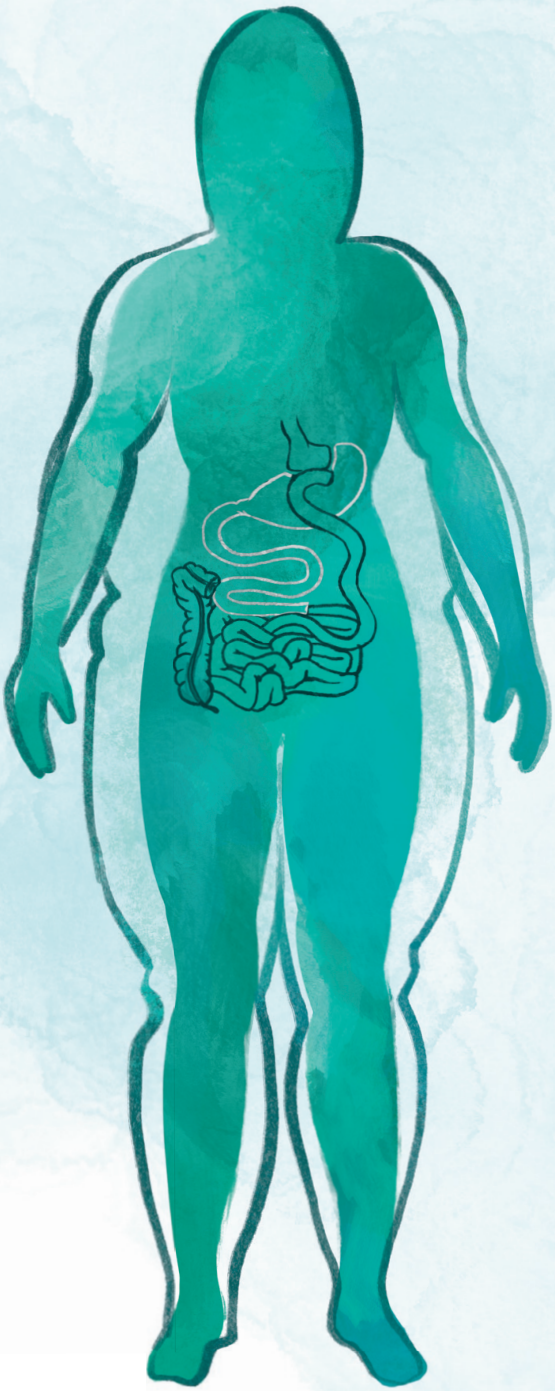
Clinical and Immunological Changes After Laparoscopic Roux-en-Y Gastric Bypass for Morbid Obesity



Kristel Wijngaarden

Clinical and Immunological Changes After Laparoscopic Roux-en-Y Gastric Bypass For Morbid Obesity

Leontine Henriëtte Wijngaarden

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Clinical and Immunological Changes After Laparoscopic Roux-en-Y Gastric Bypass For Morbid Obesity

Klinische en immunologische veranderingen na een laparoscopische Roux-en-Y gastric bypass voor morbide obesitas

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Promotor	Prof. dr. J.N.M. IJzermans
Overige leden	Prof. dr. E.F.C. van Rossum Prof. dr. C.C. Baan Prof. dr. E.J. Hazebroek
Copromotoren	Dr. ing. R.W.F. de Bruin Dr. E. van der Harst

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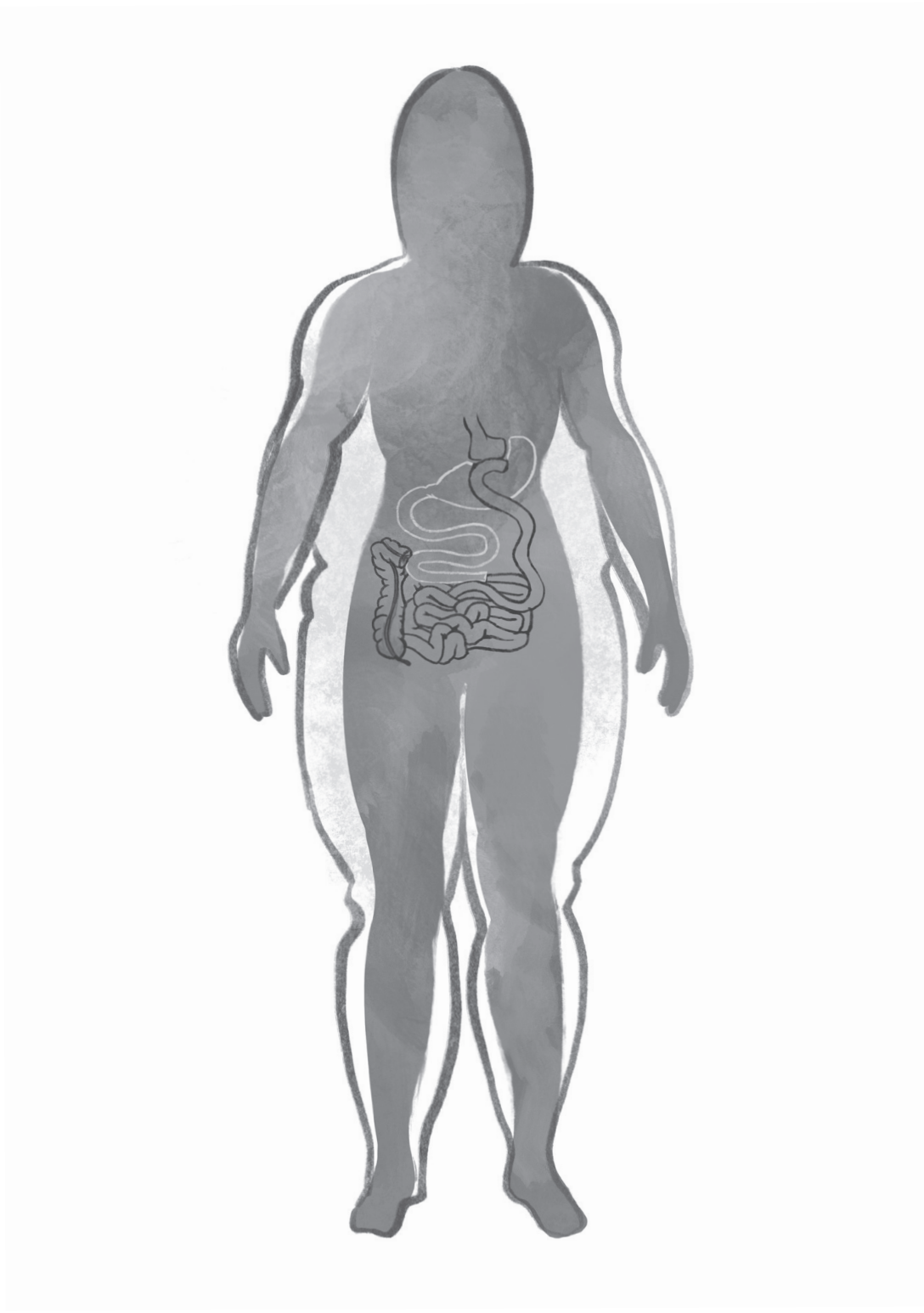


Chapter 1

General introduction and outline of this thesis

General introduction

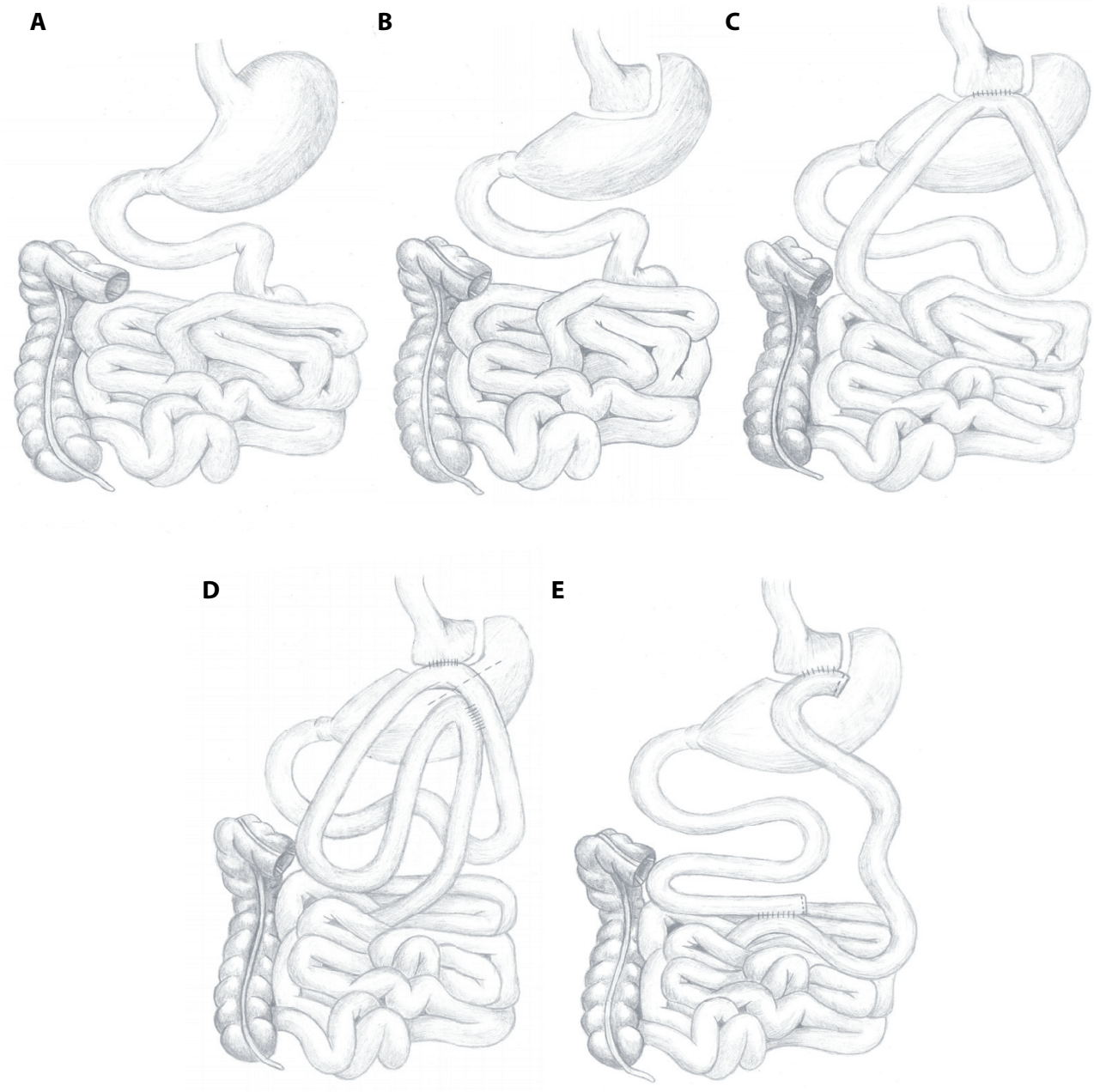
Worldwide obesity has increased rapidly since the last 5 decades[1]. The worldwide prevalence of obesity increased from 3.2% in 1975 to 10.8% in 2014 in men, and from 6.4% to 14.9% in women[2]. As the prevalence of obesity has increased, the prevalence of morbid obesity has obviously increased as well. Morbid obesity is defined as a person with a Body Mass Index (BMI) ≥ 35 kg/m2 with the presence of at least one of the obesity-related comorbidities, or a BMI ≥ 40 kg/m2 either with or without the presence of obesity-related comorbidities. Morbidly obese individuals have an increased risk for the development of those obesity-related comorbidities, such as hypertension, hypercholesterolemia, type 2 diabetes mellitus, obstructive sleep apnea, arthritis and cancer[3 4]. All of these comorbidities eventually lead to a reduced life expectancy. Additionally, morbid obesity is associated with infertility in women and may lead to higher rates of complications during pregnancy[5]. The reduced life expectancy in the morbidly obese population can be decreased by treating the obesity-related comorbidities. However, it is preferable to prevent the development of these obesity-related comorbidities by treating the cause of it, so to treat obesity itself.

Morbid obesity can be treated both non-surgically and surgically. The most effective non-surgical treatment of obesity is a combined lifestyle intervention. This consists of behavioral therapy combined with professional support leading to dietary adjustments and increased physical activity[6]. Combined lifestyle intervention has proven to reduce weight and leads to a reduction of obesity-related comorbidities[7]. However, long-term results are disappointing, as maintenance of the adjusted lifestyle is found difficult. Studies have shown that more than half of the patients return to their baseline weight within 5 years after the start of the intervention[8 9].

Contrary to the disappointing long-term results of the conservative treatment of morbid obesity, the surgical treatment of morbid obesity has shown promising long-term results. Bariatric surgery leads to substantial weight loss and remission or resolution of obesity-related comorbidities[10]. Several techniques for bariatric surgery have been described, such as laparoscopic adjustable gastric banding (LAGB), laparoscopic Roux-en-Y gastric bypass (LRYGB) and laparoscopic sleeve gastrectomy (LSG)[11]. The LRYGB technique is the most performed technique worldwide, consisting of 46.6% of all bariatric surgeries in 2011[12 13]. LRYGB is a combined restrictive and malabsorptive procedure, leading to an expected excess weight loss of 60-70%, a total weight loss of approximately 30%, and significant improvement or even resolution of obesity-related comorbidities[14]. Several studies have shown that LRYGB has superior results in terms of weight loss and improvement of comorbidities as compared to other bariatric procedures[15-18]. Therefore this thesis mainly focuses on LRYGB for the surgical treatment of morbid obesity.

The procedure of LRYGB consists of several steps, as shown in Figure 1. Figure 1A shows the normal anatomy of the stomach, the small intestines and the colon until the hepatic flexure. The first step of the Roux-en-Y gastric bypass procedure is the creation of a gastric pouch of 25 cc[Figure 1B]. Subsequently, a biliopancreatic limb is measured 50 centimeters from Treitz and then attached to the gastric pouch creating the gastrojejunostomy [Figure 1C]. Afterwards, a side-to-side anastomosis with a 150 centimeters alimentary limb is created and a transection between both anastomoses of the jejunum is performed [Figure 1D]. By this, the Roux-en-Y gastric bypass is created [Figure 1E][19].

Figure 1. Laparoscopic Roux-en-Y gastric bypass procedure



Outline of this thesis

Part I of this thesis focuses on cardiovascular changes after LRYGB. Morbid obesity increases the risk on the development of hypertension and dyslipidemia[20 21]. The combination of hypertension and dyslipidemia increases the cardiovascular risk in morbidly obese individuals, leading to atherosclerosis, coronary artery disease, heart stroke, left ventricular hypertrophy and eventually heart failure[22 23]. A representative marker of atherosclerotic disease and the development of cardiovascular disease is the carotid intima media thickness (CIMT). The CIMT is measured by a non-invasive and reliable ultrasonic technique[24 25]. The CIMT is increased in morbidly obese patients, but several studies have shown that bariatric surgery may reduce the CIMT[26-28]. As those study populations were relatively small and measurements were only performed in specific study population groups, we have investigated whether this reduction the CIMT, and thus the improvement of the cardiovascular risk, is also seen after bariatric surgery in a large study population with different age groups. **Chapter 2** of this thesis describes the results of this prospective study using the ultrasonic CIMT measurements. Subsequently to the increase of the CIMT and cardiovascular risk, morbid obesity can lead to cardiovascular disease. This can result in changes of both cardiac structure and function, such as left ventricle hypertrophy and a decrease in cardiac function[29 30]. This obesity-induced decrease of cardiac function seems to improve after bariatric surgery[31 32]. However, these improvements have primarily been investigated in patients with symptomatic cardiomyopathy, while obese patients may have asymptomatic changes in cardiac function for several years. To our knowledge, cardiac function after bariatric surgery in patients without symptomatic cardiomyopathy has been poorly investigated. Therefore, **Chapter 3** focuses on changes in the cardiac function after Roux-en-Y gastric bypass in morbidly obese patients without cardiac history.

Part II investigates immunological changes after LRYGB. The white adipose tissue in obese individuals causes an increase of pro-inflammatory cytokine secretion, leading to a chronic, low-grade inflammation[33 34]. It is suggested that this results in accelerated ageing of the immune system, which is also called immunosenescence. Indeed, several studies have shown accelerated deterioration of the phenotype and function of the T cells, B cells and NK cells in obese subjects as compared with lean subjects[35-37]. The presence of metabolic syndrome in obese patients is a suggested risk factor for immunosenescence and gives an even more differentiated T cell compartment[38]. Most studies that have shown obesity-induced immunosenescence, have mainly focused on one specific immune cell type. Additionally, most study populations were relatively small. Therefore we have investigated changes in the cellular immune system in a larger study population with morbid obesity as compared with lean individuals, which is described in **Chapter 4**. Thus **Chapter 4** focuses on changes in the composition of the immune system. Morbid obesity also leads a change in immune cell function, with an increased secretion of pro-

inflammatory cytokines such as interleukins (IL)-6, tumor necrosis factor (TNF)-α and interferon (IFN)-γ and a decreased secretion of IL-10[35 39 40]. Studies have shown that the pro-inflammatory cytokine secretion decreases after substantial weight loss induced by bariatric surgery[41 42],however, results are contradicting[43]. Therefore, in **Chapter 5** we have investigated changes in T and B cell function before and after LRYGB in our study cohort, as compared with lean individuals.

The final part of this thesis, **Part III**, focuses on long-term results and complications after bariatric surgery. Although bariatric surgery has been proven to be an excellent short-term treatment for obesity, persisting weight loss and long-term remission of comorbidities are not guaranteed. For instance, the short term results after LAGB are good in terms of excess weight loss; however, there are high rates of failure after LAGB and weight regain occurs in up to 51% of patients who underwent LAGB[44-47]. Failure of the LAGB is described as either insufficient weight loss, weight regain, pouch dilation or band-related problems[48]. One of the surgical options after LAGB failure is a conversion to LRYGB[49], but weight loss results after revisional LRYGB for LAGB failure appear to be inferior to primary LRYGB[50 51]. **Chapter 6** of this thesis investigates the impact of initial response to LAGB on these inferior outcomes after revisional LRYGB. The occurrence of weight regain is not only described after LAGB, but is also seen in a small group of patients after LRYGB[52]. Weight regain after LRYGB can either be patient related (e.g. dietary non-compliance, physical inactivity or hormonal/metabolic factors) or surgery related (e.g. enlarged pouch, gastro-gastric fistulas or dilation of the gastrojejunostomy)[53]. Several techniques for the surgical treatment of weight regain after LRYGB due to either an enlarged pouch or dilation of the gastrojejunostomy have been described[54-57]. Even though revisional bariatric surgery is performed more often nowadays, there is no standard of care for a specific revisional technique yet. In **Chapter 7**, two techniques for resizing the gastric pouch are compared. The first technique is a sleeve resection of the gastrojejunostomy and gastric pouch, and the second technique is a resection of the gastrojejunostomy with reduction of the gastric pouch and creation of a new anastomosis. We investigated whether one of the two techniques is superior in the treatment of weight regain after LRYGB, in order to suggest a standard of care technique for revisional bariatric surgery. **Chapter 8** of this thesis focuses on internal herniation, one of the long-term complications after LRYGB. Internal herniation can occur through the mesenteric defect of Petersen's space (between the mesenteries of the transverse colon and the alimentary limb) and/or the mesenteries of the jejunojejunostomy[58]. An incidence rate up to 9.3% is described if the mesenteric defects are not closed during the LRYGB procedure[59 60]. Most patients with an internal herniation typically complain of postprandial, intermittent upper abdominal pain which is sometimes accompanied by nausea and vomiting[61], but some patients may complain of non-specific symptoms[62]. The treatment for internal herniation is closure of the mesenteric defects during an explorative laparoscopy[63]. However, the outcome of pain relief after the

closure of mesenteric defects seems to be unpredictable. Therefore we have investigated patient-related factors and intraoperative findings in patients with suspected internal herniation, in order to predict which patients may benefit from laparoscopic closure of the mesenteric defects. **Chapter 9** gives a summary of this thesis with conclusions, discussion and future perspectives.

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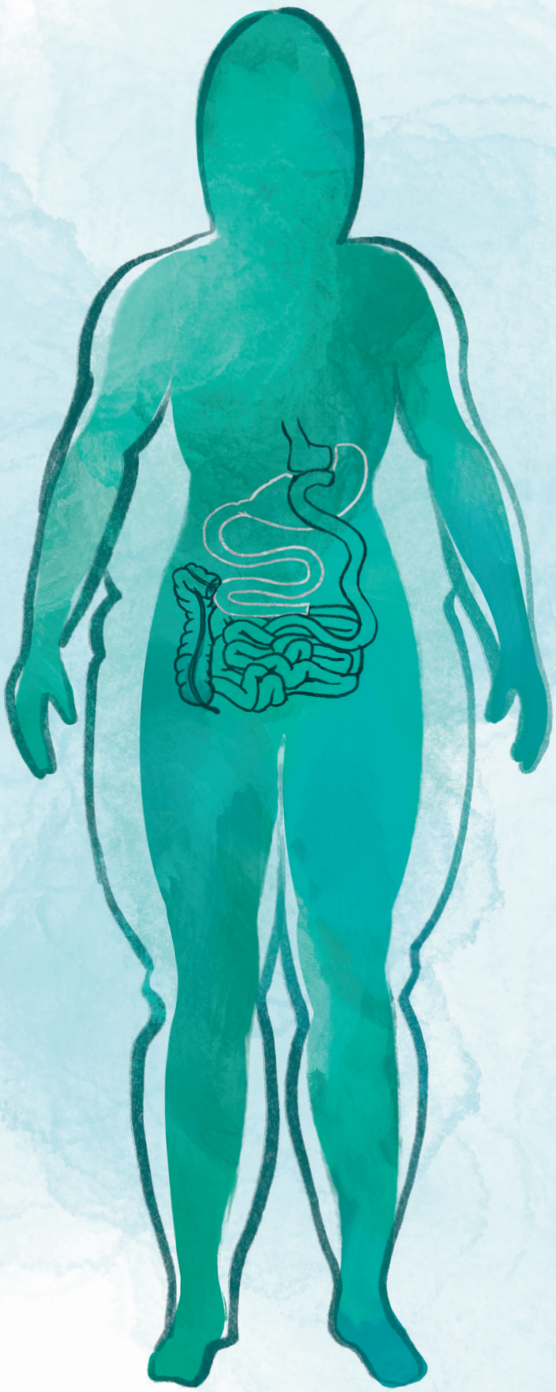
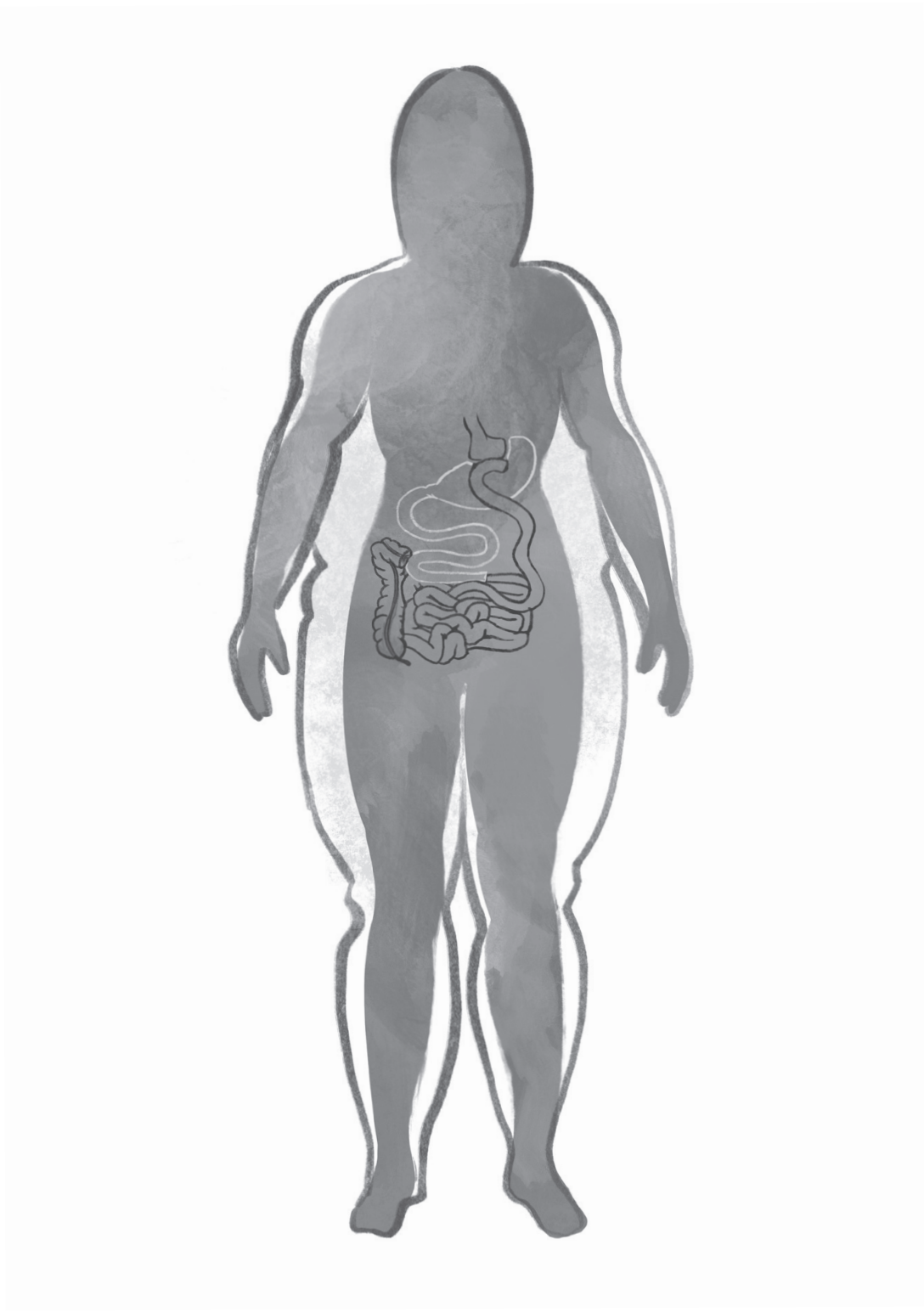


Part I

Cardiovascular changes after laparoscopic Roux-en-Y gastric bypass



Chapter 2

Age-related effects of bariatric surgery on early atherosclerosis and cardiovascular risk reduction

F.H.W. Jonker, V.A.A. van Houten*, L.H. Wijngaarden, R.A. Klaassen, A.A.E.A. de Smet, A. Niezen, L.J.D.M. Schelfhout, T.A. Bruning, E. van der Harst*

** Authors contributed equally*

Obesity Surgery Volume 28, Issue 4, April 2018, Pages 1040 – 1046

Abstract

Background

Carotid intima-media thickness (CIMT) is increasingly used as a prognostic indicator for early atherosclerosis and the development of cardiovascular disease. The objective of this study is to assess the exact effects of bariatric surgery on CIMT reduction in different age groups.

Methods

CIMT was measured just proximal to the bifurcation of the carotid artery in 166 patients with mean body mass index of 43.4 kg/m² before and at 6 and 12 months after bariatric surgery. Preoperative CIMT and Framingham Risk Score (FRS) were compared to measurements at 6 and 12 months postoperatively. Impact of age on CIMT change and cardiovascular risk reduction was analyzed.

Results

Median follow-up was 12 months; 12% were lost to follow-up. Mean CIMT values at 12 months after bariatric surgery were significantly lower compared to baseline (0.619mm vs. 0.587mm, p=0.005 in women and 0.675mm vs. 0.622mm, p=0.037 in men, respectively), and these effects were statistically significant in all age groups. The mean reduction of CIMT for patients <50 years at 12 months was 0.043mm (-7.0%), while CIMT was reduced with 0.013mm for patients ≥50 years (-1.9%, p=0.022). At 12 months after bariatric surgery, FRS had decreased with 52% in patients <50 years as compared with 35% in patients ≥50 years (p=0.025).

Conclusions

Bariatric surgery resulted in a significant CIMT decrease in patients with morbid obesity in all evaluated age categories. These beneficial effects of bariatric surgery were more pronounced in younger patients, while cardiovascular risk reduction by bariatric surgery appeared inferior in patients of 50 years and older.

Introduction

Obesity and its associated morbidities remain one of the greatest public health concerns worldwide[1]. Obesity has been solidly identified as an independent risk factor for the development of atherosclerosis, and is strongly related to cardiovascular morbidity and mortality[2]. Cardiovascular disease is currently the primary cause of death in the United States, and prevention and treatment of risk factors including obesity, are crucial for reducing cardiovascular mortality rates[3 4]. With longer follow-up, conservative treatment of morbid obesity, such as life style changes and pharmacological therapy, has been proven to be less effective than surgical intervention[5]. Bariatric surgery, including laparoscopic Roux-Y gastric bypass (RYGB), has been well established as a successful approach to reduce morbid obesity and its associated cardiovascular morbidity and mortality[6-8].

The carotid intima-media thickness (CIMT) can be used as a representative marker of early atherosclerosis and for the development of cardiovascular disease. Ultrasonic CIMT measurement is a validated non-invasive technique, which has been proven to be reliable and inexpensive[9-12]. Currently, reversibility of early atherosclerosis by clinical interventions remains a matter of scientific debate. Bariatric surgery may result in decreased CIMT, as suggested in small case-series[13-19]. However, it is unclear if such early atherosclerotic changes occur in all patients after successful reduction of excess weight loss. The objective of the present study is to assess the exact effects of bariatric surgery on CIMT in different age groups, in order to determine to which extent premature atherosclerosis is reversible in the individual obese patient after bariatric surgery.

Materials and methods

Patients eligible for a primary bariatric procedure according to the International Federation for the Surgery of Obesity and Metabolic Disorders (IFSO) guidelines[20], were prospectively enrolled at the Maasstad Hospital Rotterdam, the Netherlands, between May 2012 and January 2014. Two experienced bariatric surgeons performed all bariatric procedures, which included both laparoscopic RYGB (n=157) and laparoscopic gastric sleeve (n=7).

Patients underwent ultrasonic CIMT measurements prior to bariatric surgery (baseline) and at 6 and 12 months after surgery. CIMT measurements were performed with patients lying in supine position, while connected to a 3-leads ECG. The intima-media thickness of the near and far wall of the common carotid artery just proximal of the carotid bulb was measured during diastole by two trained physicians, using CIMT software on the Aloka Prosound α6 (Hitachi, Tokyo, Japan). CIMT was measured in the common carotid artery and near the carotid bifurcation as well, but only CIMT measurements proximal

of the carotid bulb were used for analysis, in accordance with previous reports[9-12]. Two independent physicians obtained CIMT measurements in order to ensure reproducibility. Intraclass correlation coefficients for intra- and inter-observer variability were 0.768 and 0.829 for CIMT (just proximal of the bulb).

In addition, weight, length, body mass index (BMI), abdominal and hip circumference, abdominal visceral fat were measured, and laboratory tests including fasting glucose, (HDL/LDL) cholesterol and triglycerides values were determined during follow-up. The Human Ethics Committee of the Maasstad Hospital in Rotterdam approved this study; all patients provided written informed consent to participate.

The mean CIMT measurements based on the left and right CIMT were used for statistical analysis. The paired-sample Student's t-test was used to compare the mean CIMT between baseline and at 6 and 12 months follow-up, separately. Repeated measures ANOVA and post hoc pairwise comparisons with Bonferroni adjustment was used to compare differences over time. Univariate and multivariate linear regression analyses were used to investigate interaction of CIMT and covariates. A two-sided P-value smaller or equal to 0.05 was considered as statistically significant. All analyses were performed using SPSS version 20 for Windows (SPSS, Chicago, IL, USA).

Results

Baseline characteristics

A total of 166 patients with morbid obesity, including 38 men (16.9%) and 128 women (83.1%) with a mean age of 42.5 (19.4 – 62.1) years were enrolled. Men were significantly taller and heavier compared to women, but no differences in BMI were observed at baseline (table 1). Eighty-five of our 166 patients (51.2%) met the criteria for metabolic syndrome, and 22 patients 13.3% were using lipid-lowering medication.

No significant differences between right and left carotid artery CIMT were observed at baseline; mean values of both measurements were used for further analysis. Mean CIMT just proximal to the bifurcation of the carotid artery at baseline was 0.675 ± 0.10 mm in men and 0.619 ± 0.11 mm in women ($p = 0.009$). Patients who met the criteria for metabolic syndrome had a significant higher CIMT at baseline than patients who did not meet the criteria (0.649± 0.12 mm vs. 0.614± 0.10 mm, $p < 0.05$).

Table 1. Baseline characteristics

	Total (n = 166)	**Women (n = 128)**	**Men (n = 38)**
Age (y)	42.5 (19.4 – 62.1)	41.9 (20.1 – 62.1)	44.4 (19.4 – 61.4)
Weight (kg)	125.2 ± 19.1	120.3 ± 16.8	141.8 ± 17.0*
Length (cm)	169.6 ± 8.9	166.5 ± 6.9	180.1 ± 5.9*
BMI (kg/m2)	43.4 ± 4.8	43.3 ± 4.7	43.7 ± 5.1
Abdominal circumference (cm)	129.7 ± 14.0	127.3 ± 13.9	138.0 ± 11.2*
Hip circumference (cm)	128.3 ± 10.5	129.2 ± 10.7	125.3 ± 9.2*
Systolic BP (mmHg)	134 ± 15	132 ± 15	139 ± 14*
Diastolic BP (mmHg)	79 ± 10	78 ± 10	82 ± 9*
Abdominal fat diameter (cm)	8.3 ± 2.2	7.8 ± 1.9	10.2 ± 2.4*
CIMT (mm)	0.632 ± 0.11	0.619 ± 0.11	0.675 ± 0.10*
Comorbidity			
Hypertension	63 (38)	46 (35.9)	17 (44.7)
Diabetes	27 (16.3)	20 (15.6)	7 (18.4)
Hypercholesterolemia	25 (15.1)	17 (13.3)	8 (21.1)
OSAS	22 (13.3)	8 (6.3)	14 (36.8)
Osteoarthritis	40 (24.1)	31 (24.2)	9 (23.7)
COPD	16 (9.6)	14 (10.9)	2 (5.3)
Smoking			
Current	6 (3.6)	4 (3.1)	2 (5.3)
Past	79 (47.6)	59 (46.1)	20 (52.6)
BP lowering medication	57 (34.3)	44 (34.3)	13 (34.2)
Lipid lowering medication	22 (13.3)	16 (12.5)	6 (15.8)
Glucose lowering medication	27 (16.3)	20 (15.6)	7 (18.4)
Bariatric procedure			
Roux-Y Gastric Bypass	157 (94.6)	121 (94.5)	36 (94.7)
Sleeve Gastroplasty	9 (5.4)	7 (5.5)	2 (5.3)

BMI = body mass index; BP = blood pressure; CIMT = carotid intima-media thickness

* Statistically significant difference

Anthropometric and CIMT measurements during follow-up

Median follow-up was 12 months (range 1 to 14 months). Twenty patients (12%) were lost to follow-up at 12 months, mainly because patients declined follow-up examinations (n=16), because of pregnancy (n=2), death due to cancer (n=1), and one patient was excluded for further analysis because of pre-existent occlusion of the right common carotid artery.

Mean weight loss in female patients at 12 months was 36.8 kg, corresponding with a 75.2% Excess Weight Loss (EWL), as compared to a mean weight loss of 39.2 kg and a

66.3% EWL in men. All other anthropometric measurements including abdominal and hip circumference, abdominal fat diameter and blood pressure, significantly decreased during follow-up in both female as male patients (table 2). The Framingham Risk Score (FRS) decreased significantly by 43% in female patients and 37% in male patients (table 2).

From baseline to 6 months postoperatively, CIMT did not change significantly. At 12 months after bariatric surgery, a significant decrease in mean CIMT of 0.032 mm in female patients (95% CI, 0.008 to 0.056 mm, p=0.005) and 0.052 mm in male patients (95% CI, 0.003 mm to 0.102 mm, p=0.037) was observed (table 2).

Table 2. Anthropometric and CIMT measurements during follow-up after bariatric surgery

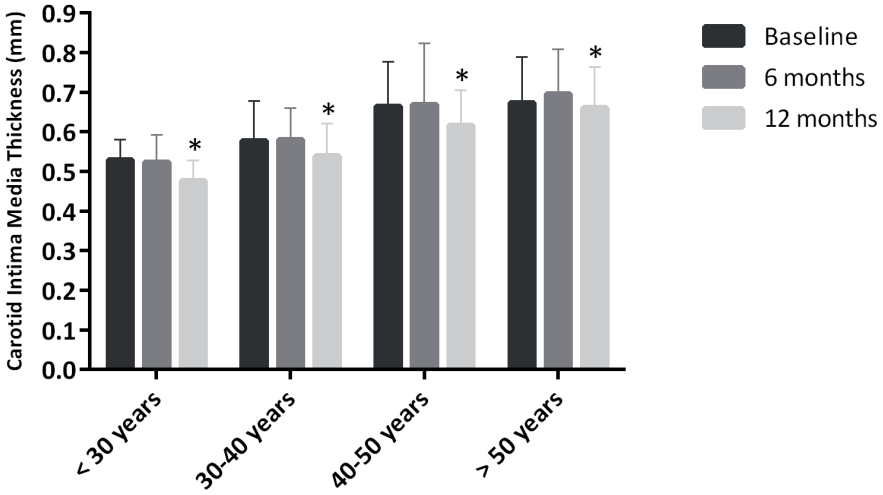
	Baseline (n = 128)	**6 months (n = 116)**	**12 months (n = 111)**	**Change in 12 months (%)**	**P value**
Women					
Weight (kg)	120.3 ± 16.8	91.3 ± 15.8	83.5 ± 16.0	-36.8 (31)	< 0.001
BMI (kg/m²)	43.3 ± 4.7	33.0 ± 4.9	30.1 ± 5.1	-13.2 (30)	< 0.001
EWL (%)		59.4 ± 17.4	75.2 ± 21.7		-
Abdominal circumference (cm)	127.3 ± 13.9	103.5 ± 13.8	97.6 ± 14.5	-29.7 (23)	< 0.001
Hip circumference (cm)	129.2 ± 10.7	112.0 ± 11.4	107.8 ± 11.6	-21.4 (17)	< 0.001
Abdominal fat diameter (cm)	7.8 ± 1.9	4.6 ± 1.9	4.0 ± 1.7	-3.8 (49)	< 0.001
Systolic BP (mmHg)	132 ± 15	120 ± 12	118 ± 13	-14 (11)	< 0.001
Diastolic BP (mmHg)	78 ± 10	74 ± 9	72 ± 10	-6 (8)	< 0.001
Framingham Risk Score	9.14 ± 11.0	-	5.16 ± 7.7	3.9 (43)	<0.001
CIMT (mm)	0.619 ± 0.11	0.622 ± 0.11	0.587 ± 0.10	-0.03 (5)	0.005
Men					
Weight (kg)	141.8 ± 17.0	109.2 ± 14.4	102.6 ± 12.8	-39.2 (28)	<0.001
BMI (kg/m²)	43.7 ± 5.1	33.5 ± 4.4	31.6 ± 4.2	-12.1 (28)	<0.001
EWL (%)		56.0 ± 13.0	66.3 ± 14.3		-
Abdominal circumference (cm)	138.7 ± 11.2	113.3 ± 12.9	106.4 ± 10.1	-32.3 (23)	<0.001
Hip circumference (cm)	125.3 ± 9.2	109.7 ± 9.7	107.7 ± 9.3	-17.6 (14)	<0.001
Abdominal fat diameter (cm)	10.2 ± 2.4	5.6 ± 2.0	5.1 ± 1.8	-5.1 (50)	<0.001
Systolic BP (mmHg)	139 ± 14	124 ± 15	126 ± 9	-13 (9)	<0.001
Diastolic BP (mmHg)	82 ± 9	73 ± 9	74 ± 8	-8 (9)	<0.001
Framingham Risk Score	15.17 ± 13.2	-	9.53 ± 9.1	5.6 (37)	<0.001
CIMT (mm)	0.675 ± 0.10	0.679± 0.12	0.622 ± 0.11	-0.05 (8)	0.037

BMI = body mass index; EWL = excess weight loss; BP = blood pressure; CIMT = carotid intima-media thickness

CIMT had significantly decreased at 12 months in all age categories, in both female (figure 1) as male patients (figure 2). Mean CIMT decreased most dramatically in patients younger than 30 years; women < 30 years: -0.053 mm (-10.0%); men < 30 years: -0.083 mm (-13.5%). In patients older than 50 years, the smallest change in CIMT was observed; women > 50 years: -0.013 mm (-1.9%); men > 50 years: -0.014 mm (-1.9%).

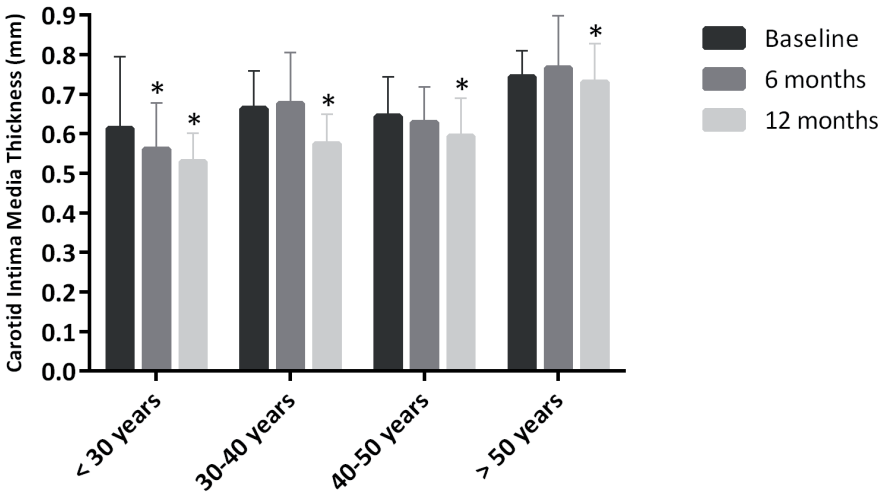
Linear regression analysis could not identify other variables that significantly affected the decrease in CIMT, although patients with at least ten pack years of smoking tended to exhibit less decrease in CIMT (-0.006 mm vs. -0.042 mm, p=0.066).

Figure 1. Age-related effects of bariatric surgery on CIMT in women



** Statistically significant difference*

Figure 2. Age-related effects of bariatric surgery on CIMT in men



***** *Statistically significant difference*

Improvement of cardiovascular risk in patients < and ≥ 50 years

The mean FRS at baseline was 6.9 for patients younger than 50 years, as compared to 21.7 for patients ≥ 50 years (p<0.001). At 12 months after bariatric surgery, the FRS had decreased with 52.4% in patients < 50 years as compared with 34.9% in patients ≥ 50 years (p=0.025). Abdominal fat diameter decreased with 3.96 cm in patients < 50 years, as compared to 5.01 cm in patients ≥ 50 years (p=0.048). Overall, the mean reduction of CIMT for patients younger than 50 years was -0.043mm (-7.0%), while for patients ≥ 50 years the change in CIMT was -0.013 mm (-1.9%, p=0.022).

Table 3. Change of anthropometric measurements and cardiovascular risk for patients < and ≥ 50 years at 12 months after bariatric surgery

	Change in patients < 50 years	**Change in patients ≥ 50 years**	**P value**
Weight (kg)	-49.4 ± 30.3	-43.1 ± 30.3	0.254
BMI (kg/m²)	-16.9 ± 10.2	-15.7 ± 10.7	0.527
EWL (%)	74.4 ± 20.0	69.5 ± 21.8	0.214
Abdominal circumference (cm)	-43.0 ± 34.4	-39.3 ± 31.9	0.542
Hip circumference (cm)	-35.5 ± 37.7	-29.9 ± 34.5	0.402
Abdominal fat diameter (cm)	-3.96 ± 3.0	-5.01 ± 2.6	0.048
Systolic BP (mmHg)	-13.7 ± 12.6	-14.6 ± 20.9	0.760
Diastolic BP (mmHg)	-7.2 ± 9.7	-4.9 ± 12.8	0.268
Framingham Risk Score (%)	-52.4 ± 35.8	-34.9 ± 31.9	0.025
CIMT (mm)	-0.043 ±0.02	-0.013 ±0.01	0.022

BMI = body mass index; EWL = excess weight loss; BP = blood pressure; CIMT = carotid intima-media thickness

Discussion

In the present study, CIMT and FRS decreased significantly in both men and women after bariatric surgery, in all age categories. The effects of bariatric surgery on CIMT were more pronounced in younger patients, which may suggest that the reversibility of atherosclerosis and cardiovascular risk reduction may diminish with ageing of patients.

The Swedish Obese Subjects study has demonstrated that bariatric surgery results in a significant decrease in cardiovascular mortality[7 8]. A recent meta-analysis including 14 studies with 29208 surgical and 166200 non-surgical controls with a follow-up of up to 15 years showed a 50% risk reduction on cardiovascular events (myocardial infarction and stroke, and composite adverse CVD events) after bariatric surgery[21].

CIMT is an established marker for the development of cardiovascular disease and related events[9-12], and previous reports have shown that CIMT decreases after bariatric surgery[13-19]. However, these studies were not able to perform subgroup analyses because of limited patient numbers. Therefore, it remained unclear whether risks of atherosclerosis and associated cardiovascular morbidity could be reduced in all patients after bariatric surgery, or whether some subgroups might benefit more from this surgical intervention than others.

Overall, CIMT had decreased with approximately 0.04 mm at 12 months after surgery, which corresponds with a reduction of 5% in female and 8% in male patients. This is a dramatic improvement of the arterial wall composition, and this impact of bariatric surgery appears superior as compared to medical treatment with statins for 2 years, which results in a mean CIMT reduction of 0.029 mm[22]. Reduction of CIMT after bariatric surgery did not occur directly, since there was even a slight increase in CIMT at 6 months after the intervention, followed by a significant decrease at 12 months. We do not have an explanation for this delayed improvement of the arterial wall. Interestingly, while elderly patients did lose more abdominal fat, bariatric surgery in patients of 50 years and older resulted in a less pronounced decrease in CIMT as compared to patients younger than 50 years. In addition, the FRS, a recognized tool to predict coronary heart disease[23], had decreased with 52.4% in patients younger than 50 years, whereas in patients ≥ 50 years, this decrease was "only" 34.9% at 12 months after surgery. Notably, FRS at baseline was significantly higher in older patients, so the decreased reduction in FRS may still be clinically relevant. The inferior improvements of these cardiovascular risk factors may suggest that long-lasting exposure of the arterial wall to lipids and ongoing "athero-inflammation" in older obese patients, leads to less reversible atherosclerotic plaque changes. Although reduction of excess weight loss in bariatric patients above 50 years does lead to a significant decrease in CIMT and FRS, younger patients showed more dramatic improvement of the arterial wall and associated cardiovascular risk. Early atherosclerosis may therefore still be reversible, particularly in younger obese patients undergoing bariatric surgery, however, with ageing this reversibility seems to diminish.

While cardiovascular benefits after reduction of excess weight loss appear superior in young obese patients after surgery, risks of bariatric surgery generally increase with age[24 25]. In an analysis of 20,308 laparoscopic bariatric procedures, Sanni and colleagues found that odds of postoperative complications increase by 2% with each additional year of age[24]. In addition, younger patients appear to experience a significantly greater and prolonged BMI decrease during follow-up after bariatric surgery[26]. Although bariatric surgery can still be safely performed in older patients[27], these results may suggest that surgical indications in obese patients older than 50 years should be carefully weighed.

Linear regression analysis could not identify other patient characteristics than age that significantly affected the decrease in CIMT, although patients with at least ten pack years of smoking tended to exhibit less decrease in CIMT. Prolonged smoking may results in irreversible atherosclerotic changes of the arterial wall, which may not be affected by significant weight loss induced by bariatric surgery.

Since the objective of the present study was to assess age-related effects of bariatric surgery on CIMT, median follow-up of the cohort was only 1 year, so we could not analyze actual cardiovascular outcomes in different age groups. Our cohort of 166 obese patients was probably too small as well for such analyses. Nevertheless, the current cohort represents the largest evaluation of CIMT after bariatric surgery ever described and therefore the best available evidence regarding the impact of weight loss on the reversibility of atherosclerosis. The study was adequately powered and subgroup analyses for different age categories were performed, in contrast to previous studies describing the CIMT only in smaller groups of predominantly female patients with obesity, not stratified by age or gender[13-19]. In addition, another strength of the present study was the intraclass correlation coefficients of 0.768 and 0.829 for intra- and inter-observer variability, suggesting high reproducibility of the results.

Conclusion

Bariatric surgery resulted in a significant decrease in CIMT and FRS in patients with morbid obesity in all evaluated age categories. The effects of bariatric surgery on CIMT were more pronounced in young patients, which may suggest that the reversibility of atherosclerosis may diminish with ageing of patients. Cardiovascular risk reduction by bariatric surgery appears inferior in patients of 50 years and older.

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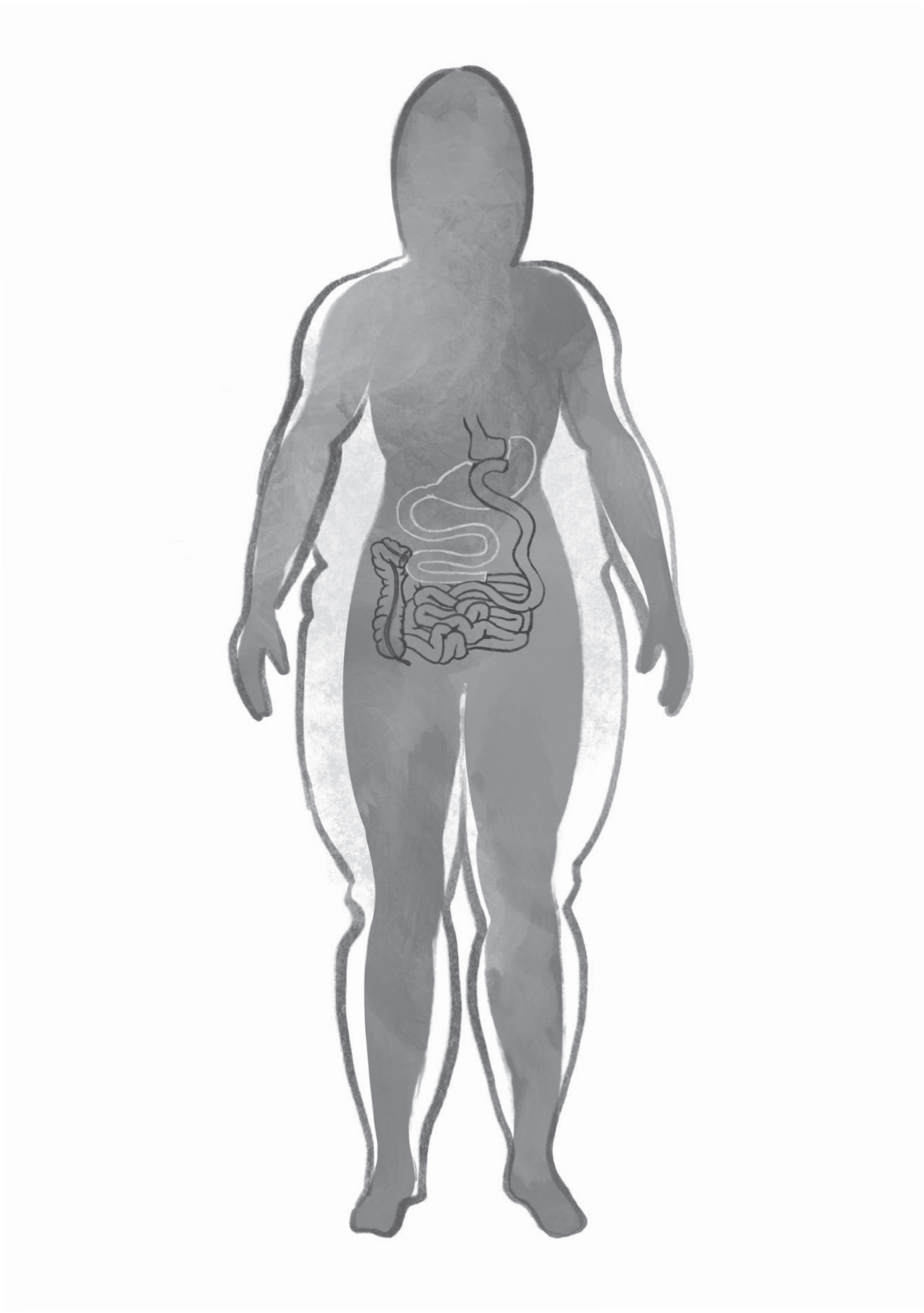

Chapter 3

Improvement of cardiac function after Roux-en-Y gastric bypass in morbidly obese patients without cardiac history measured by cardiac MRI

D. de Witte, L.H. Wijngaarden, V.A.A. van Houten, M.A. van den Dorpel, T.A. Bruning, E. van der Harst, R.A. Klaassen, R.A. Niezen

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Abstract

Purpose

Metabolic syndrome in patients with morbid obesity causes a higher cardiovascular morbidity, eventually leading to left ventricular hypertrophy and decreased left ventricular ejection fraction (LVEF). Roux-en-Y gastric bypass (RYGB) is considered the gold standard modality for treatment of morbid obesity and might even lead to improved cardiac function. Our objective is to investigate whether cardiac function in patients with morbid obesity improves after RYGB.

Materials and methods

In this single center pilot study, 15 patients with an uneventful cardiac history who underwent RYGB were included from May 2015 – March 2016. Cardiac function was measured by Cardiac Magnetic Resonance Imaging (CMRI), performed preoperatively and 3, 6 and 12 months postoperative. LVEF and myocardial mass and cardiac output were measured.

Results

A total of 13 patients without decreased LVEF preoperative completed follow-up (mean age 48.0 ±8.8). There was a significant decrease of cardiac output 12 months postoperative (8.3 ±1.8 preoperative vs 6.8 ±1.8 after 12 months, $P = 0.001$). Average myocardial mass declined by 15.2% ($P < 0.001$). After correction for body surface area (BSA), this appeared to be non-significant ($P = 0.36$). There was a significant improvement of LVEF/BSA at 6 and 12 months postoperative (26.2 ± 4.1 preoperative vs 28.4 ±3.4 and 29.2 ±3.6 respectively, both $P = 0.002$). Additionally, there was a significant improvement of stroke volume/BSA 12 months after surgery (45.8 ±8.0 versus 51.9 ±10.7, P = 0.033).

Conclusion

RYGB in patients with morbid obesity with uneventful history of cardiac disease leads to improvement of cardiac function.

Background

Morbid obesity is characterized by multiple pathophysiological processes leading to changes in metabolism and eventually functional impairment [1]. One of the obesity-related comorbidities is cardiac morbidity, including left ventricular hypertrophy (LVH) as well as diminished left ventricular ejection fraction (LVEF) [2]. Bariatric surgery is a well-established and effective treatment for morbid obesity, including improvement of obesity-related comorbidities such as hypertension, dyslipidemia and type 2 diabetes mellitus [3]. Several studies have been performed to analyze changes in cardiac function in patients with preoperative cardiomyopathy, showing improvement in cardiac function after Roux-en-Y gastric bypass (RYGB) [4-6]. As measured by cardiac ultrasound (CUS), it has been stated that cardiac function may also benefit from bariatric surgery in patients without cardiac history, eventually resulting in improved left ventricular function (LVF) and diminished left ventricle mass (LVM) and diameter. This potentially leads to a decrease of LVH and an increase of LVEF [4 7-9]. The decrease in body mass index (BMI) after bariatric surgery seems to be correlated with the decrease in LVM [2]. Cardiac magnetic resonance imaging (CMRI) is less seriously influenced by subcutaneous fat than CUS, and can determine functional parameters, such as dimensions of the left ventricle (LV), LVM and LVEF [7 10-13]. Therefore, the gold standard for measurement of cardiac function in patients with morbid obesity should be CMRI. Previous studies to assess changes in cardiac function in patients without a history of cardiac disease have been performed using CUS, and therefore we performed a pilot study to investigate whether cardiac function in patients with morbid obesity with uneventful cardiac history improves after RYGB as measured by CMRI [14-16].

Methods

Study population

A total of 15 patients who underwent RYGB at the Maasstad Hospital in Rotterdam from September 2015 to May 2016 were included in this study. CMRI could not be performed in two patients as they were claustrophobic. Therefore, the data of these patients were excluded from this study.

Surgical procedure

All procedures were performed by experienced bariatric surgeons. First, a gastric pouch of 25 cc was created. A 50-cm biliopancreatic limb was measured and the gastrojejunostomy was created using an endostapler and a continuous, absorbable suture. A side-to-side jejunojejunostomy was created using an endostapler and a continuous, absorbable suture, with an alimentary limb of 150 cm. Afterwards, a transection between both anastomoses of the jejunum was performed.

Postoperative care

Postoperative care was performed by our standard postoperative care protocol. In this protocol, all patients were seen at the outpatient clinic 2 weeks, 3, 6, 9 and 12 months postoperative. All patients were counseled by a dietician, consisting of two group sessions and four individual consultations in the first year postoperative. All patients were advised to consume a calorie-restricted, high-protein diet consisting of approximately 1000 calories per day and 60-80 grams of protein per day. All patients were advised to do moderate-intensity physical activities for at least thirty minutes per day. In addition, patients were advised to exercise for one hour at least twice a week.

Cardiac magnetic resonance imaging

Imaging was performed with a 1.5 Tesla Siemens Somatom Definition scanner (Siemens AG, Erlangen, Germany). Short axis multislice cine TRUE FISP series of the heart were obtained for LV function analysis. In addition, post contrast series using gadolinium contrast agent (Bayer AG, Leverkusen, Germany) were obtained for the detection of late enhancement, a parameter to objectify ischemic changes of the myocardium.

CMRI data analysis

CMRI data analysis was performed using Siemens syngo.via versions 10 and 11 (Siemens AG, Erlangen, Germany). By drawing the endocardial and epicardial contours of the myocardium a 3D model was obtained. Via this model the LVEF, end diastolic volume (EDV), end systolic volume (ESV), stroke volume (SV), cardiac index (CI), myocardial mass (MM; at end diastolic phase), peak ejection rate (PER) and peak filling rate (PFR) were calculated.

In addition to functional CMRI studies, we obtained blood samples to evaluate the effects of RYGB on the metabolic syndrome in these patients, such as kidney function, liver function and lipid spectrum. We also determined leptin and ghrelin and the cardiac NT-pro BNP and vWF antigen.

Statistical analysis

Body surface area (BSA) was measured using the Du Bois formula [17]:

$$BSA = 0.007184 \times Weight\ (in\ kg)^{0.425} \times Height\ (in\ cm)^{0.725}$$

Statistical analysis was performed with IBM SPSS Statistics, version 23 (SPSS, Chicago, IL). Continuous data are presented as the mean ± standard deviation. Percentage excess weight loss (%EWL) was measured with the ideal weight defined by the weight corresponding to a BMI of 25 kg/m². Analysis of repeated measures was performed using linear mixed models. A P value < 0.05 was considered significant. Missing data were addressed with pairwise deletion of missing data.

Results

Clinical characteristics

A total of 13 patients with a mean age of 48.0 ± 8.8 years were included in this study, of whom 8 (61.5%) were female. There was a significant increase of %EWL of 46.4%, 67.8% and 84.5% % at 3, 6 and 12 months after surgery respectively ($P < 0.001$). As a result, BSA decreased significantly after 3, 6 and 12 months, from 2.3 m² pre-operative to 2.0 m² after 12 months. Heart rate and systolic blood pressure decreased significantly after 6 and 12 months ($P < 0.001$), Table 1.

Table 1. Clinical characteristics

Variable	Preoperative	Postoperative (months)		
		3	6	12
Pulse (/min)	83.8 ± 16.2	72.3 ± 12.8	67.5 ± 9.5*	66.3 ± 12.2*
Systolic BP	143.3 ± 22.6	126.3 ± 10.5*	127.2 ± 11.9*	133.1 ± 22.7*
Diastolic BP	86.6 ± 14.3	87.9 ± 13.2	88.9 ± 10.1	89.4 ± 14.8
BMI (kg/m²)	40.1 ± 2.1	33.2 ± 2.6*	30.0 ± 2.7*	27.5 ± 3.8*
BSA	2.3 ± 0.2	2.1 ± 0.2*	2.1 ± 0.1*	2.0 ± 0.2*
%TWL		17.0 ± 4.2	25.1 ± 5.5†	31.2 ± 8.1
%EWL		46.4 ± 14.0	67.8 ± 17.2†	84.5 ± 23.2†

BP = blood pressure; BMI = body mass index; BSA = body surface area; %TWL = percentage total weight loss; %EWL = percentage excess weight loss

* Significant ($P < 0.05$) versus preoperatively

† Significant as compared to %TWL or %EWL after 3 months using the paired Student T-test

Changes in cardiac function

Cardiac output declined significantly 12 months after bariatric surgery (8.3 ± 1.8 versus 6.8 ± 1.8, $P = 0.001$). Heart rate declined significantly at 6 and 12 months after bariatric surgery (67.5 ± 9.5 and 66.3 ±12.2, $P < 0.05$). The average MM declined by 15.2% ($P < 0.001$), Table 2. However, after correction for changes in BSA, no significant decline was seen 12 months postoperative ($P = 0.36$).

LVEF declined significantly only at 3 months postoperative (56.6 ± 6.6, $P < 0.05$). However, after correction for BSA there was a significant increase in LVEF/BSA ratio 6 and 12 months postoperative (both, $P = 0.002$), Figure 1. SV did nog change significantly. Additionally, there was a significant increase in SV/BSA ratio after 12 months follow-up (45.8 ± 8.0 versus 51.9 ± 10.7, $P = 0.033$). These results did not change after exclusion of one female patient in whom a right bundle branch block was found by coincidence. No patients had delayed myocardial enhancement. Detected by CMRI, all patients had

hepatic steatosis preoperative, which completely disappeared 3 to 6 months postoperative in all study subjects.

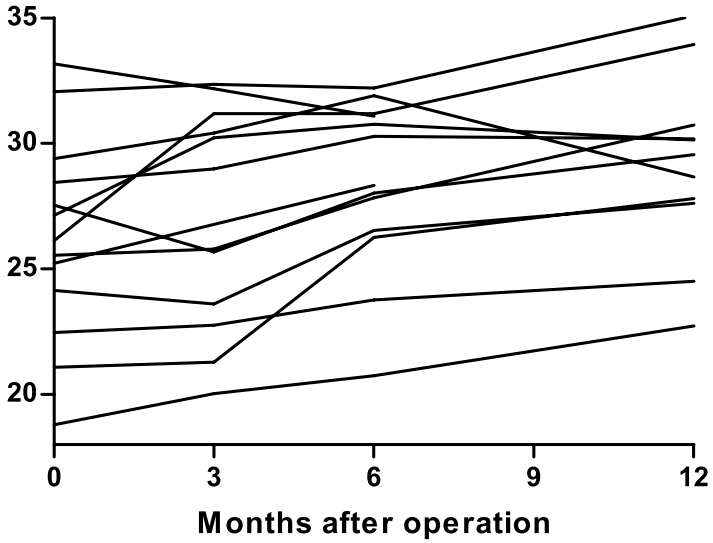
Table 2. Cardiac function based on magnetic resonance imaging

Variable	Preoperative	Postoperative (months)		
		3	6	12
MRI				
LVEF (%)	60.2 ± 6.9	56.6 ± 6.6*	58.3 ± 6.1	58.0 ± 6.3
ED volume (ml)	177.6 ± 35.0	176.8 ± 44.9	183.5 ± 46.3	180.1 ± 43.6
ES volume (ml)	71.3 ± 20.1	77.2 ± 24.0	76.8 ± 22.5	71.3 ± 20.1
Stroke volume (ml)	106.3 ± 22.2	99.5 ± 25.9	106.7 ± 28.1	104.8 ± 29.7
Cardiac output (l/min)	8.3 ± 1.8	6.7 ± 1.3*	6.6 ± 1.4*	6.8 ± 1.8*
Myocardial mass (ED, in g)	127.4 ± 35.5	113.2 ± 33.3*	111.7 ± 31.1*	111.8 ± 34.0*
Myocardial mass (Average, in g)	140.6 ± 35.7	121.2 ± 34.3*	115.8 ± 30.6*	119.2 ± 36.0*
Peak ejection rate (ml/s)	-505.7 ± 135.3	-479.5 ± 93.7	-471.0 ± 92.6	-471.3 ± 116.2*
Peak ejection time (ms)	113.5 ± 20.8	125.3 ± 34.9	131.9 ± 26.0*	146.6 ± 28.3*
Peak filling rate (ml/s)	501.5 ± 78.8	447.0 ± 116.6	438.3 ± 143.7*	443.8 ± 113.5*
Peak filling time (ms)	546.5 ± 101.5	660.4 ± 198.9	527.0 ± 209.2	622.0 ± 180.1
MRI / BSA				
LVEF/BSA (%/m²)	26.2 ± 4.1	27.0 ± 4.4	28.4 ± 3.4*	29.2 ± 3.6*
ED volume/BSA (ml/m²)	76.5 ± 12.0	83.0 ± 16.5*	88.4 ± 18.0*	89.3 ± 15.2*
ES volume/BSA (ml/m²)	30.7 ± 8.0	36.1 ± 9.1*	37.1 ± 9.6*	37.4 ± 7.6*
Stroke volume/BSA (ml/m²)	45.8 ± 8.0	46.9 ± 10.5	51.4 ± 11.1*	51.9 ± 10.7*
Cardiac index (ml/min/m²)	3.6 ± 0.6	3.1 ± 0.5*	3.2 ± 0.6*	3.4 ± 0.7
Myocardial mass/BSA (ED, in g/m²)	54.4 ± 11.1	52.9 ± 12.1	53.7 ± 12.0	55.3 ± 13.5
Myocardial mass/BSA (Average, in g/m²)	60.1 ± 10.6	56.7 ± 12.2*	55.7 ± 11.4*	58.7 ± 13.5
Peak ejection rate/BSA (ml/s/m²)	-216.4 ± 43.1	-226.0 ± 36.1	-227.5 ± 35.6	-233.7 ± 40.8*
Peak filling rate/BSA (ml/s/m²)	216.7 ± 26.9	211.6 ± 54.6	211.3 ± 65.1	220.2 ± 41.9
Additional findings				
Late enhancement (n)	0	0	0	0
Hepatic steatosis	13	2	0	0

MRI = magnetic resonance imaging; LVEF = left ventricular ejection fraction; ED = end diastolic; ES = end systolic; BSA = body surface area

* Significant (P<0.05) versus preoperative

Figure 1. Changes in left ventricular ejection fraction / body surface area ratio



LVEF = left ventricular ejection fraction; BSA = body surface area

Correlation of blood test results and cardiac function

Even though there is a significant decrease of LDL and triglycerides and significant increase of HDL at 12 months after surgery (Table 3), there was no correlation between the improvement of the lipid spectrum and the increase of the LVEF/BSA ratio ($P = 0.105$, $P = 0.127$, $P = 0.197$ and $P = 0.767$ respectively). Additionally, the significant decrease of leptin levels does not seem to influence the LVEF/BSA ratio ($P = 0.072$).

Table 3. Blood test results

Variable	Preoperative	Postoperative (months)		
		3	6	12
Lipid spectrum				
Cholesterol	5.2 ± 1.2	4.6 ± 1.1*	4.7 ± 0.9	4.7 ± 0.9
HDL	1.2 ± 0.3	1.2 ± 0.4	1.3 ± 0.4	1.4 ± 0.5*
LDL	3.2 ± 0.8	2.6 ± 1.0*	2.6 ± 0.9*	2.5 ± 0.8*
Triglycerides	2.6 ± 1.4	1.7 ± 0.7*	1.8 ± 1.0*	1.8 ± 1.4*
Metabolic biomarkers				
Leptin	90.6 ± 44.5	33.9 ± 22.0*	26.6 ± 15.0*	18.9 ± 7.7*
Ghrelin	650.5 ± 146.1	691.7 ± 124.3	725.4 ± 260.2	675.6 ± 165.8

HDL = high-density lipoprotein; LDL = low-density lipoprotein

* Significant ($P<0.05$) versus preoperative

Discussion

This is the first CMRI study to assess cardiac changes after RYGB performed in patients with an uneventful cardiac history. We found a significant increase in LVEF, even after correction for BSA (LVEF/BSA) after RYGB. Additionally, we found a significant decrease in non-corrected cardiac output and absolute LV mass 12 months postoperatively. SV/BSA significantly improved after 12 months and none of the patients showed signs of myocardial ischemia.

Due to the necessary increase of cardiac output, needed for enhanced blood supply to the excess peripheral tissue, obesity is associated with a chronic higher cardiac workload as compared to healthy individuals. This will eventually lead to LVH as described by multiple studies [2 18 19]. One of these studies was obtained in a group of patients with a BMI >50, in contrast to our test group with a median BMI of 40.1 preoperatively [20]. As the blood supply to the peripheral tissue decreases after significant weight loss, it is expected that the cardiac workload will change and therefore the cardiac function will improve after bariatric surgery. In our study, there was no significant change in non-BSA-corrected LVEF after bariatric surgery. Two other studies have found comparable results [21 22]. It could be possible that in the presence of depressed wall mechanics, ejection fraction is sustained by increased concentricity of LV geometry. A simultaneous reduction of concentricity with improvement in mid wall mechanics is expected to leave ejection fraction unchanged. Nevertheless, due to the significant decrease in BSA, a significant change in LVEF/BSA was seen after 6 and 12 months, despite the small study population. In line with this, there was a significant increase in SV/BSA-ratio after 12 months of follow-up. Furthermore, a significant decrease in heart rate and systolic blood pressure was found as a result of loss of volume and thus a decrease of cardiac afterload. Eventually there was a significant decrease in cardiac output 12 months postoperatively.

After three months, some patients showed a (slight) decrease in EDV and SV. Theoretically this might be due to lipolysis and/or the cardiodepressive effect of released free fatty acids (FFA) and its associated cardiotoxicity. However, in our population there was a decrease in serum triglycerides [23]. An explanation for this could be the release of lipid droplets (LDs) which can become cardiotoxic [24].

Besides these theories it is conceivable that in the first three months postoperative, there is a derangement of a stable but adipose state to a katabolic state, which alone has a cardiodepressive effect[25]. The temporary increase in FFA after surgery (due to lipolysis by the acute weight loss) could have a cardiotoxic effect on heart function, just like diabetic cardiomyopathy[26]. Furthermore there are multiple mechanical changes in cardiac function load and LVF in patients with morbid obesity, for example increased RV load and OSAS.

Because the relative onset of all these cardiac changes are due to the biggest weight loss, the emphasis is on BSA-corrected values. When BSA-corrected values are used, there is an overall improvement as seen in other studies [27].

We detected a 15.2% reduction of LV mass in our patients, which is comparable to other studies measured by CUS [18 19 21 28]. These studies reported mass reductions of 16-22%. However, after correction for changes in BSA, no significant decline was found 12 months postoperatively. There is no evidence that the degree of increase in LVEF and decrease in LV mass is determined by the type of bariatric surgical procedure [29].

In all patients, a significant decrease of BMI (up to 31%) and BSA was found as compared to the preoperative condition, which is to be expected after RYGB. In our study, we have corrected the cardiac function outcomes for BSA as heart function is correlated with BSA and without correction the LVEF would change dramatically. Correction for BSA using the Du Bois formula is known for an underestimation of the BSA in patients with obesity of 3% in male patients and 5% in female patients [30]. BSA is generally accepted and widely used to assess cardiac function [31]. The most accurate correction, however, would be with the measurement of the patient's volume using a 3-dimensional body scanner [32]. Unfortunately, this technique was not available at our hospital during our study.

In a larger study with 312 patients with higher BMI's, Brownell et al. reported that the presence of LVH was independently associated with BMI >50 and female sex, after adjusting for age, diabetes, hypertension and pulmonary hypertension [20]. As there was no significant LVH preoperatively in our group, we cannot confirm this. A possible explanation for the differences in LVH between these test groups and our test group could be the lower BMI in our test group.

In one study (n=10) adenosine-induced sub-endocardial ischemia was reported at baseline [9]. Half of the patients in this study underwent bariatric surgery, resulting in complete normalization of ischemia in 3 out of 5 patients and partial improvement in the remaining 2 patients. As determined by CMRI, none of the patients in our study had signs of previous infarction. For logistic reasons we couldn't use adenosine CMRI for detection of reversible stress induced myocardial ischemia.

Hepatic steatosis is closely linked to obesity. This linkage is based on the fact that obesity results in marked enlargement of the intra-abdominal visceral fat depots. The eventual development of insulin resistance leads to continuous lipolysis within these depots, releasing fatty acids into the portal circulation, where they are rapidly translocated to the liver and reassembled into triglycerides [33]. All of our 13 patients had hepatic steatosis preoperatively. This disappeared in 11 patients 3 months after the bariatric procedure.

In the other 2 patients, hepatic steatosis decreased significantly and disappeared after 6 months. This all is a direct consequence of diminished intra-abdominal visceral fat depots after RYGB.

There was a significant decrease of LDL and triglyceride levels and increase of HDL 12 months after RYGB. An improved lipid spectrum after RYGB is associated with an improvement of the cardiovascular risk profile [34-36]. However, in our study, no correlation was found between the improvement of the cardiac function and the improvement of the lipid spectrum, which has also been shown in a study in patients with preoperative heart failure [37]. Although cardiovascular risks decline due to an improved lipid spectrum, it does not seem to be related to the improvement of cardiac function. Priester et al. reported that weight loss achieved through bariatric surgery is associated with less coronary calcification and this effect, which appears to be independent of changes in LDL-C, may contribute to lower cardiac mortality in patients with successful gastric bypass [38]. Additionally, Jonker et al. demonstrated that bariatric surgery results in a significant decrease in carotid intima-media thickness in all evaluated age categories, resulting in an improvement of cardiovascular risks [39].

Glucose, leptin and ghrelin levels couldn't consequently be measured after fasting due to logistic limitations in CMRI planning (mostly at the end of the day) and patient comorbidity like diabetes. Therefore, the results of glucose, leptin and ghrelin outcomes could not be used for analysis.

Our study is limited by the small study population. We started with 15 patients, but 2 patients had claustrophobia, even though they had a test visit to the MRI before the study started. The MRI protocol of 45 minutes was well-tolerated by the other 13 non-claustrophobic patients. Almost all patients stated the breath-holding technique was easier to perform after weight loss. The quality of the CMRI was good and there were no distractions due to the subcutaneous fat. Therefore we conclude that CMRI is a good technique to assess cardiac function in the population with morbid obesity. Further research in a larger study population is recommended in order to have a better insight of the correlations of different factors in relation to the improvement of cardiac function. It is also recommended to obtain a 3D whole body scan to measure the whole body volume for correction of the cardiac function instead of the BSA.

In conclusion, this study shows that CMRI is an effective imaging technique to objectively analyze cardiac functional changes in patients with morbid obesity. Also, an improvement of cardiac function after RYGB is seen in patients with morbid obesity without a history of cardiac disease.

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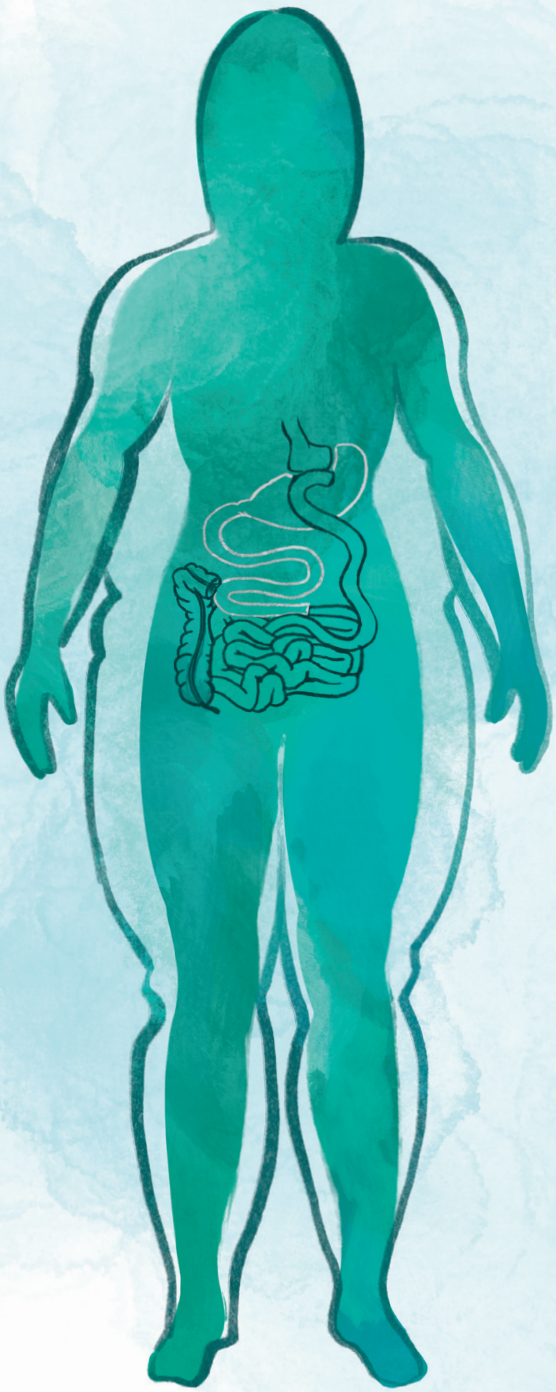
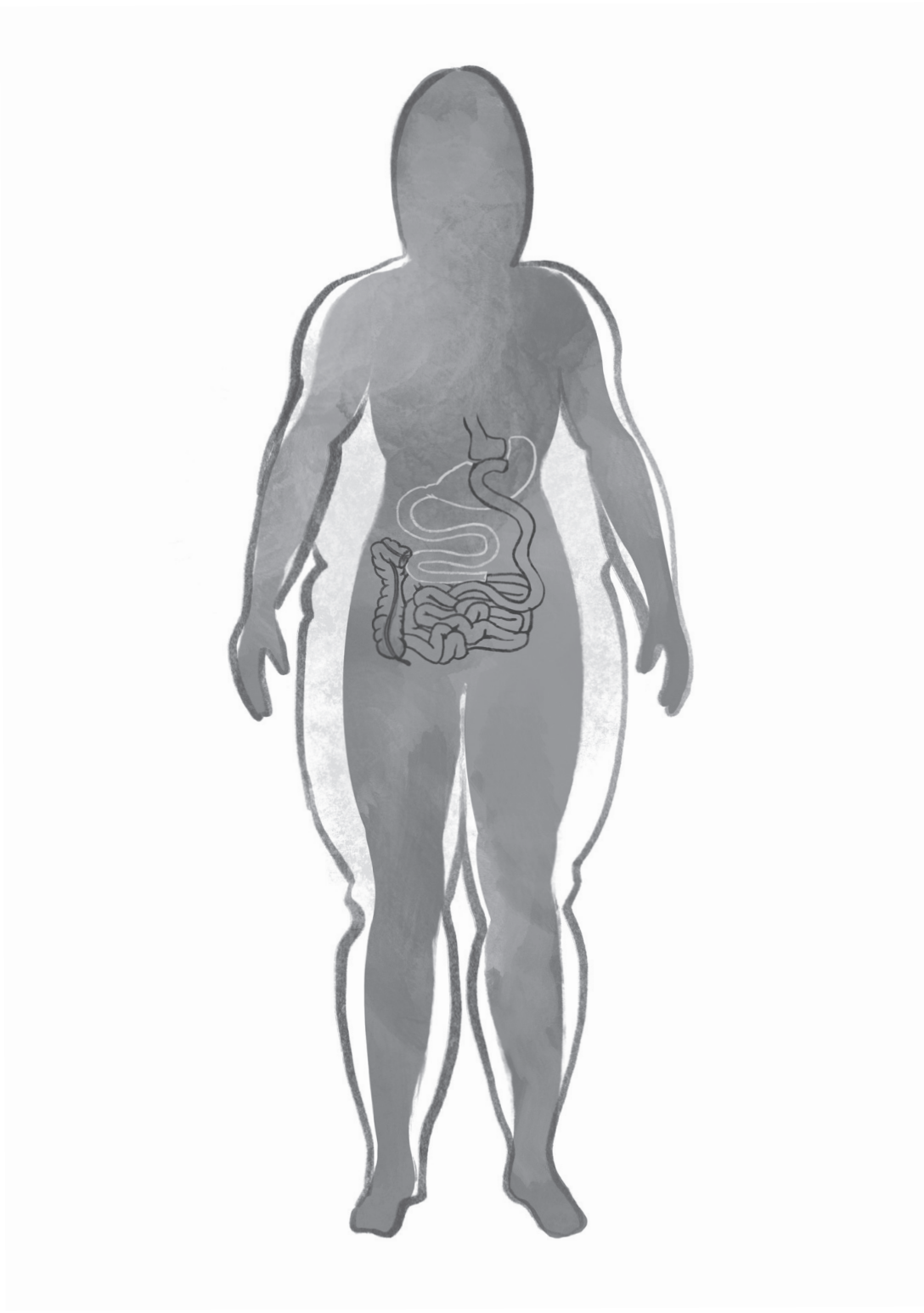


Part II

Immunological changes after laparoscopic Roux-en-Y gastric bypass



Chapter 4

Effects of morbid obesity and metabolic syndrome on the composition of circulating immune subsets

L.H. Wijngaarden, E. van der Harst, R.A. Klaassen, M. Dunkelgrun, T.M. Kuijper, M. Klepper, G. Ambagtsheer, J.N.M. IJzermans, R.W.F. de Bruin, N.H.R. Litjens**

** Authors contributed equally*

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Abstract

Morbid obesity is characterized by chronic, low-grade inflammation, which is associated with 'inflamm-aging'. The presence of metabolic syndrome (MetS) might accelerate this phenomenon of metaflammation. In this study, we assessed the effects of morbid obesity and MetS on the composition of a broad spectrum of immune cells present within the circulation. A total of 117 morbidly obese patients (MOP) without MetS (MetS-), 127 MOP with MetS (MetS+) and 55 lean controls (LC) were included in this study. Absolute numbers of T cell, B cell, NK cell and monocyte subsets were assessed within peripheral blood using flow cytometry. Both absolute cell numbers and proportion of cells were evaluated correcting for covariates age, body mass index and cytomegalovirus serostatus. Although the absolute number of circulating CD4+ T cells was increased in the MetS+ group, the CD4+ T cell composition was not influenced by MetS. The CD8+ T cell and B cell compartment contained more differentiated cells in the MOP, but was not affected by MetS. Even though the absolute numbers of NK cells and monocytes were increased in the MOP as compared to LC, there was no difference in proportions of NK and monocyte subsets between the three study groups. In conclusion, although absolute numbers of CD4+ and CD8+ T cells, B cells, NK cells and monocytes are increased in MOP, obesity-induced effects of the composition of the immune system are confined to a more differentiated phenotype of CD8+ T cells and B cells. These results were not affected by MetS.

Introduction

Morbid obesity is characterized by a state of chronic, low-grade inflammation [1]. This systemic inflammation, also called metaflammation, is caused by the high number of adipocytes in the white adipose tissue. Especially metabolic overload leads to adipocyte dysfunction [2]. This secretes pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukins (IL-) 2 and 6, and C-reactive protein (CRP) [3]. Metaflammation is associated with accelerated aging, referred to as 'inflamm-aging' [1 4]. This phenomenon is especially described in morbidly obese individuals with metabolic syndrome (MetS), which is characterized by dyslipidemia, dysglycemia, an elevated blood pressure and an increased abdominal waist circumference [5 6]. Clinical consequences of metaflammation include a decreased vaccination efficacy, an increased risk for developing cardiovascular diseases and type 2 diabetes (T2D), and an increased mortality rate [4 7 8].

Aging affects different components of the adaptive as well as the innate immune system, the former being more extensively studied. In the aging population, there is a phenotypic shift of the lymphoid to the myeloid lineage [9]. This eventually contributes to immune dysfunction in the older population. For the adaptive immune system, thymic involution caused by aging leads to a decrease in circulating recent thymic emigrants (RTEs), which can be identified by CD31-expression within naive circulating T cells [10]. Furthermore, there is a shift from $CD45RO^{-}CCR7^{+}$ naive T cells to $CD45RO^{+}$ memory and $CD45RO^{-}CCR7^{-}$ terminally differentiated effector memory (EMRA) T cells [1 11]. Additionally, loss of CD28 on the membrane of T cells leads to an increase in advanced differentiated $CD28^{null}$ T cells in the aging population. A comparable shift is seen in B cell subpopulations, resulting in a decrease of $CD24^{high}$ transitional and $CD27^{-}$ naive B cells, and an increase in a more differentiated phenotype of B cell subsets, including $CD27^{+}$ switched and non-switched B cells, $CD27^{-}IgD^{-}$ double negative B cells and an increase in $CD27^{high}$ plasma blasts [12 13]. For the innate immune system, an age-related phenotypic change of NK cells and monocytes is described. Whereas the immunomodulatory $CD56^{bright}$ NK cells do not seem to be influenced by aging, an increase in mature, cytotoxic $CD56^{dim}$ NK cells has been described [14 15]. Additionally, in aging there is a shift from the pro-inflammatory classical $CD14^{+}CD16^{-}$ monocytes to the anti-inflammatory non-classical $CD14^{dim}CD16^{+}$ monocytes [16].

Several studies have investigated the effect of obesity on immunosenescence, and found a phenotypically aged profile among morbidly obese individuals [17-23]. However, most studies investigated only one or two specific immune cell subsets instead of a broad spectrum of circulating immune cells. Additionally, study populations were relatively small and studies were performed in specifically chosen study populations, which do not reflect the general population at the outpatient clinic. In addition, not all studies included a lean healthy control group. To our knowledge, we were the first to study the influence of MetS and

corrected for confounders such as cytomegalovirus (CMV) seropositivity [24]. Undoubtedly, correction for CMV status should be performed as CMV seropositivity is associated with an increase in differentiated memory T cells, and thus an aged immune profile [25]. Therefore, CMV seropositivity can influence the outcomes of our research question.

In summary, the aim of this study was to assess the effects of morbid obesity and MetS on phenotypical changes of the adaptive as well as the innate immune system in a large cohort of morbidly obese patients as compared to lean subjects, with correction for CMV status.

Materials and Methods

Patient selection

Morbidly obese patients who were scheduled for laparoscopic Roux-en-Y gastric bypass (LRYGB) in the Maasstad Hospital and Sint Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands between June 2018 and October 2019 were invited to participate in this prospective cohort study. To be eligible for LRYGB, patients had to fulfill the criteria for bariatric surgery of the International Federation for the Surgery of Obesity and Metabolic Disorders (IFSO) [26]. Exclusion criteria were lack of basic understanding of the Dutch or English language, or previous bariatric surgery in the medical history. In order to reflect the general bariatric population of the outpatient clinic, there were no exclusion criteria based on comorbidities, medication use prior to bariatric surgery, use of supplements or dietary intake. Between December 2018 and April 2019, blood donors at the Sanquin blood bank were invited to participate in this study as lean, healthy controls. Controls with a Body Mass Index (BMI) $\geq$ 30 kg/m^2 or with the presence of MetS were excluded from this study. BMI was calculated using a person's height and weight with the following formula: . Lean controls were included to analyze the effect of morbid obesity (with a distinction between morbidly obese patients with versus without MetS) on the immune system, as lean controls do not have an accumulation of white adipose tissue leading to metaflammation and thus affect the phenotype of the immune system. A sample size calculation was performed prior to the start of this study. According to this sample size calculation, the aim was to include 125 patients in each morbidly obese groups and 60 lean controls. The local medical ethical committee (MEC), being the Medical research Ethics Committees United, approved the study (MEC number: MEC-2018-06). All participants of this study gave written informed consent. This study was conducted in accordance with the Declaration of Helsinki and the Declaration of Istanbul and in compliance with the International Conference on Harmonization/Good Clinical Practice regulations.

Metabolic syndrome

Patients were clinically diagnosed with MetS if they had the presence of three or more of the following risk factors [6 27]:

- An increased waist circumference (≥ 102 cm in males, ≥ 88 cm in females)
- Elevated triglycerides (≥ 150 mg/dL or 1.7 mmol/L) or drug treatment for elevated triglycerides
- Reduced HDL cholesterol (<40 mg/dL or 1.0 mmol/L in males, <50 mg/dL or 1.3 mmol/L in females) or drug treatment for reduced HDL cholesterol
- Elevated blood pressure (systolic ≥ 130 mmHg and/or diastolic ≥ 85 mmHg) or antihypertensive drug treatment
- Elevated fasting glucose (≥100 mg/dL) or antidiabetic drug treatment

Blood pressure was measured with the patient sitting in an upright position with the back supported for at least five minutes and the arm supported at the level of the heart, using an automatic sphygmomanometer (Welch Allyn, Hillrom Holdings, Inc., Chicago, IL, USA). The triglycerides, HDL cholesterol and fasting glucose were measured directly in serum obtained by vena puncture.

Blood collection

In the morbidly obese population, venous blood samples were obtained at the clinic on the day of surgery, prior to the surgical intervention. Blood samples were collected in either two 10.0 mL or four 6.0 mL Lithium-Heparin tubes (BD, Franklin States NJ, USA). The blood samples were stored at room temperature and were analyzed within 8 hours after blood collection. In the lean control population, blood was collected in two 10.0 mL Lithium-Heparin tubes prior to blood donation. The blood samples were stored at room temperature and were analyzed within 8 hours after blood collection.

CMV serology

The diagnostic department of Virology assessed the CMV serology of all included participants. This was performed by determining the presence of plasma IgG antibodies to CMV with an enzyme immune assay (Biomerieux, VIDAS, Lyon, France). An outcome of ≥ 6 arbitrary units/mL (AU/mL) was considered as positive.

Immune cell phenotyping

Whole blood stainings were performed using fluorescently-labelled antibodies to identify and determine frequencies as well as absolute numbers of the different circulating immune cells and their differentiation status. Supplementary Table 1 lists the circulating immune cells we measured and the markers used for their identification.

Briefly, Trucount tubes (BD Pharmingen, Erebodegem, Belgium) containing a fixed number of beads were used to determine absolute numbers of leukocytes. Whole blood was incubated for 15 minutes at room temperature with blue violet 510 (BV510)-labeled anti-CD3, Pacific Blue (PacBlue)-labeled anti-CD4, fluorescein isothiocyanate (FITC)-labeled anti-CD8, phycoerythrin/cyanine7 (PE-Cy7)-labeled anti-CD19, allophycocyanin (APC)-labeled anti-CD45, PE-labeled anti-CD16, peridin chlorophyll protein (PerCP)-Cy7-labeled anti-CD56 (Biolegend Europe B.V. Uithoorn, Netherlands), and APC-H7-labeled anti-CD14 (BD). Subsequently, cells were lysed with Pharm Lyse solution (BD, diluted 10x with Milli-Q water) for 15 minutes. Afterwards, cells numbers were determined on a FACSCanto II equipped with 3 lasers (Blue laser harboring 4 detectors, red laser harboring 2 detectors and violet laser harboring 2 detectors; BD Biosciences, Erembodegem, Belgium) using FACSDiva software version 8 (BD).

An additional staining was performed to identify the different T cell subsets within CD4+ and CD8+ T cells. Whole blood staining using BV510-labeled anti-CD3, PacBlue-labeled anti-CD4, APC-Cy7-labeled anti-CD8, APC-labeled anti-CD45RO, PE-Cy7-labeled anti-CCR7, PE-labeled anti-CD31 (Biolegend), and PerCP-Cy5.5-labeled anti-CD28 (BD) were added to whole blood. The different B cell subsets were determined in a separate staining, using BV510-labeled anti-CD19, PE-Cy7-labeled anti-CD27, APC-Cy7-labeled anti-IgD, APC-labeled anti-CD24 (Biolegend), and BV421-labeled anti-CD38 (BD). Both staining tubes were incubated for 15 minutes at room temperature. Subsequently, cells were lysed for 10 minutes at room temperature using BD FACS lysing solution (BD), centrifuged for 5 minutes at 2000 RPM and washed twice using FACS flow solution (BD). Afterwards, cells were measured on a FACSCanto II (BD) using FACSDiva software version 8 (BD).

The cells were analyzed using Kaluza Analysis Software version 2.1 (Beckman Coulter, Indianapolis, USA). A typical example of the flow cytometric analysis and gating strategy used is depicted in Supplementary Figure 1.

Statistical analysis

A sample size calculation was performed, by which we aimed to include 125 patients in both morbidly obese patients groups and 60 patients in the lean control group. Baseline characteristics are reported using simple descriptive statistics. Comparisons between groups were performed using Pearson's chi –square test for categorical data and Mann-Whitney U test for continuous data. A mixed negative binomial regression model with a random intercept for each subject was used for the statistical analysis of cell counts and cell subset composition. A Dirichlet multinomial mixed model was used for statistical analysis of cell subset composition in percentages [28]. Additionally, the effects of covariates were investigated by including interactions for cell type and covariates age, BMI and CMV (yes/no). BMI was centered at the medians of the respective groups

to allow for selective adjustment of within-group differences only. Thus, effects due to between-group differences in BMI were captured by the indicator variables for groups. Age was centered at the overall median to allow for easier interpretation of coefficients. The dispersion parameter was modeled as a function of the expected mean. Significance of differences in cell counts was tested by multivariate Wald tests in a sequential fashion. First, an overall test was done to assess differences for any cell type between groups. If significant, separate follow-up tests were performed for each cell type. Statistical analysis was performed using Stata version 14.2 (StataCorp, Texas, USA) and R version 3.6.1 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). A two-sided *P*-value <0.05 was used to indicate statistical significance.

Results

Baseline characteristics

The study population consisted of 55 lean controls (LC), 117 morbidly obese patients without metabolic syndrome (MetS-) and 127 morbidly obese patients with metabolic syndrome (MetS+). The immune status of 60 lean controls were analyzed, however, five lean controls were excluded from this study as they had a BMI > 30 kg/m². The LC were significant younger than the MetS- and MetS+ groups (*P*<0.001). Baseline characteristics are shown in Table 1. The BMI of LC was significantly lower than that of morbidly obese patients (*P*<0.001), however, there was no significant difference in BMI between the MetS- and MetS+ groups (*P*=0.095). There were no significant differences in CMV seropositivity between the three study groups. Four MetS+ patients had a BMI <35 kg/m² on the day of surgery. These patients all had a BMI >35 kg/m² on their first presentation at the outpatient clinic. As a result of participation in an intervention program, consisting of psychological, dietetic and physiotherapeutic support to adjust their life style prior to surgery, they had already lost weight preoperatively.

Morbid obesity induces an aged CD8+ T cell compartment

Morbidly obese patients had an increased number of circulating CD3+ T cells when compared to lean controls (*P*=0.010). The median [interquartile range] numbers of the circulating cells are shown in Table 2a. CMV seropositivity was significantly associated with higher overall CD3+ T cell counts (coefficient 0.110, *P*=0.014). The difference in number of circulating T cells was mainly caused by an increase in CD4+, but not CD8+, T cells in the MetS+ group. The increase in CD4+ T cells was not influenced by any of the confounders. Whereas the absolute number of circulating CD4+ T cells was increased in the MetS+ group, the presence of MetS was not associated with the composition of the CD4+ T cell subsets with respect to absolute numbers (*P*=0.156). In contrast to the CD4+ T cell subsets, the CD8+ T cell compartment contained more differentiated cells in the

morbidly obese patients (*P*<0.001). This was reflected by an increase in CD8+ EMRA T cells in the morbidly obese patients as compared to lean controls (LC vs MetS- *P*=0.004, LC vs MetS+ *P*<0.001). MetS did not have an additional effect on CD8+ T cell differentiation (*P*=0.519), Figure 1A and 1B. There were no significant differences in CD4+ T cell subsets in terms of percentages (*P*=0.070), while CD8+ T cell subsets in terms of percentages were significantly different (*P*=0.033), as shown in Figure 2A and 2B. This was in both lean controls versus MetS- (*P*=0.043) and LC versus MetS+ (*P*=0.004).

Table 1. Baseline characteristics

	Lean controls (n= 55)	**MetS- (n=117)**	**MetS+ (n= 127)**	**P-value**
Age (median and range, in years)	31 [25;52]	42 [35;50]	50 [39;56]	<0.001
Weight (median and range, in kg)	75 [70;83]	115.5 [107.1;125.1]	119.5 [107.3;131.2]	<0.001
BMI (median and range, in kg/m²)	24.4 [22.5;26.8]	41.5 [40.0;43.4]	40.6 [37.4;43.6]	<0.001
BMI group (number, %)				<0.001
< 30 kg/m²				
30 – 34.9 kg/m²	55 (100%)	0 (0%)	0 (0%)	
35 – 39.9 kg/m²	0 (0%)	0 (0%)	4 (3.2%)	
40 – 44.9 kg/m²	0 (0%)	29 (24.8%)	50 (39.4%)	
> 45 kg/m²	0 (0%)	66 (56.4%)	48 (37.8%)	
	0 (0%)	22 (18.8%)	25 (19.7%)	
CMV seropositivity (number, %)	28 (50.9%)	61 (52.1%)	71 (55.9%)	0.767
Comorbidities (number, %)				
T2D		0 (0%)	43 (33.9%)	<0.001
HT		17 (14.5%)	68 (53.5%)	<0.001
HC		11 (9.4%)	35 (27.6%)	<0.001
OSAS		14 (12.0%)	27 (21.3%)	0.052

MetS- = morbidly obese patients without metabolic syndrome, Mets+ = morbidly obese patients with metabolic syndrome, BMI = body mass index, CMV = cytomegalovirus, T2D = type 2 diabetes, HT = hypertension, HC = hypercholesterolemia, OSAS = obstructive sleep apnea syndrome

The CD4+/CD8+ ratio is not influenced by metabolic syndrome

Although there was a trend towards an increased CD4+/CD8+ ratio in the morbidly obese patients as compared to lean controls (2.06 [1.63;2.99] in LC, 2.31 [1.84;3.20] in MetS- and 2.57 [1.94;3.60] in MetS+), this was not significantly different after correction for the covariates (*P*=0.518).

Figure 1. Composition of immune cell subsets in absolute cell counts

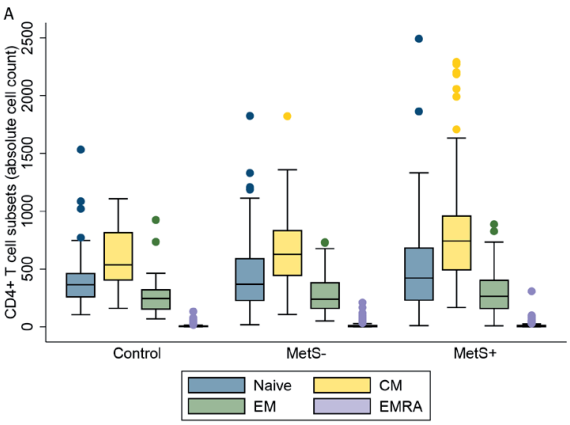

Figure 1A. CD4+ T cell subsets

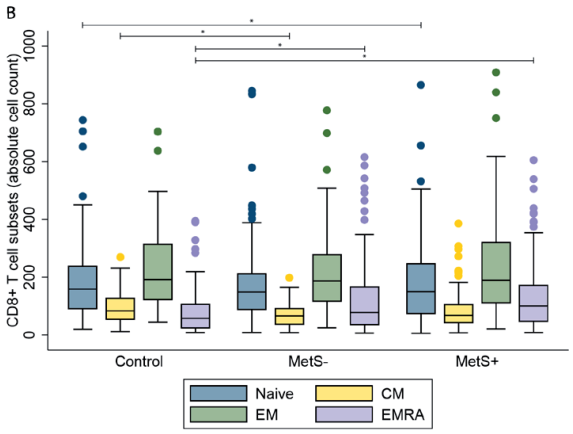

Figure 1B. CD8+ T cell subsets

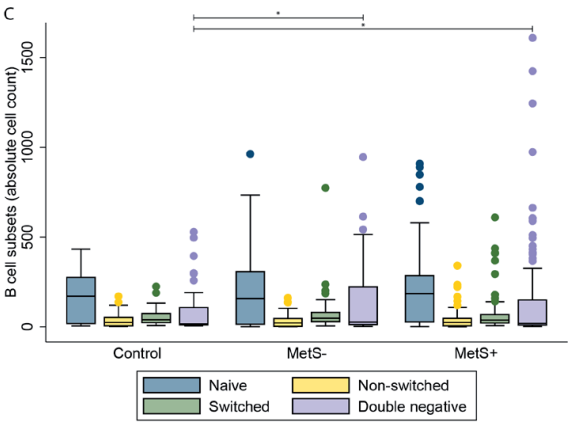

Figure 1C. B cell subsets

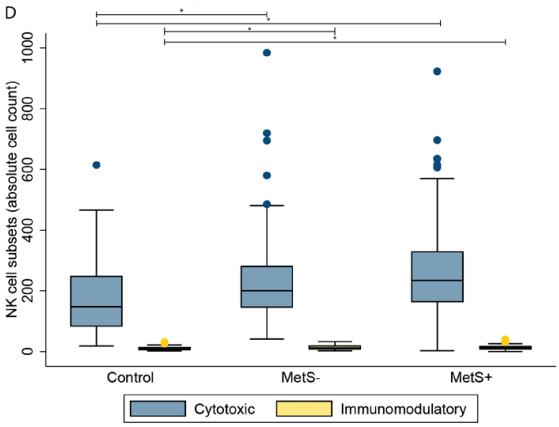

Figure 1D. NK cell subsets

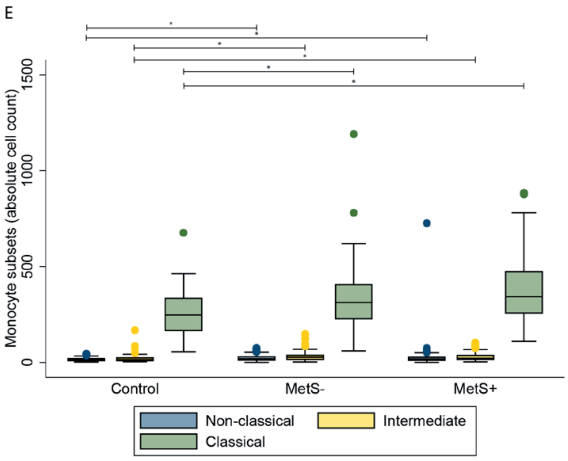

Figure 1E. Monocyte subsets

MetS- = morbidly obese patients without metabolic syndrome; MetS+ = morbidly obese patients with metabolic syndrome; CM = central memory; EM = effector memory; EMRA = terminally differentiated effector memory

*$P < 0.05$

Figure 2. Composition of immune cell subsets in percentages

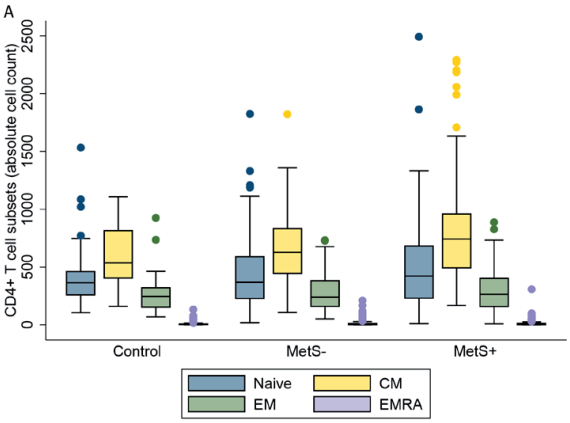

Figure 2A. CD4+ T cell subsets

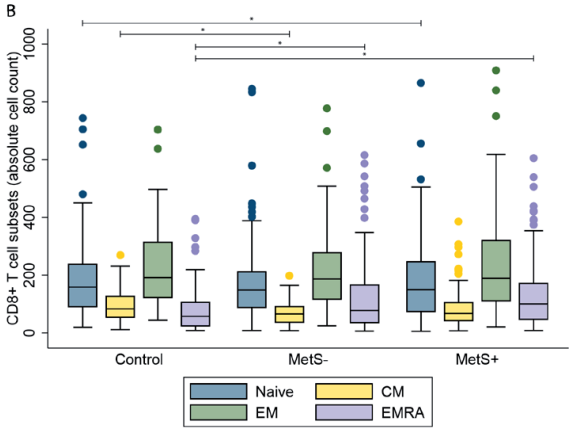

Figure 2B. CD8+ T cell subsets

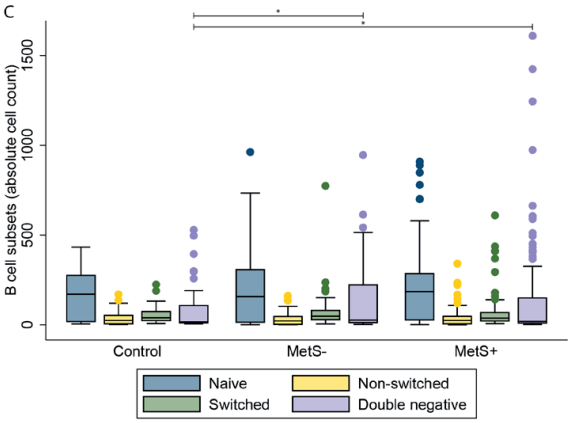

Figure 2C. B cell subsets

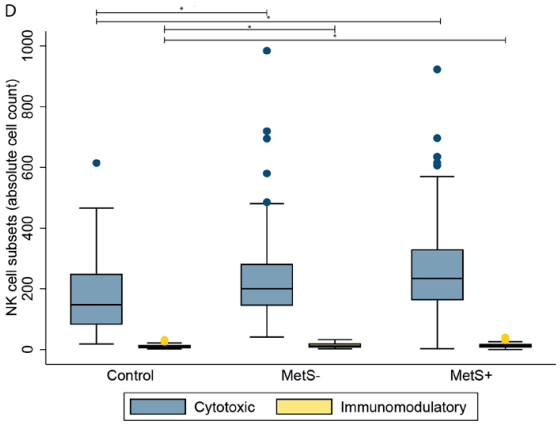

Figure 2D. NK cell subsets

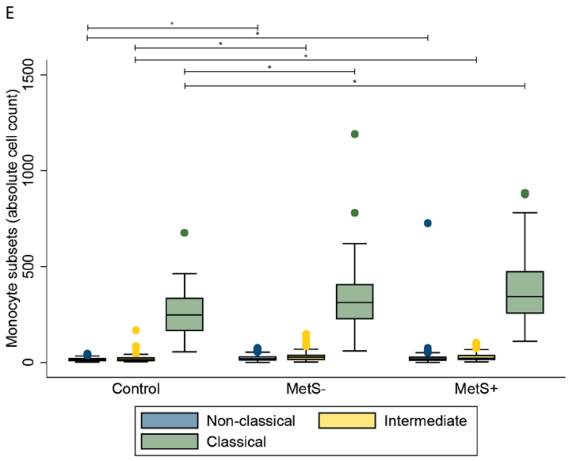

Figure 2E. Monocyte subsets

MetS- = morbidly obese patients without metabolic syndrome; MetS+ = morbidly obese patients with metabolic syndrome; CM = central memory; EM = effector memory; EMRA = terminally differentiated effector memory

*$P < 0.05$

Morbidly obese patients have increased numbers of differentiated DN B cells

Along with the absolute number of T cells, the absolute number of B cells was significantly different between the three study groups (*P*=0.001), as presented in Table 2b. Additionally, there was a significant difference in the absolute number of B cell composition (*P*=0.037). This was reflected by an increase of DN B cells in morbidly obese patients as compared to LC (LC vs MetS- *P*=0.011, LC vs MetS+ *P*<0.001), Figure 1C. Both age (*P*=0.010) and BMI (*P*=0.025) influenced this difference positively. Also, there was a significant increase in mature plasma blasts in the morbidly obese patients as compared to the lean controls (*P*<0.001). None of the confounders showed an additional effect on this increase in plasma blasts. Nevertheless, the composition of B cell subsets in percentages was not significantly different between the three study groups (*P*=0.152), Figure 2C.

Absolute numbers of NK cells are increased in morbidly obese patients

Absolute numbers of NK cells were increased in the morbidly obese patients as compared to lean controls (*P*<0.001), as shown in table 2c. These differences were not significantly affected by the confounders that we corrected for. There was no difference in absolute numbers of NK cells between the MetS- and MetS+ group (*P*=0.182). When comparing the two different phenotypes of the NK cells, there was an increase in absolute numbers of both cytotoxic ($CD56^{dim}$) and immunomodulatory ($CD56^{high}$) NK cells (*P*<0.001), Figure 1D. These differences were not seen when comparing the MetS- with the MetS+ groups (*P*=0.459). Proportions of the various subsets were not significantly different between the three study groups (*P*=0.089), as shown in Figure 2D.

Monocyte phenotype is not affected in morbidly obese patients

Similarly to the T cells, B cells and NK cells, the absolute number of monocytes was increased in the morbidly obese patients as compared to the lean controls (*P*<0.001), Table 2D. This was, however, not significantly different between the MetS- and MetS+ groups (*P*=0.163). Whereas there was no phenotypic change in monocytes with respect to percentages (*P*=0.832), Figure 2E, there was a significant increase absolute number of all three monocyte subsets between the LC and morbidly obese patients (*P*=0.002), Figure 1E. However, there were no differences between the MetS- and MetS+ groups.

Table 2a. Absolute numbers of the T cell subsets

	Lean controls (n=55)	MetS- (n=177)	MetS+ (n=127)	P-value
CD3+	1975 [1448;2440]	2032 [1517;3451]	2255 [1596;2840]	0.010
CD4+	1278 [897;1539]	1286 [1023;1775]	1552 [1124;1990]	0.003
CD4+ naive $CD31^{+}$	856 [675;1153]	968 [729;1314]	1070 [708;1393]	0.003
CD4+ naive	365 [258;460]	369 [228;589]	422 [230;682]	0.156
CD4+ CM	535 [404;815]	628 [445;833]	742 [493;958]	0.156
CD4+ EM	246 [152;320]	240 [160;381]	263 [158;402]	0.156
CD4+ EMRA	3 [2;7]	3 [1;11]	3 [1;11]	0.156
CD4+ $CD28^{null}$	10 [4;43]	6 [2;75]	8 [2;64]	0.234
CD8+	604 [340;772]	554 [389;770]	561 [398;816]	0.231
CD8+ $CD31^{naive}$	574 [315;761]	547 [386;753]	540 [383;795]	0.237
CD8+ naive	159 [91;238]	149 [88;212]	150 [74;247]	< 0.001
CD8+ CM	83 [54;127]	66 [37;91]	68 [43;105]	< 0.001
CD8+ EM	192 [123;314]	187 [117;277]	190 [112;320]	< 0.001
CD8+ EMRA	57 [24;106]	77 [36;166]	100 [47;172]	< 0.001
CD8+ $CD28^{null}$	120 [59;216]	145 [76;286]	152 [79;329]	0.060

MetS- = morbidly obese patients without metabolic syndrome; MetS+ = morbidly obese patients with metabolic syndrome; CM = central memory; EM = effector memory; EMRA = terminally differentiated effector memory. All absolute numbers are presented as median [interquartile range]. *P*-values are after correction for covariates using a binomial mixed regression model.

Table 2b. Absolute numbers of the B cell subsets

	Lean controls (n=55)	MetS- (n=177)	MetS+ (n=127)	P-value
CD19+ B cells	340 [217;432]	368 [273;484]	374 [264;579]	0.001
Transitional B cells	16 [9;25]	17 [11;27]	16 [11;28]	0.744
Naive B cells	170 [17;276]	158 [14;306]	184 [28;286]	0.037
Non-switched B cells	24 [5;52]	22 [3;46]	24 [5;47]	0.037
Switched B cells	39 [23;74]	48 [29;80]	37 [21;69]	0.037
Double negative B cells	16 [10;108]	26 [11;222]	19 [10;150]	0.037
Plasma blasts	12 [7;19]	16 [9;30]	15 [9;31]	< 0.001

MetS- = morbidly obese patients without metabolic syndrome; MetS+ = morbidly obese patients with metabolic syndrome.
All absolute numbers are presented as median [interquartile range]. *P*-values are after correction for covariates using a binomial mixed regression model.

Table 2c. Absolute numbers of the NK cell subsets

	Lean controls (n=55)	MetS- (n=177)	MetS+ (n=127)	P-value
CD56+ NK cells	163 [90;264]	219 [171;313]	257 [182;360]	< 0.001
Immunomodulatory NK cells	9 [7;14]	11 [8;19]	12 [8;17]	< 0.001
Cytotoxic NK cells	147 [84;248]	200 [146;281]	234 [164;328]	< 0.001

MetS- = morbidly obese patients without metabolic syndrome; MetS+ = morbidly obese patients with metabolic syndrome; NK = natural killer
All absolute numbers are presented as median [interquartile range]. *P*-values are after correction for covariates using a binomial mixed regression model.

Table 2d. Absolute numbers of the monocyte subsets

	Lean controls (n=55)	MetS- (n=177)	MetS+ (n=127)	P-value
CD14+ monocytes	313 [232;414]	392 [293;507]	423 [315;558]	< 0.001
Non-classical monocytes	14 [9;19]	18 [12;29]	18 [11;27]	0.002
Intermediate monocytes	14 [9;23]	26 [15;37]	21 [15;36]	0.002
Classical monocytes	248 [166;334]	312 [228;405]	343 [257;473]	0.002

MetS- = morbidly obese patients without metabolic syndrome; MetS+ = morbidly obese patients with metabolic syndrome.
All absolute numbers are presented as median [interquartile range]. *P*-values are after correction for covariates using a binomial mixed regression model.

Discussion

In this study, cells of both the adaptive as well as the innate immune system proved to be affected by morbid obesity. Whereas MetS only induced an increase in CD4+ T cells, the absolute number of CD3+ T cells was also increased in morbidly obese patients as compared to lean controls. This increase in CD3+ T cells was amplified by CMV seropositivity. Furthermore, the CD8+ T cell differentiation was enhanced in morbidly obese patients, which was not affected by MetS and CMV seropositivity. For the innate immune system, absolute numbers of both monocytes and NK cells were increased in morbidly obese patients. However, this was not significantly different between the MetS- and MetS+ groups. Additionally, neither morbid obesity or MetS induced a phenotypic change in the NK cell and monocyte subsets. CMV seropositivity did not influence these results.

Ageing is associated with changes in composition of immune cell subsets, including the loss of naive CD8+ T cells and an increase in differentiated CD8+ T cells [29]. We found a differentiated composition of CD8+ T cells in the morbidly obese patients as compared with lean controls, which is comparable to the increase of differentiated CD8+ T cells in the aging population. In contrast to an aging population, there was no loss of naive and

RTE CD8+ T cells in our study population [30]. The increase in both CD8+ EMRA T in the morbidly obese patients is comparable to what has been described in literature, in which immunological changes due to obesity were most pronounced in the CD8+ T cells [24 31]. In this study, this accelerated differentiation of CD8+ T cells was not only associated with morbid obesity, but also with increasing age. The accelerated aging of CD8+ T cells was not influenced by CMV seropositivity, which is in contrast to what has been described in literature [32]. It might be that this accelerated differentiation is not further enhanced by CMV.

In elderly populations, a more differentiated profile of B cells with an increase of DN B cells has been described [33]. In a study performed by Frasca et al., an increase of late/exhausted memory B cells was described among young individuals with obesity as compared to elder individuals with obesity and lean young and elderly controls [23]. In our study, a comparable increase of DN B cells was seen in both MetS- and MetS+ groups as compared to the lean controls.

In this study, we have found a specific differentiated profile of the adaptive immune system of CD8+ T cells and B cells in morbidly obese patients, suggesting that there is obesity induced metaflammation. This aging profile consisted of an increase in more differentiated immune cells, being CD8+ EMRA T cells and DN B cells, while the number of immature immune cells was similar between the three study groups. Therefore, our data suggests that the production of immature immune cells is not disturbed by obesity. However, obesity does induce accelerated differentiation of CD8+ T cells and B cells. In our previous study, we found that T cell aging is partially reversed after bariatric surgery [24]. Thus a long-term follow-up study in morbidly obese patients who will undergo bariatric surgery is suggested, in order determine whether excessive weight loss can reverse the aged composition of the different immune cell subsets.

An increased age is associated with an inverted CD4/CD8 ratio [34]. In contrast to this, we found an increase in the CD4/CD8 ratio in the morbid obese patients, which confirms earlier studies in obesity [35 36]. The increase in CD4/CD8 ratio is especially explained by the increase in total CD4+ T cell numbers, whereas the CD8+ T cell numbers remains comparable between obese and lean subjects.

Despite the increase in absolute numbers of NK cells and monocytes, both cell types did not show an aged subset profile. Literature reports contradicting results on NK cell subset composition. Some studies show an increase in cytotoxic NK cells in morbidly obese patients, whereas other studies show an increase in immunomodulatory NK cells [19 21 37]. These studies consisted of study populations of less than 20 patients. In our study, which was performed in a large study cohort, there was an increase of both cytotoxic and immunomodulatory NK cells. However, no obesity-induced senescence of NK cells was found.

We found an increase in CD14^{dim} monocytes in morbidly obese patients that what comparable to what has been observed previously [38 39]. Similarly to Poitou et al., this increase was seen in morbidly obese patients as compared to lean controls, but was not seen in MetS- as compared to MetS+. Thus, MetS does not seem to influence aging of monocytes. Poitou et al. describes a decrease of CD14^{dim} monocytes after LRYGB, suggesting that the monocyte aging is reversible. However, that study group only consisted of 36 patients, and it would therefore be interesting to duplicate this study in a larger cohort.

Although, to our knowledge, this is the first large and comprehensive study investigating immunosenescence in morbidly obese patients, a limitation of this study is that we have only focused on the composition of the immune system. Previous studies have shown an increase in proinflammatory and a decrease of anti-inflammatory cytokine production in morbidly obese individuals, which causes DNA damage and is associated with age-related diseases and mortality [29 40]. Therefore a study to the functioning of the immune system in this large population of morbidly obese patients as compared to lean controls is recommended.

Oxidative and nitrosative stress play an important role in the development of metabolic diseases [41]. Increased levels of serum myeloperoxidase were previously observed in patients with metabolic syndrome, indicating that inflammation is intensified in this patient population [42]. Glutathione deficiency gives oxidative stress, leading to an accelerated aging and diseases such as T2D. Glutathione oxidation was increased in patients with obesity and hypertension, but not specifically in patients with metabolic syndrome [42]. Bariatric surgery reduces protein glycoxidation and nitrosative stress [43]. It would be interesting to also identify the markers on our large study cohort, and additionally assess these markers in the cohort after bariatric surgery.

In conclusion, obesity-induced effects on the composition of the immune system are confined to shifting of the CD8+ T cell and B cell compartment to a more differentiated phenotype. Further research is required to evaluate whether bariatric surgery reverses this differentiated phenotype, as well as research into the function of the immune cells of morbidly obese individuals, both before and after bariatric surgery.

References

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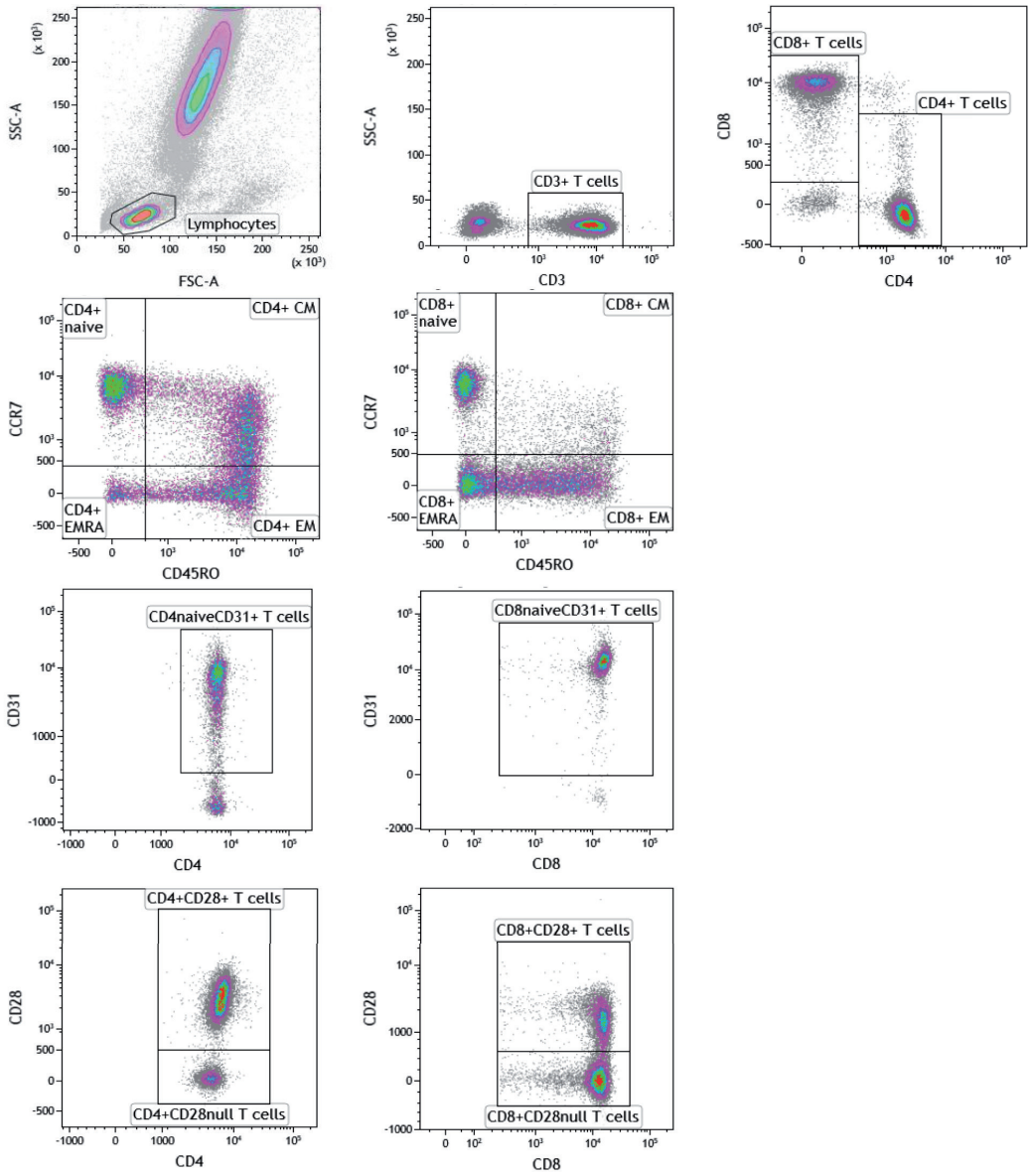
Supplementary Material

Supplementary Table 1. Immune cells and their corresponding markers

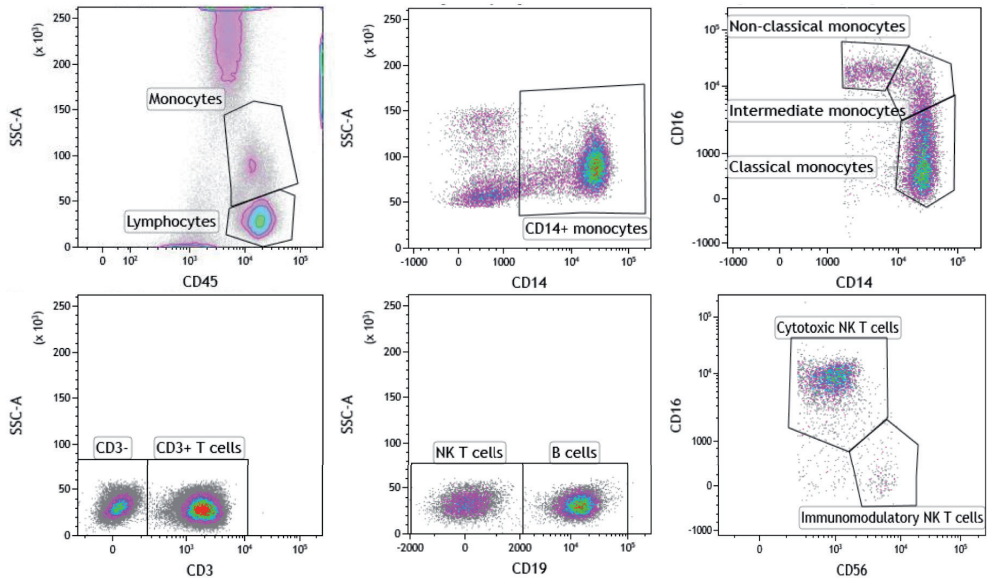
Immune cell	Marker
T cell subsets	
Recent thymic emigrants (RTEs)	$CD31^{+}$ $CD45RO^{-}CCR7^{+}$
Naive T cells	$CD45RO^{-}CCR7^{+}$
Central memory T cells (CM)	$CD45RO^{+}CCR7^{+}$
Effector memory T cells (EM)	$CD45RO^{+}CCR7^{-}$
Terminally differentiated effector memory T cells (EMRA)	$CD45RO^{-}CCR7^{-}$
Advanced differentiated T cells	$CD28^{null}$
NK T cell subset	
Immunomodulatory NK cells	$CD56^{bright}CD16^{-}$
Cytotoxic NK cells	$CD56^{dim}CD16^{+}$
B cell subsets	
Naive B cells	$CD27^{-}IgD^{+}$
Non-switched B cells	$CD27^{+}IgD^{+}$
Switched B cells	$CD27^{+}IgD^{-}$
Double-negative B cells	$CD27^{-}IgD-$
Transitional B cells	$CD24^{high}CD38^{ihgh}$
Plasma blasts	$CD27^{high}CD38^{high}$
Monocyte subset	
Classical monocytes	$CD14^{+}CD16^{-}$
Non-classical monocytes	$CD14^{+}CD16^{+}$
Intermediate monocytes	$CD14^{dim}CD16^{+}$

Supplementary Figure 1. Flowcytometry gating strategies



A. Flow cytometry gating strategy for CD4+ and CD8+ T cell subsets

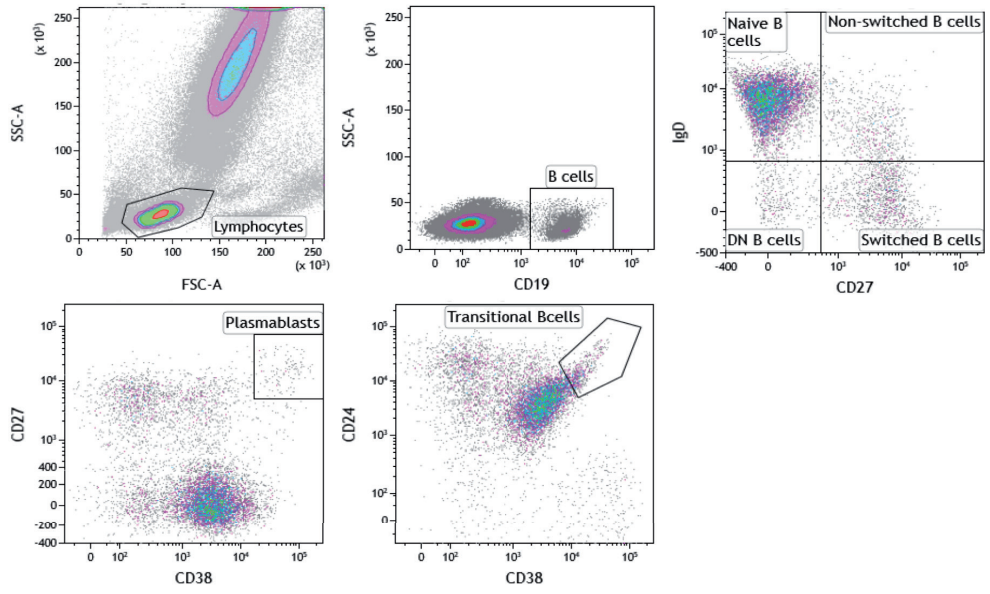
Lymphocytes were gated and then viable CD3+ T cells were selected. These were then selected for either CD4+ or CD8+ T cells. Afterwards, the CD4+ and CD8+ T cells were subdivided into the main T cell subsets using CCR7 and CD45RO into CD45RO^{-}CCR7^{+} naive, CD45RO^{+}CCR7^{+} CM, CD45RO^{+}CCR7^{-} EM and CD45RO^{-}CCR7^{-} EMRA T cells. Within naive T cells, CD31^{+} RTEs were then selected. Additionally, CD4+ and CD8+ T cells were plotted against CD28 and the CD28$^{\mathbf{null}}$ T cells were gated.



B. Flow cytometry gating strategy for NK cell and monocyte subsets

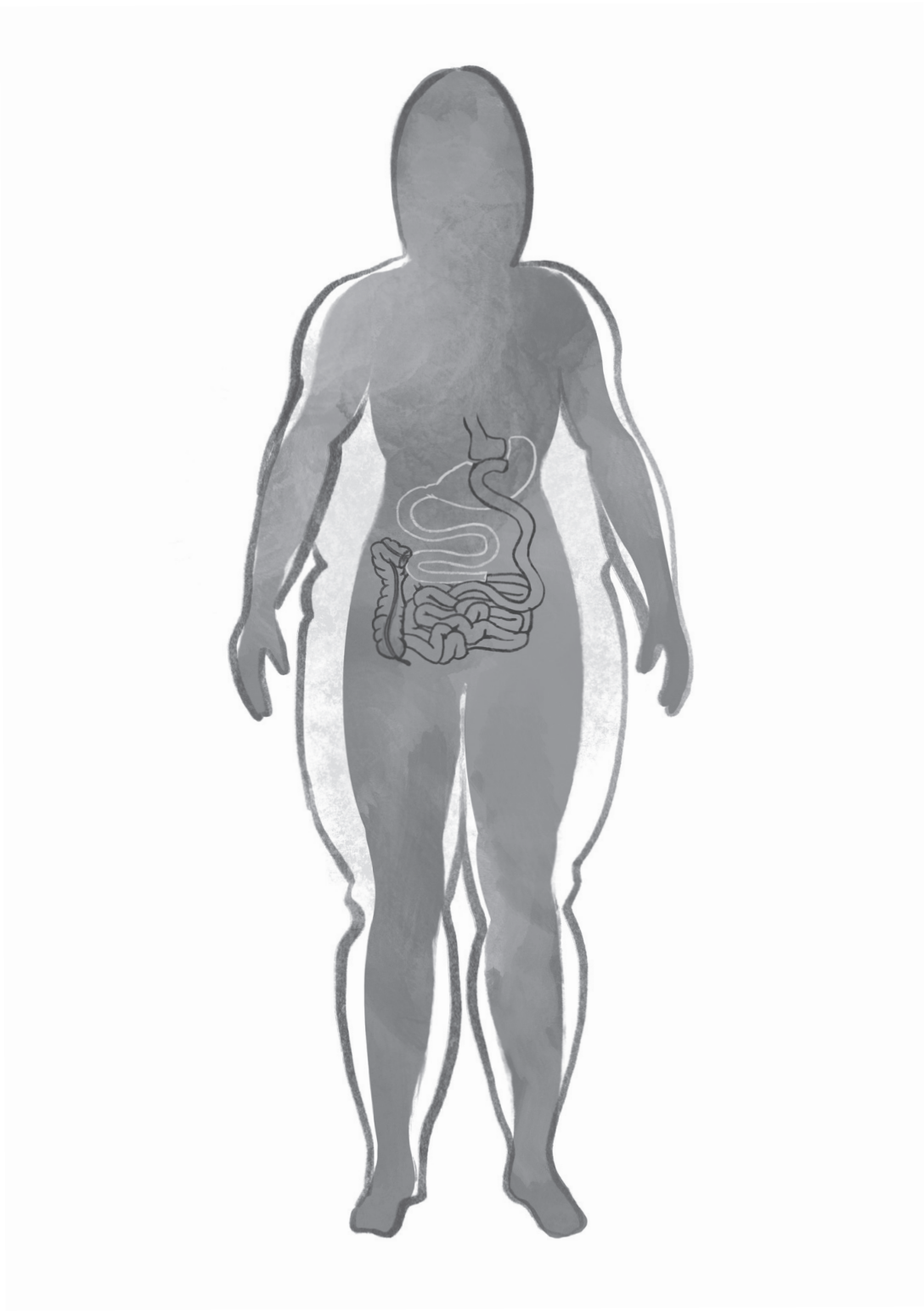
First, the monocytes were gated using SSC and CD45. Then, monocytes were selected using CD14 and afterwards the monocytes were subdivided into $CD14^{dim}CD16^{bright}$ non-classical, $CD14^{+}CD16+$ intermediate and $CD14^{+}CD16^{-}$ classical monocytes.

Second, the viable lymphocytes were gated and then the $CD3^{-}$ cells were selected. These cells were plotted against CD19 and $CD19^{-}$ NK cells were then gated. Afterwards, the NK cells were subdivided into $CD56^{dim}CD16^{+}$ cytotoxic and $CD56^{bright}CD16^{-}$ immunomodulatory NK cells.



C. Flow cytometry gating strategy for B cell subsets

Viable lymphocytes were gated and then plotted against CD19. $CD19^{+}$ B cells were then gated and subdivided into the main B cell subsets using CD27 and IgD into $CD27^{-}IgD^{+}$ naive, $CD27^{+}IgD^{+}$ non-switched, $CD27^{+}IgD^{-}$ switched and $CD27^{-}IgD^{-}$ double negative B cells. Additionally, the B cells were plotted against CD27 and CD38 and $CD27^{high}CD38^{high}$ plasmablasts were gated. At last, the B cells were plotted against CD24 and CD38 and the $CD24^{high}CD38^{high}$ transitional B cells were gated.



Chapter 5

T and B cell composition and cytokine producing capacity before and after bariatric surgery

L.H. Wijngaarden, F. Nuijten, E. van der Harst, R.A. Klaassen, T.M. Kuijper, F. Jongbloed, G. Ambagtsheer, M. Klepper, J.N.M. IJzermans, R.W.F de Bruin , N.H.R. Litjens

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Abstract

Morbid obesity is associated with a chronic state of low-grade inflammation, which may lead to accelerated differentiation of T and B cells. These differentiated immune cells are strongly cytotoxic and have an increased pro-inflammatory cytokine producing capacity. Furthermore, the anti-inflammatory function of the T and B cells decreases. The aim of this study was to evaluate the effect of morbid obesity on the subset profile and cytokine producing capacity of T and B cells. Subsequently we assessed whether bariatric surgery affected the subset profile and cytokine producing capacity of these cells. We determined proportion of T and B cell subsets and their cytokine producing capacity in peripheral blood collected from 23 morbidly obese patients before and three months after bariatric surgery using flow-cytometry. We compared this with the results of 25 lean controls. Both CD4+ and CD8+ T cells showed a more differentiated subset profile in morbidly obese patients as compared to lean controls, which was not reversed three months after bariatric surgery. The B cell composition of morbidly obese patients after bariatric surgery adjusted towards the profile of lean controls. However, the IL-2 and IFN-γ producing capacity of CD8+ T cells and the IL-2, IFN-γ, TNF-α and IL-10 producing capacity of B cells was not restored three months after bariatric surgery. In conclusion, the data suggest that the immune system has the capacity to recover from the detrimental effects of morbid obesity after bariatric surgery. The restoration of the immune system after bariatric surgery is a gradual process.

Introduction

Morbid obesity increases the risk for the development of obesity-related comorbidities, such as hypertension, type 2 diabetes mellitus (T2D) and cancer [1 2]. Morbid obesity is defined as a Body Mass Index (BMI) ≥35 kg/m² with the presence of at least one obesity-related comorbidity or a BMI ≥40 kg/m² either with or without the presence of obesity-related comorbidities. Furthermore, morbid obesity is associated with a chronic state of low-grade inflammation [3 4]. The high number of adipocytes in white adipose tissue of morbidly obese individuals secrete pro-inflammatory cytokines (such as tumor necrosis factor-alpha (TNF-α), interferon gamma (IFN-γ) and interleukins (IL-) 2 and 6), which causes this systemic inflammation [5]. This phenomenon is more pronounced in morbidly obese individuals with metabolic syndrome (MetS), which is characterized by dyslipidemia, dysglycemia, an elevated blood pressure and an increased abdominal waist circumference [6 7]. This pro-inflammatory environment may lead to a shift in immune cell composition and immune function in morbidly obese patients as compared to lean individuals [8].

In the adaptive immune system, shifting of the T cell subset composition towards a more differentiated profile has been reported as a consequence of morbid obesity. This shifting typically shows similarities with the T cell composition of elderly individuals [8-10]. A shift towards more differentiated memory T cells, such as terminally differentiated effector memory T cells (T_{EMRA}) and $CD28^{null}$ T cells, has been described [11 12]. These cells produce increased amounts of pro-inflammatory cytokines IFN-γ and TNF-α [13 14], and decreased levels of IL-2 [15].

Similar changes in subset composition have been described in B cell populations, in which a more differentiated B cell profile is seen in morbidly obese individuals [16 17]. An increase in double negative (DN) B cells has been described in morbidly obese individuals, which is comparable to the cell subset composition found in elderly individuals [16 18]. Additionally, a change in B cell function in morbidly obese individuals has been described. Several studies have reported an increased production of pro-inflammatory cytokines such as IL-6, TNF-α and IFN-γ and a decrease in the production of the anti-inflammatory cytokine IL-10 [18 19].

These dysfunctional T and B cells in morbidly obese individuals have several clinical consequences. The enhanced TNF-α and IFN-γ production in morbidly obese individuals leads to insulin resistance, causing a higher risk of T2D development [20-22]. Additionally, as the IL-10 production is decreased in morbidly obese individuals, the IL-10 regulation of insulin sensitivity is lowered. This consequently leads to a higher risk of T2D development as well [23]. Another clinical consequence of the chronic state of low-grade inflammation is the development of cardiovascular pathology. The increased production of TNF-α and IFN-γ by CD4+ and CD8+ T cells leads to atherosclerosis and hypertension, eventually

resulting in cardiovascular diseases [24 25]. Subsequently, the lower levels of IL-2 lead to a decreased regulatory T cell function, which contributes to a persisting inflammation [15]. Moreover, the decreased IL-2 producing capacity by both T and B cells in morbidly individuals leads to a decreased humoral response to vaccinations [18 26].

Bariatric surgery is regarded as an effective treatment for morbid obesity, resulting in significant weight loss and improving, or even resolving, obesity-related comorbidities [27-30]. Only a few studies have been performed to assess the effects of bariatric surgery on the T and B cell function. One study found an increase in IL-10 production by B cells after laparoscopic Roux-en-Y gastric bypass (LRYGB), measured in peripheral blood [31]. Another study found that the T cells did not differ in number after LRYGB, although the cytokine producing capacity of the T cells did change after LRYGB [32]. This resulted in a decreased IFN-γ, IL-2, IL-4 and IL-17 secretion by T cells and an increased IL-10 secretion by B cells. To our knowledge, studies to assess the T and B cell function were performed in very small study populations, results are contradicting, and the cytokine producing capacity of the T and B cells was not compared to that of lean controls.

Therefore, the aim of this study was to investigate the T and B cell composition and cytokine producing capacity of morbidly obese patients and lean controls, and to study the effect of bariatric surgery on T and B cell cytokine producing capacity. Our hypothesis is that bariatric surgery decreases the pro-inflammatory environment and restores the cytokine production by T and B cells to the cytokine production of lean controls.

Methods

Patient selection

Morbidly obese patients who were scheduled for a laparoscopic Roux-en-Y gastric bypass (LRYGB) or laparoscopic sleeve gastrectomy (LSG) between March 2014 and August 2015 in the Maasstad Hospital, Rotterdam, the Netherlands, were invited to participate in this non-randomized prospective cohort study. To be eligible for LRYGB or LSG, patients had to fulfil the criteria for bariatric surgery of the International Federation for the Surgery of Obesity and Metabolic Disorders (IFSO). Patients were excluded if their morbid obesity was caused by genetic defects or if they had previous bariatric surgery in their medical history. All patients gave written informed consent before inclusion.

Blood donors at the Sanquin blood bank were invited to participate in this study as lean, healthy controls. The lean controls were aged between 18 to 65 years. Controls with a BMI > 30 kg/m2 and/or with the presence of metabolic syndrome were excluded from this study. Lean controls were informed about the study and were asked if two blood samples

of 10mL each could be collected for this study. No informed consent was required. Blood samples were obtained between December 2018 and April 2019.

The local medical ethical committee (MEC) approved the study (MEC number: MEC-2018-06 for lean controls and MEC 2012-51 for morbidly obese patients). All participants of this study gave written informed consent. This study was conducted in accordance with the Declaration of Helsinki and the Declaration of Istanbul and in compliance with the International Conference on Harmonization/Good Clinical Practice regulations.

Metabolic syndrome

Metabolic syndrome was defined as the presence of at least three of the following symptoms [6]:

1. Fasting blood glucose ≥ 5.6 mmol/L (100 mg/dL) or drug treatment for elevated blood glucose.
2. HDL cholesterol < 1.0 mmol/L (40 mg/dL) in men, < 1.3 mmol/L (50 mg/dL) in women or drug treatment for low HDL cholesterol.
3. Blood triglycerides ≥ 1.7 mmol/L (150 mg/dL) or drug treatment for elevated triglycerides.
4. Waist circumference ≥ 102 cm for men or ≥ 88 cm for women.
5. Blood pressure ≥ 130 mmHg systolic or ≥ 85 mmHg diastolic or antihypertensive drug treatment.

Surgical procedures

In this study, morbidly obese patients underwent either LRYGB or LSG. In the LRYGB procedure, first a gastric pouch with a volume of 25-30 cm^3 was created using an Endostapler (Medtronic, Minneapolis, MN). Next, a biliopancreatic limb was measured 50 cm distal from the ligament of Treitz and stapled to the gastric pouch with an Endostapler, creating the posterior wall of the gastrojejunostomy. A continuous absorbable suture was used to close the anterior aspect of the gastrojejunostomy. A side-to-side jejunojejunostomy with an alimentary limb of 150 cm was created with an Endostapler and a continuous absorbable suture. Hereafter, a transection between both anastomoses was performed [33]. During LSG, a tubular sleeve was created using a 35 Fr bougie. The greater curvature was dissected starting 4-5 cm from the pylorus and up to the angle of His and was then removed using an endobag [34].

Blood collection

In morbidly obese patients, blood was obtained prior to surgery to determine the immune status. Three months postoperatively, blood collection took place during a routine outpatient clinic visit. Blood was also collected from lean controls during their visit at the

Blood bank. Blood was collected in 10.0 mL BD Lithium-Heparin tubes (Franklin Lakes, NJ, USA), with a maximum of two tubes per time point.

CMV seropositivity

CMV seropositivity is associated with age-related changes in the circulating T cell compartment, such as an increased CD8+ T cell differentiation status and decreased CD4+/ CD8+ T cell ratio [35 36]. To avoid confounding, CMV infection status was assessed in all participants at the diagnostic Department of Virology of Erasmus University Medical Centre by determining the presence of plasma IgG antibodies to CMV using an enzyme immunoassay (Biomerieux, VIDAS, Lyon, France). An outcome of ≥ 6 arbitrary units per mL (AU/mL) was considered positive [37].

PBMCs isolation

Ficoll™ gradient centrifugation was used to isolate peripheral blood mononuclear cells (PBMCs) from heparinized blood samples, as described in detail by Litjens et al [26]. After isolation, samples were stored in liquid nitrogen with $10x10^6$ cells per vial. A vial of PBMCs was thawed at 37°C and added dropwise to a mixture containing 5 mL DNAse medium and 1 mL of normal human serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) afterwards. The suspension was then centrifuged for 5 minutes at 2000 rounds per minute (rpm), after which the supernatant was discarded and the pellet resuspended in 5 mL of DNAse medium and 1mL of human serum. After a second centrifugation and discarding of the supernatant, the pellet was resuspended in 2 mL of HCM (90% RPMI 1640 + 10% humane serum (Gibco)). Cells were then incubated overnight at 37°C and 5% CO_2 to allow the cells to recover. Following an overnight recovery, cells were centrifuged and the remaining pellet was suspended in 3 mL of HCM and the number and viability of cells were assessed. PBMCs were brought to a concentration of $2x10^6$ cells/ml.

Assessment of maximal T and B cell cytokine producing capacity

Maximal cytokine producing capacity was assessed for T and B cells by stimulating $1x10^6$ cells/mL PBMCs with a cocktail of phorbol myristate acetate (PMA, 50 ng/mL, Sigma Aldrich, St. Louis, MO, USA) and ionomycin (1 µM, Sigma Aldrich) for 5 hours, of which the last 4 were in presence of the cytokine secretion inhibitor monensin (Golgistop, BD, Erembodegem, Belgium). To control for spontaneous cytokine production, PBMCs were left unstimulated. Cytokine producing capacity was analyzed separately for T cells and B cells.

After stimulation, frequencies of cytokine producing cells were visualized using a modification of flow cytometric based assay [38]. Briefly, the cell surface was stained using antibodies directed to T and B cells and including 7-AAD, a marker to exclude dead cells (Supplementary Table 1); upon fixation and permeabilization, cytokines were stained intracellular using antibodies directed to IL-10, TNF-α, IFN-γ and IL-2 (BioLegend;

Supplementary Table 2). Cytokine producing cells were determined by measuring the samples on a BD FACSCanto II (BD) using FACSDiva software version 8 (BD). Analysis of the data was performed using Kaluza Analysis Software version 2.1 (Beckman Coulter, Indianapolis, USA) in order to determine the percentages of T and B cell subsets and frequencies of cytokine producing cells. Representative images of the gating strategies of flow cytometry analyses are shown in Supplementary Figure 1.

Table 1. Study population characteristics

	Lean controls (n=25)	**Morbidly obese patients (n=23)**		**P-value**
		Preoperatively	**Postoperatively**	
Age (median and range, in years)	29 [25-37]	40 [31-55]		0.010
Weight (median and range, in kg)	73 [68-82]	129 [114.9-140]	106 [90.5-113.8]	<0.001
BMI (median and range, in kg/m²)	23.7 [22.4-24.6]	43.4 [38.5-47.9]	34.0 [30.2-37.7]	<0.001
Presence of MetS (number, %)	0 (0%)	12 (52.2%)		<0.001
CMV seropositivity (number, %)	12 (48%)	12 (52.2%)		0.733

BMI = body mass index; MetS = metabolic syndrome; CMV = cytomegalovirus

Statistical analysis

Baseline characteristics are reported using descriptive statistics. Comparisons between the three groups (lean controls, morbidly obese patients preoperatively, and morbidly obese patients three months postoperatively) were performed using Pearson's chi – square test for categorical data, Mann Whitney-U test for unpaired continuous data and the Wilcoxon matched-pairs signed rank test for paired continuous data. The Dirichlet multinomial mixed model was used for statistical analysis of cell subset composition in percentages and frequencies of cytokine producing capacity [39]. Additionally, the effects of covariates were investigated by including interactions for cell type and covariates age, BMI, CMV (yes/no) and MetS (yes/no). BMI was centered at the medians of the respective groups to allow for selective adjustment of within-group differences only. Thus, effects due to between-group differences in BMI were captured by the indicator for group. Age was centered at the overall median to allow for easier interpretation of the coefficients. The dispersion parameter was modeled as a function of the expected mean. Significance of differences in cell counts was tested by multivariate Wald tests in a sequential fashion. Statistical analysis was performed using Stata version 16.0 (StataCorp, Texas, USA) or R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). Figures were made using Stata version 16.0 (StataCorp, Texas, USA). A two-sided P-value <0.05 was used to indicate statistical significance.

Results

Baseline characteristics

Forty-eight participants were included in this study, consisting of 23 morbidly obese patients and 25 lean controls. Twenty-one morbidly obese patients underwent LRYGB and two underwent LSG. Baseline characteristics are shown in Table 1. Twelve (52.2%) morbidly obese patients were clinically diagnosed with MetS preoperatively. The age of the morbidly obese patients was significantly higher than the lean controls (*P*=0.010). Additionally, both weight and BMI of the morbidly obese patients was significantly higher at both measurement points as compared to lean controls (*P*<0.001 for weight and *P*<0.001 for BMI). There was a significant decrease in both weight and BMI three months after bariatric surgery in morbidly obese patients (P<0.001). There was no significant difference in CMV seropositivity between lean controls and morbidly obese patients (P=0.733).

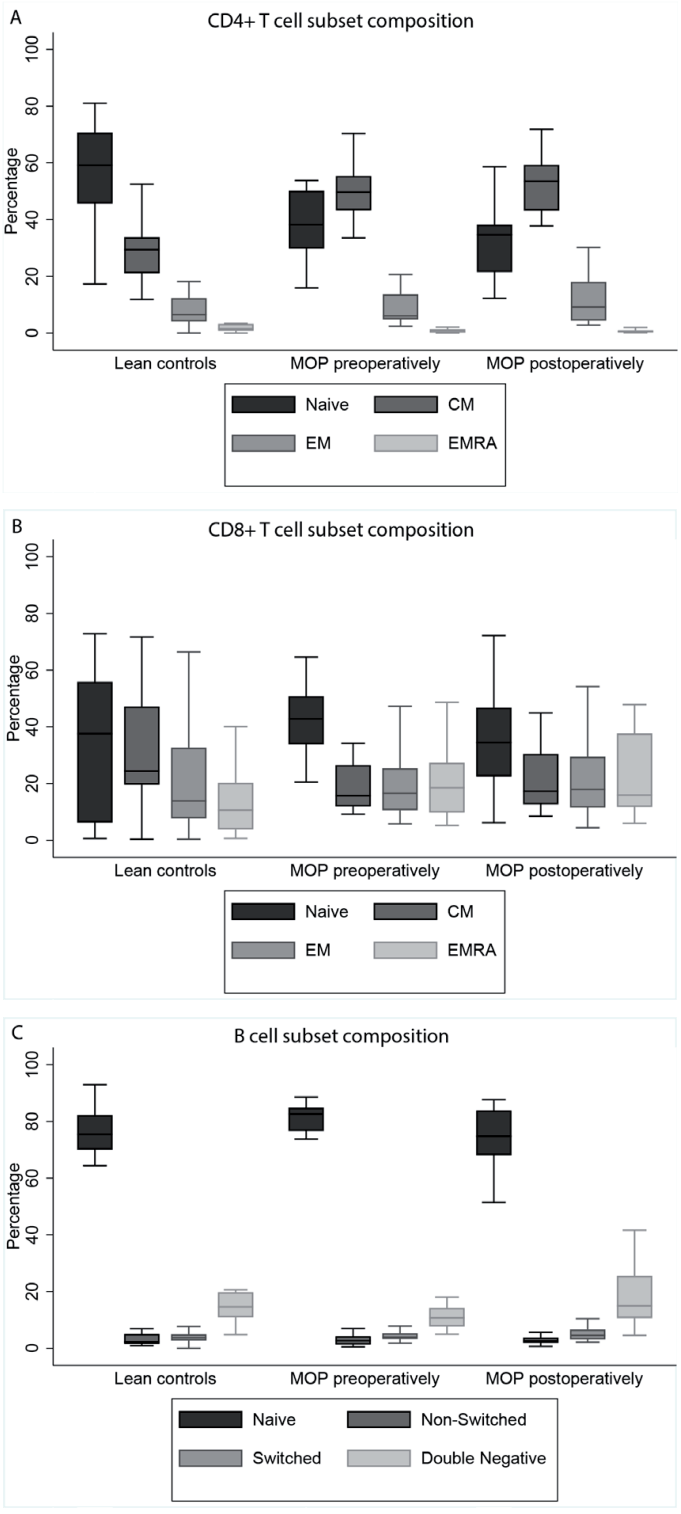
Morbid obesity decreases the total IL-2 producing capacity of CD8+ T cells and the single IL-2 producing capacity of B cells

A statistical difference in CD4+ T cell subset composition was found between lean controls and morbidly obese patients before bariatric surgery (*P*<0.001). Data are depicted in Supplementary Table 3a. A decrease in naive CD4+ T cells and $T_{EMRA,}$ and an increase in T_{CM} was observed in morbidly obese patients before bariatric surgery as compared to lean controls, Figure 1A. The total cytokine producing capacity of these cells was not significantly different between the lean controls and morbidly obese patients, Table 2a.

The CD8+ T cell subset composition was also significantly different between the lean controls and morbidly obese patients preoperatively (*P*<0.001). This was reflected as an increase in naive CD8+ T cells and T_{EM} and T_{EMRA}, and a decrease in the percentage of T_{CM}, Figure 1B. In contrast to the CD4+ T cells, the total cytokine producing capacity of CD8+ T cells was significantly lower in the morbidly obese patients as compared to lean controls (*P*<0.001), Table 2a. This was seen in both single IL-2 and single IFN-γ producing CD8+ T cells.

The subset composition of B cells was also significantly different (*P*=0.005). The morbidly obese patients had a significantly higher percentage of naive B cells, while the percentage of DN B cells was decreased, Figure 1C. There was a difference in the percentages of cytokine producing cells (*P*<0.001). The single IL-2 and single IL-10 production was increased in morbidly obese patients, while the single TNF-α production was decreased, Table 2b. The single IFN-γ producing capacity was relatively the same between the two different groups.

Figure 1. T and B cell subset compositions



Morbid obesity causes a significant decrease in percentage of naive CD4+ T cell (**A**). The CD8+ T_{EMRA} cells were significantly higher in morbidly obese patients, both preoperatively and postoperatively (**B**). Both percentages of non-switched and switched B cells were increased in morbidly obese patients (**C**). Percentages and significances are depicted in Supplementary Table 3a.
MOP = morbidly obese patients; CM = central memory; EM = effector memory; EMRA = terminally differentiated effector memory

Metabolic syndrome increases the total cytokine producing capacity of CD4+ T cells and decreases the single IL-2 producing capacity of B cells

The effect of covariates was analyzed between the three study groups. It showed that the presence of CMV seropositivity led to a decrease in total cytokine producing capacity of the CD8+ T cells (*P*=0.003). Metabolic syndrome led to an increase in total IFN-γ and IL-2 producing capacity of CD4+ T cells (*P*=0.044). Additionally, the presence of MetS led to an increase in single TNF-α production by B cells, while it decreased the IL-2 producing capacity of B cells (*P*=0.009). An increased BMI led to a decrease in single IL-2 cytokine production by CD4+ T cells (*P*<0.001) and B cells (*P*=0.021). Furthermore, the single TNF-α (*P*=0.021), single IL-10 and single IFN-γ (both *P*=0.005) producing capacity of B cells was decreased when BMI was increased. Age only influenced the cytokine producing capacity of T cells, where an increased age led to an increase in single IFN-γ production by both CD4+ (*P*<0.001) and CD8+ (*P*=0.030) T cells.

Bariatric surgery does not improve the cytokine producing capacity of T and B cells

After bariatric surgery, the CD4+ T cell subset composition significantly changed (*P*=0.013). This difference was seen as a decrease of the percentage naive CD4+ T cells, while the percentages of T_{EM}, T_{CM} and T_{EMRA} cells increased. However, the subset composition was not reversed towards the that of lean controls. Notably, bariatric surgery increased the single and total IL-2 and IFN-γ producing capacity of the CD4+ T cells in morbidly obese patients, which was comparable to the cytokine producing capacity of the lean controls (*P*=0.103).

The CD8+ T cell composition was not influenced by bariatric surgery (*P*=0.186). Particularly, the CD8+ T cell subset composition after bariatric surgery remained significantly different as compared to the lean controls (*P*=0.019). In contrast to the CD4+ T cell cytokine producing capacity, the CD8+ T cell cytokine producing capacity was not altered by bariatric surgery (*P*=0.094). However, there was a slight increase in single IFN-γ producing CD8+ T cells and a decrease of single IL-2 producing CD8+ T cells after bariatric surgery as compared to the lean controls.

After bariatric surgery, the B cell subset composition adjusted towards the profile of lean controls, Supplementary Table 3b. Even though B cell compartment was reversed by bariatric surgery, the cytokine producing capacity was not reversed after bariatric surgery. When comparing the cytokine producing capacity of morbidly obese patients after bariatric surgery with the lean controls, it remains significantly different; this was comparable to the difference between morbidly obese patients before bariatric surgery and lean controls, Table 2b.

Table 2a. Maximal frequencies of cytokine producing capacity by T cells

T cell subtype	Specific cytokine producing subset	Lean controls (n=25)	Morbidly obese patients (n=23)		P-value		
			Preoperatively	Postoperatively	LC vs MOP preoperatively	MOP preoperatively vs MOP postoperatively	LC vs MOP postoperatively
CD4+					0.851	<0.001	0.103
	Single IL-2	12.5 [9.0-20.0]	12.8 [7.6-16.6]	17.1 [9.5-22.3]			
	Single IFN-γ	6.1 [4.3-8.8]	5.1 [4.3-7.8]	7.3 [5.4-10.7]			
	IL-2 and IFN-γ	5.2 [2.6-11.6]	4.8 [3.0-8.1]	9.0 [6.3-13.5]			
CD8+					<0.001	0.094	0.003
	Singe IL-2	4.1 [2.6-8.1]	2.8 [2.0-5.8]	3.1 [1.7-5.0]			
	Single IFN-γ	32.4 [23.4-40.5]	29.8 [20.4-35.8]	37.3 [24.9-49.2]			
	IL-2 and IFN-γ	6.2 [2.9-8.8]	3.6 [1.4-5.2]	4.0 [1.8-8.5]			

MOP = morbidly obese patients; LC = lean controls; IL-2 = interleukin 2; IFN-γ = interferon gamma
All numbers are presented as percentages of cytokine producing cells in median [interquartile range]. *P*-values are after correction for covariates using a Dirichlet multinomial mixed model.

Table 2b. Maximal cytokine producing capacity by B cells

Specific cytokine producing B cell subset	Lean controls (n=25)	Morbidly obese patients (n=23)		P-value		
		Preoperatively	Postoperatively	LC vs MOP preoperatively	MOP preoperatively vs MOP postoperatively	LC vs MOP postoperatively
				<0.001	0.108	<0.001
Single TNF-α	21.9 [15.5-32.3]	14.8 [11.4-21.0]	20.2 [13.5-26.4]			
Single IL-2	1.2 [0.8-1.7]	3.4 [2.1-5.2]	4.4 [2.8-6.6]			
TNF-α and IL-2	1.27 [1.0-2.5]	1.6 [1.0-2.4]	2.2 [1.1-3.0]			
				<0.001	0.183	<0.001
Single IL-10	0.5 [0.3-1.0]	2.8 [1.9-3.6]	2.9 [2.4-4.3]			
Single IFN-γ	2.6 [1.9-6.6]	2.3 [1.3-2.9]	2.0 [1.2-2.9]			
IL-10 and IFN-γ	0.8 [0.5-1.3]	1.0 [0.7-2.4]	1.2 [0.7-3.3]			

MOP = morbidly obese patients; LC = lean controls; TNF-α = tumor necrosis factor-alpha; IL = interleukin 2; IFN-γ = interferon-gamma
All numbers are presented as percentages of cytokine producing cells in median [interquartile range]. *P*-values are after correction for covariates using a Dirichlet multinomial mixed model.

Discussion

In this study, we compared a cohort of morbidly obese patients to that of lean controls, and evaluated the effects of bariatric surgery with respect to composition and cytokine producing capacity of T and B cells. The main findings include a shift towards a more differentiated CD4+ and CD8+ T cell compartment in morbidly obese patients as compared to lean controls; three months after bariatric surgery, this had not changed towards the profile of lean controls. The IL-2 and IFN-γ producing capacity of CD8+ T cells was significantly decreased by morbid obesity, which was not influenced by bariatric surgery. The B cell subset composition of morbidly obese patients adjusted towards the profile of lean controls three months after bariatric surgery. Nonetheless, the cytokine producing capacity of these cells was not reversed by bariatric surgery.

The decrease in naive CD4+ T cells in morbidly obese patients is similar to previously described findings in mice [10 14]. The chronic inflammation in morbid obesity might lead to accelerated aging of the immune system [40 41]. In our study, we found an increase in T_{CM} CD4+ T cells, while the T_{EM} CD4+ T cells seemed unaffected. Although the findings were significantly different, the clinical implication is debatable as the difference was just one percent. In contrast to our study, a study performed in morbidly obese individuals described an increase in both naive and memory T cells [9]. In this study, this increase in naive T cells was explained as a reaction to the antigenic load. An alternative explanation for the increase of naive T cells is that the thymic production of T cells is not affected by morbid obesity.

We found an increase in naive B cells, which has been described before and can be explained by the chronic inflammation in morbidly obese patients [18 42]. This chronic inflammation causes mobilization of developing B cells from the bone marrow into peripheral immune organs and peripheral blood. There was a decrease in DN B cells, which is in contrast to what we expected. We expected that the chronic low-grade inflammation in morbidly obese patients would lead to an increase in the more differentiated DN B cells [18]. A possible explanation for this finding is that there was such a large increase of naive B cells, that the DN B cells in the B cell composition in percentages decreased. Nevertheless, much remains unclear about the origins of the differences in B cell subset composition, and further research into this topic is therefore recommended.

Jongbloed et al. described enhanced CD8+ T cell differentiation in morbidly obese patients, which was mainly related to the presence of MetS [37]. The CD8+ T cell differentiation state was comparable to what we found, however, CD8+ T cell differentiation was not affected by MetS. These different findings can be explained by the larger population of morbidly obese patients and thus bigger subpopulations. Even so, the presence of MetS seemed to

increase the IFN-γ and IL-2 producing capacity of CD4+ T cells and the TNF-α producing capacity of B cells, and to decrease the IL-2 producing capacity of B cells. Bariatric surgery had an effect on the producing capacity of CD4+ T cells, but not on that of CD8+ T cells and B cells. In literature, it is suggested that changes in cellular immunity after weight loss is linked to metabolic improvement [43]. As our follow-up period was three months, not all patients with MetS prior to surgery might have recovered from this during the short follow-up period. This might explain why the cytokine producing capacity of CD8+ T cells and B cells was not restored in our study.

We found an increase in IL-2 and IFN-γ secretion by CD4+ T cells. This is in contrast with an earlier published article by Zhan et al., in which follicular helper T cells were identified [32]. Three months after LRYGB, the follicular helper T cells had an altered function, resulting in a decrease in IFN-γ, IL-2, IL-4 and IL-17 secretion and an increase of IL-10 secretion. This study included a total of eight patients and all patients were diagnosed with T2D, whereas only six patients (26%) in our study were diagnosed with T2D preoperatively. Lips et al. showed an increase in TNF-α secretion by T cells three months after LRYGB [44]. However, they have not investigated the B cell cytokine producing capacity.

One of the limitations of this study is the follow-up period of three months. Although weight loss follows rapidly after bariatric surgery, the total expected excess weight loss after bariatric surgery is typically achieved after twelve to eighteen months [45]. Moreover, the immune system might need a longer period to recover from the alterations caused by morbid obesity. In a previous study, a decrease in CD8+ T_{EM} cells was found three months after bariatric surgery, while a decrease of CD4+ T_{EM} and T_{EMRA} was found six months after bariatric surgery [37]. These findings indicate that alterations of the immune system caused by morbid obesity could be restored after bariatric surgery, but that some changes are reached after a longer period of time. We would therefore suggest additional research into the effect of bariatric surgery on the immune system for a follow-up period of at least eighteen months. Another limitation of this study is the study population. Although our study is larger than most other studies [31 32], a larger cohort could lead to a better distinction between the observed differences between the study groups. By this, the influence of MetS on the immune system can be investigated more thoroughly. As an increased age leads to a more differentiated subset composition, age-matched controls are recommendable. As we received the blood collections from the Sanquin blood bank, we could not obtain samples in the exact same age. However, we performed a mixed model analysis with correction for age, and age only influenced the cytokine producing capacity of T cells. Besides this, it would be interesting to compare the postoperative data with lean controls who have undergone surgery as well. Furthermore, the analysis of the immune system in this study was performed on lymphocytes from the peripheral blood. Some studies have reported effects of morbid obesity in T and B cells in adipose tissue,

such as an increase of proinflammatory cytokine production by adipose-resident T cells and a decrease of IL-10 production by B cells [14 46]. It would therefore be interesting to compare the T and B cell subset composition and function in both peripheral blood and the adipose tissue. In the present study, we have only studied the cytokine producing capacity of six cytokines. It would be interesting to expand the studied cytokines. Furthermore, it would be interesting to investigate the vaccination response of morbidly obese patients before and after bariatric surgery, as this is indicative for the quality of the immune system.

Our data suggest accelerated differentiation of CD4+ and CD8+ T cells in morbidly obese patients as compared to lean controls. Even though this did not influence the cytokine producing capacity of CD4+ T cells, the IL-2 and IFN-γ production of CD8+ T cells was decreased in morbidly obese patients. Bariatric surgery changed CD4+ T cell and B cell subset composition towards the profile of lean controls, and the IL-2 and IFN-γ producing capacity of CD4+ T cells was increased three months after bariatric surgery. However, the cytokine producing capacity of CD8+ T cells and B cells was not restored three months after bariatric surgery. A longer follow-up period after bariatric surgery is recommended as the immune system might need more than three months to recover from immune changes caused by morbid obesity, as well as patients might need more time to recover from MetS.

Altogether, these data suggest that the immune system has the capacity to recover from the detrimental effects of morbid obesity after bariatric surgery, but full restoration may take more than three months.

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Supplementary Material

Supplementary Table 1. Antibodies for surface staining

T cells				
Antibody	**Clone**	**Dilution**	**Titer/100 µl cell suspension**	**Firm**
CD3 BV510	OKT3	1:10	10 µl	Biolegend
CD4 PacBlue	RPA-T4	1:40	10 µl	Biolegend
CD8 APC-Cy7	SK1	1:40	10 µl	Biolegend
CD45RO APC	UCHL1	1:10	10 µl	Biolegend
CCR7 PE-Cy7	G043H7	Undiluted	5 µl	Biolegend
7AAD		Undiluted	5 µl	Biolegend
B cells				
Antibody	**Clone**	**Dilution**	**Titer/100 µl cell suspension**	**Firm**
CD19 BV510	HIB19	Undiluted	5 µl	Biolegend
CD27 PE-Cy7	O323	1:40	10 µl	Biolegend
IgD APC-Cy7	IA6-2	1:10	10 µl	Biolegend
CD38 BV421	HIT2	1:10	10 µl	BD
CD24 APC	ML5	Undiluted	2 µl	Biolegend
7AAD		Undiluted	5 µl	Biolegend

Supplementary Table 2. Antibodies for intracellular staining

T cells				
Antibody	**Clone**	**Dilution**	**Titer/100 µl cell suspension**	**Firm**
IL2 FITC	MQ1-17H12	1:10	10 µl	Biolegend
IFN-γ PE	B27	1:20	10 µl	Biolegend
B cells mix 1				
Antibody	**Clone**	**Dilution**	**Titer/100 µl cell suspension**	**Firm**
IL2 FITC	MQ1-17H12	1:10	10 µl	Biolegend
TNF-α PE	MAb11	Undiluted	2 µl	Biolegend
B cells mix 2				
Antibody	**Clone**	**Dilution**	**Titer/100 µl cell suspension**	**Firm**
IL10 PE	JES3-9D7	Undiluted	5 µl	Biolegend
IFN-γ FITC	4S.B3	Undiluted	5 µl	Biolegend

Supplementary Table 3. T and B cell subset compositions in percentages

Supplementary Table 3a. T cell subsets in percentages

T cell subtype	Lean controls (n=25)	Morbidly obese patients (n=23)		P-value		
		Preoperatively	Postoperatively	LC vs MOP preoperatively	MOP preoperatively vs MOP postoperatively	LC vs MOP postoperatively
CD4+ composition				<0.001	0.013	<0.001
CD4+ naive	59.2 [45.7-70.7]	38.2 [29.8-50.2]	34.7 [21.5-38.3]			
CD4+ CM	29.4 [21.1-33.8]	49.7 [43.3-55.4]	53.5 [43.2-59.3]			
CD4+ EM	6.5 [4.1-12.3]	6.1 [4.8-13.7]	9.2 [4.5-18.0]			
CD4+ EMRA	1.6 [0.9-3.3]	0.4 [0.2-1.3]	0.5 [0.2-1.0]			
CD8+ composition				<0.001	0.186	0.019
CD8+ naive	37.6 [6.3-55.9]	42.8 [33.8-50.8]	34.5 [22.5-46.8]			
CD8+ CM	24.2 [19.6-47.2]	15.7 [12.0-26.5]	17.2 [12.7-30.4]			
CD8+ EM	13.9 [7.8-32.6]	16.6 [10.6-25.4]	17.9 [11.6-29.4]			
CD8+ EMRA	10.6 [3.9-20.3]	18.5 [9.8-27.3]	15.9 [11.8-37.7]			

MOP = morbidly obese patients; LC = lean controls; CM = central memory; EM = effector memory; EMRA = terminally differentiated effector memory.

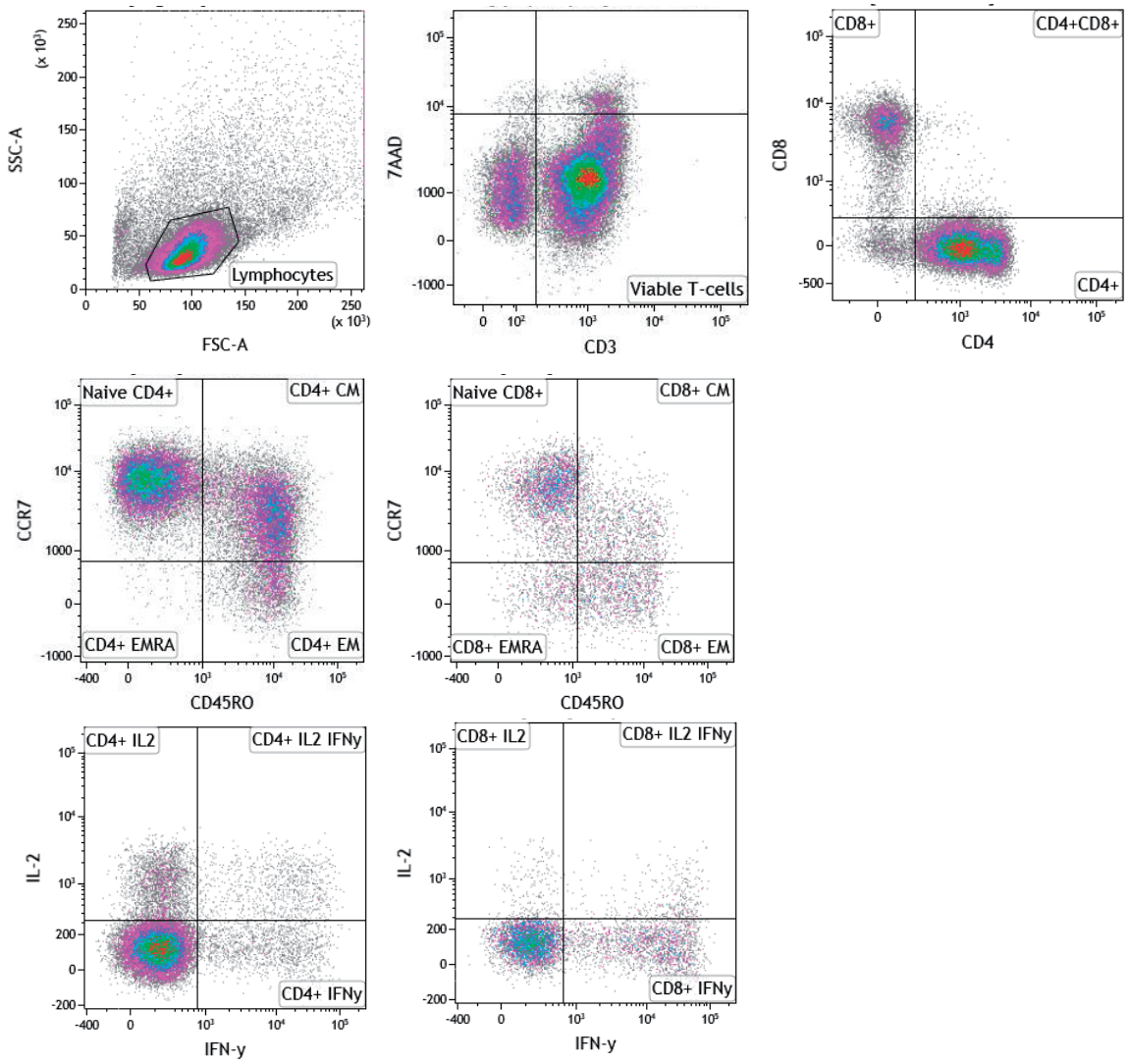
All percentages are presented as median [interquartile range]. *P*-values are after correction for covariates using a Dirichlet multinomial mixed model.

Supplementary Table 3b. *B* cell subsets in percentages

B cell subtype	Lean controls (n=25)	Morbidly obese patients (n=23)		P-value		
		Preoperatively	Postoperatively	LC vs MOP preoperatively	MOP preoperatively vs MOP postoperatively	LC vs MOP postoperatively
Composition				0.005	<0.001	0.095
Naive	75.4 [70.0-82.2]	82.6 [76.6-84.8]	74.7 [68.1-83.8]			
Non-switched	2.2 [1.5-5.0]	2.6 [1.3-4.3]	2.5 [1.9-3.7]			
Switched	3.7 [2.7-4.9]	4.0 [3.3-5.3]	4.5 [3.1-6.6]			
Double negative	14.6 [10.9-19.7]	10.7 [7.7-14.2]	14.9 [10.6-25.5]			

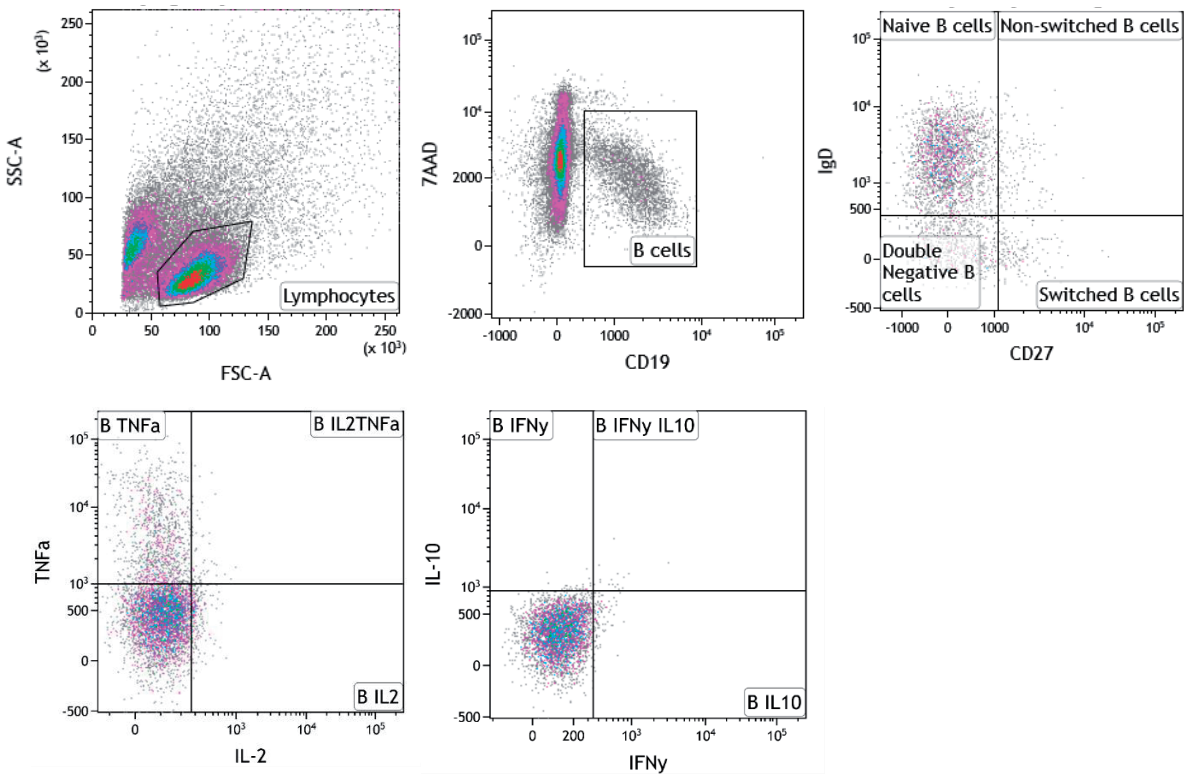
MOP = morbidly obese patients; LC = lean controls
All percentages are presented as median [interquartile range]. *P*-values are after correction for covariates using a Dirichlet multinomial mixed model.

Supplementary Figure 1. Typical examples of the flow cytometric gating strategies

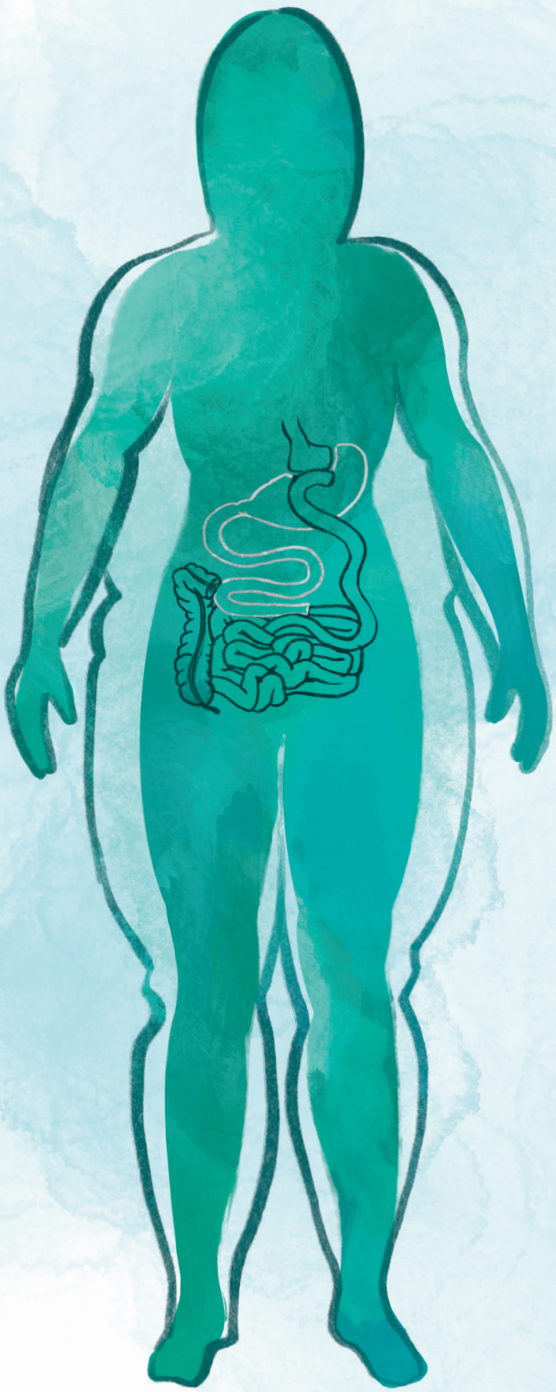


A. Flow cytometric gating strategy for CD4+ and CD8+ T cell subsets and frequencies of cytokine producing cells

First, lymphocytes were gated and then viable CD3+ T cells were selected. From these viable T cells, CD4+ or CD8+ T cells were selected. The CD4+ and CD8+ T cells were then subdivided into the main T cell subsets based on the expression of CCR7 and CD45R0, with $CD45R0^{-}CCR7^{+}$ being naive T cells, $CD45R0^{+}CCR7^{+}$ central memory T cells (CM), $CD45R0^{+}CCR7^{-}$ effector memory (EM) and $CD45R0^{-}CCR7^{-}$ terminally differentiated effector memory (EMRA) T cells. Additionally, CD4+ and CD8+ T cells were subdivided based on the expression of IL-2 and/or IFN-γ.

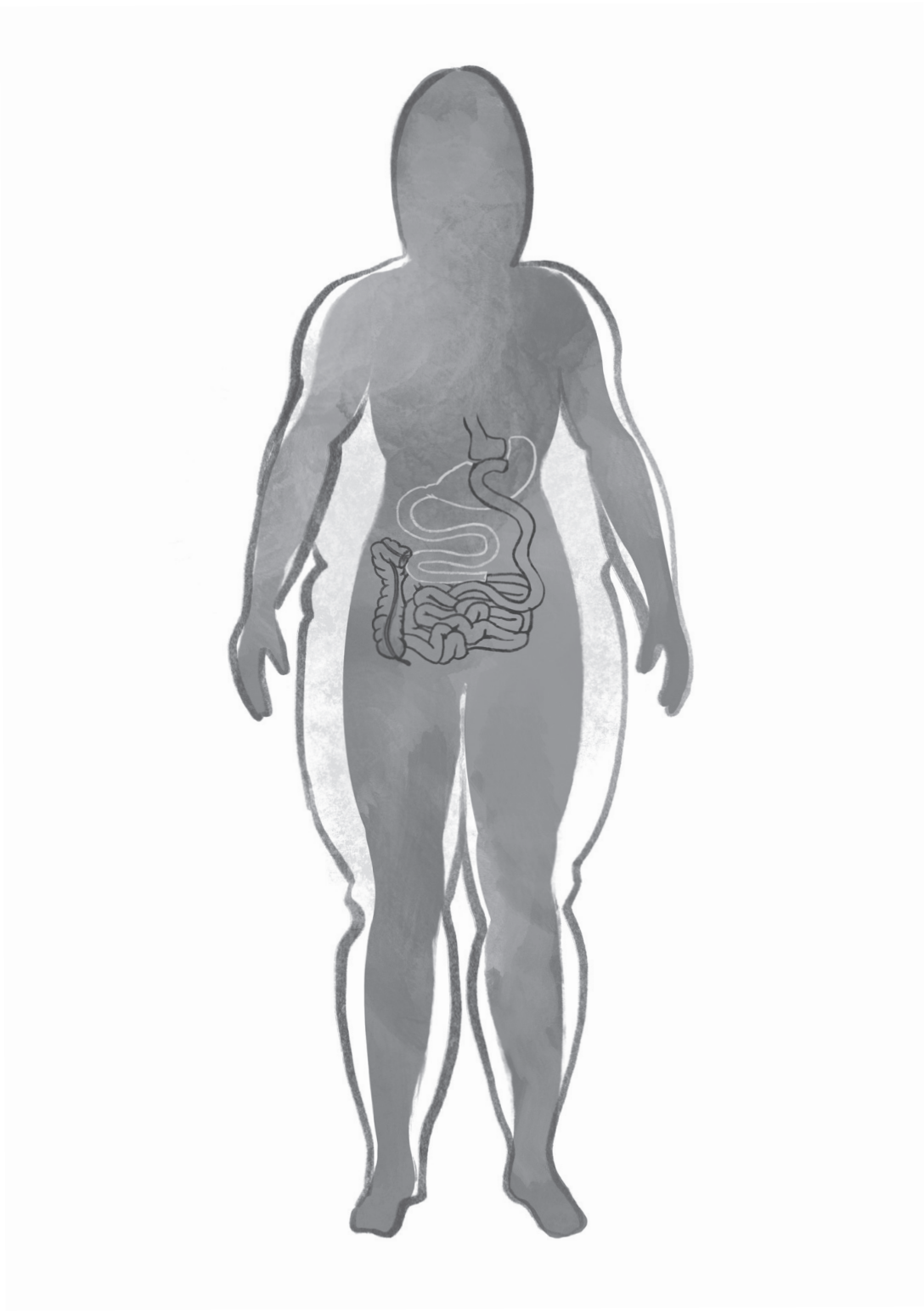


B. Flow cytometric gating strategy for B cell subsets and frequencies of cytokine producing cells
First, lymphocytes were gated and then viable CD19+ B cells were selected. These B cells were then subdivided into B cell subsets based on the expression of CD27 and IgD, with CD27-IgD+ being naive, CD27+IgD+ non-switched, CD27+IgD- switched and CD27-IgD- double negative (DN) B cells. Furthermore, B cells were subdivided based on the expression of TNF-α and/or IL-2 and of IFN-γ and/or IL-10.



Part III

Long-term complications after bariatric surgery



Chapter 6

Impact of initial response of laparoscopic adjustable gastric banding on outcomes of revisional laparoscopic Roux-en-Y gastric bypass for morbid obesity

L.H. Wijngaarden, F.H.W. Jonker, J.W. van den Berg, C.C. van Rossem, E. van der Harst, R.A. Klaassen

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Abstract

Background

Failed laparoscopic adjustable gastric banding (LAGB) can be converted to laparoscopic Roux-en-Y gastric bypass (LRYGB), which is currently the gold standard for bariatric surgery. Revisional LRYGB (rLRYGB) is associated with inferior results compared to primary LRYGB (pLRYGB), but the exact influence of the initial response to LAGB is unclear.

Objectives

To compare follow-up outcomes after pLRYGB with rLRYGB in nonresponders of LAGB and rLRYGB in responders of LAGB.

Setting

General-community teaching hospital, Rotterdam, the Netherlands

Methods

All patients who underwent pLRYGB and rLRYGB after LAGB were reviewed in an observational study. Postoperative outcomes, excess weight loss (%EWL), total weight loss (%TWL), success and failure rate were compared in patients after pLRYGB and rLRYGB (both responders and nonresponders of LAGB) at 12, 24 and 36 months.

Results

285 primary patients, 96 nonresponders and 120 responders were included. The median follow-up was 33.9±18.0 months. After 36 months, the mean %EWL was significantly lower in the nonresponding group compared to the responding and primary groups (48.1% versus 58.2% versus 72.8%, $P < .001$), %TWL showed the same trend. The success rate was 38.2% versus 61.0% versus 81.6% respectively, $P < .001$. The failure rate was significantly higher after rLRYGB compared to pLRYGB (10.9% nonresponders, 8.5% responders and 2.5% primary, $P = .001$).

Conclusions

Nonresponders of LAGB show inferior weight loss results after rLRYGB compared to responders of LAGB and pLRYGB at all moments of follow-up.

Introduction

Due to increasing rates of obesity worldwide, bariatric surgery is being performed more often and has been established as the primary treatment for morbid obesity[1]. Bariatric surgery has been shown to be a safe and successful treatment for reduction of weight and associated comorbidities[2-4]. As long term results of laparoscopic adjustable gastric banding (LAGB) showed disappointing results with failure rates of up to 51%, laparoscopic Roux-en-Y gastric bypass (LRYGB) has become the standard of care in bariatric surgery[5-9]. Failure of LAGB is defined as insufficient excess weight loss (EWL<25%), dilatation of the pouch or distal esophagus, or band related problems[10]. Surgical options after LAGB failure are a revision or removal of the LAGB or conversion to LRYGB or laparoscopic sleeve gastrectomy (LSG).

Revisional LRYGB (rLRYGB) after LAGB is safe and typically results in 57-65% excess weight loss (%EWL)[11-16], which is inferior to the results of primary LRYGB (pLRYGB)[13 17-20]. Potential causes of failure of rLRYGB may be both operative, as well as patient related. However, obvious reasons of these inferior outcomes remain unclear.

The initial response in weight loss after LAGB could be a possible patient related cause of failure of rLRYGB. Nonresponders to LAGB, defined as insufficient weight loss after LAGB, hypothetically also have a lower %EWL after rLRYGB than patients with gastric band complications but who did have an adequate response in weight loss after LAGB.

The aim of this study was to compare outcomes after rLRYGB of LAGB nonresponders to LAGB responders and pLRYGB.

Methods

Patient selection

Patients who underwent a primary or revisional laparoscopic Roux-en-Y gastric bypass for morbid obesity according to the IFSO criteria from January 2009 to December 2013 in our hospital were included in this observational cohort study. Indications for rLRYGB were gastric band slippage, band erosion, band leakage, obstruction symptoms, pouch formation, insufficient weight loss or weight regain.

Patients undergoing revisional surgery after a previous sleeve gastrectomy or Masons procedure were excluded. Patients were divided into three subgroups: pLRYGB, rLRYGB after nonresponding of LAGB (<25 %EWL), and rLRYGB after (initially) responding of LAGB.

Surgical procedure

All procedures were performed by bariatric surgeons or experienced trainees supervised by a bariatric surgeon.

First, a gastric pouch of 25 cc was created using an endostapler. A biliopancreatic limb was measured 50 cm from Treitz and stapled to the gastric pouch with an endostapler, creating the posterior wall of the side-to-end anastomosis. The anterior aspect of the gastrojejunostomy was closed with a continuous, absorbable suture. A side-to-side jejunojejunostomy with a 150 cm-alimentary limb was created using an endostapler and a continuous, absorbable suture. A transection between both anastomoses of the jejunum was performed.

Revisional LRYGB was performed similar to a primary procedure.

One- or two-step approach

A 1-step approach with removal of the LAGB combined with revisional LRYGB was only performed in patients without band-related complications such as pouch formation and only when few adhesions were present intraoperative. In all other cases, a 2-step approach was performed with removal of the LAGB and rLRYGB in a second procedure after several weeks.

End points and statistical analysis

The % excess weight loss (%EWL), % total weight loss (%TWL), Body Mass Index (BMI) loss, success rate and failure rate of 12, 24 and 36 months after LRYGB were compared between pLRYGB patients, responders and nonresponders to LAGB. The %EWL was calculated based on the initial weight and the ideal body weight as the equivalent to a BMI of 25 kg/m^2. Success was defined as EWL ≥ 50% and failure was defined as <25% EWL.

Statistical analysis was performed with IBM SPSS Statistics, version 23 (SPPS, Chicago, IL). The ANOVA test was used for continuous data analysis; the Chi square test with z-test and Bonferroni correction was used for categorical data analysis. Complications and re-interventions during follow-up were compared using the Kaplan-Meier with log rank test. A P value < .05 was considered significant. Missing data was addressed with case-wise deletion in follow-up analysis.

Results

Patient selection

A total of 1501 patients were identified including 1285 pLRYGB (85.6%), 96 nonresponders to LAGB (6.4%) and 120 responders to LAGB (8.0%). The initial BMI (before LAGB) was significantly higher in the nonresponding group compared to both pLRYGB and the responder group (46.5 versus 43.7 versus 44.3 kg/m^2, P < .001). Responders underwent

significantly more often a 1-step revision than the nonresponder group (91.7% versus 71.9%, $P < .001$). Baseline characteristics are shown in table 1.

Postoperative outcomes

A total of 128 (8.5%) patients had an early complication of which 18.8% was defined as severe (table 2). One patient died 20 days after rLRYGB because of pulmonary embolism. In 18 patients (1.2%) a reoperation was performed within 30 days postoperative. The main reasons for early reoperations were bleeding (8 patients), anastomotic leak (4 patients), abscess drainage (2 patients) and internal herniation (2 patients). There was no difference in re-interventions between the three groups. Pouch dilatation as indication for revision of the gastrojejunostomy was significantly more frequent in the nonresponder group compared to the responders and pLRYGB, (7.5% versus 2.1% versus 0.7%, $P < .001$).

Table 1. Baseline characteristics of pLRYGB and rLRYGB

Characteristics	pLRYGB (n = 1285)	Nonresponders (n = 96)	Responders (n = 120)	P value
Gender				.663
Female	1054(82.0%)	81 (84.4%)	104 (86.7%)	
Male	213 (18.0%)	15 (15.6%)	16 (13.3%)	
Mean age (years)	40.4 ±10.0	42.4 ±8.4	41.5 ±8.8	.226
ASA classification				.985
2	1222(95.2%)	92 (95.8%)	115 (95.8%)	
3	61 (4.8%)	4 (4.2%)	5 (4.2%)	
BMI (kg/m²)				
Initial	43.7 ±4.7	46.5 ±5.8	44.3 ±5.7	< .001
Prerevisional		43.7 ±6.1	39.5 ±5.7	< .001
Comorbidities				
T2D	250 (19.5%)	14 (14.6%)	14 (11.7%)	.176
HT	407 (31.7%)	23 (24.0%)	34 (28.3%)	.270
HC	187 (14.6%)	13 (13.5%)	13 (10.8%)	.721
OSAS	124 (9.6%)	5 (5.2%)	2 (1.7%)	.012

pLRYGB=primary laparoscopic Roux-en-Y gastric bypass; rLRYGB=revisional laparoscopic Roux-en-Y gastric bypass; ASA = American Society of Anesthesiologists; T2D = type 2 diabetes mellitus; HT = hypertension; HC = hypercholesterolemia; OSAS = obstructive sleep apnea syndrome.

Table 2. Operative characteristics and postoperative outcomes after pLRYGB and rLRYGB

Characteristic	pLRYGB (n = 1285)	Nonresponders (n = 96)	Responders (n = 120)	P value
Revision				
Two-step approach		69 (71.9%)	110 (91.7%)	< .001
Laparoscopic	1284 (99.9%)	95 (99.0%)	117 (97.5%)	< .001
Operating time (min)	89.1 ±27.7	116.2 ±36.0	104.2 ±28.9	< .001
Duration of hospital stay (days)	2.5 ±3.2	2.5 ±1.1	2.5 ±1.1	.997
Peroperative complications	13 (1.0%)	2 (2.1%)	1 (0.8%)	.595
Early postoperative complications	104 (8.1%)	11 (11.5%)	12 (10.0%)	.427
Severe early complications	22 (1.7%)	1 (1.0%)	1 (0.8%)	.690
Early reinterventions	16 (1.2%)	1 (1.0%)	1 (0.8%)	.915
Total reinterventions	83 (6.5%)	7 (7.3%)	16 (13.3%)	.060

pLRYGB=primary laparoscopic Roux-en-Y gastric bypass; rLRYGB = revisional laparoscopic Roux-en-Y gastric bypass

Table 3. Follow-up results after pLRYGB and rLRYGB

Outcome measurement	pLRYGB (n = 1285)	Nonresponders (n = 96)	Responders (n = 120)	P value
12 months FU	*n = 1153(89.7%)*	*n = 81 (84.4%)*	*n = 106 (88.3%)*	
%EWL	74.6 ±20.4	52.3 ±19.6	66.8 ±21.7	< .001
%TWL	31.0 ±0.2	23.7 ±1.0	28.0 ±0.2	< .001
BMI loss*	13.6 ±3.8	11.2 ±4.8	12.7 ±4.9	< .001
Success (%)	88.6	59.3	75.5	< .001
Failure (%)	0.7	12.3	0.9	< .001
24 months FU	*n = 922 (71.7%)*	*n = 58 (60.4%)*	*n = 79 (65.8%)*	
%EWL	77.1 ±22.7	53.7 ±23.2	63.8 ±22.9	< .001
%TWL	32.2 ±0.3	23.8 ±1.3	26.8 ±0.3	< .001
BMI loss*	14.1 ±4.5	11.1 ±4.9	12.1 ±5.3	< .001
Success (%)	88.9	55.2	72.2	< .001
Failure (%)	1.2	8.6	1.3	< .001
36 months FU	*n = 674 (52.5%)*	*n = 55 (57.3%)*	*n = 59 (49.2%)*	
%EWL	72.8 ±24.2	48.1 ±24.5	58.2 ±24.1	< .001
%TWL	30.3 ±0.4	21.5 ±1.5	25.3 ±1.5	< .001
BMI loss*	13.3 ±4.7	10.1 ±5.5	11.5 ±6.2	< .001
Success (%)	81.6	38.2	61.0	< .001
Failure (%)	2.5	10.9	8.5	.001

* in kg/m²

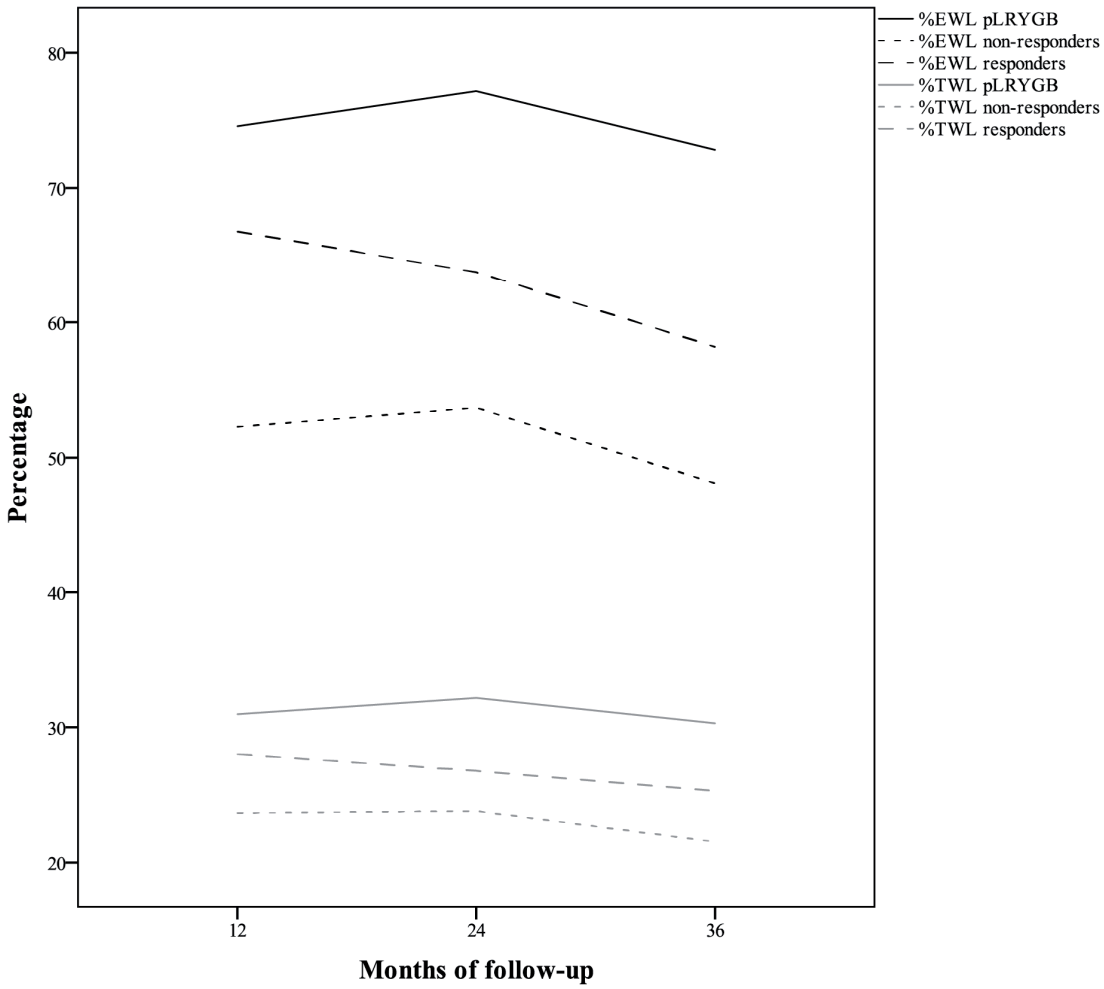
pLRYGB = primary laparoscopic Roux-en-Y gastric bypass; rLRYGB = revisional laparoscopic Roux-en-Y gastric bypass; FU = follow-up; %TWL = percentage total weight loss; %EWL = percentage excess weight loss; BMI = body mass index.

Weight reduction

The median follow-up was 33.9±18.0 months, follow-up was completed in 52.5%, 57.3% and 49.2% of pLRYGB, nonresponding and responding groups respectively, as shown in table 3. There was a significant higher %EWL in the responder group compared to the nonresponder group (66.8% versus 52.3%, *P* < .001 at 12 months; 63.8% versus 53.7%, *P* = .012 at 24 months; 58.2% versus 48.1%, *P* = .029 at 36 months; Fig. 1). pLRYGB showed a higher %TWL as well as BMI loss as compared to rLRYGB (*P* < .001).

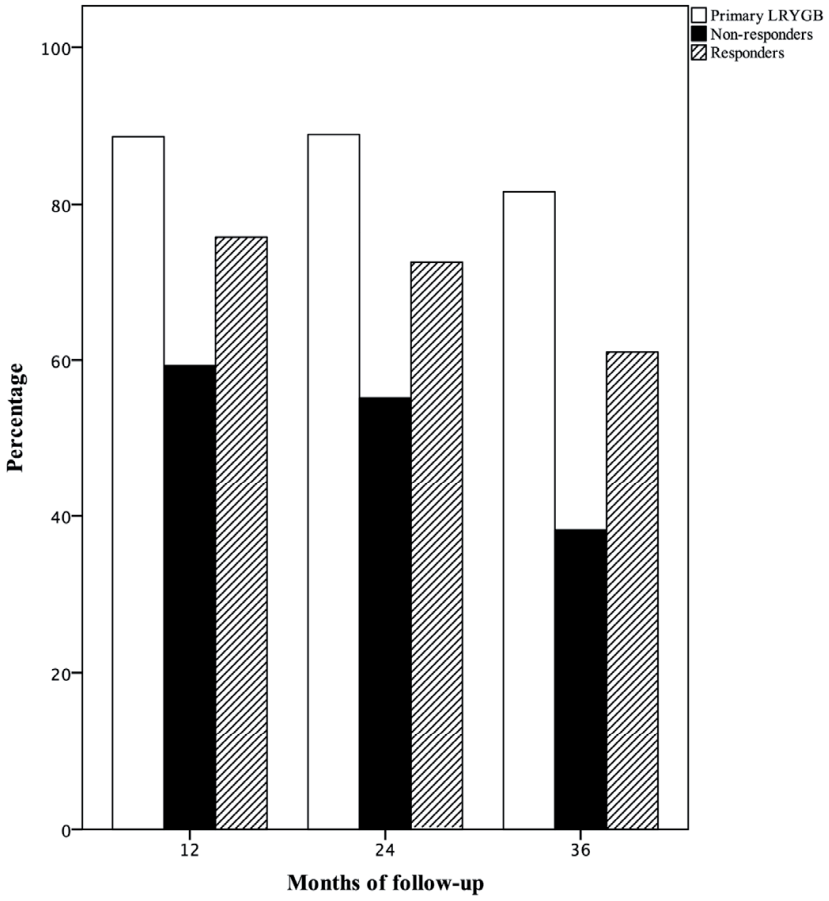
The success rate was significantly higher in both the primary and responding groups compared to the nonresponders at 36 months follow-up (81.6%, 61.0%, 38.2% respectively, *P* < .001; Fig. 2). After 36 months, the failure rate was significantly lower in the primary group compared to both revisional groups (2.5% versus 10.9% and 8.5%, *P* = .001).

Figure 1. %EWL and %TWL during follow-up after pLRYGB and rLRYGB



pLRYGB = primary laparoscopic Roux-en-Y gastric bypass; rLRYGB = revisional laparoscopic gastric bypass; %EWL = percentage excess weight loss; %TWL = percentage total weight loss

Figure 2. Success rate during follow-up after pLRYGB and rLRYGB



pLRYGB = primary laparoscopic Roux-en-Y gastric bypass; rLRYGB = revisional laparoscopic gastric bypass

Discussion

rLRYGB of responders to LAGB was associated with significantly higher %EWL and success rates compared to nonresponders at all evaluated moments during follow-up. In addition, after 12 months %TWL was significantly higher in the responding group compared to the nonresponding group. However, the %TWL remained significantly higher after pLRYGB compared to rLRYGB during the entire follow-up.

In contrast to several other studies, we showed a significant higher %EWL after pLRYGB compared to rLRYGB at all moments of follow-up[14,19,21]. Little is known regarding the impact of initial response to LAGB on the outcomes of revisional LRYGB. We observed a significant difference in %EWL between nonresponders and responders to LAGB. To our knowledge, only one previous study stratified the rLRYGB on initial response after LAGB, showing a significant lower %EWL in the group with lower weight loss after LAGB[12]. However, the authors did not assess whether there was initial response to the LAGB, but stratified the

groups based on the weight before revision. Another study divided rLRYGB patients into two subgroups, but only showed the results of the patients that underwent rLRYGB due to unsuccessful weight loss after LAGB[22]. In this group, 67.6% EWL one year after revision to LRYGB was reported, which was higher than our findings (52.3% EWL). Notably, the study population existed of 8 nonresponders only. Our results showed a 36-months success rate of 61.0% of the responders compared to 38.2% of the nonresponders ($P < .001$), based on the results of a larger study population. Therefore it appears that responders to LAGB show superior weight reduction after conversion to LRYGB compared to nonresponders. A potential explanation for the inferior results among nonresponders is the higher mean initial BMI of the nonresponding group compared to the primary and responding groups[23]. However, we also calculated the %TWL to compare the different study groups. A similar trend in %TWL and %EWL was found between the nonresponders compared to the responders and the primary LRYGB.

The 1-step approach was performed significantly more often in the responder group compared to the nonresponder group. A study showed inferior %EWL after a 1-step approach than a 1-step approach (53% versus 67%)[24]. Notably, in this study, the reason for revision in the 1-step approach group was more often due to band related complications as compared to the 1-step approach, so it is most likely that most patients of the 1-step approach are nonresponders to the LAGB. As the study does not address whether the patients in the group are responders or not, it is plausible that a higher nonresponder rate was present among the 1-step approach group as compared to the 1-step approach, which can have attributed to the worse %EWL results. The 1-step approach was more likely performed in the beginning of our study cohort. The 1-step approach is preferred, as the stomach can recover after the removal of the LAGB, so that pouch dilatation after the revision is less likely.

Our early complication rate is comparable to previously reported results[11]. According to the Clavien-Dindo score, there are no significant differences in the occurrence of serious complications between the three study groups[25]. The number of re-interventions did not differ significantly between the groups. Pouch dilatation was diagnosed significantly more often after rLRYGB compared to pLRYGB, which might explain the inferior outcomes. However, an upper gastrointestinal tract fluoroscopy was only performed to assess pouch dilatation among patients who failed to lose weight after rLRYGB or pLRYGB. An additional study could be recommended to assess pouch dilatation when the LRYGB fails including a control group with successful excess weight loss, as successful patients might also have pouch dilatation.

Even though the responder group had pouch dilatation diagnosed more frequently compared to the nonresponders, the responders showed more weight reduction than the nonresponders.

A limitation of our study is the follow-up compliance. A minimum follow-up rate of 61% is recommended for each time interval reported after surgical treatment for obesity[26]. Unfortunately, we only reached this minimum follow-up rate at 12 and 24 months of follow-up. However, at 36 months follow-up, the follow-up rate was comparable among the groups.

There are several possible explanations for our differences in outcomes between the three study groups. It seems there is an obvious relation between the response of LAGB and the response after rLRYGB. There could either be a genetic component that is responsible for the inadequate weight loss after both procedures in the nonresponder group[27]. Another explanation could be the inability of nonresponders to adjust their diet habits and lifestyle, which could be the reason they did not respond to the LAGB, and we expect they will not be able to adjust their lifestyle after rLRYGB as well. These patients are unsuitable candidates for bariatric surgery in general.

Therefore, we recommend adequate routine screening for rLRYGB candidates. For nonresponding patients to LAGB, we recommend a pathway with both cognitive and dietary support. In case such patients would still not be able to reduce weight, rLRYGB is not advisable. If nonresponding patients would show the ability of adjusting diet and lifestyle, rLRYGB could be considered.

Conclusion

Nonresponders to LAGB appear to have a lower success rate after revisional LRYGB compared to responders to LAGB. The %EWL and %TWL of pLRYGB remain significantly higher compared to both rLRYGB groups.

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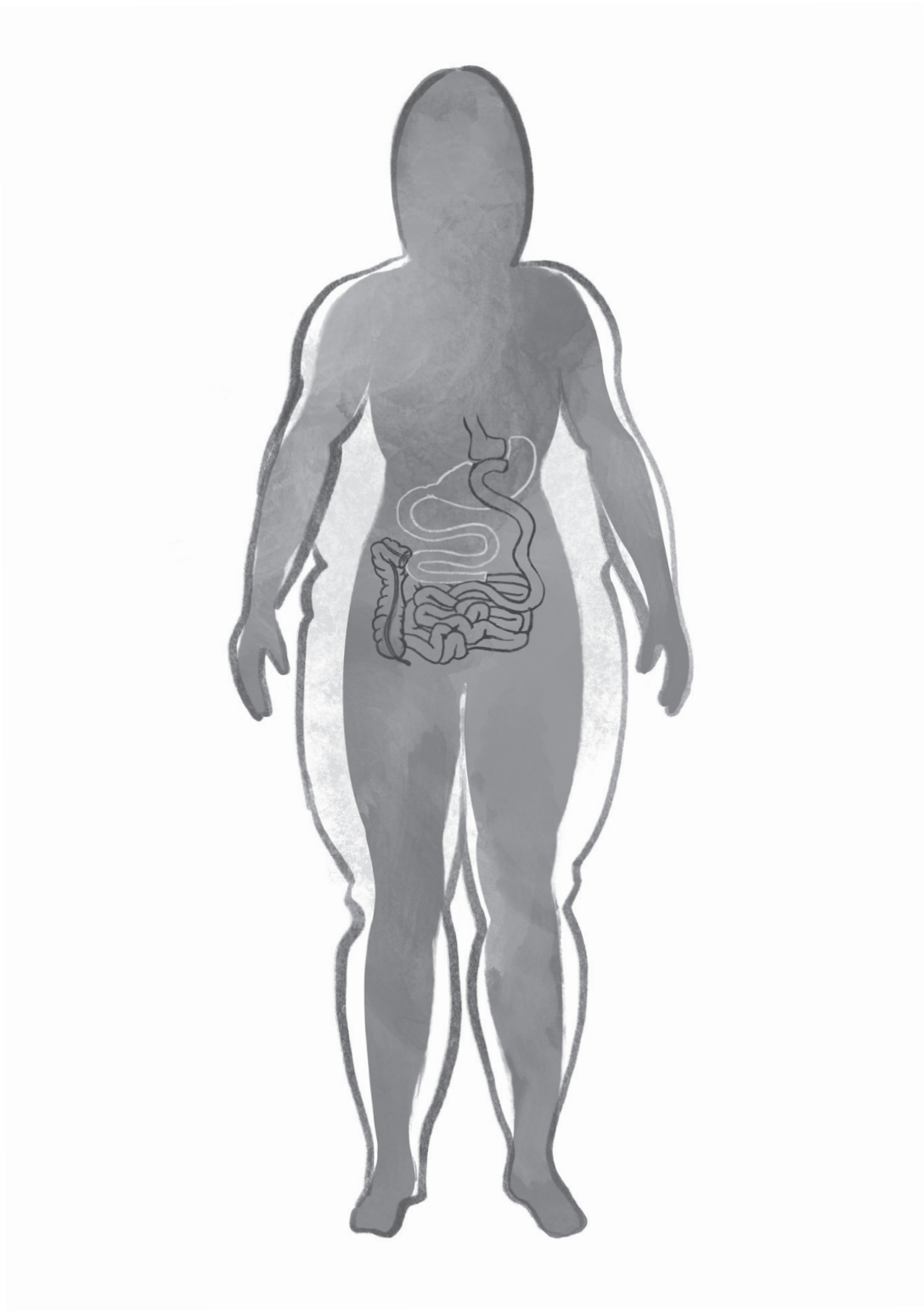

Chapter 7

Resizing a large pouch after laparoscopic Roux-en-Y gastric bypass: comparing the effect of two techniques on weight loss

L.H. Wijngaarden, B.M.M. Reiber*, F. Yousufzai, A. Demirkiran, R.A. Klaassen*

** Authors contributed equally*

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Abstract

Background

Insufficient weight loss or weight regain has many causes including a large gastric pouch. A large gastric pouch may be due to the surgical technique or can be patient related (dilation). Resizing the gastric pouch may lead to additional weight loss. Currently, there is no gold standard for the revisional surgical technique. Therefore this study was performed to determine which surgical technique for revisional bariatric surgery (BS) has superior outcomes in terms of weight loss: sleeve resection of the gastrojejunostomy and and gastric pouch (SGP), or resection of the gastrojejunostomy with resizing of the pouch and creation of a new anastomosis (RGJ).

Methods

All patients who underwent revisional BS for insufficient weight loss or weight regain as a result of an enlarged pouch after LRYGB from April 2014 to June 2018 in our hospitals were included in this observational cohort study. Outcomes were measured in percentage total weight loss (%TWL).

Results

A total of 37 patients who underwent SGP and 21 patients who underwent RGJ as revisional BS were included in this study. The median body mass index before revisional BS was 37.6 kg/m² versus 35.7 kg/m² (SGP vs RGJ respectively, *P*=0.115). There was no significant difference in %TWL between the two cohorts one and two years after revisional BSrespectively; SGP 14.5% vs RGJ 11.0%, P=0.885 and SGP 12.3% vs RGJ 10.8%, P=0.604. Comparing %TWL based on weight at LRYGB, there was also no significant difference two years after revisional BS (SGP 22.0% vs RGJ 22.2%, P=0.885). The average use of surgical disposables for the SGP technique were lower compared to the RGJ technique.

Conclusions

Resizing a large pouch leads to additional weight loss. Both techniques have comparable outcomes in terms of weight loss. However, based on average surgical costs, the SGP technique may be preferable.

Introduction

Laparoscopic Roux-en-Y gastric bypass (LRYGB) has been proven to be an effective treatment of morbid obesity. It leads to substantial excess weight loss and reduction or even remission of metabolic comorbidities[1-3]. Unfortunately, insufficient weight loss or weight regain after LRYGB have been described as well[4]. Insufficient weight loss and weight regain may have multifactorial causes. It can either be patient related (e.g. dietary non-compliance, physical inactivity or hormonal/metabolic factors) or surgery related (e.g. enlarged pouch due to construction of a large pouch at the primary LRYGB, gastro-gastric fistulas or dilation of the gastrojejunostomy)[4 5].

Several studies have shown the importance of the size of the pouch in the primary surgery on weight loss[6-8]. In case of an anatomical cause of insufficient weight loss or weight regain, surgical treatment is challenging and controversial. Several techniques have been proposed for revisional bariatric surgery if insufficient weight loss or weight regain is caused by an enlarged pouch[9]. Laparoscopic resizing of the gastric pouch and gastrojejunostomy show good results on percentage excess weight loss (%EWL) with low reoperation rate and no mortalities[10 11]. Two techniques for resizing of the gastric pouch and gastrojejunostomy are described in literature: sleeve resection of the gastrojejunostomy and gastric pouch (SGP)[11 12], and resection of the gastrojejunostomy with creation of a smaller pouch and a new anastomosis (RGJ)[13 14]. So far only short term results in small groups have been described.

The aim of this study is to determine whether a sleeve resection of the gastrojejunostomy and gastric pouch or a revision of the gastrojejunostomy is the superior technique for additional weight loss after LRYGB.

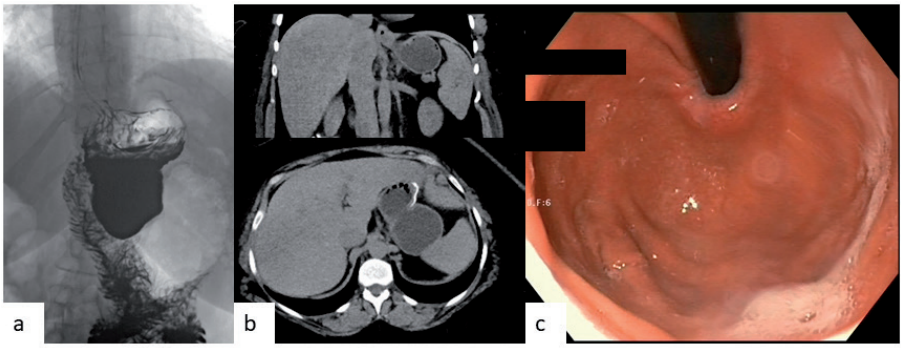
Materials and Methods

Patient selection

All patients who underwent revisional bariatric surgery for insufficient weight loss or weight regain after LRYGB from April 2014 to June 2018 in two expert centers for bariatric surgery were included in this observational cohort study. Patients after primary LRYGB as well as patients who underwent previous bariatric surgery before LRYGB were included. Previous bariatric surgery was either laparoscopic adjustable gastric banding (LAGB) or laparoscopic sleeve gastrectomy (LSG). The SGP technique was performed in one of the centers, and the RGJ technique was performed in the other center. The performed revisional technique was based on the preference of the bariatric surgeons. Both techniques are described below.

Before qualifying for revisional bariatric surgery, all patients with insufficient weight loss or weight regain followed an obligatory and standardized trajectory led by a team of psychologists, dietitians and psychotherapists in order to optimize the patients motivation and compliance to an adjusted life style. Patients were only eligible for revisional bariatric surgery after failing this standardized trajectory in terms of additional weight loss combined with no sensation of restriction and diagnostics tests showing a large pouch. Diagnostic tests to evaluate a large pouch were either barium swallow test (BST) in combination with gastroscopy or a three dimensional gastric computed tomography (3D-GCT) scan. A pouch was defined as dilated if the pouch was >5 cm on the BST in combination with gastroscopy, based on the difference in length measured from the gastrojejunostomy to the Z-line, or if the pouch volume was >50 ml on the 3D-GCT. Examples of pouch enlargement signs on these tests are depicted in Figure 1. Patients were excluded from this study if the insufficient weight loss or weight regain was caused by a gastro-gastric fistula or if revision of the gastrojejunostomy was due to a marginal ulcer.

Figure 1. Examples of an enlarged pouch evaluated through barium swallow test (a), computed tomography (CT) scan (b) and gastroscopy (c).



Data collection

Baseline characteristics (i.e. age, sex, presence of metabolic comorbidities) and surgical characteristics (i.e. type of primary operation, duration of surgery and early surgical complications) were collected. The initial weight and body mass index (BMI) before the primary bariatric surgery, the initial weight response to the primary bariatric surgery, and the weight before revisional bariatric surgery were recorded.

During follow-up, outcomes were collected at three, six, twelve and twenty four months. Additionally, patients with comorbidities were seen by the internal medicine specialist, who evaluated whether metabolic comorbidities persisted, improved or resolved. This evaluation was based on general use of medication. For type 2 diabetes mellitus specifically HbA1c levels, for hypertension specifically blood pressure and screening for concomitant organ damage, for hypercholesterolemia specifically cholesterol levels and

lastly for obstructive sleep apnea an evaluation of the pulmonologist was included. The amount of weight loss was described as the percentage of total weight loss (%TWL) and was calculated as ((operative weight – follow up weight)/operative weight) · 100%. BMI was calculated as weight (kg)/height (m)².

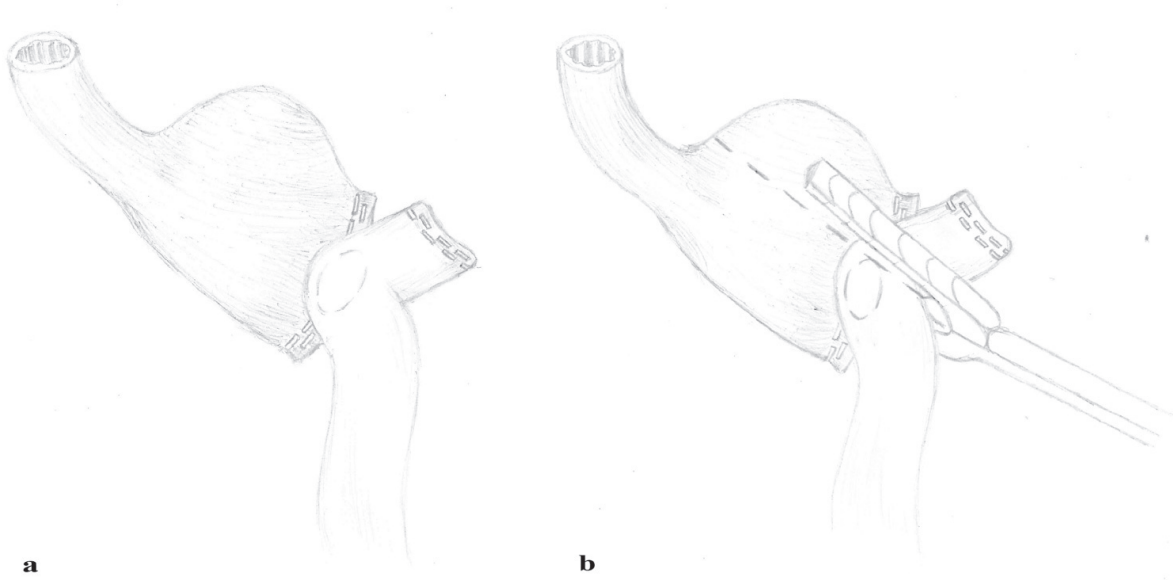
Weight data were analyzed for patients undergoing primary LRYGB and undergoing LRYGB as conversion from LAGB or LSG separately. In addition, all weight results were presented for the entire group.

Surgical techniques

Laparoscopic sleeve resection of the gastro-jejunostomy and gastric pouch (SGP)

Laparoscopic sleeve resections of the gastrojejunostomy, gastric pouch and blind afferent limb were performed using a 60 millimeter endoscopic stapler (Figure 2). A 34-gauge gastric tube was used to calibrate the size of the revised gastric pouch that was transected longitudinally 6 centimeters from the diaphragm. The resected tissue was removed using an endoscopic bag.

Figure 2. Laparoscopic sleeve resection of the gastro-jejunostomy



2a. Roux-en-Y gastric bypass with an enlarged gastric pouch **2b.** Laparoscopic sleeve resection of the gastrojejunostomy, gastric pouch and blind afferent limb

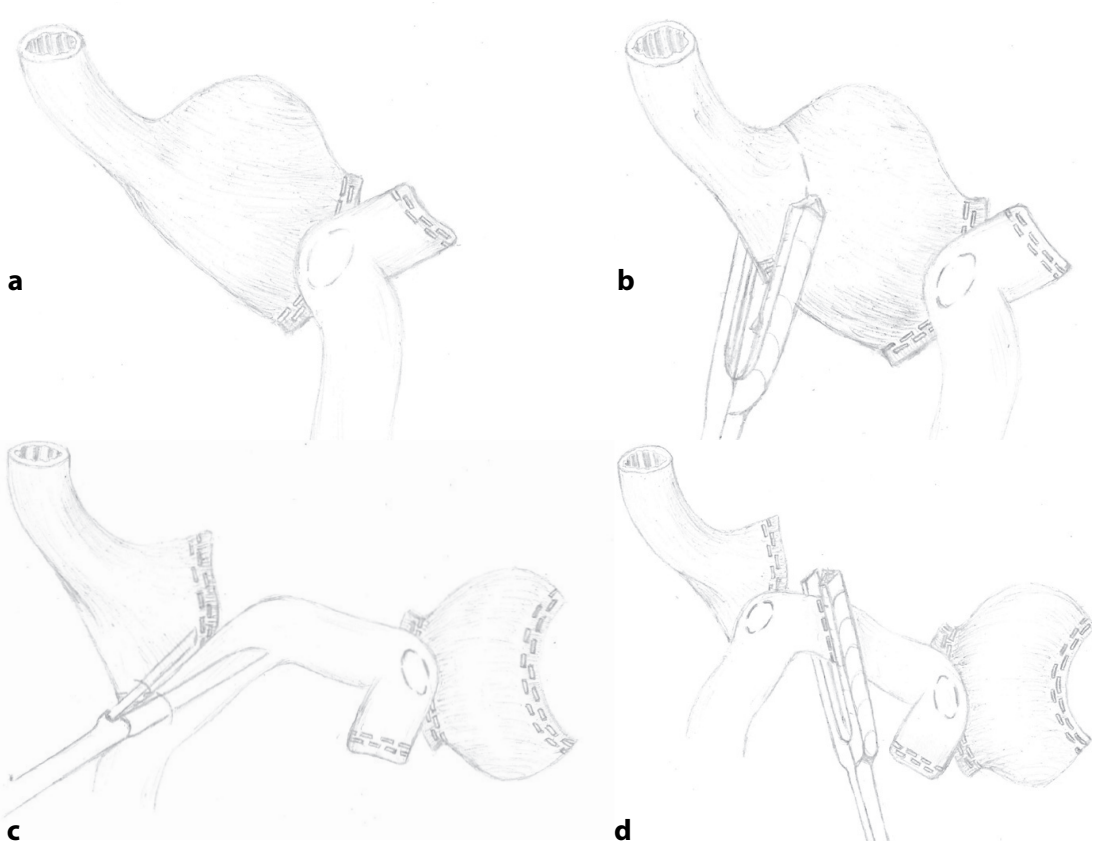
Laparoscopic revision of the gastrojejunostomy (RGJ)

In this technique, the gastric pouch was transected above the level of the anastomosis approximately 6 centimeters from the diaphragm and reduced by a 60-millimeter endoscopic stapler, using a 34-gauge gastric tube for calibration (Figure 3). A new 30-millimeter linear gastrojejunostomy was created and the remaining defect was

laparoscopically sutured. Subsequently, the jejunum was transected below the anastomosis. The resected tissue was then removed using an endoscopic bag.

All operations were performed laparoscopically with the use of one 12-millimetre vision port for the camera, two 12-millimeter working ports, one 5-millimeter working port and a liver retractor. The average use of surgical disposables was calculated and operation time were compared for both techniques.

Figure 3. Laparoscopic revision of the gastrojejunostomy



3a. Roux-en-Y gastric bypass with an enlarged gastric pouch **3b.** Transection and reduction of the gastric pouch **3c.** Creation of a new gastrojejunostomy **3d.** Transection of the jejunum and removal of the old anastomosis

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics, version 24 (SPSS, Chicago, IL). Continuous data are presented as median and interquartile range (IQR) and were compared by the Mann-Whitney U-test according to normality. The Chi square test was used for categorical data analysis. A P value < 0.05 was considered significant. Missing data were addressed with a pair wise deletion in follow-up analysis.

Results

A total of 37 patients who underwent SGP and 21 patients who underwent RGJ as revisional bariatric surgery were included in our study. The baseline characteristics are presented in Table 1.

Table 1. Baseline characteristics

Characteristic	SGP, n=37 (number (%) or median [interquartile range])	RGJ, n=21 (number (%) or median [interquartile range])	P-value
Female gender	29 (78.4%)	21 (100%)	0.022*
Age at gastric bypass	41.6 [32.5;49.4]	49.0 [38.8;53.7]	0.079
Time between LRYGB and rBS in years	4.3 [3.1;5.7]	3.2 [1.9;3.9]	0.005*
Previous bariatric intervention			
LAGB	17 (45.9%)	6 (28.6%)	0.194
LSG	1 (2.7%)	0 (0.0%)	0.447

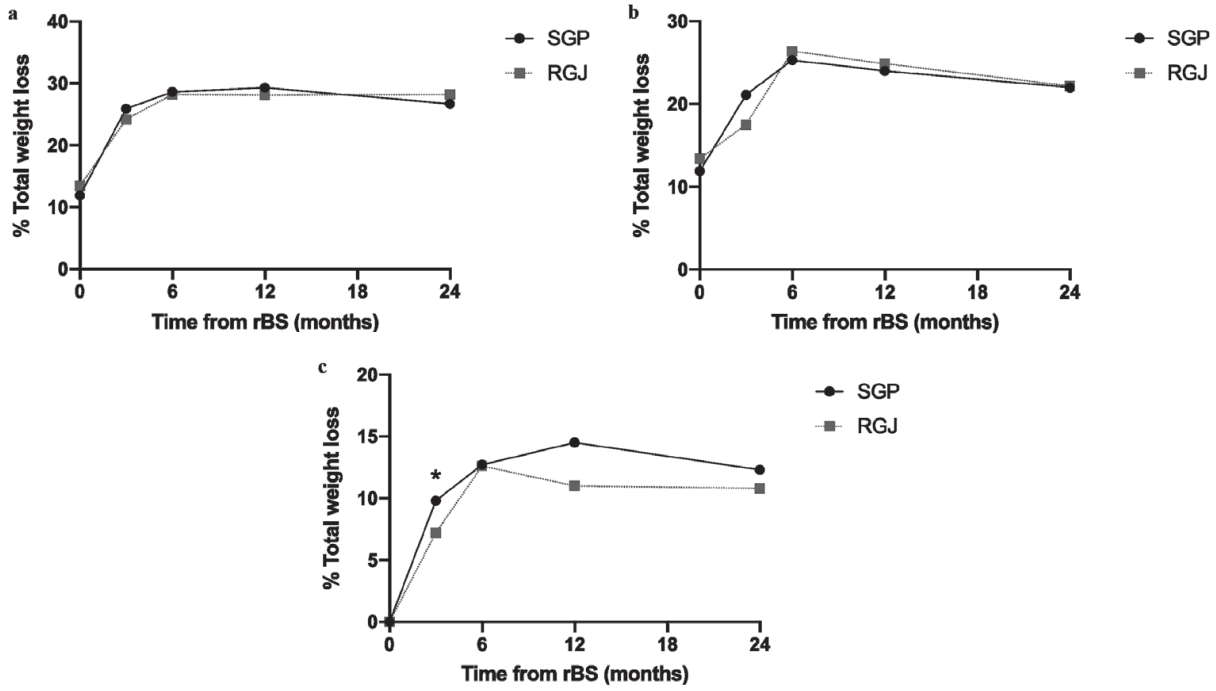
SGP = sleeve resection of the gastrojejunostomy and gastric pouch; RGJ = revision of the gastrojejunostomy; LRYGB = laparoscopic Roux-en-Y gastric bypass; rBS = revisional bariatric surgery; LAGB = laparoscopic adjustable gastric banding; LSG = laparoscopic sleeve gastrectomy
* Significantly different

Weight loss after resizing the pouch

Weight data are presented in Table 2. Median BMI at primary bariatric surgery was significantly different but no differences were found in BMI at revisional bariatric surgery. There were no statistical differences in %TWL between the two techniques during follow-up, as presented in Figure 3. %TWL was determined based on weight at primary bariatric surgery (Figure 4a), at LRYGB (Figure 4b) and at revisional bariatric surgery (Figure 4c). Based on weight at revisional bariatric surgery, the 24-month %TWL was 12.3% [5.8;14.5] in the SGP cohort versus 10.8% [3.4;22.1] in the RGJ cohort (P = 0.604). The total %TWL based on weight at primary bariatric surgery was %TWL [IQR] was 22.0% [16.4;30.3] in the SGP cohort and 22.2% [11.7;32.1] in the RGJ cohort (P = 0.885).

Figure 5 shows the trend of overall %TWL based on weight at primary bariatric surgery for patients who had undergone LYRGB as primary bariatric surgery (Figure 5a) and patients who had undergone LRYGB as a conversion from LAGB or SG to LRYGB (Figure 5b) respectively.

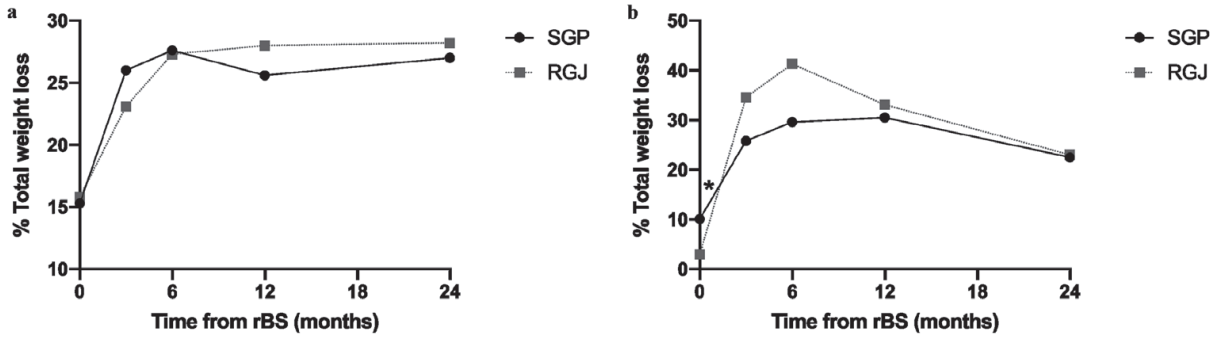
Figure 4. Percentage total weight loss during follow-up



4a. %TWL based on weight at primary bariatric surgery
4b. %TWL based on weight at LRYGB
4c. %TWL based on weight at revisional bariatric surgery

SGP = sleeve resection of the gastrojejunostomy and gastric pouch; RGJ = revision of the gastrojejunostomy; rBS = revisional bariatric surgery; %TWL = percentage total weight loss; LRYGB = laparoscopic Roux-en-Y gastric bypass
* Significantly different ($P < 0.005$)

Figure 5. Percentage total weight loss during follow-up



5a. %TWL based on weight at primary bariatric surgery in patients who underwent LRYGB as primary bariatric procedure
5b. %TWL based on weight at primary bariatric surgery in patients who underwent LRYGB as a revisional bariatric procedure after LAGB or LSG

SGP = sleeve resection of the gastrojejunostomy and gastric pouch; RGJ = revision of the gastrojejunostomy; rBS = revisional bariatric surgery; %TWL = percentage total weight loss; LRYGB = laparoscopic Roux-en-Y gastric bypass; LAGB = laparoscopic adjustable gastric banding; LSG = laparoscopic sleeve gastrectomy
* Significantly different ($P < 0.005$)

Table 2. Prerevisional and postrevisional weight characteristics of patients who underwent revisional bariatric surgery after primary LRYGB as well as the entire study population

	Primary LRYGB			Total population		
	SGP (n=19)	**RGJ (n=17)**	**P-value**	**SGP (n=37)**	**RGJ (n=21)**	**P-value**
Prerevisional characteristics						
BMI at intake primary operation	-	-	-	45.8 [41.9;51.8]	42.5 [39.9;47.3]	0.024
BMI at intake LRYGB	42.7 [40.7;47.1]	41.9 [38.6;45.3]	0.656	44.4 [41.5;49.1]	41.3 [38.8;45.3]	0.015
Best BMI after LRYGB	28.4 [25.9;30.4]	32.5 [29.8;34.7]	0.056	32.1 [28.8;39.6]	33.5 [29.4;35.8]	0.936
Best %TWL after LRYGB	30.6 [24.01;42.1]	28.3 [18.3;38.1]	0.098	24.8 [15.2;33.2]	18.9 [11.0;24.4]	0.032
%TWL at time of revision	19.6 [12.8;25.6]	20.1 [15.9;23.0]	0.973	11.9 [8.4;21.2]	13.4 [5.5;18.7]	0.777
Postrevisional characteristics						
BMI (in kg/m2)						
At revision	34.3 [32.6;37.2]	34.9 [30.7;35.4]	0.784	37.6 [33.6;42.5]	35.7 [33.5;38.3]	0.115
6 months after rBS	30.1 [28.6;30.4]	30.0 [28.8;31.1]	0.953	31.0 [30.0;37.1]	30.1 [28.8;32.1]	0.100
12 months after rBS	30.1 [27.2;33.9]	28.4 [26.9;31.2]	0.978	33.6 [29.8;36.9]	30.4 [27.8;31.9]	0.087
24 months after rBS	29.8 [27.8;33.9]	28.8 [27.9;34.1]	0.913	34.9 [29.7;37.7]	31.4 [28.4;35.8]	0.094
%TWL based on weight at revisional bariatric surgery						
6 months after rBS	14.0 [9.6;17.9]	12.6 [3.7;18.1]	0.411	12.7 [9.2;17.2]	12.6 [9.3;17.2]	0.956
12 months after rBS	12.8 [5.1;19.4]	14.5 [2.9;23.9]	0.978	14.5 [9.3;17.4]	11.0 [6.2;19.2]	0.885
24 months after rBS	12.8 [11.1;16.1]	10.0 [-1;21.1]	0.419	12.3 [5.8;14.5]	10.8 [3.4;22.1]	0.604
%TWL based on weight at primary bariatric procedure						
6 months post rBS	32.3 [21.4;37.4]	27.3 [22.7;27.7]	0.599	25.3 [18.1;32.7]	26.4 [21.5;29.8]	0.781
12 months post rBS	34.8[19.3;39.1]	28.9 [23.5;40.1]	1.00	24.0 [18.5;34.4]	24.9 [11.5;32.7]	0.864
24 months post rBS	29.7 [22.2;37.6]	26.9 [17.6;37.8]	0.744	22.0 [16.4;30.3]	22.2 [11.7;32.1]	0.885

LRYGB = laparoscopic Roux-en-Y gastric bypass; SGP = sleeve resection of the gastrojejunostomy and gastric pouch; RGJ = revision of the gastrojejunostomy; BMI = Body Mass Index; %TWL = percentage total weight loss; rBS = revisional bariatric surgery. All results are presented as median [interquartile range]

Table 3. Early postoperative complications

	SGP	RGJ
Anastomotic leakage	1 (2.7%)	1 (4.8%)
Superficial wound infection	1 (2.7%)	1 (4.8%)
Subcutaneous hematoma	1 (2.7%)	0 (0.0%)

SGP = sleeve resection of the gastrojejunostomy and gastric pouch; RGJ = revision of the gastrojejunostomy

Surgical factors, early postoperative complications and surgical costs

Operation time was significantly higher in the RGJ procedure (57.0 min SGP vs 74.0 min RGJ, $P = 0.003$). At the RGJ group, one patient underwent both revisional surgery, laparoscopic cholecystectomy and closure of the mesenteric defects within one procedure. After exclusion of this patient, operation time was still significantly higher in the RGJ cohort (73.5 min, $P=0.006$). Three early postoperative complications occurred in the SGP group (8.1%) as compared to two complications in the RGJ group (9.5%), as demonstrated in Table 3. According to the Clavien-Dindo classification of surgical complications[15], in the SGP group two grade I and one grade IIIb complications occurred as compared to one grade I complication and one grade IIIB complication in the RGJ group. In both study groups, one patient had an anastomotic leakage (2.7% in the SGP group and 4.8% in the RGJ group). In the SGP group, a jejunal tube was placed endoscopically for enteral feeding to treat the anastomotic leakage. In the RGJ group, a relaparoscopy was performed to close the defect of the anastomotic leakage. Both patients recovered without any negative residual effects. The average surgical costs for the SGP technique were approximately €337 lower than for the SGJ technique (Table 4).

Table 4. Surgical disposables*

	SGP		RGJ	
Operation time	57 min	€ 855	73.5 min	€ 1022
Stapler devices	1	€ 300	1	€ 300
Reload of staplers	4	€ 600	5	€ 750
Barbed sutures	0	€ 0	1	€ 20
Total costs		**€ 1755**		**€ 2092**

SGP = sleeve resection of the gastrojejunostomy and gastric pouch; RGJ = revision of the gastrojejunostomy
*Costs that were different between the two revisional surgery techniques are shown in this table. It should be noted that costs that were equal between both techniques, such as standard surgery instruments and anesthesiology equipment, are not shown.

Obesity-related comorbidities

In both groups, all patients achieved either improvement or remission of DM2 after revisional bariatric surgery (Table 5). Hypertension improved or even resolved in 60% of the patients in the SGP group as compared to 66.7% in the RGJ groups. Two patients

(50%) achieved remission of hypercholesterolemia in the SGP groups as compared to one patient (50%) in the RGJ group. There was no remission or improvement of OSAS achieved in both study groups.

Table 5. Prerevisional presence and postrevisional improvement or remission of comorbidities

Comorbidities	SGP (n=37)	RGJ (n=21)	P-value
DM2			
Prerevisional	5 (13.5%)	4 (19.0%)	0.576
Improvement*	3 (60%)	1 (25%)	
Remission	2 (40%)	3 (75%)	
Hypertension			
Prerevisional	10 (27.0%)	6 (28.6%)	0.899
Improvement*	3 (30%)	3 (50%)	
Remission	3 (30%)	1 (16.7%)	
Hypercholesterolemia			
Prerevisional	4 (10.8%)	2 (9.5%)	0.877
Improvement*	0 (0%)	0 (0%)	
Remission	2 (50%)	1 (50%)	
OSAS			
Prerevisional	2 (5.4%)	2 (9.5%)	0.552
Improvement*	0 (0%)	0 (0%)	
Remission	0 (0%)	0 (0%)	

SGP = sleeve resection of the gastrojejunostomy and gastric pouch; RGJ = revision of the gastrojejunostomy; DM2 = type 2 diabetes mellitus; OSAS = obstructive sleep apnea syndrome

* Total number of patients with improvement only, patients with complete remission are not included in this number

Discussion

Resizing a large gastric pouch after LRYGB leads to additional weight loss and has a positive effect on obesity-related comorbidities. The two techniques, SGP and RGJ, were equally effective in terms of weight loss. However, the SGP technique did result in less usage of disposables and a shorter operation time compared to the RGJ technique.

Based on weight prior to resizing the gastric pouch the median %TWL at 24 months after revisional bariatric surgery was 12.3% [5.8;14.5] in the SGP cohort versus 10.8% [3.4;22.1] in the RGJ cohort ($P = 0.604$). When we compare the %TWL based on weight prior to the first bariatric procedure, the %TWL is 22.0% in the SGP cohort and 22.2% in the RGJ cohort ($P = 0.885$), which is comparable to previous studies[16-19]. Considering these results it should be noted that the additional effect on %TWL of revisional surgery was relatively small. This study, however, shows that the additional effect may be well worth

the effort as all patients now achieved %TWL of more than 20% and additional resolution of comorbidities.

Previous studies have shown that the clinical effect on comorbidities in revisional bariatric surgery is similar to primary bariatric surgery[20 21]. In this study, there seems to be an improvement of comorbidities in both groups. As the sample size was small, this study could not demonstrate a statistically significant reduction or improvement of comorbidities between the two techniques. Nevertheless, it can be concluded from this study that revision of a large pouch can exert a positive effect on obesity-related comorbidities. Therefore the continuous presence of obesity-related comorbidities should be considered as an indication for revisional bariatric surgery.

Although this study did not intend to perform a full cost effectiveness analysis, we did find lower surgical costs of the SGP technique as compared to the RGJ technique. This difference was a result of a shorter operating time and less use of disposables in the SGP technique.

The effect of pouch size on the achieved weight loss after LRYGB remains controversial. Even though some studies have shown that a small pouch size results in higher achieved weight loss[6-8], others could not demonstrate a correlation[22 23]. In this study, no calibration for pouch size was used at the primary LRYGB. However, revisional bariatric surgery for a large pouch can lead to additional weight loss. Thus we might suggest the use of a calibration tube for LRYGB in order to prevent insufficient weight loss or weight regain due to a large pouch.

In this study, there was no consistent diagnostic technique used for pouch volume measurement, as no standardization for pouch volume measurement is defined in literature yet. The barium swallow test (BST) with upper gastrointestinal series has been used to measure pouch volume after LRYGB[6 14]. However, it is challenging to calculate a three-dimensional pouch volume from two-dimensional radiological imaging. Therefore two other suggested techniques are 3D-GCT and upper endoscopy. The volumes of the gastric pouch and the diameter of the gastrojejunal anastomosis can be measured exactly in these two techniques[24 25]. Unfortunately, the exact pouch size was not measured according to a standardized protocol preoperatively in this retrospective cohort study. However, all patients had demonstrated a large pouch, either diagnosed by BST, upper endoscopy combined with BST or 3D-GCT. Patients were excluded from this study if the diagnostic technique showed other causes for insufficient weight loss or weight regain, such as gastro-gastric fistula.

The revisional bariatric surgery technique was based upon the preference of the surgeon. Because of this, two bariatric surgeons performed the RGJ and three bariatric surgeons performed the SGP technique. There was no crossover in treatments between the surgeons. As a consequence, there were significant differences at baseline: gender, BMI before LRYGB, BMI before primary bariatric surgery and best %TWL after primary bariatric surgery and BMI before primary gastric bypass. In multivariate analysis, only best %TWL after primary bariatric surgery was a positive predictor of %TWL twelve months post-revisional. However, as standardization of revisional bariatric surgery is needed, we analyzed the results in order to assess whether one technique is preferable. Further research in a randomized controlled trial is recommended in order to prevent selection bias.

In conclusion, both SGP and RGJ techniques are feasible to perform and achieve adequate weight loss after revisional bariatric surgery for insufficient weight loss or weight regain as a consequence of a large pouch after LRYGB. There was no statistical difference in %TWL between either procedures during follow-up, and both techniques showed improvement of obesity-related comorbidities. However, the average surgical costs of the SGP technique were lower and may therefore be the preferred revisional bariatric technique.

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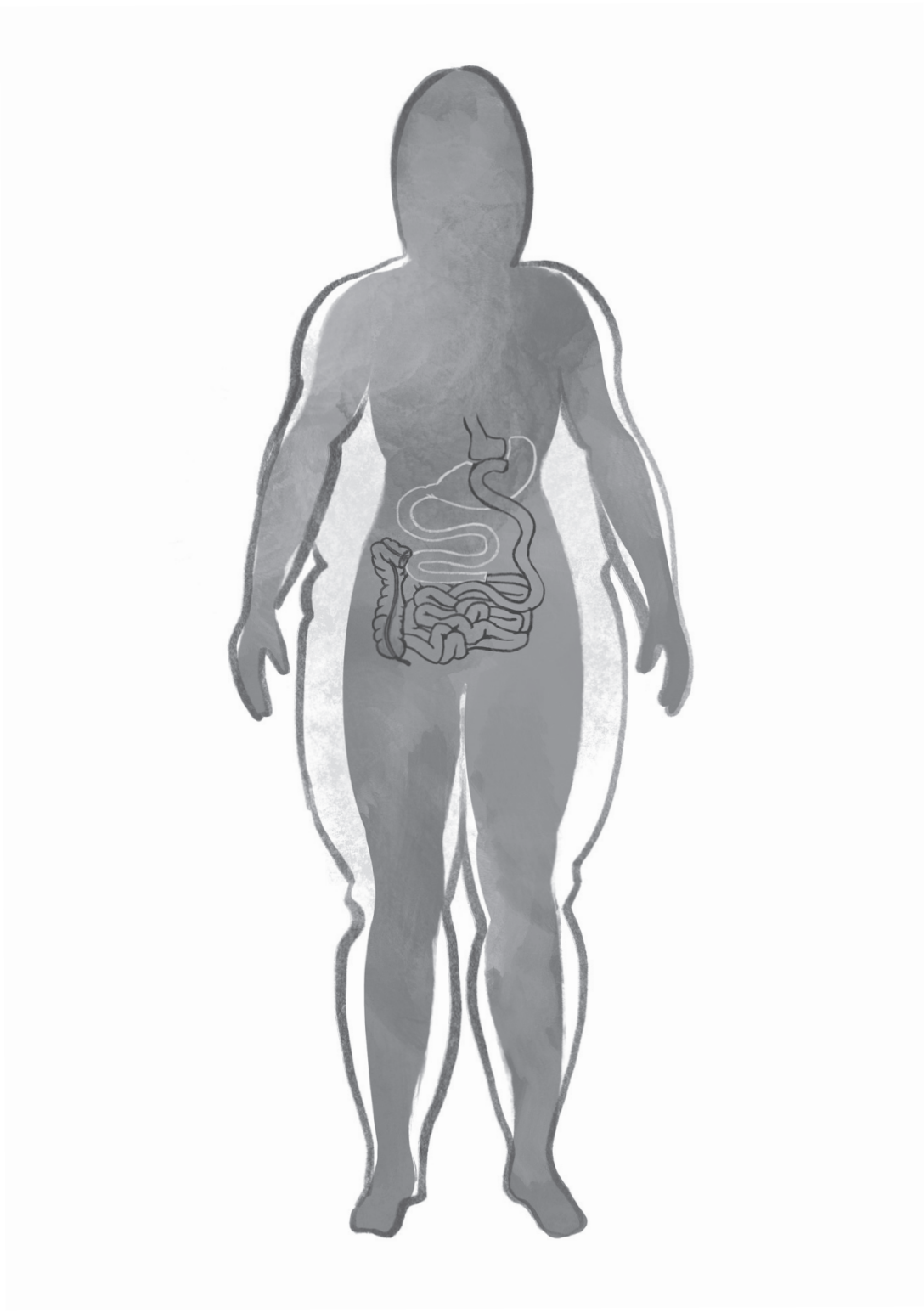

Chapter 8

Predicting symptom relief after reoperation for suspected internal herniation after laparoscopic Roux-en-Y gastric bypass

L.H. Wijngaarden, S.L. van Veldhuisen, R.A. Klaassen, E. van der Harst, C.C. van Rossem, A. Demirkiran, S.M.M. de Castro, F.H.W. Jonker

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Abstract

Background

Internal herniation (IH) is one of the most common long-term complications after laparoscopic Roux-en-Y gastric bypass (LRYGB). Diagnosis of IH may be difficult and not all patients with suspected IH will have full relief of symptoms after closure of both mesenteric defects.

Objectives

To investigate possible predictive factors for relief of symptoms in patients with suspected IH.

Methods

All patients that underwent reoperation for (suspected) IH after LRYGB from June 2009 to December 2016 were retrospectively evaluated in this multicenter cohort study. Logistic regression analysis was used to identify predictive factors for pain relief after closure of the mesenteric defects.

Results

A total of 193 patients underwent laparoscopy for (suspected) IH during the study period. The median interval between LRYGB and reoperation was 18.3±19.0 months. In 40.2% of cases, IH was identified on computed tomography (CT), and IH was objectified during surgery in 61.1%. Postoperative symptom relief was observed in 146 patients (77.2%). For patients in which IH was present during surgery 82.8% had relief of pain postoperatively, as compared to 68.5% for those procedures in which no IH was found. The only significant predictor for postoperative pain relief was a swirl sign on CT (OR 4.24, 95% CI 1.63 – 11.05).

Conclusions

Pain relief after closure of the mesenteric defects for IH remains unpredictable. A positive CT for IH was a predictive factor for symptom relief after reoperation for (suspected) IH after LRYGB. However, many patients benefit from closure of the mesenteric defects, irrespective of perioperative presence of IH.

Introduction

The laparoscopic Roux-en-Y gastric bypass (LRYGB) has become a common bariatric procedure leading to satisfying long-term results in both weight reduction as well as reduction or even remission of comorbidities of morbid obesity. [1-4] However, due to altered bowel anatomy after LRYGB, internal herniation (IH) can occur through either the mesenteric defect of Petersen's space or the mesenteric defect of the jejunojejunostomy (JJ-stomy) during follow-up. [5] The reported incidence of internal herniation varies widely between 1.6 and 9.3%. [5-9] The typical presentation of patients with an internal herniation is intermittent, postprandial, upper abdominal pain, sometimes accompanied by nausea and vomiting. [10 11] Less frequently acute intestinal obstruction with or without bowel strangulation may occur, in which case emergency surgery is indicated. The mean interval between LRYGB and presentation of IH varies between 15 to 26 months in larger series. [12 13]

The presence of a 'swirl sign', caused by rotation of the mesenteric vessels on computed tomography (CT), is the golden standard to diagnose an IH, albeit varying sensitivity outcomes of CT have been reported. [14-16] Typically, management of IH consists of a reoperation with repositioning of the herniating bowel and closure of both mesenteric defects. [17 18]

Since IH may present with non-specific symptoms, preoperative diagnosis may be difficult and negative explorations have been described. [8] In some patients with typical intermittent pain symptoms and a clear 'swirl sign' on CT scan, actual visible IH may be absent during surgery. In case of open mesenteric defects without objectified IH, patients may still benefit from closure of the mesenteric defects. Even more strikingly, some asymptomatic patients may have IH clearly visible on abdominal CT or during reoperation but do not benefit from closure. Therefore, outcome of pain- and symptom relief after mesenteric defect closure seems to be highly unpredictable in literature.

The aim of this study is to investigate patient related factors and intraoperative findings in patients with delayed closure of mesenteric defects, in order to predict postoperative symptom relief after reoperation in patients with suspected IH after LRYGB.

Methods

Patient selection

Mesenteric defects were not routinely closed during LRYGB at our institutions until January 2017. Generally, we differentiate between patients readmitted with acute symptoms of IH, possibly with abdominal tenderness and hemodynamic instability, and patients

with a more chronic or intermittent presentation. In case of acute symptoms, urgent abdominal CT is typically performed, with subsequent laparoscopy if IH is suspected or if serious abdominal symptoms persist without any diagnosis. Patients with a more chronic or intermittent presentation first undergo treatment with increased dosage of proton pomp inhibitors (PPI) and mucosal protective drugs (MPD). If complaints are persistent, an abdominal CT, gastroscopy (EGD) and/or reoperation are performed for suspected IH. For this analysis, all patients who underwent reoperation after LRYGB for suspected acute or chronic IH from June 2009 until December 2016 at the three bariatric institutions were retrospectively reviewed. Patients were excluded if other abnormalities were found during reoperation that could explain the complaints (e.g. obstructing adhesions) or in case mesenteric defects turned out to be closed during exploration (e.g. by adhesions).

All CT scans were interpreted both by a radiologist and an experienced bariatric surgeon. We defined a CT scan as positive if a swirl sign with an estimated amount of swirl of at least 180˚ were seen. Two examples of a positive swirl sign on CT are shown in figure 1 and 2. Intraoperative findings were investigated and postoperative pain relief was assessed for all patients. Subsequently, predictors of internal herniation during reoperation and predictors of postoperative pain relief were investigated. Pain relief was scored positive if the patient did not have postprandial, upper abdominal pain three months after reoperation.

Figure 1. Transverse CT scan through mesentery shows a subtle swirl sign of the mesenteric vessels of a 45-year-old woman with internal herniation.

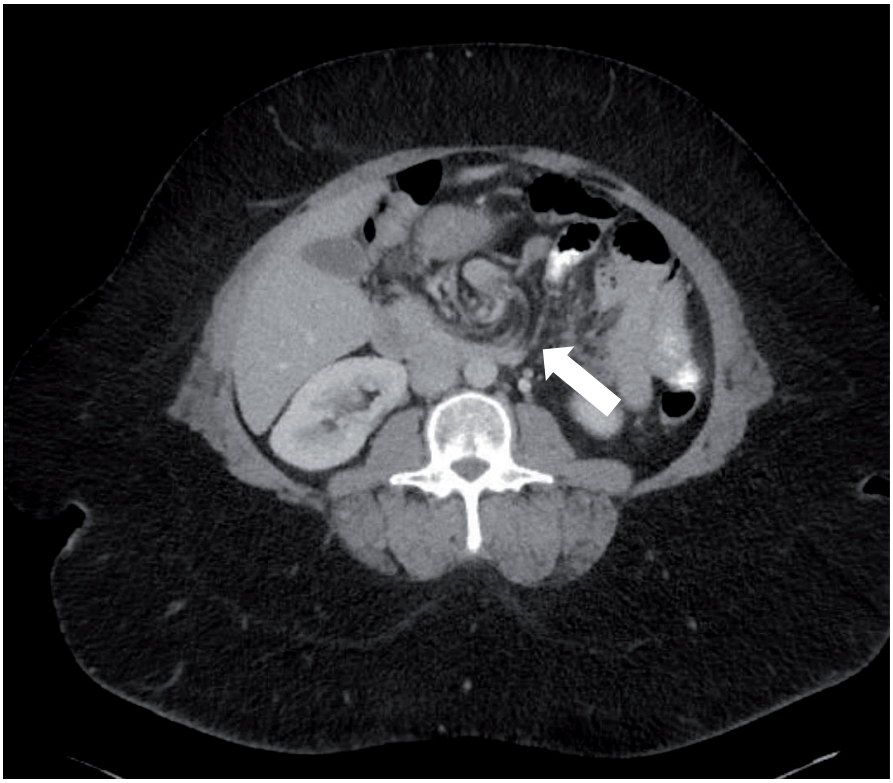
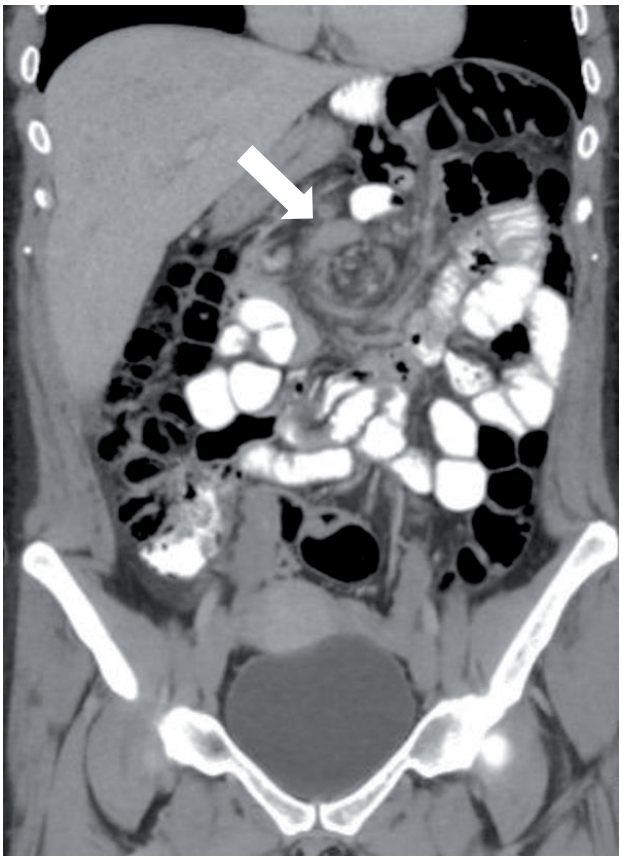


Figure 2. Sagittal CT scan of a 360˚ swirl sign in the mesenteric vessels of a 34-year-old woman with internal herniation.



Surgical procedure

In our institutions, LRYGB was performed without primary closure of the mesenteric defects. The LRYGB was performed with an antecolic approach. After division of the jejunum, the biliopancreatic limb was positioned to the left of the alimentary limb while rotating the alimentary limb to the right. The jejunojejunal limb and biliopancreatic limb were positioned to the left of the alimentary limb.

Reoperations were performed by an experienced bariatric surgeon using laparoscopy with inspection of the bowel anatomy of the alimentary limb, the biliopancreatic limb and the common limb. If internal herniation in Petersen's space or through the defect of the jejunojejunostomy was present, the bowel was repositioned and the mesenteric defects were either closed with running, non-absorbable sutures or with non-absorbable staples (EndoHernia™, Medtronic, Minneapolis, MN), depending on the surgeon's preference.

End points and statistical analysis

Statistical analysis was performed with IBM SPSS Statistics, version 23 (SPSS, Chicago, IL). Univariate logistic regression was used to investigate predictors of IH during reoperation and postoperative pain relief. Crosstabs were used to calculate sensitivity and specificity of

the interpretation of the CT-scan. We have used LOWESS local regression to determine the point on which %TWL could be a predictor for pain relief. Multivariate logistic regression was performed to determine independent predictors for pain relief. A p-value <0.05 was considered statistically significant.

Results

A total of 193 reoperations were performed for (suspected) IH, with an estimated incidence of 2.8% (a total of 6896 LRYGB were performed in our institutions during the study period). Mean age of patients at reoperation was 41.5 ±9.6 years and 171 (88.6%) patients were female. The median interval between gastric bypass and reoperation was 18.3 ±19.0 months. Laparoscopic cholecystectomy was performed between LRYGB and reoperation for suspected IH in 16.0% of the study cohort, table 1. In 35.2% of the patients, gastroscopy was performed before reoperation for suspected IH. In 28 (14.5%) patients, gastroscopy was performed after reoperation, in 4 cases a marginal ulcer was found.

Of all procedures, 72 (37.3%) were performed in an acute setting. Preoperative abdominal CT was performed in 144 patients, and in 56 patients (38.9%) signs of internal herniation were found. The sensitivity of the swirl sign found on CT for suspected IH after interpretation of a radiologist and an experienced bariatric surgeon was 50.0% and the specificity was 83.0%.

An IH was found intraoperatively in 118 (61.1%) patients, there was no preference for the JJ-stomy or Peterson's space, table 2. There were no cases of intestinal ischemia. In 75 (38.9%) patients, no abnormalities were found intraoperatively. Nevertheless, mesenteric defects were closed in these patients in order to prevent future IH -absorbable sutures were used more frequently than staples to close the mesenteric defects (164 vs. 22). In the remaining 7 patients, absorbable sutures were used. A total of 37 patients underwent reoperation for recurrence of the postprandial, upper abdominal complaints. Four of them had recurrence of IH after previous closure with absorbable sutures, 14 had previous closure with non-absorbable sutures and 1 had a recurrence after closure with staples. In 18 patients with symptom recurrence, there was no perioperative sign of IH. Three of them had a marginal ulcer during gastroscopy. Complete postoperative symptom relief was observed in 146 patients (77.2%). For patients in which IH was present during surgery, 82.8% had relief of pain postoperatively, as compared to 68.5% for those procedures in which no IH was found.

Table 1. Baseline characteristics

Variable	Number/Mean	Range/percentage
Demographics		
Age at reoperation (years)	41.5 ±9.6	21 – 61
Female gender	171	88.6%
BMI before gastric bypass[1]	42.4 ±5.4	26 – 63
BMI at reoperation[1]	28.9 ±5.7	19 – 56
BMI loss between LRYGB and reoperation[1]	13.6 ±5.3	0 – 27
% TWL	31.4 ±11.4	-9.9 – 54.4
Interval LRYGB and reoperation (months), median	18.3 ±19.0	0 – 99
Presentation and imaging		
Acute presentation	72	37.3%
CT abdomen performed	145	74.6%
CT normal	87	60.4%
Internal herniation on CT	58	40.2%
Days between CT and reoperation, median	9.5 ±97.1	0 – 535
Days between IH on CT and reoperation, median	1.0 ±21.4	0 – 135
Medical history		
PPI use	124	64.2%
Smoking	52	26.9%
Previous cholecystectomy		
No	114	59.1%
Before LRYGB	21	10.9%
After LRYGB but before re-operation	31	16.1%
During reoperation	19	9.8%
After re-operation	8	4.1%
Gastroscopy		
No	97	50.3%
Before reoperation	68	35.2%
After reoperation	21	10.9%
Before and after reoperation	7	3.6%

[1] In kg/m²

BMI = body mass index; LRYGB = laparoscopic Roux-en-Y gastric bypass; CT = computed tomography; IH = internal herniation; PPI = proton-pump inhibitor

Table 2. Operative characteristics

Variable	Number (n=193)	(%)
Conversion/laparotomy	6	3.1%
Internal herniation during surgery	118	61.1%
Petersen's space hernia	54	45.8%
JJ-stomy hernia	55	46.6%
Peterson's space and JJ-stomy hernia	9	7.6%
Closure technique[1]		
Non-absorbable suture	164	85.0%
Staples	22	11.4%
Postoperative symptoms		
Relief of symptoms[2]	146	77.2%
Additional reoperations		
For suspected recurrence IH	37	19.2%
Proven recurrence IH	19	9.8%

JJ-stomy = jejunojejunostomy; IH = internal herniation

[1] In seven patients, absorbable sutures were used

[2] Missing data of 4 patients, so total population to answer this question is 190 patients.

Table 3. Predictors of intraoperative presence of internal herniation

Variable	Internal herniation (%)	OR	95%CI	P value
Age > 45 (n=75)	46 (61.3%)	1.01	0.56 – 1.84	0.965
Female gender (n=171)	101 (59.1%)	0.42	0.15 – 1.20	0.107
Male gender (n=22)	17 (77.3%)	2.36	0.83 – 6.69	0.107
Reoperation after 2014 (n=137)	87 (64.0%)	1.49	0.80 – 2.79	0.214
BMI ≥ 30 at reoperation (n=73)	47 (65.3%)	1.34	0.73 – 2.46	0.340
BMI loss ≥ 20 (n=25)	15 (60.0%)	1.04	0.45 – 2.43	0.926
%TWL >40 (n=44)	30 (68.2%)	1.54	0.75 – 3.14	0.753
Acute surgery (n=73)	57 (79.2%)	3.74	1.91 – 7.31	<0.001*
Elective surgery (n=121)	61 (50.4%)	0.27	0.14 – 0.52	<0.001*
CT normal (n=87)	43 (47.3%)	0.20	0.09 – 0.45	<0.001*
Internal herniation on CT (n=58)	48 (82.8%)	4.91	2.11 – 10.93	<0.001*

BMI = body mass index; %TWL = percentage total weight loss; CT = computed tomography

* Significant difference

Predictors of internal herniation during reoperation and relief of symptoms

There was no significant difference in the presence of IH between females and males (p=0.107), table 3. When internal herniation was visible on CT, IH was found present perioperative in 82.8% of procedures (OR 4.78; 95%CI 2.09-10.93). In patients with normal

abdominal CT, internal herniation was found in 47.3% of procedures (OR 0.20; 95%CI 0.09-0.45). In acute surgery, perioperative IH was seen more frequently than in elective surgery (OR 3.74, 95%CI 1.91-7.31).

A predictive factor for pain relief after delayed closure of mesenteric defects was IH on CT (OR 4.24, 95%CI 1.63-11.05). Presence of IH perioperatively affected postoperative pain relief (OR 2.21, 95%CI 1.11-4.40), table 4. In multivariate analysis, a positive CT scan for IH was the only independent predictor for pain relief. Time from initial LRYGB to reoperation, the location of IH and the closure technique did not seem to affect postoperative pain relief. There was no significant correlation between smoking status and postoperative pain relief, table 4.

Table 4. Predictors of symptom relief after reoperation

Variable	Symptom relief (%)	OR	95%CI	P value
Age > 45 (n=73)	56 (76.7%)	0.95	0.47 – 1.91	0.889
Female gender (n=167)	127 (76.0%)	0.50	0.14 – 1.78	0.286
Male gender (n=22)	19 (86.4%)	2.00	0.56 – 7.09	0.286
%TWL> 40 (n=44)	34 (77.0%)	1.02	0.46 – 2.28	0.962
Acute surgery (n=70)	57 (81.4%)	1.48	0.71 – 3.07	0.295
Elective surgery (n=119)	89 (74.8%)	0.68	0.33 – 1.41	0.295
Smoking (n=50)	41 (82.0%)	1.48	0.65 – 3.34	0.352
CT normal (n=86)	57 (66.3%)	0.24	0.09 – 0.61	0.003*
Internal herniation on CT (n=56)	50 (89.3%)	4.24	1.63 – 11.05	0.003*
Internal herniation during surgery (n=116)	96 (82.8%)	2.21	1.11 – 4.40	0.024*
Petersen's space (n=53)	42 (79.2%)	1.18	0.54 – 2.55	0.683
JJ-stomy (n=54)	45 (83.3%)	1.68	0.75 – 3.80	0.210
Petersen's space and JJ-stomy[1] (n=9)	9 (100%)			0.095
Closing technique				
Non-absorbable suture (n=160)	123 (76.9%)	0.78	0.28 – 2.22	0.645
Staples (n=22)	17 (77.3%)	1.00	0.35 – 2.89	0.998

%TWL = percentage total weight loss; CT = computed tomography; JJ-stomy = jejunojejunostomy

* Significant difference

[1] As all patients with an internal herniation at both spaces had postoperative symptom relief, Odds ratio could not be calculated and therefore Pearson's Chi square was used.

Discussion

A swirl sign on CT was predictive for both perioperative presence of IH as well as for postoperative pain relief after delayed closure of mesenteric defects. Actual visible IH during laparoscopy appeared more common in an acute setting than when surgery was performed electively. Perioperative presence of IH was a predictive factor for pain relief postoperative; however, the location of the IH did not seem to affect postoperative pain relief.

The number of re-laparoscopies and performed CT scans for suspected IH increased considerably over the years in our clinics. A possible explanation for this trend may be increased knowledge and awareness regarding long-term complications of LRYGB. The median interval between LRYGB and re-operation for suspected IH in our study was comparable to other studies. [5 12 13] Previous studies reported rapid excess weight loss (EWL) as a predictive factor for the incidence of IH, in which the risk of developing IH was twice as high in patients with rapid EWL. [19 20] We have used LOWESS local regression to determine the point on which %TWL could be a predictor for pain relief, which was 40%. In our study, %TWL ≥ 40% as compared to a %TWL < 40% did not seem to affect the intraoperative presence of IH or of postoperative pain relief. As our study was retrospective, we did not have the exact weight loss per time period whether there was rapid weight loss could not be determined.

The presence of a swirl sign on CT was a predictor for both intraoperative presence of IH as well as postoperative symptom relief. However, a varying sensitivity of CT scans for diagnosing IH has been described in literature, ranging between 61 – 83%. [14 16] The existence of intermitting IH could be an explanation for these low sensitivity rates, as the CT scans were not always performed at the moment when a patient experiences pain.

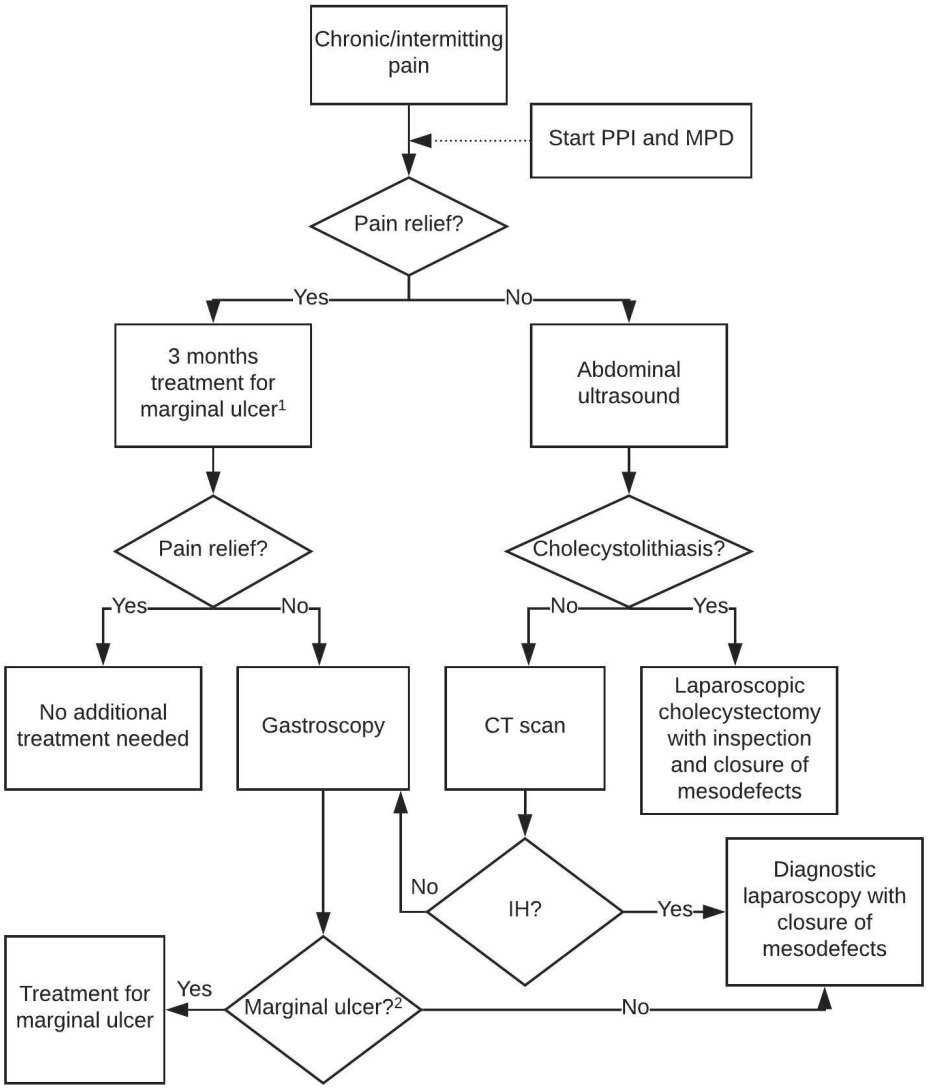
Notably, in some patients with a proven IH on CT there was a long interval between CT and reoperation. In three patients, CT was performed in a non-bariatric hospital, where they did not acknowledge the pain as IH. Once the patients arrived in one of our institutions, the IH was seen on CT and reoperation was performed soon afterwards. Also, in two patients, symptoms had dissolved at the time of interpretation of the CT, and therefore underwent elective surgery. However, we would advise to perform a reoperation when IH is seen on CT as soon as possible to reduce symptoms and prevent potential incarceration.

We recommend that in all patients with chronic and/or intermittent postprandial, upper abdominal pain a treatment with PPI and mucosal protective drugs is started. If this does not give pain relief, the presence of cholecystolithiasis should first be excluded by ultrasound. If there are no gallstones detected or if the patient does not have a gall bladder anymore, we would advise to perform a CT scan in order to rule out IH. If there is no swirl

sign on CT, gastroscopy should be performed to exclude the presence of a marginal ulcer. If the gastroscopy is negative as well and symptoms persist, we would advise to perform a diagnostic laparoscopy to close the mesenteric defects. Our recommended treatment algorithm for chronic and/or intermittent complaints can be found in figure 3.

Overall, IH was present during surgery in only 61.3% of procedures. Surprisingly, 77.2% of all patients did report postoperative pain relief after closure of mesenteric defects. In 68.5% of all procedures in which no IH was found perioperatively, postoperative pain relief was reported. Possible explanations for this observation are the intermittent presence of IH or a placebo effect of reoperation.

Figure 3. Treatment algorithm for patients with chronic and/or intermittent postprandial upper abdominal pain.



[1] Treatment with PPI (=proton pump inhibitor) and MPD (= mucosal protective drugs) for gastric irritation, gastritis or marginal ulcer

[2] If a marginal ulcer is not found during gastroscopy and a CT scan has not been performed yet, a CT scan prior to diagnostic laparoscopy is recommended to exclude other abdominal pathologies

Closure of mesenteric defects with sutures or with staples during initial LRYGB appears to result in lower incidence of IH as compared to no closure. [6 20 22] In the present study, there is no significant difference in the odds of symptom relief after closure of mesenteric defects with sutures as compared to staples.. A limitation of this study is the small number of patients in whom staples were used to close the mesenteric defects. Further research to the difference in the use of staples versus non-absorbable sutures is recommended.

In conclusion, pain relief after closure of the mesenteric defects for (suspected) IH remains unpredictable. A swirl sign on CT was the only significant predictor of pain relief after reoperation for (suspected) IH after delayed closure of mesenteric defects of LRYGB. However, many patients benefit from closure of the mesenteric defects, irrespective of perioperative presence of IH, and therefore reoperation for suspected IH is recommended if no marginal ulcer was found during gastroscopy.

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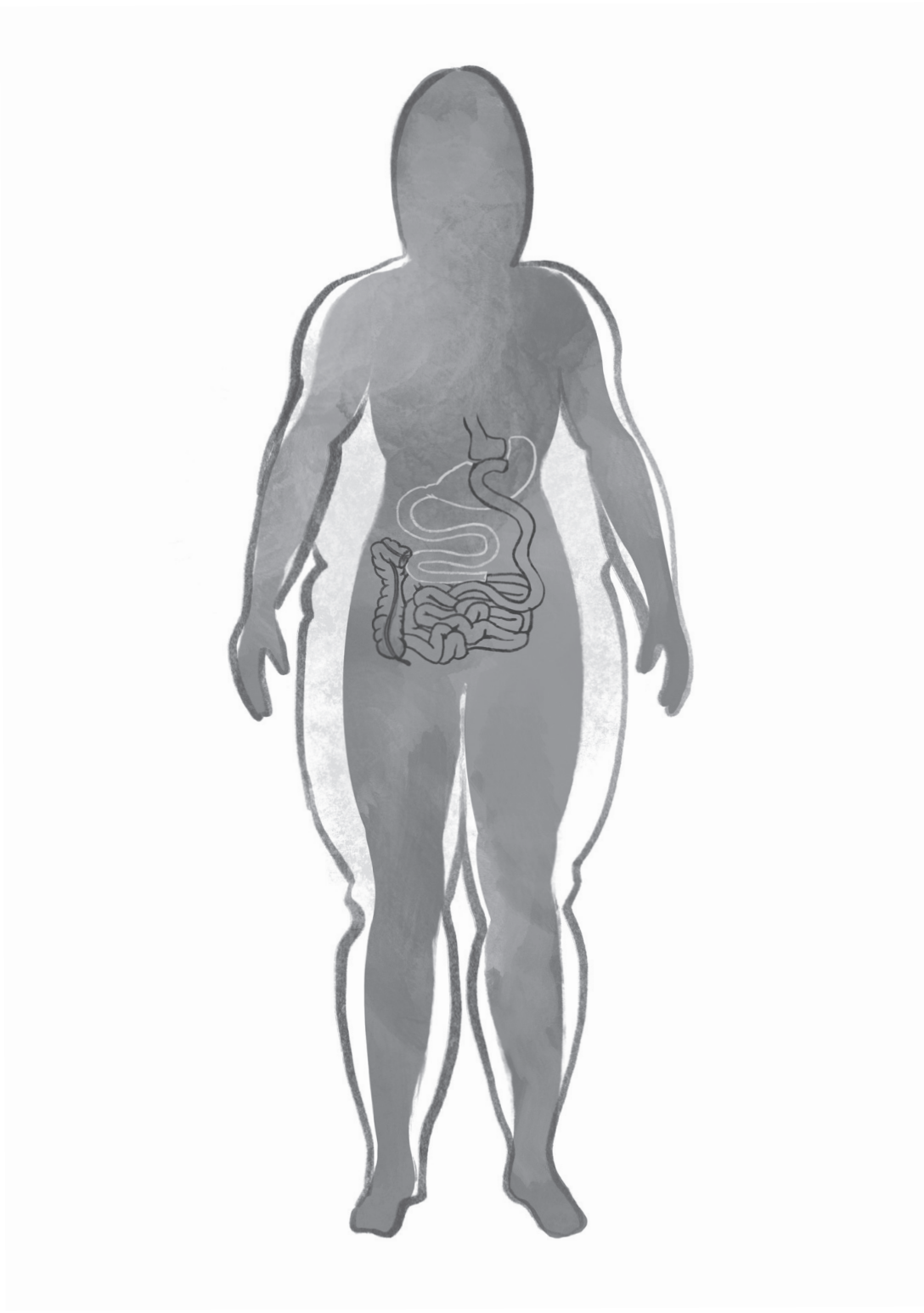

Chapter 9

General discussion and future perspectives

General discussion

In spite of the risen awareness of the negative health consequences of morbid obesity, the worldwide prevalence of obesity has nearly tripled between 1975 and 2016[1]. In 2019, half of the Dutch population aged eighteen or older had overweight and as many as 14.7% was diagnosed with morbid obesity[2]. If this trend of the past decades continues, the prevalence of morbid obesity will continue to rise.

We currently live in a world with food in abundance and we have developed into a sugar consuming society, which is highly responsible for the increasing prevalence of morbid obesity. The first step in obesity treatment is adjustment of lifestyle. This generally consists of dietary changes with reduction of caloric intake and an increase of physical activity. In some cases this will be combined with psychological therapy in order to motivate patients for lifestyle adjustment. Although lifestyle adjustment initially leads to substantial weight loss, most individuals fail to maintain the body weight beyond the intervention period[3]. Weight regain that even surpasses the weight before lifestyle intervention might occur, also known as weight cycling or the Yoyo effect. It has been hypothesized that there is an obesogenic memory that drives weight regain in order to maintain a previous status quo of body weight, in order to protect the body from weight loss[4 5]. This set-point theory helps us understand why lifestyle intervention often fails on the long-term.

For decades, doctors have been searching for the treatment of morbid obesity. In 1983, Kuzmak invented the first gastric band. Ten years later, Belachew placed the first adjustable gastric bands in four voluntary patients[6]. Since the publication of these results, bariatric surgery emerged. Optimization of the gastric band followed quickly, leading to increased weight loss and a decrease of postoperative complications[7]. Additionally, new bariatric surgical techniques were developed to increase weight loss with better long-term effects and with more improvement of obesity-related comorbidities. As a result of this evolution, laparoscopic adjustable gastric banding (LAGB) is barely performed nowadays. Instead of LAGB, the laparoscopic Roux-en-Y gastric bypass (LRGYB) and laparoscopic sleeve gastrectomy (LSG) are the two most performed bariatric techniques as they have better short- and long-term results. It has even been proposed that LRYGB and LSG can reset the obesogenic memory[8], which could explain why successful long-term weight loss is achieved after bariatric surgery and not after lifestyle adjustment.

As bariatric techniques have improved over the past few decades, bariatric surgery is performed more often worldwide. Nowadays, approximately 12.000 bariatric interventions are performed annually in the Netherlands only[9]. Even though the high numbers of surgeries performed annually, many physiological side-effects of bariatric surgery remain

uninvestigated. Hence, the aim of this thesis was to address less known effects of bariatric surgery in large patient cohorts.

The first part of this thesis focuses on cardiovascular changes after Roux-en-Y gastric bypass in a study population without cardiac history. The second part of this thesis concentrates on the composition of immune subsets in morbidly obese patients and the effect of bariatric surgery on the immune function. The last part of this thesis focuses on two long-term complications after bariatric surgery: insufficient weight loss after bariatric surgery and internal herniation.

Part I. Cardiovascular changes after Roux-en-Y gastric bypass

Bariatric surgery has proven to reduce morbid obesity and its associated cardiovascular morbidity and mortality in patients known with cardiovascular disease[10 11]. However, it remains unclear whether improvement of cardiovascular morbidity also occurs in patients without cardiovascular disease. Therefore the aim of this part was to investigate whether premature atherosclerosis can be reversed by bariatric surgery and if it can improve the cardiac function in morbidly obese individuals without cardiac history.

Chapter 2 of this thesis addresses the reversibility of early atherosclerotic changes after bariatric surgery in the individual obese patient. This was assessed by the measurement of the carotid intima-media thickness (CIMT) in different age groups before and after bariatric surgery. In this study, we found a decrease in CIMT and an improvement of cardiovascular risk reduction after bariatric surgery. The results were more pronounced in patients aged 50 or younger. The minor improvements of cardiovascular risk factors in older patients may suggest that long-lasting exposure to 'athero-inflammation' leads to less-reversible atherosclerotic plaque changes. The risks of bariatric surgery generally increase with age, while the cardiovascular benefits decrease. Nonetheless, the CIMT did improve in all patients, suggesting that the risk for cerebral vascular accidents might diminish after bariatric surgery. Overall, the indication for bariatric surgery should be carefully weighed in patients older than 50 years.

A limitation of this study was the relative short follow-up period of one year. We would recommend to additionally assess the long-lasting effects of bariatric surgery on cardiovascular improvements in both age groups. A strength of this study was the adequate power calculation, by which subgroup analysis for different age categories was possible. Another strength was the high intra- and inter-observer variability of the CIMT measurement (0.768 and 0.829 respectively), suggesting high reproducibility of the results.

Chapter 3 of this thesis focused on the effects of bariatric surgery on cardiac function in patients without a history of cardiac disease specifically. We found a significant decrease in left ventricle ejection fraction after LRYGB, which was also decreased after correction for body surface area. Additionally, cardiac output and absolute left ventricular mass decreased twelve months postoperatively.

The use of cardiac magnetic resonance imaging (CMRI) to assess cardiac changes was one of the strengths of this study. In previous studies, mostly cardiac ultrasound was performed to assess cardiac function. However, the measurement by ultrasound can be highly influenced by subcutaneous fat, which is obviously present in our morbidly obese study population. Therefore, CMRI is suggested as gold standard for the measurement of cardiac function in patients with morbid obesity. The small study population was a limitation of this study. To our knowledge, there was no literature on changes of cardiac function in patients without cardiac history. Therefore, we first investigated the effects of bariatric surgery in pilot study in a small study population. Further research in a larger study population is recommended in order to obtain better insight of the correlations of covariates resulting in improvement of cardiac function.

Future perspectives

Part I of this thesis confirms that prevention is better than cure, as the young population achieved more improvement of cardiovascular risk factors than the older population. Moreover, cardiac function even improved in patients without any signs of cardiovascular disease preoperatively.

The eligibility for bariatric surgery is currently based upon body weight and the presence of obesity-related comorbidities[12-14]. These guidelines focus on cure rather than prevention. If a person has a BMI of 35-40 kg/m^2 this patient should also have the presence of at least one obesity-related comorbidity in order to be eligible for surgery. In other words, the damage of obesity should have already been done before a person is allowed bariatric surgery. Moreover, the type of fat distribution influences the risk on the development of obesity-related comorbidities. It has been proven that obese individuals with mainly visceral fat mass have a higher risk for comorbidity development as compared to obese individuals with peripheral fat distribution[15-18]. With this in mind, assessing the eligibility for bariatric surgery on BMI and the presence of obesity-related comorbidities seems outdated. De Lorenzo et al. suggested new obesity classification criteria for the assessment of eligibility for bariatric surgery[19]. In this paper, it is recommended to include the assessment of fat distribution in the determination whether someone is eligible for bariatric surgery. When this assessment is included in the eligibility guidelines, bariatric surgery can also become a preventive rather than curative intervention only. This part of the thesis has specifically proven that bariatric surgery is prevents the development of

cardiovascular disease, as the cardiovascular system improves even before patients show signs of cardiovascular disease. To conclude, it is recommended to develop a patient specific system for the assessment of eligibility for bariatric surgery. In these guidelines, not only BMI and the presence of obesity-related comorbidities should be taken into account, but also fat distribution and age.

Part II. Immunological changes after Roux-en-Y gastric bypass

Visceral fat mass is not only known for a higher risk for the development of obesity-related comorbidities. The high number of adipocytes in in the white adipose tissue of morbidly obese patients leads to a chronic, low-grade inflammation[20 21]. This might lead to accelerated aging of the immune system and a decrease of immune function[21-25]. However, most studies that investigated the influence of morbid obesity and bariatric surgery on the immune system were performed in small study populations. Moreover, results were not always compared with lean controls. Therefore, the aim of this part was to assess the immune system in a large morbidly obese patient cohort as compared to lean individuals, and to evaluate the effect of bariatric surgery on the immune function.

Cells of the adaptive as well as the innate immune system are affected by morbid obesity, as shown in **Chapter 4**. These obesity-induced effects were confined to shifting of the CD8+ T cell and B cell compartment to a more differentiated phenotype, which is comparable to what has been described in the elderly population[26 27]. However, the differences were relatively small, and thus it might be questioned whether those differences have clinical consequences. We hypothesized that patients with metabolic syndrome (MetS) would have a more differentiated phenotype of immune cells, as MetS is associated with more visceral fat and obesity-related comorbidities. Thus, we expected increased inflammation in patients with MetS as compared to patients without MetS. In our study, MetS only affected the CD4+ T cell compartment, which is in contrast to our expectations and to previously published literature[28 29]. As our study was performed in a large population consisting of 117 patients without MetS and 127 patients with MetS, we suggest that the accumulation of white adipose tissue in morbidly obese patients causes the inflammation, and not the presence of MetS.

Notably, there was no decrease in naive T and B cells, suggesting that the thymic function is not compromised in morbidly obese individuals. This also suggests that aging of the immune system in morbidly obese patients might be reversed by bariatric surgery. Accelerated aging of the immune cells was halted by substantial weight loss[29], thus phenotypic shifting might be reversed to a less differentiated phenotype. Consequently, bariatric surgery might not only lead to substantial weight loss and reduction of obesity-related comorbidities but might also rejuvenate the immune system. Further research

into the effect of bariatric surgery on the immune system is recommended, as it remains unclear if bariatric surgery can induce reversal of the immune phenotype.

To our knowledge, this was the first large and comprehensive study investigating immunosenescence in morbidly obese patients. As we only used minor exclusion criteria, the results reflect the general population of morbidly obese patients visiting the outpatient clinic. However, we have only focused on the composition of the immune system. To obtain a full scope of the effects of morbid obesity on the immune system, a study to additionally assess the functioning of the immune system in this large population is recommended.

Chapter 5 addresses this immune function by assessing the cytokine producing capacity of T and B cells in morbidly obese patients before and after bariatric surgery. In this study, the IL-2 and IFN-γ production of CD8+ T cells only was decreased in morbidly obese patients as compared to lean controls. Three months after bariatric surgery, the IL-2 and IFN-y producing capacity of CD4+ T cells was slightly increased; however, that of CD8+ T- and B cells was not restored. Literature suggested that weight loss leads to metabolic improvement, eventually leading to changes in cellular immunity[30]. However, we could not reproduce those results. Additionally, preoperative presence MetS did not seem to affect postoperative changes in cytokine producing capacity. Since the few differences that were discovered were relatively small, the clinical relevance of these changes could be questioned.

The short follow-up period of this study could be an explanation for the minor changes we have seen after bariatric surgery. As the total expected excess weight loss after bariatric surgery is typically achieved after twelve to eighteen months[31], the immune system might also need a longer period to recover from the alterations caused by morbid obesity. Therefore, further research with a longer follow-up period is recommended.

Another limitation of this study is that we have only investigated the lymphocytes of the peripheral blood. As some studies have reported effects of morbid obesity in T and B cell function in adipose tissue particularly[32 33], it would be interesting to compare the T and B cell function in both peripheral blood and adipose tissue. Also, we investigated a small selection of cytokines, so the entire cytokine producing profile of T and B cells was not covered in this paper.

Future perspectives

In this part, the impact of morbid obesity on the induction of accelerated aging of the immune system in the lymphocytes of the peripheral blood was assessed. It would be interesting to compare the T and B cell subset composition and the cytokine producing

capacity of these immune cells in lymphocytes of the peripheral blood with that of the adipose tissue. Particularly because the white adipose tissue is known for increased cytokine production, creating a chronic and low-grade inflammatory state[21 22].

Additionally, it would be interesting to investigate whether accelerated aging of the cells is reflected by telomere length or telomerase activity. Telomeres play an important role in the protection of DNA damage in humans[34]. Telomere length is decreased in the aging population. A decrease of telomere length is known for more DNA damage, leading to an increased risk of tumor growth[35]. Telomerase activity can increase the telomere length in cells, and thus increases the protective character of the telomeres. Rat studies have shown that aging leads to shorter telomere length and decreased telomerase activity in liver cells[36]. In a study performed in humans, both skeletal muscle telomere length and leukocyte telomere length decreased with age; however, muscle telomere length was always longer than leukocyte telomere length[37]. This could be explained by the fact that leukocytes divide more often than muscle cells, and telomere length is shortened by cell division.

In morbidly obese patients, similar results were presented in telomere length attrition as compared to aging individuals. In a study performed in 7827 humans, obesity was related with a shorter telomere length in leukocytes in young participants[38]. This relationship diminished with an increasing age. A study performed in morbidly obese patients who underwent bariatric surgery, attrition of telomere length in leukocytes was seen preoperatively, which was temporarily reversed after bariatric surgery[29]. Another study confirms the attrition of telomere length of morbidly obese subjects, but this study showed an additional attrition after bariatric surgery instead of an improvement[39]. As results on the effect of bariatric surgery on telomere length are still contradicting, it is interesting to repeat these studies in a large study cohort with a follow-up period of at least eighteen months, as the catabolic state after bariatric surgery is often restored after this period. Additionally, it is recommended to assess the telomere length in peripheral blood, liver tissue, muscle tissue and adipose tissue. By doing so, the effect of obesity on the different tissues can be evaluated.

Besides the aging markers that were investigated in this thesis, it would be interesting to look at additional biological age markers in morbidly obese patients in order to evaluate the effect of bariatric surgery on aging. The first marker that would be interesting to investigate is DNA methylation. Methyl groups are added to the DNA molecule, which is called DNA methylation. By this, the activity of the DNA segment can change. In aging, there is a change in DNA methylation. The DNA methylation levels can be used to accurately estimate the age in all sources of DNA, also known as the epigenetic clock[40]. DNA methylation status of leukocytes, liver tissue, adipose tissue and muscle tissue

can be determined and compared to each other, and to age matched lean controls. Importantly, the DNA methylation biomarkers can detect epigenetic changes. Thus, the effect of bariatric surgery on DNA methylation in morbidly obese patients can be assessed[41]. One study assessed DNA methylation after bariatric surgery. In this cohort of 40 patients, a decrease in biological age and epigenetic age acceleration was seen. It would be recommended to repeat this study in a larger study cohort. The other option is to assess a panel of metabolic markers that are associated with aging. Deelen et al. performed metabolic biomarker profiling in 44,168 individuals, and by this identified a panel of 14 markers[42]. This panel was developed to estimate all-cause mortality based on metabolic biomarkers. However, it could also be used to estimate biological age. This can be estimated in morbidly obese patients and compared with age-matched lean controls. Additionally, the effect of bariatric surgery on biological age can be assessed using this panel of biomarkers.

Part III. Long-term complications after bariatric surgery

Even though bariatric surgery has been shown to be a safe and successful treatment of morbid obesity and obesity-related comorbidities[43 44], it is also associated with long-term complications. Literature demonstrates intervention and reoperation rates after bariatric surgery from 8.9% to 19.5%[45 46]. For all bariatric procedures, long-term complications such as nutritional and vitamin deficiencies, incisional hernias and cholelithiasis are described[47]. For LRYGB specifically, anastomotic stricture, marginal ulceration, dumping syndrome, gastrogastric fistula and internal hernia have been described. For LSG particularly, sleeve stricture and gastroesophageal reflux disease have been described. Moreover, insufficient weight loss or weight regain can occur after bariatric surgery. As this might lead to revisional bariatric surgery, insufficient weight loss or weight regain should be considered as long-term complications too[48 49].

Both **Chapter 6** and **Chapter 7** of this thesis focus on the treatment of insufficient weight loss or weight regain after bariatric surgery. In **Chapter 6** the effect of revisional LRYGB after failure of laparoscopic adjustable gastric banding (LAGB) was assessed. In this study, we distinguished between responders and nonresponders to LAGB. Patients were identified as nonresponders if they had achieved less than 25% excess weight loss (EWL) after LAGB, while responders initially achieved more than 25% EWL after LAGB. Notably, responders to LAGB achieved significantly higher %EWL after revisional LRYGB as compared to nonresponders (58.2% versus 48.1% respectively, $P<0.001$). A genetic component could be responsible for the differences in weight loss between nonresponders and responders to LAGB[50]. It could also be explained by the inability for nonresponders to adjust their lifestyle after LAGB, and we could expect that they will not be able to adjust their lifestyle after revisional LRYGB as well. Therefore, it is important to screen all patients routinely, and

to assess whether they have adequately adjusted their lifestyle in terms of diet habits and sufficient physical activity.

Without a doubt, it is a strength that we included a total of 1501 patients in this study. Unfortunately, follow-up data on obesity-related comorbidities were not registered adequately and could therefore not be used in this retrospective cohort study. Data on the comorbidities is interesting as bariatric surgery is not only performed in order to achieve sufficient weight loss. Without a doubt, reduction of obesity-related comorbidities is a very important outcome measurement as well. The reduction rate of obesity-related comorbidities after revisional LRYGB might not be as high as after primary LRYGB[51], but several studies have shown that comorbidities did improve after revisional LRYGB[52 53]. Thus, the evaluation of success after revisional LRYGB should not only be based upon weight loss, but upon reduction of comorbidities as well.

To conclude, adequate routine screening of revisional LRYGB candidates is recommended. Although revisional LRYGB in nonresponders after LAGB is not advisable in terms of weight loss, revisional LRYGB should be considered if substantial reduction of comorbidities is to be expected. However, we would advise to always adequately weigh the pros and cons of revisional bariatric surgery, as revisional LRYGB gives additional risks on postoperative complications.

Whereas **Chapter 6** focused on revisional LRYGB after failure of LAGB, **Chapter 7** focused on revisional LRYGB after insufficient weight loss or weight regain after LRYGB due to pouch enlargement. In this study, two revisional bariatric techniques for the resizing of the pouch were compared. Outcome measurements were achieved weight loss, reduction of comorbidities and average surgical costs. The two techniques were equally effective in terms of weight loss and reduction of comorbidities; however, the average surgical costs for sleeve resection of the gastrojejunostomy and gastric pouch were lower as compared to resection of the gastrojejunostomy with a new creation of the gastric pouch and anastomosis.

Reduction of the gastric pouch size resulted in substantial weight loss in both techniques. Therefore our data suggest that a small pouch will result in higher achieved weight loss. However, the effect of pouch size on the achieved weight loss remains controversial[54-57]. Even though all patients had proven pouch enlargement in our study, the exact sizes were not measured according to a standardize protocol preoperatively. Postoperative pouch size was not measured as well. It would, however, be interesting to assess whether pouch size could be related to achieved additional weight loss after revisional surgery. Another limitation of this study is that the revisional technique was based upon preference of the

surgeon. Even though there was no crossover in treatment between the surgeons, further research in a randomized controlled trial is recommended in order to prevent selection bias.

Chapter 8 discusses possible predictors of pain relief after reoperation for (suspected) internal herniation (IH), which is one of the most common long-term complications after LRYGB. Both a swirl sign on CT and perioperative presence of IH were predictive factors for postoperative pain relief. Notably, the achieved percentage total weight loss and the location of the IH were no predictors for symptom relief.

IH can occur through the mesenteric defect of Petersen's space or the mesenteric defect of the jejunojejunostomy. The treatment for (suspected) IH is repositioning of the bowel and closure of the mesenteric defects. Literature has shown that the incidence of IH decreases if the mesenteric defects are primarily closed during LRYGB[58-60]. Closing the mesenteric defects could be performed with either non-absorbable sutures or staples[59]. However, there is still no consensus on which technique is the most efficient and the choice for material is based upon the surgeon's preference.

Remarkably, 66.3% of the patients with a normal CT scan had symptom relief after reoperation for suspected IH. Torensma et al. have shown that using the CT scan in suspected IH is not useful if mesenteric defects were not closed, while it is a diagnostic tool for the presence of IH if mesenteric defects were closed[61]. Thus, in patients with typical complains of IH but without any signs for it on CT, reoperation with closure of the mesenteric defects is recommended if mesenteric defects were not primarily closed.

Future perspectives

Prevention of long-term complications after bariatric surgery is always better than cure. Unfortunately, surgical procedures will always come with a risk for the development of complications. Even though the bariatric procedures have improved immensely over the past decades, complications still occur.

Internal herniation is a complication that usually develops after at least 18 months after LRYGB. The typical presentation of patients with internal herniation is intermittent, postprandial upper abdominal pain; nonetheless, internal herniation may also present with non-specific symptoms. Therefore, we have developed a treatment algorithm for patients with chronic and/or intermittent postprandial upper abdominal pain, as presented in **Chapter 8**. The CT scan remains the most common diagnostic tool for the confirmation of an internal herniation; however, varying sensitivities have been reported[62 63]. Luckily, the radiologists have developed a structured reporting with ten signs that are used to aid CT diagnosis of internal herniation[64]. By this, sensitivity of the CT scan is increased. But like said before, it is better to prevent the development of a complication. The incidence

of internal herniation was definitely reduced after closure of the mesenteric defects was introduced[60]. After the publication of **Chapter 8** in 2018, we decided to primarily close the mesenteric defects during LRYGB. It would be interesting to assess the incidence of internal herniation since the mesenteric defects were routinely closed and compare it with the results presented in **Chapter 8**. The type of closure, either non-absorbable sutures or staples, was based upon the surgeon's preference. It would be very interesting to compare those two techniques, to find out if one of the two types of closure leads to a lower incidence rate of internal herniation. Undoubtedly, a randomized controlled trial would give the most valuable outcomes. However, as the incidence of internal herniation was already low before we started closing the mesenteric defects (2.8%), the number of patients that should be included in this study is high.

As new mesenteric defects might develop over time despite closure during LRYGB, inspection of the mesenteric defects every time a laparoscopy is performed is recommended. For instance, during laparoscopic cholecystectomy, a procedure that is often performed after bariatric surgery, inspection of the mesenteries should be performed, and possible defects should be closed.

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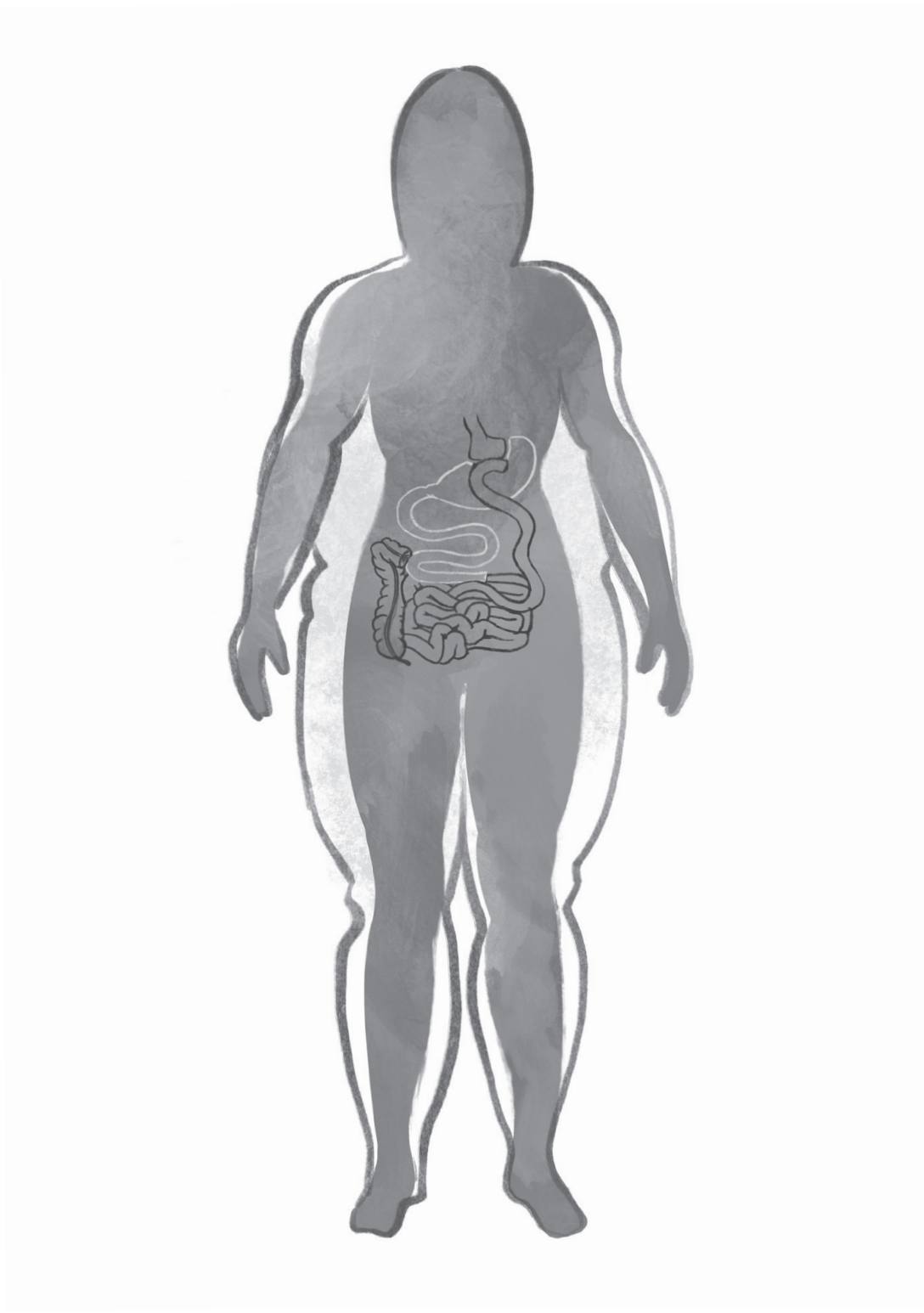


Chapter 10

Summary

Summary

The prevalence of morbid obesity has increased rapidly over the past decades. Morbid obesity increases the risk for the development of obesity-related comorbidities such as hypertension, type 2 diabetes and hypercholesterolemia. Those obesity-related comorbidities eventually lead to a reduced life expectancy. Bariatric surgery is the most effective treatment of morbid obesity and its related comorbidities. Laparoscopic Roux-en-Y gastric bypass (LRYGB) is currently the most performed bariatric intervention in the Netherlands. The aim of this thesis was to assess several clinical and immunological effects of bariatric surgery on morbidly obese individuals. A general introduction on these topics was provided in **Chapter 1**.

Part I described the cardiovascular changes after LRYGB.

In **Chapter 2**, the effects of bariatric surgery on carotid intima-media thickness (CIMT) were assessed in different age groups. Ultrasonic CIMT measurements were performed in 166 patients, prior to bariatric surgery, at six months and at twelve months after surgery. The CIMT was significantly decreased in all age groups after bariatric surgery, but these effects were most pronounced in patients aged under 50. This suggests that the reversibility of atherosclerosis and cardiovascular risk reduction by bariatric surgery diminishes with aging.

In **Chapter 3,** we investigated whether cardiac function improves after LRYGB in morbidly obese patients without a cardiac history. This single center pilot study included fifteen patients. Cardiac magnetic resonance imaging was used to measure cardiac function before LRYGB and at three, six, and twelve months after surgery. A significant increase in left ventricle ejection fraction/body surface area ratio was found at six and twelve months postoperative. Additionally, cardiac output and absolute left ventricle mass were significantly decreased after twelve months. Thus, LRYGB leads to an improvement of cardiac function in patients without a history of cardiac disease.

Part II focused on the effects of morbid obesity on the immune system and the immunological changes after LRYGB.

The effects of morbid obesity and metabolic syndrome (MetS) on the composition of a broad spectrum of circulating immune cells was assessed in **Chapter 4**. The immune subset composition was compared between 117 morbidly obese patients without MetS, 127 morbidly obese patients with MetS and 55 lean controls. Flow cytometry was used to assess absolute cell numbers and proportions of T cells, B cells, NK cells and monocyte subsets within peripheral blood. Morbidly obese patients had increased absolute numbers

of CD4+ and CD8+ T cells, B cells, NK cells and monocytes as compared to lean controls. However, obesity-induced phenotypic differentiation was only found in CD8+ T cells and B cells. Remarkably, the number of immature immune CD8+ T cells and B cells was similar between the three study groups. The presence of MetS did not affect the results, neither in absolute numbers, nor in the immune subset compositions of the investigated immune cells.

In **Chapter 5**, it was investigated whether bariatric surgery affected the subset profile and cytokine producing capacity of T and B cells in 23 morbidly obese patients, and these results were compared with that of 25 lean controls. Morbidly obese patients showed a more differentiated subset profile in CD4+ and CD8+ T cells, which was not reversed towards the profile of lean controls three months after bariatric surgery. The interleukin (IL-) 2 and interferon gamma production of CD8+ T cells was significantly decreased in morbidly obese patients; three months after bariatric surgery this was not restored. On the contrary, the B cell subset composition of morbidly obese patients that underwent bariatric surgery adjusted towards the profile of lean controls. However, the increased IL-2 and IL-10 producing capacity by B cells in morbidly obese patients remained unchanged after bariatric surgery.

Part III addressed long-term complications after bariatric surgery.

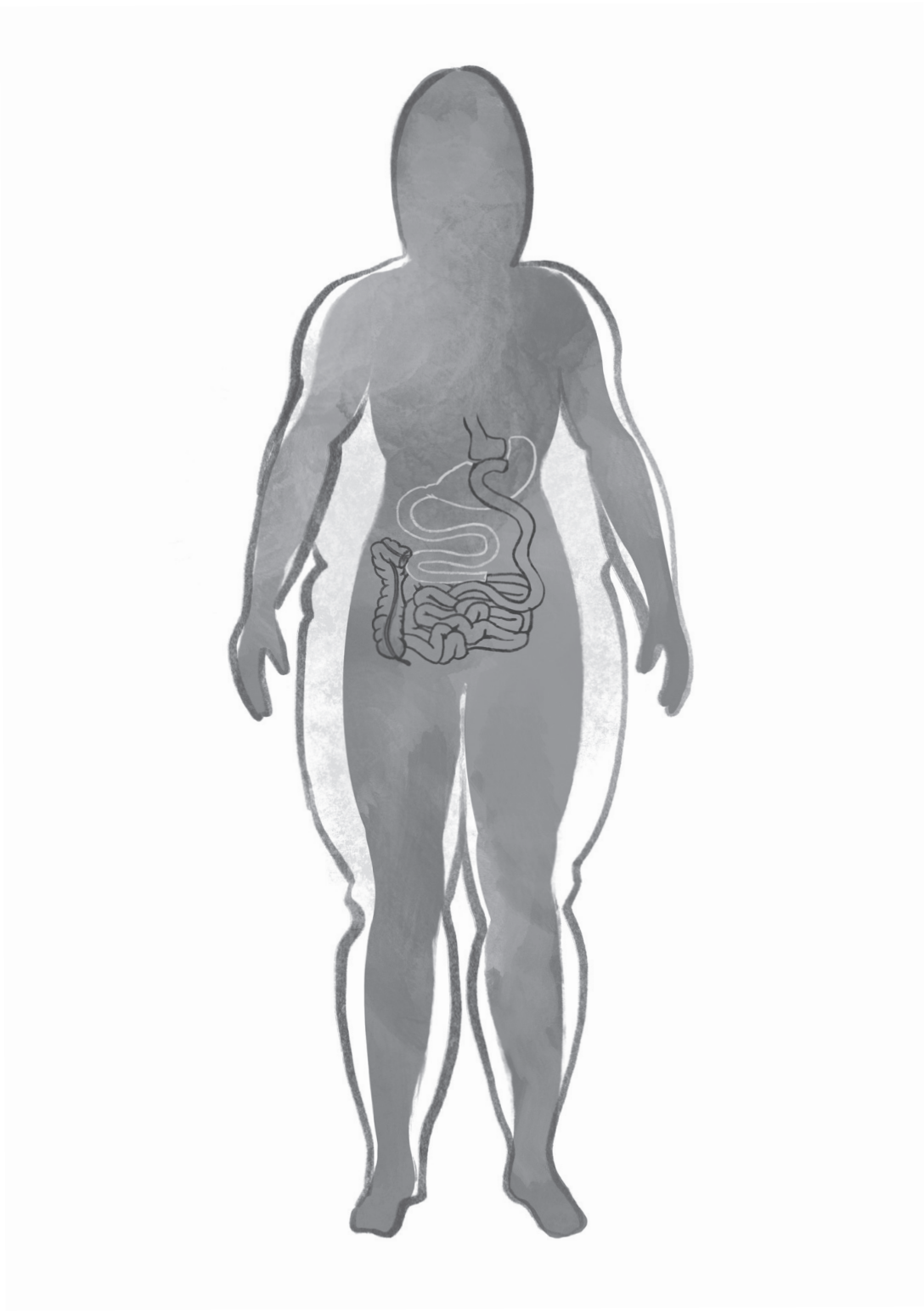
The impact of initial response of laparoscopic adjustable gastric banding (LAGB) on weight loss results after revisional LRYGB was assessed in **Chapter 6**. This retrospective cohort study included 1285 primary LRYGB patients, 96 nonresponders to LAGB and 120 responders to LAGB. After 36 months, the mean percentage excess weight loss (%EWL) was significantly lower in the nonresponding group, compared to the responding and primary groups (48.1% versus 58.2% and 72.8% respectively, $P<0.001$). Both nonresponders and responders to LAGB achieved substantial weight loss after revisional LRYGB. Nonetheless, %ELW and percentage total weight loss (%TWL) remained significantly higher after primary LRYGB as compared to revisional LRYGB. Additionally, the success rate after revisional LRYGB in nonresponders was significantly lower compared to responders (38.2% versus 61.0%, $P<0.001$). Therefore, revisional LRYGB is not advisable for nonresponders to LAGB and adequate routine screening for revisional LRYGB candidates is recommended.

In **Chapter 7**, two surgical techniques for the treatment of a large gastric pouch after LRYGB were compared. In this retrospective, multicenter cohort study, 37 patients underwent a laparoscopic sleeve resection of the gastrojejunostomy and gastric pouch (SGP), and 22 patients underwent laparoscopic revision of the gastrojejunostomy with resizing of the pouch and creation of a new anastomosis (RGJ). After two years, the %TWL based on weight at primary bariatric surgery was 22.0% for the SGP cohort en 22.2%

for the RGJ cohort (*P*=0.885). Both techniques showed improvement of obesity-related comorbidities, however, the study population was too small to draw conclusions. The average surgical costs of surgical disposables were lower for the SGP technique and may therefore be the preferred revisional bariatric technique.

In **Chapter 8**, we described the effects of laparoscopy for (suspected) internal herniation (IH) on symptom relief. In this multicenter cohort study, 193 patients who underwent laparoscopy for (suspected) IH were evaluated. Mesenteric defects were closed during laparoscopy in all patients, whether IH was objectified during surgery or not. After closure of the mesenteric defects, symptom relief was reported 77.2% of the patients. IH was objectified during surgery in 61.1% of the patients, of whom 82.8% had postoperative pain relief. However, many patients benefit from closure of the mesenteric defects, irrespective of the perioperative presence of IH. The only significant predictor for pain relief was a swirl sign on CT (OR 4.24, 95%CI 1.63-11.05).

The findings of the presented studies were discussed and future perspectives were described in **Chapter 9**.



Chapter 11

Nederlandse samenvatting

Nederlandse samenvatting

De afgelopen tientallen jaren is de prevalentie van morbide obesitas snel toegenomen. Morbide obesitas verhoogt het risico op het ontwikkelen van obesitas-gerelateerde comorbiditeit, zoals hypertensie, diabetes mellitus type 2 en hypercholesterolemie. Deze obesitas-gerelateerde comorbiditeit kan leiden tot een verminderde levensverwachting. Bariatrische chirurgie is de meest effectieve behandeling van morbide obesitas en gerelateerde comorbiditeit. Laparoscopische Roux-en-Y gastric bypass (LRYGB) is momenteel de meest uitgevoerde bariatrische ingreep van Nederland. Dit proefschrift beschrijft effecten van bariatrische chirurgie bij patiënten met morbide obesitas op verschillende klinische en immunologische parameters van gerelateerde ziekten. Een algemene introductie over de behandelde onderwerpen van dit proefschrift is beschreven in **Hoofdstuk 1**.

Deel I beschrijft cardiovasculaire veranderingen na LRYGB.

De effecten van bariatrische chirurgie op de carotis intima-media dikte (CIMD) in verschillende leeftijdsgroepen worden beschreven in **Hoofdstuk 2**. Echografische CIMD-meting werd verricht bij 166 patienten, zowel vóór als zes en twaalf maanden na bariatrische chirurgie. De CIMD nam significant af in alle leeftijdsgroepen na bariatrische chirurgie, maar de resultaten waren het meest uitgesproken in patiënten jonger dan 50 jaar. Dit suggereert dat de omkeerbaarheid van atherosclerose en cardiovasculaire risico reductie afneemt bij veroudering.

In **Hoofdstuk 3** wordt de cardiale functie na LRYGB in morbide obese patiënten zonder een cardiale voorgeschiedenis onderzocht, uitgaande van een verwachte verbetering. In deze single-center pilotstudie werden vijftien patiënten geïncludeerd. Door middel van een cardiale magnetische resonantie scan werd de cardiale functie twee tot drie maanden vóór, drie, zes en twaalf maanden na bariatrische chirurgie gemeten. Zes en twaalf maanden na chirurgie werd er een significante verhoging van de linker ventrikel ejectie fractie per lichaamsoppervlakte gevonden. Twaalf maanden na chirurgie was er bovendien een significante afname in het hartminuutvolume en de absolute linker ventrikel massa. Wij concludeerden dat LRYGB leidt tot een verbetering van cardiale functie in patiënten zonder cardiale voorgeschiedenis.

In **Deel II** worden de effecten van morbide obesitas op het immuunsysteem en de mogelijke immunologische veranderingen na LRYGB bestudeerd.

De effecten van morbide obesitas met metabool syndroom (MetS) op de samenstelling van circulerende immuuncellen worden beschreven in **Hoofdstuk 4**. De samenstelling

werd vergeleken tussen 117 morbide obese patiënten zonder MetS , 127 morbide obese patiënten met MetS en 55 gezonde controles. De absolute cel aantallen en verhoudingen van T-, B- en NK-cellen en monocyten in het perifere bloed werden bepaald met flowcytometrie. Wij vonden een toename van absolute aantallen CD4+ en CD8+ T-, B- en NK-cellen en monocyten in morbide obese patiënten in vergelijking met gezonde controles. Een obesitas-geïnduceerde fenotypische differentiatie werd echter alleen gevonden in CD8+ T-cellen en B-cellen. Het was opmerkelijk dat het aantal onrijpe CD8+ T-cellen en B-cellen gelijk was tussen de drie studiegroepen. De aanwezigheid van MetS had geen invloed op de absolute aantallen en de verhoudingen van de onderzochte immuuncellen.

In **Hoofdstuk 5** is de invloed van bariatrische chirurgie op het immuun subgroep profiel en het cytokine producerend vermogen van T- en B-cellen in 23 morbide obese patiënten en 25 gezonde controles onderzocht. Morbide obese patiënten lieten een gedifferentieerder subgroep profiel van CD4+ en CD8+ T-cellen zien, wat drie maanden na bariatrische chirurgie niet was teruggedraaid richting het profiel van gezonde controles. De interleukine (IL)-2 en interferon gamma productie van CD8+ T-cellen was significant lager in morbide obese patiënten. Drie maanden na bariatrische chirurgie herstelde dit niet. Daarentegen waren de B-cel subgroep verhoudingen van morbide obese patiënten na bariatrische chirurgie wel hersteld richting het profiel van gezonde controles. Het verhoogde IL-2 en IL-10 producerend vermogen van B-cellen in morbide obese patiënten werd echter niet beïnvloed door bariatrische chirurgie.

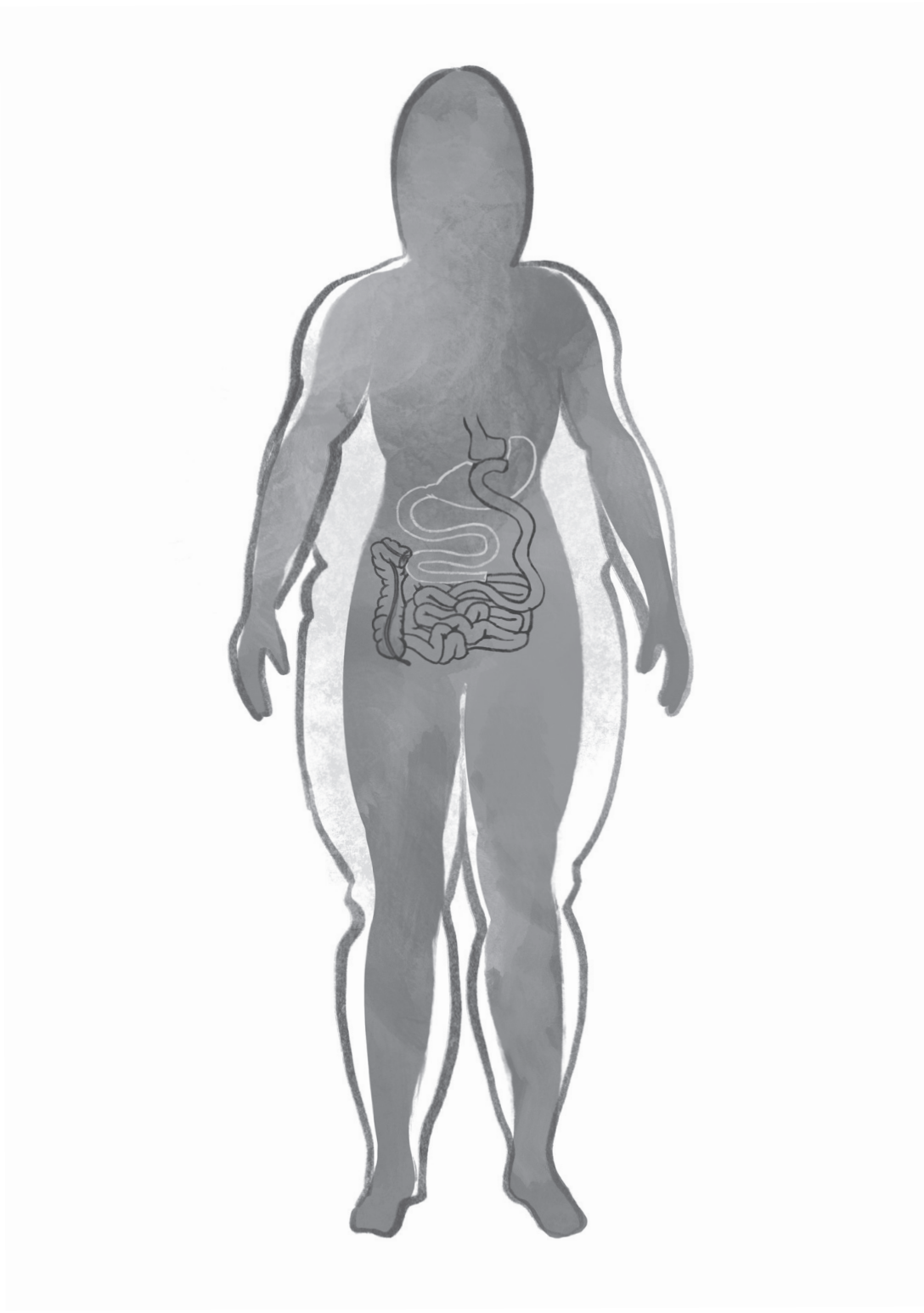
Deel III beschrijft lange-termijn complicaties na bariatrische chirurgie.

Hoofdstuk 6 bestudeert het gewichtsverlies na revisie van een maagband door een LRYGB en het effect van de initiële respons op een maagband op dit gewichtsverlies na revisie. In deze retrospectieve cohort studie werden 1285 primaire LRYGB patiënten geïncludeerd, 96 'nonresponders' op een maagband en 120 'responders' op een maagband. Na 3 jaar was het gemiddelde Excess Weight Loss percentage (%EWL) significant lager in de nonresponder groep vergeleken met de responder en primaire groep (48.1% versus 58.2% en 72.8% respectievelijk, $P<0.001$). Zowel nonresponders als ook responders op een maagband bereikten een wezenlijk gewichtsverlies na revisie door middel van een LRYGB. Desondanks waren het %EWL en het totaal gewichtsverlies percentage (%TWL) significant hoger na primaire LRYGB. Overigens was het succes ratio na revisie door middel van LRYGB bij nonresponders significant lager dan die bij responders (38.2% versus 61.0%, $P<0.001$). Derhalve wordt revisie door middel van LRYGB niet aangeraden voor nonresponders op een maagband en wordt een adequate routine screening geadviseerd voor de beoordeling van revisie LRYGB kandidaten.

In **Hoofdstuk 7** worden twee chirurgische technieken voor de behandeling van een vergroot maagreservoir (maagpouch) na LRYGB vergeleken. In een retrospectieve, multicenter cohort studie, ondergingen 37 patiënten een laparoscopische sleeve resectie van de gastrojejunostomie en het maagreservoir (SGM) en ondergingen 22 patiënten een laparoscopische revisie van de gastrojejunostomie met het verkleinen van het maagreservoir en het creëren van een nieuwe anastomose (RGJ). Twee jaar na de revisie was het %TWL gebaseerd op het gewicht vóór de primaire bariatrische ingreep 22.0% voor het SGM cohort en 22.2% voor het RGJ cohort (*P*=0.885). Beide technieken suggereerden een verbetering van obesitas-gerelateerde comorbiditeit zien, echter was de studiepopulatie te klein om hier conclusies uit te trekken. De SGP techniek is de voorkeurstechniek voor revisie bariatrie, omdat de gemiddelde chirurgische kosten voor wegwerpinstrumenten lager waren voor deze techniek.

Hoofdstuk 8 beschrijft de effecten van laparoscopie voor (vermoedelijke) inwendige herniatie (IH) op het gebied van symptoomverlichting. Er werden 193 patiënten die laparoscopie ondergingen voor (vermoedelijke) IH geëvalueerd in een retrospectief, multicenter cohort studie. De mesenteriale defecten werden in alle patienten gesloten, ongeacht de perioperatieve aanwezigheid van IH. Na sluiting van de mesenteriale defecten ervaarden 77.2% van de patiënten symptoomvermindering. IH werd in 61.1% van de patiënten geobjectiveerd tijdens operatie, waarvan 82.8% postoperatieve pijnvermindering ervaarden. Veel patienten hadden baat bij het laparoscopisch sluiten van de mesenteriale defecten, ongeacht de perioperatieve aanwezigheid van IH. De aanwezigheid van een 'swirl sign' op CT bleek de enige significante voorspeller van pijnverlichting na het sluiten van de mesenteriale defecten (OR 4.24, 95%-BI 1.63-11.05).

De bevindingen van de studies van dit proefschrift en toekomstperspectieven worden bediscussieerd in **Hoofdstuk 9**.



Chapter 12

Appendices

List of publications

Impact of initial response of laparoscopic adjustable gastric banding on outcomes of revisional laparoscopic Roux-en-Y gastric bypass for morbid obesity
L.H. Wijngaarden, F.H.W. Jonker, J.W. van den Berg, C.C. van Rossem, E. van der Harst, R.A. Klaassen
Surgery for Obesity and Related Diseases; Volume 13, Issue 4, April 2017, Pages 594–599

Age-related effects of bariatric surgery on early atherosclerosis and cardiovascular risk reduction
F.H.W. Jonker, V.A.A. van Houten, **L.H. Wijngaarden**, R.A. Klaassen, A.E.A. de Smet, A. Niezen, L.J.D.M. Schelfhout, T.A. Bruning, E. van der Harst
Obesity Surgery; Volume 28, Issue 4, April 2018, Pages 1040 – 1046

Predicting symptom relief after reoperation for suspected internal herniation after laparoscopic Roux-en-Y gastric bypass
L.H. Wijngaarden, S.L. van Veldhuisen, R.A. Klaassen, E. van der Harst, C.C. van Rossem, A. Demirkiran, S.M.M. de Castro, F.H.W. Jonker
Obesity Surgery; Volume 28, Issue 12, December 2018, Pages 3801 – 3808

Improvement of cardiac function after Roux-en-Y gastric bypass in morbidly obese patients without cardiac history measured by cardiac MRI
D. de Witte, **L.H. Wijngaarden**, V.A.A. van Houten, M.A. van den Dorpel, T.A. Bruning, E. van der Harst, R.A. Klaassen, R.A. Niezen
Obesity Surgery; Volume 30, Issue 7, July 2020, Pages 2475 – 2481

Effects of morbid obesity and metabolic syndrome on the composition of circulating immune subsets
L.H. Wijngaarden, E. van der Harst, R.A. Klaassen, M. Dunkelgrun, T.M. Kuijper, M. Klepper, G. Ambagtsheer, J.N.M. IJzermans, R.W.F. de Bruin*, N.H.R. Litjens* (*Authors contributed equally to this manuscript)
Frontiers in Immunology, June 2021 [Epub ahead of print]

Resizing a large pouch after laparoscopic Roux-en-Y gastric bypass: comparing the effect of two techniques on weight loss
L.H. Wijngaarden*, B.M.M. Reiber*, F. Yousufzai, A. Demirkiran, R.A. Klaassen (*Authors contributed equally to this manuscript)
Surgical Endoscopy, July 2021 [Epub ahead of print]

Submitted manuscripts

T and B cell composition and cytokine producing capacity before and after bariatric surgery

L.H. Wijngaarden, F. Nuijten, E. van der Harst, R.A. Klaassen, T.M. Kuijper, F. Jongbloed, G. Ambagtsheer, M. Klepper, J.N.M. IJzermans, R.W.F de Bruin , N.H.R. Litjens

Frontiers in Immunology

Manuscript in preparation

The association between dietary protein intake and fat free mass six months after laparoscopic Roux-en-Y gastric bypass: a cross-sectional study

A.J. Boes, **L.H. Wijngaarden**, R.A. Klaassen

PhD portfolio

PhD student: Leontine Henriëtte Wijngaarden
PhD period: December 2017 – March 2021
Erasmus MC Department: Surgery
Promotor: Prof. dr. J.N.M. IJzermans
Copromotors: Dr. ing. R.W.F. de Bruin and dr. E. van der Harst

PhD training	Year	ECTS
Courses		
BROK (Basiscursus Regelgeving Klinisch Onderzoek), NFU	2018	1.8
Research integrity, Erasmus MC	2018	0.3
Biomedical English Writing Course, MolMed	2018	2.0
GraphPad Prism, MolMed	2020	0.3
Scientific presentations		
IFSO congress	2017, 2019	4.0
DSMBS congress	2017	2.0
Wetenschapsdag Maasstad Ziekenhuis	2018, 2019	4.0
Attendance at (inter)national conferences		
Wetenschapsdag Maasstad Ziekenhuis	2017	1.0
NVvH Chirurgendagen	2017, 2018	2.0
Wetenschapsdag Heelkunde	2018	1.0
DSMBS congress	2018, 2019	1.0
Nationaal Obesitas Symposium	2020	1.0
Supervising students		
Master Thesis Health Sciences (A.J. Boes)	2018	1.0
Student Biological medical analysis (Y. Goos)	2018-2019	3.0
Student Biological medical analysis (D. Huijbregts)	2019-2020	2.0
Master Thesis Molecular Medicine (F. Nuijten)	2020	1.0
Other		
Student coach of medical students in their bachelor	2018-2020	2.0
Maasstad Wetenschapsvoucher	2019	1.0
Total		**30.4**