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# $^{99m}$Tc-sestamibi muscle scintigraphy to assess the response to neuromuscular electrical stimulation of normal quadriceps femoris muscle

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***Objectives:*** Neuromuscular electrical stimulation (NMES) is widely used for improving muscle strength by simultaneous contraction in the prevention of muscle atrophy. Although there exist many clinical methods for evaluating the therapeutic response of muscles, $^{99m}$Tc-sestamibi which is a skeletal muscle perfusion and metabolism agent has not previously been used for this purpose. The aim of our work was to ascertain whether $^{99m}$Tc-sestamibi muscle scintigraphy is useful in the monitoring of therapeutic response to NMES in healthy women.
***Methods:*** The study included 16 women aged between 21 and 45, with a mean age of 32.7 ± 6.4. Both quadriceps femoris muscles (QFM) of each patient were studied. After randomization to remove the effect of the dominant side, one QFM of each patient was subjected to the NMES procedure for a period of 20 days. NMES was performed with an alternating biphasic rectangular current, from a computed electrical stimulator daily for 23 minutes. After measurement of skinfold thickness over the thigh, pre- and post-NMES girth measurements were assessed in centimeters. Sixty minutes after injections of 555 MBq $^{99m}$Tc-sestamibi, static images of the thigh were obtained for 5 minutes. The thigh-to-knee uptake ratio was calculated by semiquantitative analysis and normalized to body surface area (NUR = normalized uptake ratio).
***Results:*** The difference between the pre and post NMES NUR values was significant (1.76 ± 0.31 versus 2.25 ± 0.38, p = 0.0000). The percentage (%) increase in NUR values also well correlated with the % increase in thigh girth measurements (r = 0.89, p = 0.0000).
***Conclusion:*** These results indicated that $^{99m}$Tc-sestamibi muscle scintigraphy as a new tool may be useful in evaluating therapeutic response to NMES.

**Key words:** neuromuscular electrical stimulation, $^{99m}$Tc-sestamibi scintigraphy, muscle

## INTRODUCTION

NEUROMUSCULAR ELECTRIC STIMULATION (NMES) has been widely used for many years in the rehabilitation of muscles.[1] This training technique can increase the functional activity of muscles by improving their physiological, morphological, biochemical and motor performance properties.[2] Chronic electrical stimulation has been reported to improve muscle strength and endurance,[3–5] prevent denervation atrophy,[6] cause muscle hypertrophy,[2] and increase capillary density in animal muscles,[7] increase muscle cell mitochondrial fraction,[8,9] succinic dehydrogenase[10] and protein synthesis.[11] The strengthening effects of NMES can be evaluated by many different evaluation methods. Isokinetic dynamometry (Cybex) can be used for the evaluation of the strength of muscles, EMG for the evaluation of motor unit potentials and basal metabolism measurements for the evaluation of the strength exerted. Manual muscle strength testing and some clinical scoring scales can also be used. But none of these techniques can assess the muscle from a perfusion and/or metabolism point of view.

$^{99m}$Tc-sestamibi is a lipophilic cation primarily used as a myocardial perfusion imaging agent in the assessment

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of coronary artery disease and it has also been employed in the evaluation of skeletal muscle perfusion and metabolism in patients with peripheral artery and other muscle diseases.[12–21]

The aim of this study was to ascertain whether $^{99m}$Tc-sestamibi muscle scintigraphy is useful in the monitoring of therapeutic response of muscles to NMES therapy in healthy women.

## MATERIALS AND METHODS

*Patients*

This study was approved by the local ethical committee and written informed consent was obtained from all volunteers. Sixteen healthy women aged 21–45 years (32.7 ± 6.4) were included in the study. Patients with systemic diseases, such as generalized muscle disease, diabetes mellitus, lupus erythematosus, polyarteritis nodosa, systemic sclerosis, amyloidosis, carcinomatosis, renal failure and cardiovascular disease were excluded from the study. Also patients with painful knee joints and those performing regular exercises were not included in the study. We selected only female patients to study for two reasons: to eliminate the effect of sex differences and because of the lack of individual fibril type variations in female patients.[22,23]

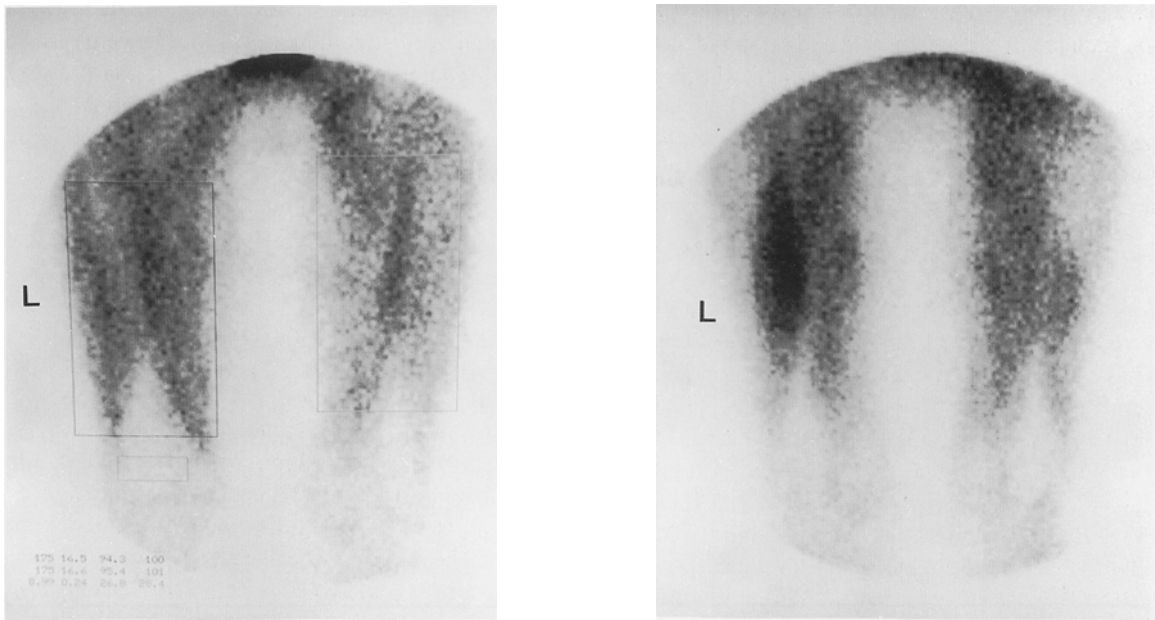
*NMES application*

After randomization to remove the effect of the dominant side, the selected QFM of the patients (right or left) were subjected to the NMES procedure with a COMPEX PM device, a computed electrostimulator. The time and the modulation of stimulation procedures were directed by stimulation programs preserved in the hard disc of the device. Electrical stimulation was applied through three channels by a monopolar, large negative electrode and four small, positive electrodes on the motor stimulation points of the quadriceps muscles. These electrodes were applied with gels to increase the impulse transmission. They were fixed with velcro tapes to the thigh. NMES was applied to the patient in the sitting position near the examination table and the thigh of the patient was fixed manually to the table by the physician in order to prevent knee movement during strong contractions. Motor unit stimulation and strong, tetanic isometric muscle contractions were formed by bipolar and symmetrical square waves. Programs with frequencies of 75 and 85 hertz were selected to obtain maximal contractions in fast muscle fibrils. The intensity was increased by verbal motivation of the patient, after every three or four contractions according to the compliance and tolerance of the patient. Impulses of 20–25 amperes were used for the beginning and these values were increased to 50–55 amperes until the end of the training set. The programs specific for strengthening of large muscle groups were selected in the first training week. Programs with higher impulse frequencies and intensities were applied in the second and third weeks. Each training session consisted of three parts. The first part was for warming and lasted for four minutes. The second part was for training and lasted for 15 minutes. The third part was a four minute period for relaxation.

**Table 1** Values NURs, girth, skinfold for pre- and post-NMS patients and % uptake, % girth increases

| Patient no. | Age/ site | PrNT NURs | NNCT NURs | PoNT NURs | NNCT NURs | UI (%) | PrNT Girth (cm) | PoNT Girth (cm) | GI (%) | PrNTS (mm) | PoNTS (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 32/R | 2.31 | 2.12 | 2.89 | 2.09 | 25 | 34.7 | 35.1 | 1.2 | 20 | 20 |
| 2 | 40/R | 2.17 | 1.96 | 2.87 | 2.04 | 32 | 33.5 | 34.0 | 1.5 | 19 | 18 |
| 3 | 21/R | 1.30 | 1.21 | 1.91 | 1.11 | 46 | 49.3 | 50.5 | 2.5 | 50 | 52 |
| 4 | 32/R | 1.47 | 1.62 | 1.96 | 1.58 | 33 | 37.1 | 37.8 | 1.9 | 38 | 38 |
| 5 | 29/R | 2.09 | 1.52 | 2.47 | 1.57 | 18 | 37.8 | 38.1 | 1.0 | 55 | 57 |
| 6 | 45/R | 1.39 | 1.45 | 1.78 | 1.42 | 28 | 42.0 | 42.7 | 1.7 | 38 | 37 |
| 7 | 29/R | 2.00 | 1.60 | 2.40 | 1.66 | 20 | 30.5 | 30.8 | 1.2 | 30 | 29 |
| 8 | 25/R | 1.81 | 1.72 | 2.27 | 1.77 | 25 | 33.2 | 33.6 | 1.4 | 31 | 29 |
| 9 | 34/L | 1.86 | 1.95 | 2.39 | 1.91 | 29 | 44.2 | 44.8 | 1.5 | 12 | 12 |
| 10 | 28/L | 1.92 | 2.04 | 2.55 | 2.09 | 33 | 35.8 | 36.3 | 1.6 | 7 | 8 |
| 11 | 37/L | 2.01 | 2.12 | 2.77 | 2.18 | 38 | 9.40 | 40.5 | 2.8 | 5 | 6 |
| 12 | 26/L | 1.58 | 1.72 | 1.86 | 1.76 | 18 | 37.8 | 38.1 | 0.9 | 10 | 9 |
| 13 | 30/L | 1.85 | 1.94 | 2.23 | 1.93 | 21 | 47.6 | 48 | 0.9 | 17 | 16 |
| 14 | 35/L | 1.48 | 1.66 | 1.98 | 1.64 | 34 | 44.7 | 45.7 | 2.4 | 20 | 20 |
| 15 | 29/L | 1.67 | 1.75 | 2.05 | 1.70 | 23 | 44.4 | 44.8 | 1.1 | 24 | 23 |
| 16 | 41/L | 1.32 | 1.37 | 1.67 | 1.40 | 27 | 36.7 | 37.3 | 1.8 | 20 | 19 |
| mean | 32.7 | 1.76 | 1.73 | 2.25 | 1.74 | 28.12 | 37.41 | 39.88 | 1.58 | 24.75 | 24.56 |
| ± s.d. | 6.4 | 0.31 | 0.27 | 0.38 | 0.29 | 7.67 | 9.27 | 5.64 | 0.57 | 14.64 | 15.03 |

PrNT, Pre-NMES thigh; NNCT, non-NMES contrelateral thigh; PoNT, post-NMES thigh; NNCT, non-NMES contrelateral thigh; UI, uptake increase; PrNT, pre-NMES thigh; PoNT, post-NMES thigh; GI, girth increase; PrNTS, pre-NMES thigh skinfold; PoNTS, post-NMES thigh skinfold




a b

**Fig. 1** Pre- and post-NMES $^{99m}$Tc-sestamibi images of a subject, L: left. (a) Symmetrical rectangular ROIs were placed over the both muscular (thigh) and nonmuscular (knee) region. Difference of activitiy was not seen between both thigh muscles at pre-NMES $^{99m}$Tc-sestamibi image (a) whereas there was visually increased uptake in the left thigh muscles at post-NMES image (b).

*$^{99m}$Tc-sestamibi imaging*

All study patients were fasted and were maintained at rest for at least 30 min before the intravenous administration of $^{99m}$Tc-sestamibi [555 MBq]. Scintigraphic images were obtained approximately 60 min later. Twenty days after NMES, second scintigraphic images were performed. Studies were performed with a large field of view gamma camera (PHILIPS diagnost tomo) equipped with a low-energy, parallel-hole, high-resolution collimator, and a 20% energy window centered at 140 keV. Images were acquired in a 128 × 128 pixel matrix until 400,000 counts/view were obtained or for 5 min. The semiquantitative analysis was performed in a blinded fashion 1 week later. Symmetrical rectangular regions of interest (ROIs) for both thighs were drawn around muscular [thigh (T)] and nonmuscular [knee (K)] areas (Fig. 1a). The uptake ratios (T/K) were computed by dividing the mean counts in the muscular ROI by mean counts in the nonmuscular ROI.[20,24] The contralateral thigh on which NMES was not performed was used as a control. Because these ratios could be affected by increases in muscle mass during the NMES therapy period, the normalized uptake ratio (NUR) was calculated by dividing these ratios by the patient's body surface area.[25] Intraobserver variations were assessed by reanalyzing all data 1 week later.

*Thigh girth measurements*

Thigh girths were measured in centimeters, around the thigh region localized 10 centimeters above the apical tip point of the patella. Then the thigh skinfold measurement (mm) was performed and used for determining the fatty free girth of the thigh (fatty free thigh girth (cm) = thigh girth − ($\pi$ × thigh skinfold value/10).

*Statistical analysis*

Dependent Student's t-test was used to analyze differences between pre- and post-NMES thigh NURs. Comparisons between NMES performed and contralateral thighs were made using independent Student's t-test. Data are the expressed as mean ± standard deviation. Correlations between parameters were calculated by means of Pearson's correlation coefficient. A p value below 0.05 was considered significant.

## RESULTS

The pre- and post-NMES theraphy NURs, girth, skinfold and % uptake, % girth increases are shown in Table 1. No significant difference was seen between the two pre-NMS thigh NURs (1.76 ± 0.31 vs. 1.73 ± 0.27, p = 0.58). Statistical analysis of the pre- and post-NMES NURs showed a significant increase with respect to the NMES performed thigh (1.76 ± 0.31 vs. 2.25 ± 0.38, p = 0.0000) (Fig. 1a, b). A significant difference was also seen between the post-NMES and contralateral thigh NURs (2.25 ± 0.38 vs. 1.74 ± 0.29, p = 0.0000).

A good positive correlation was found between % girth increase and % uptake increase (r = 0.89, p = 0.0000). The

mean of post-NMES girth values was also higher than the pre-NMES value, even though statistical significance was not reached (37.41 ± 9.27 vs. 39.88 ± 5.64, p = 0.21). There was no difference between pre- and post-NMES thigh skinfold values (24.75 ± 14.64 vs. 24.56 ± 15.03, p = 0.53).

## DISCUSSION

Neuromuscular electric stimulation has been used for many years in the rehabilitation of muscles.[1] Many reports have supported the physiological, morphological, biochemical and muscle force improving effect of NMES on muscles.[2] This method is also well known for its denervation atrophy prevention effect as well as its muscle strength and endurance improvement effect.[3–6] There are many studies in the literature which demonstrate the underlying mechanisms responsible for these effects. It has been suggested that capillary blood flow and oxygen delivery to tissues increase during contractions with the application of NMES.[26] Blood flow was observed to increase by 20%, according to the results of a study in which the flow in gastrochinemius muscle during NMES application was measured by Doppler ultrasonography.[27] And, intermittent electrical stimulation with high frequency waves has been shown to cause intensitive capillary growth prominently in the glycolytic fibrils of muscle[7] beginning from the seventh day of the intervention. Twenty-one days of NMES application to triceps surae muscle resulted in increases in capillary number and density, as well as the capillary/fibril ratio in another previous study.[28] The increase in blood flow after NMES seems to be related with this capillary growth in muscles.

Other studies also confirm that the onset of hemodynamic and oxidative-glycolytic enzyme profile changes with NMES usually occur between the 4th and 7th days of the application.[7] At the end of 2.5 weeks of NMES application to rabbit tibialis anterior muscle, oxidative enzymes such as citrate hexokinase and 3-oxoacid coatransferase were found to increase between two and ten folds. It was shown in the same study that the NMES could alter the mitochondrial content of a muscle cell.[29] The reports of another study suggesting increases in nucleus size and number, nuclear DNA content and fibril size as well as increases in the mitochodrial fraction in type 2 fibrils, after 19–21 days of NMES application to the gastrochinemius muscle in healthy population confirmed the results of previous studies.[8,9] Moreover NMES has been shown to increase succinic dehydrogenase activities and muscle protein synthesis.[11,30] We applied 15 sessions of NMES in our study, and that seemed to be sufficient to bring out these changes.

Recently, $^{99m}$Tc-sestamibi has been found to be an appropriate radiopharmaceutical for noninvasive imaging of the perfusion of the lower extremities. Cellular uptake and trapping of $^{99m}$Tc-sestamibi are related not only to regional blood flow but also to mitochondrial metabolic conditions and viability.[31] The tracer is driven across both plasma and mitochondrial membranes in response to progressively larger negative transmembrane potentials, and trapped within the mitochondrial layer.[32] According to our previously studies, $^{99m}$Tc-sestamibi scintigraphy can assess the perfusion and viability of muscle tissue, and the changes in skeletal muscle metabolism induced by therapy can also be tracked noninvasively.[19–21]

Using treadmill stress testing with an injection of $^{99m}$Tc-sestamibi at peak exercise in a normal person, it was shown that the muscular $^{99m}$Tc-sestamibi uptake in extremity muscle increased significantly from rest to exercise.[12,13] Therefore, exercise $^{99m}$Tc-sestamibi leg scintigraphy has been found to be useful for assessment of ischemic extremities.[12–15,18] In our study, a significant difference was seen between pre- and post-NMES uptake values, and also the % uptake increase correlated closely with the % girth increase. Muscle cell hypertrophy and consequent extremity diameter increases have been reported after NMES application.[2] Treadmill exercises acutely increase the blood flow in muscle tissue by leading to vasodilation, and so $^{99m}$Tc-sestamibi uptake increases, but in our study NMES therapy increased $^{99m}$Tc-sestamibi uptake in a long time period of three weeks, by increasing the metabolism and consequently the mass of muscle tissue. Since NMES helps strengthen or maintain mucle mass, maintain or increase the range of motion, facilitate voluntary motor control and reduce the effects of spasticity,[33] it can be used in the period of rehabilitation of traumas, such as sport injuries, surgical operations, osteoarthritis of joints, structural abnormalities such as scoliosis, paresis and paralysis observed in upper and lower motor neuron lesions and rheumatological diseases such as rheumatoid arthritis and ankylosing spondylitis. Thus, the clinical significance of our findings must nevertheless be confirmed in further studies to assess the influence of NMES treatment.

## REFERENCES


1. Romero JA, Sanford TL, Schroeder RV. The effects of electrical stimulation of normal quadriceps on strength and girth. *Med Sci Sports Exerc* 1982; 14: 194–197.
2. Cabric M, Appell HJ. Effect of electrical stimulation of high and low frequency on maximum isometric force and some morphological characteristics in men. *Int J Sports Med* 1987; 8: 256–260.
3. Laughmann RK, Youdas JW, Garrett TR, Chao EYS. Strength changes in the normal quadriceps femoris muscle as a result of electrical stimulation. *Physşcal Theraphy* 1983; 63: 494-499.
4. Balogun JA, Onari OO, Akiju OA. High voltage electrical stimulation in the augmentation of muscle strength: effects of pulse frequency. *Arch Phys Med Rehabil* 1993; 74: 910–916.

5. Edgerton VR. Mammalian muscle fiber types and their adaptability. *Am Zool* 1978; 18: 113–125.
6. Boumann HD, Shaffer KJ. Physiological basis of electrical stimulation of human muscle and its clinical application. *Phys Ther Rev* 1957; 37: 207–223.
7. Hudlicka O, Tyler KR. The effect of long-term high-frquency stimulation on capillary density and fibre types in rabbit fast muscles. *J Physiol* 1984; 353: 435–445.
8. Cabric M, Appell HJ, Resic A. Fine structural changes in electrostimulated human skeletal muscle. Evidence for predominant effects on fast muscle fibres. *Eur J Appl Physiol* 1988; 57: 1–5.
9. Cabric M, Appell HJ, Resic A. Effects of different frequencies on the myonuclei and fiber size in human muscle. *Int J Sports Med* 1987; 8: 323–326.
10. Eriksson E. Sports injuries of the knee ligaments: their diagnosis, treatment, rehabilitation, and prevention. *Med Sci Sports* 1976; 8: 133–144.
11. Lake DA. Neuromuscular electrical stimulation: an overview and its application in the treatment of sports injuries. *Sports Med* 1992; 13: 320–336.
12. Sayman HB, Urgancioglu I. Muscle perfusion with technetium-MIBI in lower extremity peripheral arterial disease. *J Nucl Med* 1991; 32: 1700–1703.
13. Miles KA, Barber RW, Wraight EP, Cooper M, Appleton DS. Leg muscle scintigraphy with Tc99m-MIBI in the assessment of peripheral vascular [arterial] disease. *Nucl Med Commun* 1992; 13: 563–603.
14. Bostrom PA, Diemer H, Leide S, Lilja B, Bergqvist D. $^{99}Tc^{m}$-sestamibi uptake in the leg muscles and in the myocardium in patients with intermittent claudication. *Angiology* 1993; 44 (12): 971–976.
15. Wann LS, Hellman C, Dorros G. Evaluation of leg perfusion during exercise using technetium 99m sestamibi. A new test for peripheral vascular disease. *Echocardiography* 1992; 9 (5): 547–552.
16. Scopınaro F, Manni C, Micchelli A, Mossa R, Vincentıs GD, Schillachi O, et al. Muscular uptake of Tc-99m MIBI and Tl-201 in Duchenne muscular dystrophy. *Clin Nucl Med* 1996; 21: 792–796.
17. Cittanti C, Colamussi P, Giganti M, Orlandi C, Uccelli L, Manfrini S, et al. Technetium-99m sestamibi leg scintigraphy for non-invasive assessment of propionyl L-carnitine induced changes in skeletal muscle metabolism. *Eur J Nucl Med* 1997; 24: 762–766.
18. Edwards PD, Miles KA, Owens SJ, Kemp PM, Jenner JR. A new non-invasive test for the detection of compartment syndromes. *Nucl Med Commun* 1999; 20 (3): 215–218.
19. Aygıt C, Sarıkaya A. Technetium-99m sestamibi scintigraphy for non-invasive assessment of muscle flap viability. *Ann Plas Surg* 1999; 43 (3): 338–340.
20. Sarıkaya A, Şen Ş, Çermik TF, Birtane M, Berkarda Ş. Evaluation of skeletal muscle metabolism and erythropoietin treatment response in patients with chronic renal failure with $^{99m}$Tc-sestamibi leg scintigraphy. *Nucl Med Commun* 2000; 21 (1): 83–87.
21. Sarıkaya İ, Aygıt AC, Candan L, Sarıkaya A, Türkyılmaz M, Berkarda Ş. Assessment of tissue viability in frostbite injury by $^{99m}$Tc sestamibi scintigraphy in an experimental rabbit model. *Eur J Nucl Med* 2000; 27: 41–45.
22. Johnson MA, Polgar J, Weightman D. Data on the distribution of fiber types on thirthy-six human muscles. *J Neurol Sci* 1973; 18: 111–129.
23. Prince FP, Hikida RS, Hagerman FC. Muscles fiber types in woman athletes and non-athletes. *Pflugers Arch* 1977; 371: 161–165.
24. Siegel ME, Stewart CA. Peripheral vascular disease. In *Principles of Nuclear Medicine*, Wagner HN (ed), 2nd ed., W.B. Saunders Comp., 1995: 859–880.
25. Kim CK, Gupta NC. Dependency of standardized uptakes values of fluorine-18 fluorodeoxyglucose on body size: Comparison of body surface area correction and lean body mass correction. *Nucl Med Commun* 1996; 17: 890–894.
26. Shenton DW, Heppenstall RB, Chance B, Glasgow SG, Schnall MD, Sapega AA. Electrical stimulation of human muscle studied using $^{31}$P-Nuclear Magnetic Resonance Spectroscopy. *J Orthop Research* 1986; 4: 204–211.
27. Currier DP, Petrilli CR, Threlkeld AJ. Effect of graded electrical stimulation on blood flow to healthy muscle. *Phys Ther* 1986; 66: 937–943.
28. Cabric M, Appell HJ, Resic A. Stereological analysis of capillaries in electrostimulated human muscles. *Int J Sports Med* 1987; 8: 327–330.
29. Henriksson J, Salmons S, Lowry OH. Chronic stimulation of mammalian muscle: enzyme and metabolic changes in individual fibres. *Biomed Biochim Acta* 1989; 48: 445–454.
30. Ericsson E, Haggmark T, Kiessling KH. Effect of electrical stimulation on human skeletel muscle. *Int J Sports Med* 1981; 2: 18.
31. Beanlands RSB, Dawood F, Wen WH, McLaughlin PR, Butany J, D'amati G, et al. Are the kinetics of technetium-99m methoxyisobutyl isonitrile affected by cell metabolism and viability? *Circulation* 1990; 82: 1802–1814.
32. Piwnica-Worms D, Kronauge JF, Chiu ML. Uptake and retention of hexakis[2-methoxyisobutylisonitrile]technetium[I] in cultured chick myocardial cells: mitochondrial and plasma membrane potential-dependence. *Circulation* 1990; 82: 1826–1838.
33. Baker LL. Clinical uses of neuromuscular electrtical stimulation. In: Nelson RM, Currier DP (eds), *Clinical Electrotherapy*, Second Edition. Norwalk, Appleton and Lange, 1991: 143–170.