Variability of Urinary Albumin Excretion in Patients With Microalbuminuria

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OBJECTIVE — To estimate the within-person variability (SD_i) for the overnight urinary albumin excretion rate (AER) in diabetic patients with persistent microalbuminuria.

RESEARCH DESIGN AND METHODS — Thirteen normotensive diabetic patients in stable medical control, with normal renal function and without any associated cardiovascular or other clinical disorders, collected overnight urines at monthly intervals during one year. AER was determined by radioimmunoassay.

RESULTS — Analysis of individual series of AER showed a significant trend ($P \leq 0.01$) in three cases. The remaining patients (eight men, two women; seven with insulin-dependent diabetes mellitus) had a median mean AER 102 μg/min (range 30–238 μg/min). Because the individual mean AERs were significantly associated with their respective SD_is, the data was $\log_e$ transformed. $\text{Log}_e SD_i$ was estimated as 0.420, and its 90% probability range (0.353–0.490) was calculated using the bootstrap method.

CONCLUSIONS — The high within-person variance for AER means that only people with an initial AER in the range of 53–76 μg/min have a high probability ($P \geq 0.95$) of being classified as microalbuminuric (20–200 μg/min) on a subsequent specimen. However, subjects with an initial AER ≥53 or ≥80 μg/min have a 95 and 99% probability of persistent microalbuminuria. The large variability of AER limits its potential as a serial marker to detect any gradual deterioration of established renal dysfunction.

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AER, albumin excretion rate; SD_i, within-person variability; ACE, angiotensin-converting enzyme; dBP, diastolic blood pressure; BMI, body mass index; RIA, radioimmunoassay; UTI, urinary tract infection; IDDM, insulin-dependent diabetes mellitus; LL, lower limit; UL, upper limit.

We define microalbuminuria as an overnight albumin excretion rate (AER) of 20 to 200 μg/min. Persistent microalbuminuria is a marker for development of overt nephropathy, proliferative retinopathy, and cardiovascular morbidity/mortality (1). Assessment of the significance of changes in urinary albumin excretion requires knowledge of the within-person variation (SD_i) in the diabetic population and the analytical imprecision of the method (2). However, current estimates of the within-person variability vary widely (3–7), and many have been derived over short periods and/or in heterogenous groups with differing degrees of albuminuria.

In this study, we have estimated the intra-individual variability of AER over a 12-month period in a group of medically stable normotensive diabetic subjects with persistent microalbuminuria.

RESEARCH DESIGN AND METHODS

— Subjects ($n = 13$) were placebo-controlled in a double-blind, randomized multicenter trial that evaluated treatment with an angiotensin-converting enzyme (ACE) inhibitor (8). Inclusion criteria were an AER of 20–200 μg/min on at least 2 of 3 occasions, GHb <13.5%, mean sitting diastolic blood pressure (dBP) ≤95 mmHg on at least 2 of 3 occasions, body mass index (BMI) ≤32 kg/m^2, and compliance ≥80%. Patients were excluded if they had cardiovascular or certain other medical diseases, were taking medication affecting blood pressure, or had a history of any contraindication to ACE inhibitors.

Timed overnight urine specimens (mean 12.3, range 9–13) were collected from each subject at monthly intervals, and the albumin concentrations were analyzed at a single center using a commercial radioimmunoassay (RIA) kit (Pharmacia South Seas, Sydney, Australia). The long-term total (intra- plus inter-) assay variability was 10, 4.2, and 7.8% at urinary albumin concentrations of 3.6, 27, and 52 mg/L, respectively. Although it is

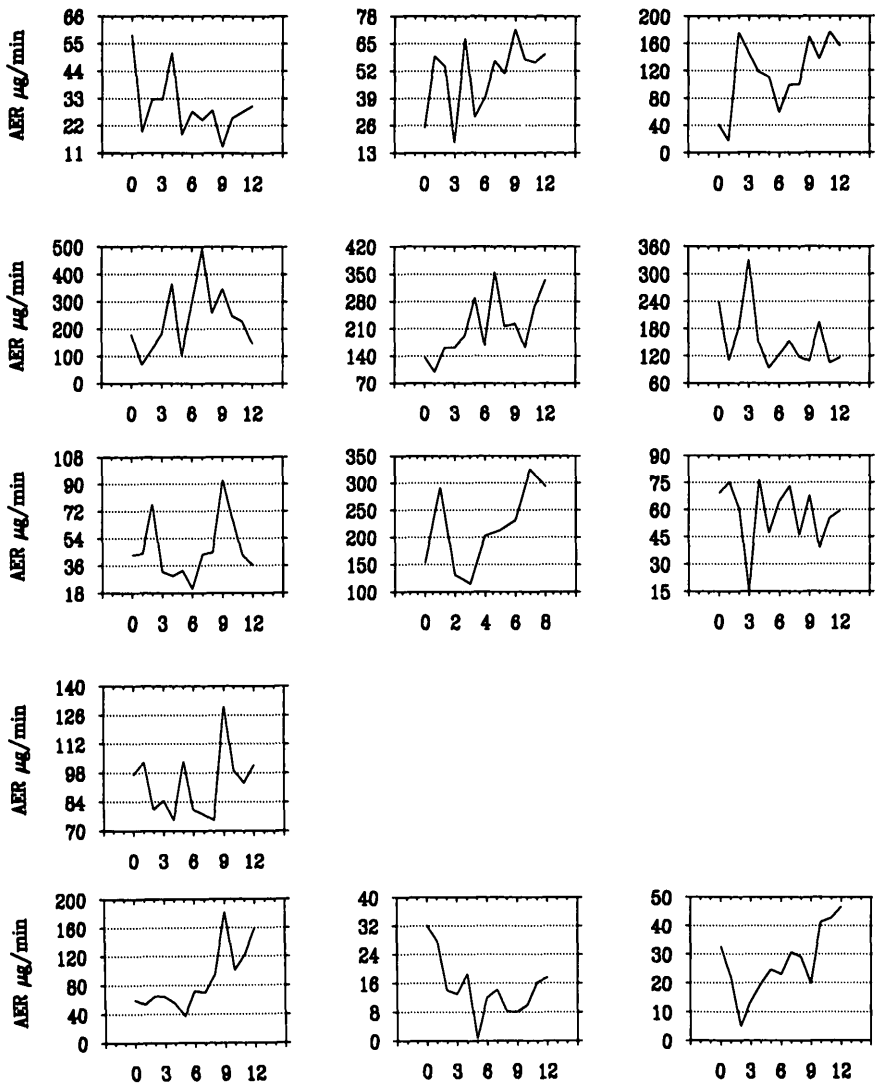

Figure 1—*Plots of AER (µg/min) versus time (months) for 13 normotensive diabetic subjects. The bottom panels of three plots are subjects for which the serial AER values had a significant trend.*

recognized that nondiabetic renal or systemic disease, including hypertension, urinary tract infections (UTIs), congestive heart disease, exercise, or use of anti-inflammatory drugs may significantly alter AER, the design of the study excluded these possibilities except for UTI. Each specimen was tested for UTI, and, if it was found, we requested a follow-up specimen when the UTI had been resolved. The importance of accurate timing and complete urine collection was stressed by the study personnel and reinforced by written materials.

Each AER series was tested, using the Grub procedure (9), and shown to have no outliers ($P \leq 0.01$). The ratio of the mean square successive difference to the variance was calculated for each individual AER data set and compared with the published values (10) to test for a significant trend. The bootstrap technique (11) was used to estimate the magnitude of deviation of SD_i from the true value. This method draws numerous artificial samples from a random sample to determine the accuracy of a statistical estimator (mean SD_i).

RESULTS — Because the derivation of within-person variability assumes random, independent fluctuations about a set point, any systematic change in the mean will increase the estimate of the standard deviation. Accordingly, all serial AER sets were tested for trend, which was statistically significant in three cases (two men/one woman; two with insulin-dependent diabetes mellitus [IDDM]). The AER data for all subjects is shown in Fig. 1.

The 10 subjects (eight men/two women) whose AER data showed no trend included 7 with IDDM. This group had a median age of 47 years; duration of diabetes of 16 years; GHb of 11.5%; BMI of 26.8 kg/m^2; and mean dBP of 74.2 mmHg. The median of mean plasma creatinine concentrations during the study was 81 µM, and the range of means was 66–93 µM (normal range is 50–120 µM). No significant relationship was noted (Spearman r, $P \geq 0.4$, $n = 10$) between AER and the respective age, duration of diabetes, BMI, GHb, plasma creatinine, or dBp where the individual values for AER, GHb, creatinine, and dBP were the corresponding subject means. Considering an association between increased AER and many of the latter variables has been shown (12), our negative findings undoubtedly reflect both the small sample size and the particular subject cohort selected.

The median mean AER was 102 µg/min (range 30–238 µg/min). Because SD_i was found to be significantly associated (Spearman $r = 0.879$, $P \leq 0.001$) with the mean, the data was $\log_e$ transformed, and the resulting SD_is were established as independent of the respective means ($r = -0.0424$). The mean $\log_e SD_i$ for the sample was 0.420, with a 90% probability of the true value being between 0.353 and 0.490 (11). When the entire data set ($n = 13$) was analyzed, the mean $\log_e SD_i$ increased to 0.473 and the associated 90% probability range was 0.398–0.556.

Given the estimate of $\log_e SD_i$ as 0.42 and the defined range of microalbuminuria as between the lower limit of 20

μg/min (LL; $\log_e$ = 2.9957) and the upper limit of 200 μg/min (UL; $\log_e$ = 5.2983), specific AER thresholds may be computed, above or below which there is a low likelihood ($P \leq 0.05$, one-tail; Z = 1.64) that a second measurement will fall outside 20–200 μg/min. For example, the lower threshold is estimated as 53 μg/min using the formula $LL + \sqrt{2} \cdot Z \cdot SD_i$ ($2.9957 + 1.414 \cdot 1.64 \cdot 0.42 = 3.9698$). Similarly, the upper threshold is calculated as 76 μg/min ($UL - \sqrt{2} \cdot Z \cdot SD_i$). Therefore, if a subject has an AER ≥53 μg/min, the probability is ≤5% that a subsequent specimen will be ≤20 μg/min. Alternatively, if the AER value is ≤76 μg/min, then the likelihood a second specimen is ≥200 μg/min is ≤5%. A subject may also be classified as having persistent microalbuminuria on a single estimation of the AER if the respective value is ≥53 ($P \geq 0.95$) or ≥80 ($P \geq 0.99$) μg/min.

CONCLUSIONS — We observed some significant distinctions between this study and previous studies (3–7) of AER variability in subjects with diabetes. These differences relate to short-term sampling protocols (4,5,7), combining patients with normo- and microalbuminuria (5,6), lack of definition of patient blood pressure status (3,5,7), restriction to patients with IDDM (3,4,6), and failure to analyze AER series for significant trends (1,6). Furthermore, during this study, all patients were in stable medical control, with no episodes of acute illness or hospitalization.

As also noted by Watts et al. (6), the variability and range of AER values requires $\log_e$ transformation of the data before calculation of the within-person variance. Other studies (3–5,7) provide estimates of SD_i based on parametric data despite the significant index of heterogeneity noted (5). Accordingly, meaningless critical differences (>100%) are reported for a significant change (5). This study establishes that only AER values within a narrow range of 53–76 μg/min are associated with a high probability that a subsequent estimation will also be within limits (20–200 μg/min) that define microalbuminuria.

Watts et al. (6) estimated $\log_e SD_i$ at 0.594, using a 3-month sampling interval in subjects with IDDM and normo- or microalbuminuria. This is considerably higher than the $\log_e SD_i$ of 0.420 calculated in this study or even our estimate of the 95th percentile (0.490). We attribute our lower SD_i estimate to several factors, including the medical stability of the patients, restriction to patients with persistent microalbuminuria, a more frequent sampling schedule, and exclusion of all individual AER series showing a significant trend. Our estimate of SD_i is likely to be exceeded in routine clinical practice, and clinicians should be aware of the limitations of AER in diagnosing and especially monitoring diabetic nephropathy.

The high variance of AER restricts its application as a sensitive longitudinal measure of progressive renal dysfunction; the variance could be improved only by multiple sampling, which is normally impractical. Even with triplicate sampling, the 95% confidence interval for an AER of 100 μg/min would remain at 62 to 161 μg/min. In contrast, the respective within-person variation for plasma creatinine in the same group was estimated at ~7.0%, indicating that an 18% increase in successive creatinine values would be statistically significant ($P \leq 0.05$) (G.P., P.J.P., unpublished observations).

Confirmation of persistent microalbuminuria for diabetic subjects is an important finding with long-term prognostic significance for the development of diabetic nephropathy (1). The results suggest that a single AER measurement ≥80 μg/min is confirmatory for persistent microalbuminuria. The detection of any gradual and systematic deterioration in established renal dysfunction may require serial AER measurements and more complex time series analysis (13) or frequent sequential monitoring of plasma creatinine.

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