Surg Endosc (2016) 30:3499–3504
DOI 10.1007/s00464-015-4638-3

Effect of bariatric surgery on systemic and adipose tissue inflammation

Valerie G. Sams[1,3] · Camille Blackledge[1] · Nadeeja Wijayatunga[2] · Patrick Barlow[1] · Matthew Mancini[1] · Gregory Mancini[1] · Naima Moustaid-Moussa[1,2]

Received: 11 May 2015 / Accepted: 17 October 2015 / Published online: 30 October 2015

Abstract
Background Obese patients are predisposed to developing insulin resistance and associated metabolic diseases such as diabetes and cardiovascular disease. The objective of this study was to determine the effect of bariatric surgery on adipose-derived inflammatory cytokines (adipokines), which play a key role in insulin resistance and obesity. We hypothesized that there is a significant increase in serum and tissue anti-inflammatory adiponectin with a decrease in circulating pro-inflammatory TNF-α and MCP-1, leading to reduced inflammation post-bariatric surgery.
Methods In this study, we investigated the effects of laparoscopic Roux-en-Y gastric bypass (LRYGB) and laparoscopic gastric band on serum and tissue levels of adiponectin and serum levels of MCP-1 and TNF-α. Samples of serum and adipose tissue were collected at the time of surgery, 2 weeks and 6 months postoperatively. Adipokine levels were assayed by ELISA kits.
Results A significant increase in adiponectin levels 2 weeks after surgery was observed in the subcutaneous adipose tissue in both groups combined. Serum adiponectin in LRYGB patients showed an increasing trend, while MCP-1 showed a decreasing trend post-surgery. There was no difference in TNF-α among the groups. The number of patients enrolled did not allow for statistical power to be reached.
Conclusion Our results show significant and rapid increases in subcutaneous adipose adiponectin as early as 2 weeks post-bariatric surgery demonstrating reduced inflammation and possibly reduced insulin resistance. Future studies are warranted in larger cohorts with additional measurements of insulin sensitivity and inflammation.

Keywords Insulin resistance · Bariatric surgery · Adipokines

Obesity is a major health problem in the USA with an increasing prevalence to 16.9 % in youth and 34.9 % in the adult population in 2011–2012 [1]. These patients are at risk of developing conditions such as type 2 diabetes, cardiovascular disease, cancer and stroke [2]. Furthermore, all grades of obesity carry a higher all-cause mortality than normal weight people. Obesity grades 2 (BMI $\geq$ 35–39) and 3 (BMI $>$ 40) are associated with higher all-cause mortality [3]. Bariatric surgery is a more effective and durable treatment for obesity and associated comorbidities compared to non-surgical interventions such as lifestyle modifications and pharmacological therapies [4]. The exact mechanism that leads to these effects is not well understood, but the effects are independent of weight loss [5]. Since the discovery of the adipocyte as a "metabolically active organ" which secretes hormones, cytokines and chemokines, there is growing interest in understanding its functions in obesity and weight loss [6]. Adipose tissue secretes several pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and monocyte chemoattractant protein (MCP-1). It also

✉ Valerie G. Sams
Valerie.g.sams@gmail.com; Valerie.g.sams.mil@mail.mil

[1] Department of Surgery, University of Tennessee Medical Center, Knoxville, TN, USA

[2] Department of Nutritional Sciences, College of Human Sciences and The Obesity Research Cluster, Texas Tech University, Lubbock, TX, USA

[3] San Antonio Military Medical Center, 26 Campedn Circle, San Antonio, TX 78209, USA

secretes anti-inflammatory adipokines such as adiponectin which is known to enhance insulin sensitivity. Chronic low-grade inflammation is identified as a hallmark in obesity with dysregulation of adipose tissue-derived cytokines (adipokines) production. These are detected in both serum and adipose tissue and are active factors that modulate the effects of obesity and related comorbidities [7].

Adiponectin levels are decreased in obese people compared to normal individuals [7, 8]. Studies have shown an inverse relationship with serum adiponectin and weight loss. As patients lose weight, they have a concurrent increase in adiponectin, and resolution of comorbidities such as diabetes [9]. Pro-inflammatory adipokines such as TNF-α, IL-6, leptin and MCP-1 are elevated in obese patients and have been shown to be associated with increased insulin resistance [10].

The purpose of this study was to determine the effect of bariatric surgery on adipose-derived adipokines, such as adiponectin, TNF-α and MCP-1, which play a key role in insulin resistance and obesity. We hypothesized that there is a significant increase in serum and tissue anti-inflammatory adiponectin with a decrease in circulating pro-inflammatory TNF-α and MCP-1, leading to reduced inflammation post-bariatric surgery.

Materials and methods

Institutional review board approval was obtained at the University of Tennessee. Patients undergoing laparoscopic Roux-en-Y gastric bypass (LRYGB) and laparoscopic adjustable gastric banding (LAGB) were chosen to participate in the study. The selection of the surgical procedure to be performed was based on the patients' preference. The procedures were performed by two bariatric surgeons at the University of Tennessee Bariatric Surgery Center. Written, informed consent was obtained from all the study participants. Blood was drawn preoperatively the day of surgery, and intraoperative omental and subcutaneous adipose tissue samples were obtained. Serum was separated immediately by centrifugation. The patients were followed in the clinic at 2 weeks and 6 months, and at each time point, serum and subcutaneous tissue samples were obtained. The subcutaneous tissue was collected by means of fine needle aspiration using instillation of sterile water and an 18-gauge needle.

Frozen adipose tissue was homogenized using Tissue Lyser LT Adapter (Qiagen) in modified radioimmunoprecipitation assay (modified RIPA) buffer (20 mmol/L Tris–HCl (pH 7.5), 150 mmol/L sodium chloride, 1 mmol/L PMSF, 0.05 % Tween-20) and a protease inhibitor cocktail tablet (Roche). After homogenization, tissue lysate was centrifuged at 17,000 rpm for 10 min at 4 °C. Upper aqueous phases were collected and aliquoted. Protein concentration of tissue samples was determined by the Bradford protein quantification assay and was used to normalize adiponectin measurements in adipose tissue. Adipokine levels in serum (adiponectin, TNF-α and MCP-1) were assayed by ELISA kits (Ray Biotech).

Results

We ultimately enrolled 20 patients who underwent LRYGB and 5 who underwent LAGB. Patient demographics are illustrated in Table 1.

We investigated the effects of bariatric surgery on omental and subcutaneous tissue levels of adiponectin in 10 patients (8 LRYGB and 2 LAGB), both diabetic and non-diabetic obese patients, for whom we had a complete set of data for the three time points (time of surgery, 2 weeks post-surgery and 6 months post-surgery). Adiponectin levels were normally distributed, both in the omental and in the subcutaneous tissue. When performing a simple independent *t* test, there was no significant difference in the adiponectin levels in omental adipose tissue at the time of surgery between the two surgery groups (LRYGB and LAGB) ($p = 0.8$). Comparison of subcutaneous tissue adiponectin levels (1) across time, (2) between groups and (3) the interaction between group and time was performed

Table 1 Patient demographic characteristics

Variable	Mean (SD) or frequency (%)	
	LRYGB ($n = 20$)	LAGB ($n = 5$)
Age	37.25 (11.68)	38.33 (12.94)
BMI		
Baseline	47.24 (6.58)	48.34 (54.24)
2 weeks post	43.65 (6.42)	45.93 (6.19)
6 months post	34.54 (6.07)	44.34 (6.03)
Sex		
Male	5 (31.2 %)	1 (20 %)
Female	11 (68.8 %)	4 (80 %)
Race		
Caucasian	15 (6.2 %)	4 (80 %)
African-American	1 (93.8 %)	1 (20 %)
Smoking Hx		
No	11 (68.8 %)	4 (80 %)
Former	5 (31.2 %)	1 (20 %)
Hypertension		
Yes	14 (73.7 %)	1 (20 %)
Diabetes		
Yes	6 (31.6 %)	3 (60 %)

using mixed analysis of variance. A significant increase in adiponectin levels 2 weeks after bariatric surgery was observed in the subcutaneous adipose tissue ($p = 0.007$), but no difference between groups or interaction between groups and time ($p > 0.05$) was observed (Table 2).

In the same cohort of 10 patients, we analyzed serum adiponectin, TNF-α and MCP-1 for all three time points. Since all of those variables were not normally distributed and were skewed, log-transformation was performed. There was no significant difference across time within the surgery groups, between surgery groups or with the interaction between surgery groups and time for any of the serum cytokines following exploratory analysis of variance (p value >0.05). It is interesting to note that serum adiponectin in LRYGB patients showed an increasing trend in log-transformed values, while MCP-1 showed a decreasing trend post-surgery though not statistically significant ($p > 0.05$). TNF-α did not show any trends (Table 3).

Finally, we analyzed the changes in serum adiponectin and MCP-1 at baseline ($n = 20$ LRYGB and $n = 5$ LAGB), 2 weeks post-surgery ($n = 16$ LRYGB and $n = 5$ LAGB) in a subgroup of patients and TNF-α for a subgroup of patients ($n = 11$ LRYGB and $n = 4$ LAGB). Exploratory analysis of variance findings was similar to the analysis above for up to 6 months, and we did not find significant differences over time, between groups or the interaction of groups and time when studied for 2 weeks post-surgery (p value >0.05) (Table 4). Again we observed that serum adiponectin showed an increasing trend, while MCP-1 decreased in the LRYGB group by 2 weeks post-surgery even though not statistically significant ($p > 0.05$) (Table 4). All serum cytokine trends in each group over time are illustrated in Fig. 1.

Discussion

Bariatric surgery leads to significant weight loss through restriction, malabsorption or both in morbidly obese patients [11–13]. In addition, it reduces insulin resistance, cardiovascular disease and mortality, independent of weight loss [14]. Thus, bariatric surgery is considered the most effective treatment option for morbidly obese patients [15]. Nearly 85 % of patients who undergo gastric bypass surgery experience remission of their diabetes prior to and independent from the weight loss [13]. This suggests that there is a surgery-specific effect of glucose homeostasis which may involve modulation and regulation of insulin sensitivity [15].

There are many proposed mechanisms to explain this phenomenon. The inflammatory mechanism of insulin resistance involves an early phase of improved insulin sensitivity and a late phase of decreased inflammatory mediators. In humans, the rapid improvement in carbohydrate homeostasis following gastric bypass is likely secondary to an increase in insulin sensitivity followed later by an increase in insulin secretion [16].

Animal studies demonstrate that overexpression of pro-inflammatory cytokines such as MCP-1 induces insulin resistance [17], while a reduction in inflammatory mediators such as TNF-α is protective from high-fat-diet-induced insulin resistance [18–21]. Furthermore, overexpression of anti-inflammatory cytokines such as adiponectin has a protective role in high-fat-diet-induced insulin resistance [22]. The exact mechanisms resulting in type 2 diabetes remission after gastric bypass is complex and has not been fully discerned. Rearrangement of gastrointestinal anatomy has some effect beyond food intake and weight loss [23]. Several studies have shown significant changes in gut hormones that may also mediate some of the beneficial effects of bariatric surgery [24]. Obesity-induced insulin resistance may result from an imbalance in the expression of these pro- and anti-inflammatory adipokines [10].

In this pilot study, we demonstrated an increase in adipose tissue adiponectin which could contribute to increased insulin sensitivity and decreased inflammation postoperatively. Our data also suggest that serum and adipose adiponectin levels are differentially regulated. We did not observe significant trends in the pro-inflammatory mediator, TNF-α, consistent with others that did not find

Table 2 Omental and subcutaneous tissue adiponectin levels

Adiponectin level (μg/μl)	Group mean (SD)		p value[a]		
	LRYGB ($n = 8$)	LAGB ($n = 2$)	Time	Group	Group × time
Omental	6585.64 (3692.15)	5869.92 (625.36)	–	0.800	–
SQ tissue					
Baseline	6934.56 (2750.53)	6183.55 (212.72)	0.007	0.670	0.615
2 weeks post	13,878.18 (4522.25)	14,059.85 (2932.93)			
6 months post	15,741.88 (6553.53)	12,451.69 (5309.15)			

[a] No comparison for time or group × time was performed for omental adiponectin levels because this value was only taken at baseline

Table 3 Exploratory analysis of variance for log-transformed outcome variables

Variable/time point	Group mean (SD)[a]		*p* value		
	LRYGB ($n = 8$)	LAGB ($n = 2$)	Time	Group	Group × time
Serum adiponectin					
Baseline	4.12 (1.8)	4.62 (0.43)	0.993	0.950	0.749
2 weeks post	4.39 (1.03)	4.27 (0.75)			
6 months post	4.65 (0.91)	4.16 (0.33)			
TNF-α					
Baseline	6.59 (0.39)	6.64 (0.39)	0.802	0.974	0.933
2 weeks post	6.57 (0.26)	6.54 (0.33)			
6 months post	6.56 (0.18)	6.56 (0.34)			
MCP-1					
Baseline	3.49 (1.17)	2.67 (0.1)	0.481	0.088	0.905
2 weeks post	3.3 (0.75)	2.28 (0.34)			
6 months post	2.77 (0.59)	2.04 (0.45)			

[a] Values represented on a log-normal scale

Table 4 Exploratory analysis of variance for log-transformed outcome variables using full sample

Variable/time point	Group mean (SD)[a]		*p* value		
	LRYGB ($n = 20$)	LAGB ($n = 5$)	Time	Group	Group × time
Serum adiponectin					
Baseline	3.83 (1.46)	4.36 (1.29)	0.818	0.719	0.167
2 weeks post	4.18 (1.1)	4.1 (1.13)			
TNF-α					
Baseline	6.68 (0.39)	6.48 (0.3)	0.310	0.424	0.472
2 weeks post	6.49 (0.26)	6.44 (0.22)			
MCP-1					
Baseline	3.46 (0.98)	3.01 (0.59)	0.52	0.121	0.578
2 weeks post	3.18 (0.61)	2.51 (0.47)			

[a] Values represented on a log-transformed scale

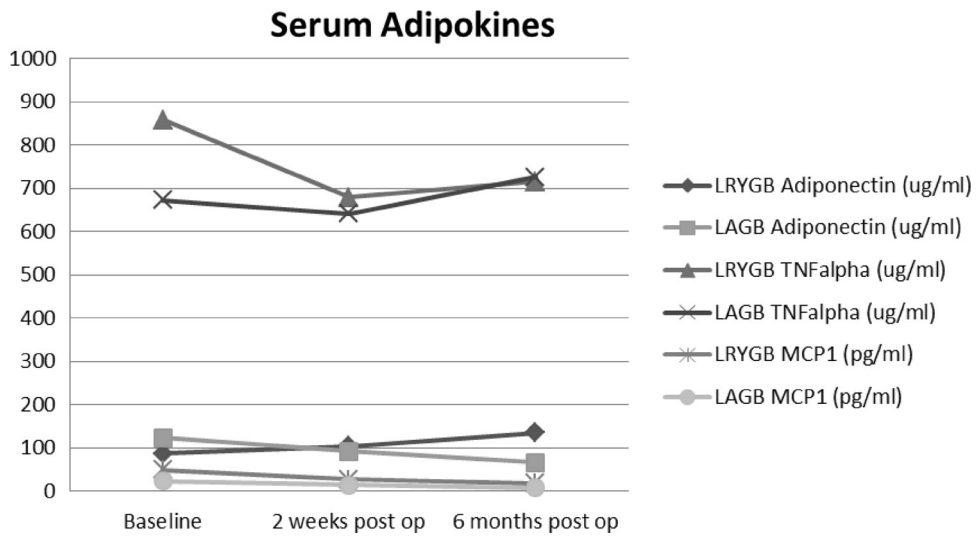

Fig. 1 Serum adipokine levels to include adiponectin (μg/ml), TNF-α (μg/ml) and MCP-1(pg/ml) for LRYGB and LAGB from baseline, 2 weeks postoperatively and 6 months postoperatively

correlations between TNF and changes in inflammation [25]. However, there was a demonstrable trend in reduction in pro-inflammatory MCP-1 in both groups. Overall, findings of our study suggest a reduction in inflammation following bariatric surgery which could be playing a major role in the reduction in obesity-associated comorbidities such as type 2 diabetes and in the reduction in mortality.

It is also worthwhile noting that our subject population had very high BMIs but did not exhibit severe diabetes based on hemoglobin A1c values for those diagnosed with diabetes. This may explain smaller differences in adipokines compared to other studies where patients had more severe comorbidities.

The major strength of this pilot study was the availability of both serum and subcutaneous adipose tissue at time of surgery and at follow-up at 2 weeks and 6 months for the measurement of adiponectin. However, low number of subjects and unequal numbers of subjects in each group due to drop out during follow-up of patients were part of our study limitations. A power analysis was performed prior to the study using G*Power version 3.1 (HHU, 2011) with a beta of 0.20 and an alpha of 0.05. Assuming that a mean difference of 22.5 pg/ml adiponectin exists between patient and control groups at posttest, 23 subjects would be needed in each arm. Unfortunately, low patient compliance with the 2-week follow-up and an even lower compliance with the 6-month follow-up resulted in fewer subjects with samples for all three time points. Overall, we ended up with 30 specimens (10 patients for which all 3 points, time 0, 2 weeks and 6 months) were available. The data represented here are considered pilot data only as we did not reach the numbers needed to obtain statistical power. A larger sample of patients undergoing bariatric surgery would help to understand the effects of bariatric surgery on inflammation and the relationship between inflammation and bariatric surgery.

There were several limitations to our study. Our initial intent was to measure fasting outcomes; however, most patients were not compliant in fasting at the follow-up visits, so HOMA IR measurements would have been inaccurate. Another measure of insulin sensitivity is HbA1C from whole blood; however, this measurement was not possible as the blood samples were all prepared for serum analyses reported here. We intend in future studies to more closely monitor and facilitate compliance and conduct fasting measurements for both HbA1C and other metabolic biomarkers and assess medication therapy for diabetes pre- and post-surgery.

A limitation for the comparison of the two groups was that the study size was too small. So, although we recognize that accounting for other variables or comorbidities to compare between the groups would be preferable, it was not statistically feasible given the small and pilot nature of the study sample. We plan in future larger studies with more patients to conduct such comparisons.

Another limitation of this small pilot study was that while several cytokines were analyzed, they did not demonstrate a difference or only showed trends. This may also be related to the pilot nature of our study, and we may obtain more significant changes with a larger study with more patients.

Finally, the design was originally to compare a bypass group to a control group (the gastric bands); however, the number of gastric bands performed after we began enrollment dramatically declined as the number of sleeve gastrectomies increased. So the choice to not include sleeve gastrectomies when we designed this study was twofold: (1) When the pilot study was developed, our sleeve gastrectomy practice was just beginning; (2) we were not familiar with any existing data regarding the inflammatory and hormonal effects of sleeve gastrectomy. Future studies should include a sleeve gastrectomy arm with a review of the literature evaluating the physiologic effects of the procedure.

In summary, our study demonstrates beneficial effects of bariatric surgery. Despite the small number of subjects, our results show significant and rapid increases in subcutaneous adipose adiponectin as early as 2 weeks post-bariatric surgery demonstrating reduced inflammation and possibly reduced insulin resistance, which is a novel finding. Future studies are warranted in larger cohorts with additional measurements of insulin sensitivity and inflammation.

Acknowledgments We would like to acknowledge Dr. Nalin Siriwardhana for his contributions in the initiation of the study and sample collection. We also thank the Physicians Medical Education Fund (PMERF) at the University of Tennessee Medical Center for awarding us the Grant to carry out this research and to the staff of the University of Tennessee Medical Center Bariatric Center. This study was also partially funded by start-up funds from Texas Tech University (College of Human Sciences and Office of the Vice president for Research).

Compliance with ethical standards

Disclosures Drs. Valerie Sams, Camille Blackledge, Naima Moustaid-Moussa, Patrick Barlow, Matthew Mancini, Greg Mancini and Nadeeja Wijayatunga have no conflict of interest or financial ties to disclose.

References

1. Ogden CL, Carroll MD, Kit BK, Flegal KM (2013) Prevalence of obesity among adults: United States, 2011–2012. NCHS Data Brief 131:1–8
2. Poirier P, Cornier M-A, Mazzone T et al (2011) Bariatric surgery and cardiovascular risk factors: a scientific statement from the American Heart Association. Circulation 123(15):1683–1701

3. Flegal KM, Kit BK, Orpana H, Graubard BI (2013) Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. JAMA 309(1):71–82
4. Colquitt JL, Pickett K, Loveman E, Frampton G (2014) Surgery for weight loss in adults. Cochrane Database Syst Rev 8: CD003641
5. Knop FK, Taylor R (2013) Mechanism of metabolic advantages after bariatric surgery: it's all gastrointestinal factors versus it's all food restriction. Diabetes Care 36(Supplement 2):S287–S291
6. Feng B, Zhang T, Xu H (2013) Human adipose dynamics and metabolic health. Ann N Y Acad Sci 1281(1):160–177
7. Ouchi N, Parker JL, Lugus JJ, Walsh K (2011) Adipokines in inflammation and metabolic disease. Nat Rev Immunol 11(2): 85–97
8. Whitehead JP, Richards AA, Hickman IJ, Macdonald GA, Prins JB (2006) Adiponectin—a key adipokine in the metabolic syndrome. Diabetes Obes Metab 2:264–280
9. Faraj M, Havel PJ, Phélis S, Blank D, Sniderman AD, Cianflone K (2003) 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 88(4):1594–1602
10. Kwon H, Pessin JE (2013) Adipokines mediate inflammation and insulin resistance. Front Endocrinol 4:71
11. Mun EC, Blackburn GL, Matthews JB (2001) Current status of medical and surgical therapy for obesity. Gastroenterology 120:669–681
12. Sjostrom L, Lindroos AK, Peltonen M et al (2004) Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. N Engl J Med 351:2683–2693
13. Buchwold H, Avidor Y, Braunwald E et al (2004) Bariatric surgery: a systematic review and meta-analysis. JAMA 292: 1724–1737
14. Shah M, Simha V, Garg A (2006) Long-term impact of bariatric surgery on body weight, comorbidities, and nutritional status. J Clin Endocrinol Metab 91(11):4223–4231
15. Thaler JP, Cummings DE (2009) Minireview: hormonal and metabolic mechanisms of diabetes remission after gastrointestinal surgery. Endocrinology 150(6):2518–2525
16. Andreelli F, Amouyal C, Magnan C, Mithieux G (2009) What can batriatric surgery teach us about the pathophysiology of type 2 diabetes? Diabetes Metab 35(6pt2):499–507
17. Kalupahana NS, Voy BH, Fletcher S et al (2009) Mechanisms linking overproduction of angiotensinogen by adipose tissue to inflammation, glucose intolerance and insulin resistance. Obesity 17(suppl2):S58
18. Kanda H, Tateya S, Tamori Y et al (2006) MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis on obesity. J Clin Investig 116:1494–1505
19. Sato C, Shikata K, Hirota D et al (2011) P-selectin glycoprotein ligand-1 deficiency is protective against obesity-related insulin resistance. Diabetes 60:189–199
20. Weisberg SP, Hunter D, Huber R et al (2006) CCR2 modulates inflammatory and metabolic effects of high-fat feeding. J Clin Investig 116:115–124
21. Hotamisligil GS, Shargil NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259:87–91
22. Luo N, Liu J, Chung BH et al (2010) Macrophage adiponectin expression improves insulin sensitivity and protects against inflammation and atherosclerosis. Diabetes 59:791–799
23. Rubino F, Schauer PR, Lee KM, Cummings DE (2010) Metabolic surgery to treat type 2 diabetes: clinical outcomes and mechanisms of action. Annu Rev Med 61:393–411
24. Pournaras DJ, Aasheim ET, Bueter M et al (2012) Effect of bypassing the proximal gut on gut hormones involved with glycemic control and weight loss. Surg Obes Relat Dis 8(4):371–374
25. Miller GD, Nicklas BJ, Fernandez A (2011) Serial changes in inflammatory biomarkers after Roux-en-Y gastric bypass surgery. Surg Obes Relat Dis 7(5):618–624