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Vertical Sleeve Gastrectomy Restores Glucose Homeostasis in Apolipoprotein A-IV KO Mice

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OBESITY STUDIES

Bariatric surgery is the most successful strategy for treating obesity, yet the mechanisms for this success are not clearly understood. Clinical literature suggests that plasma levels of apolipoprotein A-IV (apoA-IV) rise with Roux-en-Y gastric bypass (RYGB). apoA-IV is secreted from the intestine postprandially and has demonstrated benefits for both glucose and lipid homeostasis. Because of the parallels in the metabolic improvements seen with surgery and the rise in apoA-IV levels, we hypothesized that apoA-IV was necessary for obtaining the metabolic benefits of bariatric surgery. To test this hypothesis, we performed vertical sleeve gastrectomy (VSG), a surgery with clinical efficacy very similar to that for RYGB, in whole-body apoA-IV knockout (KO) mice. We found that VSG reduced body mass and improved both glucose and lipid homeostasis similarly in wild-type mice compared with apoA-IV KO mice. In fact, VSG normalized the impairment in glucose tolerance and caused a significantly greater improvement in hepatic triglyceride storage in the apoA-IV KO mice. Last, independent of surgery, apoA-IV KO mice had a significantly reduced preference for a high-fat diet. Altogether, these data suggest that apoA-IV is not necessary for the metabolic improvements shown with VSG, but also suggest an interesting role for apoA-IV in regulating macronutrient preference and hepatic triglyceride levels. Future studies are necessary to determine whether this is the case for RYGB as well.

Bariatric surgery is currently the most effective therapy for sustained weight loss and improvements in metabolic comorbidities in obese and/or type 2 diabetic patients (1,2). Understanding the mechanisms associated with the success of bariatric surgery provides an opportunity to gain greater understanding of the contribution of the gastrointestinal (GI) tract in regulating energy and metabolic homeostasis. In turn, this understanding provides opportunities to pursue less invasive, and thus more widely implementable, treatment solutions for obesity and type 2 diabetes.

In this effort, we have established a mouse model of the vertical sleeve gastrectomy (VSG), a surgery where 80% of the stomach along the greater curvature is removed. Although less widely studied, the use of VSG is increasing by 30% per year and may soon surpass the frequency of use of the Roux-en-Y gastric bypass (RYGB); a more complex surgery which involves stomach size reduction and rearrangement of the GI tract. This is because VSG is less complicated, but the degree of weight loss and improvements in obesity-associated comorbidities are quite similar to those for RYGB (1,3). Indeed, the same molecular and physiological mechanisms have been proposed for both surgeries (4).

We have demonstrated similar weight loss–independent improvements in glucose homeostasis between VSG and RYGB performed in rats that are due to both increased insulin sensitivity and secretion (5). GLP-1, one of the most highly studied GI peptides that stimulates insulin secretion, is increased ~10-fold after both RYGB and VSG in both humans and rodents (5–9). Despite this, we (6) and others (10,11) have found that GLP-1 receptor activation is not required to achieve the metabolic benefits of either VSG or RYGB, respectively. This leaves open the

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question of the mechanism for the enhanced insulin secretion seen after both RYGB and VSG.

Both RYGB and VSG also improve plasma lipid profiles (12–15), but the mechanisms remain unknown. Recent clinical work demonstrates that patients who undergo RYGB have increased plasma levels of apolipoprotein A-IV (apoA-IV) over presurgical values, an effect not demonstrated in patients who underwent lifestyle intervention for weight loss and glucose control (16,17). However, this result is controversial as another study demonstrated an early (3 month) significant decrease in plasma apoA-IV levels, with a return to baseline values by 6 months postoperatively (18). apoA-IV is an apolipoprotein synthesized by the intestine and liver, and the physiological role of apoA-IV is strikingly similar to the physiological changes that occur with bariatric surgery. For example, in the intestine apoA-IV is packaged into chylomicrons by enterocytes after lipid ingestion (19); regulates satiety (20–23), intestinal lipid handling (24), and hepatic lipid handling (25); and has also been suggested to serve as an incretin to increase insulin secretion (26). At this time, it remains unknown whether apoA-IV levels change after VSG. However, the majority of the effects of apoA-IV are ones that are similar to the potent effects of VSG. Given the potential increase in apoA-IV seen with RYGB, and the striking parallels in the effects of RYGB and VSG, the purpose of the current study was to determine whether apoA-IV is a critical mechanism underlying the benefits of VSG on glucose and lipid homeostasis.

RESEARCH DESIGN AND METHODS

Animals

apoA-IV homozygote knockout (KO) mice were provided by Dr. J.L. Breslow (Rockefeller University, New York, NY) and were subsequently backcrossed >15 generations against a C57BL/6 background. Adult, age-matched, 8-week-old C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were maintained on a 12:00 A.M./12:00 P.M. lights on/lights off cycle, and at 8 weeks of age they were individually housed and given ad libitum access to a high-fat butter diet (4.54 kcal/g; 41% fat; Research Diets, New Brunswick, NJ) for a total of 6 weeks. Animals were then assigned to counterbalanced surgical groups within each genotype (n = 21 controls; n = 19 apoA-IV KO mice) based on fat mass (VSG or sham surgery) assessed by an EchoMRI whole-body composition analyzer (Echo Medical Systems, Houston, TX). All procedures for animal use were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Surgical Procedures

VSG and sham surgeries were performed under isoflurane anesthesia as described previously (6,27). Briefly, the lateral 80% of the stomach was excised, leaving a tubular gastric remnant in continuity with the esophagus proximally and the pylorus distally. The sham procedure involved the opening of the peritoneal cavity and the application of manual pressure on the stomach with blunt forceps along a vertical line between the esophageal sphincter and the pylorus. Mice consumed a liquid diet (Osmolite 1 Cal) for the first 4 postoperative days and were reintroduced to solid food (high-fat diet) on day 4. Postoperative deaths, mainly within the first week, yielded final group numbers of n = 10 for WT mice that had undergone sham surgery, n = 8 for WT mice that had undergone VSG, n = 8 for apoA-IV KO mice that had undergone sham surgery, and n = 6 for apoA-IV KO mice that had undergone VSG. The mice were maintained on high-fat diet after surgery except during the immediate postoperative period, and during diet choice testing, as noted. Body weight and food intake were measured daily for the first 2 weeks and then weekly after surgery.

Glucose Tolerance Tests

Oral (7 weeks postoperatively) and intraperitoneal (5 weeks postoperatively) glucose tolerance tests (IPGTTs) were performed to determine gut-dependent and gut-independent impacts on glucose tolerance, respectively. At 7:00 A.M., mice were fasted for 4 h, and a baseline blood sample was taken (0 min). Thereafter, 25% of 2 g/kg dextrose was delivered by intragastric gavage or intraperitoneal injection for the oral glucose tolerance test (OGTT) and IPGTT, respectively. Blood glucose was measured at 0, 15, 30, 45, 60, and 120 min after glucose administration on duplicate samples using ACCU-CHEK glucometers and test strips (Roche, Indianapolis, IN). All blood samples were obtained from the vein in the tip of the tail of freely moving mice.

In order to minimize stress, a separate OGTT was performed with a gavage of 2 g/kg dextrose plus acetaminophen (100 mg/kg); acetaminophen was used as a marker for the gastric emptying rate. This time point was used in previous studies to represent peak acetaminophen levels (28). Approximately 55 μL of blood was collected at baseline and after 15 min to measure blood glucose, plasma insulin, and acetaminophen levels. Blood was cold centrifuged, and plasma was stored at −80°C until insulin was assessed by ELISA (Crystal Chem, Inc., Downers Grove, IL) and acetaminophen was assessed with a spectrophotometric assay (Sekisui Diagnostics, Stamford, CT).

Macronutrient Selection

Macronutrient preference was assayed 8 weeks postoperatively using a macronutrient selection paradigm in which diets of pure carbohydrate (Teklad TD.02521; Harlan), fat (Teklad TD.02522; Harlan), and protein (Teklad TD.02523; Harlan) were presented in separate containers but simultaneously to the animals. The containers were weighed daily with the first 2 days of data considered as acclimation. Data from days 3–6 were collected, and the total caloric intake per day and the intake of each macronutrient per day were calculated and averaged over the 4 days.

Plasma Lipids

Eight weeks postoperatively, plasma lipids were determined in ad libitum–fed animals just prior to the onset of

dark; food was removed, and blood was sampled again after a 24-h fast. Blood was collected from the tail in heparin-coated microtubes and spun for 5 min. Plasma triglyceride and cholesterol levels were determined using Randox TG kits (Randox Laboratories, Crumlin, U.K.) and Infinity cholesterol kits (Thermo Electron, Noble Park, Victoria, Australia), respectively. Plasma phospholipids and nonesterified fatty acids were analyzed using phospholipid C and HR Series NEFA-HR(2) kits (Wako Diagnostics), respectively.

Gene Expression

Mice that had been fasted for 4 h were killed by CO_2 asphyxiation, and pieces from the liver, duodenum, jejunum, and ileum were flash frozen and stored at −80°C until RNA isolation. Total RNA was isolated, and cDNA was generated as described previously (29). Predesigned and validated Taqman PCR primer probes were used to assess apoA-IV (Mm00431814_m1), cholecystokinin (CCK) (Mm00446170_m1), and carnitine palmitoyl transferase 1A (CPT1a) (Mm01231183_m1) gene expression (Applied Biosystems, Carlsbad, CA). Apolipoprotein CIII (apoCIII) (forward, 5′GCATCTGCCCGAGCTGAAGAG3′; reverse, 5′CTGAA GTGATTGTCCATCCAGC3′) and sterol regulatory element-binding transcription factor 1 (SREBF1) (forward, CACAC CAGCTCCTGGATCG; reverse, GGCAGATAGCAGGATGC CAA) expression with 36B4 (forward, ATCCCTGACGCA CCGCCGTG; reverse, GCGCATCATGGTGTTCTTGC) as a housekeeping gene were analyzed using SYBR Green detection.

Western Blot Analysis

Ad libitum–fed plasma apoA-IV protein levels were determined via Western blot analysis as described previously (24). Briefly, 20 μL 2× SDS sample buffer was added to each sample, boiled for 5 min, and then loaded onto a 10% Tris-HCl gel, with a 4% stacking gel (Bio-Rad Laboratories, Hercules, CA). Gels were run at a constant voltage (80 V) until the protein standards were well separated. Proteins were then transferred to a polyvinylidene fluoride membrane (Bio-Rad Laboratories) for 1 h at a constant current of 350 mA. After nonspecific binding sites on the membranes were blocked for 1 h with a 5% solution of nonfat milk in Tris-buffered saline with 0.1% Tween (TBS-T), membranes were then incubated with goat anti-rat/mouse apoA-IV antibodies diluted 1:12,000 overnight at 4°C. After incubation, the blots were subsequently washed with nonfat milk in TBS-T, and then incubated with either horseradish peroxidase–conjugated goat anti-rabbit antibodies or with horseradish peroxidase–conjugated rabbit anti-goat antibodies (Dako, Glostrup, Denmark) diluted 1:20,000 with 2.5% nonfat milk in TBS-T at room temperature for 30 min. Detection was achieved by using the enhanced chemiluminescence system (ECL Western Blotting Detection Reagents, Amersham Biosciences, Buckinghamshire, U.K.), and X-OMAT AR films (Kodak) were used for development and visualization of the membranes.

Statistical Analysis

All data were analyzed by appropriate one- or two-way ANOVAs with a Tukey post hoc analysis to detect where significant differences lie. Data are presented as the mean ± SEM, and significance was accepted at $P < 0.05$.

RESULTS

Body Mass

Presurgical body mass was significantly lower in apoA-IV KO versus wild-type (WT) controls after 6 weeks on a high-fat diet (36.2 ± 1.0 g vs. 42.6 ± 0.7 g; $P < 0.05$). After surgery, animals that had undergone VSG, regardless of genotype, were significantly lighter compared with their counterparts that had undergone sham surgery (Fig. 1). It is important to note that mice continue to add both fat and lean mass throughout their life span, and bariatric surgery clearly did not stunt this growth curve.

Macronutrient Preference

Regardless of genotype, surgery reduced fat and increased carbohydrate intake (the main effect of surgery; $P < 0.05$; Fig. 2*A* and *B*). However, apoA-IV KO animals increased their intake of protein compared with WT animals and independent of surgery. When expressed as a percentage of total intake, the percentage of fat was lower in apoA-IV KO vs. WT mice and lower with VSG vs. sham surgery. Total caloric intake during the macronutrient preference test was significantly greater in apoA-IV KO mice vs. WT controls, regardless of surgery (main effect of genotype; $P < 0.05$; Fig. 2*C*).

Glucose Homeostasis

Intraperitoneal (Fig. 3*A* and *B*) but not oral (Fig. 3*C*) glucose tolerance was impaired in apoA-IV KO vs. control animals. Further, surgery improved both intraperitoneal (Fig. 3*A*; time × surgery interaction; $P < 0.05$) and oral glucose tolerance (Fig. 3*C*) compared with sham surgery among both WT and apoA-IV KO animals. In fact, after VSG, apoA-IV KO animals had similar glucose excursions after the intraperitoneal glucose load was administered compared with the WT animals that had undergone VSG, suggesting that the surgery normalized glucose homeostasis (Fig. 3*A* and *B*).

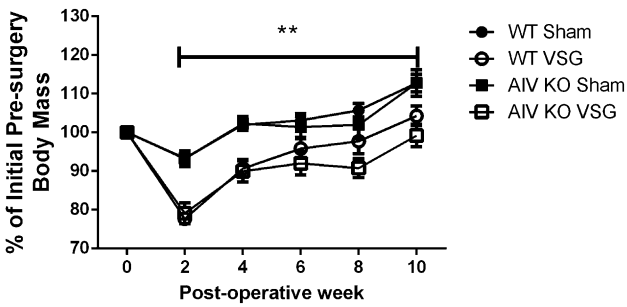

Figure 1—Body mass changes after VSG. Regardless of genotype, surgery caused a significant decrease in the percentage of presurgical body mass. $^{**}P < 0.01$, surgery × time interaction from 2 to 10 weeks postoperatively.

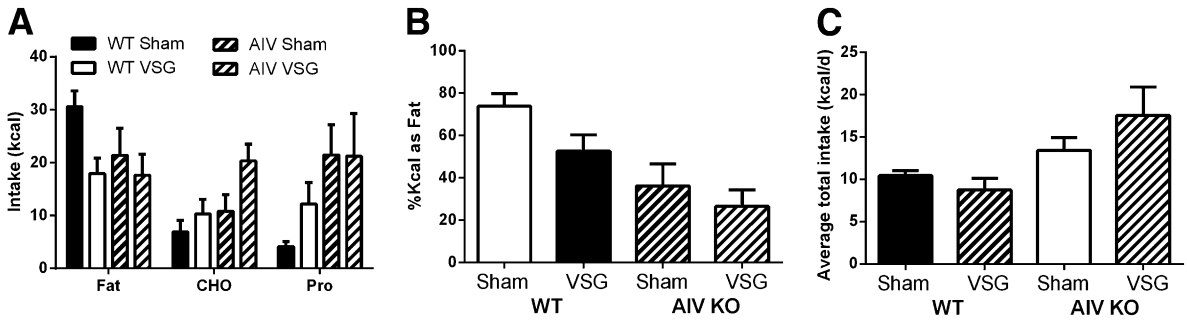

Figure 2—Macronutrient preference after VSG. *A*: Caloric intake of fat, carbohydrate, and protein when given a choice among the three macronutrients. Surgery reduced fat intake and increased carbohydrate intake, regardless of genotype ($P < 0.05$ for main effect surgery). However, the apoA-IV KO mice, regardless of surgery, had reduced fat intake and increased protein and carbohydrate intake ($P < 0.05$ for main effect of genotype). *B*: When calculated as a percentage of the total intake, fat preference was significantly reduced after VSG and in apoA-IV KO mice ($P < 0.05$ for main effects of surgery and genotype).

We separately examined glucose, insulin, and gastric emptying rates in apoA-IV KO versus WT animals after VSG and sham surgeries. Fifteen minutes after an oral glucose load, a time point when glucose levels were similar among the four groups (Fig. 3*D*), apoA-IV KO animals had significantly reduced plasma insulin responses ($P < 0.05$; main effect of genotype) regardless of surgery (Fig. 3*E*). The apoA-IV KO animals that had undergone VSG also had significantly increased gastric emptying rates compared with those of apoA-IV KO and WT mice that had undergone sham surgery and mice that had undergone VSG (Fig. 3*F*).

Lipid Homeostasis

Eight weeks postoperatively, fasting lowered plasma triglycerides and phospholipids in both genotypes and in both sham and VSG surgeries (Fig. 4*A* and *C*; main

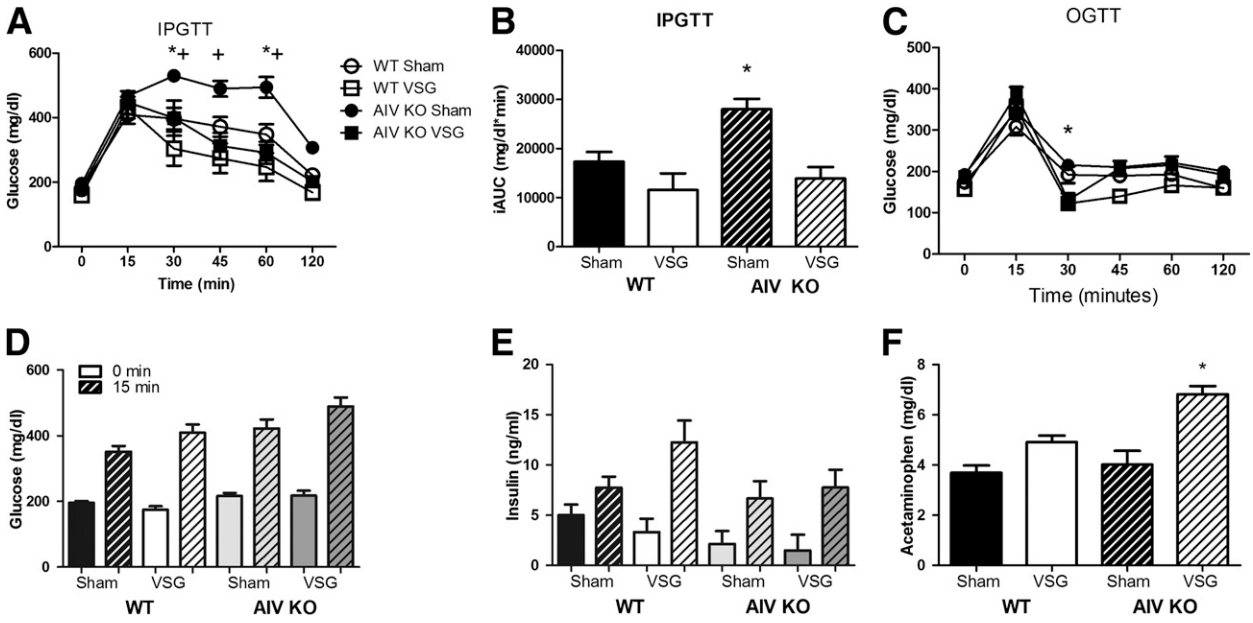

Figure 3—Glucose homeostasis after VSG. *A*: Five weeks postoperatively, the glucose excursion after a glucose load (2 g/kg i.p.) was greater in apoA-IV KO animals compared with WT animals at 30 and 60 min. VSG reduced the glucose excursion at 35–60 min in both WT and apoA-IV KO animals (*$P < 0.05$, time × genotype interaction; +$P < 0.05$ time × surgery interaction). *B*: apoA-IV KO animals that had undergone sham surgery had the greater glucose excursion when calculated as the integrated area under the curve (iAUC) compared with all other groups (*$P < 0.05$, genotype × surgery interaction). *C*: Seven weeks postoperatively, glucose excursion after an oral glucose load (2 g/kg) was significantly reduced by surgery at 30 min after the gavage (*$P < 0.05$, surgery × time interaction). *D*: On another occasion, glucose (2 g/kg) and acetaminophen (100 mg/kg) were gavaged, and glucose, insulin, and acetaminophen levels were measured 15 min after the gavage. Glucose levels were similar 15 min after the gavage. *E*: Insulin was increased 15 min after the gavage but was reduced overall in apoA-IV KO mice (main effect of genotype and time). *F*: Acetaminophen appearance in the plasma after a gavage is representative of the gastric emptying rate. Plasma acetaminophen levels were significantly greater after VSG in apoA-IV KO mice compared with all other groups (*$P < 0.05$, genotype × surgery interaction).

effect of time; $P < 0.05$). In addition, the apoA-IV KO mice had significantly reduced levels of plasma triglycerides, cholesterol, phospholipids, and nonesterified free fatty acids compared with WT animals (main effect of genotype; $P < 0.05$; Fig. 4*A–D*). However, there was also a main effect of surgery on plasma cholesterol and phospholipid levels (Fig. 4*B* and *D*).

Hepatic triglyceride levels were significantly lower in apoA-IV KO mice (main effect of genotype) and after VSG (main effect of surgery; Fig. 4*E*). When expressed as a percentage of sham-operated mice, apoA-IV KO mice had a significantly greater reduction in hepatic triglyceride levels compared with WT KO mice (51.0 ± 12.9% vs. 17.6 ± 3.6% in WT vs. apoA-IV KO mice, respectively; *t* test $P = 0.04$). There was no significant impact of genotype or surgery on hepatic cholesterol levels (Fig. 4*F*).

Gene and Protein Expression

As expected, and in confirmation of the genotyping, apoA-IV KO mice were devoid of apoA-IV mRNA in the duodenum, jejunum, and ileum (Fig. 5*A–C*). There was also no significant effect of surgery on intestinal apoA-IV gene expression (Fig. 5*A–C*). However, WT-VSG animals had a significant decrease in plasma protein and hepatic gene expression of apoA-IV compared with sham-operated WT animals (Fig. 6*A* and *B*).

To understand whether there was developmental compensation to account for the normal response to VSG in apoA-IV KO animals, we examined the gene expression of CCK and apoCIII. We found that apoA-IV KO animals had significantly reduced duodenal CCK and hepatic apoCIII gene expression that was independent of surgery (Fig. 7*A* and *B*). While we found no significant changes in hepatic CPT1a expression (Fig. 7*C*), we observed a trend toward a significant reduction in hepatic Srebf1 expression with surgery regardless of genotype ($P = 0.06$; Fig. 7*D*). Last, confirming previous reports (22), we found that both apoA-IV and apoCIII positively correlated with hepatic triglyceride levels (Fig. 8*A* and *B*).

DISCUSSION

apoA-IV is an intestinally derived protein that has demonstrated incretin effects (26) and is also critical in lipid metabolism (24,30). Although not entirely consistent, recent clinical research has suggested that RYGB could increase plasma apoA-IV levels (16). Moreover, many of the known metabolic effects of apoA-IV parallel the metabolic effects of VSG. While it is unknown whether, like RYGB, VSG also increases plasma apoA-IV levels in the clinical setting, because of the high degree of similar metabolic outcomes between the two surgeries, we hypothesized that apoA-IV would be integral to VSG-mediated benefits on glucose and lipid homeostasis. Surprisingly, we found that plasma apoA-IV levels decreased and apoA-IV deficiency did little to prevent the metabolic benefits of VSG. Consistent with our previous findings, VSG, in and of itself, improved body weight, glucose, and lipid metabolism, and reduced the preference

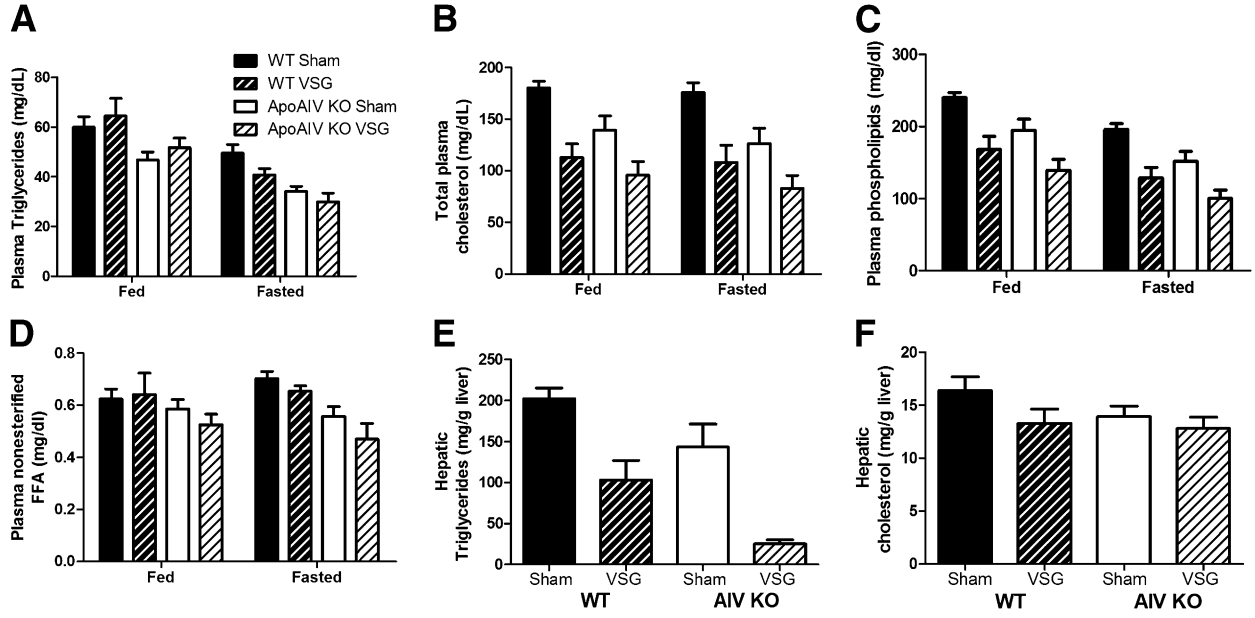

Figure 4—Lipid homeostasis after VSG. *A*: Eight weeks postoperatively, plasma triglyceride levels were reduced by fasting and were lower in apoA-IV KO vs. WT mice ($P < 0.05$ for main effect of postprandial state and genotype). *B*: Plasma cholesterol levels were reduced by VSG and in apoA-IV KO vs. WT mice ($P < 0.05$ for main effect of surgery and genotype). *C*: Plasma phospholipid levels were reduced by fasting, in response to surgery, and in apoA-IV KO vs. WT mice ($P < 0.05$ for main effects of postprandial state, genotype, and surgery). *D*: Plasma nonesterified fatty acids were reduced in apoA-IV KO vs. WT mice ($P < 0.05$ for main effect of genotype). *E*: Hepatic triglyceride levels were reduced by surgery and in apoA-IV KO mice ($P < 0.05$ for main effect of surgery and genotype). *F*: Hepatic cholesterol levels were similar between WT and apoA-IV KO mice, and did not change with surgery.

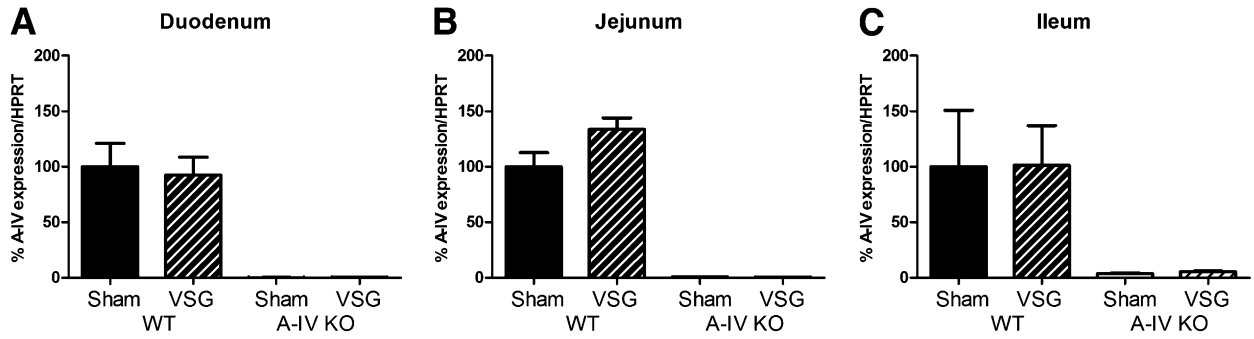

Figure 5—Intestinal gene expression of apoA-IV. *A–C*: apoA-IV expression was significantly greater in WT vs. apoA-IV KO animals in all three sections of the intestine ($P < 0.001$ for main effect of genotype).

for fat ingestion; however, there was no significant interaction between genotype and surgery. Unfortunately, due to faulty equipment we were unable to report body fat levels here; however, we (6,27,31) and others (32,33) have shown previously that VSG reduces body fat and maintains lean mass in mice. While we also confirmed previous work that apoA-IV deficiency impairs intraperitoneal glucose tolerance, notably, VSG was able to normalize this response. This change in glucose tolerance may be related to the fact that VSG induced a greater reduction in hepatic triglycerides in apoA-IV KO versus WT animals. These data suggest that apoA-IV deficiency and VSG have either additive or synergistic effects on reducing hepatic triglyceride levels. However, these data do not support the hypothesis that apoA-IV is necessary for the metabolic benefits of VSG.

One intriguing new finding, unrelated to surgery, is that when apoA-IV KO animals are given a choice among fat, carbohydrate, or protein ingestion, they have a reduced preference for fat compared with WT animals. This may not be surprising given that several studies have linked GI peptides to fat intake. Enterostatin, a peptide cosecreted with pancreatic lipase, and thus secreted during lipid ingestion, decreases the preference for a high-fat versus a low-fat diet (34). Exendin 4, a long-acting GLP-1 agonist, and peptide YY have also been found to increase carbohydrate but not fat preference (35,36); conversely, ghrelin stimulates fat preference (37). In addition, animals administered mercaptoacetate, a pharmacological inhibitor of intestinal fat oxidation (38,39), and animals null for CD36 (40), a fatty acid transporter, exhibited a decreased preference to ingest fat. This presents a model in which lipid-stimulated GI peptides as well as the inability to use lipids both serve as a negative feedback signal to the brain to prevent further lipid ingestion. As such, given that apoA-IV–deficient animals have a reduction in fat preference, our data suggest that a rise in intestinal apoA-IV levels is a signal to the central nervous system regarding lipid use rather than lipid appearance.

After VSG, both WT and KO mice had improved intraperitoneal glucose tolerance; in fact, the glucose curve in the apoA-IV KO mice is indistinguishable from that of the WT mice. VSG also improved oral glucose tolerance 30 min after the gavage in both groups of mice, but this effect is complicated by the fact that VSG

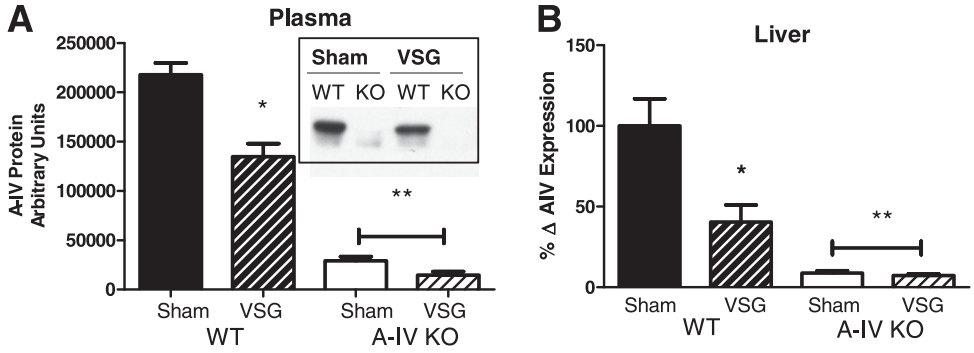

Figure 6—Plasma protein and hepatic expression levels of apoA-IV. *A*: Plasma levels of apoA-IV were significantly reduced in apoA-IV KO animals vs. WT animals, and after VSG in WT animals. Inset: Representative Western blot of sham-operated and VSG WT and KO animals. *B*: Hepatic gene expression of apoA-IV was significantly reduced by surgery in WT animals and was significantly lower in apoA-IV KO vs. WT animals. *$P < 0.05$ in WT VSG vs. WT sham-operated animals; **$P < 0.05$ apoA-IV vs. WT animals, genotype × surgery interaction.

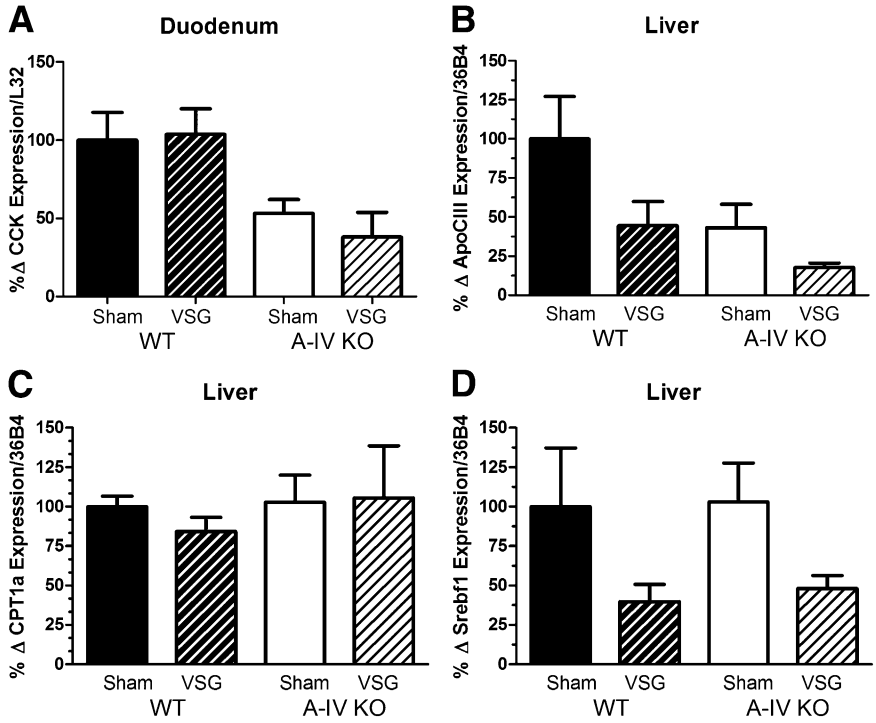

Figure 7—Gene expression changes after surgery. *A*: Duodenal CCK expression was significantly lower in apoA-IV KO vs. WT animals, regardless of surgery ($P < 0.05$ for main effect of genotype). *B*: apoCIII level was significantly reduced in apoA-IV KO vs. WT animals, regardless of surgery ($P < 0.05$ for main effect of genotype). The expressions of CPT1a (*C*) and Srebf1 (*D*) were not significantly different between groups.

increases the gastric emptying rate in rats (41), an effect that is seen clinically as well (42). Based on previous literature (28,43), and based on our findings that 100% of gastric contents were emptied within 5 min of a gavage after VSG in rats (41), we only measured plasma acetaminophen levels at one time point, which may have limited our ability to detect an effect of VSG on gastric emptying in WT mice. Regardless, the apoA-IV KO mice had a stronger increase in the gastric emptying compared with WT animals after VSG, but this was not sufficient to offset the mild improvement in OGTT. One report (44) has demonstrated that CCK has inhibitory effects on gastric emptying rate, and we observed a reduction in the expression of CCK in the apoA-IV KO mice, which may have minimal effects on gastric emptying rate under basal conditions but becomes important when CCK is combined with surgery. Overall, these data demonstrate that both surgery and apoA-IV act on multiple aspects of glucoregulation, and, interestingly, that VSG is able to correct deficiencies caused by a lack of apoA-IV.

In the WT animals, we found that VSG did not alter intestinal gene expression, but did significantly decrease plasma protein and hepatic expression levels of apoA-IV. apoA-IV is secreted from the intestine by being packaged into chylomicrons. apoA-IV then rapidly disassociates from the chylomicron and is found in the lipoprotein-free plasma fraction and in the HDL pool (45). Thus, the level of plasma apoA-IV is as an indicator of chylomicron

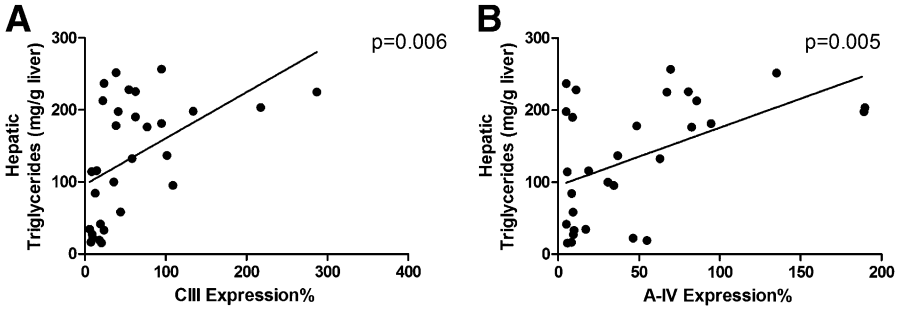

Figure 8—Regression analysis of hepatic triglycerides and hepatic gene expression. apoCIII (*A*) and apoA-IV (*B*) expressions were significantly correlated with hepatic triglyceride levels ($P < 0.01$).

production. Since VSG lowers intestinal chylomicron production (12), the lower apoA-IV level in the plasma could simply be representative of lower intestinal secretion of dietary lipids. Further, we measured plasma apoA-IV levels in ad libitum–fed animals during the middle of the light cycle. Since animals that have undergone VSG eat smaller, more frequent meals (46), the reduction in apoA-IV level could simply reflect the smaller meals, rather than being a direct effect of the surgery. However, a role for the reduction in hepatic expression of apoA-IV in the reduction in plasma levels of apoA-IV cannot be ruled out.

The physiological relevance of the reduction in hepatic apoA-IV gene expression after VSG may be related to the changes in hepatic triglyceride levels themselves. Genetic deletion and transgenic overexpression of apoA-IV in mouse models of hepatic steatosis lead to decreased and increased hepatic triglyceride levels, respectively (25). Multiple reports (25,47,48) also demonstrate that levels of hepatic apoA-IV and hepatic triglycerides rise and fall in parallel. In addition, apoCIII, another apolipoprotein in the same gene cluster as apoA-IV, which functions to reduce both hydrolysis and clearance of triglyceride-rich lipoproteins (49,50), was also significantly reduced in the livers of apoA-IV KO mice, and there was a trend for it to be reduced by VSG. Consistent with this, a reduction in hepatic triglyceride levels has also been seen in association with a downregulation of apoCIII (51). Indeed, we saw significant correlations of hepatic apoA-IV and apoCIII levels with hepatic triglyceride levels. Although it remains unclear what comes first, a reduction in apoA-IV and apoCIII levels or the change in hepatic triglyceride levels, it is possible that the reduction of both of these apolipoproteins may simply be secondary to the fall in hepatic triglyceride levels that occurs with VSG. While our data indicate that a decrease in apoA-IV is not necessary for the changes in hepatic triglycerides, they do not preclude a role for apoCIII, which will be an interesting target for further study.

Our data are in contrast to a couple of studies (16,18) where fasting apoA-IV plasma levels were found to be increased following RYGB surgery. However, this is not a universal finding. Similar to the current study with VSG, another study (17) found significantly reduced plasma apoA-IV levels at 1 and 3 months after RYGB, yet, at 6, 9, and 12 months postoperatively, apoA-IV levels were not significantly different than baseline. Regardless of this controversy, and despite the wide range of metabolic similarities between VSG and RYGB (4), it may be that apoA-IV is an important contributor to the benefits of RYGB but not VSG. Interestingly, if humans have reduced levels of apoA-IV after VSG, as was observed here in mice, this reduction could actually undermine the weight and metabolic benefits of VSG. This brings up the intriguing possibility that apoA-IV replacement therapy could be a therapeutic option for patients who have suboptimal results from VSG.

On the other hand, apoA-IV is a part of a gene cluster consisting of apolipoprotein AI, apoCIII, and apolipoprotein A-V, genes encoding apolipoproteins that are important for regulating lipid metabolism. Although contradictory data exist (52,53), human polymorphisms in this gene cluster, specifically a threonine-to-serine substitution at residue 347, is associated with reduced plasma levels of apoA-IV (54), and increased BMI and plasma lipid levels (55–60). These data highlight the relevance of this gene to human physiology and indicate that obese patients with this polymorphism would be better served with VSG rather than RYGB.

Taken together, the current data indicate a potentially interesting additive effect of hepatic apoA-IV deficiency and hepatic triglyceride levels, but it remains that apoA-IV is not necessary to realize the full benefits of VSG on body mass or glucose and lipid homeostasis. Further, VSG was able to normalize the gross impairment in intraperitoneal glucose tolerance in apoA-IV KO animals. These data suggest that patients with apoA-IV gene abnormalities may respond well to VSG. Future research is needed to determine whether apoA-IV is a distinguishing mechanism between VSG and RYGB.

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