3

Vascular Function and Mild Renal Impairment in Stable Coronary Artery Disease

Pim van der Harst[1,2], Tom D.J. Smilde[1], Hendrik Buikema[2], Adriaan A. Voors[1], Gerjan Navis[3], Dirk J. van Veldhuisen[1], Wiek H. van Gilst[2]

Arterioscler Thromb Vasc Biol
2006;26:379-84

[1]Department of Cardiology, [2]Department of Clinical Pharmacology, and [3]Department of Internal Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

Abstract

Objective – In patients with coronary artery disease, the concomitant presence of renal function impairment is associated with decreased survival. We aimed to assess whether in coronary artery diseased patients, renal function impairment is associated with systemic vascular function, functional parameters of the renin-angiotensin system or inflammation as potential mediators for cardiovascular risk.

Methods and Results – We studied 125 patients, 87% male, with a mean age of 62.2±8.2 years, 72% had 3-vessel disease, and mean renal function was 74±13 mL/min/1.73m^2. Internal thoracic artery rings were sampled during coronary bypass surgery and used for in vitro vascular measurements. We could not establish an association between endothelium dependent vasorelaxation (response to methacholine) and renal function. In addition, vascular response to potassium chloride, phenylephrine and angiotensin II were not associated with renal function. Finally, serum ACE activity, usage of ACE inhibitors, C-reactive protein (CRP) and soluble intercellular adhesion molecule 1 (sICAM) were not related to renal function.

Conclusions – In coronary artery diseased patients, mild renal function impairment is not associated with systemic vasomotor responsiveness, inflammation or functional systemic parameters of the renin-angiotensin system. The relation between systemic endothelial dysfunction and mild renal insufficiency might be more complicated than previously thought.

Introduction

The concomitant presence of renal function impairment in patients with various forms of cardiovascular disease is associated with a worse prognosis.[1-5] Limited data are available on the pathophysiologic mechanisms linking mild renal function impairment to the marked increase in cardiovascular events. A recent study in essential hypertension demonstrated an association between mild renal function impairment and endothelial dysfunction, suggesting that renal function impairment might be a marker of generalized vascular dysfunction, as a possible mediator of cardiovascular risk.[6] However, since hypertension can be expected to elicit endothelial and renal dysfunction this assumption needs to be tested in a non-essential hypertensive population as well. Other factors affecting the prognosis of coronary artery disease involve alterations of the renin-angiotensin system and inflammation.[7-9] However, whether these factors are associated with renal function remains to be determined.
In this report, we analyzed the potential relationship between renal function and endothelium dependent vasodilatation in subjects with severe coronary artery disease. Second, we assessed whether renal function was associated with the vascular responses of several vasoconstrictors, systemic parameters of the renin-angiotensin system and systemic markers of inflammation.

Methods

Study Population

Patients in the present study participated in the QUinapril On Vascular Ace and Determinants of Ischemia Study (QUO VADIS). A total of 187 patients were included into QUO VADIS, and methods and results of this study were published elsewhere.[10] We studied all 125 subjects undergoing CABG of whom endothelium dependent vasodilatation or vascular responsiveness to Angiotensin II was assessed. The glomerular filtration rate (GFR) was estimated by the Modification of Diet in Renal Disease (MDRD) equitation[11] and, additionally, the Cockcroft-Gault (GFRc) corrected for body surface area.[12] The Institutional Review Board approved this study, and written, informed consent was obtained from each subject.

Organ Chamber Experiments

During coronary artery bypass grafting segments of internal thoracic arteries were collected as excess graft material and transported immediately to the laboratory, where in vitro vascular measurements were performed within 6 hours of harvesting as published previously.[10;13] Endothelial dependent relaxation was studied by cumulative concentrations of methacholine. Endothelium independent relaxation was studied by the response to a single high concentration of sodium nitrite ($NaNO_2$; 10 mmol/L; yields approximately

Table 1. Clinical and biochemical data of patients divided on the basis of 3 tertiles of endothelium dependent vasodilatation*

	Maximal Endothelial Dependent Vasodilatation				
	Tertile 1 (n=37)	Tertile 2 (n=38)	Tertile 3 (n=37)	P*	r (P) †
Age, y	64.2±6.9	60.9±7.9	60.3±9.6	0.09	0.21 (<0.05)
Male gender, n (%)	28 (76)	34 (89)	35 (95)	0.05	0.30 (<0.05)
Diabetes	4(10)	2(5)	1(3)	0.36	0.10 (0.29)
3-vessel disease, n (%)	26 (70)	25 (66)	30 (81)	0.32	-0.15 (0.11)
Body mass index, kg/m2	26.7±3.5	26.5±2.9	27.2±2.7	0.58	-0.06 (0.53)
Systolic blood pressure, mmHg	144.9±22.3	138.2±18.7	137.4±21.6	0.24	-0.04 (0.51)
Diastolic blood pressure, mmHg	80.5±7.8	80.8±8.4	80.7±7.0	0.99	-0.04 (0.67)
Heart rate, bpm	72.0±15.3	70.1±13.5	70.3±13.2	0.82	-0.02 (0.81)
Current smokers, n (%)	4 (11)	5 (13)	5 (14)	0.93	0.05 (0.64)
Total cholesterol, mmol/L	6.3±1.2	6.0±1.1	6.2±1.5	0.66	0.11 (0.27)
sICAM, µg/L	176±211	142±40	124±63	0.47	0.18 (0.19)
C-reactive protein, mg/L	1.60 [0.50-4.28]	2.50 [1.10-5.35]	1.55 [0.70-5.20]	0.36	-0.07 (0.64)

* On the basis of maximal response to ME, patients were ranked and grouped into 3 tertiles (first tertile: lower response; second tertile: intermediate response; third tertile: high response). † Correlation coefficient R and P value of relationship between maximal response to ME (as a continuous variable) and each variable listed in the table. C-reactive protein is expressed as median [interquartile range], all other data are expressed as mean ±SD or as percent frequency, and comparisons among groups were made by 1-way ANOVA or $\chi 2$ test, as appropriate.

10 nmol/L nitric oxide).[14] Vascular responsiveness to Angiotensin II was studied with cumulative concentrations as described previously.[10;13] Maximal vasoconstriction was evoked by stimulation with 60 mmol/L potassium chloride.

ACE Activity Determination

Plasma ACE activity was measured 1 day before CABG as described previously.[15] Briefly, using 35 times diluted plasma 10 minutes of incubation with 7 mmol/L hippuryl-L-histidyl-L-leucine (Hip-His-Leu) at 37°C the production of hippuric acid (nM His-Leu/min/mL) was measured spectrophotometricly.

Inflammatory markers

Of our 125 patients, inflammatory markers were determined in a subset of 62 patients, solely defined by the availability of a stored (at -80°C) blood sample taken before surgery.[16] This subgroup was comparable to the whole study population with regard to the variable listed in table 1. Soluble intercellular adhesion molecule 1 (sICAM) and C-reactive protein (CRP), were determined by ELISA in duplicate as described previously.[16]

Statistical Analysis

Data are expressed as mean±SD or presented as frequency, unless indicated different. Non-normally distributed variables were log-transformed for statistical comparisons. Comparisons between groups were made by 1-way ANOVA or the χ^2 test, as appropriate. Relationships between paired parameters were analyzed by Pearson product moment correlation coefficient. Dose response curves of methacholine and Ang II were performed of each of the GFR tertiles and the differences of these curves were tested using repeated measures analysis of

Table 2. Correlation coefficients for renal function*

Variable	R	P
Vascular function		
Endothelial dependent vasodilatation	-0.049	0.61
Endothelial independent vasodilatation	-0.113	0.24
Phenylephrine contraction	0.089	0.35
Potassium chloride contraction	0.071	0.43
Renin-Angiotensin system parameters		
Angiotensin II contraction	0.011	0.91
Serum ACE-activity	0.186	0.21
ACE inhibitor usage	0.014	0.88
Inflammatory parameters		
C-reactive protein	0.058	0.66
sICAM	0.009	0.94

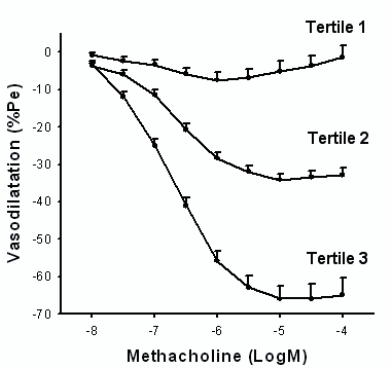
* Correlation coefficient R and P value of relationship between estimated renal function and (as a continuous variable) and each variable listed in the table.

variance test. All calculations were made with a standard statistical package (SPSS for Windows version 12.0.1).

Results

The population consisted of predominantly (87%) male patients, 72% had 3 diseased coronary arteries. The estimated mean renal function was 74±13 mL/min/1.73^2 (range 34-112). At time of CABG, 58% of the patients were on ACE inhibitor therapy, 75% on aspirin, and 40% on lipid lowering medication.
All patients showed normal, dilatory response, to endothelium independent vasodilatation (sodium nitrite). On the basis of maximal response to methacholine, patients were grouped into 3 tertiles. As shown in figure 1 there was a considerable difference among the tertiles of endothelium dependent vasodilatation. As shown in table 1, patients in the first tertile (ie, those with less endothelium dependent vasodilatation) were older, with a lower prevalence of males than for patients in the other 2 tertiles. The 3 tertiles did not differ with regard body mass index, arterial pressure and heart rate, serum cholesterol, diabetes or the proportion of smokers.
Serum creatinine was not different among the tertiles of endothelium dependent vasodilatation, neither was (MDRD) estimated GFR. Univariate linear regression analysis, using continues variables, confirmed maximal endothelium dependent vasorelaxation not to be associated with serum creatinine or estimated GFR (figure 2). Since diabetic patients, with relative glomerular hyperfiltration, are likely to mask these associations, we repeated additional analyses with the diabetic patients excluded. However, this did not change our results (data not shown). A posteriori we computation showed statistical power to detect a correlation of $r^2 \geq 0.07$ (based on 2-sided test; n=112; $P<0.05$; Power=0.80).[17]
Additional analysis with correction for maximal achieved vasodilatation with sodium nitrite (%SN) instead of phenylephrine (%PE) did not change these findings (data not shown). Estimation of the GFR with the Cockcroft-Gault formula did not change these findings. In addition, serum creatinine and

Figure 1.



3 tertiles of endothelium dependent vasodilatation, response to methacholine. Data (mean±SEM) are expressed as percentage of phenylephrine (PE) precontraction. Differences among groups are significant (*P*<0.001).

estimated GFR were identical when patients were grouped according endothelium independent vasodilatation (maximal response to sodium nitric) (*P*=NS).

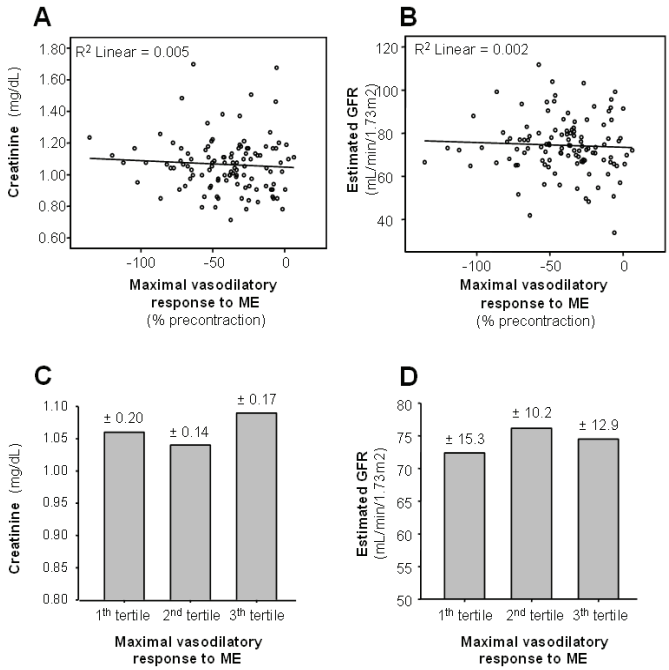
Next, we studied the vascular responsiveness to the vasoconstrictors phenylephrine, potassium chloride and to Ang II. We did not observe a relation between these vasoconstrictors and estimated GFR (table 2). To further examine the possible involvement of the systemic RAS, we considered the relation between serum ACE activity and usage of ACE inhibitors with renal function. ACE activity and ACE inhibitor usage were also not associated with differences in renal function. Furthermore, performing stratified analysis for ACE inhibitor therapy did not reveal an association between vascular function and renal function parameters (data not shown). Finally, we evaluated the possible role of inflammatory markers with renal function. Both CRP and sICAM were not related with estimated GFR (figure I please see www.ahajournals.org).

To evaluate to possible the relation of mild-moderate renal function impairment with vascular function, we grouped patients according to the median and, in addition, two clinical relevant renal function categories (estimated GFR <90 versus GFR >90, and GFR <60 versus GFR >60 mL/min/1.73m^2).[18] Dose-response curves for endothelial dependent relaxation and vascular responsiveness to Ang II for these renal function categories were constructed (for median, see figure 3). Repeated measurement analysis did not reveal any differences among these categorizations.

Discussion

In these patients with severe coronary artery disease, we observed a wide range of endothelium dependent vasodilatation of the internal thoracic artery. Compromised endothelium results in the loss of a powerful defense mechanism

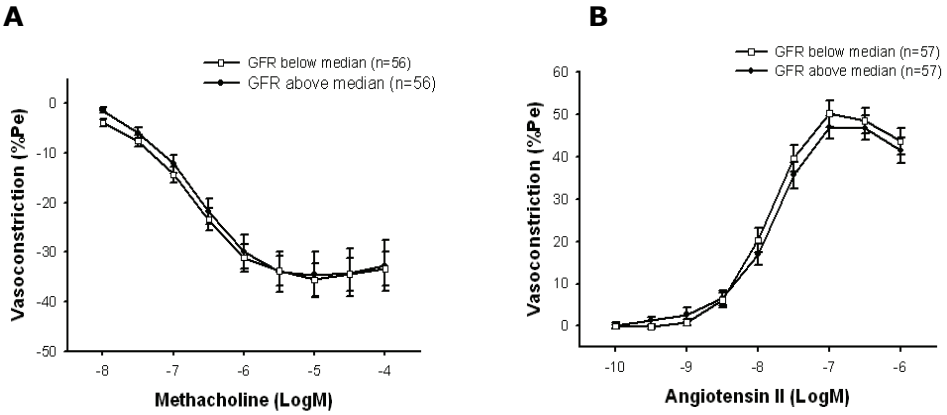
Figure 2.



A.) Relationship between maximal vasodilatory response to methacholine (ME) with serum creatinin. B.) Relationship between maximal vasodilatory response to methacholine (ME) with estimated GFR. C.) Relationship between the tertiles of maximal vasodilatory response to methacholine (ME) and serum creatinine. D.) Relationship between the tertiles of maximal vasodilatory response to methacholine (ME) and estimated GFR.

against vascular and renal damage.[19-21] Although the differences in endothelial function were considerable, it did not enable us to observe any associations with serum creatinine or estimated GFR. Furthermore, we did not found significant associations between the response to the potent vasoconstrictors phenylephrine, potassium chloride or Ang II with serum creatinine or estimated GFR. In addition to vascular response to Ang II, the other parameters of the RAS serum ACE activity and use of ACE inhibitors were also not associated with renal function. Additionally, inflammatory markers were not related to renal function in our patients. Overall, this study did not demonstrate a relation between systemic vascular measurements and renal function in CABG patients. The present study has a number of strengths. We performed detailed vascular measurements consisting of a 9-point dose-response curve for endothelium dependent vasodilatation and angiotensin II responsiveness in more than 100 patients. The range of endothelium dependent vasodilatation was considerable, enhancing the detection of potential differences in renal function parameters. In addition to vasodilatation, we assessed several potent vasoconstrictors to assess smooth muscle cell properties. The vascular measurements were all performed within one structured protocol by experienced biotechnicians. The a posteriori computation of statistical power showed that this study could detect a

Figure 3.



correlation with an r^2 of 0.07, i.e. a relationship where approximately 7% of the variability in renal function was explained by endothelial function. Thus, our negative findings are unlikely to be due to a lack of power. Most prior studies focused on the relation between renal and endothelial function in patients with chronic renal failure or severe renal function impairment.[22-24] Only recently, Perticone *et al* studied untreated essential hypertensive patients with normal to mild renal function.[6] In that study, endothelial dependent vasodilatation was correlated with serum creatinine levels and estimated renal function (r^2=0.10 and r^2=0.04, respectively, both P<0.001). In addition, they observed a relation between serum CRP with estimated GFR. We studied patients with similar renal function, carried out similar statistics and grouping of patients, and estimated GFR with the same formula. In addition, we performed more and detailed vascular measurements. In vitro assessment of vessels allowed us to determine, in addition to endothelium dependent and independent relaxation, the vascular responsiveness to potent vasoconstrictors. In contrast to the study of Perticone, the present study demonstrated no association of endothelial dependent vasodilatation on serum creatinine levels or estimated renal function. Furthermore, we did not observe a relation between CRP and estimated GFR. Several factors might explain these apparently discrepant findings. One of the major differences is the difference in the study population. Although both studies focused on patients in which endothelial function plays a crucial pathophysiological role, the origin of endothelial function impairment was dissimilar.[25;26] Whereas Perticone studied uncomplicated, untreated essential hypertensive patients, we studied patients with severely diseased coronary arteries with a lower incidence of hypertension and lower blood pressure levels. Of note, the kidney is a well known target of hypertension. The previously reported association between endothelial function and renal function might therefore not result from one-other, but instead, from the common denominator

hypertension. Although some adjustments were made for blood pressure levels in the study of Perticone, other hypertension characteristics known to influence renal function were not taken into account, i.e. the duration of hypertension.[27] In addition, the individual susceptibility to develop renal insufficiency might be traced back to disturbances in the way the kidney autoregulates.[28] Increased susceptibility for impaired renal autoregulation might merely be the renal manifestation of a more widespread abnormality in endothelial function. In that case, the kidney is less well protected from sustained elevations in arterial pressure, potentially making some subjects more vulnerable to subtle acquired injury, particularly in the setting of hypertension. Together, the study by Perticone, and the present study are suggesting that haemodynamic, i.e. blood pressure, may lead to renal injury instead of systemic endothelial dysfunction per se. The influence of atherosclerosis on renal function is not fully elucidated and remains to be established. Systemic endothelial dysfunction associated with atherosclerosis might not be a critical determinant contributing to mild renal dysfunction, in contrast to essential hypertension.

The current study provides two new pathophysiological insights into the relation between vasomotor function and renal function impairment in coronary artery diseased patients. First, in atherosclerotic patients endothelium dependent dilatory function of the internal thoracic artery is not associated with renal function. Second, vasoconstrictor responses to phenylephrine and potassium chloride and, in addition, several markers of the RAS, namely vascular responsiveness to Ang II, serum ACE activity and use of ACE inhibitors, were also not associated with renal function in these patients. Thus, our findings suggest that in CABG patients renal function is, in contrast to hypertensive patients, not related to the functional integrity of the systemic endothelium.

The present study has its limitations. This study provides cross-sectional observational data and therefore can only be used to generate new hypotheses. We were unable to determine local renal vascular responses to a NO agonist or antagonist and Ang II. Endothelial function has been demonstrated to be parallel in various vascular beds and endothelial dysfunction is considered a generalized condition. However, the functional heterogeneity of the vascular bed is also well-established. Thus, we cannot exclude that the lack of association between our endothelial function estimates and renal function is due to functional disparity between the internal thoracic artery and the renal vascular bed.[26] We studied vascular function in an ex vivo set up. An advantage of this set up is that it is not influenced by the sympathetic nervous system or by circulating neurohumoral factors or factors influencing vascular function originating from or cleared by the kidney. However, this can also be viewed as a limitation, as a possible role of these factors cannot be analyzed and thus will remain undetected. For example, the endogenous L-arginine analogue asymmetric dimethylarginine (ADMA) concentrations are raised in patients with renal dysfunction. Since ADMA inhibits eNOS it can diminish endothelium dependent vasodilatation.[29] We cannot exclude that our estimation of GFR was not precise enough to find a correlation with vascular function. Indeed, the shortcomings of the MDRD formula, as well as other renal function equations in populations with only mildly impaired renal function is increasingly recognized.[30] However, the association between mild renal function impairment and cardiovascular risk was established in several studies that also used the MDRD, or Cockcroft Gault

equation and considering tha lack of a generally accepted better estimate of renal function it would be best to comply with common practice in the literature, for sake of comparability.[1;6;31]

Furthermore, no urine samples were taken so we have no information on urinary albumin excretion. Urinary albumin excretion is considered a more sensitive measure of early renal damage than glomerular filtration rate.[32;33] Thus, the current study cannot exclude a possible association between urinary albumin excretion and systemic vascular function in this population, but this does not affect the conclusion on absence of a relationship between reduced glomerular filtration rate and endothelial function.

Nevertheless, the present data are unique in their kind and provide novel pathophysiological insights. We are not aware of a larger series of detailed vascular assessment performed within a single structured protocol.

In conclusion, this is the first study to study the relation between vascular function and renal function in patients with severe coronary artery disease. We did not found a relation between diminished endothelium-dependent relaxation and the presence of mild to moderate reduced renal function impairment. In addition, functional parameters of the systemic RAS, smooth muscle properties and inflammatory markers were not associated with renal function. The relation between vascular function and mild renal function impairment might be more complicated than previously thought.

Acknowledgements

P.v.d.H. is supported by Zon-MW (grand 920-03-236) of The Netherlands Organization for Health Research and Development. D.J.v.V is a Clinical Established Investigator of the Netherlands Heart Foundattion (Grand D97-017).

References

24. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med*. 2004;351:1296-1305.
25. Hillege HL, Girbes AR, de Kam PJ, Boomsma F, de Zeeuw D, Charlesworth A, Hampton JR, van Veldhuisen DJ. Renal function, neurohormonal activation, and survival in patients with chronic heart failure. *Circulation*. 2000;102:203-210.
26. Anavekar NS, McMurray JJ, Velazquez EJ, Solomon SD, Kober L, Rouleau JL, White HD, Nordlander R, Maggioni A, Dickstein K, Zelenkofske S, Leimberger JD, Califf RM, Pfeffer MA. Relation between renal dysfunction and cardiovascular outcomes after myocardial infarction. *N Engl J Med*. 2004;351:1285-1295.
27. Smilde TD, Hillege HL, Voors AA, Dunselman PH, van Veldhuisen DJ. Prognostic importance of renal function in patients with early heart failure and mild left ventricular dysfunction. *Am J Cardiol*. 2004;94:240-243.
28. Van de Wal RM, van Brussel BL, Voors AA, Smilde TD, Kelder JC, van Swieten HA, van Gilst WH, van Veldhuisen DJ, Plokker HW. Mild preoperative renal dysfunction as a predictor of long-term clinical outcome after coronary bypass surgery. *J Thorac Cardiovasc Surg*. 2005;129:330-335.
29. Perticone F, Maio R, Tripepi G, Zoccali C. Endothelial Dysfunction and Mild Renal Insufficiency in Essential Hypertension. *Circulation*. 2004.
30. Ridker PM, Hennekens CH, Roitman-Johnson B, Stampfer MJ, Allen J. Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. *Lancet*. 1998;351:88-92.
31. Van der Harst P, Volbeda M, Voors AA, Buikema H, Wassmann S, Bohm M, Nickenig G, van Gilst WH. Vascular response to angiotensin II predicts long-term prognosis in patients undergoing coronary artery bypass grafting. *Hypertension*. 2004;44:930-934.
32. Dickstein K, Kjekshus J. Effects of losartan and captopril on mortality and morbidity in high-risk patients after acute myocardial infarction: the OPTIMAAL randomised trial. Optimal Trial in Myocardial Infarction with Angiotensin II Antagonist Losartan. *Lancet*. 2002;360:752-760.
33. Oosterga M, Voors AA, Buikema H, Pinto YM, Haber HE, Ebels T, Morshuis WJ, Kingma JH, Crijns HJ, van Gilst WH. Angiotensin II formation in human vasculature after chronic ACE inhibition: a prospective, randomized, placebo-controlled study. QUO VADIS Investigators. *Cardiovasc Drugs Ther*. 2000;14:55-60.
34. Manjunath G, Sarnak MJ, Levey AS. Prediction equations to estimate glomerular filtration rate: an update. *Curr Opin Nephrol Hypertens*. 2001;10:785-792.

35. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron*. 1976;16:31-41.
36. Buikema H, Grandjean JG, van den BS, van Gilst WH, Lie KI, Wesseling H. Differences in vasomotor control between human gastroepiploic and left internal mammary artery. *Circulation*. 1992;86:II205-II209.
37. Feelisch M. The Biochemical Pathways of Nitric-Oxide Formation from Nitrovasodilators - Appropriate Choice of Exogenous No Donors and Aspects of Preparation and Handling of Aqueous No Solutions. *J Cardiovasc Pharmacol*. 1991;17:S25-S33.
38. Santos RA, Krieger EM, Greene LJ. An improved fluorometric assay of rat serum and plasma converting enzyme. *Hypertension*. 1985;7:244-252.
39. Van Haelst PL, Tervaert JW, van Geel PP, Veeger NJ, Gurne O, Gans RO, van Gilst WH. Long term angiotensin converting enzyme-inhibition in patients after coronary artery bypass grafting reduces levels of soluble intercellular cell adhesion molecule-1. *Eur J Vasc Endovasc Surg*. 2003;26:387-391.
40. Kendall MG, Stulak JM. The Advanced Theory of Statistics. 1963. Harner Publishing Company, New York.
41. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr., Jones DW, Materson BJ, Oparil S, Wright JT, Jr., Roccella EJ. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA*. 2003;289:2560-2572.
42. Gschwend S, Buikema H, Navis G, Henning RH, de Zeeuw D, van Dokkum RP. Endothelial dilatory function predicts individual susceptibility to renal damage in the 5/6 nephrectomized rat. *J Am Soc Nephrol*. 2002;13:2909-2915.
43. King AJ, Brenner BM. Endothelium-derived vasoactive factors and the renal vasculature. *Am J Physiol*. 1991;260:R653-R662.
44. Stroes ES, Joles JA, Chang PC, Koomans HA, Rabelink TJ. Impaired endothelial function in patients with nephrotic range proteinuria. *Kidney Int*. 1995;48:544-550.
45. Stam F, van Guldener C, Schalkwijk CG, ter Wee PM, Donker AJ, Stehouwer CD. Impaired renal function is associated with markers of endothelial dysfunction and increased inflammatory activity. *Nephrol Dial Transplant*. 2003;18:892-898.
46. Bolton CH, Downs LG, Victory JG, Dwight JF, Tomson CR, Mackness MI, Pinkney JH. Endothelial dysfunction in chronic renal failure: roles of lipoprotein oxidation and pro-inflammatory cytokines. Nephrol Dial Transplant. 2001;16:1189-1197.
47. Jacobson SH, Egberg N, Hylander B, Lundahl J. Correlation between soluble markers of endothelial dysfunction in patients with renal failure. *Am J Nephrol*. 2002;22:42-47.
48. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. *Circulation*. 2004;109:III27-III32.
49. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. *Arterioscler Thromb Vasc Biol*. 2003;23:168-175.
50. Leoncini G, Viazzi F, Parodi D, Vettoretti S, Ratto E, Ravera M, Tomolillo C, Del Sette M, Bezante GP, Deferrari G, Pontremoli R. Mild renal dysfunction and subclinical cardiovascular damage in primary hypertension. *Hypertension*. 2003;42:14-18.
51. Palmer BF. Disturbances in renal autoregulation and the susceptibility to hypertension-induced chronic kidney disease. *Am J Med Sci*. 2004;328:330-343.
52. Perticone F, Sciacqua A, Maio R, Perticone M, Maas R, Boger RH, Tripepi G, Sesti G, Zoccali C. Asymmetric dimethylarginine, L-arginine, and endothelial dysfunction in essential hypertension. *J Am Coll Cardiol*. 2005;46:518-523.
53. Bostom AG, Kronenberg F, Ritz E. Predictive performance of renal function equations for patients with chronic kidney disease and normal serum creatinine levels. *J Am Soc Nephrol*. 2002;13:2140-2144.
54. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Kidney Disease Outcome Quality Initiative. *Am J Kidney Dis*. 2002;39:S1-246.
55. Pinto-Sietsma SJ, Janssen WM, Hillege HL, Navis G, de Zeeuw D, de Jong PE. Urinary albumin excretion is associated with renal functional abnormalities in a nondiabetic population. *J Am Soc Nephrol*. 2000;11:1882-1888.
56. Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H. Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. *Lancet*. 1982;1:1430-1432.