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Statin therapy improves brachial artery endothelial function in nephrotic syndrome

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Background: Patients with nephrotic syndrome have impaired endothelial function probably related to dyslipidemia. This study evaluated the effects of statin therapy on dyslipidemia and endothelial function in patients with nephrotic syndrome.

Methods: A sequential, open-label study of the effects of statins on endothelial dysfunction in 10 nephrotic patients treated with an angiotensin-converting enzyme (ACE) inhibitor or angiotensin II (Ang II) receptor antagonist. Endothelial function was assessed at baseline, after 12 weeks of treatment with statins, and after an 8-week washout. Brachial artery endothelial function was measured as post-ischemic flow-mediated dilation (FMD) using ultrasonography. Endothelium-independent, glyceryl trinitrate-mediated vasodilation (GTNMD) also was measured.

Results: Serum lipids were significantly lower following statin: total cholesterol mean 8.2 ± 0.4 (standard error) mmol/L versus 5.2 ± 0.3 mmol/L, triglycerides 2.6 ± 0.4 mmol/L versus 1.6 ± 0.2 mmol/L, non-HDL-cholesterol 6.7 ± 0.4 mmol/L versus 3.7 ± 0.2 mmol/L (all $P < 0.001$). There was a trend to an increase in serum albumin (31.0 ± 1.3 g/L vs. 33.8 ± 1.5 g/L; $P = 0.078$) and FMD improved significantly following treatment (3.7 ± 1.1% vs. 7.0 ± 0.8%, $P < 0.01$). After washout, FMD deteriorated significantly to 3.5 ± 1.4% ($P < 0.05$) versus week 12 FMD. GTNMD was unchanged. In multivariate regression, reduction in non-high-density lipoprotein (HDL)-cholesterol (β - 0.736, $P = 0.027$) and increase in serum albumin (β 0.723, $P = 0.028$), but not the on-treatment level of non-HDL-cholesterol, were significant independent predictors of improvement in FMD after adjusting for change in resting brachial artery diameter. Changes in serum lipoprotein and albumin concentrations off treatment were not associated with deterioration in FMD.

Conclusion: Statin therapy significantly improves dyslipidemia and brachial artery endothelial function in patients with nephrotic syndrome. Improvement in brachial artery endothelial function may be in part related to a non-lipid effect of statins. The findings also suggest a role for dyslipidemia in endothelial dysfunction and the risk for cardiovascular disease in nephrotic syndrome.

Key words: nephrosis, HMGCoA reductase inhibitors, dyslipidemia, cardiovascular disease, proteinuria, end-stage renal disease.

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Patients with nephrotic syndrome have a 5.5-fold increased relative risk of myocardial infarction and a 2.8-fold increased relative risk of coronary death [1]. Possible etiological factors include hyperlipidemia, hypoalbuminemia and a hypercoagulable state [2], but their relative contribution to cardiovascular risk remains uncertain. The role of hyperlipidemia, in particular increased low-density lipoprotein (LDL) cholesterol, as a cardiovascular risk factor in the general population is well established [3]. Both primary and secondary prevention studies of cholesterol lowering with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) demonstrate significant risk reduction of coronary risk [4]. More recently, the importance of triglyceride-rich lipoproteins or non-high density lipoproteins in atherogenesis also has been recognized [5, 6]. Although these studies may support a role for lipid-modifying therapy in patients with nephrosis, there is no evidence that such therapy lowers cardiovascular event rates in this population.

Endothelial dysfunction is an early phase of atherogenesis, associated primarily with abnormal nitric oxide physiology [7]. It can be detected non-invasively using high-resolution ultrasonography to measure post-ischemic flow-mediated dilation of conduit arteries [8]. Brachial artery endothelial dysfunction correlates with coronary endothelial dysfunction [9], cardiovascular risk factors such as hypercholesterolemia and diabetes mellitus [10, 11] and predicts coronary events [12]. In nephrotic syndrome, forearm microcirculatory endothelial function is impaired [13] possibly due to increased content of lysophosphatidylcholine (lysoPC) in LDL-cholesterol, an oxidation by-product of cholesterol known to impair endothelial function of cultured endothelial cells [14]. Recently we have shown that brachial artery endo-

thelial function is impaired also in nephrotic syndrome to a similar extent as in patients with primary hypercholesterolemia matched for lipid and lipoprotein levels, supporting an etiological role for nephrotic dyslipoproteinemia [15]. However, no study has determined the effect of lipid-modifying therapy on endothelial dysfunction in nephrosis.

Nephrotic hyperlipidemia is characterized by increased LDL as well as triglyceride-rich lipoproteins [16, 17]. The possibility of increased oxidative stress as a consequence of hypoalbuminemia [18] and increased pro-inflammatory cytokines [19, 20] may be additive to the effects of hyperlipidemia on cardiovascular risk in nephrosis. Lipid-modifying therapy with statins has been shown to improve endothelial function in non-proteinuric populations [21–23]. Mechanisms involved include LDL-cholesterol lowering as both oxidized LDL and naive LDL reduce nitric oxide bioavailability [24, 25]. Furthermore, statins exhibit pleiotropism and may improve endothelial function by their anti-inflammatory, anti-thrombogenic and anti-oxidant effects [26, 27]. Statins also have significant triglyceride-rich lipoprotein lowering effects of particular relevance to the mixed hyperlipidemia of nephrotic syndrome [28]. Potentially statins could have a profound influence on cardiovascular risk in this patient population due to a combination of these effects. Therefore, the aim of this study was to examine the effects of lipid-lowering therapy with a statin on brachial artery endothelial function in patients with nephrotic syndrome. A secondary aim was to examine changes in plasma lipoproteins and apolipoproteins and markers of inflammation to help identify mechanisms that may contribute to endothelial dysfunction.

METHODS

Subjects and study design

Fifteen patients aged 18 to 75 years who had nephrotic syndrome with a primary glomerular disorder were recruited from Perth renal clinics over a period of two years. Patients with a serum creatinine >300 μmol/L or significant deterioration in renal function in the past six months, diabetes mellitus, malignancy, hypothyroidism, secondary cause for proteinuria, excess ethanol consumption, macrovascular atherosclerotic disease, or patients on aspirin, immunosuppressive therapy, including steroids, allopurinol, fish oils, or multivitamin and antioxidant vitamin preparations were excluded from the study. One patient (Patient No. 3) was a smoker of 20 cigarettes/day. Patients were studied in an outpatient clinic setting and were not excluded if they were taking angiotensin-converting enzyme inhibitors (ACEi) or a stable dose of any other anti-hypertensive agent. The Ethics Committee at Royal Perth Hospital approved the study, and all volunteers gave written consent.

An open-label sequential study design was used to examine the effects of lipid-lowering therapy with atorvastatin on endothelial function. All patients had a six-week run-in period during which they were off all lipid-lowering drugs and were advised to adhere to a low-fat, low-cholesterol diet. Patients had endothelial function assessed at baseline, after 12 weeks on lipid-lowering therapy and after an 8-week washout period. Patients were commenced on 10 mg of atorvastatin at baseline, interim lipid levels were assessed after six weeks, and therapy was adjusted aiming for LDL-cholesterol ≤3.4 mmol/L and triglyceride ≤1.8 mmol/L. Safety of therapy was assessed by clinical monitoring and laboratory monitoring (liver and muscle enzyme determinations: alanine aminotransferase and creatine kinase) before and during treatment.

Clinical and laboratory methods

All patients had a medical examination and 12-lead electrocardiogram at study entry. Resting blood pressure was measured using a Dinamap (Critikon Ltd., Tampa, FL, USA). Patients provided 24-hour urine collections for measurement of protein excretion and creatinine clearance. Venous blood samples were obtained after a 12-hour fast and with minimal venous stasis. The biochemical variables were measured by standard laboratory techniques unless otherwise stated. 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 triglyceride >4.5 mmol/L. Lipoprotein(a) [Lp(a)] and apolipoprotein B (apoB) were assayed by immunonephelometric methods and apolipoprotein C-III (apo C-III) using an immunoturbidimetric assay. Non-HDL-cholesterol was derived from the equation (total cholesterol − HDL cholesterol). C-reactive protein (CRP) was assayed using a high sensitive immunonephelometric method (Dade Behring Marburg GmbH, Marburg Germany). Tumour necrosis factor-α (TNFα) was measured using an immunometric assay (Immulite® TNFα; Diagnostic Products Corporation, Los Angeles, CA, USA). Interleukin-6 (IL-6) was measured using a high sensitivity quantitative enzyme immunoassay (Quantikine® HS; R&D Systems Inc., Minneapolis, MN, USA). The inter-assay coefficients of variation (CV) were all <6%.

Brachial artery ultrasonography

Brachial artery ultrasonography (Acuson Aspen 128 ultrasound device; Acuson Corporation, Mountain View, CA, USA) was carried out in patients after a 12-hour fast and after resting supine for at least 15 minutes in a quiet, temperature controlled room (21 to 25°C). Endothelium-dependent post-ischemic flow-mediated dilation

Table 1. Baseline characteristics of nephrotic patients

Patient no.	Age *years*	Sex	Serum creatinine *μmol/L*	Serum albumin *g/L*	Urinary protein *g/24 h*	Creatinine clearance *mL/min*	Glomerular histology	Disease duration *months*
1	38	M	137	40	1.39	73.5	Focal and segmental glomerulonephritis	48
2	67	F	89	26	6.43	103.8	Membranous glomerulonephritis	5
3	33	F	67	32	1.0	43.5	Mesangiocapillary glomerulonephritis type 1	156
4	38	F	185	31	8.02	35.7	Focal and segmental glomerulonephritis	36
5	69	M	154	26	14.95	66.7	No biopsy	2
6	56	F	70	26	6.92	108.1	Mesangioproliferative glomerulonephritis	6
7	72	M	271	33	3.7	27.2	No biopsy	24
8	43	M	90	33	1.39	140.4	Membranous glomerulonephritis	12
9	63	M	135	28	3.27	21.0	Focal and segmental glomerulonephritis	24
10	33	F	44	35	3.4	198.9	Focal and segmental glomerulonephritis	24
Mean	51.2		124.2	31.0	5.0	81.9		33.7
SD	15.8		67.9	4.6	4.3	56.7		45.4

(FMD) and endothelium-independent glyceryl trinitrate (GTN) mediated dilation (GTNMD) were measured using methods described elsewhere [11, 15, 29] and analyzed by two experienced observers using edge-detection software validated within our department [29]. Doppler flow velocity and flow rate (mL/min) were determined during baseline scanning and during the first 15 seconds of reactive hyperemia. Maximal FMD and GTNMD responses were calculated as % change in brachial artery diameter from baseline. The analytical (intra-observer) CV of the computerized technique in our hands is in the order of 6.7%. The CV for repeated within-subject measurement was 14.7% ($N = 24$) with a mean ± standard deviation (SD) difference in FMD of 1.6 ± 1.0%.

Statistical analysis

Analyses were performed using SPSS v.10.0 (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL, USA). Results are expressed as mean ± SD for descriptive data and mean ± SE for comparative data. Skewed variables were log transformed prior to analysis and are presented as geometric mean (95% confidence interval; 95% CI). Repeated measures analysis of variance (r-ANOVA) was used to compare data at baseline, after statin and at the end of washout. If the r-ANOVA was significant ($P < 0.05$), paired t tests were performed with Bonferroni adjustment for a three-way comparison. Linear regression analysis was used to identify significant correlations between changes in FMD and other biochemical variables.

RESULTS

Of the 15 patients entered in the study, two patients were withdrawn as they went into spontaneous complete remission within six weeks of commencing lipid-lowering therapy, one patient commenced high-dose prednisolone for minimal change glomerulonephritis, one was withdrawn as a combination of gemfibrozil, fish oil and atorvastatin for the treatment of severe mixed hyperlipidemia was required, and one withdrew for psychosocial reasons. Ten patients completed the study, of whom nine were on atorvastatin (median 20 mg/day, range 20 to 40) and one was converted to simvastatin 40 mg/day following development of an urticarial rash related to commencing atorvastatin. One patient (Patient No. 1), although nephrotic at screening with a diagnosis of focal and segmental glomerulonephritis on renal biopsy, was in partial disease remission for the duration of the study. This patient was included in our previous study [15] and this study, since moderate proteinuria, dyslipidemia and endothelial dysfunction persisted, and complete remission did not occur. FMD was studied at baseline, after 12 weeks of statin and after an 8-week washout. GTNMD was studied in all patients at baseline and in only five out of the ten patients at subsequent visits due to patient refusal or medical unsuitability related to hypotension. We have previously shown GTNMD is unimpaired in patients with nephrotic syndrome compared with healthy controls [15].

Table 1 shows the baseline characteristics, including glomerular histology and renal function of the ten patients that completed the study. Patients had a mean age of 51.2 ± 15.8 years and half were men. Two patients did not have a biopsy-proven diagnosis for the study duration. Median disease duration was 24 months (range 2 to 156). Patients had nephrotic range proteinuria 5.0 ± 4.3 g/day with hypoalbuminemia 31.0 ± 4.6 g/L and serum creatinine 124.2 ± 67.9 μmol/L. Eight patients were on ACE-I (perindopril 2, trandolapril 2, quinapril 1, enalapril 1, captopril 1, lisinopril 1) and the remainder were on an Ang II receptor antagonist (irbesartan), the doses of which were unaltered through the study.

Table 2 shows the changes in blood pressure, renal function, serum glucose and indices of nephrosis with statin therapy and following washout. Body weight, systolic and diastolic blood pressure, serum creatinine, creatinine clearance and serum glucose were not signifi-

Table 2. Clinical and biochemical characteristics of nephrotic patients pre-statin, following statin therapy and post-statin

Parameters	Pre-statin	Statin	Post-statin	ANOVA *P* value
Weight *kg*	69.9 ± 4.4	70.6 ± 4.5	70.6 ± 4.2	0.685
SBP *mm Hg*	129.5 ± 6.5	133.3 ± 7.4	126.7 ± 4.0	0.479
DBP *mm Hg*	74.5 ± 2.8	77.5 ± 4.5	74.6 ± 2.4	0.623
Serum creatinine *μmol/L*	124.2 ± 21.5	131.6 ± 25.3	133.7 ± 26.1	0.244
Creatinine clearance *mL/min*	81.9 ± 17.9	89.3 ± 15.6	88.4 ± 14.9	0.587
Serum albumin *g/L*	31.0 ± 1.3	33.8 ± 1.5	33.8 ± 1.8	0.069
Urinary protein[a] *g/24 h*	3.7 (2.0–6.8)	3.2 (1.9–5.7)	2.6 (1.3–5.0)	0.123
Serum glucose *mmol/L*	5.2 ± 0.3	5.2 ± 0.3	5.1 ± 0.2	0.785

Mean ± standard error shown except [a] geometric mean (95% confidence interval).
Abbreviations are: SBP systolic blood pressure; DBP diastolic blood pressure.

Table 3. Blood lipids, lipoproteins and markers of inflammation in nephrotic patients pre-statin, following statin therapy and post-statin

Parameters	Pre-statin	Statin	Post-statin	ANOVA *P* value
Total cholesterol *mmol/L*	8.2 ± 0.4[a]	5.2 ± 0.3[b]	7.7 ± 0.5	<0.001
LDL-cholesterol *mmol/L*	5.5 ± 0.4[a]	3.0 ± 0.3[b]	4.8 ± 0.4	<0.001
HDL-cholesterol[e] *mmol/L*	1.4 (1.1–1.7)[a]	1.4 (1.1–1.8)	1.3 (1.0–1.6)	0.027
Triglycerides[e] *mmol/L*	2.3 (1.6–3.4)[a]	1.5 (1.1–2.1)[c]	2.8 (1.8–4.2)	<0.001
Non-HDL-cholesterol *mmol/L*	6.7 ± 0.4[a]	3.7 ± 0.2[b]	6.3 ± 0.5	<0.001
ApoB *g/L*	1.6 ± 0.1[a]	1.0 ± 0.05[b]	1.5 ± 0.1	<0.001
Lp(a)[e] *g/L*	0.39 (0.17–0.87)[d]	0.24 (0.09–0.62)	0.17 (0.06–0.46)	0.002
ApoCIII[e] *g/L*	0.2 (0.16–0.26)[a]	0.18 (0.15–0.22)[d]	0.23 (0.19–0.29)	<0.001
CRP[e] *mg/L*	1.3 (0.7–2.1)	1.2 (0.7–2.1)	1.0 (0.5–2.2)	0.763
IL-6[e] *pg/mL*	3.0 (1.8–4.8)	2.9 (2.0–4.1)	2.4 (1.4–4.0)	0.297
TNF-α *pg/mL*	5.0 ± 0.8	4.7 ± 0.8	5.1 ± 0.8	0.656

Mean ± standard error shown except egeometric ([e] geometric) mean (95% confidence interval).
Abbreviations are: LDL, low-density lipoprotein; HDL, high-density lipoprotein; ApoB, apolipoprotein B; Lp(a), lipoprotein(a); ApoCIII, apolipoprotein CIII; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.
[a] *P* not significant compared to post-statin
[b] $P < 0.001$ compared to pre-statin and post-statin
[c] $P < 0.01$ compared to pre-statin and post-statin
[d] $P < 0.01$ compared to post-statin
[e] geometric

cantly different among the three study visits. There was a non-significant trend to an increase in serum albumin (r-ANOVA, $P = 0.069$) and a non-significant reduction in 24-hour protein excretion over the 20-week study period (r-ANOVA = 0.123).

Changes in serum lipids and lipoproteins are shown in Table 3. Overall, lipid-lowering therapy resulted in a significant reduction in total cholesterol, LDL-cholesterol, non-HDL-cholesterol, apoB and triglycerides (all $P < 0.001$). The increase in HDL-cholesterol did not reach statistical significance. There was a non-significant reduction in apoC-III ($P = 0.09$) with statin therapy but a significant increase in apoC-III ($P < 0.01$) following washout. Lp(a) was not significantly reduced with lipid-lowering therapy, but at washout it was significantly lower than at baseline ($P < 0.01$; Table 3). Except for Lp(a), none of the washout serum lipid and lipoprotein levels were significantly different to baseline values. Exclusion of the patient receiving simvastatin does not alter these results significantly. Table 3 also shows changes in inflammatory markers with statin therapy. There was no statistically significant change in CRP, IL-6 or TNFα with statin treatment or at the end of washout.

Statin therapy was well tolerated by the group, and there were no complaints of gastrointestinal intolerance or myalgias at the doses employed. Muscle enzyme, creatine kinase (CK), did not change significantly among visits (pre-statin 116.8 ± 23.3 U/L, statin 151.0 ± 31.4 U/L, post-statin 113.5 ± 21.2 U/L; r-ANOVA, $P = 0.110$). The liver enzyme alanine amino transferase (ALT) increased significantly on treatment (pre-statin 18.7 ± 1.8 U/L, statin 29.5 ± 3.6 U/L, post-statin 22.3 ± 4.2 U/L; r-ANOVA, $P = 0.034$). Only two patients had ALT levels above the laboratory reference range (normal <36 U/L) with the highest ALT reached being 50 U/L, and two patients had asymptomatic elevations of CK above the laboratory reference range (normal <200 U/L) with the highest CK reached being 316 U/L.

Results of brachial ultrasonography and post-ischemic FMD are shown in Figure 1 and Table 4. Baseline brachial artery diameter, basal Doppler blood flow, and hyperemic Doppler blood flow values were not signifi-

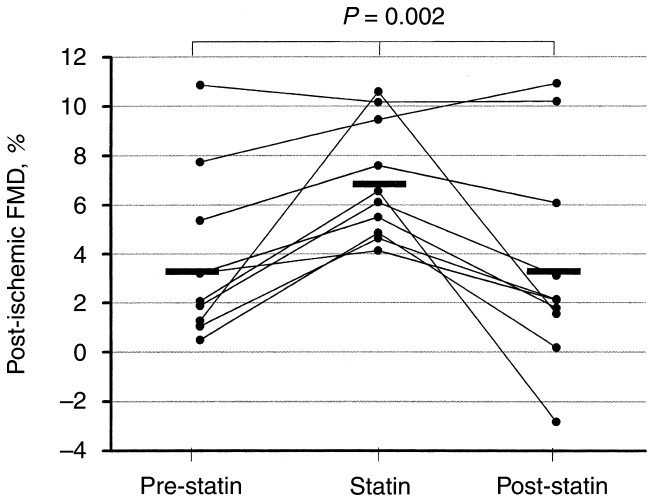

Fig. 1. Post-ischemic flow-mediated dilation (FMD) in nephrotic patients pre-statin, following statin therapy and post-statin. The *P* value refers to repeated measures ANOVA; horizontal bars represent mean FMD.

cantly different between visits. Post-ischemic FMD increased significantly on lipid-lowering therapy (pre-statin 3.7 ± 1.1%, statin 7.0 ± 0.8%, post-statin 3.5 ± 1.4%; r-ANOVA, $P = 0.002$; $P = 0.013$ between pre-statin and statin; $P = 0.039$ between statin and post-statin; $P = 1.0$ between pre- and post-statin). Exclusion of the patient in partial remission (Patient No. 1) did not alter the significant change in FMD with statin treatment (pre-statin 4.0 ± 1.1%, statin 7.2 ± 0.8%, post-statin 3.9 ± 1.4%; r-ANOVA $P = 0.007$, $N = 9$). GTNMD was not significantly different on statin therapy (pre-statin 16.8 ± 1.0%, statin 15.1 ± 1.5%; paired *t* test, $P = 0.467$).

In univariate regression, only reduction in non-HDL-cholesterol with statin therapy (regression coefficient −1.9 ± 1.0, β −0.560, $P = 0.092$) and increase in serum albumin (regression coefficient 0.5 ± 0.2, β 0.552, $P = 0.098$) showed a trend to an association with improvement in FMD. None of the other predictor variables [either change in, or absolute levels achieved on statin therapy, of LDL-cholesterol, triglycerides, apoB, Lp(a), proteinuria, or inflammatory markers] were significantly associated with improvement in FMD. In multivariate regression, both reduction in non-HDL-cholesterol with statin therapy ($P = 0.027$) and increase in serum albumin during 12 weeks of statin therapy ($P = 0.028$) independently and significantly predicted improvement in FMD after adjusting for changes in resting brachial artery diameter (model adjusted $R^2 = 0.58$; Table 5). While exclusion of the patient in partial remission (Patient No. 1) limits multivariate regression due to small sample size, the regression model continues to show a significant trend for an association between change in non-HDL cholesterol with statin treatment and improvement in FMD (regression coefficient −2.4 ± 1.0, β −0.663; $P = 0.063$) and increase in serum albumin during statin therapy (regression coefficient 0.6 ± 0.2, β 0.737; $P = 0.040$; model adjusted $R^2 = 0.57$). The fall in FMD during washout was not significantly associated with any of the predictor variables, including increase in non-HDL-cholesterol off statin therapy.

DISCUSSION

To our knowledge this is the first study to examine the effects of statin therapy on vascular function in patients with nephrotic syndrome. There was a significant improvement in flow-mediated dilation of the brachial artery, a measure of endothelial function, with statin therapy in eight of the ten nephrotic patients studied. The remaining two patients had preserved basal FMD responses off treatment and showed minimal change in FMD with statin therapy. Furthermore, this improvement in brachial FMD was significantly correlated with reduction in non-HDL-cholesterol achieved with statin therapy. These findings support the hypothesis that dyslipidemia contributes to increased risk for cardiovascular disease [1] and endothelial dysfunction [15] in nephrotic patients.

Plasma lipid abnormalities in the nephrotic syndrome are a consequence of a combination of increased synthesis and reduced clearance [30, 31]. They consist of increased concentrations of total cholesterol and apoB containing lipoproteins, namely LDL cholesterol, as well as raised triglycerides particularly in those patients with heavier proteinuria and severe hypoalbuminemia [16, 32]. More recently, elevated triglyceride-rich lipoproteins (TRL) including very low-density (VLDL) and intermediate-density lipoproteins (IDL), and apolipoprotein C-III (apoC-III), a remnant lipoprotein marker, have been reported as elevated in nephrotic patients [17, 33]. Although LDL cholesterol is recognized as the predominant lipid-related cardiovascular risk factor [3], recent evidence supports a role for triglyceride-rich lipoproteins as well [5, 6, 34]. In our study, statin therapy was associated with a significant reduction in apoB containing lipoproteins as well as non-HDL-cholesterol that includes LDL-cholesterol, as well as TRL's and apoC-III containing lipoproteins. Furthermore, reduction in non-HDL-cholesterol was a significant independent predictor of improvement in endothelial function as measured by FMD, implying a collective role for VLDL, IDL and LDL-cholesterol in inducing endothelial dysfunction and the increased risk of cardiovascular disease in nephrotic syndrome.

A further finding of our study was that absolute levels of non-HDL-cholesterol obtained on treatment did not significantly predict improvement in FMD and the increase in non-HDL-cholesterol in the post-statin period

Table 4. Blood flow, resting brachial artery diameter and post-ischemic flow-mediated dilation (FMD) in nephrotic patients pre-statin, on statin therapy and following post-statin

Parameters	Pre-statin	Statin	Post-statin	ANOVA *P* value
Resting brachial artery diameter *mm*	3.7 ± 0.2	3.7 ± 0.3	3.8 ± 0.3	0.247
Basal Doppler blood flow *mL/min*	131.4 ± 25.6	118.1 ± 16.9	172.4 ± 12.8	0.108
Hyperemic Doppler blood flow *mL/min*	743.3 ± 151.1	722.7 ± 136.4	721.2 ± 80.9	0.975
FMD %	3.7 ± 1.1	7.0 ± 0.8	3.5 ± 1.4	0.002

Data are mean ± standard error.

Table 5. Multivariate regression model showing association between improvement in brachial FMD and reduction in non-HDL-cholesterol and increase in serum albumin during statin therapy, adjusted for change in resting brachial artery diameter

Predictor variable	Regression coefficient (standard error)	Standardized coefficient β	*P* value
Reduction in non-HDL-cholesterol *mmol/L*	−2.6 (0.9)	−0.736	0.027
Increase in serum albumin *g/L*	0.6 (0.2)	0.723	0.028
Change in resting brachial artery diameter	−5.2 (2.6)	−0.563	0.098

Adjusted $R^2 = 0.58$. Abbreviations are: HDL, high-density lipoprotein; FMD, post-ischemic flow-mediated dilation.

did not predict fall in FMD, suggesting an additional non-lipid mediated effect of statin therapy on endothelial function. Statins are known to have a wide range of pleiotropic effects beyond lipid-lowering that may contribute to improved vascular function and cardiovascular outcomes [26, 35]. This pleiotropism includes up-regulation of nitric oxide synthase and anti-oxidant, anti-inflammatory or anti-thrombotic effects [26, 27, 35–37]. The development of endothelial dysfunction on withdrawal of statins in our study supports mechanisms that involve functional changes, such as improved nitric oxide bioavailability, in preference to structural changes in the arterial wall. This mechanism also is suggested by studies showing early rapid improvement in endothelial function with statins [22, 38]. Although statin therapy was not associated with significant changes in inflammatory markers previously shown to be elevated in NS (such as TNFα [19]), our sample size was small and does not allow us to rule out a role for pleiotropic effects. Hence, the precise mechanisms by which statins improve vascular function in nephrotic syndrome remain to be determined.

We have previously shown that nephrotic patients exhibit endothelial dysfunction compared with normoalbuminemic, normolipidemic controls, but that serum albumin is not a significant predictor of endothelial dysfunction [15]. An unexpected finding of the present study was the significant independent correlation between improvement in FMD and increase in serum albumin during statin therapy. This increase in serum albumin was associated with a decrease in proteinuria and is consistent with a trend to disease remission with time. Nephrotic hypoalbuminemia is associated with reduced plasma anti-oxidant potential [18] and an increased content of lysophosphatidylcholine (lysoPC) in LDL-cholesterol, which is known to inhibit acetylcholine-mediated relaxation of cultured endothelial cells [14]. It is possible that an increase in serum albumin as a consequence of partial disease remission contributes to improvement in FMD by these anti-oxidant mechanisms or by mechanisms related to its vasorelaxant and nitric oxide binding properties [39]. Partial remission of nephrosis may marginally improve dyslipidemia thereby contributing to improved FMD. However, decrease in serum concentrations of Lp(a), also shown to be associated with disease remission [31], were not a significant predictor of improvement in FMD. Furthermore, in the post-statin period FMD deteriorated significantly in the absence of a significant deterioration of serum albumin concentration. This suggests a minimal contributory role for serum albumin in nephrotic endothelial dysfunction.

We have previously shown that GTNMD is unimpaired in nephrotic syndrome [15] and in this study GTNMD did not change with statin therapy in five patients. This suggests that the improvement in FMD with statin therapy in nephrotic patients specifically represents enhanced endothelial function, which in conduit arteries is felt to be predominantly nitric oxide mediated [40, 41]. Endothelial dysfunction itself is an early risk marker for cardiovascular disease [7, 8] and improved nitric oxide bioavailability has anti-atherogenic effects [7]. Furthermore, impaired brachial and coronary endothelial function predicts future risk for coronary events [12, 42, 43]. These studies support the concept that improving endothelial dysfunction with statin therapy should reduce the known cardiovascular risk associated with nephrotic syndrome. However, the prospective studies showing increased coronary events in subjects with impaired FMD also showed impaired GTNMD in

the same subjects [12], indicating that impaired FMD in these groups may be either an endothelial or endothelium-independent response, and thus the findings have limited generalizability. Hence, in the absence of prospective studies looking at the impact of improving conduit vessel FMD on coronary events, the clinical implications of improved FMD and endothelial function remain uncertain and the data should be interpreted with caution.

The open-labeled sequential study designed to be conducted in the out-patient setting has obvious limitations, but was chosen due to limited availability of cases for a parallel design and risk of the confounding effects of spontaneous remission or the introduction of immunosuppressive therapies for nephrotic syndrome with a longer crossover study. There was also physician bias to promote lipid-lowering therapy in nephrotic patients and a reluctance to participate in a blinded placebo-controlled trial. The sample size was small but adequate based on the repeatability of technique. With ten patients in a paired design the study had 90% power with α 0.05 to detect a minimum 3% change in FMD with treatment allowing for the larger standard deviation of paired differences in FMD seen in this study. Although inclusion of the patient in partial remission (Patient No. 1) is questionable, his exclusion does not alter the significance of these results. Correlation and regression analyses are limited by the small sample size and we may have missed other potential predictors of improvement in FMD. Specifically, the importance of LDL-cholesterol independent of TRLs as a mediator of endothelial dysfunction in nephrotic syndrome may have been missed. The relationship between LDL cholesterol and endothelial dysfunction in nephrotic syndrome would be better tested using an intervention that selectively lowers LDL cholesterol without pleiotropic effects. Although one patient received simvastatin instead of atorvastatin, the LDL cholesterol and triglyceride goals were achieved to test the effects of lowering lipid levels on FMD.

In conclusion, the findings of this study provide supportive evidence for statin therapy in nephrotic syndrome. Improvement in endothelial function associated with lowering of non-HDL cholesterol in nephrosis is in keeping with larger primary and secondary prevention studies of cholesterol lowering [4]. This study also provides evidence for both the safety and efficacy of atorvastatin in nephrotic syndrome. Definitive evidence is needed from larger clinical trials evaluating the role of lipid regulating therapy, including combination therapy, in reducing the risk of cardiovascular events in nephrotic syndrome.

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