Muscle ATP turnover rate during isometric contraction in humans

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KATZ, A., K. SAHLIN, AND J. HENRIKSSON. *Muscle ATP turnover rate during isometric contraction in humans.* J. Appl. Physiol. 60(6): 1839–1842, 1986.—ATP turnover and glycolytic rates during isometric contraction in humans have been investigated. Subjects contracted the knee extensor muscles at two-thirds maximal voluntary force to fatigue (mean ± SE, 53 ± 4 s). Biopsies were obtained before and after exercise and analyzed for high-energy phosphates and glycogenolytic-glycolytic intermediates. Total ATP turnover was 190 ± 7 mmol/kg dry muscle, whereas the average turnover rate was 3.7 ± 0.2 mmol·kg dry muscle^{-1}·s^{-1}. The average ATP turnover rate was positively correlated with the percentage of fast-twitch fibers in the postexercise biopsy ($r = 0.71$; $P < 0.05$) and negatively correlated with contraction duration to fatigue ($r = -0.88$; $P < 0.05$). At fatigue, phosphocreatine ranged from 1 to 11 mmol/kg dry muscle (86–99% depletion of value at rest), whereas lactate ranged from 59 to 101. The mean glycolytic rate was 0.83 ± 0.05 mmol·kg dry muscle^{-1}·s^{-1} and was positively correlated with the rate of glucose 6-phosphate accumulation ($r = 0.83$; $P < 0.05$). It is concluded that *1*) a major determinant of the ATP turnover rate is the muscle fiber composition, which is probably explained by a higher turnover rate in fast-twitch fibers; *2*) fatigue is more closely related to a low phosphocreatine content than to a high lactate content; and *3*) the increase in prephosphofructokinase intermediates is important for stimulating glycolysis during contraction.

high-energy phosphates; economy of contraction; fiber composition; glycolytic intermediates; fatigue; glycolysis

WHEN SKELETAL MUSCLE AT REST is maximally stimulated, it can increase its energy expenditure several hundredfold. The different biochemical pathways available for ATP production are oxidation of carbohydrate and fat, glycolysis (resulting in lactic acid formation), and phosphocreatine (PCr) degradation. During isometric contraction at high forces, when the blood flow is occluded, oxidation of substrates for ATP production is insignificant and ATP is derived only from PCr breakdown and glycolysis.

The ATP turnover rate has been reported to increase linearly with the relative contraction force (7). The ATP turnover rate may also be influenced by the fiber composition, since, in mouse, the rate of phosphagen utilization per force × time integral is greater in fast- than in slow-twitch muscle (6). The purpose of the present investigation was to determine the relationship between ATP turnover rate and fiber composition in humans during isometric contraction to fatigue. An additional aim was to examine the relationship between ATP turnover rate and the maximal time the subjects could maintain the contraction.

MATERIALS AND METHODS

Subjects. Seventeen healthy volunteers (11 men, 6 women), whose mean (range) age, height, and weight were, respectively, 22 (18–21) yr, 177 (157–195) cm, and 73 (52–87) kg, participated in the study. The participants were generally active physical education students, some of whom competed at the Swedish National level. The subjects were informed of the possible risks involved in the experiments before giving their voluntary consent. The study was approved by the Ethical Committee of Huddinge Hospital.

Experimental design. Isometric maximal voluntary contraction (MVC) force of the knee extensor muscles was determined before the experiment, using a Cybex dyanometer (Lumex, New York City), as previously described (15). On the day of the experiment the subjects reported to the laboratory and rested for 10–15 min, after which a biopsy was obtained from the lateral aspect of quadriceps femoris muscle. Biopsies were performed with either a Weil-Blakesly chonchotome (Wisex, Mölndal, Sweden) or the Bergström needle (2) as previously described (15). Subjects were then seated in a chair and had an inflatable cuff placed about the thigh to be exercised. Subjects then performed an isometric contraction of the knee extensor muscles at 65.4 ± 4.1% (mean ± SE) MVC. When the subject demonstrated difficulty in maintaining the predetermined force output, the cuff was rapidly inflated (~2 s) to 33 kPa, and the subject continued exercise to fatigue. A postexercise biopsy was then taken, and the cuff was deflated. The cuff was used to ensure that an anaerobic environment was maintained between the end of contraction and the postexercise biopsy so that aerobic resynthesis of PCr would not occur.

Analytical methods. All biopsies were rapidly frozen (~2 s) in liquid Freon, which was maintained at its freezing point by liquid N_2. From nine subjects a portion of the postexercise biopsy was mounted for fiber type analysis (3) as previously described (1). From one subject the postexercise biopsy appeared to be taken from partially inactive muscle; hence the metabolite data were not utilized. The basis for this judgment was the comparison of the metabolite levels with those in the 1-min recovery sample (12). The remainder of the material was freeze-dried, powdered, and extracted in 0.5 M perchloric acid and neutralized with $KHCO_3$ (10). Enzymatic methods were used to analyze muscle PCr, creatine, ATP, ADP, glucose, glucose 6-phosphate (G-6-*P*), α-glycerophosphate (α-GP), pyruvate (10), and alanine (13). All metabolites, except for lactate, pyruvate, and glucose (due to their relatively large extracellular presence), have been adjusted to the highest total creatine content for each subject. All metabolites are given as millimoles per kilogram of dry muscle.

Statistics. Significant differences between means were determined with the paired *t* test. Additionally, linear and exponential regression analyses were employed. The limit for statistical significance was set at $P < 0.05$, and values are presented as means ± SE.

RESULTS

During the contraction, energy was derived from utilization of high-energy phosphates and glycolysis (Table 1). The total ATP turnover was 190 ± 7 mmol/kg dry muscle, the major portion being derived from glycolysis. The average ATP turnover rate was 3.7 ± 0.2 $\text{mmol}\cdot\text{kg dry muscle}^{-1}\cdot\text{s}^{-1}$ and was positively correlated with the percentage of fast-twitch fibers in the postexercise biopsy (Fig. 1) and negatively correlated with contraction duration to fatigue (Fig. 2, top). During anaerobic conditions the components of ATP turnover are PCr degradation and glycolysis, and the rates of the latter two processes were also negatively correlated with contraction duration to fatigue (Fig. 2, middle and bottom). From the above results it might be expected that contraction duration would also be related to the muscle fiber composition, as was previously shown in 19 subjects who performed isometric contraction at 50% MVC to fatigue (11). We could, however, not demonstrate a significant relationship between the percentage of fast-twitch fibers and exercise duration ($r = -0.43$; $P > 0.05$), which may be partially explained by too few subjects.

Changes in glycogenolytic and glycolytic intermediates are shown in Table 2. It should be noted that the change in G-6-*P* accounts for nearly one-fourth of total glycogenolysis. Muscle glucose increased from 2.6 ± 0.4 mmol/

TABLE 1. *Changes in muscle metabolite contents and corresponding ATP equivalents after isometric contraction to fatigue*

Metabolite	Δ	ATP Equivalent	%Total ATP Turnover
ATP	−4.7±0.5	8.0	4.2
ADP	1.4±0.2		
PCr	−68.7±2.5	68.7	36.2
Pyruvate	2.1±0.3	3.1	1.6
Lactate	73.5±3.0	110.2	58.0

Values are means ± SE given in mmol/kg dry muscle; n = 16 subjects. Total ATP turnover was calculated from $[2(-\Delta ATP)-\Delta ADP-\Delta PCr+1.5\ (\Delta lactate+\Delta pyruvate)]$ where PCr is phosphocreatine. Absolute metabolite values can be obtained from Katz et al. (12). Contraction duration to fatigue was 53.5 ± 3.8 s.

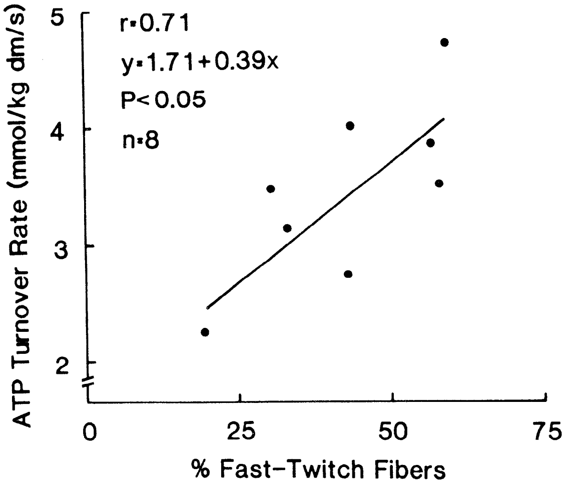

FIG. 1. Relationship between average ATP turnover rate and percentage of fast-twitch fibers in postexercise biopsy after isometric contraction to fatigue.

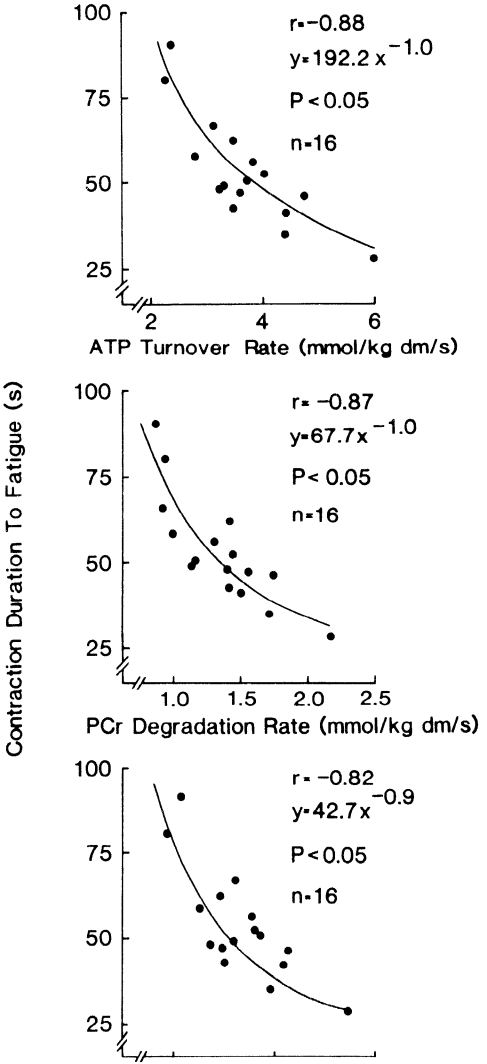

FIG. 2. Relationships between ATP turnover (*top*), phosphocreatinine (PCr) degradation (*middle*), and glycolytic (*bottom*) rates and contraction duration after isometric contraction to fatigue.

TABLE 2. *Changes in muscle glycogenolytic and glycolytic intermediates after isometric contraction to fatigue*

Metabolite	Δ	%Total Glycolysis	%Total Glycogenolysis
Glucose	1.0±0.4		1.8
G-6-*P*	12.6±1.3		22.5
α-GP	6.8±0.2	8.0	6.1
Pyruvate	2.0±0.2	2.4	1.8
Alanine	2.4±0.4	2.8	2.1
Lactate	73.4±3.0	86.8	65.7

Values are means ± SE given in mmol/kg dry muscle; n = 11–16 subjects. Total glycolysis was calculated from $(0.5)(\Delta\alpha\text{-GP} + \Delta\text{pyruvate} + \Delta\text{alanine} + \Delta\text{lactate})$, where α-GP is α-glycerophosphate, and total glycogenolysis from $\Delta\text{G-6-}P + \Delta\text{glucose}$ + total glycolysis, where G-6-*P* is glucose 6-phosphate. Absolute metabolite values can be obtained from Katz et al. (12).

kg dry muscle at rest to 3.7 ± 0.4 after contraction ($P < 0.05$). These values are similar to previous findings under the same conditions (9). If it is assumed that extracellular glucose is 5 mmol glucose/l extracellular water, and there are 0.3 liters extracellular water/kg dry muscle, then the corresponding intracellular values are 1.2 ± 0.4 and 2.2 ± mmol/kg dry muscle ($P < 0.05$). It is, however, generally accepted that under basal conditions the intracellular glucose level is very low. It is thus possible that the calculated intracellular values are overestimated due to a small error in the extracellular water content of the biopsy.

The mean glycogenolytic and glycolytic rates were, respectively, 1.09 ± 0.08 and 0.83 ± 0.05 $\text{mmol}\cdot\text{kg dry muscle}^{-1}\cdot\text{s}^{-1}$. The mean value of G-6-*P* accumulation was 0.25 ± 0.03 $\text{mmol}\cdot\text{kg dry muscle}^{-1}\cdot\text{s}^{-1}$ and was positively correlated with the glycolytic rate (Fig. 3).

DISCUSSION

ATP turnover rate. A main finding of the present study was the relationship between ATP turnover rate and fiber composition of the postexercise biopsy (Fig. 1). It has been demonstrated that fast-twitch muscle has a greater rate of phosphagen utilization per force × time integral than slow-twitch muscle (6). Our finding of a significant correlation between ATP turnover rate and percent fast-twitch fibers is consistent with the idea that fast-twitch muscle has a higher ATP turnover rate than slow-twitch muscle, possibly due to a higher rate of actomyosin turnover in the fast-twitch fibers (6).

It might be argued that an incomplete activation of the slow-twitch fibers may explain the relationship between the ATP turnover rate and the percentage of fast-twitch fibers. However, the virtual depletion of the PCr content in all subjects speaks against this possibility. Another factor that may regulate the ATP turnover rate of the knee extensor muscles is the degree of activity of the antagonist muscle group. No quantitative estimate on this parameter can, however, be made from the present data.

It might also be argued that the lower ATP turnover rate in subjects with a long contraction duration is due to an increase in the economy of ATP hydrolysis, i.e., ATP hydrolyzation per unit force, during the contraction. During electrically stimulated contraction in humans the economy of ATP hydrolysis, based on changes in muscle temperature, has been shown to increase (8). It should be noted, however, that during the latter part of a contraction the increased economy of ATP hydrolysis actually levels off rather than continues to rise (8). Thus, if there is an increased economy of ATP hydrolysis, it can hardly account for the large decrease in the ATP turnover rate with respect to time to fatigue in the present study.

ATP turnover rate and fatigue. The ATP turnover rate as well as the PCr degradation and glycolytic rates were inversely related to contraction duration to fatigue. There is, however, no reason to suggest that the rates of these processes per se are responsible for fatigue. It is more likely that the high rates are reflecting changes in the absolute levels of metabolites that may be responsible or associated with fatigue.

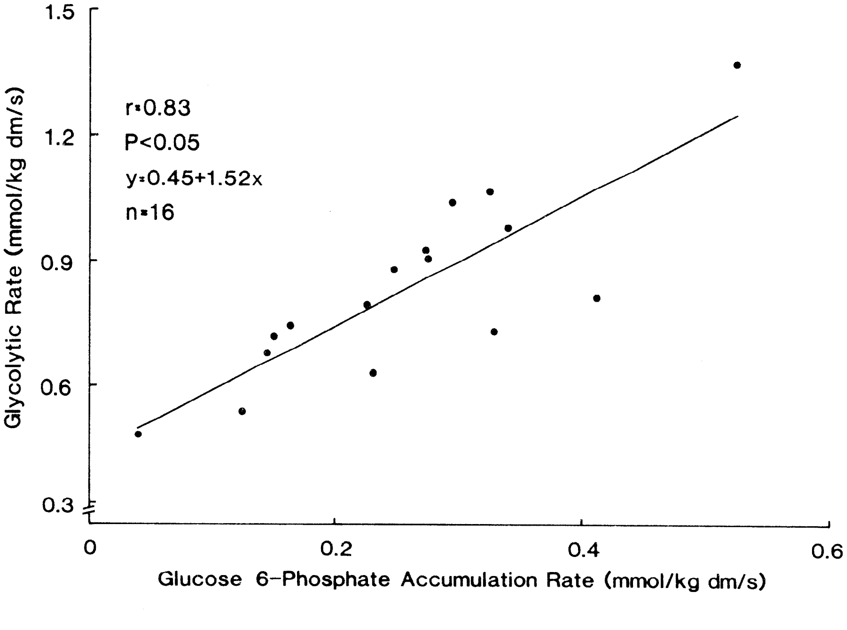

FIG. 3. Relationship between muscle glycolytic and glucose 6-phosphate accumulation rates after isometric contraction to fatigue. See legend to Table 2 for calculation of glycolytic rate.

For example, a high glycolytic rate may lead to a rapid depletion of glycogen, resulting in lack of adequate substrate for the required energy demand. If it is assumed that the glycogen level at rest was 350 mmol glucosyl units/kg dry muscle (9), then the present glycogenolytic rate should have resulted in a mean value of 292 mmol glycosyl units/kg dry muscle at fatigue. Since the Michaelis constant of phosphorylase for glycogen has been reported to be ~6 mmol glucosyl U/kg dry muscle (recalculated from Ref. 4), it is not likely that the glycogen level could have been limiting for glycolysis. On the other hand, at two-thirds MVC, glycolysis will result in high muscle lactate levels that will lead to a substantial decrease in muscle pH. The mechanisms whereby hydrogen ions may cause fatigue have been discussed elsewhere (14). The range in muscle lactate values at fatigue was, however, very large (59–101 mmol/kg dry muscle), which suggests that hydrogen ions per se are not responsible for fatigue. This conclusion is consistent with the results of a recent study which demonstrated that trained subjects maintained an isometric contraction to fatigue slightly longer than untrained subjects, although the trained group accumulated only 70% of the lactate content observed in the untrained group (15). PCr content was, however, virtually depleted in all subjects at fatigue in both groups. In the present study PCr was also nearly depleted in all subjects at fatigue (range, 1–11 mmol/kg dry muscle, which corresponds to an 86–99% decrease of the value at rest), suggesting that fatigue is related to depletion of the high-energy phosphates.

Glycolysis and G-6-P. One of the key regulatory enzymes of glycolysis, i.e., phosphofructokinase, has been shown to be inhibited at low pH (16). The rate of glycolysis is, however, constant during contraction (5, 12), although pH is constantly decreasing. It is thus evident that increases in the levels of activators-deinhibitors counteract the pH inhibition and maintain phosphofructokinase activity. The rate of G-6-*P* formation was well related to the glycolytic rate. The increase in fructose 6-phosphate, which is in equilibrium with G-6-*P* and is an activator of phosphofructokinase (17), might be one mechanism whereby phosphofructokinase could be activated during contraction. Other important activators under these conditions are AMP, ADP, and inorganic phosphate.

It is concluded that, during isometric contraction, *1)* a major determinant of the ATP turnover rate is the muscle fiber composition, which is probably explained by a higher turnover rate in fast-twitch fibers; *2)* fatigue is more closely related to a low phosphocreatine content than to a high lactate content; and *3)* the increase in prephosphofructokinase intermediates is important for stimulating glycolysis during contraction.

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