Myofilament function and body mass index

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Abstract. Body mass is reported to influence myocardial performance. Recent studies have emphasised the importance of negative inotropic adipocyte-derived factors and their impact on cardiac contractile function. However, the underlying mechanisms remain unclear. We aimed to determine whether body mass impacts cardiac force development on the level of the contractile apparatus. We examined the influence of body mass index (BMI) (3 groups: group I >25, group II 25-30, group III >30) on the myocardial performance of skinned muscle fibres. Right atrial tissue preparations of 70 patients undergoing aortocoronary bypass operation (CABG, 48 patients, group a) and aortic valve replacement (AVR, 22 patients, group b) were obtained. The fibres were exposed to a gradual increase in the calcium concentration, and the force values were recorded. The statistical analysis was performed using Pearson's correlation (P<0.05 significant). A BMI >30 (group III) was associated with less force (mean force 1.58 ± 0.1 mN, P=0.02, max force 2.24 ± 0.17 mN, P=0.02 vs. group II (mean force 1.8 ± 0.3 mN, P=0.04, max force 2.59 ± 0.2 mN, P=0.03) and group I (mean force 1.8 ± 0.1 mN, P=0,03, max force 2.62 ± 0.3 mN, P=0.03). Dividing the groups in the post-surgical procedure, the impact of BMI on force development in group III was more intense in the CABG group compared to the AVR group: 2.0 ± 0.2 mN vs. 2.4 ± 0.1 mN, P=0.04. In accordance with the literature, a BMI >30 is associated with reduced force capacities. Additionally, the underlying cardiac disease may aggravate the impact of weight on cardiac force. Further studies are needed to evaluate the clinical relevance of this experimental observation and the potential consequences for the treatment of cardiac function.

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Introduction

The obesity paradox describes the paradoxical relationship between obesity and mortality in specific populations, although obesity is a risk factor for diabetes type 2, hypertension, dyslipidemia and other cardiovascular diseases and has a negative impact on health outcomes (1). Obesity is assumed to also have an impact on myocardial function; however, although evidence exists, this has not been confirmed, as abdominal obesity induces insulin resistance, which may have a potent effect on myocardial function (2). Furthermore, cardiac function can be depressed, and the development of severe cardiomyopathy can be promoted (3). Similar to diabetic cardiomyopathy, obesity cardiomyopathy is characterised by reduced LV diastolic dysfunction, impaired diastolic LV longitudinal function and increased LV diastolic filling pressures (4). Furthermore, increased myocardial collagen content has been observed (4). However, although the relationship between obesity and heart failure is widely acknowledged, the pathophysiological mechanisms regarding how obesity affects cardiomyocytes remain unclear and have yet to be examined.

Different mechanisms affecting cardiomyocytes have been discussed: obesity as an increased hemodynamic load, which leads to ventricular remodelling and systolic dysfunction, due to prolonged left ventricular stress, or increased lipid accumulation into cardiac myocytes leading to cardiomyocyte apoptosis and dysfunction. Another proposed mechanism is the endocrine activity of adipose tissue, which releases a variety of peptides and proteins, which are shown to attenuate cardiomyocyte contraction (5,6). Leptin, for example, has been shown to play a role in cardiovascular physiology and was observed to suppress cardiac contraction in high doses (7). Different studies have shown that adipose tissue exerts highly potent cardiodepressant activity, which could also have an acute effect on cardiomyocyte contraction (5,6). This may be the missing link between heart failure and obesity, but it has not been proven.

Experimental evidence in accordance to the clinical 'entity' of obesity cardiomyopathy, which is described echocardiographically by slightly reduced diastolic LF function, increased diastolic filling pressures and higher myocardial collagen content, is hypothesised to affect myofilament function. The purpose of this study was therefore to examine the impact of different body mass index (BMI) stages on contractile apparatus.

Materials and methods

Clinical setting. The experiments were carried out at the Experimental Laboratory of the Department of Cardiothoracic and Vascular Surgery, Johannes Gutenberg University (Mainz, Germany). The Ethics Committee of the Medical Association Rheinhessen approved the study. All the patients gave written consent to use intraoperative resected tissue for further research examination. The study was performed conforming to the declaration of Helsinki.

Patients collective. This was a prospective study with patients scheduled for elective cardiac surgery. A total of 70 patients undergoing cardiac surgery were included. The patients were divided into two groups according to the operative procedure of aortocoronary bypass grafting (CABG) or aortic valve replacement procedure (AVR). The AVR group consisted of 22 patients, and the CABG group consisted of 48 patients (Table I). We performed a classification according to the WHO classification of BMI into 3 groups: group I >25, group II 25-30, and group III >30.

Exclusion criteria. We excluded patients presenting as emergency cases, endocarditis, combined procedures (CABG and valve operations), operations on the ascending aorta and patients with clinical as well as echocardiographic signs of chronic or acute right or acute left heart failure.

Experimental setting
Skinned fibre preparation. The process of skinned fibre preparation has been described in detail elsewhere (8). Briefly, the fibres were collected in the operation room after resecting the right auricle. In the no-touch technique, the auricle was transferred in an ice-cooled vial (4˚C), filled with modified cardioplegic solution (Krebs-Henseleit solution, in mmol/l: 118.07 NaCl, 11.1 $C_6H_{12}O_6$ + H_2O, 4.7 KCL, 25 $NaHCO_3$, 1.2 KH_2PO_4, 1.2 $MgSO_4$ + 7 H_2O, 1.8 $CaCl_2$ + 2 H_2O) containing BDM (30 mmol/l $C_4H_2NO_2$ Butanedione-Monoxime) as an ATP-sensitive potassium-channel inhibitor. In the laboratory, the tissue was placed in a dish, filled with a special ice-cooled (4˚C) preparation solution [contents (mM): $C_3H_4N_2$ 68.08, NaN_3 65.01; $C_{14}H_{24}N_2O_{10}$ 380.4; $C_4H_{10}O_2S_2$. 154.3; $MgCl_2$ x 6 H_2O 203.3; $C_{10}H_{14}N_5O_{13}$ P_3 Na_2 605.2]. The muscle bundles were resected out of the auricle and placed in a test tube containing the modified preparation solution with 1% Triton X-100 to permeabilise the membrane of the fibres for 24 h at 4˚C on a shaking device. The purpose of these preparatory steps was to remove the membrane-dependent properties (skinning). After this skinning process, the RA muscle bundles were transferred to a separate ice-cooled (4˚C) dish again, filled with preparation solution to prepare the single RA muscle stripes (size of 2-2.5 mm x 0.3 mm) under the microscope (x10, magnification, Leica S6D stereomicroscope; Leica Microsystems GmbH, Wetzlar, Germany).

RA pCa/force measurements. A muscle investigation system (Gradient Program; Scientific Instruments, Heidelberg, Germany) was used to expose the RA fibres to a gradual increase in the calcium concentration for force measurements. The RA fibres were fixed in the perfusion chamber and incubated with a relaxation solution [contents (mM): $C_3H_4N_2$ 68.08; $C_4H_8N_3O_5PNa_2$ + 4 H_2O 327.2; NaN_3 65.01, $C_{14}H_{24}N_2O_{10}$ 380.4, $MgCl_2$ 203.3; $C_4H_{10}O_2S_2$.154.2; $C_{10}H_{14}N_5O_{13}$ P_3 Na_2 605.2; creatine kinase 400 U/ml]. By adding $CaCl_2$ (147.02 mM) to the relaxation solution, a pCa-force curve was created. pCa is the negative decadic log of the calcium concentration. Specially designed software (Gradient Program; Scientific Instruments) was used to calculate the necessary amount of calcium to achieve a stepwise increase in the calcium concentration according to an equation proposed by Fabiato and Fabiato (8). The pCa starts with 6.5 ≤4.0 in 0.5 increments. For each patient, a set of three RA fibres underwent calcium-induced force measurements. The mean of these 3 measurements was determined.

Results

The mean and maximum force values of patients undergoing AVR were significantly higher than the force values of patients undergoing CABG (F mean 2.00±0.22 vs. 1.51±0.14 mN, P=0.03, F max 2.68±0.24 vs. 2.28±0.19 mN, P=0.02; Table II).

Table I. Patients' demographics and characteristics.

Variable	CABG group (n 48)	AVR group (n 22)	P-value
Age (years)	65.3±5.2	63.7±3.5	0.9
Height (cm)	168±9.6	171±13	0.9
BMI	27.6±4.8	26.3±6.7	0.8
Female gender; n (%)	23 (47.9)	11 (50)	0.7
DM; n (%)	29 (60)	8 (36)	0.03
Art. Hypertension; n (%)	8 (53)	5 (50)	0.9
NYHA class III-IV; n (%)	21 (43)	6 (27)	0.03
LVEF (%)	53.7±2.5	55.3±4.1	0.7

BMI, body mass index; DM, diabetes mellitus; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction.

Table II. Differences between the CABG and AVR groups.

Variable	CABG	AVR	P-value
F mean (mN)	1.51±0.14	2.00±0.22	0.03
F max (mN)	2.28±0.19	2.68±0.24	0.02
BMI <25 F max	2.35±0.34	2.89±0.57	0.07
BMI >25 F max	2.43±0.40	2.75±0.31	0.08
BMI >30 F max	2.06±0.48	2.41±0.58	0.02

CABG, coronary artery bypass grafting; AVR, aortic valve replacement; BMI, body mass index.

Table III. Summary of results.

	BMI	F mean (mN)	F max (mN)	P-value
AVR	<25 vs. >25	2.18±0.1 vs. 2.07±0.2	2.89±0.31 vs 2.75±0.26	0.08
	<25 vs. >30	2.18±0.1 vs. 1.74±0.18	2.89±0.31 vs. 2.41±0.32	0.02
	25-30 vs. >30	2.07±0.2 vs. 1.74±0.18	2.75±0.26 vs. 2.41±0.32	0.007
CABG	<25 vs. >25	1.50±0.42 vs. 1.66±0.51	2.34±0.26 vs. 2.43±0.2	0.19
	<25 vs. >30	1.50±0.42 vs. 1.36±0.3	2.34±0.26 vs. 2.06±0.2	0.01
	25-30 vs. >30	1.66±0.51 vs. 1.36±0.3	2.43±0.29 vs. 2.06±0.2	0.04

CABG, coronary artery bypass grafting; VR, aortic valve replacement; BMI, body mass index.

Regarding the differentiation of BMI indices, there were significant differences in the group BMI >30 among CABG and AVR (F mean 2.06, 2.00±0.22 0.48 mN vs. 2.41, 2.00±0.22, 0.58 mN, P=0.02). The BMI group <25 did not significantly differ from the BMI group 25-30 in the F mean and F max.

We divided the group after aortic valve replacement and coronary bypass grafting: in the AVR group, the highest mean and maximum forces were achieved by the patients with a BMI <25 (F mean 2.18±0.1 mN vs. F max 2.89±0.31 mN). The lowest force values were developed by patients with a BMI >30 (F mean 1.74±0.18 mN and F max 2.41±0.32 mN). Patients with a BMI 25-30 were in the middle between the two groups (F mean 2.07±0.2 mN, F max 2.75±0.26 mN). When comparing the mean and maximum force values among the AVR group, we found significant differences in the force development between the BMI group <25 vs. >30. This was valid for the mean forces (2.18±0.1 vs. 1.74±0.18 mN, P=0.02) as well as for the maximum forces (2.89±0.31 vs. 2.41±0.32 mN, P=0.02). Similar differences were evident when comparing the force values of patients with a BMI 25-30 vs. >30 (F mean 2.07±0.2 vs. 1.74±0.18 mN, F max 2.75±0.26 vs. 2.41±0.32 mN, P=0.007; Table III).

In the CABG group, the highest force values were obtained in the BMI group 25-30 (F mean 1.66±0.51 mN, F max 2.43±0.29 mN) and the lowest force values again in the BMI group >30 (F mean 1.36±0.3 mN, F max 2.06±0.2 mN).

When we compared the different BMI groups within the AVR and CABG group, we identified similar results: in both groups, significant differences were found between the BMI group <25 vs. >30 as well as 25-30 vs. >30: AVR: BMI <25 vs. >30: F mean 2.18 0.1 vs. 1.74±0.18 and F max 2.89±0.31 vs. 2.41±0.32, P=0.02 and BMI 25-30 vs. >30: F mean 2.07±0.2 vs. 1.74±0.18 and F max 2.75±0.26 vs. 2.41±0.32, P=0.007). In the CABG group, the following values were found: CABG: BMI 25 vs. >30: F mean 1.50±0.42 vs. 1.36±0.3 and F max 2.34±0.26 vs. 2.06±0.2, P=0.01) and BMI 25-30 vs. >30: F mean 1.66±0.51 vs. 1.36±0.3, F max 2.43±0.29 vs. 2.06±0.2, P=0.04).

Regarding the clinical characteristics of the patients, we observed significantly more patients with diabetes in the CABG group (P=0.03) and more New York Heart Association (NYHA) class II-IV in the CABG group (P=0.03). The remaining clinical features were similarly distributed in the two groups.

Discussion

The present study has shown that patients with a BMI >30 develop significant lower forces compared to patients with a BMI between 25-30 and <25. This was similar in the AVR group as well as the CABG group. These experimental observations are in concert with the literature: Adipocyte secretory products, identified as fatty acid-binding proteins (FABPs), acutely inhibited cardiomyocyte contraction in a rat model (9,10). Lamounier-Zepter furthermore observed that FABP4 concentrations correlated with cardiodepressant activity in the adipocyte-conditioned medium (10).

These results can also be seen in diabetic animal studies (11) as well as skinned fibre models (12). In the CABG group, we had more diabetic patients, which may have also influenced the observed reduced force values in patients with a BMI >30. Diabetes seems to reduce even more contractile force in humans as well as in animals. We observed this difference to be more pronounced in patients undergoing AVR, as previously identified (13).

Additionally, during the post-surgical procedure, we found more patients in the CABG group with diabetes and with NYHA III/IV. Since the Framingham study revealed that the risk of developing heart failure was independent of diabetes in overweight and obese patients, diabetes may have an additive effect on myocardial function (14), i.e., the impairment of myocardial function in obese patients may be explained by the cardiac effects of comorbidities secondary to obesity, but they may not completely explain the obesity-associated impairment of heart function (5).

In addition, the reduced cardiac status of the patients, indicated by NYHA III/IV, may aggravate the results, as studies have shown a reduced myofilament function of skinned fibres in the experimental models of impaired heart function (15). Thus, the reduced force values observed in the CABG group may also be influenced by this function.

The observation of different force values of AVR and CABG patients may also reflect the different pathological mechanisms since aortic valve diseases are an example of pressure overload and lead to different changes in the contractile properties than ischemic heart disease (16). There is some evidence in the literature that human myocardium can better tolerate pressure overload than ischemic heart disease: during acute myocardial ischemia, the sensitivity of the myofilaments to activating Ca^{2+} is significantly reduced, largely

due to the effects of intracellular acidosis on the contractile machinery (17). Previous findings of a pressure-overload rat model showed that myocytes can withstand the ultrastructural changes in pressure overload preserving Ca^{2+} signalling and contractility (18). That finding may be one explanation for the higher force values in patients with aortic valve disease compared to the CABG group. The issue of a potential influence of underlying disease in skinned human fibers cannot be excluded. Previous findings suggested that skinned human fibers from dilated cardiomyopathy are significantly more sensitive for calcium compared to those taken from patients with coronary or ischemic heart disease or mitral valve disease (19). Thus, we excluded this potential influence by skinning the fibers and removing all membrane-dependent processes. Regarding the transferability of results taken from atrial tissue to ventricular tissue, the literature reports higher maximal forces taken from rat ventricular fibers compared to those from atria, but considering that calcium sensitivity is species-dependent, it is also difficult to transfer this observation to human tissue (20). Another study gives some evidence that adult atrial tissue exhibits contractile properties similar to adult ventricular properties and differs from adult ventricle mainly in metabolic properties (21). So we believe to transfer these results were transferred to ventricular tissue always considering the origin of the fibers.

The present study is an attempt to examine the impact of diabetes on myofilament function in human tissue. Although the reduced contractile force values may have been influenced by several factors including the underlying pathology (aortic valve disease vs. ischemic heart disease) or the NYHA status, it is likely that altered myofilament function is present in obese patients.

Notably, the mechanisms that affect myofilament function in association with diabetes cannot be definitively estimated in the present study, but according to the literature obesity affects the myofilament function in a membrane-independent manner, as shown in our skinned fibre model.

Further studies are necessary to evaluate whether the observed reduced myofilament function may have clinical relevance since LV function was preserved in the examined patients. Additionally, studies suggest a relationship between obesity-associated dysfunctional adipose tissue and heart failure with a preserved ejection fraction (22).

Acknowledgements

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