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ORIGINAL ARTICLE

Reduced right atrial contractile force in patients with left ventricular diastolic dysfunction: A study in human atrial fibers—contractile force and diastolic dysfunction

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Summary *Background/Objective:* The aim of our study was to evaluate right heart contractile force in patients with diastolic dysfunction (DD) with preserved left heart ejection fraction undergoing cardiac surgery. We examined the contractile properties of skinned human fibers obtained from the right auricle in two groups (DD and controls).
Methods: Right atrial tissue from 64 patients, who were undergoing cardiac surgery, were collected before extracorporal circulation. Tissue was conserved and prepared as "skinned fibers". We exposed the dissected fibers to increasing calcium concentrations and recorded the force values.
Results: Patients with DD develop significantly less force at middle and higher calcium concentrations pCa 4.0: DD 2.58 $\pm$ 0.4 mN, controls 5.32 $\pm$ 0.4 mN, $p = 0.02$; pCa 5.5: DD 1.14 $\pm$ 0.3 mN, controls 1.45 $\pm$ 0.3 mN, $p = 0.03$. DD significantly correlates with left ventricular hypertrophy (LVH; $p = 0.03$). DD did not significantly occur more often in patients with mitral valve insufficiency, aortic insufficiency or stenosis, or coronary heart disease (all $p > 0.10$). LVH, which was associated with DD, correlated significantly with mitral valve prolapse ($p = 0.05$), aortic valve stenosis ($p = 0.02$), and mitral valve insufficiency ($p = 0.03$).
Conclusion: Contractile force is significantly reduced in right atrial skinned human fibers with DD. DD is significantly associated with LVH, but emerges independently from underlying pathologies like valve diseases or coronary heart disease. This underlines the hypothesis that

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impairment of contractile capacity directly results from DD—independent from volume or pressure overload due to valvular or ischemic heart disease.

1. Introduction

Diastolic dysfunction (DD) describes the inability of relaxation of the ventricles during diastole.[1,2] It is the main feature of heart failure with preserved ejection fraction (EF). Normal diastolic dysfunction is adequate ventricular filling without abnormal elevation in diastolic pressures.[3] DD is therefore associated with increased diastolic pressures, increased diastolic volume and/or impaired relaxation, and ventricular stiffness. But studies focusing on right ventricular (RV) function and DD are very rare. Considering that left ventricular (LV) DD aggravates ventricular filling and may lead to pulmonary congestion and edema, a right heart involvement can be present before the onset of symptoms. There is a variety of studies concerning mitral valve regurgitation, as well as pulmonary arterial hypertension, and RV function, but a precise association of DD and impairment of RV function have not been examined to date.

However, considering the accompanying pathomechanism of DD and pulmonary hypertension as increased diastolic pressures, increased diastolic volume and/or impaired relaxation, and ventricular stiffness, it seems justified to bridge the gap of missing experimental studies to DD and RV function by looking at similar pathophysiological mechanisms.

Different studies revealed that pulmonary arterial hypertension reduces biventricular vasoreactivity, and furthermore increases diastolic RV stiffness, as well as a decrease of force-generating capacity of single cardiomyocytes.[4–6] These studies detected cardiomyocyte atrophy, a reduction of the number of actin-myosin bridges, and reduced phosphorylation level of sarcomeric proteins.[4–6] Furthermore, Manders et al[7] observed a compensating increase of calcium sensitivity. These observations of a possible affliction of the RV are of academic interest.

RV function is a well-known major predictor of mortality after acute myocardial infarction and aorto-coronary bypass grafting.[3,8–10] The significance of RV function, as well as right atrial volume index, as a prognostic value[2,11] is believed to be a marker for risk prediction in patients with chronic heart failure.

Considering that studies already existed from the 1990s[12] on early involvement of the RV in patients with pulmonary arterial hypertonus due to elevated pulmonary resistance and right-sided pressures, the question is raised about whether this observation can also applied to patients with DD, which also leads to pulmonary congestion and edema and might affect force recruitment. Furthermore, if calcium mishandling occurs, which is believed to be associated with left heart DD, it also counts for impairment of right heart contractility.[8]

To prove this assumption we performed a study regarding force recruitment in patients with and without LV DD in a skinned fiber model.

2. Methods

2.1. Patients

We included 64 patients undergoing mitral valve surgery for valve regurgitation (MR) or valve stenosis: 43 patients without DD (Non-DD group) and 21 patients with DD Grade I with an impairment of relaxation (DD group). DD was measured echocardiographically before operation. The patients' clinical characteristics are summarized in Table 1. Diastolic function was defined as impaired filling of the LV that was measured with Doppler tissue imaging to estimate the mitral flow pattern. DD Grade I was defined with $_1E/E' < 6$ cm/s, $E' < 8$ cm/s and normal or slightly increased LV filling pressure. Table 2 shows echocardiographic and Swan-Ganz catheter data. All examined patients met these criteria. We excluded patients with DD Grades II or III because we wanted to focus on patients with normal LV filling pressures and normal EF, but with impaired relaxation as a sign for myocardial disease.

All patients examined were diagnosed as having DD Grade I. All patients were informed and gave written consent to use intraoperative resected tissue for further research examination.

Table 1 Overview over patient's clinical data.

	DD	Non-DD
Age (y)	67 ± 14	56 ± 13
No. of samples	19	34
Sex		
men	11	19
women	8	15
BMI	23	21
Mitral valve prolapse	4	6
Mitral regurgitation	7	12
Mitral valve stenosis	3	2
Aortic valve stenosis	5	13
Aortic valve regurgitation	1	3
Aortic valve repair	0	3
Aortic valve replacement	5	13
Mitral valve replacement	10	14
Coronary artery bypass grafting	4	4

BMI = body mass index; DD = diastolic dysfunction.

Table 2 Echocardiographic and Swan-Ganz-catheter data.

	DD	Non-DD
Age (y)	67 ± 14	56 ± 13
No. of samples	19	34
Sex		
men	11	19
Women	8	15
DD Grade I	19	34
LA dilatation	12 (35)	5 (26)
Atrial fibrillation	12 (35)	5 (26)
RA dilatation (No. of patients)	7 (36)	9 (26)
LVH	11 (57)	13 (38)
RV dilatation (No. of patients)	12 (63)	21 (61)
Mean PAP (mmHg)	20 ± 2	15 ± 2
PCWP (mmHg)	12 ± 4	9 ± 3
RA (mmHg)	8 ± 2	5 ± 1
EF (%)	51	55

Data are presented as *n* (%) or mean ± SD, unless otherwise indicated.
DD = diastolic dysfunction; EF = ejection fraction; LA = left atrium; LVH = left ventricular hypertrophy; PAP = pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; RA = right atrium; RV = right ventricular.

2.2. Tissue harvesting and preparation

Before starting with extracorporal circulation, the right auricle, which was cannulated for venous drainage, was resected. Tissue from the right auricle was routinely resected (Figure 1). The tissue was immediately put in an oxygenated, cardioplegic solution containing butanedione-monoxim 30mM and transported in a cool box to the laboratory. The tissue was then prepared in an ice-cooled petri dish, containing a preparation solution (imidazol 68. 08mM; sodium azide 65. 01mM; ethyleneglycol tetraacetic acid 380. 4mM; dithioerythriol 154. 2mM; magnesium chloride 203. 3mM; adenosine 5′-triphosphate 605. 2mM). The tissue was cut down into small bundles. Afterwards, the fibers were stored in a fresh preparation solution containing Triton X-100 for 24 hours at 4°C under permanent rotation to make sure that all membrane-dependent properties were washed out from the tissue.

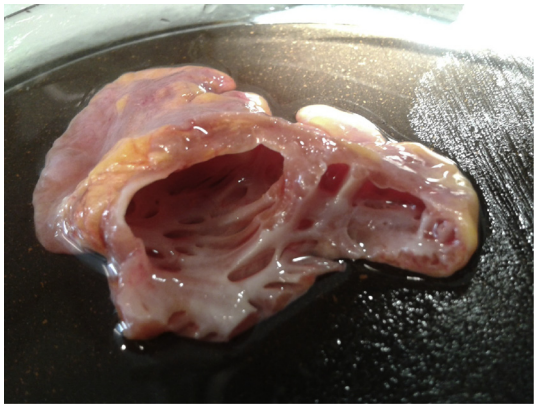

Figure 1 Right auricle.

After 24 hours, the experimental cycle was started. The fibers were cut into strips and inserted into the muscle investigation system. We took three bundles per patient and averaged the values. The muscle investigation system is a piece of equipment that measures force under defined conditions (Figure 2). In this experimental set up, the fibers are exposed to a relaxation solution (imidazol 68. 08mM; creatine phosphate 327. 2mM; sodium azide 65. 01mM; ethylene glycol tetraacetic acid 380. 4mM; magnesium chloride 203. 3mM; *dithioerythritol* 154. 2mM; and adenosine 5′-triphosphate 605, 2mM). Afterwards, the fibers were carefully prestretched until the force transducer showed a stable base line, and then exposed to a gradual increase of calcium starting at the lowest concentration (pCa 6.5) until the highest step of calcium concentration at pCa 4.0 is achieved. The calcium concentration is the negative decadic logarithm, and therefore is without any unit. This was realized by adding a contraction solution (same content as the relaxation solution, except for calcium chloride) to the cuvette containing calcium. The stepwise increase of calcium concentration leads to a contraction of muscle fibers, which was recorded by the force transducer and stored by the attached computer system.

2.3. Statistical analysis

Data shown are mean ± standard error of the mean. Gausian distribution was tested with the Shapiro-Wilk test. Because we have a different number of samples, we could not use standard tests and therefore used permutation tests. For the evaluation of the force-pCa values we used the Wilxcoxon rang sum test. The influence of the different factors was tested using permutation tests.

3. Results

Patients with DD achieve less force than patients without DD (Figure 3). This difference was significant in the higher

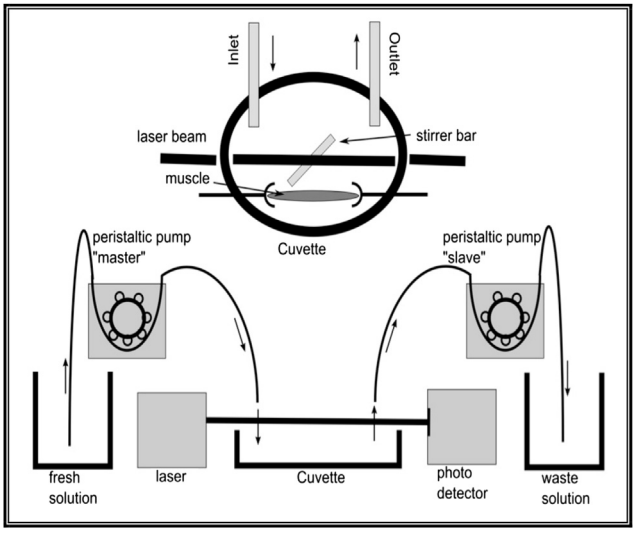

Figure 2 Scheme: O. Möller after Güth, Scientific Instruments, Heidelberg, Germany.

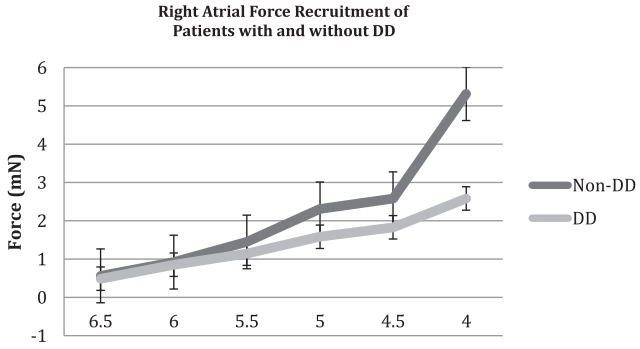

Figure 3 Comparison of force recruitment of patients with and without diastolic dysfunction. Significant less force is achieved at pCa steps 4.0—5.5. DD = diastolic dysfunction.

steps of calcium concentration: pCa 4.0: DD 2.58 ± 0.4 mN, controls 5.32 ± 0.4 mN, $p = 0.02$; pCa 5.5: DD 1.14 ± 0.3 mN, controls 1.45 ± 0.3 mN, $p = 0.03$. In the lower steps of calcium concentration (pCa 6.0 and pCa 6.5, respectively) there was no significant difference ($p = 0.6$ and $p = 0.5$, respectively).

We compared the values of patients with DD and without DD in reference to the performed surgical procedure, echocardiographic reports, as well as diagnosis, and evaluated any significant correlations.

DD correlates significantly with left ventricular hypertrophy (LVH) ($p = 0.03$). Depending on the underlying disease, patients with DD did not significantly more or less require coronary artery bypass grafting ($p = 0.8$) or repair of aortic ($p = 0.5$) or mitral valve ($p = 0.08$). DD (Figures 4—6) did not significantly occur more often in patients with aortic insufficiency ($p = 0.5$) or coronary heart disease ($p = 0.8$). However, DD was significantly associated with aortic stenosis ($p = 0.04$). LVH, however, correlated significantly with aortic valve stenosis ($p = 0.02$) and mitral valve insufficiency ($p = 0.03$).

DD was not significantly associated with mitral valve prolapse ($p = 0.05$) and annulus dilatation of mitral valve ($p = 0.05$), but with left atrial dilatation ($p = 0.02$).

In opposition to our assumption, DD did not significantly occur more often in females ($p = 0.2$). But, it was interesting to see that the EF did not correlate with the appearance of DD ($p = 0.1$).

Hypertonus was significantly associated with DD ($p = 0.04$). Further clinical diagnoses of patients with

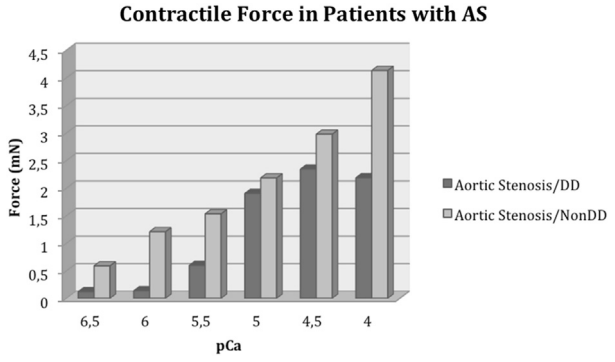

Figure 4 Contractile force of patients with aortic valve stenosis. AS = aortic stenosis; DD = diastolic dysfunction.

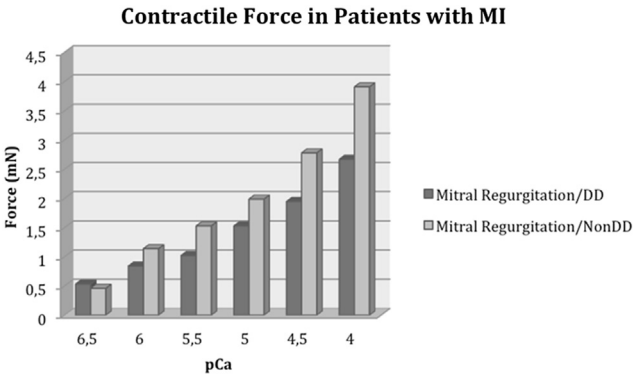

Figure 5 Contractile force of patients with mitral valve regurgitation. DD = diastolic dysfunction; MI = myocardial infarction.

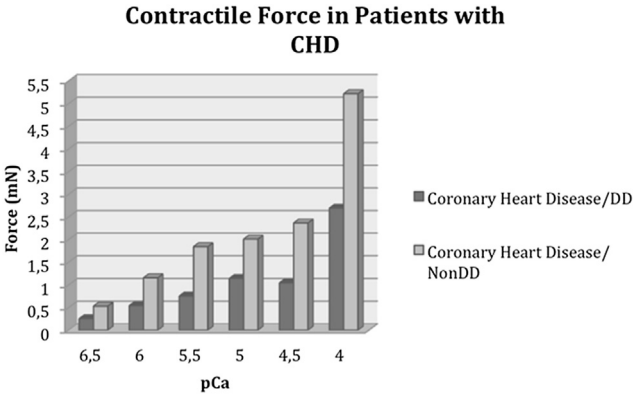

Figure 6 Contractile force of patients with coronary heart disease. CHD = coronary heart disease; DD = diastolic dysfunction.

diabetes or heart rhythm (atrial fibrillation or sinus rhythm) did not appear more often with DD.

Furthermore, we examined the calcium sensitivity in patients with and without DD and detected an increased sensitivity in patients with DD (pCa^{2+}+50 at 5.5) compared with those without DD (pCa^{2+}+50 at 5.0).

4. Discussion

DD can be caused by different mechanisms. According to Zile and Brutsaert[13] the mechanisms can be divided in two factors: myocardial and extramyocardial (hemodynamic load, early diastolic load, and afterload). The first one contains all structures and processes within the muscle cell and within the extracellular matrix. All these mechanisms that result in DD and heart failure can be caused by ischemia (myocardial infarction), pressure and or volume overload (valve disease), and by restrictive or hypertrophic cardiomyopathy.

We examined the impact of these pathologies on the level of contractile proteins. We observed that patients with DD seem to recruit less myocardial contractile force than patients without this diagnosis. In human fibers we could show that force values decreased significantly in patients with DD compared with healthy controls. This

observation could be made in higher doses of calcium concentration. Additionally calcium sensitivity also seems to be increased in patients with DD. Brixius et al[14] also made this observation and observed increased calcium sensitivity in failing human myocardium as well as ischemic cardiomyopathy.[7,14,15] They concluded that myofibrillar function is impaired by blunted length-dependent force generation and leads to compensatory increased affinity to calcium to cover the cardiac demand. These studies were performed in failing and cardiomyopathy myocytes. Similar results are also observed in patients with pulmonary arterial hypertension[4–6] and the described reduction of available myosin-based cross-bridges leads to a contractile weakness of cardiomyocytes, seen in magnetic resonance imaging analysis.[4]

Potential reasons are multifold. Referring to the cardiomyocyte changes in calcium homeostasis are considered to cause DD.[10] Zile and Brutsaert[13] describe abnormal sarcolemmal channels, abnormal sarcoplasmatic reticulum calcium reuptake, and changes in the phosphorylation state of proteins which are involved in calcium release. These changes lead to increased cytosolic diastolic calcium concentration and a decelerated decrease of cytosolic calcium.

Hamdani et al[2] also suggests in his study of dogs with induced heart failure and preserved EF, an impairment on the sarcomere level and observed compensatory increased calcium sensitivity. This observation was also made in knock-out mice. Fraysse et al[9] showed in knock-in mice, which mimic the human hypertrophic cardiomyopathy condition, that higher calcium sensitivity and DD may precede LVH and might be the first sign of cardiac function impairment. We observed similar results in human tissue and all these observations support our assumption of an early sign of reduced cardiac force.

Searching literature for similar results in human tissue, we came upon a study from Lamberts et al.[16] He described an impairment of relaxation in the right atria from diabetic patients without any systolic dysfunction. In diabetic patients this process was associated with increased fibrosis.[17] However, we could not see any significant correlation of DD to diabetes concerning force values, but we saw a correlation of LVH and DD. So the results of the study by Fraysse et al[9] in mice might also be valid for humans. But is higher calcium sensitivity accompanied by reduced force capacity as we could see in our curves?

Rain et al[6] gives a possible explanation. They showed that LV DD is characterized by increased cardiomyocyte stiffness as a consequence of functional modifications of sarcomeric protein titin. They assumed, therefore, that increased calcium sensitivity might influence lusiothropy. This is an interesting aspect, which might have an implication on our results, because an increase in sarcomere Ca^{2+}; calcium sensitivity can cause incomplete actin-myosin release—even in low dose calcium concentrations and might have great impact on relaxation,[1] as well as contraction.

Another observation was the significant correlation of LVH, which was significantly associated with DD with aortic stenosis and mitral insufficiency. We suppose that in these patients LVH might influence cardiac contractile properties, because myocardial perfusion in aortic stenosis can be associated with reduced myocardial perfusion reserve due to microvascular dysfunction and might impair LV as well as RV dysfunction.[18] Mitral regurgitation is a disease which is beneficial for diastolic filling because of volume overload, however, systolic emptying might be impaired. This might be caused by the thin-walled ventricle, which requires less filling pressures, but the relative lack of muscle mass (perhaps because of the lack of isovolumic period before aortic valve opening) seems to be associated with reduced LV function.[19,20] Spinale et al[21] concludes that this impairment is caused by loss of contractile elements and impaired calcium handling in patients with MR. This might promote the development of DD and supports our finding of reduced force properties in patients with mitral regurgitation and DD. The reason why some patients develop DD (like in the MR or valve stenosis group) and others not, is difficult to answer. Koide et al[22] developed modest concentric hypertrophy, whereas others had severe hypertrophy. He observed that dogs with modest hypertrophy had persistently higher wall stress and far less myocardial mass and assumed that there is an inhomogeneity in the hypertrophic response. We suggest that this mechanism is also present in humans and others cardiac diseases.

Patients diagnosed with DD are more likely to have atrial fibrillation.[23] Both clinical entities share some risk factors like age and hypertension. It is known that LA dilatation is a risk factor for atrial fibrillation, which contains impaired myocardial relaxation, increased LA pressure, decreased ventricular compliance, as well as deficiencies in atrial contraction.[24] So, deficiencies of force power in the DD group might also be impaired by higher prevalence of atrial fibrillation.

We summarize that patients with DD present a lowered contractile power. DD impairs not only left heart function, but also right heart function. DD is significantly associated with LVH, but emerges independently from underlying pathologies like valve disease or coronary heart disease, concluding that the impairment of contractile capacity results directly from DD independently from volume or pressure overload given in valve disease or ischemic heart disease. An increase of calcium sensitivity is a compensatory mechanism to cover the demand. Nevertheless, the challenging therapy of right heart failure and impairment underlines the importance of an early therapy when signs of DD are given in any event of reduced LV function, but also in case of reduced/impaired RV function.

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