Cardiac resynchronization therapy cures dyssynchronopathy in canine left bundle-branch block hearts

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Aims We investigated to what extent biventricular pacing (BVP) can normalize LV function and remodelling, induced by isolated left bundle branch block (LBBB).

Methods and results In 16 dogs LBBB was induced. Eight animals were followed for 16 weeks and in 8 animals BVP was started after 8 weeks. LV pressure, LV geometry (2D echocardiography), systolic circumferential shortening (CSSys, MRI tagging) and myocardial blood flow (MBF, microspheres) was measured. * and # indicate P < 0.05 compared to pre-LBBB and 8 weeks of LBBB, respectively. Data is presented relative to pre-LBBB values (mean ± SEM). BVP increased LV dP/dt|max from 78 ± 5%* to 86 ± 5%*# (immediately) and 89 ± 6%# (after 8 weeks) and normalized regional differences in CSSys and MBF. After 8 weeks of BVP, LV end-diastolic volume (EDV) was reduced from 123 ± 3%* to 109 ± 6%# and LV lateral wall mass was reduced from 128 ± 5%* to 113 ± 3%#*. The acute increase in LV dP/dt|max upon BVP correlated with LV EDV and LV wall mass after 8 weeks of BVP.

Conclusion In canine hearts with long-term isolated LBBB, BVP largely reverses global and regional functional and structural abnormalities induced by LBBB.

Introduction

Over a decade ago cardiac resynchronization therapy (CRT) has been introduced as an adjunctive therapy for patients with chronic heart failure (CHF) and ventricular conduction disturbances, especially left bundle-branch block (LBBB). In these patients CRT improves left ventricular (LV) pump function,1,2 which has been attributed to resynchronization of activation and contraction.1,3,4 CRT improves clinical status, exercise capacity, and survival in patients with NYHA functional class III and IV.5,6 Furthermore, CRT causes significant regression of LV dimensions, referred to as reverse remodelling.3,7 A recent study indicates that reverse remodelling predicts survival.8 It would therefore be important to know to what extent CRT can reverse remodelling in LBBB hearts.

Reversal of ventricular dilatation by CRT in LBBB hearts implies a causal relation between dyssynchrony and dilatation. In patients such a relationship is hard to establish because of the silent onset of LBBB and the considerable co-morbidity in patients with LBBB.9 Animal studies provided evidence that dyssynchrony not only induces ventricular dilatation, but also asymmetric hypertrophy. Data from various studies suggests that dilatation and asymmetric hypertrophy is due to a combination of reduction in global pump function and regional differences in myocardial work-load.10–12,14 In LBBB hearts workload is lowest in the septum and highest in the LV lateral wall. As a consequence, hypertrophy and molecular alterations are most pronounced in the lateral wall.10,13,14 We regard this combination of abnormalities specific for dyssynchronously activated hearts and define it as ‘dyssynchronopathy’.

The aim of the present study was to investigate the effect of CRT on hearts with long-term isolated LBBB. Based on the above-mentioned experimental findings that LBBB results in dyssynchronopathy, we hypothesize that CRT can cure dyssynchronopathy. This would imply acute restoration of LV pump function and the abnormal distribution of regional LV blood flow and mechanical work, followed by reversal of the LBBB-induced LV dilatation and asymmetric hypertrophy back to values before induction of LBBB. These hypotheses were investigated in canine hearts. After induction of LBBB by radio-frequency (RF) ablation, the animals were treated with biventricular pacing (BVP) after 8 weeks (LBBB+BVP group).
Methods

Animal handling was performed according to the Dutch Law on Animal Experimentation (WOD) and the European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EU). The protocol was approved by the Experimental Committee of the Maastricht University.

Experimental protocol

LBBB was induced by RF ablation in 16 adult mongrel dogs of both sex and unknown age. Anaesthesia during this procedure was induced with pentothal and maintained by ventilation with O₂ and infected with midazolam and sufentanil. A catheter tip manometer was used to measure LV pressure. For myocardial blood flow (MBF) measurements, using the fluorescent microsphere technique, a catheter was placed in the LV for microsphere injection.

Subsequently, the dogs were divided into control LBBB dogs (n = 8) and dogs undergoing biventricular pacemaker implantation 8 weeks after induction of LBBB (LBBB+VBP group) using the same anaesthesia as used in the previous procedure. The implantation procedure is described in more detail below. Time-wise, the first four dogs belonged to the LBBB group, the remaining dogs were randomized between LBBB and LBBB+VBP. Data on the LBBB group were already published elsewhere. All dogs were anaesthetized again 16 weeks after induction of LBBB and sacrificed. Finally, the heart was removed and stored at −20°C together with the reference blood samples for microsphere analysis.

LV pressure and ECG measurements as well as fluorescent microspheres injections were performed before and shortly after (30–60 min) induction of LBBB (at week 0), before and acutely after onset of BVP (week 8, in the LBBB+VBP group only) and at week 16. At week 16 measurements were performed during LBBB (which was reverted in the LBBB+VBP group by turning off the pacemaker) and during BVP.

Two-dimensional echocardiographic images were made before and every 2 weeks after onset of LBBB and BVP.

Magnetic resonance (MR) tagging measurements were performed 1 week before, 6–8 weeks after induction of LBBB, as well as after 8 weeks of BVP under the same anaesthetic conditions as mentioned above using manual ventilation with room air. Multi-slice short axis cine and MR tagging images were acquired during breath hold at a frame rate of 15 ms, as previously described. In order to prevent disturbances of pacemaker action due to the magnetic field of the MR scanner the implanted pacemaker was removed. The pacemaker leads were exteriorized and connected to an external pacemaker. BVP was performed in D00 mode with a lower heart rate just above sinus rhythm.

Pacemaker implantation procedure

During a minimally invasive procedure, an LV epicardial screw-in pacing lead (Medtronic model 6917A, Minneapolis, MN, USA) was placed on the LV lateral wall. The lead was tunneled to the neck and the thorax was subsequently closed. Cut down of the left jugular vein in the neck provided access to the venous system to position the right atrial (Medtronic model 5568) and right ventricular (RV) lead (Medtronic model 5068).

An extensive pacing protocol was performed in VDD mode at various atrioventricular (AV)-delays and interventricular (VV)-intervals using an external pacemaker (Medtronic AV pacing System Analyzer Model 5311B) to identify pacing configuration with optimal LV dP/dt max. Optimal LV dP/dt max could be achieved at a relatively short AV delay (62 ± 1 ms) and VV delay of zero. Finally, the permanent internal pacemaker was implanted (Medtronic, InSync II). After the dogs had been allowed to recover from surgery, ventricular pacing was started using the AV-delay and VV-interval that resulted in best LV dP/dt max.

Data analysis

Data analysis was performed as described previously. LV end-diastolic volume (EDV) and total and regional LV wall mass were determined from short axis two-dimensional echocardiographic images (Figure 1). Values were normalized to pre-LBBB values. Regional geometry was determined within four LV wall regions: septum, posterior, lateral, and anterior wall. On short-axis M-mode echocardiographic images, the time delay between the earliest inward movement of the septum and opposite LV lateral wall (septal-to-lateral wall motion delay, Figure 1), was calculated as a measure of intraventricular mechanical asynchrony.

MR tagging image analysis was performed off-line using home-made software for MATLAB 5.3.1 (MathWorks, Natick, MA, USA), as previously described in more detail. Myocardial shortening was determined in 32 midwall regions of five to seven short-axis sections of the LV. From this, systolic circumferential shortening (CSsys) was calculated as the change in segment length during the ejection phase. Begin and end ejection were determined from the radial displacement of the LV wall during the cardiac cycle. Values of regional CSsys were normalized to the mean LV CSsys. Furthermore, the time delay of maximal systolic shortening between the septum and LV lateral wall was obtained with MR Tagging (Sep-Lat shortening delay).

For MBF measurements, tissue samples were taken from two cross-sectional slices, each slice being divided into transmural sectors of the septum, anterior, lateral, and posterior wall. Microspheres were isolated from the tissue by digestion (ethanol KOH). Fluorescence was extracted from the beads by 2-ethoxyethylacetate and determined using spectrofluorometry. Values of regional MBF were normalized to the mean MBF in the entire LV wall.

Statistical analysis

The sample size in the present study was based on previous experience in our laboratory. Significant differences in echocardiographic variables, as well as MBF and circumferential shortening between the LBBB group and LBBB+VBP group were analysed at 16 weeks by unpaired t-test. Within the LBBB group and the LBBB+VBP group significant differences in echocardiographic variables, MBF, and circumferential shortening were analysed between 16 weeks and 8 weeks by paired t-test. Changes over time of echocardiographic and haemodynamic variables presented in Table 1 were evaluated using ANOVA for repeated measures. We used fixed effect ANOVA, as provided by Graphpad Prism software, using group and time as fixed variables and the echocardiographic and haemodynamic variables as dependent variables. Each time point was given equal weight. If ANOVA indicated a significant difference between time points, a Bonferroni post hoc test was performed to compare selected pairs of time-points. Comparison between the acute haemodynamic improvement and remodelling after 8 weeks of BVP was made by linear regression analysis. All measured values are described as mean ± SD. For all analyses, a P-value < 0.05, two-sided, was considered statistically significant.

Results

Haemodynamics, electrocardiography, and M-mode echocardiography

LBBB doubled QRS duration and increased the septal-to-lateral wall motion delay and the Sep-Lat shortening delay. LBBB acutely decreased LV dP/dt max to 78 ± 5% of pre-LBBB values (Table 1). The values obtained immediately after induction of LBBB were not significantly different from those after 8 weeks (LBBB+VBP group) and 16 weeks of LBBB (LBBB group; Table 1). BVP reduced QRS duration and restored the septal-to-lateral wall motion delay as well as the Sep-Lat shortening delay. BVP increased LV dP/dt max.
to 86 ± 5% of pre-LBBB values, without affecting LV systolic or end-diastolic pressure. After 8 weeks of BVP, LV dP/dt\textsubscript{max} was 89 ± 5% of pre-LBBB values (Table 1).

**Differences in septum vs. left ventricular lateral wall**

Figure 2 presents myocardial circumferential shortening tracings in eight regions along the circumference in a mid-basal short-axis LV slice. Before LBBB, the time course and amplitude of myocardial shortening were similar in the various LV regions. During LBBB, the early-activated septum showed early-systolic myocardial shortening followed by paradoxical motion. In the late-activated LV lateral wall, early systolic pre-stretch was followed by pronounced systolic shortening. BVP largely restored the abnormal contraction pattern induced by LBBB.

Induction of LBBB decreased CSsys in the septum to almost zero and approximately doubled CSsys in the LV lateral wall (Figure 3). This redistribution of CSsys remained unchanged over 16 weeks of LBBB (LBBB group). However, BVP virtually normalized the redistribution of regional CSsys (Figure 3).

Along with CSsys, induction of LBBB resulted in an immediate and persistent redistribution of regional MBF (Figure 4). LBBB decreased MBF in the septum and increased MBF in the LV lateral wall to 88 ± 4% and 114 ± 4%, respectively. BVP immediately returned MBF distribution close to the pre-LBBB state (septal MBF 100 ± 5%; LV lateral wall MBF 97 ± 3%, Figure 4). During 8 weeks of BVP MBF distribution remained uniform. Turning off BVP after 8 weeks in the LBBB+BVP group resulted again in an immediate redistribution of MBF to values similar to those seen after induction of LBBB.

**Left ventricular (reverse) remodelling**

Eight weeks of LBBB resulted in an increase of both LVEDV and LV wall mass. At the regional level, wall mass was increased in the LV lateral wall but not in the septum, indicating asymmetrical hypertrophy (Figures 1 and 5). In the LBBB group, LVEDV and LV wall mass levelled-off between 8 and 16 weeks of LBBB.

Figure 5 shows that within 8 weeks of BVP, LVEDV returned to pre-LBBB values (from 123 ± 3 to 109 ± 7% of pre-LBBB values), indicating reversal of LV dilatation. BVP did not significantly affect total LV mass (from 117 ± 5 to 110 ± 2%). However, at the regional level a significant decrease was observed in LV lateral wall mass (from 128 ± 5% to 113 ± 3%), whereas septal wall mass did not change during BVP (Figure 5 lower panel). Consequently, the LV septal-to-lateral wall mass ratio decreased to 84 ± 2% of the pre-LBBB values after 8 weeks of LBBB and increased to 98 ± 3% after a subsequent 8 weeks of BVP, indicating reversal of asymmetrical LV hypertrophy.

The relative improvement in LV dP/dt\textsubscript{max} acutely after onset of BVP proved to be related to the relative decrease in LVEDV and LV wall mass during 8 weeks of BVP (Figure 6). When plotting the changes in LV dimensions as a function of the changes in LV dP/dt\textsubscript{max} for all dogs in the study, significant correlations were found between Δ% LVEDV and Δ% LV dP/dt\textsubscript{max} (r = −0.69; P < 0.05) and Δ% LV wall mass (r = −0.73; P < 0.05).

**Discussion**

The present study demonstrates that in LBBB hearts ventricular resynchronization by BVP largely restores LV pump function. Moreover, as indicated by the uniform distribution...
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Acute LBBB</th>
<th>8 weeks LBBB</th>
<th>8 weeks LBBB + BVP</th>
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<tr>
<td>Heart rate (b.p.m.)</td>
<td>101 ± 17</td>
<td>121 ± 47</td>
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<td>QRS duration (ms)</td>
<td>108 ± 23</td>
<td>117 ± 13*</td>
<td>112 ± 11*</td>
<td>108 ± 8*</td>
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<tr>
<td>PQ time (ms)</td>
<td>106 ± 8</td>
<td>117 ± 14</td>
<td>129 ± 22</td>
<td>74 ± 10*</td>
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<td>LV dP/dt max (mmHg/s)</td>
<td>1803 ± 909</td>
<td>1313 ± 464</td>
<td>1552 ± 580</td>
<td>2033 ± 580*</td>
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<tr>
<td>LVSP (mmHg)</td>
<td>101 ± 17</td>
<td>102 ± 19</td>
<td>97 ± 27</td>
<td>116 ± 3</td>
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<tr>
<td>LVEDP (mmHg)</td>
<td>8 ± 6</td>
<td>6 ± 7</td>
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<tr>
<td>Sep-Lat shortening delay (ms)</td>
<td>91 ± 15</td>
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<td>236 ± 31*</td>
<td>N.A.</td>
</tr>
<tr>
<td>Sep-Lat shortening delay (ms)</td>
<td>34 ± 29</td>
<td>N.A.</td>
<td>200 ± 20</td>
<td>173 ± 53*</td>
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LV dP/dt max, maximal rate of increase in left ventricular (LV) pressure; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; Sep-Lat shortening delay, time delay from inward movement of septum to LV lateral wall; N.A., data not available. Values are presented as mean ± SD.

*P < 0.05 compared with pre-LBBB.
†P < 0.05 compared with 8 weeks LBBB.
‡P < 0.05 between 16 weeks LBBB and 8 weeks LBBB + 8 weeks BVP.
patients during RV apex pacing\(^2\) and in patients with LBBB.\(^9\) Zhang et al.\(^{21}\) did not find asymmetric hypertrophy in their study and no regional difference in the change of local wall mass. This lack of regional change in wall mass could be explained by the small size of the study and/or the measuring technique; measuring the thickness at a certain point. We employed integration over entire wall sectors rather than measuring local thickness; the latter can be expected to be relatively inaccurate due to the irregular endocardial wall. The present study and also an earlier echocardiographic study on LBBB patients\(^9\) indicates that the ratio of septum to LV lateral wall thickness may be a parameter to assess (reversal of) asymmetric hypertrophy. Also, because presumably asymmetric hypertrophy is an expression of long-lasting and significant redistribution of workload due to asynchronous activation, asymmetric hypertrophy could be an additional diagnostic indication of the success of CRT.

Regression of hypertrophy in the most hypertrophied part of the LV wall may be functionally relevant. Asynchronous ventricular activation in failing hearts, induced by rapid RV pacing, resulted in myocardial protein dysregulation selectively in the late-activated LV lateral wall, such as down-regulation of SR Ca-ATPase, phospholamban, and connexin 43.\(^{14}\)

Relation between pump function and reverse remodelling

Ventricular remodelling in asynchronously activated hearts is characterized by global enlargement (LVEDV and LV mass) and an asymmetric distribution of LV mass.\(^9,10,13\) We have previously proposed that overall LV enlargement is related to the reduction in pump function.\(^{12}\) Consequences of reduced pump function, such as a right-ward shift in the LV pressure–volume relation\(^{13}\) and neurohumoral
activation,24 are established triggers for hypertrophy. The finding in the present study, that the acute improvement of LV pump function by BVP predicts the degree of LV reverse remodelling, supports the above-mentioned relation between pump function and global LV enlargement.

The finding that the acute improvement of LV pump function by BVP predicts LV reverse remodelling should, however, be extrapolated to the clinical situation with care. Evidence available so far indicates that the relation between haemodynamic improvement and reverse remodelling is less clear in patients.25 The number of observations in the present animal study is too low to be used for prediction in a large population. Moreover, reversibility of remodelling may be less in patients with long-standing remodelling than in healthy dogs with 2 months of LBBB.

Lower reversibility of reverse remodelling and the observation that CRT can already reverse remodelling in LBBB hearts without overt heart failure could plea for starting CRT in less-advanced heart failure. This idea is supported by previous studies. Stellbrink et al.25 observed that ‘volume responders’ had smaller baseline LVEDV and ESV than non-responders. Secondly, Bleeker et al.26 showed larger relative reduction in NYHA II than in NYHA class III patients. The regionally different contraction patterns in the asynchrony heart cause significant regional differences in stretch and subsequently workload, being the most likely explanation for the asymmetry of hypertrophy.4,10,13 Similarly, the normalization of the contraction patterns by BVP, thereby normalizing regional differences in workload, explain the disappearance of the asymmetry of hypertrophy during BVP.

The immediate change in MBF distribution in the present study indicates that this change can predominantly be attributed to the distribution of regional workload upon changing the sequence of activation. PET studies on MBF in CRT patients were performed at least 3 weeks after onset of CRT, leaving open the possibility that the change in blood flow distribution is due to reverse remodelling.27–29 The parallel change of blood flow and oxygen uptake distribution in CRT patients further supports the redistribution of mechanical work by the change in activation sequence.28–30

**Dog model**

The present study was performed in an animal model of LBBB. There may be various differences between this animal model and patients with conduction disturbances. In the present study LBBB was induced by RF ablation in the animals, whereas a proximal block of the left bundle may not be present in all patients with LBBB.31,32 Also, the animals lack any other co-morbidity, which may be of influence in the response to BVP. Nevertheless, the relative increase in QRS duration and septal-to-lateral wall motion delay in the present study was the same as the difference between control and LBBB patients.16 Also, the redistribution of blood flow during LBBB and BVP in the present study was similar to that of non-ischaemic LBBB patients.28

Like patients with LBBB showing lower septal perfusion28 and mechanical ventricular asynchrony,16,19 all our dogs were ‘responders’ in the sense that BVP improved LV pump

![Figure 5](https://academic.oup.com/eurheartj/article-abstract/28/17/2148/425515/2153)

**Figure 5** Relative changes in (A) LV end-diastolic volume (LVEDV), (B) total LV wall mass, (C) septal (squares), and LV lateral wall (circles) mass in the LBBB (open symbols) and LBBB + BVP group (closed symbols). *P < 0.05 (unpaired t-test) LBBB group vs. LBBB + BVP group at 16 weeks. †P < 0.05 (paired t-test) 16 weeks vs. 8 weeks within LBBB + BVP group. Values are presented as mean ± SD.

![Figure 6](https://academic.oup.com/eurheartj/article-abstract/28/17/2148/425515/2153)

**Figure 6** Correlation between the acute haemodynamic improvement during optimal BVP (Δ% LV dP/dt(max)) and relative reduction of LV end-diastolic volume (Δ% LVEDV) and LV wall mass (Δ% LV wall mass) after 8 weeks of BVP.
function, normalized blood flow redistribution, and reduced LV dilatation. Moreover, a previous study showed that the relation between interventricular asynchrony and LV pump function during ventricular pacing was similar in our LBBB animals when compared with CRT candidates.20 Thus, despite potential differences between the LBBB animals and patients, our LBBB animal model has many aspects of asynchrony and resynchronization in common with patients with LBBB with and without resynchronization.

Conclusion
In canine hearts with isolated LBBB, BVP can reverse most of the LV ventricular remodelling, induced by LBBB. Reverse remodelling consisted of reduction of LV dilatation as well as regression of hypertrophy in the most hypertrophied LV lateral wall. LV reverse remodelling appeared to be related to the more uniform distribution of myocardial workload.

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Conflict of interest
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