

## METABOLIC RESPONSE AND MUSCLE GLYCOGEN DEPLETION PATTERN DURING PROLONGED ELECTRICALLY INDUCED DYNAMIC EXERCISE IN MAN

C. K. Kim, PhD,<sup>2\*</sup> J. Bangsbo, Dr Sci,<sup>2</sup> S. Strange, MD,<sup>2</sup> J. Karpakka, MD<sup>1\*\*</sup> and B. Saltin, MD<sup>1</sup>

From the <sup>1</sup>Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden, and <sup>2</sup>August Krogh Institute, University of Copenhagen, Copenhagen, Denmark

**ABSTRACT.** Muscle glycogen depletion pattern and metabolic responses during voluntary (VOL) and functional electrical stimulated (FES) dynamic knee-extensor exercise with one leg were evaluated. Seven healthy men exercised for 60 minutes at 30 W with an pulmonary oxygen uptake of 0.8 and 1.0 l min<sup>-1</sup>, and respiratory exchange ratios of 0.90 and 0.95 in VOL and FES, respectively. Heart rate reached a level around 90 beats min<sup>-1</sup> (VOL) and up to 110 beats min<sup>-1</sup> (FES). Muscle glycogen decreased in FES with 260 and 290 mmol kg<sup>-1</sup> d.w. in vastus lateralis and m. rectus femoris, respectively, compared with 45 and 160 mmol kg<sup>-1</sup> d.w. in VOL ( $p < 0.05$ ). In FES the percentage of empty and almost empty fibres determined by periodic acid-Schiff staining in vastus lateralis and rectus femoris was 50 and 77% of type I, 63 and 90% of type IIa, and 59 and 84% of type IIb fibres, respectively, whereas in VOL it was 24 and 26% of type I, 7 and 19% of type IIa, and 2 and 3% of type IIb fibres. Muscle lactate reached 30 mmol kg<sup>-1</sup> d.w. in FES and was 9 mmol kg<sup>-1</sup> d.w. lower in VOL. The changes in blood lactate and NH<sub>3</sub> during the exercise were slightly higher in FES than in VOL, whereas the alterations in glucose, FFA, and K<sup>+</sup> were small in both exercise modes. The pressure in the two muscle portions at different locations (proximal-distal) and depths was always higher (~50%) in FES than in VOL, reaching levels around 55 mmHg. It is concluded that with FES the entire quadriceps muscle was engaged and all fibre types were recruited, resulting in a quite significant energy turnover and a more pronounced metabolic response compared to VOL. Furthermore, FES can be used for prolonged dynamic exercise.

*Key words:* dynamic exercise, electromyostimulation, oxygen uptake, glycogen depletion, intramuscular pressure, muscle metabolism.

## INTRODUCTION

Electrically induced exercise may be advantageous in rehabilitation and to minimize muscle wasting in prolonged immobilization of a limb as well as in weightlessness (6, 17). There are, however, several unanswered questions with regard to the metabolic response to functional electrical stimulation (FES) as compared with voluntary dynamic muscle contraction (VOL) in man. During short lasting exercise the anaerobic metabolic response was more pronounced in FES than VOL at submaximal exercise levels, and the heart rate was also more elevated with FES (8). This could be due to a larger contribution of type II fibres in FES, as large size motor axons are more easily excited than the small size axons which innervate type I fibres. An additional explanation could be that with electrical stimulation only a small fraction of the whole muscle may be activated, thus, the recruited motor units may be intensely contracting and then demand a metabolic turnover close to their upper limit. More knowledge of the recruitment pattern and the metabolic response to FES would be of value to optimize the use of FES.

Thus, the aim of the present study was to elucidate whether there is a selective recruitment of fibre types when muscle contraction is performed by direct electrical stimulation, and to evaluate to what extent various portions of the muscle are engaged in the contractions.

Present addresses:

\* Human Physiology, Korean National University of Physical Education, Seoul, Korea.

\*\* Department of Sport Medicine, Deacones Institute, Oulu, Finland.



## MATERIALS AND METHODS

### Subjects

Seven healthy volunteers participated in the study. They had a mean weight of 74 (71–79) kg and height 180 (176–183) cm, and they were 28 (24–34) years of age. All were physically active and occasionally participated in some sports activity. The subjects were fully informed of any risks and discomfort associated with the experiment before they volunteered to participate in the study, which was approved by the Ethics Committees of Copenhagen University and the Karolinska Institute.

### Protocol

Subjects performed one-legged knee extension exercise in a sitting position on a specially designed Krogh ergometer which permitted exercise to be confined to the thigh muscles (1). On separate days, the subjects exercised with FES and VOL for 60 min at a work rate of 30 W. Prior to the experiment, a catheter was inserted into an arm vein for blood sampling. Heart rate was measured, expired air and blood sample were collected at rest and after 10, 20, 40, and 60 min of exercise. In some of the experiment catheters were placed to record pressure in superficial and deep portions of both m. vastus lateralis and m. rectus femoris. Muscle biopsies were obtained before and immediately after the exercise from vastus lateralis and rectus femoris (4). The biopsies were taken at a depth of 3–4 cm within 2 cm from the tip of a deeply placed catheter for the pressure recording.

### Electrical stimulation

The apparatus consisted of an arbitrary waveform generator (Kron-Hite 5920) and battery powered amplifier (Biomedical Engineering, NASA). Two large electrodes (76 × 114 mm) were applied proximally and distally to the thigh. The muscle was stimulated to contract with a bi-phasic pulse mode which reduced irritation between the electrodes and skin interface because no net charge was delivered to the subject. With each contraction the characteristics of used pulse train was as follows;

pulse shape:	bi-phasic (sine wave)
pulse width:	500 $\mu$ sec
pulse frequency:	50 Hz
pulse amplitude:	variable
train length:	320 msec
train frequency:	1 Hz
ramp duration:	50 msec

A pulse width of 500  $\mu$ sec was used and train length was variable but less than 320 msec. Train amplitude was adjusted manually to give a kicking frequency of 1 Hz. The computer controlled stimulating device was connected to a microswitch on the Krogh cycle ergometer which was activated when the pedal arm passed the horizontal position and the leg was in the right position for a new contraction. Activation of the microswitch triggered the electrical stimulation of the muscle. Variation in kicking frequency was minimized by a feedback loop from ergometer to computer. Thus, the train length was increased or decreased automatically if the kicking frequency decreased or increased. In order for ensuring smooth muscle contraction the pulse frequency was increased from 0 to 50 Hz during a 50 msec linear ramp at the beginning of each contraction.

### Oxygen uptake, ventilation, and heart rate

Pulmonary oxygen uptake and ventilation were determined by collecting expelled air in Douglas bags. The volume of air was measured in a Tissot spirometer and the O<sub>2</sub> and CO<sub>2</sub> concentrations were determined with Servomex and Beckman LB-2 analyzers, respectively. Electrodiagram was monitored continuously, and heart rate (HR) was determined.

### Blood samples

The concentration of blood lactate and glucose was immediately assayed using YSI (Yellow Springs Instruments Co. Yellow Springs, OH). Plasma concentration of potassium (K<sup>+</sup>) was determined with flame photometer (Radiometer FLM3) with lithium as internal standard, and plasma ammonia (NH<sub>3</sub>) was measured by the method of Lowry & Passonneau (9). Plasma free fatty acid concentration (FFA) was measured fluorometrically (13).

### Intramuscular pressure

For intramuscular pressure measurement, 2–3 Atos myopress catheters were placed at various sites at a depth of either 1–2 cm or 3–5 cm underneath the fascia of vastus lateralis and rectus femoris (cf. 14). During exercise the pressure was continuously recorded from the catheters with only brief interruption for infusions of small amount of saline to maintain the tip of the catheters open.

### Muscle fibre type composition and glycogen depletion pattern

One portion of the muscle biopsy was mounted with embedding medium (O.T.C. compound) for histochemistry. Serial transverse sections (10  $\mu$ m) were cut with a microtome at –20°C and histochemically stained for myofibrillar ATPase activity, after pre-incubation at different pH intensities for fibre type classification into type I, type II and its subunits (5). To visualize glycogen depletion pattern sections were stained with the periodic acid-Schiff (PAS) staining method (10). The glycogen content of single fibres was divided into four classes with respect to the intensity of PAS staining: filled, slightly filled, almost empty, and empty.

### Muscle glycogen and lactate

Another portion of the muscle sample was immediately frozen in liquid nitrogen and stored at –80°C. It was, then, freeze-dried, dissected free from blood, vessels and all visible fat and connective tissues. A part of the freeze-dried sample (1–2 mg) was hydrolyzed at 100°C for 2 hours and the muscle glycogen content was determined by the hexokinase method (9). Another part of the sample (1–2 mg) was extracted in 3 M HClO<sub>4</sub> and neutralized with 2 M KHCO<sub>3</sub> for muscle lactate analysis, and immediately assayed in duplicate using a fluorometer (9).

### Statistics

Values represent means  $\pm$  standard error of mean. The Friedman test was used to evaluate statistical significances. Level of significance was set at  $p < 0.05$ .

## RESULTS

Pulmonary oxygen uptake was  $0.811\text{min}^{-1}$  after 10 min of exercise in both VOL and FES, thereafter oxygen uptake gradually increased in FES reaching  $1.011\text{min}^{-1}$  at the end of exercise (Fig. 1). The ventilation was  $26\text{--}29\text{min}^{-1}$  in FES and  $21\text{--}23\text{min}^{-1}$  in VOL ( $p < 0.05$ ). The respiratory exchange ratios were

higher in FES than VOL, especially early in the exercise with an overall mean of  $0.95$  ( $0.92\text{--}1.00$ ) and  $0.90$  ( $0.86\text{--}0.91$ ) in FES and VOL, respectively ( $p < 0.05$ ). After 10 min the heart rate was  $92\text{bpm}$  during FES which was  $8\text{bpm}$  higher than during VOL ( $p < 0.05$ ; Fig. 1). A further difference developed during the exercise and the heart rate was  $110\text{bpm}$  (FES) and  $88\text{bpm}$  (VOL) at  $60\text{min}$  ( $p < 0.05$ ).

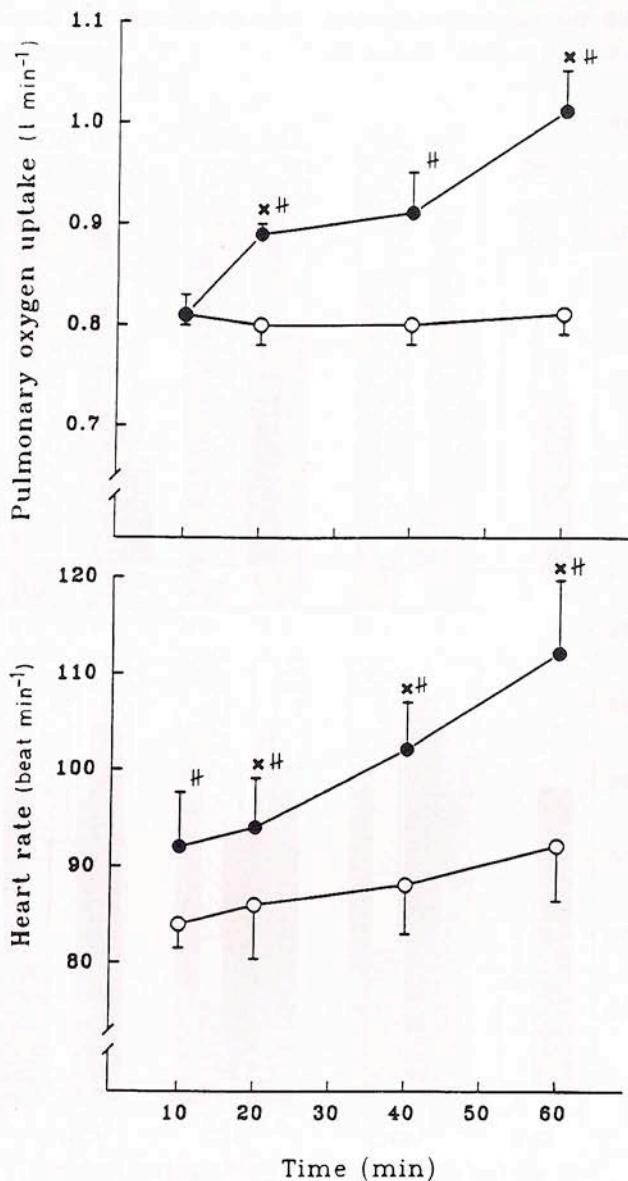


Fig. 1. Pulmonary oxygen uptake (upper panel) and heart rate response (lower panel) during 60 min dynamic exercise at 30 W with FES (filled circle) and VOL (open circle). \*: Significant difference ( $p < 0.05$ ) between two consecutive means. #: Significant difference ( $p < 0.05$ ) between VOL and FES. Values are mean  $\pm$  S.E.



In FES muscle glycogen was reduced with  $260 \text{ mmol kg}^{-1} \text{ d.w.}$  in vastus lateralis as compared with  $45 \text{ mmol kg}^{-1} \text{ dry weight (d.w.)}$  in VOL ( $p < 0.05$ ; Fig. 2). The difference between FES and VOL was smaller in rectus femoris but almost  $290 \text{ mmol kg}^{-1} \text{ d.w.}$  was utilized in FES compared with  $160 \text{ mmol kg}^{-1} \text{ d.w.}$  in VOL ( $p < 0.05$ ). The lowest muscle glycogen concentration of  $40 \text{ mmol kg}^{-1} \text{ d.w.}$  was found in rectus femoris after FES. The glycogen depletion pattern determined by PAS staining was in line with the quantitative muscle

glycogen data (Fig. 3). All muscle fibre types revealed some degree of glycogen depletion in both muscles studied and with both modes of inducing the contraction, but the decreases were more pronounced with FES. The depletion of both fibre types with FES was slightly greater in rectus femoris than in vastus lateralis with 80–90% and 50–60% being empty/almost empty of glycogen, respectively, after 60 min of exercise in rectus femoris.

Before the exercise started the lactate concentration in vastus lateralis and rectus femoris was higher in

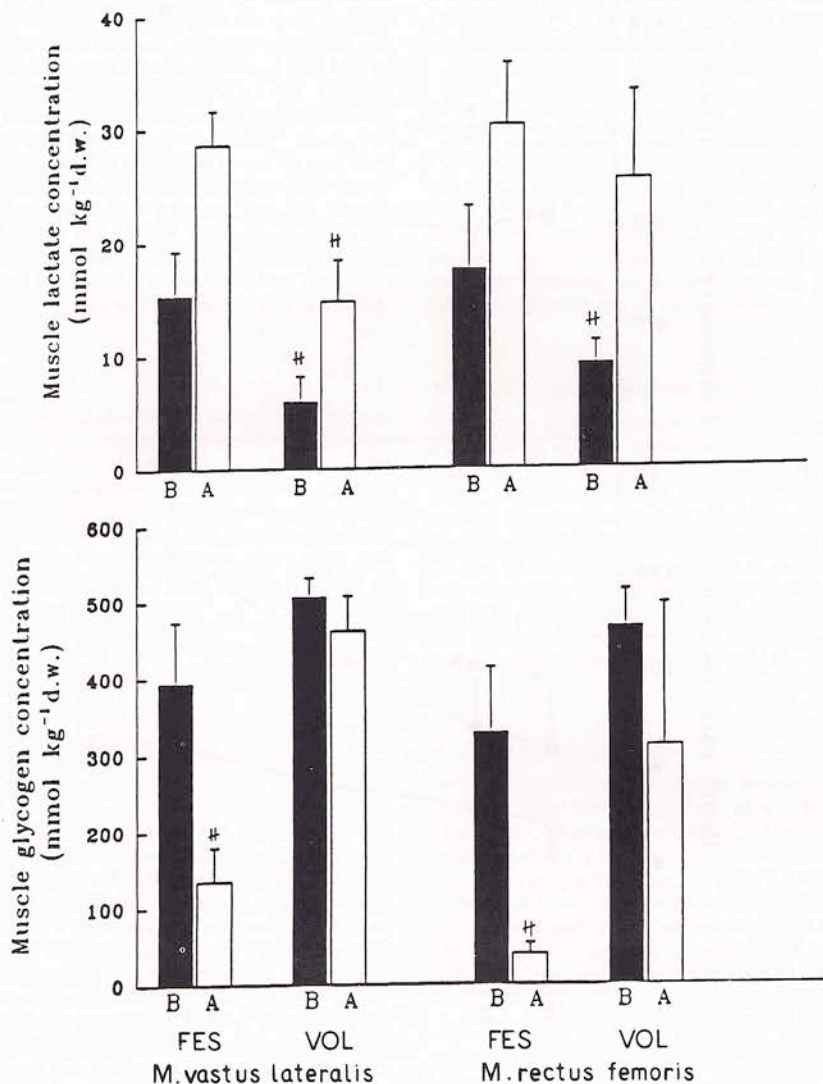


Fig. 2. Muscle lactate (upper panel) and glycogen (lower panel) concentrations before (B) and after (A) 60 min of dynamic exercise at 30 W with FES and VOL in m. vastus lateralis and m. rectus femoris. #: Significant difference ( $p < 0.05$ ) between VOL and FES. Values are mean  $\pm$  S.E.

FES than in VOL ( $p < 0.05$ ; Fig. 2). This was probably due to the fact that some contractions had been made before the pre-exercise muscle biopsies were obtained in order to confirm proper activation of the quadriceps muscle. The lactate concentrations in vastus lateralis and rectus femoris at the end of the exercise was about  $30 \text{ mmol kg}^{-1}$  d.w. with FES, which was  $9 \text{ mmol kg}^{-1}$  d.w. higher than the corresponding values in VOL ( $p < 0.05$ ). The elevation in lactate concentration in vastus lateralis and rectus femoris from rest to exercise was not significantly different in either mode of exercise.

The blood concentration of lactate was unchanged throughout exercise in VOL, whereas it increased to  $2.8 \text{ mmol l}^{-1}$  after 10 min in FES (Fig. 4). It remained at this level until 20 min of exercise, thereafter it gradually declined to  $1.9 \text{ mmol l}^{-1}$  at 60 min of exercise in FES.  $\text{NH}_3$  concentration in VOL was unaltered during the exercise, while a small elevation of  $12\text{--}20 \mu\text{mol l}^{-1}$  was observed in FES (Fig. 4). FFA and glucose concentrations were unchanged during the exercise in both FES and VOL (Fig. 5).  $\text{K}^+$  concentration increased from  $4.1\text{--}4.2 \text{ mmol l}^{-1}$  at rest to  $4.6\text{--}4.7 \text{ mmol l}^{-1}$  both in FES and VOL (Fig. 5).

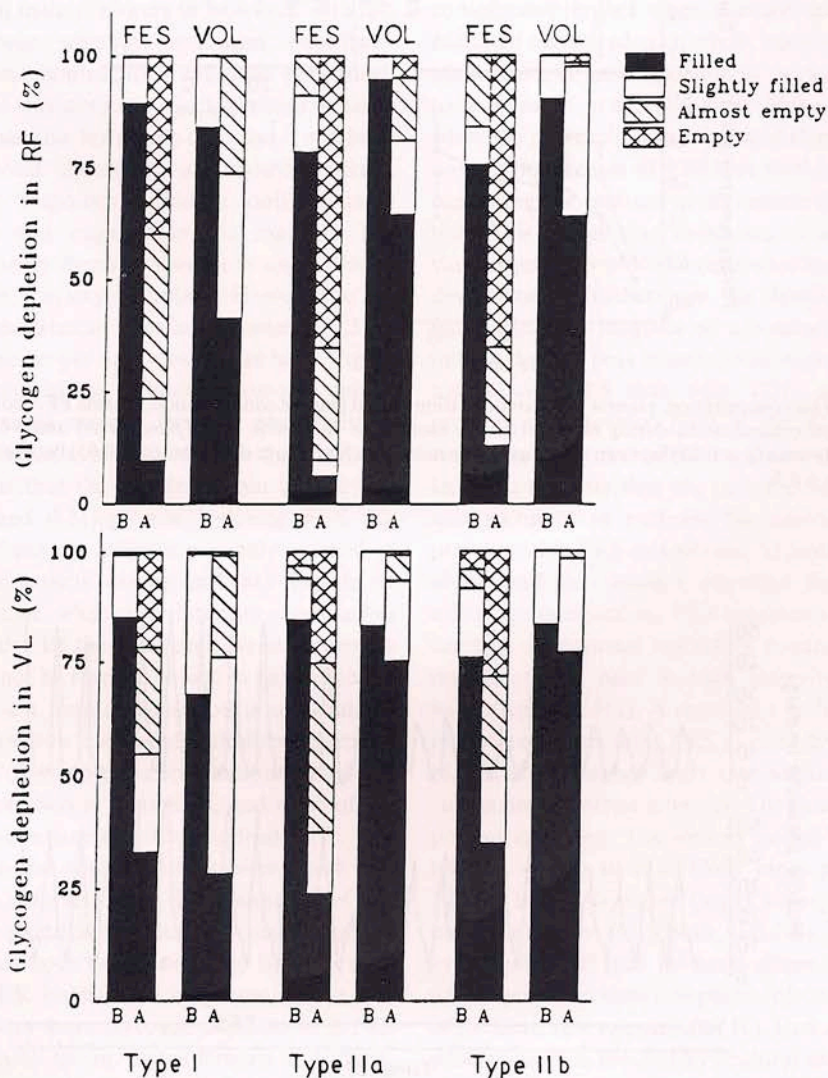


Fig. 3. Muscle glycogen depletion pattern in the various fibre types in m. rectus femoris (upper panel) and m. vastus lateralis (lower panel) before (B) and after (A) 60 min of dynamic exercise at 30 W with FES and VOL. The relative occurrence of type I, IIa and IIb fibres in vastus lateralis was 62%, 31%, and 7%, and in rectus femoris 50%, 39%, and 11%, respectively.



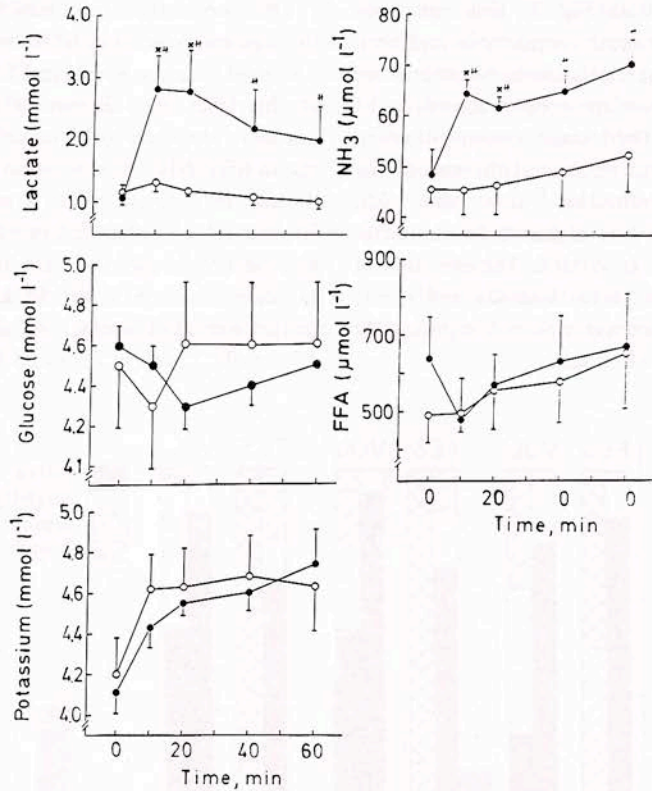


Fig. 4. Blood lactate concentration, plasma NH<sub>3</sub> concentration, blood glucose concentration, plasma FFA concentration, and plasma potassium concentration during 60 min dynamic exercise at 30 W with FES (filled circle) and VOL (open circle). \*: Significant difference ( $p < 0.05$ ) between two consecutive means. #: Significant difference ( $p < 0.05$ ) between VOL and FES. Values are mean  $\pm$  S.E.

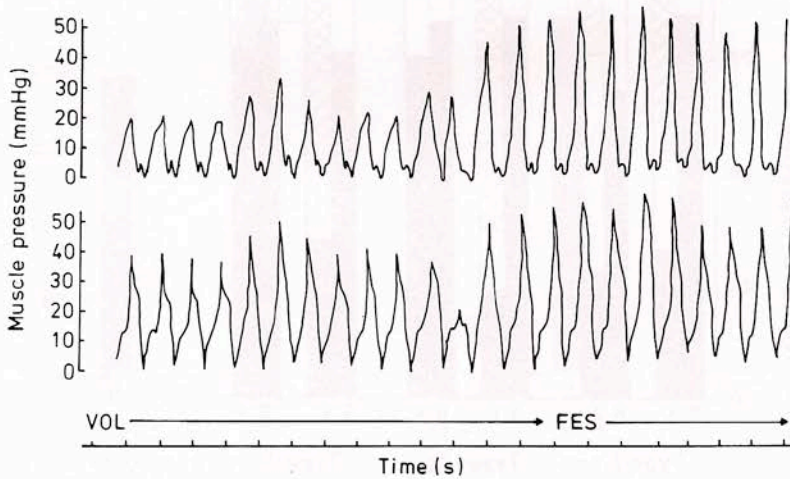


Fig. 5. Muscle pressure in m. rectus femoris (upper panel) and m. vastus lateralis (lower panel) during voluntarily (VOL) and electrically induced contractions (FES). The tips of the catheter were at similar depths in the two muscle portions and were placed approximately in the middle of the muscle.

At rest, the pressure in the muscle was between 4–8 mmHg in the different locations studied. During exercise the peak pressure in the muscle was elevated to 23–53 mmHg, with the highest value in the rectus femoris with FES. Generally, the peak pressure was 50% (or more) higher during FES than VOL in both portions of the muscle throughout the exercise, but as the contraction induced by FES was more synchronous, the contraction and the elevation in muscle pressure were of shorter duration (Fig. 5).

## DISCUSSION

There were two main problems to be solved with this study. One was whether prolonged electrically induced dynamic contractions could be performed. A clear answer was obtained. Such exercise is easily induced and tolerable for a long time and it results in pronounced local metabolic and some systemic cardiovascular responses although only a small muscle group was engaged in the exercise. The whole body energy demand was quite small, barely reaching  $1 \text{ l min}^{-1}$  in oxygen uptake. However, as the mass of the knee extensor muscle amounted to 2.5 kg, the energy turnover per unit muscle was high. Eighty to ninety percent of the pulmonary oxygen uptake during submaximal exercise intensity above resting level can be assumed to be utilized in the thigh muscles (2). This means that the muscle oxygen uptake was probably around  $0.21 \text{ kg}^{-1} \text{ min}^{-1}$  during FES (8). Such a rate of oxygen utilization is only reached in the quadriceps muscle during ordinary bicycle or treadmill exercise when the intensity approaches maximum. With FES the same peak exercise intensity as in VOL cannot be reached, which in part is related to the fact that a burning sensation is unavoidable when the contraction is induced with direct electrical stimulation. With the present stimulation procedures the burning sensation is minimized, and none of the subjects became exhausted after 1 hour at 30 W.

This leads to the other major problem which was studied. How complete was the activation of the muscle when electrically induced? One important finding was that both type I and type II fibres were recruited in FES. Indeed, not only type II, but also type I fibres were more glycogen depleted with FES than VOL in both the m. rectus femoris and the m. vastus lateralis muscles. An additional indication of the effectiveness of the activation with FES was the finding of respiratory exchange ratios of around 0.95

and a rate of glycogen utilization of  $4\text{--}5 \text{ mmol kg}^{-1} \text{ min}^{-1}$  d.w. in both rectus femoris and vastus lateralis. Such a rate of glycogen utilization corresponds to a relative work rate of more than 75% of maximal oxygen uptake during cycle or treadmill exercise (7, 11). The anaerobic glycogenolysis may have amounted to less than 1/5 of the total carbohydrate turnover, indicating a very high rate of aerobic utilization of glycogen in FES. At such an exercise intensity the accumulation of lactate in the muscle during whole body exercise is more pronounced, than observed in the present study. One possible explanation for the difference is that the muscle perfusion is considerably higher when a small muscle group is exercised compared with whole body exercise, which allows for a larger proportion of the lactate produced to be released to the blood (3). This is also the case when the contractions are induced electrically (8).

The effectiveness of FES was further evaluated by measuring the pressure in the muscle during contraction. This can give an indication of whether or not various portions of the muscle contribute to the force development. Furthermore, the elevation in pressure may reflect the intensity of a contraction (15). The finding that the peak muscle pressure was significantly higher with FES than with VOL, and that peak pressure was elevated both in the deep and in the superficial regions for both rectus femoris and vastus lateralis, suggests that the entire quadriceps muscle was recruited to perform the exercise. The peak pressure with FES was around 53 mmHg which was about half the pressure recorded during maximal voluntary contraction. This represents a higher percentage of maximal voluntary contraction than is thought to be used in high intensity submaximal bicycle exercise (12). A consistent finding in this and two other studies using FES to elicit dynamic exercise (8, 16) is the higher heart rate response at a given submaximal exercise intensity. The cause for this is at present unknown. The sensory output from the contracting muscle is most likely more pronounced in FES as lactate is higher (= pH lower) and so is the muscle pressure (18). Both variables are known to excite group III and IV nerve fibres in the muscle, which have been shown to play a role in the regulation of the heart rate response (cf. 16). However, when this sensory input is blocked by epidural anaesthesia, and the muscles are made to contract by FES, heart rate is nevertheless elevated to the anticipated level (16). The blood pressure is lowered in this situation. Thus, in the



experiments with epidural anaesthesia, the arterial baroreceptors may drive the heart rate response, but that cannot be the explanation in the present experiments as the blood pressure during FES was normal or slightly exaggerated (8). Left is the venous return which via the Bainbridge reflex can cause parasympathetic withdrawal. That this should explain the elevation in heart rate response and the difference between FES and VOL is unlikely. It is more likely that the sensory input from the muscle indeed has a functional role.

In conclusion, the present study has demonstrated that electrically induced contraction (FES) can produce forceful dynamic contractions, which can be sustained over a considerable time. The rate of glycogen and anaerobic energy production is high during electrically elicited contractions and both type I and II muscle fibres are engaged in the exercise. The heart rate response is substantial, which could be a function of the forceful contractions eliciting a more powerful muscle reflex, which has been suggested to be a modulator of the cardiovascular response to exercise.

#### ACKNOWLEDGEMENTS

This work was supported by NASA Grant #199-26-11-6A-SD2511-SD511 and Swedish Medical Research Council Grant B91-14X-09480-01A.

#### REFERENCES

- Andersen, P., Adams, R. P., Sjøgaard, G., Thorboe, A. & Saltin, B.: Dynamic knee extension as a model for the study of an isolated exercising muscle man. *J Appl Physiol* 59: 1647-1653, 1985.
- Andersen, P. & Saltin, B.: Maximal perfusion of skeletal muscle in man. *J Physiol* 366: 233-249, 1985.
- Bangsbo, J., Gollnick, P. D., Graham, T. E., Juel, C., Kiens, B., Mizuno, M. & Saltin, B.: Anaerobic energy production and O<sub>2</sub> deficit-debt relationship during exhaustive exercise in humans. *J Appl Physiol* 422: 539-559, 1990.
- Bergström, J.: Muscle electrolytes in man. *Scand J Clin Lab Invest Suppl* 68: 1-110, 1962.
- Brooke, M. H. & Kaijser, K. K.: Three "myosin adenosine triphosphatase" systems: The nature of their pH lability and sulphhydryl dependence. *J Histochem Cytochem* 18: 670-672, 1970.
- Eriksson, E. & Häggmark, T.: Comparison of isometric muscle training and electrical stimulation supplementing isometric muscle training in the recovery after major knee ligament surgery. *Am J Sports Med* 7: 169-171, 1979.
- Hultman, E. & Spriet, L. L.: Dietary intake prior to and during exercise. In: *Exercise, Nutrition and Energy Metabolism* (eds.: Horton, E. S. & Terjung, R. L.), pp 132-149, MacMillan, N.Y., 1988.
- Kim, C. K., Strange, S., Bangsbo, J. & Saltin, B.: Skeletal muscle perfusion in electrically induced dynamic exercise in man. *Acta Physiol Scand*, In Press, 1994.
- Lowry, O. H. & Passonneau, J. V.: *A flexible system on enzymatic analysis*. Academic Press, New York, 1972.
- Pearse, A. G. E.: *Histochemistry—Theoretical and Applied*, Appendix 9, p. 832, Little Brown, Boston, Mass., 1961.
- Saltin, B. & Karlsson, J.: Muscle glycogen utilization during work of different intensities. In *Muscle Metabolism During Exercise*. Plenum Press, N.Y. 11: 395-399, 1971.
- Saltin, B.: Muscle fibre recruitment and metabolism in prolonged exhaustive dynamic exercise. Human muscle fatigue: Physiological mechanisms. The 1982 Ciba Foundation Symposium, Pitman Medical, London: 41-58, 1981.
- Shimizu, S., Inoue, K., Tani, Y. & Yamada, H.: Enzymatic microdetermination of serum free fatty acids. *Anal Biochem* 98: 341-345, 1979.
- Sjøgaard, G., Kiens, B., Jørgensen, K. & Saltin, B.: Intramuscular pressure, EMG and blood flow during low-level prolonged static contraction in man. *Acta Physiol Scand* 128: 475-484, 1986.
- Sjøgaard, G.: Exercise induced muscle fatigue: the significance of potassium. *Acta Physiol Scand* 140, Suppl. 593: 1-63, 1990.
- Strange, S., Secher, N. H., Pawelczyk, J. A., Karpakka, J., Christensen, N. J., Mitchell, J. H. & Saltin, B.: Neural control of cardiovascular responses and of ventilation during dynamic exercise in man. *J Physiol* 470: 693-704, 1993.
- Wigerstad-Lossing, I., Grimby, G., Jonsson, T., Morelli, B., Peterson, L. & Rebström, P.: Effects of electrical muscle stimulation combined with voluntary contractions after knee ligament surgery. *Med Sci Sports Exer* 20: 93-98, 1988.
- Williamson, J. W., Mitchell, J. H., Olesen, H. L., Raven, P. B. & Secher, N. H.: Reflex increase in blood pressure induced by leg compression in man. *J Physiol* 475: 351-357, 1994.

Address for offprints:

Jens Bangsbo  
August Krogh Institute  
University of Copenhagen  
13 Universitetsparken  
DK-2100 Copenhagen  
Denmark