Cardiac torsion-strain relationships in fatigued Primary Biliary Cirrhosis patients show accelerated ageing: a pilot cross-sectional study

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Authors’ contributions

KGH analysed the cardiac tagging data and some of the cardiac cine data, analysed the data, drafted the manuscript and revised it for critical content. MGDB analysed the data required for the repeatability analyses and assisted with critical revision of the manuscript.

GAG, AMB, JLN assisted with data interpretation, the drafting of the manuscript and its critical revision. DEJ and RT assisted with data interpretation and the critical revision of the
manuscript. LM assisted with data analysis of the cine data, data acquisition and the critical revision of the manuscript. All authors read and approved the final manuscript.

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Abstract (243/250 words)

The autoimmune liver disease Primary Biliary Cirrhosis (PBC) is associated with life-altering fatigue in approximately 50% of patients. Previous work suggests that fatigued PBC subjects have evidence of autonomic dysfunction and may be at a higher risk of sudden cardiac death. The manifestation of this risk is not clear. This pilot study investigated whether alterations in cardiac torsion and strain could be detected in fatigued or non-fatigued early-stage PBC patients. We performed cardiac tissue tagging and anatomical cine-imaging in 13 early stage PBC patients (including 7 with significant fatigue) and 10 control subjects to calculate cardiac torsion and strain throughout systole and diastole. From the cardiac tagging, we calculated the torsion-to-shortening ratio (TSR), a measure of subepicardial torsion exerting mechanical advantage over subendocardial shortening. Autonomic function testing was performed to evaluate baroreceptor effective index on standing. TSR was markedly increased in the fatigued PBC patients (0.70 ± 0.13) compared to both controls (0.46 ± 0.11, p=0.002) and non-fatigued PBC patients (0.44 ± 0.12, p=0.003). Decreased baroreceptor effective index on standing strongly correlated with increased TSR within the whole PBC group (r=-0.71, p=0.007). Fatigued PBC patients demonstrate a redistribution of myocardial strain characteristic of a reduced relative contribution to contraction from the subendocardium. This is analogous to the changes found in healthy ageing for subjects approximately 16 years older than the fatigued PBC patients. Hence the hearts of fatigued PBC patients may be subject to processes of accelerated ageing.

Keywords: Primary biliary cirrhosis, torsion, strain, cardiac, MRI
Abbreviations

BEI = baroreceptor effectiveness index
BMI = body mass index
BP = blood pressure
BRS = baroreceptor sensitivity
CSPAMM = complementary spatial modulation and magnetization
ELISA = enzyme-linked immunosorbent assay
FIS = fatigue impact score
MRI = magnetic resonance imaging
PBC = Primary Biliary Cirrhosis
TSR = torsion to shortening ratio
Introduction

The autoimmune cholestatic liver disease primary biliary cirrhosis (PBC) is associated with increased all-cause mortality rates in comprehensive, well-characterised patient groups, with a significant component of this increased mortality coming from non-liver related causes (14, 15, 29). Data strongly supports that this excess mortality is related to cardiac deaths, particularly in light of studies confirming that PBC is associated with dysfunction of the autonomic nervous system (10, 17, 18, 25, 26) with specifically sympathetic dysfunction. In our recent work (16) we demonstrated that PBC patients had impaired cardiac energetic function using magnetic resonance spectroscopy. This has led to the suggestion that those with PBC have a primary cardiac abnormality that accounts for at least some of their symptoms and the excess cardiac mortality.

While standard cine-magnetic resonance imaging (MRI) provides the gold standard measures of cardiac morphology and function, it cannot give detailed information about myocardial mechanics such as wall strain (percentage shortening during contraction) and torsion (a measure of “twist”), which may be affected by energetic deficits before becoming apparent as a clinical impairment. This additional information can however be measured using the modification of cine MRI known as cardiac-tagged MRI (8). This MR based method works by nulling signal from the myocardium in diastole in a rectangular grid pattern and tracking the deformation of these tags through the rest of the cardiac cycle (figure 1a). By tagging two parallel planes it is possible to calculate myocardial torsion (figure 1b) while in-plane analysis allows circumferential strains to be calculated across the myocardial wall. The technique has been used to examine cardiac function during healthy ageing (4, 11, 22, 27), where gradual subclinical differences in systolic and diastolic function are expected, as well as in conditions with definite cardiac involvement (12, 33).

Given the observation of abnormalities of cardiac function in PBC (16) we used cardiac-tagged MRI to study a well-characterised group of PBC patients, with reference to a group of
age- and gender-matched controls, to determine whether there were any abnormalities of cardiac strain or torsion.
Methods

Subjects

13 female PBC Stage I-II patients and 10 healthy female subjects were recruited as controls, to obtain groups with equivalent age, weight, body mass index and blood pressure (table 1). PBC patients were consecutive early-stage (non-cirrhotic) patients identified from a specialist disease clinic with normal liver synthetic function (albumin, bilirubin, and prothrombin time). All had definite or probable PBC defined according to previously validated criteria (at least 2 of cholestatic biochemical parameters, compatible liver histology and supportive serology (24)). All participants in the present study were antimitochondrial antibody positive by immunofluorescence at a titre of 1:40 with M2 presence confirmed by enzyme-linked immunosorbent assay (ELISA). Normal control subjects were recruited via notices in the local press. Patient and control subject fatigue severity was assessed by means of a validated questionnaire, the Fatigue Impact Score (FIS) (30). The FIS is a 40 item symptom specific measure of health related quality of life, commonly used in medical conditions in which fatigue is a prominent symptom. The scale allows patients to rate each item on a scale of 0 to 4, with 0 representing no problem and 4 representing an extreme problem, summed to provide a total score running from 0 (no fatigue) to 160 with higher scores indicating worse fatigue. Patients were divided into two groups: those without significant fatigue (defined as FIS < 25) and those with severe fatigue (FIS > 50). All controls had FIS < 25. The same experimental subjects had had phosphorus-31 ($^{31}$P) magnetic resonance spectroscopy (MRS) of the heart carried out at the same experimental session and this has been reported elsewhere (16). Written informed consent from all participants and institutional ethic approval were obtained.
Cardiac Magnetic Resonance Cine Imaging

Cardiac examinations were performed using a 3T Philips Intera Achieva scanner (Best, NL). A dedicated 6-channel cardiac coil (Philips, Best, NL) was used with the subjects in a supine position and electrocardiogram gating. Cardiac magnetic resonance cine imaging was acquired to assess cardiac morphology, and systolic and diastolic function. A stack of balanced steady-state free precession images was obtained in the short axis view during breath holding covering the entire left ventricle (field of view = 350mm, repetition time/echo time = 3.7/1.9ms, turbo factor 17, flip angle 40°, slice thickness 8mm, 0mm gap, 14 slices, 25 phases, resolution 1.37mm, temporal duration approx. 40ms per phase dependent on heart rate): perpendicular long axis views were also acquired. Image analysis was performed using the cardiac analysis package of the ViewForum workstation (Philips). Manual tracing of the epicardial and endocardial borders was performed on the short axis slices at end-systole and end-diastole. The algorithm for contour selection and subsequently calculating left ventricular mass, systolic and diastolic parameters, including the ratio of early to late ventricular filling velocity (E/A ratio) and early filling percentage, have been detailed elsewhere (16). The ratio of the left ventricular (LV) mass to the end-diastolic volume was calculated as this parameter is a measure of concentric remodelling (5). Preload, afterload, contractility and ventricular-arterial coupling were also estimated: preload was determined by the end-diastolic volume, afterload by arterial elastance ($E_a = end$-systolic pressure (systolic blood pressure x 0.9) / stroke volume (normalised to body surface area), contractility by $E_{es} = end$-systolic pressure / end-systolic volume (normalised to body surface area), and ventricular-arterial coupling by the ratio of $E_{es}/E_a$

Cardiac Tagging

Tagged short axis images were acquired at the same session as the morphological imaging (figure 1a). A turbo-field echo sequence with acceleration factor 9 was used (repetition time/echo time/flip angle/number of averages = 4.9/3.1/10°/1, SENSE factor 2, field of view
350x350mm, voxel size 1.37x 1.37mm with an orthogonal CSPAMM grid with tag spacing of 7mm) (8). Two adjacent short-axis slices of 10mm thickness were acquired at mid-ventricle with a 2mm gap. The Cardiac Image Modelling package (Auckland UniServices Ltd, New Zealand) was used to analyse the tagging data by aligning a mesh on the tags between the endo- and epi-cardial contours. Circumferential strain and the rotation of the two planes were calculated throughout the cardiac cycle. Circumferential strain is quoted for both the whole myocardial wall and the endocardial third of the wall thickness. The epicardial torsion between the two planes (taken as the circumferential-longitudinal shear angle defined on the epicardial surface, $\gamma$, figure 1b) was calculated (4).

In the healthy heart, torsion occurs such that there is homogeneity of fiber shortening across the myocardial wall and is a marker of the dominance of epicardial fibers over endocardial fibers as a consequence of the greater radius in the epicardium. This can be quantified by a ratio of the peak torsion (in radians), defined as the shear angle between two planes on the epicardial surface (22), and the peak circumferential strain in the endocardial third of the myocardium and is referred to as the torsion to shortening ratio, TSR (22, 33). This ratio has been shown to be near constant amongst healthy subjects of the same age, and to increase with both healthy ageing and disease. The rate at which torsion dissipates after systole is an important measure for good diastolic function and this was assessed by calculating the residual torsion at 150% of the end-systolic time (27), as measured from the cine imaging. Where this time-point fell between two tagging acquisition times, linear interpolation of the nearest results was used. Longitudinal shortening was determined from long axis cine-MRI by determining the perpendicular distance from the plane of the mitral valve to the apex in systole and diastole. The myocardial wall thickness at systole and diastole was determined at the same level as the cardiac tagging and hence radial thickening was calculated.
Assessment of the Autonomic Nervous System: Baroreceptor Function

In view of previous studies linking impaired cardiac function with sympathetic nervous system dysfunction, all PBC participants underwent autonomic nervous system assessment at rest over 10 minutes and in response to standing using established approaches (16, 28). Subjects refrained from ingesting caffeine or smoking on the day of their assessment. Each participant’s evaluation occurred during the morning, after a light breakfast only and in a quiet room.

Cardiovascular measurements were performed using continuous heart rate and beat-to-beat blood pressure (BP) measurement (Task Force, CNSystems). Baroreceptor function was assessed during a 10 minute supine rest by evaluation of baroreceptor sensitivity (BRS) and the baroreceptor effectiveness index (BEI). BRS assesses the appropriate cardiovascular responses to the spontaneous changes in heart rate and blood pressure during rest whilst BEI is the ratio of spontaneous systolic BP changes to the corresponding changes in the R-R interval and details the number of times the baroreflex response is effective (2). The baroreflex slope is measured using the sequence technique which identifies sequences of progressive increases in systolic BP followed by appropriate pulse interval delays or vice versa (7).

Heart rate variability was measured using spectral analysis to derive low frequency (predominantly sympathetic nervous system function) and high frequency (predominantly parasympathetic nervous system function) heart rate variability(1).

Inter- and intra-observer variability

We had previously established the reliability of MRI measures of our torsion, myocardial strain and diastolic function measurements by Bland-Altman analysis, comparing values derived from myocardial contours by two independent observers (KGH and MGDB), and then redrawn after one month (MGDB) in eight subject datasets. Inter-observer and intra-observer
limits of agreement were respectively -0.19 ± 0.31° and 0.06 ± 0.51° for torsion, 0.74 ± 1.31% and 1.53 ± 1.08% for peak endocardial circumferential strain, 0.001 ± 0.11 and 0.08 ± 0.16 for E/A ratio and –0.54 ± 1.58% and 0.68 ± 2.84% for early filling percentage.

**Statistical Analysis**

Image analysis was performed blinded to the status of patients and controls. Statistical comparisons were made using SPSS version 17. Data are presented as mean and standard deviation. Data were tested for normality using the Shapiro-Wilk test and comparisons were drawn between groups using ANOVA (or Kruskal-Wallis where non-parametric) with post-hoc Bonferroni correction for multiple comparisons. Correlations were executed as two-tailed tests using the Pearson correlation method (or Spearman’s rank if non-parametric). Statistical significance level was set at p<0.05.
Results

Cardiac MR data for the groups are presented in tables 1 and 2.

Cardiac tagging strain and torsion measurements

Peak left ventricular torsion was increased by 27% in the fatigued PBC group compared to the control group and peak endocardial circumferential strain was reduced by 9% in the fatigued PBC group, though these changes did not reach individual statistical significance (table 2). The torsion to shortening ratio (TSR) was significantly increased in the fatigued PBC group compared to either the age-matched control group (by 52%, p = 0.002) or the non-fatigued PBC group (by 59%, p = 0.003, figure 2). There was no difference in torsion, strain or TSR between the controls and the non-fatigued PBC group. Longitudinal shortening and radial thickening were not different between the three groups.

There were no significant differences in diastolic function as measured by the ratio of the early to late filling (E/A ratio) or the early filling percentage (table 2). The residual torsion at 150% of the end-systolic time was significantly longer in the PBC group as a whole (3.1 ± 1.6°) compared to the control group (1.7 ± 0.7°, p = 0.02): there was no significant difference between the non-fatigued and fatigued PBC groups (figure 3), with the greatest difference between the control group and the fatigued PBC patients (p = 0.07). There was no correlation between the residual torsion at 150% of the end-systolic time and either measure of diastolic function (EFP, E/A ratio) or the TSR.
Relationship between cardiac tagging measurements and autonomic function tests

There was a strong inverse relationship between baroreflex effectiveness index upon standing and the TSR ($r = -0.71$, $p = 0.007$, figure 4) in the PBC group as a whole. The baroreflex effective index on standing (which measures the number of times that the baroreflex is effective in overcoming the non-baroreflex influences that regulate the sinus node) indicated that the baroreflex was less effective in those with the most impaired ratio between torsion and endocardial strain at rest.

There was also a strong inverse relationship between low frequency beat-to-beat interval variance on standing and the TSR ($r = -0.70$, $p = 0.007$, figure 5). The low frequency beat-to-beat interval variance after orthostatic loading represents peripheral sympathetic activity/sympathetic vasomotor control and this association indicated that those patients with the most abnormal TSR in the resting state also had the greatest impairment of sympathetic vasomotor control.

There was no correlation between TSR and autonomic function results with patients at rest.

Cardiac Morphology and function by standard cine-MRI

As reported previously, parameters relating to cardiac morphology (LV mass, end-diastolic and end-systolic volume) were not significantly different between the control group and PBC patients regardless of fatigue status. Table 1 summarises the main parameters, which can be found in more detail in (16): there was no significant difference in systolic or diastolic blood pressures between any groups. When the PBC group was divided into those with and without significant fatigue, there was no significant difference between LV mass, LV index or mass to volume ratio, eliminating the possibility of overt concentric hypertrophy in the fatigued PBC group. Likewise, ejection fractions, stroke volume, cardiac output, heart rate,
arterial elastance, contractility and ventricular-arterial coupling were equivalent in the three groups.
Discussion

This study demonstrated that there are abnormalities in the ratio of torsion to endocardial circumferential strain in a group of severely fatigued PBC patients compared to groups of matched controls and non-fatigued PBC patients. This finding arose from raised peak torsion and lower circumferential strain. The release of left ventricular torsion in early diastole was delayed in PBC patients compared to controls, despite no significant defect in diastolic filling.

In our previous work (11) on healthy subjects in young (mean age 31 ± 6y), middle-aged (mean age 48 ± 6) and older groups (mean age 62 ± 2y), we found that the TSR remains constant between the young and middle-aged groups (mean 0.43 and 0.48 respectively), while it was raised by 44% in the oldest group (mean 0.62). Other authors have noted increases in torsion and TSR, notably [12], where a 38% increase in TSR and 33% increase in torsion were noted between two groups of healthy volunteers with mean ages of 23 and 68 years’ old. Our group of fatigued PBC patients (mean age 54) demonstrated an increase in TSR (52% compared to controls) in excess of that of a group of subjects who were 8 years older on average. Under an assumption of linearly increasing TSR with age (as we reported in healthy control subjects (11)), we would estimate that the fatigued PBC hearts belonged to healthy individuals of mean age 70, rather than their true mean of 54 years, representing sixteen years of accelerated ageing.

The timely release of torsion and strain during diastole is crucial for good diastolic function, and we evaluated this, as in other studies (27), by measuring the residual torsion at 150% of the end-systolic time. The PBC group as a whole had 89% more residual torsion at this time than the controls, though there was no significant difference between the non-fatigued and fatigued groups. By contrast, our work on healthy controls (11) indicated a 45% increase in residual torsion between mean age 48 and 62. Similarly, a study with a wider age range (27) found a 56% increase in residual torsion between the ages of 22 and 69 years' old. This
residual torsion did not significantly correlate with any measurement of cardiac morphology, function or autonomic function in this study, though subject numbers are limited.

The increase in torsion and TSR in older adults indicated that there was an alteration in the transmural distribution of strain, and, in particular, that subendocardial fibers are not making a proportionate contribution to systolic ejection as compared to the subepicardial fibers, with a consequent increase in torsion. It has been observed that the endocardium is particularly sensitive to insult, as has been shown in histopathology of ischemic tissue (13), by studies of transmural wall motion in stunned myocardium that has recovered from an ischemic insult (3, 23) and in patients with aortic valve stenosis (33).

Observation of raised TSR is not confined to healthy ageing, and has been shown to occur in overt cardiac diseases where relative subendocardial contractile performance may be impaired. Compared to our observation of a 52% increase in TSR, in adult patients with severe aortic valve stenosis (33), TSR was increased by 91% compared to controls, while in patients 3 months after valve replacement, TSR was only 39% increased compared to controls. In asymptomatic children with aortic stenoses, TSR was increased by 38% (6). In left ventricular hypertrophy, a comparable measure of TSR was found to be increased by 24% in HCM mutation carriers without hypertrophy (31) while a study of those with hypertrophy (34) found an approximate 50% increase in torsion compared to strain (though they did not calculate TSR itself). The magnitude of the changes we find are therefore comparable with examples of subclinical and overt disease.

Previous studies exploring potential biomarkers for cardiac risk have suggested that reduced baroreceptor function and reduced heart rate variability have the potential to identify those at increased risk of cardiac mortality (19, 20, 32). Interestingly reductions in these parameters on standing were both associated with increased TSR at rest in the PBC patient group,
indicating reduced relative subendocardial contribution. This provides a direct link between changes in baroreceptor function under stress and general contractile performance, confirming the potential role that TSR may have in predicting cardiac mortality. We acknowledge that we cannot draw conclusions about causation from this study, but it is interesting to speculate that increased TSR in PBC arises either as a consequence or cause of reduced baroreceptor function.

Previously we had observed that myocardial energetics were impaired in two thirds of the PBC cohort compared to a matched control group (16). There was no association between the abnormally high TSR found and the myocardial ratio of phosphocreatine to adenosine triphosphate in this group. Abnormalities in myocardial energetics may precede the development of motion abnormalities as has been found in other diseases (9, 21).

The limitations of this study included that we did not perform tagging studies of the entire left ventricle, and so we are unable to comment on differences that may exist in torsion and strain characteristics between apex and base. A small number of subjects were examined in this pilot study. To reduce the burden of the examination (which was one part of a larger MR protocol and medical assessment), we did not perform tagging with as fine a time resolution as other studies, so we are unable to detect small changes in the timing of the onset and release of torsion and systole. Given the findings of potentially altered contractile function, a study of a larger cross-sectional cohort of PBC subjects is indicated. We are unable to say how such differences may develop within the time course of PBC. This would demand an extensive longitudinal experiment: such experiments would now be highly desirable to establish the time course of changes.
Conclusions

This study has established that there are differences in myocardial mechanics present in both non-fatigued and fatigued PBC subjects, and that a larger characterization study is warranted, particularly focusing on those PBC subjects with fatigue and the relationship between cardiac torsion, strain and autonomic function.
Competing interests

The authors have no competing interests to declare.

Acknowledgements and Funding

The study was funded by MRC and the Newcastle UK NIHR Biomedical Research Centre in Ageing and Age Related Diseases. KGH is funded by an MRC New Investigator Research Grant (G1100160). MGDB is funded by a Wellcome Trust Research Training Fellowship (BH092142).

We thank the patients and volunteers for contributing to this study. In addition we would like to acknowledge the significant contribution from Carol Smith, Research Radiographer, Jessie Pairman and Katherine Wilton, Research Nurses.
References


20. **La Rovere MT, Pinna GD, Hohnloser SH, Marcus FI, Mortara A, Nohara R, Bigger JT, Jr., Camm AJ, and Schwartz PJ.** Baroreflex sensitivity and heart rate variability


**Figure Legends**

**Figure 1**: (a) Cardiac cine-imaging (*top*) and cardiac tagging (*bottom*) at diastole (*left*) and systole (*right*), showing how a rectangular grid of nulled signal applied at diastole remains with the tissue through the cardiac cycle, allowing calculation of strain and torsion. (b) Tagging in two parallel short axis sections allows the calculation of the torsion (the longitudinal-circumferential shear angle $\gamma$) between the two planes.

**Figure 2**: Torsion to shortening ratio in the control, non-fatigued and fatigued PBC groups

**Figure 3**: Residual torsion at 150% of end-systolic time in control, non-fatigued and fatigued PBC groups

**Figure 4**: Decreased baroreflex effectiveness index on standing correlates strongly with increased TSR in the PBC cohort ($r = -0.71$, $p = 0.007$)

**Figure 5**: Reduced low frequency beat-to-beat interval variance on standing correlates strongly with increased TSR in the PBC cohort ($r = -0.70$, $p = 0.007$)
The chart shows the torsion to shortening ratio (rad) for different groups:

- **Controls**
- **PBC low fatigue**
- **PBC high fatigue**

The chart indicates a significant difference in the torsion to shortening ratio among these groups, with p-values of **0.002** and **0.003** respectively.

The ratio values for each group are as follows:

- **Controls**: Between 0.2 and 0.8 radians
- **PBC low fatigue**: Around 0.2 radians
- **PBC high fatigue**: Between 0.6 and 0.8 radians
Residual torsion at 150% of end-systolic time (degrees)

Controls  PBC low fatigue  PBC high fatigue

p = 0.07
Torsion to shortening ratio (rad)

Baroreflex effectiveness index (%)

$r = -0.71, p = 0.007$
Table 1: Morphological parameters for controls and PBC subjects (mean ± s.d.).

Where ANOVA indicates significant differences between at least two groups, the letters (a), (b) and (c) are used to specify the post-hoc Bonferroni corrected significance between two specific groups. "ns" indicates no significant difference between any pair of groups.

<table>
<thead>
<tr>
<th></th>
<th>Control (a)</th>
<th>PBC non-fatigued (b)</th>
<th>PBC fatigued (c)</th>
<th>ANOVA sig.</th>
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<tbody>
<tr>
<td>N</td>
<td>10</td>
<td>6</td>
<td>7</td>
<td>ns</td>
</tr>
<tr>
<td>Age (y)</td>
<td>51 ± 8</td>
<td>51 ± 10</td>
<td>54 ± 12</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>27 ± 3</td>
<td>26 ± 3</td>
<td>25 ± 5</td>
<td>ns</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71 ± 7</td>
<td>69 ± 9</td>
<td>63 ± 14</td>
<td>ns</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>121 ± 13</td>
<td>126 ± 23</td>
<td>116 ± 8</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77 ± 11</td>
<td>73 ± 7</td>
<td>80 ± 8</td>
<td>ns</td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>96 ± 16</td>
<td>93 ± 6</td>
<td>88 ± 13</td>
<td>ns</td>
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<tr>
<td>LV index (g/m²)</td>
<td>54 ± 7</td>
<td>53 ± 3</td>
<td>53 ± 7</td>
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<tr>
<td>Heart rate (bpm)</td>
<td>64 ± 12</td>
<td>65 ± 6</td>
<td>65 ± 6</td>
<td>ns</td>
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<tr>
<td>Ejection fraction (%)</td>
<td>62 ± 7</td>
<td>65 ± 5</td>
<td>65 ± 4</td>
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<td>Mass to end diastolic volume (g/ml)</td>
<td>0.78 ± 0.07</td>
<td>0.75 ± 0.09</td>
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<td>Stroke volume (ml)</td>
<td>76 ± 13</td>
<td>81 ± 10</td>
<td>68 ± 14</td>
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<td>Cardiac output (l/min)</td>
<td>4.8 ± 0.6</td>
<td>5.3 ± 1.0</td>
<td>4.4 ± 0.8</td>
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<tr>
<td>End diastolic volume (ml)</td>
<td>123 ± 21</td>
<td>124 ± 14</td>
<td>105 ± 22</td>
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</tr>
<tr>
<td>End-systolic volume (ml)</td>
<td>47 ± 14</td>
<td>43 ± 9</td>
<td>37 ± 10</td>
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Table 2: Wall motion, diastolic function and autonomic function parameters for controls and PBC subjects (mean ± s.d.)

Where ANOVA indicates significant differences between at least two groups, the letters (a), (b) and (c) are used to specify the post-hoc Bonferroni corrected significance between two specific groups. "ns" indicates no significant difference between any pair of groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (a) N=10</th>
<th>PBC non-fatigued (b) N=6</th>
<th>PBC fatigued (c) N=7</th>
<th>ANOVA sig</th>
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<tr>
<td>Torsion to shortening ratio</td>
<td>0.46 ± 0.11</td>
<td>0.44 ± 0.12</td>
<td>0.70 ± 0.13</td>
<td>p=0.002 a/c</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>p=0.003 b/c</td>
</tr>
<tr>
<td>Peak torsion (°)</td>
<td>6.2 ± 1.7</td>
<td>5.6 ± 1.4</td>
<td>7.9 ± 1.4</td>
<td>p = 0.05 b/c</td>
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<td>Residual torsion at 150% ES (°)</td>
<td>1.7 ± 0.7</td>
<td>2.9 ± 1.0</td>
<td>3.4 ± 2.0</td>
<td>p = 0.07 a/c</td>
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<td>Peak endocardial circumferential strain (%)</td>
<td>23.2 ± 2.7</td>
<td>22.9 ± 3.0</td>
<td>21.1 ± 1.8</td>
<td>ns</td>
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<tr>
<td>Longitudinal shortening (%)</td>
<td>18.3 ± 3.1</td>
<td>18.6 ± 2.3</td>
<td>19.8 ± 3.0</td>
<td>ns</td>
</tr>
<tr>
<td>Radial thickening (%)</td>
<td>61 ± 17</td>
<td>60 ± 16</td>
<td>59 ± 15</td>
<td>ns</td>
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<tr>
<td>E/A ratio (-)</td>
<td>1.73 ± 0.60</td>
<td>1.95 ± 0.60</td>
<td>1.47 ± 0.52</td>
<td>ns</td>
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<tr>
<td>Early filling percentage (%)</td>
<td>72 ± 5</td>
<td>73 ± 4</td>
<td>67 ± 6</td>
<td>ns</td>
</tr>
<tr>
<td>Arterial elastance (mmHg m²/ml)</td>
<td>2.6 ± 0.5</td>
<td>2.5 ± 0.5</td>
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<td>ns</td>
</tr>
<tr>
<td>Contractility (mmHg m²/ml)</td>
<td>4.5 ± 1.1</td>
<td>4.7 ± 1.4</td>
<td>4.9 ± 1.1</td>
<td>ns</td>
</tr>
<tr>
<td>Ventricular-arterial coupling (-)</td>
<td>0.6 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>ns</td>
</tr>
<tr>
<td>Baroreflex effectiveness index on standing (%)</td>
<td>55.5 ± 21.5</td>
<td>71.9 ± 14.1</td>
<td>47.7 ± 23.1</td>
<td>ns</td>
</tr>
<tr>
<td>Low frequency heart rate variability on standing (ms²)</td>
<td>241 ± 239</td>
<td>631 ± 283</td>
<td>272 ± 257</td>
<td>p = 0.06 a/b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p = 0.08 b/c</td>
</tr>
</tbody>
</table>