Eur J Appl Physiol (2007) 102:119–126
DOI 10.1007/s00421-007-0542-4

ORIGINAL ARTICLE

Effect of a single session of electrical stimulation on activity and expression of citrate synthase and antioxidant enzymes in rat soleus muscle

Aurélio da Silva Pimenta · Rafael Herling Lambertucci · Renata Gorjão · Leonardo dos Reis Silveira · Rui Curi

Accepted: 26 July 2007 / Published online: 26 October 2007

Abstract The aim of our study was to investigate the effect of a single high intensity session of muscle contractions on the activity and expression of citrate synthase (CS) and of the following major antioxidant enzymes: Mn-superoxide dismutase (Mn-SOD), Cu,Zn-superoxide dismutase (Cu,Zn-SOD), catalase (CAT), and glutathione peroxidase (GPX). To accomplish this, soleus muscles of male Wistar rats were subjected to contractions using a intense electrical stimulation (ES) protocol. Soleus muscles were isolated either immediately or 1 h after the contractions and utilized for enzyme activity determination, and for analysis of gene expression by quantitative PCR. A significant increase in maximal activity (63%) and expression (80%) of CS was observed in stimulated soleus muscles, isolated 1 h after ES as compared to controls. However, this effect was not observed in muscles isolated immediately after ES. By using macroarray and Real Time RT-PCR analysis, an increase in expression of Mn-SOD, Cu,Zn-SOD, CAT, and GPX was also found. Interestingly, of these enzymes, only CAT activity was significantly increased (44%) 1 h after ES in soleus muscle. These results indicate that acute ES up-regulates activity and expression of CS and CAT in soleus muscles. This increase in expression of CAT may play an important role in counteracting the potential deleterious effects of elevated oxidative stress induced by a high oxidative demand in skeletal muscles subjected to exercise training.

Keywords Antioxidant enzymes · Citrate synthase · Electric stimulation · Soleus muscle

A. da Silva Pimenta · R. H. Lambertucci · R. Gorjão · L. dos Reis Silveira · R. Curi (✉)
Department of Physiology and Biophysics,
Institute of Biomedical Sciences,
University of São Paulo,
Avenida Professor Lineu Prestes 1524,
sala 105 Butantan, 05508-900 São Paulo, SP, Brazil
e-mail: ruicuri@icb.usp.br

Introduction

It is well established that endurance training causes an increase in the activity of oxidative enzymes (Fernstrom et al. 2004; Holloszy et al. 1970; Ji et al. 1988). One of these enzymes is citrate synthase (CS), which is localized on the inner mitochondrial membrane and promotes the condensation of acetyl-CoA and oxaloacetate, generating citrate in the Krebs cycle (Wiegand and Remington 1986). The increase in CS activity with physical training has been associated with increments in mitochondrial protein content (Booth and Thomason 1991; Holloszy and Booth 1976). However, although 6-7 days of exercise training increases CS activity (Green et al. 1999; Spina et al. 1996), no evidence has been provided that mitochondrial number and/or size are also increased after this short training period. In fact, CS activity increases in skeletal muscles after acute exercise (single session) in humans (Fernstrom et al. 2004; Jacobs et al. 1987; Leek et al. 2001; Roepstorff et al. 2005; Tonkonogi et al. 1997) and in rats (Siu et al. 2003), which is compatible with the increase in CS activity observed in short periods of exercise training.

The increase in CS activity after an acute exercise bout indicates that there is a higher metabolic demand toward the oxidative pathway, which may also lead to an increase in the production of reactive oxygen species (ROS). ROS production during exercise has been associated with an increase in the activity of antioxidant enzymes such as

Mn-superoxide dismutase (Mn-SOD) (Oh-ishi et al. 1997; Ortenblad et al. 1997), Cu,Zn-superoxide dismutase (Cu,Zn-SOD) (Navarro-Arevalo et al. 1999; Oh-ishi et al. 1997), catalase (CAT) (Leeuwenburgh et al. 1994), and glutathione peroxidase (GPX) (Leeuwenburgh et al. 1994) in skeletal muscle. However, the studies that investigated the effect of acute exercise on the expression of antioxidant enzymes in skeletal muscle have reported conflicting results (Hollander et al. 2001; Itoh et al. 2004; Oh-ishi et al. 1997; Ohishi et al. 1998). Furthermore, the effect of a high intensity acute exercise on the expression and activity of antioxidant enzymes and CS have not been investigated. In order to address this issue, in this study, we investigated the effect of exercise on the activity and expression of CS and antioxidant enzymes in rat skeletal muscle. We used a high frequency electrical stimulation (ES) protocol that resembles high intensity resistance exercise. ES was chosen due to several reasons: (1) it allows a pattern of motor units recruitment that cannot be obtained by voluntary exercise where maximal contraction is prevented by neural mechanisms and motivation (Hainaut and Duchateau 1992); (2) it allows direct stimulation of only one limb preventing circulatory limitations potentially imposed by cardiovascular adjustments induced by exercise, and (3) because it imposes a strenuous challenge to the muscle and potently increases O_2 consumption by the active muscle. Importantly, most studies that used low/moderate intensity exercise have not found increase of activity of CS in animals. And, there is evidence that the increase in the expression of antioxidant enzymes is related to oxygen consumption. In this context, we hypothesize that a high intensity muscle contraction could have effect in these variables.

Materials and methods

Reagents

The following reagents were used: sodium pentobarbital (Cristalia, Itapira, SP, Brazil); Tris–HCl (Inlab, São Paulo, SP, Brazil); Tris/aminomethane, Trizol, Sybr Green, Random Primers, PCR buffer, Taq DNA polymerase, dCTP, dGTP, dTTP, dATP, $MgCl_2$, DNAse buffer, DNAse (Invitrogen, Carlsbad, CA, USA); 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), EDTA, acetyl-CoA, Triton X-100, oxaloacetic acid, cytochrome c, xanthine, xanthine oxidase, NADPH, glutathione reductase, reduced glutathione, *t*-butyl hydroperoxide (Sigma, St. Louis, MO, USA); sodium phosphate, potassium cyanide (Merck, Darmstadt, Germany); primers for glyceraldehyde 3-phosphate dehydrogenase (G3PDH), CS, Mn-SOD, Cu,Zn-SOD, CAT e GPX (IDT, Coralville, IA, USA); express hyb solution, termination mix (Clontech Laboratories, Mountain View, CA, USA); reverse transcriptase *Revertaid*™ M-MuLV (MBI Fermentas, Burlington, ON, Canada) and ^{33}P-labeled ATP (GE Healthcare, Waukesha, WI, USA).

Animals

Male adult (9 weeks of age) albino rats (Wistar strain) from the Institute of Biomedical Sciences of the University of São Paulo were used. The experimental protocol used was approved by the Ethics Committee of the Institute of Biomedical Sciences of the University of São Paulo. The rats were housed 5 per cage at 20–23°C in a reversed 12 : 12 h light-dark cycle, and had ad libitum access to rat chow (Nuvilab CR1, Nuvital Nutrientes, Curitiba, PR, Brazil) and water.

Electrical stimulation

Rats ($n = 7$) were anaesthetized with sodium pentobarbital (75 mg kg^{-1} b.w.) and had their right sciatic nerve exposed through a lateral section on the thigh where a platinum electrode was connected. Afterwards, the rats were fastened on an acrylic platform with a metallic bar crossing the right knee to fix the limb. Another metallic bar was fixed at the Achilles tendon, connecting the hind-foot to a force transducer (Myograph F-2000, Narco Bio-Systems, Austin, TX, USA) that indicated the generated tension by using a polygraph (Narco Bio-Systems). The contralateral limb was fastened to the platform by using an adhesive tape. Rats were kept under external warming to maintain core temperature during the entire procedure.

Rats were subjected to an intense ES protocol as previously described (Wojtaszewski et al. 1996; Silveira et al. 2007). Briefly, the stimulus consisted of 200 ms trains (24 ± 3 V) of 100 Hz with 0.1 ms pulses, delivered each second for 1 h. In order to reach maximum force output, the muscle rest length and the stimulation voltage were adjusted in the beginning of each experiment. When the force output assessed by the polygraph could not be increased, even with further adjustments of voltage and muscle length, it was considered as maximum.

Subsequently, soleus muscles were removed either immediately after ES or 1 h later. Control rats were subjected to the same conditions as the experimental group but with no ES. The tissues were extracted and immediately frozen in liquid nitrogen and kept at −70°C for the assays. Rats were killed by cervical dislocation.

Enzyme activity assays

In order to determine the maximal activities of CS, Mn-SOD, Cu,Zn-SOD, CAT, and GPX, soleus muscles were

homogenized for 20 s on ice, using a tissue homogenizer (Ultra-Turrax T8, Ika-Werke, Staufen, Germany) in the respective extraction solutions, depending on the enzyme assay.

Citrate synthase activity of soleus muscle was determined under the following conditions: (1) immediately after ES, and (2) 1 h after ES. The extraction buffer contained 0.5 mM Tris–HCl and 1.0 mM EDTA, pH 7.4, and the assay buffer contained Tris/aminomethane (100 mM), DTNB (0.2 mM), acetyl-CoA (0.1 mM), and Triton X-100 (0.1% v/v), pH 8.1. The reaction was initiated by the addition of 10 µl of the tissue extract and 50 µl of oxaloacetic acid (10 mM final concentration). Absorbance at 412 nm (25°C) was spectrophotometrically measured during 5 min as described (Srere et al. 1963).

The activities of Mn-SOD, Cu,Zn-SOD, CAT, and GPX were measured as previously described (Aebi 1984; Flohe and Otting 1984; Wendel 1981). The extraction buffer for Mn-SOD, Cu,Zn-SOD, CAT, and GPX assays contained 0.1 M sodium phosphate, pH 7.0. Mn-SOD and Cu,Zn-SOD activities were determined according to the method of Flohe and Otting (1984) by measuring, at 25°C, the decrease in the rate of cytochrome c reduction in a xanthine–xanthine oxidase superoxide generating system consisting of 10 µM cytochrome c, 100 µM xanthine, 50 mM sodium phosphate buffer (pH 10.0), and the necessary quantity of xanthine oxidase units to yield a variation of 0.025 absorbance per min at 550 nm. Mn-SOD activity was determined by the addition of 1 mM KCN to the assay of total SOD activity. This drug suppresses the activity of CuZn-SOD (Flohe and Otting 1984). CAT activity was determined by measuring the breakdown of hydrogen peroxide at 230 nm (Aebi 1984). GPX activity was determined as described by Wendel (1981), following the rate of NADPH oxidation, at 340 nm, 37°C, in an assay medium containing 50 mM phosphate buffer (pH 7.0), 0.3 mM NADPH, glutathione reductase (0.25 U ml^{-1}) and 5 mM reduced glutathione. The reaction was initiated by the addition of *t*-butyl hydroperoxide (1.5 mM). Activities of all enzymes are expressed on the basis of mg protein.

Real time RT-PCR analysis

Total RNA was extracted from soleus muscles as previously described (Sambrook et al. 2001). Briefly, 60–70 mg of soleus muscle was lysed using 1 ml Trizol reagent. After 5 min incubation at room temperature, 200 µl of chloroform were added to the tubes and centrifuged at 12,000×*g*. The aqueous phase was transferred to another tube and the RNA was pelleted by centrifugation (12,000×*g*) with cold ethanol and dried in air. RNA pellets were eluted in RNase-free water and treated with DNase I. The RNA preparation was then stored at −70°C. The RNA content was measured in duplicate at 260 nm. The purity of the RNA preparation was assessed by the 260/280 nm ratio and on a 1% agarose gel stained with ethidium bromide at 5 µg ml^{-1}.

Total RNA (4 µg) was reverse transcribed to cDNA using reverse transcriptase *Revertaid*™ M-MuLV. Expression of G3PDH, CS, Cu,Zn-SOD, Mn-SOD, CAT, and GPX was determined by real-time PCR (Higuchi et al. 1992) with a Rotor Gene-3000 equipment (Corbett Research, Mortlake, VIC, Australia), using Sybr Green as the fluorescent dye. The sequences of the utilized primers are described in Table 1.

Quantification of expression was performed by the comparative cycle threshold method, using G3PDH expression as inner control (Livak and Schmittgen 2001). Efficiency of the amplification reaction was calculated by using the LinRegPCR Analysis of Real Time PCR Data software Version 7.5 (Ramakers et al. 2003).

Analysis of gene expression by macroarray

Synthesis of cDNA probes

A pool of total RNA from three soleus muscles of each group (control and stimulated, isolated 1 h after ES) was prepared using 10 µg of RNA from each muscle, as above described. The cDNA probes were synthesized using the pure total RNA labeling system Atlas Kit™ according to the manufacturer's recommendations (Clontech Laboratories).

Table 1 Sense and antisense sequences of the primers and respective amplicons' sizes used in the real time RT-PCR assays

Gene	Sense	Antisense	Size (bp)
G3PDH	5′TGCCATCACTGCCACTC3′	5′CTGCTTCACCACCTTCTTG3′	256
CS	5′CGGTTCTTGATCCTGATGAGGG3′	5′ACTGTTGAGGGCTGTGATGGC3′	284
Cu,Zn-SOD	5′CCAGCGGATGAAGAGAGG3′	5′CCAATCACACCACAAGCC3′	230
Mn-SOD	5′GACCTGCCTTACGACTATG3′	5′TACTTCTCCTCGGTGACG3′	119
CAT	5′ATTGCCGTCCGATTCTCC3′	5′CCAGTTACCATCTTCAGTGTAG3′	105
GPX	5′GTTCGGACATCAGGAGAATGG3′	5′GGGTTCGATGTCGATGGTGC3′	331

G3PDH glyceraldehyde 3-phosphate dehydrogenase, *CS* citrate synthase, *Cu,Zn-SOD* Cu,Zn-superoxide dismutase, *Mn-SOD* Mn-superoxide dismutase, *CAT* catalase, *GPX* glutathione peroxidase

Briefly, 15 μg of total RNA pool from soleus muscles of each group and 2 μl of primer mix (a mixture of primers relative to the genes spotted in the macroarray membrane) were heated at 70°C for 5 min in a Techne-Genius Thermal cycler (Oxford, UK). The temperature was decreased to 50°C and 13.5 μl of the mix of the following reagents were added: 4 μl reaction buffer 5×, 0.5 μl 100 mM DTT, 2 μl 10× dNTP mix (dCTP, dGTP, dTTP, and dATP), 5 μl [α-^{33}P] ATP (at 10 μCi μl^{-1}) and 2 μl of reverse transcriptase enzyme. The mixture was incubated for 25 min at 50°C and stopped by adding 2 μl of Termination Mix. The ^{33}P-labeled probe was purified from unincorporated nucleotides by passing the reaction mixture through a push column (NucleoSpin Extraction Spin Column, Clontech Laboratories).

Macroarray hybridization

The Rat Toxicology Array II (PT3567-3, Clontech Laboratories) was used. A list of the 464 genes in the macroarray membranes is available at the Clontech website (www.clontech.com/clontech/atlas/genelists/). The membrane was pre-hybridized for 30 min at 68°C in Express Hyb containing 50 μg of freshly denaturated salmon sperm DNA. Subsequently, the membrane was hybridized during 18 h at 68°C with ^{33}P-labeled denaturated probe (2×10^6 cpm ml^{-1}). The membrane was washed four times at 68°C with 1× SSC, 0.1% SDS; followed by one washing in 1× SSC, 1% SDS and then exposed to phosphor screen for 48 h and scanned in the Storm 840 (Molecular Dynamics, Sunnyvale, CA, USA).

Analysis of macroarray results

Changes in expression from electrical stimulated soleus muscles were analyzed by comparison with the results of expression observed in the soleus muscle from control rats using the software Array-Pro™ Analyzer, Version 4 (Media Cybernetics, Silver Spring, MD, USA). The results were presented as mean of the normalizations performed by using the housekeeping genes G3PDH and α-tubulin, present in the membrane. Triplicate hybridizations using separate sets of nylon membranes were performed for all conditions. Only signals that differed from the control by at least twofold in the three independent experiments were considered as significantly regulated. A similar procedure was used in previous studies (Verlengia et al. 2004; Yamazaki et al. 2002).

Protein determination

Total tissue protein was determined by the method of Bradford (1976) using bovine serum albumin as standard.

Statistical analysis

The differences between groups were assessed by using Student's *t*-test or one-way ANOVA with Tukey's post hoc test. For the assays of mRNA content of the antioxidant enzymes, Welch's correction was used since the data did not present a Gaussian distribution. Significance was set at $P < 0.05$. Results are presented as mean ± SEM. Analysis were performed by using GraphPad Prism Version 4.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

Results

There was no change of CS activity in the stimulated soleus muscle isolated immediately after ES as compared to control. However, an increase of 63% in CS activity was observed in the stimulated soleus muscle 1 h after ES (Fig. 1).

The values of CS mRNA levels were normalized by the respective controls of each experiment. CS mRNA levels were determined in rat soleus muscles immediately after ES and 1 h afterwards. An increase of 80% in CS mRNA levels was observed 1 h after ES as compared with controls (Fig. 2).

The analysis by macroarray revealed an increase in expression of several genes 1 h after ES (data not shown). Among these genes, expression of Mn-SOD (SOD-2), Cu,Zn-SOD (SOD-1), CAT, and GPX were increased by 31.8-, 23.9-, 14-, and 6.2-fold, respectively (Table 2).

Real time RT-PCR was performed for antioxidant enzymes in soleus muscle isolated 1 h after ES. A marked increase of mRNA levels of Mn-SOD, Cu,Zn-SOD, CAT, and GPX by 43-, 3-, 21-, and 0.6-fold, as compared to the control values, respectively, was observed (Fig. 3).

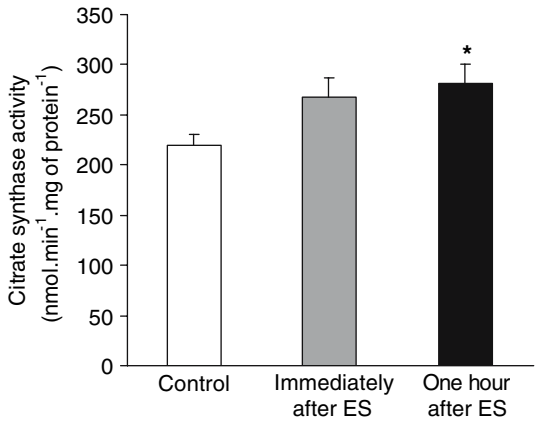

Fig. 1 Citrate synthase activity from soleus muscles control and stimulated isolated immediately after the electrical stimulation and 1 h after. *Different from control ($P = 0.005$; $n = 7$ in each group)

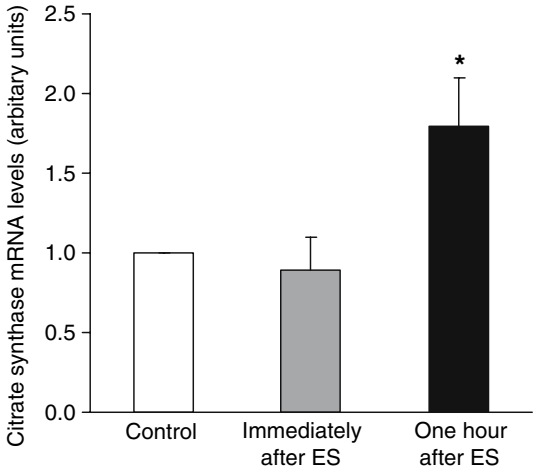

Fig. 2 Citrate synthase mRNA levels in soleus muscle obtained from rats immediately after the electrical stimulation and 1 h after. Data are presented as the ratio of respective controls, which received an arbitrary value of 1 in each experiment. *Different from controls ($P = 0.0088$; $n = 6$ in each group)

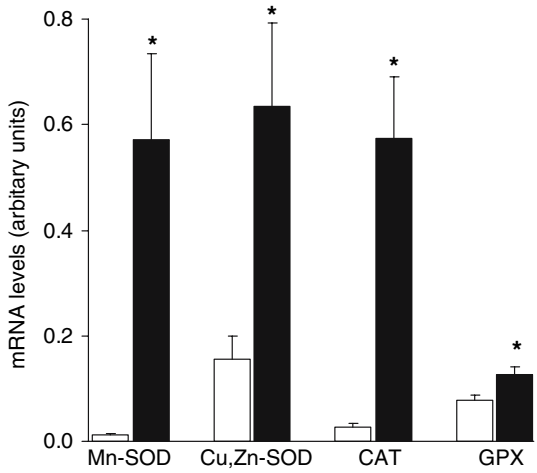

Fig. 3 mRNA levels of Mn-superoxide dismutase (*SOD*), Cu,Zn-SOD, catalase (*CAT*) and glutathione peroxidase (*GPX*) in control (*white bars*) and stimulated (*black bars*) soleus muscles, 1 h after electrical stimulation. *Different from respective controls, $P = 0.018$, 0.034, 0.002, and 0.017 for Mn-SOD, Cu,Zn-SOD, CAT, and GPX, respectively ($n = 6$ in each group)

Table 2 Increase in expression of the antioxidant enzymes in stimulated soleus muscle isolated 1 h after electrical stimulation

Access number	Gene name	S/C
X56600	Superoxide dismutase 2	31.8
Y00404	Superoxide dismutase 1	23.9
M11670	Catalase	14.0
X12367	Glutathione peroxidase 1	6.2

Gene expression was increased in stimulated soleus muscle (S) as compared to control from unstimulated muscle (C). In S/C column, the values are expressed as times of increase in comparison to control. The results were presented as mean of the normalizations performed by using the housekeeping genes G3PDH and α-tubulin, present in the membrane

Catalase activity in soleus muscles was increased by 44% 1 h after ES (Fig. 4). However, there was no alteration in the activities of Mn-SOD, Cu,Zn-SOD, and GPX.

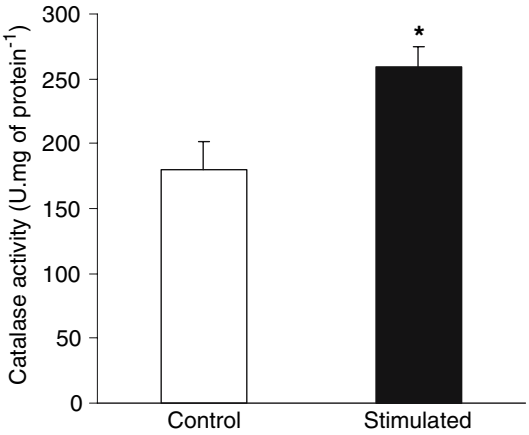

Fig. 4 Catalase activity from soleus muscle control and 1 h after electrical stimulation. *Different from control, $P = 0.020$ ($n = 6$ in each group)

Discussion

Skeletal muscle has a remarkable capacity to adapt to different stimulus imposed by contraction (Atherton et al. 2005; Neufer and Dohm 1993). Endurance training is highly associated with elevated muscle oxidative capacity, activity of mitochondria enzymes and increased muscle oxygen uptake (Atherton et al. 2005; Nader and Esser 2001). In contrast, resistance training is associated with increased muscle mass, fiber hypertrophy, and gain in strength (Nader and Esser 2001).

Several reports have shown that acute muscle contraction induces marked increase in CS activity in both humans and rats (Tonkonogi et al. 1997; Leek et al. 2001; Fernstrom et al. 2004; Jacobs et al. 1987), an effect similar to that observed in chronic endurance training. Most of these studies were performed using moderate intensity exercise, which is closely related to endurance exercise adaptation (Booth and Thomason 1991; Holloszy and Booth 1976). In our study, we did not find any effect of an acute moderate-intensity muscle contraction induced by ES on CS activity (data not shown). In contrast, a single session of high-intensity muscle contraction caused a marked increase in CS activity.

The mechanisms behind this phenomenon still remain unclear. Different hypotheses have been raised to explain the increase in CS activity after acute exercise as the existence of an alternative CS isoform which is transcriptionally

regulated, or an enzyme covalent modification (Leek et al. 2001; Roepstorff et al. 2005; Siu et al. 2003). However, none of these hypotheses has been confirmed. In addition to the mechanisms mentioned above, a decrease in allosteric inhibition by citrate has been proposed (Leek et al. 2001). The effect of oxaloacetate to enhance acetyl-CoA binding to the catalytic site of CS and to inhibit the effect of citrate on this enzyme is well described in in vitro assays (Beeckmans 1984). However, in our experiments, the activity of CS was assayed in a reaction medium containing saturated concentrations of substrates (Srere et al. 1963). Therefore, changes in intramuscular concentrations of oxaloacetate, acetyl-CoA, and citrate during contractions cannot be accounted for the results obtained.

The absence of alteration in CS activity immediately after ES is suggestive that the exercise-induced increase in the activity of this enzyme depends on transcriptional regulation. This was confirmed by our findings showing a marked increase in CS expression 1 h after induction of muscle contraction, but not immediately afterwards. Although the length of time from the beginning of muscle contraction to the point of removal of the muscle was relatively short (2 h), we observed that exercise induced a significant increase in CS expression.

Other studies (Neufer and Dohm 1993; Vissing et al. 2005) found that acute exercise increases CS expression in skeletal muscle but the activity of this enzyme was not measured. In rats, only Siu et al. (2003) found an increase in activity and expression of this enzyme in rat soleus muscle 1 h after one session of treadmill exercise. However, differently from our study, the rats submitted to acute exercise were previously trained and they were compared to a sedentary group. This is critical because the increase of both CS activity and expression induced by acute exercise could not be distinguished from the adaptations potentially promoted by chronic training.

Atherton et al. (2005) proposed that high intensity muscle contraction as obtained by resistance exercise results in activation of the PKB-TSC2-mTOR pathway leading to muscle hypertrophy. On the other hand, oxidative exercise results in activation of specific intracellular signaling steps mediated by the AMPK/PGC-1α pathway, which promotes oxidation in skeletal muscle. The experimental protocol used in the present study has been demonstrated to cause a marked reduction in the content of ATP, phosphocreatine (Wojtaszewski et al. 1996) in soleus and gastrocnemius (glycolytic type II) muscles. In our experiments, the high intensity stimulus promoted by ES led to a decrease of almost 60% force output after the first minute of contraction followed by additional reductions in force production as time of exercise progressed. In fact, after 4 min of contraction, force output corresponded to only 30% of maximum and remained at this level afterwards (data not shown). This is in agreement with previous observations that fatiguing muscle contractions caused elevated metabolic stress and significant reductions in force production during resistance exercise in humans (Sahlin and Ren 1989).

Increases in activities of oxidative enzymes, such as CS and β-hydroxyacyl CoA dehydrogenase, have been shown to occur after resistance training in human vastus lateralis muscle (Tang et al. 2006; Braith et al. 2005), indicating that high-intensity training might induce alterations in oxidative metabolism in skeletal muscle. Unfortunately, the lack of a specific antibody against CS prevented us from determining whether the increase in the expression of CS also led to and increase in the content of this protein in the muscles subjected to ES.

Analysis by Real Time RT-PCR revealed a marked increase in the expression of Mn-SOD, Cu,Zn-SOD, CAT, and GPX 1 h after ES protocol. These findings confirm our observations obtained by macroarray analysis showing increases in expression of these genes. Only few studies have investigated the effect of acute exercise on expression of antioxidant enzymes in rat skeletal muscle (Hollander et al. 2001; Itoh et al. 2004; Oh-ishi et al. 1997; Ohishi et al. 1998). With respect to CAT and GPX, it has been reported that no alterations in the expression of these enzymes occurred in rat diaphragm muscle after 1 h of treadmill exercise (Itoh et al. 2004). Inconclusive results have been presented regarding the responses of Mn-SOD and Cu,Zn-SOD expression after acute exercise. There are data reporting increase (Itoh et al. 2004), decrease (Oh-ishi et al. 1997; Ohishi et al. 1998), or no alteration (Hollander et al. 2001) in the expression of these enzymes in skeletal muscle after an acute treadmill exercise.

The marked increase in expression of the antioxidant enzymes in soleus muscle was probably due to the high metabolic demand induced by ES. The elevation in Mn-SOD expression has been associated with activation of NF-κB and AP-1, which is induced by ROS produced during muscle contraction (Hollander et al. 2001). NF-κB and AP-1 bind to the promoter region of the Mn-SOD gene leading to an increase in expression of this enzyme. Although we have not assessed these parameters, it is expected that the high metabolic demand induced by ES causes an increase in ROS production, which, in turn, could lead to expression of the antioxidant enzymes.

Most of the studies that investigated the effect of acute exercise on skeletal muscle have shown no alteration in activities of Mn-SOD, Cu,Zn-SOD or total SOD (Cooper et al. 1986; Hollander et al. 2001; Itoh et al. 2004; Ji et al. 1990, 1992; Lawler et al. 1993; Ohishi et al. 1998). CAT activity seems not to be changed by acute exercise (Itoh et al. 2004; Ji et al. 1988, 1990, 1992; Ohishi et al. 1998), except for two studies (Ji and Fu 1992; Oh-ishi et al. 1997)

where this enzyme activity was increased. Most studies have found an increase of GPX activity in skeletal muscle after a single session of exercise (Itoh et al. 2004; Ji et al. 1998, 1990, 1992; Ji and Fu 1992; Lawler et al. 1993, 1994; Oh-ishi et al. 1997). Interestingly, in the studies of Itoh et al. (2004) and Oh-ishi et al. (1997) where there were increases in GPX and Cu,Zn-SOD activities, respectively, these alterations were not associated to elevations in mRNA levels. This suggests a non-transcriptional mechanism for the increase in the activities of these enzymes, such as reduction of protein degradation. On the other hand, Khassaf et al. (2001) observed an increase of total SOD activity, up to 3 days after acute intense aerobic exercise in vastus lateralis muscle as compared to basal. This finding suggests that up-regulation of gene expression is an important mechanism for the increased activities of antioxidant enzymes induced by exercise. In the present study, although the expression of the four antioxidant enzymes was increased, only CAT activity was significantly raised 1 h after ES. In fact, according to our results and from the others (Hollander et al. 2001; Itoh et al. 2004), the increased expression of the antioxidant enzymes not always result in a concomitant augment of their activities. Therefore, it is possible that a longer period of time is necessary to have protein translation and so elevation of the activities of Mn-, Cu,Zn-SOD, and GPX.

In summary, evidence is provided herein that high-intensity muscle contraction promoted an increase of activity and expression of CS. This protocol also increased the expression of the antioxidant enzymes (Mn-SOD, Cu,Zn-SOD, CAT, and GPX). The increase in CS and CAT activities may be associated to high expression of these proteins in stimulated soleus muscle but other mechanisms for stimulation of enzymes cannot be ruled out. The increase in expression of CAT may play an important role in counteracting the potential deleterious effects of elevated oxidative stress induced by a high oxidative demand in skeletal muscles subjected to intense contraction. Taken together these findings point out that a high-intensity muscle contraction session modulates expression of important enzymes of cell metabolism.

Acknowledgments The authors are indebted to the constant technical assistance of E. P. Portiolli, T. C. Alba, J. R. Mendonça, and G. de Souza. This study is supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Atherton PJ, Babraj J, Smith K, Singh J, Rennie MJ, Wackerhage H (2005) Selective activation of AMPK-PGC-1alpha or PKB-TSC2-mTOR signaling can explain specific adaptive responses to endurance or resistance training-like electrical muscle stimulation. FASEB J 19(7):786–788

Beeckmans S (1984) Some structural and regulatory aspects of citrate synthase. Int J Biochem 16:341–351

Braith RW, Magyari PM, Pierce GL, Edwards DG, Hill JA, White LJ, Aranda JM Jr (2005) Effect of resistance exercise on skeletal muscle myopathy in heart transplant recipients. Am J Cardiol 15; 95(10):1192–1198

Booth FW, Thomason DB (1991) Molecular and cellular adaptation of muscle in response to exercise: perspectives of various models. Physiol Rev 71:541–585

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Cooper MB, Jones DA, Edwards RH, Corbucci GC, Montanari G, Trevisani C (1986) The effect of marathon running on carnitine metabolism and on some aspects of muscle mitochondrial activities and antioxidant mechanisms. J Sports Sci 4:79–87

Fernstrom M, Tonkonogi M, Sahlin K (2004) Effects of acute and chronic endurance exercise on mitochondrial uncoupling in human skeletal muscle. J Physiol 554:755–763

Flohe L, Otting F (1984) Superoxide dismutase assays. Methods Enzymol 105:93–104

Green H, Grant S, Bombardier E, Ranney D (1999) Initial aerobic power does not alter muscle metabolic adaptations to short-term training. Am J Physiol 277:E39–E48

Hainaut K, Duchateau J (1992) Neuromuscular electrical stimulation and voluntary exercise. Sports Med 14:100–113

Higuchi R, Dollinger G, Walsh PS, Griffith R (1992) Simultaneous amplification and detection of specific DNA sequences. Biotechnology (N Y) 10:413–417

Hollander J, Fiebig R, Gore M, Ookawara T, Ohno H, Ji LL (2001) Superoxide dismutase gene expression is activated by a single bout of exercise in rat skeletal muscle. Pflugers Arch 442:426–434

Holloszy JO, Booth FW (1976) Biochemical adaptations to endurance exercise in muscle. Annu Rev Physiol 38:273–291

Holloszy JO, Oscai LB, Don IJ, Mole PA (1970) Mitochondrial citric acid cycle and related enzymes: adaptive response to exercise. Biochem Biophys Res Commun 40:1368–1373

Itoh M, Oh-Ishi S, Hatao H, Leeuwenburgh C, Selman C, Ohno H, Kizaki T, Nakamura H, Matsuoka T (2004) Effects of dietary calcium restriction and acute exercise on the antioxidant enzyme system and oxidative stress in rat diaphragm. Am J Physiol Regul Integr Comp Physiol 287:R33–R38

Jacobs I, Esbjornsson M, Sylven C, Holm I, Jansson E (1987) Sprint training effects on muscle myoglobin, enzymes, fiber types, and blood lactate. Med Sci Sports Exerc 19:368–374

Ji LL, Dillon D, Wu E (1990) Alteration of antioxidant enzymes with aging in rat skeletal muscle and liver. Am J Physiol 258:R918–R923

Ji LL, Fu R (1992) Responses of glutathione system and antioxidant enzymes to exhaustive exercise and hydroperoxide. J Appl Physiol 72:549–554

Ji LL, Fu R, Mitchell EW (1992) Glutathione and antioxidant enzymes in skeletal muscle: effects of fiber type and exercise intensity. J Appl Physiol 73:1854–1859

Ji LL, Stratman FW, Lardy HA (1988) Enzymatic down regulation with exercise in rat skeletal muscle. Arch Biochem Biophys 263:137–149

Khassaf M, Child RB, McArdle A, Brodie DA, Esanu C, Jackson MJ (2001) Time course of responses of human skeletal muscle to oxidative stress induced by nondamaging exercise. J Appl Physiol 90:1031–1035

Lawler JM, Powers SK, Van Dijk H, Visser T, Kordus MJ, Ji LL (1994) Metabolic and antioxidant enzyme activities in the diaphragm: effects of acute exercise. Respir Physiol 96:139–149

Lawler JM, Powers SK, Visser T, Van Dijk H, Kordus MJ, Ji LL (1993) Acute exercise and skeletal muscle antioxidant and metabolic enzymes: effects of fiber type and age. Am J Physiol 265:R1344–R1350

Leek BT, Mudaliar SR, Henry R, Mathieu-Costello O, Richardson RS (2001) Effect of acute exercise on citrate synthase activity in untrained and trained human skeletal muscle. Am J Physiol Regul Integr Comp Physiol 280:R441–R447

Leeuwenburgh C, Fiebig R, Chandwaney R, Ji LL (1994) Aging and exercise training in skeletal muscle: responses of glutathione and antioxidant enzyme systems. Am J Physiol 267:R439–R445

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408

Navarro-Arevalo A, Canavate C, Sanchez-del-Pino MJ (1999) Myocardial and skeletal muscle aging and changes in oxidative stress in relationship to rigorous exercise training. Mech Ageing Dev 108:207–217

Nader GA, Esser KA (2001) Intracellular signaling specificity in skeletal muscle in response to different modes of exercise. J Appl Physiol 90(5):1936–1942

Neufer PD, Dohm GL (1993) Exercise induces a transient increase in transcription of the GLUT-4 gene in skeletal muscle. Am J Physiol 265:C1597–C1603

Oh-ishi S, Kizaki T, Nagasawa J, Izawa T, Komabayashi T, Nagata N, Suzuki K, Taniguchi N, Ohno H (1997) Effects of endurance training on superoxide dismutase activity, content and mRNA expression in rat muscle. Clin Exp Pharmacol Physiol 24:326–332

Ohishi S, Kizaki T, Ookawara T, Toshinai K, Haga S, Karasawa F, Satoh T, Nagata N, Ji LL, Ohno H (1998) The effect of exhaustive exercise on the antioxidant enzyme system in skeletal muscle from calcium-deficient rats. Pflugers Arch 435:767–774

Ortenblad N, Madsen K, Djurhuus MS (1997) Antioxidant status and lipid peroxidation after short-term maximal exercise in trained and untrained humans. Am J Physiol 272:R1258–R1263

Ramakers C, Ruijter JM, Deprez RH, Moorman AF (2003) Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci Lett 339:62–66

Roepstorff C, Schjerling P, Vistisen B, Madsen M, Steffensen CH, Rider MH, Kiens B (2005) Regulation of oxidative enzyme activity and eukaryotic elongation factor 2 in human skeletal muscle: influence of gender and exercise. Acta Physiol Scand 184:215–224

Sahlin K, Ren JM (1989) Relationship of contraction capacity to metabolic changes during recovery from a fatiguing contraction. J Appl Physiol 67:648–654

Sambrook LE, Fritisch EF, Maniatis T (2001) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York

Silveira LR, Hirabara SM, Alberici LC, Lambertucci RH, Peres CM, Takahashi HK, Pettri A, Alba-Loureiro T, Luchessi AD, Cury-Boaventura MF, Vercesi AE, Curi R (2007) Effect of lipid infusion on metabolism and force of rat skeletal muscles during intense contractions. Cell Physiol Biochem 20(1–4):213–226

Siu PM, Donley DA, Bryner RW, Alway SE (2003) Citrate synthase expression and enzyme activity after endurance training in cardiac and skeletal muscles. J Appl Physiol 94:555–560

Spina RJ, Chi MM, Hopkins MG, Nemeth PM, Lowry OH, Holloszy JO (1996) Mitochondrial enzymes increase in muscle in response to 7–10 days of cycle exercise. J Appl Physiol 80:2250–2254

Srere PA, Brazil H, Gonen L (1963) The citrate condensing enzyme of pigeon breast muscle and moth flight muscle. Acta Chemica Scandinavica 17:129–134

Tang JE, Hartman JW, Phillips SM (2006) Increased muscle oxidative potential following resistance training induced fibre hypertrophy in young men. Appl Physiol Nutr Metab 31(5):495–501

Tonkonogi M, Harris B, Sahlin K (1997) Increased activity of citrate synthase in human skeletal muscle after a single bout of prolonged exercise. Acta Physiol Scand 161:435–436

Verlengia R, Gorjao R, Kanunfre CC, Bordin S, de Lima TM, Martins EF, Newsholme P, Curi R (2004) Effects of EPA and DHA on proliferation, cytokine production, and gene expression in Raji cells. Lipids 39:857–864

Vissing K, Andersen JL, Schjerling P (2005) Are exercise-induced genes induced by exercise? Faseb J 19:94–96

Wendel A (1981) Glutathione peroxidase. Methods Enzymol 77:325–333

Wiegand G, Remington SJ (1986) Citrate synthase: structure, control, and mechanism. Annu Rev Biophys Biophys Chem 15:97–117

Wojtaszewski JF, Hansen BF, Urso B, Richter EA (1996) Wortmannin inhibits both insulin- and contraction-stimulated glucose uptake and transport in rat skeletal muscle. J Appl Physiol 81:1501–1509

Yamazaki K, Kuromitsu J, Tanaka I (2002) Microarray analysis of gene expression changes in mouse liver induced by peroxisome proliferator- activated receptor alpha agonists. Biochem Biophys Res Commun 290:1114–1122