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
Table 1. Demographic and Clinical Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy Control</th>
<th>ESRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>29</td>
<td>42</td>
</tr>
<tr>
<td>Age, y</td>
<td>48±13</td>
<td>44±12</td>
</tr>
<tr>
<td>Sex, male:female</td>
<td>18:11</td>
<td>30:12</td>
</tr>
<tr>
<td>Etiology of ESRD</td>
<td>Not applicable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glomerular disease: 18 (43%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HTN: 5 (12%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Type 2 diabetes: 5 (12%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PCKD: 4 (10%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other: 9 (21%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unknown: 1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>Not applicable</td>
<td>30</td>
</tr>
<tr>
<td>Baseline hyperparathyroidism (PTH &gt;30 pmol/L), %</td>
<td>Not applicable</td>
<td>40</td>
</tr>
<tr>
<td>Antihypertensive medications</td>
<td>Not applicable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACEI or ARB: 36%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcium channel blocker: 25%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alpha blocker: 11%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beta-blocker: 14%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vasodilator: 14%</td>
<td></td>
</tr>
</tbody>
</table>

Data represented as mean±SD.

ESRD indicates end-stage renal disease; PTH, parathyroid hormone; HTN, hypertension; PCKD, polycystic kidney disease; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Table 2. Clinical Variables Before and After Conversion From CHD to NHD

<table>
<thead>
<tr>
<th>Variable</th>
<th>CHD</th>
<th>NHD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>eKt/V per session</td>
<td>1.20±0.2</td>
<td>2.2±0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Predialysis urea, mmol/L</td>
<td>19±6</td>
<td>13±6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Postdialysis urea, mmol/L</td>
<td>6±3</td>
<td>2±1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>132±20</td>
<td>124±14</td>
<td>0.07</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>81±11</td>
<td>73±10</td>
<td>0.01</td>
</tr>
<tr>
<td>Plasma hemoglobin, g/L</td>
<td>114±15</td>
<td>125±12</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Darbepoetin alpha dose, µg/wk</td>
<td>31±7</td>
<td>34±13</td>
<td>0.84</td>
</tr>
<tr>
<td>Predialysis calcium, mmol/L</td>
<td>2.4±0.06</td>
<td>2.4±0.10</td>
<td>0.99</td>
</tr>
<tr>
<td>Predialysis phosphate, mmol/L</td>
<td>1.42±0.4</td>
<td>1.24±0.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PTH, pmol/L</td>
<td>51±67</td>
<td>24±37</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Data represented as mean±SD.

CHD indicates conventional hemodialysis; NHD, nocturnal hemodialysis; eKt/V, equilibrated Kt/V (where K=delivered clearance, t=dialysis time, and V=urea distribution volume); PTH, parathyroid hormone.

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. A description of our study patient cohort is outlined in Table 1.

Normal subjects were derived from healthy subjects referred for echocardiography to evaluate minor symptoms or murmurs who had normal echocardiographic findings and healthy volunteers. Twenty-nine subjects matched for age and sex distribution (18 male:11 female; age, 48±13 years) were included in the present study (Table 1).

Dialysis Protocol

Patients on NHD received hemodialysis at home for 6 to 8 hours 5 to 6 nights per week. Vascular access was achieved through either a long-term internal jugular catheter (Uddall Catheter; Cook Critical Care, Bloomington, IN) or an arteriovenous fistula. Dialysate flow rate of 350 mL/min and blood flow rate of 200 to 300 mL/min were used. F80 polysulfone dialyzers (Fresenius Medical Care, Lexington, MA) or Exceltra 120 dialyzers (Baxter, Chicago, IL) were used. Patients on CHD received hemodialysis for 4 hours 3 times per week through similar vascular access. A blood flow rate of 400 mL/min, a dialysate flow rate of 500 to 750 mL/min, and F80 polysulfone dialyzers (Fresenius Medical Care) were used. Unfractionated heparin was used for anticoagulation on CHD and NHD.

Dialysis dose per treatment was estimated by equilibrated Kt/V (eKt/V) as described by Daugirdas and colleagues in which eKt/V =spKt/V−0.6(spKt/V)/t+0.03 (spKt/V=single pool Kt/V; K=delivered clearance; t=dialysis time; and V=urea distribution volume).24 Single pool Kt/V was determined using blood urea reduction ratio.25

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.25 Left atrial (LA) pressure was estimated using the Naghieh method.25

Strain, Strain Rate, and Rotation Evaluation

Measurements were performed using velocity vector imaging software (Siemens Medical Systems, Mountainview, CA).16,18,24 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.46

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.47 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 by enzymatic digestion using 0.1 mg/mL collagenase (Type III, Sigma). Cell suspensions were collected 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.

Gene Expression Profiling
Total RNA was isolated from cells (46 samples) using Trizol Reagent (GIBCO/BRL) following the manufacturer’s protocol on Day 5. The quality of total RNA was assessed by an Agilent 2100 Bioanalyzer (Version A.02.01S1232; Agilent Technologies). Only RNA with the following the manufacturer’s protocol on Day 5. The 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.47 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 by enzymatic digestion using 0.1 mg/mL collagenase (Type III, Sigma). Cell suspensions were collected 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.

Gene Expression Profiling
Total RNA was isolated from cells (46 samples) using Trizol Reagent (GIBCO/BRL) following the manufacturer’s protocol on Day 5. The quality of total RNA was assessed by an Agilent 2100 Bioanalyzer (Version A.02.01S1232; Agilent Technologies). Only RNA with the outer diameter ratio of 1.99 to 2.0 at 260/280 was used for microarray analysis. A total of 16 hybridizations was performed on the Rat GeneChip ST (Affymetrix, Santa Clara, CA). Samples were prepared for hybridization according to standard Affymetrix instructions and performed at the Genomic Core Facility at the Hospital for Sick Children, Toronto, Ontario, Canada.

Analysis of all data derived from arrays was performed using data obtained from GCOS (GeneChip Operating software). The pivot table was imported into GeneSpring GX 11 (www.chem.agilent.com). Hierarchical clustering (condition tree) using a Pearson correlation similarity measure with average linkage was used to produce a dendrogram. The pivot table was also imported into Partek Genomics Suite software (Partek Inc, St Louis, MO) to produce a principal components analysis plot as a second test for grouping of biological replicates. Statistical analysis was based on a regression model and analysis of variance to examine for signiﬁcant genes between conditions. False discovery rate corrections were applied to reduce the total number of false-positives. Gene ontology was used for categorizing expressed genes with respect to gene function, including molecular 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 relative gene expression (fold change with respect to baseline) was estimated with the 2−ΔΔCT method.30

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 sessional 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.
compared with controls. However, regional LV apical circumferential strain improved, and basal rotation decreased after conversion to NHD and approximated normal values (Figure 1). The change in LV rotation was associated with the change in PTH values ($r=0.37$, $P=0.02$) and to the change in LV mass ($r=0.31$, $P=0.046$).

Results from differential gene profiling by microarray examination and confirmed by quantitative real-time polymerase chain reaction were displayed in Table 4 and Figure 2, respectively. Overall, intensification of hemodialysis dose was associated with a coordinated downregulation of genes, which are responsible for apoptosis and fibrosis (Cdkn1a [cyclin-dependent kinase inhibitor 1A], Cdkn1c [cyclin-dependent kinase inhibitor 1C], Fas, Bax [Bcl2-associated X protein]). In addition, a gene associated with cardiac contractility, S100a1 (S 100 calcium binding protein A1), was increased after escalating dialysis dose and frequency.

### Discussion

Regression of left ventricular mass has been observed with frequent hemodialysis. This study represents the first attempt to examine the impact of NHD on LV myocardial mechanics and cardiac gene signatures. These data add to the emerging benefits of frequent hemodialysis by (1) quantifying LV mechanics before and after conversion to NHD; (2) documenting a unique genomic signature in cardiomyocytes exposed to uremic sera under CHD and NHD conditions; and (3) identifying associations between PTH control and the evolution of LV mass and its functional consequences.

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. 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. 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. 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.

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. 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. 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;
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.

References


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

Christopher T. Chan, Sara Arab, Shemy Carasso, Gil Moravsky, Guo Hua Li, Peter P. Liu and Harry Rakowski

_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

Copyright © 2012 American Heart Association, Inc. All rights reserved.

Print ISSN: 1941-9651. Online ISSN: 1942-0080

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://circimaging.ahajournals.org/content/5/4/474

Data Supplement (unedited) at:

http://circimaging.ahajournals.org/content/suppl/2012/05/30/CIRCIMAGING.111.971606.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation: Cardiovascular Imaging_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:

http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation: Cardiovascular Imaging_ is online at:

http://circimaging.ahajournals.org/subscriptions/
## Supplemental Table. Primers used for Real Time Quantitative PCR

<table>
<thead>
<tr>
<th></th>
<th>PF</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdkn1a</td>
<td>5’ – GCAGACCAGCCTGACAGATTT – 3’</td>
<td>5’ – CTCCAGACCCACACGAGAAGA – 3’</td>
</tr>
<tr>
<td>Cdkn1c</td>
<td>5’ – AATCAGCCAGCCTTCGACCAT – 3’</td>
<td>5’ – TGGGAAGGTATCGCTGGAGG – 3’</td>
</tr>
<tr>
<td>Fas</td>
<td>5’ - CGGTGGTATTTTTTATGGTTCTG - 3’</td>
<td>5’ - TGAACCTCACGGAGTTCTGCCA - 3’</td>
</tr>
<tr>
<td>Bax</td>
<td>5’ - TGGAGCTGCAGAGGATGATGG - 3’</td>
<td>5’ - CCCAGTTGAAGTTGCCATCAG - 3’</td>
</tr>
<tr>
<td>S100a1</td>
<td>5’- CCAACCCTGTGCTGCTGAA –3’</td>
<td>5’- TTTGTCCCTTCCTTGCCC - 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