Exercise-Induced Proteinuria in Well-Trained Athletes

Aldo Clerico, Carlo Giammattei, Luigi Cecchini, Amalia Lucchetti, Lorella Cruschelli, Giuseppe Penno, Giovanna Gregori, and Ottavio Giampietro

We studied the rate of urinary excretion of albumin, α_1-microglobulin (as an indicator of the renal tubular involvement), sodium, potassium, and creatinine in the basal state (overnight urine collection) and after physical exercise (training session) in 10 professional cyclists, to verify whether protein excretion is increased even in well-trained athletes after physical effort. In addition, we wanted to understand whether the origin of exercise-induced proteinuria was glomerular, tubular, or both. Compared with the basal state (overnight collection), exercise significantly ($P < 0.01$) increased the excretion rate of albumin (4.2 ± 2.6 µg/min vs 18.1 ± 10.6 µg/min, mean ± SD), Na, and K, and also the urinary volume. Creatinine output was not affected by exercise. The mean (±SD) overnight excretion rate of albumin by athletes was quite similar to that found for 91 healthy nonathletes at rest (4.6 ± 2.7 µg/min). The mean exercise-related excretion of α_1-microglobulin by the athletes significantly exceeded the overnight value (6.6 vs 0.3 mg/L, $P = 0.037$). Our study indicates that (a) albuminuria furnishes the greater contribution to the increase in exercise-induced proteinuria; (b) the exercise proteinuria is both glomerular and tubular in origin, and is reversible; (c) the enhanced protein requirement of athletes may in part be due to the recurrent excretion of proteins in the urine after physical effort.

In the last few years, measurement of urinary albumin excretion has become widely used for early assessment of renal glomerular involvement in different pathological conditions such as arterial hypertension (1–3) or post-transplant kidney rejection (4). In diabetics the evaluation of the urinary albumin excretion rate (AER) has recently assumed a central role in prevention and follow-up of diabetic nephropathy (5). The screening and follow-up of proteinuria and (or) albuminuria in diabetic patients demands a complete knowledge of the pathophysiological mechanisms that can affect the excretion of urinary proteins (functional proteinuria) in healthy and diabetic people.

Physical effort causes proteinuria in healthy adults (6, 7), children, and adolescents (8), as well as in football, basketball, and handball players (9) and long-distance runners (10). Physical exercise as a provocative test for proteinuria has been proposed to unmask early changes of diabetic renal disease (11). So far, the causes of increased excretion of urinary proteins during physical exercise in healthy subjects and diabetic patients are still unknown. In particular, reports have been conflicting on the type of proteinuria involved—i.e., glomerular, tubular, or mixed in origin (12).

We measured the urinary excretion of albumin, α_1-microglobulin (as an indicator of the renal tubular dysfunction) (12), in the basal state (overnight collection) and after physical exercise (training session) in 10 professional cyclists. Urinary excretions of sodium, potassium, and creatinine were also measured, substances well known to be subject to glomerular filtration and tubular rearrangement, although in different degrees and manners. The aim of our study was therefore to verify whether protein excretion is increased after exercise, even in the case of well-trained athletes, and to elucidate whether the exercise-induced proteinuria is glomerular or tubular (or both) in origin.

Materials and Methods

Urinary albumin excretion. We measured urinary albumin excretion in urine samples with a commercial RIA (Albumin RIA 100; Pharmacia AB, Uppsala, Sweden). This RIA method makes use of purified human serum albumin as standard, ^{125}I-labeled human serum albumin as tracer, and a specific antiserum to human serum albumin. Bound albumin is separated from free by use of a solid-phase system (anti-rabbit IgG, raised in sheep, bound to Sepharose). The standard curve is prepared with use of solutions containing known amounts of purified human serum albumin (Figure 1). We performed the assay according to the manufacturer's instructions. The analytical characteristics of this RIA method have been reported (13). In brief, we added to all tubes 50 µL of the standard solutions or urine samples, 50 µL of the tracer solution (about 30 000–40 000 counts/min of ^{125}I-labeled human serum albumin), and 2.0 mL of a mixture of antiserum to human serum albumin; mixed; then incubated the tubes for 1 h at room temperature (18–22 °C). After centrifugation (1500 × g, 10 min, 4 °C), the supernates were aspirated and discarded, and the

B/Bo, %
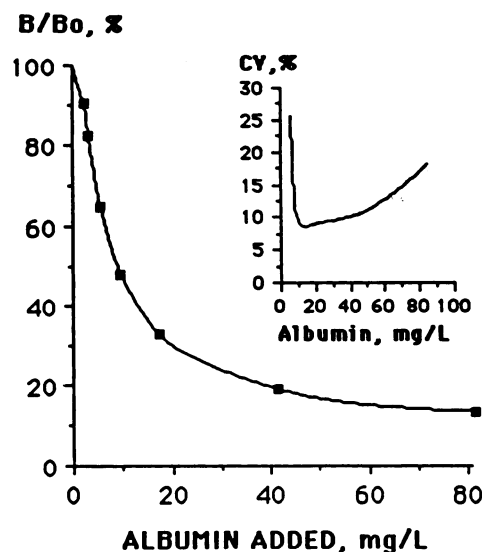
CV, %

ALBUMIN ADDED, mg/L

Fig. 1. Standard curve and precision profile of the RIA method for assay of urinary albumin

Precision profile (within-run CV) calculated from the error of replicates (14)

C.N.R. Institute of Clinical Physiology, Cattedra di Malattie del Ricambio, Istituto di Clinica Medica II, University of Pisa, Via Savi 8, 56100 Pisa, Italy.

radioactivity in the pellets was counted in a well-type gamma counter. The RIA was not significantly subject to interference from bovine serum albumin, transferrin, or human immunoglobulins.

All sample values were obtained and the index of sensitivity, the precision profile, and the quality control for the RIA were evaluated according to a previously described computer program (14). The interpolation of the dose-response curve was calculated with use of a four-parameter logistic function (14).

All urine samples from the 10 athletes were assayed in the same RIA experiment. The standard curve and the imprecision profile (within-run variability) of this RIA experiment are shown in Figure 1. The sensitivity (lower detection limit) of this standard curve was 0.38 mg/L. The reproducibility (between-run variability) of the RIA was determined by measuring three pooled specimens of urine in several consecutive assays. The CVs were 8.19% (albumin concentration 4.74 ± 0.39 mg/L, mean ± SD, n = 18), 7.38% (18.63 ± 1.37 mg/L, n = 26), and 10.53% (43.49 ± 4.58 mg/L, n = 17), respectively.

Other methods. The α_1-microglobulin was measured by a radial immunodiffusion method (LC-Partigen α_1-microglobulin; Behringwerke AG, Marburg, F.R.G.). The detection limit for this assay was 0.9 mg/L. Creatinine, sodium, and potassium in urine were measured by automated methods (Astra 4; Beckman Instruments, Brea, CA 92621).

Subjects. We studied 10 male professional cyclists (ages 21–26 years) during an intense training period. Each subject voided the bladder before going to bed and upon rising, collecting the latter. Collection times were exactly recorded (timed overnight specimen). In addition, a timed specimen of urine was also collected from all athletes during a training session lasting 5 h (road race of 153 km, energy expenditure ranging from 60% to 90% of maximum O_2 expenditure during training). Every subject voided urine just before the start of training session (this specimen was discarded) and soon after (within 15 min) the end of the training (exercise specimen). For every subject, after the volumes of the overnight and exercise specimens had been accurately measured, the urines were divided into various aliquots and stored at −20 °C until assay. Bacterial growth was routinely excluded from all urines. For each urine from each subject we calculated the excretion rate for albumin (AER, μg/min), creatinine (mg/h), and the electrolytes Na^+ and K^+ (mmol/h). The excretion rate for α_1-microglobulin was not calculated, because this protein was not detectable in half of the urines collected.

We also studied samples of overnight urine specimens from 91 healthy adults (15) and from 22 healthy subjects who had collected their urine for 24 h in several portions while maintaining their normal activity. None of these subjects was participating in physical training programs. The first urine specimen on the morning of the day of the 24-h collection (specimen 0) was put in a separate container. Subsequently, the complete 24-h urinary collection consisted of specimen 1, from waking to lunch (generally 5 h, from 0800 to 1300 hours); specimen 2, from lunch to dinner (generally 6 h, from 1300 to 1900 hours); specimen 3, from dinner to bedtime; and specimen 4, overnight, at rest. The volume of each portion was recorded, as was the time of collection.

Statistical analysis. The statistical analysis for the calculation of mean values, Student's *t*-test for paired and unpaired data, Mann–Whitney *U*-test, simple and stepwise

regression analysis, and interpolation of the standard curve of the RIA was carried out by a Macintosh SE personal computer with use of the "Stat-View 512" program.

Results

Figure 2 shows the mean (± SEM) urinary excretion rates for albumin, Na, K, and creatinine, and the mean urine volumes, calculated both for the overnight and the exercise specimens from the 10 athletes. Compared with the basal state (overnight collection), physical effort did provoke a significant ($P <0.01$, *t*-test for paired data) increase in the excretion rate of albumin, Na, and K, and it increased the urinary volume, whereas urinary creatinine output was not significantly affected by exercise. The exercise-induced increase in Na and K and in the urinary volume was 1.5–2.5 times less than that of albumin.

The mean (± SD) AER of 10 athletes, measured in the overnight samples, was 4.2 ± 2.6 μg/min, a value not significantly different ($P >0.05$) from that found at rest for the 91 healthy nonathletic subjects (4.6 ± 2.7 μg/min) (15). For the 22 healthy subjects who collected a 24-h urine specimen during a day with "normal" activity, the mean (± SD) AER was 7.4 ± 3.9 μg/min (range 1.8–17.0 μg/min), significantly greater than the AER found at rest for the overnight urine sample (sample 4, 5.54 ± 2.64 μg/min, $P <0.01$).

The mean (± SD) AER for the 10 athletes during exercise (18.1 ± 10.6 μg/min) was significantly greater ($P <0.01$, both by *t*-test for unpaired data and by the Mann–Whitney *U*-test) than those measured in the 22 control subjects in the "morning" (specimen 1) and "afternoon" (specimen 2) periods (8.7 ± 5.9 and 8.2 ± 5.5 μg/min, respectively) and during the entire 24-h collection (7.4 ± 3.9 μg/min).

Table 1 lists the correlation coefficients (*r*) between the variables studied, calculated for all the urine specimens from the athletes (overnight + exercise collections, n = 20). Because the correlation coefficients for simple linear regressions were all highly significant ($P \leq 0.01$), we performed stepwise multiple regression analysis to assess the separate contribution of each variable to the regression. Only urinary volume significantly contributed to explaining the variability of AER in the stepwise multiple regres-
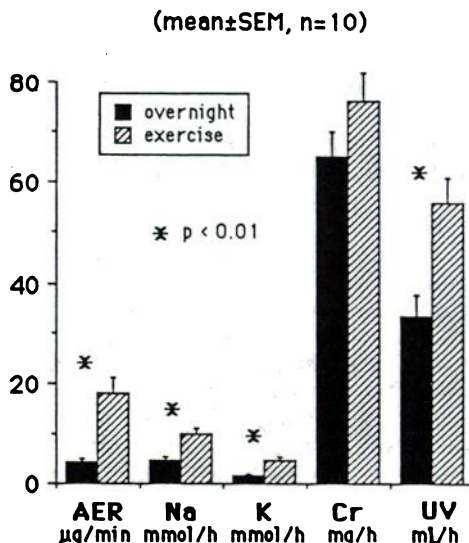
(mean±SEM, n=10)

Fig. 2. Values for albumin (AER), Na, K, and creatinine (Cr) excretion rate and urine volume (UV) measured for the overnight and the exercise specimens

Table 1. Correlation Coefficients between the Variables

r[a]

	Na	K	Creatinine,	Urine vol,
	mmol/h		mg/h	mL/h
AER	0.70	0.762	0.528	0.786
Na		0.779	0.594	0.92
K			0.552	0.834
Creatinine				0.801

[a] All r values were highly significant ($P \leq 0.01$).

sion analysis, whereas Na, K, and creatinine excretion rates did not.

The urinary concentration of α_1-microglobulin was undetectable in the overnight urine specimens from nine of the athletes and ranged from 0.9 to 25.7 mg/L (mean ± SEM 6.6 ± 2.5 mg/L) during exercise; only in a single athlete was α_1-microglobulin detectable in the overnight specimen (2.8 mg/L) and undetectable in the exercise sample. The mean value of α_1-microglobulin in the exercise specimen was significantly greater than that in the overnight specimen (6.6 vs 0.3 mg/L, P = 0.037, n = 10, t-test for paired data).

Discussion

Overt diabetic nephropathy, defined as proteinuria >500 mg/24 h, is preceded by a silent phase characterized by a persistent increase in AER without clinical proteinuria (5). According to several recent prospective studies, this so-called microalbuminuria strongly predicts the future development of overt diabetic nephropathy (5). Measurement of AER is also of interest in the management of hypertensive patients (1–3, 7). In both situations, the screening and (or) follow-up programs involving large groups of patients require accurate standardization of the procedures for AER evaluation.

In our study, the mean exercise-induced AER in well-trained athletes was 18 μg/min, a value that clearly exceeds our upper normal limit (11.5 μg/min) (15). Kramer et al. (7) recently reported that the 24-h albumin excretion from 30 healthy subjects was not significantly affected by a maximal bicycle ergometer test. However, the mean exercise AER value they reported (10.3 ± 0.9 mg/24 h) tended to be greater than the AER measured for the urines collected without exercise (8.5 ± 0.7 mg/24 h) (7). Use of a longer period of exercise might have enhanced the albumin excretion in that study. Our well-trained athletes excreted on the average about 5.4 mg of urinary albumin during 5 h of training. This corresponds to 50.5% of the mean whole 24-h amount of albumin excreted at rest by control subjects (10.7 mg). Diabetic patients excrete more albumin in urine after exercise than do healthy untrained subjects (16, 17). Hence, the physical activity may significantly affect the determination of AER in screening or follow-up programs of diabetic and (or) hypertensive patients.

Although in this study albuminuria furnished the greater contribution to the exercise-induced proteinuria, which fits with reports of others (9, 18), nine of 10 athletes also showed an increased excretion of α_1-microglobulin during exercise. Conceivably, physical activity also provokes a tubular proteinuria. Thus, exercise proteinuria may be tubular in origin, in addition to glomerular, as indicated by significant correlations between AER and

urinary excretions of Na, K, and creatinine (Table 1).

Our findings indicate that exercise proteinuria is rapidly reversible, because these athletes, whom we studied at the end of an intense one-month period of training, showed no greater AER values at rest (i.e., in the overnight collection) than did the normal controls.

Finally, our data suggest that the increased protein requirements of athletes may be at least partly attributed to the repeated loss of proteins in the urine (19).

References

1. Parving HH, Jensen HA, Mogensen CE, Evrin PE. Increased urinary albumin excretion rate in benign essential hypertension. Lancet 1974;i:1190–2.
2. Pedersen EB, Mogensen CE. Effect of antihypertensive on urinary albumin excretion, glomerular filtration rate, and renal plasma flow in patients with essential hypertension. Scand J Clin Lab Invest 1976;36:231–7.
3. Losito A, Zampi L, Fortunati L, Del Favero A. Glomerular hyperfiltration and albuminuria in essential hypertension. Nephron 1988;49:84–5.
4. Woo J, Floyd M, Cannon DC, Kaman B. Radioimmunoassay for urinary albumin. Clin Chem 1978;24:1464–7.
5. Viberti GC, Walker JD. Diabetic nephropathy: etiology and prevention [Review]. Diabetes/Metabolism Rev 1988;4:147–62.
6. Fletcher DJ. Athletic pseudonephritis [Letter]. Lancet 1977;i:910–1.
7. Kramer KB, Kernz M, Ress KM, et al. Influence of strenuous exercise on albumin excretion. Clin Chem 1988;34:2516–8.
8. Huttunen NP, Kaar ML, Pietilainen M, Vierikko P, Reinila M. Exercise-induced proteinuria in children and adolescents. Scand J Clin Lab Invest 1981;41:583–7.
9. Campanacci L, Faccini L, Englaro E, et al. Exercise-induced proteinuria. Contrib Nephrol 1981;26:31–41.
10. Mogensen CE, Vittinghus E. Urinary albumin excretion during exercise in juvenile diabetes. Scand J Clin Invest 1975;35:295–300.
11. Gilli P, De Paoli Vitali E, Tataranni G, Farinelli A. Exercise-induced urinary abnormalities in long-distance runners. Int J Sport Med 1984;5:237–40.
12. Waller KV, Ward KM, Mahan LD, Wilsmatt DK. Current concepts in proteinuria [Review]. Clin Chem 1989;35:755–65.
13. Giampietro O, Clerico A, Cruschelli L, et al. Measurement of urinary albumin excretion rate (AER) in normal and diabetic subjects: comparison of two recent radioimmunoassays. J Nucl Med Allied Sci 1987;31:321–6.
14. Pilo A, Zucchelli GC, Malvano R, et al. Main features of computer algorithms for RIA data reduction. Comparison of some different approaches for the interpolation of dose-response curve. J Nucl Med Allied Sci 1982;26:235–48.
15. Giampietro O, Miccoli R, Clerico A, et al. Urinary albumin excretion in normal subjects and in diabetic patients measured by a radioimmunoassay: methodological and clinical aspects. Clin Biochem 1988;21:63–8.
16. Viberti GC, Jarrett RJ, McCartney M, Keen H. Increased glomerular permeability to albumin induced by exercise in diabetic subjects. Diabetologia 1978;14:293–300.
17. Feldt-Rasmussen B, Baker L, Deckert T. Exercise as a provocative test in early renal disease in Type 1 (insulin-dependent) diabetes: albuminuric, systemic and renal haemodynamic responses. Diabetologia 1985;28:389–96.
18. Poortmans J, Jeanloz RW. Quantitative immunological determination of 12 plasma proteins excreted in human urine collected before and after exercise. J Clin Invest 1968;47:386–93.
19. Dohm LG. Protein nutrition for the athlete. Clin Sports Med 1984;3:595–604.