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Myoglobin content in human skeletal muscle and myocardium: relation to fibre size and oxidative capacity

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SUMMARY Myoglobin, muscle fibre diameter, and citrate synthase activity were measured in leg muscle of untrained and trained men and in the myocardium from the apex of the left ventricle and from papillary muscle in patients subjected to open heart surgery. The citrate synthase (CS) activity was 60% higher in trained than in untrained skeletal muscle. In the myocardium it was around four times greater than in untrained skeletal muscle but there was no difference between the apex of the left ventricle and papillary muscle. The fibre diameter varied almost threefold between the different groups of muscles with the largest diameter in untrained skeletal muscle and the smallest in papillary muscle. The myoglobin content in trained skeletal muscle did not differ from that of untrained muscle. In the left ventricle it was only 40% of that found in untrained muscle while papillary muscle had almost twice as high a myoglobin content as did the left ventricle. The ratio between myoglobin and fibre diameter, however, was of similar magnitude in skeletal muscle and the left ventricle while it was twice as high in papillary muscle as in the other muscles.

In conclusion, the diffusion distance in terms of fibre diameter decreased with increased oxidative capacity (CS activity), when comparing the statistical means of the four different groups. The capacity for oxygen diffusion in relation to oxygen demand measured as the ratio of myoglobin to fibre diameter appeared to be of a similar magnitude in skeletal muscle and left ventricle but was higher in papillary muscle.

Myoglobin is an intracellular haeme-protein operative in skeletal muscle and the myocardium. Its main function is thought to be to facilitate the diffusion of oxygen into and within the cell, making the oxygen available for the oxidative process in the mitochondria. The terminal step in the electron transport chain, cytochrome oxidase, has its minimum effective pO_2 at 5 mmHg. At this pressure 60% of the myoglobin is saturated and the dissociation curve is steep.[1] The implication is that at this critical pO_2 large quantities of oxygen will be released resulting in an increased oxygen diffusion. At the same time the myoglobin functions as a short term oxygen store buffering fluctuations in the rate of flow of oxygen to the mitochondria during contraction, for example in the beating heart.

An increased myoglobin concentration has been demonstrated to occur in two experimental situations, the first one being high altitude hypoxia both in man and animals[2 3] and the second one, based on animal studies, as a response to physical training, which additionally is known to increase the oxidative capacity of the muscle tissue.[4]

However, in previous studies we found: (1) that skeletal muscle myoglobin did not increase with physical training although the oxidative capacity measured as succinate dehydrogenase or citrate synthase was significantly increased[5 6]; and (2) that the human myocardial myoglobin concentration is approximately half of that in human skeletal muscle in spite of the three to four times higher citrate synthase activity.[7] The low myoglobin in human myocardium has been reported earlier.[8] However, it was realised that oxygen transport from the capillaries to the

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mitochondria in muscle fibres is dependent not only on the myoglobin concentration but also on the diffusion distance.[9] The oxygen transport capacity from the capillaries to the mitochondria was thus analysed, in the present study, in terms of fibre diameter and myoglobin concentrations and correlated with the oxidative capacity in terms of the citrate synthase activity. To get a wide range in oxidative capacity, biopsies from untrained and trained skeletal muscle and from the left ventricle and the papillary muscle of the human myocardium were compared.

Methods

SKELETAL MUSCLE BIOPSIES

Six untrained and five trained male subjects were studied. The untrained subjects were unaccustomed to habitual physical activity, and their mean and range, for age, height, and weight were 34 (27 to 42) years, 183 (178 to 192) cm, and 79 (60 to 110) kg respectively. The trained subjects were Swedish elite long-distance runners. Their mean and range for age, height and weight were 35 (24 to 43) years, 168 (158 to 176) cm, and 56 (49 to 64) kg, respectively. Part of the results from these subjects has been reported elsewhere.[10]

MYOCARDIAL BIOPSIES

Biopsies were taken from the apex of the left ventricle (eight patients) and from the removed papillary muscle (six patients). Both groups of patients were subjected to open heart surgery due to aortic or mitral valve disease. The mean age and range for patients from which myocardial biopsies were taken were 62 (52 to 75). The study was approved by the Ethical committee of the Karolinska Hospital.

Procedure and analyses

Muscle samples were obtained at rest from m. quadriceps femoris vastus lateralis using the percutaneous needle biopsy technique.[11] Each biopsy was rapidly divided into two pieces. One of these was immediately frozen in liquid nitrogen and stored at –80°C until biochemically analysed. After freeze drying each sample was dissected free from blood and connective tissue under a dissection microscope and weighed at a temperature of 22°C and a relative humidity less than 35%. Samples were thereafter homogenised (dilution 1:100) in ice-cooled 0.1 mol·litre^{-1} phosphate buffer pH 7.7 with 0.5% BSA. The citrate synthase activity was analysed by a modified enzymatic fluorometric procedure[12] according to the principles of Lowry and Passoneau.[13] The myoglobin concentration was determined on a further dilution of the homogenate (total dilution 1:25 000) by a radioimmunoassay.[14 15] The other piece of the biopsy was used for histochemical analysis and mounted on OCT compound embedding medium (Tissue-Tek II) frozen at –150°C in isopentane, precooled by liquid nitrogen and stored at –80°C until analysed. The skeletal muscle biopsies were sectioned (10 μm) at –20°C and stained for myofibrillar ATPase at pH 9.4[16] after preincubation at pH 10.3 (9 min at 37°C in 0.05 mol·litre^{-1} glycine buffer, 0.03 mol·litre^{-1} $CaCl_2$-0.05 mol·litre^{-1} NaCl and for NADH-tetrazolium reductase (NADH-TR, Novikoff *et al*[17]).

Skeletal muscle fibres were classified into type I or type II based on the myofibrillar ATPase stain. The cross-sectional area for each fibre type was measured from NADH-TR stained sections by a grid method and based on the mean value of 20 fibres.[18] The mean fibre area was thereafter calculated by the formula 0.01 × (% type I × type I area + % type II × type II area). Myocardial biopsies were transversely sectioned and stained for NADH-TR. The cross-sectional fibre area was measured from prints of these sections by cutting out, pooling and weighing all fibres within a selected area including at least 20 fibres. By weighing photopaper with known areas, a fibre averaged area could be calculated.[19] The mean fibre diameter was thereafter calculated for both the skeletal and myocardial biopsies.

Values are expressed as mean with standard deviation. Statistical analysis was performed by Student's *t* test.

Results

Citrate synthase (CS) activity was approximately 60% higher in trained than in untrained skeletal muscle (table). In both left ventricle and papillary muscle it was around 4 times higher than in the untrained skeletal muscle.

Myoglobin concentration was not found to be different when comparing trained and untrained skeletal muscle (table). In left ventricle and papillary muscle it was 42% and 73% respectively, of that found in skeletal muscle.

Fibre diameter was not significantly different between trained and untrained skeletal muscle (table).

The fibre diameter of left ventricle and papillary muscle was 40% and 36% respectively of that found in the untrained skeletal muscle.

A decreasing fibre diameter was found to be parallel to an increasing citrate synthase activity when looking at means for the four groups of muscle (table).

The myoglobin/fibre diameter ratio was not found to

TABLE *Citrate synthase activity, myoglobin concentration, fibre diameter and the ratio of myoglobin to fibre diameter in untrained and trained skeletal muscle and in myocardium from the apex of the left ventricle and from papillary muscle*

Muscle	*n*	*Citrate synthase* ($\mu kat \cdot g_{dw}^{-1} \cdot min^{-1}$)	*Myoglobin* ($\mu mol \cdot g_{dw}^{-1}$)	*Fibre diameter* (μm)	*Myoglobin/fibre diameter* ($\mu mol \cdot g_{dw}^{-1} \cdot \mu m^{-1} \cdot 10^{-2}$)
Skeletal muscle					
Untrained (U)	(6)	0.68±0.21	1.21±0.22	81.3±13.2	1.59±0.14
		*(U *vs* T)	NS	NS	NS
Trained (T)	(5)	1.09±0.21	1.19±0.14	74.4±6.0	1.51±0.37
		**(T *vs* V)	***	***	NS
Myocardium					
Left ventricle (V)	(8)	2.55±0.53	0.50±0.17	32.0±8.2	1.70±0.77
				(35.7±4.1)	(1.48±0.55)
		NS (V *vs* P)	*	NS	*
				(**)	(**)
Papillary muscle (P)	(6)	2.73±0.52	0.88±0.27	29.3±2.0	3.02±1.06

Values within parenthesis for fibre diameter indicate mean value when one markedly deviant value (16.5) was excluded. Statistical differences between groups are indicated as *$p<0.05$; **$p<0.01$; ***$p<0.001$ and NS=non-significant. n denotes the number of subjects or patients.

differ when comparing trained and untrained skeletal muscle and left ventricle (table). Papillary muscle however, showed a ratio of approximately twice that of the other muscles. When comparing papillary muscle with left ventricle the higher ratio for papillary muscle was dependent on both its higher myoglobin concentration and its smaller fibre diameter.

Discussion

Within the four different kinds of human muscle analysed there was almost a threefold variation in fibre diameter. This observation probably is of considerable importance with relation to the oxygen transport capacity of the tissue with emphasis on diffusion distance. Of course it should be of great interest also to consider the role of capillarisation in regard to oxygen transport. However, determination of capillary density in human myocardial biopsies by means of histochemical methods and light microscopy has so far not been technically satisfying in our hands. It is also likely that the muscle myoglobin concentration must be of substantial importance in facilitating the diffusion of oxygen. It has been puzzling, therefore, that skeletal muscle myoglobin concentrations do not increase with physical training in man. Likewise the fact that the myoglobin concentration in the left ventricle, which is known to have a higher oxidative capacity than skeletal muscle is only about 40% of that found in skeletal muscle. However, the papillary muscle in the present study contains almost twice as much myoglobin as the apex of the left ventricle.

When the ratio of myoglobin to fibre diameter was considered no differences were detected between trained and untrained skeletal muscle and left ventricle. This similar myoglobin/fibre diameter ratio might indicate a similar balance between oxygen transport capacity and oxygen demand in trained and untrained skeletal muscle as well as in apical myocardium. This in spite of the high oxygen demand of the left ventricle as indicated by the almost fourfold higher level of citrate synthase activity compared with untrained skeletal muscle.

The oxidative capacity of papillary muscle in the present material was of the same size as found in the apex of the left ventricle. However, the myoglobin concentration was almost twice as high and the fibre diameter smaller compared with the apex of the left ventricle. In this set of papillary muscles the oxygen supply might be more critical than in the analysed apical myocardium. A larger capacity for oxygen transport and storage might be needed to meet the oxygen demands during contraction in papillary muscles than in the left ventricle. In this context, the calculation that the myoglobin oxygen reserve is only a short term store sufficient for 1 to 2 beats might be of interest.

It might be argued that the results of the present study do not represent the normal heart as biopsies were taken from hearts with severe valvular disease with or without diagnosed hypertrophy. Of course there may be definite differences both in fibre diameter and myoglobin content between hypertrophied and non-hypertrophied myocardium. However, a low myocardial myoglobin content compared with skeletal muscle has been documented elsewhere,[8] and also a smaller fibre diameter in myocardium than in skeletal muscle.[20] Thus in general, lower myoglobin concentration and smaller fibre diameters are present in the human myocardium than in the human skeletal muscle.

In concusion this study demonstrates the relation between myoglobin concentration and diffusion distance (estimated by fibre diameter) in different

types of human muscles. This relation is not directly related to the oxidative capacity of the cell but might adapt to the special physiological or pathophysiological situation in order to adjust oxygen supply to oxygen demand.

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