plied another concentration and nonimmune steps. The reaction mixture, (R-1) was mixed. After 3 min, 1.2 mL of Reagent 2 (R-2) was added to the mixture, and the absorbance change at 650 nm was recorded at 37°C.

1.1.3.21), 195 U of peroxidase (EC 1.11.1.7), 150 U of ascorbate oxidase (EC 1.10.3.3), 0.21 mmol of ATP, and 60 mg of sodium 3,5-dimethoxy-N-ethyl-N-(2-hydroxy-3 sulfo-propyl)aniline (DAOS) dissolved in 100 mL of 200 mmol/L 2-(N-morpholino)ethanesulfonic acid buffer (pH 6.5). In our modified method, we additionally dissolved 11.8 mg of 4-aminoantipyrine in the 100 mL of Triglyceride. In Reagent 2, 30 mg of 4-aminoantipyrine and 270 kU of lipoprotein lipase (EC 3.1.1.34) are dissolved in 100 mL of 200 mmol/L 2-(N-morpholino)ethanesulfonic acid buffer (pH 6.5).

Our modified enzymatic method is performed in two steps. The first step is the color development to monitor the high concentration of FG. Endogenous FG is reacted with glycerol kinase and glycerol-3-phosphate oxidase to form hydrogen peroxide and then with peroxidase to form quinonimine dye. The second step is as in the routine enzymatic assay: lipoprotein lipase acts on TG to form glycerol, and the glycerol is measured as in the first step of the procedure.

The first-step reaction reaches maximum at an FG concentration of 1.50 g/L. Thus, samples containing FG >1.50 g/L show increased absorbance at 600 nm at the initiation of the second reaction. This value is fixed as a baseline value in TBA-80S analyzer; any further increase in absorbance in the second step is read as TG (Figure 1).

Within-assay CVs were 0.89–1.83% and between-assay CVs were 2.69–3.47%. Linearity was observed for TG concentrations up to at least 32.0. Values obtained by this modified method correlated well (r = 0.998) with those obtained by conventional enzymatic assay. An error signal indicating the need to dilute samples was observed when we used serum obtained from patients receiving glycerol treatment. We obtained similar results when we used another commercial kit, Triglyceride Color (Boehringer Mannheim Yamanouchi Co. Ltd., Tokyo, Japan), and applied this method to a Cobas-FARA centrifugal analyzer (F.

Hoffmann-La Roche & Co. Ltd., Basel, Switzerland). Thus, our modified method for TG is applicable to other automated analyzers and should be used for samples from patients receiving medication containing glycerol.

References

Impact of Inhibition of Angiotensin-Converting Enzyme on Urinary Excretion of Proteins in Chronic Heart Failure, G. Ellekleilade,1 J. Holm,2 L. Hemmingsen,2 B. Koczula,1 and F. E. von Eyben1 (Depts. of Intern. Med.1 and Clin. Chem.,2 Central Hospital Nykøbing Falster, Nykøbing Falster 4800, Denmark)

Chronic heart failure (CHF) is often associated with a reduction of renal blood flow (1). Hence, concentrations of serum creatinine are commonly increased in CHF patients (1). Abnormalities in the renal handling of proteins, i.e., increased urinary excretion rates of albumin (a marker of glomerulopathy) and retinol-binding protein (RBP; a marker of proximal tubular dysfunction reflecting impaired reabsorption of low-M proteins), are also seen in CHF (2). Angiotensin-converting enzyme (ACE) inhibitors, which increase renal blood flow, are commonly used as supplementary medication to treat CHF (1, 3). Here, we report a study to monitor the impact of ACE inhibitor treatment on the urinary excretion pattern of proteins in CHF.

We studied 19 patients, 16 men (median age 75 years, range 68–87) and 3 women (ages 56, 67, and 78 years), consecutively admitted to our hospital with a diagnosis of CHF and dyspnea during physical exercise (New York Heart Association groups II and III). Exclusion criteria for the study were diabetes mellitus, arterial hypertension, myocardial infarction within six months, valvular disorder, acute pulmonary edema, primary renal disorder, unstable angina, and treatment with ACE inhibitors. All 19 patients received conventional treatment for CHF, i.e., diuretics alone or in combination with digoxin, before and during hospitalization. Serum creatinine concentrations were normal in 13 patients (3 women ≤115 μmol/L; 10 men ≤133 μmol/L) but above normal in 6 men (134–200 μmol/L). All urine specimens were Albustix negative.

Treatment with the ACE inhibitor (Captopril, Bristol-Myers Squibb Jægersborgvej 64–66 2800 Lyngby, Denmark) was begun during hospitalization (25–75 mg/day). Albumin and RBP concentrations were determined by immunochemical assays (4, 5) in overnight urine specimens collected before and after the start of treatment. Creatinine in serum and urine was determined by a routine method (kinetic Jaffé, with a Hitachi 717 analyzer).

As shown in Table 1, the urinary excretion of albumin decreased significantly from days 0 (before ACE inhibitor treatment) to 7 after initiation of therapy. The decrease seemed to continue on days 14 and 30, although the between-day changes were not statistically significant. The proportion of patients with above-normal urinary albumin
Table 1. Urinary Excretion of Albumin and RBP in 19 CHF Patients Treated with ACE Inhibitor

<table>
<thead>
<tr>
<th>Day of treatment</th>
<th>Albumin (μmol/mol creatinine)</th>
<th>Retinol-binding protein (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>67 (4-1918)</td>
<td>0.64 (0.11-103.67)</td>
</tr>
<tr>
<td>7</td>
<td>36 (0-8875)*</td>
<td>0.92 (0.16-48.23)</td>
</tr>
<tr>
<td>14</td>
<td>17 (2-475)*</td>
<td>0.41 (0.24-33.14)</td>
</tr>
<tr>
<td>30</td>
<td>11 (0-759)*</td>
<td>0.59 (0.20-52.39)*</td>
</tr>
<tr>
<td>Ref. group*</td>
<td>7 (3-29)</td>
<td>0.44 (0.07-1.37)</td>
</tr>
</tbody>
</table>

*Significantly different from excretion on day 0: P = 0.01. 
**Values from 22 apparently healthy subjects.

excretion values decreased from two-thirds on day 0 to one-third on day 30.

The impact of ACE inhibitor treatment on RBP urinary excretion was slower and less pronounced (Table 1). A statistically significant decrease in the excretion of RBP from day 0 was not seen until day 30, and the proportion of patients with above-normal values decreased from only one-fifth on day 0 to one-tenth on day 30.

The median (and range) serum creatinine concentrations remained constant during the observation period: 114 (62-200) μmol/L on day 0 and 118 (75-182) μmol/L on day 30. All patients were normotensive. Systolic and diastolic blood pressures remained constant during the study, as did body weights.

Increased glomerular perfusion pressure accompanied by changes in glomerular permeability may cause glomerular sclerosis with progressive renal failure (1). A mechanism of that type could explain the increased urinary excretion of albumin in CHF (2), where the glomerular perfusion pressure is increased (1). The antiproteinuric effect of ACE inhibition in hypertension and diabetes is associated with glomerular hemodynamic alterations, which result in a decreased glomerular perfusion pressure (1). Supplementary treatment with ACE inhibitor in CHF had no effect on body weight, serum creatinine concentration, and blood pressure, but the urinary excretion of RBP and in particular albumin decreased. The present data do not lead to any conclusions as to the exact mechanisms underlying the antiproteinuric effect of ACE inhibition in CHF. However, obvious parallels can be drawn to the conditions in diabetes mellitus and arterial hypertension (6).

We appreciate the valuable technical assistance of Mrs. Hanne Bredder.

References