Determinants of baroreflex function in juvenile end-stage renal disease

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Arterial baroreflex sensitivity (BRS) is markedly reduced in middle-aged patients with end-stage renal disease (ESRD), due to the combined effects of aging, arterial stiffening, and autonomic neuropathy. Much less is known about the effects of ESRD on arterial baroreflex in juvenile patients. Therefore, we investigated baroreflex function and its relation to carotid artery elasticity and heart rate variability in children and young adults with ESRD. We studied 42 subjects (9–30 years): 14 patients on maintenance hemodialysis (HD), 14 renal transplant recipients (RT), and 14 healthy control subjects (C). Baroreflex function was determined by pharmacological (BRS) and spontaneous (sequence and spectral indices) techniques. Carotid artery elasticity was characterized by stiffness index β. Heart rate variability was assessed using time and frequency domain measures. Data are expressed as mean $\pm$ s.d. BRS was markedly reduced in HD as compared to C (10.0 $\pm$ 4.2 vs 25.7 $\pm$ 5.9 ms/mm Hg); spontaneous indices were reduced to similar extent. Carotid artery stiffness was $\sim$50% higher in HD than in C and was inversely related to BRS. Heart rate variability was also compromised in HD, and was directly related to spontaneous indices. No significant differences existed in any of these variables between RT and C. Decreased baroreflex function in juvenile HD is partly due to loss of carotid artery elasticity and partly due to impaired heart rate variability. Renal transplantation may partly prevent impairment or improve compromised baroreflex function in young patients with ESRD.

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Cardiovascular disease is the leading cause of mortality both in young and in elderly patients on maintenance hemodialysis (HD).[1,2] This excess cardiovascular mortality is partly attributed to hypertension,[3] vascular stiffening,[4] and cardiovascular autonomic dysfunction.[5] Cardiovagal baroreflex function, which is important for the overall integrity of cardiovascular autonomic control, was found to be impaired in middle-aged HD.[6–10] At that age, however, impairment caused by uremia is superimposed on age-related physiologic decline in baroreflex function.[11] It is not known how the uremic state affects baroreflex function in the late teens and early 20s, when baroreflex functions with the highest efficacy in healthy individuals.[12]

Blood pressure,[13] carotid artery compliance,[14] and vagal tone[15] are among predictors of baroreflex function in healthy subjects. Therefore, chronic hypertension, stiffening of the carotid artery, and efferent parasympathetic neuropathy may contribute to depressed reflex sensitivity in HD. This issue has been addressed previously in studies on adult patients with end-stage renal disease (ESRD), but information is scarce on children and young adults.[16–22] In adult ESRD patients, renal transplantation was found to be associated with improved baroreflex function[9] and enhanced heart rate variability,[23] but without change in large artery elasticity.[24] In juvenile transplant recipients, neither baroreflex function nor its relation to carotid artery elasticity or heart rate variability has been investigated.

Therefore, the aim of the present study was to determine in children and in young adults: (1) whether cardiovagal baroreflex function is impaired in juvenile HD; (2) if decreased baroreflex function is explained by hypertension, stiffening of the carotid artery, or impaired cardiac vagal tone; and (3) if baroreflex function is affected by renal transplantation. To this end, we determined BRS, carotid artery elastic parameters, and heart rate variability in young hemodialyzed and renal transplant patients and in age- and gender-matched healthy controls in a cross-sectional design.

RESULTS

Subjects' anthropometric and resting hemodynamic data are given in Table 1. Age and gender distribution were not different in the three groups. HD had significantly higher mean arterial pressure and heart rate than renal transplant

Table 1 | Anthropometric and hemodynamic parameters in healthy controls, hemodialyzed patients and renal transplant recipients

	C	HD	RT
Number of subjects	14	14	14
Male/female	7/7	7/7	7/7
Age (year)	20.6±5.5	22.1±5.0	18.2±6.7
Height (cm)	168±13	165±12	152±15[a,b]
Weight (kg)	59±13	59±12	51±11
Mean arterial pressure (mm Hg)	85±9	105±13[a]	88±12[b]
Heart rate (beat/min)	69±11	81±11[a]	79±16

C, control subjects; HD, hemodialyzed patients; RT, renal transplant recipients. Data are given as mean±s.d.
[a]Significantly different from C at $P<0.05$.
[b]Significantly different from HD at $P<0.05$.

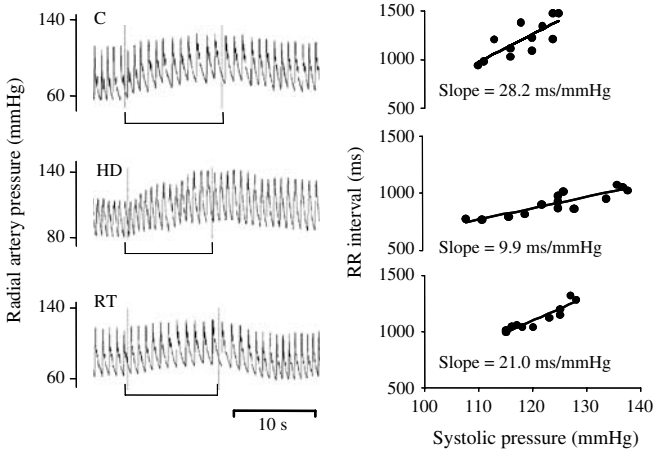

Figure 1 | Representative blood pressure recordings and calculated baroreflex gains in a healthy control subject (C), a hemodialyzed patient (HD), and a renal transplant recipient (RT) after the bolus injection of 200 μg phenyleprine.

recipients (RT) and control subjects (C), but no difference was observed between RT and C.

Baroreflex function

Baroreflex sensitivity (BRS) was markedly lower in HD (10.0±4.2 ms/mm Hg), as compared with both C (25.7±5.9 ms/mm Hg, $P<0.001$) and RT (19.2±5.9 ms/mm Hg, $P=0.006$), but no difference was found between RT and C ($P=0.064$, non-significant) (Figures 1 and 2). Spontaneous indices were also lower in HD than in C, while in RT they fell in between those of HD and C. Differences in heart rate variability indices were similar to those observed in spontaneous indices (Table 2).

Carotid artery elasticity

Carotid artery blood pressure, vessel dimensions, and elastic variables are given in Table 3. HD had elevated systolic and diastolic carotid pressures as compared to RT and C. Carotid artery end diastolic diameter was not different in the three groups. Pulsatile distension was smaller in HD as compared with both C and RT, while carotid pulse pressure was of similar magnitude in the three groups. Distensibility coefficient was 50 and 20% less in HD and RT, respectively, than in C, indicating impaired pressure sensitivity of baroreceptors in

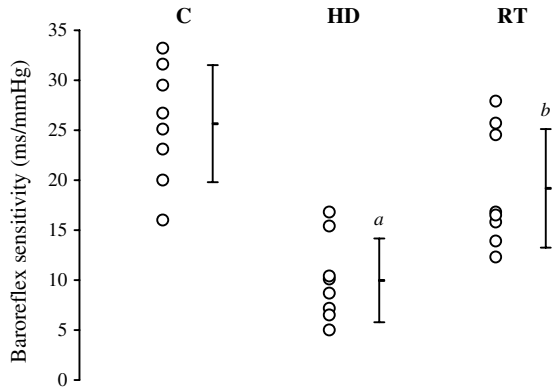

Figure 2 | Individual BRS data in young adult healthy controls (C), in hemodialyzed patients (HD), and in renal transplant recipients (RT) ($n=8$ in each group). Bar graphs indicate mean±s.d. (**a**) Significantly different from C at $P<0.05$. (**b**) Significantly different from HD at $P<0.05$.

Table 2 | Time- and frequency-domain spontaneous autonomic indices and measures of heart rate variability in healthy controls, hemodialyzed patients and renal transplant recipients

	C	HD	RT
Seq (ms/mm Hg)	24.3±14.3	10.9±4.8[a]	15.6±7.3
LF_{gain} (ms/mm Hg)	16.8±8.4	6.6±2.9[a]	11.4±6.7
RMSSD (ms)	45 (29–71)	14 (12–36)[a]	25 (14–44)
LF (ms^2)	802 (352–1312)	204 (92–290)[a]	225 (189–551)
HF (ms^2)	431 (116–890)	121 (578–366)	207 (79–450)

C, control subjects; HD, hemodialyzed patients; HF, high (0.15-0.4 Hz) frequency power of RR interval variability; LF, low (0.05-0.15 Hz) frequency power of RR interval variability; LF_{gain}, cross-spectral transfer gain in the low-frequency range; RMSSD, root mean square of successive RR interval differences; RT, renal transplant recipients; Seq, spontaneous sequence index. Data are given as mean±s.d. for normally distributed and medians (quartiles) for non-normally distributed variables.
[a]Significantly different from C at $P<0.05$.

Table 3 | Carotid artery systolic and diastolic pressures, vessel dimensions and elastic variables in healthy controls, hemodialyzed patients and renal transplant recipients

	C	HD	RT
Systolic pressure (mm Hg)	108±15	131±15[a]	113±10[b]
Diastolic pressure (mm Hg)	67±9	83±12[a]	70±12[b]
End-diastolic diameter (μm)	6212±397	6670±934	6243±503
Pulsatile distension (μm)	857±131	581±170[a]	741±158[b]
Intima-media thickness (μm)	505±62	559±77	520±73
Distensibility coefficient (10^{-3}/mm Hg)	7.2±1.5	3.6±0.8[a]	5.6±1.3[a,b]
Incremental elastic modulus (10^3*mm Hg)	1.5±0.4	2.8±0.7[a]	1.9±0.6[b]
Stiffness index β	3.5±1.0	5.5±1.0[a]	4.2±0.8[b]

C, control subjects; HD, hemodialyzed patients; RT, renal transplant recipients. Data are given as mean±s.d.
[a]Significantly different from C at $P<0.05$.
[b]Significantly different from HD at $P<0.05$.

Table 4 | Correlation coefficients for relations between measures of baroreflex function (BRS, Seq, LF_{gain}) and mean arterial pressure, carotid artery stiffness index β and heart rate variability indices (RMSSD, HF) across adult subjects ($\geqslant$18 years, n=24) and across all subjects (n=42) determined by single linear regression analysis

	Mean arterial pressure		Stiffness index β		RMSSD		HF	
	Adults	All	Adults	All	Adults	All	Adults	All
BRS	−0.66[a]	—	−0.75[a]	—	0.44[c]	—	NS	—
Seq	−0.59[b]	−0.50[b]	−0.51[c]	−0.42[b]	0.87[a]	0.83[a]	0.77[a]	0.73[a]
LF_{gain}	−0.63[a]	−0.52[a]	−0.60[b]	-0.46[b]	0.79[a]	0.73[a]	0.66[a]	0.59[a]

BRS, baroreflex sensitivity; HF, high (0.15–0.4 Hz) frequency power of RR-interval variability; LF_{gain}, cross-spectral transfer gain in the low-frequency range; NS, non-significant; RMSSD, root mean square of successive RR-interval differences; Seq, spontaneous sequence index.
[a]$P<0.001$.
[b]$P<0.01$.
[c]$P<0.05$.

ESRD patients. Both stiffness index β and incremental elastic modulus were markedly higher in HD as compared with C, but were not different in RT and C, suggesting that stiffness of the carotid artery in HD was at least partly independent of elevated blood pressure and altered vessel wall geometry. Intima–media thickness did not differ between groups.

Determinants of baroreflex function

In simple regression analysis, BRS, sequence index (Seq) and low-frequency transfer function gain (LF_{gain}) were related to mean arterial pressure, to carotid artery stiffness, and to root mean square of successive RR-interval differences (RMSSD) (Table 4), but not to age. In a forward stepwise multivariate analysis, only stiffness index β proved to be an independent predictor of BRS, whereas stiffness index β and RMSSD both proved to be independent predictors of Seq and LF_{gain} (Figure 3, Table 5).

Within-group relations

Within HD, BRS was related only to stiffness index β (Figure 4). Patients being on dialysis for a longer time had stiffer carotid arteries (Figure 4) and a tendency towards lower BRS. This latter correlation, however, was not significant ($P=0.09$, non-significant), probably due to the limited number of subjects ($n=8$). Spontaneous Seq correlated with heart rate variability indices ($0.65\leqslant r\leqslant 0.95$, $P<0.05$ for all correlations) and with mean arterial pressure ($r=-0.81$, $P<0.001$).

Within RT, measures of baroreflex function did not correlate with the time spent on dialysis prior to transplantation or the time spent after transplantation, and they were not related to carotid stiffness either. Spontaneous indices, however, were strongly related to heart rate variability indices ($0.67\leqslant r\leqslant 0.93$, $P<0.01$ for all correlations), and heart rate variability indices to mean arterial pressure ($-0.69\leqslant r\leqslant -0.82$, $P<0.01$ for all correlations).

Within C, similarly to RT, spontaneous indices were related to heart rate variability indices ($0.66\leqslant r\leqslant 0.73$, $P\leqslant 0.01$ for all correlations) and heart rate variability indices to mean arterial pressure ($-0.54\leqslant r\leqslant -0.57$, $P<0.05$ for all correlations).

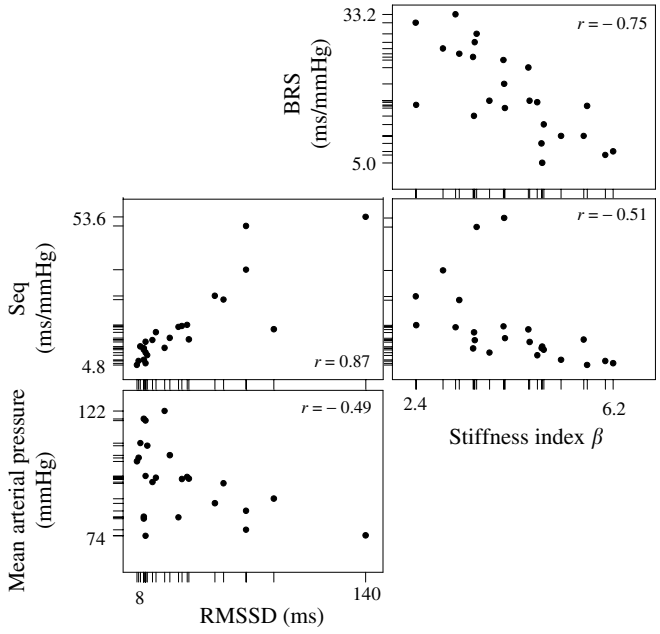

Figure 3 | Rug-plot representation of relationships between BRS, stiffness index β, Seq, RMSSD, and mean arterial pressure across adult subjects ($n=24$).

Table 5 | β-weight values for independent relations among BRS, spontaneous indices (Seq, LF_{gain}), carotid artery stiffness index β and heart rate variability index RMSSD across adult subjects ($\geqslant$18 years, n=24) and across all subjects (n=42) determined by multiple forward stepwise regression analysis

	Stiffness index β		RMSSD	
	Adult subjects	All subjects	Adult subjects	All subjects
BRS	−0.89[a]	—	NI	—
Seq	−0.29[c]	−0.25[b]	0.82[a]	0.77[a]
LF_{gain}	−0.41[b]	−0.33[b]	0.66[a]	0.66[a]

BRS, baroreflex sensitivity; LF_{gain}, cross-spectral transfer gain in the low-frequency range; NI, variable not included in the model; RMSSD, root mean square of successive RR interval differences; Seq, spontaneous sequence index. Variables are expressed as z-scores.
[a]$P<0.001$.
[b]$P<0.01$.
[c]$P<0.05$.

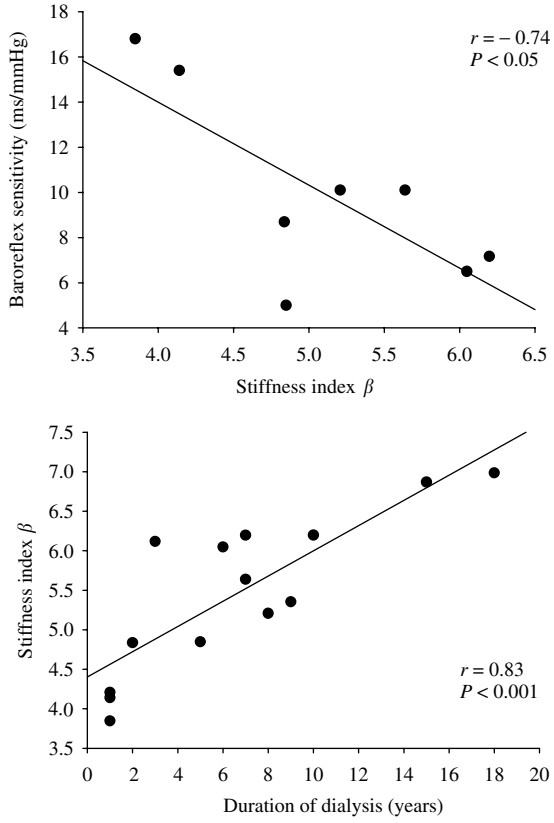

Figure 4 | Relationship between BRS, stiffness index β and the duration of dialysis in HD. Upper panel – relationship between stiffness index β and BRS in young adult HD ($n = 8$). Lower panel – relationship between duration of dialysis and stiffness index β in all juvenile HD ($n = 14$).

DISCUSSION

This is the first study in which predictors of BRS were defined in young patients either on hemodialysis or after renal transplantation. We found that: (1) baroreflex function was impaired considerably in juvenile uremia, as both BRS and spontaneous indices were more than 50% smaller in HD than in C; (2) carotid artery stiffness was about 50% higher in HD than in C and a strong negative correlation existed between BRS and stiffness index β both in HD and across all subjects; (3) heart rate variability was also compromised in HD, and was related to spontaneous indices both in HD and across all subjects; (4) the influence of mean arterial pressure on BRS and spontaneous indices was affected through impaired carotid artery elasticity and reduced heart rate variability; (5) no significant differences existed in baroreflex function, carotid artery stiffness, and heart rate variability indices between RT and C.

Baroreflex function in ESRD

In the early 70s, Pickering *et al.*[6] were the first to report impaired BRS in chronic renal failure in middle-aged hemodialyzed patients. The observation of compromised baroreflex function has been confirmed by a number of investigators, by employing invasive and non-invasive techniques.[7–10] Most previous data, however, were obtained in middle-aged or elderly patients, at ages when BRS and spontaneous indices are already reduced even in healthy individuals.[11,13] In healthy populations, BRS and spontaneous indices are the highest in the late teens and early 20s.[12] Our data demonstrate that long-term hemodialysis is associated with markedly reduced baroreflex function even at younger ages, as BRS was reduced by 60%, and spontaneous indices by 55–60% in our HD cohort. Since baroreflex function in these patients will decline further due to aging and in some of them this decline will be accentuated by hypertension, BRS and spontaneous indices might eventually be reduced to extremely low values, and are likely to contribute to the excess cardiovascular mortality of adult ESRD.

Cardiovascular morbidity and mortality remain a considerable problem even after renal transplantation both in adult and in juvenile ESRD patients,[1,25] but the contribution of compromised baroreflex function to this persistent high mortality is not known. The only study that examined BRS after renal transplantation showed an improvement from 3.95 to 7.46 ms/mm Hg 6 months following transplantation.[9] In another study on RT and C in their mid-30s, sequence indices were found to be of similar magnitude.[10] Though we observed a tendency towards lower BRS and spontaneous indices in juvenile RT, there was no significant difference as compared to C. It appears, therefore, that factors other than decreased baroreflex function may explain the high cardiovascular mortality in RT.

Determinants of baroreflex function

Arterial stiffening has been suggested as a contributing factor to impaired baroreflex function observed in ESRD.[18] Previously, Groothoff *et al.*[17] found abnormal carotid artery function in patients with renal disease since childhood, but they investigated older subjects. In our present work, we demonstrated a profound reduction in carotid elastic function in juvenile HD, and close to normal carotid elasticity in RT. The transduction of blood pressure into baroreceptor stimulus was markedly impaired in HD, since distensibility coefficient was lower by almost 50% as compared to C. Only a part of this difference is attributed to the elevated arterial pressure, as stiffness index β – a pressure-independent measure of arterial elasticity – was higher in HD than in C. We also found that stiffness index β was comparable in juvenile RT and in C. In a recent study, Mitsnefes *et al.*[26] reported decreased carotid elasticity in RT children; however, this was observed only in hypertensive RT, and normotensive RT and C had similar carotid elastic variables.

We have shown earlier that carotid artery distensibility correlated with BRS and spontaneous indices in young healthy adults.[14,27] It has also been demonstrated that in elderly HD the Seq correlated with a global measure of arterial elasticity.[18] Relationship between baroreflex function and carotid artery elasticity in juvenile ESRD patients, however, has never been studied. One of the fundamental new findings of this study is that carotid artery stiffness was

strongly correlated with BRS in HD, and it was the only predictor of BRS in a forward multiple stepwise analysis across all subjects. Presumably due to the combined effects of aging, time spent on dialysis, and time spent since renal trans plantation, we could not demonstrate this relationship within RT. Carotid artery stiffness was also an independent predictor of spontaneous indices, but, according to β-weights, its relative importance to predict spontaneous indices was considerably lower than that predicting BRS.

Decreased heart rate variability may also contribute to impaired baroreflex function in patients with ESRD. Several studies have reported compromised vagal function in HD, which improved after renal transplantation.[19–21] In a longitudinal study, Yildiz *et al.*[23] found in patients of 16–50 years that both time and frequency domain heart rate variability indices improved following transplantation. The recovery may not be complete, however, as Tory *et al.*[22] reported smaller low- and high-frequency RR interval (RRI) variability in RT children as compared to C. Our present data are in line with earlier observations, as we also found decreased heart rate variability in HD, and heart rate variability of RT fell in between those in HD and in C.

In our study, heart rate variability indices closely correlated with spontaneous indices both across and within groups. According to β-weights, the contribution of heart rate variability indices was 1.6–2.8 times higher than the contribution of carotid stiffness to spontaneous indices. On the other hand, heart rate variability indices did not contribute to BRS. Thus, it may be concluded that heart rate variability, while influencing the coupling between spontaneous oscillation of blood pressure and RRI, does not contribute significantly to RRI responses to intravenous bolus phenylephrine.

HD in our study had higher resting blood pressure as compared to RT and C. Hypertension is known to be associated with impaired baroreflex function.[11] Although mean arterial pressure exhibited negative correlation with both BRS and spontaneous indices across all subjects in our study, it was not an independent predictor, and presumably exerted its deleterious effect on baroreflex function through contributing to decreased pressure sensitivity of baroreceptors and compromising heart rate variability.

Limitations

Our negative findings, describing similarity between RT and C, are probably due to the limited number of subjects studied. Indeed, measures of baroreflex function were $\sim$20–35% lower and carotid stiffness was $\sim$20% higher in RT as compared to C. Though none of these differences reach statistical significance, the tendencies may indicate that renal transplantation does not prevent or restore ESRD-associated loss of carotid elasticity and baroreflex function completely. On the other hand, our main conclusions on differences between HD and C were based on highly significant effects. Comparisons between HD and RT, however, were made using a cross-sectional design, which represents another limitation of this study.

Due to ethical considerations, we characterized baroreflex function by measuring BRS with intravenous bolus phenylephrine only in young adult subjects ($\geqslant$18 years), and in children we measured only spontaneous indices. Spontaneous indices are widely used to assess baroreflex function;[28,29] however, they do not correspond with BRS,[30] and, in contrast with BRS, they are highly dependent on heart rate variability.[30,31]

Since HD and RT were on different medications, we must consider this as a confound. Cyclosporine A was found to depress baroreflex function both in animals and in humans.[32,33] Antihypertensive drugs can also modify our variables investigated. We suspended antihypertensive therapy for 12 h prior to study, but we did not intend to stop treatment for any longer period because of ethical reasons.

Conclusion

In conclusion, we found markedly decreased baroreflex function in children and young adults on maintenance hemodialysis, which was partly due to the loss of carotid artery elasticity and impaired heart rate variability. BRS and spontaneous indices were close to normal in young RT, suggesting that renal transplantation in childhood may partly prevent impairment in baroreflex function in juvenile ESRD.

MATERIALS AND METHODS

Subjects

We studied 42 children and young adults aged between 9 and 30 years: 14 patients on maintenance hemodialysis (HD), 14 renal transplant recipient (RT), and 14 healthy controls (C). In all, 24 subjects (eight in each study group) were young adults ($\geqslant$18 years; aged 23.8 ± 3.7 years). All subjects were non-smokers. The causes of renal failure in HD and RT were chronic obstructive uropathy ($n=2$), glomerulonephritis ($n=10$), polycystic kidney disease ($n=5$), nephrosclerosis ($n=5$), interstitial nephritis ($n=4$), and others ($n=2$).

Hemodialyzed patients. In all HD, hemodialysis access was by arteriovenosus graft on one of the arms. The average time spent on dialysis prior to the study was 6.6 ± 5.2 years. HD were dialyzed for 4–6 h three times a week. Investigations were performed before the midweek dialysis. Twelve HD were taking antihypertensive medications (10 receiving calcium channel blockers, 8 receiving diuretics, 6 receiving β-blockers, and 4 receiving angiotensin-converting enzyme inhibitors).

Renal transplant recipients. Inclusion criteria for RT were as follows: absence of primary cardiovascular disease, functioning graft for $\geqslant$1 year with estimated glomerular filtration rate >40 ml/min per 1.73 m^2, and no evidence of rejection. RT were transplanted 6.1 ± 4.6 years prior to the study, and they spent 7.7 ± 5.6 years on HD prior to transplantation (not different from HD). Twelve individuals received kidneys from cadaver and two from living donors. Serum creatinine level was 1.6 ± 0.7 mg/dl, and estimated glomerular filtration rate was 64 ± 21 ml/min per 1.73 m^2. Immunosuppressive drug treatment consisted of steroids in 11 patients, cyclosporine A in 6 patients (dose adjusted to maintain trough levels between 120 and 150 ng/ml), tacrolimus in 7 patients (dose adjusted to maintain trough levels between 5 and 10 ng/ml), and myco-

phenolate mofetil in 10 patients. One RT has undergone bilateral native kidney nephrectomy. Eight RT were receiving antihypertensive medications at the time of the present study (seven receiving calcium channel blockers, five receiving diuretics, four receiving β-blockers, and four receiving angiotensin-converting enzyme inhibitors).

Control subjects. Control subjects were matched for age and gender, were non-obese, normotensive, free of overt autonomic and cardiovascular disease, and were not taking any regular medication.

Subjects gave written consent to participate in the study, which was approved by the Ethical Committee of the Semmelweis University.

Measurements

Radial artery pressure was monitored continuously with an automated tonometric device (Colin CBM-7000, Colin Medical Technology Corporation, Komaki, Japan) for determination of BRS and spontaneous indices of autonomic function. During data collection, the servo-reset mechanism of the apparatus was turned off to permit continuous data acquisition. Systolic and diastolic blood pressure values measured on the brachial artery by an automatic microphonic sphygmomanometer built in the device were used to calibrate the radial pressure pulse.

Common carotid artery pressure was measured by applanation tonometry (SPT-301, Millar Instruments, Houston, TX, USA) for determination of carotid artery elastic parameters. The carotid pulse wave recording was calibrated by brachial diastolic and the electronically determined mean radial pressure values. Diastolic brachial pressure was assigned to the minimum value of the carotid pressure pulse wave and mean pressure to its electrically averaged value. This calibration of the tonometric signal was based on the assumption that mean pressure did not change in the large conduit arteries and that diastolic pressure was not substantially different in the brachial and carotid arteries.[34]

Common carotid artery diameter, its change with the arterial pressure pulse and intima–media thickness were measured 1.5 cm proximal to the bifurcation by ultrasonography using a vessel wall-tracking system combined with a conventional ultrasound scanner (7.5 MHz linear array, Scanner 200 Pie Medical, Maastricht, The Netherlands) previously described in detail.[35,36]

RRI were measured from R wave threshold crossings on continuously recorded ECGs and respiration was recorded with an inductive system (Respitrace System, Ambulatory Monitoring Inc., Ardsley, NY, USA).

Protocol

All subjects were studied in the morning at least 2 h postprandial and at least 24 h after refraining from strenuous exercise and consumption of caffeine or alcohol. After supine subjects were instrumented, they were asked to synchronize their respiratory rate to a metronome beating at 0.25 Hz. RRI and beat-by-beat radial artery pressure were recorded for a 10-min period to determine heart rate variability and spontaneous autonomic indices. Then, carotid artery tonometric pressure and carotid diameter were recorded in five epochs, each containing 4–8 pressure and distension pulses to determine carotid artery elastic parameters. In young adult subjects, BRS was determined using the Oxford technique;[12,15] ECG and beat-by-beat arterial pressure were recorded during the pressure rise induced by intravenous bolus administration of 200 μg phenylephrine. This was repeated three times, with at least 15 min between trials.

Data analysis

Cardiovagal baroreflex function. In young adults ($n = 24$), we determined BRS with the phenylephrine method. After the threshold and/or saturation regions of the sigmoid systolic blood pressure (SBP)–RRI relationship were excluded, the slope of the linear regression line relating RRI to SBP was taken as an index of BRS (Figure 1). Only trials with $r^2 > 0.6$ were used for analysis. All subjects had at least two valid trials, and trials within each subject were averaged to provide a single measure of BRS.

Since determination of BRS was not feasible in minors, we used spontaneous indices to characterize baroreflex function both in children and also in young adults ($n = 42$). The coupling between spontaneous fluctuations in RRI and SBP was determined by the sequence method and by spectral analysis.[28,29] Recordings of 10 min duration were digitized with a commercial software (WinCPRS, Absolute Aliens Oy, Turku, Finland) using a sampling rate of 500 Hz and stored in a personal computer for subsequent off-line analysis. The software detected the ECG R-wave, computed RRI and radial artery SBP time series, and identified spontaneously occurring sequences in which SBP and RRI concurrently increased over three or more consecutive beats. Minimal accepted change was 1 mm Hg for SBP and 5 ms for RRI. Seq was calculated as the mean slope of the regression lines applied to individual sequences with a correlation coefficient > 0.85. After the signals were interpolated, resampled, and their power spectra were determined using fast Fourier transform-based methods, the software calculated LF_{gain} that shows RRI and SBP cross-spectral magnitude in the 0.05–0.15 Hz frequency band, where coherence is > 0.5.

Carotid artery elastic parameters. Carotid artery distensibility coefficient was calculated as $(2\Delta D^{*}D + \Delta D^2)/(\Delta P^{*}D^2)$, where ΔD, D, and ΔP represent pulsatile distension, end-diastolic diameter, and carotid artery pulse pressure, respectively.[37] Distensibility coefficient was used to characterize pressure sensitivity of the baroreceptors. Since distensibility coefficient is highly dependent on blood pressure, we also determined stiffness index β, a pressure-independent measure of carotid artery elasticity. Stiffness index β was calculated as $\beta = (\ln[\mathrm{SBP}/\mathrm{DBP}])/(\Delta D/D)$, where SBP and DBP represent carotid artery systolic and diastolic blood pressures, respectively.[17] We also calculated incremental elastic modulus, a measure of stiffness of the vessel wall material, as $[3 \times (1 + \mathrm{LCSA}/\mathrm{IMCSA})]/\mathrm{DC}$, where LCSA, IMCSA, and DC represent the lumen cross-sectional area, the intima-media cross-sectional area, and distensibility coefficient, respectively.[4]

Heart rate variability. From 10-min time series of RRI, the following time and frequency domain measures of heart rate variability were calculated: the root mean square of successive RRI differences, and the low (0.05–0.15 Hz) and high frequency (0.15–0.4 Hz) powers of RRI variability.[38]

Statistical analysis. Group comparisons were made by one-way analysis of variance and *post hoc* Tukey test. Differences in parameters with non-normal distribution were assessed with Kruskal–Wallis one-way analysis of variance on ranks. Relationships between variables were determined by simple linear regression analysis. To determine independent predictors of BRS and spontaneous indices, we performed multiple stepwise linear regression analysis with variables expressed as z-scores. This method provided β-coefficients, which reflect the relative importance of each independent variable in explaining variance of BRS and of spontaneous indices. Significance was accepted at $P < 0.05$. Data

are expressed as mean ± s.d., non-normally distributed parameters are presented as median (quartiles).

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