Gastric bypass surgery is associated with reduced subclinical myocardial injury and greater activation of the cardiac natriuretic peptide system than lifestyle intervention

Running head: Bariatric surgery and cardiac markers

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Conflicts of interests

Dr. Aakre has served on advisory boards for Roche Diagnostics and received lecture fees from Siemens Healthineers. Dr. Omland has served on advisory boards for Abbott Diagnostics and Roche Diagnostics and has received research support from Abbott Diagnostics, Novartis, Roche Diagnostics, Singulex and SomaLogic via Akershus University Hospital, in addition to a speaker's honoraria from Roche Diagnostics and Siemens. Dr. Holven reports grants and/or personal fees from Tine SA, Mills DA, Olympic Seafood, Amgen, Sanofi, Kaneka and Pronova, none of which are related to the content of this manuscript. Dr. Nordstrand, Dr. Gjevestad, Dr. Lyngbakken and Dr. Hjeltnes report no conflicts of interest.

Abstract

Aims: Morbid obesity is a risk factor for cardiovascular disease. The relative effects of Roux-en-Y gastric bypass surgery (GBS) and intensive lifestyle intervention (ILI) on subclinical myocardial injury, the activity of the cardiac natriuretic system, and systemic inflammation remain unclear.

Methods: In a 59-week non-randomized clinical trial that included 131 patients with morbid obesity, we compared the effects of GBS and ILI on concentrations of cardiac troponin T (cTnT) and I (cTnI), N-terminal pro-B-type natriuretic peptide (NT-proBNP) and C-reactive protein (CRP).

Results: In the GBS and ILI group, median body mass index (BMI) was reduced by 14.4 kg/m² versus 3.9 kg/m², respectively (p value < 0.001). Cardiac troponins decreased after GBS, p=0.014 (cTnT) and p=0.065 (cTnI) and increased significantly in those treated with ILI (p values ≤ 0.021) (between-group differences for deltas: p ≤ 0.003). NT-proBNP increased in both groups, but significantly more in the GBS than in the ILI group (between-group differences for deltas: p=0.008). CRP decreased significantly within the GBS and the ILI group, with this change significantly greater in the GBS group (between-group differences for deltas p < 0.001). The dominating mediator of the biomarker changes was weight loss. Prior coronary artery disease and diabetes were predictive of the magnitude of the changes in cTnI and NT-proBNP, respectively.

Conclusion: Compared to ILI, GBS was associated with reduced subclinical myocardial injury and systemic inflammation, and enhancement of the cardiac natriuretic peptide system. The biomarker changes were predominantly mediated by weight loss.

Keywords: Troponin, NT-proBNP, CRP, weight loss, cardiovascular risk, Roux-en-Y gastric bypass

Introduction

Obesity affects approximately 20-30% of adults in the industrialized world, with its prevalence also increasing rapidly in the third world (1). Obesity is an independent risk factor for cardiovascular (CV) disease (2), and the risk may be amplified by co-morbidities and other risk factors. Comprehensive lifestyle intervention, including diet, physical activity, and behavior modification, is the cornerstone of obesity treatment. In addition, both bariatric surgery and adjunctive therapies using weight reducing drugs may be appropriate for people who fail to achieve sufficient weight loss or are unable to maintain weight loss by means of lifestyle intervention alone (3).

Subclinical chronic myocardial injury is characterized by a stable, low-grade increase in cardiac troponin concentrations. Multiple studies have shown a link between chronically elevated troponin concentrations and poor long-term CV prognosis. Importantly, an association with risk is evident at concentrations below the 99th percentile of high-sensitivity assays (4-8). In a previous study, we found that gastric bypass surgery, and to a lesser extent lifestyle intervention, was associated with a decrease in cardiac troponin I (cTnI) concentrations (9). Recent studies have revealed interesting biological differences between cardiac troponin T and I (cTnT and cTnI), with cTnI seemingly more closely associated with future MI, stable ischemic cardiovascular disease and left ventricular mass index, while cTnT is strongly associated with non-CV mortality (7, 10, 11). The relative response of cardiac troponin I and T to weight loss induced by bariatric surgery and lifestyle intervention remains unknown.

Natriuretic peptides exert vasodilatory, natriuretic, growth-inhibitory and anti-fibrotic actions that are considered beneficial for the cardiovascular system (12). Interestingly, genetic variants associated with higher natriuretic peptide concentrations (within the normal range) seem to be associated with

reduced cardiovascular risk (13-15). Recent studies indicate that natriuretic peptides also play an important role in the regulation of glucose and lipid metabolism (12), and high-normal concentrations are linked to reduced risk of type 2 diabetes mellitus and metabolic syndrome. N-terminal pro-B-type natriuretic peptide (NT-proBNP) is measured at lower concentrations in individuals with obesity than normal weight individuals (16), reflecting the higher percentage of glycosylation at residue threonine 71, inhibiting the production of BNP and NT-proBNP (17). Both bariatric surgery and physical activity may increase NT-proBNP concentrations (18). The mediating factors of this increase remain unclear.

White adipose tissue produces different cytokines, which induce production of CRP in the liver (19). Multiple studies, including a recent meta-analysis, have shown that bariatric surgery and the accompanying weight loss lead to a significant reduction in CRP (20), but lifestyle interventions like dietary interventions or physical activity may also lower CRP concentrations substantially (21-23).

As such, the aim of this study was to compare the effect of a one-year intensive life-style intervention (ILI) program that included caloric restriction and physical activity with the effect of Roux-en-Y gastric bypass surgery (GBS) on biomarkers of subclinical myocardial injury, neuro-hormonal activation and systemic inflammation. Further, we attempted to identify the major factors mediating the effect of these interventions.

Methods

The study is a sub-study of a 59-week non-randomized clinical trial comparing the effects of ILI with an initial 7-week low-calorie diet followed by laparoscopic GBS. The details of the main study have been described earlier (24, 25). The study was approved by the local ethical committee (REK S-

05175) and registered at Clinical Trials.gov (NCT00626964). All procedures were conducted according to the Declaration of Helsinki and written informed consent was given by all participants.

Participants

All participants were recruited from the Morbid Obesity Center at Vestfold Hospital Trust, with the study taking place between February 2008 and June 2012 (24, 25). This is a tertiary care center treating people with morbid obesity. The decision regarding the type of intervention was made prior to inclusion to the present study, with patients either assigned to an ILI program or to an initial low-caloric diet followed by GBS. Inclusion criteria were BMI ≥ 35 kg/m² with at least one obesity related comorbidity, or a BMI ≥ 40 kg/m². Patients were excluded if they had decompensated heart failure, cardiac arrhythmias, unstable angina, cardiac pacemakers, intra-cardiac devices, a recent cerebrovascular event or myocardial infarction (within the past 6 months), end-stage renal disease, bleeding disturbances, serious psychiatric disorders or serious eating disorders.

A complete biomarker set (baseline and follow-up cardiac troponins, NT-proBNP and CRP) was available for 137 of the 159 patients who completed the original study (25). Of these, six patients were excluded as outliers (see Supplemental data table 1), as the deviation between measurements suggested a non-acknowledged acute clinical condition influencing one of the results; absolute delta values were 68 mg/L (CRP), 192 ng/L (cTnT), 52-630 (cTnI) and 700 ng/L (NT-proBNP). Accordingly, 131 patients (62 in the ILI and 69 in the GBS group) were included in the final statistical data analysis.

Intervention

The ILI included both a dietary and physical intervention (24, 25). The first 12 weeks included treatment sessions three days per week. On each occasion, the patients participated in two supervised training sessions (60-90 min) and attended lectures on nutrition, physical activity, and motivation. Patients were recommended an energy restricted diet of 1000 kcal per day based on the calculated total energy expenditure at baseline. During weeks 13-52 patients were advised to perform physical activity for 60-90 minutes per day, and received monthly follow-up, alternating between group based and individual sessions every other month.

Patients in the GBS-group completed a 7-week low calorie diet (900 kcal per day) before undergoing surgery. A standardized regimen of dietary supplements and a proton pump inhibitor were prescribed to all patients after surgery.

Blood sampling, analysis and other measures

Venous blood samples were collected after overnight fasting. Serum samples were frozen at -80 degrees Celsius until thawed and analyzed in one run for high-sensitivity cTnT, NT-proBNP and CRP (Roche Diagnostics, Basel, Switzerland), high-sensitivity cTnI (Centaur, Siemens Healthineers, Erlangen, Germany). The cTnT assay had a 20% CV_A at 2.8 ng/L (lowest reported concentration was 3 ng/L) a 10% CV_A at 5 ng/L and a 99th percentile of 14 ng/L. The cTnI assay had a 20% CV_A at 2.5 ng/L (lowest reported concentration), a 10% CV_A at 6 ng/L and a 99th percentile of 45 ng/L (26). Values below the lowest reported concentration were assigned a value corresponding to 50%, i.e. 1.5 ng/L and 1.25 ng/L respectively.

Detailed descriptions of the remaining data sources and measurements have been published previously (24, 25). In brief, analyses of serum glucose, creatinine and blood lipids were performed

using dry reagent slide technology on the Vitros FS 5.1 (Ortho-Clinical Diagnostics, NY, USA). Low-density lipoprotein (LDL) cholesterol concentrations were estimated using the Friedewald equation and estimated GFR was calculated by the applicable CKD-EPI equation for a creatinine method traceable to isotope-dilution mass spectrometry. Hemoglobin A1c (HbA1c) was analyzed using high performance liquid chromatography on Tosoh HLC-723 G7 (Tosoh Corporation, Tokyo, Japan) and insulin was analyzed using an immunoassay from Linco Research Inc., St. Charles, MO.

Arterial hypertension was defined by either a systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg or the use of antihypertensive medication. Coronary artery disease (CAD) was defined as a history of stable CAD, percutaneous coronary intervention, coronary artery bypass graft surgery, or prior myocardial infarction. Type 2 diabetes was diagnosed in patients who had a prior history of type 2 diabetes, received antidiabetic drugs, or who had a fasting serum glucose concentration ≥ 7.0 mmol/L (27). Analyses of body composition were performed using bioelectrical impedance analysis (Inbody 720, Body Composition Analyzer, Biospace, Seoul, South Korea). Resting blood pressure was measured using an electronic blood pressure recorder with an appropriately sized cuff (DinamapVR, ProCare Series, G.E. Medical Systems, Buckinghamshire, UK) with the patient sitting in an upright position. Mean arterial pressure (MAP) was calculated as $[(\text{diastolic pressure} \times 2) + \text{systolic pressure}]/3$ (28). Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated as $[\text{fasting serum glucose (mmol/L)} \times \text{fasting serum insulin (pmol/L)}]/135$ (29). The SphygmoCorVR system (AtCor Medical, Sidney, Australia) and a single high-fidelity applanation tonometer (MillarVR) were used to measure carotid femoral pulse-wave velocity (cfPWV). The travel distance was calculated by subtracting the distance between the carotid artery and the sternal notch

from the distance between the sternal notch and the femoral artery (30). The subtracted travel distance measured at baseline was used in the calculations of cfPWV at follow-up.

Statistical analysis

Continuous variables are reported as median with 25 and 75 percentiles. Between group comparisons were made by the Mann-Whitney U test. Categorical data are reported as absolute numbers and percentages and were compared between groups using the Chi Square or Fisher exact test, as appropriate. Differences in delta values between groups were compared using Mann-Whitney U test, and biomarker changes within group were compared using the Wilcoxon Signed Rank test. A sensitivity analysis comparing the differences in delta values within groups was performed for patients with baseline troponin concentrations ≥ 5 ng/L.

For the troponin data we also evaluated the percentage of patients within the two groups for whom the delta value exceeded the random variation that may be seen in two consecutive results, doing so using the concept of reference change values (RCV) (31). RCV combine within-individual biological variation, analytical variation and a z-value to give an estimate of random variation when two consecutive results are compared. Earlier studies have shown that the long-term within-individual biological variation of troponins is approximately 15% (32). The analytical variation at the low concentrations measured in our study was approximately 10% (see above). Based on these two numbers we could calculate a RCV (95% CI) value of approximately $\pm 50\%$. This value is similar to the one used for evaluation of significant changes in the LIPID study (33). Percentages of patients who exceeded these limits were calculated within each group, and compared using Chi-square tests.

As this was a non-randomized trial, we investigated if the changes in biomarkers were associated with the intervention type or with differences in baseline characteristics. This was achieved by a multiple linear regression analysis with change in biomarkers as dependent variables and intervention type as the main independent variable, adjusting for baseline variables that differed between the groups. If baseline variables were related (Spearman rank correlation >0.4), the variable most strongly associated with the dependent variable was selected.

A series of trivariate regression models were undertaken to evaluate potential mediators of the effect of the intervention. The biomarker changes (delta cardiac troponins, NT-proBNP and CRP concentrations from 59 weeks to baseline) were assigned as the dependent variables and the intervention and the potential mediator of the intervention effect were assigned as the independent variables. If variables were related (Spearman rank correlation >0.4), the variable most strongly associated with the dependent variable was selected for the analysis. Independent variables that changed the beta coefficient of the intervention $>10\%$ were considered potential mediators of the intervention effect. SPSS statistics 24.0 was used for all statistical analyses.

Results

Compared with the GBS group, the ILI group had a significantly higher prevalence of CAD, higher median systolic blood pressure, and lower median BMI, fat mass, HOMA-IR, and CRP, while gender distribution, age, cardiac troponins and NT-proBNP did not differ significantly between the groups (Table 1). During the study period, cTnT concentrations significantly decreased and the cTnI concentrations were non-significantly reduced within in the GBS group, while both cTnT and cTnI increased significantly in the ILI group (between-group differences for deltas: $p \leq 0.003$) (Figure 1,

Table 2). Of note, these changes seemed to be confined to patients in whom baseline troponin concentrations were above 5 ng/L (Figure 2), and the sensitivity analysis including patients with baseline concentrations ≥ 5 ng/L supported this. For both troponin I and T the concentrations were significantly decreased in the GBS group and stable or non-significantly (cTnI) increased for ILI patients (Table 2).

When individual deltas were compared to the RCV values ($\pm 50\%$) we found that 20% vs. 11% of cTnI deltas increased more than 50% in the ILI vs. GBS group, respectively (p-value 0.161). Significantly more GBS patients compared to ILI showed declining values of more than 50%; 19% vs. 3% (p-value 0.002). For cTnT we found that 13% of ILI patients increased their delta by more than 50%, compared to 5% of GBS patients (p-value 0.104), and similarly 3% (ILI) vs. 5% (GBS) showed declining values of more than 50% (p-value 0.563).

CRP decreased and NT-proBNP increased significantly in both intervention groups, but changes were greater after GBS (Table 2).

We investigated the impact of between-group differences in baseline characteristics on the magnitude of changes in the concentration of cardiac troponins, NT-proBNP and CRP. Table 3 shows a multiple linear regression analysis with biomarker changes as the dependent variable and the interventions and the baseline variables that differed between the groups as independent variables. History of CAD was associated with increasing concentrations of cTnI following the interventions, while high HOMA-IR was associated with a larger reduction in cTnI. Diabetes mellitus at baseline was associated with

greater increase in NT-proBNP concentrations. High baseline CRP was associated with larger reductions in CRP.

Supplemental Table 2 summarizes the changes seen in previously reported variables (25) according to study group. All weight variables (BMI, waist-to-hip ratio, fat mass, fat free mass, skeletal muscle mass) showed significantly greater reductions in the GBS than in the ILI group. Changes in all metabolic biomarkers and eGFR were more favorable in the GBS group, while blood pressures, arterial stiffness indices and urinary albumin-creatinine ratios were comparable between groups.

Bivariate correlations between the delta values of cardiac troponins, NT-proBNP and CRP and changes in the other continuous data are shown in Supplemental table 3, and the subsequent mediation analyses are shown in Table 4. For cTnT, changes in fat mass, HbA1c and eGFR altered the beta-coefficient of the intervention variable (ILI vs GBS) more than the 10% predefined threshold, with changes in fat mass being the dominating mediator. For cTnI, change in BMI appeared to be the major mediator, with LDL-cholesterol change showing a smaller effect. For NT-proBNP, changes in fat mass, HbA1c and to lesser extent LDL-cholesterol altered the beta-coefficient more than the 10% predefined threshold, with change in fat mass being the major apparent mediator. For CRP, only change in BMI changed the beta-coefficient of the intervention more than 10%.

Discussion

The principal findings of this study were that GBS, as compared with ILI, was associated with significantly greater changes in cardiac troponins, NT-proBNP and CRP concentrations, mirroring the greater magnitude of weight loss in the surgical group. Cardiac troponin levels decreased

significantly more in the GBS group than in the ILI group during the study period. Within-group data showed that cardiac troponins decreased in the GBS group and increased or stabilized after ILI. It is noteworthy that these changes were predominately seen in patients with cardiac troponin concentrations above 5 ng/L. This suggests that the cardiac troponin change associated with intervention is unlikely to occur in those with very low concentrations, i.e. those without any indication of subclinical myocardial injury. NT-proBNP increased and CRP significantly decreased within both groups, but these changes were also significantly less pronounced in the ILI than in the GBS group. Accordingly, weight loss induced by GBS seems to be beneficial to cardiovascular health, as reflected in the observed changes in biomarkers of subclinical myocardial injury, inflammation and the cardiac natriuretic peptide system. Although the changes in cardiac troponins were predominantly observed within the normal range and may appear modest, prior studies have shown that even cardiac troponin concentrations within the normal range reflect CV risk (34, 35) and troponin concentrations below the detection level of high sensitivity assays reflect cardiovascular health.

The effect of weight loss on subclinical myocardial injury

Chronic, subclinical myocardial injury, as evidenced by stable, low-grade increase in cardiac troponin concentration, is strongly associated with the incidence of heart failure and cardiovascular death (4-8). In epidemiological studies, obesity has been associated with higher concentrations of cardiac troponins, as well as with increased risk of heart failure, suggesting that obesity may cause subclinical myocardial injury that may progress to heart failure over time. In accordance with this mechanistic model, weight loss following GBS has been associated with reduced incidence of heart failure (36),

and we have previously shown that GBS is associated with a significant reduction in cTnI (9). However, several questions have remained unanswered within the academic literature. Recent large-scale studies have provided evidence that cTnI and cTnT are differentially associated with cardiovascular risk factors and also seem to provide differential and complementary prognostic information regarding future ischemic cardiovascular disease, left ventricular mass and non-CV mortality (7, 10, 11). Whether cTnI and cTnT respond differently to weight loss induced by GBS or ILI has not previously been assessed. The current data demonstrate that GBS was associated with a roughly similar reduction in cTnT and cTnI, while ILI was associated with a comparable increase in both markers, suggesting a weight dependent response pattern for both markers.

Improved physical fitness and statin therapy has been linked to an attenuation or reduction in the age-related expected increase in cardiac troponins (33, 37-40). ILI comprising both measures to reduce weight (9) and to increase physical fitness, would therefore be expected to reduce cardiac troponin concentrations. Contrary to our expectations, we observed that ILI was associated with stable or a slightly increase in cTnT and cTnI concentrations. The reasons for this observations remain unclear. Exercise is associated with an acute and transient increase in cardiac troponins (41), but shorter (60-90 minutes) activity should not lead to chronic elevations. The increase in troponins was limited to ILI patients with baseline troponin concentrations above 5 ng/L, indicating that patients with severe obesity and subclinical myocardial injury may have another response to lifestyle modifications compared with those with a healthier myocardium. Alternative explanations for the different responses in the GBS and ILI groups should also be considered. Of note, lipids and glucose tolerance indices were greatly improved in the GBS group. HbA1c and LDL showed some mediating effects

on changes in cardiac troponins. A more favorable metabolic milieu may potentially lead to reduced myocardial injury and explain why cardiac troponins decreased in the GBS group. Clearly, more information is needed on the isolated effect of physical activity in the morbidly obese, normal weight individuals and in patients with different degrees of myocardial injury and metabolic risk factor burden.

The effect of weight loss on markers of myocardial stress

Somewhat paradoxically, obesity is associated with lower circulating concentration of natriuretic peptides, including inactive fragments of natriuretic peptide prohormones such as NT-proBNP. The lower concentration is linked to increased insulin resistance and hyperglycemia, increased RAAS stimulation and further unfavorable lipid accumulation (12). Our findings of low median baseline NT-proBNP concentrations and increasing concentrations after treatment therefore correspond with previous data (16, 18). Interestingly, we observed that the magnitude of the NT-proBNP increase was largest in the GBS group (18) and was mediated by a profound weight loss. Although the exact mechanisms responsible for the association between obesity and lower natriuretic peptide concentrations remain to be explored, a similar pattern has been observed for both B-type natriuretic peptide (BNP) and NT-proBNP. BNP and NT-proBNP are degraded via different pathways, suggesting that reduced production in the presence of a metabolically unhealthy milieu, e.g. due to increased glycosylation (17), may contribute to this association. Accordingly, a recent study showed both a negative regulation of natriuretic peptides after an oral lipid tolerance test and reduced concentrations of natriuretic peptide receptor A after fatty acid stimulation, indicating reduced production and effect of natriuretic peptides when abundant lipids circulate.(42) A baseline diagnosis

of diabetes predicted a larger increase in NT-proBNP, and reduction in HbA1c mediated the NT-proBNP increase, strengthening the theory that natriuretic peptides are closely linked to glucose metabolism (12).

Changes in CRP

Several studies have measured the effect of GBS or ILI on CRP concentrations (22). Our data are in keeping with a recent meta-analysis of GBS including more than 100 studies, showing an overall reduction in CRP of 5.3 mg/L after surgery (20, 22). Another meta-analysis showed that lifestyle interventions were associated with a significant weight dependent reduction in CRP (23). Data from the JUPITER trial showed a linear relationship between reduction in CRP and future MI or coronary death; the lowest event rate was seen at CRP concentrations below 1 mg/L (43). The CANTOS trial showed that post-myocardial infarction anti-inflammatory treatment with canakinumab significantly reduced future cardiovascular events if the treatment induced CRP reductions below 2 mg/L. The observed CRP reductions seen in our data may thus translate into potential improved CV outcome, particularly in the GBS patients who achieved a median CRP concentration < 1 mg/L. That the largest effect was seen in those with the highest baseline CRP concentration is to be expected, as the largest changes usually occur in those with the most extreme baseline measurements (regression to the mean).

Strengths and limitations

The strengths of this study include the prospective design and the follow-up period of 59 weeks. A large number of co-morbidities, metabolic and CV risk factors were monitored and included in the

statistical analysis. Additionally, we performed a head-to-head comparison of several biomarkers used for predicting CV risk, including inflammatory and myocardial injury and function encompassing different pathophysiological mechanisms involved in CV disease. The major limitation in the current study is the non-randomized design. We have compensated for this using multiple linear regression analysis correcting for several baseline factors that differed between the groups. Randomization is difficult in this setting as the success of ILI is highly influenced by the patients' motivation, while GBS is associated with short- and long- term risk of complication that could not be neglected (44). The sample size was rather small and our findings need to be confirmed in a larger data set and with longer follow-up assessing CV events. Even though there appears to be a clear trend that GBS reduces cardiac troponin concentrations compared to ILI, (table 2, the RCV data, table 3 and 4), the study is probably underpowered to provide robust answers. Our study did not include compliance data in the ILI group, which could underestimate the effect of ILI if patients were less compliant. Nor did we perform a measurement of peak oxygen consumption, echocardiography or cardiac MRI, which may have provided valuable insight into changes in fitness or cardiac function.

Conclusion

Our findings confirm and extend the results of prior studies on the effect of GBS and ILI on cardiovascular and inflammatory biomarkers (9, 18, 20, 22), demonstrating that compared to ILI, GBS leads to significantly greater beneficial changes in biomarkers signaling cardiac injury, hemodynamic and metabolic stress and inflammation. It is noteworthy that markers of low-grade myocardial injury decreased after GBS and increased after ILI. The effects of intervention appear to be mediated through a more pronounced weight loss in GBS patients, with cardiac troponins and NT-

proBNP also to a lesser extent influenced by changes in glucose and lipid variables. Although the observed biomarker changes in the GBS group are considered favorable, future studies should investigate if the current findings translate into reduced long-term CV risk in patients surgically treated for obesity.

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Figure legends

Figure 1. Median, 10 and 90 percentile for the changes (59 weeks minus baseline values) in CRP, troponins and NT-proBNP in the ILI and GBS group.

Figure 2. The delta (from 59 weeks to baseline) troponin changes; median, 10 and 90 percentile are stratified according to baseline troponin concentrations and type of intervention. ILI; black bars, GBS; grey bars. The first bar shows non-measurable concentrations, the remaining data are presented as quartiles.

Table 1. Patients characteristic.

Patient characteristics	GBS (n = 62)	ILI (n= 69)	p-value
Age, median (25-75 percentile)	42 (36 – 50)	44 (36 – 55)	0.147
Female, %	62.9	68.1	0.530
Caucasian, %	95.2	97.1	0.563
Type 2 diabetes mellitus, %	33.9	18.8	0.050
Hypertension, %	62.9	76.1	0.102
Coronary artery disease, %	3.3	14.7	0.026
Smoking, %	21.0	13.0	0.226
BMI, kg/m ²	45.7 (41.8 – 48.4)	41.6 (38.9 – 45.1)	< 0.001
Waist-to-hip ratio	1.00 (0.93 – 1.07)	0.98 (0.91 – 1.07)	0.364
Fat mass, kg	64.8 (56.3 – 75.5)	56.4 (50.4 – 64.1)	<0.001
Skeletal muscle mass, kg	37.5 (34.0 – 43.5)	36.0 (31.7 – 41.5)	0.125
Fat free mass, kg	66 (61.0 – 77.1)	64.5 (57.0 -73.6)	0.116
Heart rate	74 (67 – 84)	78 (69 -87)	0.311
Systolic blood pressure, mm Hg	136 (128 – 145)	144 (133 – 152)	0.030
Diastolic blood pressure, mm Hg	79 (71 – 87)	80 (73 – 86)	0.597
Pulse pressure, mm HG	54 (46 – 70)	60 (53 -72)	0.026
Mean arterial pressure, mm HG	98 (90 – 107)	101 (95 – 108)	0.115
Carotid-femoral pulse wave velocity, m/s	8.2 (7.5 – 9.9)	8.1 (7.1 – 9.6)	0.359
Augmentation index	24.2 (14.2 – 29.4)	23.7 (14.0 – 29.1)	0.982
β-Blocker, %	16.1	18.8	0.684
Calcium-channel blocker, %	12.9	15.9	0.622
RAAS inhibitor, %	33.9	34.8	0.913
Diuretics, %	17.7	21.7	0.567
Other blood pressure lowering drugs, %	4.8	8.7	0.384
Statins, %	16.1	18.8	0.684
Insulin, %	1.6	2.9	0.623
Non-insulin glucose lowering therapy, %	26.7	23.2	0.648
Sibutramin therapy, %	0	1.4	0.341
HbA1c, %	5.8 (5.3 – 6.3)	5.7 (5.4 – 6.0)	0.323
HOMA-IR	4.4 (2.4 – 6.7)	3.4 (2.0 – 5.0)	0.049
Glucose, mmol/L	5.5 (5.1 – 7.1)	5.3 (4.9 – 6.0)	0.053
Total Cholesterol, mmol/L	4.9 (4.2 – 5.7)	5.1 (4.4 – 5.7)	0.256
LDL-Cholesterol, mmol/L	3.0 (2.5 – 3.7)	3.0 (2.4 – 3.7)	0.903
HDL-Cholesterol, mmol/L	1.1 (0.9 – 1.2)	1.2 (1.0 – 1.4)	0.062
Triglyceride, mmol/L	1.5 (1.0 -1.9)	1.4 (1.1 - 2.0)	0.888
eGFR _(CKD-EPIcr)	105 (92 – 113)	101 (92 – 110)	0.567
ACR, mg/mmol	0 (0 – 0.8)	0 (0 – 0.8)	0.229
cTnT, ng/L	4.6 (3.2 – 7.4)	4.2 (3.3 – 7.0)	0.980
cTnI, ng/L	3.5 (1.3 – 6.0)	1.3 (1.3 – 4.9)	0.202
NT-proBNP, ng/L	40.7 (17.8 – 75.6)	28.0 (15.2 – 64.4)	0.257
CRP, mg/L	7.6 (3.4 – 11.4)	4.7 (2.4 – 8.7)	0.033

GBS: Roux-en-Y gastric bypass surgery, ILI: Intensified lifestyle intervention, BMI: Body mass index, HbA1c: Hemoglobin A1c, HOMA-IR: Homeostatic model assessment for Insulin Resistance, LDL: Low-density lipoprotein, HDL: High density lipoprotein, eGFR: estimated glomerular filtration rate, CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration, ACR: Albumin to Creatinine Ratio, CRP: C-reactive protein, cTnT: Cardiac troponin T, cTnI: Cardiac troponin I, NT-proBNP: N-terminal pro-B-type natriuretic peptide

Table 2. Median concentrations of the biomarkers at baseline and 59 weeks, stratified according to intervention group. 25 and 75 percentile in brackets. The sensitivity analysis include patients with baseline troponin concentrations $\geq 5\text{ng/L}$. Number within each group is presented in brackets.

	Baseline		59 weeks	p-value
All patients				
GBS group (n=62)				
cTnT, ng/L	4.6 (3.2 – 7.4)		4.7 (3.2 – 6.0)	0.014
cTnI, ng/L	3.5 (1.3 – 6.0)		2.7 (1.3 – 4.0)	0.065
NT-proBNP, pmol/L	40.7 (17.8 – 75.6)		69.4 (35.9 – 120.4)	< 0.001
CRP, mg/L	7.6 (3.4 – 11.4)		0.8 (0.5 – 1.8)	< 0.001
ILI group (n=69)				
cTnT, ng/L	4.2 (3.3 – 7.0)		5.1 (3.3 – 6.9)	0.021
cTnI, ng/L	1.3 (1.3 – 4.9)		3.0 (1.3 -7.0)	0.001
NT-proBNP, pmol/L	28.0 (15.2 – 64.4)		35.9 (20.7 – 74.2)	0.032
CRP, mg/L	4.7 (2.4 – 8.7)		2.9 (1.4 – 5.8)	< 0.001
Patients with baseline troponin concentrations $\geq 5\text{ng/L}$				
	Baseline		59 weeks	p-value
GBS group				
cTnT _{BL} $\geq 5\text{ ng/L}$ (n=27)	7.7 (6.2 – 11.9)		6.2 (5.4 – 8.7)	< 0.001
cTnI _{BL} $\geq 5\text{ ng/L}$ (n=20)	7.9 (6.0 – 9.1)		5.0 (1.7 -7.8)	0.004
ILI group				
cTnT _{BL} $\geq 5\text{ng/L}$ (n=29)	7.7 (6.4 – 12.1)		7.6 (6.1-13.7)	0.417
cTnI _{BL} $\geq 5\text{ng/L}$ (n=17)	9.2 (7.7 – 12.6)		11.5 (8.4 – 26.2)	0.055

GBS: Roux-en-Y gastric bypass surgery, ILI: Intensified lifestyle intervention, CRP: C-reactive protein, cTnT: Cardiac troponin T, cTnI: Cardiac troponin I, NT-proBNP: N-terminal pro-B-type natriuretic peptide

Table 3. Multiple linear regression analysis testing if variables that were significantly different at baseline would predict the magnitude of the biomarker changes. Biomarker delta value (59 weeks – baseline concentration) as dependent variable.

Delta cTnT_{59w} – cTnT_{BL}, ng/L					
	B (unstandardized)	SE	Beta (standardized)	t	P value
GBS vs ILI	-1.187	0.450	-0.257	-2.640	0.009
Delta cTnI_{59w} – cTnI_{BL}, ng/L					
	B (unstandardized)	SE	Beta (standardized)	t	P value
GBS vs ILI	-1.291	0.669	-0.172	-1.930	0.056
CAD	4.889	1.106	0.372	4.420	<0.001
HOMA-IR	-0.191	0.070	-0.250	-2.714	0.008
Delta NT-proBNP_{59w} – NT-proBNP_{BL}					
	B (unstandardized)	SE	Beta (standardized)	t	P value
GBS vs ILI	22.118	10.616	0.203	2.083	0.039
Type 2 diabetes mellitus	26.882	12.248	0.213	2.195	0.030
Delta CRP_{59w} - CRP_{BL}, mg/L					
	B (unstandardized)	SE	Beta (standardized)	t	P value
Bariatric surgery vs ILI	-3.257	0.823	-0.244	-3.956	<0.001
CRP at baseline	-0.689	0.061	-0.682	-11.376	<0.001

In the CRP model: Bariatric surgery, coronary artery disease, diabetes mellitus, fat mass, pulse pressure, HOMA-IR, and CRP.

In the cTnT model: Bariatric surgery, coronary artery disease, diabetes mellitus, BMI, SBP, HOMA-IR, and CRP.

In the cTnI model: Bariatric surgery, coronary artery disease, diabetes mellitus, fat mass, pulse pressure, HOMA-IR, and CRP.

In the NT-proBNP model: Bariatric surgery, coronary artery disease, diabetes mellitus, fat mass, SBP, HOMA-IR, and CRP.

cTnT: Cardiac troponin T, 59w: 59 weeks, BL: Baseline, GBS: Roux-en-Y gastric bypass surgery, ILI: Intensified lifestyle intervention, cTnI: Cardiac troponin I, CAD: coronary artery disease, HOMA-IR: Homeostatic model assessment for Insulin Resistance, NT-proBNP: N-terminal pro-B-type natriuretic peptide, CRP: C-reactive protein.

Table 4. Mediator analysis. Variables that changed the unstandardized B more than 10% are marked* below. Biomarker delta value as dependent variable.

Delta cTnT_{59w} – cTnT_{BL}, ng/L					
	B (unstandardized)	SE	Beta (standardized)	t	P value
GBS vs ILI	-1.442	0.390	-0.309	-3.692	<0.001
+/- 10% B (-1.586 to -1.298)					
GBS vs ILI	-0.913	0.662	-0.192	-1.380	0.170
Delta Fat mass, kg*	0.025	0.021	0.168	1.205	0.230
GBS vs ILI	-1.200	0.402	-0.257	-2.984	0.003
Delta HbA1c*	0.540	0.256	0.182	2.108	0.037
GBS vs ILI	-1.399	0.404	-0.300	-3.461	0.001
Delta Triglyceride	0.124	0.289	0.037	0.429	0.668
GBS vs ILI	-1.234	0.397	-0.265	-3.109	0.002
Delta eGFR _{CKD-EPI} *	-0.040	0.019	-0.184	-2.166	0.032
Delta cTnI_{59w} – cTnI_{BL}, ng/L					
	B (unstandardized)	SE	Beta (standardized)	t	P value
GBS vs ILI	-2.254	0.657	-0.289	-3.431	0.001
+/- 10% B: -2.479 to -2.029					
GBS vs ILI	-1.719	1.130	-0.220	-1.521	0.131
Delta BMI*	0.057	0.090	0.091	0.631	0.529
GBS vs ILI	-2.355	0.663	-0.301	-3.553	0.001
Delta DBP	0.027	0.025	0.092	1.086	0.280
GBS vs ILI	-2.223	0.659	-0.283	-3.372	0.001
Delta Augmentation index	-0.064	0.032	-0.168	-2.005	0.047
GBS vs ILI	-2.347	0.672	-0.297	-3.490	0.001
Delta Carotid-femoral pulse wave velocity, m/s	0.377	0.259	0.124	1.456	0.148

GBS vs ILI	-2.534	0.733	-0.323	-3.458	0.001
Delta LDL-Cholesterol*	-0.404	0.448	-0.084	-0.902	0.369
Delta NT-proBNP_{59w} – NT-proBNP_{BL}					
	B (unstandardized)	SE	Beta (standardized)	t	P value
GBS vs ILI	26.535	9.634	0.236	2.754	0.007
+/- 10% B: 23.88 – 29.189					
GBS vs ILI	10.206	15.615	0.094	0.654	0.515
Delta Fat mass, kg*	-0.630	0.487	-0.185	-1.293	0.198
GBS vs ILI	26.566	9.564	0.235	2.778	0.006
Delta SBP	0.482	0.223	0.183	2.159	0.033
GBS vs ILI	27.945	9.673	0.245	2.889	0.005
Delta Carotid-femoral pulse wave velocity, m/s	10.060	3.731	0.228	2.696	0.008
GBS vs ILI	26.679	9.806	0.235	2.721	0.007
Delta Augmentation index	0.295	0.472	0.054	0.625	0.533
GBS vs ILI	18.917	9.810	0.168	1.928	0.056
Delta HbA1c*	-17.018	6.250	-0.237	-2.723	0.007
GBS vs ILI	22.718	10.075	0.202	2.255	0.026
Delta Total cholesterol*	-7.439	5.884	-0.113	-1.264	0.208
GBS vs ILI	24.551	9.953	0.218	2.467	0.015
Delta Triglyceride	-5.760	7.113	-0.072	-0.810	0.420
GBS vs ILI	23.936	9.922	0.213	2.412	0.017
Delta eGFR _{CKD-EPI}	0.506	0.467	0.095	1.083	0.281
GBS vs ILI	26.834	10.122	0.235	2.651	0.009
Delta ACR	0.795	1.491	0.047	0.533	0.595
Delta CRP_{59w} – CRP_{BL}, mg/L					

	B (unstandardized)	SE	Beta (standardized)	t	P value
GBS vs ILI	-5.457	1.062	-0.412	-5.138	<0.001
+/- 10% B: -6.003 to -4.911)					
GBS vs ILI	-1.363	1.776	-0.103	-0.767	0.444
Delta BMI*	0.404	0.141	0.383	2.868	0.005
GBS vs ILI	-5.395	1.112	-0.407	-4.850	<0.001
Delta HbA1c	0.139	0.709	0.016	0.196	0.845
GBS vs ILI	-5.918	1.182	-0.444	-5.006	<0.001
Delta LDL-Cholesterol					
GBS vs ILI	-5.390	1.100	-0.407	-4.900	<0.001
Delta Triglyceride	0.195	0.786	0.021	0.248	0.804

cTnT: Cardiac troponin T, 59w: 59 weeks, BL: Baseline, GBS: Roux-en-Y gastric bypass surgery,

ILI: Intensified lifestyle intervention, HbA1c: Hemoglobin A1c, eGFR: estimated glomerular

filtration rate, CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration, cTnI: Cardiac

troponin I, BMI: Body mass index, DBP: Diastolic blood pressure, LDL: Low-density lipoprotein,

NT-proBNP: N-terminal pro-B-type natriuretic peptide, SBP: Systolic blood pressure, ACR:

Albumin to Creatinine Ratio

SUPPLEMENTAL DATA

Table 1. Six patients were excluded as outliers due to large deviation between measurements results.

Patient group	Baseline	59 weeks
ILI	cTnT 207 ng/L / cTnI 646 ng/L	cTnT 15 ng/L / cTnI 16 ng/L
ILI	cTnI 48 ng/L	cTnI 116 ng/L
ILI	cTnI 84 ng/L	cTnI 136 ng/L
ILI	cTnI 265 ng/L	cTnI 189 ng/L
GBS	NT-proBNP 36 ng/L	NT-proBNP 736 ng/L
ILI	CRP 6 mg/L	CRP 74 mg/L

ILI: Intensified lifestyle intervention, GBS: Roux-en-Y gastric bypass surgery, Cardiac troponin T, cTnI: Cardiac troponin I, NT-proBNP: N-terminal pro-B-type natriuretic peptide, CRP: C-reactive protein.

Table 2. Delta biomarker concentration (59 weeks minus baseline value), stratified according to intervention (median (25-75 percentile)).

	GBS (n = 62)	ILI (n= 69)	P value for delta value
Delta BMI	-14.4 (-16.5 to -12.2)	-3.9 (-6.8 to -1.4)	< 0.001
Delta Waist-to-hip ratio	-0.06 (-0.10 to -0.03)	0 (-0.1 – 0.2)	< 0.001
Delta Fat mass, kg	-35.7 (-40.0 to -29.0)	-8.1 (-16.5 to -3.2)	< 0.001
Delta Fat free mass, kg	-7.6 (-9.2 to -4.9)	-1.1 (-3.1 – 0.3)	< 0.001
Delta Skeletal muscle mass, kg	-4.9 (-5.8 to -3.5)	-0.8 (-2.0 – 0.1)	< 0.001
Delta SBP mm Hg	-7.0 (-16.0 – 7.0)	-7.0 (-16.5 – 2.5)	0.641
Delta DBP, mm Hg	1.0 (-12.5 - 8.5)	-3.0 (-9.5 – 3.5)	0.405
Delta PP, mm HG	-2.5 (-17.0 - 7.8)	-4.5 (-13.0 – 3.8)	0.760
Delta Carotid-femoral pulse wave velocity, m/s	-0.1 (-1.0 – 0.9)	0.1 (-0.7 - 0.6)	0.956
Delta Augmentation index	2.5 (-3.4 – 9.6)	0 (-6.0 – 8.0)	0.246
Delta HbA1c, %	-0.5 (-1.0 to -0.2)	-0.2 (-0.5 to -0.1)	0.001
Delta HOMA-IR	-3.1 (-5.6 to -1.3)	-0.8 (-2.1 to -0.1)	< 0.001
Delta Glucose	-0.8 (-1.8 to -0.4)	-0.2 (-0.6 – 0.1)	< 0.001
Delta Total Cholesterol	-0.7 (-1.2 – 0)	0 (-0.6 – 0.6)	< 0.001
Delta LDL- Cholesterol	-0.8 (-1.2 to -0.2)	0 (-0.4 - 0.5)	< 0.001
Delta HDL- Cholesterol	0.3 (0.2 – 0.5)	0.1 (0 – 0.2)	< 0.001
Delta Triglyceride	-0.6 (-0.9 to -0.2)	-0.2 (-0.7 - 0.1)	0.001
Delta eGFR _(CKD-EPIcr)	2.0 (-2.3 – 9.0)	-1.0 (-0.5 – 3.0)	0.004
Delta ACR	0 (-0.5 - 0)	0 (0.7 – 0)	0.212

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, PP: Pulse pressure, HbA1c: Hemoglobin A1c, HOMA-IR: Homeostatic model assessment for Insulin Resistance, LDL: Low-density lipoprotein, HDL: High density lipoprotein, eGFR: estimated glomerular filtration rate, CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration, ACR: Albumin to Creatinine Ratio

Table 3. Associations between change in biomarker concentrations from 59 weeks to baseline and potential predictor variables was assessed using Spearman rank correlation. If related variables show evidence of collinearity (Spearman rank correlation >0.4), the variable strongest associated with the dependent variable was selected for the mediator analysis.

	Delta CRP, r	p-value	Delta cTnT, r	p-value	Delta cTnI, r	p-value	Delta NT- proBNP, r	p-value
Delta BMI	0.513	<0.001	0.270	0.002*	0.300	<0.001	-0.192	0.027*
Delta Waist-to-hip ratio	0.143	0.139	0.156	0.106	0.115	0.233	-0.145	0.132
Delta Fat mass, kg	0.437	<0.001*	0.291	0.001	0.264	0.002*	-0.234	0.008
Delta Fat free mass, kg	0.462	<0.001*	0.230	0.009*	0.281	0.001*	-0.028	0.752*
Delta Skeletal muscle mass, kg	0.471	<0.001*	0.233	0.008*	0.297	0.002*	-0.092	0.298*
Delta SBP mm Hg	-0.059	0.501	-0.076	0.387	0.095	0.277	0.205	0.018
Delta DBP, mm Hg	-0.130	0.137	-0.058	0.506	0.171	0.049	0.113	0.194
Delta PP, mm HG	-0.030	0.733	-0.040	0.647	-0.042	0.632	0.155	0.078*
Delta Carotid-femoral pulse wave velocity, m/s	0.032	0.715	0.032	0.720	0.224	0.010	0.178	0.043
Delta Augmentation index	-0.137	0.118	-0.128	0.144	-0.144	0.100	0.151	0.083
Delta HbA1c	0.220	0.011	0.164	0.058	0.066	0.452	-0.290	0.001
Delta HOMA-IR	0.344	<0.001*	0.207	0.019*	0.192	0.029*	-0.185	0.036*
Delta Glucose	0.112	0.011*	0.094	0.281	0.072	0.407	-0.215	0.013*
Delta Total Cholesterol	0.176	0.042*	0.071	0.415	0.137	0.113	-0.216	0.012
Delta LDL-Cholesterol	0.225	0.009	0.074	0.398	0.148	0.090	-0.210	0.016*
Delta HDL-Cholesterol	-0.250	0.004*	-0.102	0.242	-0.079	0.362	0.039	0.652
Delta Triglyceride	0.149	0.086	0.162	0.061	0.075	0.390	-0.224	0.009
Delta eGFR _(CKD-EPI_{cr})	0	0.999	-0.236	0.006	-0.046	0.599	0.159	0.066
Delta ACR	-0.120	0.181	0.082	0.358	0.054	0.548	0.212	0.017

*not included in further analysis due to collinearity with another variable

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, PP: Pulse pressure, HbA1c: Hemoglobin A1c, HOMA-IR: Homeostatic model assessment for Insulin Resistance, LDL: Low-density lipoprotein, HDL: High density lipoprotein, eGFR: estimated

glomerular filtration rate, CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration, ACR:

Albumin to Creatinine Ratio

■ ILI
■ GBS