RESEARCH ARTICLE

Sleeve Gastrectomy Decrease Body Weight, Fasting Blood Glucose, and Gene Expression of TNF-α and IL-1 in The Abdominal Aorta of Rats with Obesity and Diabetes Mellitus

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Abstract

BACKGROUND: Sleeve gastrectomy (SG) is one option to significantly reduce body weight while also protect the cardiovascular system by controlling hyperglycemia and inflammatory markers. Secretion of tumor necrosis factor (TNF)-α and interleukin (IL)-1 could induce obesity- and diabetes mellitus (DM)-related inflammation, however its association with SG procedure has not been elucidated well. Therefore, TNF-α and IL-1 gene expression on the abdominal aorta of obese and DM rats that went through SG procedure were evaluated.

METHODS: Fifteen rats were divided into 3 groups: lean-non-DM rats model (C1 group), obese-DM rats model (C2 group), and obese-DM rats model underwent SG (T group). Before and 10 days after the SG procedure, rats' body weight and fasting blood glucose (FBG) were measured. Ten days after the procedure, TNF-α and IL-1 gene expression were also evaluated by PCR.

RESULTS: In the end of study, mean body weight and FBG levels in C1 group (231.80±4.32 gram; 68.60±2.07 mg/dL) and T group (232.00±5.33 gram; 114.40±3.20 mg/dL) were significantly lower than in C2 group (264.60± 3.28 gram; 271.00±6.89 mg/dL). TNF-α and IL-1 gene expressions were also found to be significantly lower in the C1 group (1.01±0.01 rfu; 1.01±0.01 rfu) and T group (1.97±0.57 rfu; 1.21±0.78 rfu) compared to the C2 group (224.12±47.59 rfu; 1.85±0.73 rfu).

CONCLUSION: SG could decrease body weight and FBG, as well as TNF-α and IL-1 gene expression in the abdominal aorta of rats with obesity and DM, hence SG could be a useful method in reducing body weight and controlling hyperglycemia and inflammatory markers.

KEYWORDS: sleeve gastrectomy, TNF-α, interleukin-1, obesity, diabetes mellitus

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Introduction

Changes in human environment, behavior, and lifestyle are contributing in the increases of diabetes mellitus (DM) and obesity prevalence.(1) Insulin action and secretion from pancreatic β-cells are compromised in DM and could induce inflammation through the secretion of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6 and IL-8.(2-4) It has also been demonstrated that obesity causes inflammation due to the increase in adipose tissue, which releases proinflammatory cytokines and are thought to be the primary causes of inflammation in chronic vascular disease, a feature of atherosclerosis.(5)

Sleeve gastrectomy (SG) is found to be a new, safe, and efficient method for the treatment of obesity with higher survival rates among patients. As the gold standard in restrictive bariatric surgery with better result than other procedures, SG has captured remarkable surgical interest due to its technique that removes the need for gastrointestinal anastomosis or intestinal bypass.(6,7) American Society for Metabolic and Bariatric Surgery (ASMBS) itself has approved SG as the primary bariatric procedure, demonstrating its technical viability, preservation of normal anatomical shape, decreased risk of nutritional deficiencies, and efficient primary weight loss.(8) The patient outcome from this treatment were observed to be ideal, reported a mean excess weight loss of 76% at 5 years and 67% at 8 years after SG.(9)

SG also plays a significant role in protecting patients from cardiovascular disease by regulating hyperglycemia, blood pressure, and inflammatory markers.(10) Regarding glucose metabolism and variability, it has been described in a previous research that both of these aspects could be improved significantly as fast as 1 week after surgery.(11) A significant decrease of TNF-α pre-surgery to post-surgery and a significant decrease of TNF-α in type 2 DM patients 6 months later were also reported.(12,13) Not only TNF-α, significantly lower IL-1 was also found in rats with induced obesity and type 2 DM from.(14) Previous studies also suggests that SG procedure had the same effect on TNF-α and IL-1 as other common bariatric procedures and when combined with other techniques.(15,16) Hence, it could be assumed that SG could produce better medical outcomes for the patient, however the association with TNF-α and IL-1 has not been elucidated well enough. Therefore, this study was aimed to determine the weight loss, reduced fasting blood glucose (FBG), as well as the decrease of TNF-α and IL-1 gene expression in the abdominal aorta of obese and DM rats undergoing SG procedures.

Methods

Animal Models

Fifteen male Sprague Dawley rats, aged 4-6 weeks and weighing 150-200 grams, were used in this study. The rats were housed at room temperature with access to drinking water and fed a high-fat diet (HFD) for 8 weeks (56 days) before the SG surgery.(17) Ethical approval for the study protocol was obtained from The Health Research Ethics Commission, Faculty of Medicine, Diponegoro University (No. 66/EC/H/FK-UNDIP/VII/2022).

Obesity and DM Induction

All rats were randomly divided into 3 groups: C1 group (control 1: lean-non-DM rats), C2 group (control 2: obese and DM rats), and T group (treatment: obese and DM rats underwent SG). To promote obesity, rats in the C2 and T groups were fed a high fat diet (HFD) that included 8% maize oil, 44% sweetened condensed milk, and 48% purine rat food for 8 weeks. To create an animal model of DM, rats in the C2 and T groups were given intraperitoneal injections of streptozotocin (STZ) at a dose of 40 mg/kg BW dissolved in citrate buffer pH 4.5 and 120 mL/kg BW nicotinamide dissolved in phosphate-buffered saline for the last 5 consecutive days in the 8th week.(18)

To confirm whether obesity had been successfully obese-induced or not, Lee index was calculated as follows: Lee Index = $\sqrt[3]{}$ body weight (g) / nose-to-anus length (cm) × 1000. And to confirm whether the rats has been successfully DM-induced, FBG analysis were performed.

SG Procedure

After five consecutive days of STZ induction, rats from the T group were fasted for 4-6 hours before undergoing general anesthesia with 2 mg/kg BW ketamine and a midline supra-umbilical incision to open the abdominal cavity was performed. The stomach was injected with saline solution to dilate the volume and cut above the mayor curvature line until only half of its volume remained. The line was clamped before excision to minimize the bleeding. The residual stomach was then closed and stitched with PGA 5.0 thread running suture.

FBG Analysis

The blood was collected from the rat tail veins 5 days after STZ injection (to confirm the DM-induction) and 10 days after SG procedure. Rats' tail tip was cleaned and amputated, and blood was taken and measured using the DR Bio-sensor glucometer strip (All Medicus, Anyang, Korea).

Analysis of TNF-α and IL-1 Gene Expression

Ten days after SG (day 66th), all rats were terminated using chloroform and dissected from chest to abdomen to obtain the abdominal aorta. The gene expression of TNF-α and IL-1 was measured using the polymerase chain reaction (PCR) procedure according to the manufacturer's instructions.(19,20) Several steps were carried out, starting from RNA extraction through centrifuge; RNA concentration measuring using Quantifluor RNA solution; cDNA synthesis by processing microtubes in a thermal cycler (PCR) which had been filled with 1 μL RNA sample, 1 μL Deoxynucleotide

mix (dTNP), and Oligo (dT)23 primer that would further be added with 2 μL 10X buffer for eAMV-RT, 1 μL Enhanced avian RT, 1 μL RNase inhibitor, and 6 μL Water PCR reagent; and finally, the PCR process itself. The final 20 μL of master mix consisted of 10 μL KAPA SYBR FAST qPCR Master Mix (2X), 0.4 μL Forward Primer, 0.4 μL Reverse Primer, 1 μL cDNA Template and Water PCR reagent. The conditions for the TNF-α and IL-1 PCR reactions carried out were enzyme activation at 95°C for 3 minutes 1 cycle, denaturation at 95°C for 10 seconds in 40 cycles, annealing at 50°C for 30 seconds in 40 cycles, and melt curve at 50°C if needed. The results were obtained and compared with standard, no template control (NTC), and positive control.

Statistical Analysis
SPSS version 26.0 (IBM Corporation, Armonk, NY, USA) was used to analyze the data. Data on the body weight, FBG level, as well as TNF-α and IL-1 gene expression were reported in mean and standard deviation (mean±SD). The Shapiro-Wilk test was used for the normality test and the Levene test was used for the homogeneity test. For normally distributed and homogeneous data, a comparative analysis between groups was performed using the parametric ANOVA test, followed by LSD post hoc test. Meanwhile, for data that are not normally distributed and not homogeneous, a comparative analysis between groups was performed using the Kruskal-Wallis non-parametric test, followed by the Mann-Whitney post hoc test. Comparisons were considered significant if $p<0.05$.(21)

Results

Sample Characteristic
Body weight, FBG levels, TNF-α and IL-1 gen expressions of rats in each groups on the first day of the experiment, after

8 weeks of inducing obesity and diabetes (day 55th), and ten days after SG procedure (day 66th) were presented in Table 1. The body weight measurement in the first day showed that there were no differences between group ($p=0.054$), but after 56 days, there were significant differences between the three groups ($p=0.009$). Measurement of Lee Index at day 56th showed significant difference between C1, C2, and T groups ($p=0.009$), and the follow up test showed significant differences between each group, and that C2 and T groups were successfully induced to be obese. FBG was also measured at day 56th, and significant differences between each group ($p=0.007$) indicated the successful induction of diabetes in rats of C2 and T groups.

SG Procedure Reduce Rats' Body Weight
The results ANOVA test of body weight between the three groups on day 66th showed a significant difference ($p=0.000$) (Figure 1, Table 1). The C2 group had the highest mean body weight, followed by T and C1 groups. The LSD post hoc test showed that the mean body weight of the T group was significantly different from the C2 group which did not undergo the SG procedure ($p=0.000$). This shows that SG can significantly reduce weight in obese and DM rats.

SG Procedure Reduce Rats' FBG Level
The C2 group expressed the highest FBG level compared to the T and C1 groups (Figure 2). ANOVA test showed that there were significant ($p=0.000$) differences in FBG between all groups (Table 1). LSD post hoc test showed that compared to C2 group, the SG procedure (T group) significantly reduced FBG in obese and DM rats ($p=0.000$).

SG Procedure Reduce TNF-α Gene Expression in Rats
The results of TNF-α measurement from each group were shown in the real-time graph as seen on Figure 3A, 3B,

Table 1. Characteristics of rats in each group on day 1st, 56th, and 66th.

Day	Parameter	C1 (n=5)	C2 (n=5)	T (n=5)	*p*-value
1	Body weight (g)	166.60±4.03	172.60±3.05	169.60±3.20	0.054
56	Body weight (g)	221.60±4.39	265.80±3.49	266.60±3.49	0.009[#]
	Lee Index	278.40±5.89	318.40±5.77	319.40±7.53	0.009[#]
	FBG (mg/dL)	68.00±1.58	270.20±6.83	265.40±4.15	0.007[#]
66	Body weight (g)	231.80±4.32	264.60±3.28	232.00±5.33	0.000*
	FBG (mg/dL)	68.60±2.07	271.00±6.89	114.40±3.20	0.000*
	TNF-α (rfu)	1.01±0.01	224.12±47.59	1.97±0.57	0.002[#]
	IL-1 (rfu)	1.01±0.01	1.85±0.73	1.21±0.78	0.025[#]

*$p<0.05$, tested with one-way ANOVA; #$p<0.05$, tested with Kruskal-Wallis Test.
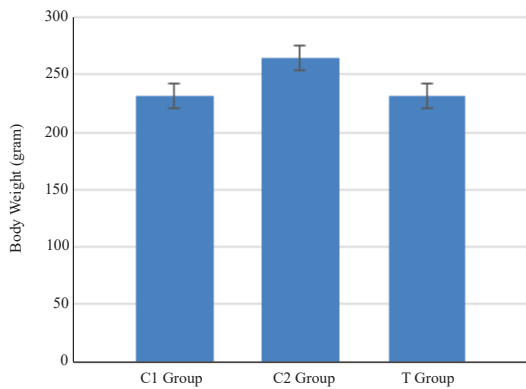
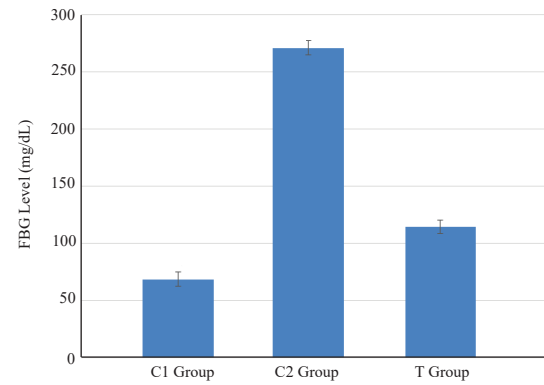**Figure 1. Body weight of rats in each group on day 66th.**

Figure 2. FBG level in each group on day 66th.

and 3C. The C2 group expressed the highest TNF-α gene expression compared to the T and C1 groups (Figure 3D). Similarly, the T group expressed slightly higher TNF-α than the C1 group. The results of the Kruskal Wallis test showed that there were significant differences in TNF-α gene expression in all groups (p=0.002) (Table 1). The follow up Mann Whitney U test showed that SG significantly decreased TNF-α expression in obese and DM rats (p=0.009).

SG Procedure Reduce IL-1 Gene Expression in Rats
The results of IL-1 measurement from each group were shown in the real-time graph as seen on Figure 4A, 4B, and 4C. The C2 group expressed the highest IL-1 gene expression compared to T and C1 groups (Figure 4D). The

T group also expressed higher IL-1 gene expression than the C1 group. Results of the Kruskal Wallis test showed that there were significant differences in IL-1 gene expression in all groups (p=0.025) (Table 1). The follow up Mann Whitney U test showed that there was significant decrease of IL-1 gene expression T group, which show that SG procedure can lower the expression in obese and DM rats (p=0.021).

Discussion

According to previous research, weight loss following bariatric surgery reduced systemic inflammation and boosted metabolism. Three months after having bariatric
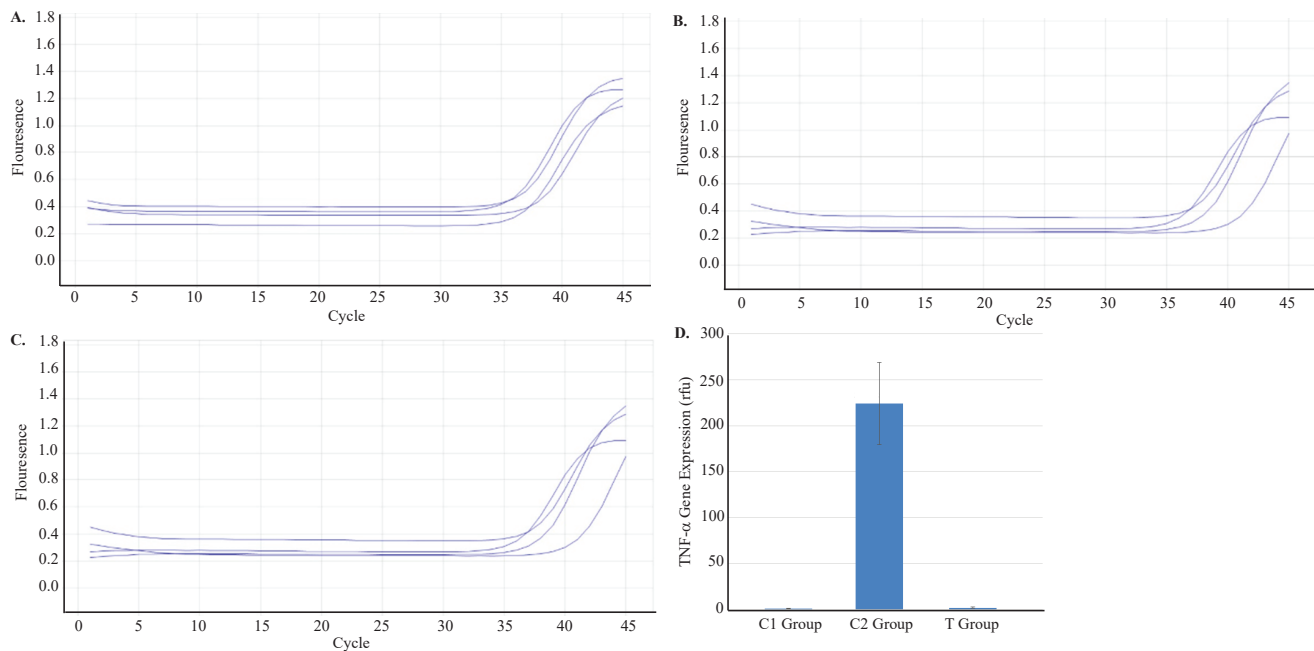

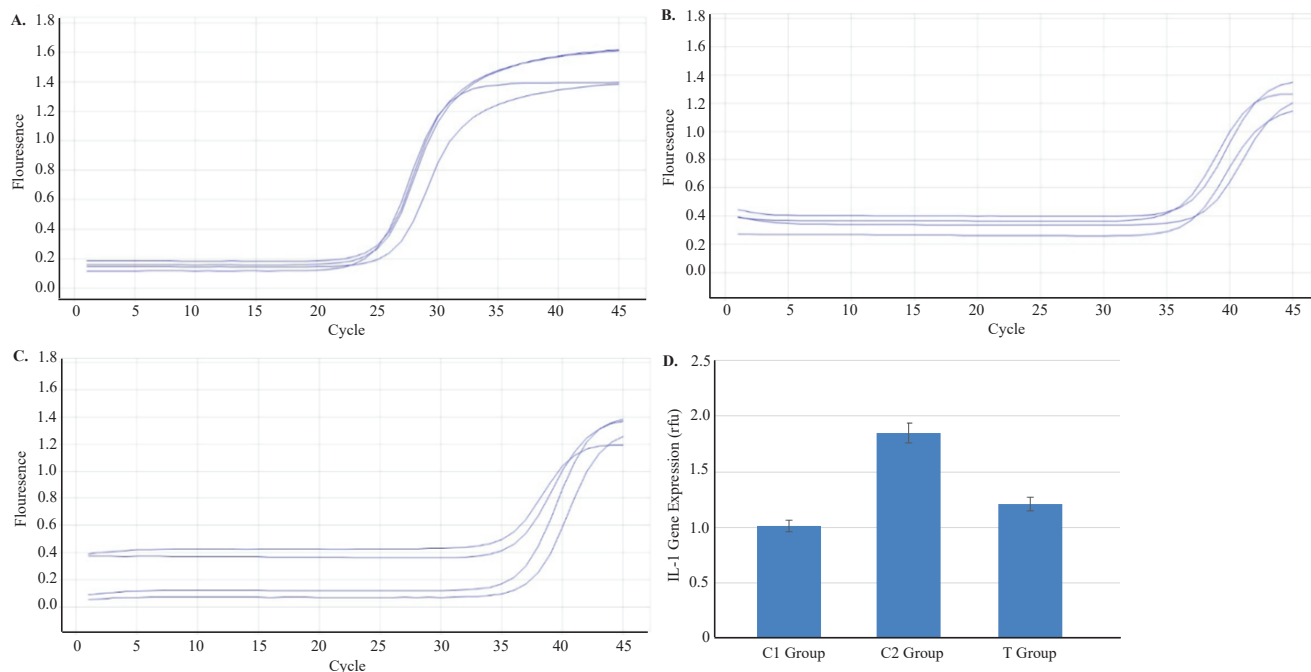**Figure 3. TNF-α gene expression in each group on day 66th.** A: Real time graph of TNF-α gene expressions in C1 group. B: Real time graph of TNF-α gene expressions in C2 group. C: Real time graph of TNF-α gene expressions in T group. D: TNF-α gene expression on day 66th.

Figure 4. IL-1 gene expression in each group on day 66th. A: Real time graph of IL-1 gene expressions in C1 group. B: Real time graph of IL-1 gene expressions in C2 group. C: Real time graph of IL-1 gene expressions in T group. D: IL-1 gene expression on day 66th.

surgery, the HOMA-IR index, glucose, and insulin levels are much lower than they were before the procedure.(22) In this study, there was a significant difference in body weight and FBG levels between the obese and DM rats group, and the obese and DM rats underwent SG, where SG can reduce body weight and FBG levels in the obese and DM rats.

In our study, the obese and DM rats group's TNF-α gene expression levels were higher than those in the lean-non-DM rats group. According to the notion, obesity-related increases in adipose tissue can raise levels of proinflammatory mediators. This study found that obese and DM rats underwent SG significantly lower TNF-α gene expression than the obese and DM rats group. Based on research that was conducted before, the SG procedure has been shown to reduce visceral adipose tissue, repair T lymphocyte and macrophage infiltration, as well as decrease other inflammatory cytokines in arterial adipose tissue and improve endothelial function.(23)

Other study mentioned that there is a correlation between TNF-α and insulin resistance as well as body mass index, which is supported by the fact that type 2 DM patients have much higher TNF-α levels than lean-non-DM individuals. The study also found that obese diabetic patients had much higher levels of TNF-α than did non-obese diabetic patients and lean-non-DM individuals.(24) Therefore, increasing TNF-α gene expression may cause the inflammatory process to worsen. Additionally, this study found a positive correlation between TNF-α gene

expression and the atherosclerosis-causing process of inflammation in blood vessel walls. Not only that, the obese and DM rats group had higher IL-1 gene expression than the lean-non-DM rat group. ILs, a specific subset of cytokines, are expressed in atherosclerotic lesions. The previous study found that many cytokines, including IL-1, IL-12, and IL-18, were consistently proatherogenic and were thought to be the primary causes of atherosclerosis.(25)

SG significantly reduces adipose tissue mass and has been shown to decrease TNF-α by upregulating adiponectin. It was confirmed in this study that there was a decrease of TNF-α in the obese and DM rats who underwent SG compared to the obese and DM rats group. Besides that, reduced IL-1 gene expression were also observed. These results are supported by other research which reported a decrease in IL-1 gene expression in obese patients 6 months after surgery.(26) A similar case where the concentrations of the proinflammatory cytokines TNF-α, IL-6, IL-8, and IL-1, decreased 6 months after surgery was also reported.(22) Other research conclude that 12 months after SG, there was no discernible change in the expression of IL-1 in lean-non-DM individuals and obese patients.(27)

Pro-inflammatory cytokine markers used in this study, which were TNF-α and IL-1 gene expression in the abdominal aorta, as indicators to see the degree or severity of the atherosclerotic process in obesity and DM could be biased due to the role of other cytokines that overlap each other and complexly related. In this study, the

histopathological examinations were also not carried out in different group of rats to see if there were differences in the appearance of the abdominal aorta microscopically. Relevant examinations, such as physical and other laboratory examinations were not conducted as well. Further studies to analyze the histopathological differences would be useful to understand microscopic benefits of SG.

Conclusion

SG could reduce the body weight, fasting blood glucose, and levels of TNF-α and IL-1 gene expression in the abdominal aorta of rats with obesity and diabetes mellitus. SG can aid in the prevention of atherosclerosis risk in people with obesity and diabetes.

Authors Contribution

All authors were involved in planning of the study. AS was involved in processing the experimental data, performing the analysis, drafting the manuscript, and designing the figures. SAP, MAS, AM and YAP aided the interpretation of results and worked on the manuscript. All authors discussed the results and commented on the manuscript.

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