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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

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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, β_2-microglobulin and creatinine along with the activity of N-acetyl-β-D-glucosaminidase (EC 3. 2. 1. 30). Concentrations of total protein, albumin, β_2-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, β_2-microglobulin and creatinine concentrations, but not between the β_2-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 celluose 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, β_2-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-α_1-glycoprotein (9), α_1-acid glycoprotein (10) and α_2-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 β_2-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, β_2-microglobulin

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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 β_2-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[a]

Components		Before exercise	After exercise[b]	Difference
Urine volume	(ml)	68.2 ± 23.7	47.8 ± 18.2**	− 20.4 ± 5.5
Specific gravity		1.0324 ± 0.0058	1.0324 ± 0.0055	−
Concentrations determined				
Total protein	(mg/dl)	19.03 ± 7.84	32.44 ± 19.79*	13.4 ± 15.2
Albumin	(mg/dl)	6.26 ± 3.29	17.94 ± 14.17*	11.7 ± 12.1
β_2-Microglobulin	(mg/dl)	2.82 ± 1.94	4.30 ± 3.12*	1.5 ± 1.9
Creatinine	(mg/dl)	212.32 ± 52.96	257.56 ± 63.52*	45.2 ± 31.1
N-acetyl-β-D-glucosaminidase	(U/dl)	59.7 ± 31.1	58.2 ± 28.3	− 1.5 ± 31.0
Concentrations corrected for specific gravity[c]				
Total protein	(mg/dl)	13.95 ± 4.63	23.55 ± 13.88*	9.84 ± 11.25
Albumin	(mg/dl)	4.43 ± 2.23	12.81 ± 10.20*	8.79 ± 9.00
β_2-Microglobulin	(mg/dl)	2.03 ± 1.28	3.15 ± 2.15*	1.11 ± 1.35
Creatinine	(mg/dl)	156.12 ± 23.46	188.94 ± 27.96*	32.97 ± 27.23
N-acetyl-β-D-glucosaminidase	(U/dl)	43.6 ± 20.5	42.8 ± 18.5	− 6.1 ± 33.0
Concentrations relative to the amount of creatinine[d]				
Total protein	(g)	0.089 ± 0.023	0.155 ± 0.049*	0.032 ± 0.039
Albumin	(g)	0.028 ± 0.012	0.062 ± 0.043*	0.031 ± 0.035
β_2-Microglobulin	(g)	1.258 ± 0.818	1.491 ± 0.986*	0.430 ± 0.575
N-acetyl-β-D-glucosaminidase	(U)	29 ± 11	21 ± 8	− 21 ± 18

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: Signiaicantly 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 sodio-m-cresolsulfonphthaleinyl-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 Na_2CO_3. Densitometry 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 proteins, albumin, β_2-microglobulin and creatinine 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 expressed relative to the amount of creatinine.

The concentrations of total protein, albumin, β_2-microglobulin and creatinine increased significantly ($p < 0.01$, 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, β_2-microglobulin and creatinine and NAG of the activity before and after exercise. Positive correlations were observed in all pairs of concentrations, except for between the β_2-microglogulin 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.

Table 2 Correlation matrix of compounds in the urine before and after physical exercise.

	Before exercise				
After exercise	Total protein	Albumin	β_2-Micro-globulin	Creatinine	NAG[a]
Total protein	——	0.925	0.644	0.714	0.400
Albumin	0.971	——	0.583	0.695	0.376
β_2-Micro-globulin	0.685	0.596	——	0.474	0.215
Creatinine	0.729	0.706	0.574	——	0.490
NAG	0.397	0.313	0.200	0.525	——

a: N-acetyl-β-D-glucosaminidase.

Table 3 Isoenzyme activities of urinary N-acetyl-β-D-glucosaminidase before and after physical execise[a]

Isoenzyme	Activity (Units/liter) (%)	
	Before exercise	After exercise
A-form	2.84 ± 1.32 (76.47 ± 5.17)	2.79 ± 1.32 (79.36 ± 6.33)
B-form	0.84 ± 0.40 (23.51 ± 5.17)	0.80 ± 0.49 (20.50 ± 6.44)
Total	3.68 ± 1.65	3.59 ± 1.73

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

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-

centage 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 β_2-microglobulin. In the present experiment, no correlation between urine β_2-microglobulin and urine NAG was observed. Nevertheless, β_2-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.

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