Angiotensin-Converting Enzyme Genotype Modulates Pulmonary Function and Exercise Capacity in Treated Patients With Congestive Stable Heart Failure

M. Roselle Abraham, MD; Lyle J. Olson, MD; Michael J. Joyner, MD; Stephen T. Turner, MD; Ken C. Beck, PhD; Bruce D. Johnson, PhD

Background—The gene encoding ACE exhibits an insertion/deletion polymorphism resulting in 3 genotypes (DD, ID, and II), which affects serum and tissue ACE activity as well as other vasoactive substances. Pulmonary function is frequently abnormal in patients with congestive heart failure (CHF), the mechanism of which has not been completely characterized. ACE inhibition has been shown to improve diffusion across the alveolar-capillary membrane and to improve exercise capacity and gas exchange in CHF. The aim of the current study was to determine if ACE genotype is associated with altered pulmonary function and exercise intolerance in patients with treated CHF.

Methods and Results—Fifty-seven patients (stratified according to ACE genotype as 17 DD, 28 ID, 12 II) with ischemic and dilated cardiomyopathy, left ventricular ejection fraction (LVEF) <35%, and <10 pack-years of smoking history were studied. All patients were receiving standard therapy for left ventricular systolic dysfunction. Pulmonary function, LVEF, serum ACE, plasma angiotensin II, atrial natriuretic peptide, and brain natriuretic peptide were measured at baseline. Peak VO2 and gas exchange measurements were assessed with graded exercise. Resting LVEF was similar among the genotype groups (25% to 28%), and no differences were observed in diastolic function or pulmonary artery pressures (P>0.05). Mean peak VO2 and forced vital capacity (% Pred) were significantly reduced (P<0.05), whereas mean serum ACE activity and plasma angiotensin II concentration were highest in DD homozygotes. Subjects homozygous for the D-allele also demonstrated higher mean ventilatory equivalents for carbon dioxide (VE/VCO2) during exercise (P<0.05).

Conclusions—ACE DD genotype is associated with decreased exercise tolerance in CHF, possibly mediated by altered pulmonary function. Pharmacological strategies effecting more complete inhibition of serum and tissue ACE and/or potentiation of bradykinin may improve exercise capacity in patients with CHF and ACE DD genotype. (Circulation. 2002;106:1794-1799.)

Key Words: heart failure ■ angiotensin ■ renin ■ lung

Exercise intolerance is a hallmark of the clinical syndrome of congestive heart failure (CHF). However, previous studies have shown poor correlation between exercise capacity and estimates of resting cardiac function.1 In stable, treated patients with CHF, pulmonary function varies considerably and does not correlate well with estimates of resting left ventricular (LV) function.2 Several studies have suggested that decreased pulmonary function may contribute to exercise limitation in patients with CHF: Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weakness are common in CHF. Baseline restrictive and obstructive pulmonary function changes as well as respiratory muscle weak
CHF.\textsuperscript{10,11} In humans, the gene encoding ACE, which is located on chromosome 17, exhibits an insertion/deletion polymorphism characterized by either insertion (allele I) or deletion (allele D) of a 287 base-pair marker in intron 16, resulting in 3 genotypes (DD or II homozygotes or ID heterozygotes). Subjects homozygous for the D-allele have been shown to have serum ACE concentrations and activity levels 48\% higher than those homozygous for the insertion allele II\textsuperscript{10,12} and decreased survival with LV systolic dysfunction.\textsuperscript{13,14} Moreover, studies have suggested a more rapid rebound of serum ACE activity acutely in DD homozygotes after a dose of ACE inhibitors; whether this is true with chronic ACE inhibitor treatment (ACE escape) is unclear.\textsuperscript{15} Accordingly, it seems possible that variation in pulmonary function and exercise capacity observed in treated patients with CHF may in part be attributable to the ACE I/D polymorphism. Consequently, in the current study, we examined pulmonary function and exercise capacity in relation to ACE I/D polymorphism in medically treated patients with stable CHF. We hypothesized that the ACE DD genotype would be associated with greater ACE activity and A-II concentration and decreased pulmonary function and exercise capacity.

Methods

Patient Selection
Fifty-seven white patients (36 men, 19 women) with a diagnosis of CHF were recruited from the Heart Failure and Cardiovascular Health Clinics in the Division of Cardiovascular Diseases of the Mayo Clinic, Rochester, Minn, from November 1999 to March 2001. Patients with a history of ischemic or idiopathic dilated cardiomyopathy were studied. Inclusion criteria included stable CHF symptoms (>3 months), ejection fraction \( \leq 35\% \), duration of CHF symptoms >1 year, body mass index <35, smoking history <10 pack-years, and exercise not limited by joint pain, peripheral vascular disease, or chest pain. Exclusion criteria included pacemaker dependency, atrial fibrillation or history of dangerous ventricular arrhythmias. Medications included ACE inhibitors (78\%), \( \beta \)-blockers (58\%), digoxin (80\%), diuretics (62\%), A-II receptor blockers (15\%), and amiodarone (7\%).

The Mayo Institutional Review Board approved the study. Informed consent was obtained from each patient before participation. All study measurements were completed over a 2- to 3-day period. Informed blood samples were drawn for ACE genotype, serum ACE, hemoglobin, hematocrit, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and A-II under resting conditions. After blood was drawn, all subjects performed pulmonary function testing (PFTs), had resting echocardiograms, and completed a cardiopulmonary exercise test.

Blood Tests

ACE Genotyping
Blood samples drawn were anticoagulated with EDTA, and buffy coat was stored at \(-80^\circ C\). Genomic DNA was subsequently extracted from thawed buffy coat by a salting-out procedure. Previously described methods were followed for genotyping of the ACE insertion-deletion polymorphism.\textsuperscript{12,16,17}

Serum ACE Activity, A-II, ANP, BNP
Blood was drawn into Vacutainer tubes with and without EDTA and chilled until centrifuged at 4°C at 2500 rpm for 10 minutes. Serum and plasma aliquots (1 mL each) were transferred into 12×75 mm polystyrene tubes and frozen until assayed. Serum ACE activity was determined according to the spectrophotometric methods described by Holmquist et al.,\textsuperscript{18} using reagents purchased from Sigma (St Louis, Mo). A-II, ANP, and BNP were assessed according to previously published methods.\textsuperscript{19,20}

Pulmonary Function Testing
Pulmonary function measurements included an assessment of lung volumes (forced vital capacity [FVC] and forced expiratory volume in 1 second [FEV\(_1\)] and an assessment of expiratory flows (mean forced expiratory flow between 25\% and 75\% of the FVC, FEF25\%-75\%). Subjects also performed a single breath-diffusing capacity of the lung for carbon monoxide (DLCO). Spirometry and DLCO data were collected in accordance with American Thoracic Society (ATS) standards.\textsuperscript{21}

Echocardiography
LV ejection fraction (LVEF) was measured by 2-dimensional echocardiography. LV and atrial size were also determined along with estimates of right ventricular pressures and indexes of diastolic function.\textsuperscript{22}

Exercise Testing
Each subject underwent graded treadmill testing to volitional fatigue. Before testing, they were instrumented to measure heart rate (ECG), gas exchange (mouthpiece and nose clip), and oxygen saturation (pulse oximetry). The exercise protocol consisted of an initial treadmill speed and grade of 2.0 mph and 0\%, respectively, and speed/grade were adjusted every 2 minutes to yield ~2 MET increase per work level.\textsuperscript{23} Gas exchange measurements were obtained with a Medical Graphics metabolic cart validated with classic gas collection techniques.\textsuperscript{24} Subjects were encouraged to reach a maximal or near maximal effort by monitoring the respiratory exchange ratio ([RER] >1.15) and perceived exertion ([RPE] >18 on the Borg 6 to 20 scale).\textsuperscript{25}

Statistics
The ANOVA method was used to assess the statistical significance of differences in means across genotype groups. The Scheffé multiple comparison test was used to determine differences between specific genotypes (II versus DD, DD versus ID, ID versus DD). Comparisons of pulmonary function among patients were based on percent predicted values to adjust for potential differences among groups attributable to differences in age, height, and sex. Regression analysis was used to determine the variation in \( V_\text{O2} \) and lung function that could be attributed to serum ACE activity and ACE genotype.

Results

Subject characteristics and hormonal intermediates stratified by genotype are summarized in Table 1. Figure 1 shows the percent of subjects on a given class of medication across the genotypes. Mean age and underlying cause of CHF did not differ significantly between genotypes. Compared with the ID and DD genotypes, subjects with the II genotype had decreased mean serum ACE activity, a trend toward lower mean A-II concentration (\( P<0.07 \)), and a higher proportion of subjects with New York Heart Association class I symptoms and treatment with \( \beta \)-blockers (75\% versus 50 and 48\% of subjects, respectively). Mean resting LVEF, cardiac index, ANP, and BNP levels did not differ significantly among the 3 genotype groups, although BNP levels tended to be higher in the DD subjects relative to ID and II subjects. No significant differences in mean diastolic function or pulmonary artery systolic pressures were detected by echocardiography between the different genotypes.

Figure 2 shows the mean baseline PFTs (% predicted [Pred]) and peak exercise \( V_\text{O2} \) (mL/kg per minute) according to ACE genotype; Figure 3 shows the changes in \( V_\text{E}/V_\text{O2} \)
TABLE 1. Characteristics of Patients With CHF According to ACE Genotype

<table>
<thead>
<tr>
<th></th>
<th>II</th>
<th>ID</th>
<th>DD</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (% female)</td>
<td>12 (33)</td>
<td>28 (32)</td>
<td>17 (35)</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>58±4</td>
<td>57±3</td>
<td>53±3</td>
<td>NS</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>83±6</td>
<td>87±4</td>
<td>86±4</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27±2</td>
<td>29±1</td>
<td>29±2</td>
<td>NS</td>
</tr>
<tr>
<td>NYHA class 1, 2, 3, 4, %</td>
<td>42, 16, 42, 0*#</td>
<td>21, 36, 39, 4</td>
<td>17, 35, 47, 0</td>
<td>0.01</td>
</tr>
<tr>
<td>CHF cause (% ischemic)</td>
<td>66</td>
<td>68</td>
<td>64</td>
<td>NS</td>
</tr>
<tr>
<td>LV mass index</td>
<td>158±17</td>
<td>155±8</td>
<td>171±16</td>
<td>NS</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>26±3</td>
<td>27±2</td>
<td>25±2</td>
<td>NS</td>
</tr>
<tr>
<td>RV pressure, mm Hg</td>
<td>40</td>
<td>37</td>
<td>41</td>
<td>NS</td>
</tr>
<tr>
<td>MV deceleration time, m/s</td>
<td>207</td>
<td>185</td>
<td>202</td>
<td>NS</td>
</tr>
<tr>
<td>MV e/a ratio</td>
<td>1.13</td>
<td>1.19</td>
<td>1.14</td>
<td>NS</td>
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<tr>
<td>Cardiac index (Q/BSA)</td>
<td>2.7±0.2</td>
<td>2.3±0.1</td>
<td>2.7±0.2</td>
<td>NS</td>
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<tr>
<td>ACE, U/L</td>
<td>3.4±1.1*</td>
<td>6.7±1.2</td>
<td>9.1±2.1</td>
<td>0.03</td>
</tr>
<tr>
<td>A-II, pg/mL</td>
<td>11.1±3.2</td>
<td>15.0±3.4</td>
<td>19.1±3.8</td>
<td>0.07</td>
</tr>
<tr>
<td>ANP, pg/mL</td>
<td>167±70</td>
<td>220±41</td>
<td>176±45</td>
<td>NS</td>
</tr>
<tr>
<td>BNP, pg/mL</td>
<td>692±336</td>
<td>854±408</td>
<td>1042±560</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Pairwise comparisons: *II different from DD, #II different from ID.

Discussion

The main observations of our study were that stable, treated patients with CHF with the ACE-DD genotype had more restrictive pulmonary changes, a reduced lung diffusing capacity, and poorer exercise tolerance compared with the patients with CHF with the ACE II genotype. The DD subjects also demonstrated a more tachypneic breathing pattern with a reduced breathing efficiency (↑ VE/VC0₂), possibly contributing to their poorer exercise capacity.

CHF causes greater RAS activation in DD individuals and may account for the observed differences in pulmonary function and exercise capacity among the genotypes. The DD subjects had greater serum ACE activity as well as A-II concentration despite similar treatment with ACE inhibitors, which is consistent with variation in serum ACE activity and A-II levels in healthy subjects according to ACE genotype. However, ACE activity and A-II concentration were suppressed relative to those found in healthy subjects consistent...
with ACE-I therapy. Furthermore, circulating ACE and A-II levels accounted for only a small fraction of the variation in pulmonary function or peak VO₂. Previous studies have suggested a significant improvement in exercise capacity with high-dose ACE inhibitor therapy compared with low-dose therapy, without a clear influence on baseline plasma ACE levels. These observations suggest that circulating ACE and A-II may not be good markers of the magnitude of RAS activation in treated subjects or that local tissue concentration may contribute significantly to the variation in pulmonary function and exercise capacity observed between the ACE genotypes in our study.

ACE-I also limits bradykinin degradation, which may play a role in modulating endothelium-dependent relaxing factors. Although not measured in our study, it seems possible that bradykinin levels may be increased both in the plasma and tissue of the subjects homozygous for the ACE I allele. A role for bradykinin modulation of pulmonary function is also implied from studies that suggest that salicylate (prostaglandin inhibitor) used in conjunction with ACE inhibitors adversely affects the alveolar-capillary membrane and gas exchange of patients with CHF.

Although it is possible that effects of the ACE polymorphism on pulmonary function and exercise gas exchange are due to cardiac-related differences between groups (eg, diastolic dysfunction), this seems less likely given the findings of echocardiography. It seems more likely these alterations are due to genotype associated effects on pulmonary function mediated by A-II or bradykinin. ACE is highly localized in the lung and is the main site of conversion of A-I to A-II, and A-II is known to affect pulmonary vascular tone, permeability, tissue fibrosis, pulmonary artery smooth muscle, and microvascular plasma leakage in the trachea and lungs; it also appears to inhibit pulmonary ATP-sensitive potassium channels. As described by Figure 3, our data suggest that reduced lung volumes and diffusion impairment may negatively impact exercise capacity in CHF. This probably is a result of limiting ventilatory reserve, reduced lung compliance, and reduced breathing efficiency (increased Vo₂/VCO₂) rather than from a true gas exchange abnormality (ie, hypoxemia).

Previous studies have suggested that although DLCO is reduced in CHF, typically it is reduced commensurate with the fall in cardiac output so that circulation time in the pulmonary capillaries is likely preserved and oxygen desaturation is rarely observed. It is more likely that the reduced breathing efficiency (increased Vo₂/VCO₂) combined with smaller, stiffer lungs increases the work and cost of breathing so that competition for blood flow between the respiratory muscles and locomotor muscles is increased. Previous work by Musch et al demonstrated a preferential recruitment of blood flow by the respiratory muscles during exercise in a rat model of CHF, suggesting a blood flow “steal” phenomenon. Similar findings have been observed in healthy humans during heavy exercise with a local reflex vasoconstriction in response to increased ventilatory work that significantly compromises locomotor muscle blood flow.

In a prior study of the relation between ACE genotype and survival in heart failure, McNamara et al. observed that the presence of the D-allele was associated with increased risk for death or heart transplantation. This effect was primarily evident in patients not treated with β-blockers, suggesting a pharmaco-
genetic interaction between ACE polymorphism and β-blocker therapy. Interestingly, ACE inhibitor therapy has been reported to increase myocardial β-receptor density in patients with CHF, although changes in other tissues such as the lungs are unclear.37 CHF is also associated with a shift from β₁ to β₂ receptors, and transgenic overexpression of β₂-adrenoceptors has been shown to increase cardiac contractility without causing cardiomyopathy, whereas overexpression of β₁-adrenoceptors results in early cardiomyopathy.38,39 It is possible that ACE genotype influences β-receptor density and subtype differentially, with the II subjects (lowest serum and tissue ACE activity) having greater increases in β₂-receptor density with consequently more favorable effects on airway function and chronotropic responses to exercise. Despite increased β-blocker therapy in the ACE II subjects, they did tend to have a greater increase in heart rate with exercise. Hence, DD subjects may derive more benefit than II subjects from treatment with β-blockers. It is also possible that the DD subjects may have had improved hemodynamics with exercise if they had been equally treated. Additional investigations will be required to identify other potentially favorable pharmacogenetic interactions between the ACE I/D polymorphism and therapeutic agents for CHF.

Gene Dosage Influence
Our data also suggest the possibility of a gene dosage effect on neurohumoral mediators (Table 1) as well as pulmonary function and exercise parameters (Table 2), as implied by the generally ordered values obtained for many of these variables. However, limitations of sample size precluded our making statistically meaningful distinctions between alternative models of the estimated allelic effects.

Limitations
Although our data suggest a direct influence of altered pulmonary function on exercise tolerance in CHF, we did not have invasive hemodynamic data, and it is clear that resting estimates of LV function may not reflect cardiac function during exercise. It is possible that ACE genotype influences cardiac compliance and/or sensitivity to neurohumoral stimulation. Thus it is plausible that the ACE DD subjects have higher filling pressures, which in turn influences pulmonary vascular pressures and lung fluid balance resulting in alterations in lung stiffness (influencing lung volumes) and diffusion. It is also possible that ACE genotype influences other important parameters of performance, such as peripheral blood flow and/or gas exchange at the muscle.

In summary, in patients with stable CHF treated with standard therapy, the ACE DD genotype is associated with diminished exercise capacity mediated, in part, by impaired pulmonary function. Further studies are necessary to determine if this association is primarily a consequence of variation in the generation of A-II or is modulated via differences in bradykinin pathways.

Acknowledgments
This study was supported by the Mayo Foundation, HHS grant M01-RR00585, GCRC, Division of Research Resources, NIH, and

<table>
<thead>
<tr>
<th>Measurement</th>
<th>II Mean±SEM</th>
<th>ID Mean±SEM</th>
<th>DD Mean±SEM</th>
<th>ANOVA P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise time, min</td>
<td>8.0±0.5*</td>
<td>6.5±0.5</td>
<td>6.2±0.7</td>
<td>0.05</td>
</tr>
<tr>
<td>VO₂, mL/min</td>
<td>1760±210*</td>
<td>1493±117</td>
<td>1380±143</td>
<td>0.04</td>
</tr>
<tr>
<td>VCO₂, mL/min</td>
<td>1962±240</td>
<td>1710±145</td>
<td>1548±166</td>
<td>0.06</td>
</tr>
<tr>
<td>Fb, bpm</td>
<td>31±2*</td>
<td>32±1</td>
<td>36±2</td>
<td>0.05</td>
</tr>
<tr>
<td>V₉, L</td>
<td>2062±229</td>
<td>1838±120</td>
<td>1605±132</td>
<td>0.06</td>
</tr>
<tr>
<td>O₂ pulse</td>
<td>12.9±1.6</td>
<td>12.3±0.9</td>
<td>11.1±0.9</td>
<td>NS</td>
</tr>
<tr>
<td>VO₂/HR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, bpm</td>
<td>143±10</td>
<td>128±7</td>
<td>128±6</td>
<td>0.09</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>149±10</td>
<td>137±9</td>
<td>134±9</td>
<td>NS</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>80±4</td>
<td>71±5</td>
<td>69±4</td>
<td>NS</td>
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</table>

Pairwise comparisons, *II different from DD, #II different from ID. P<0.05, P>0.10 (NS).

Figure 4. Relation of FVC (left) and DLCO (right) to peak exercise VO₂.
National Heart Lung and Blood Institute grant HL71478. We would like to thank Kathy O’Malley and Sue Nelson, LPN, for their help in patient recruitment, Becky Hughes-Borst for help in data management, and Renee Blumers for help in manuscript preparation.

References


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_Circulation_. 2002;106:1794-1799; originally published online September 9, 2002;
doi: 10.1161/01.CIR.0000031735.86021.79
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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