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CHANGES IN HISTOCHEMICAL PROFILE OF MUSCLE AFTER LONG-TERM ELECTRICAL STIMULATION IN PATIENTS WITH IDIOPATHIC SCOLIOSIS

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ABSTRACT. Adolescent patients with idiopathic scoliosis were treated with long-term electrical stimulation (30 Hz) at the posterior axillary line on the convex side of the curvature in order to correct the spinal deformity. The patients were also followed with muscle biopsies from the latissimus dorsi of the stimulated side taken before, after 3 and 6 months of electrical stimulation. There was a tendency for an increase in the percentage of type I and especially the type II C (undifferentiated) fibers after stimulation. The mean muscle fiber area and the fiber areas of the various fiber types did not change significantly. Histopathological findings were generally rare before as well as after 3 months of electrical stimulation, the only noticeable finding being a somewhat increased frequency of atrophic fibers in groups after 6 months of stimulation. In all studied patients the enzymatic activity of citrate synthase increased after 3 months and further in three studied patients after 6 months of stimulation. The present study gives some evidence of an adaptive process caused by electrical stimulation towards a more fatigue-resistant muscle.

Key words: Electrical stimulation, muscle morphology, muscle enzymes, scoliosis

The present report has a twofold background: 1) It is part of a clinical study on the long-term use of electrical muscle stimulation in patients with idiopathic scoliosis in order to prevent further development of the curvature without the use of braces. It was considered important to exclude any significant damage to the muscle structure in these otherwise healthy subjects; 2) to gain unique information on the effect of long-term electrical stimulation in human muscle and look for confirmation of previous reports from animal studies on muscle fiber transformation.

In the present report the main emphasis will be placed on the effects on the muscle structure, whereas the effect on the scoliotic deformity of the treatment with electrical stimulation in a larger material will be reported in detail elsewhere (Nordwall—in preparation).

There are a number of experimental reports (17) on the effect of electrical stimulation on skeletal muscle. By chronic low frequency stimulation a fast twitch muscle will be transformed to a slow twitch muscle with increased aerobic enzymatic activity and capillary density (5, 8, 13, 14, 15). Changes in isometric contractile properties will occur with increased time to peak twitch tension (14) and also an increased resistance to fatigue (10). The overwhelming evidence points to a transformation within the individual fiber and not to proliferation of slow-twitch (type I) fibers with concomitant atrophy and degeneration of fast-twitch (type II) fibers (17), e.g. in the experimental long-term stimulation where no signs of extensive degeneration or regenerative changes have been found and a mosaic fiber pattern was seen.

Relatively few studies have been reported on the effect of electrical stimulation on muscle structure and function in humans, and then usually only over a relatively short period of up to two to three months. Munsat et al. (12) found, after stimulation for 5–12 weeks at 33 Hz, an increase in the percentage of the type I fibers of 4–48% in the quadriceps muscle of four patients with extreme muscle atrophy due to disuse. The oxidative enzymatic activity and fiber size also increased.

We will in the present report give some evidence of changes in the fiber population and an increase of the oxidative enzymatic activity after three to six months' electrical stimulation with no major histopathological findings.

MATERIAL

The study was performed in 10 young patients (9 girls and 1 boy) with recently detected moderate, progressive idiopathic scoliosis. All curves were thoracic with the curve vertex at T7 or T8. Mean chronological age of the patients was 12 years and 9 months (range 11.0–13.2 years) and the mean skeletal age was 12 years and 5 months. None of the

girls had had menarche. The mean scoliosis curve was 31.0 degrees, and needed immediate treatment to prevent further progression.

In four patients the scoliosis progression was arrested, these patients reached skeletal maturity after a mean stimulation time of 32 months. Their average scoliosis curve at start of treatment was 32.3°, at end of treatment it was 33.8°. In four patients the scoliosis curve progressed 5° or more in spite of treatment and the stimulation was discontinued. Another two patients preferred other types of treatment after half a year's treatment with electrical stimulation.

With the consent of the patient and the parents, treatment with electrical muscle stimulation was instituted with close, regular observation, clinical and roentgenological, of the scoliosis curve development throughout the treatment.

METHODS

Electrical stimulation of the trunk muscles on the convex side of the scoliotic curvature was performed (2), using surface electrodes placed approximately 10 cm apart in the posterior axillary line and centered over the apex of the curvatures. The muscles primarily stimulated were the latissimus dorsi and the intercostal muscles on the convex side of the curvature. Disc shaped carbon-rubber electrodes with a diameter of 5 cm were utilised. An adhesive coupling medium was used between the skin and the electrodes. The electrical current was provided by a portable pulse generator (Scolitron^{Tm}) with rechargeable batteries. The apparatus supplied square wave pulses of a duration of 0.2 msec at a frequency of 30 pulses per second. The pulses were supplied in trains with 6 sec on and 6 sec off. The stimulation was used only at night time, i.e. approximately 9 hours per day. The current amplitude was chosen individually to be accepted for this night-time stimulation, usually in the order of 60 mA.

Muscle biopsies were taken under local anaesthesia with alligator forceps technique from the latissimus dorsi at the mid point between the center of the electrodes. These biopsies were performed before the treatment was started, and after 3 months of night-time stimulation and in some patients also after 6 months of stimulation. The specimens were divided into two parts. One part was frozen immediately in liquid nitrogen and used for enzyme activity and glycogen analyses. The other part of the sample was trimmed, mounted and frozen in cooled isopentan and used for histochemical analysis. Both parts were stored at −80°C until analyzed.

For histochemical analysis serial transverse sections (10 μm) were cut with a cryotom at −20°C. The myofibrillar ATP-ase method was used for muscle fiber classification into type I and II fibers. The reaction was carried out at pH 9.4 following alkaline preincubation (pH 10.3). The type II fibers were further subclassified into type IIA, type IIB and type IIC using preincubations at pH 4.6 and 4.3. The average number of fibers counted in each subject was 613±21.

For the histopathological evaluation haematoxylin-eosin and modified Gomori-trichrome staining was also used as well as periodic acid Schiff (PAS) reaction for glycogen. Group atrophy was defined as groups of three or more adjacent fibers in the field at light microscopy. Type grouping (not seen in any specimens) was defined as at least 16 fibers of a certain fiber type grouped together.

Measurements of the fiber areas were made on photos of NADH activity-stained transverse sections. An optical illumination device ("particle size analyser", Carl Zeiss, West Germany) projecting the muscle fibers as circles of varying size was used and the total fiber area was approximated. This technique has shown good agreement with planimetric area measurement as demonstrated by Aniansson et al. (1) (see also for reference of their methods) and the standard error of the single determination expressed in per cent of the mean being 1.8%.

The enzyme activity determinations were performed by means of fluorimetric techniques using a Farrand ratio-fluorimeter-2 (Farrand Optical Co., N.Y.). The reactions catalyzed by the enzymes under investigation were coupled to NAD-NADP-linked reactions and determined according to the principles given by Lowry & Passoneau (11). The enzymes analyzed were citrate synthase (CS), lactate dehydrogenase (LDH), triosephosphate dehydrogenase (TPDH), myokinase (MK). The content of protein and glycogen was determined and expressed on wet weight. The enzymatic activities were expressed per g protein.

Conventional statistical methods were used to calculate the mean and standard error of mean (SEM). The comparison between the dependent samples was carried out using Wilcoxon test for paired samples.

RESULTS

As seen in Table I muscle fiber composition did not change signficiantly with respect to the relationship between type I and type II fibers in the whole samples of eight patients, who were followed for three months. However, two of these patients (MA and RA) reported that they did not use the stimulator quite regularly and were those, who did not show an increase in the relative occurrence of type I fibers. For the remaining six patients there was a significant increase after three months in percentage type I fibers ($p<0.05$). An example of muscle biopsy before and after 3 months of electrical stimulation is shown in Fig. 1. Unfortunately, data is only available in two patients after six months' electrical stimulation. In one of these patients the relative occurrence of type I fibers was markedly higher after three (87%) as well as after six months (95%) compared to before electrical stimulation (58%). The other patient is RA mentioned above. Whether this points to a change in fiber composition with long-term electrical stimulation, or is a finding due to the sampling technique is naturally impossible to define from just one patient, although the observation is intriguing. It is also interesting to note that the type II C (undifferentiated) fibers increased in four of six patients after three months' of stimulation, where this dif-

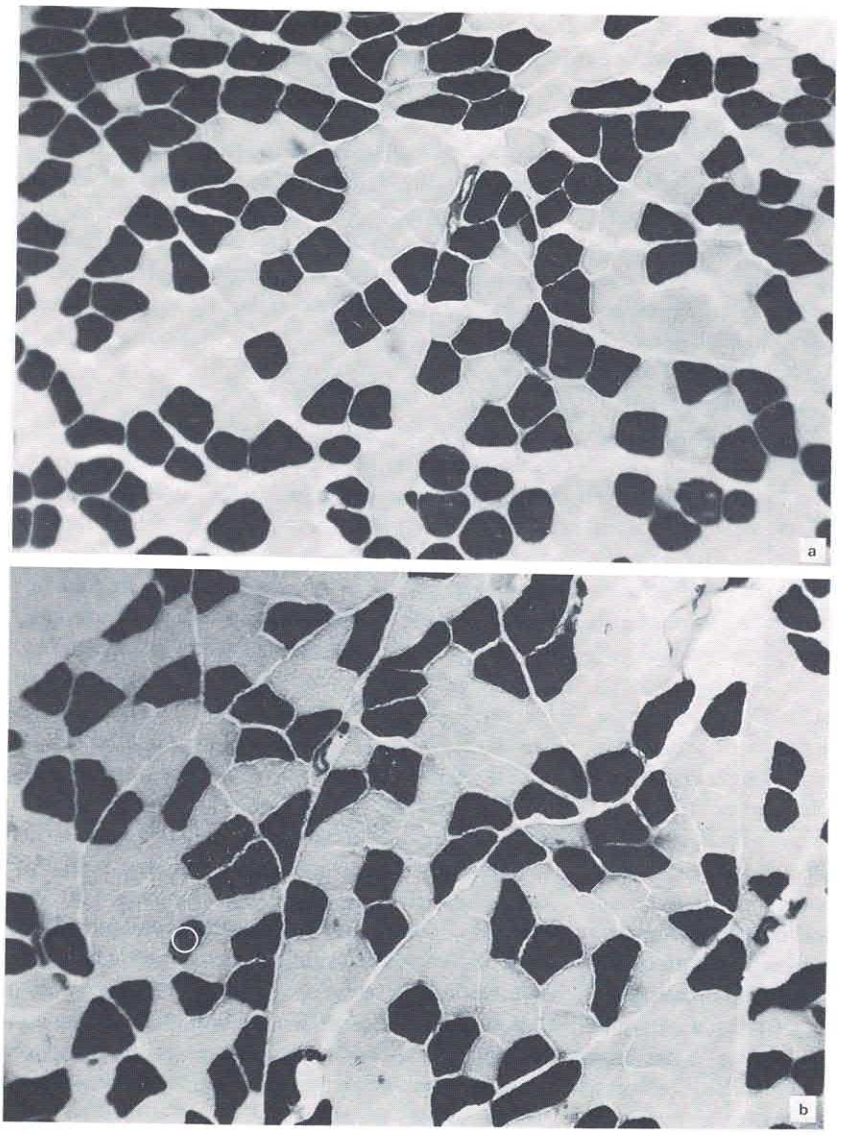

Fig. 1. Cross-sections from latissimus dorsi (convex side) of muscle biopsies taken before (*a*) and after (*b*) three months of electrical stimulation in one of the patients (E. F.). Staining for actomyosin ATPase after preincubation at pH 10.3. The relative occurrence of type I fibers (lightly stained) was 55% before and 68% after electrical stimulation. Type II fibers are dark. Note a type II C fiber with an intermediate staining in Fig. 1 *b* (marked with a circle).

ferentiation of type II fibers could be made. The high number of type II C fibers was also seen in two other patients with data only available after stimulation.

Neither the mean muscle fiber area (Table I), the areas of the various fiber types or the area ratio between type I and II fibers changed significantly with electrical stimulation.

Histopathological findings were generally rare before as well as after 3 months of electrical stimulation. Of special interest are changes described as evidence of neuropathy and myopathy. Single atrophic fibers were seen in the specimens of 200–1 000 fibers in six patients and more than 10 atrophic fibers in three patients before stimulation with no increase in occurrence after stimulation. Group atrophy was seen in four patients before stimulation, but only single groups, and only in one patient after stimulation. Type grouping was not seen in any of the specimens. Internal nuclei were only seen in few fibers in one of the patients after stimulation. Five of the patients showed evidence of fiber splitting before stimulation in the same number of specimens although not always in the same patients after stimulation. In a few of these subjects splitting was seen in more than ten fibers.

Table I. *Relative occurrence of fiber types and mean fiber area before and after electrical stimulation*

Mean values and standard error of the mean (SEM) are given for those with data before as well as after three months' stimulation (n=8)

Patient		Percent fiber type				Mean fiber area $\mu m^2 \times 10^3$
		I	II A	II B	II C	
T. A.	Before	47	26	26	0	5.72
	3 months	58	19	5	18	5.27
A. C.	6 month	51	27	4	18	4.53
A.-C. P.	3 months	67	27	5	1	6.40
J. S.	Before	35	28	36	1	3.09
	3 months	37	25	34	3	2.29
E. F.	Before	55	18	27	0	2.21
	3 months	68	9	22	0	2.98
M.-L. E.	Before	40	←	60[a]	→	1.31
	3 months	39	30	25	6	2.18
M. P.	Before	46	28	26	0	1.35
	3 months	81	2	1	16	3.29
C. S.	Before	58	20	22	0	2.51
	3 months	87	5	1	7	2.40
	6 months	95	2	1	1	2.23
M. A.	Before	77	23	1	0	1.06
	3 months	64	35	0	0	1.95
R. A.	Before	66	←	34[a]	→	2.24
	3 months	60	←	40[a]	→	2.25
	6 months	55	←	45[a]	→	2.30
Before	n	8	6	6	6	8
	Mean	53.0	23.8	23.0	0.2	2.4
	SEM	4.9	1.7	4.8	0.2	0.5
After 3 month's stimul.	n	8	6	6	6	8
	Mean	61.8	15.9	10.5	7.3	2.80
	SEM	6.3	5.2	5.8	3.2	0.40

[a] Total number of type II fibers.

Three of the five patients who had biopsies after six months' stimulation had an increased amount of atrophic fibers (both type I and type II) arranged in groups. It is notable that one of the patients (TA) with high number of type II C fibers after three months' stimulation (data not available after six months) and one of the patients with high number of type II C fibers after six months' stimulation (AC) had also increasing number of atrophic fibers after stimulation. In one biopsy after six months of stimulation there was a small area with increased amount of endomysial connective tissue. In this area the muscle fibers showed great variation in diameter and had internally placed nuclei.

Table II. *Muscle enzymatic activities, protein and glycogen concentrations before and after 3 months of electric stimulation in seven patients*

Mean values and standard error of the mean (SEM) are given in μmoles × g protein^{-1} × min^{-1} for the enzymes

	Before		After 3 months	
	Mean	SEM	Mean	SEM
Citrate synthase	29.3	±2.6	36.1	±3.9*
Lactate dehydrogenase	691	±41	700	±90
Triosephosphate dehydrogenase	1 326	±77	1 219	±94
Myokinase	670	±57	698	±67
Protein mg × g^{-1} (w.w)	190	±4.6	187	±3.6
Glykogen μmoles g^{-1} (w.w)	64.3	±9.1	66.6	±4.8

* $p<0.05$.

Electrical stimulation resulted in an increase in the enzymatic activity of citrate synthase (CS) in the seven patients where data are available before as well as after stimulation (Table II). In three patients with values also available after six months' stimulation there was a further increase in the CS activity. No general tendency in changes of enzymatic activity was noted for the other studied enzymes (MK, TPDH, LDH). Glycogen and protein concentrations did not differ before and after stimulation.

DISCUSSION

The present study had as its primary aim the analysis of any positive or negative effects on muscle during the clinical usage of electrical stimulation in patients with idiopathic scoliosis. At the same time it gave an opportunity to collect information of general biological interest on the response of skeletal muscle to long-term electrical stimulation. The results point in the direction of an adaptive process with transformation of muscle fibers and increased oxidative enzymatic activity. There is, however, not a consistent change in fiber composition, yet in more than half of the patients a relatively high number of type II C fibers were seen after stimulation, resembling findings also seen in stimulation experiments in animals (14). This fiber type is looked upon as intermediate, that is a fiber in transformation, as demonstrated by immunohistochemical data (3).

No significant pathological changes were seen after electrical stimulation in our study. The only noticeable finding was a somewhat increased frequency of atrophic fibers in groups after six months of stimulation. This was seen in two of the patients with a high frequency of the intermediate type II C fibers and may represent a neurogenic lesion. In one biopsy a small area of fibrosis was seen after six months of stimulation and it cannot be excluded that this is a result by a previous biopsy in the same area. The intermediate fibers did not show any signs of regeneration and were not atrophic. It is, thus, not likely that they represent regenerating fibers after the previous biopsies.

The results thus give evidence of the metabolic plasticity of the muscle as seen also with endurance training, for review see e.g. Salmons & Henriksson, (17) Saltin & Gollnick (18). There are a number of animal studies on chronic low frequency stimulation of fast-twitch skeletal muscles, demonstrating an ultimate transformation to a slow-twitch muscle. Changes seem to appear with the first evidence of metabolic conversion after a few days with later changes in contractile characteristics appearing after some weeks of stimulation. The fiber numbers do not seem to change, but there may be reductions in cross-sectional area (14). From animal experiments there is, however, no reason to assume that this is degenerative in nature, as the muscle shows a normal histological picture and high resistance to fatigue (17).

In one of the few studies on humans by Munsat et al. (12) 5–12 weeks intermittent stimulation of a higher frequency (33 Hz) as in the animal experiments was used. In five patients stimulated for six hours per day a variable degree of increase in the relative number of type I fibers was noted, a finding parallel to what was seen in some of the patients in the present study. In contrast there was, however, an increase in the fiber size of both main fiber types. The intensity of the muscle contractions were probably less in the present study, as it was set to be comfortable during sleep. The individual variation in the changes in fiber population in the present study may also be due to variable and submaximal stimulation. Exact data concerning the duration and intensity of the stimulation was not possible to collect in a clinical study as the present one.

As demonstrated in several studies (e.g. 6, 19), there is a predominance of type I fibers in multifidus muscle on the convex side of the curvature in patients with idiopathic scoliosis. Whether this also could be true in the latissimus dorsi is uncertain and, furthermore, in the present study changes in histochemical profile in the same individual were analyzed.

Thus, the present study gives some evidence of an adaptive process brought about by electrical stimulation towards a more fatigue-resistant muscle. In that respect electrical stimulation in the scoliotic patients may induce changes which increase the ability for postural stabilizing muscle activity in the spine. Whether this is a contributing factor to the clinical effect of electrical stimulation in these patients reducing further development of the scoliotic curvature is naturally impossible to judge. In 60% of the patients in this study the muscle stimulation was successful in arresting the further progression of the scoliosis curve, which can be compared to 72% of the patients in a large multicenter trial (4), who had either reduced or stabilized their scoliosis (mean treatment time 12 months). At the time being, our

results give evidence of an appropriate muscle adaptation at electrical stimulation with no direct adverse effects.

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