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*MOLECULAR MARKERS OF WEIGHT LOSS IN
SLEEVE GASTRECTOMY PATIENTS: A
PROSPECTIVE COHORT STUDY*

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Thesis submitted August 2014 for the degree of

Master of Medicine/Surgery, UNDA.

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II. ACKNOWLEDGEMENTS:

I submit that this thesis is my own work and contains no material which has been accepted for the award of any other degree or diploma in any university or other institution. To the best of my knowledge, this thesis contains no material previously published or written by another person.

Tamara Catherine Preda 10/8/2014

I would like to thank my supervisors Prof. R V Lord and Dr. M. Swarbrick for their guidance in this project. I would also like to thank Dr. Sally Lord for her input and sage advice throughout the duration of this Masters course and thesis preparation.

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III. ABSTRACT:

Background:

Obesity is a polymorphic chronic disease that has reached epidemic proportions. Bariatric surgery including sleeve gastrectomy (SG) has an increasingly important role in long-term management of these patients. The molecular mechanisms post SG are complex and not fully understood.

Aims:

The primary study aim is to investigate the hormonal mechanisms by which SG effects weight loss and related health benefits by examining the association between SG weight loss and biochemical/hormone levels. A secondary aim was to assess the improvements in obesity related chronic disease states following SG.

Methods:

We conducted a prospective cohort study of obese patients undergoing SG and 2:1 age and sex matched non obese controls undergoing non- bariatric procedures during the study period from a single bariatric surgeon in Sydney. Height, weight, body mass index (BMI) and percentage excess body weight (%EBW) were determined for each subject at baseline, 3 and 6 months post SG.

Plasma samples were obtained and key biochemical markers measured (NEFA (non-essential fatty acids), C-peptide, Ghrelin, GIP (Gastric Inhibitory Peptide), Glucagon Like Peptide -1 (GLP-1), insulin, resistin, visfatin, glucagon, leptin, Plasminogen Activator Inhibitor-1 (PAI-1)). Comparisons of baseline levels between obese and non obese subjects; and pre and post surgery levels and clinical factors in the SG cohort at 0, 3, 6 months post SG were performed using unpaired and paired t-tests respectively on Graph-Pad PRISM © software.

Results:

16 SG patients and 32 controls were included with 3 month clinical follow up available for all SG subjects and 3 month biochemical follow-up available for 11 SG subjects. In the SG cohort, the mean BMI at baseline was 43.5 +/- 1.8 kg/m² SEM. Males undergoing SG were heavier than females. The mean %EBW loss was 42.3% +/- 8.4SD at 3 months and 51.5% +/- 18.5SD at 6 months. There was a statistically significant incremental weight loss between 3 and 6 month time points from baseline, $p < 0.0001$ and $p < 0.0009$ respectively.

A statistically significant decrease in levels of NEFA, ghrelin, GLP-1, glucagon, leptin and PAI-1 was observed between baseline and 3 months post-operative ($p < 0.05$). This reduction remained statistically significant at 6 months for NEFA and ghrelin. Due to small numbers at 6 months it is unclear if there are further changes in these hormone levels compared to 3 months.

No statistically significant difference was found for C-peptide, GIP, insulin or resistin between baseline and 3 months. Mean visfatin and resistin levels differed between subjects and controls at baseline (time 0). There was no difference in mean baseline BMI and %EBW lost for the patients who completed clinical and biochemical follow up versus those who had clinical follow-up alone.

In the SG cohort, secondary co-morbidities improved, with patients less dependent on oral hypoglycaemic agents for T2DM and improvements in hypertension, gastro-esophageal reflux disease and obstructive sleep apnea.

Conclusions:

We demonstrate significant weight loss and hormone changes post SG surgery. Our research adds to the literature to identify markers that are associated with surgical weight loss that may provide insights into the endocrine mechanisms or effects of surgical weight loss.

IV. INTRODUCTION:

Substantial efforts are now focusing on combating the obesity epidemic. Obesity is a polymorphic chronic disease that has reached epidemic proportions and is now one of the most important public health issues in the Western world(1). Although there is considerable evidence that body weight and fat mass are highly heritable traits(2), the rise in the prevalence of obesity has been driven by environmental factors; an abundance of calorie rich food and lack of physical activity(3). Obesity is epigenetic in origin, with genetic and environmental components to this problem. At a patient management and therapeutic level, bariatric surgery is increasingly being relied upon as the most effective way to enable significant and sustained weight loss in patients failing dietary and lifestyle measures and those who are severely obese. The Australian government guidelines from the Department of Health and Ageing support this approach.(4)

With weight reduction comes a fall in cardiovascular risk profile; improved control of associated comorbidities such as dyslipidaemia, type II diabetes mellitus (T2DM), hypertension (HTN), obstructive sleep apnea (OSA), non-alcoholic steatohepatitis (NASH) as well as an overall drop in mortality (5, 6). Recent literature now indicates that there has been sufficient time latency to demonstrate risk reversibility for these conditions in morbidly obese cohorts undergoing bariatric surgery. This is in addition to a reduction in overall cancer risk (e.g. colon, endometrial, post-menopausal breast cancers, oesophageal adenocarcinoma and thyroid cancer)(7).

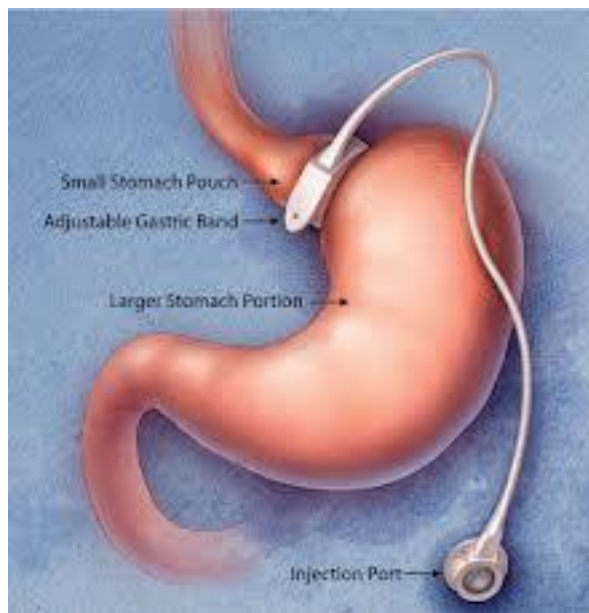
V. LITERATURE REVIEW:

The three main bariatric options are, in increasing order of complexity:

1. Laparoscopic adjustable gastric band (LAGB) surgery (Fig. 1a)
2. Sleeve Gastrectomy (SG) (Fig. 1c)
3. Roux en Y Gastric Bypass (RYGB) (Fig.1b) or duodenal switch procedure (4, 8)

SG is an operation that is increasingly preferred to gastric band as it demonstrates more substantial and sustained weight loss and to gastric bypass operations or duodenal switch which carry a higher attendant risk of morbidity and mortality.

Figure 1a. Laparoscopic Adjustable Gastric Band Surgery:

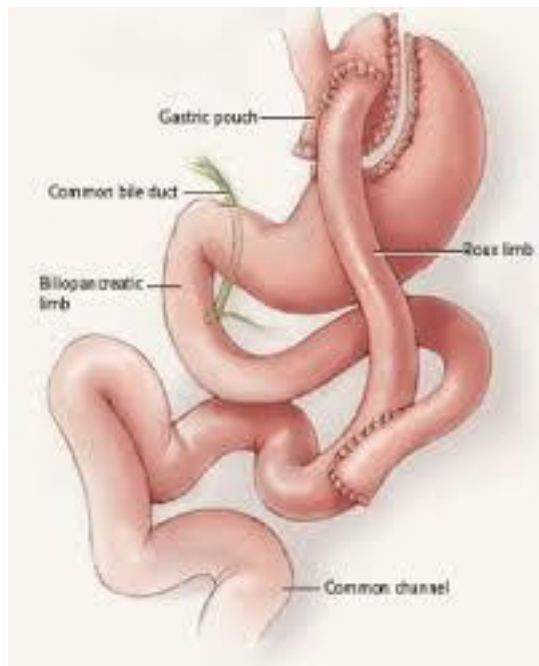


www.ucsfhealth.org

LAGB involves placing a silicon band around the upper portion of the stomach to create a small gastric pouch. The tightness of the band is increased by adding fluid through a subcutaneous port. Early satiety and so decreased food intake occurs. The main advantage is that this is a quick, safe and fully reversible procedure.

Disadvantages include a slower and lower weight loss than with other bariatric procedures (maximum 50-60% EBWL at the rate of approximately 1kg/week); the risk of band erosion or dislodgement, exacerbation of reflux and the need for adjustments over time.

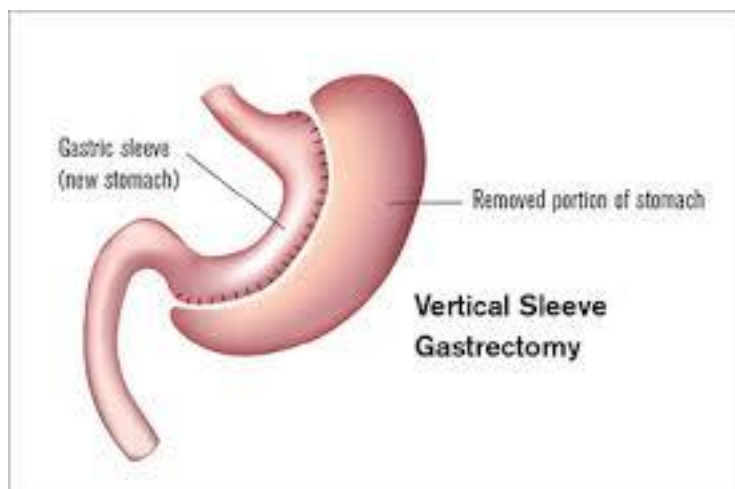
Figure 1b. Roux en Y Gastric Bypass Surgery:



www.utswmedicine.org

RYGB is the most complex of the bariatric procedures and has a multifactorial mechanism of action. A small gastric pouch is created followed by a proximal small intestinal bypass or 're-routing' that confers a malabsorption syndrome, predominantly for fats. Weight loss is the result of volume restriction, malabsorption and a number of hormonal changes including decreased leptin and leptin resistance, increased peptide YY, GLP-1 and reduced GIP. Specific complications include higher perioperative mortality than LAGB, anastomotic leak, Vitamin B12, iron and calcium deficiencies, 'dumping' syndrome and the need for vitamin and mineral replacement lifelong.

Figure 1c. Sleeve Gastrectomy:



www.lakewoodweightloss.com

The hormonal/endocrinological changes that occur post LAGB and RYGB have been extensively studied but relatively less is known about changes post SG.

To date, there is limited literature available on the underlying molecular and physiological mechanisms responsible for the health benefits observed with surgical weight loss in SG patients. Plausible biological explanations for improvement in cardiovascular and cancer risk profiles consequent to a fall in general body adiposity (as measured by percentage of excess body weight lost (%EBWL)) post-bariatric surgery include; changes in insulin/IGF-1, thyroid stimulating hormone (TSH), sex steroids, bile acids, gut microbiota and adipokines/inflammatory cytokines (9). It is this abnormal hormonal milieu and increased release of adipokines and alteration in gastrointestinal and adipose-derived biomarkers that we wish to explore pre and post bariatric, specifically SG surgery.

The objective of this study is to measure changes in a panel of markers (insulin, glucagon, ghrelin, visfatin, resistin, Glucagon Like Peptide-1 (GLP-1), Gastric

Inhibitory Peptide (GIP), leptin, C-peptide, Plasminogen Activator Inhibitor-1 (PAI-1) and NEFA (non essential fatty acids), to ascertain whether a change in the levels can be correlated to weight loss. This would help better understand the mechanisms by which surgically induced weight reduction and associated health benefits occurs.

Sleeve Gastrectomy (SG) (sometimes referred to as vertical sleeve gastrectomy), is usually a laparoscopic procedure that involves stapled resection of approximately 80% of the stomach including the fundus and greater curvature. First described in 1998 as the initial step in a bilio-pancreatic diversion/duodenal switch procedure, its capacity to enable significant weight loss as a stand alone intervention was observed. Additional benefits include gastric conduit for future endoscopic surveillance/intervention, avoidance of malabsorption syndromes and a low risk of future operations being required as well as a low overall mortality rate of 0.39% (10).

SG has a multifactorial mechanism of action. A restrictive component was postulated as the predominant factor initially, although the finding that much more weight is lost following SG than LAGB despite the gastric pouch in LAGB being much smaller than the SG remnant stomach refutes this idea.(11)

A variety of hormones have been studied for their role in weight loss and obesity related health conditions and a summary of those relevant to our study follows below.

Adiponectin is an adipocyte-derived hormone that links visceral adiposity with many of the negative health sequelae of obesity, such as insulin resistance, dyslipidaemia and atherosclerosis. It acts to increase glucose utilization by the liver and skeletal muscle, increase fatty acid oxidation via activation of AMP

kinase and acetyl-coA carboxylase. In addition, adiponectin has anti-inflammatory properties in cultured human endothelial cells and is inversely proportional to inflammatory markers in situ, such as C-peptide(12). It exists in high, medium and low molecular weight forms. The high molecular weight form is the most important. Low levels are associated with 'fat states'- that is, obesity, type 2 DM and NASH. More than 20 studies to date have shown increases in adiponectin after bariatric surgery, but only one included SG patients and to our knowledge no studies have to date examined the different multimers. (13-16)

Ghrelin is also known as the 'hunger peptide' [13]. It is an appetite stimulating hormone mainly produced by the oxyntic glands of the gastric body and fundus. The bulk of this portion is surgically removed in sleeve gastrectomy. Ghrelin's concentration is increased in starvation, binding to and activating the growth hormone sensing receptor in the hypothalamus, where it stimulates growth hormone release from the anterior pituitary; in turn promoting food intake, carbohydrate utilization and adiposity. It also has a direct action on adipocytes to promote lipid storage. Ghrelin levels are thought to be reduced post SG.(17)

Gastric inhibitory polypeptide (GIP) may also promote energy storage.

Ghrelin, peptide YY, gastric inhibitory polypeptide (GIP), GLP-1, cholecystokinin, pancreatic polypeptide and amylin are released from the gastrointestinal tract and pancreas in response to food ingestion. All except ghrelin and peptide YY are negative feedback hormones that inhibit food intake (18). There have been conflicting data in the literature concerning ghrelin levels post bariatric surgery.

Leptin was the first fat cell (adipocyte)-derived hormone to be discovered

in the 1960's. It acts via its receptor in the hypothalamus - the region of the brain known to regulate appetite, food intake and body weight. It is a surrogate indicator of fat stores and gene mutations have been shown to result in obesity [16]. The protein product leptin, is an anorexigenic agent. That is, it acts to reduce food intake and increase energy expenditure. [13,17]
Circulating leptin levels are lower than expected for body weight following SG.(17)

Resistin is an adipokine, found to induce insulin resistance in mice. Increased levels are correlated with increased fat mass. It is found in white fat which is known to play a significant role in the pathogenesis of insulin resistance and to promote the chronic inflammatory state that is conferred by obesity. (19)

Visfatin, is an adipokine involved in inflammatory phenomena, atherosclerosis, and possibly in insulin secretion. It is an established marker of visceral adiposity with direct correlation to the risk of developing type 2 diabetes and the metabolic syndrome. (20)

Other metabolic parameters:

C-peptide is the connecting peptide in the proinsulin molecule and is co-secreted with insulin and can be used to assess insulin secretion.

NEFAs are free fatty acids released by adipocytes in response to lipolytic stimuli like weight loss.

Co-morbidities and the effect of obesity surgery:

Recent studies have reinforced the credence that bariatric surgery is a beneficial treatment option for the improvement of type 2 diabetes mellitus (T2DM) in obese individuals. *Schauer et al.* compared the effects of bariatric surgery (Roux-

en-Y gastric bypass or SG) in combination with medical therapy to medical therapy alone on glycaemic control (measured by glycosylated haemoglobin) in obese patients with advanced T2DM(21). Glycaemic control was improved in all three study groups, however, the most marked reduction was observed for patients who underwent gastric bypass, followed by SG (42% and 37% of patients achieving glycosylated haemoglobin of < 6%, respectively, versus 12% for medical therapy alone group. $P < 0.05$ for all comparisons). The authors postulated that this improvement in glycaemic control observed for patients who underwent surgery is due to a rise in insulin sensitivity, which may in turn be linked to a reduction in chronic inflammation. Similarly, *Mingrone* and colleagues found that remission of T2DM (defined as a glycosylated haemoglobin level of < 6.5%, without concomitant pharmacologic therapy) occurred in 75% and 95% of severely obese patients who underwent gastric bypass or bilio-pancreatic diversion respectively, with no remission observed for patients who received standard medical therapy ($P < 0.001$) (22). In keeping with previous reports, it was shown that there was no correlation between the degree of weight loss and normalisation of glycaemia, suggesting that bariatric surgery may exert its positive effect on T2DM via mechanisms that are independent of weight. A recent analysis of the Swedish Obese Subjects (SOS) study results demonstrates that bariatric surgery exerts a preventative effect, with regard to the incidence of T2DM (23). This longitudinal study, carried out over 15 years on an obese, non-diabetic patient cohort showed that T2DM developed in 392 patients who received regular treatment for obesity, versus 110 patients who underwent bariatric surgery. Interestingly, it was found that baseline BMI is not predictive of a beneficial outcome of bariatric surgery, in terms of the onset of type 2 diabetes. The results of this study again suggest that bariatric surgery results in sustained weight loss over time, however whether it is this weight loss directly, or other factors influencing T2DM progression, such as incretin and other adipokine levels are as yet not known.

VI. AIMS:

The primary research questions we pose are:

What changes occur in the circulating levels of peripheral hormones involved in the homeostatic regulation of body weight pre versus post sleeve gastrectomy (SG)?

Does amount of weight lost correlate to the change in hormone/biomarker levels?

The study aims to find if there exists a difference in circulating levels of peripheral hormones in:

- i) obese patients pre-operatively compared to normal controls
- ii) in obese patients pre and post operatively (three and six months after bariatric intervention).

These analyses will allow us to explore whether these hormones could be responsible for conferring a physiochemical component to weight loss and/or the health benefits associated with weight loss in obese patients.

Biomarkers studied were: NEFA, C-peptide, ghrelin, GIP, GLP-1, glucagon, insulin, leptin, PAI-1, resistin and visfatin.

Primary outcomes were weight loss and change in biochemical profiles as measured by bioassay.

Outcome measures were percentage of excess body weight lost and biochemical levels of markers involved in obesity (mM for NEFA and pg/ml for all others). This involved the **primary endpoint** of change in hormone levels related to obesity as listed above. **Other endpoints** were percentage of excess

body weight lost (%EBWL). Where ideal body weight equates to a BMI=25 and excess body weight is calculated as actual minus ideal body weight in kilograms. **Surrogate endpoints** were indicators of an improvement in health outcomes post SG, i.e improvement and reversal of clinical conditions related to obesity; diabetes (by HbA1C, need for insulin or oral hypoglycaemic medications); arthritis and obstructive sleep apnoea. The **measure of effect** was studied by comparing the groups before and after surgery; % EBWL, percentage change of hormone levels and comparing the SG group to controls for hormone profile mean differences. These endpoints were measured at timing intervals of zero, three and six months.

VII. METHODS:

Study design:

This is a prospective cohort study performed over a six month duration as a pilot study to inform the design of a larger study to investigate the hormones involved in the homeostatic regulation of body weight.

Setting (Site) and participant selection with controls:

Patients presenting to a single upper gastrointestinal and bariatric surgeon (RVL) for weight loss surgery were assessed for eligibility. Eligibility criteria were: Age 18-75 years old, having exhausted non-surgical options for weight loss, body mass-index (BMI) greater than 30. Persons with untreated malignancy or clinically significant illness precluding general anaesthesia and those taking medications known to affect body weight were excluded.

Included patients were voluntary participants who had decided in consultation with their primary physician and treating surgeon that SG was their best surgical option.

Patients were recruited who had an operative admission date between April 1st and June 30th, 2013. Informed written consent was obtained from all and stored securely in the research office. Withdrawal from the study at any time was possible at the request of the patient.

Surgery was performed at two private hospitals (MUH and SVPH, Sydney) after institutional Ethics Board Approval (bariatric surgery not being covered

under Medicare provision of services at public hospitals in Australia except in special circumstances).

Ethical approval:

Collection of patient data at SVPH/SVC was undertaken as part of a larger multi-institutional obesity study (Episcope). SVH Ethical Board approval was granted for this. Patients were educated on the goals of the study and nature of the blood samples taken; also that samples would be stored for future use. Signed consent was obtained for all and stored in a locked research office facility.

Process for revocation of consent at any time was also made clear to each participant.

Under the title '*The biology, epigenetics and genetics of adipose tissue in human health and disease*' Macquarie University /Macquarie University Hospital Ethics Board granted approval for this project in accordance with NHMRC/HREC committee guidelines. Similarly Notre Dame University (Sydney) ethics approval was obtained.

Patients who had undergone non-bariatric upper gastrointestinal procedures (surgery or endoscopy) were used as controls. Patients were age and sex matched from an established gastroesophageal research specimen bank, ensuring they had a normal BMI (18-25), no known malignancy and were not diabetic. This was performed with a 2:1 (control:case) ratio.

Bias:

This was a cohort study with 2:1 control to case ratio. Controls were age and sex matched to help reduce the risk of selection bias in our assessment of the effect of obesity on baseline hormone levels.

All blood samples used for the extraction of biochemical analytes in the hormonal assays were de-identified prior to analysis. Hence laboratory staff performing the assays were blinded to the nature of the samples to reduce the risk of measurement bias. Biochemical analysis was undertaken on a single platform to improve the reliability of the assay.

Anthropometric measurements were standardized by using the same scales and height chart to reduce the risk of random error.

The statistical analyses were conducted un-blinded to case status.

iii) Study Size:

This study was designed as a pilot study to provide preliminary data to inform the design of a further larger study needed to provide more definitive evidence, therefore power calculations were not performed. The sample size of 16 patients reflects the number of eligible patients available for recruitment from one surgeon during the study period.

iv) Data Collection:

Once patients agreed to participate, a questionnaire regarding co-morbidities attributable to obesity was completed. This was done in rooms at the initial consult and filed with the patient notes.

- Physical examination (t = 0, 3, 6, months) was performed by one individual (to avoid inter-observer error). Weight (kilograms) and height (centimeters) were measured on the same scales which were calibrated

and with the same chart to measure height. Thus the accuracy of BMI was ensured.

- Some serum peripheral blood samples were kept in storage in a secure research freezer for each study patient and control to ensure that experiments could be repeated and data recalculated if necessary. Further analysis can also therefore occur. This was according to the protocol developed by Dr. M. Swarbrick, quoted in prior accepted literature (14).

On scheduled clinical review at three and six months, body weight, weight loss (%EBWL) and body mass index were again measured and recorded on the same chart, as was assessment of improvement or resolution of symptoms of obesity related co-morbidities; for example, glycaemic control (as measured by need for an adjusted dose of oral hypoglycaemic medication and HbA1C/fasting blood glucose level), hypertension (blood pressure >140/90mmHg; need for ongoing pharmacologic therapy), obstructive sleep apnoea (need for ongoing CPAP) or osteoarthritis (patient reported improvement in symptoms over time and exercise capacity).

Initial fasting blood samples on the day of surgery (post induction of general anaesthesia) were collected.

Three and six monthly fasting blood samples were collected by the same pathology service and transferred to a dedicated gastrointestinal research lab for processing and storage.

Blood tests included; fasting blood glucose levels, HbA1C, lipid profile full blood count, liver and renal function tests, zinc, copper, iron, vitamin B12,

folate and calcium (processed in the hospital pathology lab). Levels of C-peptide, ghrelin, GIP, GLP-1, glucagon, insulin, leptin, PAI-1, resistin and visfatin were performed using a Bio-Rad Human Diabetes Bioplex panel with analysis undertaken at the Australian Proteome Analysis Facility (Macquarie University) using a Bio-Plex 200 instrument (both from Bio-Rad, Hercules, CA, USA).

NEFA were measured in plasma using reagents from Wako Diagnostics (Richmond, VA, USA). These were supervised by an experienced laboratory scientist.

For bio-assays, 20 mL whole blood was collected and divided between two EDTA tubes. It was stored on ice until transfer to the laboratory as a de-identified sample with lab specific code. The blood was centrifuged and plasma removed for assay. In the bariatric group, circulating biomarker levels immediately pre-operatively and at three and six months after surgery were quantified. These same markers were measured in the plasma samples of patients in the control group - considered to represent a 'lean normal value'.

The units are mM for NEFA and all hormone levels are in pg/mL.

Note visfatin due to analyte availability difficulties was not measured in all SG patients at three and six months.

Follow up continued until February 2014.

Data analysis/ Statistical Methods:

Paired t-tests were used to compare study factors pre and post surgery at time intervals three and six months post SG.

Independent (unpaired) sample t-tests were used to compare anthropometric results such as BMI and hormone levels between control and SG patients prior to surgery. All tests were two tailed with a significance level of $p=0.05$.

Statistical analysis to compare baseline patient characteristics of SG patients completing biochemical follow up (blood sampling) and their counterparts who did not were made, to determine whether patients lost to follow up were similar to those completing the study.

Data was analysed using Excel and GraphPad Prism Statistical Analysis software.

VIII. RESULTS:

AI Clinical Data:

Clinical data were available on 16 (100%) of consenting patients at three months and 13 (81.3%) of patients at six months. One patient having moved interstate; a set of clinical notes being misplaced and a third patient being lost to follow up.

A biochemical profile of 11 (68.8%) patients at 3 months and 4 (25%) of patients at 6 months was obtained.

The baseline characteristics of SG cohort and control patients are summarized in Table 1. SG cohort and controls were age and sex matched.

Table 1: Baseline demographics of SG cohort and controls who went on to have hormone tests.

	SG cohort (N=13)		Control (N=26)	P value (0.05)
Age - Range (years)	29-71		27-66	0.99
- Mean (SD)	52 +/- 12.7SD		51.5+/-11.9SD	
Sex - M	7		14	1
- F	6		12	
Weight (kg) (All)	119.7 +/-8.4 SEM		74.3+/-2.4SEM	P<0.0001
Ideal Body Weight (kg)	70 +/-8 SD		N/A as controls were within normal weights	
BMI - Range (kg/m ²)	32.5 to 55		18.5 to 25.5	P<0.0001
- Mean (+/- SEM)	42.5 +/-1.8		23.5 +/- 0.5	
Weight (kg)	Male	Female		P<0.03
	133.9 +/-	102.7 +/-		
	10.16 SEM	8.07 SEM		

Serial assessment of obesity related co-morbidities was conducted at clinical review three and six months following surgery. The results are as summarized in Table 2.

Table 2: Obesity related co-morbidities over time, SG cohort (N =13)

Condition and number (x) of SG patients affected at time 0	Improvement at 3months	Resolution (^) at 3 months	Improvement at 6 months	Complete resolution at 6 months
Diabetic (4) - 4 on oral hypoglycaemic agents (OHG)	-	4 off OHG	-	4 ceased OHG
Hypertension (6) (BP >140/90)	-	1	-	2
Obstructive Sleep Apnoea (OSA) (7) -6 requiring CPAP	1	-	3	1
Gastroesophageal reflux (GORD)(6)	-	1	1	1
Osteoarthritis (6)	1			1
Hypercholesterolaemia (3) (Total cholesterol >5.5mmol/L & meeting statin guidelines))				1

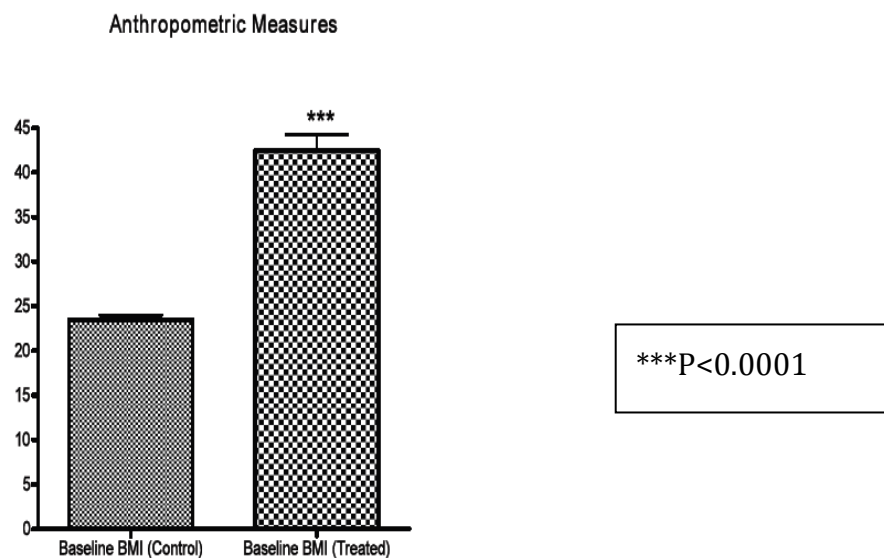
^ *Resolution* as defined by an HbA1C <6.0% and or random blood glucose recordings <7mmol/L

B] BMI and Weight Data:

The following graphs depict

- BMI of case and control patients at baseline.
- BMI, weight and percentage of excess body weight loss in the sleeve gastrectomy patients over time
- A subgroup analysis for weight loss and gender was also performed

Figure 2. SG cohort vs. control BMI:



Unpaired t test:

P value $P<0.0001$ ($p<0.05$)

Two-tailed

t, df $t=13.38$ $df=37$

Magnitude of difference

Mean \pm SEM of column A 23.42 ± 0.4831 $N=26$

Mean \pm SEM of column B 42.52 ± 1.794 N=13
Difference between means -19.11 ± 1.428
95% confidence interval -22.00 to -16.21
R squared 0.8287

Figure 3a. SG patient's BMI over time (0, 3, 6, months)

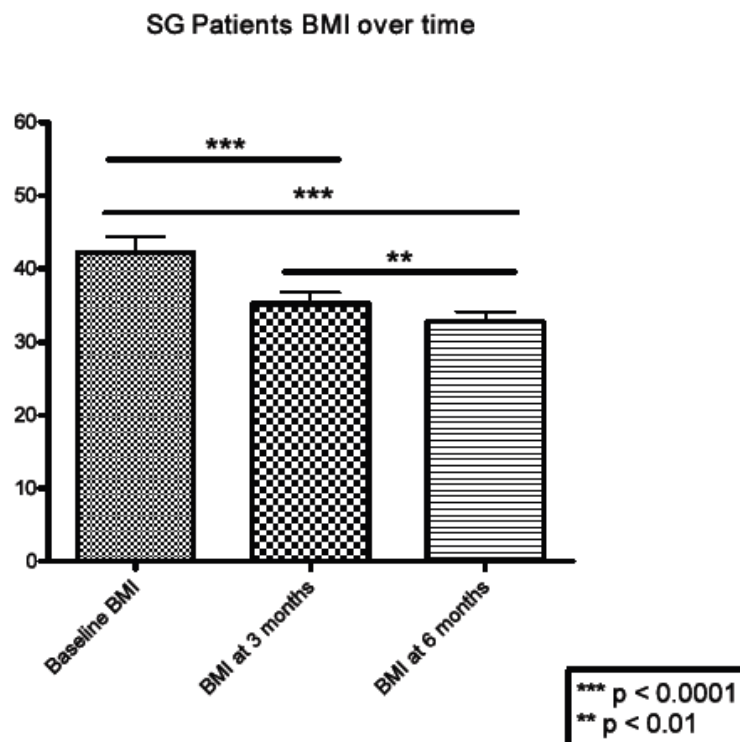


Figure 3b. SG Patient's Weight (kg) over time (0,3,6 months)

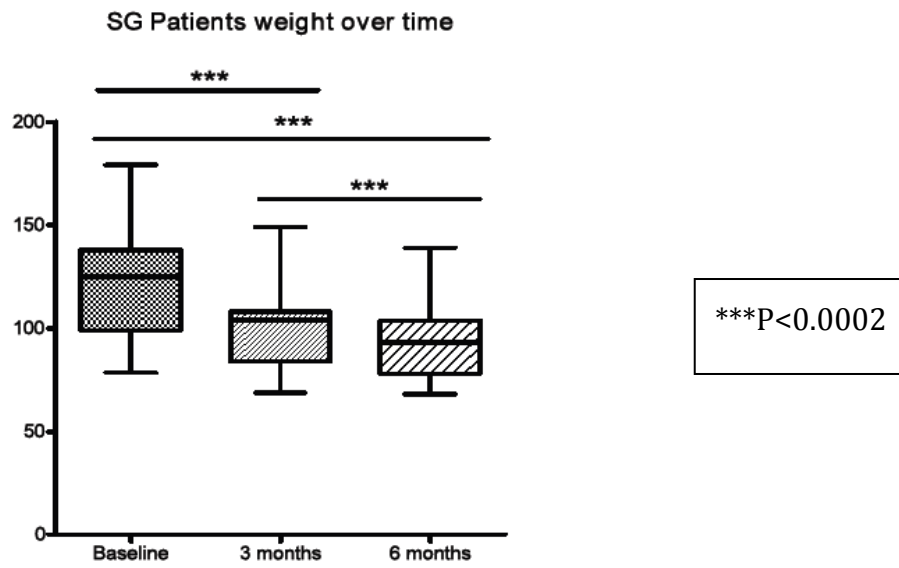
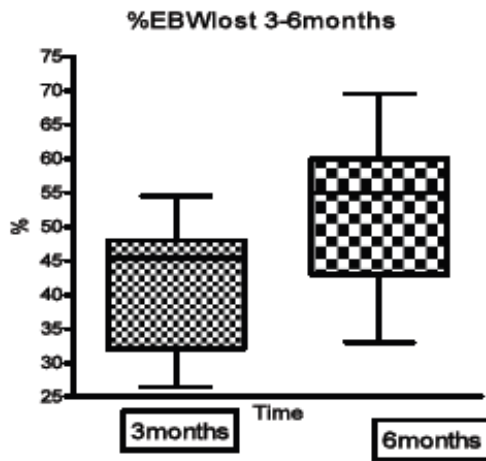


Figure 3c. SG patient's %EBWL at 3 and 6 months



Column A %EBWL at 3 months vs. Column B % EBWL at 6 months

Paired t test:

P value 0.0002 (p<0.05)

Two tailed

t, df t=5.632 df=10

Number of pairs 11

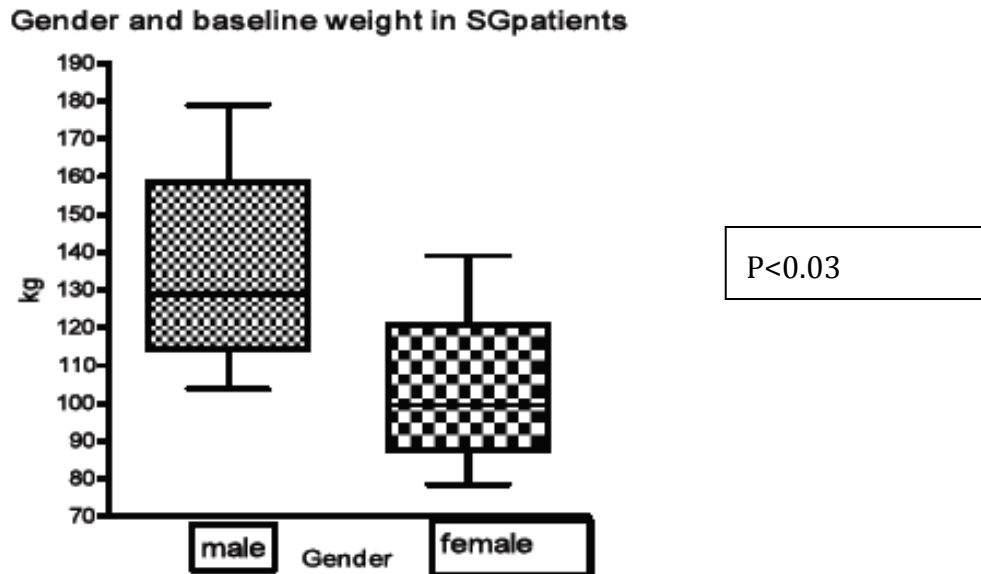
Magnitude of difference?

Mean of differences -9.636

95% confidence interval -13.45 to -5.824

R squared 0.7603

Figure 3d. Baseline weight (kg) and Gender



Column A male weight (kg) vs. Column B female weight (kg)

Unpaired t test:

P value 0.0373 ($p < 0.05$)

Two-tailed

t, df $t=2.401$ $df=10$

Magnitude of difference?

Mean \pm SEM of column A 133.9 ± 10.16 $N=6$

Mean \pm SEM of column B 102.7 ± 8.076 $N=6$

Difference between means 31.17 ± 12.98

95% confidence interval 2.245 to 60.09

R squared 0.3657

C] Hormone/Biomarker Levels:

For each of the following hormones, SG patient plasma levels were analysed at zero, three and six months following surgery.

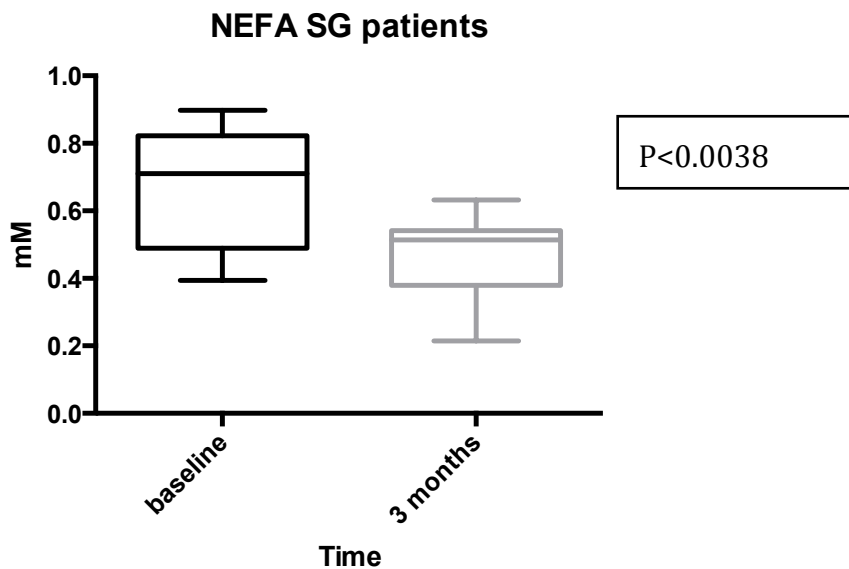
- i) NEFA
- ii) C-peptide
- iii) Ghrelin
- iv) GIP
- v) GLP-1
- vi) Glucagon
- vii) Insulin
- viii) Leptin
- ix) PAI-1
- x) Resistin
- xi) Visfatin
- xii) Fasting blood glucose

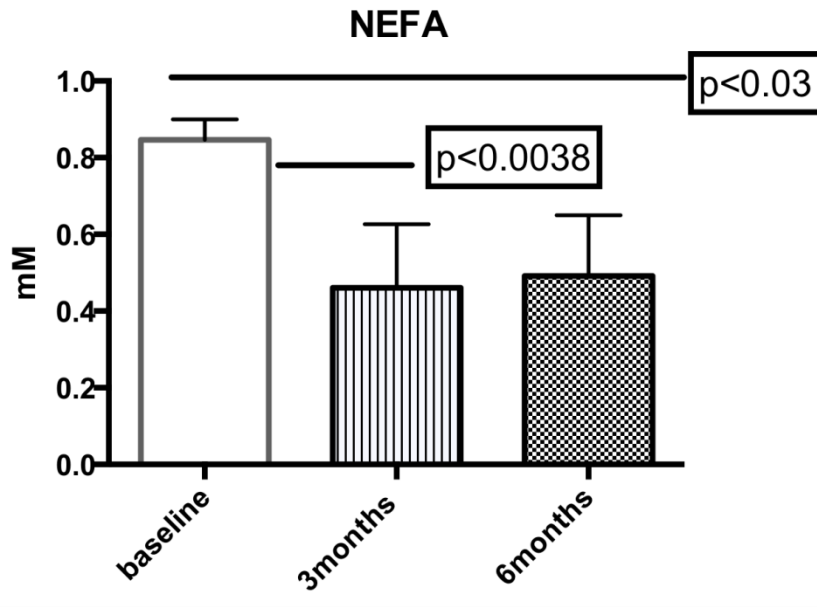
i) NEFA:

There was no statistically significant difference in NEFA levels between SG patients at baseline to controls $p < 0.65$

There was a statistically significant difference in NEFA levels in SG patients from baseline to 3 months $p < 0.0038$ ($p < 0.05$). There was a statistically significant difference in NEFA levels in SG patients from baseline to 6 months $p < 0.03$ ($p < 0.05$)

Figure 4. NEFA Assays: baseline vs. 3 months (N=13, 11) vs. 6 months (N=4)





ii) C-peptide:

Levels were not statistically significant between controls and baseline SG patients ($p < 0.71$)

There was no statistically significant change in C-peptide level in SG patients from baseline to 3 months ($p < 0.75$), ($p < 0.05$)

Nor was there a statistically significant change in these hormone levels in SG patients from 3 to 6 months ($p < 0.97$)

Magnitude of difference:

Mean of differences -22.18 SD of differences 217.9 SEM of differences 65.70 95% CI -168.6 to 124.2

iii) Ghrelin:

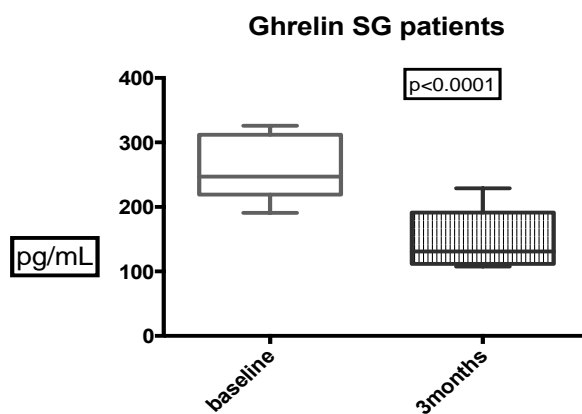
There was a highly statistically significant difference in baseline Ghrelin levels of SG patients compared to controls $p < 0.0001$ ($p < 0.05$)

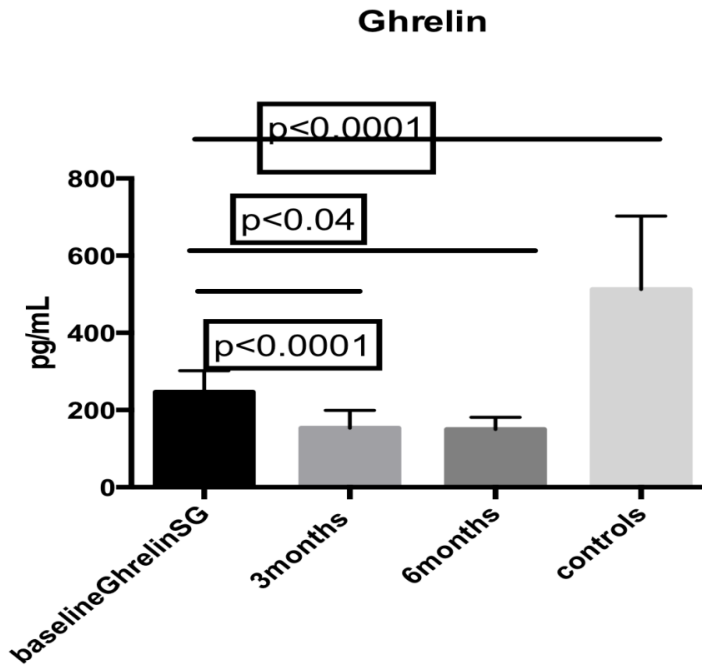
There was a highly statistically significant difference in ghrelin levels measured

in SG patients from baseline to 3 months $p < 0.0001$ ($p < 0.05$)

There was a statistically significant difference in SG patients from baseline to 3 months and to 6 months (one way ANOVA) $p < 0.04$ ($p < 0.05$)

Figure 5. Ghrelin Assays - Baseline vs. 3 months (N=13, 11) vs. 6 months (N=4)





Magnitude of difference:

Mean of differences -109.9

SD of differences 51.53

SEM of differences 16.30

95% CI -146.8 to -73.08

R squared 0.8349

iv) GIP:

There was no statistically significant difference in GIP values in SG patients at baseline from levels measured in matched controls $p < 0.059$ ($p < 0.05$)

There was no statistically significant difference in GIP values in SG patients from baseline to 3 months $p < 0.61$ ($p < 0.05$)

Magnitude of difference:

Mean of differences 17.58

SD of differences 109.5

SEM of differences 33.02

95% CI -55.98 to 91.15

R squared 0.02758

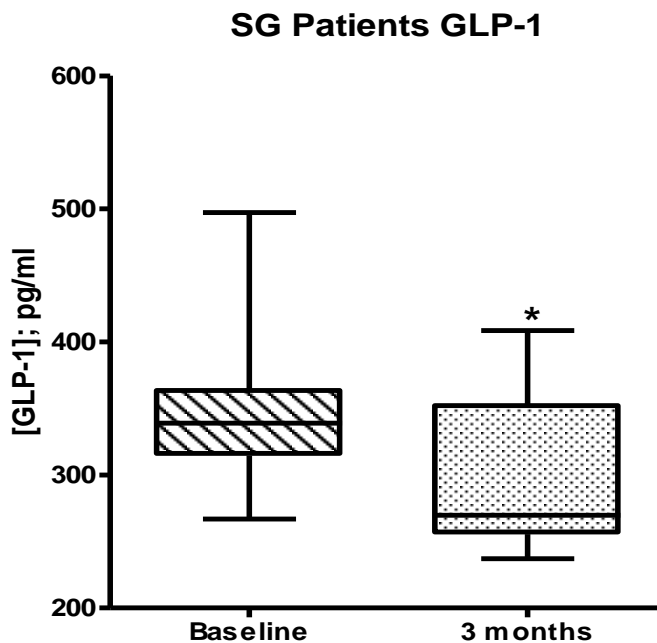
There was no statistically significant difference in GIP values in SG patients from baseline to 3 months and to 6 months (ANOVA) $p < 0.36$ ($p < 0.05$)

v) GLP-1:

There was no statistically significant difference in GLP-1 levels between baseline SG patients and controls. $P < 0.31$ ($p < 0.05$)

There was a statistically significant difference in GLP-1 levels in SG patients from baseline to 3 months $p < 0.05$ ($p < 0.05$)

Figure 6. GLP-1 Assays. Baseline vs. 3 months (N= 13, 11)



Magnitude of difference:

Mean of differences 346.8

SD of differences 58.62

SEM of differences 17.68

95% CI 307.4 to 386.1 at baseline.

Mean of the differences at 3 months 302.2

SD 62.5

SEM 18.86

CI 260 to 344.2

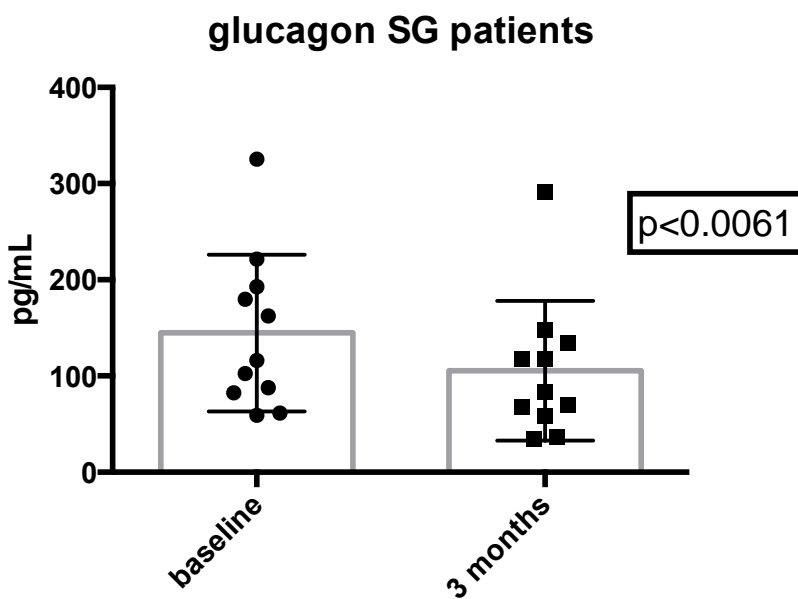
There was no statistically significant difference in GLP-1 levels of SG patients from baseline to 3 months to 6 months $p < 0.66$ ($p < 0.05$) ANOVA

vi) Glucagon:

There was no statistically significant difference in glucagon levels in SG patients from baseline compared to controls $p < 0.77$ ($p < 0.05$)

There was a statistically significant difference in glucagon levels in SG patients from baseline to 3 months $p < 0.0061$ ($p < 0.05$)

Figure 7. Glucagon Assays. Baseline vs. 3 months (N=13, 11)



Magnitude of difference:

Mean of differences -39.18

SD of differences 37.51

SEM of differences 11.31

95% CI -64.38 to -13.98

R squared 0.5455

There was no statistically significant difference in glucagon levels in SG patients from 0 to 6 months $p < 0.28$ ($p < 0.05$)

vii) Insulin:

There was a no statistically significant difference in insulin levels in SG patients and their controls at baseline. $P < 0.07$ ($p < 0.05$)

There was a no statistically significant difference in insulin levels in SG patients from baseline to 3 months $p < 0.12$ ($p < 0.05$)

Magnitude of difference:

Mean of differences -94.33

SD of differences 186.0

SEM of differences 56.09

95% CI -219.3 to 30.64

There was a no statistically significant difference in insulin levels in SG patients from baseline to 6 months $p < 0.76$ ANOVA ($p < 0.05$) or paired t test 0 and 6 months $p < 0.5$ ($p < 0.05$)

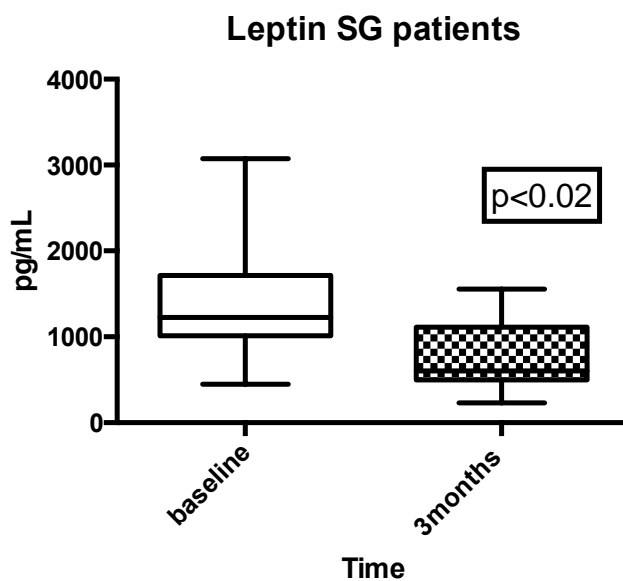
viii) Leptin:

There was no statistically significant difference in leptin levels in SG patients from controls at baseline $p < 0.10$ ($p < 0.05$)

There was a statistically significant difference in leptin levels in SG patients from baseline to 3 months $p < 0.02$ ($p < 0.05$)

There was no statistically significant difference in leptin levels in SG patients from baseline to 6 months $p < 0.09$ ($p < 0.05$)

Figure 8. Leptin Assays. Baseline vs. 3 months (N=13, 11)



Magnitude of difference:

Mean of differences -662.9

SD of differences 756.3

SEM of differences 239.2

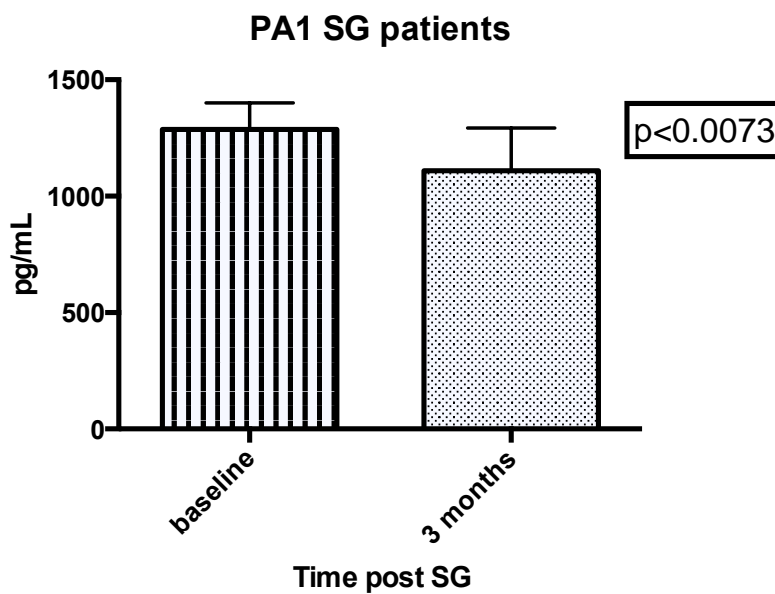
95% CI -1204 to -121.9

ix) PAI-1:

There was no statistically significant difference in PAI levels in SG patients from controls at baseline $p < 0.28$ ($p < 0.05$)

There was a statistically significant difference in PAI levels in SG patients from baseline to 3 months $p < 0.0073$ ($p < 0.05$)

Figure 9. PAI Assays. Baseline vs. 3 months (N= 13, 11)



Magnitude of difference:

Mean of differences -177.6

SD of differences 175.4

SEM of differences 52.88

95% CI -295.4 to -59.78

There was no statistically significant difference in PAI levels in SG patients from

baseline to 6 months $p < 0.17$ ($p < 0.05$) ANOVA

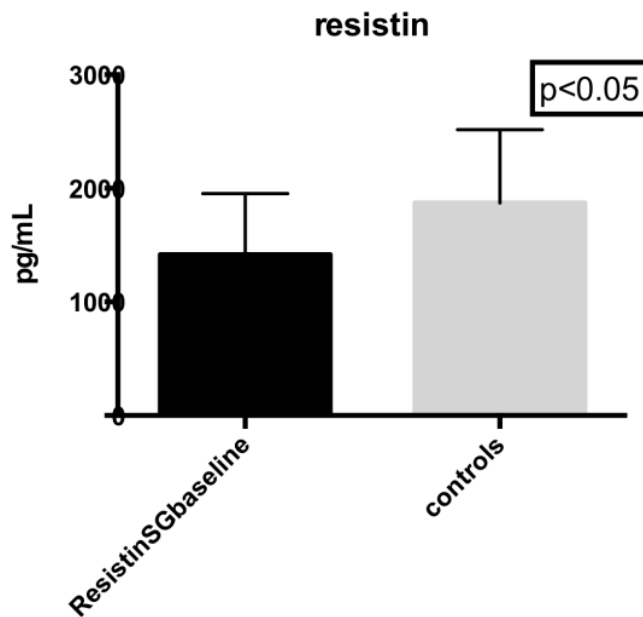
x) Resistin:

There was a statistically significant difference in resistin levels in SG patients from controls at baseline $p < 0.05$ ($P < 0.05$)

There was no statistically significant difference in resistin levels in SG patients from baseline to 3 months $p < 0.1332$ ($p < 0.05$)

There was no statistically significant difference in resistin levels in SG patients from baseline to 6 months $p < 0.57$ ($p < 0.05$)

Figure 10. Resistin Assays. Baseline vs Controls (N= 13, 26)



Magnitude of difference:

Mean of differences -305.8

SD of differences 586.0

SEM of differences 185.3

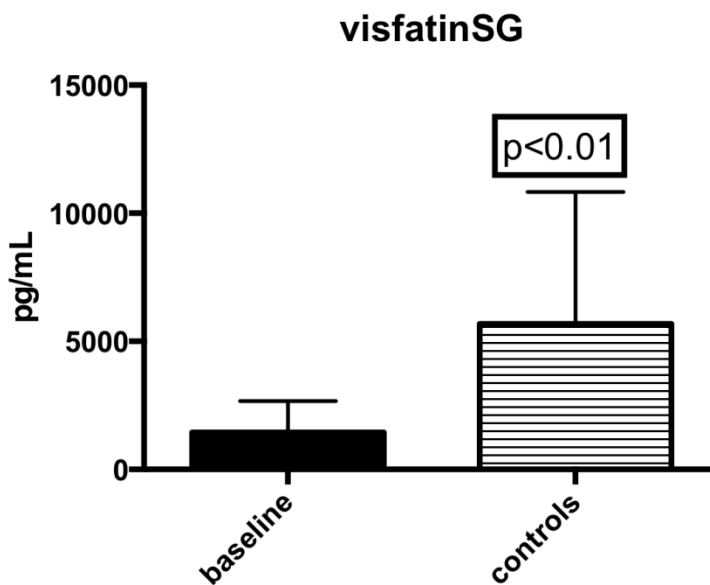
95% CI crosses 1

xi) Visfatin :

Note - although planned, analysis was not able to be performed on the platform due to lack of analyte availability in the lab at time 3 and 6 months, however baseline values were possible for all subjects and controls.

There was a statistically significant difference in visfatin levels in SG patients versus controls at baseline .

Figure 11. Visfatin Assays. Baseline SG vs Controls (N= 13, 26)

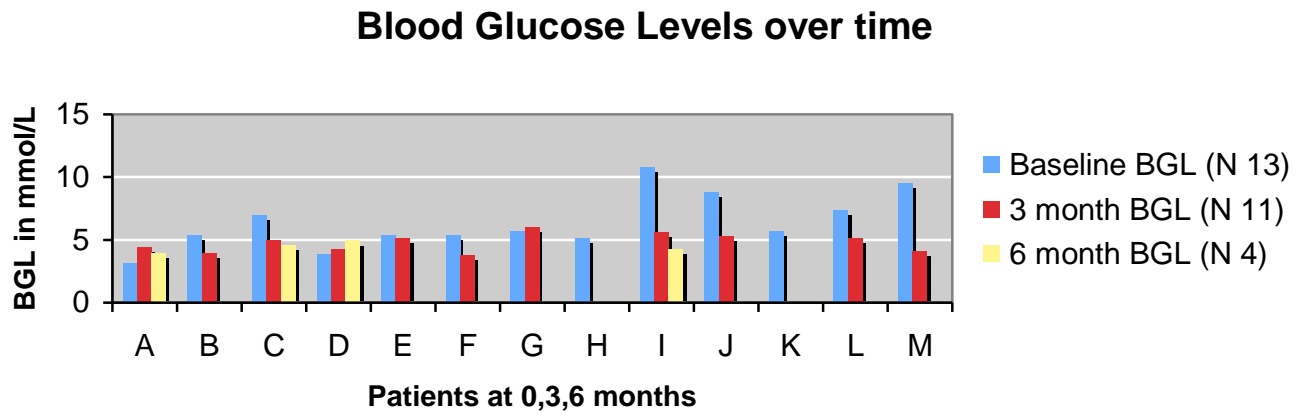


xii) Fasting blood glucose levels:

Four patients in our sleeve gastrectomy cohort were diabetic with a fasting blood glucose level (BGL) of greater than 7.0mmol/L. (Average BGL was 9.12mmol/L) at baseline. All four of these patients had resolution of their diabetes as indicated by a decrease in fasting blood glucose level to within the

normal range ($<7.0\text{mmol/L}$) with a new average fasting BGL in this group of 5.04mmol/L . (Definition of diabetes as per Diabetes Australia Guidelines).

Figure 12- Blood Glucose levels over time (N = 13, 11, 4 at 0, 3, 6 months)



IX. CONCLUSIONS:

There was a statistically significant difference between the SG patient's baseline anthropometric characteristics of weight and BMI, confirming that in this respect the cohorts were different from each other. Age and gender p values approached 1 between the SG cohort and controls, indicating good matching, with no difference between the groups. Males in the SG cohort were significantly heavier at baseline than females undergoing SG.

Our primary outcome measures were percentage excess body weight lost at 3 and 6 months post-operatively and change in hormone profiles.

Percentage excess body weight lost (%EBWL) at three months had a mean of 42.3% +/- 8.4 SD. Percentage of excess body weight lost at six months had a mean of 51.5% +/- 18.5 SD of total EBWL. There was a statistically significant ($p < 0.05$) incremental weight loss between three and six month time points from baseline, $p < 0.0001$ and $p < 0.0009$ respectively.

There was a statistically significant ($p < 0.05$) incremental weight loss from baseline and 3 months ($p < 0.0001$) and between 3 and 6 month time points ($p < 0.0009$).

A statistically significant ($p < 0.05$) reduction in levels of NEFA, ghrelin, GLP-1, glucagon, leptin and PAI-1 were seen in subjects between baseline and 3 month post-operative plasma assays.

No statistically significant association was determined in the hormone levels of C-peptide, GIP, insulin or resistin in SG subjects at baseline and three months post surgery.

There was a statistically significant ($p<0.05$) reduction in levels of NEFA and ghrelin at 6 months.

However due to small sample numbers at 6 months it is unclear if a further change in these hormone levels in subjects at 6 months compared to 3 months occurs.

A baseline difference between plasma hormone levels in subjects and controls at time 0 was only evident for resistin and visfatin.

Table 3: Hormone level changes in SG patients at baseline vs. 3 months post SG

Hormone	Change baseline to 3 months	Significant Y – Yes, N-No	($p<0.05$ is significant)
NEFA	Decrease	Y	$P<0.0038$
Ghrelin	Decrease	Y	$P<0.0001$
GLP-1	Decrease	Y	$P<0.05$
Glucagon	Decrease	Y	$P<0.0061$
Leptin	Decrease	Y	$P<0.02$
PA1	Decrease	Y	$P<0.0073$
C-peptide		N	$P<0.75$
GIP		N	$P<0.61$
Insulin		N	$P<0.12$
Resistin		N	$P<0.1332$
Visfatin		Not available	

There was no difference in the characteristics of the patients who completed clinical and biochemical follow up versus those who had clinical follow up alone.

X. DISCUSSION:

Obesity is a major health challenge with bariatric surgery being increasingly relied on to enable weight loss. In Australia, Medicare Benefits Schedule (MBS) item numbers reflect this. Total bariatric operations performed have increased from 6,557 in 2005-6 to 13,600 in 2009-10. (4)

Figure 4:

ADoHA		Total number of MBS surgical treatment of obesity claims, by year				
MBS item number		2005-06	2006-07	2007-08	2008-09	2009-10
30511						
Number of MBS items claimed		6,080	7,531	11,350	14,139	12,221
% growth			23.9%	50.7%	24.6%	-13.6%
30518						
Number of MBS items claimed		238	322	515	895	1,097
% growth			35.3%	59.9%	73.8%	22.6%
30512						
Number of MBS items claimed		239	241	214	231	282
% growth			0.8%	-11.2%	7.9%	22.1%

Source: DoHA (data) Deloitte Access Economics calculations.

The table above is taken from a report by the Australian Department of Health and Ageing to assess economic impact of obesity, given 17.5% of the population were obese in the 2008 Australian census. From 2005-2010, spending on bariatric surgery has more than tripled to \$19.3 million in 2009). This figure still pales in comparison to the cost of treating long term complications of obesity; diabetes, cardiovascular disease, cancer, osteoarthritis and decreased productivity which was estimated to be \$8.3 billion in 2008. (4)

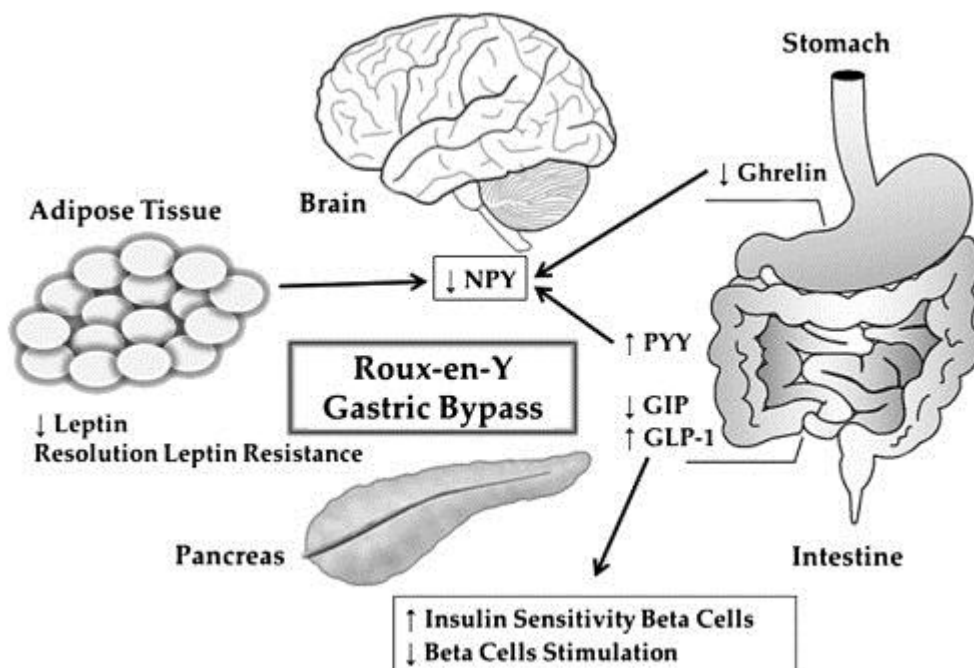
LAGB and RYGB have been well studied and good knowledge exists as to their mechanism of inducing weight loss.

LAGB relies on volume restriction to promote early satiety and a decrease in caloric intake. The advantages of this operation include relative ease of

placement with short operating time, fast recovery and return to normal activities. It relies on behavioural modification and 'sensible food choices' as well as adequate exercise for maximum benefit. Weight loss following LAGB is slower and lower than other forms of bariatric surgery. Generally 1kg/week resulting in 40-50% EBWL at 18 months is described. (25)

RYGB was first described by Mason and Ito in 1966 and has become the most commonly performed bariatric operation in the USA. The creation of a small gastric pouch, a gastrojejunostomy and a Roux limb of variable length cause volume restriction and a malabsorption syndrome. The hormonal mechanisms of weight loss are indicated in the diagram below.

Figure 13:



The mechanisms of weight loss in RYGB surgery. Surg Clin N Am 91 (2011) 1149-1161.

Physiology of weight loss surgery. Park C, Torquati A.

60-75% EBW is lost in the 18-24 months following RYGB (25). Vitamin and mineral deficiencies, anastomotic leak or stricture, dumping syndrome (diarrhea, abdominal pain, diaphoresis and pre-syncope that occur following

high sugar intake) are the disadvantages to this procedure. (25) The resolution of comorbidities including the amelioration of diabetes and improvements in insulin resistance and glucose tolerance is well established. Bypassing the antrum, duodenum, and jejunum during RYGB may provide additional benefits in the treatment of diabetes by altering gut signaling mechanisms that are beneficial to treating insulin resistance or glucose tolerance when compared with restrictive operations alone (26)

Sleeve gastrectomy surgery is currently considered the 'happy medium' between LAGB and RYGB with notable improvements in BMI and metabolic parameters post operatively. Stapled resection of the greater curvature of the stomach results in volume reduction and series of metabolic feedback responses which are currently the topic of investigation. It is a technically easier operation than RYGB with the advantage of maintained intestinal continuity so preventing nutritional deficiencies. Disadvantages include staple line bleed, leak or stricture. (25)

Molecular/Hormonal changes known to occur post SG include:

- decrease in ghrelin levels
- rapid nutrient transit into the distal bowel mediated by peptide YY and GLP-1 (27)
- Other hormonal mechanism yet to be established including alterations in bile acid binding and interaction with gut flora.

Our study has confirmed rapid and durable weight loss as characterized by percentage excess body weight lost (%EBWL) at 3 and 6 months post sleeve gastrectomy surgery.

The mean %EBW loss was 42.3% +/- 8.4SD at 3 months and 51.5% +/- 18.5SD at 6 months. There was a statistically significant incremental weight loss between 3

and 6 month time points from baseline, $p < 0.0001$ and $p < 0.0009$ respectively. This compares favourably to other studies for example a meta-analysis by Shi et al in *Obesity Surgery 2010* demonstrates a EBWL of 35% at 6 months. (24)

A number of hormonal changes were observed in these patients (Table 3 of results). Of statistical significance are a fall in ghrelin, GLP-1, glucagon, PAI-1 and leptin at 3 months. A continued significant drop at 6 months was observed for ghrelin.

The literature to date demonstrates largely conflicting data for the hormonal changes following sleeve gastrectomy surgery. Our study adds to the body of evidence already available on what hormonal changes are most likely to predominate in subjects following this procedure. The majority of studies are of small sample size and it may prove insightful to undertake a meta-analysis to determine what the overall trend is.

Other effects of gastric sleeve surgery include increased resolution of obesity related co-morbidities, such as obstructive sleep apnoea and T2DM. This is in keeping with other studies.

Successful management of obesity requires safe, effective, long term treatments that resist the compensatory mechanisms of the body to desire to store food. Bariatric surgery in the form of sleeve gastrectomy has been shown to be very successful in this (9).

A strength of our study is that it also demonstrates in SG specifically the hormone profile of changes that induce more than a mere mechanical component to weight loss.

This study confirms and adds to the literature on the success of SG surgery in achieving and maintaining profound weight loss for obese patients. To date there is no equivalent medical pharmacotherapy to achieve such success for

these patients. Sleeve gastrectomy offers a more effective weight loss strategy than LAGB and carries less surgical risk and nutritional disruption than RYGB.

XI. LIMITATIONS:

There are several limitations of this study.

i) Timing for patients and follow up:

Our attrition rate was high, not uncommon for studies of long term weight loss. The possibility that the changes in hormone levels differed in patients who discontinued the study can not be excluded. As part of this attrition rate, in terms of timing of the study, our time line of data collection was limited. As such, there is a need for longitudinal data in this cohort, which would add to the literature base and experience in SG patient studies.

ii) Multiplex bioassay platform:

The use of a multiplex bioassay platform results in measurements of individual hormones that are less accurate and precise than those obtained with an individualized optimized assay. However, this is likely to minimise the detection of changes occurring post SG with weight loss and serve to minimise the attributable detection of change.

XII. FUTURE DIRECTIONS:

Circulating hormonal mediators of regulation of body weight are altered significantly in a number of instances but do not revert to pre-surgical levels of 'normal' at three or six months post operatively. Further future research work in our cohort and others, could involve investigating additional markers, such as acylated ghrelin and high and low molecular weight forms of adiponectin. FXR and other bile acid pathway metabolites would also be interesting to study. Longitudinal data in our SG cohort out to 12 months and beyond would also be interesting to document anthropometric and hormonal changes.

To date, bariatric surgery with SG as the technique of choice. Having a low morbidity and mortality, it represents the most efficient and durable mode of weight loss for the ever increasing number of morbidly obese patients. Our results confirm quick and sustained weight loss that correlates to a change in profile of a variety of hormones that are known to be associated with weight gain/loss. It suggests a number of potential hormonal pathways for pharmacological targets. Our research adds to the literature to encourage further research to evaluate if a primary hormonal defect can be found to be responsible for promoting weight gain and so its counter could promote weight loss. It may be feasible in the long term to exploit this physicochemical imbalance and develop a pharmacological means of achieving swift and sustained weight loss without the risks of surgery.

XIII. REFERENCES:

1. Wang Y, Beydoun MA, Liang L, Caballero B, Kumanyika SK. Will all Americans become overweight or obese? estimating the progression and cost of the US obesity epidemic. *Obesity (Silver Spring)*. 2008 Oct;16(10):2323-30.
2. Stunkard AJ, Sorensen TI, Hanis C, Teasdale TW, Chakraborty R, Schull WJ, et al. An adoption study of human obesity. *N Engl J Med*. 1986 Jan 23;314(4):193-8.
3. World Health Organisation.
<http://www.who.int/mediacentre/factsheets/fs311/en/2014> May 2014.
4. Australian Department of Health and Ageing
http://www.health.gov.au/internet/main/publishing.nsf/Content/Obesity_+Review2011.
5. Sjostrom L, Lindroos AK, Peltonen M, Torgerson J, Bouchard C, Carlsson B, et al. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N Engl J Med*. 2004 Dec 23;351(26):2683-93.
6. Sugerman HJ, Wolfe LG, Sica DA, Clore JN. Diabetes and hypertension in severe obesity and effects of gastric bypass-induced weight loss. *Ann Surg*. 2003 Jun;237(6):751-6; discussion 7-8.
7. Christou NV, Lieberman M, Sampalis F, Sampalis JS. Bariatric surgery reduces cancer risk in morbidly obese patients. *Surg Obes Relat Dis*. 2008 Nov-Dec;4(6):691-5.
8. Neff KJ, Olbers T, le Roux CW. Bariatric surgery: the challenges with candidate selection, individualizing treatment and clinical outcomes. *BMC Med*. 2013;11:8.
9. Ryan KK, Tremaroli V, Clemmensen C, Kovatcheva-Datchary P, Myronovych A, Karns R, et al. FXR is a molecular target for the effects of vertical sleeve gastrectomy. *Nature*. 2014 May 8;509(7499):183-8.

10. Moy J, Pomp A, Dakin G, Parikh M, Gagner M. Laparoscopic sleeve gastrectomy for morbid obesity. *Am J Surg*. 2008 Nov;196(5):e56-9.
11. Melissas J, Koukouraki S, Askoxylakis J, Stathaki M, Daskalakis M, Perisinakis K, et al. Sleeve gastrectomy: a restrictive procedure? *Obes Surg*. 2007 Jan;17(1):57-62.
12. Sowers JR. Endocrine functions of adipose tissue: focus on adiponectin. *Clin Cornerstone*. 2008;9(1):32-8; discussion 9-40.
13. Faraj M, Havel PJ, Phelis S, Blank D, Sniderman AD, Cianflone K. Plasma acylation-stimulating protein, adiponectin, leptin, and ghrelin before and after weight loss induced by gastric bypass surgery in morbidly obese subjects. *J Clin Endocrinol Metab*. 2003 Apr;88(4):1594-602.
14. Swarbrick MM, Austrheim-Smith IT, Stanhope KL, Van Loan MD, Ali MR, Wolfe BM, et al. Circulating concentrations of high-molecular-weight adiponectin are increased following Roux-en-Y gastric bypass surgery. *Diabetologia*. 2006 Nov;49(11):2552-8.
15. Gass M, Beglinger C, Peterli R. Metabolic surgery-principles and current concepts. *Langenbecks Arch Surg*. 2011 Oct;396(7):949-72.
16. Woelnerhanssen B, Peterli R, Steinert RE, Peters T, Borbely Y, Beglinger C. Effects of postbariatric surgery weight loss on adipokines and metabolic parameters: comparison of laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy--a prospective randomized trial. *Surg Obes Relat Dis*. 2011 Sep-Oct;7(5):561-8.
17. Stefater MA, Wilson-Perez HE, Chambers AP, Sandoval DA, Seeley RJ. All bariatric surgeries are not created equal: insights from mechanistic comparisons. *Endocr Rev*. 2012 Aug;33(4):595-622.
18. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, et al. Long-term persistence of hormonal adaptations to weight loss. *N Engl J Med*. 2011 Oct 27;365(17):1597-604.

19. Stofkova A. Resistin and visfatin: regulators of insulin sensitivity, inflammation and immunity. *Endocr Regul.* 2010 Jan;44(1):25-36.
20. Retnakaran R, Youn BS, Liu Y, Hanley AJ, Lee NS, Park JW, et al. Correlation of circulating full-length visfatin (PBEF/NAMPT) with metabolic parameters in subjects with and without diabetes: a cross-sectional study. *Clin Endocrinol (Oxf).* 2008 Dec;69(6):885-93.
21. Schauer PR, Kashyap SR, Wolski K, Brethauer SA, Kirwan JP, Pothier CE, et al. Bariatric surgery versus intensive medical therapy in obese patients with diabetes. *N Engl J Med.* 2012 Apr 26;366(17):1567-76.
22. Mingrone G, Panunzi S, De Gaetano A, Guidone C, Iaconelli A, Leccesi L, et al. Bariatric surgery versus conventional medical therapy for type 2 diabetes. *The New England journal of medicine.* 2012 Apr 26;366(17):1577-85.
23. Carlsson LM, Peltonen M, Ahlin S, Anveden A, Bouchard C, Carlsson B, et al. Bariatric surgery and prevention of type 2 diabetes in Swedish obese subjects. *N Engl J Med.* 2012 Aug 23;367(8):695-704.

24. Shi X, Karmali S, Sharma AM et al. A review of laparoscopic sleeve gastrectomy for morbid obesity. *Obesity Surgery* 2010; 20 (8): 1171-7

25. Needleman B, Happel L. Bariatric Surgery: Choosing the optimal Procedure. *Surg Clin N Am* 88 (2008) 991-1007.

26. Rubino F, Gagner M. Potential of surgery for curing type 2 diabetes mellitus. *Ann Surg* 2002;236(5):554-9.

27. Brethauer S. Sleeve Gastrectomy. *Surg Clin N Am* 91(2011) 1265-1279.



