Evaluation of Skeletal Muscle Capillary Basement Membrane Thickness in Congestive Heart Failure*

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Although it has been demonstrated that during isotonic grip exercise patients with chronic congestive heart failure have an abnormally reduced forearm oxygen consumption resulting from a depressed forearm blood flow, there may be additional etiologies of this abnormality. To explore this possibility biopsies of the pronator teres muscle were taken in eight control subjects with normal cardiac hemodynamics and in seven severely decompensated chronic congestive heart failure subjects. Each sample was fixed, stained, and photomicrographs of the sections determined. The control capillary basement membrane thickness was 3028±187 Å (mean ± SEM) compared to the congestive heart failure thickness of 4924±538 Å (p<.01). It is possible that the increased basement membrane thickness in congestive heart failure may result from or actually cause the depressed oxygen consumption by altering diffusion.

Patients with congestive heart failure have been observed to have reduced blood flow to skeletal muscle both at rest and during submaximal dynamic exercise. Since during the resting state an increased oxygen extraction occurs, the forearm muscle oxygen consumption remains normal. This increased oxygen extraction may be facilitated by a prolonged capillary transit time as well as by an increased P50 of the oxygen-hemoglobin disassociation curve which is secondary to increased red blood cell 2, 3-diphosphoglycerate content and a relative tissue acidosis. During dynamic exercise in heart failure the increase in skeletal muscle blood flow is inadequate since the arterioles respond poorly to metabolic vasodilator stimuli. Although there is a further enhancement in oxygen extraction during exercise, forearm oxygen consumption falls and becomes inadequate to maintain aerobic metabolism. Although it has been assumed that the relative arteriolar stiffness as well as increased sympathetic discharge could account for a shift to anaerobic metabolism, the possibility of a diffusion defect at the capillary level has not been explored. This study does not look at capillary diffusion directly but rather looks for possible ultrastructural abnormalities that might alter diffusion.

**METHODS**

Seven patients with congestive heart failure (NYHA functional class 3-4) 35 to 72 years old (mean age: 55.4±4.3, SEM) were compared with eight control subjects 28 to 53 years old (mean age 43.6±2.8) who underwent diagnostic cardiac catheterization and were found to have minimal or no heart disease. Six of the heart failure patients had rheumatic valvular heart disease and one had constrictive pericarditis. Three normal subjects had very mild valvular heart disease, three had mild coronary artery disease and two were normal. Only subjects who were found to have normal glucose tolerance tests were studied. All studies were approved by the Chancellor's Advisory Committee for Research Involving Physiological or Clinical Studies on Human Subjects and in all instances informed consent was obtained. All subjects required complete hemodynamic studies as well as left heart catheterization by brachial arteriotomy to evaluate their heart disease. Intracardiac pressures were measured by standard catheter manometer systems (Statham P23Db pressure transducers) and recorded on a Hewlett Packard 8800 series system optical recorder model no. 4578A. Cardiac index was determined by the dye dilution technique in duplicate.

Following exposure the brachial artery was gently retracted with a rubber Penrose drain to expose the pronator teres muscle. A 1 ml biopsy specimen of this muscle was obtained by sharp dissection and was immediately rinsed in cold phosphate buffer to remove all blood products. Sections were fixed 0.15 M phosphate buffered 1.6 percent glutaraldehyde solution with an osmolarity of 356 for one hour. This was followed by a phosphate buffered 1 percent osmium tetroxide fixation for one hour. The sections were
Table 1—Hemodynamics in Control Subjects and Patients with Congestive Heart Failure

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Congestive Heart Failure</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects in each group</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Right atrial pressure (mm Hg ± SEM)</td>
<td>4.5 ± 0.7</td>
<td>13.6 ± 3.2</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Right ventricular end diastolic pressure (mm Hg ± SEM)</td>
<td>5.6 ± 0.9</td>
<td>15.9 ± 2.9</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Left ventricular end diastolic pressure (mm Hg ± SEM)</td>
<td>11.5 ± 1.5</td>
<td>27.0 ± 3.7</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Cardiac index (L/min/M² ± SEM)</td>
<td>3.42 ± 0.22</td>
<td>1.65 ± 0.22</td>
<td>&lt;.01</td>
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then carefully oriented in Lufts Epon plastic with aid of a 40 X magnification dissecting scope to obtain only cross sections of capillaries. They were then stained in 2 percent aqueous uranyl acetate (pH=4.8) for five minutes followed by Reynolds lead citrate stain (pH=12) for five additional minutes. A Zeis model 9S electron microscope was then used to examine the sections and obtain photographs at a magnification of 2500. Initially, all micrographs of capillaries were carefully screened to separate lymphatic from blood capillaries. This was done by observation of a clear basement membrane or a pericyte or both surrounding a capillary which are typical for blood capillaries only. Thus, only blood capillaries which appeared to be cut in cross section were evaluated. The plane of section was determined by several procedures. Since muscle fibers have capillaries which run predominantly parallel with the myofibers, both were oriented so that the fibers' as well as the capillaries' longitudinal axis were predominately perpendicular to the plane of section. All capillaries which appeared in a shape which was not predominately circular were not considered. In all sections myofibrils could be viewed and if these did not appear to be cut in cross section, the micrographs were discarded. The method of Siperstein and co-workers was used to determine average basement membrane thickness. Each negative was projected onto a translucent screen via an opaque projector together with a plastic overlay consisting of 20 equidistant lines radiating from a central point. With the central point or intersection of these lines in the center of each capillary, the distance of the perpendicular from the point at which each line touched the luminal side of the basal lamina to the interstitial surface of the lamina was used as the basement membrane thickness at that point. For each capillary, 20 measurements could be taken to obtain an average single basement membrane thickness. All negatives that were not in focus or those with unclear basement membrane borders were rejected. At least 14 (average=18) capillaries were averaged for each individual to help rule out individual capillary variations. Longitudinal sections of skeletal muscle caused too much disruption of basement membranes to make accurate measurements; however, it was determined from these sections that capillaries did not appear to be more tortuous in heart failure than in normal.

RESULTS

The hemodynamic status of the heart failure patients and the control subjects is presented in Table 1. The patients with congestive heart failure had significant right heart failure as evidenced by an elevated right atrial pressure and right ventricular end diastolic pressure. Similarly, the subjects had significantly altered left heart hemodynamics with elevated left ventricular end diastolic pressures as well as reduced cardiac indices. The eight control
subjects had a mean basement membrane thickness of 3028±187 Å. This was significantly less than the basement membrane for the seven patients with congestive heart failure who had a mean basement membrane thickness of 4924±538 Å, p<.01 (Fig 1). In three patients with class 2 and early class 3 congestive failure, basement membrane thickness averaged 3352±67 Å.

**DISCUSSION**

An increased basement membrane thickness has been noted in a variety of conditions. These have included diabetes mellitus, prediabetes, myxedema and polymyositis. The basement membrane thickness of all our subjects were greater than those previously reported. This may reflect the relatively older age of our subjects, especially those with congestive heart failure. However, an increased basement membrane thickness with age is not universally accepted. The larger values might be accounted for by the different muscle sampled. The pronator teres was used because of the convenience in sampling at the time of cardiac catheterization. Since sampling and processing techniques for both groups of subjects were similar, the large differences between the two groups in basement membrane thickness appears to be real. The additional basement membranes evaluated from heart failure patients in functional class 2 or early 3 (NYHA) demonstrated only a slight increase in thickness from those of normal subjects and were much thinner than basement membranes of late class 3 or 4 patients suggesting that if congestive failure is a major factor inducing these changes in basement membrane thickness, it must be of a severe degree. One theory suggests that an increased basement membrane thickness may be the result of local hypoxia and cell death. With the formation of new cell components, the new basement membrane material may oppose itself to the old lamina leading to the appearance of significant thickening. In congestive heart failure a high degree of local hypoxia undoubtedly occurs especially during exercise. Therefore, if local hypoxia were a factor contributing to increased basement membrane thickness, the finding of this abnormality in severe heart failure is not unexpected. Alternatively, the chronic elevation of venous pressure seen in heart failure might also be contributory.

In this study it was not determined whether the increased thickness of the capillary basement membrane in severe congestive heart failure patients' skeletal muscles was continuous or segmental in nature, due to a need to restrict the biopsy to a very small sample size which could be easily yet quickly obtained at the time of cardiac catheterization. However, the random sampling method in this study of an average of 18 capillaries per individual was utilized as an effort to prevent inadequate sampling of a few unrepresentative capillaries.

Recently electrolyte diffusion has been evaluated in diabetes, and it has been suggested that the increased basement membrane thickness facilitates rather than retards diffusion. On the other hand measurements of oxygen diffusion, which is not dependent upon electrostatic charges in the ground substance as is electrolyte diffusion, has not been determined. Another abnormality present in severe congestive heart failure is the peripheral edema which could theoretically retard diffusion by increasing the capillary to cell diffusion distance. Clearly further studies are needed to determine whether or not the increased basement membrane thickness and edema seen in severe congestive heart failure retards oxygen diffusion and may partially explain the reduced muscle oxygen consumption when such patients perform dynamic exercise.

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