Is Nonalbuminuric Renal Insufficiency in Type 2 Diabetes Related to an Increase in Intrarenal Vascular Disease?

RICHARD J. MACISAAC, BSC(HONS), PHD, MBBS, FRACP[1]
SIANNA PANAGIOTOPOULOS, BSC(HONS), PHD[1]
KAREN J. MCNEIL, MBBS, FRACP, MPH+TM[1]
TRUDY J. SMITH, BSC, MAPPSC[1]
CON TSALAMANDRIS, MBBS[1]
HUMING HAO, MD, DMU[2]
P. GEOFFREY MATTHEWS, MBBS, PHD, DDU, FRACP[2]
MERLIN C. THOMAS, MBCHB, FRACP, PHD[3]
DAVID A. POWER, FRACP, PHD[4]
GEORGE JERUMS, MBBS, FRACP, MD[1]

OBJECTIVE — To investigate the role of intrarenal vascular disease in the pathogenesis of nonalbuminuric renal insufficiency in type 2 diabetes.

RESEARCH DESIGN AND METHODS — We studied 325 unselected clinic patients who had sufficient clinical and biochemical information to calculate an estimated glomerular filtration rate (eGFR) using the Modified Diet in Renal Disease six-variable formula, at least two estimations of urinary albumin excretion rates (AER), and a renal duplex scan to estimate the resistance index of the interlobar renal arteries. The resistance index, measured as part of a complications surveillance program, was compared in patients with an eGFR < or ≥60 ml/min per 1.73 m^2 who were further stratified into normo- (AER <20), micro- (20–200), or macroalbuminuria (> 200 μg/min) categories.

RESULTS — Patients with an eGFR <60 ml/min per 1.73 m^2 had a higher resistance index of the renal interlobar arteries compared with patients with an eGFR ≥60 ml/min per 1.73 m^2. However, the resistance index was elevated to a similar extent in patients with an eGFR <60 ml/min per 1.73 m^2 regardless of albuminuric status (normo- 0.74 ± 0.01, micro- 0.73 ± 0.01, and macroalbuminuria resistance index 0.75 ± 0.11). Multiple regression analysis revealed that increased age ($P < 0.0001$), elevated BMI ($P = 0.0001$), decreased eGFR ($P < 0.01$), and decreased diastolic blood pressure ($P < 0.01$), but not an increased AER, were independently associated with an elevated resistance index in patients with impaired renal function.

CONCLUSIONS — Subjects with type 2 diabetes and reduced glomerular filtration rate had similar degrees of intrarenal vascular disease, as measured by the intrarenal arterial resistance index, regardless of their AER status. The pathological mechanisms that determine the relationship between impaired renal function and AER status in subjects with type 2 diabetes remain to be elucidated.

Diabetes Care 29:1560–1566, 2006

Traditionally, microvascular disease resulting in a glomerulopathy and an increase in albumin excretion rate (AER) is believed to be the only significant mechanism by which diabetic renal disease develops. However, recent results have challenged the concept that a decline in renal function in patients with diabetes is always accompanied by an increased AER. Results from our group (1,2) and from the Third National Health and Nutrition Survey (NHANES III) (3,4) have suggested that the finding of nonalbuminuric renal insufficiency is not an uncommon discovery for subjects with diabetes, especially those with type 2 diabetes.

The structural basis of nonalbuminuric renal insufficiency in type 2 diabetes remains to be elucidated. However, the use of techniques such as duplex Doppler ultrasound allows for the rapid, noninvasive evaluation of the intrarenal vasculature (5). In particular, the presence of intrarenal vascular disease can be documented by the use of established methods such as the calculation of the resistance index (5,6). Intrarenal arteriosclerosis, as opposed to other forms of renal damage, has been shown to be an independent risk factor for an increased intrarenal resistance index in nondiabetic subjects and even after adjustment for multiple clinical and biochemical parameters, including creatinine clearance, the intrarenal resistance index of subjects with diabetes is greater than the resistance index in subjects with nondiabetic renal disease (7,8). Furthermore, subjects with type 2 diabetes, microalbuminuria, and preserved renal function who have typical and atypical renal structure have a higher intrarenal resistance index when compared with those who have near normal renal structure (9). This suggests that even when subjects have similar levels of impaired renal function, the type of underlying renal disease, especially the degree of intrarenal arteriosclerosis, may influence the intrarenal resistance index.

It is possible that a reduced GFR in normoalbuminuric subjects with type 2 diabetes occurs predominantly due to an increase in intrarenal arteriosclerosis as opposed to the well-described pathological changes that affect the glomerulus in diabetic subjects with reduced GFR and an elevated AER. However, currently it is not known whether the degree of intrarenal vascular disease is greater in low-GFR subjects with normaoalbuminuria compared with those with an elevated AER.

We therefore compared the intrarenal arterial resistance index in patients with

From the [1]Endocrine Centre and Department of Medicine, University of Melbourne, Austin Health, Heidelberg West, Victoria, Australia; the [2]Vascular Laboratory, Austin Health, Heidelberg, Victoria, Australia; the [3]Danielle Alberti Memorial Centre for Diabetic Complications, Baker Institute, Melbourne, Victoria, Australia; and the [4]Department of Nephrology, Austin Health, Heidelberg, Victoria, Australia.

Address correspondence and reprint requests to R.J. MacIsaac, Endocrine Centre, Austin Health, Heidelberg Repatriation Hospital, Waterdale Road, Heidelberg West, Victoria 3081, Australia. E-mail: r.macisaac@unimelb.edu.au.

Received for publication 22 September 2005 and accepted in revised form 14 April 2006.

Abbreviations: AER, albumin excretion rate; eGFR, estimated glomerular filtration rate; GFR, glomerular filtration rate; MDRD, Modified Diet in Renal Disease; RAS, renin-angiotensin system.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

DOI: 10.2337/dc05-1788

type 2 diabetes according to their GFR estimation (eGFR), i.e., < or ≥60 ml/min per 1.73m^2, who were further classified according to albuminuria status, i.e., normo- (<20), micro- (20–200), or macroalbuminuria (>200 μg/min). Clinical and biochemical associations with the intrarenal arterial resistance index for patients with an eGFR <60 ml/min per 1.73m^2 were also sought.

RESEARCH DESIGN AND METHODS

— We studied 325 patients attending the diabetes clinics at Austin Health in 2001 who had sufficient clinical and biochemical information to calculate an eGFR using the Modified Diet in Renal Disease (MDRD) six-variable formula, at least two estimations of AER, and a renal duplex scan to estimate intrarenal vascular resistance, i.e., the resistance index of the interlobular arteries. The Ethics Committee of Austin Health approved this study, and informed consent was obtained from the study participants. The following parameters were measured in the study population: age, duration of diabetes, systolic and diastolic blood pressure (blood pressure − mean of three readings), HbA_{1c} (A1C), plasma creatinine, urea, albumin, cholesterol, and triglyceride levels. Mean arterial and pulse pressure were also calculated, as well as a history of antihypertensive agent use, including inhibitors of the renin-angiotensin system (RAS), any class of retinopathy, and any history of a macrovascular event (including stroke, coronary artery disease, and peripheral vascular disease) was also recorded. Height and weight were also measured, and BMI was calculated as weight (in kilograms) divided by the square of height (in meters).

Measurement of intrarenal arterial resistance index

Patients with known renal artery stenosis, nondiabetic renal disease, or those with abnormal urinary sediment or microscopic hematuria were not included in this resistance index study. The resistance index was measured using a color Doppler ultrasound machine (Phillips HDI-5000). All measurements were made after an overnight fast and during suspended respiration at the end of inspiration. Apart from fasting overnight, the use of antihypertensive agents, including RAS inhibitors, was not suspended before the resistance index evaluation. We did not specifically screen patients for the presence of undiagnosed renal artery stenosis by performing a Doppler evaluation of the main renal arteries but did assess renal size and blood flow at the renal hilum. None of the 325 patients that were included in this study had an hilar acceleration time >100 mm/s or had evidence of kidney atrophy (<9 cm in length), parameters that are suggestive of renal artery stenosis <60% (10,11). Furthermore, no patients with renal insufficiency were included in this study if they had a mean resistance index value <0.45, a value associated with bilateral renal artery stenosis, or a difference in resistance index values >8% between the two different kidneys, a value associated with the presence of unilateral renal artery stenosis (12). We also assessed the effects on resistance index values of a >25% difference in kidney size (estimated by length × width × 0.5). One patient with impaired renal function and normoalbuminuria (right resistance index 0.76, length 9.3 cm and left resistance index 0.76, length 10.4 cm) not eliminated on the above criteria was later diagnosed with bilateral renal artery stenosis by angiography when investigated for poor blood pressure control.

The Doppler frequency (<3.5 MHz) and window area was kept to a minimum. The angle between the Doppler beam and vessels examined was kept <60°. All measurements were performed by the same examiner (M.H.) who was also unaware of the patient's eGFR and albuminuria status. Three different interlobar arteries from the upper and lower poles of each kidney were randomly selected and examined. The mean value from the two kidneys was then calculated and averaged. The resistance index was calculated as previously described (5), i.e., resistance index = (peak systolic velocity − end diastolic velocity)/peak systolic velocity.

Laboratory methods

AER was measured using fresh 24-h urine collections. On completion of each collection, a midstream specimen of urine was examined by microscopy and culture to exclude urinary tract infection and hematuria. AER and biochemical parameters were measured as previously described (2). Serum creatinine was measured by the modified Jaffe reaction on a Hitachi 911 autoanalyzer, and eGFR was calculated using the six-variable MDRD formula (13,14).

Since one of the MDRD formulae were used to estimate GFR, the method for creatinine measurement used in this study was calibrated with that used in the MDRD study (15). Creatinine measurements on the Hitachi machine had a good correlation with the Beckman-Coulter CX3 machine, the analyzer used to measure serum creatinine levels in the MDRD study (i.e., slope = 1.074, y-intercept = 0.003, r = 0.995). The mean creatinine measurement of 46 creatinine samples assayed by both methods was 152 μmol on the Hiatchi (range 37–659 μmol/l) and 166 μmol on the Beckman Coulter machine (30–710 μmol/l). Although mean creatinine values were 14 μmol higher on the Hitachi machine compared with the Beckman Coulter machine, there was a tendency for the Hitachi machine to underestimate creatinine levels at low (<5 μmol/l) concentrations compared with the Beckman Coulter machine. As the Hitachi machine had an error of approximately ±7% in comparison to the Beckman Coulter and methods that produce creatinine values falling within ±15% of the reference MDRD method are considered acceptable with respect to bias and precision by the Australian working group on chronic kidney diseases and automatic reporting of eGFR (16), we did not adjust serum creatinine concentrations in this study.

Patient classification

Patients were divided on the basis of their GFR estimation, i.e., < or ≥60 ml/min per 1.73m^2, and further classified according to albuminuria status, i.e., normo- (<20), micro- (20–200), or macroalbuminuria (>200 μg/min) as determined by the geometric mean of at least two measurements collected within the same 12-month period as the eGFR measurement. In patients with a GFR <60 ml/min per 1.73 m^2 and normoalbuminuria, the results of all previous AER measurements were reviewed to identify any patients whose normoalbuminuric status was possibly related to RAS inhibitor use before the start of this study. After exclusion of these patients, an adjusted prevalence of a GFR <60 ml/min per 1.73 m^2 and normoalbuminuria was also calculated.

Statistics

Continuous data are expressed as means ± SE and categorical data as number (%), except where specified. Serum creatinine, AER, and triglyceride values were expressed as median (interquartile range). Logarithmic transformation of AER and triglycerides was performed be-

Table 1—*Clinical and laboratory characteristics for 93 patients with type 2 diabetes and an eGFR <60 ml/min per 1.73 m²*

Variable	Normoalbuminuria	Microalbuminuria	Macroalbuminuria	*P*
n	39	29	25	
Age (years)	72 ± 1	73 ± 2	67 ± 2	0.02
Sex (% female)	49	28	28	0.12
eGFR (ml/min per 1.73m²)	45 ± 2	46 ± 2	35 ± 3	<0.001
Creatinine (μmol/l)	118 (93–154)	129 (108–144)	142 (127–242)	<0.001
AER (μg/min)	9 (8–14)	60 (40–101)	1,234 (317–1,954)	<0.001
BMI (kg/m²)	31.8 ± 0.9	29.1 ± 0.7	32.8 ± 1.1	0.02
Duration of diabetes (years)	17 ± 2	15 ± 2	15 ± 1	0.72
Systolic blood pressure (mmHg)	143 ± 3	147 ± 3	146 ± 3	0.49
Diastolic blood pressure (mmHg)	77 ± 2	79 ± 2	77 ± 2	0.48
Mean arterial pressure (mmHg)	99 ± 2	102 ± 2	100 ± 2	0.42
A1C (%)	7.2 ± 0.2	7.5 ± 0.3	8.1 ± 0.4	0.07
Antihypertensive agent (%)	95	93	96	0.89
Anti-RAS inhibitor (%)	82	76	96	0.12
Retinopathy (%)	46	43	52	0.83
Macrovascular disease (%)	58	55	72	0.40
Total cholesterol (mmol/l)	4.3 ± 0.1	4.5 ± 0.1	4.5 ± 0.2	0.53
LDL cholesterol (mmol/l)	2.3 ± 0.1	2.6 ± 0.1	2.5 ± 0.2	0.28
HDL cholesterol (mmol/l)	1.15 ± 0.05	1.15 ± 0.06	1.10 ± 0.09	0.83
Triglycerides (mmol/l)	1.6 (1.3–2.1)	1.4 (1.1–1.9)	1.7 (1.2–2.8)	0.33

Data are means ± SE or median (interquartile range). Patients were stratified on the basis of AER, i.e., normo- (<20), micro- (20–200), and macroalbuminuria (>200 μg/min). Macrovascular disease includes a history of coronary heart disease, cerebrovascular disease, and peripheral vascular disease.

fore statistical analysis. Differences in continuous variables were compared using one-way ANOVA (three or more groups) with subgroups then compared by Fisher's partial least-squares difference post hoc test. Differences in categorical variables were compared using χ^2 analysis. Univariate and multivariate analysis was performed with resistance index as the dependent variable. We focused on associations with the resistance index in patients with an eGFR <60 ml/min per 1.73 m² because of the inherent bias and lack of accuracy of the MDRD formula for estimating GFR values above this cutoff.

RESULTS

Intrarenal resistance index measurements divided on the basis of GFR and AER status

In total, 93 (29%) patients had renal insufficiency defined as an eGFR <60 ml/min per 1.73 m². Of these patients, 39 (42%) had normo-, 29 (31%) had micro-, and 25 (27%) had macroalbuminuria. As shown in Table 1, macroalbuminuric patients were younger and had a lower eGFR and higher serum creatinine level than normo- or microalbuminuric patients. There was also a preponderance of females in the normoalbuminuric low-eGFR group, but overall there were no significant differences in sex between the normo-, micro-, and macroalbuminuric groups. There were significant differences in BMI for the three groups of patients, but there was no apparent trend for BMI to alter in parallel with albuminuric status. There were no significant differences in duration of diabetes, blood pressure values, A1C levels, use of antihypertensive agents, use of RAS inhibitors, prevalence of retinopathy or macrovascular disease, and lipid levels between the groups.

Patients with an eGFR <60 ml/min per 1.73 m² ($n = 93$) had a higher resistance index of the renal interlobar renal arteries compared with patients with an eGFR ≥60 ml/min per 1.73 m² ($n = 232$) when matched for AER status (Fig. 1). Furthermore, the resistance index was elevated to a similar extent in patients with an eGFR <60 ml/min per 1.73 m² regardless of albuminuria status (normo- 0.74 ± 0.01, micro- 0.73 ± 0.01, and macroalbuminuria resistance index 0.75 ± 0.11). These resistance index values for patients with an eGFR <60 ml/min per 1.73 m² were well above the previously reported upper end of the range for resistance index (i.e., < 0.7) in healthy subjects (6). For patients with impaired renal function, the lowest mean resistance index value was 0.54 and the mean difference between resistance index values for paired kidneys (left versus right) was 0% (range −4.9 to +6.6%). The mean difference in kidney size (left versus right) was 5.9% (−47 to +163%). After excluding patients ($n = 22$) with a >25% difference in kidney size and the one patient who was subsequently diagnosed with bilateral renal artery stenosis, resistance index results were still similar for patients with impaired renal function regardless of their albuminuric status (normo- 0.74 ± 0.01, micro- 0.74 ± 0.01, and macroalbuminuria 0.75 ± 0.11).

Associations with intrarenal resistance index for low-GFR subjects

For subjects with an eGFR <60 ml/min per 1.73 m² ($n = 93$), there were significant univariate associations between the intrarenal arterial resistance index and age ($r = +0.36$, $P < 0.001$), eGFR ($r = -0.45$, $P < 0.0001$), diastolic blood pressure ($r = -0.44$, $P < 0.001$), mean arterial pressure ($r = +0.28$, $P < 0.01$), pulse pressure ($r = +0.22$, $P = 0.03$), BMI ($r = +0.29$, $P < 0.01$), diabetes duration ($r = +0.28$, $P < 0.01$), and the reciprocal of serum creatinine levels ($r = +0.23$, $P = 0.03$). There was also a significant sex difference in resistance index values for low-GFR patients (females 0.76 ± 0.01 vs. males 0.73 ± 0.01, $P = 0.05$). However, multivariate analysis revealed that only four variables were independently associated with resistance index in this group, i.e., age ($P < 0.0001$), BMI ($P = 0.0001$), eGFR ($P < 0.01$), and diastolic blood pressure ($P < 0.01$). These four variables explained 43% of the variance in resistance index (adjusted $R^2 = 0.43$, $P < 0.0001$). The relationship between resistance index and age, eGFR, diastolic blood pressure, and BMI for patients with impaired renal function and normo-,

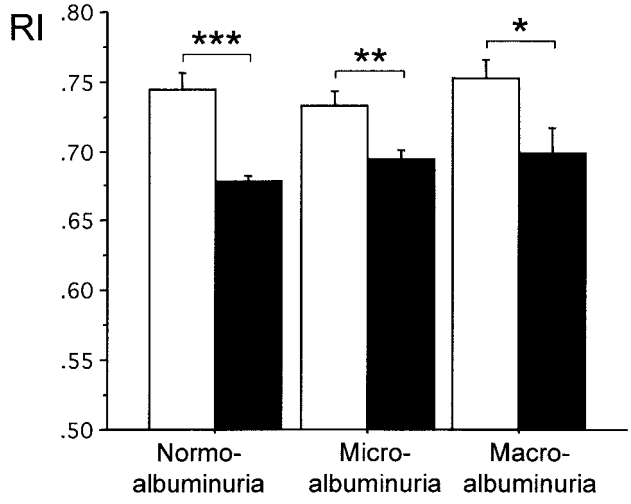

Figure 1—*Intrarenal arterial resistance index (RI) in 325 patients with type 2 diabetes stratified according to eGFR, i.e., < or ≥60 ml/min per 1.73 m^2, and albuminuric status, i.e., normo- (AER <20), micro- (20–200), or macroalbuminuria (> 200). □, GFR <60 ml/min per 1.73 m^2 (n = 93); ■, eGFR ≥60 ml/min per 1.73 m^2 (n = 232). *P < 0.05, **P < 0.01, ***P < 0.001.*

micro-, and macroalbuminuria is shown in Fig. 2. There were no associations between resistance index and AER, systolic blood pressure, A1C, cholesterol (total, LDL, or HDL) or triglyceride levels. Similar associations were also observed for all patients irrespective of their GFR (n = 325) with age ($P < 0.0001$), eGFR ($P < 0.0001$), and pulse pressure ($P < 0.0001$) emerging as independent predictors of resistance index.

The effect of RAS inhibition on intrarenal resistance index

The prevalence of an eGFR <60 ml/min per 1.73 m^2 and normoalbuminuria was also calculated after excluding 22 of 39 patients whose normoalbuminuric status was possibly altered by the use of an RAS inhibitor (i.e., the 13 patients on an RAS

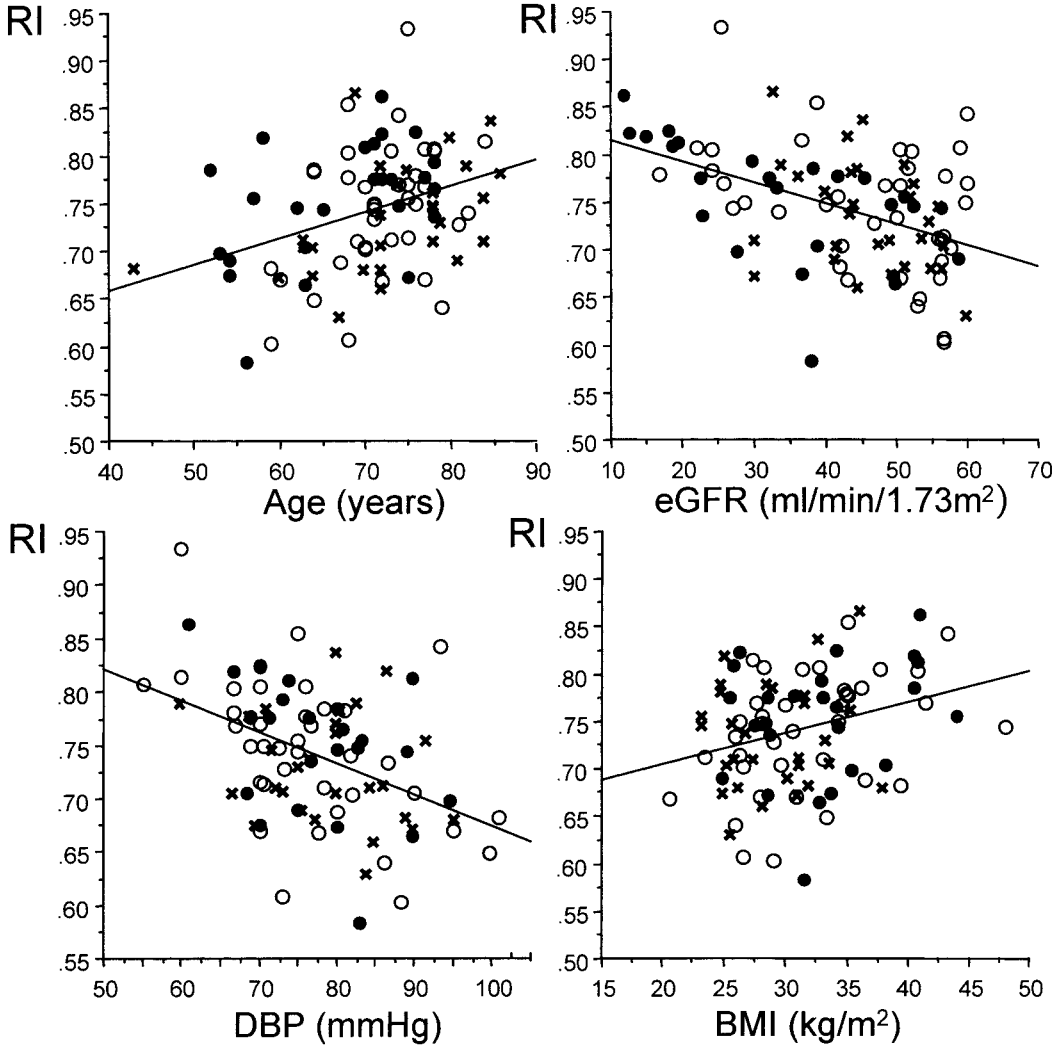

Figure 2—*The relationship between the intrarenal arterial resistance index (RI) versus age (r = +0.36, P < 0.000), eGFR (r = −0.45, P < 0.0001), diastolic blood pressure (DBP) (r = −0.44, P < 0.0001), and BMI (r = +0.29, P < 0.01) for 93 patients with an eGFR <60 ml/min per 1.73 m^2 and normo- (AER <20), micro- (20–200), or macroalbuminuria (> 200 μg/min). ○, normoalbuminuric patients; ✕, microalbuminuric patients; ●, macroalbuminuric patients.*

inhibitor before attending the clinic and the 9 patients with micro- or macroalbuminuria before the initiation of therapy). After this adjustment, the prevalence of an eGFR <60 ml/min per 1.73 m^2 and normoalbuminuria was 17/74 (22%). The age difference (normo- 74 ± 1, micro- 73 ± 2, and macro- 67 ± 2 years; $P <$ 0.02) and lack of differences between other clinical and biochemical factors among patients with an eGFR <60 ml/min per 1.73 m^2 and various levels of albuminuria were preserved after the exclusion of normoalbuminuric patients without a documented history of normoalbuminuria before the initiation of an RAS inhibitor.

Resistance index values that were observed for the entire population of low-eGFR subjects with normo-, micro-, and macroalbuminuria, regardless of RAS inhibitor use (Fig. 1), were not significantly different from those observed after excluding low-eGFR normoalbuminuric subjects who did not have a documented history of normoalbuminuria before the initiation of an RAS inhibitor (i.e., normo- 0.75 ± 0.02, micro- 0.73 ± 0.01, and macroalbuminuria 0.75 ± 0.11). To further evaluate the effects of RAS inhibition on the resistance index, we compared the resistance index in all low-eGFR subjects ($n = 93$) according to their RAS inhibition status. Normo-, micro-, and macroalbuminuric patients were evaluated together because of the small number of subjects that were not taking RAS inhibitors ($n =$ 15). There was no significant difference between resistance index values for subjects taking or not taking RAS inhibitors (0.75 ± 0.01 vs. 0.73 ± 0.07). Furthermore, the use of antihypertensive agents or RAS inhibitors did not emerge as independent predictors of the intrarenal resistance index for low-eGFR patients.

CONCLUSIONS — Since it is becoming increasingly apparent that a considerable proportion of subjects with type 2 diabetes can develop renal impairment in the absence of an increase in albuminuria, it is most important that the pathogenesis of normoalbuminuric renal insufficiency is fully elucidated. It has been suggested that premature senescence of the diabetic kidney, interstitial fibrosis, ischemic vascular disease, or cholesterol microemboli, as opposed to classical diabetic glomerulosclerosis, may contribute to the development of normoalbuminuric renal impairment in type 2 diabetes (4,17). In this study, we found that the resistance of the intrarenal arteries, as estimated by the calculation of a resistance index, was increased to a similar extent in subjects with type 2 diabetes and impaired renal function regardless of their albuminuric status.

Functional as well as structural parameters are known to influence the intrarenal resistance index (18–20). For example, an acute captopril challenge will decrease intrarenal resistance index values in diabetic subjects (20). We therefore evaluated the use of antihypertensive agents and RAS inhibitors in our study subjects as determinants of the resistance index. Of note, the resistance index was evaluated in our study while subjects were still taking their normal medications. The use of antihypertensive agents and RAS inhibitors was similar in normo-, micro-, or macroalbuminuric subjects with impaired renal function. We found that the chronic use of antihypertensive agents or RAS inhibitors did not emerge as independent predictors of intrarenal resistance index values. Furthermore, the resistance index values were similar for low-eGFR subjects taking and those not taking RAS inhibitors. Differences in the intrarenal resistance index response to acute and chronic RAS inhibition remain to be explained.

It is recognized that acute changes in afferent or efferent arteriolar diameter can have effects on GFR and intraglomerular pressure, which may affect AER independent of glomerular injury. However, resistance index as measured in this study reflects predominantly arterial resistance at the whole-kidney level rather than changes at the single-nephron level (5–7). This may explain the lack of a significant difference in resistance index for subjects taking or not taking RAS inhibitors.

One of the weaknesses of this study was that we did not specifically screen for the presence of renal artery stenosis in patients with impaired renal function by performing a Doppler examination of the main renal artery or by conducting an angiographic investigation. However, patients with known renal artery stenosis were excluded, and we accounted for Doppler intrarenal indexes that have been previously reported to be associated with the presence of hemodynamically significant renal artery stenosis (12). Also, eliminating patients with asymmetrical kidney size (>25%) did not alter the finding that resistance index values were elevated to a similar extent in subjects with renal insufficiency regardless of albuminuric status.

Renal artery stenosis is known to lower resistance index values, and the possibility exists that a higher prevalence of extrarenal arterial disease (involving the main renal arteries) masked a higher prevalence of intrarenal vascular disease or dysfunction in patients with impaired renal function and normoalbuminuria compared with those with a similar degree of renal impairment and elevated AERs. Using intrarenal Doppler indexes and kidney size criteria, we did not diagnose any new cases of renal artery stenosis in this population of patients from which known cases of renal artery stenosis had already been excluded. Independent of the current study, one patient with impaired renal function was later diagnosed with severe bilateral renal artery stenosis. The prevalence of renal artery stenosis in our study population with impaired renal function was therefore 1/93 (1.1%). It has recently been reported that only 2/171 (1.2%) subjects with diabetes, hypertension, and microalbuminuria may have hemodynamically significant renal artery stenosis when extra- and intrarenal Doppler screening techniques are used (9).

Consistent with previous studies, we found that the resistance index of the intrarenal arteries correlated with age, eGFR, and BMI in subjects with type 2 diabetes (21,22). Unlike a previous study, duration of diabetes was not found to correlate with resistance index values (22). This difference may possibly be explained by the characteristics of our study population that consisted of subjects with established diabetes and renal impairment.

Our results also support the finding that increases in intrarenal arterial resistance index are not related to albuminuria (21,23). Decreases in GFR in subjects with type 2 diabetes have been associated with increases in carotid intimal-medial thickness, carotid stiffness, and increases in the intrarenal arterial resistance index (23). This has led to the suggestion that the decline in GFR in type 2 diabetes is in part due to generalized increase in arteriosclerosis. We also found that diastolic blood pressure values were negatively correlated with resistance index values. This suggests that an increase in the resistance index of the intrarenal vasculature reflects a generalized increase in arteriosclerosis and a widening of pulse pressure. In support of this suggestion, it has been shown that there is a relationship

between the resistance index of the main renal artery and a nonsignificant correlation ($P = 0.072$) of the intrarenal resistance index with systemic pulse wave velocity in subjects with diabetic and nondiabetic renal disease (8). The mechanisms responsible for the increase in intrarenal resistance index and a reduction in systemic arterial compliance, at least in diabetes, are not fully understood but may involve impaired glycemic control, the glycation of structural proteins within the arterial wall, angiotensin-stimulated collagen deposition in vessel walls, autonomic nervous system dysfunction, or endothelial dysfunction (24).

The renal pathologies that explain the relationship between declining GFR and AER in type 2 diabetes are not well characterized. Renal structural changes that are typical and atypical of diabetic glomerulopathy have been described for subjects with type 2 diabetes, microalbuminuria, and preserved renal function. The most common renal changes reported in this clinical context include normal or near-normal renal structure and atypical patterns or injury including tubulointerstitial lesions, advanced glomerular arteriolar hyalinosis, and global glomerular sclerosis (25). For macroalbuminuric subjects with various degrees of renal impairment, structural changes apart from those of typical diabetic glomerulopathy are also not uncommon (26–30). At present, very little information is available regarding the renal morphology of normoalbuminuric patients with type 2 diabetes regardless of GFR status.

Recently an animal model of type 2 diabetic normoalbuminuric renal insufficiency has been described (31). The Cohen diabetic rat is known to develop progressive impairment in renal function with morphological changes that are typical of diabetic glomerulosclerosis but without proteinuria. These rats also develop retinal pathology that is consistent with nonproliferative diabetic retinopathy. Also, normoalbuminuric patients with type 1 diabetes and reduced GFR have more advanced glomerular lesions than normoalbuminuric subjects with normal renal function, particularly if retinopathy or hypertension are also present (32).

These latter findings suggest that changes in glomerular pathology at the molecular, rather than the microscopic, level possibly determine AER status. For example, maintenance of normal expression of nephrin and other proteins that are essential for the integrity of the slit diaphragm (33–35) may explain why AER is not elevated in the setting of a microscopic glomerulopathy. In contrast, a reduced GFR may be associated with pathological changes, including, but not exclusively related to, morphological changes that result in an increase in intrarenal vascular disease. Indeed, at least in subjects with type 1 diabetes and normoalbuminuric renal insufficiency, glomerular structural parameters correlate with a decline in GFR (32), but glomerular pathological changes are not thought to have a major influence on the intrarenal resistance index (5,7,32). The pathological processes that result in a decline in GFR in normaolbumiuric subjects with type 2 diabetes remain unknown, but our results suggest that all subjects with type 2 diabetes and a low eGFR have a similar burden of intrarenal vascular disease regardless of their AER status.

In the present study we found that the prevalence of normoalbuminuric renal insufficiency, defined as an eGFR <60 ml/min per 1.73 m^2 (estimated by the MDRD six-variable formula) and after accounting for the use of RAS inhibitors, was 23% for clinic subjects with type 2 diabetes. We have previously reported an almost identical prevalence of normoalbuminuric renal insufficiency when GFR was estimated by the accepted gold standard for measuring GFR in routine clinical practice, i.e., the plasma disappearance of a radioisotopic marker, in a similar population of clinic subjects (1). The concordance between the prevalence of normoalbuminuric renal insufficiency when GFR is measured either by the MDRD six-variable formula or by an isotopic method suggests that using the MDRD six-variable formula may prove to be a useful and accurate method for detecting renal insufficiency in normoalbuminuric subjects with diabetes.

Subjects with impaired renal function and macroalbuminuria tended to be older and female and as mentioned had lower absolute eGFR values when compared with those with renal insufficiency and normoalbuminuria. However, consistent with our previous report, there were no significant differences in diabetes duration, blood pressure measurements, treatment for hypertension, use of RAS inhibitors, prevalence of macrovascular disease, and prevalence of retinopathy among subjects with impaired renal function and normo-, micro-, and macroalbuminuria (1). We did not specifically match subjects with impaired renal function for clinical and biochemical parameters that are known to influence the resistance index. However, it is unlikely that the small clinical differences in age and absolute eGFR values that were observed for macro- compared with normoalbuminuric subjects masked any true difference in the resistance index values for these groups of patients.

In conclusion, subjects with type 2 diabetes and an eGFR <60 ml/min per 1.73 m^2 had a greater degree of intrarenal vascular disease, as measured by the intrarenal resistance index, compared with subjects with an eGFR ≥60 ml/min per 1.73m^2, but these differences were independent of AER status. An increase in intrarenal vascular disease may be related to a decline in GFR in nonalbuminuric renal insufficiency. However, we did not find that there was a preponderance of intrarenal vascular disease in normoalbuminuric compared with micro- and macroalbunuric subjects who had renal impairment. The pathological mechanisms that determine the relationship between impaired renal function and AER status in subjects with type 2 diabetes remain to be elucidated.

Acknowledgments— This work was supported by the National Health and Medical Research Council of Australia (grant no. 266505) and the Austin Hospital Medical Research Foundation. R.J.M. was a recipient of Cardio-Vascular-Lipid research grant from Pfizer.

We thank Judy Winikoff and Aysel Akdeniz for their assistance in measuring urinary albumin.

References

1. MacIsaac RJ, Tsalamandris C, Panagiotopoulos S, Smith TJ, McNeil KJ, Jerums G: Nonalbuminuric renal insufficiency in type 2 diabetes. *Diabetes Care* 27:195–200, 2004
2. Tsalamandris C, Allen TJ, Gilbert RE, Sinha A, Panagiotopoulos S, Cooper ME, Jerums G: Progressive decline in renal function in diabetic patients with and without albuminuria. *Diabetes* 43:649–655, 1994
3. Garg AX, Kiberd BA, Clark WF, Haynes RB, Clase CM: Albuminuria and renal insufficiency prevalence guides population screening: results from the NHANES III. *Kidney Int* 61:2165–2175, 2002
4. Kramer HJ, Nguyen QD, Curhan G, Hsu CY: Renal insufficiency in the absence of albuminuria and retinopathy among adults with type 2 diabetes mellitus. *JAMA*

289:3273–3277, 2003
5. Platt JF, Ellis JH, Rubin JM, DiPietro MA, Sedman AB: Intrarenal arterial Doppler sonography in patients with nonobstructive renal disease: correlation of resistive index with biopsy findings. *AJR Am J Roentgenol* 154:1223–1227, 1990
6. Platt JF, Rubin JM, Ellis JH: Diabetic nephropathy: evaluation with renal duplex Doppler US. *Radiology* 190:343–346, 1994
7. Ikee R, Kobayashi S, Hemmi N, Imakiire T, Kikuchi Y, Moriya H, Suzuki S, Miura S: Correlation between the resistive index by Doppler ultrasound and kidney function and histology. *Am J Kidney Dis* 46: 603–609, 2005
8. Ohta Y, Fujii K, Arima H, Matsumura K, Tsuchihashi T, Tokumoto M, Tsuruya K, Kanai H, Iwase M, Hirakata H, Iida M: Increased renal resistive index in atherosclerosis and diabetic nephropathy assessed by Doppler sonography. *J Hypertens* 23:1905–1911, 2005
9. Nosadini R, Velussi M, Brocco E, Abaterusso C, Carraro A, Piarulli F, Morgia G, Satta A, Faedda R, Abhyankar A, Luthman H, Tonolo G: Increased renal arterial resistance predicts the course of renal function in type 2 diabetes with microalbuminuria. *Diabetes* 55:234–239, 2006
10. Baxter GM, Aitchison F, Sheppard D, Moss JG, McLeod MJ, Harden PN, Love JG, Robertson M, Taylor G: Colour Doppler ultrasound in renal artery stenosis: intrarenal waveform analysis. *Br J Radiol* 69: 810–815, 1996
11. Nazzal MM, Hoballah JJ, Miller EV, Sharp WJ, Kresowik TF, Corson J: Renal hilar Doppler analysis is of value in the management of patients with renovascular disease. *Am J Surg* 174:164–168, 1997
12. Riehl J, Schmitt H, Bongartz D, Bergmann D, Sieberth HG: Renal artery stenosis: evaluation with colour duplex ultrasonography. *Nephrol Dial Transplant* 12: 1608–1614, 1997
13. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D: A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation: Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 130:461–470, 1999
14. Thomas MC, MacIsaac RJ, Tsalamandris C, Power D, Jerums G: Unrecognized anemia in patients with diabetes: a cross-sectional survey. *Diabetes Care* 26:1164–1169, 2003
15. Coresh J, Astor BC, McQuillan G, Kusek J, Greene T, Van Lente F, Levey AS: Calibration and random variation of the serum creatinine assay as critical elements of using equations to estimate glomerular filtration rate. *Am J Kidney Dis* 39:920–929, 2002
16. Mathew T on behalf of the Australasian Creatinine Consensus Working Group: Chronic kidney disease and automatic reporting of estimated glomerular filtration rate: a position statement. *Med J Aust* 183: 138–141, 2005
17. Ritz E: Type 2 diabetes: absence of proteinuria does not peclude loss of renal function (Editorial). *J Am Soc Nephrol* 16: 284–285, 2004
18. Miller JA: Impact of hyperglycemia on the renin angiotensin system in early human type 1 diabetes mellitus. *J Am Soc Nephrol* 10:1778–1785, 1999
19. Miller JA, Floras JS, Zinman B, Skorecki KL, Logan AG: Effect of hyperglycaemia on arterial pressure, plasma renin activity and renal function in early diabetes. *Clin Sci (Lond)* 90:189–195, 1996
20. Taniwaki H, Ishimura E, Kawagishi T, Matsumoto N, Hosoi M, Emoto M, Shoji T, Shoji S, Nakatani T, Inaba M, Nishizawa Y: Intrarenal hemodynamic changes after captopril test in patients with type 2 diabetes: a duplex Doppler sonography study. *Diabetes Care* 26:132–137, 2003
21. Boeri D, Derchi LE, Martinoli C, Simoni G, Sampietro L, Storace D, Ponte L, Calvi C, Repetto M, Robaudo C, Maiello M: Intrarenal arteriosclerosis and impairment of kidney function in NIDDM subjects. *Diabetologia* 41:121–124, 1998
22. Ishimura E, Nishizawa Y, Kawagishi T, Okuno Y, Kogawa K, Fukumoto S, Maekawa K, Hosoi M, Inaba M, Emoto M, Morii H: Intrarenal hemodynamic abnormalities in diabetic nephropathy measured by duplex Doppler sonography. *Kidney Int* 51:1920–1927, 1997
23. Taniwaki H, Nishizawa Y, Kawagishi T, Ishimura E, Emoto M, Okamura T, Okuno Y, Morii H: Decrease in glomerular filtration rate in Japanese patients with type 2 diabetes is linked to atherosclerosis. *Diabetes Care* 21:1848–1855, 1998
24. Woodman RJ, Watts GF: Measurement and application of arterial stiffness in clinical research: focus on new methodologies and diabetes mellitus. *Med Sci Monit* 9:RA81–RA89, 2003
25. Fioretto P, Mauer M, Brocco E, Velussi M, Frigato F, Muollo B, Sambataro M, Abaterusso C, Baggio B, Crepaldi G, Nosadini R: Patterns of renal injury in NIDDM patients with microalbuminuria. *Diabetologia* 39:1569–1576, 1996
26. Gambara V, Mecca G, Remuzzi G, Bertani T: Heterogeneous nature of renal lesions in type II diabetes. *J Am Soc Nephrol* 3:1458–1466, 1993
27. Mazzucco G, Bertani T, Fortunato M, Bernardi M, Leutner M, Boldorini R, Monga G: Different patterns of renal damage in type 2 diabetes mellitus: a multicentric study on 393 biopsies. *Am J Kidney Dis* 39:713–720, 2002
28. Olsen S, Mogensen CE: How often is NIDDM complicated with non-diabetic renal disease? An analysis of renal biopsies and the literature. *Diabetologia* 39:1638–1645, 1996
29. Parving HH, Gall MA, Skott P, Jorgensen HE, Lokkegaard H, Jorgensen F, Nielsen B, Larsen S: Prevalence and causes of albuminuria in non-insulin-dependent diabetic patients. *Kidney Int* 41:758–762, 1992
30. Schwartz MM, Lewis EJ, Leonard-Martin T, Lewis JB, Batlle D: Renal pathology patterns in type II diabetes mellitus: relationship with retinopathy: The Collaborative Study Group. *Nephrol Dial Transplant* 13: 2547–2552, 1998
31. Yagil C, Barak A, Ben-Dor D, Rosenmann E, Bernheim J, Rosner M, Segev Y, Weksler-Zangen S, Raz I, Yagil Y: Nonproteinuric diabetes-associated nephropathy in the Cohen rat model of type 2 diabetes. *Diabetes* 54:1487–1496, 2005
32. Caramori ML, Fioretto P, Mauer M: Low glomerular filtration rate in normoalbuminuric type 1 diabetic patients: an indicator of more advanced glomerular lesions. *Diabetes* 52:1036–1040, 2003
33. Bonnet F, Cooper ME, Kawachi H, Allen TJ, Boner G, Cao Z: Irbesartan normalises the deficiency in glomerular nephrin expression in a model of diabetes and hypertension. *Diabetologia* 44:874–877, 2001
34. Langham RG, Kelly DJ, Cox AJ, Thomson NM, Holthofer H, Zaoui P, Pinel N, Cordonnier DJ, Gilbert RE: Proteinuria and the expression of the podocyte slit diaphragm protein, nephrin, in diabetic nephropathy: effects of angiotensin converting enzyme inhibition. *Diabetologia* 45:1572–1576, 2002
35. Toyoda M, Suzuki D, Umezono T, Uehara G, Maruyama M, Honma M, Sakai T, Sakai H: Expression of human nephrin mRNA in diabetic nephropathy. *Nephrol Dial Transplant* 19:380–385, 2004