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Article

Early Changes in Left and Right Ventricular Function in Obese Patients after Bariatric Surgery

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Abstract

Background: Severe obesity causes hemodynamic changes in the circulatory system, which leads to the development of heart failure with either preserved or reduced ejection fraction. Weight loss achieved through bariatric surgery is likely to possibly reverse many of hemodynamic and structural abnormalities caused by obesity. **Methods:** Detailed echocardiographic parameters assessing left and right ventricular function in severely obese patients undergoing bariatric surgery were analyzed. The following parameters were compared in the examinations performed before surgery and 3 and 6 months after surgery: left ventricular ejection fraction (LVEF), left ventricle (LV) and right ventricle (RV) global longitudinal strain (GLS and GSRV) with right ventricular free wall strain (FWSRV), peak early (e') and late (a') diastolic annular velocities (lateral and septal) with calculation of E/e' ratios, left atrium volume index (LAVI), left atrial strain (LAS) including LAS conduit (LAS-cd) and LAS tricuspid regurgitation velocity (TRV), tricuspid annular plane systolic excursion (TAPSE), maximum systolic velocity of the lateral part of the tricuspid annulus-s' and accelerated pulmonary time (AcT). **Results:** Forty consecutive obese patients undergoing bariatric surgery from December 2022 till June 2023 were enrolled to the study. Finally 39 patients were included. The study population consisted of 76% women with mean BMI of 40.3 (SD 5.6) and mean age of 42.4 (SD 11.9). BMI after 3 months was 35.3 kg/m², after 6 months was 31.2 kg/m² (SD 5.1). At 3- and 6-month follow-up after bariatric surgery there was a reduction in left ventricular mass (109.7 vs. 99.1 vs. 87.4 kg/m²; $p < 0.001$), LVEDV (47.1 vs. 30.0 vs. 43.7; $p < 0.001$) and SV (29.7 vs. 24.3 vs. 26.3; $p = 0.05$). Simultaneously an improvement in GLS (-14.38 vs. -16.79 vs. -18.01) and an increase in LAS parameters (reservoir: 22.5 vs. 28.0 vs. 31.1; $p < 0.001$ and conduit: -12.8 vs. -16.5 vs. -19.6) were observed. Comparison of right ventricular parameters before and after bariatric surgery showed improvement in GSRV (-15.9 vs. -18.8 vs. -18.38%; $p = 0.005$), FWS (-18.38 vs. -19.70 vs. -19.50; $p = 0.042$) and reduction in TRV (1.84 vs. 1.67 vs. 1.46±0.52; $p = 0.01$). **Conclusions:** Weight loss contributes to rapid improvement in left and right ventricular function. Some changes of echocardiographic parameters: LVEDV, LAS-r, LAS-cd, GSRV and TAPSE correlate with the degree of weight loss. New echocardiographic parameters earlier detect subclinical hemodynamic changes associated with obesity and weight loss after bariatric surgery.

Keywords: obesity; heart failure; bariatric surgery; echocardiography

1. Introduction

Obesity is a global health problem with a steadily increasing prevalence. It is associated with numerous cardiovascular complications, including the development of left ventricular hypertrophy (LVH), diastolic and systolic dysfunction of the left ventricle, as well as pulmonary hypertension and right ventricular (RV) dysfunction [1–3,5]. Surgical treatment of obesity, such as bariatric surgery, leads to significant weight reduction and may induce beneficial changes in cardiac structure and

function [1,2,4,6]. This study aimed to assess early changes in left and right ventricular function after bariatric surgery based on echocardiographic data and to relate these findings to results reported in the literature.

2. Methods

We analyzed echocardiographic data of bariatric patients who underwent surgical obesity treatment. The study included 40 adult patients hospitalized at the Department of General, Oncological, and Endocrine Surgery between December 2022 and June 2023. One patient withdrew their consent, and finally 39 were included in the analysis. Inclusion criteria were BMI >35, performing bariatric surgery and echocardiographic examinations before and after surgery. Exclusion criteria included pregnancy, postpartum period, alcohol dependence, and cancer.

Parameters of left and right ventricular function were assessed before surgery, and at 3 and 6 months postoperatively. All patients underwent standard transthoracic echocardiography via a GE Vivid E95 ultrasound diagnostic system equipped with an M5s 3.5–5 MHz transducer (GE Vingmed Ultrasound, Horten, Norway) by experienced sonographer. Two-dimensional, color Doppler, pulsed wave Doppler, and standard high-frame-rate (>60/s) apical 4-, 2- and 3-chamber views from five consecutive cycles were archived for offline analysis (EchoPAC version: 204, GE Vingmed Ultrasound, Norway). We evaluated left and right ventricular function parameters, including left ventricular ejection fraction (LVEF), left ventricle (LV) and right ventricle (RV) global longitudinal strain (GLS and GSRV), right ventricular free wall strain (FWSRV), peak early (e') and late (a') diastolic annular velocities (lateral and septal) with calculation of E/e' ratios, left atrium volume index (LAVI), left atrial strain (LAS) including reservoir phase (LAS-r), conduit phase (LAS-cd) and contraction phase LAS-ct, tricuspid regurgitation velocity (TRV), tricuspid annular plane systolic excursion (TAPSE), maximum systolic velocity of the lateral part of the tricuspid annulus- s' and accelerated pulmonary time (AcT).

Left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), and left ventricular ejection fraction (LVEF) were determined using the biplane Simpson method. The left atrial volume (LAV) was measured by averaging the values in the apical 4- and 2-chamber views, and then the LAV index (LAVI) was calculated.

Diastolic dysfunction was defined per ASE/EACVI guidelines [7] to emphasize the identification of elevated LVEDP and the differentiation of diastolic dysfunction grades. The peak early and late diastolic mitral annular velocities (E and A, respectively) were measured by pulsed-wave Doppler, and the E/A ratio was then calculated. The peak early (e') and late (a') diastolic annular velocities were obtained by averaging the values at the septum and lateral positions using TDI, and then E/e' was calculated. The TRV parameter was also used to assess left ventricular diastolic function.

Global longitudinal strain was determined from apical images by maximizing the frame rate (from 50 to 70 frames per second) by narrowing the sector to isolate individual walls. Apical 4-chamber, 3-chamber and 2-chamber views were obtained to assess the longitudinal strain in each wall at the basal, mid and apical level in each of these views. For each view, five cardiac cycles were recorded and saved digitally for further study. Automatic functional imaging (AFI) tracked the LV endocardial and epicardial boundaries in the three apical dynamic images. Tracking was accepted or rejected according to the quality of image. The global longitudinal strain of the left ventricle was calculated systematically from the average value of the three views, including 18 segments of the myocardium.

Left atrial strain measurements were performed using the apical 4-chamber and 2- chamber views. The left atrium was contoured automatically with manual correction. For each patient, strain during the reservoir phase (LAS-r), during the conduction phase (LAS-cd) and during the atrial contraction phase (LAS-ct) was calculate.

The conventional echocardiographic assessment of RV function of tricuspid annular plane systolic excursion (TAPSE), maximum systolic velocity of the lateral part of the tricuspid annulus- s'

and accelerated pulmonary time (AcT) were measured according to current guidelines (7). The maximum amplitude was used for TAPSE measurement.

A RV focused four-chamber apical view was used to assess GSRV. The right ventricular endocardium was automatically contoured using AFI, with manual corrections.

Patient signed informed consent and the study was approved by the local bioethics committee (No 70/2021 from 30 November 2021).

Statistical Methods

Repeated-measures ANOVA was used to assess the significance of parameter changes over time. When the assumptions of this test were not met, the nonparametric Friedman test was applied as an alternative. Post hoc analyses included pairwise t-tests or Wilcoxon signed-rank tests, with Bonferroni correction for multiple comparisons. Relationships between changes in parameters and changes in body mass were evaluated using linear models, including hybrid within-between models and independent t-tests where appropriate. Linear mixed-effects models were not feasible due to the relatively small sample, which led to convergence issues. The hypothesis regarding the relationship between overall changes in right ventricular function and body mass was tested using multivariate ANOVA (MANOVA) with Wilks' lambda, supported by principal component analysis (PCA). A p-value below 0.05 was considered statistically significant.

3. Results

The study group consisted of 32 women and 7 men aged 42.3 (±12) years. Among the comorbidities, arterial hypertension was the most common (43.59% of patients). The most commonly used type of procedure was sleeve gastrectomy (87.18%) (Table 1).

Table 1. Baseline characteristic of the study group.

| Category | Count (%) |
|--|-------------|
| Gender | |
| Women | 32 (82.05%) |
| Men | 7 (17.95%) |
| Comorbidities | |
| Hypertension (HTN) | 17 (43.59%) |
| Ischemic Heart Disease (IHD) | 3 (7.69%) |
| Diabetes | 9 (23.08%) |
| Hypothyroidism | 11 (28.21%) |
| Type of procedure | |
| Sleeve Gastrectomy | 34 (87.18%) |
| Balloon | 3 (7.69%) |
| Mini-Gastric Bypass | 1 (2.56%) |
| Single Anastomosis Sleeve Ileal Bypass | 1 (2.56%) |

3.1. Left Ventricular and Left Atrium Parameters

During the 3- and 6-month follow-up period after bariatric surgery, no significant changes in left ventricular thickness were observed, but a significant decrease in LV mass was observed (Table 2, Figure 1).

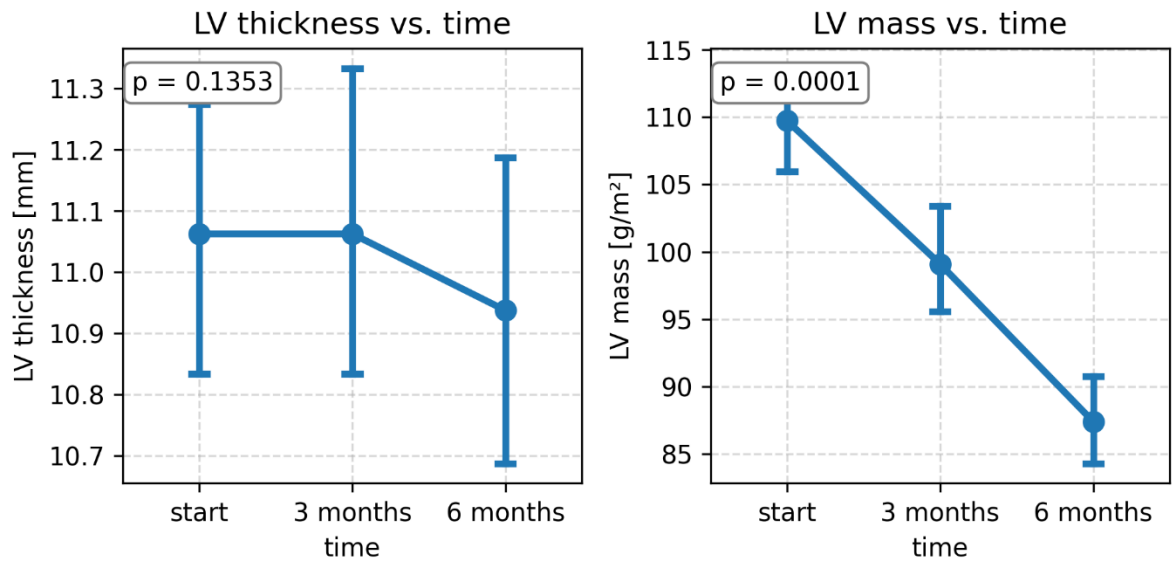


Figure 1. Changes in left ventricular thickness and mass over time.

We found no statistically significant association between change in LV wall thickness (as well as change in LV mass) and body weight loss. (Figure 2).

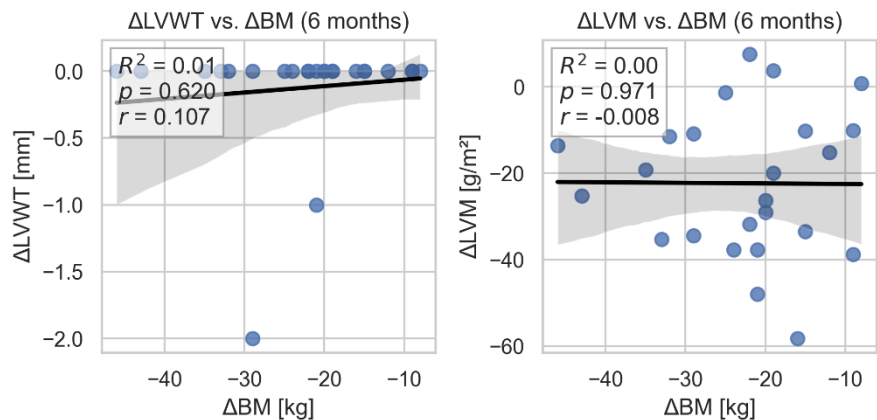


Figure 2. Linear regression models assessing the relationships between LV wall thickness, LV mass, and change in body weight. LVWT- left ventricular wall thickness; LVM- left ventricular mass.

GLS significantly improved from -14.38 ± 2.47 before surgery to -18.01 ± 2.32 at 6 months ($p=0.001$). LVEF increased slightly from 59% to 61.3%, but this change was not statistically significant ($p=0.167$), however, a significant reduction in LVEDV was observed, especially after 3 months. No significant changes were observed in E/e' sept and lat, e' sept and lat, or LAVI. Analysis of left atrial strain parameters showed a significant improvement in LAS-r and LAS-cd components, with no significant changes in LAS-ct (Table 2).

Table 2. Changes in left ventricular parameters after bariatric surgery.

| Left ventricle Parameters | Before surgery | 3 months after surgery | 6 months after surgery | P value |
|---------------------------|----------------|------------------------|------------------------|---------|
| LV wall thickness | 11.1 +/- 1.2 | 11.1 +/- 1.2 | 10.9 +/- 1.2 | 0.14 |
| LV mass index (g/m2) | 109.7 +/- 20.3 | 99.1 +/-19.4 | 87.4 +/- 16.2 | <0.001 |
| LVEDV | 47.1 +/- 13.2 | 30.0 +/- 15.0 | 43.7 +/- 11.0 | <0.001 |

| | | | | |
|----------------|----------------|----------------|----------------|--------|
| LVESV | 18.7 +/- 5.4 | 16.1 +/- 5.3 | 16.9 +/- 3.8 | 0.18 |
| SV | 29.7 +/- 7.8 | 24.3 +/- 7.6 | 26.3 +/- 3.7 | 0.05 |
| LVEF (%) | 59±4.8 | 61±4.8 | 61.3±4.8 | 0.167 |
| GLS (%) | -14.38±2.47 | -16.79±2.21 | -18.01±2.32 | 0.001 |
| E/A | 1.1 +/- 0.3 | 1.1 +/- 0.4 | 1.2 +/- 0.3 | 0.38 |
| T dec | 194.8 +/- 33.4 | 214.8 +/- 45.2 | 197.1 +/- 33.9 | 0.06 |
| e' sept (cm/s) | 9.88±2.54 | 10.04±2.31 | 11.00±2.65 | 0.15 |
| E/e' sept | 8.17±1.69 | 7.58±2.36 | 7.46±1.69 | 0.39 |
| e' lat (cm/s) | 13.5±2.70 | 13.46±3.51 | 15.21±4.42 | 0.13 |
| E/e' lat | 6.71±1.52 | 6.58±1.61 | 6.04±1.27 | 0.14 |
| LAVI (ml/m2) | 24.3±9.17 | 23.9±8.77 | 22.8±7.19 | 0.32 |
| LAS-r | 22.5 +/- 7.5 | 28.0 +/- 6.1 | 31.1 +/- 8.6 | <0.001 |
| LAS-cd | -12.8 +/- 6.3 | -16.5 +/- 5.3 | -19.6 +/- 7.0 | <0.001 |
| LAS-ct | -8.3 +/- 7.0 | -11.5 +/- 3.8 | -10.8 +/- 5.8 | 0.18 |

GLS- global longituginal strain; LAVI- left atrium volume index; LV- left ventricle; LVEDV- left ventricular end- diastolic volume; LVESV- left ventricular end-systolic volume, SV- stroke volume; LVEF- left ventricular ejection fraction.

Significant changes were found in the volumetric parameters of the left ventricle. After 3 months of follow-up, LVEDV, LVESV, and SV decreased, while after 6 months, a slight increase in these parameters was observed (Figure 3). We did not find any significant relationship between changes in volumetric parameters of LV and change in body weight, but the within-between model revealed that after adjusting for changes in body mass, the change in LVEDV was significantly higher in the late period (from 3 months to 6 months after surgery) than in the early period (from baseline to 3 months after surgery) (Figure 4).

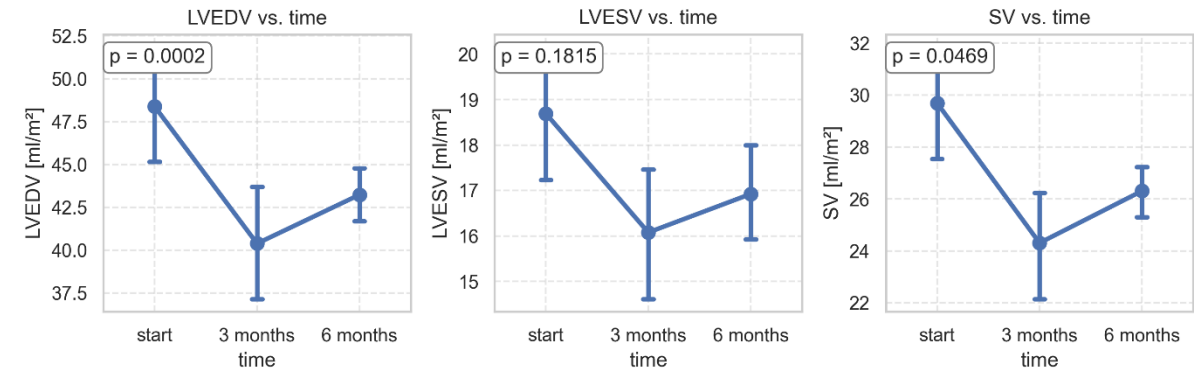


Figure 3. Changes in volumetric parameters of the left ventricle 3 and 6 months after bariatric surgery. LVEDV- left ventricular end-diastolic volume; LVESV- left ventricular end-systolic volume; SV- stroke volume.

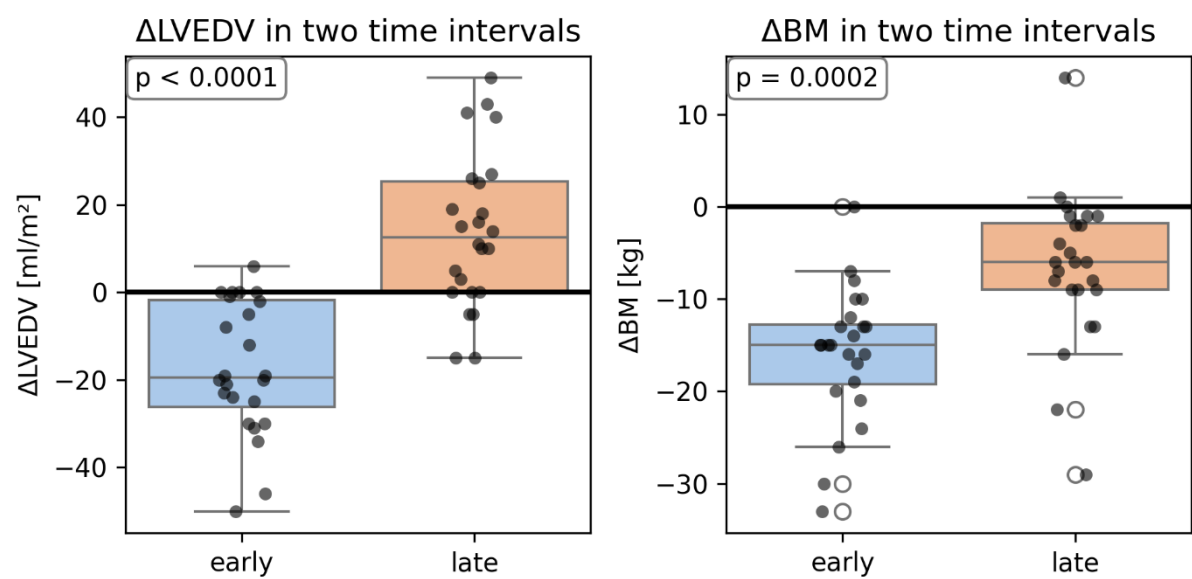


Figure 4. Changes in LVEDV in the early and late period compared to similar changes in BM. LVEDV- left ventricular end-diastolic volume; BM- body mass.

During the 3- and 6-month follow-up after bariatric surgery, significant improvement in left atrial strain parameters: LAS-r and LAS-cd were observed. We found statistically significant associations between 6-month changes in these parameters and the corresponding change in body weight. Of note, adjustment for time did not materially alter the statistical significance of the relationships (Figure 5, Figure 6). No significant changes were found in LAS-ct.

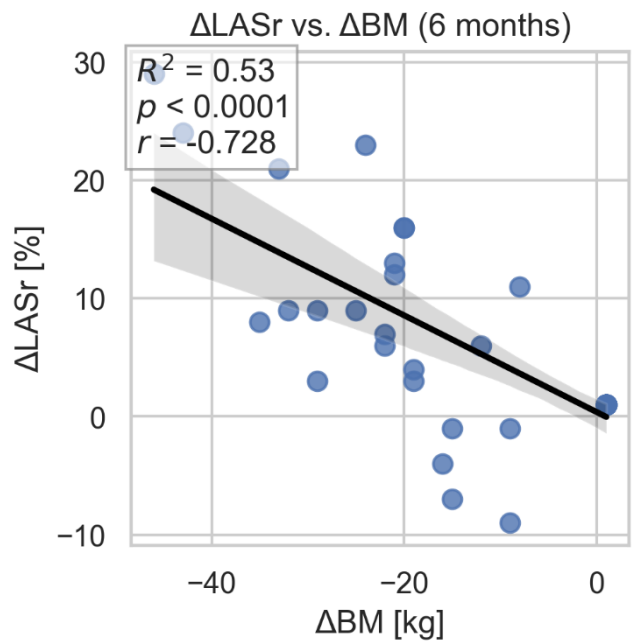


Figure 5. Correlation between changes in LAS-r and loss of weight after bariatric surgery. LAS-r- left atrial strain-reservoir; BM- body mass.

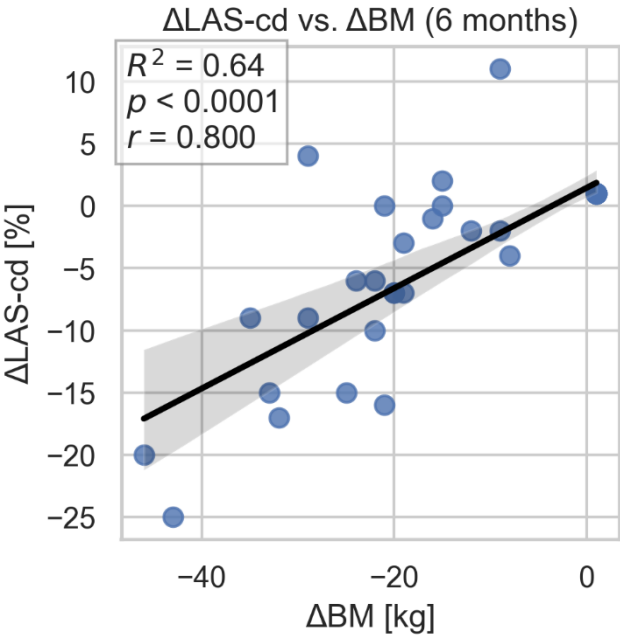


Figure 6. Correlation between changes in LAS-cd and loss of weight after bariatric surgery. LAS-cd- left atrial strain- conduit; BM- body mass.

3.2. Right Ventricular Function

During the study period, GSRV and FWSRV changed significantly, while TAPSE did not show a significant change. GSRV increased significantly from -15.9 ± 5.29 to -18.38 ± 3.25 at 6 months ($p=0.005$). FWSRV improved from -16.9 ± 6.17 to -19.5 ± 5.18 ($p=0.042$). TRV max significantly decreased from 1.84 ± 0.62 to 1.46 ± 0.52 at 6 months (Table 3, Figure 7).

Table 3. Changes in right ventricular parameters after bariatric surgery.

| Right ventricle Parameters | Before surgery | 3 months after surgery | 6 months after surgery | P-value |
|----------------------------|-----------------|------------------------|------------------------|---------|
| GSRV [%] | -15.9 ± 5.29 | -18.8 ± 4.03 | -18.38 ± 3.25 | 0.005 |
| FWSRV [%] | -18.38 ± 6.17 | -19.7 ± 5.00 | -19.5 ± 5.182 | 0.042 |
| TAPSE [mm] | 25.4 ± 3.3 | 25.8 ± 4.0 | 26.5 ± 3.3 | 0.36 |
| TRV max (m/s) | 1.84 ± 0.62 | 1.67 ± 0.59 | 1.46 ± 0.52 | 0.01 |
| s' [cm/s] | 14.7 ± 3.52 | 14.2 ± 2.9 | 14.9 ± 2.32 | 0.55 |
| AcT [ms] | 108.4 ± 13.5 | 110.3 ± 12.3 | 109.9 ± 12.3 | 0.43 |

AcT- accelerated pulmonary time; GSRV- global longitudinal strain of the right ventricle; FWSRV- free wall strain of the right ventricle; TAPSE- tricuspid annular plane systolic excursion; TRV- tricuspid regurgitation velocity.

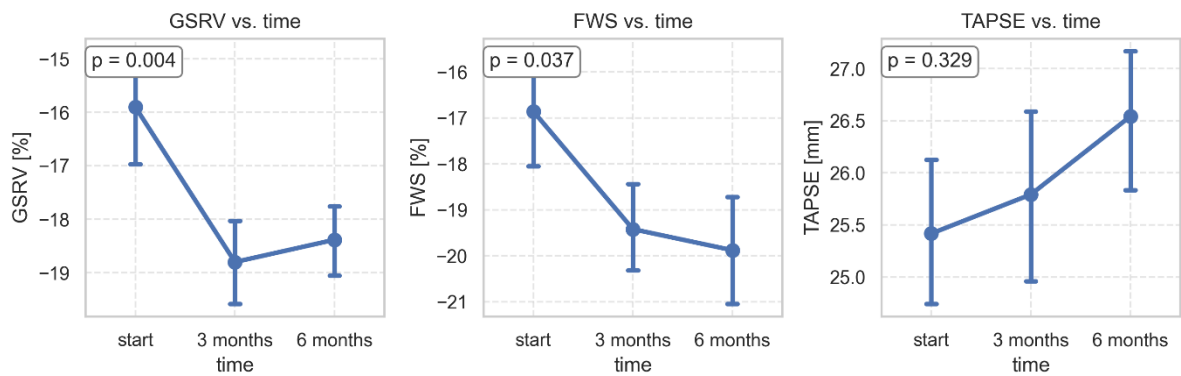


Figure 7. Changes in parameters of the right ventricle 3 and 6 months after bariatric surgery. GSRV- global longitudinal strain of the right ventricle; FWSRV- free wall strain of the right ventricle; TAPSE- tricuspid annular plane systolic excursion.

Additionally, a significantly greater change in GSRV was observed in the second time interval compared to the first period, adjusted for change in body weight. (Figure 8).

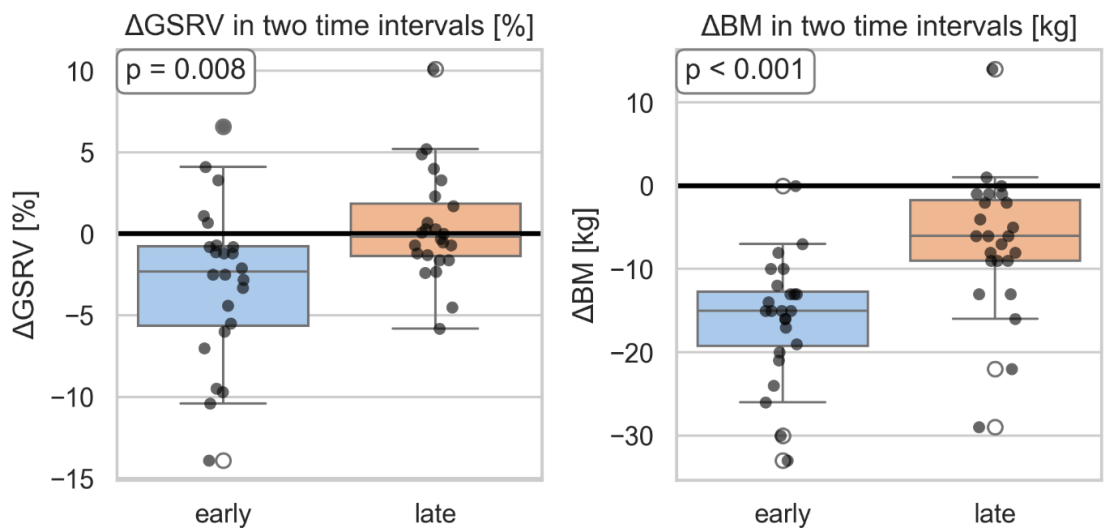


Figure 8. Change in global strain of the right ventricle and correlation with body mass in two time intervals. GSRV-global longitudinal strain of the right ventricle; BM- body mass.

The study showed a correlation between improvement in GSRV and TAPSE parameters and weight loss. However, we did not find evidence of a similar, statistically significant association between improvement in FWS and change in body weight. (Figures 9–11).

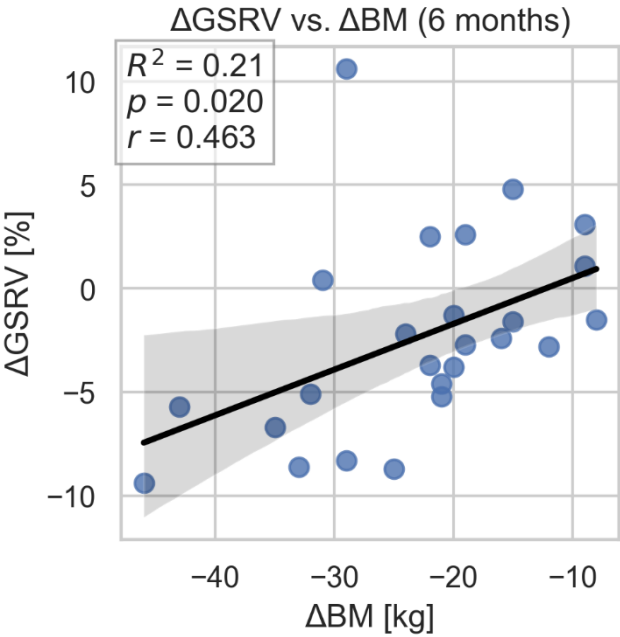


Figure 9. Correlation between GS RV change and weight loss. GSRV-global longitudinal strain of the right ventricle; BM- body mass.

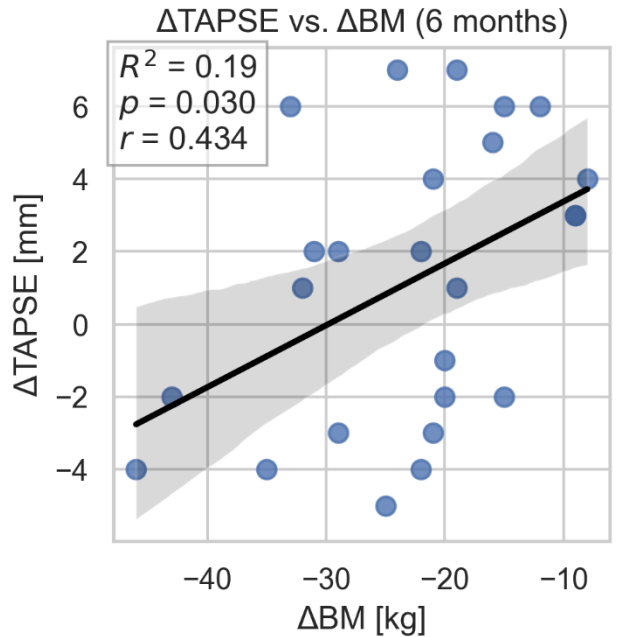


Figure 10. Correlation between TAPSE change and weight loss. TAPSE- tricuspid annular plane systolic excursion; BM- body mass.

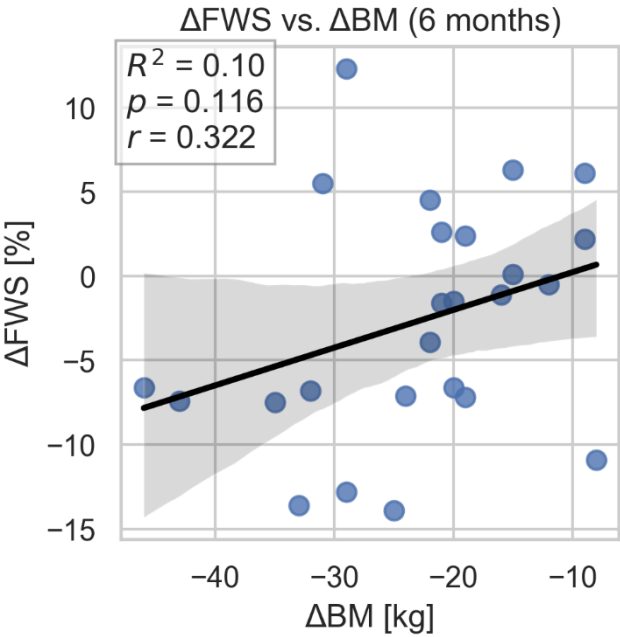


Figure 11. Correlation between FWSRV change and weight loss. FWS- free wall strain of the right ventricle; BM- body mass.

On top of individual regression models for each of the aforementioned parameters (GSRV, FWS and TAPSE), a multivariate analysis of variance (MANOVA) was performed to test whether the combined change in these parameters was associated with weight loss. The overall MANOVA was statistically significant (Wilks' $\lambda = 0.673$, $p = 0.037$), indicating that change in body weight was related to the multivariate profile of these right ventricular parameters. In the follow-up canonical discriminant analysis, ΔGSRV (-0.285) had the largest standardized weight in separating groups of low vs. high body mass change, followed by ΔTAPSE (-0.166) and ΔFWS (0.101) (Figure 12).

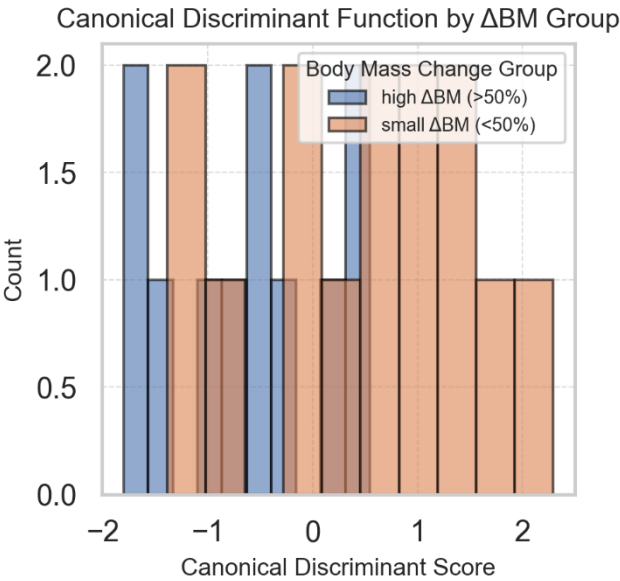


Figure 12. Canonical discriminant analysis (CDA) plot showing separation between participants with low and high change in body weight based on the combined change in GSRV, FWS and TAPSE.

4. Discussion

Obesity, especially severe obesity, can cause changes in cardiac hemodynamics, cardiac morphology and atrial and ventricular function that may predispose to heart failure (HF). Pathophysiological mechanisms responsible for the development of HF in patients with obesity include hemodynamic changes, neurohormonal activation, the influence of adipose tissue on the endocrine and paracrine systems, and lipotoxicity [8]. Excessive adipose accumulation is associated with increased total and central blood volume and increased cardiac output. Higher cardiac output leads to left ventricular dilatation and subsequent hypertrophy secondary to increased wall stress [9]. The above hemodynamic changes mainly affect the diastolic function of the left ventricle, while studies examining LV systolic function in obese individuals have shown mixed results. Most studies have demonstrated normal or above-normal left ventricular ejection fraction in obese people. Moreover, most reports have not shown a significant difference in left ventricular systolic function between obese and normal weight individuals. It is believed that the presence of severe LV systolic dysfunction in obese patients is associated with co-morbidities - most often coronary artery disease [10]. Additionally LVEF may not be an optimal marker for the evaluation of cardiac dysfunction in the obese population. In the present study, LVEF was normal at baseline and unchanged at follow-up. However, the current study confirmed a reduction in LV end-diastolic volume after bariatric surgery, correlating with body weight loss. The results of the current analysis also showed that despite normal LVEF, abnormal GLS parameters were observed in the study population. According to previous reports, tissue Doppler imaging studies in obese individuals have demonstrated evidence of subclinical left ventricular dysfunction, even with normal left ventricular ejection phase indices. Compared with ejection fraction, myocardial mechanical index global longitudinal strain can detect subclinical reduced left ventricular systolic function earlier and reflect myocardial systolic function abnormalities more sensitively. LV longitudinal strain mostly represents the function of subendocardial longitudinal fibers, which could be more sensitive to change of wall stress according to obesity status. Therefore, GLS is likely a better parameter for assessing the left ventricle in obese patients, as it allows for early detection of left ventricular systolic-diastolic dysfunction [3,11–14]. It is also very important that the current study demonstrated a significant improvement in LV GLS in patients shortly after bariatric surgery. These findings are consistent with few previous reports in the literature. Shin et al. demonstrated that approximately 15 months after bariatric surgery, there is a reduction in LV mass and improvement in longitudinal strain, with unchanged LVEF [3]. Another study assessing myocardial mechanics in obese patients undergoing bariatric surgery also confirmed a significant improvement in GLS over a 23-month follow-up period with unchanged LVEF parameters [15]. Subsequent studies have shown improvement in GLS as early as 1 and 6 months after bariatric surgery - these results are consistent with those shown in the present analysis [16,17].

Obesity is considered an independent factor in the development of left ventricular diastolic failure [10,18]. Most studies demonstrating impaired LV diastolic function in obesity have reported a high prevalence of left ventricular hypertrophy (LVH), with progressive impairment of LV diastolic function with increasing LV mass (LVM) (presumably due to muscle, fibrosis and intra-myocardial fat) [19–21]. In the present study, the average cardiac muscle thickness was 11.2 mm, and the average cardiac muscle mass was 205 g/m². After bariatric surgery, a significant reduction in LV mass was observed, but no correlation was found between LV mass reduction and body weight loss. Several studies have assessed the association between the degree of obesity and left ventricular mass measurements and the reduction in LV mass after weight loss [22–24]. These studies also demonstrated the coexistence of obesity with left ventricular hypertrophy and a reduction in left ventricular mass after weight loss, with no correlation between LV mass and BMI.

Previous reports have shown improvement in left ventricular diastolic function shortly after bariatric surgery [25–28]. In the current study, no significant changes after bariatric surgery were found in typical echocardiographic parameters used to assess left ventricular filling, however, a significant improvement in left atrial strain was demonstrated. According to previous reports, obese patients had a significant reduction in LAS parameters compared with non-obese controls. Obese

patients without known cardiovascular disease have significantly decreased LA function in all three LA functional components compared to non-obese controls. There was no difference in the prevalence of diastolic dysfunction evaluated using current guidelines, suggesting that left atrial dysfunction assessed by LAS allows for earlier detection of left ventricular diastolic dysfunction [29]. According to another study, obesity is associated with a functional LA phenotype by reduced conduit and reservoir function, with an increase in booster function, which may be compensatory [30]. In the current study, a reduction in all LAS parameters was demonstrated, while during the 6-month follow-up period, significant improvement was observed only in reservoir and conduit function. These changes correlated with weight loss. Similar results were presented by Strzelczyk et al. [31]. The 12-month follow-up after bariatric surgery showed a significant increase in LA-r and LA-cd with a decrease in LA-ct. According to the authors, the decrease in LASct is associated with improved early left ventricular filling during diastole, which may lead to a relative decrease in the contribution of atrial contraction.

The current study demonstrated significant improvement in right ventricular function after bariatric surgery. According to previous reports, subclinical right ventricular dysfunction is often found in patients with obesity. Right ventricular dilation caused by volume overload may increase myocardial oxygen consumption and ventricular wall stress [32]. In studies assessing the right ventricle before and after bariatric surgery, significant improvements in RV function were observed, demonstrated by various parameters [33,34]. The current study demonstrated significant improvements in GSRV and FWS parameters. To date, there are few studies assessing right ventricular strain in obese patients. Yuksel et al. showed in a short-term study that weight reduction in obese patients improved diastolic parameters and strain of both the left and right ventricles [35]. In turn, the meta-analysis by Esparham et al. [6] showed an improvement in most left ventricular parameters, while the right ventricular structure changed to a lesser extent. Sorimachi et al [4] highlighted long-term benefits, including LV mass reduction, decreased epicardial adipose tissue thickness, and improvement in left and right ventricular strain, even though LA volume and preload pressures in the LA increased over time. Additionally, regarding right ventricular function and pulmonary circulation pressure, Büber et al [36] demonstrated that as early as 6 months after bariatric surgery, pulmonary artery stiffness decreased and TAPSE improved, indicating better RV function [8]. In our study, the combined analysis of right ventricular strain and TAPSE proved important. We found that improvement in these parameters correlated best with weight loss after bariatric surgery.

In summary, in the present study, significant changes in left and right ventricular function were demonstrated shortly after bariatric surgery.

5. Conclusions

In the short term, bariatric surgery leads to significant improvement in global longitudinal strain of the left and right ventricles in obese patients, with relative stability of LVEF and diastolic parameters. These results suggest early improvement in subclinical systolic function parameters following weight reduction in obese patients. The best echocardiographic parameters for monitoring hemodynamic changes in this group of patients are GLS, RVFWS, and TRV.

Further studies with longer follow-up are needed to assess the durability of these changes and their prognostic significance.

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Data Availability Statement: All data were included in the manuscript.

Conflicts of Interest: Authors declare no conflict of interest.

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