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Sleeve Gastrectomy in Rats Improves Post-Prandial Lipid Clearance by Reducing Intestinal Triglyceride Secretion

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Abstract

Background & Aims—Post-prandial hyperlipidemia is a risk factor for atherosclerotic heart disease and is associated with the consumption of high-fat diets and obesity. Bariatric surgeries result in superior and more durable weight loss than dieting. These surgeries are also associated with multiple metabolic improvements, including reduced plasma lipid levels. We investigated whether the beneficial effects of vertical sleeve gastrectomy (VSG) on plasma lipid levels are weight-independent.

Methods—VSG was performed on Long-Evans rats with diet-induced obesity and pair-fed and ad libitum-fed rats that received sham operations (controls). We measured fasting and post-prandial levels of plasma lipid. To determine hepatic and intestinal triglyceride secretion, we injected the lipase inhibitor poloxamer 407 alone, or before oral lipid gavage. ¹³C-Triolein was used to estimate post-prandial uptake of lipid in the intestine.

Results—Rats that received VSG and high-fat diets (HFDs) had markedly lower fasting levels of plasma triglyceride, cholesterol, and phospholipid than obese and lean (pair-fed) controls that were fed HFD. Rats that received VSG had a marked, weight-independent reduction in secretion of

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intestinal triglycerides. VSG did not alter total intestinal triglyceride levels or size of the cholesterol storage pool, nor did it affect the expression of genes in the intestine that control triglyceride metabolism and synthesis. VSG did not affect fasting secretion of triglyceride, liver weight, hepatic lipid storage, or transcription of genes that regulate hepatic lipid processing.

Conclusions—VSG reduced post-prandial levels of plasma lipid, independently of body weight. This resulted from reduced intestinal secretion of triglycerides following ingestion of a lipid meal and indicates that VSG has important effects on metabolism.

Keywords

bariatric surgery; VLDL; bile acid; meal pattern; weight reduction

Background

Cardiovascular disease in obese patients is a leading cause of mortality, which can arise from dyslipidemia associated with consumption of high-fat diets. In fact, even mild obesity has been reported to increase risk of coronary heart disease by 50%, with more severe obesity increasing risk as much as 3-fold¹. Cardiovascular disease is currently the leading cause of death in the United States². Obesity-related dyslipidemia commonly includes elevated total cholesterol, LDL, and triglycerides and reduced levels of HDL cholesterol³. Dyslipidemia in addition to excess fat, is therefore an important goal of any ideal weight loss strategy.

Bariatric surgery improves cardiovascular health by reducing symptoms of and risk factors for cardiovascular disease⁴. Dyslipidemia is a potent cardiovascular risk factor shown to be reduced in at least 70% of patients after bariatric surgery⁵. Improvements have been documented following RYGB⁶⁻⁹, gastric banding^{10, 11}, biliary-intestinal bypass¹¹, duodenal-jejunal bypass¹², biliopancreatic diversion¹³, ileal interposition¹⁴, and vertical sleeve gastrectomy (VSG)¹⁵⁻¹⁷. The degree of improvement to plasma lipids appears to vary with each procedure, but it is yet unknown which gastrointestinal manipulations are most effective to reverse dyslipidemia. Importantly, for each of these procedures, it is also unclear whether improvements are due to weight loss or to an independent effect of the surgery. In this study we focused on VSG, where a reduction in gastric volume is created by the removal of gastric mucosa along the greater curvature and fundus. We have previously shown that this surgical model produces profound and persistent weight loss in rats¹⁸. We hypothesized that VSG would also reverse dyslipidemia secondary to altered gastrointestinal function. Here, we demonstrate that VSG improves dyslipidemia in a weight-independent manner and that this reduction is due to attenuated postprandial triglyceride production by the intestine.

Methods

Animals

Male Long-Evans rats (Harlan Laboratories, Indianapolis, IN; 250-300 g) were fed a high-fat butter oil-based diet (HFD, Research Diets, New Brunswick, NJ, D12451; 45% fat; 4.73 kcal/g) or standard chow (Harlan-Teklad, Indianapolis, IN) for 8 weeks prior to surgery and maintained on this diet post-surgery. Rats were housed under controlled conditions (12:12-h light-dark cycle, 50-60% humidity, 25° C, free access to water and food except where noted). A subgroup of sham-operated rats was pair-fed to the VSG group; amount of food eaten by each VSG rat during the previous 24 h was given to each PF rat at a random time during the light/dark cycle. For the tracer study, all animals were fed 15 g of HFD on the day prior to the study, divided into two meals given at 0 and 6 hours after the onset of the

dark cycle. Fat and lean tissue masses were measured using nuclear magnetic resonance (NMR, Echo MRI: Echo Medical Systems, Houston, TX). At the end of each study, animals were placed briefly in a CO₂ chamber and then sacrificed by decapitation. All procedures for animal use were approved by the University of Cincinnati Institutional Animal Care and Use Committee. A summary of cohorts is provided in Supplemental Table 1.

Surgical Procedures

For VSG, a laparotomy incision was made. The stomach was isolated outside the abdominal cavity. Loose gastric connections to the spleen and liver were released along the greater curvature and the suspensory ligament supporting the upper fundus was severed. The lateral 80% of the stomach was excised using an ETS 35-mm staple gun (Ethicon Endo-Surgery, Cincinnati, Ohio), leaving a tubular gastric remnant in continuity with the esophagus and duodenum. Following reintegration of the stomach into the abdominal cavity, the abdominal wall was closed in layers. Sham surgery was laparotomy, isolation of the stomach, and application of blunt pressure using forceps along a line between the esophageal sphincter and the pylorus. Post-surgery, rats received post-operative care for 3 days (Supplemental Figure 1), consisting of twice-daily subcutaneous injections of 10 mL warm saline and 0.3 mL buprenorphine. Daily subcutaneous meloxicam was administered for 2 days postoperatively. Rats were given access only to Osmolite OneCal liquid diet from 24 h pre-operatively until solid food was returned 3 days after surgery.

Measurement of lipids

All blood was sampled from the tip of the tail except when taken as trunk blood during sacrifice. Plasma cholesterol, NEFA, phospholipid, and triglycerides were measured via colorimetric assays using Infinity Reagents (Thermo Fisher Scientific, Inc., Waltham, MA). For the study of triglyceride production rate, rats were fasted for 24 h and baseline blood samples were collected prior to i.p. injection of 1 g/kg poloxamer 407 (P-407; Sigma-Aldrich, St. Louis, MO) and blood was taken at various timepoints thereafter. For the experiment described in Figure 3, an intragastric gavage of 0.5 mL/kg olive oil was delivered 1 h prior to P-407 injection. For apolipoprotein profiles, trunk blood was collected into EDTA after 24 h of fasting and stored at 4°C for fractionation via fast protein liquid chromatography (FPLC) within 7 days.

For measurement of triglycerides and cholesterol in liver and intestine, tissues from 24-h-fasted rats were flash-frozen in isopentane and lipid from 50 mg of tissue was extracted in 2:1 chloroform:methanol. Triglyceride and cholesterol content were measured as described above, using colorimetric assays. Hepatic cholesterol esters were measured using a Cholesterol/Cholesteryl Ester Quantitation Kit (EMD Chemicals, Gibbstown, NJ) from 10 mg tissue homogenates.

Plasma total sample bile acid concentrations were determined using a kit from BioQuant (San Diego, CA).

Lipid absorption

Dietary lipid absorption of a 5 mL/kg intragastric lipid emulsification (20% soybean oil, 1.2% egg phospholipid, 2.5% glycerin, 2.5% sucrose polybehenate) was measured as previously described¹⁹. Briefly, 24-h-fasted rats received the emulsion via intragastric gavage. 10 mg fecal samples were collected 24 h later. Fecal lipid content was assayed by gas chromatography of fatty acid methyl esters. Dietary lipid absorption was estimated using a ratio of total fecal fatty acids to sucrose polybehenate.

Stable isotope study

On the day prior to the study, all rats were placed in clean cages and given 10 g HFD at the onset of and 5 g HFD 6 hours into the dark phase of the light-dark cycle. All food was consumed prior to sacrifice. On the day of the study, 1 g/kg P-407 was administered 1 hour prior to a 0.28 g intragastric lipid gavage containing 0.18 g olive oil and 0.10 g 1,1,1-¹³C₃-Triolein. Rats were sacrificed 4 hours later. Intestines were removed immediately after sacrifice and dissected into 4 equal-length sections (M1, M2, M3, and M4 from proximal to distal, respectively). After removal of 1.5 cm from each segment for PCR and histology, each gut segment was cleaned thoroughly by removing mesenteric fat and rinsing inner surface in 1% SDS followed by PBS. Each segment was placed in 10 mL chilled PBS for immediate homogenization on ice. Lipids were extracted from homogenate aliquots using the method described by Folch et. al.²⁰. Ratio of ¹³C to ¹²C in the lipid fraction was measured using gas-chromatography combustion isotope ratio mass spectroscopy (GCC-IRMS) and reported as excess enrichment above baseline, as measured using samples from pluronic-treated, ungavaged animals.

Intestinal and liver gene expression

Tissue harvested 6 hours after a lipid gavage was homogenized in RLT buffer using a tissue lyser (QIAGEN, Inc., Valencia, CA). RNA was extracted using a QIAGEN miniprep RNA extraction kit and cDNA was made using an iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). Quantitative PCR was performed using TaqMan gene expression assays (Supplemental Table 2).

Histology

To visualize intestinal morphology, tissue samples were fixed in 4% paraformaldehyde and stored in 30% sucrose. 7 µm cryosections were stained with Cresyl Violet. Digital images of sections were acquired using a digital camera attached to a Zeiss microscope (Zeiss, Thornwood, NY).

Biochemical Assays

Western blots were used to measure plasma ApoB48 and ApoB100 content after a lipid gavage in the presence of i.p. P-407. Plasma samples were denatured in one volume of RIPA buffer (Santa Cruz Biotechnologies, Santa Cruz, CA) plus three volumes Laemmli buffer (Bio-Rad Laboratories, Hercules, CA) and diluted with distilled water. Following 10 minutes at 95°C, samples were applied to a 4-15% Tris-HCl polyacrylamide gel (Bio-Rad Laboratories, Hercules, CA) and run at 50 V for 4.5 hours. Proteins were transferred to nitrocellulose overnight at 30 V. Blots were blocked at room temperature in 5% BSA. Goat anti-human apolipoprotein B antibody (Millipore, Temecula, CA) was diluted 1:5000 in 5% BSA and incubated overnight at 4°C. Blots were incubated in secondary antibody, 1:5000 HRP-conjugated rabbit anti-goat IgG (Millipore, Temecula, CA) in 5% milk for 1 h at room temperature. Immunoreactivity was quantified using Alpha Ease software (Alpha Innotech Corporation, San Leandro, CA).

Statistical Analysis

All data are expressed as mean ± SEM. Body weight, food intake, NMR, and changes in plasma triglyceride or cholesterol levels over time were analyzed via 2-way ANOVA (Variables: treatment & time) with a Bonferroni *post hoc* test where appropriate. Area-under-the-curve comparisons, triglyceride production rates, plasma lipid analytes at single time points, plasma bilirubin and bile acid measurements, single-gene expression comparisons, comparison of gavage sizes, single-day body weight and body composition measurements, stable isotope enrichment in intestinal lipid samples, and total tissue

cholesterol and triglyceride content were analyzed using 1-way ANOVA followed by a Tukey post-hoc test where appropriate. Two-tailed, unpaired t-tests were used where indicated.

Results

VSG reduces plasma lipids

4-h fasting plasma lipids were measured 50 d post surgery, when rats were weight-stable and obese, sham-operated (SHAM) animals were heavier compared to VSG, sham-operated, chow-fed (CHOW), and sham-operated, pair-fed (PF) groups (Figure 1A). Weight loss after VSG is a selective loss of fat mass (Ref 17 and Figure 1B). All animals in PF, SHAM, and VSG groups were maintained on HFD for the duration of the study. Plasma cholesterol (Figure 1C, $P<0.001$), triglycerides (Figure 1D, $P<0.01$), and phospholipids (Figure 1E, $P<0.001$), but not non-esterified fatty acids (NEFA) (Figure 1F) concentrations were decreased after VSG as compared to SHAM controls. Importantly, this observed reduction after VSG is weight-independent, since cholesterol (Figure 1C, $P<0.05$), triglycerides (Figure 1B, $P<0.01$), and phospholipids (Figure 1E, $P<0.001$) were all reduced after VSG versus PF animals, both groups exhibiting similar body weight (Supplemental Figure 2). Furthermore, levels of all four lipids measured (cholesterol, triglycerides, phospholipids and NEFA) were similar in CHOW and VSG groups (Figures 1A-D) despite lower body weight and fat mass in the CHOW group (Figures 1A-B), underscoring a weight-independent effect of VSG on dyslipidemia.

In order to explore whether reductions in plasma triglycerides were dependent on the duration of fasting, we measured triglycerides in plasma sampled after 0, 4, 8, and 24 hours of fasting. All animals consumed 15 g of HFD on the day prior to the study. Body weight was significantly reduced in VSG and PF animals on the day of the study (body weight, food intake, and body composition are presented in Supplemental Figure 2). Consistent with the data from Figure 1C, triglyceride levels were reduced in unfasted plasma from VSG animals as compared with SHAM ($P<0.001$) and PF ($P<0.01$). This difference was gradually diminished along the course of the fast (Figure 2A). At 4 and 8 hours of fasting, plasma triglycerides were lower in VSG animals than SHAM ($P<0.001$ at both time points) but no longer significantly different from PF. By 24 hours of fasting, no significant differences existed between any of these groups. However, area under the curve for triglycerides during the 24-hour experiment was reduced for VSG compared to PF ($P<0.05$) or SHAM ($P<0.001$; Figure 2B). At the same time we did not detect significant differences in plasma cholesterol levels among the three groups (Figure 2C) and thus area under the curve for 24-hour cholesterol was unaffected by treatment (Figure 2D). As reflected by total plasma cholesterol levels after 24 h of fasting (Supplemental Figure 3), VSG improved fasting hypercholesterolemia by reducing both VLDL and HDL cholesterol (Figure 2E). Apolipoprotein size did not appear to be altered by VSG.

Triglyceride production rate and postprandial lipid clearance

To determine whether reduced plasma triglyceride levels after VSG are due to attenuated VLDL production and/or secretion, we administered an intraperitoneal injection of the lipase inhibitor poloxamer 407 (P-407) (1 g/kg) to 24 h-fasted animals. We found no changes in rate of triglyceride appearance in the plasma across groups following P-407 administration (Figures 3A-B). These results indicate that VLDL production/ secretion rate is similar between the different experimental groups.

Based on the observation that plasma triglycerides were reduced most dramatically in VSG animals after short periods of fasting, we hypothesized that VSG might enhance postprandial

lipid metabolism. We next investigated whether intestinal chylomicron assembly and secretion are affected by VSG. To answer this question, we administered P-407 to 24-hour fasted rats 1 hour prior to a 0.5 mL/kg olive oil gavage (average fat content: 0.28 ± 0.004 g) and measured plasma triglycerides for 6 hours after the gavage. We found a significantly reduced rate of triglyceride appearance in the blood for VSG animals from 4–6 hours after the gavage and which remained low throughout the 6-hour duration of the study (treatment $\times$ time interaction, $P < 0.0001$; Figure 3C). Rate of triglyceride appearance during the 6-hour experiment was lower in VSG animals than in either SHAM ($P < 0.01$) or PF ($P < 0.001$) animals (Figure 3D). Postprandial plasma ApoB48 and ApoB100 content was assayed in order to assess whether reduced postprandial plasma lipid concentration might be due to the secretion of smaller or to fewer triglyceride-rich particles (TRPs). No significant changes to ApoB48 or to ApoB100 content in the plasma were detected in samples collected at the 6-hour time point (Figure 3E). Triglyceride-to-total ApoB content also did not differ among groups at this time point, although a trend toward increased particle size was observed for PF animals relative to SHAM and VSG animals (Figure 3E).

VSG does not affect hepatic lipid storage

Although fasting and thus hepatic triglyceride secretion appears to be unchanged after VSG, other metabolic changes might occur in the liver to affect lipid homeostasis. For example, the liver secretes bile acids that are critical for digestion and absorption of fats. Recent evidence also suggest that bile acids serve to activate nuclear transcription factors that can directly influence genes regulating lipid metabolism, glucose metabolism, and gut peptide secretion²¹. Serum bile acids have been reported to be increased following bariatric surgery^{22, 23} but have not previously been measured following VSG. We found that plasma bile acids were decreased in SHAM animals compared to CHOW, VSG, and PF animals (Figure 4A). Plasma bilirubin levels were unaffected by weight or diet (Figure 4B). We did not detect any significant differences in expression of hepatic CYP7A1, CYP27A1, HMG-CoA reductase (HMGCR), or scavenger receptor class B, member 1 (SCARB1; Table 1), genes that regulate bile acid circulation and metabolism. Neither hepatic triglyceride (Figure 4D) nor cholesterol (Figure 4E) content was affected by treatment in this study. Ratio of cholesterol ester content to total cholesterol did not differ among groups (Figure 4F), indicating that long-term hepatic cholesterol storage was also unaffected by either VSG or pair-feeding. Consistent with this result, we did not detect any differences in whole liver weight among groups (Figure 4G). We next assayed the expression of several genes (Table 1) related to hepatic lipid uptake (CD36, FATP4, L-FABP) and esterification (ACAT2), intracellular triglyceride synthesis and packaging (MGAT2, DGAT1, MTP), lipoprotein composition (ApoA2 and ApoB), and lipoprotein lipase (LPL) activity. VSG was not found to have any effect on hepatic gene expression. ApoB expression was found to be reduced in PF vs. SHAM with no effect of VSG (ANOVA, $P = 0.0181$; PF vs. SHAM, $P < 0.05$).

VSG does not affect the size of intestinal lipid storage pools

Enterocytes of the upper small intestine are known to contain lipid storage pools²⁴. Because VSG seemed to have minimal effect on hepatic lipid processing, we hypothesized that reduced postprandial intestinal triglyceride secretion after VSG might be due to enhanced intestinal lipid storage. Following P-407 administration and a 1,1,1-¹³C3-Triolein-enriched olive oil gavage containing a total of 0.28 g of lipid, uptake of lipids into the proximal intestine was comparable among groups (Figure 5A). We did not find any differences in intestinal weight (Figure 5B) or in triglyceride (Figure 5C) or cholesterol content (Figure 5D) from whole intestinal segment homogenates. Cresyl violet-stained sections from M2 were examined, revealing no obvious differences in villus length or morphology, which could affect lipid absorption (Figure 5E). No differences in plasma triglycerides or cholesterol were detected at this time point (Supplemental Figure 6). Analysis of the

expression of genes known to control intestinal triglyceride metabolism and chylomicron synthesis did not reveal any significant changes in VSG-operated animals. Samples from M1 and M2 represent duodenum and jejunum, respectively, and so any changes to chylomicron formation or secretion are expected to be effected in these regions. Gene expression studies within these regions (Table 2) did not reveal any significant changes to transcripts known to affect intestinal lipid uptake (CD36, FATP4, L-FABP, I-FABP, and ACAT2), triglyceride synthesis and packaging (DGAT1, MGAT2, and MTP), chylomicron composition and size (ApoAIV and ApoB), and LPL activity (ApoC2 and ApoC3).

Discussion

It has been speculated that plasma lipids are reduced in humans after bariatric surgery in part due to conscious dietary changes. Here, we show that the improvement to lipid homeostasis after VSG is instead a physiological consequence of the surgery. We report lower plasma lipid levels in VSG-operated rats than weight-matched (PF) rats despite the fact that both groups were maintained on the same amount of HFD.

Our data show that plasma triglyceride levels are markedly reduced in VSG-operated rats, specifically after short periods of fasting, suggesting that VSG primarily affects postprandial lipid metabolism. Our findings indicate that VSG improves postprandial triglyceride clearance through an intestinal mechanism. While endogenous triglyceride secretion is not changed by VSG (Figure 3), the secretion of dietary-derived triglyceride-rich particles is significantly reduced in VSG animals as compared either with SHAM or PF controls. The ratios of triglyceride levels to ApoB concentrations in plasma 6 hours after oral lipid gavage indicate that VSG does not significantly alter the size of TRLs secreted postprandially. However, the data do trend towards the secretion of larger TRLs in PF animals than in either SHAM or VSG animals, and towards the secretion of fewer TRLs in PF and VSG vs. SHAM animals.

Intestinal enterocytes contain lipid storage pools which may be acutely mobilized by the consumption of a meal²⁴. Thus, the degree of postprandial lipidemia depends not only on intestinal lipid absorption, re-synthesis, and secretion, but also on the extent of intestinal lipid storage. Here, we demonstrate that VSG does not alter intestinal storage capacity for a dose of oral fat known to produce reduced postprandial lipidemia in VSG rats (Figure 5). We did not detect any changes in the expression of a large panel of genes known to regulate intestinal triglyceride metabolism and/or chylomicron synthesis (Table 2). These data lead us to the hypothesis that VSG does not induce long-term changes to intestinal lipid metabolism. Importantly, gene expression was assayed at a single postprandial timepoint. While we would expect that earlier transcriptional changes would be maintained at the 6-hour time point, we cannot exclude the possibility that key transcriptional changes occur at other timepoints. However the current data are consistent with the hypothesis that smaller, more frequent meals in VSG rats¹⁸ might reduce postprandial plasma lipids by altering the dynamics of lipid flux through the intestine.

In humans, it has been speculated that small, frequent meal intake may have benefits for metabolic health. Although the degree of lipidemia observed postprandially is determined by the fat content of the meal²⁵, it has recently been shown that enterocytes abide by a “last in-last out”²⁶ phenomenon whereby triacylglycerols consumed at breakfast may not appear in the plasma until after lunch. Our data raise the possibility that smaller meals consumed by VSG animals¹⁸ may reduce plasma lipid excursions by reducing the size of the lipid pool which is released from the enterocyte postprandially. This would also explain why VSG-operated rats exhibit lower plasma triglycerides following a lipid gavage designed to eliminate inter-group differences in meal size since the preceding meal was smaller in the

VSG animals. Thus, we hypothesize that eating smaller, more frequent meals may alter the rate by which stored intestinal lipids are mobilized, in effect “metering out” triglycerides more gradually as they are released in smaller, more frequent boluses and leading to weight-independent reductions in plasma lipid levels in VSG rats during *ad libitum* feeding conditions. In humans, reduced post-meal lipid levels might translate into reduced cardiovascular risk in patients receiving VSG, as high postprandial lipid levels have been shown to promote atherosclerosis²⁷.

We were surprised to find malabsorption in VSG animals following a large intragastric lipid bolus (Supplemental Figure 4). This malabsorption was despite a lack of intestinal malabsorption during a 24-hour period of *ad libitum* HFD consumption¹⁸. The fat content of the gavaged bolus did not exceed the size of meals consumed *ad libitum*: VSG animals freely consume small meals of about 5.77 kcal (1.27 ± 0.10 grams of HFD per meal, with a caloric density of 4.54 kcal/g¹⁸), whereas the malabsorbed gavage dose contained an average of 4.5 kcal (0.5 fat g). However, the nonphysiologic nature of the gavaged meal may be responsible for impaired lipid absorption in VSG animals. Intragastric gavage bypasses the cephalic response to food and is delivered more rapidly to the stomach than a meal consumed *ad libitum*. Furthermore, meal size appears to have a relationship to the degree of malabsorption, as a smaller dose (0.28g lipid; Figures 3 and 5) minimized inter-group differences and increased absorption in all groups. Equivalent post-meal levels of isotope-labeled lipid across groups (Figure 5) eliminates the possibility that malabsorption might account for inter-group differences. However, at larger doses of gavaged lipid, certain hypothesized features of VSG, including accelerated gastric emptying²⁸⁻³⁰, might promote malabsorption. Future exploration into the effects of VSG on GI physiology will help to clarify these issues in the future.

Our data highlight several important points regarding weight loss and lipid metabolism. First, weight loss either via caloric restriction (PF) or VSG does not appear to alter the expression of several target genes known to be affected by HFD consumption. High-fat diets have been reported to increase intestinal absorptive capacity by enhancing the expression of several genes including FATP4, I-FABP, L-FABP, CD36, ApoC2, ApoAIV, and MTP³¹. We did not detect any weight-dependent changes to the expression of these genes in the proximal intestine, suggesting that weight loss alone (without changes to dietary composition) cannot reverse the hyperabsorptive effect of HFD on the intestine, at least at the level of the transcriptional machinery.

Second, our data show, for the first time, that both VSG and caloric restriction (PF) enhance plasma bile acid levels (Figure 4) relative to diet-induced obese (SHAM) animals. We have not defined a mechanism for weight-related alteration to plasma bile acids, but we do report reduced expression of hepatic CYP7A1, the rate-limiting step in hepatic bile acid synthesis, in VSG animals (Table 1). Plasma bile acid levels are influenced by a number of variables, including hepatic bile acid synthesis and ileal bile acid reabsorption efficiency. Plasma bile acids might become elevated in VSG vs. CHOW or PF animals due to distinct mechanisms, such as enhanced ileal reabsorption secondary to accelerated delivery of bile acids to ileal transporters, but the current data cannot distinguish among these explanations.

Collectively, these data provide critical insights into an important secondary benefit to VSG as one model of bariatric surgery. Atherosclerosis can be caused by high postprandial lipid levels²⁷ which are characteristic of obesity. As we determined herein, the improvements to lipid homeostasis observed in VSG-operated animals are most dramatic in the postprandial state. Understanding mechanisms by which VSG may elicit this improvement may thereby lead to the development of novel therapies for atherosclerotic cardiovascular disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. VSG reduces plasma lipids in a weight-independent manner

A: Blood from 4-h fasted animals was sampled on post-operative day 50. At this time, VSG animals weighed less than SHAM ($P < 0.05$) animals. CHOW rats were lighter than SHAM ($P < 0.001$), VSG ($P < 0.05$), and PF ($P < 0.01$) rats on the day of study. B: Fat mass was reduced in VSG ($P < 0.001$) and PF ($P < 0.05$) animals as compared with SHAM. CHOW animals had reduced fat mass as compared with SHAM ($P < 0.001$), VSG ($P < 0.05$), and PF ($P < 0.001$) animals. Lean mass was unaffected by surgery, as compared with SHAM and PF groups. CHOW animals had reduced lean mass as compared with SHAM ($P < 0.05$) and VSG ($P < 0.05$) animals. C: Plasma triglyceride levels were reduced in VSG and CHOW animals as compared with SHAM ($P < 0.01$ vs. VSG, $P < 0.001$ vs. CHOW) and PF ($P < 0.01$ vs. VSG, $P < 0.001$ vs. CHOW) rats. D: Plasma cholesterol levels were reduced by VSG as compared with SHAM ($P < 0.001$) and PF ($P < 0.05$). E: Phospholipid levels were reduced in plasma from VSG and CHOW animals as compared with either SHAM or PF rats ($P < 0.001$ for all comparisons). F: Plasma NEFA levels were reduced in CHOW animals as compared to PF ($P < 0.05$) but were not reduced significantly by VSG.

Figure 2. Reductions in plasma lipids are most dramatic during short periods of fasting
 Triglyceride and cholesterol levels were measured in plasma sampled at 0, 4, 8, and 24 hours of fasting. A: In unfasted blood, triglycerides were reduced by VSG as compared with SHAM ($P < 0.001$) and PF ($P < 0.01$). Triglycerides were also reduced in VSG animals after 4 and 8 hours of fasting ($P < 0.001$ vs. SHAM at each time point), but this reduction was not weight-independent ($P > 0.05$ vs. PF). B: Area under the curve for plasma triglycerides across 24 hours of fasting was reduced for VSG animals as compared with either SHAM ($P < 0.001$) or PF ($P < 0.05$). C: Plasma cholesterol did not differ significantly between groups across the 24 hour fast. D: Area under the curve for plasma cholesterol across the 24 hour study was unchanged by surgery or weight loss. E: Cholesterol in fractionated plasma from VSG rats did not reveal a shift in the size of plasma lipoproteins. However, VSG improved fasting hypercholesterolemia observed in SHAM animals, as demonstrated by reduced cholesterol content in VLDL and HDL peaks. The reduction in HDL cholesterol content appears to be unique to VSG vs. PF or CHOW groups.

Figure 3. VSG impairs dietary triglyceride secretion without affecting fasting triglyceride secretion

A: Under fasted conditions, plasma triglycerides were increased along a similar trajectory in all animals after a 1 g/kg i.p. dose of P-407 (treatment X time, interaction $P=0.6684$; effect of treatment, $P=0.7272$; effect of time, $P<0.0001$). B: VSG did not affect the rate of appearance of triglycerides in the blood across the 24-hour experiment (ANOVA: $P=0.6053$). C: After an i.p. injection of 1 g/kg of P-407, a 0.47 g/kg lipid gavage resulted in an attenuated appearance of triglycerides in the plasma of VSG animals as compared with PF ($P<0.001$ at 4- and 6-hour timepoints) and SHAM ($P<0.01$ at 4-hour timepoint and $P<0.001$ at 6-hour timepoint). Interaction of treatment and time, $P<0.0001$. D: The rate of appearance of triglycerides in the plasma during the 6-hour experiment was reduced by VSG as compared with SHAM ($P<0.01$) and PF ($P<0.001$) animals. E: Plasma ApoB48 ($P=0.1193$), ApoB100 ($P=0.8792$), and total ApoB ($P=0.6486$) content were unchanged 6 hours after the oral lipid load. VSG did not significantly alter the ratio of triglyceride to total ApoB at this time point ($P=0.3502$).

Figure 4. VSG does not affect hepatic lipid storage

A: Plasma bile acid content is increased by weight loss (vs. SHAM: $P < 0.05$ for VSG, $P < 0.05$ for PF, and $P < 0.001$ for CHOW; $P = 0.0006$). B: Plasma bilirubin levels did not differ among groups ($P = 0.0776$). C: Hepatic triglyceride content was unaffected by VSG ($P = 0.3902$). D: Cholesterol content was unchanged in livers from VSG animals ($P = 0.3920$). E: No differences in the ratio of hepatic cholesterol ester to total cholesterol content were detected ($P = 0.7771$). F: Wet liver weight (expressed as a ratio to body weight) did not differ among groups (0.2831).

Figure 5. VSG does not affect the size of intestinal lipid storage pools

A: ^{13}C enrichment in M1 and M2 intestinal samples after ^{13}C -Triolein gavage did not differ between groups (M1, $P=0.1274$; M2, $P=0.9866$). B: Weight of neither M1 ($P=0.5527$), M2 ($P=0.7453$), M3 ($P=0.9788$), nor M4 ($P=0.8089$) was affected by VSG surgery or by weight loss. C: Triglyceride content did not differ among groups for any quartile of the small intestine (M1, $P=0.6202$; M2, $P=0.9445$; M3, $P=0.2394$; M4, $P=0.4164$). D: Differences in cholesterol content were not detected in any gut region (M1, $P=0.5310$; M2, $P=0.8361$; M3, $P=0.3177$; M4, $P=0.7376$). E: No obvious differences in the morphology of intestinal villi in cresyl violet-stained M2 sections were observed.

Table 1

Expression of genes known to affect hepatic lipid uptake.

Gene	SHAM	VSG	PF	P
CD36	100.0 ± 21.14	79.16 ± 10.78	54.30 ± 10.40	0.0975
L-FABP (1)	100.0 ± 14.82	71.11 ± 6.121	79.15 ± 11.60	0.1598
FATP4	100.0 ± 6.224	89.97 ± 5.556	81.45 ± 5.817	0.1329
ACAT2	100.0 ± 16.91	165.8 ± 33.36	78.03 ± 30.15	0.0981
DGAT1	100.0 ± 5.066	93.62 ± 4.983	79.03 ± 7.389	0.0656
MGAT2	100.0 ± 13.39	66.71 ± 9.168	67.41 ± 8.861	0.0507
MTP	100.0 ± 10.59	92.46 ± 4.408	85.04 ± 10.40	0.4932
APOB	100.0 ± 7.986	79.74 ± 6.043	67.55 ± 6.164	0.0107*
APOA2	100.0 ± 9.322	106.6 ± 6.865	102.4 ± 4.575	0.7981
APOC2	100.0 ± 8.895	85.2 ± 5.433	94.64 ± 10.82	0.4287
APOC3	100.0 ± 9.444	75.71 ± 5.019	85.82 ± 8.247	0.0801
CYP7A1	100.0 ± 27.04	46.71 ± 9.300	85.96 ± 25.74	0.1687
CYP27A1	100.0 ± 19.38	122.0 ± 7.334	109.3 ± 13.47	0.4755
HMGCR	100.0 ± 15.36	129.3 ± 20.20	68.08 ± 11.27	0.0528
SCARB1	100.0 ± 9.981	101.1 ± 8.118	82.61 ± 9.082	0.2892

Table 2

Expression of genes affecting intestinal lipid metabolism are unaffected by VSG.

Gene	M1				M2			
	SHAM	VSG	PF	P	SHAM	VSG	PF	P
CD36	100±18.52	69.96±23.94	128.5±39.27	0.3469	100±21.17	57.96±16.3	81.32±14.71	0.2392
FATP4	100±13.16	76.42±17.40	89.75±23.22	0.6533	100±15.05	92.31±12.17	88.34±10.62	0.8235
L-FABP (1)	100±20.39	61.03±21.58	96.29±20.55	0.3477	100±20.28	62.23±17.45	76.87±23.23	0.4131
L-FABP (2)	100±20.46	144.7±63.31	163.4±60.26	0.7320	100±21.08	63.34±16.14	83.79±18.52	0.3653
ACAT2	100±13.62	89.71±16.28	93.44±18.63	0.9084	100±15.52	133.9±16.27	105.1±20.25	0.3200
DGAT1	100±17.05	79.43±15.15	112.3±24.94	0.4614	100±19.05	81.38±14.06	88.65±14.14	0.6997
MGAT2	100±10.10	75.01±8.65	93.52±12.85	0.2234	100±13.61	86.7±9.580	98.8±11.78	0.6455
MTP	100±15.62	65.73±14.41	108.3±25.57	0.2356	100±18.59	93.10±16.06	94.89±12.97	0.9531
APOA4	100±30.44	97.55±61.22	162.6±59.35	0.6469	100±25.90	81.49±31.97	113.7±32.12	0.7584
APOB	100±22.71	87.68±30.61	132.4±41.95	0.6143	100±24.86	72.78±19.96	96.54±17.76	0.5927
APOC2	100±24.87	74.03±32.52	137.4±42.51	0.4229	100±20.32	91.04±32.43	104.1±21.4	0.9388
APOC3	100 ± 17.77	63.4 ± 17.27	89.96±24.15	0.3952	100±19.68	72.68±23.36	96.73±20.79	0.6156