



Long-Term Changes in Cytokine and Ghrelin Levels Following Laparoscopic Sleeve Gastrectomy in Obese Individuals

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Abstract

Obesity is a complex disease considered as a global epidemic which has been associated with elevated levels of inflammatory cytokines and Ghrelin. Of all the bariatric procedures, laparoscopic sleeve gastrectomy is the most widely used procedure across the globe. This study sought to assess the efficiency of laparoscopic sleeve gastrectomy surgery by measuring long-term changes in inflammatory cytokines and ghrelin levels, in visceral and subcutaneous adipose tissue, at protein and gene expression level. The results showed that protein and gene expressions of inflammatory cytokines in visceral and subcutaneous adipose tissues fall significantly 5 years postoperatively ($p < 0.05$). At gene expression level, Ghrelin concentration may decrease by 37% and 31% in VAT and SAT tissues, respectively. However, the protein expression of Ghrelin in VAT tissue may increase by approximately 44%, 5 years after LSG surgery ($p > 0.05$). To the best of our knowledge, this is the first study to demonstrate the efficiency of the laparoscopic sleeve gastrectomy surgery by investigating the concentration of inflammatory cytokines and ghrelin, 5 years after surgery.

Introduction

Obesity is a chronic disease with increasing prevalence in adults, adolescents and children. At present, obesity is increasing at a rapid rate since it affects 604 million adults across the globe [1,2]. According to recent epidemiologic data, over 30% of adults reported themselves as obese with a Mean Body Mass Index (BMI) greater than 30 kg/m² in the United States [3]. Further, the obesity rate has increased by approximately 27.2% in low- to middle-income countries [2]. Obesity contributes significantly to the associated comorbidities, such as cancer, cardiovascular diseases, and diabetes mellitus, making this modern epidemic one of paramount importance [4,5].

In general, management of obesity can be mainly achieved *via* two approaches, namely the conservative approach (i.e. diet and exercise) and the surgical approach (i.e. bariatric surgery). Bariatric surgery has been considered an effective treatment option for obese people with a variety of procedures available to date [6]. Surgical options include Roux-en-Y gastric bypass, adjustable gastric banding and Laparoscopic Sleeve Gastrectomy (LSG) surgery. LSG remains the most favourable option due to its low cost and efficiency in weight loss [7]. The underlying mechanisms associated with weight loss include reduction of gastric volume and most importantly, its hormonal effect on appetite and energy homeostasis [8,9]. It has been noted that several hormones are major regulators of hunger and appetite, which in turn, are affected by bariatric operations such as LSG.

In this study, Ghrelin (Ghr) and inflammatory cytokine (IL-6, IL-1b, TNFa) level changes have been measured in visceral (VAT) and subcutaneous (SAT) tissue samples at protein and gene expression (mRNA) level following LSG surgery. To the best of our knowledge, this is the first study to assess and demonstrate the efficiency of the LSG technique 5 years post-surgery.

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Materials and Methods

Study subjects and tissue specimens

A multicenter cross-sectional study was conducted in order to evaluate the postoperative physiological changes in obese patients undergoing bariatric surgery. The study was performed at Hippokratation General Hospital, Evgenidion Clinic of Athens and General Hospital of Athens “Georgios Gennimatas” between 2014 and 2020. The protocol obtained approval from the Local Research Ethics Committee. Written informed consent/assent was obtained from all participants.

Thirty-five patients who underwent LSG surgery and subsequently abdominoplasty was included in this study (Group A). The BMI values of the study group were greater than 40 kg/m² or 35 kg/m² with obesity associated comorbidities. Although exercise and improved dietary habits was emphasized before the procedure, all participants were unsuccessful to permanently lose weight with conservative treatment. These aspects were closely observed through the process. Prolonged weight loss, prevention of weight regains and remission of obesity-induced co morbidities was achieved by increasing awareness towards these factors. Patients with uncontrolled psychiatric disease, malignancies or chronic inflammatory conditions, with alcohol or drug abuse, underage, pregnant women and patients who did not discipline with the dietary habits and intake frequency were excluded. The control group (Group A, BMI<30 kg/m²) consisted of 15 participants who underwent elective abdominal surgery.

Samples of SAT and VAT adipose tissue were obtained from the abdominal incision sites. First, elective abdominal surgery was performed to obtain samples of gastric tissue, fundus, fat and rectus abdominis muscle (Group A). Second, the aforementioned samples were obtained during LSG surgery (Group A). Further, these samples were also obtained during abdominoplasty surgery (Group B), 5 years after the initial procedure (i.e. LSG). All samples were washed in saline buffer, frozen in liquid nitrogen and stored at -80°C until analysis.

Human inflammatory Cytokine multiplex ELISA kit (Arigo Biolaboratories, Taipei, Taiwan) and Human Ghr ELISA kit (MyBioSource Inc., San Diego, CA, USA) were utilized to accurately measure cytokines and Ghr, respectively. Samples were tested together with standards diluted with a similar matrix provided with each kit. Optical Density (O.D) vs. cytokine concentration (pg/mg) was produced with the kit. Concentration of cytokines was determined by comparing the O.D. to the standard curve. Total RNA was extracted from the adipose tissue samples using Invitrogen.

cDNA was synthesized using SuperScript™ Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Quantitative RT-PCR amplifications of IL-1b, IL-6, TNFa and Ghr were carried out using PCR detection systems mix kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Specificity analysis was performed with High Resolution Melt curves (HRM).

Statistical analysis

All statistical analyses were performed using MS-Excel (Microsoft, Redmond, WA, United States). Control group (Group A) and study group (Group B) were compared by performing a Student’s t-test. All the tests applied were two-tailed. The summary statistics for variables were reported as mean ± Standard Error of the Mean (SEM). The level of statistical significance was set at 5% level (i.e. p value <0.05).

Results

All patients were screened by measuring weight, height and waist circumference (Table 1). In this study, control group (Group A) was assumed to be normal and it was consisted of 15 patients. A total of 11 males (31.2%) and 24 females (68.5%) were included in group B, with no difference in gender distribution between the two groups (p>0.05). As shown in Table 1, all patients were screened by measuring weight, height and waist circumference. As can be seen in Table 1, weight loss percentage 5 years following LSG surgery was substantially notable and well sustained.

Inflammatory cytokines, such as IL-6, TNFa and IL-1b, Ghr were analyzed at the protein and gene level (mRNA) in adipose tissues. The presented data show that pro-inflammatory cytokine levels associated with obesity subside after surgery. (Figure 1a, 1b) reveals that protein concentration values of TNFa in the VAT and SAT tissues were lower, by approximately 47.7% and 88.8%, respectively, when compared to IL-6 in the study group. In addition, it was observed that protein concentration values of IL-1b in the VAT and SAT tissues were also lower, by approximately 92% and 78.1%, respectively, when compared to IL-6 in the study group.

At the protein level, IL-6 showed a substantial decline in VAT as well as SAT tissues 5 years postoperatively when compared to the pre-op (LSG surgery), especially in VAT tissues, IL-6 is lower by 60% while in certain cases IL-6 may decrease by up to 72% (Figure 2) (p<0.05). Furthermore, Ghr at protein level, 5 years after LSG, showed a significant increase by 43.5% in VAT tissue (Figure 3). It was observed that Ghr in VAT tissue may increase by up to 400% in certain cases. TNFa and IL-1b also decreased in VAT and SAT tissues after LSG surgery, but not as significantly as IL-6 (Table 2). Notably, the values of Ghr, 5 years postoperatively in VAT and SAT tissues fell

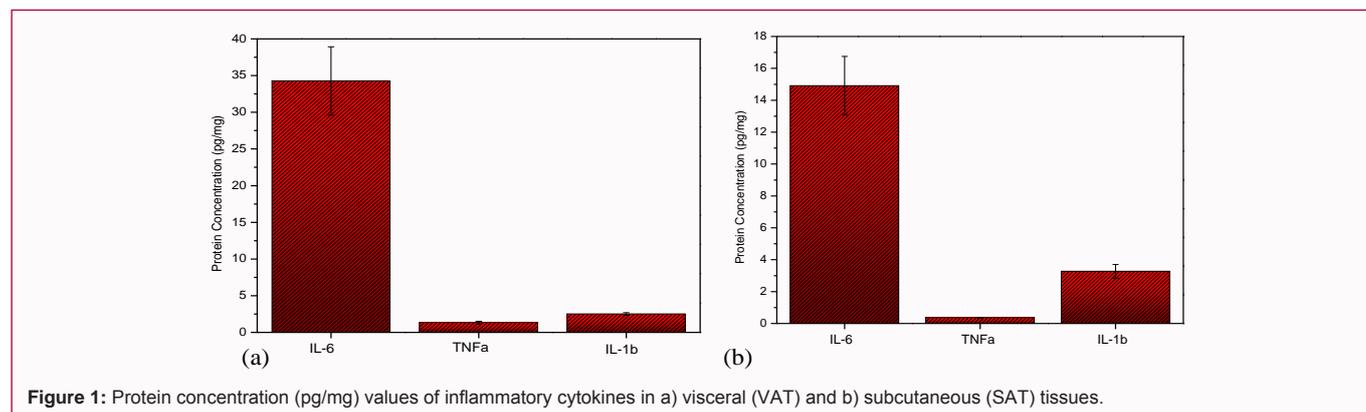


Figure 1: Protein concentration (pg/mg) values of inflammatory cytokines in a) visceral (VAT) and b) subcutaneous (SAT) tissues.

Table 1: Demographics and weight loss percentage 5 years following Laparoscopic Sleeve Gastrectomy (LSG).

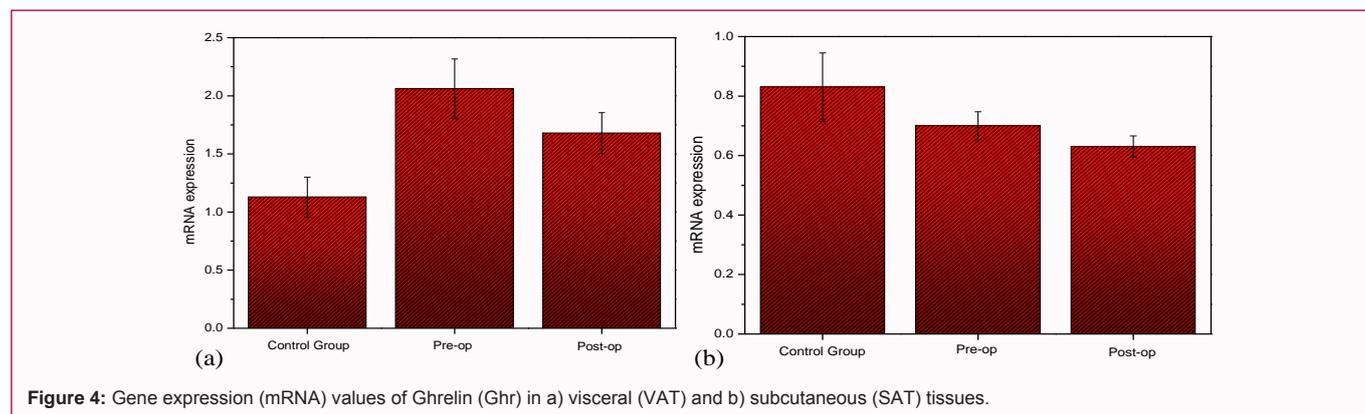
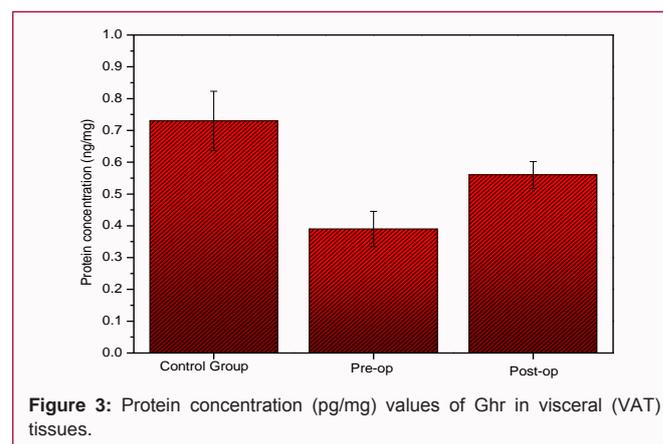
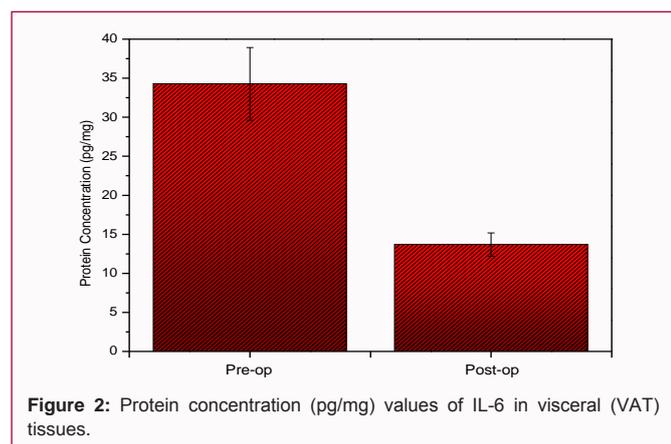
Patients	Number of Patients	Age	BMI (kg/m ²)	Waist circumference (cm)	Weight loss percentage (%)
Male	11	36.82 ± 2.04	41-49.2	130.1-150.8	41.23 ± 2.7
Female	24	41.63 ± 0.99	38.2-48.9	112.5-137	38.17 ± 3.1
Control Group	15	44.27 ± 1.11	20.7-27.2	74.5-92.9	

Table 2: Protein expression levels in control and study group.

Tissue Sample	Visceral			Subcutaneous		
	Control Group	Pre-op	Post-op	Control Group	Pre-op	Post-op
IL-6	3.89 ± 0.594	34.27 ± 4.658	13.68 ± 1.502	16.94 ± 2.406	14.91 ± 1.840	11.34 ± 1.758
TNFa	0.41 ± 0.095	1.31 ± 0.199	1.20 ± 0.182	0.25 ± 0.049	0.36 ± 0.020	0.33 ± 0.022
IL-1b	1.34 ± 0.105	2.51 ± 0.167	2.35 ± 0.158	1.12 ± 0.143	3.27 ± 0.429	2.98 ± 0.386
GHR	0.73 ± 0.093	0.39 ± 0.055	0.56 ± 0.042	0.43 ± 0.057	0.49 ± 0.031	0.54 ± 0.035

Table 3: Gene expression (mRNA) levels in control and study group.

Tissue Sample	Visceral			Subcutaneous		
	Control Group	Pre-op	Post-op	Control Group	Pre-op	Post-op
IL-6	4.31 ± 1.188	8.02 ± 0.895	6.26 ± 0.652	3.29 ± 0.666	29.97 ± 3.426	22.71 ± 3.182
TNFa	3.61 ± 0.543	7.19 ± 0.813	6.89 ± 0.722	0.54 ± 0.044	17.94 ± 3.540	14.42 ± 2.934
IL-1b	0.75 ± 0.052	3.60 ± 0.643	2.88 ± 0.492	0.84 ± 0.116	14.24 ± 1.581	11.47 ± 1.378
GHR	1.13 ± 0.171	2.03 ± 0.258	1.68 ± 0.175	0.83 ± 0.115	0.7 ± 0.047	0.63 ± 0.036



within the range of the values of the control group. In certain cases, the values of IL-6 in SAT tissue and Ghr in VAT tissue were even lower when compared to the values of the control group ($p > 0.05$).

At mRNA level, the inflammatory cytokines (i.e. IL-6, TNF α and IL-1b) showed a decline 5 years postoperatively in both VAT and SAT tissues (Table 3) ($p < 0.05$). A large proportion of obese patients

expressed low levels of transcripts for IL-6, IL-1b, TNF α markers that have been associated with chronic inflammation related to obesity. Ghr expression also expressed low transcript levels (Figure 4a, 4b). In particular, significant decrease of the inflammatory cytokines was observed mainly in SAT tissue. For instance, IL-6, TNF α and IL-1b in SAT tissue, may decrease by approximately 24%, 20% and 19%, respectively. In contrast to Ghr at protein level which showed

an increase in SAT and VAT tissues, Ghr at mRNA level showed a decrease in both tissues. The decrease of Ghr after LSG may be up to 37% and 31% in VAT and SAT tissues, respectively. It should be noted that Ghr values 5 years postoperatively in SAT tissue, fell within the range of the values of the control group (Table 3) ($p>0.05$).

Discussion

LSG surgery has been successfully applied in long-term weight loss and sustainability [10-13]. LSG promotes weight loss through restriction of gastric volume (mechanical) but mainly, through hormonal alterations (endocrine) to the advantages of weight loss [8,9]. Tissue sample values for both gene expression (mRNA) and protein levels have been analyzed, in order to assess the efficiency of the technique along with the sustained cytokine and Ghr changes, 5 years after LSG surgery. In this study, the protein concentration levels of pro-inflammatory cytokines subside postoperatively, while Ghr showed a significant increase in adipose tissues. At mRNA level, proinflammatory cytokines (IL-6, IL-1b, TNFa) as well as Ghr showed a decrease in both VAT and SAT tissues. The results of cytokines are in agreement with similar findings by Rakotoarivelo [14].

Proinflammatory cytokines play a significant role in the development of obesity and the associated disorders, namely type 2 diabetes, dysmetabolic syndrome X and hypertension. These cytokines regulate energy balance and carbohydrate metabolism [15,16]. They are expressed in VAT and SAT adipocytes [17]. IL-6 is responsible for immune response activation and is related to the synthesis of acute phase proteins in liver. IL-1b is a cytokine produced within cells of the innate immune system, such as monocytes and macrophages and it is responsible for host-defence responses to infection and injury [18]. TNFa, another proinflammatory cytokine, is mainly secreted by macrophages and lymphocytes in response to cell damage [19]. The functions of TNFa include cytotoxic and cytostatic effects against cancer cells [20]. Ghr is produced from the stomach fundus and proximal intestine [21]. Ghr levels increase prior to meals and are suppressed postprandially [22]. Obese individuals have lower fasting levels and reduced postprandial Ghr suppression compared to normal weight individuals [23].

The relationship between inflammation and obesity is supported by the post-bariatric surgery decrease of those markers, which subsequently results in weight loss and mainly, improvement in metabolic function [15,24,25]. Obesity-associated inflammation is complex and mediated from a variety of cytokines within and outside the adipose tissues. There are several reports providing evidence for the presence of a low-grade systemic inflammation in adipose tissue of obese individuals with an increase in the values of different adipokines [26-28]. Numerous studies [23-25] assessing the effects of various bariatric surgery techniques with correlation between levels of cytokines and Ghr have been conducted, in order to elucidate the mechanism that leads to sustained weight loss. According to previous work [23,29-31], the concentration of Ghr is known to decrease after LSG procedures. In contrast to previous studies, simultaneous analysis of IL-6, IL-1b, TNFa and Ghr in VAT and SAT tissues was performed in obese patients who underwent LSG surgery. Findings suggest that 5 years postoperatively, inflammatory cytokines subside at protein level and their expression is downregulated. In this study, data come in agreement with past findings of Rakotoarivelo et al. [14] who has shown reduced protein levels and inflammatory gene expression of IL-6, IL-1b after bariatric surgery. Ghr showed an increase of up to 450% at protein level following surgery, while Ghr gene expression

was downregulated [32,33]. Even though gene expression levels of Ghr were similar with previous studies [31], protein concentration of Ghr levels showed significant increase 5 years following LSG surgery. The reason for this increase in Ghr expression is probably due to altered expression of Ghr-regulating molecules and/or from glucose levels along with improved dietary habits [34].

Findings suggest that chronic inflammation associated with obesity subsides 5 years following sleeve gastrectomy surgery and that can be seen by the decrease in gene expression and protein levels of classical pro-inflammatory cytokines. Furthermore, Ghr protein concentration levels in adipose tissues (i.e. VAT, SAT) fell within the values of control group. Ghr postoperative alteration levels seem to play a key role to weight reduction and maintenance following sleeve gastrectomy.

Conclusion

It has been shown that LSG surgery is an effective weight loss surgery in obese individuals. At protein and mRNA expression levels, cytokines fall significantly in both VAT and SAT tissues 5 years postoperatively. For instance, the protein expression of IL-6 in VAT tissues may decrease by up to 72% ($p<0.05$).

In contrast to previous studies, the protein expression of Ghr in VAT tissue may increase by 43.5%, 5 years after LSG surgery. The reason for the increase is probably due to altered expression of Ghr-regulating molecules and/or from glucose levels along with improved dietary habits. It has also been found that the protein concentration levels of Ghr in adipose tissues fell within the range of the control group. In certain cases, the concentration of Ghr in VAT tissue was lower when compared to the values of the control group ($p>0.05$).

At mRNA level, Ghr showed a decrease by 37% and 31% in VAT and SAT tissues, respectively. In particular, mRNA expression of Ghr in SAT tissue, fell within the range of the values of the control group ($p>0.05$). However, further studies are required to elucidate better the long-term changes in Cytokine and Ghr levels after LSG.

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References

- Di Cesare M, Bentham J, Stevens G. Trends in adult body-mass index in 200 countries from 1975 to 2014: A pooled analysis of 1698 population-based measurement studies with 19.2 million participants. *Lancet*. 2016;387(10026):1377-96.
- The GBD Collaborators. Health effects of overweight and obesity in 195 countries over 25 years. *N Engl J Med*. 2017;377:13-27.
- Elflein J. Obesity prevalence adults by country 2020. Statista.
- National Task Force on the Prevention and Treatment of Obesity. Overweight, obesity, and health risk. *Arch Intern Med*. 2000;160(7):898-904.
- Makris M, Alexandrou A, Papatsoutsos E, Malietzis G, Tsilimigras DI, Guerron AD. Ghrelin and obesity: Identifying gaps and dispelling myths. A reappraisal. *In Vivo*. 2017;31(6):1047-50.
- Ponce J, DeMaria E, Nguyen N, Hutter M, Sudan R, Morton JM. American Society for Metabolic and Bariatric Surgery estimation of bariatric surgery procedures in 2015 and surgeon workforce in the United States. *Surg Obes Relat Dis*. 2016;12:1637-9.
- Buchwald H, Oien D. Metabolic/bariatric surgery worldwide. *Obes Surg*.

- 2013;23(4):427-36.
8. Arble DM, Sandoval DA, Seeley RJ. Mechanisms underlying weight loss and metabolic improvements in rodent models of bariatric surgery. *Diabetologia*. 2014;58(2):211-20.
 9. Abdemur A, Slone J, Berho M, Gianos M, Szomstein S, Rosenthal RJ. Morphology, localization, and patterns of ghrelin-producing cells in stomachs of a morbidly obese population. *Surg Laparosc Endosc Percutan Tech*. 2014;24(2):122-6.
 10. Korner J, Bessler M, Cirilo L, Conwell IM, Daud A, Restuccia NL. Effects of Roux-en-Y gastric bypass surgery on fasting and postprandial concentrations of plasma ghrelin, peptide YY, and insulin. *J Clin Endocrinol Metab*. 2005;90(1):359-65.
 11. Roux CW le, Aylwin SJB, Batterham RL, Borg CM, Coyle F, Prasad. Gut Hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters. *Ann Surg*. 2006;243:108.
 12. Rubino F, Gagner M, Gentileschi P, Kini S, Fukuyama S, Feng J, et al. The early effect of the Roux-en-Y gastric bypass on hormones involved in body weight regulation and glucose metabolism. *Ann Surg*. 2004;240(2):236-42.
 13. Roth C, Reinehr T, Scherthaner G, Kopp H-P, Kriwanek S, Scherthaner G. Ghrelin and obestatin levels in severely obese women before and after weight loss after Roux-en-Y gastric bypass surgery. *Obes Surg*. 2009;19(1):29-35.
 14. Rakotoarivelo V, Lacraz G, Mayhue M, Brown C, Rottembourg D, Fradette J. Inflammatory cytokine profiles in visceral and subcutaneous adipose tissues of obese patients undergoing bariatric surgery reveal lack of correlation with obesity or diabetes. *EBioMedicine*. 2018;30:237-47.
 15. Frühbeck G, Gómez-Ambrosi J, Muruzábal FJ, Burrell MA. The adipocyte: A model for integration of endocrine and metabolic signaling in energy metabolism regulation. *Am J Physiol Endocrinol Metab*. 2001;280(6):827-47.
 16. Grunfeld C, Feingold K. The metabolic effects of tumor necrosis factor and other cytokines. *Biotherapy*. 1991;3:143-58.
 17. Fried S, Bunkin D, Greenberg A. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: Depot difference and regulation by glucocorticoid. *J Clin Endocrinol Metab*. 1998;83(3):847-50.
 18. Dinarello CA. Biologic basis for interleukin-1 in disease. *Blood*. 1996;87(6):2095-147.
 19. Beutler B, Cerami A. The biology of cachectin/TNF--a primary mediator of the host response. *Annu Rev Immunol*. 1989;7:625-55.
 20. Vassalli P. The pathophysiology of tumor necrosis factors. *Ann Surg*. 1992;10:411-52.
 21. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*. 1999;402(6762):656-60.
 22. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. Preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*. 2001;50(8):1714-9.
 23. Karamanakos S, Vagenas K, Kalfarentzos F, Alexandrides TK. Weight loss, appetite suppression, and changes in fasting and postprandial ghrelin and peptide-YY levels after Roux-en-Y gastric bypass and sleeve gastrectomy: A prospective, double blind study. *Ann Surg*. 2008;247(3):401-7.
 24. Gumbs AA, Modlin IM, Ballantyne GH. Changes in insulin resistance following bariatric surgery: Role of caloric restriction and weight loss. *Obes Surg*. 2005;15(4):462-73.
 25. Pardina E, Ferrer R, Baena-Fustegueras J. Only C-reactive protein, but not TNF- α or IL6, reflects the improvement in inflammation after bariatric surgery. *Obes Surg*. 2012;22(1):131-9.
 26. Schmidt FM, Weschenfelder J, Sander C, Minkwitz J, Thormann J, Chittka T. Inflammatory cytokines in general and central obesity and modulating effects of physical activity. *PLoS One*. 2015.
 27. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- α : Direct role in obesity-linked insulin resistance. *Science*. 1993;259(5091):87-91.
 28. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante Jr AW. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest*. 2003;112(12):1796-808.
 29. Langer F, Bohdjalian A, Felberbauer F, Fleischmann E, Reza Hoda MA, Ludvik B. Does gastric dilatation limit the success of sleeve gastrectomy as a sole operation for morbid obesity? *Obes Surg*. 2006;16(2):166-71.
 30. Adami G, Cordera R, Marinari G, Lamerini G, Andraghetti G, Scopinaro N. Plasma ghrelin concentratin in the short-term following biliopancreatic diversion. *Obes Surg*. 2003;13(6):889-92.
 31. Meek CL, Lewis HB, Reimann F, Gribble FM, Park AJ. The effect of bariatric surgery on gastrointestinal and pancreatic peptide hormones. *Peptides*. 2016;77:28-37.
 32. Poitou C, Perret C, Mathieu, Truong V, Blum Y, Durand H. Bariatric surgery induces disruption in inflammatory signaling pathways mediated by immune cells in adipose tissue: A RNA-Seq study. *PLoS One*. 2015;10:e0125718.
 33. Seim I, Crisp G, Shah ET, Jeffery PL, Chopin LK. Abundant ghrelin gene expression by monocytes: Putative implications for fat accumulation and obesity. *Obes Med*. 2017;5:1-3.
 34. Akalu Y, Molla MD, Dessie G, Ayelign B. Physiological effect of ghrelin on body systems. *Int J Endocrinol*. 2020;1385138.