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HIGHLIGHTED TOPIC | *Regulation of Protein Metabolism in Exercise and Recovery*

Decreased muscle ACE activity enhances functional response to endurance training in rats, without change in muscle oxidative capacity or contractile phenotype

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Habouzit E, Richard H, Sanchez H, Koulmann N, Serrurier B, Monnet R, Ventura-Clapier R, Bigard X. Decreased muscle ACE activity enhances functional response to endurance training in rats, without change in muscle oxidative capacity or contractile phenotype. *J Appl Physiol* 107: 346–353, 2009. First published April 30, 2009; doi:10.1152/japplphysiol.91443.2008.—In the present study, we tested the hypothesis that chronic ANG I-converting enzyme (ACE) inhibition could improve the training-induced improvement in endurance exercise performance and that this could be related to enhanced skeletal muscle metabolic efficiency. Female Wistar rats were assigned to four groups comprising animals either maintained sedentary or endurance trained (Sed and Tr, respectively), and treated or not for 10 wk with an ACE inhibitor, perindopril ($2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) (Per and Ct, respectively) ($n = 8$ each). Trained rats underwent an 8-wk treadmill training protocol that consisted of 2 h/day running at 30 m/min on a 8% decline. Before the start of and 1 wk before the end of experimental conditioning, the running time to exhaustion of rats was measured on a treadmill. The training program led to an increase in endurance time, higher in Tr-Per than in Tr-Ct group (125% in Tr-Ct vs. 183% in Tr-Per groups, $P < 0.05$). Oxidative capacity, measured in saponin-permeabilized fibers of slow soleus and fast plantaris muscles, increased with training, but less in Tr-Per than in Tr-Ct rats. The training-induced increase in citrate synthase activity also was less in soleus from Tr-Per than Tr-Ct rats. The training-induced increase in the percentage of the type IIa isoform of myosin heavy chain (MHC) (45%, $P < 0.05$) and type IIx MHC (25%, $P < 0.05$) associated with decreased type IIb MHC (34%, $P < 0.05$) was minimized by perindopril administration. These findings demonstrate that the enhancement in physical performance observed in perindopril-treated animals cannot be explained by changes in mitochondrial respiration and/or MHC distribution within muscles involved in running exercise.

mitochondria; myosin heavy chain; angiotensin I-converting enzyme inhibitor; perindopril; running performance

ENDURANCE TRAINING leads to molecular and cellular adaptations that mainly occur in the cardiovascular system and skeletal muscles and that improve performance during prolonged exercise. Skeletal muscle adaptation to endurance training includes quantitative and qualitative changes in mitochondria,

marked development of muscle capillary network, and transition in isoforms of myosin heavy chain. Such training-induced changes contribute to improve aerobic capacity and reliance on oxidative metabolism to provide energy (for review, see Refs. 9 and 14). Individual responses to exercise training result from environmental factors (training program, altitude, nutrition) and are also obviously influenced by genetic factors (5). Recent advances in the sequencing of the human genome have strengthened efforts made to understand genetic differences that may explain individual responses to training, especially to endurance training (18, 19). Although it is unlikely that the genetic component of endurance performance phenotype might be explained by DNA sequence variations of only a few genes, some studies attempted to relate whether the polymorphism of candidate genes might be associated with some aspects of physical performance.

The most studied of the DNA sequence variations potentially associated with endurance performance and response to physical training is the *I/D* polymorphism of the ANG I-converting enzyme (ACE) gene (for review, see Ref. 11). The absence (deletion, *D* allele) of a 287-bp fragment in intron 16 of the *ACE* gene is associated with increased ACE activity, known to influence blood pressure through both the generation of the vasoconstrictor factor ANG II deriving from ANG I, and inactivation of the vasodilatator bradykinin. Conversely, the presence (insertion, *I* allele) of this fragment is associated with lower ACE activity in serum (20). Moreover, local renin-angiotensin systems exist in many tissues including skeletal muscle, and these systems might affect muscle metabolic efficiency, likely through bradykinin availability (7). In human, the ACE genotype-dependent variation in ACE activity may thus influence endurance performance through a variety of mechanisms, including altered circulatory homeostasis (11) and/or skeletal muscle metabolism (7), two determinant factors of physical performance.

Although controversial, several human studies including elite groups of athletes suggested that the *I* allele and low circulating ACE levels were associated with endurance athletic performance (for review, see Ref. 11). Maximum exertional oxygen uptake ($\dot{V}_{O_{2max}}$) is determined by changes in oxygen transport and consumption. Performance in endurance events is limited by $\dot{V}_{O_{2max}}$ but also by the proportion of $\dot{V}_{O_{2max}}$ that can be sustained and muscular efficiency, both factors determined

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by skeletal muscle characteristics (4). It has been shown that the putative association between the *I* allele, and thus low ACE activity in plasma and tissue, and endurance performance could be due rather to local muscle factors than central cardiovascular effects (21, 31). Moreover, the ACE *I* allele is mostly associated with an increased percentage of fibers with a low energy cost per unit of force, i.e., type I fibers in human skeletal muscle (34). Taken together, these findings suggest that low ACE activity could have positive effects on muscle efficiency during exercise. This hypothesis has been previously tested in sedentary rats and ACE inhibition failed to affect the endurance capacity of animals during running exercise and/or the maximal oxidative capacity of skeletal muscles (3).

The putative advantage of the II genotype on endurance performance has never been reported in sedentary subjects. However, the hypothesis that the *ACE* gene I/D polymorphism could influence the adaptive responses to endurance training, especially muscular responses, has been previously tested. The mechanical efficiency of human muscle has been shown to increase in response to training, but only in subjects of the II genotype, those with low ACE activity (30). However, the cellular and molecular mechanisms responsible for the greater improvement in muscle efficiency reported in subjects with low ACE activity after endurance training remain to be elucidated. We thus studied the effects of ACE inhibition on training-related changes in the capacity of muscle mitochondria to oxidize energetic substrates via oxidative phosphorylation and on the shift toward type I fibers.

The purpose of this study was to examine whether or not muscular ACE activity influences the adaptive responses of skeletal muscle to endurance training. We hypothesized that chronic ACE inhibition combined with endurance training could improve endurance performance more than training alone and that this association could positively affect skeletal muscle metabolic efficiency. To address this issue, we measured the endurance performance of perindopril-treated rats, either maintained sedentary or submitted to 8-wk treadmill training. In addition to training-induced changes in running performance, we examined the associated alterations in muscle oxidative capacity and contractile phenotype. In the present study, oxidative capacity was assessed through respiratory parameters of the total mitochondrial population measured in situ in fresh saponin-skinned fibers as previously described (15, 28).

MATERIALS AND METHODS

Animals. Female Wistar rats initially weighing 175–200 g were housed two per cage in a thermoneutral environment (22 ± 2°C) on a 12:12-h light-dark period and were provided with food and water ad libitum throughout the study period. This study was performed in accordance with the Helsinki accords for humane treatment of laboratory animals and the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe no. 129, Strasbourg, 1985) and was approved by the Animal Ethics Committee of the Centre de Recherches du Service de Sante des Armees.

Experimental design. After 4 days of acclimatization to the animal facility, all animals were familiarized with rodent treadmill and running exercise. Rats were running onto a rodent treadmill with a stainless steel grid, 10–25 m/min for 10 min/day, at 0–8° grade. Mild electrical shock stimulation was applied, if necessary, only to maintain the running motivation.

To measure the maximal endurance performance all rats performed a bout of exhaustive running exercise. The animals were exercised to exhaustion at 25 m/min up a 8° gradient, 48 h after the last session of running acclimatization. Running time to exhaustion was measured when animals were unable to longer maintain the treadmill speed and lost the righting reflex.

After this first endurance test, animals were randomly assigned to one of four groups, designed as either sedentary control (Sed-Ct, $n = 8$), sedentary perindopril-treated (Sed-Per, $n = 8$), trained control (Tr-Ct, $n = 8$), and trained perindopril-treated (Tr-Per, $n = 8$). Perindopril was given orally (in drinking water, 2 mg·kg^{-1}·day^{-1}) for 10 wk; all animals were weighted regularly to adjust perindopril dose. To begin the endurance training program with low ACE activity, running exercises started 2 wk after beginning the perindopril treatment and lasted 8 wk. Animals ran on a motorized treadmill 5 days/wk using an exercise program involving both progressive intensity and duration. Briefly, the rodents were initially running trained at 10 m/min for 15 min, and by the end of 2 wk, rats were running at 30 m/min on an 8% grade for 2 h/day. This intensity and duration were maintained for the remaining 6 wk of the training program. Electrical shocks were used sparingly to encourage the animals to run. This protocol was selected because it has been shown that an increase in mitochondrial oxidative activity.

One week before the end of the training program and/or perindopril administration, the running time to exhaustion of rats of all experimental groups was assessed. Trained animals stayed 48 h resting before exhaustive running exercise.

Surgical procedures. Forty-eight hours after the last endurance test, animals were anesthetized with pentobarbital sodium administered intraperitoneally (50 mg/kg body wt). Soleus (Sol) and plantaris (Pla) muscles of both hindlimbs were immediately excised. Right muscles were put in Krebs solution composed of 118 mM NaCl, 25 mM NaHCO$_3$, 4.7 mM KH$_2$PO$_4$, 1.2 mM MgSO$_4$, and 4.7 mM KCl for mitochondrial respiration experiments. Left ones were quickly frozen in liquid nitrogen until biochemical assays were performed.

Determination of plasma and skeletal muscle ACE activity. The efficacy of perindopril administration was assessed on both plasma and muscle ACE activity. Perindopril-induced alterations in ACE activity were first tested through the accumulation of acetyl-Ser-Asp-Lys-Pro (AcSDKP), a tetrapeptide that is cleared from plasma by ACE-mediated hydrolysis (2). Plasma AcSDKP levels were determined by a competitive enzyme immunoassay, as previously reported (2).

Skeletal muscle ACE activity was determined in a crude muscle homogenate, using a modified sensitive fluorometric assay according to Morrel et al. (17). Plantaris muscle samples were thawed and homogenized at 4°C in Tris (0.05 M, pH 7.4)-buffered saline (0.15 M NaCl), and homogenates were then centrifuged at 1,000 *g* for 15 min at 4°C. One-hundred microliters of 5 mM Phe-His-Leu substrate, 50 mM Tris, pH 7.5, and 150 mM NaCl were added to crude homogenates. The reaction was stopped after 60 min at 37°C by addition of 750 µl 0.28 M NaOH. Fifty microliters of *o*-phthalaldehyde (1 mg in 100 µl methanol) was added for 10 min, and the final reaction was then stopped by addition of 100 µl 2 N HCl. The fluorescence of the samples was measured with a fluorescence spectrometer (Kontron Instrument Win25, Montigny le Bretonneux, France) at an emission wavelength of 424 nm and an excitation wavelength of 354 nm. Samples were run in duplicate, and blanks (all reagents except homogenates) and samples incubated in the presence of the ACE inhibitor captopril were included. The results were expressed as microunits (mU) per milligram of protein, where 1 mU represents the generation of 1 nmol His-Leu/min. The protein concentration was determined by using the Bradford method.

In situ study of mitochondrial respiration. Respiratory parameters of the total mitochondrial population were studied in situ in saponin-skinned fibers, using the method described earlier (15, 28). Thin fibers bundles were excised from skeletal muscles and incubated with intense shaking for 30 min at 4°C in *solution S* (see below) containing

saponin (2.5 mg/ml) and rinsed 10 min in *solution S* alone. Five- to 10-mg fiber bundles were then rinsed in *solution R* or *solution R_O* (see below) to wash out adenine nucleotides and PCr. Respiration rates were determined using a Clark electrode in an oxygraphic cell (Hansatech Oxygraph Instrument, Norfolk, UK) containing 1.5 ml of *solution R* or R_O at 22°C with continuous stirring. After measurements, fibers bundles were carefully removed, dried (10 min at 110°C), and weighed. Respiration rates and substrates consumption were expressed as micromoles O_2 per minute per milligram dry weight.

Solutions S, R, and R_O were designed to mimic the intracellular milieu and were previously accurately described (28). The protocol was designed to determine the dependences of respiration on external [ADP] in the presence or in the absence of creatine (Cr) and with appropriate concentrations of glutamate and malate as substrates. Respiration rates were recorded in *solution R*. After addition of fibers, basal oxygen consumption ($\dot{V}_0$) due to proton leak was measured. Then, respiration rate was accelerated by addition of successive [ADP] until it reached the maximal oxygen consumption ($\dot{V}_{max}$). The ADP-stimulated respiration above $\dot{V}_0$ was then measured under the presence of saturating amount of ADP as phosphate acceptor and glutamate-malate as mitochondrial substrates ($\dot{V}_{GM}$) (28). Apparent K_m for ADP was calculated using a nonlinear fitting of the Michaelis-Menten equation. The acceptor-to-control ratio (ACR) was calculated as $\dot{V}_{GM}/\dot{V}_0$ and represented the functional coupling between oxidation and phosphorylation.

Citrate synthase activity. Frozen tissue samples from soleus and plantaris muscles were weighed and homogenized into ice-cold buffer (50 mg/ml) containing 5 mM HEPES (pH = 8.7), 1 mM EGTA, 0.1% Triton X-100, and 1 mM DTT. They were incubated for 60 min at 0°C to ensure complete enzyme extraction. Determination of citrate synthase (CS) activity was assayed at 30°C (pH = 7.5), using coupled enzyme systems as previously described (23). CS activity was given as micromoles per minute (i.e., international units, IU).

Analysis of myosin heavy chain proteins. Skeletal muscles were subjected to the analysis of myosin heavy chain (MHC) isoforms as described previously (25). A small section was taken from the midregion of the muscle. Tissue samples were minced with scissors, and myosin was extracted in seven volumes of an appropriate buffer. After 1 h of gentle shaking at 4°C, the mixture was centrifuged at 13,500 *g* for 15 min, and the supernatant containing myosin was diluted with one volume of glycerol. Extracts were stored at −20°C until the separation process. Electrophoresis was performed using a Mini Protean II system (Bio-Rad, Marne-la-Coquette, France). Separating gel solution contained 30% glycerol, 8% acrylamide-bis (50:1), 0.2 M Tris, 0.1 M glycine, and 0.4% sodium dodecyl sulfate (SDS). Stacking gel was composed of 30% glycerol, 4% acrylamide-bis (50:1), 70 mM Tris, 4 mM EDTA, and 0.4% SDS. Myofibril samples were denatured using a sample buffer containing 5% 2-β-mercaptoethanol, 100 mM Tris-base, 5% glycerol, 4% SDS, and bromophenol blue. Gels were run at constant voltage (72 V) for 31 h and then silver stained (1). The MHC protein isoform bands were scanned and quantified using a densitometer system equipped with an integrator (GS-700, Bio-Rad).

Statistical analysis. Data were expressed as means ± SE. A two-way ANOVA was used to determine the global effects of perindopril treatment and running training, and interactions between those factors. When appropriate, differences between groups were tested with a Newman-Keuls post hoc test. Within each experimental group, the comparison of K_m values with and without creatine was performed using a Student's paired *t*-test. Statistical significance was set at $P < 0.05$.

RESULTS

Body weight and muscle mass. At the start of the study, animals in all groups had similar body weight values. Final body weight remained unaffected by the experimental conditioning (Table 1). Soleus muscle weight, expressed as either absolute values or relative to body weight, slightly increased after endurance training (global effect of training, $P < 0.05$), while plantaris as fast-twitch muscle remained unaffected. Perindopril treatment failed to affect muscle mass.

Plasma and muscle tissue ACE activity. No effect of endurance training was shown on plasma and muscle ACE activity. The effectiveness of perindopril administration was thus examined in plasma and muscle samples from both Sed and Tr animals. As expected, perindopril-treated rats showed plasma AcSDKP levels approximately four times higher than those observed in control nontreated animals (Fig. 1*A*).

Mean tissue ACE activity in plantaris muscle was 13 times lower in perindopril-treated rats than in nontreated animals (Fig. 1*B*). ACE activity in plantaris muscles from Per groups corresponded to a residual tissue activity level of 11% of that measured in muscles from control nontreated rats.

Exercise capacity. At the beginning of experimental conditioning, female rats ran ~180 min before becoming exhausted, without difference between groups (Table 2). Ten weeks after, the running time to exhaustion decreased significantly in sedentary rats (54 and 53% in Sed-Ct and Sed-Per groups, respectively, $P < 0.01$), without effect of perindopril administration. In contrast, the training program led to a marked increase in the duration of the exhaustive running exercise (119 and 154% in Tr-Ct and Tr-Per groups, respectively, $P < 0.01$). Interestingly, the running time to exhaustion was 14% higher in Tr-Per than in Tr-Ct group ($P < 0.05$).

CS activity. There was a global effect of endurance training that increased CS activity in both soleus and plantaris muscles ($P < 0.001$) (Table 3). The training-induced increase in CS activity occurred in both perindopril-treated animals (25% and 33% in soleus and plantaris muscles, respectively, $P < 0.05$) and control nontreated rats (39% and 46% in soleus and plantaris muscles, respectively, $P < 0.05$). However, CS ac-

Table 1. *Anatomic data of sedentary control or perindopril-treated rats, and trained control or perindopril-treated rats*

	Sed-Ct ($n = 8$)	Sed-Per ($n = 8$)	Tr-Ct ($n = 8$)	Tr-Per ($n = 8$)	Global Effect of Tr	Global Effect of Per
Final body wt, g	259±6	283±6	274±11	274±6	NS	NS
Sol muscle weight, mg	112±7	128±4	134±9	138±10	$P < 0.05$	NS
Pla muscle weight, mg	216±17	257±8	235±8	231±7	NS	NS
Tibia length, cm	3.64±0.04	4.00±0.05	3.81±0.06	3.70±0.07	NS	NS
Sol/body wt, mg/g	0.43±0.03	0.45±0.01	0.49±0.03	0.50±0.03	$P < 0.05$	NS
Pla/body wt, mg/g	0.83±0.06	0.90±0.02	0.77±0.11	0.85±0.03	NS	NS

Values are means ± SE. Sed-Ct, sedentary control rats; Sed-Per, sedentary perindopril-treated rats; Tr-Ct, trained control rats; Tr-Per, trained perindopril-treated rats; Sol, soleus muscle; Pla, plantaris muscle; NS, not significant.
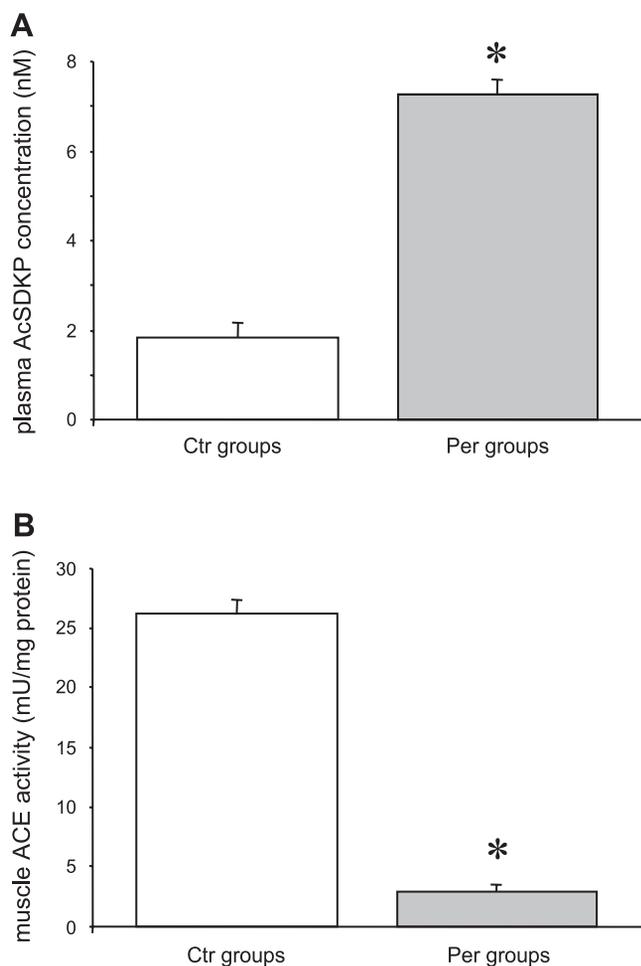
Fig. 1. Plasma acetyl-Ser-Asp-Lys-Pro (AcSDKP) levels (*A*) and plantaris muscle ANG I-converting enzyme (ACE) activity (*B*) measured in control (Ctr) and perindopril-treated animals (Per). Values are means ± SE. *Significance vs. Ctr groups, $P < 0.001$.

In plantaris muscle, endurance training increased both basal and maximal ADP-stimulated respiration rates ($\dot{V}_0$ and $\dot{V}_{GM}$, respectively) in control nontreated animals (36% and 38%, respectively, compared with Sed-Ct rats $P < 0.05$) (Fig. 2). Surprisingly, perindopril administration alone significantly increased $\dot{V}_0$ and $\dot{V}_{GM}$ in plantaris muscle taken from sedentary animals ($P < 0.05$). These alterations were not observed in trained animals. Like in soleus muscle, ACR values were slightly decreased by perindopril administration in both sedentary and physically trained rats (global effect $P < 0.05$) (Table 4). As expected in fast-twitch glycolytic muscles, values of the K_m for ADP were low in the presence or absence of creatine. In the absence of creatine, K_m values for ADP increased after 10 wk of perindopril administration in sedentary rats ($P < 0.05$), but values were still on the same order as those expected in fast-twitch glycolytic muscles. Endurance training failed to affect the mitochondrial affinity for ADP in either control or perindopril-treated rats.

MHC distribution. In Wistar rats, soleus muscle is known to comprise more than 95% type I MHC. The MHC distribution remained unaffected by either endurance training or repeated perindopril administration (data not shown).

In the fast-twitch plantaris muscles from sedentary rats, perindopril administration led to only a slight but significant decrease in the percentage of type IIb MHC (12%, $P < 0.05$) (Fig. 3). As expected, training alone in control nontreated rats induced an increase in the percentage of type IIa MHC (45%, $P < 0.05$) and type IIx MHC (25%, $P < 0.05$), at the expense of type IIb MHC (decrease by 34%, $P < 0.05$). Perindopril treatment associated with endurance training minimized the training-induced increase in type IIa MHC, and fully cancelled the expected increase in type IIx percentage and decrease in type IIb percentage, compared with Sed-Per animals. As a consequence, the relative contents of type IIa and type IIx MHC were lower, and the relative content of type IIb was higher in Tr-Per than in the Tr-Ct group (Fig. 3). No detectable change was shown in the percentage of type I MHC as a result of endurance training in either control or perindopril-treated rats.

DISCUSSION

Because the ACE genotype has never been associated with endurance performance in the untrained state, it has been suggested that the *I* allele and the decreased tissue ACE activity could be mainly related to greater enhancement in muscle metabolic efficiency in response to endurance training, rather than improving basal performances (11, 30). The present

tivity in soleus muscle remained 19% lower in perindopril-treated than in control nontreated rats after endurance training ($P < 0.05$).

Mitochondrial function. In soleus muscle, basal respiration rate ($\dot{V}_0$) increased by ~50% with endurance training, but only in the control nontreated group ($P < 0.05$) (Fig. 2). The training-induced increase in the basal respiration rate was not observed in perindopril-treated rats, so that $\dot{V}_0$ values in Tr-Per rats were 26% lower than in Tr-Ct group ($P < 0.05$). The ADP-stimulated respiration of in situ mitochondria using glutamate-malate as substrates (i.e., $\dot{V}_{GM}$) was higher in soleus muscles from Tr-Ct than Sed-Ct animals (20%, $P < 0.05$). In contrast $\dot{V}_{GM}$ values were lower in trained perindopril-treated rats than in Tr-Ct animals ($P < 0.05$) (Fig. 2). The oxidation-phosphorylation coupling, expressed by ACR, slightly decreased with running training in both control and perindopril-treated rats (24% and 27%, respectively, $P < 0.05$) (Table 4). Moreover, repeated perindopril administration also decreased mean ACR values in both sedentary and trained animals (19% and 22%, $P < 0.05$). The mitochondrial affinity for ADP in the absence ($K_m\text{ADP}_{-Cr}$) or in the presence of creatine ($K_m\text{ADP}_{+Cr}$) remained unaffected either by endurance training or perindopril treatment.

Table 2. *Running time to exhaustion measured before and after the experimental protocol in sedentary control or perindopril-treated rats and trained control or perindopril-treated rats*

	Sed-Ct (*n* = 8)	Sed-Per (*n* = 8)	Tr-Ct (*n* = 8)	Tr-Per (*n* = 8)
Running time to exhaustion, min				
Before protocol	187±16	187±18	182±13	179±20
After protocol	86±6*	88±14*	398±20*†	454±16*†‡

Values are means ± SE *Significantly different from values measured before the protocol, $P < 0.05$. †Significantly different from corresponding Sed group, $P < 0.001$. ‡Significantly different from Tr-Ct group, $P < 0.05$.

Table 3. *CS activity for soleus and plantaris muscles of sedentary or trained rats, either control or perindopril treated*

	Sed-Ct ($n = 8$)	Sed-Per ($n = 8$)	Tr-Ct ($n = 8$)	Tr-Per ($n = 8$)	Global Effect of Tr	Global Effect of Per
CS activity, IU/g wet wt						
Sol	38.6±2.5	34.9±2.4	53.5±1.0*	43.5±2.1*†	$P < 0.001$	NS
Pla	31.1±2.0	31.1±1.8	45.4±3.3*	41.4±2.6*	$P < 0.001$	NS

Values are means ± SE. CS, citrate synthase; IU, international units. *Significantly different from values measured in corresponding Sed group, $P < 0.05$. †Significantly different from Tr-Ct group, $P < 0.05$.

study was thus undertaken to test this hypothesis in a validated animal model and examine the cellular mechanisms of such enhanced response to endurance training.

The effectiveness of perindopril administration on ACE activity was first examined. Because ACE is the main enzyme involved in AcSDKP metabolism, plasma AcSDKP was expected to increase after long-term perindopril administration (13). It is now a well-known fact that AcSDKP accumulates in plasma during chronic ACE inhibitor administration (2). The perindopril-induced increase in plasma AcSDKP observed in the present study is consistent with this finding and similar to that previously reported after captopril administration (13). Moreover, plantaris muscle ACE activity was markedly de-

creased after chronic perindopril administration, to a residual level similar to that reported after administration of one of the most efficient ACE inhibitors on tissue ACE activity (8). There is thus evidence that chronic perindopril administration decreased tissue ACE activity to residual levels within skeletal muscle.

The present study revealed that decreased ACE activity after repeated administration of the ACE inhibitor perindopril in trained rats was associated with a higher increase in the running exercise capacity than in control nontreated trained animals. Female rats were used in the present study regarding their natural higher ability to run and lower body weight growth rate than male rats (26). Enhanced exercise tolerance in
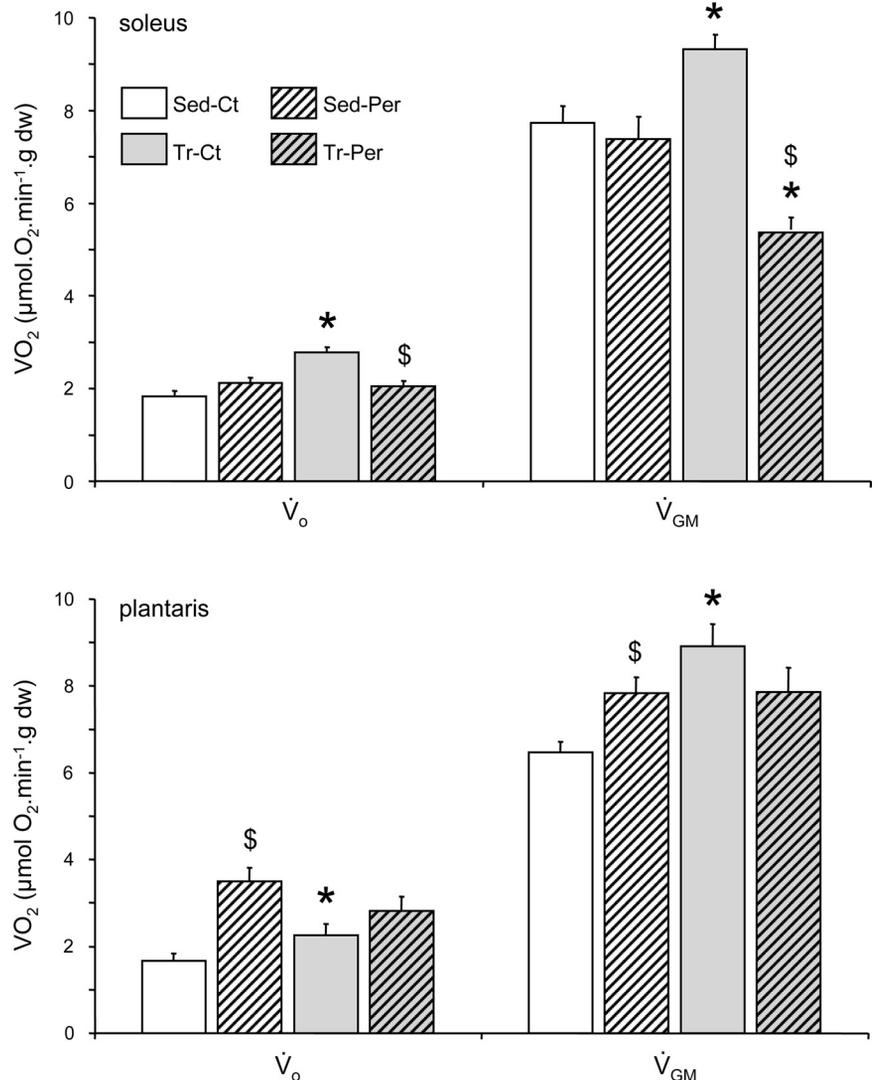

Fig. 2. Effects of endurance training and/or perindopril administration on basal ($\dot{V}_0$) and maximal ADP-stimulated respiration rates of in situ mitochondria from soleus (*top*) and plantaris muscles (*bottom*) using glutamate-malate as substrates ($\dot{V}_{GM}$). Sed-Ct, sedentary control nontreated rats; Sed-Per, sedentary perindopril-treated rats; Tr-Ct, trained control nontreated rats ; Tr-Per, trained perindopril-treated rats. Values are means ± SE. *Significance vs. corresponding Sed group, $P < 0.05$. $ Significance vs. corresponding Ct nontreated group, $P < 0.05$.

Table 4. *Effects of endurance training and perindopril treatment on acceptor-to-control ratio ($\dot{V}_{GM}/\dot{V}_0$) and K_m for ADP of in situ mitochondria from soleus and plantaris muscles*

	Sed-Ct($n = 8$)	Sed-Per($n = 8$)	Tr-Ct($n = 8$)	Tr-Per($n = 8$)	Global Effect of Tr	Global Effect of Per
Sol muscle						
ACR	4.5 ± 0.3	3.7 ± 0.3†	3.4 ± 0.1*	2.7 ± 0.2*†	$P < 0.01$	$P < 0.05$
K_mADP$_{-Cr}$, μM	338 ± 64	291 ± 46	303 ± 37	231 ± 35	NS	NS
K_mADP$_{+Cr}$, μM	76 ± 9	64 ± 12	67 ± 12	47 ± 9	NS	NS
Pla muscle						
ACR	4.5 ± 0.3	2.5 ± 0.2†	4.3 ± 0.3	3.0 ± 0.3†	NS	$P < 0.05$
K_mADP$_{-Cr}$, μM	15 ± 3	46 ± 9†	29 ± 6	25 ± 9	NS	NS
K_mADP$_{+Cr}$, μM	16 ± 4	26 ± 8	23 ± 4	42 ± 9	NS	NS

Values are means $\pm$ SE. K_m values were calculated by nonlinear fit of the Michaelis-Menten equation of respiration rates obtained for increasing ADP concentrations in absence (K_mADP$_{-Cr}$) or presence (K_mADP$_{+Cr}$) of creatine. ACR, acceptor-to-control ratio ($\dot{V}_{GM}/\dot{V}_0$, where $\dot{V}_0$ is basal respiration rate and $\dot{V}_{GM}$ is maximal ADP-stimulated respiration rate with glutamate and malate as substrates). *Significantly different from values measured in corresponding Sed group, $P < 0.05$. †Significantly different from corresponding Ct group, $P < 0.05$.

trained perindopril-treated rats could be related to direct effects of ACE inhibition on the acute responses to exercise and/or the extent of adaptive changes elicited by endurance training. However, the pharmacological inhibition of ACE activity failed to enhance physical performance in sedentary rats, a finding consistent with previous results (3). It is thus unlikely that the increased endurance capacity reported in the present study after endurance training in perindopril-treated rats could be related to more favorable acute responses to a bout of exercise. Rather than improving the acute responses to exercise, decreased ACE activity would enhance the adaptive changes in response to endurance training.

Many previous studies showed that ACE inhibitors such as perindopril have two primary effects, first a decrease in the conversion in ANG I to the vasoconstrictor ANG II, and second a decreased bradykinin degradation into inactive peptides, leading to increased bradykinin action (27). It is thus likely that the cellular effects of ACE inhibition on skeletal muscle can involve both reduced ANG II action and increased bradykinin influence. A reduction in ANG II formation during exercise favors vasodilatation and then potential substrate delivery to active skeletal muscles (24), while bradykinin likely has a synergistic role in mediating vasodilatation during muscular contraction (6).

Respiration rate in skinned fibers is a unique way to examine the muscle oxidative capacity by measuring oxygen consumption of the entire mitochondrial population within its cellular environment. Whether the pharmacological inhibition of ACE affects the mitochondrial function has been previously studied. Prolonged ACE inhibition completely prevented the decreased skeletal muscle oxidative capacity expected in patients with chronic heart failure (10) and in rats with heart failure secondary to myocardial infarction (32, 35). However, this protective effect of ACE inhibition remains controversial, and perindopril administration failed to restore muscle oxidative capacity and mitochondrial function in a rat model of heart failure induced by aortic stenosis (16) or in a rat model of type I diabetes (22). Taken together, these previous findings suggested that ACE inhibitors at best improve the mitochondrial dysfunction that characterizes skeletal muscle myopathy associated with chronic diseases. However, whether perindopril affects the muscle mitochondrial responses to endurance training in normal rats remained to be examined. The present study, for the first time, shows that the training-induced changes in CS activity and maximal mitochondrial respiration of saponin-skinned fibers with glutamate-malate as substrates ($\dot{V}_{GM}$) were less in perindopril-treated rats than in control animals. These findings, together with the slight decrease in the functional
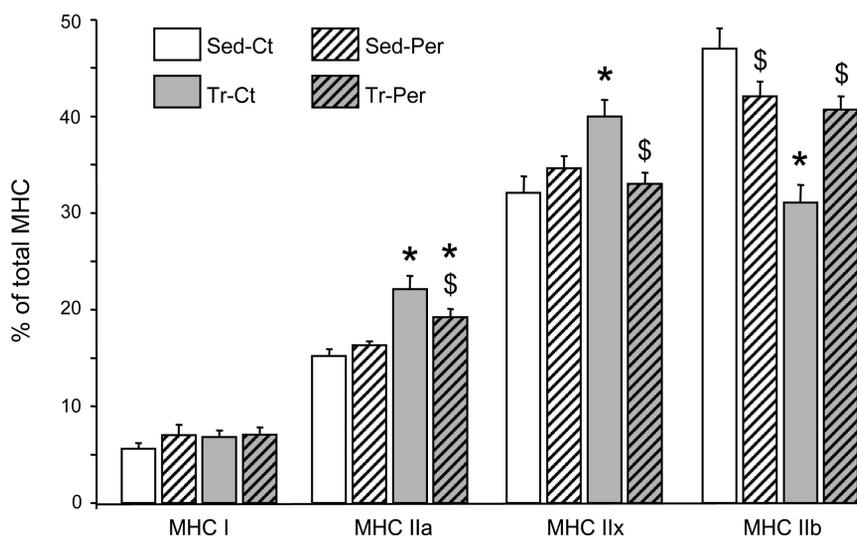

Fig. 3. Distribution of myosin heavy chain (MHC) isoforms in plantaris muscles from after an endurance-training program and/or perindopril administration. Sed-Ct, sedentary control nontreated rats ; Sed-Per, sedentary perindopril-treated rats; Tr-Ct, trained control nontreated rats; Tr-Per, trained perindopril-treated rats. Values are means $\pm$ SE. *Significance vs. corresponding Sed group, $P < 0.05$. $ Significance vs. corresponding Ct nontreated group, $P < 0.05$.

coupling between oxidation and phosphorylation related to perindopril administration, disagree with the hypothesis that low ACE activity could improve the efficiency of mitochondrial respiration in skeletal muscle, and then endurance capacity (for review, see Ref. 12).

Prolonged administration of ACE inhibitors has been shown to alter the myosin content toward MHC isoforms associated with oxidative fatigue-resistant fibers (29). Moreover, population studies suggested that low ACE activity resulting from the presence of the *I* allele of the ACE gene was associated with an increased percentage of type I fibers in young untrained volunteers (34). However, the heterozygous disruption of the *ace* gene in mice (ACE+/−) did not affect the percent composition of soleus muscle fibers (33). The direct effects of ACE activity on MHC expression remain thus controversial, while the results of the present study clearly suggest that the pharmacological inhibition of ACE activity failed to significantly affect the MHC composition of skeletal muscles from sedentary rats. Moreover, our results demonstrate that the shift toward MHC IIa expected with endurance training in fast-twitch muscle was less in perindopril-treated than in control animals. Under control conditions, the training-induced shift in MHC expression is consistent with low metabolic cost, high efficiency of the myosin molecule, and parallel increase in muscle oxidative capacity. In the present study, perindopril administration minimized both the training-induced shift toward more fatigue-resistant MHC isoforms and the associated low response of mitochondrial function to endurance training. Although these negative effects of perindopril on the responses of contractile phenotype and muscle oxidative capacity to endurance training are finely coordinated, exact mechanisms remain to be elucidated. Taken together, all these findings strongly suggest that it is unlikely that changes in muscle metabolic efficiency and contractile characteristics contribute to explain the marked enhancement in physical performance observed in trained perindopril-treated animals.

In conclusion, the present study demonstrates for the first time that inhibition of skeletal muscle ACE activity using a nonsulfhydryl ACE inhibitor in rats improves the functional response to endurance training. The higher endurance capacity reported in trained perindopril-treated rats, compared with trained nontreated animals, was not related to detectable changes in muscle oxidative capacity or contractile phenotype. However, performance in endurance events is mainly limited by the ability of the cardiorespiratory system to transport oxygen to skeletal muscles. The degree of peripheral oxygenation through muscle capillary bed could also be involved to account for enhanced muscle efficiency. Whether the enhanced endurance capacity of perindopril-treated rats is related to improved angiogenic responses to endurance training needs to be addressed in future studies.

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