

Alterations in Serum-based Breast Cancer Markers Following Bariatric Surgery in Rodent Models: Are They of Clinical Value?

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Abstract

Objective

Breast cancer is one of the most common malignancies in females worldwide. The percentage of adults with a body-mass index (BMI) of 25 kg/m² or greater is 38.0% in U.S. females. The increase in obesity epidemic in females is accompanied with an increase in bariatric procedures that not only lower weight but appear to lower risks of breast cancer. The objective of this paper is to explore the effects of bariatric surgery on multiple serum-based breast tumor markers in breast cancer rodent models.

Research design and methods

40 total mice were studied in C3Tag (1) transgenic model of breast cancer. Half of animals went through vertical sleeve gastrectomy (VSG) (one group on 60% HFD and one group on Chow) and were compared the other half undergoing sham surgery. Data collection was performed ~6 weeks post surgeries for both cohorts.

Results

Previously, our group has demonstrated positive impacts with VSG on glucose homeostasis, cholesterol, and body composition compared to sham treated animals. This study was performed to explore the serum growth factor alterations following bariatric surgeries. 6 weeks post-surgery, we observed changes in serum-based biomarkers post bariatric surgery.

Conclusions

Bariatric surgery may provide improvements in growth factor serum levels that might be contributors to reduced breast cancer risk.

Limitations

This study is limited by small sample size and advanced breast cancer in an aggressive tumorigenesis rodent model. Further study is warranted.

Keywords

vertical sleeve gastrectomy (VSG); bariatric surgery; breast cancer

Introduction

The prevalence of obesity continues to rise at a significant pace in most developed countries [1]. Long term consequences of obesity include cardiovascular complications and diabetes-related morbidity and mortality [2]. However, obesity more recently has been recognized as an important risk factor for the development of cancer, including breast cancer in women [3]. Strikingly, there is accumulating evidence that demonstrates that obesity is directly related to increased morbidity and mortality following cancer diagnosis [4]. Accomplishing a reduction in body weight may therefore be beneficial in reducing the risk of developing cancer but may also improve treatment outcomes following diagnosis. The most effective current strategy to induce weight loss is bariatric surgery.

The most universally implemented bariatric surgeries include: open or laparoscopic Roux-en-Y gastric bypass (RYGB) and vertical sleeve gastrectomy (VSG) [5]. These surgeries result in significant improvements in glucose and insulin profiles, with some patients being able to discontinue type 2 diabetes treatment only a few days after surgery [6].

Besides the well-documented improvements in cardiovascular health and glucose metabolism related to surgery-induced weight loss, these surgeries have also been related to a reduction in the incidence of breast cancer [7, 8]. The question remains if this reduction in breast cancer occurrence is related to weight loss alone, or if there are other mechanisms triggered following these surgeries that may be detrimental to tumor growth. Weight loss following bariatric surgery was thought to be the result of mechanically restricting nutrient intake and absorption.

However, currently it is accepted that the weight-loss and metabolic improvements induced by bariatric surgery are also caused by physiological changes of the intestinal tract [9]. Some of the physiological impacts of the surgery include changes in GI function and morphology [10], changes in secreted gut peptides such as ghrelin, GLP-1, GLP-2 and cholecystokinin (CCK) [11], bile acid levels and composition [12] and even rearrangements of microbial composition [13, 14].

Recently it has been shown that bariatric surgery reduces the inflammatory state as well as a reduction in local hypertrophy of the adipose tissue in rodents [15]. Adipocyte hypertrophy resulted from obesity leads to systemic inflammation [16, 17]. Several lines of evidence suggest that inflammation has crucial roles to the initiation, promotion, and progression and metastasis in breast cancer [18, 19]. It is suggested that the systematic inflammation, as well as the local inflamed adipose tissue of the breast could form a niche to promote a favorable environment for breast cancer [20]. Together these effects could result in lowering breast cancer risk in obese women.

Therefore, one possibility is that surgery could reduce inflammation and circulating markers of inflammation that include a variety of growth factors. In the studies described here, we performed VSG in a transgenic rodent model destined to develop breast cancer. In this model, atypia of the mammary ductal epithelium develops at 8 weeks of age, and mammary intraepithelial neoplasia (MIN) are progressed by 12 weeks. Invasive and metastatic carcinomas develop in 100% of animals by 16 weeks of age [21]. This model is an established model of mammary and prostate cancers similar to human cancers. Here we measured a variety of serum-based endpoints to determine whether they are reduced after VSG. We also performed SCFA quantification through gas chromatography, in order to identify the potential benefits of VSG on gut bacteria by products.

Methods

Animals

For all experiments we used the C3Tag (1) mouse model. The breeding animals were donated to the Seeley lab from Jeffrey E Green at the National Institute of Health (NIH). Invasive and metastatic carcinomas develop in 100% of animals by 16 weeks of age. Two cohorts of age-matched (6 weeks) C3Tag (1) female mice (Cohort one: N=30; Cohort two: N=29), body weight (22.46 +/- 2.3 g at arrival; Jeffrey E Green Laboratories, NIH, Bethesda, MD) were individually housed and maintained on a 12/12-h light/dark cycle at 25°C and 50–60% humidity. The initial total number of animals in cohort one was sixty and in cohort two was forty. In cohort one 12 animals and in cohort two 7 animals were sacrificed due to over limit tumor size before the termination point. Twenty-two animals died because of unknown reasons, likely due to surgery complications. Animals were matched by age because of the complications involved in matching the tumor developing age required for experiments' time points. Further, animals were randomized based on their body mass to minimize complicating different weight gain trajectories in response to 60% high-fat diet. Following acclimatization to the facilities, animals had *ad libitum* access to water and standard chow (manufacturer) or a palatable 60% high-fat diet (D12451, Research Diets, New Brunswick, NJ) for 2 weeks prior to surgery and maintained on the diet until the studies were terminated. Animals were assigned to receive either Sham or VSG surgery in a counterbalanced fashion by body weight. All procedures for animal use were approved by the University of Michigan Animal Care and Use Committee (Protocol number: PRO00008242) and follow the guidelines outlined in the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Surgical procedures

Four days prior to surgery, body composition was assessed using an EchoMRI™ analyzer (EchoMRI LLC, Houston, TX). Solid food was removed 24hrs prior to surgery and mice got *ad lib* access to a liquid diet (Osmolite 1.0 Cal, Abbott). Here we describe both surgical procedures in details:

Vertical Sleeve Gastrectomy

Animals were anesthetized by isoflurane inhalation and received subcutaneous injections of buprenorphine hydrochloride (0.1 mg/kg) and meloxicam (0.5 mg/kg). A midline abdominal skin incision is made, followed by an incision in the underlying muscle. The stomach is exposed and transected to form a sleeve. Sleeve formation consists of the removal of 80% of the stomach using an ETS 35-mm stapler (Ethicon Endo-Surgery), leaving behind 20% of the stomach which is a tubular gastric “sleeve”. The sleeve portion of the stomach is placed back into the abdominal cavity and the muscle and skin of abdominal wall are sutured in separate layers.

Sham Surgery

Animals were anesthetized by isoflurane inhalation and received subcutaneous injections of buprenorphine hydrochloride (0.1 mg/kg) and meloxicam (0.5 mg/kg). An abdominal laparotomy was performed, light manual pressure was applied with to the exteriorized stomach, and then the abdomen was closed in layers. After surgery, animals are put on a nutritionally complete Osmolite liquid diet which is replaced with pre-surgery solid diets on the fourth day post-operation. Post-surgical analgesia consisted of daily subcutaneous injection of an NSAID (e.g. Meloxicam) for at least 3 days.

Body weight and body composition

Measurements were collected in two cohorts for these studies. Cohort one refers to the first cohort where the main purpose was the growth factor measurements after VSG. Second cohort was used mainly for the SCFA measurements and is referred to cohort two in this paper. In all cohort of VSG and sham animals, body weights were measured daily for the first week following surgery as a post-op care routine and once weekly for the duration of the study. Body composition was determined in live animals 4 days prior to surgery, and then cohort one 5 weeks and cohort two 4 weeks following surgery via EchoMRI™ Analyzers.

Preparation of Plasma Samples

Plasma were collected in mice upon sacrificing. Plasma collection was performed using EDTA as an anti-coagulant. Samples were centrifuged for 10 minutes at 1000xg within 30 minutes of blood collection. Plasma was removed and aliquots were made to reduce freeze/thaw cycles for further analysis. Samples were stored at ≤ -80°C.

Biomarker Quantification

To identify specific hormones that change following bariatric surgeries in animal models high at risk of breast cancer, it was necessary to screen panels of growth factor hormones. Biomarker quantifications was performed by Luminex 200™ System and Luminex XY Platform™. Simultaneous quantification of several analyses was performed according to manufacture protocol in serum samples (Luminex®).

High-sensitivity detection of short-chain fatty acids in colon by gas chromatography-mass spectrometry

High-sensitivity detection of SCFA in colon was measured using the gas chromatography Agilent 69890N GC -5973 MS detector.

1) Sample preparation: ~50 mg of tissue was transferred to a pre-weighed locked Eppendorf tube and 600 uL of 30 mM hydrochloric acid containing isotopically-labeled acetate (150uM), propionate (75uM), butyrate, isobutyrate, valerate (30 uM), isovalerate, hexanoate, heptanoate and octanoate (15 uM) was added to each sample. Samples were homogenized in a Bullet Blender Gold at speed 8 for 5 minutes. Samples were vortexed for 10 seconds, incubated for 10 minutes on wet ice, and then vortexed again for 10 seconds. Samples were then centrifuged at 1500 *ref*, 4 °C for 10 minutes and the supernatant transferred to a 1 mL glass tube. 20 uL of each sample was removed to a separate glass vial to create a pooled sample for QC purposes. 300 uL of methyl tert-butyl ether (MTBE) was added to each sample and the mixture vortexed for 10 seconds to emulsify, then held at 4 °C for 5 minutes, and vortexed again for 10 seconds. Samples were centrifuged for 1 minute to separate the solvent layers and the MTBE layer was then removed to an autosampler vial for GC-MS analysis. A series of calibration standards were prepared along with samples to quantify metabolites. Post-extract, tissues were taken to dryness in speedvac and the Eppendorf tube re-weighed to obtain the dry tissue weight for normalization.

2) GC-MS analysis: GC-MS analysis was performed on an Agilent 69890N GC -5973 MS detector with the following parameters: a 1 μ L sample was injected with a 1:10 split ratio on a ZB-WAXplus, 30m x0.25mmx0.25um (Phenomenex Cat#7HG-G013-11) GC column, with him as the carrier gas at a flow rate: 1.1ml/min. The injector temperature was 240 °C, and the column temperature was isocratic at 310 °C.

3) SCFAs Data analysis: Data were processed using Mass Hunter Quantitative analysis version B.07.00. SCFAs were normalized to the nearest isotope labeled internal standard and quantitated using 2 replicated injections of 5 standards to create a linear calibration curve with accuracy better than 80% for each standard. Data were processed using Mass Hunter Quantitative analysis version B.07.00. Metabolites in the glycolysis/tca/ppp pathways were normalized to the nearest isotope labeled internal standard and quantitated using 2 replicated injections of 5 standards to create a linear calibration curve with accuracy better than 80% for each standard. Other compounds in the analysis were normalized to the nearest internal standard, and the peak areas were used for differential analysis between groups. Samples were normalized to dry sample weight after quantification.

Statistical analysis

Data are expressed as average \pm standard error of the mean (SEM). Between groups differences were analyzed for statistical significance using one-way ANOVA testing combined with post hoc Tukey when appropriate. For time dependent analysis repeated measures (RM) ANOVA were performed. Differences between data were considered statistically significant when $P < 0.05$. Statistical tests were performed in IBM SPSS v.23, all graphs were created in Graph pad Prism 7.0.

Results

Clinical data suggest that bariatric surgery reduces breast cancer risk in females [22]. To study the possible relation between VSG and carcinogenesis, two separate cohorts of the rodent model of breast cancer C3Tag (1) were generated to test the influence of VSG on 1) the composition of circulating biomarkers related to cancer in this cancer model (cohort one) and 2) the short chain fatty acid composition of the colon (cohort two). C3Tag (1) transgenic mice overexpress the 5' flanking region of the C3 (1) component of the rat prostate steroid binding protein (PSBP) and targets the expression of the SV40 large T-antigen (Tag) to the epithelium of mammary glands.

Cohort One: Effects of bariatric surgery on circulating levels of growth factor breast cancer markers

A. Body weight and composition

C3Tag (1) mice fed a high fat diet seem to be unresponsive to VSG at the level of long-term body weight regulation. C3Tag (1) mice fed a chow diet combined with VSG are lower in body weight over the duration of the study (fig 1a), but the body weight over time is only significantly different from the Sham-HFD group ($P < 0.01$, $F_{34, 459} = 2.282$, rm-ANOVA post hoc Tukey). As depicted in figure 1A, actual body weights in the VSG-Chow group are lower compared to the Sham-HFD group at days -7, 0, 1-7, 28, 49, 63, and 70 post-surgery ($*P < 0.05$, $**P < 0.01$, one-way ANOVA post hoc Tukey).

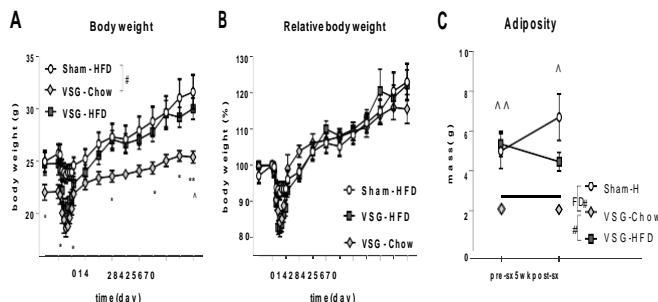


Figure 1: Cohort One, Body weight following VSG in C3Tag (1) is lower in mice on a chow fed diet.

A) Body weight dynamics over the duration of the study are different between the VSG-Chow fed mice and the Sham-HFD treated mice ($*P < 0.001$, rm-ANOVA post hoc Tukey), but not the VSG-HFD group. The chow fed VSG treated group was lower in body weight compared to the Sham-HFD group at multiple days pre- and post-surgery ($*P < 0.05$, $**P < 0.01$, post hoc Tukey). Body weight of the VSG-Chow group was only significantly lower compared to the VSG-HFD group at post-surgery day 70 ($*P < 0.05$, post hoc Tukey). B) Relative body weight change following surgery was not different between the three studied groups (rm-ANOVA post hoc Tukey). C) Total adipose tissue mass pre- and post-surgery as measured by NMR. Chow-VSG show reduced body adiposity during the period of the study compared to both Sham and VSG HFD fed groups ($*P < 0.001$, rm-ANOVA post hoc Tukey). Adiposity mass is lower pre-surgery compared to both HFD fed group, whereas at 5 weeks post-surgery the adiposity level of the VSG-Chow group is only lower compared to the Sham-HFD group ($*P < 0.05$, $**P < 0.01$, one-way ANOVA post hoc Tukey). Remarkably, only at day 70 post-surgery actual body weight between the chow and HFD fed VSG groups was significantly different ($*P < 0.05$, post hoc Tukey). When body weight over time was tested for main effects, both an effect of surgery ($P < 0.01$, $F_{17, 459} = 2.444$, rm-ANOVA) as well as an effect of diet ($P < 0.001$, $F_{17, 459} = 2.505$, rm-ANOVA) was found. In contrast, relative body weight dynamics following surgery over the duration of the study did not differ between groups (rm-ANOVA post hoc Tukey; fig. 1B). This suggests that actual body weight differences are primarily a result of the initial lower body weight of the chow fed mice at the moment of surgery. This difference in initial body weight is most likely a result of reduced adipose tissue mass in the Chow fed mice prior to surgery (Fig. 1C) compared to both HFD fed groups ($*P < 0.05$, $F_{2, 31} = 5.354$, one-way ANOVA post hoc Tukey), whereas at 5 weeks post-surgery adiposity levels in the VSG-Chow group were only reduced compared to the Sham-HFD group ($P < 0.01$, $F_{2, 31} = 7.978$, One-way ANOVA post hoc Tukey). Nonetheless a general reduction in adiposity levels was observed in the Chow-VSG group compared to both the Sham-HFD and VSG-HFD group ($P < 0.001$, $F_{2, 32} = 11.344$, rm-ANOVA post hoc Tukey).

B. Growth factors

Growth factors regulate development of the normal breast as well as the carcinogenesis of epithelium breast tissue. To assess the effects of bariatric surgery on circulating growth factors, we analyzed several of these factors that are the key mediators of breast cancer growth in serum from animals in each surgery group.

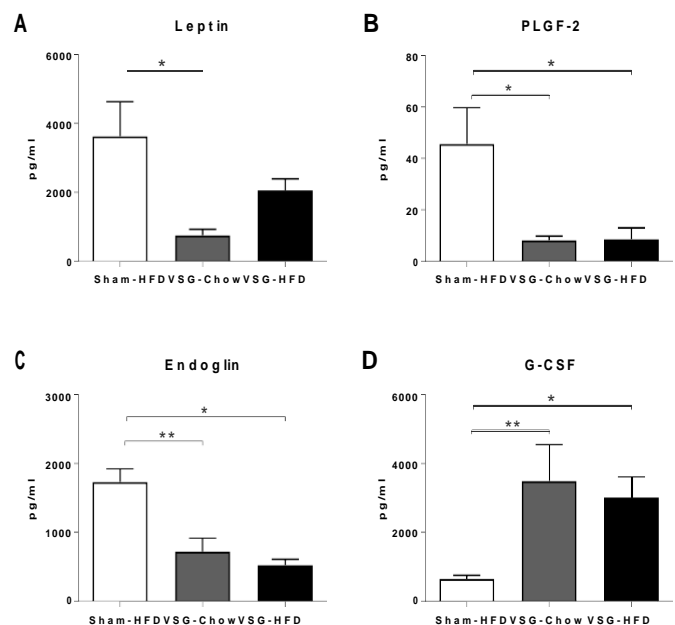


Figure 2: Cohort One, circulating growth factors in C3Tag1 mice following VSG surgery. Leptin (A), PIGF-2 (B), and endoglin (C) levels are decreased following VSG compared to Sham-HFD, whereas G-CSF (D) levels are increased following VSG surgery. $*P < 0.05$, $**P < 0.01$ one-way ANOVA post hoc Tukey

As depicted in figure 2, leptin levels (fig. 2A) were reduced in the VSG-Chow group compared to Sham-HFD ($P<0.05$, $F_{2,27}=5.095$, one-way ANOVA post hoc Tukey), additionally a main effect of surgery was observed for reduced leptin levels following VSG ($P<0.05$, $F_{1,27}=3.518$, glm-ANOVA). Compared to Sham-HFD, PIGF-2 levels (fig. 2B) were decreased in both the VSG-Chow ($P<0.01$) and VSG-HFD ($P<0.05$, $F_{2,28}=6.390$, one-way ANOVA post hoc Tukey), additionally a main effect of surgery was observed for reduced levels of PIGF-2 in the VSG groups ($P<0.05$, $F_{1,28}=7.648$, glm-ANOVA). Likewise, endoglin levels (fig. 2C) were reduced in both the VSG-Chow ($P<0.01$) and VSG-HFD ($P<0.05$, $F_{2,29}=18.744$, one-way ANOVA post hoc Tukey) groups compared to the Sham-HFD group, resulting in a main effect of surgery ($P<0.001$, $F_{1,29}=33.453$, glm-ANOVA). In contrast, circulating G-CSF levels (fig. 2D) were increased in the VSG-Chow ($P<0.01$) and VSG-HFD ($P<0.05$, $F_{2,28}=6.390$, one-way ANOVA post hoc Tukey) compared to the Sham-HFD group, resulting in a main effect of surgery ($P<0.01$, $F_{1,28}=8.070$, glm-ANOVA).

Biomarker	Sham-HFD Avg±sem	VSG-Chow Avg±sem	VSG-HFD Avg±sem	Surgery effect (P-value)
Follistatin (pg/ml)	297±116	556±118	908±261	0.035*
Interleukin-6 (pg/ml)	3.40±0.57	32.9±18.0	25.5±7.06	0.070
Prolactin (ng/ml)	16.3.9±3.31	28.5±5.50	27.4±4.93	0.080
Amphiregulin (pg/ml)	10.0±2.46	13.3±7.43	21.7±6.31	0.144
HGF (pg/ml)	451±48.7	794±168	932±323	0.180
MCP-1 (pg/ml)	17.7±2.71	24.9±8.19	25.6±7.91	0.356
KC (pg/ml)	55.8±17.3	36.3±9.71	39.7±14.4	0.448
TNF-α (pg/ml)	7.40±1.06	8.33±1.64	7.79±2.02	0.861

Table 1: Cohort one, Main-effects for the biomarkers.

Table 1 shows the average circulating levels of all biomarkers tested and additionally shows a main effect of surgery for increased levels of Follistatin ($P<0.05$, $F_{1,28}=4.969$, glm-ANOVA), whereas VSG resulted in a trend of increased levels of both IL-6 ($P=0.07$) and Prolactin ($P=0.08$).

Cohort Two: Colon derived short chain fatty acid composition is altered following vertical sleeve gastrectomy.

A. Body weight and body composition

VSG surgery induced an initial reduction in body weight compared to Sham that was completely re-gained at day 14 post-surgery and lasted throughout the rest of the study (Fig. 3A). Nonetheless, repeated measures analysis did measure a significant difference between the body weight dynamics over time between VSG and Sham ($P<0.001$, $F_{10,270}=6.641$, rm-ANOVA). Between group analysis revealed that actual body weight was only lower at post-surgery days 1 to 7 (one-way ANOVA). Similarly, the overall relative weight changes post-surgery (fig. 3B) was different between groups ($P<0.001$, $F_{10,270}=8.960$, rm-ANOVA) and relative body weight was lowered at days 1 to 6 post-surgery (one-way ANOVA) in the VSG group. Likewise, relative weight loss in the VSG was re-gained at day 7 post-surgery and persisted over time. Adipose tissue mass was assessed before and 4 weeks post-surgery. No differences between groups were observed for total adiposity (Fig. 3C).

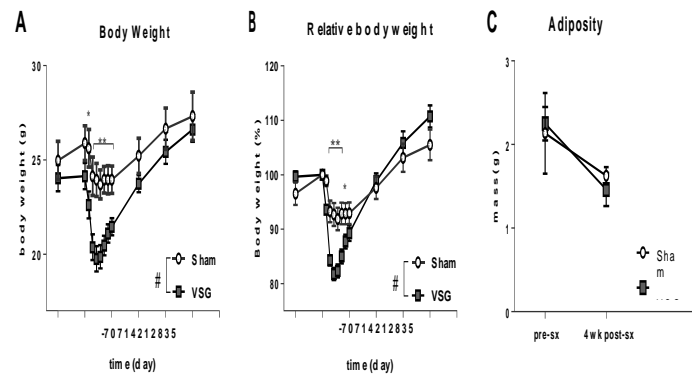


Figure 3: Body weight change following vertical sleeve gastrectomy in C3Tag1 mice. A) VSG induces a change in body weight dynamics over the duration of the study ($^{\#}P<0.001$, rm-ANOVA). Although initially VSG reduces body weight compared to Sham ($*P<0.05$, $**P<0.01$, one-way ANOVA), this initial loss in body weight was regained at day 14 post surgery and persisted over time. B) Relative weight change over the duration of the study was different between VSG and Sham ($^{\#}P<0.001$, rm-ANOVA), but relative body weight was only lower during days 1 to 6 post-surgery ($*P<0.05$, $**P<0.01$, one-way ANOVA). C) Total body adiposity assessed by EchoMRI did not reveal any difference between groups pre-surgery as well as 4 weeks post-surgery.

B. Detection of short-chain fatty acids in colon

Short-chain fatty acids (SCFAs) are produced in the colon by the gut microbiome as a result of dietary fiber fermentation [23]. SCFAs play crucial roles in health and disease states and have been implicated in some types of cancers [24]. In particular, butyrate has been associated with breast carcinogenesis [25]. No significant differences between Sham and VSG were observed for the levels of butyrate, acetate, propionate, octanoate, heptanoate and valerate in the colon (see Table 2).

SCFA (pmol/g)	Sham-HFD	VSG-HFD
Butyrate	597.0±99.7	660.8±153
Acetate	3427±418	3649±506
Propionate	597.0±99.7	688.9±121
Octanoate	0.909±0.10	0.706±0.06
Heptanoate	1.231±0.13	1.111±0.11
Hexanoate	21.87±2.36	25.41±3.59
Valerate	55.82±8.02	64.33±12.1

Table 2: Cohort two - Short chain fatty acid composition in the colon of the C3Tag1 mice following VSG surgery

However, levels of iso-valerate were significantly higher in the colon of VSG-treated mice compared to Sham ($P<0.05$, $F_{1,26}=5.370$, one-way ANOVA) (Fig. 4).

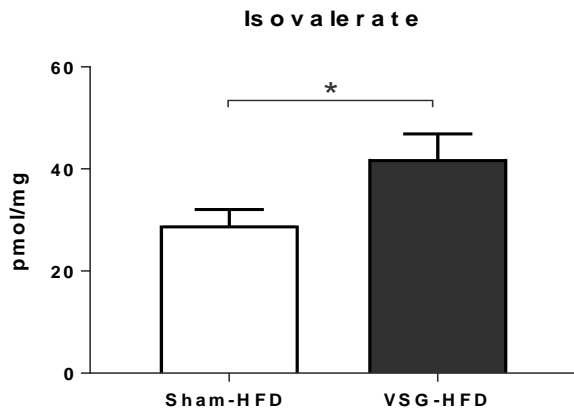


Figure 4: Cohort two, VSG increases the short chain fatty acid isovalerate in the colon tissue of C3Tag (1) mice (* $P < 0.05$, one-way ANOVA).

Discussion

VSG in mice that are genetically prone to develop mammary gland carcinomas did not significantly affect the occurrence or severity of tumor development in these mice. One explanation may be that the aggressiveness of the tumor development due to the genetic modification is too powerful to be overcome by surgical intervention or reduced adiposity due to a standard chow diet. In short, whatever one does, these mice will develop carcinomas. More remarkable, the VSG did not result in additional weight loss, therefore the weight loss that may have reduced the spontaneous cancer development could also not be achieved. The reason that the VSG did not induce additional weight loss may be partly explained by the occurrence of tumor growth in these mice and therefore all mice, including the Sham, are lean and don't have much excess weight to lose. Another reason may be that the background of the C3Tag (1) mice is FVBN, which is a mouse line known to be resistant to diet induced obesity [26]. That being said, the chow fed mice were leaner compared to their HFD fed VSG counterparts, so there could be another physiological mechanism in this strain that makes it resistant to surgery-induced weight loss. Fortunately, this provided the opportunity to study the weight-independent effects of surgery on circulating biomarkers and SCFAs.

In both cohorts, there was no significant surgery effects on weights between VSG-HFD groups compared to sham-HFD (Figure 1 and 3). This might strike some as surprising but the background strain of these C3Tag mice are FVBN. This strain is quite resistant to weight gain when exposed to a HFD and consequently their starting body weights and body adiposity levels are much lower than in most work that has been done exposing mice to VSG [27]. The advantage of this observation is that any observed physiological effects of VSG are the product of the surgical effect and not secondary to weight-loss. All the animals were maintained on their respective diet after surgery, as a result the VSG- chow group weights was significantly lower than both HFD groups.

Although a number of factors influence an individual's predisposition to breast cancer and course of progression to cancer, a growing body of evidence shows various effects of SCFAs on carcinogenesis [28, 29]. SCFAs are produced in the proximal colon through anaerobic bacteria substrate fermentation [30]. Approximately, 95% of the produced SCFAs are absorbed by the colonocytes and about 5% are lost in the feces [31, 32]. Some of the anti-cancer effects of SCFAs includes roles in differentiation, tumor growth arrest, and apoptosis [33]. In this study, we measured the SCFAs concentrations in intestinal colon after VSG in a mouse breast cancer model. There is no significant difference in the levels of primary SCFAs (propionate, acetate and butyrate). However, isovalerate concentrations were higher as a result of VSG. High levels of isovalerate suggest inadequate protein digestion. Here, we confirm that circulating leptin levels were significantly lower after VSG.

The reductions in circulating leptin levels are similar to the total adiposity levels. Several studies have shown a link between the levels of the adipocyte derived hormone leptin contributing to tumor formation and/or development [34-36]. Nonetheless, lower levels of leptin after VSG may contribute to breast cancer risk reduction.

We show a dramatic reduction of PIGF-2 levels post-VSG in both diet groups. Placenta growth factor-2 (PIGF-2) is an angiogenic protein belonging to the vascular endothelial growth factor (VEGF) family of growth factors and is upregulated mainly in pathologic conditions and cancer [37, 38]. PIGF-2 has been extensively researched clinically [39] and experimentally [40, 41] and its significance and roles in tumor progression is established [42].

Consistent with the decreased breast cancer risk observed after bariatric surgery, G-CSF levels are significantly higher after VSG both in HFD and chow. Growth factor families play crucial roles in tumor initiation, expansion, invasion and angiogenesis [43, 44]. Therefore, it is of high interest to observe that G-CSF levels are significantly higher due to VSG independent of diet. Granulocyte colony stimulating factor (G-CSF) stimulates white blood cell production in the bone marrow and contributes to the movement of stem cells from the bone marrow into the blood, strengthening the immune system [45]. The combination effects of G-CSF actions have been known to reduce inflammation and improve breast cancer therapies [46].

Conclusions

VSG results in dramatic shifts in circulating levels of growth factors and some of these effects appear to be a direct effect of VSG rather than a secondary effect of the resulting weight loss. These direct effects of surgery may contribute to the observed effect of bariatric surgery to reduce breast cancer risk and imply that some of the effects of surgery to alter breast cancer risk may not be over and above the potential benefit that accrues as a result of the substantial weight loss. Further work is needed to develop and determine the mechanisms that are responsible for alterations of SCFAs and growth factors after VSG. **Acknowledgments**

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- 2- The Metabolomics Core, University of Michigan, Ann Arbor, MI, USA.

Competing Interests

R.J.S. serves as a paid consultant for and receives research support from Ethicon Endo-Surgery (a subsidiary of Johnson & Johnson), Orexigen, Novo Nordisk, Novartis, Daiichi Sankyo, Janssen (a subsidiary of Johnson & Johnson), and Kallyope. He serves as a paid consultant for Novartis, and Scovia. R.J.F. receives research support from Zafgen, Sanofi, and MedImmune. In addition, R.J.S. is a paid-consultant and expert witness for Paul Hastings Law Firm. B.C.E.P. has declared no conflicts of interest

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