Subepicardial dysfunction leads to global left ventricular systolic impairment in patients with limb girdle muscular dystrophy 2I

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Introduction Limb girdle muscular dystrophy 2I (LGMD2I) is an autosomal recessive condition associated with mutations in the fukutin-related protein gene (*FKRP*) leading to weakness in the pelvic and shoulder girdle muscles. Previous studies have indicated variable degrees of cardiac involvement, including reduced left ventricular ejection fraction (LVEF), increased end-diastolic volume (EDV) and occasional cardiac hypertrophy [1]. There is a complex fibre architecture that varies from subepicardium through to subendocardium. In the human heart, the subepicardium fibres are oriented obliquely in a left-handed spiral at 75^{o} from the circumference of the heart and subendocardial fibres in the opposite direction at -70^{o}. Midwall fibres are oriented circumferentially. Important functional consequences occur as a result of these fibre oritentations [2]. At the subepicardium, maximal fibre shortening occurs along the fibre length, whereas at the subendocardium maximal shortening occurs at almost right angles to the fibre direction. This subendocardial 'cross-fibre' shortening is thought to be due to the greater radius and mechanical advantage of the subepicardium. Additionally due to its own contraction the subendocardium shortens in both the fibre and cross fibre directions. As there is shortening in 2 planes, there must be enhanced thickening in a third plane to preserve volume. This results in marked radial thickening, which is an essential part of normal ejection. Torsion of the left ventricle occurs through shortening of the obliquely oriented subepicardial fibres partially counteracted by subendocardial fibre shortening [3]. Thus, normal left ventricular ejection is caused by a balance of subepicardial and subendocardial strains. With respect to LGMD2I associated cardiomyopathy, we hypothesized that impaired binding of extracellular matrix components to α-dystroglycan causes injury along fibre bundles leading to a selective loss of myocardial strains in the left ventricle, based on previous animal data [4]. This could result in an imbalance between subepicardial and subendocardial forces. We used magnetic resonance imaging, tissue tagging, and spectroscopy to assess strains and energetics in the hearts of ambulant patients with LGMD2I and related these to measures of global left ventricular function.

Methods *Subjects* 11 patients (8M:3F) with a confirmed genetic diagnosis of LGMD2I with homozygous c.826C>A *FKRP* mutations were recruited prospectively: inclusion criteria were being ambulant for more than 50 metres, no ventilator requirements and able to lie flat plus no contraindications for MRI scanning. One male LGMD2I subject was subsequently excluded due to previous use of recreational drugs. 10 gender, age-, weight- and BMI- matched subjects were recruited by advertisement as controls. All control subjects were screened with a 12-lead ECG to exclude cardiac abnormalities and hypertension (> 150/90 mmHg). This study was approved by the local ethics committee and informed consent was obtained from each participant.

MRI protocol: 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 ECG gating (Philips vectorcardiogram). A stack of balanced steady-state free precession images was obtained in the short axis view during breath holding covering the entire left ventricle (FOV = 350mm, TR/TE = 3.7/1.9ms, turbo factor 17, flip angle 40^{o}, slice thickness 8mm, 0mm gap, 14 slices, 25 phases, resolution 1.37mm). Image analysis was performed using the ViewForum workstation (Philips, Best, NL). Manual tracing of the epicardial and endocardial borders was performed on the short axis slices at end-systole and end-diastole. Details of our algorithm for contour selection and our methods for subsequently calculating left ventricular mass, systolic and diastolic parameters have been described elsewhere [5]. *Cardiac tagging:* Tagged short axis images were obtained at the same session. Cardiac tagging works by applying radiofrequency pulses to cancel MR 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 [6]. A turbo-field echo sequence was used (TR/TE/FA/NEX = 4.9/3.1/10^{o}/1, SENSE factor 2, FOV 350x350mm, voxel size 1.37x 1.37mm, orthogonal CSPAMM grid [6] with tag spacing of 7mm). Two adjacent short-axis slices of 10mm thickness were acquired at mid-ventricle with a 2mm gap. The Cardiac Image Modelling package (University of Auckland) 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 was measured for both the whole myocardial wall and the endocardial third of the wall thickness. The torsion between the two planes (taken as the circumferential-longitudinal shear angle defined on the epicardial surface) was calculated [7]. The relationship between torsion and strain can be approximated by a ratio of the peak torsion and the peak circumferential strain in the endocardial third of the myocardium, the torsion to endocardial strain ratio (TSR) [8, 9]. This ratio is constant amongst healthy subjects of the same age, and increases with healthy ageing [8, 10]. Both torsion and TSR are measures of epicardial–endocardial interactions. Longitudinal shortening was determined from cine-MRI in the 4-chamber view 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 were determined at the same level as the cardiac tagging. *Cardiac ^{31}P MRS spectroscopy:* Cardiac high-energy phosphate metabolism was assessed on the same occasion. Data were acquired from prone subjects with a 10cm diameter ^{31}P surface coil (Pulseteq, UK), using a 1-dimensional spectroscopic imaging sequence with slice selective pulse to eliminate contamination from the liver, and spatial pre-saturation of skeletal muscle. Data acquisition took approximately 20 minutes. Full details of our method of estimating the ratio of PCr/ATP have been published previously [5].

Results LGMD2I subjects were found to have a significant reduction in peak cardiac torsion (3.9^{o} vs 6.4^{o}, p=0.04), and in the ratio of torsion to endocardial strain, TSR (0.31 vs 0.51, p=0.03), compared to control subjects. The impairment in torsion correlated strongly with reduction in ejection fraction (r=0.93, p<0.001). Peak circumferential strain was not reduced, either for the whole wall or for the subendocardium. Cardiac cine analysis demonstrated reduced ejection fraction (48% vs 58%, p=0.02) and stroke volume (61ml vs 81ml, p=0.04), though no evidence of left ventricular hypertrophy or of diastolic dysfunction. The ratio of phosphocreatine to adenosine triphosphate (PCr/ATP) was reduced in the LGMD2I subjects compared to controls (1.50 vs 1.94, p=0.0001), though this did not correlate with the impairment in torsion. PCr to ATP ratio was significantly lower in the older half of the LGMD2I group (1.37 vs 1.65, p=0.02).

Conclusions The loss of torsion with preservation of circumferential and longitudinal strain is a unique finding and suggests subepicardial dysfunction with abnormal transmission of force across the cardiac wall. This pattern of change is markedly different to cardiomyopathy in Duchenne muscular dystrophy, where there is early loss of circumferential strain and pre-clinical models. It also differs from the increase in torsion and reduction in subendocardial strain observed in healthy ageing.

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