Preclinical research

Left bundle branch block induces ventricular remodelling and functional septal hypoperfusion

Kevin Vernooy¹,²*, Xander A.A.M. Verbeek¹, Maaike Peschar¹, Harry J.G.M. Crijns², Theo Arts³, Richard N.M. Cornelussen¹, and Frits W. Prinzen¹

¹Department of Physiology, Cardiovascular Research Institute Maastricht, PO Box 616, 6200 MD Maastricht, The Netherlands
²Department of Cardiology, Cardiovascular Research Institute Maastricht, PO Box 616, 6200 MD Maastricht, The Netherlands
³Department of Biophysics, Cardiovascular Research Institute Maastricht, PO Box 616, 6200 MD Maastricht, The Netherlands

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Aims Left ventricular (LV) dilatation, hypertrophy, and septal perfusion defects are frequently observed in patients with left bundle branch block (LBBB). We investigated whether isolated LBBB causes these abnormalities.

Methods and results In eight dogs, LBBB was induced by radio frequency ablation. Two-dimensional echocardiography showed that 16 weeks of LBBB decreased LV ejection fraction (by 23 ± 14%) and increased LV cavity volume (by 25 ± 19%) and wall mass (by 17 ± 16%). The LV septal-to-lateral wall mass ratio decreased by 6 ± 9%, indicating asymmetric hypertrophy. After onset of LBBB, myocardial blood flow (MBF, fluorescent microspheres) and systolic circumferential shortening [CSsys, magnetic resonance (MR) tagging] decreased in the septum to 83 ± 16% and –11 ± 20% of baseline, respectively, and increased in LV lateral wall to 118 ± 12% and 180 ± 90% of baseline, respectively. MBF and CSsys values did not change over 16 weeks of LBBB. Changes in external mechanical work paralleled those in CSsys. Glycogen content was not significantly different between septum and LV lateral wall of LBBB hearts (16 weeks) and control samples, indicating absence of hibernation.

Conclusions The asynchronous ventricular activation during LBBB leads to redistribution of circumferential shortening and myocardial blood flow and, in the long run, LV remodelling. Septal hypoperfusion during LBBB appears to be primarily determined by reduced septal workload.

KEYWORDS
Left bundle branch block; Remodelling; Echocardiography; Myocardial blood flow; MR tagging

Introduction

Epidemiological studies have identified left bundle branch block (LBBB) as an independent risk factor for cardiac mortality.¹–³ While in the general population LBBB has an incidence of 1–3% at age 65, this incidence is ~30% in patients with heart failure.² Little is known of the aetiology during the early phase of LBBB, because it usually has a silent onset. Understanding LBBB is further complicated by its frequent coincidence with other cardiovascular derangements.⁴⁻⁵

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In patients, LBBB is often accompanied by left ventricular (LV) dilatation, reduced LV ejection fraction (EF),\textsuperscript{4–6} and septal perfusion defects,\textsuperscript{7,8} even in the absence of coronary artery disease. It is not known whether these abnormalities are the cause of the abnormal conduction or whether the conduction disturbance is a marker of a more progressive disease state.\textsuperscript{9}

Structural adaptation of the left ventricle could be expected with LBBB because in canine hearts, chronic pacing at the LV lateral wall, also leading to asynchronous electrical activation, has been shown to lead to asymmetric hypertrophy and ventricular dilatation.\textsuperscript{10} However, during LV pacing the activation sequence is opposite to that of LBBB and the ectopic stimulus is generated outside the Purkinje system.

Moreover, in these LV pacing experiments it was observed that the non-uniform distribution of blood flow disappeared over time,\textsuperscript{11} which seems in conflict with the septal underperfusion in patients with LBBB. Also, it is still controversial whether the redistribution of perfusion during asynchronous activation is a functional adaptation to altered mechanical work, and therefore demand, or signifies hampered perfusion due to the abnormal contraction.\textsuperscript{11,12} In the latter case, structural and functional abnormalities in underperfused regions would be anticipated.

The aim of the present study was to investigate to what extent isolated LBBB causes ventricular remodelling and loss of LV pump function and whether abnormal septal perfusion contributes to these changes. To this purpose, LBBB was induced in normal canine hearts. LV endocardial electrical activation (endocardial mapping), myocardial circumferential shortening [magnetic resonance (MR) tagging], and myocardial blood flow (MBF, fluorescent microspheres) were mapped before, shortly and chronically (16 weeks) after onset of LBBB. Ventricular remodelling was assessed by serial two-dimensional echocardiography. Tissue glycogen content was used to detect potential hibernation, a sign of longer lasting restriction of perfusion.

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 Animal Experimental Committee of Maastricht University approved the protocol.

Experimental protocol

The experiments were performed on eight adult mongrel dogs of both sexes and unknown age, with a weight of 28.0±0.5 kg. After pentothal induction, anaesthesia was maintained by ventilation with O₂ and N₂O (1:2) in combination with an infusion of midazolam (0.1 mg/kg/h iv) and sufentanyl (3 μg/kg/h iv).\textsuperscript{13} During sterile surgery, LBBB was induced by radio frequency (RF) ablation.\textsuperscript{14} Two catheter tip manometers (CD-Leycom, Zoetermeer, The Netherlands) were used to measure LV and right ventricular (RV) pressures. Cardiac output was measured by thermodilution. A basket catheter (EPT Constellation, Boston Scientific, San Jose, CA, USA) was unfolded in the left ventricle to obtain LV endocardial activation times.\textsuperscript{14} For MBF measurements, using the fluorescent microsphere technique,\textsuperscript{11} a catheter was placed in the left ventricle for microsphere injection. For each MBF measurement, 5×10⁶ fluorescent microspheres (15.5 μm±2%, Molecular Probes, Eugene, OR, USA) were injected.

Haemodynamic, ECG, and LV endocardial electrical potential measurements and microsphere injections were performed before (baseline) and shortly after (∼30 min) creation of LBBB (acute LBBB). Endocardial mapping was performed directly after haemodynamic measurements to prevent interference of the basket electrode with LV function measurements.

Two-dimensional echocardiographic images of the left ventricle were made before (baseline) and every 2 weeks after onset of LBBB up to 16 weeks (chronic LBBB). The animals were pre-medicated with acepromazine (0.6 mg/kg im).\textsuperscript{13}

MR tagging measurements were performed 1 week before (baseline), 3±1 weeks after (acute LBBB) and as well as 15±1 weeks after (chronic LBBB) creation of LBBB under the same anaesthetic conditions as mentioned above using manual ventilation.

After 16 weeks of LBBB the animals were anaesthetized again, repeating the measurements of haemodynamics, ECG, and LV endocardial mapping and MBF by microsphere injections. To investigate whether the remodelling process had caused impaired septal perfusion, microspheres were injected within 15 min after starting simultaneous RV apex and LV lateral wall stimulation (biventricular pacing) in three experiments.

Finally, the heart was removed and transmural tissue samples for glycogen analysis were taken from the septum and LV lateral wall, quickly immersed in liquid nitrogen, and stored at −80°C. Control values for myocardial glycogen content were obtained from 10 samples from sham operated control dogs,\textsuperscript{10} which were virtually equal to 69 historic control values.\textsuperscript{15} For microsphere analysis, the left ventricle was stored at −20°C together with the reference blood samples.

Magnetic resonance imaging

Cine-images were acquired on a Philips Gyroscan 1.5 T (NT, Philips Medical Systems, Best, the Netherlands). The RF receiver coil was a standard synergy body coil for thorax examinations. Breath-hold (∼12 s) was accomplished by discontinuing manual ventilation and followed by a recovery period of ∼45-60 s. Images of seven short-axis cross-sections, slice thickness 8 mm with inter-slice distance 0 mm, were obtained to capture the whole heart. Cine-images were acquired using non-tagged steady state gradient echo sequences, starting 28 ms after the R-wave on the vectorcardiogram (field of view 400 mm, image size 256×256 pixels). Thereafter a series of grid-tagged images from the same slices were obtained with time intervals of 15 ms, using balanced fast field echo (FFE) scanning.

Data analysis

Haemodynamic data analysis was performed as described previously.\textsuperscript{16} Interventricular asynchrony was calculated as the timing difference between the upslopes of simultaneously recorded LV and RV pressure curves.\textsuperscript{14} LV endocardial activation times, derived from endocardial activation maps, were used as a measure for intraventricular asynchrony.

The echocardiograms were digitized and short-axis images were analysed by use of software developed in our own laboratory by an experienced echocardiographer who was blinded for the time interval the echocardiogram was performed.\textsuperscript{14} Regional geometry was determined from end-diastolic short-axis LV images after division into four sectors: anterior, septal, posterior, and lateral wall.
LV EF was calculated from the LV end-diastolic and end-systolic volume (EDV and ESV, respectively).

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. Within the region of interest, systolic circumferential shortening (CSsys) was calculated as the change in segment length with respect to initial ejection \((L_0 - L_j)/L_0\).

External mechanical work was calculated by combining CSsys data, obtained during the MRI scan, with the LV pressure and volume data, obtained during the haemodynamic measurements immediately preceding the MRI scan. Timing of the strain, pressure, and volume curves was matched by superimposing the LV volume signal obtained from the MRI scan with that from the haemodynamic measurements.

For MBF measurements, the left ventricle was divided into three short-axis slices and then divided into 12 transmural sections. Microspheres were isolated from the tissue by digestion (ethanolic KOH). Fluorescence was extracted from the beads by 2-ethoxyethylacetate and determined using spectro-fluorometry.

To analyse regional glycan content, aliquots of ventricular tissues were freeze-dried. After adding 1 M HCl to the freeze-dried material, glycogen was hydrolysed at 100 °C for 3 h. After neutralization with TRIS/KOH saturated with KCl, the glucose residues were measured fluorometrically.

### Statistical analysis

The number of experiments in the present study was based on previous experience in our laboratory. All values are described as mean values with their corresponding standard deviations. Changes over time of electrocardiographic, haemodynamic, and echocardiographic variables, as well as myocardial blood flow and circumferential shortening, were evaluated using repeated measures of ANOVA. If ANOVA indicated a significant difference between time points, a Bonferroni post hoc test was performed to compare selected pairs of time points. Differences in myocardial shortening, work and blood flow, and glycogen content between the septum and LV lateral wall were evaluated using a paired Student's t-test. In all tests a \(P\)-value of \(<0.05\) or less (two-sided) was considered statistically significant.

### Results

#### Electrophysiological and haemodynamic changes

Creation of LBBB did not affect heart rate or PQ interval but increased QRS duration (Figure 1, upper row). Intra-LV activation time (Figure 1, second row) was prolonged leading to increased intraventricular asynchrony (Table 1). None of these variables changed significantly between acute and chronic LBBB. Interventricular asynchrony increased significantly after LBBB was established and remained stable during chronic LBBB (Table 1). LBBB immediately decreased cardiac output and LV dP/dt\text{max} without change in LV end-diastolic pressure (Table 1). Haemodynamic parameters did not change significantly between acute and chronic LBBB, but during chronic LBBB the cardiac output was no longer different from baseline.

#### Redistribution of systolic shortening

Figure 2 presents myocardial circumferential shortening as a function of time in eight regions along the circumference in a mid-basal short-axis LV slice. During baseline, the time course and amplitude of myocardial shortening were similar in the various regions. During LBBB, the early-activated septum showed pre-systolic myocardial shortening followed by some paradoxical motion, whereas the late-activated LV lateral wall showed pronounced myocardial shortening.

During baseline, CSsys was not significantly different between septum and LV lateral wall (5.9 ± 2.4% and 6.5 ± 2.6%, respectively). LBBB induced asynchrony of contraction, as measured by significant differences in timing of maximal shortening between septum and LV lateral wall (Figure 1, third row). Acute LBBB decreased septal CSsys to −0.5 ± 1.3% (−11 ± 20% of baseline, indicating some systolic stretching) and increased CSsys in the LV lateral wall to 10.1 ± 3.0% (180 ± 90% of baseline, Figure 3A). During chronic LBBB, no significant changes in CSsys were observed as compared with acute LBBB. Changes in external mechanical work paralleled those in CSsys (Figure 3B).

#### Myocardial blood flow

During baseline, MBF showed a well known heterogeneous distribution, but without significant differences between septum and LV lateral wall (0.78 ± 0.34 and 0.76 ± 0.36 mL/min/g, respectively). During acute and chronic LBBB, MBF was consistently lower in the septum than in the LV lateral wall (Figure 1, fifth row). Relative to baseline, MBF decreased by 17 ± 16% in the septum but increased by 18 ± 12% in the LV lateral wall during acute LBBB (Figure 3C). These changes resulted in a decrease of the LV septal-to-lateral MBF ratio from 1.28 ± 0.34 during baseline to 0.88 ± 0.16 and 0.66 ± 0.20 during acute and chronic LBBB, respectively. Regional MBF during chronic LBBB was not significantly different from that in acute LBBB. Immediately after starting biventricular pacing, after 16 weeks of LBBB, MBF distribution normalized as evidenced by a LV septal-to-lateral MBF ratio of 0.96 ± 0.26.

#### Structural remodelling

During 16 weeks of LBBB, LV EDV and wall mass gradually increased, indicating development of eccentric hypertrophy (Table 2 and Figure 4). Although both septal and LV lateral wall mass increased significantly during chronic LBBB (Table 2), the septal-to-lateral wall mass ratio decreased by 6 ± 9% (Figure 4B), indicating asymmetry of hypertrophy. LV EF was reduced significantly within 2 weeks of LBBB, and tended to decrease further until 16 weeks of LBBB (Table 2). The time courses of the relative changes in LV EDV, LV wall mass and LV EF are shown in Figure 4. The time courses of LV EDV and LV EF of two individual dogs in Figure 4A and 4C illustrate that the degree to which LBBB affected these variables differs between dogs.
Figure 5 shows that the tissue glycogen content, a measurement of myocardial hibernation, was not significantly different between the septum and LV lateral wall (133 ± 30 and 150 ± 34 μmol/g dry weight, respectively) and not different from control myocardium (160 ± 15 μmol/g dry weight).

Discussion

The findings in the present study demonstrate that, in otherwise normal hearts, LBBB immediately and persistently induces mechanical asynchrony. As a consequence LBBB leads to a reduction of LV EF and to redistribution...
of myocardial shortening and blood flow from the septum to the LV lateral wall. Because the patterns of redistribution of myocardial blood flow and external work are similar and remain constant over time and because there is no sign of hibernation in the septum, the septal underperfusion in LBBB hearts is most likely a functional adaptation to the reduced septal workload. Therefore, during LBBB loss of pump function and hypertrophy appear to be a greater threat to long-term prognosis than the reduced septal blood flow.

**Structural remodelling**

The main part of the 20–30% dilatation and hypertrophy and the similar relative reduction in LV ejection fraction occurs within a few weeks of LBBB, but for all three variables a further progression over time is noticed. Comparative studies in patients with LBBB showed a more pronounced LV dilatation (~75%) and hypertrophy (~40%) and more reduced LV EF (~25%) relative to a control group. This more extensive LV remodelling in patients with LBBB may be due to a longer duration of LBBB and the presence of other concomitant diseases, i.e. valvular insufficiencies and myocardial infarction. While these patient studies cannot distinguish between LBBB as cause or consequence of ventricular remodelling, our data demonstrate that LBBB can solely initiate remodelling in a normal heart. Also, asynchronous ventricular activation, induced by pacing the LV free wall, caused ventricular dilatation and asymmetric hypertrophy. Ventricular hypertrophy and dilatation are known to contribute to the development of heart failure, but the extent and duration of hypertrophy associated with onset of heart failure vary per animal model and disease. In dogs for example, pressure and volume overload hypertrophy require at least 50% hypertrophy before heart failure develops, whereas hardly any hypertrophy is present in the rapid pacing model of heart failure. The ~20% hypertrophy in our LBBB model is similar to the degree of hypertrophy induced by coronary occlusion in canine hearts leading to a 20% infarct size, but the dilution in infarcted hearts was more pronounced. The ~30% ventricular dilatation observed in our canine LBBB hearts is slightly more pronounced than the dilatation observed in patients with sinus node dysfunction treated with RV pacing, which results in a similar activation pattern to LBBB. These patients developed significantly more heart failure than patients treated with atrial pacing over a mean follow-up time of 5.5 years. Similarly, in patients with indications for implantable cardioverter defibrillator therapy, but no pacing indications, ventricular pacing increased the incidence of heart failure compared with ventricular back-up pacing. Therefore, the reduction in LV pump function and ventricular remodelling induced by long-term asynchronous ventricular activation might play a role in the high incidence of heart failure in patients with LBBB.

The observation that, even in our study, the degree of ventricular remodelling differed between dogs (Figure 4) suggests that individual hearts may have different sensitivities to abnormal conduction. After all, we created LBBB in a controlled way and interindividual variation in QRS duration, as well as inter- and intraventricular asynchrony, was small. The small but significant asymmetrical hypertrophy after 16 weeks of LBBB is most likely due to the redistribution of workload, as evidenced by the regional differences in CSys and external work (Figure 3). A similar degree of asymmetry of hypertrophy was observed in patients with LBBB and in dogs with RV pacing. Regional differences in macroscopic hypertrophy were related to regional differences in myocyte diameter without differences in regional collagen content, indicating that the hypertrophy is due to a proportional increase of myocyte and collagen volume.
Septal perfusion

Using the gold standard for regional MBF measurements (microspheres), the present study demonstrates considerable and consistent redistribution of MBF due to LBBB. A similar MBF redistribution has been observed during ventricular pacing, indicating the role of the asynchrony of activation. Both situations reveal reduced blood flow in early-activated regions and increased blood flow in late-activated regions. In contrast to long-term pacing at the LV lateral wall, the distribution of blood flow in the present study did not normalize during chronic LBBB. This may be related to the more pronounced asymmetry of hypertrophy during long-term LV pacing. This large asymmetry (~40%) matched the regional differences in initial blood flow reduction and mechanical load. Therefore, the small degree of asymmetry of hypertrophy during LBBB explains why septal perfusion remains reduced over time. It is not well understood why asymmetry of hypertrophy differs between chronic LBBB and LV pacing, because the degree of mechanical differences as well as acute blood flow changes appear to be similar.

The reduced septal blood flow in LBBB patients has frequently been described, although the degree of reduction of septal perfusion varies between studies. $^{13}$N-ammonia PET perfusion studies showed virtually normal septal perfusion, whereas in nuclear (thallium, SPECT) perfusion studies the reduction was similar to that observed in the present study. Methodological factors, such as the kind of tracer and image analysis employed and the use of exercise or a vasodilating agent, may underlie these differences. In the present study, measurements were made at rest and without a vasoactive agent.

The functional implication of reduced septal perfusion in LBBB hearts is under debate. Some investigators suggest that reduced perfusion in early activated regions is due to restriction of blood flow by the abnormal contraction pattern. To some extent such restriction might play a role, since underperfusion is more pronounced at higher heart rates, probably because at high heart rates the asynchronous contraction leaves less diastolic time for perfusion. However, considerable and/or frequent restriction of septal perfusion would have led to stunning or hibernation. The present study shows that stunning is unlikely to occur, because systolic shortening does not deteriorate during longer lasting LBBB. There are also no biochemical signs of hibernation. Hibernation, characterized by myocyte dedifferentiation due to longer lasting mild to moderate underperfusion, can increase myocardial glycogen three-fold. No signs of glycogen accumulation were observed in this study, where measurement error was <10% of normal glycogen values. The lack of hibernation is in agreement with a good balance between oxygen supply and demand in asynchronously activated ventricles which has previously

Table 2 Echocardiographic parameters

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<th>Baseline</th>
<th>2 weeks LBBB</th>
<th>Chronic LBBB</th>
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<tr>
<td>LV EDV (mL)</td>
<td>104 ± 31</td>
<td>110 ± 36</td>
<td>135 ± 53</td>
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<tr>
<td>LV wall mass (g)</td>
<td>126 ± 31</td>
<td>135 ± 37</td>
<td>145 ± 30</td>
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<td>LV EF (%)</td>
<td>43 ± 4</td>
<td>37 ± 8</td>
<td>33 ± 6</td>
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LV EDV = left ventricular end-diastolic volume, EF = ejection fraction. *P < 0.05 compared with baseline.

Figure 3 (A) Regional LV systolic circumferential shortening, (B) external work, and (C) myocardial blood flow during baseline, acute and chronic LBBB in the septum (Sep, white bars) and the LV lateral wall (Lat, black bars). Data are normalized to regional baseline values.
been found during acute ventricular pacing. Throughout paced ventricles regional systolic shortening, mechanical work, and oxygen uptake are mutually related to each other. Moreover, under these conditions no lactate release occurs\(^3\) and complete vasodilation attenuates the abnormal blood flow distribution,\(^3\) suggesting that coronary autoregulation is responsible for the effects. This is further supported by the immediate normalization of blood flow distribution upon resynchronization of the activation in chronic LBBB hearts.

Therefore, the reduced septal contractility in the presence of reduced contractile performance, as frequently reported in patients, does not exclude adequate septal perfusion. Rather, the reduced septal perfusion during LBBB appears to be the result of autoregulation following a reduction in local oxygen demand in early activated myocardium.

**Limitations**

The present study shows that, in normal dog hearts, LBBB can reduce septal perfusion and can cause ventricular remodelling. However, these findings do not exclude the possibility that ventricular remodelling, induced by other disease processes, can cause LBBB or that septal underperfusion can cause LBBB, but clearly indicate that LBBB should be considered as a mechanism of these changes.

In this animal model, LBBB is generated by ablation of the proximal left bundle branch. The LV endocardial activation and the myocardial shortening maps indicate that this ablation was sufficient to force the electrical impulse to be conducted through the LV wall by predominantly slow myocardial conduction as opposed to the rapid conduction through the Purkinje system. In patients, however, variable patterns of LBBB are present.\(^3\) Differences in the sequence of activation of the ventricles are likely to affect the distribution of myocardial shortening and blood flow, but to what extent this would also affect ventricular performance or remodelling is not clear.

Although no untreated control group was used, it is known from previous animal experiments in our laboratory that MBF and LV geometry remain constant over time.\(^1\)^\(^1\)

**Conclusions**

LBBB induces immediate and persistent LV asynchronous electrical activation, reduced LV pump function and redistribution in LV MBF, CS\(_{sys}\), and external work. The parallel decrease in septal blood flow and myocardial work, their constancy over time, and the absence of hibernation in the septum indicates that the reduced septal work appears to be the major determinant of septal hypoperfusion during LBBB. Therefore, the contractile inco-ordination during LBBB appears to play an important role in the development of LV dilatation and hypertrophy.
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