Albumin Excretion Rate and Cardiovascular Risk

Could the Association Be Explained by Early Microvascular Dysfunction?

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Elevated albumin excretion rate (AER) independently predicts total and cardiovascular mortality in a variety of conditions, although the exact mechanisms are unknown. Laser Doppler fluximetry was used to study associations with risk factors and renal damage (AER calculated from a timed overnight urine collection) in 188 people without diabetes and 117 individuals with diabetes. Skin flow (flux) in response to arterial occlusion (ischemia) was measured. Three distinct patterns of postischemic peak flow were observed: *1*) gradual rise to peak (normal), *2*) nondominant early peak, and *3*) dominant early peak. Those with a dominant early peak were more likely to have diabetes ($P = 0.01$), hypertension ($P = 0.001$), and obesity ($P < 0.001$) and had a higher AER (12.6 μg/min [95% CI 7.8–20.2] vs. 7.2 [5.5–9.5] nondominant early peak group and 3.7 [3.2–4.1] normal group; $P < 0.001$ for trend). This could not be accounted for by conventional cardiovascular risk factors ($P < 0.001$ after adjustment). A rapid peak flow response after ischemia is associated with an elevated AER and increased cardiovascular risk. This may represent shared mechanistic pathways and causative or consequential changes in the microvasculature and supports the hypothesis that microvascular dysfunction may contribute to large vessel pathophysiology. *Diabetes* 54: 1816–1822, 2005

Elevated albumin excretion rate (AER) is a hallmark of microvascular damage in those with diabetes and independently predicts total and cardiovascular mortality (1). Similar observations in hypertensive patients (2), postmenopausal women (3), elderly patients (4), and the general (largely nondiabetic) population, right into the normal range of AER (5–7), have led to speculation regarding the link between AER and the etiopathogenesis of cardiovascular disease. The relationship is unlikely to be causal. AER may be a marker of unmeasured risk factors or an index of microvessel damage in other vascular beds, which in turn either results in or is associated with macrovascular disease. This association is supported by the observation that microvascular damage in other organ beds, such as the eye, is highly predictive of future cerebrovascular disease independent of conventional risk factors (8). Furthermore, in a population of healthy women, microvascular responsiveness was inversely related to Framingham risk score (9), suggesting that the link between microvascular dysfunction and cardiovascular risk may span into the healthy population.

Therefore we investigated the hypothesis that abnormalities in AER reflect abnormalities of microvascular function that are mirrored in other vascular beds, by examining the patterns of skin microvascular perfusion after an ischemic stimulus in a population with AERs ranging from normal to elevated. Potential explanations for any links observed were also explored.

RESEARCH DESIGN AND METHODS

This was a secondary analysis of data designed to compare microvascular function in people with and without diabetes. Men and women from a family practitioner register, aged 40–65 years and of African Caribbean and European descent, were randomly sampled and stratified by age-group, sex, and ethnicity to yield 188 nondiabetic and an additional 117 participants with type 2 diabetes. The family practitioner register provides a comprehensive sampling frame, because more than 97% of the population is registered (10), and notification of diabetes status is a requirement under the family practitioner contract and the English National Service Framework (http://www.doh.gov.uk/nsf/diabetes, accessed 21 August 2003). The following groups of people were excluded: those with cancer or major psychiatric illness, which would have precluded travel and attendance for investigation; those with known HIV or who were hepatitis B positive, because of the infection hazard posed as a consequence of blood sampling; and those with sickle cell disease or atrial fibrillation, which would interfere with the vascular tests. Respondents attended the Hammersmith Hospital for examination. The protocol was approved by the medical research ethics committee of the Hammersmith Hospital.

All participants gave informed written consent and completed a questionnaire on demographics, medical history, medications, and lifestyle behaviors. Ethnicity was self-assigned and cross-checked against patients' own and parental country of birth. Measures to a standard protocol included height, weight, and waist-to-hip ratio (11). Blood and urine samples were analyzed by the on-site laboratory, which is a member of the appropriate U.K. National Quality Assessment Scheme. Glucose and lipid levels were determined using the AU600 Olympus Diagnostic analyzer, and insulin was determined by the Abbott Axsym immunometric assay. All participants attended in the morning, after fasting from at least midnight the night before and having refrained from smoking, drinking tea or coffee, and taking any medications. The mean of

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AER, albumin excretion rate.

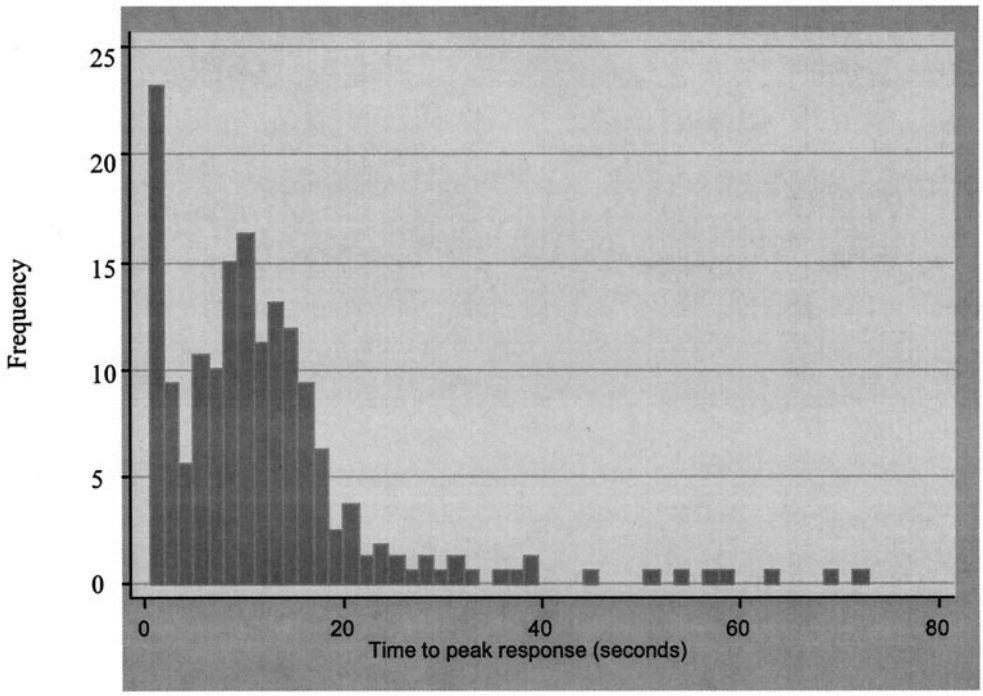

FIG. 1. Distribution of time to peak responses.

three supine resting brachial and ankle blood pressure readings using an automated device (Omron 705CP) was taken as the representative value of brachial and ankle blood pressures, from which ankle-brachial indexes were calculated. Pulse wave velocity, a measure of arterial stiffness, was measured in the arterial segment between the femoral and the dorsalis pedis pulse to assess arterial function within the supply vessels to the foot using the Complior device (COLSON, Paris, France) according to a standard protocol (12). This was performed to explore whether abnormalities at the microvascular level were associated with macrovascular dysfunction or localized to the microcirculation.

Microvascular assessment was performed using laser Doppler fluximetry (DRT4; Moor Instruments, Axminster, U.K.; time constant, 0.1 s; bandwidth, 22 kH_z) with the probe sited on the dorsum of the foot. Subjects were allowed to acclimatize for a minimum of 40 min lying supine in a quiet temperature-controlled room (at 22°C). A 3-min period of ischemia was induced by inflation of a sphygmomanometer cuff around the calf to a suprasystolic level. Adequate occlusion was determined as the loss of pulsatile flux on visual inspection of the trace, followed by decline of flow to a steady constant level (the biological zero). Rapid release of cuff pressure prompted a reactive increase in flux (flow) to an eventual peak, which was recorded digitally using the DRT4 For Windows Version 1.2 software (Moor Instruments) sampling at 40 Hz. Before release of the cuff, subjects were instructed to remain as still as possible while the cuff was released and for the following 2 min. However, if involuntary movement was observed, the tracing was discarded. The tracing was not repeated because it has been suggested that a second arterial occlusion within a short time frame may result in a different response. Flux and the time to reach the peak flux were recorded.

A timed overnight urine sample was collected, and albumin and creatinine concentrations were measured using the immunoturbometric method, with a lower level of sensitivity of 5 μg/dl. All values below this were coded as 5 μg/dl. AER and albumin-to-creatinine ratio were calculated according to standard formulas.

Analysis. Skewed variables were log transformed. Statistical significance for categorical variables was calculated using the χ^2 test for trend and the Student's t test for continuous variables. ANCOVA was used to determine explanations for differences in key variables between the three peak response curve morphologies, with tests for interaction. In keeping with the recommendations of Cupples et al. (13) and Rothman (14), the measured significance of the variables of interest is reported without adjustment for multiple testing. Bonferroni's adjustment is only relevant for additional covariates within the models, and where significance of these variables is described, this is after adjustment. Analyses were performed using STATA (version 7). Analyzable microvascular recordings were available for 271 of 305 participants. The rest were discarded because of poor quality either because of patient factors or technical difficulties (29 because of movement artifact identified at the time of recording, 4 because of lost data, and 1 because of inability to occlude the artery). The demographics of the 34 patients with discarded data did not differ from those of the remaining 271 patients. There was no ethnic difference in the distribution of the peak response curve morphologies or in the correlates thereof; thus the ethnic groups were combined.

A frequency distribution plot of times to peak flux postischemia indicated the presence of at least two distinct subgroups (Fig. 1). A detailed visual inspection of each trace, masked to all patient data apart from randomly allocated study number, was then performed. The principal criteria used were the presence of a transient peak in the first 2 s and whether this peak or a subsequent later peak was the tallest (Fig. 2). This analysis revealed the presence of a group of individuals with a "normal" graded rise to peak response curve (with a peak at 9.7 s [95% CI 8.5–11.1]) and a group with a dominant early peak within 2 s, which declined rapidly and was then followed by a lesser "normal" peak at 11.9 s (9.7–14.0). An "intermediate" group, those also with an early peak within 2 s but which was lower than the subsequent "normal" graded rise to peak at 11.7 s (10.1–13.5), was also identified. These three groups were labeled according to their reactive hyperemia peak response curve morphology, as "group 1-normal peak response curve morphology," "group 2-nondominant early peak," and "group 3-dominant early peak" (Fig. 3). The intraobserver repeatability of this visual analysis and categorization was 100% (n = 25; time separation, 3 months). In an additional 22 individuals studied on two separate occasions (10 with proven cardiovascular disease and 12 normal control subjects studied by two separate investigators) at least 24 h apart, the within-subject repeatability of category membership was also high (κ = 0.90; $P < 0.0001$). The investigators were blinded to the results of the previous analysis when these repeatability studies were performed.

RESULTS

Cardiovascular disease risk factor status varied by the microvascular response to ischemia. There was a gradation in the severity of cardiovascular risk factors across the microvascular response groups (Table 1), such that those individuals with a dominant early peak (group 3) had higher blood pressures and glucose levels, had greater obesity, and were more likely to be ex-smokers or current smokers than those with normal peak response curve morphology (group 1). In a multivariate model, independent associates of trend across the three peak response curve morphology groups were mean resting systolic blood pressure (β-regression coefficient [95% CI] across the three groups 0.0058 [0.0005–0.0110]; P = 0.03), noctur-

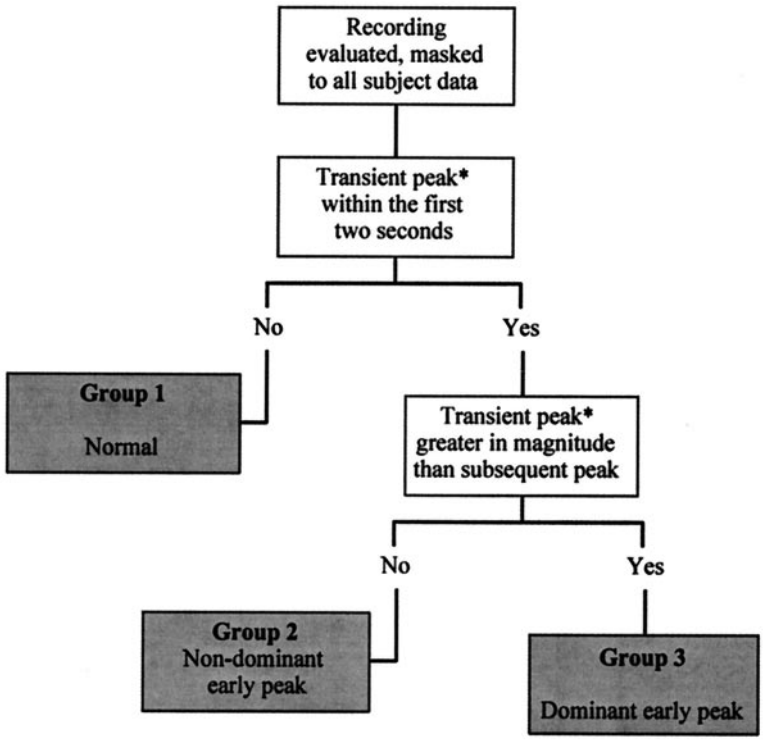

FIG. 2. Algorithm for the assessment of time to peak tracings. *Transient peak: a peak that has reached its zenith within the first 2 s and has a total duration of <4 s.

nal blood pressure dip (1.06 [0.22–1.91]; $P = 0.01$), weight (0.011 [0.004–0.018]; $P = 0.003$), and smoker status (0.218 [0.088–0.347]; $P = 0.001$). There was no association with arterial stiffness of the supply vessels in the femoral-dorsalis pedis region or ankle-brachial index. There was no difference in peak flux (flow) between the three groups using the measured peaks or the "traditionally measured" peak value (data not shown).

Mean AER increased across the three groups, again with the highest levels in the dominant early peak group (Table 2). This association between reactive peak response curve morphology and AER was also observed in analyses stratified by diabetes status, hypertension, and the use of antihypertensive therapies. In exploring explanatory factors for this association, potential confounding due to age, sex, smoking, body size, blood pressure indexes, and diabetes

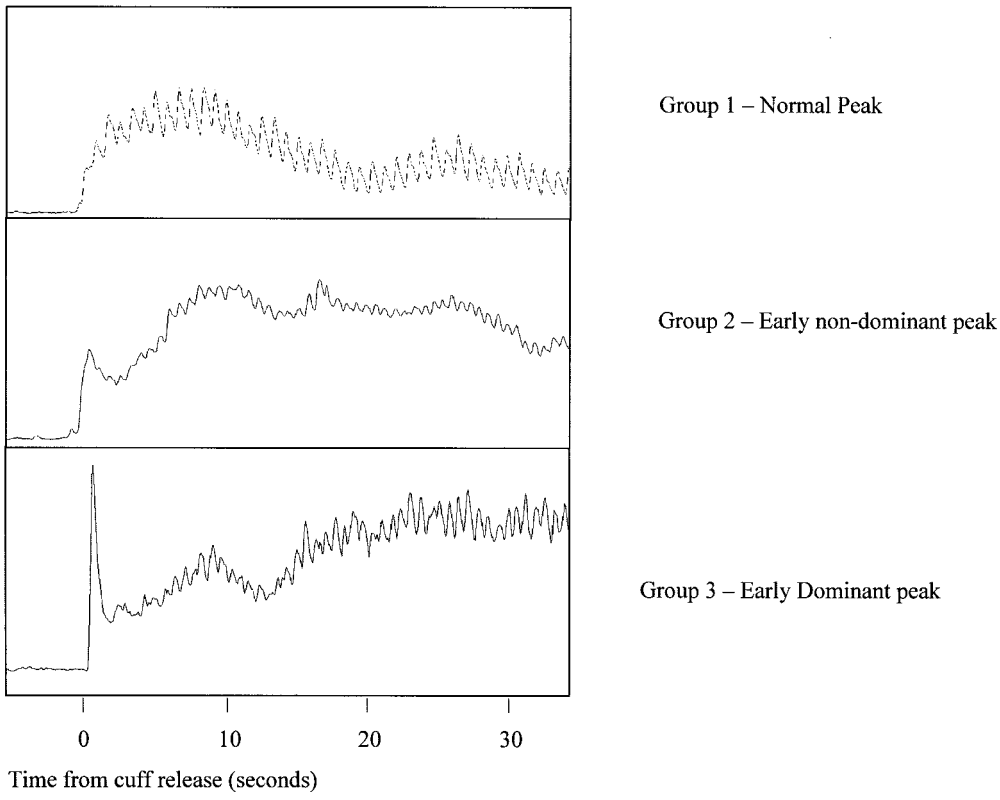

FIG. 3. The three peak response curve morphology groups.

TABLE 1
Baseline characteristics stratified by peak response curve morphology group

Variable	Group 1: normal peak	Group 2: early nondominant peak	Group 3: early dominant peak	*P* for trend
n	137	93	41	
Sex (% male)	43	40	64	0.06
Age (years)	53 ± 7.8	55 ± 7.0	55 ± 8.5	0.053
Ethnicity (% European)	55	44	51	0.2
Blood pressure measures				
Resting brachial systolic (mmHg)	133 ± 20	142 ± 24	146 ± 26	0.001
Resting brachial diastolic (mmHg)	81 ± 9	84 ± 11	85 ± 11	0.01
Ankle systolic (mmHg)	148 ± 17	155 ± 22	158 ± 26	0.002
Ambulatory daytime systolic (mmHg)	113 ± 12	119 ± 19	122 ± 15	0.001
Ambulatory nighttime systolic (mmHg)	104 ± 14	107 ± 17	104 ± 31	0.5
Ankle-brachial index	1.12 ± 0.11	1.11 ± 0.11	1.09 ± 0.15	0.2
Femoral-dorsalis pedis PWV (m/s)	10.9 ± 2.0	11.4 ± 2.8	10.6 ± 2.5	0.8
Height (cm)	167 ± 9	167 ± 9	169 ± 10	0.07
Weight (kg)	81 ± 15	84 ± 16	90 ± 19	<0.001
Waist (cm)	92 ± 12	95 ± 13	99 ± 20	0.005
Fasting insulin (IU/dl)*	6.3 (4.4–9.4)	6.8 (4.3–8.7)	7.4 (4.3–18.4)	0.18
Fasting glucose (mmol/l)*	5.6 (4.9–6.0)	6.1 (4.9–6.8)	6.6 (4.9–10.1)	0.001
Cholesterol (mmol/l)	5.2 ± 0.9	5.1 ± 1.1	5.1 ± 1.3	0.7
HDL (mmol/l)	1.30 ± 0.35	1.25 ± 0.33	1.20 ± 0.35	0.08
Fasting triglyceride (mmol/l)*	1.08 (0.78–1.40)	1.15 (0.72–1.52)	1.23 (0.92–1.85)	0.1
Diabetes (%)	31	42	51	0.012
Statin therapy (%)	12	15	20	0.5
Antihypertensive therapy (%)	25	30	40	0.2
Smokers (current/ex/never) (%)	14/37/49	24/23/53	32/37/32	0.02
Peak response (au)	47.2 (33.5–69.4)	50.3 (34.9–76.2)	46.9 (34.7–70.4)	0.8

Data are means ± SD except where data are skewed, for which geometric mean and 25th and 75th percentiles are shown. *Log-transformed data. PWV, pulse wave velocity.

were considered. In bivariate analyses (age adjusted), blood pressure (β between AER [log transformed], mean systolic blood pressure = 0.015 [0.008–0.021]; $P < 0.001$), and obesity indexes (β for weight = 0.017 [0.009–0.026]; $P < 0.001$) played significant roles in explaining differences in AER. In multivariate analyses, however, the only significant variable in the final model to account for variations in AER was the reactive hyperemia peak response curve morphology, with all other conventional cardiovascular risk factors, including blood pressure, obesity, diabetes, age, and smoking, rendered nonsignificant (Table 3). In this model, the peak response curve morphology alone accounted for nearly half of the total model variance explained and displaced the significance of blood pressure and weight (Table 3).

DISCUSSION

These data demonstrate, for the first time, that individuals at increased cardiovascular risk have a distinct morphological change in the microvascular response to ischemia, with an immediate and abrupt rise to peak flux, as com-

TABLE 2
AER by peak response curve morphology group

Model	Normal peak	Early nondominant peak	Early dominant peak	*P* for trend	R^2 (%)
AER (μg/min)					
Unadjusted	3.69 (3.20–4.07)	7.22 (5.47–9.53)	12.56 (7.80–20.20)	<0.001	18.3
Age and sex adjusted	3.80 (3.20–4.50)	6.73 (5.84–7.76)	11.94 (9.07–15.72)	<0.001	24.2
Fully adjusted model*	4.17 (3.45–5.04)	7.34 (6.28–8.59)	12.93 (9.45–17.70)	<0.001	29.5
Fully adjusted AER in selected populations (μg/min)					
No diabetes	4.49 (3.78–5.34)	6.71 (5.72–7.87)	10.03 (7.20–13.97)	<0.001	26.4
With diabetes	3.71 (2.33–6.17)	7.56 (5.38–10.62)	15.38 (7.98–29.63)	0.004	44.1
Normotensive	3.71 (3.03–4.53)	5.60 (4.58–6.84)	8.45 (5.64–12.66)	0.001	18.2
Hypertensive	4.90 (3.31–7.24)	9.70 (7.54–12.49)	19.22 (11.73–31.47)	<0.001	34.5
No anti-HTs	3.70 (2.96–4.54)	5.59 (4.57–6.84)	8.44 (5.62–12.66)	0.002	18.2
No DM/no anti-HTs	1.43 (2.26–7.68)	5.78 (3.09–10.81)	8.01 (3.86–16.59)	0.009	24.7

Data are means (95% CI) after adjustment for potential confounding and mechanistic factors stratified by peak response curve morphology group. R^2, total variance explained by the model; hypertensive, systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg or taking antihypertensive agents (HTs); no anti-HTs, patients taking no vasoactive medications (including antihypertensives, antianginals, sulfonylureas, or thiazolidinediones); no DM/no anti-HTs, patients with no diagnosis of diabetes and taking no vasoactive medications. *Adjusted for age, sex, daytime ambulatory systolic blood pressure, weight, fasting glucose level, fasting triglyceride level, and smoker status.

TABLE 3
Contribution and significance of the various mechanistic factors to the prediction of AER within the same model without and with peak response curve morphologies included

Variable	β-Regression coefficient between variable and AER in model without peak response curve morphology included in model	*P*	β-Regression coefficient between variable and AER in model that includes peak response curve morphology	*P*
Peak response curve morphology			0.568 ± 0.105	<0.001
Age (years)	0.017 ± 0.010	0.09	0.008 ± 0.009	0.4
Sex (1 = male, 2 = female)	−0.196 ± 0.157	0.2	−0.142 ± 0.151	0.3
Daytime ambulatory systolic blood pressure (mmHg)	0.011 ± 0.005	0.03	0.008 ± 0.005	0.1
Weight (kg)	0.014 ± 0.005	0.007	0.008 ± 0.005	0.1
Fasting glucose (mmol/l)*	0.332 ± 0.251	0.2	0.289 ± 0.249	0.2
Fasting triglycerides (mmol/l)*	−0.143 ± 0.146	0.3	−0.119 ± 0.141	0.4
Smoking status (current/ex/never)	−0.142 ± 0.097	0.1	−0.708 ± 0.924	0.4
Variance explained by the total model	16%		29%	

Data are means ± SE. *P*, significance of contribution of variable to the overall model. *Log-transformed variable.

pared with a graduated rise observed in those with a lesser degree of cardiovascular risk. This morphological pattern was strongly related to AER, accounting for much of its variance, independently of such risk factors as glucose and blood pressure. (The latter being the major cause of any elevations in AER in a population sample.)

The presence of these different early peak patterns in skin microvascular functional response has not previously been described. There are several reasons why this could be the case. First, it may be that such a pattern has been observed but disregarded as artifactual. On visual inspection, however, the appearance of movement artifact and the early dominant or nondominant peaks are distinctly different, supported by the repeatability of their occurrence. Our repeatability studies demonstrated a high degree of intraobserver and intrasubject repeatability of response patterns to ischemia. The latter included an independent sample of 10 patients with angiographically proven cardiovascular disease and 12 nondiseased control subjects to ensure sample heterogeneity, and it was performed by two investigators, excluding the possibility of a systematic error in data collection by a single observer. Second, an explanation may be in our bandwidth settings. Data were collected at a 22-kHz bandwidth in the present study to optimize detection of transient high values of perfusion. In contrast, previous authors have collected at 14, 12, 10, or 4 kHz, which may be less sensitive to these response patterns (15–18). Finally, our time constant smoothing coefficient was set to 0.1 s, which demonstrated a large beat-to-beat variability in flux (Fig. 4*A*). Others often use a higher time constant of 0.5, 1.0, or 1.5 s, which produces a much smoother curve and makes assessment of conventional peaks much easier (17–19). However, when this smoothing takes place, the rapid early peak is obliterated (Fig. 4*B*). Interestingly, Wilson et al. (15), using a 0.2-s time constant, reported the time to peak occlusive reactive hyperemic response to be unsuitable as an indicator of early diabetic microangiopathy because several of the responses were complicated by brief (1- to 2-s) subsidiary maxima, making accurate timing of the peak response difficult. These subsidiary maxima may be similar to those described here; however, Wilson et al. (15) did not describe them further.

Reactive hyperemia, the increase in perfusion after the temporary interruption of blood flow, is a complex response that potentially involves myogenic, neurogenic, metabolic, and physical factors (16,20–24). The precise mechanisms involved and their interactions and roles at different times in the response are, however, still not clearly understood. Vasodilation of downstream vessels during occlusion has been attributed to the myogenic response (20), extracellular (22,23) and potentially intracellular (25) changes in metabolite concentration, release of vasodilators from endothelial cells (24), and viscoelastic wall properties (16).

The abnormal reactive hyperemia peak response morphologies described in this study differ from the classic descriptions in the early, immediate postcuff release, phase of the response. Which and how many of the factors (described above) are abnormal is unclear. At cuff release, the most likely abnormality relates to an impairment in the myogenic vasoconstriction or its modulation via the endo-

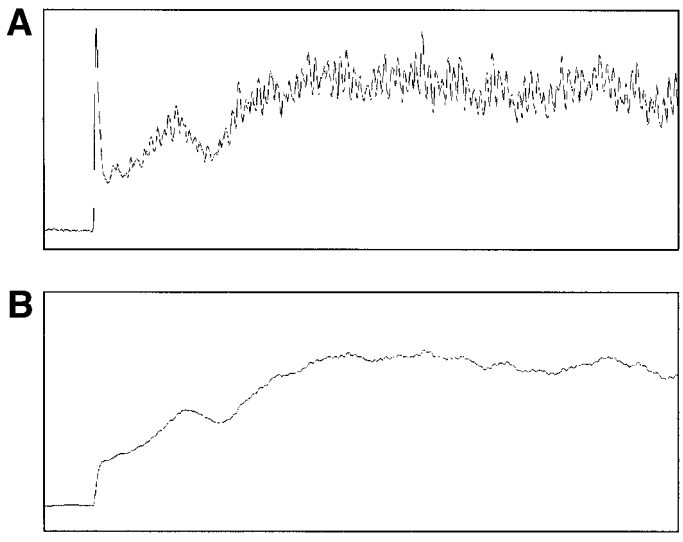

FIG. 4. The "smoothing" effect of the time constant algorithm. A tracing of a patient with an early dominant peak. *A*: The time constant is set to 0.1 s, and there is a clear early peak followed by a very pulsatile trace. *B*: The time constant is set to 1 s. Note that the dominant early peak has been lost, and the curve is much smoother. Both are on the same time and flux axis.

thelium or nerves, which would lead to a very rapid unmodulated increase in flow. Because wall stress is the stimulus for the myogenic response, thickened wall structure, such as that occurring in diabetes or hypertension, will reduce the response at a given pressure (26) and may delay the onset of the myogenic response so that an early increase in flow is observed before the myogenic vasoconstriction. A reduction in skin sympathetic activity, such as in diabetic autonomic neuropathy, may further enhance the hyperemia, although whether this affects early dominant peaks is unknown (21).

The rapid dominant early peak pattern observed in this study is likely to represent an alteration in the microvascular regulatory mechanisms that lead to transmission of all minor pressure fluctuations to the capillary bed. If this occurs in the renal microvascular bed, alterations in pressure may lead to hemodynamic-induced changes within the glomerulus such as mesangial expansion (27), adhesion molecule expressions (28), oxidative stress (29), or reduction in podocyte foot processes (30) in the at-risk group. In keeping with this suggestion, abnormalities of renal morphology analogous to those seen in diabetes have been described in at-risk individuals with obesity (31,32). One of the mechanisms proposed to explain these obesity-related renal changes is glomerular hypertension (33). The dominant early peak pattern (and subsequent exposure of the vascular bed to increased pressure fluctuations) may thus represent an early stage in the mechanistic pathway of microcirculatory damage that ultimately results in renal tissue damage and by similar mechanisms simultaneous deleterious effects on multiple microvascular beds. If this is the case, microalbuminuria is simply a proxy for microcirculatory damage occurring throughout the vascular tree (8).

Together, these observations support the hypothesis that microvascular damage is more involved in the pathogenesis of cardiovascular disease than has previously been thought. Given that most of the values of AER measured here are in the normal range and the association with other conventional cardiovascular risk factors, this emphasizes the fact that AER may be a significant cardiovascular risk factor below what is conventionally termed "microalbuminuria" at 30–300 mg in a 24-h period.

Microalbuminura, one of the strongest independent cardiovascular risk factors, is linked to an early transient skin microvascular hyperemia after the release of ischemia. This association is independent of traditional cardiovascular risk factors. Thus, it appears that those with increased cardiovascular risk have abnormalities of microvascular regulatory mechanisms that are not confined to the renal bed. The role of such microvascular abnormalities in the pathogenesis of cardiovascular disease requires further investigation and may also help in directing research into novel therapeutic interventions.

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