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Metabolic Effects of Bariatric Surgery in Mouse Models of Circadian Disruption

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Abstract

Background/Objectives—Mounting evidence supports a link between circadian disruption and metabolic disease. Humans with circadian disruption (*e.g*., night-shift workers) have an increased risk of obesity and cardiometabolic diseases compared to the non-disrupted population. However, it is unclear if the obesity and obesity-related disorders associated with circadian disruption respond to therapeutic treatments as well as individuals with other types of obesity.

Subjects/Methods—Here, we test the effectiveness of the commonly used bariatric surgical procedure, Vertical Sleeve Gastrectomy (VSG) in mouse models of genetic and environmental circadian disruption.

Results—VSG led to a reduction in body weight and fat mass in both *Clock*$^{\Delta 19}$ mutant and constant-light mouse models ($P < .05$), resulting in an overall metabolic improvement independent of circadian disruption. Interestingly, the decrease in body weight occurred without altering diurnal feeding or activity patterns ($P > .05$). Within circadian-disrupted models, VSG also led to improved glucose tolerance and lipid handling ($P < .05$).

Conclusions—Together these data demonstrate that VSG is an effective treatment for the obesity associated with circadian disruption, and that the potent effects of bariatric surgery are orthogonal to circadian biology. However, since the effects of bariatric surgery are independent of circadian disruption, VSG cannot be considered a cure for circadian disruption. These data have important implications for circadian-disrupted obese patients. Moreover, these results reveal new

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Supplementary information is available on IJO's website.

information about the metabolic pathways governing the effects of bariatric surgery as well as of circadian disruption.

Introduction

More than 33% of Americans are obese and at risk for metabolic-associated diseases including heart disease, stroke and type-2 diabetes[1]. Despite the known health risk, relatively few treatment options are successful at producing substantial and sustained weight loss. Uniquely, bariatric surgery leads to significant and sustained weight loss. For example, patients receiving a Roux-en-Y Gastric Bypass lose ~30% body weight after surgery, and weight loss is maintained for periods of 10 years or more[2]. This and other bariatric surgical procedures, such as Vertical Sleeve Gastrectomy (VSG), additionally improve glucose and lipid metabolism independent of weight loss[3] suggesting mechanisms of action which go beyond caloric restriction[2].

Unlike other treatment options, bariatric surgery did not begin in animal models, but instead was pioneered in the clinical setting by surgeons. With increasing clinical data available, subsets of patients are observed to have very high or very low weight loss after surgery [4]. However, it is unknown if certain sub-populations are associated with differing weight loss outcomes and little is known about the effect of bariatric surgery in specific obese populations.

An emerging factor associated with obesity and cardiometabolic disorders is circadian disruption. Humans who experience on-going disruptions to their day-night cycles, such as night-shift workers, have a greatly increased risk of obesity and diabetes[5] and in a single published report have less weight loss 3, 6, and 12 months post-surgery compared to non-shift workers undergoing the same surgical procedure[6]. However, this study was small, did not include a substantial control group, and did not carefully characterize the circadian disruption of these patients. With the development of animal models for VSG, we can systematically determine the effects of surgery in obese animal models corresponding to different sub-populations of obese individuals, such as circadian disrupted patients.

Circadian disruption can be mimicked in animal models using both genetic and environmental perturbations that induce metabolic impairment. For example, the *Clock*$^{\Delta 19}$ mutant mouse (hereafter, *Clock*) expresses a whole-body mutation in the core conical circadian gene, *Clock*, which results in a lengthened circadian period and often less circadian rhythmicity under constant darkness conditions. Accompanying this altered circadian rhythm is a spectrum of metabolic disorders including an increase in body fat, hyperphagia, hyperglycemia, hypoinsulinemia and hyperlipidemia, and a blunted diurnal feeding pattern[7]. Circadian disruption can also occur when behaviors of genetically-normal individuals become desynchronized from the environmental light:dark (LD) cycle. Such disruptions are common in humans (*e.g.*, disruptions in the sleep/wake cycle and/or feeding/fasting patterns) and have also been associated with weight gain[8-11]. Using mouse models, environmental circadian disruption can be triggered by housing animals in constant light (LL). LL leads to arrhythmic activity, sleep, and feeding behaviors after approximately 4 weeks[12, 13] and, when combined with a high-fat diet, leads to increased body weight and

caloric intake, lowered energy expenditure, and impaired insulin sensitivity compared to animals maintained on a normal LD cycle[14].

Despite the growing evidence demonstrating that circadian disrupted individuals are more likely to be obese, little is known about how they specifically respond to weight loss treatment, such as bariatric surgery. Indeed, the observed variation in weight loss outcomes after bariatric surgery may be a direct or indirect consequence of including patients with undocumented circadian disruption which, in turn, may have altered responses to surgical interventions. Therefore, the current research uses our established mouse model of VSG to test the metabolic effects of the surgery in two models of circadian disruption: a genetic model, the *Clock* mutant mouse, and an environmental model, a diet-induced obese mouse exposed to constant light. Determining if bariatric surgery is successful in an animal model of circadian disruption can provide critical insight into the clinical treatment of the circadian disrupted, obese subpopulation as well as drive future research on the mechanisms underlying bariatric surgery and obesity induced by circadian disruption.

Materials and Methods

Study Design

For the outlined experiments, sample size was determined based on previous validated mouse studies using the VSG model[15]. For all Sham surgery groups, n=10. For VSG groups, pre-surgery group sizes were 12-13. Some VSG mice did not recovery from the surgery, dropping group sizes to a minimum of 8/group. For data calculations, group sizes for VSG were as follows, *Clock* (n=13), WT (n=12), LD (n=9), and LL (n=8). VSG Primary endpoints were set in advance and included body weight, body composition, food intake, and glucose tolerance. The objective of the research was to determine if VSG could successfully treat circadian disruption-associated obesity and glucose tolerance. To create circadian disruption, we use two approaches: genetic (via the *Clock* mouse) and environmental (via LL exposure). In the case of the environmental disruption, mice were disrupted before the surgical intervention and remained on LL even after the VSG surgery. Similarly, all mice were maintained on the same diet before and after surgery. In general, mice recover quickly and well from the VSG surgery. However, we did note that mice in the LL surgery group showed slower recovery times compared to animals housed in the LD group. Given both VSG and circadian disruption have well-documented metabolic effects, we hypothesized that VSG may work through a similar mechanism. Thus, if circadian disruption-associated obesity impaired metabolism through a similar mechanism to which VSG improved metabolism, we should expect VSG to fail to reduce body weight and improve glucose in the genetic model and environmental disruption. For all collected measurements, except body weight and daily food intake measures, the experimenter was blinded to the experimental group. For measurement of body weight and food intake, it is necessary to know the group as an additional health check for recovery after the surgery.

Animals

Circadian disruption models were selected to encompass both genetic and environmental disruption. *Clock*$^{\Delta 19}$ mutant mice were obtained from Northwestern University and bred in

house at the University of Cincinnati to obtain homozygous and wild type littermate control mice. For the environmental disruption experiments, WT mice were ordered from the Jackson Laboratory (JAX) at 5-6 wk of age. All mice were singly housed and fed a 45% high-fat diet (45% fat, 4.54kcal/g, D12451 Research Diets, New Brunswick, NJ) both before and after surgery. For all studies, groups were age-matched and divided to ensure equal body weights among similar genotyped animals prior to surgery intervention. All studies were approved by and performed according to the guidelines of the Institutional Animal Care and Use Committee of the University of Cincinnati (approval number 05-04-1203).

Light protocols

Mice were housed in a standard vivarium room with a minimum of 80 lux during the light period. Standard 12L:12D (LD) consisted for 12-h of light followed by 12-h of dark. Constant light (LL) occurred in a neighboring room in which the lights were on continuously but with otherwise similar environmental conditions.

Automated behavioral measurements

Animals were singly housed, maintained on the appropriate light:dark cycle, and placed into an automated system equipped with metabolic chambers (TSE Systems International Group, Chesterfield, MO) to measure data in 10-min increments. Four weeks after surgery, energy expenditure (measured using indirect calorimetry and calculated as kcal/h per g of lean body mass), activity counts (via beam break), and feeding patterns were measured simultaneously over the course of 4-7 d. Data analysis began after animals acclimated to the metabolic chambers for at least 48 h. A meal bout was determined by at least 0.1 g of food consumed within a 10min span separated by at least a 10-min fast before and after.

Glucose Tolerance Test (GTT)

For the oral GTT, all animals were fasted for 6 h prior to an oral gavage of 200 ul Ensure Plus (Abbott Laboratories). For the i.p. GTT, all animals were fasted for 6 h prior to an i.p. injection of 25% dextrose at a dose of 8 ul/g. All glucose was measured by gluocometer prior to gavage/injection (time 0), and then 15, 30, 60, and 120 min post gavage/injection. Blood was collected from the tip of the tail by cutting a small amount of the tail and gently massaging the blood out. Animals were excluded from analysis based on pre-established criteria, *i.e*. if blood glucose did not rise significantly over the 2-h period, indicating a failed glucose injection.

Vertical Sleeve Gastrectomy

Surgery was performed as indicated previously[16]. Briefly, all mice were maintained on the high-fat diet at least 6 wk prior to surgery and exposed to liquid Osmolite before surgery. Surgery began when mice were 13-15 wk old. VSG mice had ~80% of the stomach resected along the major curvature while Sham mice had their stomach exposed and manipulated but not cut. Following surgery, mice were maintained on liquid Osmolite for 4 d, solid food was returned on the 4^{th} d along with Osmolite, and on the 5^{th} d mice were only provided solid food from then on. Metacam was provided daily for 4 d post-surgery for pain.

Lipid analysis

Lipid analysis was performed by the Metabolic Core at the University of Cincinnati. Blood samples from the tail were taken from animals during an *ad libitum* fed state as well as during fasting.

Body weight and food intake measurements

Body weight and food intake measurements were measured over the course of the experiment daily or 3x/wk. Food was measured by manually removing food from cage top to determine weight.

Macronutrient Preference

Food choice was assayed for seven days, eight weeks after surgery using a macronutrient selection paradigm in which three pure macronutrient diets (TD.02521 [carbohydrate], TD. 02522 [fat], and TD02523 [protein] all Harlan Teklad, Indianapolis, IN) were presented simultaneously in separate containers.

Statistics

Statistical analysis was performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, California, USA). Statistical significance was determined either by two-way ANOVA followed by Bonferroni's multiple comparison post hoc test, or a repeated measures ANOVA followed by Bonferroni's multiple comparison post hoc test. Results were considered statistically significant when $P < .05$.

Results

GENETIC MODEL OF CIRCADIAN DISRUPTION

Body Weight and Fat Mass—As expected, *Clock* mutant mice were heavier (~5.4 g) than their wildtype (WT) littermates prior to surgery, $P < .001$. In response to VSG, both *Clock* and WT lost weight compared to their genotyped Sham controls (Figure 1A,B, $P < .01$). Interestingly, despite the *Clock* mouse starting at a higher baseline body weight and thus having more potential weight to lose, the two genotypes lost comparable weight following VSG, demonstrating independent effects of the surgery and circadian disruption. In both *Clock* and WT, weight loss was achieved almost exclusively through a reduction in fat mass (Figure 1C). There were no changes in lean mass in *Clock* Sham, *Clock* VSG, WT Sham, or WT VSG: 0.00 ± 0.15g, 0.04 ± 0.12g, −0.03 ± 0.10g, 0.01 ± 0.12g, respectively ($P < .05$).

Food Intake and Meal Patterns—As previously described [7], *Clock* mice are hyperphagic and consume more calories than WT mice ($P < .001$). VSG reduced total caloric intake in both *Clock* and WT mice in an orthogonal fashion (Figure 1D, $P < .001$). Indeed, VSG normalized the *Clock* caloric intake to that of a WT Sham control, but did not lower caloric intake to that of the WT VSG control. Additionally, *Clock* mice displayed smaller day-night differences in food consumption compared to WT mice (Figure 2A,B). Our data support previous reports[17], that VSG leads to changes in meal patterns, including

an increase in the number of feeding bouts in the dark phase (Figure 2C, $P < .05$) and a trend toward smaller meals consumed during these bouts (Figure 2D). This effect on meal patterns occurred in both WT and *Clock* mice. In terms of diurnal feeding rhythms, there was a main effect of VSG ($P < .05$) and genotype ($P < .01$) to alter the percentage of dark phase feeding. However, there was no interaction between surgery and genotype (Figure 2E, $P > .05$).

Locomotor Activity and Activity Patterns—Both WT and *Clock* mice displayed a diurnal rhythm of activity while housed on a normal 12-light:12-dark cycle (Supplemental Figure 1A,B). There was no difference in average 24-hour activity among groups (Supplemental Figure 1C, $P > .05$), implying that the elevated body weight of *Clock* mice was due to increased food intake and/or reduced energy expenditure. Surgery had no effect on the diurnal activity patterns of either *Clock* or WT mice (Supplemental Figure 1D, $P > .05$); however, there was an effect of genotype to alter diurnal activity, which was lower in the *Clock mice* ($P < .01$).

Energy Expenditure—We used indirect calorimetry to assess energy expenditure (EE) in *Clock* and WT mice. To account for the differences in mass between genotypes and surgical groups, we normalized EE to g of lean mass as measured by NMR (kcal/hr* $g_{\text{lean mass}}$). Consistent with previous reports[17], we found no main effect of VSG to alter EE in either *Clock* or WT mice (Supplemental Figure 1E,F, $P > .05$).

Glucose Analysis—In response to an oral gavage of glucose, there was an overall main effect of surgery to improve glucose tolerance (Figure 3A, $P < .05$). However, *Clock* VSG mice did not differ from *Clock* Sham mice in glucose tolerance (Figure 3A, $P < .05$). Moreover, *Clock* VSG mice had less improvement than WT VSG mice (Figure 3A), perhaps due to the decreased insulin response in *Clock* VSG mice 10 min post glucose gavage (Figure 3B, $P < .01$). There was a main effect of VSG to lead to increased insulin levels at 10 min ($P < .001$) and an interaction with genotype ($P < .05$).

Lipid Analysis—Since *Clock* mice have hyperlipidemia, for the genetic circadian disruption experiments we also analyzed lipids in *Clock* and WT mice under Sham and VSG conditions. Triglycerides and NEFA were reduced in the WT with VSG ($P < .05$), but surgery had no effect in the *Clock* mouse in these same parameters (Figures 3C,D, $P > .05$). For triglycerides, there was an interaction ($P < .05$) between fasting state and animal group, with WT VSG having reduced triglycerides ($P < .05$) in the Fed and 24-h Fasted state compared to WT Sham. There was no difference in triglycerides comparing *Clock* VSG to *Clock* Sham at any feeding state ($P > .05$). For NEFA, there was a group effect ($P < .01$), with WT VSG having reduced NEFA ($P < .05$) in the Fed state compared to WT Sham. There was no difference in NEFA comparing *Clock* VSG to *Clock* Sham at any feeding state ($P < .05$).

Fasting levels of phospholipids and cholesterol were reduced in both *Clock* and WT mice following VSG (Figure 3E,F, $P < .05$). Although each genotype had a significant reduction, cholesterol and phospholipids were not reduced by the same magnitude in the *Clock* mouse with VSG compared to the WT with VSG. For phospholipids, there was a group effect ($P < .001$), with WT VSG having reduced phospholipids ($P < .001$) in all feeding states compared

to WT Sham. Within the Clock, VSG only reduced phospholipids during the 6-h fasting time point ($P < .05$). For cholesterol, there was a group effect ($P < .001$) and interaction ($P < .01$), with WT VSG having reduced cholesterol ($P < .01$, $P < .001$) in all feeding states compared to WT Sham. *Clock* mice with VSG also had a reduction in phospholipids at all points except the 24-h fast, but to a lesser extent than within WT mice ($P < .05$, $P < .01$).

ENVIRONMENTAL MODEL OF CIRCADIAN DISRUPTION

Body Weight and Fat Mass—DIO mice exposed to constant light (LL) gained more body weight than those exposed to a normal light-dark schedule (LD, main effect of light exposure, $P < .05$; Figure 4A). In response to VSG, both LL and LD mice lost a comparable amount of weight (Figure 4B, $P < .05$), which can be accounted for by loss in fat mass specifically (Figure 4C, $P < .001$). There was also a main effect of the LL to increase both fat and lean mass independent of surgical condition (Figure 4C,D, $P < .05$).

Food Intake and Meal Patterns—VSG similarly reduced caloric intake in both LL and LD models (Figure 4E, $P < .01$). Moreover, LL resulted in a blunting of the 24-h meal patterns (Figure 5A,B, $P < .05$), and affected meal patterns. Overall, there was a main effect of VSG to increase the number of feeding bouts (Figure 5C, $P < .05$) and a trend to decrease the amount consumed per bout (Figure 5D). LL led to a reduction in the diurnal organization of meals ($P < .001$) but there was no effect of VSG to alter diurnal meal organization (Figure 5E, $P > .05$).

Locomotor Activity and Activity Patterns—As expected, LL altered locomotor activity predominately by ablating diurnal activity patterns (Supplemental Figure 2A,B). Overall, there was no difference in total activity counts among the groups (Supplemental Figure 2C, $P > .05$). There was an effect of LL to cause a reduction in diurnal activity ($P > .05$) but there was no effect of the surgery to either increase or decrease dark-phase activity (Supplemental Figure 2D, $P > .05$).

Energy Expenditure—In agreement with the *Clock* data above, VSG did not alter EE (kcal/hr* $g_{\text{lean mass}}$) in WT mice under normal LD conditions (Supplemental Figure 2E, $P < .05$). In agreement with other reports[14], mice housed in LL had decreased EE compared to those held in LD ($P < .05$). The combination of LL and VSG led to a further decrease in EE compared to LL Sham controls (Supplemental Figure 2F, $P < .05$). Moreover, the 24-h EE profiles of LL mice lacked circadian rhythmicity, unlike mice exposed to LD that displayed a clear, daily increase in energy expenditure.

Glucose Analysis—In response to an oral gavage of glucose, both LL VSG and LD VSG mice had similar improvements in glucose tolerance (Figure 6A,B, $P < .05$) compared to the response in Sham controls. Similarly, VSG improved glucose tolerance in response to an i.p injection of glucose, $P < .05$. In contrast to the oral gavage, an i.p. injection of glucose revealed a significant effect of LL to impair glucose tolerance regardless of surgery condition (Figure 6C,D, $P < .05$). The difference in glucose metabolism observed during an i.p. but not an oral gavage suggests that incretins may mitigate some glycemic effects of LL.

Macronutrient preference—Since both mice housed in LL and those housed in LD had similar total kcal intake, for the environmental disruption experiments we additionally examined the macronutrient preference of the mice. Mice housed in LL and LD were evaluated for macronutrient preference by being provided a choice between 3 pure macronutrients: fat, protein, and carbohydrate. LL Sham mice consumed more calories from fat than LD Sham mice (Figure 6E, $P < .05$), an effect due to the circadian disruption. In agreement with previous reports[18], VSG caused a profound reduction in fat preference and increase in carbohydrates consumption in LD and this was also observed in LL mice ($P < .05$). Preference for carbohydrates was unaffected by circadian disruption and was comparable between LL and LD Sham and between LL and LD VSG groups (Figure 6F, $P > .05$). These data demonstrate that VSG can overcome the macronutrient fat preference caused by environmental circadian disruption.

Discussion

Circadian disruption has been associated with adverse health conditions, including those related to obesity and diabetes. Despite the documented relationship between circadian disruption and obesity, no published data addresses the proportion of patients with a BMI ≥40 that also have circadian disruption. This could be in part due to the diverse causes of circadian disruption and the difficulties in assessing the degree of circadian disruption. Based on independent estimates of patients with Night-Eating Syndrome[19, 20] and shift workers[6], the population of circadian-disrupted individuals could be as high as 28% of the severely obese population, or ~20 million people in the United States. Thus, there is a need to understand the relationship and possible interactions between circadian disruption and obesity treatments.

To examine this relationship under controlled experimental conditions, we compared the effects of VSG in two mouse models of circadian disruption, the genetic *Clock* mutant mouse and an environmental model of constant light in a DIO WT mouse. Overall, our data indicate that VSG is effective at significantly decreasing body weight and food intake, and improving glucose tolerance within both of these models. However, given that the improvements from VSG are either similar or smaller in magnitude compared to mice with genetic or environmental circadian disruption, we conclude that mice with circadian disruption combined with VSG are not as metabolically fit as control mice receiving VSG. These data are in agreement with the observation that shift-workers tend to lose less excess body weight than non-shift workers after bariatric surgery[6]. Thus, while VSG is effective at improving overall metabolic health, by itself the surgery cannot completely eliminate the effects of circadian disruption and should not be considered a “cure” for metabolic impairments associated with circadian disruption. Indeed, this conclusion calls for further research on how to treat the circadian disrupted population.

Interestingly, VSG failed to cause significant changes in diurnal feeding or activity patterns in either genetic or environmental models of circadian disruption, and yet significant weight loss occurred. The *Clock* mouse, for example, continued to display a reduction in dark-phase feeding compared to WT mice after the surgery but lost significant weight. In the case of environmental LL exposure, WT mice continued to display both blunted activity and feeding

patterns after VSG but still lost significant weight. Importantly, weight loss is possible within circadian-disrupted models and these changes in body weight occur even when the disrupted circadian rhythms in locomotor activity and feeding are not restored. While some research indicates that VSG can correct some kinds of circadian-disrupted feeding[21], it remains unclear if the combination of bariatric surgery and meal pattern modification may be sufficient to completely alleviate metabolic impairments in an otherwise circadian-disrupted individual or animal model.

Most instances of human circadian disruption are due to voluntary activities and/or environmental disruption, making our animal model in constant light particularly relevant to the human experience. Within this model, we observed a difference in glucose tolerance between the oral and i.p. glucose tolerance tests, highlighting a potential interaction between incretins and the metabolic impact of circadian disruption. Indeed, recent reports suggest that incretins are altered with circadian misalignment[22]. It is possible that constant light could lead to an increase in incretin release that works to limit glucose intolerance.

Our data also indicate that macronutrient preference for fat increases in mice with LL-induced circadian disruption. These data are in agreement with a recent human study of simulated night work[23], but contradict macronutrient preferences observed in human shift workers, which have an increased carbohydrate intake, not fat[24]. In our model, bariatric surgery completely reversed the tendency to consume more fat. Unlike the moderate changes in other metabolic endpoints, macronutrient preference was the only measure we found that was entirely reversed by bariatric surgery.

Using animal models of circadian disruption has great benefits, including the ability to amass significant numbers of animals with a specific type of circadian disruption and tightly controlled comparison groups to address metabolic questions. The current work focused on one particular source of obesity and metabolic dysregulation and examined the effects of a bariatric surgical procedure to improve a number of important metabolic endpoints, thus allowing us to ask specific, controlled questions that may be difficult to ask in a clinical setting.

Mechanistically, this study reveals that the metabolic benefit of bariatric surgery is downstream of the influence of clock biology on metabolism. Additionally, metabolic improvement from VSG is not dependent solely on the reorganization of feeding behavior. Together, these data suggest that another system(s) may be similarly altered in both circadian disrupted and control groups. One promising hypothesis points to alterations in the gut microbiota[25]. Gut microbiota populations are different in lean vs obese animals and bariatric surgery results in a microbiota population more similar to a lean control[26]. Intriguingly, circadian disruption also been shown to directly affect the microbiota[27, 28] – indicating a possible mechanism of improvement that spans obese subpopulations.

The ability of VSG to improve a wide range of metabolic endpoints has two important implications. The first is clinical: These data support the use of bariatric surgical procedures in patients where circadian disruption contributes to increased body weight and poor metabolic regulation. Moreover, these data speak to the effectiveness of a specific treatment

option within this distinct subpopulation of obese individuals, which have thus far been understudied for treatment purposes. The second is mechanistic: Given the powerful effects of bariatric surgery and circadian disruption to alter metabolic endpoints, one logical hypothesis is that these manipulations work through common underlying mechanisms. The current data do not support this hypothesis and indicate that most of the effects of bariatric surgery are orthogonal to those of circadian disruption. This is most notable as VSG does little to improve circadian behaviors yet still improves a variety of metabolic parameters.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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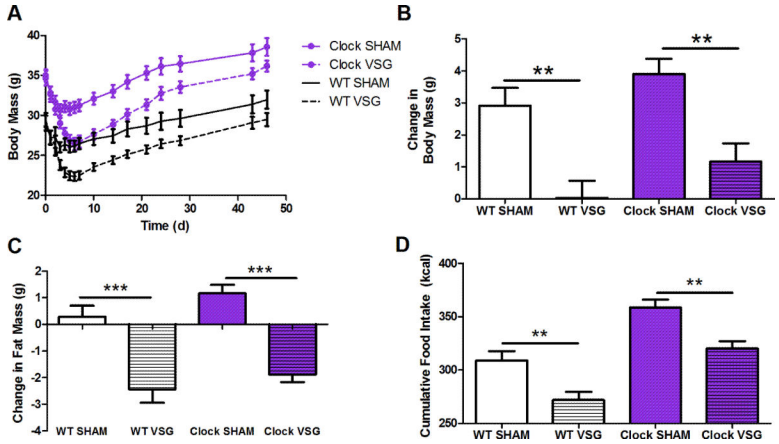

Figure 1.

Body weight, fat mass, and food intake in a genetic model of circadian disruption with VSG. (**A**) Body mass (g) of *Clock* (purple) and WT (black) mice receiving either Sham surgery (solid line) or VSG (dotted line), expressed as days after surgery. (**B**) Absolute change in body mass. (**C**) Change in fat mass (g) as measured by NMR before and after Sham or VSG. (**D**) Total food intake (g) after the surgery. Data expressed as mean ± SEM, ** $P < .01$, *** $P < .001$.

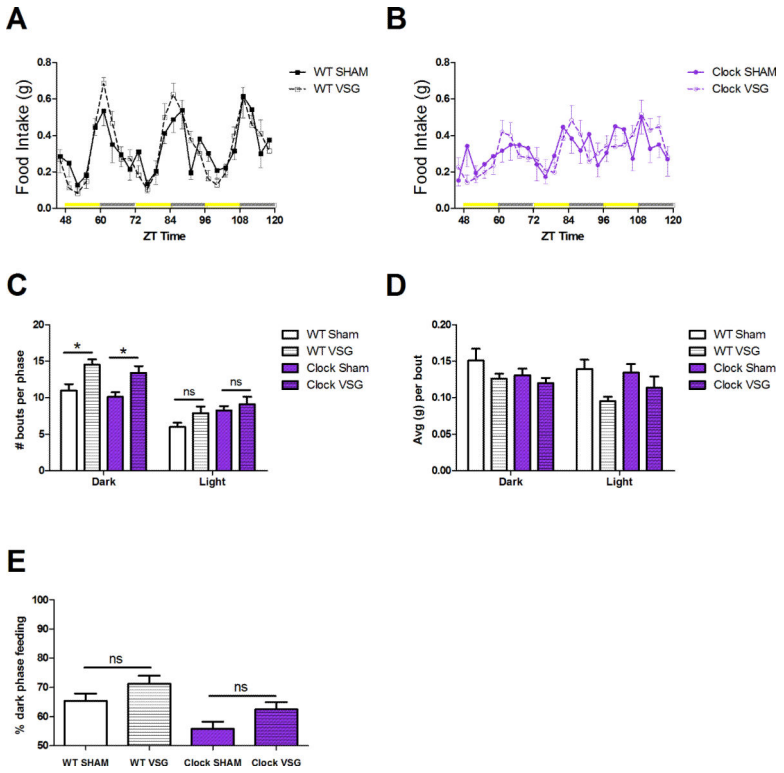

Figure 2.

24-h feeding patterns. Feeding patterns of WT (**A**) and *Clock* (**B**) mice with Sham (solid lines) and VSG (dotted lines) are plotted in 3-h increment over the course of 3 d. ZT time denotes hours after lights on. Yellow bars on the x-axis represent lights on, grey bars represent lights off periods. (**C**) Total number of meal bouts per phase, grouped by bouts during the 12-h dark and 12-h light period. (**D**) The average amount of food consumed (g) per bout in the 12-h dark and 12-h light phase. (**E**) Percentage of dark phase feeding as measured by the total calories consumed during the 12-h dark divided by the total calories consumed during the 24-h period. No significant differences are denoted by “ns”. Data expressed as mean ± SEM, **P* < .05.

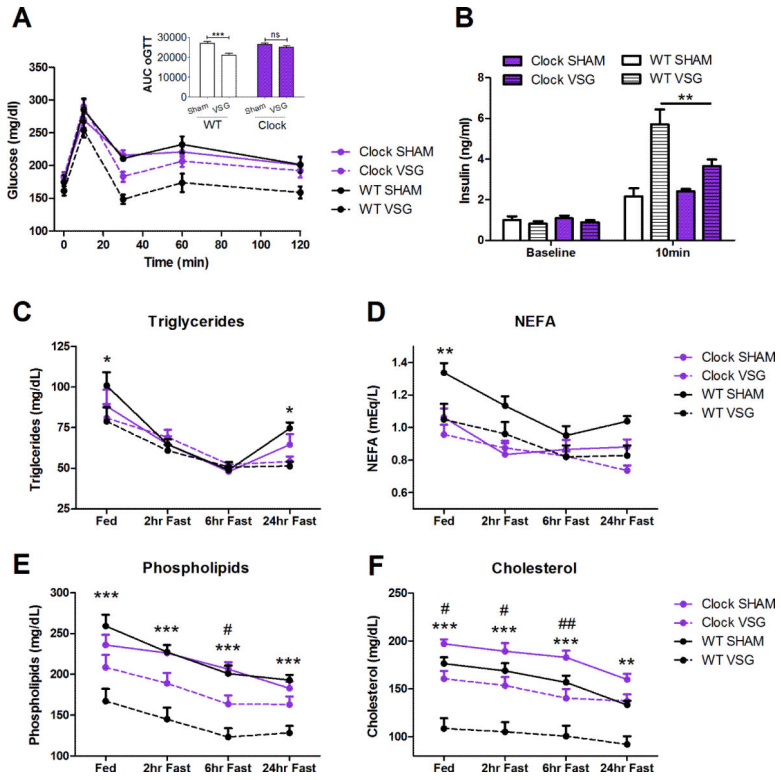

Figure 3.

Glucose homeostasis and lipid handling in a genetic model of circadian disruption with VSG. (**A**) Glucose tolerance curve and area under the curve (AUC) in response to an oral gavage of a mixed meal in both *Clock* (purple) and WT (black) mice receiving VSG (dotted line) and Sham (solid line) surgery. (**B**) Insulin levels (ng/mL) measured before the oral gavage and 10 min after. (**C-F**) Triglyceride, NEFA, phospholipids, and cholesterol in WT and *Clock* mice receiving Sham and VSG under various feeding/fasting conditions. * mark significant differences between WT surgery groups, # mark significant differences between *Clock* surgery groups. No significant differences are denoted by “ns”. Data expressed as mean ± SEM, **P* < .05, ***P* < .01, ****P* < .001, #*P* < .05, ##*P* < .01.

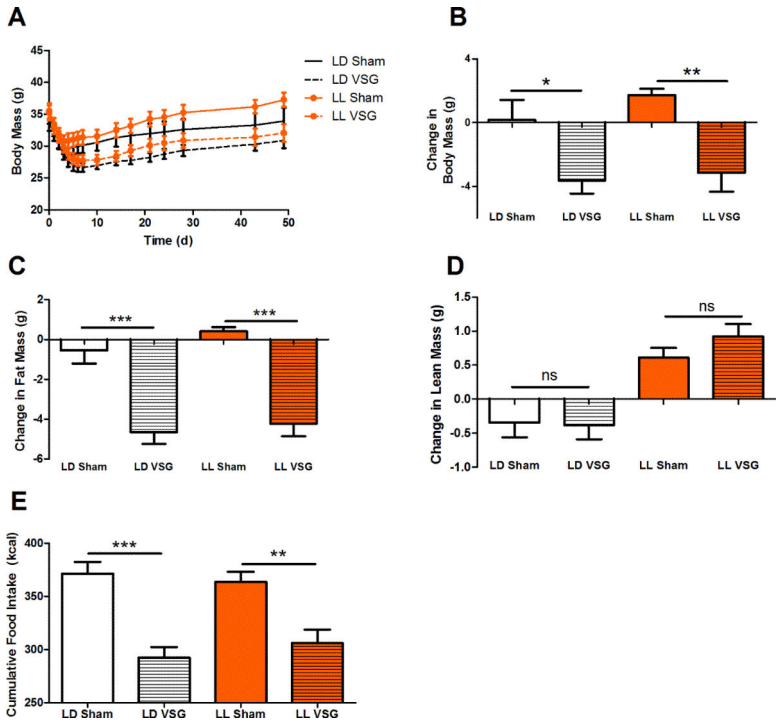

Figure 4.

Body weight, fat mass, and food intake in an environmental model of circadian disruption with VSG. (**A**) Body mass (g) of DIO WT mice in LL (orange) and LD (black) receiving either Sham surgery (solid line) or VSG (dotted line). (**B**) Absolute change in body mass. (**C**) Change in fat mass (g) as measured by NMR before and after Sham or VSG. (**D**) Change in lean mass (g) as measured by NMR before and after Sham or VSG. (**E**) Total food intake (g) after the surgery. No significant differences are denoted by “ns”. Data expressed as mean ± SEM, * $P < .05$, ** $P < .01$, *** $P < .001$.

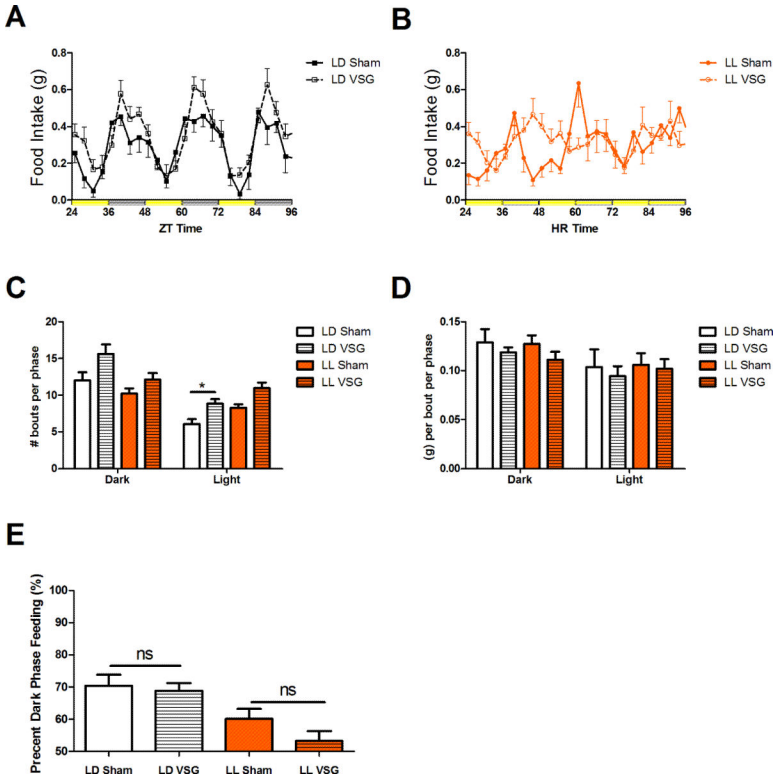

Figure 5.

24-h feeding patterns. Feeding patterns of WT mice in LD (**A**) and LL (**B**) with Sham (solid lines) and VSG (dotted lines) are plotted in 3-h increment over the course of 3 d. ZT time denotes h after lights on. HR denotes h into constant light protocol. Yellow bars on the x-axis represent lights on, grey bars represent lights off periods. (**C**) Total number of meal bouts per phase, grouped by bouts during the 12-h dark and 12-h light period. (**D**) The average amount of food consumed (g) consumed per bout in the 12-h dark and 12-h light phase. (**E**) Percentage of dark phase feeding as measured by the total calories consumed during the 12-h dark divided by the total calories consumed during the 24-h period. No significant differences are denoted by “ns”. Data expressed as mean ± SEM, **P* < .05.

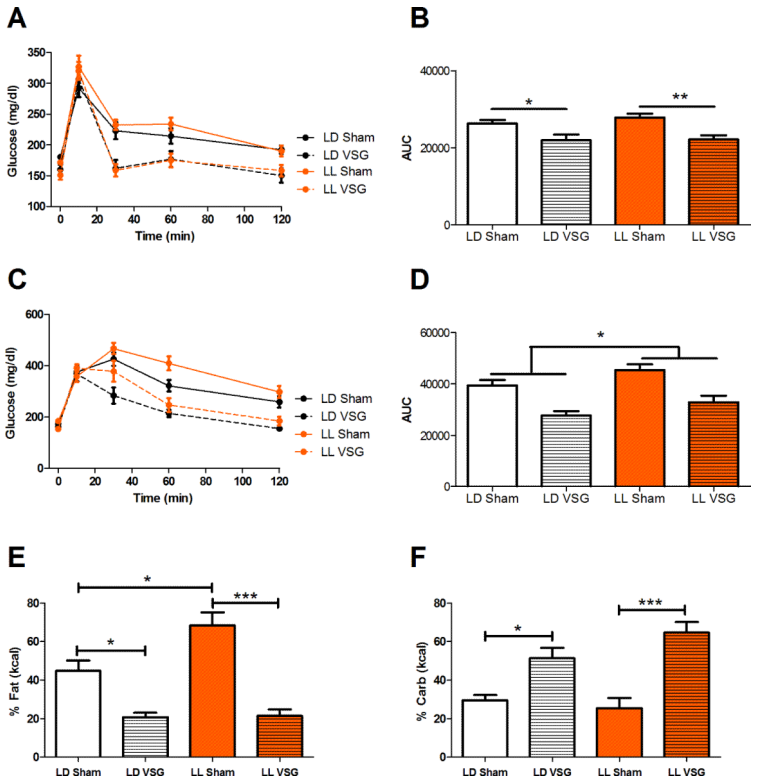

Figure 6.

Glucose homeostasis and macronutrient preference in an environmental model of circadian disruption with VSG. (**A**) Glucose tolerance curve in response to an oral gavage of a mixed meal (Ensure) in both LL (orange) and LD (black) housed mice receiving VSG (dotted line) and Sham (solid line) surgery. (**B**) Area under the curve (AUC) of the calculated glucose curves for the oral gavage. (**C**) Glucose tolerance curve in response to an i.p. glucose injection. (**D**) Area under the curve (AUC) of the calculated glucose curves for the i.p. injection. (**E**) Macronutrient preference for fat in LD and LL mice with surgery. (**F**) Macronutrient preference for carbohydrates in LD and LL mice with surgery. No significant differences are denoted by “ns”. Data expressed as mean ± SEM, **P* < .05, ***P* < .01, ****P* < .001.