Does ACE inhibition enhance endurance performance and muscle energy metabolism in rats?

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Submitted 1 April 2003; accepted in final form 25 August 2003

Bahi, L., N. Koulmann, H. Sanchez, I. Momken, V. Veksler, A. X. Bigard, and R. Ventura-Clapier. Does ACE inhibition enhance endurance performance and muscle energy metabolism in rats? J Appl Physiol 96: 59–64, 2004. First published August 29, 2003; 10.1152/japplphysiol.00323.2003.—The renin-angiotensin-aldosterone system plays an important role in the hydroelectrolytic balance, blood pressure regulation, and cell growth. In some studies, the insertion (I) allele of the angiotensin-converting enzyme (ACE) gene, associated with a lower ACE activity, has been found in excess frequency in elite endurance athletes, suggesting that decreased ACE activity could be involved in endurance performance (Myerson S, Hemingway H, Budget R, Martin J, Humphries S, and Montgomery H. J Appl Physiol 87: 1313–1316, 1999). To test this hypothesis, we evaluated whether ACE inhibition could be associated with improved endurance performance and muscle oxidative capacity in rats. Eight male Wistar rats were treated for 10–12 wk with an ACE inhibitor, perindopril (2 mg·kg⁻¹·day⁻¹), and compared with eight control rats. Endurance time was measured on a treadmill, and oxidative capacity and regulation of mitochondrial respiration by substrates were evaluated in saponin-permeabilized fibers of slow soleus and fast gastrocnemius muscles. Endurance time did not differ between groups (57 ± 5 min for perindopril vs. 55 ± 6 min for control). Absolute and relative (to body weight) left ventricular weight was 20% (P < 0.01) and 12% (P < 0.01) lower, respectively, in the treated group. No difference in oxidative capacity, mitochondrial enzyme activities, or mitochondrial regulation by ADP was observed in soleus or gastrocnemius. Mitochondrial respiration with glycerol 3-phosphate was 17% higher in soleus or gastrocnemius. Maximal oxidative capacity of skeletal muscles. This suggests that ACE activity has no implication in endurance capacity and only minor effects on mitochondrial function in sedentary animals.

skinned fibers; mitochondrial respiration; endurance performance; angiotensin-converting enzyme inhibition; energy metabolism; skeletal muscle

INDIVIDUAL VARIATIONS IN HUMAN physical performances reflect both environmental factors (training, nutrition, altitude, and so forth) and genetic elements interaction. Efforts to document genetic differences that may account for human variations in physical performance and in responses to training have been recently revived by advances in the sequencing of the human genome (30).

Among different gene polymorphisms, a relationship between a polymorphism in the gene encoding the angiotensin-converting enzyme (ACE) and human physical performance has been highlighted (6, 22, 23). The ACE cleaves angiotensin I into angiotensin II, a strong vasoconstrictor that, at physiological concentrations, potentiates vascular contractile tone (11). The polymorphism of the human ACE gene has been identified related to either the presence (insertion, I allele), or the absence (deletion, D allele) of a 287-bp segment in intron 16 (33). The I allele is associated with reduced levels of ACE activity in both serum and tissues (3, 42). Recent studies suggest that the I variant of the human ACE gene is associated with greater endurance performance in some athletes and with improved adaptive responses to physical training (23). On the other hand, the D allele is associated with higher ACE activity in tissues, and a significant excess of the D allele was found in athletes of power-oriented sports and in elite short-distance athletes compared with sedentary subjects (25). This relationship between ACE genotype and sports performances appears, however, somewhat controversial. In large cohorts of elite endurance athletes (31), Australian Olympic athletes (39), and British Olympic athletes (23), the allele and genotype frequencies of the I/D polymorphism did not differ from those of sedentary controls (see Refs. 29, 30 for review). These discrepancies could result in part from heterogeneous cohorts of mixed athletic ability and discipline. Nazarov and coworkers (25), investigating the ACE I/D polymorphism in outstanding Russian athletes, did not observe a difference with the control group, but examination of subgroups showed a significantly higher frequency of the D allele in short-distance runners and of the I allele in middle distance athletes. Because of the controversy and disparate findings, a clear link between endurance performance and ACE activity remains to be established. At present, all studies aimed at establishing a link between ACE genotype and exercise performance have been based on correlation studies in humans. Assessing the effects of low ACE activity on endurance capacity and skeletal muscle bioenergetics in an animal model could be of valuable interest.

Endurance capacity relies on central and peripheral factors. The maximal oxygen consumption (\(\dot{V}O_{2\text{max}}\)), one of the physiological variables related to endurance performance, is the best index of the ability of the cardiorespiratory system to deliver oxygen and substrates to exercising muscles. In women athletes, the ACE II genotype is associated with a 25% greater cardiac output at submaximal exercise (10). There is, however, clear evidence that endurance performance can vary among individuals with equal values of \(\dot{V}O_{2\text{max}}\) (2). Factors other than those related to the central cardiorespiratory system are clearly...
involved. It has been hypothesized that association between the I allele and endurance performance could rather be due to a local muscle effect (47). This is supported by an increased maximal arteriovenous O2 difference during maximal exercise in subjects with the ACE II genotype and low serum ACE activity (9). Endurance performance also relies on the capacity of muscle mitochondria to oxidize energetic substrates via oxidative phosphorylation process to produce a sufficient ATP flux. Endurance capacity is thus associated with a high mitochondrial density and oxidative capacity, a metabolic switch from glucose to fatty acid utilization, and an increased capillary density (13, 14, 36). Moreover, muscle adaptation to endurance training involves an increased reliance of mitochondrial respiration on ADP regeneration by mitochondrial creatine kinase. This allows a better matching between energy production by mitochondria and energy utilization by ATPases involved in excitation-contraction coupling, through the creatine kinase system (40, 45, 48, 49).

Decreased ACE activity can be produced in animal models by chronic ACE inhibition. In genetically normotensive rats, ACE inhibition by perindopril significantly decreases local vascular resistance as well as systolic and diastolic blood pressure (18). In humans, ACE inhibitors decrease local vascular resistance of arterioles and large conductance vessels, leading to increased muscle blood flow and a favorable redistribution of cardiac output toward the muscle (7). Conversely, the deletion genotype DD, leading to elevated plasma and tissue ACE activity, is associated with an increased vascular reactivity to vasoconstrictors (12), suggesting in turn a reduced reactivity for the insertion genotype II. Moreover, ACE inhibition is known to improve angiogenesis in skeletal muscles (38). This suggests that low ACE activity could have a positive effect on substrate and oxygen delivery to peripheral tissues and particularly to skeletal muscles during exercise.

Because the insertion genotype II is likely related to the capacity of muscle to oxidize energetic substrates, the influence of different ACE levels on the mitochondrial oxidative capacity, regulation of respiration, and substrate utilization needs to be examined. Only a few studies evaluated the effects of low levels of serum ACE, through repeated administration of ACE inhibitors, on skeletal muscle energy metabolism. In spontaneously hypertensive rats, ACE inhibition decreases peripheral resistance and systolic blood pressure (32) and improves tissue blood circulation. This could favor oxygen and substrate delivery to exercising skeletal muscles.

We thus hypothesized that chronic ACE inhibition could improve endurance performance and positively affect skeletal muscle metabolic efficiency in sedentary rats. To investigate this issue, we measured the running capacity of perindopril-treated rats on a treadmill and we noted the associated differences in muscle oxidative capacity and mitochondrial substrate utilization. Respiratory parameters of the total mitochondrial population were studied in situ in fresh saponin-skinned fibers as previously described (34, 44).

**MATERIALS AND METHODS**

**Animals and experimental protocols.** All procedures were performed in accordance with institutional guidelines for the use of animals. Sixteen adult male Wistar rats (~230 g) were randomly divided into two groups housed per cage in temperature-controlled room (22°C), with a 12:12-h light-dark cycle. The control group (n = 8) received only water, whereas the perindopril-treated group (n = 8) was given oral administration of 2 mg kg⁻¹ day⁻¹ for 10–12 wk. Perindopril was a kind gift from Servier Laboratories. All animals were given food ad libitum and were weighted regularly to adjust perindopril dose and record the body weight evolution.

To measure the exercise capacity, rats were placed onto a treadmill that had a stainless-steel grid at the end of the lane. This grid supplied a slight electrical stimulus to keep them running on the lane. After 1 wk of treadmill acclimation (10–15 mm/min for 10 min/day at 0% grade), the endurance test was performed at 20 mm/min and 5% grade. Animals were removed from the treadmill when they could no longer maintain the treadmill speed, and the exercise duration was immediately noted. This protocol permitted assessment of exercise tolerance without progression to an excessively high intensity that would precipitate early fatigue.

**Surgical procedures.** Forty-eight hours after exercise tolerance test, the animals were anesthetized with pentobarbital sodium, administered intraperitoneally (50 mg/kg body mass). The soleus and gastrocnemius muscles were excised immediately before exsanguination. The right muscles were put in Krebs solution composed of 118 mM NaCl, 25 mM NaHCO₃, 4.7 mM KH₂PO₄, and 1.2 mM MgSO₄, bubbled with O₂ 95%–CO₂ 5%, for mitochondrial respiration experiments, and the left ones were quickly frozen in liquid nitrogen for biochemical assays.

In situ study of mitochondrial respiration. Oxygen consumption of saponin-skinned fibers has been described previously (34, 44). Briefly, bundles of muscle fibers were isolated and incubated for 30 min in the skimming solution containing 2.77 mM CaK₂EGTA, 7.33 mM K₂EGTA, 6.56 mM MgCl₂, 5.7 mM Na₂ATP, 15 mM phosphocreatine (PCr), 20 mM taurine, 0.5 mM dithiothreitol (DTT), 50 mM K methyl sulfate, 20 mM imidazole (pH 7.1), and 50 μg/ml of saponin. Fiber bundles were transferred into the respiration solution (with the same composition as the skimming solution but containing 3 mM K₂HPO₄, and with or without 5 mM glutamate and 2 mM malate instead of PCr and ATP) for 10 min to wash out adenine nucleotides and PCr. All these steps were carried out at +4°C with continuous stirring. Respiration of 10–15 mg of skinned fibers was registered at 22°C in 1.5 ml of the respiration solution with or without substrates and containing 2 mg/ml bovine serum albumin using Hansatech Oxigraph Instruments. After measurement, the bundles were removed, dried, and weighed. Rates of respiration were given in micromoles of oxygen per minute per gram of dry weight.

Two different experimental protocols were used. The first protocol was to determine the dependencies of respiration on external ADP concentration in the presence or the absence of creatine. The ADP-stimulated respiration above basal oxygen consumption was plotted by using the nonlinear fitting of the Michaelis-Menten relationship to determine the apparent Kₘ for ADP. The second one was to assess the effects of various substrates on mitochondrial respiration in the presence of 2 mM ADP. Experiments were started in the respiration solution without glutamate and malate. For the soleus, an oxidative muscle, substrates were sequentially added as follows: 4 mM glycerol 3-phosphate, 4 mM malate, 0.4 mM octanoylcarnitine, 1 mM pyruvate, 2 mM dichloroacetate (to maximally stimulate pyruvate dehydrogenase), and 10 mM glutamate. For the gastrocnemius muscle, the glycerol 3-phosphate was administered after octanoylcarnitine. Each substrate was applied for 3–4 min.

**Biochemical studies.** Frozen tissue samples were weighed, homogenized in ice-cold buffer (50 mM) containing 5 mM HEPES (pH 8.7), 1 mM EGTA, 1 mM DTT, 5 mM MgCl₂, and 0.1% Triton X-100 and incubated for 60 min at 0°C to ensure complete enzyme extraction. Determination of citrate synthase and cytochrome-c oxidase activities was assayed at 30°C (pH 7.5) by using coupled enzyme systems as previously described (18, 21).

**Statistical analysis.** Data are expressed as means ± SE. Student’s t-test was used to determine the statistical difference of means be-
also considerably lower in gastrocnemius than in soleus (Table 2). As expected, creatine dramatically increased the sensitivity to ADP only in soleus muscle, showing the reliance of mitochondrial respiration on mitochondrial creatine kinase in this oxidative muscle. No differences were found for basal and ADP-stimulated respiration, or ADP sensitivity in treated group compared with control.

Substrates are differently utilized according to the muscle type. The soleus, an oxidative muscle, is able to actively oxidize fatty acids, whereas the gastrocnemius, a predominantly glycolytic muscle, prefers the glycolysis-derived energy. This can be observed at the level of mitochondria (Fig. 1). Soleus mitochondria are not able to use glycerol 3-phosphate as substrate, whereas, in the presence of malate, octanoylcarnitine, a medium-chain fatty acid, greatly enhanced mitochondrial respiration to 70% of maximal respiration obtained when pyruvate and then glutamate were added to the solution. In contrast, octanoylcarnitine hardly enhanced mitochondrial respiration of gastrocnemius muscle, whereas the addition of glycerol 3-phosphate increased respiration to 62% of maximal respiration, showing the tissue specificity of substrate utilization by mitochondria.

A significantly higher mitochondrial respiration was observed in treated soleus with octanoylcarnitine as substrate (14%; \( P < 0.01 \)). A higher mitochondrial respiration was also observed with glycerol 3-phosphate in gastrocnemius muscle of perindopril treated group (17%; \( P < 0.05 \)) compared with control (Fig. 1).

Activities of citrate synthase, a marker of the Krebs cycle, and of cytochrome-c oxidase, a marker of the respiratory chain,
were higher in soleus compared with gastrocnemius (58 and 80%, respectively) but did not significantly differ between control and perindopril-treated animals.

DISCUSSION

It has been suggested that the low-activity ACE II allele could be associated with increased endurance capacity in humans. The aim of the present investigation was to establish whether inhibition of ACE activity by chronic oral administration of perindopril for 10–12 wk may be associated with improved physical exercise capacity and skeletal muscle energy metabolism in sedentary rats. The results can be summarized as follows: 1) ACE inhibition had no effect on the endurance capacity of the animals; 2) no difference between soleus and gastrocnemius muscle oxidative capacity, mitochondrial regulation and enzyme activities were detected, although 3) a small but significantly higher fatty acid utilization for soleus, and glycerol 3-phosphate use for gastrocnemius muscles, were observed in perindopril-treated rats; 4) there was a slight cardiac atrophy in the perindopril-treated rats. In summary, ACE inhibition was not associated with improved endurance performance and oxidative capacity of skeletal muscles. This suggests that the renin-angiotensin-aldosterone system (RAAS) has no major implication in endurance capacity and mitochondrial function, at least in sedentary rats.

Angiotensin has been involved in muscle cell growth. In the perindopril-treated group, our data indicated a 12% lower absolute soleus weight that was, however, not significant when normalized either to body weight or tibia length. Accordingly, it was shown that although ACE inhibition induces a strong inhibitory effect on overload-induced hypertrophy in skeletal muscle, it has no effect on normalized muscle weight in basal conditions (8). On the other hand, heart weight was clearly lower in perindopril-treated animals, whether absolute or relative weights are considered, as expected from chronic ACE inhibition. A similar absolute and relative cardiac atrophy was previously reported together with a decrease in systolic blood pressure in perindopril-treated rats (18). This could be due either to the trophic effects of angiotensin II on cardiomyocytes (19) or to the decreased afterload that results from decreased vascular tone (16, 27).

Genetic studies in humans have shown that a higher frequency of the I allele, associated with a lower ACE activity, was encountered in high-level, endurance-trained, well-performing athletes (23). We thus evaluated the endurance capacity of control and perindopril-treated rats, running on motorized treadmill until exhaustion. Three conditions are necessary to consider a physical exercise as an endurance activity: low intensity, long duration, and mainly aerobic energy production. In this study, a constant speed of 20 m/min with 5% grade was chosen to maintain the exercise power test under the \( V_{O_2\ max} \) conditions (4). On the other hand, the duration of run exercise was consistent with the characteristics of an endurance exercise. However, the results did not evidence any difference in the time to exhaustion between control and perindopril-treated animals. This shows that ACE inhibition has no marked effect on endurance time in sedentary animals.

At the muscle level, endurance capacity mainly relies on high oxidative capacity, increased use of fatty acids as substrate, high muscle capillarity, and increases in substrate and oxygen delivery (1, 13, 14). Moreover, a tight coupling between energy production and energy utilization within the fibers is commonly associated with high endurance capacity (41, 48, 49). Mitochondrial energy production is the only source for long-term endurance exercise. Measurement of oxygen consumption of permeabilized muscle fibers can directly assess oxidative capacity of a given muscle. In these conditions, mitochondria are kept in their cellular environment and the respiration capacity of the whole mitochondrial population can be measured. Moreover, within the cellular architecture, mitochondria retain native regulatory properties that are lost after isolation (35). Assessment of the mitochondrial function of predominantly glycolytic (superficial gastrocnemius) or highly oxidative (soleus) muscles showed that the later exhibited higher oxidative capacity, had lower ADP sensitivity, and was more dependent on mitochondrial creatine kinase than the glycolytic muscle, as previously described (17, 43, 48). However, perindopril treatment had no effects on oxidative capacity and mitochondrial regulation by ADP and creatine. This was confirmed by measuring the activity of one Krebs cycle enzyme (citrate synthase) and of one complex of the respiratory chain (cytochrome-c oxidase). None of these enzymes were affected by long-term perindopril treatment. Thus ACE inhibition did not alter oxidative capacity, in line with the maintained endurance capacity of the animals. Similarly, in trained human subjects with or without mild hypertension, ACE inhibition does not affect endurance exercise hemodynamics and \( V_{O_2\ max} \) or parameters of energy metabolism (26, 28).

Another important determinant of endurance performance, strongly involved in the resistance to fatigue and adaptation to endurance exercise, is substrate utilization. Substrate utilization differs according to muscle phenotype. The soleus, an oxidative muscle, is able to actively oxidize fatty acids, whereas the gastrocnemius, a predominantly glycolytic muscle, produces energy mainly from glucose use. In addition to substrate specific import and transport systems in the cytosol, substrate specificity can also be observed at the level of mitochondria. It has been shown that preferential substrate utilization by mitochondria varies according to the fiber type (15). Mitochondria isolated from fast fibers preferentially uses glycerol 3-phosphate, whereas slow type I fibers mainly use fatty acids as substrates. We thus designed muscle specific protocols to assess substrate utilization in permeabilized muscle fibers. Substrates were added sequentially in a tissue specific manner. The results show that octanoylcarnitine, a medium-chain fatty acid, was able to induce 70% of maximal respiration in soleus muscle, whereas these mitochondria could not use glycerol 3-phosphate. On the opposite, glycerol 3-phosphate could induce more than 60% maximal respiration in superficial gastrocnemius muscle composed of mainly type IIb fibers, whereas no oxidation of fatty acid could be observed. This muscle type-specific preferential substrate utilization by mitochondria was slightly but significantly higher in the perindopril-treated group. A 14% (\( P < 0.01 \)) higher mitochondrial respiration with octanoylcarnitine was observed in soleus of perindopril-treated rats, whereas a 17% (\( P < 0.05 \)) higher mitochondrial respiration was observed with glycerol 3-phosphate in gastrocnemius muscles compared with controls (Fig. 1). So, perindopril treatment resulted in an improved tissue specificity of substrate utilization. This could be due to in-
increased transport and/or oxidation processes of the respective substrates.

A clear correlation between the frequency of the I allele of the ACE gene, associated with lower activity of the enzyme, and human physical performance is still a matter of controversy (see Introduction). It was suggested that it might depend on whether homogeneous subgroups vs. heterogeneous cohorts of mixed athletic abilities were compared (25, 30). The results of this animal study show that low ACE activity is not associated with any major differences in endurance performance and intrinsic fast or slow muscle metabolic properties. As long as this can be extrapolated to humans, it indicates that decreased ACE activity in sedentary subjects bearing the I allele would not affect their basal endurance capacity. This could be due to the low basal level of the RAAS in normal sedentary animals or humans. Indeed, the beneficial effects of ACE inhibition on cardiac and skeletal muscle energy metabolism are unmasked for example during heart failure when there is an overactivation of angiotensin II levels (20, 24, 37). Moreover, the RAAS and thus angiotensin II levels are activated during exercise and play a primary role in the physiological response of electrolyte balance and cardiovascular function (5). In these circumstances, the ACE genotype could be important in determining the capacity of adaptation to endurance training. Interestingly, it was shown that the improvement in maximum duration of elbow flexion following training is 11-fold greater for individuals with the II (low ACE activity) than with the DD allele (22). Moreover, the effect of endurance training on the efficiency of muscular contraction is more effective in II genotype carrying athletes than in others (46). Accordingly, influences of ACE genotype on endurance performances have been described mainly for well-trained and well-performing athletes in a given discipline (6, 22, 25). Thus, rather than determining basal performances, the advantages of the II genotype may be unmasked only when the RAAS is activated during repeated exercise bouts, that would permit the II genotype athletes to perform in endurance physical activities better than those with the DD genotype. More work is thus needed to assess the influence of ACE inhibition on the adaptive response to endurance training.

GRANTS

This study was supported by Fondation de France. R. Ventura-Clapier is supported by “Centre National de la Recherche Scientifique” and L. Bahi by “Conseil Régional d’Ile de France.”

DISCLOSURES

Servier Laboratories is acknowledged for the gift of perindopril.

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