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Selective beneficial cardiometabolic effects of vertical sleeve gastrectomy are predominantly mediated through glucagon-like peptide (GLP-1) in Zucker diabetic fatty rats

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HIGHLIGHTS

- GLP-1 increases post-VSG 30 min after glucose load.
- Post-VSG GLP-1 secretion is associatged with lower cholesterol and triglycerides.
- Bile acids and L-PGDS increase post-VSG and are inhibited in the presence of GLP-1 antagonist.
- Heart rate, blood pressure and myograph profile remain unchanged.

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ABSTRACT

Background: Glucagon-like peptide-1 (GLP-1) level was significantly increased post Vertical Sleeve Gastrectomy (VSG), an effect believed to contribute to its beneficial cardiometabolic effects.
Objective: To validate the beneficial GLP-1 mediated cardiometabolic effects post VSG using GLP-1 antagonist (exendin 9-39) in Zucker diabetic fatty rats.
Methods: Animals were divided into three (n = 5) groups: (i) sham, (ii) VSG, and (iii) VSG received exendin 9–39 (GLP-1 receptor antagonist). The study was performed over 12 weeks and parameters were measured 12 weeks post-surgery.
Results and discussion: As expected, fasting blood glucose and insulin levels were improved post VSG due to enhanced GLP-1 secretion. However, both fasting glucose and insulin levels were impaired in the presence of GLP-1 antagonist. Baseline total cholesterol level pre-surgery was 100±1 mg/dl which remained unchanged in the VSG group but significantly increased to 140±8 mg/dl in the presence of antagonist. Interestingly, post-surgery there was a nearly 70% reduction in triglyceride level in the VSG group compared to sham which was overcome in the presence of antagonist. Myographic studies using aortic rings showed no significant change between groups. Additionally, blood pressure and heart rate also remained unchanged in all groups. Serum bile acid and L-PGDS levels increased post VSG but significantly decreased in the presence of antagonist, suggesting a strong association with GLP-1 and a novel mechanism of action.
Conclusion: Enhanced GLP-1 secretion post VSG imparted beneficial cardiometabolic effects on blood glucose, insulin, total cholesterol, triglyceride, bile acids and L-PGDS levels which were abated in the presence of GLP-1 antagonist.

1. Introduction

Obesity has become a pandemic of the twenty first century for western countries affecting nearly one-third of the US population

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[1]. Along with other metabolic risk factors, obesity substantially increases the likelihood for cardiovascular disease [2]. Traditional obesity management strategies remain ineffective in long term weight management [3]. Bariatric surgery remains the most effective and durable treatment to date [4]. Furthermore, there are significant reductions in mortality from heart disease and *type 2* diabetes mellitus (T2DM) with bariatric surgery [5]. In fact, many have viewed this procedure as "metabolic" surgery, but not as weight loss surgery [6]. Even more striking, is that these beneficial cardiometabolic effects, are speculated as weight-independent [7–9].

Bariatric surgery encompasses various surgical procedures which mainly work through two different mechanisms: **restriction** or **malabsorption**. What has also been observed but not well understood, are the alterations in gastrointestinal hormones seen with the Roux-en-Y gastric bypass (RYGB), a popular weight loss surgery. Even less understood are similar gastrointestinal hormones and metabolic changes observed with the vertical sleeve gastrectomy (VSG), another increasingly common bariatric surgery. Both bariatric surgeries result in variable degrees of metabolic outcomes due to difference in their anatomical alterations. Briefly, RYGB is gastric bypass where a portion of small intestine is bypassed and attached to the stomach which ultimately reduces the intestinal absorption. VSG involves 70–80% of stomach removal which target different mechanisms, possibly alterations in gastric hormones and reduction in food intake [10]. Interestingly, among all gastrointestinal hormones, glucagon-like peptide-1 (GLP-1) has been observed to be most elevated postprandially after bariatric surgery [8,11]. Beyond their metabolic action, GLP-1 receptor agonists have been reported to function as weight loss, antioxidant and endothelium-protective agents [8]. However, the precise mechanism of action responsible for the improvement in cardiometabolic disease is unknown.

The current study specifically identifies GLP-1 regulated cardiometabolic effects. Elevated levels of this hormone alone could explain the weight-independent effects on metabolism and cardiovascular disease. Additionally, VSG was chosen because of its unusual effectiveness, as well as its increasing popularity. This study attempts to identify whether cardiometabolic improvements post VSG are indeed mediated through GLP-1 dependent mechanisms which is validated using the GLP-1 receptor antagonist exendin 9-39.

2. Materials & methods

2.1. Animals

Male Zucker diabetic fatty (ZDF *fa/fa*) rats weighing (250–300 g) at 8 weeks old were purchased from Charles River Laboratories (Wilmington, MA). Animals were housed individually in wire-mesh cages at a constant temperature of 21–23 °C with a 12-h light-dark cycle (lights on 07:00, off at 19:00).

2.2. Preoperative care and anesthesia

ZDF rats were fasted for 12 hours prior to surgery and housed in suspended wire mesh caging to prevent the rats from consuming bedding or coprophagia. The rats were anesthetized with isoflurane using a calibrated vaporizer equipped with a device for properly scavenging waste anesthetic gas. An induction chamber was used with an initial vaporizer flow rate of 3–5%. After induction, animals were removed from the chamber and anesthesia was maintained using a rodent-specific nose cone at the same flow rate. Ceftriaxone (25mg/kg) and Ketoprofen (5mg/kg) were administered subcutaneously. The abdomen was shaved in a remote location prior to transfer to the operative field. The abdomen was prepped and draped aseptically. Heating pads were used throughout the operation to maintain the core body temperature. The isoflurane vaporizer flow rate was reduced to 1–2% after an abdominal incision was made. Rats were monitored for signs of either pain or respiratory depression and the flow rate adjusted accordingly. Sham operations were conducted with identical preoperative and operative care conditions. Abdominal contents were mobilized and manipulated to parallel those of the VSG procedure. Additionally, there was a similar amount of time that the abdomenal cavity was exposed, as well as the time spent closing the wounds.

2.3. Vertical sleeve gastrectomy procedure

Animals were randomized for VSG and sham surgery and treated equally. Half of the animals underwent VSG and other half underwent sham surgery. All the outcomes were measured pre- and post-VSG. VSG surgery was performed using isoflurane anesthesia. The lateral 80% of the stomach was excised, leaving a tubular gastric remnant in continuity with the esophagus superiorly and the pylorus and duodenum inferiorly. The sham procedure involved analogous isolation of the stomach followed by manually applying pressure with blunt forceps along a vertical line between esophageal sphincter and pylorus. Animals consumed liquid diet (Osmolite) two day prior to the surgery and also continued for 6 days post-surgery. On the 7th day post-surgery, animals were reintroduced to Purina 5008 diet.

2.4. Diets

To render them obese, rats were given access for 3 weeks to a diet consisting of Purina 5008 (fat 15%, carbohydrate 56%, protein 26%) (Purina Animal Nutrition, Gray Summit, MO). After vertical sleeve gastrectomy, only Ensure (Fat 9%, carbohydrate 14%, protein 18%) (Abbott, Abbott Park, IL) a liquid meal supplement was available for the first 6 days, and then from the seventh day forward, switched back to the Purina 5008 diet. All the protocols involved in this study were approved by the Institutional Animal Care and Use Committee in accordance with guidelines established by the National Institutes of Health.

2.5. Antagonist dosing

Exendin (9–39), GLP-1 receptor antagonist dose (3μg/250g body weight) was chosen because it is the most commonly used dose in the literature [12] and injected intraperitoneally once daily for three months.

2.6. Weight loss measurement

All the rats were weighed weekly using a standard balance in the animal care facility and data was plotted as Mean ± standard error of mean (SEM).

2.7. Fasting blood collection

Fasting blood glucose level was measured using hand held Abbott Freestyle Lite glucometer (Abbott, Abbott Park, IL) per manufacturer's instructions. At the same time, fasting blood was also collected in tube containing protease inhibitors for the measurement of hormones.

2.8. Oral glucose tolerance test

Rats were fasted for 12h prior to the test. Blood glucose was

measured at 0 and 30 min post-glucose load (2g/kg).

2.9. Fasting plasma hormones levels

Plasma insulin and GLP-1 level was measured using Millipore Milliplex (Millipore Corporation, Billerica, MA, USA) metabolic hormone panels and read using a Bio-Plex 200 (Bio-Rad, Hercules, CA, USA) spectrophotometer. Sensitivity for insulin and GLP-1 as indicated by the manufacturer was 14pg/ml and 28pg/ml respectively. Intra assay coefficient of variation was less than 3% and inter-assay variability was less than 13%. Per the manufacturer, all antisera are highly specific and display insignificant cross-reactivity to other analytes within the panel.

2.10. Measurement of fasting bile acids levels

Bile acids were measured using the total bile acids Assay Kit (Calorimetric; BQ Kits, San Diego, CA) according to the manufacturer's instructions [13]. Briefly, all the contents supplied in the kit were pre-warmed at room temperature before reconstitution. Diaphorase was reconstituted with the phosphate buffer which remains stable for one week at 4 °C after reconstitution. 150 μl of Diaphorase and 20 μl of sample or standards were mixed and incubated at 37 °C for 4 minutes. After 4 minutes incubation, 30 μl of 3-α-HSD was added, mixed well and read immediately at 540 nm as A1. Samples were again incubated for 5 minutes and absorbance was read again at 540 nm as A2. Values were calculated by subtracting the change in absorbance A1from A2. Total bile acid concentrations were calculated using the equation below:

Δ Absorbance 540 (sample)/Δ Absorbance 540 (standard) × standard (35μmole/L)

2.11. Measurement of fasting L-PGDS levels

L-PGDS level was determined using ELISA kit supplied by MyBiosource. Assay procedure was followed as directed in the kit. Briefly, all reagents were brought to room temperature prior to the assay. 100μl of standard or sample were added to the wells, covered with the adhesive strip and incubated for 2 hours at 37 °C. After 2 hours, liquid was removed (note: do not wash) and 100μl of Biotin-antibody was added into each well, covered with the adhesive strip and incubated again for 1 hour at 37 °C. Solution in each well was mixed gently until solution appeared uniform. All the wells were washed three times using 200μl wash buffer. After the last wash, plate was inverted and blotted against clean paper towels. 100μl of HRP-avidin was added to each well and the microtiter plate was covered with a new adhesive strip and again incubated for 1 hour at 37 °C. After 1 hour, wells were rinsed 6 times; 90μl of TMB Substrate was added to each well and incubated for 15–30 minutes at 37 °C. (Note: Protect from light). Reaction was stopped using 50μl of stop solution. The optical density was determined within 5 minutes, using a microplate reader at 450 nm and 540 nm. absorbance was calculated by subtracting the values at 570 nm from those at 450nm. L-PGDS concentration was calculated using the standard curve and plotted

2.12. Fasting cholesterol measurement

A total Cholesterol and triglyceride level was measured with Cardio Check PA using the Lipid Panel test strip (PTS, Indianapolis, IN, USA).

2.13. Blood pressure and heart rate measurement

Blood pressure and heart rate was measured using the CODA Monitor, non-invasive blood pressure equipment purchased from Kent Scientific (Torrington, CT). The method of measurement has been adapted from Daugherty et al. [14].

2.14. Myograph studies

At the end of the study, ZDF rats were euthanized and aortas (3 mm ring) were harvested. Aortas were marked with water-resistant ink on their surface, and the distances between two given markers were measured. Aortas were excised quickly and placed in ice-cold physiological saline solution (PSS) containing (in mmol/l) 119 NaCl, 4.7 KCl, 24 $NaHCO_3$, 1.17 KH_2PO_4, 1.17 $MgSO_4$, MgCl 1.17, 1.6 CaCl, and 5.5 glucose, gassed by 95% O_2–5% CO_2. After a 45 minute rest period, PSS was removed and activation solution (KPSS) was added which replaces NaCl with KCl_2 (124mM) for 15 min and then a PSS wash for an additional 15 min. Experimentation started with concentrations (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M) with 5 minutes interval at each concentration using Angiotensin II and change in mV recorded. The vessel was washed with KPSS for 15 minutes followed by concentration response curve of acetylcholine (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M) with 5 minutes interval at each concentration and similarly change in mV recorded. Similarly, the vessel was then washed with KPSS for 15 min followed by a concentration response curve of sodium nitroprusside (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M) with 5 minutes interval at each concentration and change in mV recorded.

2.15. Statistical analysis

All data are presented as mean ± standard error of mean (SEM) and analyzed using *t*-test and one-way analysis of variance, ANOVA, with Bonferroni post hoc test for multiple comparisons wherever appropriate. A value of $p < 0.05$ was considered to be statistically significant. Statistical analyses were performed using Graph Pad Prism 5.0 (GraphPad Software Inc, San Diego, CA, USA).

3. Results

3.1. Effect of VSG on GLP-1 and insulin levels

In order to determine the effect of VSG on GLP-1 and insulin secretion, we performed vertical sleeve gastrectomy (n = 5/group) and compared results to the sham (n = 5/group) surgery group. GLP-1 level was measured initially and 30 minutes post oral glucose (2 gm/kg) load at pre- and 12 weeks post-surgery. Average fasting GLP-1 level pre-surgery was found to be 3.25 ± 0.44 pM, which reached to 4.39 ± 0.93 pM and 2.41 ± 0.25 pM in sham final and VSG final, respectively, after 12 weeks with no significant difference. Since, GLP-1 levels first starts rising up in circulation at 15–30 minutes post meal and determines the degree of insulin sensitivity [15], therefore, we decided to measure the GLP-1 level in plasma at 30 min. As shown in Fig. 1A, GLP-1 level in VSG final group at 30 minute was found significantly elevated that reached to 37.08 ± 11.48 pM while the sham final group at 30 minutes had only 6.04 ± 1.01 pM.

Given the glucose dependent insulinotropic properties of GLP-1 [16] we decided to measure insulin levels. As shown in Fig. 1B, the average fasting insulin level before surgery was found to be 2943 ± 707 ng/ml that reached to 3420 ± 561 ng/ml after 30 minutes during glucose tolerance test. Similarly, insulin level was measured at 0 and 30 minutes post oral glucose load at 12 weeks post-surgery and levels were found to 2434 ± 331 ng/ml and 2548 ± 783 ng/ml respectively. As expected, insulin levels in the VSG final group had 2535 ± 229ng/ml and 3713 ± 302ng/ml at 0 and 30 min respectively, clearly showing a significant increase in

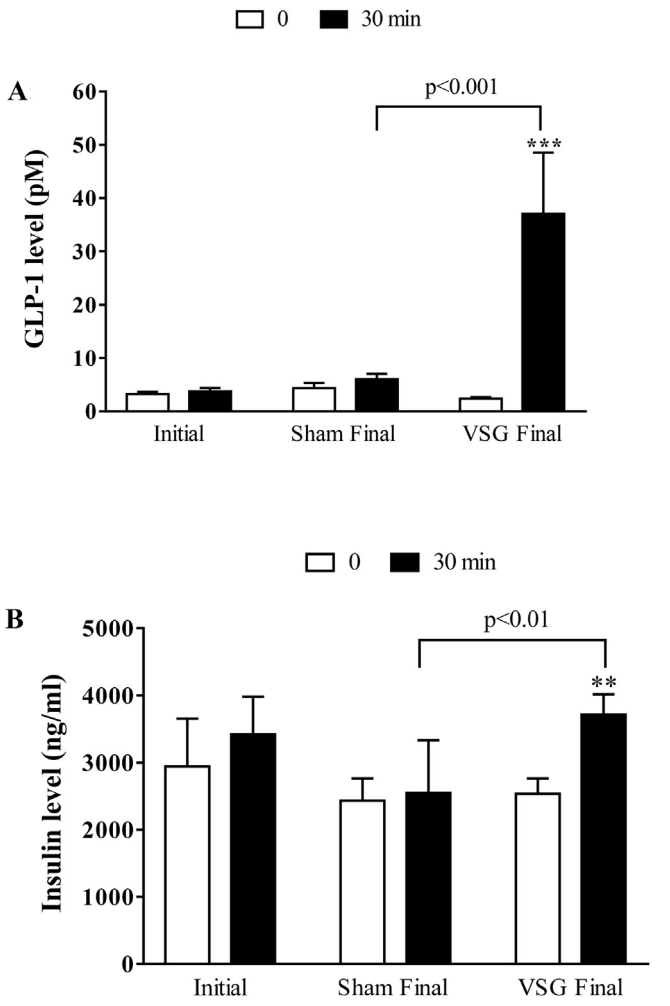

Fig. 1. Effect of VSG on GLP-1 and insulin. In order to determine the effect of VSG on GLP-1, we performed vertical sleeve gastrectomy (n = 5/group) and compared to the sham (n = 5/group) surgery group. GLP-1 (pM) (A) or insulin (B) levels were measured at 0 and 30 minutes post oral glucose (2 gm/kg) load before surgery and at 12 weeks post surgery. White bar represents baseline or initial values and black bars represent 12 week values. All the data are represented as mean ± SEM and significance was carried out based on ANOVA followed by Bonferroni' multiple comparisons test (*** $p < 0.001$ and ** $p < 0.01$).

insulin at 30 minutes unlike the sham groups at 12 weeks post surgery which did not show significant increase.

3.2. Effect of GLP-1 receptor antagonist (exendin 9-39) on fasting glucose level after VSG

In order to determine the importance of GLP-1 in response to VSG, we measured fasting blood glucose level prior to and 12 weeks post-surgery in the presence of the GLP-1 receptor antagonist, exendin 9-39. As shown in Fig. 2, the average blood glucose level before surgery was 73.0 ± 7.3mg/dl. Expectedly, the glucose levels were significantly increased to 387.8 ± 21.8 mg/dl in the sham group and the VSG group had a significantly decreased glucose level of 252.0 ± 41.5 mg/dl. Interestingly, however, the VSG group developed glucose intolerance, causing blood glucose level return to 334.3 ± 28.1 mg/dl. These data clearly suggest that GLP-1 is an essential component responsible for the beneficial glucose homeostasis observed after VSG.

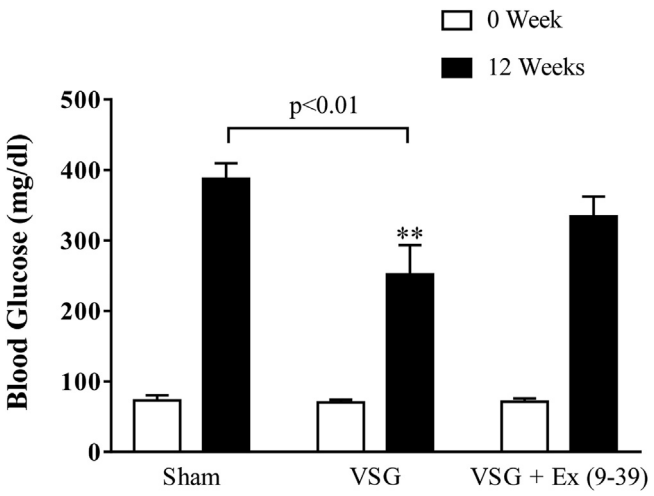

Fig. 2. Effect of GLP-1 receptor antagonist (Exendin 9–39) on fasting glucose level on VSG. In order to determine the effect of Exendin 9-39 on fasting blood glucose in ZDF rats underwent VSG, we measured fasting blood glucose level before and 12 weeks post surgery and compared with the sham group. VSG group received exendin 9-39 had significantly higher blood glucose level than the VSG group. White bar represent baseline or initial values and black bars represents 12 week values. All the data are represented as mean ± SEM and significance was carried out based on ANOVA followed by Bonferroni' multiple comparisons test (** $p < 0.01$).

3.3. Effect of GLP-1 receptor antagonist on body weight and food intake after VSG

Since VSG is considered a weight loss surgery [17]; we were interested in determining the effect of GLP-1 receptor antagonist on body weight in VSG group. We measured body weight prior to and 12 weeks post-surgery. As shown in Fig. 3A, the average body weight prior to surgery for all the three groups was 292.9 ± 9.3g. Post-surgery, the sham group increased to 495.4 ± 23.2 g and the VSG group increased to 445.3 ± 10.2 g, which was slightly significantly lower when compared to sham (Fig. 3A). However, the VSG group receiving exendin 9-39 remained elevated at 489 ± 24.6 g. This finding suggested that the beneficial effect of VSG on body weight counteracted by the antagonist suggests GLP-1 may be considered a contributing factor in sustaining weight loss post VSG.

We were interested in determining if body weight had any impact on food intake in our study since they are so strongly connected [18]. We measured food consumption 24 h before and 12 weeks post-surgery. As shown in Fig. 3B, average food consumption prior to surgery for all the three groups was 21.4 ± 4.1 g. Post surgery, the sham group increased food intake to 35.5 ± 1.8 g but in the VSG group reduced to 22.8 ± 1.8 g. However, the average food consumption in the VSG group received exendin 9-39 maintained to 23.4 ± 1.4 g. The obtained results clearly show that the antagonist seems to be unrelated to food intake, suggesting changes in food intake post sleeve gastrectomy is probably not modulated by GLP-1.

3.4. Effect of GLP-1 receptor antagonist on fasting insulin levels after VSG

Vertical sleeve gastrectomy improves insulin sensitivity and glucose tolerance during the meal tolerance test [19]. But its fasting insulin levels will provide the degree of insulin resistance. Therefore, we measured the fasting insulin levels pre and post-surgery in the presence and absence of GLP-1 receptor antagonist. Average fasting insulin level of the sham group prior to surgery was 3118 ± 566 ng/ml which reached 5849 ± 1856 ng/ml at 12 weeks post-surgery (Fig. 4). The VSG group had an initial fasting insulin

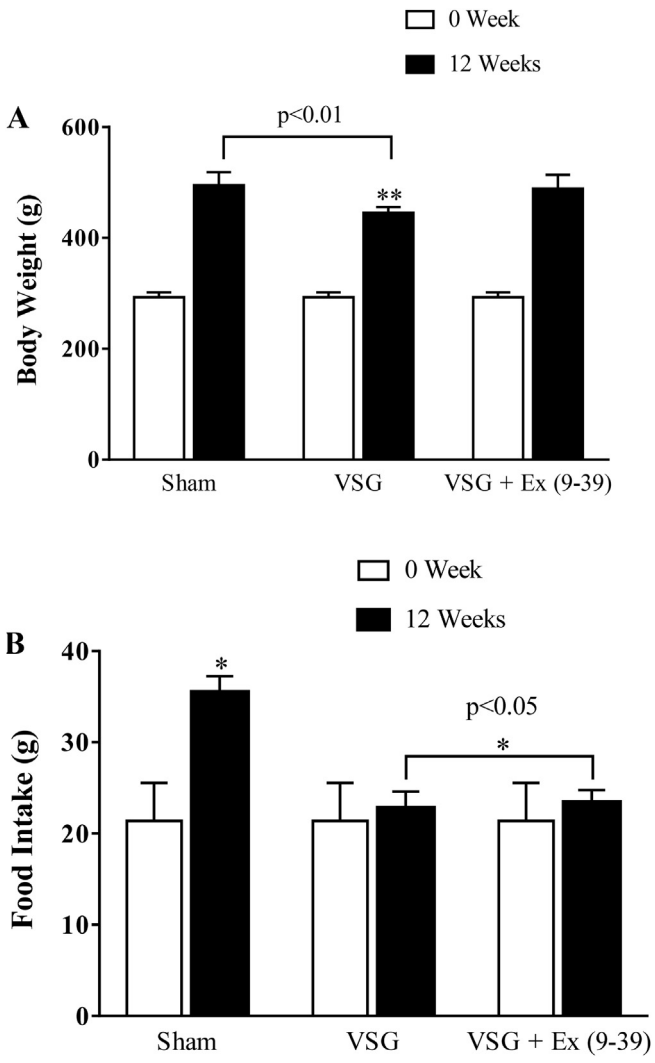

Fig. 3. Effect of GLP-1 receptor antagonist (Exendin 9–39) on body weight and food intake after VSG. In order to determine the effect of Exendin 9-39 on body weight (A) and food intake (B) in ZDF rats after VSG data was collected before and 12 weeks post surgery and compared with the sham group. White bar represent baseline or initial values and black bars represents 12 week values. All the data are represented as mean ± SEM and significance was carried out based on ANOVA followed by Bonferroni' multiple comparisons test (* $p < 0.05$ and ** $p < 0.01$).

level of 4120 ± 1107 ng/ml which reduced to 3327 ± 808 at 12 weeks post VSG. However, in the VSG group receiving GLP-1 antagonist the initial fasting insulin level 3855 ± 830 ng/ml was significantly increased to 11987 ± 2865 ng/ml after 12 weeks post-surgery (Fig. 4). These results clearly demonstrate that blocking the GLP-1 receptor significantly blunts the beneficial effect of VSG by increasing insulin resistance.

3.5. Effect of GLP-1 receptor antagonist on heart rate and blood pressure after VSG

GLP-1 has shown beneficial cardiovascular effects in clinical and preclinical studies [20], but VSG mediated GLP-1 augmentation and cardiovascular association remains unclear. Therefore, in our study we measured the effect of GLP-1 antagonist on heart rate pre and 12 weeks post-surgery. As shown in Fig. 5A, the average initial heart rate was 387 ± 22 bpm. At 12 weeks post-surgery there was no significant change in heart rate between sham and VSG animals,

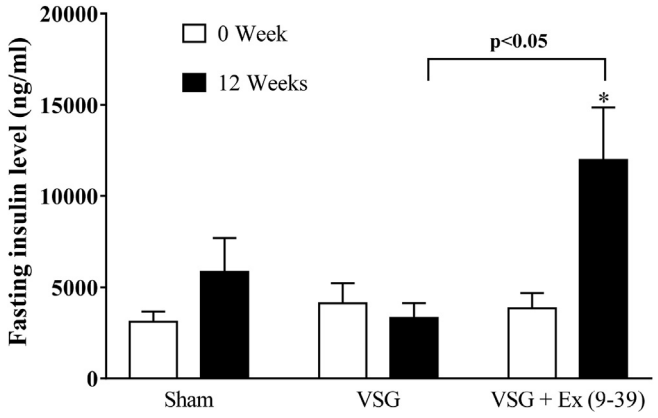

Fig. 4. Effect of GLP-1 receptor antagonist (Exendin 9–39) on insulin on VSG. In order to determine the effect of Exendin 9-39 on insulin level in ZDF rats undergoing VSG, we measured insulin level before and 12 weeks post-surgery in the presence and absence of exendin 9-39 and compared values to the VSG and sham groups. White bar represent baseline or initial values and black bars represents 12 week values. All the data are represented as mean ± SEM and significance was carried out based on ANOVA followed by Bonferroni' multiple comparisons test (* $p < 0.05$).

371 ± 9 and 401 ± 27 bpm respectively. The heart rate of the VSG group receiving GLP-1 antagonist was slightly increased to 430 ± 27 bpm but not significantly (Fig. 5A). As shown in Fig. 5B and C, average initial systolic/diastolic blood pressure was 142/89 mm/Hg which increased to 190/130 post 12 weeks surgery. Similarly, average systolic/diastolic blood pressure in VSG group was found to be 197/132. However, the VSG group received antagonist had 161/129 where systolic blood pressure found decreased compare to the sham and VSG group but diastolic blood pressure remained unchanged.

3.6. Effect of GLP-1 receptor antagonist on aortic vasorelaxation after VSG

Endothelial dysfunction is a significant biomarker of early stage of cardiovascular disease, which can be detected functionally as changes in vasomotor responses [21]. Therefore, we decided to perform myograph studies using aorta. The effect of exendin 9-39 on vasorelaxation mediated by acetylcholine (Fig. 6A) and sodium nitroprusside (Fig. 6C) in ZDF rats after VSG compared to the sham or VSG group in the presence or absence of GLP-1 antagonist. Based on the obtained results, there was no significant difference among the groups. Similarly, we measured the effect of exendin 9-39 on vasoconstriction in Fig. 6B and there were no significant differences found in response to VSG or GLP-1 antagonist.

3.7. Effect of GLP-1 receptor antagonist on total cholesterol and triglyceride after VSG

We were also interested in determining whether exendin 9–39 has any effect on total cholesterol. We measured total cholesterol prior to and 12 weeks post-surgery. As shown in Fig. 7A, average total cholesterol for all the three groups before surgery was 100 ± 00 mg/dl which increased to 162 ± 13.2 mg/dl in the sham group. Interestingly, the VSG group maintained total cholesterol of 100 mg/dl even 12 week post-surgery and the VSG group that received GLP-1 antagonist had total cholesterol of 140 ± 7.9 mg/dl 12 weeks post-surgery. Based on the obtained results, it was found that GLP-1 antagonist could disrupt the beneficial effect of VSG on total cholesterol.

Similarly, we were interested to determine whether exendin

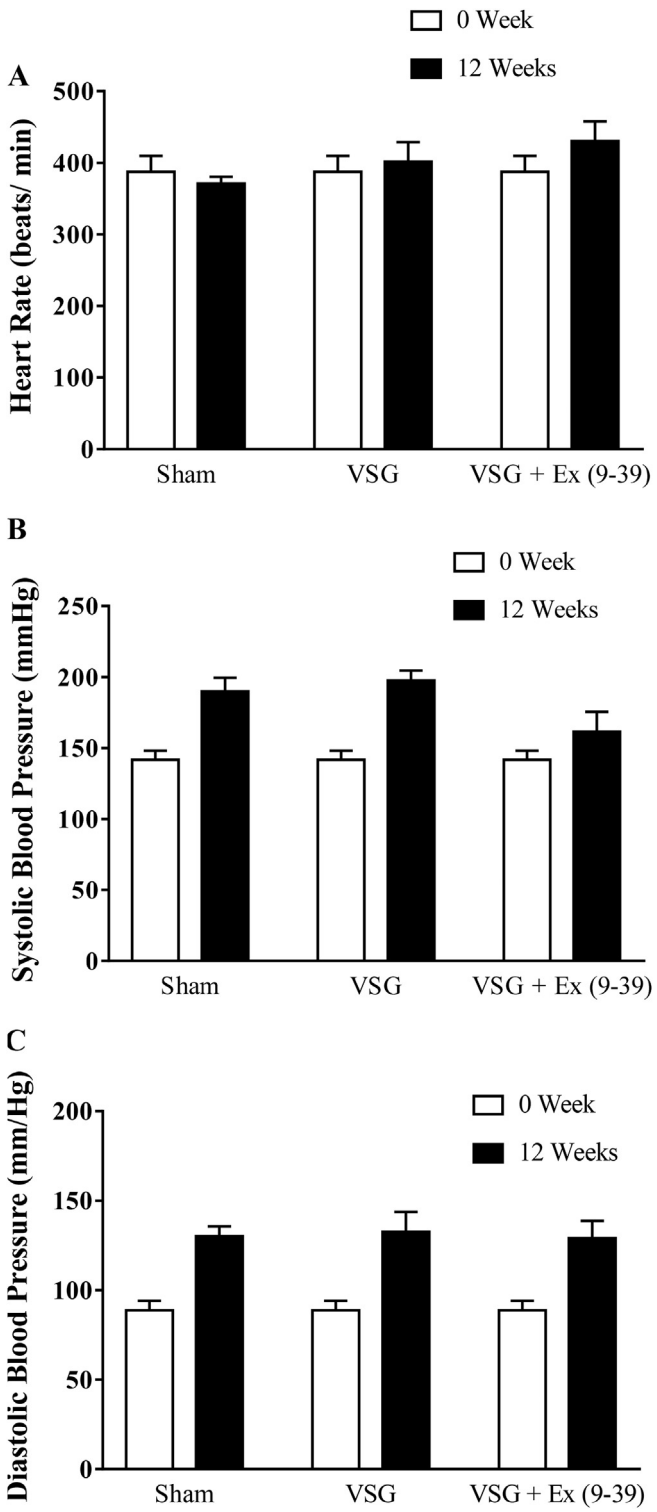

Fig. 5. Effect of GLP-1 receptor antagonist (Exendin 9–39) on heart rate and blood pressure after VSG. In order to determine the effect of GLP-1, we measured heart rate (A), systolic (B) or diastolic blood pressure (C) before and 12 weeks post-surgery in the presence and absence of exendin 9-39 and compared to the VSG and sham groups. White bar represent baseline or initial values and black bars represents 12 week values. All the data are represented as mean ± SEM and significance was carried out based on ANOVA followed by Bonferroni' multiple comparisons test.

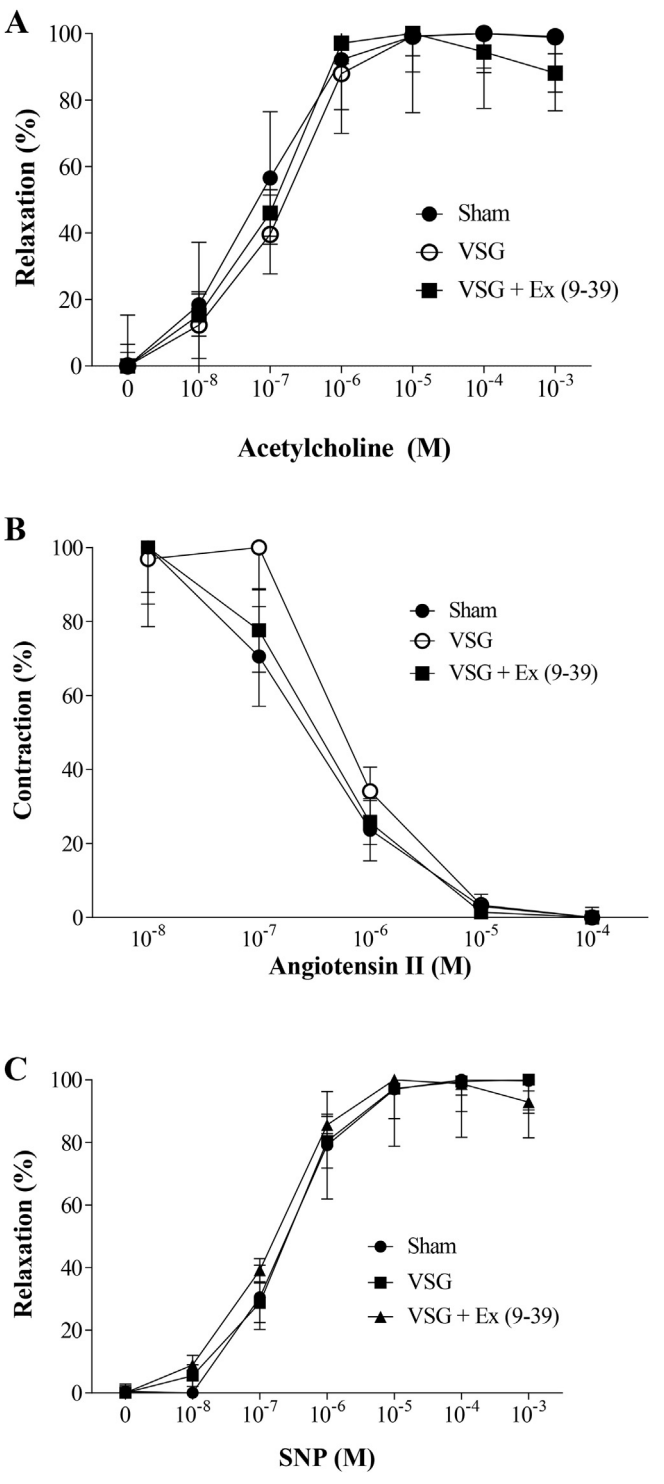

Fig. 6. Effect of GLP-1 receptor antagonist (Exendin 9–39) on Vasorelaxation or Vasocontraction (aorta) in ZDF rats underwent VSG. (A) Effect of exendin 9-39 on vasorelaxation induced by acetylcholine in ZDF rats underwent VSG compared to the sham or VSG **(B)** Effect of exendin 9-39 on Vasocontraction induced by angiotensin II in ZDF rats underwent VSG compared to the sham or VSG **(C)** Effect of exendin 9-39 on vasorelaxation induced by sodium nitroprusside in ZDF rats underwent VSG compared to the sham or VSG. Black circle, white open circle and white open square represent Sham, VSG and VSG group received exendin 9-39 respectively. All the data are represented as mean ± SEM and significance was carried out based on ANOVA followed by Bonferroni' multiple comparisons test.

9–39 has any effect on triglyceride. We measured triglyceride levels pre- and 12 weeks post-surgery. As shown in Fig. 7B, the average triglyceride before surgery was found to be 384 ± 25.5 mg/dl, which increased to 441 ± 40.6 mg/dl in the sham group. Interestingly, the VSG group had a significantly reduced triglyceride

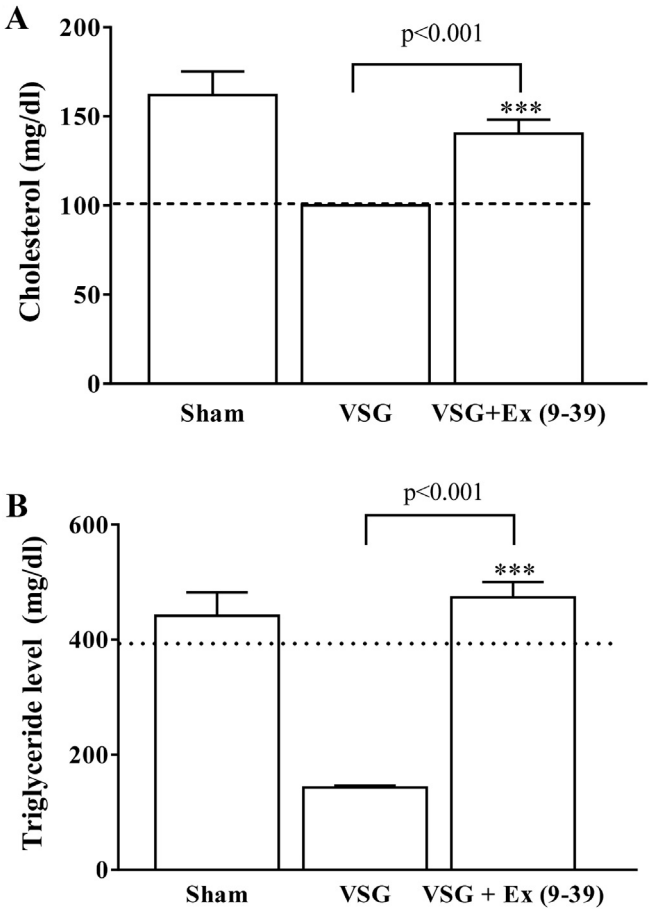

Fig. 7. Effect of GLP-1 receptor antagonist (exendin 9–39) on total cholesterol and triglyceride after VSG. Vertical sleeve gastrectomy (n = 5/group) was performed in the presence of exendin 9-39 for 12 weeks and total cholesterol (A) or triglyceride (B) levels compared to the VSG (n = 5/group) group and sham (n = 5/group). Dotted line represents baseline or initial values and bars represent 12 week values. All the data are represented as mean ± SEM and significance was carried out based on ANOVA followed by Bonferroni' multiple comparisons test (*** $p < 0.001$).

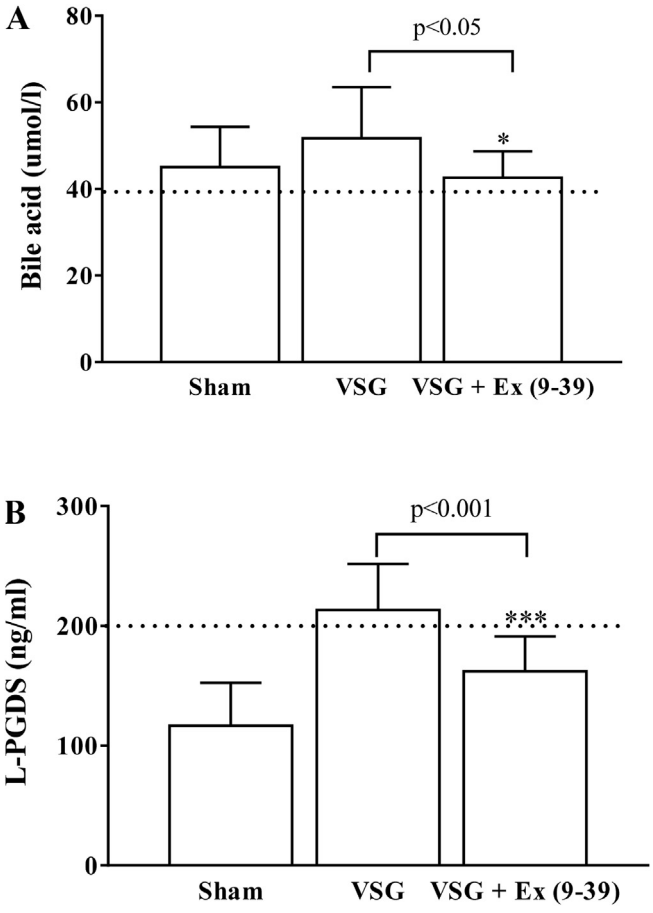

Fig. 8. Effect of GLP-1 receptor antagonist (Exendin 9–39) on serum bile acid and L-PGDS after VSG. We measured serum bile acid (A) and L-PGDS (B) levels after VSG group in the presence of exendin 9-39 (n = 5/group) and compared it to the VSG group (n = 5/group) group. Dotted line represents baseline or initial values and bars represent 12 week values. All the data are represented as mean ± SEM and significance was carried out based on ANOVA followed by Bonferroni' multiple comparisons test (* $p < 0.05$, *** $p < 0.001$).

level of 143 ± 3.5 mg/dl even at 12 weeks post-surgery. Remarkably, the VSG group that received antagonist had returned to a triglyceride level of 474 ± 26.3 mg/dl 12 weeks post-surgery (Fig. 7B). Based on these results, we believe that GLP-1 is directly involved in lowering triglyceride levels observed in response to VSG.

3.8. Effect of GLP-1 receptor antagonist (exendin 9-39) on serum bile acid and L-PGDS levels after VSG

Since bile acid and cholesterol metabolism is closely associated [22], we were interested to determine the whether exendin 9–39 has any effect on serum bile acid modulation. We measured bile acid levels before and 12 weeks post-surgery. As shown above in Fig. 8A, average bile acid level for all the three groups before surgery was found to be 38.8 ± 1.7μM which slightly increased to 45.0 ± 9.3 μM in the sham group 12 week post-surgery. Bile acid levels were found to be elevated to 51.7 ± 11.7 μM in the VSG group 12 weeks post-surgery. In the presence of GLP-1 antagonist, however, the VSG group serum bile acid concentration returned to 42.6 ± 6.1 μM representing no significant difference compared to the initial suggesting that GLP-1 may have a slight impact on bile acid secretion (Fig. 8A).

Given that L-PGDS knockout animals develop insulin resistance and glucose tolerance [23], and moreover that VSG in L-PGDS knockout mice does not impart it's beneficial metabolic effects [24], we were interested to find any relation between GLP-1and L-PGDS. We measured serum L-PGDS levels before and after VSG in all groups. The average fasting L-PGDS level was 264.7 ± 21.3 ng/ml which was reduced to 116.7 ± 35.7 ng/ml 12 weeks post surgery in the sham group as shown in Fig. 8B. L-PGDS levels were found to be slightly elevated at 213.3 ± 38.2 ng/ml 12 weeks post-surgery. However, the VSG group receiving GLP-1 antagonist had a serum L-PGDS concentration of only 162.2 ± 29.0 ng/ml, suggesting a possible association between GLP-1 and L-PGDS.

4. Discussion

Our previous study demonstrated that Roux-en-Y gastric bypass attenuates the progression of cardiometabolic complications in obese diabetic rats via alteration of gastrointestinal hormones [9]. Other studies have supported a similar concept in both animals and humans [25–27]. GLP-1 has emerged as a key player having a significant role mediating the beneficial metabolic and cardiovascular effects observed after bariatric surgery [28]. The 5- to 10-fold elevation of serum GLP-1 observed after RYGB and VSG appears to correlate well with improvement of diabetes, while its direct role on cardiovascular improvement remains less clear [11]. In the current study, we hypothesized that administration of the GLP-1

receptor antagonist (exendin 9-39) to ZDF rats undergoing VSG would blunt the beneficial cardiometabolic effects of the surgery if mediated by GLP-1.

GLP-1 levels (Fig. 1A) and insulin levels (Fig. 1B) increased significantly in response to VSG. Based on these findings, our study was designed using ZDF male rats which underwent VSG in the presence or absence of a GLP-1 antagonist (exendin 9-39) for 12 weeks and then the data was compared to the sham group. Since reduction in blood glucose level is the immediate outcome of insulin and GLP-1 release [16], fasting blood glucose levels were measured in all the groups pre- and 12 weeks post-VSG. Fasting blood glucose levels of the VSG group was significantly reduced compared to the sham group (Fig. 2). However, the VSG group receiving GLP-1 antagonist had significantly higher glucose levels compared to the VSG group alone. This finding implies the beneficial metabolic effects of VSG are mediated through GLP-1 signaling. The antagonist group also had higher insulin levels than the sham group (Fig. 4) which suggests increased insulin resistance [29] and strengthens our argument for GLP-1 involvement.

GLP-1 receptor agonists have been strongly associated with significant weight loss in diabetic patients [30]. Our results support this hypothesis, showing reduced body weight in the VSG group compared to the sham group (Fig. 3A). Interestingly, the antagonist group post-VSG did not show any significant difference in weight compared to the sham group. Generally, weight loss is generally associated with food intake, especially in context of post-bariatric surgery [31]. As shown in Fig. 3B, the VSG group had significantly lower food consumption compared to the sham group but the GLP-1 antagonist group did not show any significant change. Based on these results, it can be inferred that the reduction in food intake in the VSG group was not mediated through GLP-1 signaling and may involve some other mechanism.

GLP-1 is commonly studied as an anti-diabetic agent [32] with recent studies also reporting some beneficial cardioprotective effects of GLP-1 receptor agonists [33]. Incretin based therapy, especially GLP-1 receptor agonists, have become a prime therapeutic approach in treating *type 2* diabetes [34]. The direct effect of GLP-1 on the cardiovascular system remains controversial due to inconsistent *in-vitro* and *in-vivo* results [20]. We were interested in investigating whether enhanced GLP-1 secretion post-VSG has any cardiovascular benefits. Since, higher resting heart rate is closely associated with increased cardiovascular complications and may cause premature death in *type 2* diabetic patients [35]. We measured heart rate pre- and 12 weeks post-surgery. We did not find any significant change in heart rate in the VSG group compared to the sham group (Fig. 5A). However, the VSG group receiving GLP-1 antagonist had an elevated heart rate at 12 weeks post-surgery. Although the precise mechanism of action is still not known, these data imply that endogenous GLP-1 may have a protective effect which begins to disappear upon administration of the GLP-1 antagonist.

Similarly, there was no difference in blood pressure between the sham and VSG groups (Fig. 5B and C). But surprisingly, the VSG group receiving GLP-1 antagonist had a decreased systolic blood pressure when compared with the sham and VSG groups. These controversial results remain ambiguous because GLP-1 agonist has been associated with reduction in blood pressure [36]. This finding requires further study in detail.

Insulin resistance and hypertension appear to have a complex association with endothelial dysfunction [37,38]. Myograph studies were performed using aortic rings to measure vasorelaxation or vasoconstriction in response to acetylcholine, sodium nitroprusside or angiotensin II but no significant difference among all groups studied was found (Fig. 6). This is consistent with our blood pressure and heart rate data which also showed no significant difference (Fig. 5). These data are consistent with the lack of hemodynamic differences observed in all groups studied.

Previous studies have also reported the beneficial effects of GLP-1 on lipid metabolism [39], which prompted us to investigate the effect of enhanced GLP-1 secretion post-VSG on lipid profile. As shown in Fig. 7A, total cholesterol was increased significantly 12 weeks post-surgery in the sham group. Interestingly, total cholesterol in the VSG group remained unchanged 12 weeks post-surgery despite keeping animals on a high fat diet. More importantly, the VSG group that received GLP-1 antagonist had significantly increased total cholesterol compared to pre-surgery, which suggests that GLP-1 plays an important role in lipid metabolism. Additionally, we measured triglyceride which represents a potential indicator of cardiovascular risk [40]. As shown in Fig. 7B, triglyceride levels were increased in the sham group 12 weeks post-surgery. However, the VSG group had a significantly reduced triglyceride level which shows the clear benefit of vertical sleeve gastrectomy. This significant reduction in triglyceride levels post-VSG is speculated to result from reduced postprandial triglyceride secretion from the intestine into circulation and not due to intestinal lipid malabsorption, possibly a common mechanism in RYGB [10]. We performed only VSG which does not involve any alteration in intestine but reduces the size of stomach which ultimately reduces the food intake and possibly impacts the diminished postprandial rise in triglyceride. However, the exact mechanism of action of reducing triglyceride level post VSG needs be elaborated in detail [41]. Further, the VSG group that received the GLP-1 antagonist in our study showed no change in triglyceride levels compared to the VSG group. Therefore, based on the obtained results, it can be assumed that the reduced triglyceride level in the VSG group was not modulated through GLP-1 signaling. However, further studies will be needed to elucidate the precise mechanism.

Another factor which facilitates lipid absorption in the intestine and regulates cholesterol homeostasis is bile acids [42]. Our previous study has shown increases in bile acids post-surgery as compared to sham group [43]. It is also known that bile acid triggers GLP-1 and helps in glucose metabolism [44]. We decided to determine whether exendin (9–39) has any effect on bile acid modulation. Our results showed a slight increase in total bile acid levels in the VSG group compared to the sham group, but not significant (Fig. 8A). This was somewhat surprising as we expected bile acid levels to increase. The possible reason could be surrounded by some anatomical difference between both the surgeries which modulates the degree of alteration in bile acid levels. Our previous results showed significant increase in bile acid level post-RYGB which supports this concept [43]. Interestingly, there was a significant reduction in bile acid levels with usage of the GLP-1 antagonist which hints the possible involvement of GLP-1 in bile acid modulation.

Given that L-PGDS plays an important role as a transporter for small lipophilic molecules including bile acid [45], we decided to measure the level of L-PGDS post-VSG. As shown in Fig. 8B, the level of L-PGDS was found significantly increased at 12 weeks post-VSG compared to the sham. Our previous study had also demonstrated that higher concentrations of L-PGDS significantly reduce the secretion of glucagon in the alpha cells [46]. Even though, we did not measure insulin we can still speculate the increase in insulin secretion was triggered through GLP-1 signaling. Furthermore, the VSG group that received exendin (9–39) had a significantly reduced L-PGDS level suggesting a possible association between GLP-1 and L-PGDS. This hypothesis is supported by our previous finding which shows that L-PGDS knockout animals develop glucose intolerance and insulin resistance [23] and can't be resolved even post-VSG possibly by blocking the GLP-1 signaling

[24]. Collectively, these findings make a stronger case for the association of GLP-1 signaling and L-PGDS.

4.1. Conclusion

Based on the results, it can be concluded that beneficial metabolic effects on blood glucose, insulin, total cholesterol, triglyceride, bile acids and L-PGDS levels were mediated through GLP-1 receptor signaling. Direct cardiovascular benefits were not identified in the results of heart rate, blood pressure and vasomotor activities but significant reductions in total cholesterol and triglyceride levels mediated through GLP-1 are believed to be a significant contributing factor in the improvement of cardiovascular complications. The novelty of this study comes from GLP-1 mediated effects on alteration in bile acid and L-PGDS levels which will lead us in understanding their involvement in cholesterol metabolism in our future studies.

4.2. Limitation

The study was limited by sample size and the use of only rodent animal models.

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Author contribution

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