Dysregulated appetitive leptin signaling in male rodent offspring from post-bariatric dams

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ABSTRACT

Bariatric surgery produces significant positive benefits to recipients such as resolution of various obesity-related comorbidities, including improved reproductive function. Females of childbearing age seek bariatric surgical remedies to improve their chance of successful pregnancy; however, limited knowledge exists on the impact of surgical weight loss to subsequently born offspring.

We previously reported that circulating leptin levels were reduced in pregnant females having previously received vertical sleeve gastrectomy (VSG) in comparison to control dams having received Sham surgery. Furthermore, the levels of leptin receptors in the VSG placenta were also reduced in comparison to controls in. These data suggest there may be a significant difference in leptin signaling during pregnancy that may produce an altered developmental environment for the offspring.

Here, we investigate the adult offspring of dams having received VSG or Sham-VSG prior to pregnancy. Endogenous fasting plasma leptin levels were not different between Sham and VSG offspring. Fasting leptin receptor mRNA in the medial basal hypothalamus (MBH) was elevated in VSG offspring in comparison to Sham. Intraperitoneal administration of exogenous leptin produced reductions in acute food intake in male Sham offspring, but did not reduce food intake at any time point measured in male VSG offspring. Using Western blot, we identified elevated pSTAT3 and pSTAT3/STAT3 ratios in the MBH of post-VSG offspring in comparison to controls. Using immunohistochemistry, we found an increased number of pSTAT-positive cells in the arcuate nucleus in the Sham offspring in comparison to VSG. In contrast, within the paraventricular and ventromedial hypothalamic nuclei the VSG offspring had elevated numbers of pSTAT-positive cells in comparison to controls.

Collectively, these data support our hypothesis that leptin signaling is dysregulated in VSG offspring and may be partially responsible for the long-term impact of maternal bariatric surgery on the metabolic health of offspring.

1. Introduction

Leptin is an adipocyte-derived hormone present in circulation in proportion to the amount of adipose present (Friedman, 2002) and acts to regulate body weight by inhibiting hunger (Friedman, 2002). Leptin is significantly elevated in obesity (Considine et al., 1996); however, these high levels of leptin fail to suppress feeding as a result of leptin resistance due to several potential mechanisms (Münzberg and Myers, 2005). Bariatric surgery reduces body weight by preferentially reducing adipose mass (Stefater et al., 2010) and concomitantly reduces the levels of circulating leptin (Stefater et al., 2010). In the rodent model, even when the overall body weight of rats having received bariatric surgery is matched to control rats, the proportion of fat mass present in the VSG animal is lower than controls and thus, has substantially reduced leptin levels in comparison to its weight-matched control (Grayson et al., 2017). Women comprise the most significant proportion (>80%) of recipients of weight loss surgeries of which approximately half are of childbearing age (Buchwald et al., 2004; Stroh et al., 2014). Bariatric surgery in women of reproductive age is utilized as a tool to improve the probability and the health of pregnancy, and to attenuate the negative effects of obesity on reproduction (Spann and Grayson, 2020). Though bariatric surgery reduces the incidence of pregnancy-related obesity comorbidities, there is still an increased risk for stillbirths, premature births, NICU visits, and small-for-gestational age births following surgical weight loss (Spann and Grayson, 2020; Christinaioje et al., 2019; Akhter et al., 2019; Kwong et al., 2018). Using our rodent model of VSG

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pregnancy, we have consistently reported various anomalies following maternal VSG during the in utero period, early postnatal life and into adulthood (Spann and Grayson, 2020; Spann et al., 2018, 2019, 2020) that are in-line with the reported problems in human bariatric pregnancies. The long-term impact of obtaining a surgical weight loss procedure prior to pregnancy on the subsequent generation is not completely understood (Spann and Grayson, 2020).

We previously reported that circulating leptin levels during gestation in pregnancies following the bariatric surgery, vertical sleeve gastrectomy (VSG), were significantly reduced in pregnant females in comparison to lean controls (Spann et al., 2018). Furthermore, the expression of leptin receptors in placenta of VSG dams was significantly reduced in comparison to control placenta (Spann et al., 2018). We have also reported reduced litter sizes and increased resorptions in pregnancies following VSG (Spann et al., 2018). We identified in these transgenerational studies that offspring of VSG, when challenged later in life with extended consumption of high-saturated fat diet, exhibit a phenotype suggesting that the offspring are predisposed to developing metabolic syndrome, i.e., glucose intolerance, increased adiposity, and higher circulating lipid levels (Grayson et al., 2013). These data suggest the in utero environment of VSG pregnancies is different. These data also suggest that leptin signaling during gestation may influence the long-term body weight regulation of the VSG offspring.

The brain regions that are important for energy balance express leptin receptors (Leprb) include the medial basal hypothalamic (MBH) nuclei such as the arcuate nucleus (ARC), paraventricular nucleus (PVH), and the ventromedial hypothalamic nucleus (VMH). Specific populations of neurons within the hypothalamus coordinate orexigenic and anorexlic behaviors through interconnections with other relevant extra-hypothalamic appetitive brain regions. Specifically, the pro-opiomelanocortin (POMC) neurons within the ARC secrete peptides that suppress appetite and increase metabolic rate through the actions of leptin and other hormone modulators. Conversely, neurons that secrete neuropeptide Y and agouti-related protein (AgRP), stimulate eating and reduce energy use, and are also modulated by leptin. Within the VMH, leptin receptor disruption on steroidogenic factor 1 (SF-1) positive neurons can alter body-weight regulation (Dhillon et al., 2006). Thus, early life changes to the development of the wiring of this neurocircuitry can produce long-term changes to appetite regulation.

In the current studies, we investigate the leptin system in the adult male offspring of Sham and VSG dams. We focus after postnatal day 60 (PND60), a time when the Sham and VSG pups are similar in body weight, and hence, the adiposity of the animals has been controlled for. We hypothesized that the altered leptin signaling in utero would significantly alter the actions of leptin signaling within the offspring of post-VSG dams. We report circulating leptin levels at baseline and gene expression of important transcripts in the medial basal hypothalamus (MBH). We performed acute food intake studies to determine the sensitivity to exogenously administered leptin. Finally, we used pSTAT as a marker to determine the neuronal response to leptin in the ARC, PVH and VMH.

2. Methods

2.1. Animal assurance

All procedures for animal use complied with the Guidelines for the Care and Use of Laboratory Animals by the National Research Council of the National Academies. Procedures were reviewed and approved by the University of Mississippi Medical Center, Institutional Animal Care and Use Committee (IACUC #1423). In conducting research using animals, the investigators adhered to the laws of the United States of America and regulations of the Department of Agriculture.

2.2. Animals

Female Long Evans rats (200 g) (Invigo, Indianapolis, IN) were initially multiply housed and maintained in a room with a 12/12-h light/dark cycle at 25 °C and 50–60% humidity with free access to water. Initially, all animals were made obese on a 40% high-fat diet (HFD) (#D03082706, Research Diets, New Brunswick, NJ, 4.54 kcal/g; 40% fat, 46% carbohydrate, 15% protein) for 4 weeks prior to undergoing Sham or VSG surgery.

2.3. Surgical procedures

2.3.1. Pre-operative care

Four days before surgery, body composition was assessed using an EchoMRI analyzer (Houston, TX). Animals were fed Osmolite OneCal liquid diet (Abbott Laboratories, IL), but no solid-food for 24 h prior to surgery. Rats were counterbalanced by body weight to the following groups N = 10 Sham and N = 12 VSG.

2.3.2. VSG

As previously described (Spann et al., 2018, 2019; Lawson et al., 2017), VSG consisted of a midline laparotomy with externalization of the stomach. Ligaments and connective tissue were removed leaving an easily articulated stomach. The lateral 80% of the stomach was excised using an ENDO GIA Ultra Universal stapler (#EGLAUSHORT, Covidien, MA) coupled with an ENDO GIA Auto Suture Universal Articulating Loading Unit, 45 mm–2.5 mm (#930454, Covidien, MA). A gastric tube in continuity with the esophagus and duodenum remained. This gastric sleeve was then reintegrated into the abdominal cavity and the abdominal wall was closed in layers using 4–0 coated vicryl suture (#VE494, Ethicon Endo-Surgery Inc., OH) with staple reinforcements.

2.3.3. Sham-VSG

Sham surgery consisted of laparotomy with exteriorization of the stomach similar to VSG and then reintegration of the gastric tissue with closing of layers of muscle and tissue using 4–0 coated vicryl suture and stapling reinforcement.

2.3.4. Post-operative care

Following surgery, all rats received care for 3 d, consisting of once-daily subcutaneous injections of 5 mL saline, 0.10 mL Buprenex® (0.05 mg/kg), and 0.25 mL carprofen (5 mg/kg). Animals were maintained on Osmolite OneCal until food was returned 3 d following surgery.

2.3.5. Breeding protocol

Beginning six weeks after Sham or VSG surgery, females were placed in the cage of a Long Evans male (300 g) in a 1:1 or 2:1, female: male ratio initially multiply housed and maintained in a room with a 12/12-h light/dark cycle at 25 °C and 50–60% humidity with free access to water. Initially, all animals were made obese on a 40% high-fat diet (HFD) (#D03082706, Research Diets, New Brunswick, NJ, 4.54 kcal/g; 40% fat, 46% carbohydrate, 15% protein) for 4 weeks prior to undergoing Sham or VSG surgery.

2.3.6. Animals (offspring)

The offspring were weaned on postnatal day 21 (PND21) to chow (#8640, Envigo, 3.0 kcal/g; 17% fat, 54% carbohydrate, 29% protein) and multiply housed with their same sex siblings until around PND60 at which time, males were singly housed and allocated to one of three experiments reported here: Acute Food Intake, Protein for Western Blot, and pSTAT study.
2.3.7. Bodyweight, composition
Offspring animals were weighed regularly after weaning. Body lean and fat mass composition was analyzed using Echo Magnetic Resonance Imaging (echoMRI) at PND60.

2.3.8. Tail vein bleed for leptin determination
Prior to experimentation, we flipped food hoppers and fasted rats for 7 h after lights off and obtained tail vein plasma samples leptin measurement. Leptin was measured using ELISA according to the manufacturer's specifications: (#M0B00, R&D System, Minneapolis, MD).

2.3.9. Leptin acute food intake experiment
At approximately PND60, hoppers were pulled at 4 h prior to lights out. Rats were given either intraperitoneal injections of saline as the vehicle (VEH) or leptin (LEP) (1 mg/kg, PeproTech, Inc., Rocky Hill, NJ) just before lights off. Hoppers were returned to the animals, and food was measured at 30 min, 1, 2, 3, 4 and 24 h following injection.

2.3.10. Leptin administration for MBH Western blot
At approximately PND65, hoppers were removed at 1 h following lights on, and one series of littersmates were brought to the testing area. In a staggered fashion, rats received intraperitoneal injections of or LEP (1 mg/kg, PeproTech, Inc., Rocky Hill, NJ) every 5 min. Following 120 min, rats were deeply anesthetized with sodium pentobarbital, and perfused transcardially with 0.9% saline followed by 4.0% paraformaldehyde in sodium phosphate. Sections were stored in ethylene glycol cryoprotectant at –20 °C until the time of use.

2.3.11. Protein extraction and Western blot procedure
Protein was extracted using the Santa Cruz RIPA lysis buffer system (Santa Cruz Biotechnology, Dallas, TX). Concentrations were determined using a Pierce BCA protein assay kit (ThermoScientific, Rockford, IL), and spectrometry was performed with a Tecan Infinite 200 PRO (Tecan, Männedorf, Switzerland). Protein was combined at a 1:1 ratio with Laemmli sample buffer (BioRad Laboratories, Hercules, CA) and denatured at 95 °C for 5 min. Protein (30 μg) was loaded onto BioRad 4–20% polyacrylamide Mini Protean TGX gels (BioRad Laboratories, Hercules, CA), and electrophoresis was performed in a BioRad Tetra-Cell 2 g system (BioRad Laboratories, Hercules, CA). Protein was then transferred to PVDF membranes using a BioRad Trans-Turbo transfer system (BioRad Laboratories, Hercules, CA). Membranes were blocked for 1 h at room temperature with Pierce Protein-Free (TBS) Blocking Buffer (Thermo Scientific, Rockford, IL). Primary antibodies used are rabbit anti-Phospho-STAT3 (1:2000, #9145, Cell Signaling, Beverly, MA) and rabbit anti-STAT3 (1:2000, #4904, Cell Signaling, Beverly, MA). Primary antibodies were incubated overnight at 4 °C. In between incubations, membranes were washed in TBS with 0.05% Tween. Digital anti-rabbit HRP conjugate (1:5000, #R1006, Kindel Biosciences, LLC, Greenwich, CT) was used to incubate membranes for 1 h at room temperature before applying substrate solutions contained within KwikQuant Western Blot Detection Kit (#R1004, Kindel Biosciences, LLC, Greenwich, CT). Membranes were then imaged using KwikQuant Imaging (#D1001, Kindel Biosciences, LLC, Greenwich, CT) and images were analyzed using the KwikQuant Image Manager Software (#D1016, Kindel Biosciences, LLC, Greenwich, CT).

2.3.12. Leptin administration for pSTAT immunohistochemistry
At PND70, hoppers were pulled at 1 h following lights-on, and one series of littermates were brought to the testing area. In a staggered fashion, 4 h after lights-on rats received intraperitoneal injections of either VEH or LEP (1 mg/kg, PeproTech, Inc., Rocky Hill, NJ) every 5 min. Following 120 min, rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with 0.9% saline followed by 4.0% paraformaldehyde in sodium phosphate. We post-fixed brains at 4 °C for 24 h in 4.0% paraformaldehyde in sodium phosphate and stored them at 4 °C in 30% sucrose in PBS. Coronal hypothalamic sections (30 μm) were collected in 1:6 series using a freezing sliding microtome.

2.3.13. pSTAT immunohistochemistry
Briefly, tissue sections were removed from cryoprotectant and washed in 0.1 M potassium phosphate-buffered saline (KPBS). Tissue was treated at RT with 0.5% NaOH and 0.5% H2O2 in PBS for 20 min. Following KPBS washes, tissue was treated for 10 min with 0.3% glycine at RT. Tissue was subsequently washed in KPBS and followed by a treatment with 0.03% sodium dodecyl sulphate (SDS) in dH2O for 10 min at RT. After KPBS washes, tissue was pre-incubated in blocking buffer [4% normal horse serum + 0.4% Triton X-100 + 1% bovine serum albumin (BSA) in PBS] for 1 h at RT, followed by incubation with mouse anti-Phospho-Stat3 (1:1000, #4113, Cell Signaling, Beverly, MA) in blocking buffer for 1 h at RT and then overnight at 4 °C. Following washes in KPBS, tissue was incubated for 2 h in secondary antibody, Alexa Fluor™ 594 (1:200, Invitrogen, #A21203). Final KPBS washes were performed, and tissue was mounted and imaged.

2.3.14. Fluorescent microscopy and ImageJ
The Rat Brain Atlas by Paxinos was used to appropriately match hypothalamic levels –1.3 to –3.30 mm caudal to bregma. Images were taken using an Olympus BX60 F5 microscope with a Leica DFC310 FX camera and Leica Application Suite software version 4.6 (Leica Microsystems, Buffalo Grove, IL). Positively-stained cells were identified in the ventromedial hypothalamus (VMH), the arcuate nucleus (ARC), and the paraventricular nucleus of the hypothalamus (PVH). Average numbers of pSTAT positive cells across each nucleus were obtained. An individual blinded to the experimental treatment groups scored the sections. Publication quality pSTAT photos were taken using a Nikon Ni-E fluorescence microscope (Melville, NY).

2.3.15. RNA processing and real-time PCR
Previously collected PND60 MBH tissue (Spann et al., 2019, 2020) was microdissected as described previously and stored in –80 °C until further processing. RNA was extracted using a QiAGEN Miniprep RNA kit (QiAGEN, Inc, Valencia, CA), and complementary DNA was transcribed using an iScript complementary DNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). Quantitative polymerase chain reaction was performed on a StepOne software (v2.3) (Applied Biosystems) using a TaqMan inventoried gene expression assay for leptin receptor (lepr, #Rn01433205_m1), steroidealogenic factor 1 (sfr-1, #Rn01450960_m1), brain-derived neurotropic factor 1 (bdfn, #Rn02531967_s1), agouti-related protein (agrp, #Rn014 31703_g1), neuropeptide Y 1, (np1, #Rn01410145_m1), melanocortin 4 receptor (mcr-4, #Rn01491866_s1), and pro-opiomelanocortin, (ponc, Rn00595020_m1) (Life Technologies, Foster City, CA). Samples were analyzed in duplicate, and changes in Ct values from the internal control 60s ribosomal protein 32 (rpl32, #Rn00820748_g1) were calculated.

2.3.16. Statistical analysis
All statistical analyses were performed using GraphPad Prism version 8.0 (GraphPad Software, San Diego, California) USA. Students T test was used for comparisons. All results are reported as means ± SEM. Results were considered statistically significant when P < 0.05.

3. Results
3.1. Baseline characteristics of sham and VSG offspring
At postnatal day 60 (PND60), Sham and VSG male rats exhibit no differences in body weight (mean ± SEM, Sham: 320.6 ± 8.8; VSG: 310.7 ± 13.4), lean mass (Sham: 280.3 ± 6.3; VSG: 266.0 ± 11.8) or fat mass (Sham: 26.7 ± 1.8; VSG: 26.5 ± 1.9) when reared with their own dam until PND21 in a standardized eight pup litter. Prior to PND30, pups are smaller and shorter and experience catch-up growth after weaning (Spann et al., 2020; Grayson et al., 2013).
3.2. Expression of leptin in plasma and leptin receptor mRNA

Fasting plasma leptin levels were not different in PND60 VSG pups when compared to Sham pups (Sham: 64.27 ± 10.23 ng/mL; VSG: 61.54 ± 3.34 ng/mL) (Fig. 1A). Real-time PCR was used to examine leptin mRNA expression in the MBH. In contrast to the plasma leptin levels, the relative levels of leptin receptor mRNA expression in the MBH were significantly higher in PND60 VSG pups in comparison to Sham pups (Sham: 100.0 ± 4.3 relative units; VSG: 123.5 ± 7.64) (P < 0.05) (Fig. 1B). We also investigated the expression of other hypothalamic genes important to body-weight regulation. The mRNA for steroidogenic factor 1 (sf-1) trended to higher expression in the VSG pup MBH in comparison to Sham, but this was not significant (Sham: 100.0 ± 6.7; VSG: 124.6 ± 14.54) (Fig. 1C). Brain-derived neurotrophic factor (bdnf) mRNA (Sham: 100.0 ± 6.18; VSG: 123.7 ± 3.56) (P < 0.01) (Fig. 1D) were elevated in the MBH of offspring of VSG in comparison to Sham offspring. Further, we tested the expression of agouti-related protein (agrp), neuropeptide Y (npy), melanocortin 4 receptor (mc4r) and proopiomelanocortin (pomc) and observed no differences between groups (Fig. 1E).

Fig. 1. Circulating leptin levels and gene expression within the medial basal hypothalamus (MBH) in PND60 male offspring of Sham and VSG. (A) Fasting plasma leptin levels at PND60. (B) Lepr mRNA expression in MBH. (C) sf-1 mRNA expression in the MBH. (D) bdnf mRNA expression in the MBH. (E) mRNA expression for agrp, npy, mc4r and pomc. Data is presented as mean ± SEM. Student’s T test. PCR N = 7–8/group. (*P < 0.05, **P < 0.01).
3.3. Food intake after leptin injection

In order to determine whether responsivity to leptin was similar in Sham and VSG offspring, we performed an acute food intake study using an intraperitoneal injection of leptin (LEP). LEP administration reduced acute food intake in Sham male offspring by about 70% during the early time points, but did not reduce food intake at any time point measured in VSG males. Raw values were not statistically different due to inherent variability of individual food intake (Fig. 2A). When acute food intake was normalized to the average total 24 h intake of the Sham controls receiving the VEH injection, the 30 min, 2 h, and 4 h intake was reduced in LEP-injected Shams, but the food intake of LEP-injected VSG pups remained similar to VEH-injected controls (Fig. 2B).

3.4. Protein levels of downstream mediators of leptin activation in the MBH

LEP injection significantly increased pSTAT3 protein by Western blot analysis in the MBH of male VSG offspring compared to Sham offspring LEP-injected controls (Sham: 0.49 ± 0.189; VSG: 1.3 ± 0.777) (P < 0.01) (Fig. 3A). There was no difference in protein levels of STAT3 protein in the VSG offspring in comparison to Sham offspring (Sham: 0.586 ± 0.118; VSG: 0.412 ± 0.390) (Fig. 3B). LEP administration significantly increased the ratio of pSTAT3/STAT3 in the MBH of male VSG offspring in comparison to Sham (Sham: 0.747 ± 0.214; VSG: 3.53 ± 0.437) (P < 0.05) (Fig. 3C).

3.5. Nuclei-specific leptin-induced pSTAT activation in the hypothalamus

In order to determine the site of hypothalamic neuronal pSTAT activation as a consequence of LEP-injection, we performed immunohistochemistry and quantification of pSTAT in the PVH, VMH and ARC of Sham and VSG male rats after injection with LEP (Fig. 4). Baseline pSTAT-activation in each nuclei were quantified for comparison using saline-injected Sham and VSG controls (Fig. 4A–C). LEP-induced levels of pSTAT were significantly higher in the ARC and Arc of VSG offspring compared to offspring of controls, offspring of VSG maternal surgery were differentially-regulated in this model (Grayson et al., 2013), we decided in the current study to use a milder paradigm. Here, we generated animals in which both the Sham or VSG surgery was performed in high-fat diet induced obese female animals. Dams were then switched to consume chow for the remainder of the study, and the pups also consumed chow for the remainder of the experiment. Despite having similar dietary treatment, VSG offspring do have a reduced body weight during postnatal life, but by PND60, there are no differences in body weight or composition (Spann et al., 2020). At the critical period that we conducted our studies, on or after PND60, there were no differences between the body weight of the Sham and VSG offspring.

The offspring of Sham dams responded as would be expected, suppressing their food intake in the acute testing paradigm to LEP administration. Given that the VSG offspring do not have endogenous differences in plasma leptin, the response of the VSG offspring to exogenous LEP administration in the acute food intake paradigm suggests a significant lack of responsivity to leptin or “resistance” to leptin by the VSG offspring. This is a surprising potential conclusion given that there are no body weight or body fat differences between Sham and VSG pups at the time of the study and no differences in fasting circulating leptin levels.

4. Discussion

In this study, we investigated whether leptin signaling, which is altered in circulation and the placenta of the post-VSG pregnant rat, is also dysregulated in her adult offspring in comparison to offspring of Sham-VSG offspring. We posited that leptin dysregulation may contribute to the negative effect of maternal bariatric surgery on the long-term metabolic health of offspring that we previously published (Spann et al., 2019, 2020; Grayson et al., 2013). Our findings show that compared to offspring of controls, offspring of VSG maternal surgery exhibit the following changes (Friedman, 2002): increased leptin receptor expression levels in the MBH (Considine et al., 1996), reduced sensitivity to exogenously administered leptin to modulate food intake (Münzberg and Myers, 2005), increased pSTAT activation in the inhibitory VMH of the hypothalamus, and (Stefater et al., 2010) altered downstream mediators of leptin signaling within the PVN. Given these overall outcomes, we conclude that leptin signaling within the post-VSG offspring is dysregulated and may contribute to the long-term predisposition to metabolic problems in the VSG offspring.

Previously, we reported that the poor outcomes were observed in the offspring of VSG dams that were maintained on a high-saturated fat diet following surgery and during pregnancy. However, given the substantially reduced parturition rate of VSG dams consuming high-saturated fat diets (Grayson et al., 2013), and the host of other metabolic indices that were differentially-regulated in this model (Grayson et al., 2013), we decided in the current study to use a milder paradigm. Here, we generated animals in which both the Sham or VSG surgery was performed in high-fat diet induced obese female animals. Dams were then switched to consume chow for the remainder of the study, and the pups also consumed chow for the remainder of the experiment. Despite having similar dietary treatment, VSG offspring do have a reduced body weight during postnatal life, but by PND60, there are no differences in body weight or composition (Spann et al., 2020). At the critical period that we conducted our studies, on or after PND60, there were no differences between the body weight of the Sham and VSG offspring.

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The leptin receptor is highly expressed in the ARC, VMH, PVN as well as other brain regions (Elmqquist et al., 1998). The hypothalamus is normally responsive to peripheral administration of leptin, resulting in c-Fos activation in the ARC, PVN and other appetitive nuclei as early as the first week of postnatal life (Bouret et al., 2004; Gao et al., 2018). However, with increasing levels of adiposity and hence, endogenous levels of circulating leptin, the ability of the brain to respond to exogenously administered leptin diminishes and a state of resistance to leptin ensues as has been classically demonstrated in the OLEFT rat, a spontaneously mutated diabetic obese rat model (Niimi and Yokote, 1999) and the diet-induced obese mouse (Enriori et al., 2007a). The leptin receptor is highly expressed in the ARC, VMH, PVN as well as other brain regions (Elmqquist et al., 1998). The hypothalamus is normally responsive to peripheral administration of leptin, resulting in c-Fos activation in the ARC, PVN and other appetitive nuclei as early as the first week of postnatal life (Bouret et al., 2004; Gao et al., 2018). However, with increasing levels of adiposity and hence, endogenous levels of circulating leptin, the ability of the brain to respond to exogenously administered leptin diminishes and a state of resistance to leptin ensues as has been classically demonstrated in the OLEFT rat, a spontaneously mutated diabetic obese rat model (Niimi and Yokote, 1999) and the diet-induced obese mouse (Enriori et al., 2007a).

Leptin resistance can be produced with prolonged bouts of high-fat feeding (El-Haschimi et al., 2000) or high-fructose feeding (Haring and Harris, 2011); but in the present study, the animals were maintained on chow for the entirety of the study. Altered levels of inflammation, another byproduct of obesity, can alter leptin sensitivity (Mazor et al., 2018). In fact, a single peripheral injection of lipopolysaccharide (LPS) has been reported to produce signs of leptin resistance (Borges et al., 2015). Previously, we have reported elevated levels hypothalamic inflammation as evidenced by increased IBA1-positive microglial density and increased glial fibrillary acid protein (GFAP) in the ARC and PVN of PND 21 VSG pups in comparison to controls (Spann et al., 2019). In this particular study, we did not specifically investigate the ARC and PVN using immunohistochemistry at PND60 and beyond, to determine if there was a continued elevated IBA1 and GFAP by protein (Spann et al., 2019). Using the less sensitive real-time PCR measure, we suggested that it may have resolved. It is possible that elevated markers of inflammation persist to PND60 and beyond to aggravate the neural signaling within the hypothalamus. Finally, altered neuronal wiring can also contribute to leptin resistance (Bouret et al., 2008; Enriori et al., 2007b). In the current study, altered wiring may have occurred during pre- and post-natal development and could be linked to the maternal environment.

The gene expression of fasting lpr at PND60 was increased within the hypothalamus of VSG offspring and may be an attempt to compensate for the reduced ability to sense leptin. The increased expression of lpr may be the result of an altered in utero environment, and consequently, development of the hypothalamus. Given our method of measurement of lpr by dissecting the entire MBH, we were not able to determine what specific nuclei of the hypothalamus are directly expressing altered levels of lpr. Not only were lpr elevated in the MBH but also bdnf expression was elevated in VSG offspring. Acute injection of BDNF protein into the VMN of the hypothalamus decreases body weight by reducing feeding and increasing energy expenditure rats maintained on chow (Pellemounter et al., 1995) and HFD (Godar et al., 2011). Elevations of bdnf mRNA in VSG offspring may reflect compensatory mechanisms to control appetite despite the developmental changes to the brain. Lepr in the VM are largely expressed on SF-1 positive neurons and are important in the maintenance of normal body weight (Dhillon et al., 2006) and may explain why both are concomitantly increased.

We performed immunohistochemistry for pSTAT to whether there were differences in the sites of leptin activation within the hypothalamus. The pSTAT in the ARC of was increased by LEP administration in both Sham and VSG offspring above baseline and Sham rats has significantly higher levels of pSTAT than VSG offspring. The VMH exhibited pSTAT patterns that were activated differentially in Sham compared to VSG offspring with the VMG rats have substantially higher levels of activation. Under normal conditions the VMH has a heterogeneous response to leptin. Using Fura-2 calcium imaging, leptin administration in the VMH has been shown to excite about 24% of the neurons, inhibit 20% of the neurons, and has a biphasic response in 10% of VMN neurons (Irani et al., 2008). The elevated number of pSTAT positive cells within the VMH of VSG after LEP suggests a potential increase overall in the number of leptin-sensitive cells. Altered expression of LEPR in the VMH have been reported in metabolic states such as fasting (Baskin et al., 1998), pregnancy (Ladyman and Grattan, 2005), and rearing of obesity-resistant pups in an obesogenic postnatal environment (Gorski et al., 2006).

The PVN generally integrates the output of the other nuclei of the hypothalamus regarding body-weight. The PVN provides major inputs to hindbrain regions that regulate autonomic functions, and contains hypophysiotropic neurons that regulate the secretion of hormones from the anterior pituitary. In the case of the VSG offspring, LEP administration resulted in up-regulation of pSTAT positive cells in the PVN. Despite activation of the normally leptin-sensitive PVN, the appropriate behavior response: to reduce food intake was not present. Therefore, it is clear that the there is an uncoupling from the normal activation of these leptin sensitive regions to cause motivated reductions in food intake. In fact, this uncoupling may be responsible for the increased body weight of the
VSG offspring under an obesogenic diet as previously published (Grayson et al., 2013).

Leptin receptors are expressed in the hypothalamus as well as the hindbrain and VTA (Elmquist et al., 1998; Grill et al., 2002; Figlewicz et al., 2003). Binding of leptin to leptin receptors results in the activation of Janus tyrosine kinase 2 (Jak2) and the phosphorylation of cytoplasmic targets, e.g., STAT3 and the ras/mitogen-activated protein kinase (MAPK) (Morton et al., 2009; Ghilardi et al., 1996). LEP injection differentially activated pSTAT in the ARC of the Sham animals. VSG in comparison to Sham, the increase of pSTAT3 protein and pSTAT3/STAT3 ratio that was measured in the MBH block of VSG offspring must be the result of the non-ARC areas such as the PVN and VMH. Given that in our other studies, VSG offspring, when maintained on a HFD, increase in fat mass and have other metabolic problems (Grayson et al., 2013), the data presented here may support that the genesis of the VSG offspring metabolic defects may partially be a result of abnormal development of leptin circuits in the VSG offspring.

4.1. Caveats, alternate interpretations and future directions

Our current studies were performed in only males. In our pilot studies, the complex interaction of leptin with reproductive cyclicity produced a high degree of variability in response to exogenous leptin administration in females. We attribute the variability to the potential differences in the phase of estrus cyclicity in the animals that we could not control for.

In the current studies, we do not directly test whether leptin signaling is responsible for the body-weight and metabolic phenotype that we previously identified (Grayson et al., 2013). The response to exogenous leptin administration produced a phenotype of leptin “resistance” in the VSG animals in the absence of any difference in plasma leptin levels. In future studies, it would be important to determine the effect of central leptin administration on the activity of the hypothalamic circuits of the VSG offspring to elicit acute food intake behavior to determine if the leptin resistance was due to central neuronal mechanisms or ability of the leptin to traverse into the brain. Altogether, we conclude that the wiring of the leptin circuits has been altered due to different levels of leptin and sensitivity of the placental leptin receptor in utero during the VSG pregnancy. However, given that leptin levels and leptin signaling are different in VSG pregnant females in comparison to controls, in order to normalize these levels, we would need to treat by replacing leptin. This is altogether problematic. In the leptin-sensitive animal, leptin is anorexigenic, and gestational treatment with leptin would undoubtedly reduce the food intake of the VSG pregnant female, thus, further reducing macronutrient availability for the pregnancy. Future experiments may test whether leptin signaling is dysregulated in the pregnant post-bariatric female without an attempt to rescue the leptin levels during pregnancy.

Fig. 4. pSTAT immunohistochemistry in the hypothalamic nuclei. Sham and VSG offspring were injected with either vehicle or 1 mg/kg leptin. Photomicrographs of fluorescent pSTAT positive cells in the PVN for (A) Sham (B) VSG (C) quantification of positive cells following VEH or LEP. Labelled pSTAT cells in VMH for (D) Sham (E) VSG (F) quantification of positive cells following VEH or LEP. Labelled pSTAT cells in ARC (G) Sham (H) VSG (I) quantification of positive cells following VEH or LEP. Student’s t-test between leptin-injected Sham and VSG. N = 3–7/group. Data is presented as mean ± SEM. (*P < 0.05). ME = median eminence.
Reports from human post-bariatric pregnancies have mixed results but in several studies, the offspring of women who have obtained bariatric surgery before pregnancy have increased levels of obesity during early childhood years (Willmer et al., 2013; Gimesen et al., 2018). These human data suggest strongly that the work we have pursued in the rodent model recapitulates a phenomenon of worthy study to better delineate the factors that affect post-bariatric pregnancies.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Ahkter, Z., Rankin, J., Ceulemans, D., Nongalah, I., Ackroyd, R., Devlieger, R., et al., 2019. Pregnancy after bariatric surgery and adverse perinatal outcomes: a systematic review and meta-analysis. PLoS Med. 16 (8), e1002866. https://doi.org/10.1371/journal.pmed.1002866.

Baskin, D.G., Seeley, R.J., Knipijzer, J.L., Lok, S., Weigle, D.S., Erickson, J.C., et al., 1998. Increased expression of mRNA for the long form of the leptin receptor in the myeloid cells disrupts hypothalamic metabolic circuits and causes body weight increase. Molecular metabolism 7, 155–160. https://doi.org/10.1002/jom.140070203.

Chilardi, N., Ziegler, S., Wiestner, A., Stoffel, R., Hein, M.H., Skoda, R.C., 1996. Defective STAT signaling by the leptin receptor in diabetic mice. Proc. Natl. Acad. Sci. U.S.A. 93 (13), 6231–6235. https://doi.org/10.1073/pnas.93.13.6231.

Gimesen, J.C., Nicolleti, C.F., de Souza Pinhel, M.A., Cortes-Oliveira, C., Salgado Junior, W., Nonino, C.B., 2018. Nutritional status of children from women with previously bariatric surgery. Obes. Surg. 28 (4), 990–995. https://doi.org/10.1007/s11695-017-2990-9.

Godar, R., Del, D., Baintner, H., Billington, C., Kozl, C.M., Wang, C.F., 2011. Reduction of high-fat diet-induced obesity after chronic administration of brain-derived neurotrophic factor in the hypothalamic ventromedial nucleus. Neuroscience 194, 36–52. https://doi.org/10.1016/j.neuroscience.2011.07.079.

Gorski, J.N., Dunn-Meynell, A.A., Hartman, T.G., Levin, B.E., 2006. Postnatal environment overrides genetic and prenatal factors influencing offspring obesity and insulin resistance. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293 (1), R768–R778. https://doi.org/10.1152/ajpregu.00128.2006.

Grayson, B.E., Schneider, K.M., Woods, S.C., Seeley, R.J., 2013. Improved rodent maternal metabolism but reduced intrauterine growth rate after vertical sleeve gastrectomy. Sci. Transl. Med. 5 (199) https://doi.org/10.1126/scitranslmed.3006505.

Grayson, B.E., Gutierrez-Aguilar, R., Sorrell, J.E., Matter, E.K., Adams, M.R., Howles, P., et al., 2017. Bariatric surgery emphasizes biological sex differences in rodent hepatic lipid handling. Biol. Sex Differ. 8, 4. https://doi.org/10.1186/s13758-017-0012-x.

Godar, R., Del, D., Baintner, H., Billington, C., Kozl, C.M., Wang, C.F., 2011. Reduction of high-fat diet-induced obesity after chronic administration of brain-derived nerve growth factor in the hypothalamic ventromedial nucleus. Neuroscience 194, 36–52. https://doi.org/10.1016/j.neuroscience.2011.07.079.

Gorski, J.N., Dunn-Meynell, A.A., Hartman, T.G., Levin, B.E., 2006. Postnatal environment overrides genetic and prenatal factors influencing offspring obesity and insulin resistance. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293 (1), R768–R778. https://doi.org/10.1152/ajpregu.00128.2006.

Grayson, B.E., Schneider, K.M., Woods, S.C., Seeley, R.J., 2013. Improved rodent maternal metabolism but reduced intrauterine growth rate after vertical sleeve gastrectomy. Sci. Transl. Med. 5 (199) https://doi.org/10.1126/scitranslmed.3006505.

Godar, R., Del, D., Baintner, H., Billington, C., Kozl, C.M., Wang, C.F., 2011. Reduction of high-fat diet-induced obesity after chronic administration of brain-derived neurotrophic factor in the hypothalamic ventromedial nucleus. Neuroscience 194, 36–52. https://doi.org/10.1016/j.neuroscience.2011.07.079.

Gorski, J.N., Dunn-Meynell, A.A., Hartman, T.G., Levin, B.E., 2006. Postnatal environment overrides genetic and prenatal factors influencing offspring obesity and insulin resistance. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293 (1), R768–R778. https://doi.org/10.1152/ajpregu.00128.2006.

Grayson, B.E., Schneider, K.M., Woods, S.C., Seeley, R.J., 2013. Improved rodent maternal metabolism but reduced intrauterine growth rate after vertical sleeve gastrectomy. Sci. Transl. Med. 5 (199) https://doi.org/10.1126/scitranslmed.3006505.

Grayson, B.E., Gutierrez-Aguilar, R., Sorrell, J.E., Matter, E.K., Adams, M.R., Howles, P., et al., 2017. Bariatric surgery emphasizes biological sex differences in rodent hepatic lipid handling. Biol. Sex Differ. 8, 4. https://doi.org/10.1186/s13758-017-0012-x.

Godar, R., Del, D., Baintner, H., Billington, C., Kozl, C.M., Wang, C.F., 2011. Reduction of high-fat diet-induced obesity after chronic administration of brain-derived nerve growth factor in the hypothalamic ventromedial nucleus. Neuroscience 194, 36–52. https://doi.org/10.1016/j.neuroscience.2011.07.079.

Gorski, J.N., Dunn-Meynell, A.A., Hartman, T.G., Levin, B.E., 2006. Postnatal environment overrides genetic and prenatal factors influencing offspring obesity and insulin resistance. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293 (1), R768–R778. https://doi.org/10.1152/ajpregu.00128.2006.

Grayson, B.E., Schneider, K.M., Woods, S.C., Seeley, R.J., 2013. Improved rodent maternal metabolism but reduced intrauterine growth rate after vertical sleeve gastrectomy. Sci. Transl. Med. 5 (199) https://doi.org/10.1126/scitranslmed.3006505.
fatty rat with hyperleptinaemia. J. Neuroendocrinol. 11 (8), 605–611. https://doi.org/10.1046/j.1365-2826.1999.00368.x.
Pellcyemounter, M.A., Cullen, M.J., Wellman, C.L., 1995. Characteristics of BDNF-induced weight loss. Exp. Neurol. 131 (2), 229–238. https://doi.org/10.1006/exnr.1995.10045-4.
Spann, R.A., Grayson, B.E., 2020. Curbing obesity from one generation to another: the effects of bariatric surgery on the in utero environment and beyond. Reprod. Sci. 27 (10), 1821–1833. https://doi.org/10.1007/s43032-020-00221-7.
Spann, R.A., Lawson, W.J., Bidwell 3rd, G.L., Zamarripa, C.A., Maranon, R.O., Bandyopadhyay, S., et al., 2018. Rodent vertical sleeve gastrectomy alters maternal immune health and fetoplacental development. Clin. Sci. (Lond.) 132 (2), 295–312. https://doi.org/10.1042/cs20171416.
Spann, R.A., Taylor, E.B., Welch, B.A., Grayson, B.E., 2019. Altered immune system in offspring of rat maternal vertical sleeve gastrectomy. Am. J. Physiol. Regul. Integr. Comp. Physiol. 317 (6), R852-r63 https://doi.org/10.1152/ajpregu.00230.
Spann, R.A., Welch, B.A., Grayson, B.E., 2020. Ghrelin signalling is dysregulated in male but not female offspring in a rat model of maternal vertical sleeve gastrectomy. J. Neuroendocrinol., e12913 https://doi.org/10.1111/jnc.12913.
Stefater, M.A., Perez-Tilve, D., Chambers, A.P., Wilson-Perez, H.E., Sandoval, D.A., Berger, J., et al., 2010. Sleeve gastrectomy induces loss of weight and fat mass in obese rats, but does not affect leptin sensitivity. Gastroenterology 138 (7), 2436–2436 e3.
Stroh, C., Weiner, R., Wolff, S., Knoll, C., Manger, T., 2014. Influences of gender on complication rate and outcome after Roux-en-Y gastric bypass: data analysis of more than 10,000 operations from the German Bariatric Surgery Registry. Obes. Surg. 24 (10), 1625–1633. https://doi.org/10.1007/s11695-014-1252-8.
Willmer, M., Berglind, D., Sorensen, T.I., Naslund, E., Tynelius, P., Rasmussen, F., 2013. Surgically induced interpregnancy weight loss and prevalence of overweight and obesity in offspring. PloS One 8 (12), e82247. https://doi.org/10.1371/journal.pone.0082247.