoenzyme activities in type I and type II fibres dissected out of the freeze-dried muscles, approximately 45% higher cMDH/mMDH activity ratios were noted than could be expected from the same individuals' muscle homogenate values. Several control experiments were carried out to find the reason for this. Factors which could be ruled out included a possible imperfection in the assay system as well as possible effects of Triton X-100 and/or ultrasound *per se* or in combination with ethanol. Instead the experiments strongly suggested that the higher cMDH/mMDH activity ratio observed in the analysis of single fibres was due to a gradual loss of preferentially the mMDH activity upon storage of the freeze-dried fibres in vacuum in glass tubes at -80°C . This was verified by demonstrating that when new single fibres were prepared from six subjects and homogenized directly after typing and pooling, the cMDH/mMDH ratios were identical with those obtained from homogenates of frozen muscle. For future studies of MDH isoenzymes using single fibres we therefore recommend that the fibres are homogenized directly after having been typed and pooled (cf. methodological studies of storage of homogenate and frozen tissue by Schantz *et al.* 1986).

Thus, the above observations indicate that the data on MDH isoenzyme activities presented here do in all probability reflect a state of lowered mMDH activity. However, this does not rule out the possibility of making comparisons between the trained and untrained groups. This is so because all samples were freeze-dried simultaneously and for an equal length of time and thereafter treated identically. The only factor that differed was the delay from freeze-drying to dissection and from dissection to homogenization. That this factor did not affect the mMDH activity is evidenced by the observation that the differences between the cMDH/mMDH ratios obtained for homogenates of frozen muscle and the average values for the fibre types were relatively constant for the different individuals.

Separation and determination of the activities of mitochondrial and cytoplasmic aspartate aminotransferase (mASAT, cASAT). The ASAT isoenzymes were separated by horizontal thin-layer electrophoresis on an LKB 2117 Multiphor (LKB-Products, Sweden). Agarose gels 1 mm thick 1% (w/v) were prepared according to LKB Application Note 310, but with a modified buffer system. Tris 0.05 M/glycine 0.38 M, pH 8.4, was used in the gels and electrode vessels. Five microlitre samples of fibre homogenate (1:800, dry wt/v) were applied to the gel using a sample

application foil (LKB-products). Electrophoresis was carried out for 30 min at room temperature with a cooling water temperature of 5 °C. Field strength was adjusted to 25 V cm⁻¹ every fifth minute. Under these conditions, the ASAT isoenzymes separate into an anionic (cASAT), and a cationic (mASAT) band. The ASAT isoenzymes were determined according to Sakakibara *et al.* (1983). After electrophoresis, the gel zone containing the isoenzymes was covered with 6 ml of staining medium. The staining medium contained imidazole buffer 0.1 M, pH 7.5, alpha-ketoglutarate 0.01 M, L-cysteine sulphinic acid 0.2 M, 1-methoxy-PMS 0.3 mM (International Reagents Corporation, Japan), MTT 1.9 mM, EDTA 2.0 mM and dextran 20 mg ml⁻¹. After 30 min of incubation at room temperature, the gel was washed with water and immersed in 10% (v/v) acetic acid for 10 min. The stained bands were analysed quantitatively by scanning with an LKB 2202 Ultrosan Laser Densitometer (LKB-Products) at 633 nm. Four assays on different gels were performed for each sample and the bands of each were scanned at locations 1 mm apart. The means of the four assays were used. The areas under the absorbance curves obtained by scanning the isoenzyme bands were used to calculate the mASAT/cASAT scanning area ratios. From this ratio and the total ASAT activity, the absolute isoenzyme activities were calculated. Linearity between sample volume (enzyme concentration) and the area under the absorbance curves was obtained. Equations fitting the regression lines (mASAT: $y = 35.4x + 26.5$, $r = 0.99$; cASAT: $y = 25.0x + 24.4$, $r = 0.98$) indicated practically constant mASAT/cASAT area ratios along the employed range of enzyme concentrations. The relative error of the method was calculated from the differences in mASAT/cASAT ratio in duplicate samples. It amounted to 16.1% ($n = 24$) for the same samples in different gels and to 4.6% ($n = 24$) for double scannings (1 mm apart) of the same sample within a gel. Samples from both fibre types of each individual were applied to the same gel and the type I/II ratios for the mASAT/cASAT quotient were calculated. The methodological error in the type I/II ratio was 10.8% ($n = 12$) between the gels and 10.3% ($n = 12$) within the gels.

Stability of enzyme activities on freeze-drying and under dissection conditions. As already described, the preparation of single muscle fibres requires freeze-drying and exposure to a temperature of 20 °C and about 25% relative humidity during dissection. The dissection and typing of single fibres from each subject's biopsies required 30 h. Not all enzymes are stable under these conditions. Essén and co-workers (1975) showed that the succinate dehydrogenase activity declined by about 70% over a period of 24 h under conditions comparable to ours. The stability of shuttle enzyme activities as well as some other enzymes

was therefore investigated in the following manner. Frozen rat white vastus lateralis muscle was cut cross-sectionally into 16 pieces. Adjacent pieces were paired and one portion was freeze-dried (4 days at -40 °C) and the other stored at -80 °C. After freeze-drying, two of the samples were homogenized immediately and their activities were compared with that of paired frozen samples (stored at -80 °C until homogenization). The other freeze-dried samples were exposed to a temperature of 20 °C and about 25% relative humidity for 3, 6, 12, 24, 27 or 30 h and then homogenized and analysed in the same way as the other samples. These tests showed that the mGPDH activity declines by about 30% on freeze-drying and continues to decline during exposure to about 30% of the activity of the matched frozen samples after 30 h. In contrast, the activities of the other shuttle enzymes were stable under the described exposure conditions. This was also true for CS and HAD, while the activity of PFK declined by altogether about 40% on freeze-drying and 30 h of exposure (Table 1). Since the mGPDH activity is very low under normal conditions and there is a considerable blank reaction, the mGPDH activity in the fibre types could not be measured.

Statistics. Results are expressed as means $\pm$ standard error of mean (SE) or individual values. Only significant differences ($P < 0.05$) will be stated. Otherwise this has been indicated. Differences between the type I and type II fibres within the groups, as well as between the untrained and endurance-trained groups' type I and type II fibres, respectively, were tested for significance by analysis of variance with a split-plot repeated measures design (Kirk 1968). Student's unpaired *t*-test was used to test the significance of group differences in type I/type II ratios for the different enzymes. The relative error of the method was calculated from the formula:

$$\sqrt{\frac{\sum d^2}{2n} \cdot \frac{100}{\bar{x}}},$$

where d stands for the difference between duplicate determinations, n for the number of duplicate determinations, and $\bar{x}$ is the mean value of the observations.

RESULTS

Citrate synthase

Type I vs. type II. The activity of citrate synthase, marker enzyme for the citric acid cycle, was significantly higher in type I than type II fibres in both the untrained (C, 21%) and trained (T, 28%) group.

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Table 1. Effect of freeze-drying and time of exposure to a temperature of 20 °C and 25% relative humidity on enzyme activities

Enzyme	Time of exposure (h)							
	0	0	3	6	12	24	27	30
MDH	1.11	1.08	0.82	0.89	0.95	0.89	0.73	0.86
c/m MDH	1.04	1.48	0.94	0.90	1.02	0.95	1.03	1.09
ASAT	1.06	0.99	0.88	0.91	0.93	0.86	0.71	0.87
cGPDH	1.04	1.09	0.96	0.89	0.78	0.88	0.88	0.90
mGPDH ₁	0.69	0.71	0.61	0.38	0.49	0.35	0.23	0.32
mGPDH ₂	0.68	0.68	0.60	0.45	0.58	0.41	0.21	0.28
CS	0.97	1.21	0.84	0.87	1.06	0.87	0.72	1.15
HAD	0.96	1.01	1.17	0.85	1.13	0.83	—	0.99
PFK	0.87	0.90	0.72	0.78	0.52	0.76	0.60	0.61

The values represent ratios of the activity of freeze-dried samples divided by the activity of matched samples homogenized directly from the frozen state. Two separate experiments were performed on mGPDH.

both type I and type II fibres were 48% higher in T than in C (Fig. 1). The type I/type II CS ratio did not differ significantly between the groups.

Malate-aspartate shuttle enzymes

Type I vs. type II. All the malate-aspartate shuttle enzyme activities were significantly

higher in type I than type II fibres in C (mMDH: 17%, cMDH: 67%, mASAT: 21%, cASAT: 14%) and T (mMDH: 15%, cMDH: 50%, mASAT: 15%, cASAT: 26%).

Trained vs. untrained. No significant difference was seen between the cMDH and cASAT activities of the groups in either fibre type (cMDH, type I: 7% higher in T, type II: 19%, cASAT, type I: 22%, type II: 10%, n.s.), while the mMDH and mASAT activities were, on the average, 49% higher in the trained group (mMDH, type I: 46%, type II: 48%, mASAT, type I: 47%, type II: 54%) (Figs 2–5). The type I/type II enzyme activity ratios did not differ significantly between the groups.

Cytoplasmic glycerol-3-phosphate dehydrogenase

Type I vs. type II. The activity of cGPDH, marker enzyme for the alpha-glycerophosphate shuttle was, on the average, 26% lower in type I than in type II fibres (T: 33%, C: 20%).

Trained vs. untrained. No significant difference was noted between the groups in either fibre type (type I: 0%, type II: 16%, n.s.) (Fig. 6.) the type I/type II cGPDH activity ratio tended to differ (17% lower in C, $P < 0.1$) between the groups.

Enzyme ratios

Type I vs. type II. The cASAT/mASAT, cASAT/mMDH, mMDH/mASAT, mASAT/CS and cASAT/CS enzyme activity ratios did not differ significantly between the fibre types. Significantly higher enzyme activity

Fig. 1. Individual (●—●) and mean enzyme (horizontal line) activities in fibre type I and II from untrained and endurance-trained human skeletal muscle. The activity was measured in homogenate of pools of typed single fibres dissected out of freeze-dried biopsy specimens. * Significant ($P < 0.05$) and non-significant differences = n.s.

Fig. 2. mMDH activity. For explanation of symbols, see legend to Fig. 1.

Fig. 3. mASAT activity. For explanation of symbols, see legend to Fig. 1.

Fig. 4. cMDH activity. For explanation of symbols, see legend to Fig. 1.

Fig. 5. cASAT activity. For explanation of symbols, see legend to Fig. 1.

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Fig. 6. cGPDH activity. For explanation of symbols, see legend to Fig. 1.

ratios were noted in type I than in type II for cMDH/mMDH (38%), cMDH/cASAT (34%), cMDH/mASAT (36%) and cMDH/CS (30%). In contrast, the mMDH/CS ratio was slightly lower (6%) in the type I fibres.

Trained vs. untrained. The mASAT/CS, mMDH/CS, mMDH/mASAT, cMDH/CS

(type II) and cMDH/cASAT enzyme activity ratios did not differ between the groups' type I and type II fibres, respectively. Significantly lower cASAT/mASAT (24%, mean value for type I and type II), cMDH/mMDH (type I: 26%, $P < 0.05$, type II: 18%, $P < 0.1$), cMDH/mASAT (25%), cASAT/mMDH (20%) and cMDH/CS (type I: 27%, $P < 0.05$, type II: 14%, n.s.) enzyme activity ratios were noted in the trained as opposed to the untrained subjects' type I and II fibres, respectively. A tendency to a lower cASAT/CS ratio (19%, $P < 0.1$) was also noted. The type I/type II ratio in cASAT/mASAT activity ratio tended to differ significantly (15% higher in T, $P < 0.1$) and the cMDH/cASAT activity ratio differed significantly (16% lower in T) between the groups (Table 2).

DISCUSSION

Type I vs. type II fibres. There are several studies in which type I (slow-twitch) and type II (fast-twitch) fibres from human skeletal muscle have been characterized with regard to oxidative enzyme activities (Essén *et al.* 1975, Henriksson & Reitman 1976, Lowry *et al.* 1978, Chi *et al.* 1983, Essén-Gustavsson & Henriksson, 1984). These have shown that in untrained muscle the type I fibres have about 40–60% higher citric

Table 2. Enzyme activity ratios in pools of dissected type I and type II fibres from untrained and endurance-trained human skeletal muscle

Enzyme activity ratios	Untrained			Trained		
	Type I	Type II	Type I/Type II	Type I	Type II	Type I/Type II
cMDH/mMDH	2.65 ± 0.14	1.86 ± 0.13*	1.44 ± 0.06	1.97 ± 0.10†	1.52 ± 0.11*†	1.32 ± 0.07
cASAT/mASAT	0.90 ± 0.07	0.96 ± 0.08	0.95 ± 0.06	0.73 ± 0.03†	0.67 ± 0.03†	1.09 ± 0.04**
cMDH/cASAT	1.38 ± 0.06	0.94 ± 0.05*	1.46 ± 0.04	1.25 ± 0.11	1.02 ± 0.04*	1.22 ± 0.08§
cMDH/mASAT	12.1 ± 0.82	8.86 ± 0.75*	1.38 ± 0.06	8.96 ± 0.79†	6.76 ± 0.40*†	1.33 ± 0.10
mMDH/mASAT	4.60 ± 0.28	4.76 ± 0.18	0.97 ± 0.05	4.52 ± 0.25	4.49 ± 0.19	1.01 ± 0.06
cASAT/mMDH	0.19 ± 0.01	0.20 ± 0.01	0.98 ± 0.04	0.16 ± 0.01†	0.15 ± 0.01†	1.10 ± 0.06
cASAT/CS	1.63 ± 0.08	1.72 ± 0.09	0.94 ± 0.03	1.34 ± 0.11†	1.38 ± 0.19†	1.00 ± 0.07
mASAT/CS	1.86 ± 0.09	1.85 ± 0.08	1.01 ± 0.04	1.84 ± 0.10	2.10 ± 0.20	0.92 ± 0.05
cMDH/CS	22.3 ± 0.97	16.2 ± 0.90*	1.38 ± 0.02	16.3 ± 1.34†	13.9 ± 1.76*	1.21 ± 0.10
mMDH/CS	8.44 ± 0.17	8.77 ± 0.25*	0.96 ± 0.03	8.28 ± 0.55	9.12 ± 0.65*	0.91 ± 0.04

Type I/type II ratios are also included.

*Significant difference ($P < 0.05$) in enzyme activity ratios between fibre types (type I vs. type II) within the groups. †Significant difference ($P < 0.05$), and ‡ tendency to a significant difference ($P < 0.1$) in enzyme activity ratios between the groups' type I and type II fibres, respectively. § Significant difference ($P < 0.05$), and ** tendency to a significant difference ($P < 0.1$) in enzyme activity ratios between the groups' type I/type II ratios.

acid cycle enzyme levels and 40–80% higher fatty acid oxidation enzyme levels than the type II fibres. For CS, there have been reports of 29, 35 and 90% higher activities in the type I fibres (Lowry *et al.* 1978, Chi *et al.* 1983, Essén-Gustavsson & Henriksson 1984). Thus, the 25% difference in CS levels found between type I and type II fibres in the present study is in the lower range of the previously reported values. There are indications that with extensive endurance training mitochondrial enzyme levels in type II fibres may approach those in type I fibres (Jansson & Kaijser 1977, Chi *et al.* 1983). No such tendency was seen in the present material, however. The differences in mitochondrial enzyme levels described above correspond to similar differences in mitochondrial volume fraction between the fibre types (Sjöström *et al.* 1982, Howald *et al.* 1985).

The MA shuttle enzymes were found to follow the same pattern as the oxidative enzymes. Thus, 15–60% higher enzyme levels were noted in type I fibres. Interestingly enough, however, the difference in enzyme levels between the fibre types is not uniform. In comparing the ratios of the MA shuttle and CS enzyme levels in the different fibre types, 20–40% higher ratios of cMDH to the other MA shuttle enzymes and CS were noted in type I. The only other difference noted was a slightly lower mMDH/CS ratio (6%) in the type I fibres. Thus, in this group of enzymes, the cMDH levels are selectively higher in type I fibres. Variations in the proportions of mMDH and cMDH have previously been observed between flight and leg muscles of the locust (Pette 1966) as well as between rat colon and liver (Alderman & Schiller 1981). The constant ratio between mASAT and mMDH between the fibre types in the present study agrees with the findings of Pette (1966), who found constant mASAT/mMDH ratios in various species.

Owing to the instability of mGPDH on freeze drying and exposure to dissection conditions (see Subjects and Methods), only the cGPDH activity could be measured as representative of the alpha-glycerophosphate (GP) shuttle. Contrary to what was seen with the MA shuttle enzymes, the levels of cGPDH were about 25% higher in the type II fibres than in type I. Since rather stable proportions of cGPDH and mGPDH appear to exist in different rat muscles (cf. Baldwin *et al.* 1973, Winder & Holloszy

1977) the difference in cGPDH between the fibre types is also likely to be valid for mGPDH.

Lowry *et al.* (1978) noted a correlation between the cGPDH levels and the levels of several glycolytic enzymes in individual human muscle fibres but the relative range of the glycolytic enzyme activities was greater than that of cGPDH. Thus, the present findings of lower difference in cGPDH between type I and type II than the approximately two-fold difference normally reported for most glycogenolytic enzymes (Essén *et al.* 1975, Henriksson & Reitman 1976, Chi *et al.* 1983), are in general agreement with the findings of Lowry *et al.* (1978).

Trained vs. untrained. This study indicates that there might be a difference in the response of the cytoplasmic and mitochondrial MA shuttle enzymes to training. The levels of the cytoplasmic isoenzymes in the different fibre types were non-significantly higher (7–22%, n.s.) in the trained group than in the untrained one, while the corresponding values for the mitochondrial isoenzymes (46–54%) were significantly different. This was an unexpected result since earlier studies on muscle homogenates have indicated that endurance training induces rather concordant increases in levels of all MA shuttle enzymes (Schantz *et al.* 1986). Results of training in other species than man do, however, support the possibility of a differential response of the cytoplasmic and mitochondrial isoenzymes. Holloszy *et al.* (1975) noticed significant increases in all MA shuttle enzymes with training of rats, but the cytoplasmic isoenzyme increased only about half as much as the mitochondrial isoenzymes. Barnard *et al.* (1980) trained dogs and noticed significantly increased mMDH (55%) levels while those of cMDH (6%) were not statistically significant. These results, viewed jointly, favour the idea of differential control of the MA shuttle enzyme levels and thereby the possibility that the isoenzyme levels might change in a non-uniform manner. Thus, in what proportions the MA shuttle enzymes adapt to endurance training needs to be studied further.

With endurance training the adaptation normally comprises changes towards the type I enzyme profile, for example, mitochondrial enzyme levels increase. Thus, the previously reported increases in all MA shuttle enzymes with training (Schantz *et al.* 1986) are in line with the present results of higher levels of all MA

shuttle enzymes in type I fibres. Interestingly enough, however, there is no sign that the adaptation mimics the selectively higher cMDH levels of the type I fibres.

The lack of differences between the untrained and trained groups with respect to the levels of cGPDH in both fibre types is consistent with earlier studies of the effects of endurance training on humans (Schantz *et al.* 1986) and rats (Holloszy & Oscai 1969). Alterations in cGPDH and mGPDH levels have been shown to occur simultaneously with alterations in glycolytic enzyme levels (Golish *et al.* 1970, Pette *et al.* 1973, Baldwin *et al.* 1973). Thus, since endurance training does not normally induce alterations in glycolytic enzyme levels (Holloszy & Booth 1976, Salmons & Henriksson 1981), the lack of difference between the groups could be expected.

Which, then, are the metabolic implications of the different NADH shuttle enzyme levels in the type I and type II fibres? The approximately 25% higher cGPDH activity in the type II fibres indicates a greater relative importance of the GP shuttle in these fibres than in type I fibres. However, owing to low mGPDH activity in human muscle (Schantz *et al.* 1986), the overall importance of the GP shuttle can be assumed to be rather modest compared with that of the MA shuttle. Therefore, the 25% higher MA shuttle enzyme levels in the type I fibres most likely reflect a greater overall capacity for the transfer of reducing equivalents into the mitochondrion. A reasonable consequence of the higher MA shuttle enzyme levels is more effective competition for cytoplasmic NADH, by which means it is withdrawn to greater extent from the pyruvate to lactate reaction (for a fuller discussion, see Schantz 1986). In the extension of the idea of a slowing of the pyruvate reduction to lactate a reversal of this reaction is envisaged. It is known that arterial blood lactate can be taken up in working human skeletal muscle (Jorfeldt 1970). Thus, the reaction lactate to pyruvate with the accompanying formation of NADH may occur in some cells during work (Schantz 1986). With the type I fibres' higher enzyme levels of the TCA cycle, fatty acid oxidation and MA shuttle as well as a predominance of the heart specific lactate dehydrogenase subunits (Sjodin 1976), they are better suited for this lactate oxidation. Especially the selectively higher cMDH activities in the type I fibres create a condition for low

cytosolic NADH concentrations. This would enhance the lactate to pyruvate conversion.

It is concluded that type I fibres have higher MA shuttle enzyme levels than type II fibres. Greater differences between fibre types were noted in cytoplasmic malate dehydrogenase levels than in the other MA shuttle-enzymes and the citric acid cycle marker enzyme, citrate synthase. On the other hand, the GP shuttle enzymes appears to be better expressed in type II fibres. Training leads to higher levels of the MA shuttle enzymes. However, the single fibre analyses indicated significant changes only in the mitochondrial isoenzymes. No training-related changes were noted in the GP shuttle enzyme.

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REFERENCES

- ALDERMAN, J. & SCHILLER, C. 1981. Reducing equivalent transport by substrate shuttles in rat liver and colon. *Comp Biochem Physiol* 70B, 209-217.
- ALP, P., NEWSHOLME, E., & ZAMMIT, V. 1976. Activities of citrate synthase and NAD⁺-linked and NADP⁺-linked isocitrate dehydrogenase in muscle from vertebrates and invertebrates. *Biochem J* 154, 689-700.
- BALDWIN, K., WINDER, W., TERJUNG, R. & HOLLOSZY, J. 1973. Glycolytic enzymes in different types of skeletal muscle: adaptation to exercise. *Am J Physiol* 225, 962-966.
- BARNARD, J., DUNCAN, H., BALDWIN, K., GRIMDITCH, G. & BUCKBERG, G. 1980. Effects of intensive exercise training on myocardial performance and coronary blood flow. *J Appl Physiol* 49, 444-449.
- BERGMEYER, H. 1974. In: H. Bergmeyer (ed.) *Methods of Enzymatic Analysis*, vol. 1, p. 468. Verlag Chemie International, Deerfield Beach, Florida.
- BERGMEYER, H. & BERNT, E. 1974. In: H. Bergmeyer (ed.) *Methods of Enzymatic Analysis*, vol. 2, pp. 727-732. Verlag Chemie International, Deerfield Beach, Florida.
- BLOMSTRAND, E., EKBLOM, B., NEWSHOLME, E. 1986. Maximal activities of key glycolytic and oxidative enzymes in human muscle from differently trained individuals. *J Physiol* 381, 111-118.
- BÜCHER, T., LUH, W. & PETTE, D. 1964. In: Hoppe-Seyler & Thierfelder (eds) *Handbuch*

- der physiologisch- und patologisch-chemischen analyse, vol. 6, part A, p. 307. Springer-Verlag, Berlin.
- BYLUND, A.-C., BJURÖ, T., CEDERBLAD, G., HOLM, J., LUNDHOLM, K., SJÖSTRÖM, M., ÄNGQVIST, K. & SCHERSTÉN, T. 1977. Physical training in man. Skeletal muscle metabolism in relation to muscle morphology and running ability. *Eur J Appl Physiol* **36**, 151-169.
- CHI, M., HINTZ, C., COYLE, E., MARTIN III, W., IVY, J., NEMETH, P., HOLLOSZY, J. & LOWRY, O. 1983. Effects of detraining on enzymes of energy metabolism in individual human muscle fibers. *Am J Physiol* **244**, C276-C287.
- COONEY, G., TAEGTMEYER, H. & NEWSHOLME, E.A. 1981. Tricarboxylic acid cycle flux and enzyme activities in the isolated working rat heart. *Biochem J* **200**, 701-703.
- DAWSON, A.G. 1979. Oxidation of cytosolic NADH formed during aerobic metabolism in mammalian cells. *Trends Biochem Sci* **4**, 171-176.
- ENGEL, W. 1962. The essentiality of histo- and cyto-chemical studies of skeletal muscle in the investigation of neuromuscular disease. *Neurology (Minneapolis)* **12**, 778-794.
- 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.
- ESSÉN-GUSTAVSSON, B. & HENRIKSSON, J. 1984. Enzyme levels in pools of microdissected human muscle fibres of identified type. Adaptive response to exercise. *Acta Physiol Scand* **120**, 505-515.
- GOLISCH, G., PETTE, D. & PICHLMAYER, J. 1970. Metabolic differentiation of rabbit skeletal muscle as induced by specific innervation. *Eur J Biochem* **16**, 110-116.
- GREEN, H., FRASER, I. & RANNEY, D. 1983. Male and female differences in enzyme activities of energy metabolism in vastus lateralis muscle. *Med Sci Sports* **15**, 107, Abstract.
- GRIMBY, G., DANNESKJÖLD-SAMSOE, B., HVID, K. & SALTIN, B. 1982. Morphology and enzymatic capacity in arm and leg muscles in 78- to 81-year-old men and women. *Acta Physiol Scand* **115**, 125-134.
- GRIMBY, G., SALTIN, B., KAIJSER, L. & RENSTRÖM, P. 1983. Aerobic power, muscle morphology and enzymatic capacity in a follow-up study of very old still active endurance athletes. *Med Sci Sports Exerc* **15**, 105, Abstract.
- HARALAMBIE, G. 1979. Skeletal muscle enzyme activities in female subjects of various ages. *Bull Eur Physiopath Resp* **15**, 259-267.
- HENRIKSSON, J. & REITMAN, J. 1976. Quantitative measures of enzyme activities in type I and II muscle fibres of man after training. *Acta Physiol Scand* **97**, 392-397.
- HENRIKSSON, K.G. 1979. 'Semi-open' muscle biopsy technique. A simple outpatient procedure. *Acta Neurol Scand* **59**, 317-323.
- HOLLOSZY, J. & BOOTH, F. 1976. Biochemical adaptations to endurance exercise in muscle. *Ann Rev Physiol* **38**, 273-291.
- HOLLOSZY, J., BOOTH, F., WINDER, W. & FITTS, R. 1975. Biochemical adaptation of skeletal muscle to prolonged physical exercise. In: *Metabolic adaptation to Prolonged Physical Exercise*, pp. 438-447. Birkhäuser Verlag, Basel.
- HOLLOSZY, J. & OSCAL, L. 1969. Effect of exercise on alpha-glycerophosphate dehydrogenase activity in skeletal muscle. *Arch Biochem Biophys* **130**, 653-656.
- HOWALD, H., HOPPELER, H., CLAASSEN, H., MATHIEU, O. & STRAUB, R. 1985. Influences of endurance training on the ultrastructural composition of the different muscle fiber type in humans. *Pflügers Arch* **403**, 369-376.
- JANSSON, E. & KAIJSER, L. 1977. Muscle adaptation to extreme endurance training in man. *Acta Physiol Scand* **100**, 315-324.
- JORFELDT, L. 1970. Metabolism of L(+)-lactate in human skeletal muscle during exercise. *Acta Physiol Scand (Suppl. 338)*.
- KIRK, R.E. 1968. *Experimental Design: Procedures for the Behavioral Science*, pp. 245-268. Brooks/Cole, Belmont, California.
- KUBISTA, V., KUBISTOVA, J. & PETTE, D. 1971. Thyroid hormone induced changes in the enzyme activity pattern of energy supplying metabolism of fast (white), slow (red), and heart muscle of the rat. *Eur J Biochem* **18**, 553-560.
- LEHNINGER, A. 1951. Phosphorylation coupled to oxidation of dihydrodiphosphopyridine nucleotide. *J Biol Chem* **190**, 345-359.
- LOWRY, C., KIMMEY, J., FELDER, S., CHI, M., KAISER, K., PASSONNEAU, P., KIRK, K. & LOWRY, O. 1978. Enzyme patterns in single human muscle fibers. *J Biol Chem* **253**, 8269-8277.
- LOWRY, O.H. & PASSONNEAU, J.V. 1972. *A Flexible System of Enzymatic Analyses*. Academic Press, New York.
- NEWSHOLME, E. & LEECH, A. 1983. *Biochemistry for the Medical Sciences*, p. 199. John Wiley, Chichester.
- NYGAARD, E. 1981. Skeletal muscle fibre characteristics in young women. *Acta Physiol Scand* **112**, 299-304.
- OPIE, L. & NEWSHOLME, E. 1967. The activities of fructose 1,6 diphosphatase, phosphofructokinase and phosphoenolpyruvate carboxykinase in white muscle and red muscle. *Biochem J* **103**, 391-399.
- ÖRLANDER, J., KIESSLING, K.-H., LARSSON, L. & ANIANSSON, A. 1978. Skeletal muscle metabolism and ultrastructure in relation to age in sedentary men. *Acta Physiol Scand* **104**, 249-261.
- PETTE, D. 1966. Mitochondrial enzyme activities. In:

- J. Tager, S. Papa, E. Quagliariello & E. Slater (eds) *Regulation of Metabolic Processes in Mitochondria*, pp. 28–50. Elsevier, Amsterdam.
- PETTE, D., SMITH, M., STAUDTE, H. & VRBOVA, G. 1973. Effects of long-term electrical stimulation on some contractile and metabolic characteristics of fast rabbit muscles. *Pflügers Arch* **338**, 257–272.
- SAHLIN, K. 1983. NADH and NADPH in human skeletal muscle at rest and during ischaemia. *Clin Physiol* **3**, 477–485.
- SAKAKIBARA, S., SHIOMI, K., KOBAYASHI, S., IKEDA, T., INAI, S. & KAGAMIYAMA, H. 1983. A convenient and sensitive method for the determination of serum aspartate aminotransferase isoenzymes after electrophoresis. *Clin Chim Acta* **133**, 119–123.
- SALMONS, S. & HENRIKSSON, J. 1981. The adaptive response of skeletal muscle to increased use. *Muscle & Nerve* **4**, 94–105.
- SALTIN, B. & KARLSSON, J. 1971. Muscle glycogen utilization during work of different intensities. In: B. Pernow & B. Saltin (eds) *Muscle Metabolism during Exercise*, vol. 11, pp. 289–300. Plenum Press, New York.
- SCHANTZ, P. 1986. Plasticity of human skeletal muscle – with special reference to effects of physical training on enzyme levels of the NADH shuttles and phenotypic expression of slow and fast isoforms of myofibrillar proteins. *Acta Physiol Scand* (Suppl. 558).
- SCHANTZ, P., SJÖBERG, B. & SVEDENHAG, J. 1986. Malate-aspartate and alpha-glycerophosphate shuttle enzyme levels in human skeletal muscle: methodological considerations and effect of endurance training. *Acta Physiol Scand* **128**, 397–407.
- SHONK, C. & BOXER, G. 1964. Enzyme patterns in human tissues. I. Methods for the determination of glycolytic enzymes. *Cancer Res* **24**, 709–721.
- SIMONEAU, J., LORTIE, G., BOULAY, M., THIBAUT, M., THÉRIAULT, G. & BOUCHARD, C. 1985. Skeletal muscle histochemical and biochemical characteristics in sedentary male and female subjects. *Can J Physiol Pharmacol* **63**, 30–35.
- SJÖDIN, B. 1976. Lactate dehydrogenase in human skeletal muscle. *Acta Physiol Scand* (Suppl. 436).
- SJÖSTRÖM, M., ÄNGQUIST, K., BYLUND, A.-C., FRIDÉN, J., GUSTAVSSON, L. & SCHERSTÉN, T. 1982. Morphometric analyses of human muscle fibre types. *Muscle & Nerve* **5**, 538–553.
- SUOMINEN, H., HEIKKINEN, E., LIESEN, H., MICHEL, D. & HOLLMAN, W. 1977. Effects of 8 weeks of endurance training on skeletal muscle metabolism in 56–70 year old sedentary men. *Eur J Appl Physiol* **37**, 173–180.
- WINDER, W. & HOLLOSZY, J. 1977. Response of mitochondria of different types of skeletal muscle to thyrotoxicosis. *Am J Physiol* **232**, C180–C184.