Changes in the concentrations of urinary proteins after physical exercise.

Taizo Miyai*  Masana Ogata†

*Okayama University,
†Okayama University,
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Abstract

The influence of physical exercise on the urinary excretion of proteins was examined in 17 male high school baseball players. Their urine was collected before and after exercise to determine the concentrations of total protein, albumin, beta 2-microglobulin and creatinine along with the activity of N-acetyl-beta-D-glucosaminidase (EC 3.2.1.30). Concentrations of total protein, albumin, beta 2-microglobulin and creatinine increased significantly (p less than 0.01) after exercise, while N-acetyl-beta-D-glucosaminidase activity did not increase. Similar results were obtained when the concentrations of these urinary components were calculated on the basis of a urinary density of 1.024, and when they were expressed relative to the amount of creatinine. Positive correlations were seen among total protein, albumin, beta 2-microglobulin and creatinine concentrations, but not between the beta 2-microglobulin concentration and N-acetyl-beta-D-glucosaminidase activity. Isoenzyme activities of N-acetyl-beta-D-glucosaminidase in the urine were determined by electrophoresis on cellulose acetate plates. After exercise, the A-form increased slightly, and the B-form decreased slightly, but these changes were not statistically significant.

KEYWORDS: urinary protein, ?2-microglobulin, N-acetyl-?-D-glucosaminidase, isoenzyme

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Changes in the Concentrations of Urinary Proteins after Physical Exercise

Taizo Miyai* and Masana Ogata

Department of Public Health, Okayama University Medical School, Okayama 700, Japan

The influence of physical exercise on the urinary excretion of proteins was examined in 17 male high school baseball players. Their urine was collected before and after exercise to determine the concentrations of total protein, albumin, β₂-microglobulin and creatinine along with the activity of N-acetyl-β-D-glucosaminidase (EC 3.2.1.30). Concentrations of total protein, albumin, β₂-microglobulin and creatinine increased significantly (p < 0.01) after exercise, while N-acetyl-β-D-glucosaminidase activity did not increase. Similar results were obtained when the concentrations of these urinary components were calculated on the basis of a urinary density of 1.024, and when they were expressed relative to the amount of creatinine. Positive correlations were seen among total protein, albumin, β₂-microglobulin and creatinine concentrations, but not between the β₂-microglobulin concentration and N-acetyl-β-D-glucosaminidase activity. Isoenzyme activities of N-acetyl-β-D-glucosaminidase in the urine were determined by electrophoresis on cellulose acetate plates. After exercise, the A-form increased slightly, and the B-form decreased slightly, but these changes were not statistically significant.

Key words: urinary protein, β₂-microglobulin, N-acetyl-β-D-glucosaminidase, isoenzyme

One function of the kidneys is to excrete urine after filtration and reabsorption to maintain the homeostasis of the humoral circulation. It is well known that protein increases in the urine not only in persons with a renal disorder but also in healthy persons when they assume some specific posture, or when a work load is applied (1–3). Poortmans (4–6) reported the appearance of 15 kinds of serum proteins in the urine after hard work or physical exercise. Other authors also have reported that albumin (Mr = 69,000) (7, 8), Zn-α₂-glycoprotein (9), α₁-acid glycoprotein (10) and α₂-HS-globulin (10), were excreted in the urine after exercise. In most cases, a work load produced by exercise causes glomerular proteinuria with increased urinary protein consisting mainly of albumin, and tubular proteinuria with increased low molecular weight proteins, such as β₂-microglobulin (Mr = 11,800) (11). N-acetyl-β-D-glucosaminidase (EC 3.2.1.30) (NAG; Mr = 112,000) has been shown to increase in the urine in the case of tubular damage, such as tubular nephritis caused by cadmium intoxication (12). Most creatinine (13) is excreted without being reabsorbed, and its level is used as an endogenous index to determine renal clearance.

In this study, we measured concentrations of total protein (14, 15), albumin, β₂-microglobulin...
and creatinine concentrations and total and isoenzyme activities of NAG (16, 17) in the urine before and after physical exercise to clarify the influence of the work loading.

Materials and Methods

Subjects. The subjects were 17 healthy males who belonged to a high school baseball team. They ranged in age from 16 to 17 years old. During a training camp of 4 days and 3 nights, urine was collected on the second day before exercise at 10:00 a.m. (temperature, 28.5°C; humidity, 67%), and after exercise at 12:30 p.m. (temperature, 30.7°C; humidity, 65%) because the work load on the first day was relatively light. The urine was immediately subjected to the determinations of urinary components.

The exercise program. On the 1st day, the subjects did warm-up exercises for 20 min, ran for 20 min, practiced catch for 40 min, and after a 20 min rest, they ran for 20 min. On the 2nd day, after the 1st urine sample was collected, they did warm-up exercises for 30 min, ran for 20 min, practiced catch for 30 min, did muscular power training for 50 min, and ran for 20 min. Immediately after the exercise the 2nd urine sample was collected.

Measurements. The total protein concentration was determined with a protein assay kit (Otsuka Assay Laboratories, Tokyo Japan), which employs a dye-binding method (18). The β₂-microglobulin concentration was determined with an enzyme immunoassay kit (19) (Fuji Revio Inc., Tokyo, Japan). The albumin concentration was determined by single radial immunodiffusion (20). Standard human serum (Behring Co., West Germany) was used as the standard. The creatinine concentration was determined by Jaffe’s method (21) with an assay kit (Wako Pure Chemical Ind., Ltd., Osaka, Japan). NAG activity was determined using a colorimetric kit (22) (Sionogi Co., Osaka, Japan). For the

Table 1  Changes in urinary excretion of proteins after physical exercise

<table>
<thead>
<tr>
<th>Components</th>
<th>Before exercise</th>
<th>After exercise</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine volume</td>
<td>ml</td>
<td>68.2 ± 23.7</td>
<td>47.8 ± 18.2**</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>(ml)</td>
<td>1.0324 ± 0.0058</td>
<td>1.0324 ± 0.0055</td>
</tr>
</tbody>
</table>

Concentrations

<table>
<thead>
<tr>
<th>Components</th>
<th>(mg/dl)</th>
<th>19.03 ± 7.84</th>
<th>32.44 ± 19.79*</th>
<th>13.4 ± 15.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>(mg/dl)</td>
<td>6.26 ± 3.29</td>
<td>17.94 ± 14.17*</td>
<td>11.7 ± 12.1</td>
</tr>
<tr>
<td>β₂-Microglobulin</td>
<td>(mg/dl)</td>
<td>2.82 ± 1.94</td>
<td>4.30 ± 3.12*</td>
<td>1.5 ± 1.9</td>
</tr>
<tr>
<td>Creatinine</td>
<td>(mg/dl)</td>
<td>212.32 ± 52.96</td>
<td>257.56 ± 63.52*</td>
<td>45.2 ± 31.1</td>
</tr>
<tr>
<td>N-acetyl-β-D-glucosaminidase</td>
<td>(U/dl)</td>
<td>59.7 ± 31.1</td>
<td>58.2 ± 28.3</td>
<td>-1.5 ± 31.0</td>
</tr>
</tbody>
</table>

Concentrations corrected for specific gravity

<table>
<thead>
<tr>
<th>Components</th>
<th>(mg/dl)</th>
<th>13.95 ± 4.63</th>
<th>23.55 ± 13.88*</th>
<th>9.64 ± 11.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>(mg/dl)</td>
<td>4.43 ± 2.23</td>
<td>12.81 ± 10.20*</td>
<td>8.37 ± 9.00</td>
</tr>
<tr>
<td>β₂-Microglobulin</td>
<td>(mg/dl)</td>
<td>2.03 ± 1.28</td>
<td>3.15 ± 2.15*</td>
<td>1.11 ± 1.35</td>
</tr>
<tr>
<td>Creatinine</td>
<td>(mg/dl)</td>
<td>156.12 ± 23.46</td>
<td>188.94 ± 27.96*</td>
<td>32.97 ± 27.23</td>
</tr>
<tr>
<td>N-acetyl-β-D-glucosaminidase</td>
<td>(U/dl)</td>
<td>43.6 ± 20.5</td>
<td>42.8 ± 18.5</td>
<td>-6.1 ± 33.0</td>
</tr>
</tbody>
</table>

Concentrations relative to the amount of creatinine

<table>
<thead>
<tr>
<th>Components</th>
<th>(g)</th>
<th>0.089 ± 0.023</th>
<th>0.155 ± 0.049*</th>
<th>0.066 ± 0.039</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>(g)</td>
<td>0.028 ± 0.012</td>
<td>0.062 ± 0.043*</td>
<td>0.031 ± 0.055</td>
</tr>
<tr>
<td>β₂-Microglobulin</td>
<td>(g)</td>
<td>1.258 ± 0.818</td>
<td>1.491 ± 0.986*</td>
<td>0.430 ± 0.575</td>
</tr>
<tr>
<td>N-acetyl-β-D-glucosaminidase</td>
<td>(U)</td>
<td>29 ± 11</td>
<td>21 ± 8</td>
<td>-21 ± 18</td>
</tr>
</tbody>
</table>

a: Values were obtained from 17 male students before and after physical exercise and expressed as the mean ± SD.
Details are described under Materials and Methods.
b: Significantly different by paired t-test from the values before exercise; *, p < 0.01; **, p < 0.05.
c: Calculated on the basis of a specific gravity of 1.024.
d: Expressed as g or U per g of creatinine.
assay of NAG isoenzymes, 5 μl of a urine sample was
applied to a cellulose acetate plate, TITAN-III (Helena
Laboratories, USA), and electrophoresis was performed
using 0.04 M potassium phosphate buffer, pH 6.5, for 1 h
with a low current of 5 mA per support medium. The
sample was allowed to react for 40 min at 37°C with
9 mM of soda-m-cresolsulphonphthaleinyl-N-acetyl-β-D-
glucosaminide (MCP-NAG) applied to the support
medium by the sandwich method. Subsequently, the
reaction was terminated with 0.3 M Na₂CO₃. Den-
sitometry was conducted at 570 nm. The concentrations
of the 4 urinary components were calculated on the basis
of a urinary density of 1.024 and also expressed relative
to the amount of creatinine.

Results

Table 1 shows concentrations of total pro-
teins, albumin, β₂-microglobulin and creatinine

<table>
<thead>
<tr>
<th>Before exercise</th>
<th>Total protein</th>
<th>Albumin</th>
<th>β₂-Microglobulin</th>
<th>Creatinine</th>
<th>NAG*</th>
</tr>
</thead>
<tbody>
<tr>
<td>After exercise</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein</td>
<td>0.925</td>
<td>0.644</td>
<td>0.714</td>
<td>0.400</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>0.971</td>
<td></td>
<td>0.583</td>
<td>0.695</td>
<td>0.376</td>
</tr>
<tr>
<td>β₂-Microglobulin</td>
<td>0.685</td>
<td>0.596</td>
<td></td>
<td>0.474</td>
<td>0.215</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.729</td>
<td>0.706</td>
<td>0.574</td>
<td></td>
<td>0.490</td>
</tr>
<tr>
<td>NAG</td>
<td>0.397</td>
<td>0.313</td>
<td>0.200</td>
<td>0.525</td>
<td></td>
</tr>
</tbody>
</table>

a: N-acetyl-β-D-glucosaminidase.

Table 3 Isoenzyme activities of urinary N-acetyl-β-D-
glucosaminidase before and after physical exercise

<table>
<thead>
<tr>
<th>Isoenzyme</th>
<th>Activity (Units/liter) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before exercise</td>
</tr>
<tr>
<td>A-form</td>
<td>2.84 ± 1.32</td>
</tr>
<tr>
<td></td>
<td>(76.47 ± 5.17)</td>
</tr>
<tr>
<td>B-form</td>
<td>0.84 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>(23.51 ± 5.17)</td>
</tr>
<tr>
<td>Total</td>
<td>3.68 ± 1.65</td>
</tr>
</tbody>
</table>

a: Isoenzyme activities in the urine of 17 male students were
determined using cellulose acetate plates.
Details are described under Materials and Methods.

and activity of NAG in the urine of students
before and after physical exercise. As the specific
gravity of urine varied among subjects and
between before and after the exercise, the values
were calculated on a basis of a urinary density of
1.024. Table 1 also shows concentrations expres-
sed relative to the amount of creatinine.

The concentrations of total protein, albumin,
β₂-microglobulin and creatinine increased signifi-
cantly (p < 0.01, paired Student's t-test) after the
exercise, while NAG activity did not increase. Similar results were obtained when the
values were calculated on the basis of a specific
gravity of 1.024 or expressed per g of creatinine.

Table 2 shows the correlations among the
concentrations of total protein, albumin, β₂-
microglobulin and creatinine and NAG of the
activity before and after exercise. Positive corre-
lations were observed in all pairs of concentra-
tions, except for between the β₂-microglobulin
concentration and NAG activity.

NAG isoenzyme patterns in the urine were
determined by electrophoresis on cellulose acetate
plates. The percentage and amounts (U/l) of the
A-form of NAG isoenzymes in the urine of
students after exercise increased slightly, while
those of the B-form decreased slightly (Table 3),
but these changes were not statistically significant.

Discussion

The effect of exercise on humans varies
according to the type, intensity, and time of
loading. Exercise induces constriction of the
renal vascular system, which leads to an increase
in the permeability of the glomerular capillary
vessels and filtration fraction of the glomeruli (1,
2). These phenomena cause an increase in serum
protein infiltration, and a decrease in reabsorption
of proteins in the tubular tissue, leading to an
increase in urinary excretion of proteins (11).
The major component of proteinuria in athletes is
serum albumin (7). In the studies on NAG
isoenzymes in the urine after exercise, the per-
percentage of the A-form, which is derived from the glomerular tissue, was found to be higher than that of the B-form, which is derived from the tubular tissue. This result indicated that NAG in the urine of the subjects after exercise was not derived from the tubular tissue but from the glomerular tissue. The B-form has been shown to increase in urine in the case of tubular damage, such as tubular nephritis caused by cadmium intoxication (12). Thus NAG in the urine suggests some functional disturbance, but no marked damage of the tubular cells. After hard exercise, filtrated amounts of low molecular protein exceed the ability of reabsorption in tubules, which leads to an increase in proteins such as β₂-microglobulin. In the present experiment, no correlation between urine β₂-microglobulin and urine NAG was observed. Nevertheless, β₂-microglobulin increased in urine after exercise, but NAG did not increase, indicating that the tubular cells remained relatively intact and only tubular absorption was disturbed.

References


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