Relationship between declining GFR and measures of cardiac and vascular autonomic neuropathy.

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Abstract

Aim

Cardiac and vascular autonomic neuropathy contributes to increased morbidity and mortality in patients with chronic kidney disease. The aim of this study was to analyze the effects of a decline in GFR on heart rate variability (HRV) and nocturnal blood pressure dipping.

Methods

This cross-sectional study comprises 124 patients (46 women, 78 men; age 66±14 years) with CKD 3-5, not on renal replacement therapy. GFR was measured with iohexol clearance, HRV with 24-hour Holter ECG and nocturnal dipping with 24-hour ambulatory blood pressure.

Results

The GFR was 22.5±8.5ml/min/1.73 m^2. The main finding was a significant curvilinear association between the 24 hour standard deviation of NN interval (24SDNN) in the HRV analysis and GFR (p=0.01), logGFR (p=0.006), diabetes mellitus (p=0.05) and beta blocker treatment (0.03), respectively. The effect of diabetes mellitus on 24SDNN corresponded to a decline in GFR from 30 to 12 ml/min/1.73 m^2. There were significant curvilinear associations between systolic nocturnal dipping (p=0.02) and diastolic nocturnal dipping (p=0.05), respectively, and diabetes mellitus but not with GFR or logGFR.

Conclusion

In conclusion, cardiac sympathetic overdrive and decreased vagal control appear during CKD 4 and 5. The association with GFR is curvilinear. Diabetes mellitus was significantly associated with both cardiac and vascular autonomic neuropathy, as measured by heart rate variability and nocturnal blood pressure dipping, respectively. Knowing that arrhythmias, often due to sympathetic hyperactivity, are an important cause of sudden death in the dialysis population, this study contributes important knowledge on possible intervention thresholds.

Key words

ambulatory blood pressure, autonomic neuropathy, CKD, GFR, heart rate variability,

Introduction

Patients with chronic kidney disease (CKD) have an especially high risk of cardiovascular death and in particular sudden cardiac death[1,2]. Some common etiological factors are increased atherosclerosis, hypertension and autonomic neuropathy. Diabetes mellitus is common in CKD and a risk factor both for cardiovascular disease and autonomic neuropathy. Autonomic neuropathy with increased sympathetic nervous activity and down regulation of parasympathetic control has detrimental effects on cardiac and vascular morphology and function[3].

Cardiac autonomic function can be studied by analyzing heart rate variability (HRV) with a 24-hour Holter ECG. Lower HRV is indicative of higher cardiac risk[4,5]. Vascular autonomic function can be studied by analyzing 24-hour ambulatory blood pressure and especially nocturnal dipping.

There are some studies that have analyzed HRV at different levels of CKD and in patients on dialysis[4,5,6,7]. A number of reports have investigated circadian blood pressure variation in CKD[8,9,10.] None have measured GFR, thus avoiding the confounding effects of muscle mass and strength when estimating GFR.

Our hypothesis is that declining renal function per se has a progressively detrimental effect on cardiac and vascular autonomic control and that the effects of diabetes mellitus are additive. Our aim is to examine the association between measured GFR and HRV and nocturnal blood pressure dipping, respectively, in patients with CKD 3 to CKD 5 not on renal replacement therapy.

Subjects and Methods

The data presented are baseline data from the RENEXC trial, comprising 124 of a total of 176 patients screened. Patients were recruited from prevalent and incident patients from a single centre, the Nephrology Department in Lund, Skåne University Hospital. Patients, irrespective of age and number of co-morbidities, were included thus reflecting a cross-section of patients with CKD 3 to 5 in Sweden today. The cut-off point was intended to be a GFR lower than 31ml/min/1.73 m^2 and patients were selected on the basis of eGFR. However, when GFR was measured we found that a number of patients with higher values had been included, which explains the wider GFR range. Patients were excluded if they had severe orthopaedic or neurological impediments, unstable cardiovascular disease, uncontrolled hypertension, severe anaemia or electrolyte disturbances. No patient on or expected to require active renal replacement therapy within the year was included.

In the HRV analysis 70 of the 124 study patients participated, as patients with atrial fibrillation or flutter and bundle branch blocks were excluded and some declined participation. In the 24-hour blood pressure analyses 108 of the 124 study patients provided complete data, some patients declined participation and some did not have complete nocturnal registrations.

Patients underwent tests for laboratory analyses of blood chemistry and urine tests, iohexol clearance, 24-hour HolterECG recording and 24-hour ambulatory blood pressure registration.

Iohexol clearance

GFR is expressed as ml of filtered volume per minute and standardized to normal body surface area (1.73 m^2)[11]. The standardization is performed in order to be able to compare GFR between different individuals. An adjusted dose of iohexol (Omnipaque) was administered

intravenously. Serum iohexol was analyzed with HPLC (liquid chromatography). Using age, sex, weight, height, iohexol dose, time from injection to sample and the measured iohexol concentration in plasma, GFR is calculated. The optimal sampling schedule for iohexol is calculated by estimating GFR using the patient's age, sex, height, weight and a recent (no longer than one month old) plasma creatinine.

The reproducibility of the actual function test in the same patient is 6-11%[12]. To this is added: the inaccuracy of the method of measuring iohexol by HPLC, the number of points measured for calculation of clearance, measurement of weight, height, level of GFR, biological variation of GFR and inter laboratory variations. Thus the total measurement inaccuracy is estimated to be about ±15%[13].

24-hour ambulatory ECG recording and heart rate variability (HRV) assessment

For the assessment of HRV measures, all patients underwent 24-hour Holter electrocardiogram (ECG) monitoring. For the ECG recordings, 3-channel Galix GBI-3SM Recorders from GALIX Biomedical Instrumentation Inc (Florida, USA) were used. The sample rate for the GBI-3SM is 256 samples/second. Patients were asked to wear the portable device during a normal daily routine. ECG data was stored on a removable flashcard and analyzed using the WinTer Holter Analysis system. Two separate cardiologists blinded to patients' details conducted all analyses.

Time - domain measures of HRV were obtained from 24-hour ECG recordings. The measures included standard deviation of NN interval (SDNN), which reflects sympathovagal balance, and root mean square of successive NN interval differences (rMSSD), which has been demonstrated to be reliable index of vagal tone[14]. Frequency domain was not analyzed due to a change of software mid-study and incompatibility of measurements before and after the switch.

24-hour ambulatory blood pressure registration

For the assessment of 24-hour ambulatory blood pressure measurement all patients underwent monitoring with SpaceLabs Medical ABP-monitor model 90207 or 90217. The size of the blood pressure cuff was selected according to each patient's arm circumference. The blood pressure is measured using a completely automatic oscillometric device, which measured blood pressure every 20 minutes. The collected information is transferred to SpaceLabs computerized reporting system 90121-1 for Windows. The method is accredited by SWEDAC standard ISO 17025 and fulfils the criteria stipulated by the Association for the Advancement of Medical Instrumentation and the British Hypertension Society Protocol[15]. Nocturnal dipping was calculated as a fall of >10% in average nighttime systolic or diastolic blood pressure, or both, compared with daytime averages[16].

Co-morbidity

Co-morbidity for each patient was calculated using Davies' co-morbidity index by the same clinician (MH). Seven separate domains were identified: malignancy, ischemic heart disease, peripheral vascular disease, left ventricular dysfunction, diabetes mellitus, systemic collagen vascular disease and other significant pathology[17].

In the survival analysis we classified 0 and 1 co-morbid domains as '0' i.e. low co-morbidity, and 2 co-morbid domains as '1' i.e. high co-morbidity.

Statistical analyses

Data were analyzed using the R software (R Foundation for Statistical Computing, Vienna, Austria). Descriptive data are given as means ± SD for normally distributed data and as medians and interquartile ranges for non-parametric data. We chose curvilinear analyses

based on an appreciation of how biological systems work. They strive to maintain normality for as long as possible engaging compensatory systems until they tip over. This is especially relevant for the heart rate variability analyses. An increasingly higher GFR does not result in a linear increase in HRV. At a certain GFR, HRV eventually levels out. Nocturnal dipping is not so obviously curvilinear in the range of GFR studied, however, we chose to apply the same model, not wishing to have a data driven approach to choice of statistics. Thus, curvilinear regression analyses were employed with 24SDNN, 24rMSSD, systolic blood pressure nocturnal change and diastolic blood pressure nocturnal change as respective endpoints including GFR, logGFR and diabetes mellitus as explanatory variables. Since it is known that treatment with beta-blockers can affect SDNN they were added to the model. Estimates are given with 95% confidence intervals and the level of significance was set at $p < 0.05$.

Ethics committee

The study was approved by the Regional Ethical Review Board in Lund (2011/369). All subjects gave written informed consent.

Trial registration

RENEXC is registered in www.ClinicalTrials.gov, registration no: NCT02041156.

Results

This study comprised 78 men and 46 women, with an average age of 66±14 [range: 22 – 87] years, a median Davies' co-morbidity index of 2 [interquartile range: 1-2], an average GFR of 22.5 ± 8.5 [range: 8 – 55] ml/min/1.73m^2 with 23% of the patients classified as CKD 5, 62% as CKD 4 and 15% as CKD 3B. 41 patients suffered from diabetes mellitus. Of the 70

patients who underwent HRV analysis 44 were treated with beta-blockers. 22 of the patients examined suffered from diabetes of whom 19 were on beta-blockers. Of the 108 patients who underwent 24-hour blood pressure measurements 73 were treated with beta-blockers, 35 patients had with diabetes mellitus of whom 30 were on beta-blockers.

The causes of CKD were hypertensive renal disease in 50 patients (40%), diabetic nephropathy in 21 (17%), glomerulonephritis or vasculitis in 21 (17%), tubulointerstitial disease in 18 (15%), adult polycystic kidney disease in 9 (7%), and other causes in 5 (4%). Nearly all patients were on antihypertensive treatment (95%), comprising diuretics in 89 (72%), beta-blockers in 82 (65%), angiotensin converting enzyme inhibitors or angiotensin receptor blockers in 77 (62 %), calcium channel blockers in 70 (56%) and other agents in 33 (27%) patients. Many had several antihypertensive medications.

Clinical characteristics of the patients are given in table 1. The patients were clinically stable and well controlled. We did not find any statistically significant relationships between laboratory parameters such as plasma albumin, haemoglobin or parathyroid hormone levels and HRV analyses. Descriptive results from HRV analyses and 24-hour blood pressure measurements are given in tables 2 and 3, respectively, and results from the curvilinear regression analyses in table 4.

Heart rate variability

The main finding was a significant curvilinear association between 24SDNN and GFR (p=0.01), logGFR (p=0.006) and diabetes mellitus (p=0.009), respectively (table 4, figure 1). The effect of diabetes mellitus on 24SDNN corresponded to a decline in GFR from 30 to 12 ml/min/1.73 m^2. If we added beta blocker medication to the equation we found that the association between 24SDNN and diabetes mellitus (p=0.05) and beta blocker medication (p=0.03), respectively, were significant and of similar magnitude, as shown in table 4 and

figure 1. For rMSSD there were no statistical significant associations at all (table 4, figure 2).

24-hour ambulatory blood pressure

48% of the patients lacked a nocturnal dip. The was no significant association in curvilinear regression analysis between systolic blood pressure nocturnal change and GFR or logGFR, but the association with diabetes mellitus was significant (p=0.02, table 4, figure 3).
For diastolic blood pressure nocturnal change there was a similar association with diabetes mellitus only (p=0.05, table 4, figure 4).

Discussion

Our hypothesis was that the effects of uraemia on autonomic nervous balance become manifest in CKD 4 and 5. Our analyses show that the relationship between renal function and cardiac and vascular autonomic function, respectively, seems to be curvilinear. This is reasonable as both cardiac and vascular autonomic balance is dependent on numerous factors. Autonomic balance is one of the body's most important systems for maintaining homeostasis. There are a number of compensatory mechanisms in order to preserve cardiac and vascular function for as long as possible. At some critical point, however, the system can no longer compensate and the balance is lost. Above a certain level of GFR, for instance, other factors such as exercise capacity or concomitant heart disease can have a greater influence on HRV. A normal level of GFR does not result in an increasing improvement in autonomic balance. Normality works as a biological cut-off point.

We studied sympathovagal balance and vagal activity by time domain analysis of a 24-hour HRV registration in which the NN interval is analyzed. Higher HRV is indicative of a greater vagal influence, which has a modulating and stabilizing effect, decreasing the risk of

arrhythmias[4]. This study shows a curvilinear relationship between the decline in renal function, measured as GFR, and an increasing sympathovagal imbalance, measured as SDNN. The SDNN is a good estimate of overall sympathovagal balance. The depressed SDNN indicates an altered autonomic response with sympathetic hyper activation and/or vagal withdrawal [14]. This sympathetic dominance makes the heart vulnerable to arrhythmias and increases the risk for sudden death. The levels of SDNN in our study correspond with the levels found in a multi-centre cohort of patients with CKD 3-5[6]. However, they did not analyze the association between GFR and indices of HRV. A Japanese study in haemodialysis patients showed that SDNN lower than 75 ms was a strong independent predictor of all-cause mortality and cardiovascular death, respectively[18]. Another group reported a significant association between sudden cardiac death and the low frequency: high frequency ratio in the frequency domain of HRV[2]. Data from the USRDS data base report a death rate due to sudden cardiac death of 62 per 1000 patient years compared with an incidence of 1.9 per 1000 patient years in a regular out-patient population[1,19]. In haemodialysis patients increased plasma norepinephrine levels are significantly correlated to cardiovascular events as well as to all cause mortality[20].

We found a strong association between decreased levels of SDNN and diabetes mellitus. Our findings are in accordance with a study in CKD 4 patients with and without diabetes mellitus, where patients with CKD 4 and diabetes mellitus showed the lowest values for SDNN[7]. In patients with diabetes mellitus type 2 other investigators report a significant association between both higher SDNN and rMSSD and better eGFR with a quicker decline in eGFR in patients with cardiac autonomic neuropathy[21].

We found that the magnitude of influence of beta-blockers and diabetes mellitus were similar. The rMSSD was unchanged, implying relatively unchanged vagal activity in the patients studied.

The 24-hour ambulatory blood pressure monitoring showed that 48 % of the patients in this study lacked a nocturnal dip. The proportion of non-dippers in this cohort of patients with CKD 3 to 5 is lower than reported by another investigator, who found that 68% of patients with 3B and 74% in those with more advanced stages were non-dippers[10], compared with 41% with CKD 3B, 48% in CKD 4 and 54% in CKD 5 in our study. However, nearly 50% of the patients in that study suffered from diabetes compared with 33% in the present study. Non-dipping is common in diabetic patients even without CKD[22].

In our cohort of patients with CKD we found that diabetes was the factor with the greatest impact on nocturnal dipping. The relationship between GFR and nocturnal dipping seems to be curvilinear with the upward curve becoming more pronounced at GFR lower than 12 ml/min/1.73m^2. Albeit, that the curvilinear relationship for nocturnal dipping is not as obvious as for HRV. This is probably due to the biological cut-off point for nocturnal dipping in CKD occurring later in the course of CKD. In fact it seems to appear first in near endstage renal disease (ESRD). In this study, however, there were few patients approaching ESRD, which may explain why we did not find a significant association between GFR and nocturnal dipping.

This is supported by the findings of another study, although plasma creatinine and a linear model were used[8]. They reported a significant difference in prevalence in nocturnal dipping once plasma creatinine rose above 400 umol/L, which became still more significant at levels over 600 umol/L[8]. Another investigator reported a non-graded relationship between eGFR and non-dipping[10]. In a large cohort from Japan comprising patients with CKD 3 to 5 the prevalence of non-dippers was lower at stage 3 than at stages 4 and 5. They also found that the prevalence of non-dippers was significantly higher among diabetic patients[9]. Our findings are similar at the different CKD stages and for diabetics. Results from these other studies support our findings of a curvilinear relationship between GFR and nocturnal dipping.

Although, blood pressure dipping can be due to vascular autonomic control, it can also be a manifestation of other influences such as vascular calcification and arterial stiffness. This is especially prevalent in patients with diabetes. Even though patients' blood pressure in this study was reasonably well controlled, both daytime and nighttime average blood pressure were above the normal[16]. PTH, plasma calcium, phosphate and blood lipids are all risk factors for vascular calcification, albeit that they were all well controlled in the present study[23].

One weakness of this study is that frequency domain was not analyzed due to a change of software and incompatibility of measurements before and after the switch. Another weakness is a relatively large proportion of patients treated with beta-blockers, which did affect HRV. In order to compensate for the effects of beta-blocker treatment on HRV, we included it in our model.

There are also some strengths. GFR was measured rather than estimated, making the associations we found independent of muscle mass or wasting. Another strength is that the patients examined are a representative cross-section of patients from a large outpatient clinic and were included irrespective of co-morbidity and age.

In conclusion, cardiac sympathetic overdrive and decreased vagal control appear during CKD 4 and 5. The association with GFR is curvilinear. Diabetes mellitus was significantly associated with both cardiac and vascular autonomic neuropathy, as measured by heart rate variability and nocturnal blood pressure dipping, respectively. Knowing that arrhythmias, often due to sympathetic hyperactivity, are an important cause of sudden death in the dialysis population, this study contributes important knowledge on possible intervention thresholds.

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References

1. U.S. Renal Data System, USRDS 2006 Annual Data Report: Atlas of End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD. Chapter 6. Morbidity and Mortality 2006.

2. Nishimura M, Tokoro T, Nishida M, Hashimoto T, Kobayashi H, Yamazaki S, Imai R, Okino K, Iwamoto N, Takagashi H, Ono T (2010) Sympathetic overactivity and sudden cardiac death among hemodialysis patients with left ventricular hypertrophy. Int J Cardiol 142:80-86.

3. Schlaich MP, Socratous F, Hennebry S, Eikelis N, Lambert EA, Straznicky N, Esler MD, Lambert GW (2009) Sympathetic activation in chronic renal failure. J Am Soc Nephrol 20:933-939.

4. Deligiannis A, Kouidi E, Tourkantonis A (1999) Effects of physical training on heart rate variability in patients on hemodialysis. Am J Cardiol (84):197-202.

5. Kouidi E, Vergoulas G, Anifanti M, Deligiannis A (2013) A randomized controlled trial of exercise training on cardiovascular and autonomic function among renal transplant recipients. Nephrol Dial Transplant 28:1294-1305.

6. Chandra P, Sands RL, Gillespie BW, Levin NW, Kotanko P, Kiser M, Finkelstein F, Hinderliter A, Pop-Busui R, Rajagopalan S, Saran R (2012) Predictors of heart rate variability and its prognostic significance in chronic kidney disease. Nephrol Dial

Transplant 27: 700–709.

7. Mylonopoulou M, Tentolouris N, Antonopoulos S, Mikros S, Katsaros K, Melidonis A, Sevastos N and Katsilambros (2010) Heart rate variability in advanced chronic kidney disease with or without diabetes: midterm effects of the initiation of chronic haemodialysis therapy. Nephrol Dial Transplant 25: 3749–3754.

8. Farmer CKT, Goldsmith DJA, Cox J, Dally P, Kingswood JC, Sharpstone P (1997) An investigation of the effect of advancing uraemia, renal replacement therapy and renal transplantation on blood pressure diurnal variability. Nephrol Dial Transplant 12:2301-2307.

9. Iimuro S, Imai E, Nitta K, Matsuo S, Makino H, Ohashi Y, Hishida A (2013) for the Chronic Kidney Disease Japan Cohort Study. Clinical correlates of ambulatory BP monitoring among patients with CKD. Clin J Am Soc Nephrol 8:721-730.

10. Agarwal R, Light R (2009) GFR, proteinuria and circadian blood pressure. Nephrol Dialysis Transplant 24:2400-2406.

11. Dubois D & Dubois EF (1916) A formula to estimate the approximate surface area if height and weight are known. Arch Int Med 17:863-871.

12. Krützen, E, Bäck SE, Nilsson-Ehle I, Nilsson-Ehle P (1984) Plasma clearance of a new contrast agent, iohexol: a method for the assessment of glomerular filtration rate. J Lab Clin Med 104(6): 955-61.

13. Perrone RD, Steinman TI, Beck GJ, Skibinski CI, Royal HD, Lawlor M, Hunsicker LG (1990) Utility of radioisotopic filtration markers in chronic renal insufficiency. Am J Kidney Dis 16(3): 224-225.

14. Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology (Membership of the Task Force listed in the Appendix) (1996) Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. European Heart Journal 17, 354-381.

15. O'Brien E, Waeber B, Parati G, Staessen J, Myers MG (2001) Blood pressure measuring devices: recommendations of the European Society of Hypertension. BMJ 322: 531-536.

16. Head GA, McGrath BP, Mihailidou AS, Nelson MR, Schlaich MP, Stowasser M, Mangoni AA, Cowley D, Brown MA, Ruta L-A, Wilson A (2002) Ambulatory blood pressure monitoring in Australia: 2011 consensus position statement. J of Hypertension 30(1): 1-6.

17. Davies SJ, Russell L, Bryan J, Phillips L, Russell GI (1995) Comorbidity, Urea Kinetics, and Appetite in Continuous Ambulatory Peritoneal Dialysis Patients: Their Interrelationship and Prediction of Survival. Am J Kidn Dis 26(2): 353-361.

18. Oikawa K, Ishihara R, Maeda T, Yamaguchi K, Koike A, Kawaguchi H, Tabata Y, Murotani N, Itoh H (2009) Prognostic value of heart rate variability in patients with renal failure on hemodialysis. Int J of Cardiol (131)370–377.

19. Rea TD, Pearce RM, Raghunathan TE, Lemaitre RN, Sotoodehnia N, Jouven Z, Siscovick DS (2004) Incidence of Out-of-Hospital Cardiac Arrest. Am J Cardiol;93:1455–1460

20. Zoccali C, Mallamaci F, Parlongo S, Cutrupi S, Benedetto FA, Tripepi G, Bonanno G, Rapisardi F, Fatuzzo P, Seminara G, Ceteliotti A, Stancanelli B, Malatino LS (2002) Plasma Norepinephrine predicts survival and incident cardiovascular events in patients with end-stage-renal disease. Circulation 105:1354-1359.

21. Tahrani AA. Dubb T, Dubb K, Raymond NT, Begum S, Altaf QA, Sadiqi H, Piya MK, Stevens JM (2014) Cardiac autonomic neuropathy predicts renal function decline in patients with type 2 diabetes: a cohort study. Diabetologia 57:1249–1256.

22. Grossman, E (2013) Ambulatory blood pressure monitoring in the diagnosis and management of hypertension. Diabetes Care 36(Suppl2) S307-S311.

23. Goodman WG, London G on behalf of the Vascular Calcification Work Group (Amann K, Block GA, Giachelli C, Hruska KA, Ketteler M, Levin A, Massy Z, McCarron DA, Raggi P, Shanahan M, Yorioka N (2004) Vascular calcification in chronic kidney disease. Am Journal of Kidn Dis 43(3):572-579.

Table 1. Some clinical and laboratory data

	All Mean±SD or Median and interquartile range	**Women** Mean±SD or Median and interquartile range	**Men** Mean±SD or Median and interquartile range	**No DM** Mean±SD or Median and interquartile range	**DM** Mean±SD or Median and interquartile range	**CKD 5** Mean±SD or Median and interquartile range	**CKD 4** Mean±SD or Median and interquartile range	**CKD 3B** Mean±SD or Median and interquartile range
Patients (number)	124	46	78	83	41	27*	72*	18*
Age (years)	66±14	64±16	67±13	64±15	70±11	65±14	66±15	67±11
range	22 – 87	22 – 85	27 – 87	22 – 87	40 – 87	27 - 83	22 - 87	41 - 84
Sex (women: men)	46:78			35:48	11:30	8:19	24:48	10:8
BMI (%)	28±6	29±7	28±5	27±5	31±6	27±4	28±6	29±7
GFR (ml/min/1.73m^2)	22.5±8.5	23.2±8.3	22.1±8.7	22.8±8.7	21.9±8.3	12.5±1.8	22.6±3.9	37.1±6.4
P-Creatinine (umol/L)	258±103	215±94	282±101	254±102	266±107	376±109	238±66	157±38
P-Urea (mmol/L)	16±6	15±5	16±6	16±5	17±6	20±6	16±5	11±4
CRP (mg/L)	2.9	2.6	2.9	2.9	3.0	3.4	2.9	2.5
	1.4 – 5.5	0.9 – 7.2	1.5 – 4.4	1.4 – 5.8	1.4 – 4.1	1.6 – 5.8	1.5 – 5.2	1.0 – 4.4
P-Albumin	37±4	38±4	37±3	38±3	37±4	36±3	38±3	38±4

(g/L)								
P-Calcium (mmol/l)	2.33±0.13	2.38±0.2	2.31±0.12	2.33±0.13	2.33±0.13	2.36±0.13	2.32±0.13	2.34±0.12
P-Phosphate (mmol/L)	1.16±0.28	1.18±0.24	1.15±0.30	1.16±0.25	1.16±0.34	1.35±0.38	1.14±0.21	1.0±0.18
PTH (pmol/L)	12 9 – 21	11 7 – 16	13 9 – 25	11 8 – 21	14 10 – 22	16 11 - 38	13 9 – 20	9 7 - 11
Cholesterol (mmol/L)	4.8±1.3	5.2±1.3	4.6±1.3	4.9±1.3	4.6±1.2	5.0±1.6	4.7±1.2	5.2±1
Triglycerides (mmol/L)	1.9±1.0	1.9±1.1	1.8±0.9	1.8±1.0	2.0±1.0	2.0±1.0	1.8±0.9	1.9±1
HDL (mmol/L)	1.3±0.4	1.5±0.4	1.2±0.4	1.4±0.4	1.2±0.4	1.3±0.4	1.3±0.4	1.4±0.4
LDL (mmol/L)	3.0±1.1	3.2±1.1	2.8±1.1	3.1±1.1	2.8±1.1	3.0±1.2	2.9±1.1	3.2±1.0
Lipoprotein a (g/L)	108 37 – 286	176 55 – 487	98 36 – 236	114 38 – 287	99 34 – 247	193 99 – 512	85 34 - 235	109 25 - 234
Hemoglobin (g/L)	127±15	125±13	129±15	127±15	129±13	123±12	128±14	132±15
U-ACR (g/mol)	25 4 – 125	8 2 – 47	69 10 – 143	20 3 – 112	63 10 – 136	35 12 – 122	36 4 - 146	6 1 - 42

BMI= body mass index, GFR= glomerular filtration rate, CRP= C-reactive protein, PTH=parathyroid hormone, HDL= high density lipids, LDL= low density lipids, U-ACR= urine- albumin-creatinine-ratio, *Iohexol clearance was not performed in 7 patients.

Table 2. Descriptive data from 24-Heart Rate Variability analyses

	All Mean±SD or Median and interquartile range	**Women** Mean±SD or Median and interquartile range	**Men** Mean±SD or Median and interquartile range	**No DM** Mean±SD or Median and interquartile range	**DM** Mean±SD or Median and interquartile range	**CKD 5** Mean±SD or Median and interquartile range	**CKD 4** Mean±SD or Median and interquartile range	**CKD 3B** Mean±SD or Median and interquartile range
Patients (number)	70	22	48	48	22	15*	40*	12*
SDNN (ms)	115±41	112±31	116±45	124±43	96±29	93±35	128±44	105±26
rMSSD (ms)	30±21	25±12	32±24	30±24	30±15	29±27	31±20	25±15
VES (number)	354 94 – 871	331 137 – 806	417 66 – 837	293 53 – 883	452 148 – 735	268 72 - 697	430 108 - 900	385 31 - 588
VT (number)	1 0 – 8.5	6 0 – 12	0 0 – 5	1 0 – 8	1 0 – 10	0 0 - 5	1 0 - 7	6 0 - 14
24 hour heart rate (bpm)	73±11	75±8	72±12	73±11	72±12	78±16	70±9	75±6
Minimum heart rate (bpm)	51±8	54±6	50±9	51±9	51±7	56±11	47±6	55±6
Maximal heart rate (bpm)	121±22	128±22	117±21	126±21	109±19	122±24	119±23	123±18

SDNN= standard deviation NN interval, rMSSD= root mean square of successive NN interval differences, VES= ventricular extra systoles, VT= ventricular tachycardias, bpm=beats per minute, Iohexol clearance was not performed in 3 of the patients who underwent HRV.

Table 3. Descriptive data from 24-hour ambulatory blood pressure analyses

	All Mean±SD or Median and interquartile range	**Women** Mean±SD or Median and interquartile range	**Men** Mean±SD or Median and interquartile range	**No DM** Mean±SD or Median and interquartile range	**DM** Mean±SD or Median and interquartile range	**CKD 5** Mean±SD or Median and interquartile range	**CKD 4** Mean±SD or Median and interquartile range	**CKD 3B** Mean±SD or Median and interquartile range
Patients (number)	108	36	72	73	35	24*	65*	17*
Systolic BP day (mmHg)	133±15	128±15	136±15	132±14	137±17	134±17	134±15	130±15
Diastolic BP day (mmHg)	78±10	76±10	79±10	80±10	74±10	80±12	78±9	76±12
Systolic BP night (mmHg)	120±18	116±18	123±17	118±16	126±20	121±19	121±19	116±13
Diastolic BP night (mmHg)	67±10	65±9	69±10	68±10	65±10	70±12	67±10	64±9
Nocturnal dip (% dippers)	52	50	53	55	46	46	52	59

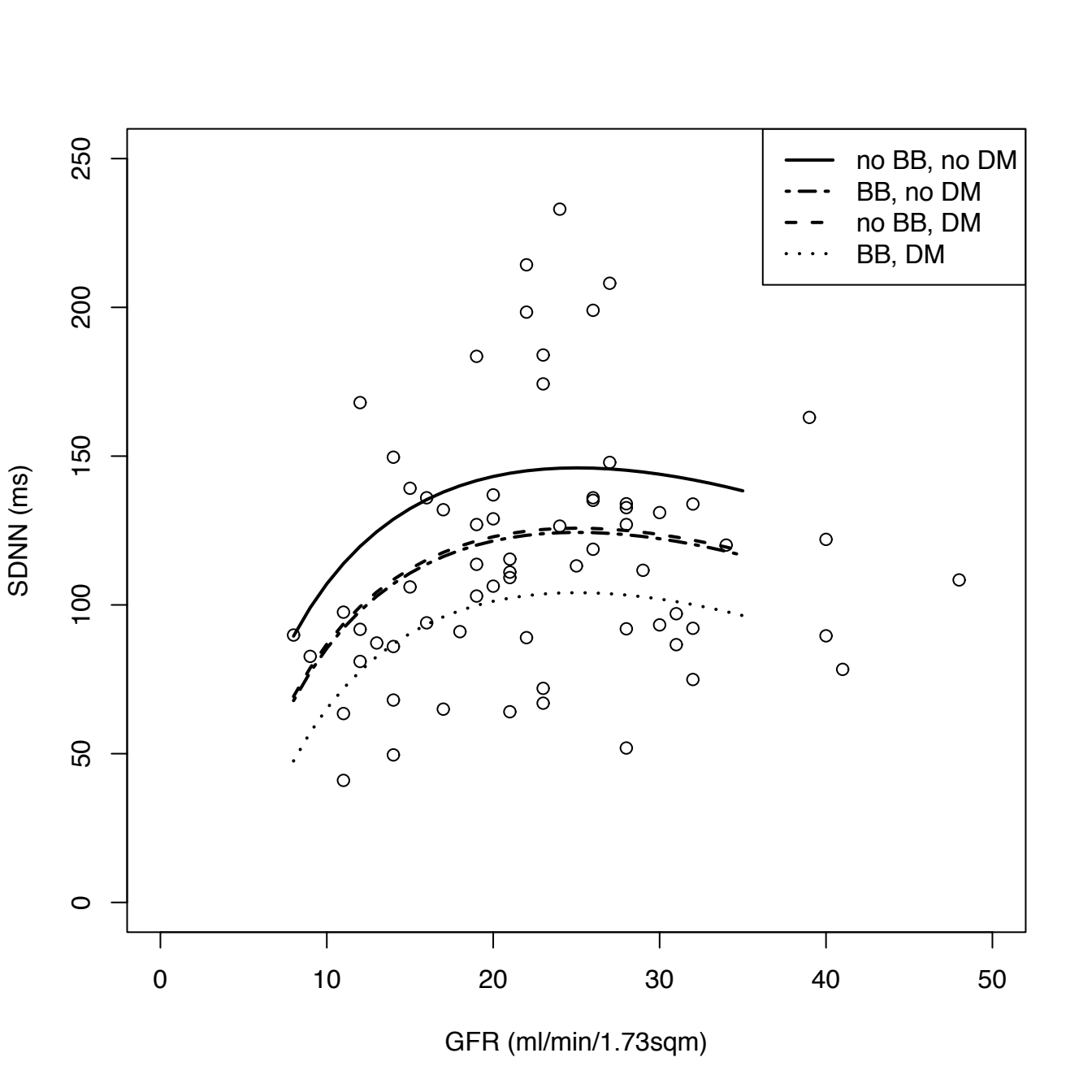
BP= blood pressure, Iohexol clearance was not performed in 2 of the patients who underwent 24-hour ambulatory blood pressure.

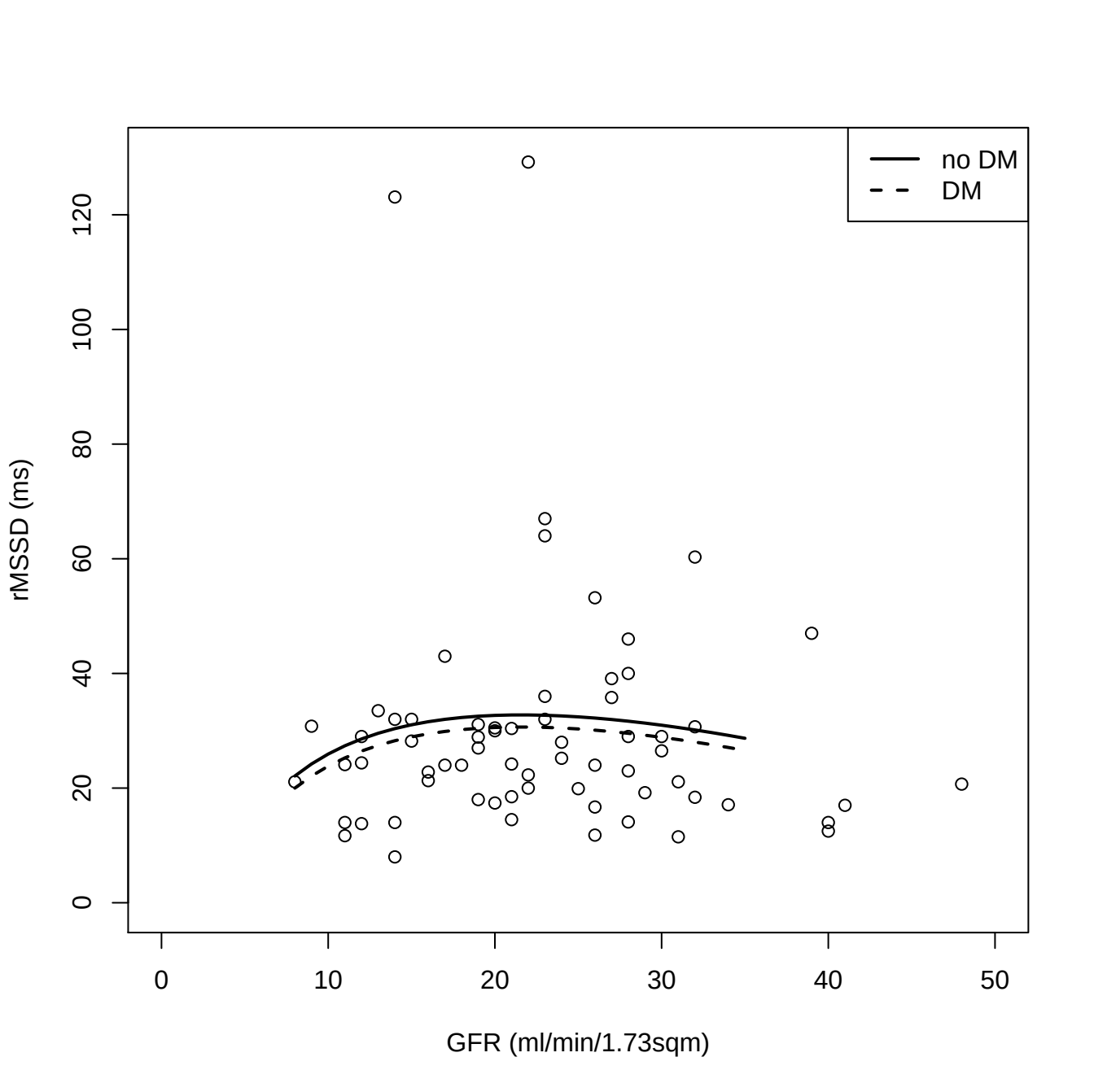
Table 4. Curvilinear regression analyses for 24SDNN, 24rMSSD, systolic and diastolic nocturnal blood pressure change with GFR, log GFR and diabetes mellitus as explanatory variables, and including beta-blocker treatment only for analysis of 24SDNN.

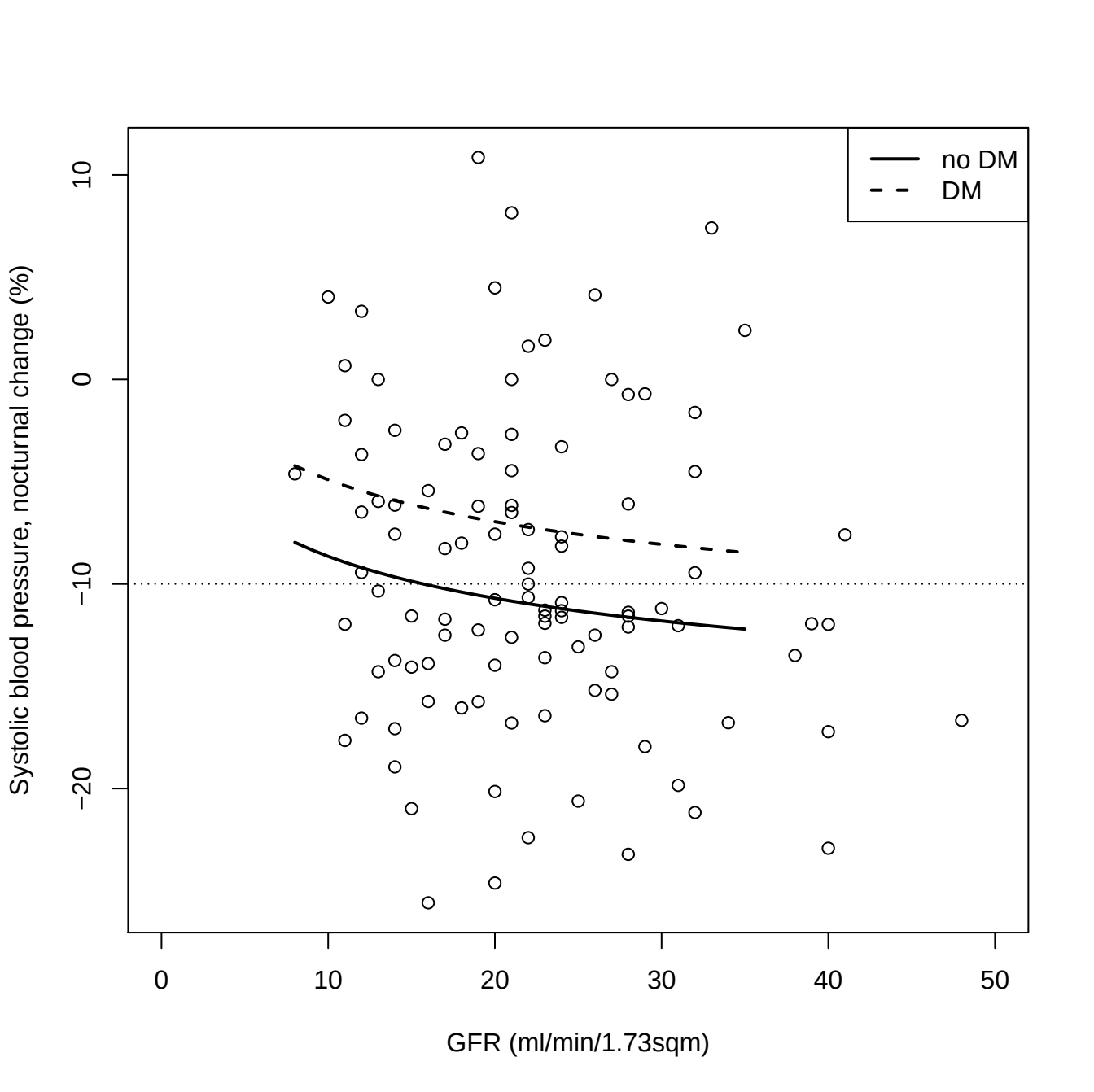
	Estimate	**Confidence interval 2.5% - 97.5%**	**P value**
		24SDNN	
GFR	-4.9	-8.7 – -1.1	0.01
logGFR	126	38 – 214	0.006
DM	-27	-47 – -7	0.009
		24SDNN	
GFR	-4.9	-8.6 – -1.2	0.01
logGFR	123	37 – 208	0.006
DM	-20	-41 – -0.04	0.05
Beta-blocker	-22	-42 – 2	0.03
		24rMSSD	
GFR	-1.4	-3.5 – 0.8	0.21
logGFR	29	-20 – 79	0.24
DM	2.1	-13 – 9	0.71
		Systolic blood pressure, nocturnal change	
GFR	0.02	-0.7 – 0.7	0.95
logGFR	-3.3	-19 – 12	0.68
DM	3.8	-0.7 – 6.8	0.02

Diastolic blood pressure, nocturnal change			
GFR	0.2	-0.5 – 1.0	0.53
logGFR	-9	-26 – 8	0.30
DM	3.3	0.1 – 6.5	0.05

DM= diabetes mellitus, SDNN= standard deviation NN interval, rMSSD= root mean square of successive NN interval differences, GFR= glomerular filtration rate


no BB, no DM
BB, no DM
no BB, DM
BB, DM
SDNN (ms)
0
50
100
150
200
250
0
10
20
30
40
50
GFR (ml/min/1.73sqm)


no DM
DM
rMSSD (ms)
0
20
40
60
80
100
120
0
10
20
30
40
50
GFR (ml/min/1.73sqm)


no DM
DM
Systolic blood pressure, nocturnal change (%)
10
0
−10
−20
0
10
20
30
40
50
GFR (ml/min/1.73sqm)

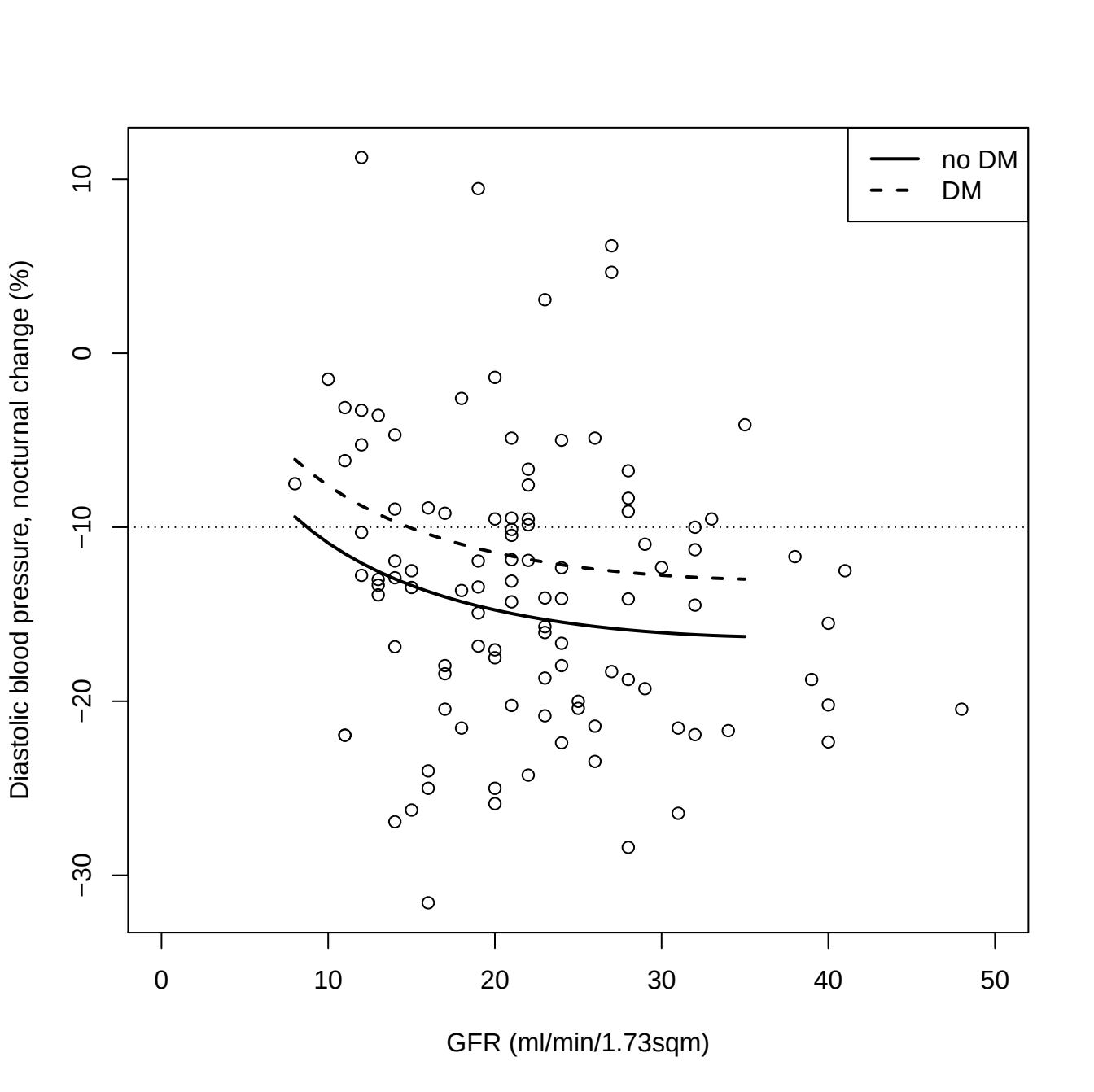

no DM
DM
Diastolic blood pressure, nocturnal change (%)
10
0
−10
−20
−30
0
10
20
30
40
50
GFR (ml/min/1.73sqm)

Figure legends

Figure 1. Relationship between 24SDNN and GFR using curvilinear regression analysis including GFR, logGFR, diabetes mellitus and beta-blocker treatment as explanatory variables. (DM= diabetes mellitus, BB=beta-blocker, SDNN= standard deviation NN interval, GFR= glomerular filtration rate)

Figure 2. Relationship between 24rMSSD and GFR using curvilinear regression analysis including GFR, logGFR and diabetes mellitus as explanatory variables. (DM= diabetes mellitus, rMSSD= root mean square of successive NN interval differences, GFR= glomerular filtration rate)

Figure 3. Relationship between systolic nocturnal blood pressure dip and GFR using curvilinear regression analysis including GFR, logGFR and diabetes mellitus as explanatory variables. (DM= diabetes mellitus, GFR= glomerular filtration rate)

Figure 4. Relationship between diastolic nocturnal blood pressure dip and GFR using curvilinear regression analysis including GFR, logGFR and diabetes mellitus as explanatory variables. (DM= diabetes mellitus, GFR= glomerular filtration rate)