Effect of vertical sleeve gastrectomy in melanocortin receptor 4-deficient rats


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The currently approved antiobesity drugs have limited efficacy, making bariatric surgery an increasingly important therapeutic option (6). Vertical sleeve gastrectomy (VSG), which is rapidly becoming the surgical procedure of choice (2, 19), results in a reduction of stomach volume by ∼80% without modification of the intestines (8, 21). Roux-en-Y gastric bypass (RYGB) is another common but more extensive bariatric procedure in which the stomach lumen is reduced by ∼90% and the remaining gastric pouch is anastomosed to the jejunum, bypassing the remaining stomach, the entire duodenum, and the proximal jejunum (8, 13, 27). Although both VSG and RYGB have been reported to produce similar reductions in weight and metabolic improvements in both humans and rodent models, VSG is technically less complex due to lack of intestinal manipulation (6, 8, 9, 13, 21, 22, 27) and has minimal malabsorptive effects (21).

The melanocortin receptor-4 (MC4R) is a critical element in the central nervous system (CNS) control of energy homeosta-

RESEARCH DESIGN AND METHODS

Animals. Age-matched experimental male rats (littermates) were obtained by partnering rats heterozygous (HET) for a nonfunctional mutation in Mc4r [Mc4r<sup>K314X</sup>] (18) that had been outcrossed for at least seven generations. Genotyping was performed by KBiosciences (Hoddesdon, UK) using the KASPar SNP genotyping system. Experimental rats (Mc4r<sup>+/+</sup> (WT), Mc4r<sup>+/−</sup> (HET), or Mc4r<sup>−/−</sup> [homozygous (HOM)]) were weaned at postnatal day (PND) 21, group-housed (2–3/cage) until PND 42, and subsequently housed individually. Rats were maintained at the Metabolic Diseases Institute of the University of Cincinnati on a 12:12-h light-dark cycle (lights off at 1800) at 23°C and 50–60% humidity. All rats had ad libitum access to water and a pelleted low-fat chow diet (LFD; LM-485 no. 7012, 25% protein, 58% carbohydrate, and 17% fat, 3.1 kcal/g) manufactured by AAF Laboratories (Madison, WI) via an in-cage dispenser until PND 56. To increase presurgical body weight, rats were given ad libitum access to water and a pelleted 40% high-fat butter/oil-based diet (HFD; D03082706, 4.54 kcal/g) manufactured by AAF Laboratories, Madison, WI, until the following day. All rats had ad libitum access to water and a pelleted low-fat chow diet (HFD; D03082706, 4.54 kcal/g) manufactured by AAF Laboratories, Madison, WI, until the following day.

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Presurgical body weight and food intake. From PND 25 onward, body weight of WT (n = 34), HET (n = 35), and HOM (n = 36) rats was followed serially once or twice per week. Home cage food intake was measured between PND 52 and 55 (LFD) and again between PND 72 and 75 (HFD). Feed efficiency was calculated as the change in body weight (g) divided by the total amount of ingested calories (kcal).

Surgical procedures. After 26 days on the HFD (PND 82), rats were counterbalanced on the basis of fat and lean mass and assigned to surgical groups (sham operation or VSG). Surgeries were performed as described previously (21), and the same surgeons conducted all surgeries. Briefly, the lateral 80% of the stomach was excised, leaving a tubular gastric remnant in continuity with the esophagus superiorly and the pylorus and duodenum inferiorly. The VSG sham procedure involved analogous isolation of the stomach, followed by applying pressure manually with blunt forceps along a vertical line between the esophageal sphincter and the pylorus.

The surgical cohort included sham-operated Mc4r+/− (WT-SHAM), VSG-operated Mc4r+/− (WT-VSG), Mc4r−/− (HET-SHAM), VSG-operated Mc4r−/− (HET-VSG), Mc4r−/− (HOM-SHAM), and VSG-operated Mc4r−/− (HOM-VSG) rats.

Pre- and postoperative care. Rats were preexposed to a liquid diet (Osmolite OneCal) twice before surgery, fasted overnight prior to surgery, and then maintained on the liquid diet for the first 3 postoperative days (POD) when they were transitioned back to HFD. Subcutaneous injections of Metacam (0.25 mg/100 g body wt once daily for 3 days), Buprenex (0.3 ml twice/daily for 3 days), and warm saline (10 ml twice/daily, day 1, and 5 ml twice/daily, days 2 and 3) were given to all rats following surgery. A wire grate was used until POD 4 to prevent the VSG groups from ingesting their bedding.

Postsurgical body weight, food intake, and body composition. Body weight and food intake were monitored daily for the first 2 wk following surgery. Subsequently, body weight and food intake were followed serially once or twice per week until POD 73. Body composition was assessed using an EchoMRI analyzer (Houston, TX) 3 days presurgery (PND 79), on POD 43 (PND 126), and on POD 73 (PND 156).

Intraperitoneal glucose tolerance test. On POD 58 (PND 141), 5-h-fasted rats were given an intraperitoneal (ip) injection of 50% dextrose (1.25 g/kg) at 1300. Blood glucose was assessed at baseline (0), 15, 30, 45, 60, and 120 min via hand-held glucose analyzer (Accuchek; Roche Diagnostics, Indianapolis, IN). Glucose area under the curve (AUC) was calculated using the AUC method. In addition, blood samples (100 μl/time point) were collected using heparinized Eppendorf tubes at 0, 15, 30, and 60 min for insulin determination. Blood was cold-centrifuged, and plasma was stored at −80°C. Plasma insulin was assayed in duplicate using an insulin ELISA (Crystal Chem, Downers Grove, IL), following the manufacturer’s instructions.

Macronutrient selection testing. Using a food preference paradigm (26), three pure macronutrient diets [TD.02521 (carbohydrate: 3.3 kcal/g, 0.1% calories protein, 99.9% calories carbohydrate, and 0% calories fat), TD.02522 (fat: 6.9 kcal/g, 0.1% calories protein, 1.3% calories carbohydrate, and 98.6% calories fat), and TD.02523 (protein: 3.2 kcal/g, 96.1% calories protein, 1.4% calories carbohydrate, and 2.6% calories fat); Harlan Teklad] were presented in separate containers simultaneously for 6 days (POD 74–80, PND 157–163). Nutrient intake was monitored daily, and data are depicted for the final 4 days.

Hemoglobin A1c assay. On POD 98 (PND 181), rats were fasted for 4 h, and tail blood was collected in EDTA tubes and stored at 4°C. Glycated hemoglobin A1c (Hb A1c) was determined using a rat Hb A1c kit (Crystal Chem), following the manufacturer’s instructions.

Human subjects. Blood samples were collected from 46 subjects at the Imperial Weight Centre (Charing Cross Hospital, London, UK) that underwent VSG through Imperial College Healthcare National Health Service Trust (London, UK) between September 2007 and June 2011. All subjects met the National Institute for Clinical Excellence guidelines [a body mass index (BMI) of >40 kg/m² or a BMI between 35 and 40 kg/m² and other significant comorbidities (1)].

Table 1. Statistical information

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Graph</th>
<th>Interaction</th>
<th>F</th>
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<tr>
<td>Presurgery body weight</td>
<td>Fig. 1A</td>
<td>time × genotype</td>
<td>F(2, 1,123) = 180.943</td>
<td>&lt;0.001</td>
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<td>Presurgery food intake on chow diet</td>
<td>Fig. 1B</td>
<td>food intake × genotype</td>
<td>F(2, 102) = 116.579</td>
<td>&lt;0.001</td>
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<td>Presurgery food intake on HFD (PND 72-75)</td>
<td>Fig. 1B</td>
<td>food intake × genotype</td>
<td>F(2, 102) = 188.092</td>
<td>&lt;0.001</td>
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<td>Presurgery feed efficiency on chow diet (PND 52-55)</td>
<td>Fig. 1C</td>
<td>feed efficiency × genotype</td>
<td>F(2, 102) = 15.813</td>
<td>&lt;0.001</td>
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<td>Presurgery feed efficiency on HFD (PND 72-75)</td>
<td>Fig. 1C</td>
<td>feed efficiency × genotype</td>
<td>F(2, 102) = 21.799</td>
<td>&lt;0.001</td>
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<td>Postsurgery body weight</td>
<td>Fig. 2A</td>
<td>time × genotype × surgery</td>
<td>F(2, 1,196) = 5.340</td>
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<td>Postsurgery food intake</td>
<td>Fig. 2B</td>
<td>genotype × surgery</td>
<td>F(2, 46) = 3.306</td>
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<td>Pre- and postlesion lean mass</td>
<td>Fig. 2D</td>
<td>time × genotype × surgery</td>
<td>F(2, 42) = 0.850</td>
<td>&lt;0.50</td>
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<td>Pre- and postlesion lean mass</td>
<td>Fig. 2D</td>
<td>main effect: genotype</td>
<td>F(2, 46) = 28.615</td>
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<td>Pre- and postlesion lean mass</td>
<td>Fig. 2D</td>
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<td>F(2, 42) = 46.355</td>
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<td>Fasting blood glucose</td>
<td>Fig. 3A</td>
<td>time × genotype × surgery</td>
<td>F(2, 42) = 2.738</td>
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<td>Blood glucose during IPGTT</td>
<td>Fig. 3B</td>
<td>time × genotype × surgery</td>
<td>F(2, 42) = 3.380</td>
<td>&lt;0.05</td>
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<td>Blood glucose during IPGTT</td>
<td>Fig. 3B</td>
<td>time × genotype</td>
<td>F(10, 210) = 1.269</td>
<td>0.25</td>
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<td>Blood glucose during IPGTT</td>
<td>Fig. 3B</td>
<td>time × genotype</td>
<td>F(10, 210) = 11.647</td>
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<td>Glucose AUC</td>
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<td>F(2, 42) = 0.483</td>
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<td>F(2, 42) = 19.560</td>
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<td>F(2, 42) = 40.779</td>
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<td>Fasting plasma insulin</td>
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<td>Plasmal insulin during IPGTT</td>
<td>Fig. 3E</td>
<td>time × genotype × surgery</td>
<td>F(6, 126) = 1.501</td>
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<td>Plasmal insulin during IPGTT</td>
<td>Fig. 3E</td>
<td>time × genotype</td>
<td>F(6, 126) = 2.632</td>
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<td>Plasmal insulin during IPGTT</td>
<td>Fig. 3E</td>
<td>main effect: surgery</td>
<td>F(2, 42) = 13.164</td>
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<td>Rat blood Hb A1c</td>
<td>Fig. 3F</td>
<td>surgery × genotype</td>
<td>F(2, 44) = 0.908</td>
<td>0.91</td>
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<tr>
<td>Rat blood Hb A1c</td>
<td>Fig. 3F</td>
<td>main effect: surgery</td>
<td>F(2, 44) = 11.412</td>
<td>&lt;0.01</td>
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<td>Total caloric intake</td>
<td>Fig. 4A</td>
<td>surgery × genotype</td>
<td>F(2, 35) = 4.015</td>
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<td>Percentage macronutrient intake</td>
<td>Fig. 4B</td>
<td>surgery × macronutrient</td>
<td>F(4, 70) = 0.885</td>
<td>0.48</td>
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<tr>
<td>Percentage macronutrient intake</td>
<td>Fig. 4B</td>
<td>surgery × macronutrient</td>
<td>F(2, 70) = 13.017</td>
<td>&lt;0.001</td>
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</table>
received preoperative psychological and medical evaluations. The London-Riverside Research Ethics Committee approved the protocols, and informed written consent was obtained from all subjects. Height, weight, and metabolic status were obtained at the initial visit and at each followup visit. Body weight is expressed as percentage of body weight in kilograms at surgery. The BMI was calculated as the weight (kg) divided by the square of the height (square meters). Subjects without a genetic variation in MC4R are indicated as non-carriers.

Sequencing. Genomic DNA was extracted from white blood cells using standard methods. Eight primers, MC4R-F1 (5’-GCAAC GCTCA GGCTG GAAAC AG-3’), MC4R-R1 (5’-AGAGG TGCAG AGAAG TGTG-3’), MC4R-F2 (5’-CCCCAG GAGGT TAAAT CAATT CA-3’), MC4R-R2 (5’-GCAAG CTTGC CAGAT ACAAC T-3’), MC4R-F3 (5’-CTGTA GCTCC TTGCT TGATC C-3’), MC4R-R3 (5’-CCAGC AGACA ACAAAA GACGC-3’), MC4R-F4 (5’-TTGCT GTCC CTCCC GCAC-3’), and MC4R-R4 (5’-CCAGT ACCCT ACAGC GAAGA-3’), were used in PCR to amplify four amplicons covering most of the 5’-untranslated region (UTR), the entire coding region, and the 3’-UTR of MC4R. The sequencing reaction was performed with the BigDye terminator kit (Applied Biosystems, Foster City, CA), using the manufacturer’s conditions. Both strands of each amplicon were sequenced on an ABI PRISM 3700 automated DNA sequencer (Applied Biosystems).

Statistics. All data are presented as means ± SE. Data were analyzed using Statistica 10 (StatSoft, Tulsa, OK). Specifically, data were analyzed analysis of variance (ANOVA) and ANOVA for repeated measurements where necessary and were followed by Fisher’s least significant difference test post hoc when appropriate to examine significant overall interactions. Values regarding the statistical analyses are presented in Table 1. The null hypothesis was rejected at the 0.05 level.

RESULTS

Loss of MC4R signaling in the rat induces hyperphagia and obesity. Compared with that of WT rats, body weight of HET rats was higher from PND 52 onward (P < 0.05), and body weight of HOM rats was higher from PND 35 onward (P < 0.05) (Fig. 1A and Table 1). Food intake was higher in HOM rats when the rats were maintained on low-fat chow (PND 52–55) and on the HFD (PND 72–75) relative to WT or HET rats (Ps < 0.001; Fig. 1B and Table 1). Food intake of HET rats was higher than that of WT rats on both LFD (P < 0.005) and HFD (P < 0.001; Fig. 1B). Feed efficiency was higher in HOM rats compared with WT rats on LFD or HFD (P < 0.001) and higher in HOM rats compared with HET rats on HFD only (P < 0.001; Fig. 1C and Table 1). Feed efficiency of HET rats was higher compared with WT rats on LFD (P < 0.001) and HFD (P < 0.01; Fig. 1C).

VSG decreases body weight independent of MC4R function. At the time of surgery, body weight of HET and HOM rats was increased relative to that of WT rats (P < 0.001), and body weight of HOM rats was higher than that of HET rats (P < 0.001; Fig. 2A and Table 1). These same relative trends persisted after surgery; i.e., rats in all three groups lost 16–17% of their presurgical weight following VSG (Fig. 2, A and C). WT-SHAM, HET-SHAM, and HOM-SHAM rats lost 7, 7, and 4% of their initial weight, respectively (Fig. 2A, C).

HOM-SHAM rats had higher total food intake following surgery (POD 4–73) than WT-SHAM rats (P < 0.001; Fig. 2B), and HET-SHAM rats were intermediate (P < 0.001 vs. HOM-SHAM, P < 0.05 vs. WT-SHAM; Fig. 2B and Table 1). WT-VSG, HET-VSG, and HOM-VSG had lower total food intake than WT-SHAM, HET-SHAM, and HOM-SHAM rats, respectively, (P < 0.001; Fig. 2B). HOM-VSG rats had higher total food intake than WT-VSG rats (P < 0.001; Fig. 2B), whereas HET-VSG rats had an intermediate phenotype (P < 0.01 vs. HOM-VSG, P = 0.06 vs. WT-VSG; Fig. 2B).

Before and following surgery (POD 43 and 73), lean mass of HOM rats was higher compared with WT rats, whereas HET rats demonstrated an intermediate phenotype (P <
Presurgery, fat mass did not differ significantly between sham and VSG rats within genotype groups (Fig. 2E and Table 1). However, presurgery fat of HOM rats was higher than that of WT rats ($P < 0.001$; Fig. 2E), whereas HET rats demonstrated an intermediate phenotype ($P < 0.001$; Fig. 2E). On both POD 43 and 73, WT-VSG, HET-VSG, and HOM-VSG had lower fat mass compared with WT-SHAM ($P < 0.05$), HET-SHAM ($P < 0.001$), and HOM-SHAM ($P < 0.001$) rats, respectively (Fig. 2E).

**VSG improves glucose metabolism independent of genotype.** Fasting blood glucose of HOM-SHAM rats was higher than that of HOM-VSG rats ($P < 0.01$; Fig. 3A and Table 1). During the intraperitoneal glucose tolerance test (IPGTT), HOM rats had higher blood glucose levels than WT and HET rats 15, 30, 45, and 60 min following glucose administration ($P < 0.001$; Fig. 3B and Table 1). HET rats had higher blood glucose than WT rats 30 min following glucose injection ($P < 0.05$; Fig. 3B). HOM rats had higher glucose AUC values compared with WT and HET rats ($P < 0.001$; Fig. 3C and Table 1). Regardless of genotype, VSG rats had lower blood glucose levels than sham rats 15, 30, 45, and 60 min following glucose injection ($P < 0.001$; Fig. 3B). Fasting plasma insulin of HOM-VSG rats was lower than that of HOM-SHAM rats ($P < 0.001$), whereas fasting plasma insulin of both HOM-
SHAM and HOM-VSG rats was higher compared with all other groups (P < 0.001; Fig. 3D and Table 1). Fasting plasma insulin of HET rats was higher compared with WT rats (P < 0.01; Fig. 3D), and in WT and HET rats we observed no effect of surgery on fasting insulin levels (Fig. 3D). HOM rats had higher plasma insulin levels during the IPGTT than WT and HET rats at 0, 15, 30, and 60 min following glucose injection (P < 0.001; Fig. 3E and Table 1). Independent of surgery, HET rats had higher plasma insulin levels than WT rats at 0, 15, 30, and 60 min following glucose injection (P < 0.05; Fig. 3E). Surgery also had an independent effect on fasting insulin, with VSG rats having lower plasma insulin levels than sham rats (P < 0.001; Fig. 3E and Table 1). Blood Hb A1c levels of VSG rats were lower than those of sham rats (P < 0.01; Fig. 3F and Table 1).

VSG affects macronutrient preference independent of MC4R. During the macronutrient selection paradigm, total caloric intake was lower in HOM-VSG than in HOM-SHAM rats (P < 0.01; Fig. 4A and Table 1). Total caloric intake was higher in HOM-SHAM than WT rats (P < 0.05) but did not differ from HET-SHAM rats (P = 0.07; Fig. 4A). The percentages of each macronutrient consumed are depicted in Fig. 4B. Independently of genotype, VSG rats had decreased preference for fat (P < 0.001), increased preference for carbohydrates (P < 0.001), and no changed preference for proteins (P = 0.82) compared with SHAM rats (Fig. 4B and Table 1). Previously, it has been shown that MC4R-deficient rats have a preference for fat (18). In our current study, sham-operated MC4R-deficient rats showed only an increased trend to prefer fat compared with WT rats. This is likely the result of differences in the testing paradigm used here to assess primarily the effect of the surgery rather than the lack of MC4R signaling.

Genetic variation in MC4R in a human VSG cohort. We sequenced the coding region and part of the 3′-UTR of MC4R in 46 subjects who had undergone VSG. This cohort was 74% female, with a mean age of 48.3 ± 1.6 yr and mean BMI of 50.8 ± 1.1 kg/m² prior to surgery. Five subjects carried common variants in the MC4R coding region. Three subjects were heterozygous, and one subject was homozygous for the common variant rs34114122 (10). Another subject was heterozygous for the common Ile251Leu (I251L; rs52820871) variant (17, 23). It is hypothesized that these common variants do not ablate but may modulate MC4R function (10, 17, 23).

Common genetic variations in MC4R do not affect the outcome of VSG in humans. BMI at the time of surgery (data not shown) and weight loss of subjects carrying the rs34114122 or I251L variants did not differ significantly from those of noncarriers (Fig. 5A). Improvements in Hb A1c levels 12 mo following surgery did not differ significantly among carriers of common variants and noncarriers (Fig. 5B).

**DISCUSSION**

Our data indicate that the beneficial effects of VSG on body weight and glucose metabolism in the rat are independent of MC4R function. VSG also affects macronutrient preference in
the rat (the present study and Ref. 26), and these effects are independent of MC4R function as well. Finally, in this small-scale human study, the improvements in body weight and Hb A1c levels after VSG in human subjects with the common variants rs34114122 or I251L are not different from those observed in noncarriers.

In this study, we replicated as well as expanded on key elements of the obesity phenotype observed in MC4R-deficient rats (18). In particular, we found that MC4R deficiency in the rat increases lean mass in addition to fat mass and induces glucose intolerance. Together with previous observations (18), our data confirm that the MC4R-deficient rat is a useful animal model to study the components of MC4R signaling that regulate feeding, body weight, and glucose regulation. We also confirmed our previous data indicating that VSG results in sustained body weight loss, lowers cumulative food intake, has little to no effect on lean tissue mass, decreases fat mass, and substantially alters macronutrient choice, increasing preference for carbohydrates and decreasing preference for fat in rats (8, 21, 26). Importantly, each of these potent effects of VSG surgery does not require MC4R signaling.

We reported previously that VSG improves glucose tolerance during an IPGTT (8). In rodents, dynamics of glucose regulation are often assessed via intraperitoneal or gavaged glucose administration, neither of which reflects the natural means of providing a glucose load in humans. However, Hb A1c levels, reflecting the glycosylation of erythrocytes, reflect long-term glucose control. Using this assessment, we observed that Hb A1c levels are improved after VSG regardless of genotype. One interesting finding is that, if anything, the improvements in body weight and glucose homeostasis were slightly larger in HOM rats. This may be a result of the greater initial body weight and body fat in HOM rats at the time of surgery.

The CNS melanocortin system is key for normal energy and glucose homeostasis. This is underscored by the extent of obesity seen with MC4R-deficient individuals, including MC4R-deficient rats. We observed previously that VSG has no weight loss-independent effects on leptin sensitivity or on the expression of neuropeptide-Y, proopiomelanocortin, agouti-related peptide, or Mc4r in the mediobasal hypothalamus (21). Since several hormonal and nutrient signals converge on the CNS melano-
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cortin system, and since gene expression does not necessarily reflect neuronal activity, MC4R deficiency provides a more persuasive probe of the role of the melanocortin system. Consistent with our previous findings, the present data indicate that, in the rat, MC4R function is not crucial for the changes in body weight, glucose metabolism, or macronutrient preference following VSG.

These data highlight the advantages of exploring the mechanisms that underlie the beneficial impact of bariatric procedures in rodent models since it allows for testing pointed hypotheses of the role of specific molecular pathways to mediate the effects of the surgery. It also allows for comparison of data among procedures. To that end, recent data made use of a mouse model of MC4R deficiency to test whether MC4R signaling is required for the effects of RYGB (14). Although the studies by Hatoum et al. (14) differ in use of species, surgical procedure, and postoperative protocol, they found that, similar to our studies with VSG, Mc4r+/− mice benefit from RYGB. Unlike the present data, these authors conclude that Mc4r−/− mice do not benefit from the surgery to the same degree as Mc4r+/− or WT mice. At face value, these data point to the hypothesis that different surgical procedures may engage the MC4R system differently to exert their beneficial effects. However, this conclusion is tempered by the differences in species and surgical approach necessitated by doing these surgeries in the mouse. The potential differences in surgical procedure are highlighted by the results from Hatoum et al. (14), where they combined data from two different institutions that had each used a different approach to the difficult task of performing RYGB in a mouse. Two of the smaller cohorts (n = 3 Mc4r+/−) showed a relatively large impact of genotype to reduce the effect of RYGB on body weight. However, the third and larger cohort (n = 7 Mc4r+/−) done with a different surgical approach showed a more modest effect of genotype. Such differences point to the possibility that surgical approach or other unknown factors could contribute to interactions between genotype and bariatric surgery outcome.

Consequently, further research exploring the differences between these two studies is warranted.

In our human cohort that underwent VSG, we observed common genetic variations in MC4R in five of the subjects. Although the precise effect of the rs3411422 variant on MC4R function is not known, it is associated with elevated BMI, fat mass, plasma ghrelin levels, energy intake, and carbohydrate intake (10). Another subject carried the I251L variant, which is also common and appears to be protective against obesity, being associated with greater weight loss after RYGB (17). We observed no difference in weight loss or HbA1c levels up to 12 mo following VSG between carriers of the common variants and noncarriers. Although the surgical procedures and mutational effects differ, our data align with published reports that heterozygous loss-of-function mutations in MC4R do not affect the outcome of RYGB (3). Since we did not detect a loss-of-function mutation in our pilot VSG cohort, a larger study would be needed to confirm that heterozygous loss-of-function mutations in MC4R do not affect the outcome of VSG.

Finally, there was only one carrier of the I251L variant such that we were unable to confirm whether the I251L variant might improve the beneficial outcome of VSG even further, as has been observed for RYGB (17). Although these data suggest that VSG is an appropriate choice for individuals where altered MC4R signaling may contribute to obesity, the data should be considered preliminary. The small number of patients available to us precludes a complete analysis of potential differences among multiple variants, and our data cannot preclude the possibility of smaller effects that cannot be detected in our restricted data set. Nevertheless, these data are consistent with available data from humans with MC4R variants undergoing RYGB, where the conclusion has been that such genetic variants do not alter the response to RYGB (3, 14).

Taken together, our data suggest that MC4R signaling is not necessary for the compelling effects of VSG on food intake, food choice, body weight, or glucose homeostasis. So although it is clear that MC4R signaling is a crucial component of the complex system that regulates energy homeostasis, alterations in MC4R signaling are not an important mechanism underlying the biological impact of VSG surgery. This work points to the viability of using rodent genetic models to identify the molecular pathways that mediate the effects of bariatric surgery and how they may be different for different procedures.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


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