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Effects of Left Gastric Artery Ligation Versus Sleeve Gastrectomy on Obesity-Induced Adipose Tissue Macrophage Infiltration and Inflammation in Diet-Induced Obese Rats

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Background: Bariatric procedures such as left gastric artery ligation (LGAL) and sleeve gastrectomy (SG) have emerged as important procedures for treating morbid obesity. In this study, we compared the effects of LGAL vs. SG on obesity-induced adipose tissue macrophage infiltration and inflammation in diet-induced obese rats.





Material/Methods: Sprague-Dawley (SD) rats were fed a high-fat diet (HFD) for 16 weeks to induce obesity. SG, GLAL, or corresponding sham surgeries were performed in anesthetized rats. Inflammatory factor expression in serum and epididymal and retroperitoneal adipose tissues were analyzed 4 weeks after surgery. Macrophage infiltration and phenotype transformation were also assessed with Western blot analysis and immunofluorescence.

Results: Both LGAL and SG strongly attenuated high-fat diet (HFD)-induced fat accumulation in retroperitoneal and epididymal tissues. The expressions of inflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-6, and monocyte chemoattractant protein (MCP)-1 were downregulated after LGAL and after SG by promoting activation of M2 macrophages, despite continued exposure to HFD. Furthermore, both LGAL and SG resulted in increased macrophage infiltration, but did not contribute to phenotype transformation of macrophages to M1.

Conclusions: LGAL and SG both reduced fat accumulation caused by HFD feeding. Therapies designed to ameliorate the inflammatory response by promoting activation of M2 macrophages may be valuable.

MeSH Keywords: **Bariatric Surgery • Diabetes Mellitus, Type 2 • Inflammation • Insulin Resistance • Macrophages • Obesity, Morbid**

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Background

Obesity, which is characterized by chronic and low-grade inflammation accompanied by macrophage infiltration into adipose tissue, eventually contributes to metabolic dysfunction such as insulin resistance (IR) and type 2 diabetes mellitus (T2DM) [1–3]. As a result of immune cells such as macrophages infiltration and accumulation in adipose tissue, the proinflammatory cytokines were markedly produced; therefore, the ensuing obesity-associated inflammation occurs. Macrophages has been hypothesized to be a critical role in the adipose tissue inflammation associated closely with obesity [4]. Increasing evidence demonstrates that activation of macrophages contributes to differentiation into M1- or M2-like macrophages, which exhibit entirely different gene expression profiles. It has been previously demonstrated that M1 macrophages initiate a direct inflammatory response producing proinflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-6, monocyte chemoattractant protein (MCP)-1, and arginase (ARG) 1, and eventually contribute to the ensuing induction of insulin resistance [5,6]. However, M2 macrophages derived from lean adipose tissue are characterized by elevated CD206, arginase-1, Mgl1, and IL-10, and are involved in tissue repair or remodeling [7–9].

Bariatric procedures, such as left gastric artery ligation (LGAL) as well as sleeve gastrectomy (SG), have emerged as important procedures for treating morbid obesity and contribute to improved metabolic function and sustained weight loss [10–12]. SG, as a relatively less invasive bariatric procedure which removes approximately 80% of the stomach, provides restrictive properties and remarkably reduces food intake [12,13]. Many reports have revealed that LGAL can regulate body weight in animal models [14,15]. However, it remains controversial whether LGAL and SG lead to comparable rates of weight loss and metabolic improvement. The effects of LGAL and SG on inflammatory response, insulin signaling, and macrophage infiltration into adipose tissues, as well as mechanisms underlying weight loss and metabolic improvements, still remain unknown. In this study, we compared postoperative outcomes, such as weight loss, inflammatory cytokines, insulin signaling pathway relative protein, adipose macrophage infiltration, and phenotype transformation, in cohorts of diet-induced obese rats that underwent LGAL and SG.

Material and Methods

Animals and ethics statement

The healthy male 8-week-old Sprague-Dawley (SD) rats were obtained from SLAC Laboratory Animal Co. (Shanghai, China) and individually maintained in the animal facility at a controlled

climate of 24–26°C and humidity of about 55% on a 12-h light-dark cycle. The male rats were allocated randomly to a control group or an HFD group fed with control diet rodent chow and high-fat diet rodent chow (60% energy from fat, Research Diets, SLAC Laboratory Animal Co.), respectively. After exposure to a high-fat diet for 16 weeks, rats in the HFD cohort were randomly assigned to the HFD Sham, HFD L, and HFD SG groups, and underwent respective surgery. All rats were sacrificed 4 weeks after surgery. All rats were treated in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The research was approved by the Animal Care and Utilization Committee of Tongji Hospital, and animal procedures strictly followed the guidelines provided by the Tongji Hospital Ethics Committee.

Surgical procedures

The obese rats were fasted the night before surgery. SG, LGAL, or corresponding sham surgeries were performed in rats anesthetized by 4% sevoflurane intraperitoneal injection, as reported previously. Briefly, for the SG procedure, approximately 80% of the stomach was removed along the greater curvature from the antrum to the fundus, as reported previously. The LGAL procedure consisted of making a 3-cm midline excision and ligation of the left gastric artery, as reported previously. The sham operation consisted of a 3-cm midline excision and stomach exposure. After surgery, rats were immediately given subcutaneous injections of sterile normal saline and administered a liquid diet for 3 days.

Serum inflammatory factor assays

The levels of serum TNF- α , IL-6, and MCP-1 were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Sigma-Aldrich, St Louis, MO, USA). All procedures were performed in accordance with the manufacturer's instructions.

Quantitative real-time PCR (qPCR)

Total RNA was extracted from the retroperitoneal and epididymal adipose tissue with TRIzol reagent (Molecular Research Center, Inc., Cincinnati, OH, USA) in compliance with the manufacturer's instruction. The cDNA was prepared from total RNA using Moloney Murine Leukemia Virus reverse transcriptase (Invitrogen, Carlsbad, CA, USA; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Primer sequences used for RT-PCR were for *IL-6*, forward: 5'-GGCGGATCGGATGTTGTGAT-3', reverse: 5'-GGACCCAGACAATCGGTTG-3'; *TNF- α* , forward: 5'-GCGGCCACAGAAAACACTC-3', reverse: 5'-CTCCAATGGTCAAGGCATC-3'; *MCP-1*, forward: 5'-CTGAGTTGACTCCTACTGTGGA-3', reverse: 5'-AGGAGTCAACTCAGCTTCTCTT-3'; *ERK*, forward: 5'-ACTGCTGGGCATAACGCTTTT-3', reverse: 5'-GAGGAGGATCTTGAGAGCCTT-3';

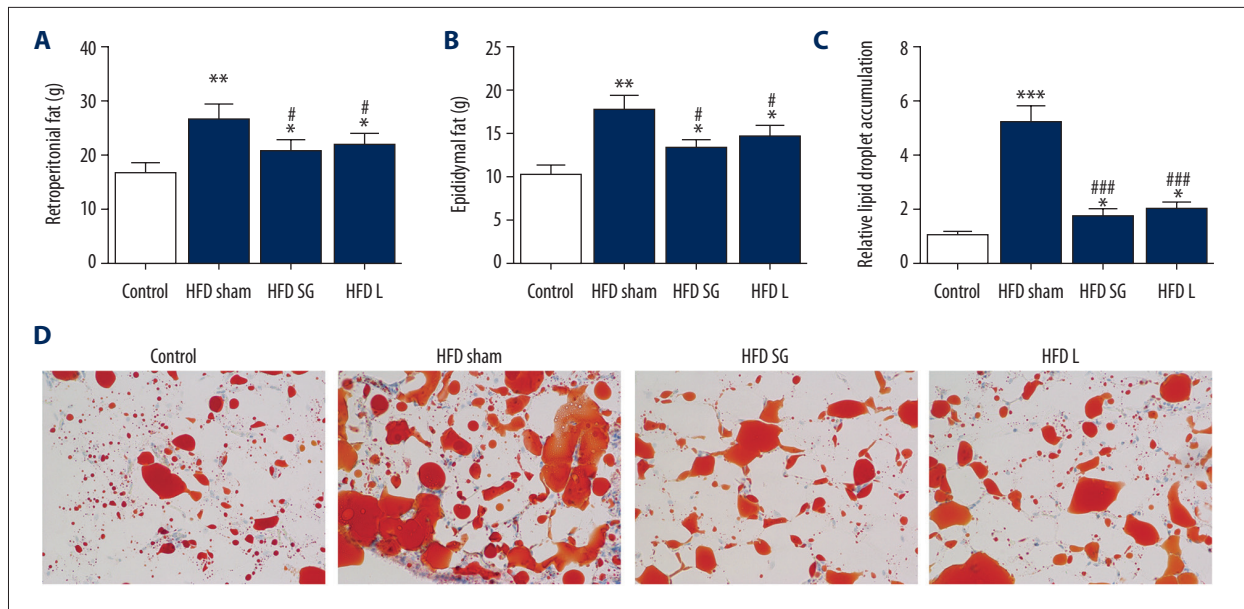


Figure 1. Surgical treatment reversed high-fat diet (HFD)-induced fat accumulation of retroperitoneal and epididymal fat. Rats were fed with an HFD for 18 weeks before treatment with gastric left artery ligation and sleeve gastrectomy for 4 weeks. Whole adipose tissue from the retroperitoneum and epididymis were separated and weighed (**A**, **B**). Weight of whole adipose tissue from the retroperitoneum (**A**) and epididymis (**B**). Data are presented as the mean \pm SD. * $p < 0.05$, ** $p < 0.01$ versus controls. # $p < 0.05$ versus the HFD group. (**C**, **D**) Oil red staining shows lipid droplet deposition in fat sections.

JNK, forward: 5'-CGCCAGTCGTGGAGGAAAA-3',
reverse: 5'-CATCGTTTGGACACATGCCAT-3';
NFκB, forward: 5'-ACACCTCTGCATATAGCGGC-3',
reverse: 5'-GCAGAGTTGTAGCCTCGTGT-3';
beta-Actin, forward: 5'-GCCCTGAGGCTCTTTCCAG-3',
reverse: 5'-TGCCACAGGATTCCATACC-3'.
Semiquantitative PCR was performed with an Applied Biosystems 7300 qPCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) with thermocycling, as previously described. The quantity of mRNA was normalized to GAPDH as an internal standard.

Western blotting

Total proteins were extracted and quantified from the cell or from the retroperitoneal and epididymal adipose tissue. Then, 30–50 μ g protein was isolated from each sample using 10% SDS-PAGE (stacking gel, 50V; separating gel, 100V), and transferred into the nitrocellulose membrane (100V, 75 min). Membranes were blocked and incubated with primary antibodies followed by secondary antibodies. Horseradish peroxidase-labeled secondary antibodies were detected by chemiluminescence and the grayscale of the protein bands was analyzed with Gel-pro Image Analysis Software (Media Cybernetics, Rockville, MD, USA). The primary antibodies against p-IRS (Cell Signaling Technology, Danvers, MA, USA), p-Akt (Cell Signaling Technology, Danvers, MA, USA), and beta-Actin (Cell Signaling Technology, Danvers, MA, USA) were used in this study.

Oil red staining protocol for the adipose tissue

The sections were dried and washed with 50% ethanol, then exposed to Oil red O ethanol dye solution for 8 min. Differentiation was terminated with 50% ethanol and tap water. Hematoxylin was used to counterstain the nucleus, with tap water back to blue and glycerin gelatin seal.

Immunofluorescence assay

Macrophage infiltrations in adipose (green) tissue from retroperitoneal and epididymal adipose tissues were detected by immunofluorescence with F4/80 and DAPI (blue) staining. F4/80 antibody is a marker of macrophages. The 4,6-diamidino-2-phenylindole (DAPI) were stained for cell nuclei. The sections were incubated with anti-F4/80 antibody (1: 100; Invitrogen, Carlsbad, CA, USA) overnight at 4°C and were incubated with a secondary antibody for 30 min, after which images were captured.

Statistical analysis

Data are presented as mean \pm SEM. The 2-tailed *t* test was performed to evaluate between-group differences using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). A *p*-value of less than 0.05 was considered to be significant.

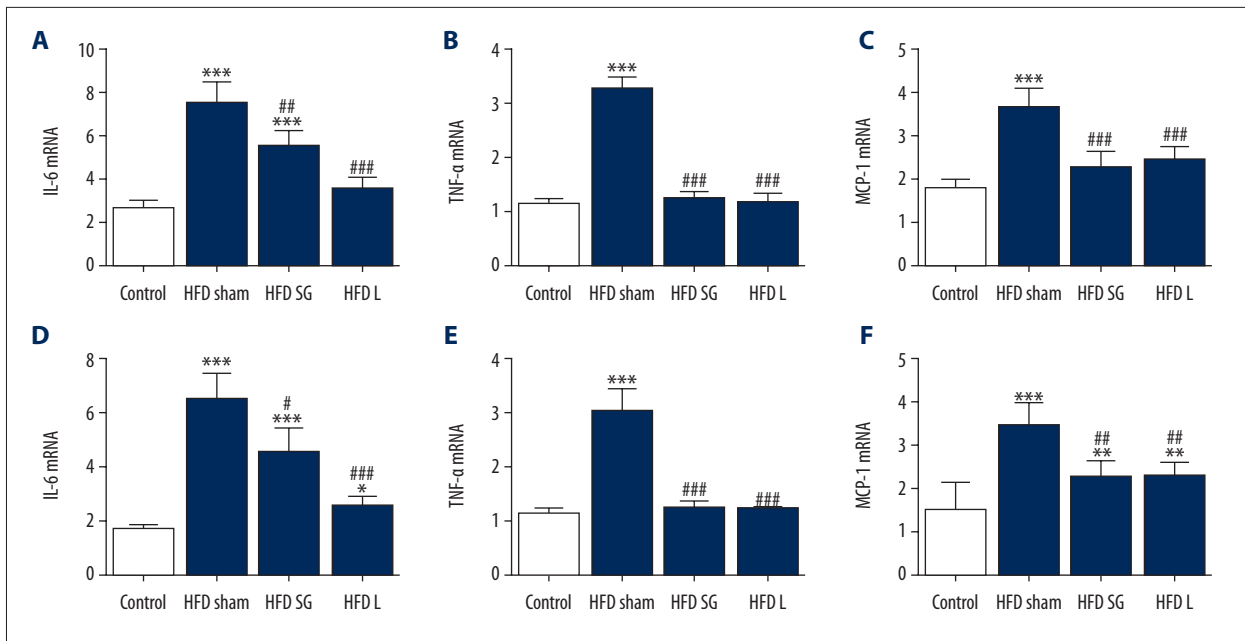


Figure 2. The expression of inflammatory factors in retroperitoneal and epididymal adipose tissue. Rats were fed with an HFD for 18 weeks before gastric left artery ligation and sleeve gastrectomy for 4 weeks. Adipose tissue from the retroperitoneum and epididymis were stripped for Rt-PCR detection. (A–C) Expression level of IL-6 (A), TNF-α (B), and MCP-1 (C) from retroperitoneal fat in different groups. Data are presented as the mean ± SD. *** p<0.001 versus controls. ## p<0.01, ### p<0.001, HFD group. (D, E) The expression level of IL-6 (D), TNF-α (E), and MCP-1 (F) from epididymal fat in different groups. Data are presented as the mean ±SD. * p<0.05, ** p<0.01, *** p<0.001 versus the control. # p<0.05, ## p<0.01, ### p<0.001 versus the HFD group.

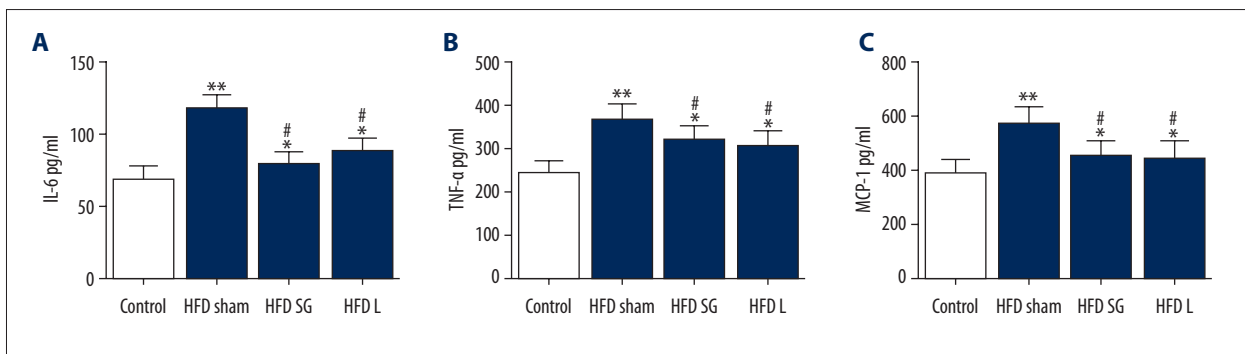


Figure 3. Inflammatory factor. (A–C) Serum inflammatory factor IL-6 (A), TNF-α (B) and MCP-1 in protein level were detection with ELISA. Data are presented as the mean ±SD. * p<0.05, ** p<0.01 versus the control. # p<0.05 versus the HFD group.

Results

LGAL and SG attenuated high-fat diet-induced fat accumulation and inflammatory factor expression in retroperitoneal and epididymal fat

The weight from retroperitoneal and epididymal fat increased significantly in diet-induced obese rats compared with the control group, while LGAL and SG decreased this weight (Figure 1A, 1B). Oil red staining further identified that surgery treatment inhibited HFD-induced lipid droplet deposition in fat sections (Figure 1C, 1D).

Adipose tissue from retroperitoneal and epididymal tissue were collected to analyze and quantify proinflammatory molecules using RT-PCR for IL-6, TNF-α, and MCP-1. Production of proinflammatory mediators significantly was decreased after surgical treatment (Figure 2). In comparing LGAL and SG treatments, a decrease in IL-6 in the LGAL group was most obvious, but TNF-α and MCP-1 expressions in the LGAL and SG treatments were not significantly different from those in the HFD groups. ELISA f showed that HFD significantly induced inflammatory factor IL-6, TNF-α, and MCP-1 expression compared with the normal diet group. LGAL and SG treatment also significantly

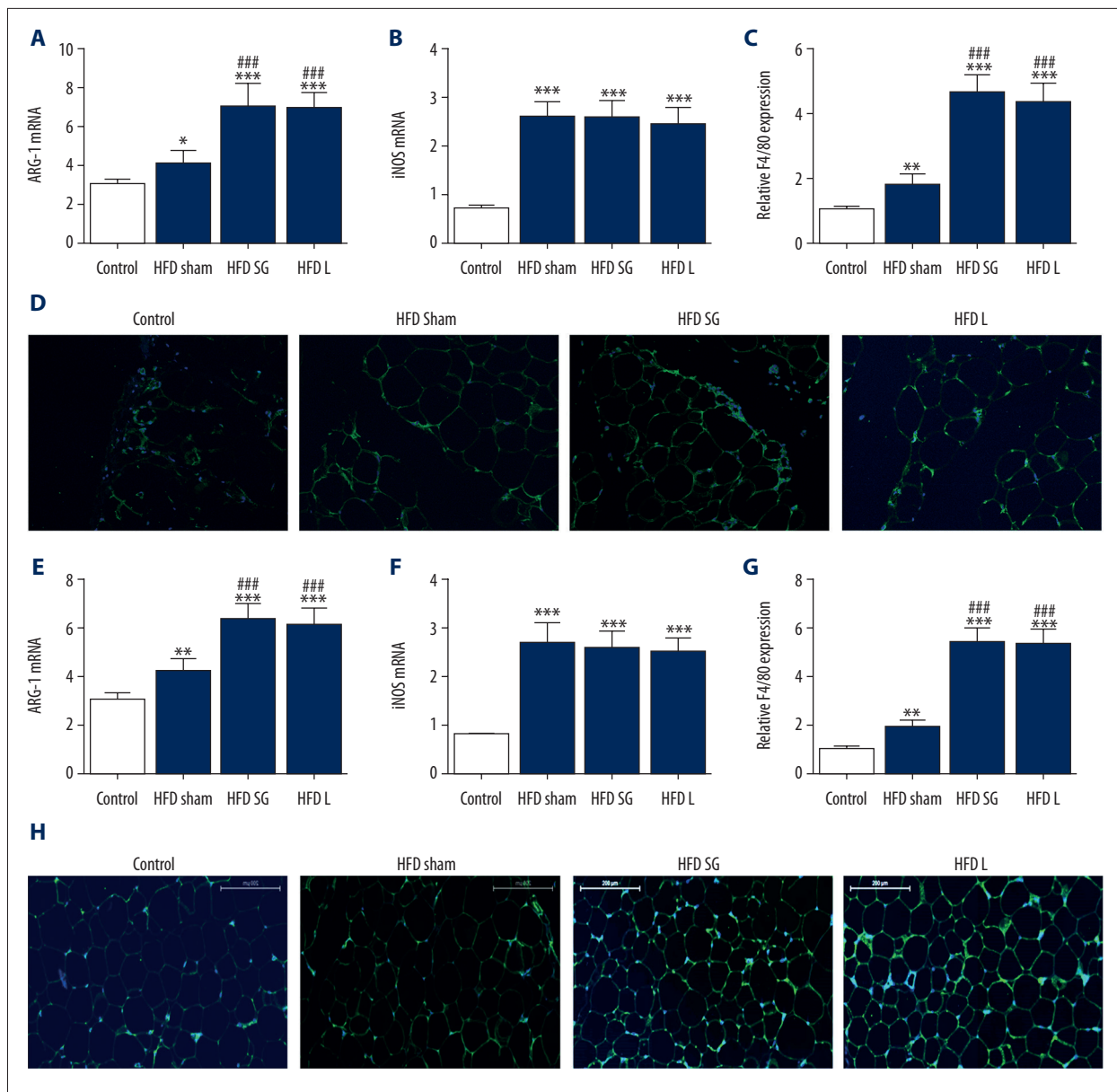


Figure 4. Macrophage infiltration and polarity conversion were detected with RT-PCR and immunofluorescence. (A, B) The expression level of M2 macrophage marker ARG-1 (A) and M1 macrophage marker iNOS (B) in adipose tissue from the retroperitoneum were detected with RT-PCR. Data are presented as the mean \pm SD. * $p < 0.05$, *** $p < 0.001$ versus controls. ### $p < 0.001$ versus the HFD group. (C, D) Macrophage infiltration in adipose (green) tissue from retroperitoneal adipose tissues were detected by immunofluorescence with F4/80 and DAPI (blue) staining. (E, F) The expression level of M2 macrophage marker ARG-1 (E) and M1 macrophage marker iNOS (F) in epididymal adipose tissue were detected with RT-PCR. Data are presented as the mean \pm SD. ** $p < 0.01$, *** $p < 0.001$ versus controls. ### $p < 0.001$ versus HFD group. (G, H) Macrophage infiltration in adipose (green) tissue from epididymal tissue was detected by immunofluorescence staining with F4/80 and DAPI (blue) staining.

suppressed HFD-induced increased inflammatory factor IL-6, TNF- α , and MCP-1 (Figure 3).

LGAL and SG suppressed macrophage infiltration and promoted macrophage M2 polarization

An increasing body of evidence shows that macrophage polarization is involved in the inflammatory response [15,16]. M2 macrophages have protection on adjacent cells by detaching cell debris and releasing trophic factors for tissue repair. However,

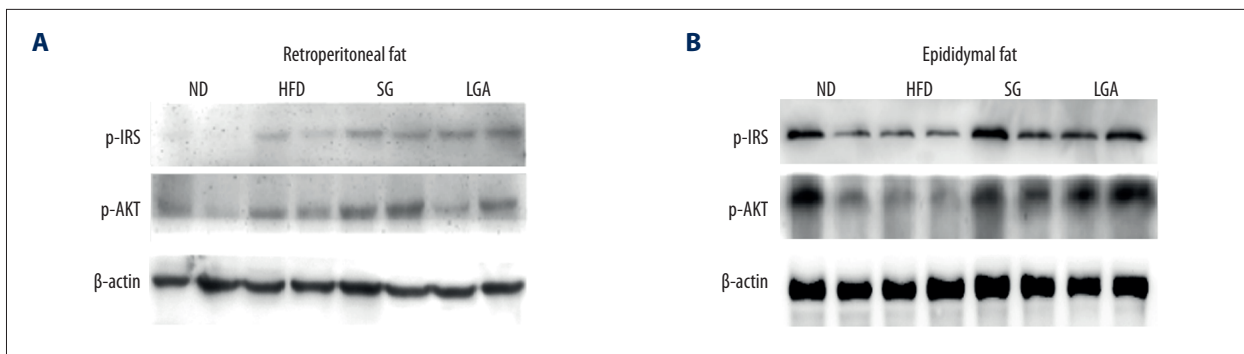


Figure 5. The effect of gastric left artery ligation and sleeve gastrectomy on p-IRS-1 and p-Akt protein expression. **(A)** Western blot detection shows the expression of p-IRS-1 and p-Akt in adipose tissue from the retroperitoneal tissue. **(B)** Western blot detection shows the expression of p-IRS-1 and p-Akt in epididymal adipose tissue.

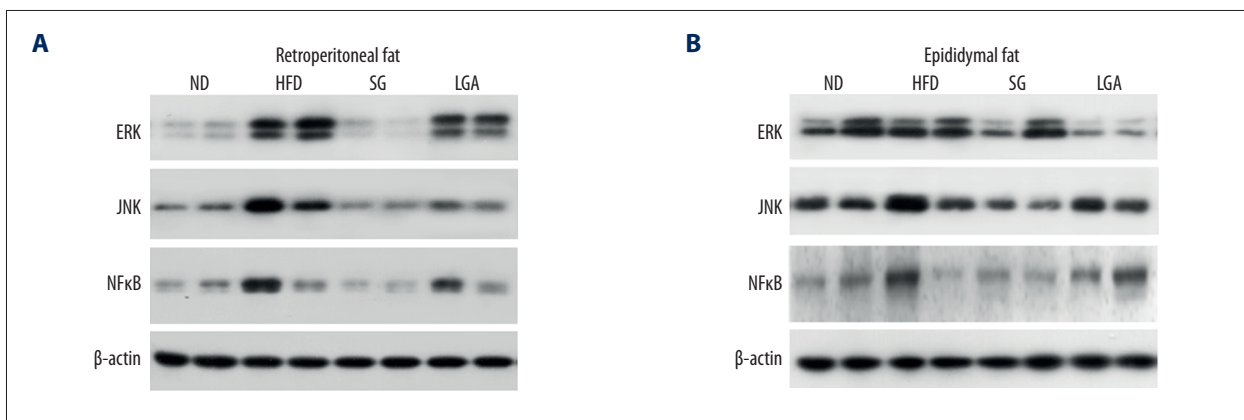


Figure 6. The effect of gastric left artery ligation and sleeve gastrectomy on inflammation and insulin signaling relative protein expression. **(A)** Western blot shows the expression of ERK, JNK, and NFκB in retroperitoneal adipose tissue. **(B)** Western blot detection shows the expression of ERK, JNK, and NF-κB in epididymal adipose tissue.

M1 macrophages may exacerbate injury. In this study, RT-PCR detection showed that HFD treatment increased expression of both ARG-1 (M2 macrophage marker) and iNOS (M1 macrophage marker) in adipose tissue from the retroperitoneum. LGAL and SG treatments both significantly increased ARG-1 expression in mRNA compared with the HFD group. However, iNOS expression was not altered. Surgical treatment significantly increased and activated M2 macrophages (Figure 4A, 4B). An immunofluorescence assay with F4/80 staining showed that surgical treatment promotes HFD-induced F4/80 expression. LGAL and SG can increase macrophage infiltration in adipose tissue from the retroperitoneum (Figure 4C, 4D). The results show that both LGAL and SG significantly increased ARG-1 expression in mRNA compared with the HFD group. The iNOS expression was not altered in adipose tissue from the epididymis (Figure 4E, 4F). Macrophage infiltration in the epididymal adipose tissue was also increased after surgery, as shown by immunofluorescence assay for F4/80 staining (Figure 4G, 4H). Taken together, the results suggest that both surgical treatments increase macrophage infiltration and promote activation of M2 macrophages.

LGAL and SG increased p-IRS and p-Akt concentrations

To examine the effect of surgery on molecules of the insulin signaling pathway, retroperitoneal and epididymal adipose tissue were collected for Western blot analysis. Both LGAL and SG increased the phosphorylation of IRS and Akt after HFD administration. These findings indicate that both LGAL and SG enhance insulin signaling via insulin receptor signaling to PI3K/AKT (Figure 5).

It is well known that insulin can reinforce peripheral glucose uptake, especially in adipose tissue, and restrain hepatic glucose production. Following insulin binding to the receptors of insulin-responsive cells, the phosphorylation of tyrosine residues on insulin receptor substrates (IRSs) is triggered by tyrosine kinase. The phosphorylated IRS 1 (p-IRS 1) is involved in docking proteins, thereby activating signaling cascades, followed by Akt phosphorylation. The glucose transporter type 4 (GLUT4) is activated by Akt phosphorylation, with ensuing glycogen synthesis [17]. The major rate-limiting step in glucose uptake is GLUT-mediated glucose transport [18,19]. GLUT4,

which is expressed primarily in muscle and adipose tissues, plays a pivotal role in glucose homeostasis. These results show that LGAL and SG both improve insulin sensitivity. It has also been shown that phosphorylation of IRS and Akt has an anti-inflammatory effect [20].

LGAL and SG decreased ERK, JNK, and NF- κ B signaling-mediated inflammatory signal activation

Western blot analysis found that HFD promoted NF- κ B, JNK, and ERK phosphorylation in retroperitoneal and epididymal adipose tissues compared with the normal diet group. LGAL and SG significantly decreased phosphorylation of NF- κ B, JNK, and ERK (Figure 6). Increasing evidence shows that JNK and ERK are involved in inflammation. The activation of JNK and ERK signaling plays an important role in modulation of inflammatory responses and regulation of macrophage phenotype [20]. Nuclear factor kappa-light-chain enhancer of activated B cells (NF- κ B) is a crucial transcription factor that is rapidly activated by various stimuli, increasing expression of proinflammatory cytokines (e.g., MCP-1 and TNF- α) and inducible nitric oxide synthase (iNOS), and it participates in inflammation [21]. Macrophages participate in obesity-induced fat tissue inflammation. Various extracellular signals stimulate intracellular pathways and alter the secretory profile of macrophages, eventually contributing to attenuation or augmentation of inflammation [22]. This suggests that LGAL and SG can promote the activation of M2 macrophages and alter the secretory profile by regulating inflammatory signaling.

Discussion

Obesity is linked to low-grade inflammation and insulin resistance, and is involved in glucose and lipid metabolism [22]. Recent findings suggest that bariatric surgery is the most effective obesity treatment available in both magnitude and durability of its effects [23,24]. Some evidence has shown that LGAL and SG can decrease obesity [25,26]. Therefore, the present study aimed to identify the effect of LGAL and SG on expression of inflammatory factors in adipose tissue. We ought to elucidate the role of inflammation and insulin resistance relative to molecular and cellular mechanisms.

We found that HFD promotes lipid accumulation in both retroperitoneal and epididymal adipose tissues. LGAL and SG reverse this phenomenon. This suggests that LGAL and SG have the same weight loss effect. SG had a minimal risk of malabsorption due to the unchanged route of nutrient flow after sleeve

gastrectomy, but compared with SG, LGAL has more advantages with regards to reduction of pain and bleeding. These results are consistent with previous reports [27].

A recent study suggests that LGAL causes the same reduction in ghrelin hormone levels as SG at 4 weeks after surgery in a rat model, and the mechanism of weight loss in SG is most likely due to restriction and to the effects of the procedure, rather than due to neurohormonal changes [26]. Our data show that LGAL and SG reversed HFD-induced inflammatory factors IL-6, TNF- α , and MCP-1 expression in serum and adipose tissues. The reduction of inflammatory factors in our study suggests a potential reduction in future risk for cardiovascular disease, since inflammation has been reported to be an independent contributor to the atherosclerotic process and predicts adverse cardiovascular events in adults [27,28]. To further assess whether macrophages are involved in inflammatory regulation, we performed immunofluorescence detection, showing that LGAL and SG promote HFD-induced macrophage infiltration in adipose tissue. Western blot analysis showed that surgical treatment promoted the activation and transformation of macrophages to the M2 phenotype. It is known that macrophage phenotypes are associated with adipose tissue inflammation, metabolic disorders, and insulin resistance in obesity [29,30]. M1 macrophages are proinflammatory and cause adipose tissue inflammation and insulin resistance, while M2 macrophages have anti-inflammatory effects and thereby alleviate obesity-induced insulin resistance [31].

Further research also found that surgical treatment enhanced insulin signaling via insulin receptor signaling to PI3K/AKT, which was suppressed by HFD induction. Surgical treatment significantly decreased phosphorylation of NF- κ B, JNK, and ERK, which resulted in decreased inflammatory gene expression [32].

Conclusions

Our data indicate that both LGAL and SG can inhibit HFD-induced chronic inflammation in adipose tissue by promoting M2 macrophages and suppressing the anti-inflammatory signal pathway. Our results also indicate that bariatric surgery can increase insulin sensitivity. In light of the safety and injury severity, LGAL has a greater clinical application potential in weight loss.

Conflicts of interest

None.

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