Kidney International, Vol. 60 (2001), pp. 182–189

Vascular function of the peripheral circulation in patients with nephrosis

GERALD F. WATTS, SUSAN HERRMANN, GURSHARAN K. DOGRA, DAVID A. PLAYFORD, JAMES D. BEST, MARK A.B. THOMAS, and ASHLEY IRISH

Department of Medicine and Western Australian Heart Research Institute, University of Western Australia, and Department of Nephrology, Royal Perth Hospital, Perth, Western Australia; and Department of Medicine, University of Melbourne and St. Vincent's Hospital, Melbourne, Victoria, Australia

Vascular function of the peripheral circulation in patients with nephrosis.

Background. Nephrotic syndrome is associated with abnormal lipoprotein metabolism and increased risk of coronary heart disease. Endothelial dysfunction, an early phase of atherogenesis that manifests as impaired flow-mediated dilation (FMD) of the peripheral circulation, may link these associations.

Methods. We examined endothelial function of the brachial artery and forearm resistance arteries in 15 patients with nephrosis (NP), 15 patients with primary hyperlipidemia (HL) alone, and 15 normolipidemic, nonproteinuric subjects (NC) matched for age, sex, and weight. The NP and HL groups had similar serum cholesterol and triglyceride concentrations. Postischemic FMD (endothelium-dependent) and glyceryl trinitrate-mediated dilation (GTNMD; endothelium-independent) of the brachial artery were studied using ultrasonography and computerized edge detection software. Postischemic forearm blood flow was also measured using plethysmography.

Results. Postischemic FMD of the brachial artery was significantly lower in the NP and HL groups compared with NC group (mean ± SE): NP 4.91 ± 0.8%, HL 4.53 ± 0.6%, NC 8.45 ± 0.5% ($P < 0.001$). There were no significant differences among the groups in baseline diameter and GTNMD of the brachial artery, nor in maximal forearm blood flow and flow debt repayment of the forearm microcirculation. Significant differences in FMD among the groups were principally related to differences in serum low-density lipoprotein cholesterol.

Conclusions. Patients with NP have abnormal endothelium-dependent but preserved endothelium-independent dilation of the brachial artery following an ischemic stimulus. Postischemic forearm microcirculatory function is unimpaired. Dyslipoproteinemia is probably the principal cause of endothelial dysfunction of conduit arteries in patients with NP and the basis for their increased risk of cardiovascular disease.

Key words: proteinuria, lipids, endothelial function, dyslipoproteinemia, cardiovascular disease, ischemia, atherogenesis.

Received for publication August 11, 2000
and in revised form January 18, 2001
Accepted for publication January 22, 2001

While the importance of proteinuria as a prognostic marker for progression of renal disease is established, its significance as a risk factor for cardiovascular disease is less widely appreciated, particularly in nondiabetic populations [1]. Nephrotic syndrome is characterized by heavy proteinuria >3.5 g/day, hypoalbuminemia, hypercoagulability, edema, and dyslipoproteinemia [2]. An increased incidence of coronary disease also has been reported in nephrotic patients, but the basis for this increase remain unclear [3].

Plasma lipid abnormalities are universal in nephrosis (NP) [2, 4]. Mixed hyperlipidemia (HL) frequently results from abnormal apolipoprotein B-100 (apoB) transport [5]. Qualitative changes in high-density lipoprotein (HDL) and elevations in plasma lipoprotein (a) [Lp(a)] may also occur [5]. Dyslipoproteinemia [6], hypoalbuminemia [7], and hyperfibrinogenemia [8], recognized cardiovascular risk factors in the community, may collectively account for the increased incidence of cardiovascular disease in NP in the absence of renal failure [3]. Their relative contributions, as well as the role of less conventional risk factors, such as insulin resistance and low-density lipoprotein (LDL) particle size, are unclear, however. Whether treating dyslipoproteinemia in NP is of clinical benefit remains to be established [4].

Endothelial dysfunction is an early phase of atherosclerosis resulting from perturbations in the physiology of several vasoactive molecules, principally nitric oxide (NO) [9, 10]. Endothelial function of peripheral conduit arteries may be measured following an ischemic stimulus using high-resolution ultrasonography [11]. Microcirculatory endothelial function may also be assessed noninvasively using venous-occlusion, strain-gauge plethysmography [12]. Endothelial dysfunction of the brachial artery correlates with abnormal vasomotor responses of the coronary circulation [13], predicts coronary events [14, 15], and is associated with conventional cardiac risk factors [16, 17]. Abnormal forearm hyperemic responses may also

reflect generalized microcirculatory dysfunction [18]. Forearm microcirculatory responses to intra-arterially administered 5-hydroxy-tryptamine (5-HT), an NO agonist, have been previously shown to be abnormal in NP [19]. Whether endothelial dysfunction extends to other vessels and the relative effects of dyslipidemia, hypoalbuminemia, and hyperfibrinogenemia on endothelial dysfunction remains to be determined.

We therefore examined endothelial function noninvasively in two peripheral arterial systems in nephrotic patients and in patients with primary HL. Using a case-control design, we aimed principally to assess the role of dyslipoproteinemia in the pathogenesis of arterial disease in NP, as well as the contributory effects of other variables, including plasma albumin, fibrinogen, and homocysteine levels.

METHODS

Subjects and study design

Fifteen patients with NP, 15 with primary HL and 15 healthy, normolipidemic, nonproteinuric (NC) subjects were recruited from renal clinics, lipid clinics, and the community, respectively. The groups were matched for age, sex, and body mass index (BMI). The NP and HL groups were selected to have similar serum total cholesterol, triglyceride (TG), and LDL cholesterol levels. In this study, NP referred to a primary glomerulopathy resulting in a classic nephrotic syndrome or an associated residual nephrotic range proteinuria. Primary HL was defined as HL in the absence of a recognized precipitating cause, with or without classic clinical stigmata and a family history of coronary disease. Subjects with cardiovascular disease, diabetes, hypothyroidism, liver disease, alcoholism, postural hypotension, intercurrent illnesses, and significant psychiatric disorders were excluded. Patients were studied off lipid-lowering drugs (for at least 4 weeks) and aspirin. Patients were not excluded for taking angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists, diuretics, or a stable dose of other antihypertensive drugs, since these treatments constitute best clinical practice and patients were studied in an outpatient setting. We carried out a cross-sectional comparison of vascular function of the peripheral circulation among the nephrotic, hyperlipidemic, and normolipidemic groups. Vascular function studies of the brachial artery and forearm microcirculation were carried out in the morning after a 12-hour fast from food and beverages and after resting in the supine position for at least 15 minutes. Smoking was not allowed on the day of the test. The study received approval from the Royal Perth Hospital Ethics Committee, and all volunteers gave informed written consent.

Clinical and laboratory methods

All patients had a medical examination and a 12-lead electrocardiogram (EKG). Resting blood pressure (BP) and heart rate were measured using a Dinamap (Critikon Ltd., Tampa, FL, USA). Urinary protein excretion was assessed in the nephrotic patients and in the other groups by 24-hour urine collection and early morning urinary protein/creatinine ratio, respectively. BMI (kg/m^2) was derived from height and weight measurements. Venous blood was obtained in the semirecumbent position after a 12-hour fast and with minimal venous stasis. Serum cholesterol and TG were measured by enzymatic methods. HDL cholesterol was estimated after precipitation of apoB-containing lipoproteins with heparin/manganese. Serum LDL cholesterol was calculated by the Friedewald equation, but was assayed directly with a commercial assay (LDL-C; Boehringer Mannheim GmBH, Mannheim, Germany) on a Hitachi 917 Biochemical Autoanalyzer (Hitachi Ltd., Tokyo, Japan) in patients with TG >4.5 mmol/L. Plasma glucose was assayed by the hexokinase method, and serum and urinary creatinines were measured by modified Jaffé reaction. Glomerular filtration rate was calculated using the Cockcroft and Gault formula. Serum albumin and urinary protein were measured on a Hitachi 917 autoanalyzer. Lp(a) and apoB were assayed by immunonephelometric methods. LDL particle diameter was determined using nondenaturing gel electrophoresis [20]. Homocysteine was measured by a fluorescence polarization immunoassay (Axis Biochemicals ASA, Oslo, Norway) and fibrinogen by the Clauss method. The interassay coefficients of variation (CVs) were all $<6\%$.

Brachial artery ultrasonography

During the ultrasound procedure, subjects rested supine in a quiet, temperature-controlled (24°C) room. The left arm was immobilized in a foam cast and supported comfortably in extension and supination. A high-resolution 12 mHz linear array transducer connected to an Acuson Aspen™ System (Acuson Pty Ltd., Mountain View, CA, USA) was employed for the majority of ultrasounds, but a few early scans were carried out using a 7.5 mHz linear-array vascular transducer connected to a Toshiba SSA-270A ultrasound system (Toshiba Corp., Tokyo, Japan). Continuous EKG monitoring was performed in all studies. The transducer was placed 5 to 10 cm proximal to the antecubital crease and fixed in position by a stereotactic clamp. After good images were obtained, the edge-to-lumen interface was further optimized using depth and gain controls, and an edge enhancement function. Images were recorded on s-VHS videotape (Sony MQSE 180) for retrospective analysis. A pneumatic tourniquet was placed around the left forearm, and after recording the baseline images for two

minutes, the cuff was rapidly inflated to 200 mm Hg for five minutes. Forearm-reactive hyperemia was induced by sudden release of the cuff. Images were recorded continuously from 30 seconds before to 4 minutes after cuff release. A second resting scan was obtained at least 10 minutes after cuff deflation to ensure that the brachial artery diameter returned to the basal level. Four hundred micrograms of glyceryl trinitrate (GTN) were administered sublingually by spray, and the images were recorded continuously for a further five minutes.

Analysis of postischemic flow and GTN-mediated dilation of the brachial artery was carried out using semiautomated edge detection software recently developed and validated within our department. During the s-VHS playback into a digital frame grabber, digital images were displayed on a personal computer. A rectangular region of interest (ROI) was drawn around the most representative section of the artery. A second ROI was selected around the EKG tracings and a third ROI for calibration of the diameter measurements. The computerized edge-detection and wall-tracking software system then automatically determined the brachial artery diameter at the end diastole, each frame corresponding to up to 300 individual diameter measurements. A third order polynomial function was applied to the curve of serial, end-diastolic diameter measurements, to derive maximal flow-mediated dilation (FMD) and glyceryl trinitrate-mediated dilation (GTNMD) of the brachial artery. Responses were calculated as the percentage of change in brachial artery diameter from baseline. All analyses were performed by two experienced observers. In our hands, the analytical CV of the computerized technique is of the order of 12% compared with at least 35% using more conventional visual estimations employing calipers. The CV for within-subject measurements ($N = 30$) in FMD is of the order of 20%.

Forearm microcirculation

Subjects were studied after 10 minutes of rest in a supine position in a quiet, temperature-controlled (24°C) room. Measurement of forearm blood flow (FABF) was carried out using venous occlusion plethysmography with mercury-in-Silastic strain gauges connected to a plethysmograph (Hokanson EC4; Hokanson, Bellevue, WA, USA) connected to MacLab/4e (ADI Instruments, Sydney, Australia). The strain gauges were calibrated electronically and placed 5 cm below the antecubital crease of the left arm. To isolate the FABF from the hand, a wrist cuff was inflated to 200 mm Hg during measurement periods. The upper arm (or collecting) cuff was inflated rapidly to 40 mm Hg to impede venous outflow but allow arterial inflow, causing swelling of the forearm and stretch of the strain gauge. The cuffs were set to inflate for 10 seconds and deflate for 7 seconds.

Forearm blood flow was obtained by recording the mean of three representative curves and was expressed as mL/100 mL of forearm/min. It was calculated in MacLab by selection of the steepest gradient of each flow curve, assuming the change in flow to be directly proportional to the change in voltage with respect to time; the intraobserver CV was <5%, but the within-subject CV was of the order of 25% [21]. BP was measured before and after the procedure using a semiautomatic sphygmomanometer (Dinamap; Critikon Ltd., Tampa, FL, USA). A sphygmomanometer cuff was placed over the left upper arm and inflated to 40 mm Hg above systolic BP to induce forearm ischemia for four minutes. After releasing the cuff, blood flow was measured for four minutes. The initial peak after the ischemic period was defined as the hyperemic response or maximal blood flow, expressed as mL/100 mL of forearm/min. Flow debt repayment was defined as the area under the blood flow curve (mL/100 mL of forearm), equivalent to the excess blood flow during hyperemia, and was derived from the hyperemic blood flow curve using nonlinear regression analysis [21]. Forearm vascular resistance (FVR) was also measured using the formula: mean arterial pressure (MAP)/maximal FABF. Forearm length (l) and forearm circumference (c) were measured in order to calculate the forearm volume: $(c^2 \times l)/4\pi$.

Statistical methods

Group comparisons were carried out using analysis of variance with Bonferroni adjustments. Group differences in postischemic FMD were adjusted for other variables by general linear modeling. Skewed variables were log transformed to normalize their distribution, and those that remained skewed were analyzed using nonparametric techniques. Associations were examined using univariate and mutivariate regression methods. Variables that were significant at the 10% level in univariate analysis or were considered, a priori, to influence vascular function were employed in the multiple regression models. Statistical significance was defined at the 5% level.

RESULTS

Table 1 shows the glomerular histology, disease duration, and renal function of the patients with NP. Two patients did not have a biopsy-proven diagnosis at the time of the study. The median (range) duration of glomerular disease was six (1 to 156) months. The mean ± SD 24-hour urinary protein excretion was in the nephrotic range at 6.3 ± 4.6 g/24 h and serum albumin decreased at 26.1 ± 7.2 g/L. Serum creatinine and glomerular filtration rates were 99.3 ± 31.9 μmol/L and 76.8 ± 33.4 mL/min, respectively. Five patients were being treated with ACE inhibitors, either enalapril, quinapril, perindopril, trandolapril, or lisinopril, and two were taking an angiotensin II receptor antagonist (irbesartan and losartan). Two patients had just commenced oral corticosteroid treatment. Eight patients had clinically detectable peripheral edema.

Table 1. Histological features, renal function and angiotensin-converting enzyme (ACE) inhibitor treatment of the patients with nephrosis

Patient no.	Glomerular histology	Disease duration *months*	Urinary protein *g/24 h*	Serum albumin *g/L*	Serum creatinine *μmol/L*	Calculated GFR *mL/min*	ACE inhibitor
1	Focal and segmental glomerulonephritis	48	1.4	40	137	56.9	yes
2	Membranous glomerulonephritis	5	6.4	26	89	67.7	yes
3	Mesangiocapillary glomerulonephritis type 1	156	1.0	32	67	69.2	no
4	Minimal change disease	2	2.2	26	56	136.6	no
5	No biopsy	2	15	26	154	36.4	yes
6	Minimal change disease	4	5.5	19	66	92.4	no
7	IgA glomerulonephritis	84	4.8	33	136	46.2	no
8	Mesangioproliferative glomerulonephritis	6	6.9	26	70	76.5	no
9	Membranous glomerulonephritis	12	1.4	33	90	90.5	yes
10	Focal and segmental glomerulonephritis	24	3.3	28	135	28.5	yes
11	No biopsy	1	7.4	15	83	73.9	no
12	Minimal change disease	7	7.5	20	102	65.3	no
13	Minimal change disease	1	16.2	20	118	70.8	no
14	Membranous glomerulonephritis	54	5.5	32	66	153.8	no
15	Minimal change disease	1	9.3	15	120	87.4	no
	Mean	27.1	6.3	26.1	99.3	76.8	
	SD	43.4	4.6	7.2	31.9	33.4	

Table 2. Demographic and clinical characteristics of the nephrotic (NP), hyperlipidemic (HL), and normolipidemic (NC) groups

	NP	HL	NC
Number	15	15	15
Sex *male/female*	10/5	11/4	8/7
Age *years*	45.5 (3.7)	41.8 (3.8)	47.6 (3.5)
Weight *kg*	76.8 (4.2)	78.8 (3.8)	73.7 (3.1)
BMI kg/m^2	27.3 (1.6)	26.1 (0.9)	25.3 (1.0)
SBP *mm Hg*	123 (3.4)[b]	114 (2.3)	112 (3.1)
DBP *mm Hg*	75 (2.6)	69 (2.2)	67 (1.8)
Pulse pressure *mm Hg*	49 (2.4)	45 (1.5)	45 (2.3)
Heart rate *bpm*	65 (2.8)	64 (2.1)	58 (2.3)
Forearm volume *mL*	1536 (96.6)	1627 (101.0)	1413 (71.0)
Urinary protein:creatinine ratio *mg/mmol*	541.6 (88.6)[a]	3.8 (1.0)	3.3 (0.5)
Smoker	2	0	0
ACE inhibitor	5	0	0

Mean (SE) shown. Abbreviations are: NP, nephrotic patients; HL, hyperlipidemic controls; NC, normolipidemic controls; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACE, angiotensin-converting enzyme.

[a] $P < 0.0001$ vs. other groups

[b] $P < 0.05$ vs. NC

The demographic and clinical characteristics of the patients with NP and of the HL and normolipidemic (NC) control groups are shown in Table 2. Age, weight, BMI, and the proportion of males to females were not statistically different among the groups. Systolic BP was only significantly higher in the NP compared with the NC group ($P = 0.03$), but was not in the hypertensive range. There were no significant differences in diastolic BP, pulse pressure, heart rate, or forearm volume among the groups. Urinary protein excretion was grossly higher in the NP compared with the HL and NC subjects, with no significant differences between the latter groups. Two of the nephrotic patients were current smokers, and all of the HL and NC subjects were nonsmokers. None of the nephrotic patients gave a history of familial HL or premature cardiovascular disease, nor were known to be hyperlipidemic prior to developing renal disease. The HL subjects had either familial hypercholesterolemia, familial combined HL, or common hypercholesterolemia.

The serum lipid, lipoprotein, glucose, insulin, creatinine, and albumin concentrations of the three groups are shown in Table 3. Cholesterol, TG, LDL cholesterol, and apoB were significantly higher in the NP and HL subjects compared with the NC controls. Lp(a) was highest in the NP group, but differed only significantly from the NC controls. Serum HDL cholesterol concentration and LDL particle size were similar among the groups. There was a trend to significantly higher glucose in both the NP and HL groups compared with the NC group ($P = 0.05$), but none of the subjects had diabetes mellitus. The nephrotic patients had a markedly lower serum albumin concentration compared with the controls ($P < 0.001$). Serum creatinine, calculated glomerular filtration rate, and plasma homocysteine did not differ significantly

Table 3. Blood lipids, lipoproteins, and other biochemical measurements in the nephrotic (NP), hyperlipidemic (HL), and normolipidemic (NC) groups

	NP	HL	NC	ANOVA *P* value
Cholesterol *mmol/L*	10.4 (0.8)[a]	8.6 (0.5)[a]	4.7 (0.2)	<0.001
Triglyceride *mmol/L*	3.6 (0.9)[a]	2.0 (0.3)[a]	0.8 (0.1)	<0.001
LDL chol *mmol/L*	6.9 (0.7)[a]	6.3 (0.5)[a]	2.8 (0.2)	<0.001
HDL chol *mmol/L*	1.6 (0.1)	1.3 (0.1)	1.5 (0.1)	0.406
ApoB-100 *g/L*	1.9 (0.2)[a]	1.5 (0.1)[a]	0.8 (0.05)	<0.001
Lp(a) *g/L*	0.6 (0.2)[c]	0.2 (0.03)	0.1 (0.04)	0.029
LDL particle size *nm*	25.8 (0.2)	26.0 (0.1)	26.2 (0.06)	0.082
Glucose *mmol/L*	5.2 (0.2)	5.2 (0.1)	4.8 (0.01)	0.050
Albumin *g/L*	26 (1.9)[ab]	42 (0.6)	44 (0.8)	<0.001
Creatinine *μmol/L*	99.3 (8.2)	87.1 (3.5)	82.9 (3.2)	0.105
Homocysteine *μmol/L*	10.8 (1.0)	8.7 (0.6)	9.2 (0.7)	0.159
Fibrinogen *g/L*	4.2 (0.5)[bc]	1.9 (0.1)	2.5 (0.2)	<0.001

Abbreviations are: LDL chol, low-density lipoprotein cholesterol; HDL chol, high-density lipoprotein cholesterol; ApoB-100, apolipoprotein B-100; Lp(a), lipoprotein (a). Mean (SE) are shown.

[a] $P < 0.001$ vs. NC

[b] $P < 0.001$ vs. HL

[c] $P < 0.05$ vs. NC

among the groups. Plasma fibrinogen was markedly elevated ($P < 0.001$) in the nephrotic patients compared with the two control groups.

There were no significant differences in prestimulatory brachial artery diameter among the three groups [NP mean 3.56 ± 0.12 mm (±SE), HL 3.80 ± 0.18, NC 3.29 ± 0.14]. The results of the poststimulatory changes in brachial artery diameter are shown in Figure 1. Postischemic FMD of the brachial artery was significantly lower in both the NP ($P = 0.002$) and HL ($P < 0.001$) groups compared with NC group, with no significant difference between the former two groups: NP 4.91 ± 0.8% (mean ± SE), HL 4.53 ± 0.6%, NC 8.45 ± 0.5% ($P < 0.001$; Fig. 1A). However, there were no significant differences in GTNMD of the brachial artery among the three groups (Fig. 1B). The findings shown in Figure 1 were also obtained after omitting the two smokers in the NP group. In a general linear model, group assignment was not a significant predictor of FMD response after adjusting for the serum LDL cholesterol and baseline brachial artery diameter. In similar models, including other variables, for example, age, gender, Lp(a), HDL or Tg, fibrinogen, glucose, albumin or BP, group assignment remained a significant predictor ($P < 0.05$) of FMD.

There were no significant differences among the groups in terms of basal FABF, maximal blood flow, flow debt repayment, or minimum vascular resistance of the forearm microcirculation (Table 4). However, mean arterial pressure at the time of the forearm test was significantly higher in the patients with NP compared with the NC group alone ($P < 0.05$; mean ± SE): NP 90.8 ± 2.7 mm Hg, HL 83.9 ± 1.9, NC 81.9 ± 2.2.

After pooling data from the three groups, postischemic FMD of the brachial artery was significantly correlated with serum total cholesterol ($r = -0.52$, $P < 0.001$), TG ($r = -0.45$, $P = 0.002$), LDL cholesterol ($r = -0.53$, $P < 0.001$), apoB ($r = -0.43$, $P = 0.004$), and baseline brachial artery diameter ($r = -0.51$, $P < 0.001$), but not with any other variables. After adjusting for other variables, only LDL cholesterol remained significantly associated ($P < 0.05$) with FMD of the brachial artery. Consistent with the study design, the association between FMD and LDL cholesterol lost significance after adjusting for group assignment. In the nephrotic group alone, postischemic FMD was not significantly correlated with other variables, including serum lipid, lipoprotein, albumin and creatinine concentrations, age, BP, or use of ACE inhibitor. There was also no significant difference in FMD between the seven patients taking ACE inhibitors or angiotensin II receptor antagonists and those not taking these agents (4.8 ± 1.3 vs. 5.0 ± 1.0%, $P = 0.937$).

DISCUSSION

Our study provides new evidence that patients with nephrotic proteinuria and preserved renal function have endothelial dysfunction of the brachial artery that is comparable to that in patients with primary HL. This abnormality was principally related to increased plasma concentrations of LDL cholesterol and did not extend to the forearm microcirculation.

While several studies have shown endothelial dysfunction in established renal failure [22–24], few have focused on vascular function in patients with proteinuria alone. Stroes et al showed impaired forearm microcirculatory responses to 5-HT in a small number of proteinuric patients [19]. They concluded that this was a consequence of a specific defect in endothelial release of NO, since the abnormality was not seen with infusion of sodium nitro-

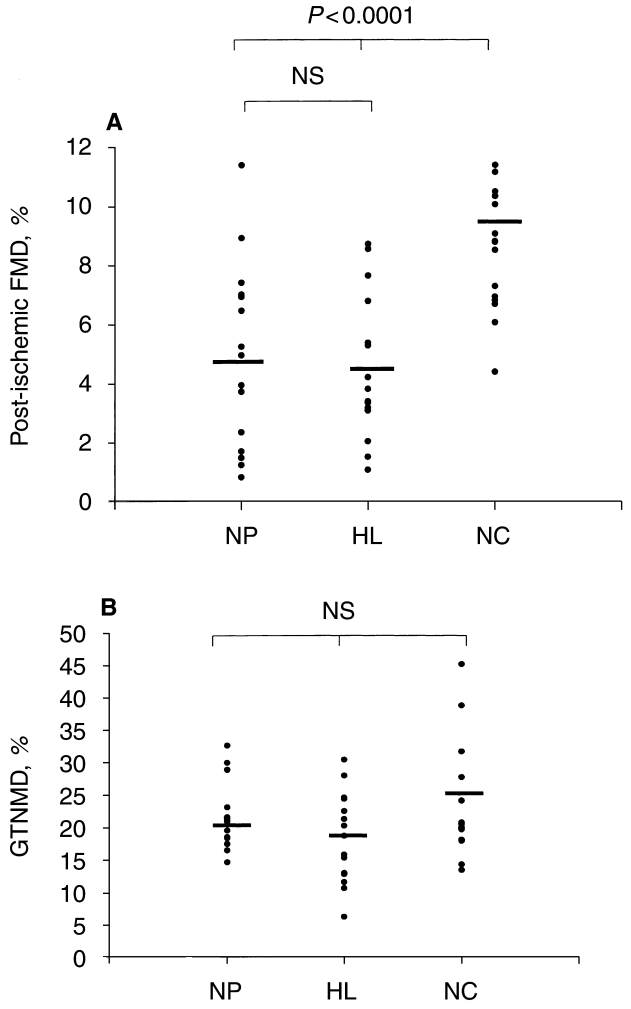

Fig. 1. Postischemic flow-mediated dilation (FMD; *A*) and glyceryl trinitrate-mediated dilatation (GTNMD; *B*) of the brachial artery in the nephrotic (NP), hyperlipidemic (HL), and normolipidemic (NC) groups. Horizontal bars are mean values, and *P* values refer to one-way analysis of variance. NS is not statistically significant.

prusside nor with coadministration of 5-HT and N^G-monomethyl-L-arginine (L-NMMA), a specific inhibitor of NO synthase. We extend this report by using a larger sample size and hyperlipidemic controls and by noninvasively testing vascular function in two peripheral arterial systems. Our suggestion that impaired vascular function in NP relates principally to elevated plasma concentrations of LDL cholesterol concurs with other findings [16].

Postischemic flow-mediated dilation of brachial and other conduit arteries results predominantly from the endothelial release of NO [12, 17, 25]. Hyperemic responses of forearm resistance arteries are mediated, however, by several agonists, including prostaglandins, adenosine, and NO, as well as by myogenic and neural mechanisms [26, 27]. Previous experiments involving coinfusion of L-NMMA have shown that in normal subjects, NO only contributes to less than 25% of forearm-reactive hyperemic blood flow [26, 27]. Given the differences in the degree of dependence on NO of the hyperemic responses measured by brachial ultrasonography and by forearm plethysmography, our results suggest that NP chiefly affects endothelium-dependent as opposed to endothelium-independent vascular function.

Table 4. Vascular function of the forearm microcirculation in the nephrotic (NP), hyperlipidemic (HL), and normolipidemic (NC) groups

	NP	HL	NC
Basal FABF *mL/100 mL/min*	1.98 (0.2)	2.13 (0.1)	2.31 (0.2)
Maximal blood flow *mL/100 mL/min*	18.07 (2.3)	21.25 (2.6)	19.26 (2.2)
Flow debt *mL/100 mL*	4.84 (1.31)	5.23 (0.7)	4.89 (0.6)
Forearm vascular resistance *mm Hg/mL/100 mL/min*	6.74 (1.2)	5.27 (1.1)	5.70 (1.2)

Mean (SE) are shown. FABF is forearm blood flow.

Extensive data suggest that oxidized LDL impairs the expression of endothelial NO synthase (eNOS), as well as the synthesis and release of NO in response to stimuli, such as increased hemodynamic flow [17, 28, 29]. Native LDL similarly may cause endothelial dysfunction by decreasing the activity of NO via a pro-oxidant effect [30], as well as by increasing the plasma concentration of asymmetric dimethylarginine (ADMA) [31], an inhibitor of eNOS. It is likely but unproven that impaired brachial artery function in both the nephrotic and hyperlipidemic subjects was due to the effect of the accumulation of LDL on the endothelial synthesis and release of NO and possibly on the oxidative catabolism of NO to peroxynitrite. The latter is not supported, however, by the normal vascular responses to GTN. Other data refute involvement of both decreased basal release of NO and a functional deficiency in L-arginine, the substrate for NO synthesis, and instead suggest a role for lysophosphatidylcholine-modified LDL [19]. Nonreversal of endothelial dysfunction with L-arginine in nephrotic patients may exclude a specific role of hypercholesterolemia, at least in the forearm microcirculation [19]. However, not all studies have demonstrated that microcirculatory endothelial dysfunction in hypercholesterolemia is reversible by L-arginine [32]. We cannot fully exclude that increased plasma concentrations of remnant lipoproteins [33] and of Lp(a) [34] contributed to the impaired brachial artery vasodilation in our patients. Small-dense LDL and low HDL are also associated with hypertriglyceridemia and endothelial dysfunction [35, 36], but did not differ significantly among our study groups.

Since brachial artery vasodilation results from the abluminal release of endothelial NO, we consider it unlikely that low serum albumin was responsible for endothelial dysfunction in our nephrotic patients, acknowledging that albumin is a reservoir for NO in plasma and is a vasorelaxant [37, 38]. Furthermore, brachial artery FMD

was similar in our patients with normal and low serum albumin concentrations. Mild hyperglycemia could also have contributed to endothelial dysfunction in the nephrotic and hyperlipidemic groups, but this effect was more likely in the forearm microcirculation [39, 40]. Hyperhomocysteinemia is associated with endothelial dysfunction in healthy individuals [41]. Its contribution, as well as that of ADMA, to endothelial dysfunction in our nephrotic patients might have been greater had we studied patients with established renal failure [42, 43]. That plasma fibrinogen was not directly correlated with endothelial dysfunction among the study groups does not exclude its role in atherogenesis in NP.

A novelty of our study was the use of an edge-detection algorithm to measure brachial artery diameter changes. In spite of the enhanced precision of measurement, our cross-sectional study was not adequately powered to detect a difference of less than 3% in FMD between the nephrotic and hyperlipidemic groups. This degree of difference in FMD may not be clinically significant, however [15]. A potential problem with estimating microcirculatory function in nephrotic subjects is that the reduction in circulating plasma volume may result in vasoconstriction. However, this was not supported by differences in either basal or poststimulatory FVR between the nephrotics and controls. A reduction in circulating fluid volume may also decrease shear-stress–mediated dilation of peripheral arteries [44]. However, our patients were not severely hypoalbuminemic and accordingly were isovolemic [45].

Our primary hypothesis would have been more rigorously tested in an intervention trial of lipid-modifying therapy. Because of limited numbers, we did not study nephrotic patients with predominant hypercholesterolemia and mixed HL separately [4]. However, it is likely that both lipid phenotypes cause comparable endothelial dysfunction in NP [17, 46]. Another potential drawback of our study was confounding due to an elevation in BP and the use of ACE inhibitors and angiotensin II receptor antagonists in the nephrotic patients. Our patients were not overtly hypertensive [47], however, and the confounding effect of these agents was not confirmed.

Our results are consistent with a primary role for disordered lipoprotein metabolism in atherogenesis and increased cardiovascular risk in NP [3]. Impaired bioavailability of NO will not only increase arterial vasotonicity, but also platelet aggregation, leukocyte chemotaxis and smooth muscle cell proliferation in the subendothelial space [9, 10]. These changes, and coexistent HL and hyperfibrinogenemia, would exacerbate atherothrombosis and acute coronary events [10]. The endothelial dysfunction demonstrated in our study probably reflects changes in coronary arteries [13] and adversely predicts coronary events [14, 15].

In conclusion, patients with nephrotic range proteinuria and patients with primary HL share similar quantitative and qualitative defects in endothelial function of the peripheral circulation. The causal role of elevated plasma LDL cholesterol in endothelial dysfunction in NP requires confirmation in an intervention study.

ACKNOWLEDGMENTS

We acknowledge the financial support of the National Health and Medical Research Council, the Australian Kidney Foundation, and the Medical Research Foundation of Royal Perth Hospital. Dr. G. Dogra was in receipt of awards from the Australian and New Zealand Society of Nephrology, the Australian Atherosclerosis Society, and Pfizer-Parke Davis for research related to this work. We are grateful to all of the physicians who referred patients for the study. A related study was also presented at the XIIth International Symposium on Atherosclerosis, Stockholm, Sweden, and published in abstract form (*Atherosclerosis* 151:208, 2000).

Reprint requests to Gerald F. Watts, M.D., Ph.D., University Department of Medicine, Royal Perth Hospital, Box X2213 GPO Perth, Western Australia 6847.
E-mail: gfwatts@cyllene.uwa.edu.au

REFERENCES

1. Kannel WB, Stampfer MJ, Castelli WP, Verter J: The prognostic significance of proteinuria: The Framingham study. *Am Heart J* 108:1347–1352, 1984
2. Cameron JS: The nephrotic syndrome: Management, complications, and pathophysiology, in *Oxford Textbook of Clinical Nephrology* (2nd ed), edited by Davison AM, Cameron JS, Grünfeld J, *et al*, Oxford, Oxford University Press, 1998, pp 461–492
3. Ordonez JD, Hiatt RA, Killebrew EJ, Fireman BH: The increased risk of coronary heart disease associated with nephrotic syndrome. *Kidney Int* 44:638–642, 1993
4. Keane WF, St. Peter JV, Kasiske BL: Is the aggressive management of hyperlipidemia in nephrotic syndrome mandatory? *Kidney Int* 42(Suppl 38):S134–S141, 1992
5. Kaysen GA, Sain-van der Velden MG: New insights into lipid metabolism in the nephrotic syndrome. *Kidney Int* 56(Suppl 71): S18–S21, 1999
6. Gordon DJ: Epidemiology of lipoproteins, in *Lipoproteins in Health and Disease*, edited by Betteridge DJ, Illingsworth DR, Shepherd J, London, Arnold, 1999, pp 587–598
7. Phillips A, Shaper AG, Whincup PH: Association between serum albumin and mortality from cardiovascular disease, cancer, and other causes. *Lancet* 2:1434–1436, 1989
8. Kannel WB, Wolf PA, Castelli WP, D'Agostino RB: Fibrinogen and risk of cardiovascular disease: The Framingham Study. *JAMA* 258:1183–1186, 1987
9. Moncada S, Palmer RM, Higgs EA: Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 43:109–142, 1991
10. Rubanyi GM: The role of endothelium in cardiovascular homeostasis and diseases. *J Cardiovasc Pharmacol* 22(Suppl 4):S1–S14, 1993
11. Sorensen KE, Celermajer DS, Spiegelhalter DJ, *et al:* Noninvasive measurement of human endothelium dependent arterial responses: Accuracy and reproducibility. *Br Heart J* 74:247–253, 1995
12. Playford DA, Watts GF: Special article: Non-invasive measurement of endothelial function. *Clin Exp Pharmacol Physiol* 25:640–643, 1998
13. Anderson TJ, Uehata A, Gerhard MD, *et al:* Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol* 26:1235–1241, 1995
14. Suwaidi JA, Hamasaki S, Higano ST, *et al:* Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 101:948–954, 2000
15. Neunteufl T, Heher S, Katzenschlager R, *et al:* Late prognostic value of flow-mediated dilation in the brachial artery of patients with chest pain. *Am J Cardiol* 86:207–210, 2000

16. Celermajer DS, Sorensen KE, Bull C, *et al:* Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol* 24:1468–1474, 1994
17. Vogel RA, Corretti MC, Gellman J: Cholesterol, cholesterol lowering, and endothelial function. *Prog Cardiovasc Dis* 41:117–136, 1998
18. Sax FL, Cannon RO III, Hanson C, Epstein SE: Impaired forearm vasodilator reserve in patients with microvascular angina: Evidence of a generalized disorder of vascular function? *N Engl J Med* 317: 1366–1370, 1987
19. Stroes ES, Joles JA, Chang PC, *et al:* Impaired endothelial function in patients with nephrotic range proteinuria. *Kidney Int* 48: 544–550, 1995
20. O'Neal D, Harrip P, Dragicevic G, *et al:* A comparison of LDL size determination using gradient gel electrophoresis and light-scattering methods. *J Lipid Res* 39:2086–2090, 1998
21. Watts GF, Herrmann S, Riches FM: Effects of diet and serotonergic agonist on hepatic apolipoprotein B-100 secretion and endothelial function in obese men. *Q J Med* 93:153–161, 2000
22. Bradley JR, Evans DB, Cowley AJ: Abnormalities of the peripheral circulation in patients with chronic renal failure. *Nephrol Dial Transplant* 3:412–416, 1988
23. Kari JA, Donald AE, Vallance DT, *et al:* Physiology and biochemistry of endothelial function in children with chronic renal failure. *Kidney Int* 52:468–472, 1997
24. Pannier B, Guerin AP, Marchais SJ, *et al:* Postischemic vasodilation, endothelial activation, and cardiovascular remodeling in end-stage renal disease. *Kidney Int* 57:1091–1099, 2000
25. Joannides R, Haefeli WE, Linder L, *et al:* Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation* 91:1314–1319, 1995
26. Tagawa T, Imaizumi T, Endo T, *et al:* Role of nitric oxide in reactive hyperemia in human forearm vessels. *Circulation* 90:2285–2290, 1994
27. Meredith IT, Currie KE, Anderson TJ, *et al:* Postischemic vasodilation in human forearm is dependent on endothelium-derived nitric oxide. *Am J Physiol* 270:H1435–H1440, 1996
28. Flavahan NA: Atherosclerosis or lipoprotein-induced endothelial dysfunction. Potential mechanisms underlying reduction in EDRF/nitric oxide activity. *Circulation* 85:1927–1938, 1992
29. Liao JK, Shin WS, Lee WY, Clark SL: Oxidized low-density lipoprotein decreases the expression of endothelial nitric oxide synthase. *J Biol Chem* 270:319–324, 1995
30. Pritchard KA Jr, Groszek L, Smalley DM, *et al:* Native low-density lipoprotein increases endothelial cell nitric oxide synthase generation of superoxide anion. *Circ Res* 77:510–518, 1995
31. Boger RH, Bode-Boger SM, Szuba A, *et al:* Asymmetric dimethylarginine (ADMA): A novel risk factor for endothelial dysfunction: Its role in hypercholesterolemia. *Circulation* 98:1842–1847, 1998
32. Casino PR, Kilcoyne CM, Quyyumi AA, *et al:* Investigation of decreased availability of nitric oxide precursor as the mechanism responsible for impaired endothelium-dependent vasodilation in hypercholesterolemic patients. *J Am Coll Cardiol* 23:844–850, 1994
33. Kugiyama K, Doi H, Motoyama T, *et al:* Association of remnant lipoprotein levels with impairment of endothelium-dependent vasomotor function in human coronary arteries. *Circulation* 97:2519–2526, 1998
34. Sorensen KE, Celermajer DS, Georgakopoulos D, *et al:* Impairment of endothelium-dependent dilation is an early event in children with familial hypercholesterolemia and is related to the lipoprotein(a) level. *J Clin Invest* 93:50–55, 1994
35. O'Brien SF, Watts GF, Playford DA, *et al:* Low-density lipoprotein size, high-density lipoprotein concentration, and endothelial dysfunction in non-insulin-dependent diabetes. *Diabet Med* 14: 974–978, 1997
36. Zeiher AM, Schachlinger V, Hohnloser SH, *et al:* Coronary atherosclerotic wall thickening and vascular reactivity in humans: Elevated high-density lipoprotein levels ameliorate abnormal vasoconstriction in early atherosclerosis. *Circulation* 89:2525–2532, 1994
37. Stamler JS, Jaraki O, Osborne J, *et al:* Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin. *Proc Natl Acad Sci USA* 89:7674–7677, 1992
38. Minamiyama Y, Takemura S, Inoue M: Albumin is an important vascular tonus regulator as a reservoir of nitric oxide. *Biochem Biophys Res Commun* 225:112–115, 1996
39. Vehkavaara S, Seppala-Lindroos A, Westerbacka J, *et al:* In vivo endothelial dysfunction characterizes patients with impaired fasting glucose. *Diabetes Care* 22:2055–2060, 1999
40. Steinberg HO, Chaker H, Leaming R, *et al:* Obesity/insulin resistance is associated with endothelial dysfunction: Implications for the syndrome of insulin resistance. *J Clin Invest* 97:2601–2610, 1996
41. Woo KS, Chook P, Lolin YI, *et al:* Hyperhomocyst(e)inemia is a risk factor for arterial endothelial dysfunction in humans. *Circulation* 96:2542–2544, 1997
42. Vallance P, Leone A, Calver A, *et al:* Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 339:572–575, 1992
43. Bostom AG, Shemin D, Lapane KL, *et al:* Hyperhomocysteinemia and traditional cardiovascular disease risk factors in end-stage renal disease patients on dialysis: A case-control study. *Atherosclerosis* 114:93–103, 1995
44. Hecker M, Mulsch A, Bassenge E, Busse R: Vasoconstriction and increased flow: Two principal mechanisms of shear stress-dependent endothelial autacoid release. *Am J Physiol* 265:H828–H833, 1993
45. Geers AB, Koomans HA, Boer P, Dorhout Mees EJ: Plasma and blood volumes in patients with the nephrotic syndrome. *Nephron* 38:170–173, 1984
46. Lewis TV, Dart AM, Chin-Dusting JP: Endothelium-dependent relaxation by acetylcholine is impaired in hypertriglyceridemic humans with normal levels of plasma LDL cholesterol. *J Am Coll Cardiol* 33:805–812, 1999
47. Cockcroft JR, Chowienczyk PJ, Benjamin N, Ritter JM: Preserved endothelium-dependent vasodilatation in patients with essential hypertension. *N Engl J Med* 330:1036–1040, 1994