Impact of Frequent Nocturnal Hemodialysis on Myocardial Mechanics and Cardiomyocyte Gene Expression

Christopher T. Chan, MD; Sara Arab, PhD; Shemy Carasso, MD; Gil Moravsky, MD; Guo Hua Li, PhD; Peter P. Liu, MD*; Harry Rakowski, MD*

Background—Regression of left ventricular mass with nocturnal hemodialysis has been observed. The influence of nocturnal hemodialysis on myocardial mechanics and cardiomyocyte gene expression is unknown.

Methods and Results—Forty-two patients (30 male:12 female; age, 44±12 years [mean±SD]) with end-stage renal disease were followed for 3.1±1.8 years before and after conversion to nocturnal hemodialysis and were compared with 29 normal subjects (18 male:11 female; age, 48±13 years). Myocardial mechanics were assessed by 2-dimensional velocity vector imaging. Uremic plasma (10%) was added to cultures of neonatal Sprague-Dawley rat ventricular myocytes. Total RNA was isolated from cell cultures and subjected to differential gene expression profiling with specific interest in genes affecting apoptosis and fibrosis. Left ventricular mass index and left atrial volume index decreased from 122.6±42.6 to 98.5±34.9 g/m² (P<0.001) and 25.9±9.1 to 22.5±9.6 cm³/m² (P=0.005), respectively. Left ventricular apical circumferential strain and basal rotation improved after conversion to nocturnal hemodialysis and approximated normal values. Nocturnal hemodialysis increased sessional dialysis dose and lowered parathyroid hormone levels (from 51±67 to 24±37 pmol/L, P<0.05) and phosphate. Under conventional hemodialysis conditions, there was an upregulation of genes leading to apoptosis and fibrosis in cardiomyocytes. The change in left ventricle rotation was associated with the change in parathyroid hormone values (r=0.37, P=0.02) and to the change in left ventricle mass (r=0.31, P=0.046).

Conclusions—Frequent hemodialysis is associated with improvement in myocardial mechanics and cardiac gene expression profile, which warrants prognostic validation. (Circ Cardiovasc Imaging. 2012;5:474-480.)

Key Words: cardiomyocyte gene expression ■ diastolic function ■ frequent hemodialysis ■ myocardial mechanics

Left ventricular hypertrophy is prevalent in end-stage renal disease (ESRD) and contributes to the high annual mortality rate seen in patients on dialysis.1 Although conventional hemodialysis (CHD; 3 times per week, 3–4 hours per session) is the standard renal replacement therapy in North America, it does not correct abnormal left ventricular geometry.

Clinical Perspective on p 480

Epidemiological and experimental studies have documented the rising burden of diastolic heart failure as a consequence of impaired left ventricular (LV) compliance.2,4 which has been in part attributed to hypertrophy and fibrosis of the left ventricle.5,9 Multiple risk factors are known to be associated with left ventricular hypertrophy in ESRD including volume and pressure overload, vascular stiffening, and neurohormonal activation.3,9 Similarly, several mediators of myocardial fibrosis have been implicated in ESRD, namely, increased myocardial collagen turnover, cardiac ischemia, norepinephrine, angiotensin II, aldosterone, and parathyroid hormone (PTH).10-13 Of interest, PTH is known to activate the interstitial cell leading to cardiac fibrosis.13 Most recently, other mediators of calcium/phosphate metabolism such as fibroblast growth factor 23 were also shown to correlate with left ventricular hypertrophy in patients with chronic kidney disease14 and were shown to be an independent predictor of death in the dialysis population.15 Hence, genes that lead to cardiomyocyte fibrosis and apoptosis are of particular mechanical interest.

Two-dimensional deformation analysis is a tool to quantify regional and global ventricular mechanics by echocardiography.16-18 LV strain and rotation derived from 2-dimensional deformation analysis have been applied in multiple cardiac populations19,20 and were shown to have significant prognostic value in high-risk individuals such as heart transplant recipients.21

Given that observational22 and randomized controlled studies23 have documented regression of left ventricular mass by nocturnal home hemodialysis (NHD; 5–6 sessions per week,
Subjects included consecutive eligible patients who were converted to NHD at the University Health Network. Patient demographic information such as age, sex, ethnicity, etiology of ESRD, and comorbid conditions was prospectively collected into a computerized clinical database. Medications were recorded and are presented in Tables 1 and 2. Clinical assessment, including weight, height, and blood pressure measurements, was performed at baseline and monthly after conversion to NHD. All blood pressure measurements were made at the patients’ home using a calibrated blood pressure cuff after 5 minutes of rest in the sitting position. Biochemical and hematologic parameters (complete blood count, urea, creatinine, albumin, calcium, phosphate, and PTH) were obtained monthly during the same time intervals. Baseline studies were performed the morning after a conventional hemodialysis day (ie, a minimum of 18 hours after dialysis). To minimize circadian variation, and replicate steady-state NHD conditions, subsequent experiments were performed at the same time of day (a minimum of 4 hours after the regular NHD session). Forty-two patients (30 male:12 female; age, 44±12 years) with ESRD were studied.

Diagnosis Protocol

This protocol was approved by the Research Ethics Board of the Toronto General Hospital, University Health Network, Toronto, Canada, and conformed to the standards established by the Declaration of Helsinki. Medically stable patients with ESRD (age between 18 and 85 years) who had received a minimum of 3 months of CHD and were training for NHD were invited to participate in this study. None of the patients had any acute illness, hospitalization, or symptomatic cardiovascular disease (including congestive heart failure and acute coronary syndrome). Normal subjects were also recruited for echocardiographic studies. Written informed consent was obtained from each patient. Pregnant patients were excluded.

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Echocardiographic Doppler Studies

Echocardiographic studies were interpreted by a cardiologist blinded to patients’ dialysis prescription. Two-dimensional, Doppler, and tissue Doppler imaging parameters were measured according to the guidelines of the American Society of Echocardiography. Left atrial (LA) pressure was estimated using the Nagueh method.

Strain, Strain Rate, and Rotation Evaluation

Measurements were performed using velocity vector imaging software (Siemens Medical Systems, Mountainview, CA). Velocity
vector imaging quantified myocardial motion from B-mode clips by automatically tracking user-defined points to define the inward and outward motion of the myocardial subendocardial regions. Two-dimensional tissue velocity was computed. Strain and strain rate were computed by the change in the relative distance between localized tracked trace points. Strain was defined as the instantaneous local trace lengthening or shortening and strain rate as the rate of lengthening shortening. Circumferential strain, strain rate, and rotation were measured at 3 parasternal short axis planes (base, mid, and apex, 6 segments per level). Longitudinal wall strains and strain rates were measured from the apical 2-, 3-, and 4-chamber views, 6 segments per view. As a measure of diastolic relaxation, we measured the early apical reverse rotation angle difference—from peak rotation to 10% into diastole.16

Intraobserver Variability

Two-dimensional strain measurements were done by one observer (S.C.). We reanalyzed 10 studies to evaluate intraobserver variability. For peak strain, the intraobserver agreement was 0.9.

Gene Expression Protocol

Neonatal Rat Ventricular Myocyte Culture Preparation

Neonatal Sprague-Dawley (Charles River, Montreal, Canada) rat ventricular myocytes were isolated and cultured as described previously.29 A single litter of 1- to 2-day-old rats was used for each experiment. Pups were sacrificed by cervical dislocation and the hearts removed quickly into filter-sterilized buffer. Using an aseptic technique, atria and blood vessels were removed and the ventricles minced. Ventricular tissue was dissociated at room temperature with 5-bromo-2-deoxyuridine (0.1 mmol/L; Sigma). Cells were incubated at 37°C in a humidified atmosphere of 1.5% CO2. After 24 hours in culture, the medium was replaced by serum-free medium and 24 hours after serum starvation, each cell group was treated with 10% human plasma (under CHD, NHD, or normal conditions) or with phenylephrine as a positive control.

Gene Expression Profiling

Total RNA was isolated from neonate cardiomyocytes as described previously after treatment with 10% human plasma (under CHD and NHD conditions; n=3 for each group). Reverse transcription was performed using Superscript II according to the manufacturer’s instruction. Gene expression levels were quantified by real-time polymerase chain reaction. All reactions were run in triplicate. The primers used for this study are shown in Table I in the online-only Data Supplement. The ratio of the level of each gene measured to glyceraldehyde-3-phosphate dehydrogenase was estimated with the ΔCT (Ct_gadp–Ct_145095) method. The relative gene expression (fold change with respect to baseline) was estimated with the 2−ΔΔCT method.10

Data Analysis

Descriptive data are presented as mean±SD. The primary outcome measure was the within-subject paired difference in LV systolic and diastolic myocardial mechanics before and after conversion from CHD to NHD. Secondary outcomes included within- and between-subject differences in LV mass, LA volume, and biochemical and hemodynamic parameters. Differentially expressed genes were identified as described before and after conversion to NHD. Wilcoxon test was used for comparison of paired continuous variables. Mann-Whitney U test was used for comparison of continuous variables between 2 groups. Analysis of variance was used for multiple comparisons of a continuous variable among 3 groups of subjects. Spearman correlation was used to investigate potential associations between variables of interest. All statistical tests were 2-tailed with a probability value <0.05 taken to indicate significance. SPSS 10 (SPSS Inc, Chicago, IL) was used for all statistical analyses.

Results

Forty-two patients (30 male:12 female; age, 44±12 years) with ESRD were studied. The mean time of follow-up was 3.1±1.8 years. Their comorbid conditions were listed in Table 1. Ninety percent of our study population had a diagnosis of hypertension and was prescribed antihypertensive medications. Twenty-nine subjects matched for age and sex distribution (18 male:11 female; age, 48±13 years) were studied. None of the normal subjects had any chronic illness. NHD increased s机关 dialysis dose and frequency doubled. In addition, NHD lowered parathyroid hormone levels (from 51±67 to 24±37 pmol/L, P<0.05) and phosphate concentration (from 1.42±0.4 to 1.24±0.4 mmol/L, P<0.05). Despite having a significant reduction in the number of antihypertensive medications (2.5–0.5 classes per patient, P<0.05), systolic blood pressure tended to fall (from 132±20 to 124±14, P=0.07) and diastolic blood pressure fell (from 81±11 to 75±10, P=0.01) after conversion to NHD (Table 2). Differences in systolic and diastolic mechanics were presented in Table 3. At baseline, compared with normal subjects, patients with ESRD exhibited greater LV mass, LA diameter, and elevated mitral E and A velocities. After conversion to NHD, LV mass index and LA volume index decreased from 122.6±42.6 to 98.5±34.9 g/m2 (P<0.001) and 25.9±9.1 to 22.5±9.6 cm3/m2 (P=0.005), respectively. Mitral E and A velocities tended to decrease (P=0.1 and P=0.08, respectively). Average longitudinal strain did not change between treatment groups and remained mildly decreased

function, biological process, and cellular component. Raw data from microarray experiments was submitted to the Gene Expression Omnibus database (www.ncbi.nlm.nih.gov/geo) and the GEO accession ID is GSE28723.

Reverse Transcription and Real-Time Quantitative Polymerase Chain Reaction

Total RNA was isolated from neonate cardiomyocytes as described previously after treatment with 10% human plasma (under CHD and NHD conditions; n=3 for each group). Reverse transcription was performed using Superscript II according to the manufacturer’s instruction. Gene expression levels were quantified by real-time polymerase chain reaction. All reactions were run in triplicate. The primers used for this study are shown in Table I in the online-only Data Supplement. The ratio of the level of each gene measured to glyceraldehyde-3-phosphate dehydrogenase was estimated with the ΔCT (Ct_gadp–Ct_145095) method. The relative gene expression (fold change with respect to baseline) was estimated with the 2−ΔΔCT method.10

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LV mass and its functional consequences.

uremic sera under CHD and NHD conditions; and (3) identifying a unique genomic signature in cardiomyocytes exposed to

LV mass, g 130±25 224±84† 184±70‡
LVMI, g/m² 71±11 121±43† 98±35‡
LA systolic diameter, cm 3.5±0.3 4.0±7† 3.7±6∗
Mitral E wave velocity, m/s 0.73±0.2 0.88±0.3† 0.80±0.2
Mitral A wave velocity, m/s 0.68±0.2 0.82±0.3† 0.74±0.2
Mitral annular E’ velocity, m/s 0.10±0.02 0.13±0.05† 0.14±0.05
LV longitudinal strain, % −20±2 −17±4† −17±4‡
LV base circumferential strain, % −25±4 −21±6∗ −21±6‡
LV apex circumferential strain, % −29±4 −26±8† −29±7∗
Base rotation,° −4.0±2.5 −5.9±2.6† −4.7±2.6∗
Apex rotation,° 7.2±3.6 5.4±3.0† 6.8±3.3∗
Reapical early diastolic (10%) reverse rotation,° 4.33±2.14 4.08±2.55 5.38±2.68∗

Variable Normal (n=29) CHD (n=42) NHD (n=42)

Data represented as mean±SD. CHD indicates conventional hemodialysis; NHD, nocturnal hemodialysis; LV, left ventricular; LVMI, left ventricular myocardial infarction; LA, left atrial.

The importance of impaired LV compliance in the ESRD population has led to extensive search for accurate noninvasive methods of quantifying its severity. To date, there is no single validated gold standard to assess for impaired LV relaxation. Traditionally, transmitral Doppler E/A ratio has been used to quantify the degree of impairment in LV relaxation. However, there is a wealth of published literature that has suggested that there is significant dependence between cardiac preload conditions and E/A ratio, especially in patients with ESRD.31,32 Given that there is a complex interaction between cardiac preload and “dry weight” in all patients with ESRD, E/A ratio may be a suboptimal measure to quantify the severity of LV noncompliance. The examination of LV mechanics may quantify the severity of impaired LV relaxation without the potential confounding effect of extracellular volume control. Furthermore, several investigators have proposed that analysis of LV myocardial mechanics may provide a more sensitive examination of LV compliance before a change in E/A ratio occurred.33,34 In the present report, we observed improvements in several indices of LV mechanics, regression of LV mass, and reduction of LA volume index. Myocardial shortening occurs in both the longitudinal and circumferential planes with a normal base to apex strain gradient with higher apical circumferential strain. Abnormalities in myocardial mechanics have been shown to occur well before changes in LV ejection fraction. In this study, with more frequent dialysis, longitudinal strain remained mildly decreased but apical circumferential strain increased in keeping with improving systolic myocardial function. Basal rotation returned to normal levels with increased apical rotation, thus improving the wringing action of the myocardium in systole. It is reasonable to speculate that left ventricular circumferential function (strain, rotation) is the principal compensatory mechanism that preserves LV systolic function as reported in other cardiac pathologies.19,33,34 Improvement in diastolic myocardial performance also occurred with faster reverse rotation in early diastole, a finding that is relatively independent of filling pressures. Structurally, myocardial fiber orientation spans vertically at the subendocardium and horizontally at the subepicardium to maintain longitudinal and circumferential LV function, respectively. Thus, localized abnormalities may interfere with longitudinal and circumferential function differentially.35–38

Our results suggest that there is a potential mechanistic link among PTH control, LV mechanics, and LV mass. Hyperphosphatemia and hyperparathyroidism are established independent cardiovascular risk factors in ESRD.39 It is generally accepted that hyperphosphatemia is an important factor in the development of uremic calcific vasculopathy, which in turn will increase cardiac afterload and contributes directly to the progression of left ventricular hypertrophy.40,41 Our present results support the notion that improved control of PTH may lead to a reduction in intermyocardial fibrosis, thereby resulting in improved LV myocardial mechanics.

Our genomic results also support the hypothesis that there are soluble factors in uremic serum, which may modify cardiac gene expression patterns. It is interesting to note that uremic serum was linked to genes responsible for apoptosis and fibrosis of cardiomyocytes, which in turn may result in impaired
LV relaxation. In fact, expressions of proapoptotic factors (eg, Bax, p53, Fas) have been linked consistently with progression of heart failure in the non-ESRD population. In addition, our results suggest that contractility may be modified by NHD through upregulation of S100A1, which is a calcium binding protein that is expressed in cardiac muscle. S100A1 modulates calcium homeostasis, energy metabolism, and contractile performance of the heart. Downregulation of S100A1 in cardiomyocytes after myocardial infarction has been linked to reduced cardiac reserve and development of heart failure. It is equally intriguing to note that in a pilot cohort study, patients with ESRD and refractory heart failure exhibited improvement in ejection fraction after conversion from CHD to NHD.

In summary, we have evidence to support the concept that augmented uremia clearance using NHD is accompanied by an improvement in LV strain, LV rotation, a reduction in LV mass, and LA volume index. Increases in frequency and duration of dialysis are also associated with a downregulation of genes responsible for cardiomyocyte apoptosis and fibrosis and an upregulation of S100A1, which may improve LV contractility. Our results are limited by their observational nature. Additional experiments using other cardiomyocyte functional assays and serum-mixing strategies are required to improve our basic understanding of the influence of uremia on cardiac function and geometry. Future work determining the influence of frequent hemodialysis on LV mass regression and the effects on cardiac gene expression is warranted. The use of a stable ESRD population and the lack of a randomized controlled design is consistent with the pilot nature of the results;

Table 4. Microarray Exploration of Genes That Have Altered Expression in Cardiomyocytes Under Different Uremic Conditions

<table>
<thead>
<tr>
<th>Gene</th>
<th>No. of Fold Change (CHD versus NHD)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdkn1a (cyclin-dependent</td>
<td>2.1-fold increase (CHD)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>kinase inhibitor 1A)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cdkn1c (cyclin-dependent</td>
<td>2.7-fold increase (CHD)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>kinase inhibitor 1C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fas</td>
<td>2.4-fold increase (CHD)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bax (Bcl2-associated X protein)</td>
<td>2.5-fold increase (CHD)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>S100a1 (S 100 calcium binding protein A1)</td>
<td>2.1-fold increase (NHD)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

CHD indicates conventional hemodialysis; NHD, nocturnal hemodialysis.
however, given the important potential clinical implications of LV geometry, function, and genomic signature in ESRD, we believe our work adds support to the growing benefits of frequent hemodialysis in patients with ESRD.

Sources of Funding
This study was supported by the Baxter Extramural Grant Program. Part of the gene expression array analysis is supported through grants from the Heart & Stroke Foundation and the Canadian Institutes of Health Research.

Disclosures
None.

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**CLINICAL PERSPECTIVE**

Diastolic dysfunction is common in patients with end-stage renal disease. Myocardial mechanics were assessed by 2-dimensional velocity vector imaging in 29 normal subjects and in 42 patients with end-stage renal disease before and after conversion from conventional hemodialysis (3 times a week, 4 hours per session) to nocturnal hemodialysis (5–6 nights a week, 6–8 hours per session). Gene expression profile from neonatal Sprague-Dawley rat ventricular myocytes was also examined before and after conversion to nocturnal hemodialysis. With nocturnal hemodialysis, left ventricular mass index and left atrial volume index decreased. Left ventricular apical circumferential strain and basal rotation improved after conversion to nocturnal hemodialysis and approximated normal values. Under conventional hemodialysis conditions, there was an upregulation of genes leading to apoptosis and fibrosis in cardiomyocytes. More frequent hemodialysis could be expected to improve left ventricular mechanics in patients with end-stage renal disease, which may further prevent important cardiovascular complications.
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_Circ Cardiovasc Imaging_. 2012;5:474-480; originally published online May 30, 2012;
doi: 10.1161/CIRCIMAGING.111.971606
_Circulation: Cardiovascular Imaging_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-9651. Online ISSN: 1942-0080

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### Supplemental Table. Primers used for Real Time Quantitative PCR

<table>
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<th>PF</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdkn1a</td>
<td>5' – GCAGACCAGCTGACAGATT – 3’</td>
<td>5’ – CTCCAGAACCACAGCAGAAGA – 3’</td>
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<tr>
<td>Cdkn1c</td>
<td>5’ – AATCAGCCAGCTTCGACCAT – 3’</td>
<td>5’ – TGGGAAGGTATCGCTGGAGG – 3’</td>
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<tr>
<td>Fas</td>
<td>5’ - CGGTGGTATTTTTTCATGGTTCTG - 3'</td>
<td>5’ - TGAACTCAGGGTAGTGTGCCA - 3’</td>
</tr>
<tr>
<td>Bax</td>
<td>5’ - TGGAGCTGAGAGGATGATTG - 3’</td>
<td>5’ - CCCAGTTGAAGTTGCCATCAG - 3’</td>
</tr>
<tr>
<td>S100a1</td>
<td>5’ - CCAACCCGTGTGCTGCTGAA –3’</td>
<td>5’ - TTTGCCCTCCTCTTGCCC - 3’</td>
</tr>
</tbody>
</table>

Cdkn1a: cyclin-dependent kinase inhibitor 1A, Cdkn1c: cyclin-dependent kinase inhibitor 1C, Bax: Bcl2-associated X protein, S100a1: S 100 calcium binding protein A1