Effect of aerobic training on ^{99m}Tc-methoxy isobutyl isonitrile (^{99m}Tc-sestamibi) uptake by myocardium and skeletal muscle: implication for noninvasive assessment of muscle metabolic profile

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Abstract

Aim: The effect of long-term endurance training on skeletal muscle and myocardial uptake of ^{99m}Tc-sestamibi, a radiopharmaceutical accumulating in the mitochondria, was investigated.

Methods: Twenty-six Wistar rats were divided into a trained (5 days week^{-1} endurance running for 14 weeks) and an untrained group. On completion of training, ^{99m}Tc-sestamibi was administered and, 2 h post-injection, the myocardium and the soleus, extensor digitorum longus (EDL) and medial gastrocnemius (MG) muscles were removed for the measurement of cytochrome *c* oxidase (CCO) activity and ^{99m}Tc-sestamibi uptake. Tissue ^{99m}Tc-sestamibi kinetics was preliminarily studied in 16 other rats for up to 2 h post-injection.

Results: Two hours post-injection ^{99m}Tc-sestamibi uptake was either stable (myocardium) or still rising (skeletal muscles). Both CCO activity and ^{99m}Tc-sestamibi uptake decreased in the same order (myocardium, soleus, EDL, MG) in the tissues examined. The CCO activity of the EDL and MG muscles was higher ($P < 0.05$) in the trained compared to the untrained group. ^{99m}Tc-sestamibi uptake in the soleus and EDL muscles was higher ($P < 0.05$) in the trained compared to the untrained rats, whereas the difference in MG was marginally significant ($P = 0.06$) in favour of the trained group.

Conclusions: Long-term endurance training, resulting in elevated skeletal muscle CCO activity, is also associated with a similar increase in ^{99m}Tc-sestamibi uptake. This finding suggests that ^{99m}Tc-sestamibi could be used in imaging assessment of skeletal muscle metabolism with possible applications in both clinical and sports medicine settings.

Keywords CCO, mitochondria, muscle metabolism, ^{99m}Tc-sestamibi, training.

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Traditional biochemical and histochemical methods used in assessing muscle metabolism for clinical or sports medicine purposes require tissue harvesting, an invasive technique. In addition, muscle biopsy is associated with sampling errors which may influence the validity of the results (Blomstrand & Ekblom 1982, Fekete 1986, Aoyagi & Shephard 1992, Coggan 1995). Magnetic resonance imaging and magnetic resonance spectroscopy have been introduced over the past decade as alternative techniques to assess muscle metabolism (Bangsbo *et al.* 1993, De Visser & Reimers 1994). In addition, positron emission tomography using metabolic tracers such as ^{18}F-fluorodeoxyglucose can provide metabolite assessment in skeletal muscle (Nuutila & Kalliokoski 2000). However, the high cost and the relatively limited availability of these modalities restrict their wide application. Therefore, the development of a simple, low-cost, noninvasive method for the evaluation of metabolism of large muscle groups in humans would be of great utility.

2-Methoxy isobutyl isonitrile (MIBI) is a lipophilic, cationic compound which, when injected to live animals, is delivered to tissues in proportion to regional blood flow and is retained within the mitochondria. Because normal cells have a negative plasma membrane potential and an even more negative mitochondrial membrane potential ($\Delta\psi_m = -150$ to -200 mV), both potentials contribute a large driving force for the sequestration of MIBI within the mitochondrial matrix (Piwnica-Worms *et al.* 1990). Labelled with metastable ^{99}technetium (^{99m}Tc), MIBI is currently used, as ^{99m}Tc-sestamibi, in nuclear medicine for myocardial perfusion studies (Maddahi *et al.* 1990), tumour imaging (Actolun *et al.* 1992) and parathyroid localization (Coakely *et al.* 1989).

The distribution of this agent in the myocardium has been extensively investigated (Piwnica-Worms *et al.* 1990, Backus *et al.* 1993). Studies in cultured myocardial cells have shown that ^{99m}Tc-sestamibi was concentrated up to 1000-fold in mitochondria compared to the cytosol (Piwnica-Worms *et al.* 1990). However, studies examining ^{99m}Tc-sestamibi uptake in skeletal muscle are limited, and only sporadic clinical publications can be found (Bostrom *et al.* 1993, Crane *et al.* 1993, Cittanti *et al.* 1997, Chang *et al.* 2005). Recently, mitochondrial dysfunction assessment by ^{99m}Tc-sestamibi imaging was applied to both human skeletal muscle (Chang *et al.* 2005) and myocardium (Matsuo *et al.* 2007).

Endurance training induces adaptive alterations in muscle metabolic profile including increases in blood flow (Sexton & Laughlin 1994) and mitochondrial size, number and enzyme activity in both animals (Murakami *et al.* 1995, Terblanche *et al.* 2001) and humans (Green *et al.* 1979, Starritt *et al.* 1999, Carter *et al.* 2001). However, no data are available concerning changes in ^{99m}Tc-sestamibi uptake by skeletal muscle or myocardium as a result of training.

Thus, the aim of the present study was to investigate the effect of long-term endurance training on muscle ^{99m}Tc-sestamibi uptake as a potential basis for the development of a noninvasive imaging technique for the assessment of metabolic changes in skeletal muscle. Our main hypothesis was that endurance training increases both the activity of skeletal muscle cytochrome *c* oxidase (CCO), a mitochondrial enzyme that is used widely as a marker of oxidative capacity, and ^{99m}Tc-sestamibi uptake.

Methods

Animals

Twenty-six male Wistar rats, aged 8–10 weeks and weighing 282 ± 8 g (mean ± SEM), were enrolled in this study. Rats were individually housed in standard Plexiglas cages on a 12-h light/dark cycle under controlled temperature (18–21 °C) and humidity (50–70%) conditions, and they had access to commercial rat chow and tap water *ad libitum*. The animals were randomly and equally divided into an exercising (trained) and an ambulatory (untrained) group. The project was approved by the institutional review board. All procedures were in accordance with the European Union guidelines for the care and use of laboratory animals, as well as the ''Principles of laboratory animal care'' (NIH publication No. 86-23, revised 1985).

Training programme

The trained group ran on a level motor-driven treadmill at 25 m min^{-1} for 60 min each day, 5 days weekly over a period of 14 weeks. To protect the animals from injury, velocity was progressively increased to the final speed over the first 5 min of each exercise session.

Tracer preparation

Labelling of tetrakis MIBI (Cardiolite®; Bristol-Myers Squibb, Medical Imaging Inc., North Billerica, MA, USA) was performed by adding 1850 MBq (50 mCi) of freshly eluted ^{99m}Tc (as pertechnetate) in a vial containing 1 mg of Cardiolite to a final volume of 2.5 mL with saline, according to the manufacturer's instructions. Radiochemical purity has been previously validated in our department (G.A., S.G., C.K.) and has been consistently found to be over 95%.

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Tracer kinetics

To investigate ^{99m}Tc-sestamibi kinetics in the organs of interest and determine the appropriate timing of tissue harvesting, a preliminary experiment was carried out using 16 other rats of similar age and body weight as the study animals. ^{99m}Tc-sestamibi uptake was measured as described below for the study animals at four time points (four rats per time point), namely at 10, 30, 60 and 120 min following ^{99m}Tc-sestamibi injection.

Tracer administration and tissue harvesting

Two days after the termination of the last training session, the radiopharmaceutical was administered to the animals under anaesthesia as described (Kyparos *et al.* 2006).

Based on data regarding ^{99m}Tc-sestamibi kinetics in rat heart and blood (Onoguchi *et al.* 2003), as well as findings from our preliminary experiment (see Results), a 2-h interval between radiopharmaceutical injection and tissue harvesting was judged as a reasonable compromise between the relatively short ^{99m}Tc half-life (6.02 h) and tracer kinetics. Difficulties in maintaining protracted anaesthesia were also taken into consideration. During this period rats lay unconscious in supine position on the operation table. To maintain their body temperature, we placed them in the proximity of a diffuse heat source and covered them with a 'blanket' made of aluminium foil. Anaesthesia was sustained through the administration of approx. 10% of the initial dose when necessary.

Two hours after tracer injection the animals were killed by exsanguination through transthoracic cardiac puncture, and the soleus, extensor digitorum longus (EDL) and medial gastrocnemius (MG) muscles from the right hindlimb, as well as the heart, were removed within 10–15 min. Tissues were dissected free of connective tissue, cleaned with saline, blotted on absorbent paper and weighed. A small piece from each tissue was then excised, weighed, snap-frozen in liquid nitrogen and stored at −80 °C for subsequent biochemical analysis, while the remainder was used immediately for radioactivity measurement. To check for potential extravasation of the radiopharmaceutical during injection, tissue blocks of approximately equal size including part of the jugular vein and surrounding tissues were bilaterally removed, weighed and measured for radioactivity.

Measurements

Cytochrome *c* oxidase activity and ^{99m}Tc-sestamibi uptake in the tissues removed were measured as described (Kyparos *et al.* 2006).

Statistics

Data were analysed using SPSS, version 14 (SPSS, Chicago, IL, USA). In our preliminary experiment designed to investigate ^{99m}Tc-sestamibi kinetics, time-dependent changes in tracer uptake by each tissue were examined by the nonparametric Kruskal–Wallis test, as sample sizes were small (four rats per time point). In the main experiment, the distribution of CCO activity and ^{99m}Tc-sestamibi uptake values in each tissue was examined by the Shapiro–Wilk test and was found not to differ significantly from normal. Thus, to evaluate the differences in CCO activity and ^{99m}Tc-sestamibi uptake between the trained and the untrained group in each tissue, one-tailed independent Student's *t*-test was applied. When all CCO activity and ^{99m}Tc-sestamibi uptake values were pooled together for the purpose of correlation, the distribution of both parameters was found to differ significantly from normal (because of large differences between the myocardium and the skeletal muscles). Thus, the correlation between ^{99m}Tc-sestamibi uptake and CCO activity was assessed using the nonparametric Spearman's rank correlation coefficient (r_s). Regression analysis between ^{99m}Tc-sestamibi uptake and CCO activity was also performed. Data are presented as mean ± SEM. The level of statistical significance was set at $\alpha = 0.05$.

Results

Tissue CCO activity in the untrained and trained rats is summarized in Table 1. In both animal groups, CCO activity had the highest value in the myocardium, followed by the slow-twitch soleus and the fast-twitch EDL and MG muscles. CCO activity was significantly higher in the EDL and MG muscles of the trained compared to the untrained rats ($P < 0.05$). No significant difference between trained and untrained rats was found in the CCO activity of the soleus muscle or the myocardium.

Table 1 CCO activity (U g^{-1}) in the tissues of untrained and trained rats

Tissue	Untrained	Trained
Myocardium	56.6 ± 2.9	59.7 ± 5.4
Soleus	21.0 ± 1.0	22.4 ± 1.3
EDL	10.5 ± 0.7	13.2 ± 1.0*
MG	7.9 ± 0.4	11.2 ± 0.7*

Values represent mean ± SEM (n = 13 rats per group). CCO, cytochrome *c* oxidase; EDL, extensor digitorum longus; MG, medial gastrocnemius.
*Significantly different from untrained rats ($P < 0.05$).

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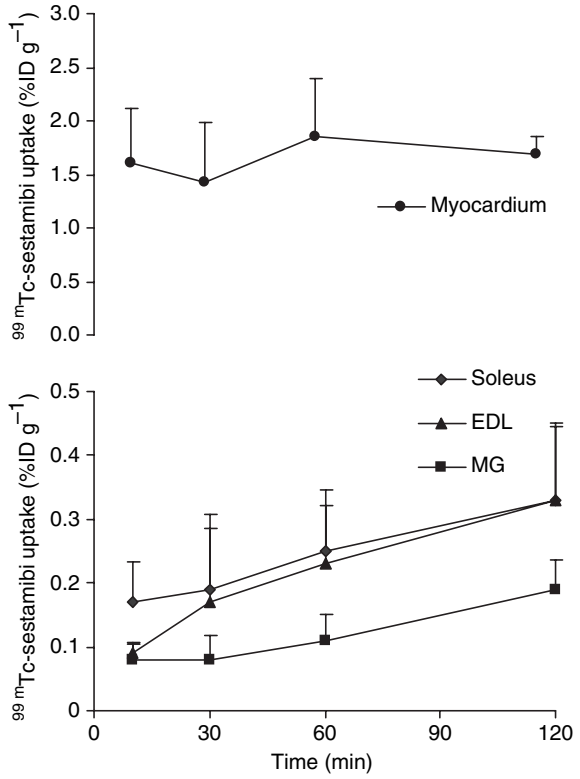

Figure 1 ^{99m}Tc-sestamibi kinetics in rat tissues. Each time point represents the mean ± SEM of four rats. ID, injected dose; EDL, extensor digitorum longus; MG, medial gastrocnemius.

Data from the preliminary experiment investigating ^{99m}Tc-sestamibi kinetics in muscle and heart are presented in Figure 1. ^{99m}Tc-Sestamibi uptake remained fairly constant in the myocardium, whereas it tended to increase gradually from 10 to 120 min in the soleus, EDL and MG muscles. This increase, however, was not statistically significant, probably because of the small number of animals examined (four per time point).

Tissue ^{99m}Tc-sestamibi uptake in the untrained and trained rats 2 h after tracer administration is summarized in Table 2. ^{99m}Tc-sestamibi uptake decreased in the tissues examined in the same order as CCO activity did. ^{99m}Tc-sestamibi uptake was significantly higher in the soleus and EDL muscles of the trained compared to the untrained rats ($P < 0.05$). ^{99m}Tc-sestamibi uptake was also marginally significantly higher in the MG muscle of the trained rats ($P = 0.06$). No significant difference was found in myocardial ^{99m}Tc-sestamibi uptake between the trained and untrained rats.

When all CCO activity values were correlated with the respective ^{99m}Tc-sestamibi uptake values, a significant correlation was observed ($r_s = 0.72$, $P < 0.01$, Fig. 2). Furthermore, the regression analysis between CCO activity and radioisotope uptake was also significant [$F(1, 102) = 249.5$, $P < 0.0001$].

Table 2 ^{99m}Tc-sestamibi uptake (% injected dose g^{-1}) in the tissues of untrained and trained rats

Tissue	Untrained	Trained
Myocardium	1.55 ± 0.18	1.81 ± 0.12
Soleus	0.24 ± 0.03	0.32 ± 0.03*
EDL	0.21 ± 0.03	0.32 ± 0.04*
MG	0.13 ± 0.02	0.17 ± 0.02†

Values represent mean ± SEM (n = 13 rats per group). EDL, extensor digitorum longus; MG, medial gastrocnemius.
*Significantly different from untrained rats ($P < 0.05$).
†Borderline significantly different from untrained rats ($P = 0.06$).

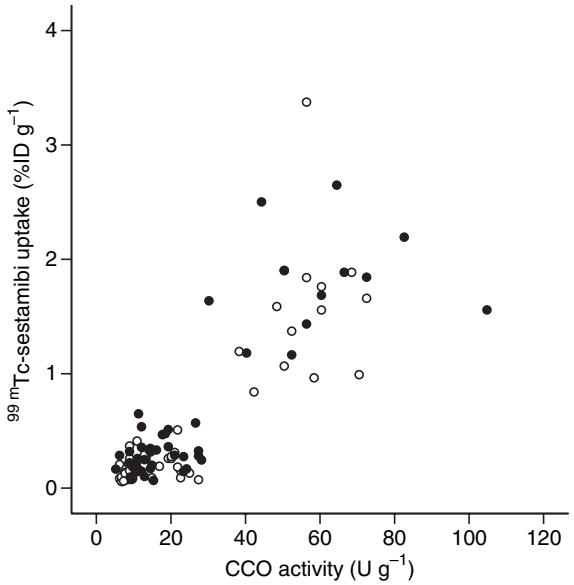

Figure 2 Correlation between cytochrome *c* oxidase (CCO) activity and ^{99m}Tc-sestamibi uptake in the tissues of untrained (○) and trained (●) rats.

Discussion

This study was designed to examine the effect of long-term endurance training on rat myocardial and muscle ^{99m}Tc-sestamibi uptake in relation to concomitant changes in CCO activity, thus evaluating ^{99m}Tc-sestamibi as a potential tool for noninvasive skeletal muscle metabolic assessment.

As expected from the fact that CCO is a mitochondrial electron transport protein, tissues characterized by high mitochondrial content, such as the myocardium and the slow-twitch, fatigue-resistant soleus muscle, had higher CCO activity than muscles with low mitochondria content, namely the EDL and MG. In support of this finding, stereological analysis of rat myocardium and hindlimb muscles has shown that mitochondrial

content was very high (36% of the myofibre volume) in myocardium (Page & McCallister 1973), intermediate (8.4%) in soleus muscle (Van Ekeran *et al.* 1992) and low (2.2%) in the vast majority of the gastrocnemius muscle fibres (Stonnington & Engel 1973).

With respect to exercise, the training protocol used in the present study was sufficient to induce aerobic adaptations, as confirmed by the higher CCO activity in those muscles of the trained animals that are characterized by low mitochondrial content. Indeed, CCO activity was higher in the predominantly composed of fast-twitch fibres EDL and MG muscles (by 26 and 42% respectively) of the trained compared to the untrained rats. CCO activity is in direct relation with the mitochondrial content of the different muscle fibre types, showing parallel alterations with other aerobic enzymes (Holloszy 1973, Davies *et al.* 1981).

The finding that endurance trained rats did not differ from their untrained counterparts in CCO activity of the slow-twitch soleus muscle and the myocardium could be attributed to the initial high mitochondrial and oxidative enzyme content of these tissues, which may limit further aerobic adaptations. Our data are consistent with those of other studies which have also reported minimal changes in muscle and myocardial CCO activity after endurance training (Morgan *et al.* 1971, Hoppeler *et al.* 1973, Tibbits *et al.* 1978, Samelman *et al.* 2000, Terblanche *et al.* 2001).

^{99m}Tc-sestamibi uptake changes in proportion (up to a certain point) with tissue blood flow. However, its intracellular retention depends on mitochondrial activity. This is evident late after ^{99m}Tc-sestamibi administration, when its blood concentration is very low and a considerable tissue/blood concentration gradient has been established. The myocardium-to-blood uptake ratio has been reported to be as high as 265 two hours after radiotracer administration (Onoguchi *et al.* 2003). At that time, despite the very low blood concentration, ^{99m}Tc-sestamibi uptake in skeletal muscles still rises (Fig. 1).

On the basis of the mitochondrial retention of ^{99m}Tc-sestamibi, one would expect similar differences in CCO activity and ^{99m}Tc-sestamibi uptake between the muscles of the trained and untrained rats. Indeed, the higher CCO activity in the EDL and MG muscles of the trained rats was paralleled by higher ^{99m}Tc-sestamibi uptake (although the statistical difference was marginal in the latter). However, this was not the case for the soleus muscle, which showed higher ^{99m}Tc-sestamibi uptake but not CCO activity in the trained rats. A possible explanation for this discrepancy could be that a higher ^{99m}Tc-sestamibi uptake, suggesting increased transmembrane potential and/or mitochondrial membrane surface, might reflect only part of the multiple adaptational alterations (such as mitochondrial size and number, aerobic enzyme activities and capillarity) induced by endurance training.

The higher uptake of ^{99m}Tc-sestamibi by tissues with increased mitochondrial content is also supported by the significant correlation between CCO activity and ^{99m}Tc-sestamibi uptake found in this study. Future studies examining whether a decrease in muscle CCO activity with detraining is accompanied by a decrease in muscle ^{99m}Tc-sestamibi uptake would further support our findings. Furthermore, it would be interesting to investigate, as indirect evidence, whether an inhibitor of the electron transport chain causes a decrease in ^{99m}Tc-sestamibi uptake, as it is the electron transport chain that generates the negative mitochondrial membrane potential which attracts ^{99m}Tc-sestamibi to the mitochondrial matrix.

In conclusion, the data of the present study illustrate that ^{99m}Tc-sestamibi uptake is related to mitochondrial activity in rat myocardium and skeletal muscle. Moreover, long-term endurance training resulted in parallel increases, or lack thereof, of CCO activity and ^{99m}Tc-sestamibi uptake in most of the tissues examined. These findings suggest that ^{99m}Tc-sestamibi could be a candidate tracer for the development of an imaging technique for the assessment of metabolic changes in skeletal muscle at the mitochondrial level. Potential applications of such a technique could expand in both the clinical and sports medicine fields.

Conflict of interest

There is no conflict of interest related to this study.

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