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Human Skeletal Muscle in Viral and Mycoplasma Infections

Ultrastructural Morphometry and Its Correlations to Enzyme Activities

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ABSTRACT

The quantitative effects of acute viral and mycoplasma infections on subcellular elements of skeletal muscle have been investigated in seven hospitalized patients, aged 20–42 years, and the findings are correlated to the activities of glyceraldehyde-3-phosphate (triosephosphate) dehydrogenase (TPD), lactate dehydrogenase (LDH), citrate synthetase (CS), and cytochrome *c* oxidase (cytox). Comparisons are made with five healthy men, aged 22–29 years, who were confined to bed for 7 days, being the mean period of confinement to bed in the patients. The muscle samples were taken from the thigh. In both patients and controls a decrease of the volume fraction of the myofibrillar compartment was observed in the acute phase of the illness when compared to 4 months afterwards. It was inversely related to the volume fraction of the sarcoplasmic space. The volume fraction of unspecified vacuols was increased in the acute phase and was greater than that of the control subjects confined to bed. The fraction of sarcoplasmic space and that of vacuols correlated negatively to the activity of CS. The activity of cytox correlated to the volume fraction of mitochondria. No correlations were found for TPD or LDH.

INTRODUCTION

In viral and mycoplasma infections the skeletal muscle of man exhibits focal deviations from normal ultrastructure (Åström et al., 1975) and a simultaneous reduction of its activities of some enzymes (Åström et al., 1976). Probably these ultrastructural deviations were not of a magnitude great enough to explain the enzymatic effects. However, there might have been quantitative alterations in the structural composition of the muscle cell, which would reflect the effects of the enzyme activities. Structural composition can be estimated, in quantitative terms, by the use of morphometric and stereological methods (Weibel, 1969 and 1972).

The present investigation contains quantitative data concerning subcellular elements, expressed as volume fractions, from muscle tissue of patients, in the acute phase of viral or mycoplasma infections, and 1.5 and 4 months thereafter. Further, equivalent data from a control group, confined to bed, are presented. The results obtained were correlated to the activities of some key enzymes of the metabolism of the muscle cell.

MATERIAL AND METHODS

Subjects

Seven hospitalized patients, all men, aged 20–42 years, were selected to participate in the investigation. Five suffered different viral infections, and two mycoplasma pneumonia. For details of clinical findings, course, and procedures an earlier report in which the present patients were included, i.e. S. J., K. E. H., L. G. H., E. E., B. S., R. M. and H. J., is referred to (Åström et al., 1976). The duration of bed rest was 7.6±1.7 days.

Five healthy men, aged 22–29 years served as the control group. They were confined to bed for 7 days in a special room on a ward for infectious diseases. They followed the same daily routine as the patients, the aim being to achieve the same degree of physical activity (clinical bed rest) and energy intake as encountered by the patients.

Biopsy

Biopsies were taken percutaneously from m. vastus lateralis by the Bergström needle technique (Bergström, 1962; Åström et al., 1976). In the patients, a biopsy was taken at the end of the acute disease (10.6±1.7 days after onset of symptoms) and follow-up biopsies after 43.6±4.4 and 134.3±7.6 days. In the healthy control subjects a biopsy was taken at the end of the bed rest period (7 days) and follow-up biopsies after 35.6±0.4 and 97.4±2.7 days. Each biopsy was divided into two parts—one for electron microscopy and the other for enzyme assays.

Table I. *Volume fractions of muscle cell components in male patients suffering viral or mycoplasma infections and in healthy controls confined to bed*

The first biopsy was taken at the end of the acute disease from the patients and after one week's bed rest from the healthy controls. The figures are mean values ± standard error. * and ** denote $p<0.05$ and $p<0.01$ when compared to the corresponding value of the other group. A p-value below a mean value denotes the level of significance when that mean value is compared to the last value in the same group. Values given in $\mu m^3/100\ \mu m^3$

No. of subjects	Time of biopsy (days)	Vacuoles	Mitochondria	Sarcoplasmic space	Myofibrils
Patients with infections					
7	10.6±1.7	0.80±0.21* $p<0.05$	3.89±0.49	24.6±1.5 $p<0.025$	70.7±1.9 $p<0.05$
7	43.6±4.4	0.82±0.25**	4.06±0.39	23.2±0.8 $p<0.05$	72.0±0.8 $p<0.05$
7	134.3±7.6	0.45±0.05	4.15±0.42	19.1±1.8	76.3±1.9
Healthy controls					
5	7	0.35±0.14*	4.70±0.65	24.3±1.8	71.5±1.9
5	35.6±0.4	0.22±0.10**	4.15±0.43	20.3±1.5	74.9±1.7
5	97.4±2.7	0.32±0.10	4.35±0.54	20.9±1.8	75.1±1.9

Electron microscopy

The part of the muscle biopsy for electron microscopy was rapidly transferred to ice-cold 2% OsO_4, according to Zetterqvist (1956) and cut into pieces smaller than 1 mm^3. The fixation continued in the same fixative for 2 hours at 0–4°C. The specimens were washed in Tyrode's solution for 30 min, dehydrated in ethanol and embedded in Epon 812. At least five different blocks were made from each biopsy.

Approximately 60–80 nm thick sections were cut in an ultramicrotome with glass knives, stained with uranyl acetate and lead citrate and examined in an electron microscope (Siemens Elmiskop 101). In order to obtain a good randomization, no orientation of the specimens was made before cutting. Photomicrographs were thus taken from longitudinal, transverse and, most frequently, oblique sections according to a randomization scheme. Altogether 40–60 micrographs were taken from at least five blocks per biopsy. Volume fractions were estimated by the point-counting technique (Weibel, 1969 and 1972) with a 160 points, coherent test lattice covering an area of 71.0 μm^2 per micrograph. The total test area per biopsy was 2.850–4.250 μm^2. The muscle tissue was divided into four compartments: mitochondria, myofibrillar space, sarcoplasmic space, and unspecified vacuoles. The latter included all kinds of vacuoles surrounded by a membrane, because it was impossible to decide from the micrographs whether a vacuole was a lysosome, autophagic vacuole, or extended sarcoplasmic reticulum. Occasionally occurring fat droplets (<0.2%) were included in the sarcoplasmic space.

Statistical evaluation of the results was performed by the Mann-Whitney U-test for independent samples.

Enzyme assays

The part of the biopsy for enzyme assays was weighed and homogenized as previously described (Åström et al., 1976). The homogenate obtained was used for estimation of the activities of citrate synthetase (CS; E.C. 4.1.3.7) glyceraldehyde-3-phosphate dehydrogenase, i.e. phosphorylating triosephosphate dehydrogenase (TPD; E.C. 1.2.1.12), lactate dehydrogenase (LDH; E.C. 1.1.1.27), and cytochrome *c* oxidase (cytox; E.C. 1.9.3.1). The methods used were, for CS according to Srere (1969), for TPD and LDH according to Bass et al. (1969), and for cytox according to Tottmar et al. (1973).

RESULTS

In the patients, no changes were found in the volume fraction of mitochondria as a result of illness,

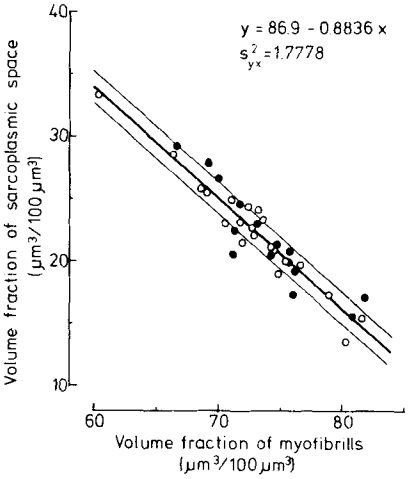

Fig. 1. Correlation between volume fractions of sarcoplasmic space (y) and myofibrillar space (x) in skeletal muscle from patients suffering viral or mycoplasma infections (open circles) and from healthy controls confined to bed (filled circles). Muscle samples taken according to Table I. The correlation coefficient (r) is −0.948.

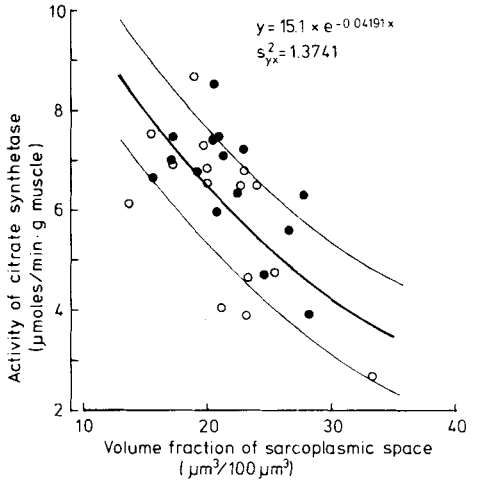

Fig. 2. Correlation between volume fraction of sarcoplasmic space (x) and the activity of citrate synthetase (y) in skeletal muscle from patients suffering viral or mycoplasma infections (open circles) and from healthy controls confined to bed (filled circles). Muscle samples taken according to Table I. The correlation coefficient (r) is -0.669 ($p<0.0001$).

on comparisons with recordings either 1.5 or 4 months thereafter, or to recordings, on corresponding occasions, in the control group confined to bed (Table I).

The volume fraction of unspecified vacuoles was increased in the acute phase of illness when compared to that of the control group confined to bed. This increased fraction of vacuoles remained unchanged 1.5 months later, but 4 months after the acute disease, it was at a similar level as that of the healthy subjects (Table I). No effect of bed rest could be observed.

In the patients, the size of the sarcoplasmic space (incl. fat droplets) was larger in the acute phase of the illness, as well as 1.5 month thereafter, when compared to 4 months afterwards. A similar although non-significant tendency was observed in the healthy control group (Table I).

The myofibrillar compartment was inversely related to the sarcoplasmic space (Fig. 1).

There was a correlation between the activity of cytochrome *c* oxidase and the volume fraction of mitochondria ($r=0.5544$; $y=0.96x+1.28$). Furthermore, there existed a negative correlation between the activity of citrate synthetase on the one hand, and the volume fraction of sarcoplasmic space (Fig. 2) and the volume fraction of vacuoles (Fig. 3), on the other. With the other two enzymes (TPD and LDH) no quantitative correlations could be found to any structural component.

DISCUSSION

The purpose of the present study was to investigate whether the enzymatic activities found in skeletal muscle in acute infectious disease and at different times thereafter (Åström et al., 1976) were associated with the relative quantities of different structural components within the muscle cell. If so, one would expect altered volume fractions of structural components in the patients, as compared to the healthy subjects. However, the volume fraction of unspecified vacuoles was the only component that showed an increase as a result of infection. Furthermore, this volume fraction decreased to a normal value afterwards (Table I).

It cannot be excluded that the change in volume fraction of sarcoplasmic space, observed from illness to the 4-month follow-up biopsy, reflected an effect of bed rest rather than of illness, since there were no differences between patients and control subjects.

The inverse relationship between the volume fractions of myofibrils and sarcoplasmic space could be explained by the fact that the fractions of the other two components, quantitatively, were on

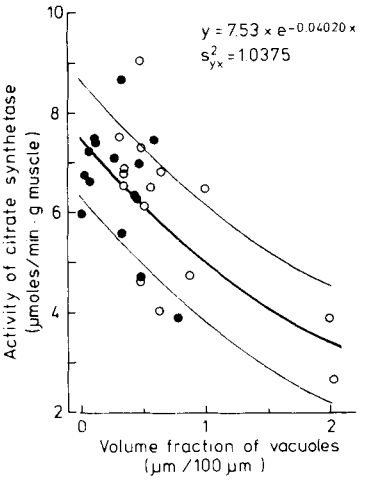

Fig. 3. Correlation between volume fraction of vacuoles (x) and the activity of citrate synthetase (y) in skeletal muscle from patients suffering viral or mycoplasma infections (open circles) and from healthy controls (filled circles). The correlation coefficient (r) is -0.709 ($p<0.0001$).

a much lower level, so that their influences were minimal.

The existence of a correlation between the activity of cytochrome *c* oxidase and the volume fraction of mitochondria is known from earlier studies (Kiessling et al., 1974).

The negative correlation found between the volume fraction of unspecified vacuoles and the activity of citrate synthetase (CS) (Fig. 3) is in agreement with the authors' previous findings of this activity to be reduced as a result of infection (Åström et al., 1976). However, it was observed that the activity of CS also decreased in the healthy subjects, as a consequence of bed rest (Åström et al., 1976) which had no corresponding effect on the volume fraction of vacuoles (Table I).

The activity of CS was also negatively correlated to the relative sarcoplasmic space (Fig. 2), suggesting that an increase of the latter might have been an effect of bed rest. The data in Table I show, for the healthy subjects, a larger sarcoplasmic space fraction in connection with bed rest than 1.5 and 4 months thereafter (the lack of statistical significance is probably due to the small number of subjects).

The reduction of the activity of CS in the patients thus seems to have a dual structural correspondence—an increase of vacuoles and an increase of sarcoplasmic space, the latter of which may be caused by the confinement to bed.

The lack of correlation of TPD to subcellular compartments might be compatible with the fact that this enzyme seemed more susceptible to the disease process showing a more pronounced decrease as a result of illness and a slower recovery than any of the other enzymes (Åström et al., 1976). The reasons for the failing correlations for LDH seem less apparent. However, the activities of LDH were found substantially variable between individuals and varied more than the other enzymes between different biopsies in the same individuals (Åström et al., 1976).

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