EFFECT OF EXERCISE ON THE RENAL CLEARANCE OF AMYLASE AND LYSOZYME IN HUMANS

J. R. POORTMANS

Laboratoire de l'Effort, Université Libre de Bruxelles, Belgium

(Received 23 December 1971)

SUMMARY

1. Total protein amount and amylase and lysozyme activities were measured in the serum and urine of healthy women and men before and after strenuous exercise.
2. Exercise produced no major difference in the serum activities of amylase and lysozyme.
3. The renal clearance of amylase was unaffected by strenuous exercise, but total protein excretion was markedly increased.
4. Very high values of renal clearance of lysozyme were observed after exercise. This implies that the proximal tubular function of protein reabsorption was specifically affected.

Key words: exercise, proximal tubule function, renal clearance.

Studies of the renal permeability to macromolecules have been made on normal human subjects (Rowe & Soothill, 1961; Poortmans & Jeanloz, 1968; Poortmans, 1969) and on patients (Blainey, Brewer, Hardwicke & Soothill, 1960; Hardwicke, 1965; Cameron & Blandford, 1966; Blainey, 1968; Bienenstock & Poortmans, 1970). Studies on the renal handling of proteins have led Schultze & Heremans (1966) and Hardwicke & Soothill (1967) to postulate a mechanism for physiological and pathological proteinuria. Analysis of different urine proteins can now indicate the way kidney function is disturbed.

Previous studies have shown that proteinuria after strenuous exercise differs from normal proteinuria at rest (Poortmans, 1964). Determination of individual proteins in urine, as well as protein clearance, provides a means of classifying 'glomerular' and 'tubular' proteinuria (Manuel, Revillard & Betuel, 1970). The specific pattern of proteinuria characterizing renal tubular disease is due to failure of reabsorption of filtered low-molecular-weight proteins (Harrison & Blainey, 1967). Therefore, a study of lysozyme (mol. wt. 15 000) and of amylase (mol. wt. 50 000) might indicate impaired tubular function.

Correspondence: Dr J. R. Poortmans, Laboratoire de l'Effort, Université Libre de Bruxelles, avenue Paul Heger 28, B-1050 Bruxelles, Belgium.
In this paper data are reported on lysozyme and amylase excretion, as well as their clearance in healthy adults, to assess the relative contribution of tubular impairment to protein excretion.

METHODS

Experimental procedure

Seventeen subjects were studied, eight women and nine men, their age being in the range 19–21 years. All subjects were in good health and all subjects consented to the study after full explanation of the purpose. They underwent strenuous exercise consisting of six 1 min periods of severe exertion, interrupted by 1 min periods of rest. This test is based mainly on anaerobic capacity and is commonly named ‘Magglinger Konditions-Test’ (Rüegsegger, 1964).

Blood samples were drawn and urine collected quantitatively by voiding, immediately before, then 2 and 30 min after exercise.

Analysis

Serum was separated within 1 h of collection and was stored at −20°C until assayed. Urine collections were timed, and specimens were preserved with sodium merthiolate (1 g/l), which does not interfere with the investigated enzyme activities. After 1 day at 4°C, urine samples were filtered and stored at −20°C until assayed.

Serum protein concentration was determined by the biuret technique (O'Brien & Ibbott, 1964) and urine protein determination was measured with Amido Black (Heremans, 1958). Serum and urinary amylase (α-1,4-glucan 4-glucanohydrolase, EC 3.2.1.1) activities were estimated by an amyloclastic method (Mark & Zimmer, 1967). The substrate was a stable soluble starch preparation (Merck). Lysozyme (muramidase or mucopeptide N-acetylmuramylhydrolase, EC 3.2.1.17) activity was estimated in serum and urine by the method of Harrison, Lunt, Scott & Blainey (1968) based on the lysis of Micrococcus lysodeikticus. The substrate was a freshly prepared suspension of dried M. lysodeikticus (Boehringer et Soehne) and the standard was crystallized egg-white lysozyme with an activity of 15 000 units/mg by the method of Shugar (1952), from Koch–Light Laboratories.

Calculations

Renal clearance of amylase and of lysozyme were expressed in µl/min. Values of P were determined by means of the Student’s t test.

RESULTS

Tables 1 and 2 show the observed data at rest, immediately after exercise and 30 min later. The values obtained at rest were within the normal ranges given in the literature. The samples obtained immediately after stopping exercise showed a significant rise in plasma and urine proteins in women and in men. The enzymic activity in plasma remained unchanged in the former group, while lysozyme activity was slightly increased in the latter. The activity of urinary amylase was slightly elevated only in men. However, high values were observed for lysozyme excretion in both sexes after exercise.

The urinary clearance of amylase was unchanged, but a significant increase of lysozyme was obtained, especially in men.
By 30 min after the end of the exercise, plasma proteins returned to initial values while urine proteins remained elevated. Amylase activity in urine continued to rise in men only. Lysozyme excretion remained high in men but increased many times in women. The clearance of lysozyme was also considerably increased, especially in men.

<table>
<thead>
<tr>
<th>Table 1. Clearances and analyses of plasma and urine of nine female subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Serum proteins (g/100 ml)</td>
</tr>
<tr>
<td>Serum amylase (units/100 ml)</td>
</tr>
<tr>
<td>Serum lysozyme (µg/100 ml)</td>
</tr>
<tr>
<td>Urine output (ml/min)</td>
</tr>
<tr>
<td>Urine proteins (mg/min)</td>
</tr>
<tr>
<td>Urine amylase (units/min)</td>
</tr>
<tr>
<td>Urine lysozyme (µg/min)</td>
</tr>
<tr>
<td>Amylase clearance (µl/min)</td>
</tr>
<tr>
<td>Lysozyme clearance (µl/min)</td>
</tr>
</tbody>
</table>

The values are the mean with the standard deviation. P values from comparisons with results before exercise are given in parentheses.

No correlation was observed between lysozyme and amylase clearances in either men or women. No relationship was obtained between the activity of either enzyme and total protein excretion in urine.
TABLE 2. Clearances and analyses of plasma and urine of eight male subjects

<table>
<thead>
<tr>
<th></th>
<th>Before exercise</th>
<th>Immediately after exercise</th>
<th>30 min after exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum proteins (g/100 ml)</td>
<td>6.34 ± 0.29</td>
<td>7.02 ± 0.48</td>
<td>6.56 ± 0.31</td>
</tr>
<tr>
<td>Serum amylase (units/100 ml)</td>
<td>112 ± 37</td>
<td>150 ± 48</td>
<td>134 ± 49</td>
</tr>
<tr>
<td>Serum lysozyme (μg/100 ml)</td>
<td>690 ± 225</td>
<td>817 ± 269</td>
<td>776 ± 214</td>
</tr>
<tr>
<td>Urine output (ml/min)</td>
<td>0.438 ± 0.325</td>
<td>0.734 ± 0.380</td>
<td>0.945 ± 0.664</td>
</tr>
<tr>
<td>Urine proteins (mg/min)</td>
<td>0.064 ± 0.018</td>
<td>0.731 ± 0.357</td>
<td>0.420 ± 0.170</td>
</tr>
<tr>
<td>Urine amylase (units/min)</td>
<td>0.82 ± 0.42</td>
<td>1.65 ± 1.14</td>
<td>2.66 ± 1.36</td>
</tr>
<tr>
<td>Urine lysozyme (μg/min)</td>
<td>0.18 ± 0.18</td>
<td>9.16 ± 12.77</td>
<td>10.61 ± 13.90</td>
</tr>
<tr>
<td>Amylase clearance (μl/min)</td>
<td>9.32 ± 4.59</td>
<td>10.95 ± 5.44</td>
<td>16.9 ± 6.24</td>
</tr>
<tr>
<td>Lysozyme clearance (μl/min)</td>
<td>34.85 ± 35.67</td>
<td>1643 ± 1902</td>
<td>2773 ± 5403</td>
</tr>
</tbody>
</table>

The values are the mean with the standard deviation. P values from comparisons with results before exercise are given in parentheses.

DISCUSSION

Studies of the renal clearances of proteins in normal human subjects remain limited by the small quantity of plasma protein present in the urine. However, approx. thirty different plasma components have been identified in normal urine obtained at rest (Berggård, 1970) and renal clearances have been established for fifteen of them (Poortmans, 1969). Most of these proteins have a molecular weight in the range from 45 000 to 160 000. Urinary excretion of plasma proteins of smaller molecular weight appears to be of less importance, even if their small size permits them to pass readily into the glomerular filtrate (Poortmans, 1969). However, these low-molecular-weight proteins are only present in very small amounts in serum, as shown by gel-filtration analyses on Sephadex G-200.
Exercise and urine enzyme clearances

It is now clear that proteinuria of renal tubular disease is due to the failure of reabsorption of filtered low-molecular-weight protein (Harrison & Blainey, 1967). For this reason several authors have chosen the urinary excretion of low-molecular-weight proteins including enzymes, as a test of renal tubular function (Blainey, 1968; Harrison et al., 1968; Barratt & Crawford, 1970). A recent report has directed attention to possible errors in the use of urine enzyme determinations in the diagnosis of renal dysfunction (Raab, 1968). Indeed, most enzymes have a prerenal origin and their presence in urine mostly reflects muscular, cardiac or hepatic diseases. This, however, is not the case with lysozyme and amylase. It has been established that the urinary excretion of lysozyme does not depend on the presence in urine of blood corpuscles, bacteria or epithelial cells (Goldberg, Chakrabarti & Filipich, 1966). Its renal threshold has been estimated as 45 µg/ml, above which lysozyme is excreted in urine (Hayslett, Perille & Finch, 1968). Several authors have used its urinary excretion to provide a simple quantitative technique for the diagnosis of proximal tubular dysfunction (Barratt & Crawford, 1970). Urinary amylase activity chiefly reflects exocrine pancreatic function (Hobbs & Aw, 1968). Blainey & Northam (1967) showed that there was little reabsorption of amylase through the renal tubules and that the amylase clearance was closely related to the creatinine clearance over a wide range.

We have postulated that proteinuria occurring after strenuous exercise is mainly due to an increased glomerular permeability associated with saturation of the tubular reabsorption (Poortmans & Jeanloz, 1968; Poortmans, 1969). These studies were based on the renal clearance of fifteen plasma proteins. Tubular impairment was thought to be absent or of very minor importance during physical stress.

Our present data on amylase and lysozyme concentration in serum and urine at rest accord with previous results (Harrison et al., 1968; Barratt & Crawford, 1970; Blainey & Northam, 1967). We have confirmed the observation of these previous authors that lysozyme and amylase excretions are not increased in the presence of pathological proteinuria. These findings suggest that the kidney handles high- and low-molecular-weight proteins differently, most of the latter being more fully reabsorbed (Bienenstock & Poortmans, 1970). We failed to observe any post-exercise disturbance of the amylase.

The lysozyme measurements, however, suggest that some failure of proximal reabsorption of filtered proteins occurs as a consequence of strenuous physical exertion. Increased filtration cannot explain the large amount of lysozyme in the urine after exercise. This indicates saturation of the capacity of tubules to bind lysozyme. Increased lysozyme in the urine usually is associated with extensive tubular damage (Wauters & Fabre, 1970). It seems improbable that strenuous exercise induces such damage of tubular cells, since this apparent dysfunction is only temporary and restricted to several hours, at the most, after stopping the exercise (Poortmans, 1969).

Thus, although the lysozyme clearance is increased, this does not give clear evidence of renal tubular dysfunction after exercise. These discrepancies between exercise and pathological conditions are at present difficult to explain and need further investigation.

ACKNOWLEDGMENTS

This research was supported by a grant from the Fonds National de la Recherche Scientifique (Belgium). We are grateful to Mrs V. Vuylsteke for determining the total protein and to Miss F. Louppe who estimated amylase and lysozyme.
REFERENCES


