Myocardial Steatosis and Left Ventricular Contractile Dysfunction in Patients With Severe Aortic Stenosis

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Background—Aortic stenosis (AS) leads to left ventricular (LV) hypertrophy and dysfunction. We hypothesized that cardiac steatosis is involved in the pathophysiology and also assessed whether it is reversible after aortic valve replacement.

Methods and Results—Thirty-nine patients with severe AS (symptomatic=25, asymptomatic=14) with normal LV ejection fraction and no significant coronary artery disease and 20 age- and sex-matched healthy controls underwent cardiac 1H-magnetic resonance spectroscopy and imaging for the determination of steatosis (myocardial triglyceride content) and cardiac function, including circumferential strain (measured by magnetic resonance tagging). Strain was lower in both symptomatic and asymptomatic AS (−16.4±2.5% and −18.1±2.9%, respectively, versus controls −20.7±2.0%, both P<0.05). Myocardial steatosis was found in both symptomatic and asymptomatic patients with AS (0.89±0.42% in symptomatic AS; 0.75±0.36% in asymptomatic AS versus controls 0.45±0.17, both P<0.05). Importantly, multivariable analysis indicated that steatosis was an independent correlate of impaired LV strain. Spectroscopic measurements of myocardial triglyceride content correlated significantly with histological analysis of biopsies obtained during aortic valve replacement. At 8.0±2.1 months after aortic valve replacement, steatosis and strain had recovered toward normal.

Conclusions—Pronounced myocardial steatosis is present in severe AS, regardless of symptoms, and is independently associated with the degree of LV strain impairment. Myocardial triglyceride content measured by magnetic resonance spectroscopy correlates with histological quantification. Steatosis and strain impairment are reversible after aortic valve replacement. Our findings suggest a novel pathophysiological mechanism in AS, myocardial steatosis, which may be amenable to treatment, thus potentially delaying onset of LV dysfunction. (Circ Cardiovasc Imaging. 2013;6:808-816.)

Key Words: aortic valve stenosis ■ cardiac MRI ■ myocardial steatosis ■ spectroscopy ■ strain

Aortic valve stenosis (AS) is characterized by pressure overload of the left ventricle (LV), resulting in compensatory LV hypertrophy (LVH). Previous studies have shown that the hypertrophied heart, such as in AS, undergoes a shift in substrate utilization, with a preference to glucose metabolism and downregulation of fatty acid (FA) oxidation.1

Under normal conditions, excess FAs are sequestered as triglycerides (TGs) and stored in adipocytes as lipid droplets, with a small amount also stored in nonadipose tissues, such as the myocardium, where levels are tightly controlled.2 However, there is increasing evidence implicating altered myocardial substrate utilization and consequent excessive TG accumulation (steatosis) in myocardial disease.2,3 Previous studies have assessed the presence of myocardial steatosis using magnetic resonance spectroscopy (MRS) and examined its functional associations in obesity, impaired glucose tolerance, type 2 diabetes mellitus, and in normal individuals when subjected to prolonged exercise and diet restrictions.4–7 These studies have shown that myocardial TG content can be an independent correlate of both systolic and diastolic function, implying a causal relationship between steatosis and such functional changes. Measuring myocardial TG content (MTC) may be invasive and unsuitable as a routine diagnostic test.8 H MRS is

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a noninvasive technique that can be used to quantify MTC in vivo and may be more suitable for routine diagnosis.

To date, no MRS studies have assessed the presence of steatosis and its relationship to indices of myocardial function in patients with AS. Although LV ejection fraction (LVEF) is the most commonly used method to assess myocardial function, it is not a sensitive measure of systolic dysfunction in AS. In contrast, myocardial strain is a measure of myocardial deformation, which represents an index of contractility, and has been shown to be impaired in AS despite normal LVEF.

Myocardial strain can be measured using noninvasive cardiovascular magnetic resonance (CMR) tagging.

Therefore, the purpose of the present study was 3-fold: first, to assess whether myocardial steatosis is present in patients with severe (both symptomatic and asymptomatic) AS, by measuring MTC using 1H MRS, validated against histological analysis. Second, to study the association between steatosis with myocardial strain as assessed by MR tagging and to test whether steatosis independently predicts myocardial dysfunction. Third, to evaluate changes in cardiac steatosis and strain after aortic valve replacement (AVR) and to test for reversibility of steatosis and strain changes.

Methods

Study Population

Thirty-nine patients with isolated severe AS (25 symptomatic, 14 asymptomatic) were prospectively recruited from the Oxford University Hospital National Health Service Trust. Symptomatic patients were being worked-up for AVR and had unobstructed coronary arteries on coronary angiography. The 14 asymptomatic patients (as judged by their treating physician) also had an exercise stress test to confirm this. Inclusion criteria included an aortic valve area ≤1.0 cm² with no more than mild aortic regurgitation or other valvular pathology, systolic blood pressure <160 mm Hg, and diastolic blood pressure <90 mm Hg. Patients who had LVEF ≤50%, contraindications to MRI, glomerular filtration rate <30 mL/min, underlying cardiomyopathy, previous myocardial infarction, coronary revascularization, or previous cardiac surgery were excluded. Twenty healthy age- and sex-matched volunteers served as control and had no history of heart disease, diabetes mellitus, hypertension, or high cholesterol and were not taking any medications. They had a normal physical examination and ECG. All subjects gave informed consent to participate in the study, which was approved by the National Research Ethics Committee system.

Study Protocol

All study subjects underwent echocardiography, cardiac MRI, cardiac MR tagging, and 1H MRS. Venous blood was drawn for nonesterified FAs, which is a measure of free FA, and for other routine laboratory parameters. Within the subsequent 2 to 8 weeks, AVR was performed on the 25 patients with symptomatic AS. For patients who consented for tissue biopsies and where it was technically feasible, endomyocardial biopsies were taken intraoperatively from the LV outflow tract. To minimize sampling inaccuracies, biopsies were taken from the LV septum where spectroscopic measurements (lipid) were acquired. The samples were sent to the histopathology laboratory for lipid analysis. One section of the heart was fixed in 10% buffered formalin and another section was placed in formalin and stained with hematoxylin and eosin. Semiquantitative analysis of lipid deposition was performed by a pathologist who was blinded to the results of lipid content obtained from MRS measurements. Lipid deposition was graded as follows: (0) no positive staining, (+1) scantly ORO-positive droplets, and (≥2) extensive ORO-positive droplets.

Statistical Analysis

All data are expressed as mean±SD and were checked for normality using Kolmogorov–Smirnov test. Categorical data are presented as numbers and percentages. Comparisons between the 3 groups were performed by 1-way ANOVA with post hoc Bonferroni corrections. The χ² test or Fisher exact test was used to compare categorical data as appropriate. Bivariable correlations were performed using the Pearson or Spearman method as appropriate. Multivariable linear regression analysis was used to identify independent correlates of systolic strain and diastolic strain rates. To generate the multivariable models, only those variables with significant correlations on bivariable analyses were entered as covariates. Comparisons between pre- and post-AVR measurements in patients with AS were performed with 2-tailed paired t test. Comparisons between patients with AS and controls at baseline and post-AVR were performed using Student post hoc Bonferroni corrections.
Results

Baseline Study Characteristics
Demographic, clinical, echocardiographic, and biochemical data are shown in Table 1. There were no significant differences in age, sex, body mass index, blood pressure, and heart rate among study groups. Blood glucose and biochemical lipid parameters were similar across all groups.

Assessment of LV Mass and Function
Cardiovascular MRI results for LV volumes and function results are summarized in Table 2. As anticipated, patients with AS had significantly higher LV mass index (LVMi) and increased LV wall thickness as a result of LV remodeling and high normal LVEF compared with the normal controls. However, despite normal LVEF, systolic circumferential strain and longitudinal strain were impaired in asymptomatic patients with AS and further deteriorated in symptomatic AS.

Assessment of LV Diastolic Function
Cardiovascular MRI results showed that circumferential and longitudinal diastolic strain rates were also impaired in AS compared with normal volunteers. Echocardiographic parameters showed that there was a stepwise increase in E/E' ratio, with the greatest increase seen in the symptomatic AS group, whereas the ratio of mitral peak velocity of early filling (E) to mitral peak velocity of late filling (A) were identical in all the study groups (Table 1).

Assessment of Myocardial Fibrosis Using Late Gadolinium Imaging
Areas of LGE were identified in the majority of patients with severe AS, with the greatest prevalence in symptomatic AS (80%) versus 64% in the asymptomatic group. The predominant pattern of LGE was patchy, involving mainly the basal inferior and inferolateral wall. For comparison, only 10% of control subjects had LGE (only seen in the left/right ventricular insertion points), which can be seen in normal subjects. None of the subjects had any evidence of previous myocardial infarction.

Measurement of Steatosis Using 1H-MRS
Patients with severe AS had significantly higher MTC than controls, with the highest level in the symptomatic patients with AS (nearly 2-fold) and a lesser, but also significant, 67% increase in the asymptomatic group. When comparing AS patients with and without LGE, those with LGE had higher MTC compared with those without: 0.82±0.39 vs 0.56±0.28, respectively, P=0.016. Patients with LGE had significantly higher LVMi and impaired systolic strain and diastolic strain rates in both circumferential and longitudinal directions (all P<0.05), despite similar aortic valve area and peak aortic valve gradient (P>0.05; results not shown). As the effects of individual medications on MTC are not known, the analysis was repeated only on patients not taking any medications. Similar MTC was obtained (symptomatic AS, 0.85±0.39%; asymptomatic AS, 0.76±0.46%), suggesting that medications were not a confounding variable.

1H-MRS Versus Histological Analysis
Biopsy samples from 10 patients with severe AS were available for analysis. There was a significant correlation between MTC and the number of ORO staining-positive myocytes (r=0.66, P=0.036). The number of ORO staining-positive

<table>
<thead>
<tr>
<th>Table 1. Clinical and Echocardiographic Characteristics</th>
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<tbody>
<tr>
<td>Severe Aortic Stenosis (n=25)</td>
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<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Male, n (%)</td>
</tr>
<tr>
<td>Symptoms, n (%)</td>
</tr>
<tr>
<td>Dyspnea</td>
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<tr>
<td>Angina</td>
</tr>
<tr>
<td>Syncope</td>
</tr>
<tr>
<td>Medical history, n (%)</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Dyslipidemia</td>
</tr>
<tr>
<td>Coronary artery disease</td>
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<tr>
<td>Medications, n (%)</td>
</tr>
<tr>
<td>Aspirin</td>
</tr>
<tr>
<td>Metformin</td>
</tr>
<tr>
<td>β-blockers</td>
</tr>
<tr>
<td>ACE-I/ARB</td>
</tr>
<tr>
<td>Statin</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
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<tr>
<td>Systolic BP, mm Hg</td>
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<tr>
<td>Diastolic BP, mm Hg</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
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<tr>
<td>Echocardiography</td>
</tr>
<tr>
<td>Peak A gradient, mm Hg</td>
</tr>
<tr>
<td>E-wave deceleration, ms</td>
</tr>
<tr>
<td>E/A ratio</td>
</tr>
<tr>
<td>E/E' ratio</td>
</tr>
<tr>
<td>Biochemical, mmol/L</td>
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<tr>
<td>Blood glucose, mmol</td>
</tr>
<tr>
<td>NEFA</td>
</tr>
<tr>
<td>Triglycerides</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
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<tr>
<td>High-density lipoprotein</td>
</tr>
</tbody>
</table>

Values are mean±SD or percentages. A indicates atrial contraction; ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blocker; AS, aortic stenosis; AV, aortic valve; BP, blood pressure; E, early diastolic filling phase; E′, mitral annular velocity; and NEFA, nonesterified fatty acids.

*P (with post hoc Bonferroni correction) <0.05 vs normal and >0.05 vs asymptomatic severe AS.
†P (with post hoc Bonferroni correction) >0.05 vs normal.
‡P value is Student t test for symptomatic vs asymptomatic aortic stenosis.
§P values for 1-way ANOVA for all groups unless specified.
myocytes was also negatively correlated with circumferential strain ($r_s=-0.79, P=0.01$; Figure 1).

### Association Between Parameters of Systolic Function and Other Study Variables

Peak circumferential and longitudinal strains represented CMR parameters of LV systolic function. Table 3 and Figure 2 show bivariable Pearson correlations and multiple regression analysis of circumferential and longitudinal strains with several parameters in all study subjects. Steatosis (MTC) and LGE were independent correlates of circumferential strain, whereas steatosis and LVMI were independent correlates of longitudinal strain.

### Association Between Parameters of Diastolic Function and Other Study Variables

Circumferential and longitudinal diastolic strain rates represented CMR parameters of LV diastolic function. For echocardiographic LV diastolic parameter, we studied the most suitable

### Table 2. Cardiovascular MRI and Spectroscopy

<table>
<thead>
<tr>
<th></th>
<th>Severe Aortic Stenosis</th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symptomatic (n=25)</td>
<td>Asymptomatic (n=14)</td>
<td>Normal Control (n=20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid/water ratio, %</td>
<td>0.89±0.42*</td>
<td>0.75±0.36†</td>
<td>0.45±0.17</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Circumferential strain, %</td>
<td>−16.4±2.5*</td>
<td>−18.1±2.9†</td>
<td>−20.7±2.0</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Longitudinal strain, %</td>
<td>−12.6±2.0*</td>
<td>−13.2±2.9†</td>
<td>−16.6±1.6</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Circumferential diastolic SR, s⁻¹</td>
<td>56±13*</td>
<td>59±12†</td>
<td>76±12</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Longitudinal diastolic SR, s⁻¹</td>
<td>43±12*</td>
<td>40±14†</td>
<td>54±12</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>75±5*</td>
<td>73±5†</td>
<td>69±4</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>LV end-diastolic volume, mL</td>
<td>137±47</td>
<td>143±27</td>
<td>143±28</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>LV end-systolic volume, mL</td>
<td>35±19</td>
<td>39±16</td>
<td>45±11</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>LV mass index, g/m²</td>
<td>94±33*</td>
<td>94±29†</td>
<td>54±13</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>LV wall thickness, mm</td>
<td>16±2*</td>
<td>15±3†</td>
<td>9±1</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>LGE present, n (%)</td>
<td>20 (80)‡</td>
<td>9 (64)</td>
<td>2 (10)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Aortic valve area, cm²</td>
<td>0.83±0.12</td>
<td>0.86±0.14</td>
<td>...</td>
<td>0.47</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD or percentages. AS indicates aortic stenosis; LGE, late gadolinium enhancement; LV, left ventricular; and SR, strain rate.

*P (with post hoc Bonferroni correction) <0.05 vs normal and >0.05 vs asymptomatic severe AS.
†P (with post hoc Bonferroni correction) <0.05 vs normal.
‡P (with post hoc Bonferroni correction) <0.05 vs asymptomatic severe AS and normal.
‖P value is Student t test for symptomatic vs asymptomatic aortic stenosis.
§P values are for 1-way ANOVA for all groups unless specified.

### Table 3. Associations of Multiple Study Parameters With Circumferential and Longitudinal Systolic Strains

<table>
<thead>
<tr>
<th></th>
<th>Circumferential Strain</th>
<th>Longitudinal Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bivariable</td>
<td>Multivariable</td>
</tr>
<tr>
<td>Age</td>
<td>0.11</td>
<td>0.41</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.29</td>
<td>0.03</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.19</td>
<td>0.15</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0.12</td>
<td>0.35</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.16</td>
<td>0.21</td>
</tr>
<tr>
<td>Aortic valve area</td>
<td>−0.07</td>
<td>0.64</td>
</tr>
<tr>
<td>Peak aortic valve gradient</td>
<td>−0.05</td>
<td>0.73</td>
</tr>
<tr>
<td>Left ventricular ejection fraction</td>
<td>0.11</td>
<td>0.43</td>
</tr>
<tr>
<td>Left ventricular mass index</td>
<td>0.45</td>
<td>0.001</td>
</tr>
<tr>
<td>Late gadolinium enhancement</td>
<td>0.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lipid/water ratio</td>
<td>0.55</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure 1. Representative photomicrographs showing Oil Red O staining in low (A), and high (B) myocardial lipid deposition from left ventricular septal biopsies of 2 patients with severe aortic stenosis. Lipid/water ratios for these patients were 0.30 and 1.10, respectively.
Thus, in AS, steatosis was a strong independent correlate of systolic dysfunction but not of diastolic dysfunction. When we further explored the relationship between MTC and diabetes mellitus status, serum glucose, TG, and high-density lipoprotein levels, no significant correlations were found between MTC and these variables (results not shown), suggesting that insulin resistance was not a confounding variable of steatosis in the AS cohort.

Changes Post-AVR
Thirteen patients had follow-up CMR scans 8.0±2.1 months after AVR. There was near-complete reversal of myocardial steatosis to control group levels (from 0.92±0.41% before surgery to 0.47±0.25% after surgery, \(P=0.04\)), with no significant differences between the post-AVR levels and normal controls \((P=0.73)\). Three examples (normal control, AS patient before and after AVR) are shown in Figure 3. There was substantial regression of LVH (LVMI from 96±35 to 69±18 g/m², \(P=0.001\)), although LVMI in patients 8 months post-AVR was still significantly higher compared with normal controls \((P=0.015)\). There was no change in LVEF (from 74±5% to 74±5%, \(P=1.0\)) post-AVR. Of the 13 patients who underwent AVR and follow-up CMR, 2 patients had new left bundle-branch block, resulting in disco-ordinate septal movement that affected strain measurements, and they were excluded from the pre- and post-AVR strain comparisons. Systolic and diastolic strain indices improved significantly post-AVR and were similar to measurements in controls: circumferential strain increased from −17.0±2.0% to −19.5±3.2%, \(P=0.04\); circumferential diastolic strain rate from 60±11 to 75±12 s\(^{-1}\), \(P=0.012\); longitudinal diastolic strain rate from 42±6 to 55±11 s\(^{-1}\), \(P=0.002\). The only exception was longitudinal strain, which showed no significant change: −13.0±2.0% to −13.6±2.1% \((P=0.26)\). These findings are presented in Figure 4.

As these results might be confounded by the presence of diabetes mellitus (6 pre-AVR and 4 post-AVR), we repeated

### Table 4. Associations of Multiple Study Parameters With Circumferential and Longitudinal Diastolic Strain Rates and E/E′

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Circumferential Diastolic SR*</th>
<th>Longitudinal Diastolic SR*</th>
<th>E/E′†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bivariable</strong></td>
<td><strong>Multivariable</strong></td>
<td><strong>Bivariable</strong></td>
<td><strong>Multivariable</strong></td>
</tr>
<tr>
<td>Age</td>
<td>−0.19 0.14</td>
<td>−0.10 0.56</td>
<td>0.21 0.13</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>−0.01 0.97</td>
<td>−0.01 0.97</td>
<td>0.28 0.03</td>
</tr>
<tr>
<td>Hypertension</td>
<td>−0.03 0.85</td>
<td>−0.03 0.86</td>
<td>0.23 0.09</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0.03 0.84</td>
<td>0.03 0.86</td>
<td>0.25 0.07</td>
</tr>
<tr>
<td>Body mass index</td>
<td>−0.07 0.58</td>
<td>0.23 0.09</td>
<td>0.30 0.03</td>
</tr>
<tr>
<td>Aortic valve area</td>
<td>0.19 0.24</td>
<td>0.10 0.54</td>
<td>−0.10 0.57</td>
</tr>
<tr>
<td>Peak AVA gradient</td>
<td>−0.10 0.55</td>
<td>0.11 0.51</td>
<td>−0.06 0.74</td>
</tr>
<tr>
<td>LVEF</td>
<td>−0.03 0.83</td>
<td>0.07 0.63</td>
<td>0.07 0.63</td>
</tr>
<tr>
<td>LV Mass index</td>
<td>−0.53 &lt;0.001</td>
<td>−0.35 0.02</td>
<td>0.31 0.02</td>
</tr>
<tr>
<td>LGE</td>
<td>−0.52 &lt;0.001</td>
<td>−0.35 0.01</td>
<td>−0.38 0.006</td>
</tr>
<tr>
<td>Lipid/water ratio</td>
<td>−0.31 0.02</td>
<td>−0.30 0.03</td>
<td>0.31 0.02</td>
</tr>
</tbody>
</table>

AVA indicates aortic valve area; E, transmitral early diastolic velocity; E′, mitral annular early diastolic velocity; LGE, late gadolinium enhancement; LV, left ventricular; LVEF, left ventricular ejection fraction; and SR, strain rate.

*Derived from cardiovascular magnetic resonance measurements.
†Derived from echocardiographic measurement.
the analysis in the subgroup of patients without diabetes mellitus before (n=19) and after AVR (n=9). The results were similar to the entire cohort (results are available in Tables I–IV in the online-only Data Supplement).

Discussion
The present study demonstrated 3 important findings: first, myocardial steatosis is pronounced in severe AS and is independently associated with impaired systolic but not diastolic strain. Second, myocardial steatosis and strain changes are reversible post-AVR. Finally, steatosis as measured by \(^1\)H MRS could be validated against histological analysis.

Myocardial Steatosis in AS
This is the first study to show the presence of myocardial steatosis in severe AS in vivo using \(^1\)H MRS. MTC determined by \(^1\)H-MRS has previously been shown to correlate with biochemical determination of myocardial TG in Zucker rats and skeletal muscle from dogs. \(^8\) We extend these findings by confirming that MTC as measured by MRS correlates closely with histological analysis of human myocardial biopsies obtained intraoperatively \((r = 0.66)\) in patients with severe AS. Steatosis existed in our AS cohort, despite no significant difference in age, sex, body mass index, serum glucose, and lipid profile compared with normal controls, all of which have been shown to contribute to myocardial steatosis. \(^5,19\) Although the mechanisms underlying cardiac steatosis are incompletely understood, it is likely that impaired myocardial FA oxidation characteristic of cardiac hypertrophy contributes to TG accumulation. \(^20\)

The important question is whether steatosis is involved in the pathophysiology of contractile dysfunction in AS or is just an epiphenomenon. The present data demonstrate that in severe AS, steatosis is independently associated with strain, similar to that described in previous animal and human studies. \(^7,16\) Furthermore, compared with other independent variables (LGE and LVMI), MTC (and ORO staining of lipid deposition from myocardial biopsies) was associated with systolic strain abnormalities with the highest statistical significance, suggesting that steatosis is at the very least a sensitive marker of myocardial contractile dysfunction. This concurs with a previous study demonstrating a significant association between high myocardial lipid staining and lower LVEF in AS patients who had metabolic syndrome. \(^21\) One explanation for this observation is that the association is causative and mediated by the generation of toxic intermediates, such as ceramides from non-oxidative FA metabolism. \(^22,23\) Excessive FA uptake in relation to oxidative requirements results in FA overload, which will lead to conversion of FA to TGs within the cardiomyocytes. When the storage capacity is exceeded, these excess FAs enter nonoxidative pathways, producing toxic intermediates leading to apoptosis, which ultimately alter myocardial structure and function. \(^22,23\) In addition, lipid vacuole infiltration has been shown to dissemble the myocardial contractile apparatus mechanically, leading to contractile dysfunction in diabetic patients. \(^24\) Thus, although our data do not prove a direct causal link, they are clearly suggestive of a pathophysiologic role of steatosis in the development of systolic dysfunction in AS.

Our data did not show any association between steatosis and LV diastolic function. LVMI and the presence of LGE were, however, independent determinants of abnormal diastolic parameters in our study. These findings are not surprising because LVH and myocardial fibrosis \(^25,26\) are well-established causes of diastolic dysfunction. In contrast, Rijzewijk et al \(^27\) and Ng et al \(^7\) demonstrated that steatosis in their diabetic cohort is independently associated with LV diastolic dysfunction using MRI-derived E/A ratio and deceleration time.
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and longitudinal strain rate measured by echocardiographic speckle tracking, respectively. The discrepancies between these reports are likely attributable to the different patient populations investigated, but warrant further study.

Reversibility of Steatosis and Strain Changes After AVR
We showed that there is a significant reduction in steatosis after AVR. Significant improvements in most parameters of myocardial function were also observed, despite incomplete LVH regression.

Improvement in myocardial metabolism and function post-AVR is likely to reflect a mitigation of the pressure overload, leading to reduced LV wall stress. Rajappan et al. using positron emission tomography, demonstrated that coronary vascular and myocardial blood flow improvements after AVR were related to reduced afterload rather than the regression of LVH. We hypothesize that the relief of LV wall stress post-AVR causes a switch in myocardial substrate metabolism from glucose back to FA, thus reducing FA overload, toxicity, and steatosis with subsequent improvement in cardiac function. Reversal of metabolic dysregulation and improved ventricular function have been reported in another model of improved LV loading after bariatric surgery for weight reduction in obese patients. Postoperative longitudinal systolic function did not improve significantly post-AVR in our AS cohort, but this is primarily determined by LVH regression, which was only partial in our study. Furthermore, it was evident from our data that LVMI was an important determinant of longitudinal systolic strain.

Clinical Implications
The present study provides novel insights into metabolic alterations and their consequences in AS. We demonstrated that steatosis and subclinical myocardial dysfunction occur, regardless of symptoms in severe AS. Furthermore, the presence of myocardial steatosis and its significant association with myocardial strain are likely to be relevant clinically. Depressed LVEF is a late phenomenon, and recognition of subtle changes in LV systolic function, while LVEF is still preserved, may permit more timely surgical intervention. It is conceivable that, in the future, steatosis may also emerge as a parameter for guidance of AVR timing. Most importantly, we showed that steatosis improved after AVR; thus, if steatosis is indeed a pathophysiological factor in AS, then a reduction in steatosis might be a potential new therapeutic target to delay the development of LV dysfunction. We are currently setting up a clinical trial that tests this intriguing possibility. Such a trial, if positive, would also provide confirmatory evidence of a causal relationship between steatosis and systolic dysfunction in AS.

Study Limitations
This study is limited by a relatively small sample size, in line with its proof-of-principle nature. Furthermore, the AS population was heterogeneous, including patients with hypertension, dyslipidemia, and diabetes mellitus, which are known to be
associated with metabolic abnormalities. However, we only included patients with well-controlled blood pressure and diabetes mellitus. We were careful to assess whether the observed association between steatosis and strain might be confounded by the presence of diabetes mellitus. However, diabetes mellitus was not predictive of strain changes when MTC, LVMi, and LGE were included in the multivariable model. Furthermore, and most importantly, steatosis was reversible post-AVR, and this group included 4 of 13 (31%) patients with diabetes mellitus. All analyses were repeated excluding the diabetic patients, and this did not affect any of the statistical significances in our study (Tables I–IV in the online-only Data Supplement).

Although it was shown that steatosis correlates with LV dysfunction, the extent to which TG accumulation represents a direct causal mechanism, in comparison with other factors such as LVH and myocardial fibrosis, is unclear. Further studies are needed to understand these complex interactions between cardiac steatosis and all these factors. Finally, although intramyocellular and extramyocellular lipids can be distinguished in skeletal muscle and animal hearts, at present we cannot reliably differentiate these 2 lipid pools in human myocardium using our methods, and therefore we measure total lipid levels.

Further work is needed to develop tools that can reliably differentiate the intramyocellular lipids (IMCL) and extramyocellular lipids (EMCL) in the human heart, but at the moment this is technical research and not ready for clinical studies. In conclusion, severe AS is characterized by pronounced myocardial steatosis and subclinical LV contractile dysfunction. Eight months post-AVR, steatosis regresses, along with normalization of myocardial strain, despite only partial regression of LVH. Myocardial lipid assessment may serve as a useful adjunct to standard clinical information for risk stratification of patients with severe AS, but future studies are needed to investigate this further.

Acknowledgments
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Disclosures
None.

References


**CLINICAL PERSPECTIVE**

The current study provides novel insights into metabolic alterations and their consequences in aortic stenosis (AS). We used 1H magnetic resonance spectroscopy to assess myocardial triglyceride content and magnetic resonance tagging to assess left ventricular strain in patients with severe AS. Our findings suggest that increased myocardial triglyceride content (steatosis) is present in both symptomatic and asymptomatic severe AS, and, importantly, steatosis correlates with the degree of left ventricular strain impairment. Furthermore, myocardial triglyceride measured by 1H magnetic resonance spectroscopy correlates with histological quantification of myocardial triglyceride of left ventricular biopsies obtained intraoperatively during aortic valve replacement. After aortic valve replacement, steatosis and strain impairment are reversible. Our study suggests that myocardial lipotoxicity may contribute to the pathophysiology of AS and that myocardial steatosis as measured by magnetic resonance spectroscopy may become a useful risk stratification tool in AS. Furthermore, substantial improvement in steatosis after aortic valve replacement suggests that myocardial steatosis is indeed a modifiable condition. Future prospective studies should determine whether steatosis regression, achieved by metabolic modulator drugs, may become a potential new therapeutic option and whether such an approach can delay the onset of symptoms and left ventricular dysfunction.
Myocardial Steatosis and Left Ventricular Contractile Dysfunction in Patients With Severe Aortic Stenosis


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Data Supplement (unedited) at:
http://circimaging.ahajournals.org/content/suppl/2013/07/05/CIRCIMAGING.113.000559.DC1
Table 1. Clinical and echocardiographic characteristics (without diabetics)

<table>
<thead>
<tr>
<th></th>
<th>Severe Aortic Stenosis</th>
<th>Normal Controls</th>
<th>P value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symptomatic (n = 19)</td>
<td>Asymptomatic (n = 14)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>68±9</td>
<td>63±16</td>
<td>63±4</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>13 (68)</td>
<td>11 (78)</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Symptoms, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>15 (78)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Angina</td>
<td>7 (28)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Syncope</td>
<td>1 (4)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Past medical history, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>6 (31)</td>
<td>3 (21)</td>
<td>–</td>
</tr>
<tr>
<td>Diabetes</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>3 (15)</td>
<td>2 (14)</td>
<td>–</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medications, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>5 (26)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Metformin</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta blockers</td>
<td>4 (16)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ACE-I/ARB-II</td>
<td>4 (21)</td>
<td>5 (36)</td>
<td>–</td>
</tr>
<tr>
<td>Statin</td>
<td>6(31)</td>
<td>3 (21)</td>
<td>–</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27±4</td>
<td>27±4</td>
<td>26±4</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>130±16</td>
<td>134±18</td>
<td>130±12</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>75±10</td>
<td>76±8</td>
<td>76±8</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>65±10</td>
<td>65±13</td>
<td>62±9</td>
</tr>
<tr>
<td>Echocardiography</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak AV gradient (mmHg)</td>
<td>82±13</td>
<td>74±14</td>
<td>–</td>
</tr>
<tr>
<td>E-wave deceleration (ms)</td>
<td>284±116</td>
<td>255±108</td>
<td>210±43</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>0.8±0.4</td>
<td>0.9±0.3</td>
<td>0.9±0.3</td>
</tr>
<tr>
<td>E/E’ ratio</td>
<td>14.4±4.7*</td>
<td>12.6±5.4†</td>
<td>9.2±2.7</td>
</tr>
<tr>
<td>Biochemical, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood glucose, mmol</td>
<td>5.1±1.0</td>
<td>5.3±0.5</td>
<td>5.1±0.9</td>
</tr>
<tr>
<td>NEFA</td>
<td>0.6±0.2</td>
<td>0.6±0.1</td>
<td>0.5±0.3</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.1±0.4</td>
<td>1.2±0.5</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
<td>2.9±1.0</td>
<td>3.1±0.2</td>
<td>3.3±0.9</td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td>1.4±0.4</td>
<td>1.5±0.3</td>
<td>1.5±0.4</td>
</tr>
</tbody>
</table>

Values are mean ± SD or percentages.

‡P-values are for one-way analysis of variance (ANOVA) for all groups unless specified.
*P (with post hoc Bonferroni correction) <0.05 vs. normal and >0.05 vs. asymptomatic severe AS.
†P (with post hoc Bonferroni correction) >0.05 vs. normal.
‡P-value is Student t test for symptomatic vs. asymptomatic aortic stenosis.
ACE indicates angiotensin-converting enzyme-inhibitors; ARB, angiotensin-receptor antagonist-II; AV, aortic valve; A, atrial contraction; BP, blood pressure; E, early diastolic filling phase; E’, mitral annular velocity; NEFA, non-esterified fatty acids.
<table>
<thead>
<tr>
<th></th>
<th>Severe Aortic Stenosis</th>
<th>Normal Control</th>
<th>P value$^\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symptomatic (n = 19)</td>
<td>Asymptomatic (n = 14)</td>
<td>(n = 20)</td>
</tr>
<tr>
<td>Lipid/water ratio (%)</td>
<td>0.90±0.46*</td>
<td>0.75±0.36†</td>
<td>0.45±0.17</td>
</tr>
<tr>
<td>Circumferential strain (%)</td>
<td>-16.6±2.6*</td>
<td>-18.1±2.9†</td>
<td>-20.7±2.0</td>
</tr>
<tr>
<td>Longitudinal strain (%)</td>
<td>-12.6±2.2*</td>
<td>-13.2±2.9†</td>
<td>-16.6±1.6</td>
</tr>
<tr>
<td>Circumferential diastolic SR (s$^{-1}$)</td>
<td>53±13*</td>
<td>59±12†</td>
<td>76±12</td>
</tr>
<tr>
<td>Longitudinal diastolic SR (s$^{-1}$)</td>
<td>42±12*</td>
<td>40±14†</td>
<td>54±12</td>
</tr>
<tr>
<td>LV ejection fraction (%)</td>
<td>74±6*</td>
<td>73±8†</td>
<td>69±4</td>
</tr>
<tr>
<td>LV end-diastolic volume (ml)</td>
<td>145±49</td>
<td>143±27</td>
<td>143±28</td>
</tr>
<tr>
<td>LV end-systolic volume (ml)</td>
<td>38±20</td>
<td>39±16</td>
<td>45±11</td>
</tr>
<tr>
<td>LV mass index (g/m$^2$)</td>
<td>97±36*</td>
<td>94±29†</td>
<td>54±13</td>
</tr>
<tr>
<td>LV wall thickness (mm)</td>
<td>16±2*</td>
<td>15±3†</td>
<td>9±1</td>
</tr>
<tr>
<td>LGE present, n (%)</td>
<td>15 (79)$^{\ddagger}$</td>
<td>9 (64)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Aortic valve area (cm$^2$)</td>
<td>0.84±0.10</td>
<td>0.86±0.14</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are mean ± SD or percentages.

$^\¶$P values are for one-way analysis of variance (ANOVA) for all groups unless specified.

$^*$P (with post hoc Bonferroni correction) <0.05 vs. normal and >0.05 vs. asymptomatic severe AS,

$^\dagger$P (with post hoc Bonferroni correction) <0.05 vs. normal.

$^\ddagger$P (with post hoc Bonferroni correction) <0.05 vs. asymptomatic severe AS and normal.

$^\chi$P-value is Student t test for symptomatic vs. asymptomatic aortic stenosis.

LV indicates left ventricular; LGE, late gadolinium enhancement; SR, strain rate.
Table 3. Cardiac MRI and spectroscopy before and after AVR in symptomatic AS after excluding diabetics (6 diabetics before and 4 after AVR).

<table>
<thead>
<tr>
<th>Cardiac MRI and Spectroscopy</th>
<th>All symptomatic AS patients§ (n=19)</th>
<th>Follow-up patients (n=9)</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre AVR</td>
<td>Post AVR</td>
<td></td>
</tr>
<tr>
<td>Cardiac lipid/water</td>
<td>0.90 ± 0.46</td>
<td>0.97 ± 0.49</td>
<td>0.45 ± 0.17</td>
</tr>
<tr>
<td>Circumferential strain</td>
<td>-16.6 ± 2.6</td>
<td>-17.4 ± 1.9</td>
<td>-20.7 ± 2.0</td>
</tr>
<tr>
<td>Circumferential diastolic strain rate</td>
<td>53 ± 13</td>
<td>56 ± 11</td>
<td>76 ± 12</td>
</tr>
<tr>
<td>Longitudinal strain</td>
<td>-12.6 ± 2.2</td>
<td>-13.2 ± 2.1</td>
<td>-16.6 ± 1.6</td>
</tr>
<tr>
<td>Longitudinal diastolic strain rate</td>
<td>42 ± 12</td>
<td>43 ± 5</td>
<td>53 ± 12</td>
</tr>
<tr>
<td>Left ventricular mass index</td>
<td>97 ± 36</td>
<td>101 ± 41</td>
<td>54 ± 13</td>
</tr>
<tr>
<td>Left ventricular ejection fraction</td>
<td>74 ± 6</td>
<td>75 ± 6</td>
<td>69 ± 4</td>
</tr>
</tbody>
</table>

§All 6 diabetics were in the symptomatic group, therefore cardiac MRI and spectroscopy parameters for asymptomatic AS remain the same as in Table 1 of the manuscript.

*p < 0.05 versus pre AVR and > 0.05 versus controls; †p > 0.05 versus pre AVR and < 0.05 versus controls; ‡p < 0.05 versus pre AVR and controls.

Note: For strain, n=8 were used for follow-up analysis as one patient was excluded due to presence of left bundle branch block post AVR.
Table 4. Cardiac MRI and spectroscopy before and after AVR in all symptomatic AS
(If needed for comparison)

<table>
<thead>
<tr>
<th>Cardiac MRI and Spectroscopy</th>
<th>All symptomatic AS patients (n=25)</th>
<th>Follow-up patients (n=13)</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre AVR</td>
<td>Post AVR</td>
<td></td>
</tr>
<tr>
<td>Cardiac lipid/water</td>
<td>0.89 ± 0.42</td>
<td>0.92 ± 0.41</td>
<td>0.47 ± 0.25*</td>
</tr>
<tr>
<td>Circumferential strain</td>
<td>-16.4 ± 2.5</td>
<td>-17.0 ± 2.0</td>
<td>-19.5 ± 3.2*</td>
</tr>
<tr>
<td>Circumferential diastolic strain rate</td>
<td>56 ± 13</td>
<td>60 ± 11</td>
<td>75 ± 12*</td>
</tr>
<tr>
<td>Longitudinal strain</td>
<td>-12.6 ± 2.0</td>
<td>-13.0 ± 1.7</td>
<td>-13.6 ± 2.1†</td>
</tr>
<tr>
<td>Longitudinal diastolic strain rate</td>
<td>43 ± 12</td>
<td>42 ± 6</td>
<td>55 ± 11*</td>
</tr>
<tr>
<td>Left ventricular mass index</td>
<td>94 ± 33</td>
<td>96 ± 35</td>
<td>69 ± 18†</td>
</tr>
<tr>
<td>Left ventricular ejection fraction</td>
<td>75 ± 5</td>
<td>74 ± 5</td>
<td>69 ± 4</td>
</tr>
</tbody>
</table>

*p < 0.05 versus pre AVR and > 0.05 versus controls; †p > 0.05 versus pre AVR and < 0.05 versus controls; ‡p < 0.05 versus pre AVR and controls.

Note: For strain, n=11 were used for follow-up analysis as two patients were excluded due to new left bundle branch block post AVR.