SEX DIFFERENCES IN EFFECTS OF ILEAL INTERPOSITION SURGERY ON GLUCOSE REGULATION IN MELANOCORTIN-4 RECEPTOR DEFICIENT RATS.

Ping Zhao[1,2], Alicia Kiechler[1], Elliott Zieman[3], Meet Maheshbhai Patel[2], Kai Xue[4] and April D. Strader[1]

[1]Department of Physiology, Southern Illinois University School of Medicine, Carbondale, Illinois 62901; [2]Department of Biology, University of North Alabama, Florence, AL 35632; [3]Department of Biological Science, Eastern Illinois University, Charleston, IL 61920; [4]State Key Laboratory of Medical Genomics, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China

Correspondence: Ping Zhao (pzhao1@una.edu)

ABSTRACT

We aimed to determine the metabolic differences between male and female rats after ileal interposition surgery and determine whether the remission of insulin resistance after ileal interposition is dependent on melanocortin-4 receptor signaling in both male and female rats. All age-matched male and female rats were treated with either sham or ileal interposition surgery. Glucose tolerance tests and body composition analysis were then performed. Our results indicated a loss of *Mc4r* function in both male and female rats induced obesity; male knockout rats with sham surgery, not female knockout rats with sham surgery, showed impaired glucose tolerance; insulin resistance of both knockout sham rats and pairfeeding sham rats is significantly higher than that of knockout interposition rats and pairfeeding interposition rats respectively in both male and female rats; interposition surgery decreased both fat percentage and fat mass in female rats, not in male rats, in the long run. In conclusion, Melanocortin-4 Receptor signaling is not necessary for the underlying beneficial effects of ileal interposition on glucose metabolism and insulin resistance. Female rats might get more stable benefits than male rats after the surgery.

INTRODUCTION

Melanocortin-4 receptor (MC4R) in the brain plays a critical role in regulating energy homeostasis [1-3]. *MC4R* gene mutations have a prevalence of 1-2.5% in people with BMI of greater than 30 and are the most frequent monogenic cause of early-onset human obesity and insulin resistance [4-7]. Obesity surgery (bariatric surgery) has been the most effective way to reduce body weight [8]. One notable and particularly interesting effect is the rapid and durable remission of insulin resistance after bariatric surgery, an effect independent of body weight loss [9]. However, it is unclear whether bariatric surgery can resolve insulin resistance caused by complete *MC4R* gene mutation in obese patients due to the limited number of the patients with this condition [10, 11]. One study about Roux-en-Y gastric bypass (RYGB) surgery, the most commonly performed bariatric procedure in the United States, in *Mc4r*-deficient mice has found that the mice do not benefit from the surgery to the same degree as *Mc4r*-heterozygous mice,

thus suggesting that *Mc4r* is required for improved glucose or insulin sensitivity [12]. However, another study of vertical sleeve gastrectomy (VSG), a different type of bariatric procedure, in *Mc4r*-deficient rats has found that VSG improves glucose metabolism in these rats, thus indicating that remission of insulin resistance is not mediated by alterations in *Mc4r* activity [13]. Both RYGB and VSG cause body weight loss that might influence the level of insulin resistance and consequently lead to conflicting results. Therefore, our study chose ileal interposition (IT) surgery (which does not result in weight loss) in *Mc4r*-deficient rats to determine whether *Mc4r* signaling is required to increase insulin sensitivity after bariatric surgery.

Ileal interposition surgery mainly improves insulin sensitivity without significant body weight loss[14, 15], which provides insight into the effectiveness of bariatric surgery [16]. Additionally, most studies use only male rats and little is known about the sexual differences between males and females after the surgeries. The sexual difference is very important because approximately 85% of the patients who have received bariatric surgery are female [17]. Therefore, we performed IT surgery in both male and female rats with *Mc4r* deficiency to determine whether the *Mc4r* signaling is necessary in resolving insulin resistance and identify the sex-specific differences after IT surgery.

METHODS

Animals

Fifty-four age-matched rats (male, n = 25; female, n = 29) were the 7th littermates from rats with a *Mc4r* heterozygous mutation (Transposagen's *Mc4r* TGEM® Rat Model from Transposagen Biopharmaceuticals, Lexington, KY, USA), an ENU-induced point mutation (K314X) that introduces a premature stop codon in the 8th helix of *Mc4r* [18]. DNA isolation and genotyping of experimental rats were performed as previously described [19]. In brief, DNA was processed from ear punches and genotyped to detect the ENU-induced single nucleotide polymorphism (SNP) in *Mc4r* (K314X) by using the KASPar SNP genotyping system (KBiosciences; Hoddesdon, UK). All experimental rats were weaned at postnatal day (PND) 21, group-housed (2/cage) until postnatal day (PND) 42, and subsequently housed individually. Rats were maintained on a 12:12-h light-dark cycle. All rats were given ad libitum access to water and a pelleted low-fat chow diet. All animal procedures and blood collection were approved by the Institutional Animal Care and Use Committee of Southern Illinois University-Carbondale; all experiments and methods in the study were performed in accordance with the guidelines and regulations approved by the Office of Sponsored Projects Administration of Southern Illinois University.

Surgery and Experimental Design

On postnatal day (PND) 56, all fifty-four rats received either sham or IT surgery by the same group of surgeons and were divided into twelve groups in total (Figure 1. Illustration of experimental design). The six male groups included four wild type rats treated with sham surgery (WT Sham); five WT rats treated with IT surgery (WT IT); four *Mc4r* knockout rats treated with sham surgery (KO Sham); four KO rats treated with IT surgery (KO IT); four KO rats treated with sham surgery and pair feeding (PF Sham); four KO rats treated with IT and pair feeding (PF IT). The six female groups included four wild type rats treated with sham surgery (WT Sham); five WT rats treated with IT surgery (WT IT); five *Mc4r* Knock-out rats treated with sham surgery (KO Sham); five KO rats treated with IT surgery (KO IT); five KO rats treated with

sham surgery and pair feeding (PF Sham); five KO rats treated with IT and pair feeding (PF IT). Body weight was measured daily until postoperative day (POD) 14 and then were monitored weekly until POD 161. On POD 28, an oral glucose tolerance test (OGTT) was completed and an EchoMRI was performed on both POD 30 and POD 161. The pair feeding ended on POD 35 and the rats were sacrificed on POD 161 after the second EchoMRI.

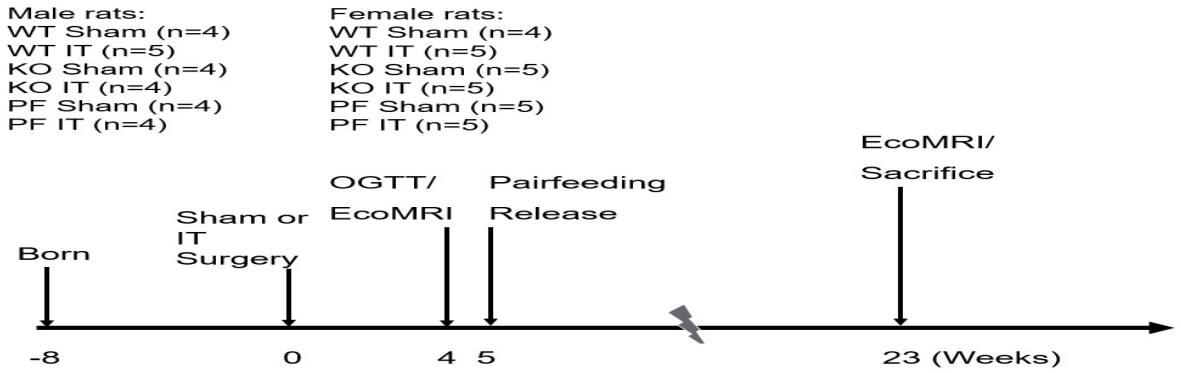


Figure 1. Illustration of experimental design.

Ileal Interposition Surgery

Rats were treated with either sham or ileal interposition (IT) as previously described [15]. Rats were anesthetized with isoflurane anesthesia (2%) during the procedure. Briefly, a midline abdominal incision was made and the caecum was externalized. Intestinal transections were made at 5 and 15 cm proximal to the ileocecal valve to isolate a 10-cm segment of ileum. A single anastomosis was made using a 7-0 silk suture (Ethicon, Cincinnati, OH, USA) at the site of the segment removal. The segment was laid aside and kept moist with warmed 0.9% saline while the remaining intestines were externalized to locate the ligament of Treitz. The jejunum was transected 5 cm distal to the ligament of Treitz and the segment was interposed using an anastomosis in an iso-peristaltic direction. The intestines were bathed in 0.9% saline and re-inserted into the abdominal cavity. Sham-operated rats were treated with three transections in the same locations as the ileal interposition group, which were immediately re-joined by anastomosis.

Oral Glucose Tolerance Test (OGTT)

The rats were fasted overnight for a period of 16 hrs, then administered an oral gavage of glucose (20% D-glucose; 1g/kg; Sigma-Aldrich, St. Louis, MO, USA). Before administration of the glucose bolus, baseline blood samples were obtained from the tail vein for measurement of fasting glucose and insulin. Blood glucose was measured with handheld glucometers in duplicate (TheraSense Freestyle Glucometers, Abbott, Chicago, IL, USA) at 15, 30, 45, 60 and 120 minutes from tail blood. Plasma was collected after centrifugation and stored at -80°C until use for measuring insulin level.

Pair Feeding (PF)

Wild type (WT) rats and knock-out (KO) rats were fed ad libitum with rodent chow. The food hoppers in the WT rat cages were weighed (to the nearest tenth of a gram) and were then subtracted from the previous day's hopper weight to determine the amount of food (grams) consumed daily. The daily food intake from WT rats averaged and was used as the amount of

respective diet given to the pair-fed (PF) rats daily. Because the food given to the PF rats was based on that consumed by the WT rats, pair-feeding began one day later for the pair-fed groups. Because PF rats were also *Mc4r* deficient, they quickly consumed all of the allotted food that they were provided. There was no remaining food left in the bedding. Pair feeding was utilized to rule out the influence of body weight differences and to determine whether the improved glucose tolerance was a result of changes in weight. Pair feeding was discontinued at POD 35.

Insulin ELISAs

Insulin concentration were measured with an Ultra-Sensitive Rat Insulin ELISA Kit (Crystal Chem Inc, Downers Grove, IL, USA), and the assay was performed according to the manufacturer's protocol. Briefly, plasma was thawed and divided into aliquots in duplicate for the measurements of plasma insulin. A total of 95 µl of sample diluent per well was dispensed into an antibody-coated microplate, and then 5 µl of the sample per well was added. The microplate was incubated for 2 hours at 4°C. After wells were washed five times with buffer solution, 100 µl of anti-insulin enzyme conjugate per well was added. After 30 minutes of incubation at room temperature, the microplate was washed seven times with wash buffer. A total of 100 µl of enzyme substrate solution per well was dispensed and the microplate was incubated at room temperature while avoiding exposure to light for 40 minutes. Then, 100 µl of enzyme reaction stop solution per well was added to stop the reaction, and measurements were collected at 450 nm and 620 nm using a Multiskan Plus plate reader (Thermo Electron Corporation, Waltham, MA, USA). The result with a correlation coefficient above 95% and a CV below 20% was accepted.

EchoMRI

Fat and lean mass and percentage were determined by nuclear magnetic resonance at both POD 30 and POD 161 (EchoMRI-900 3-in-1, Echo Medical Systems, Houston, TX, USA). Live conscious rats were inserted into an appropriate Plexiglas animal tube and placed into the EchoMRI machine.

Homeostatic Model Assessment For Insulin Resistance

Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated for all rats using the HOMA formula: HOMA-IR = Fasting insulin (mU/L) × plasma glucose (mg/dL) ÷ 405. The HOMA IR result correlated with hyperinsulinemic-euglycemic clamp results (r = 0.88) [20].

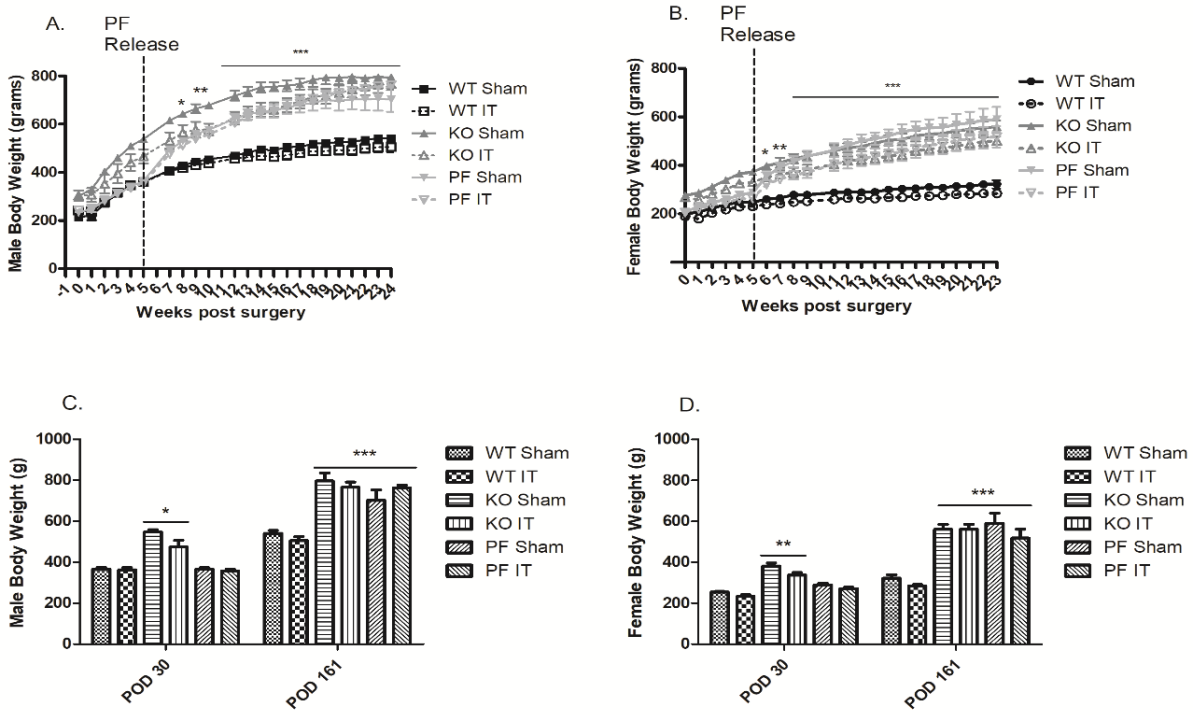
Statistics

All statistics were performed in Prism 5 statistical software by GraphPad, and significance was set at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Body weight, glucose, insulin and body composition were analyzed by two-way ANOVA with repeated measures. HOMA insulin resistance was analyzed by one-way ANOVA.

RESULTS

Loss of Mc4r function in rats induced obesity

The body weight of KO Sham, KO IT, PF Sham and PF IT was significantly higher than that of WT Sham and WT IT from the 8th week in both male rats (Figure 2A) and from the 6th week in female rats respectively (Figure 2B) (***p<0.001, **p<0.01, and *p<0.05). On POD 30, the body weight of KO Sham and KO IT was significantly higher than that of WT Sham, WT IT, PF Sham and PF IT in both male (Figure 2C) and female rats (Figure 2D) (**p<0.01, and *p<0.05). After the discontinuation of pair feeding (POD 35), the body weight in both the PF Sham and PF IT groups increased gradually to the same weight as that of the KO Sham and KO IT groups in both male (Figure 2A) and female rats (Figure 2B) on POD 161. On POD 161, the body weight of KO Sham, KO IT, PF Sham and PF IT was significantly higher than that of WT Sham and WT IT in both male (Figure 2C) and female rats (Figure 2D) (***p<0.001). The body weight of WT Sham and WT IT, KO Sham and KO IT, PF Sham and PF IT groups in both male (Supplementary figure 1A~1C) and female rats (Supplementary figure 1D~1F) were not significantly different during the experiment period. Body weight was analyzed by two-way



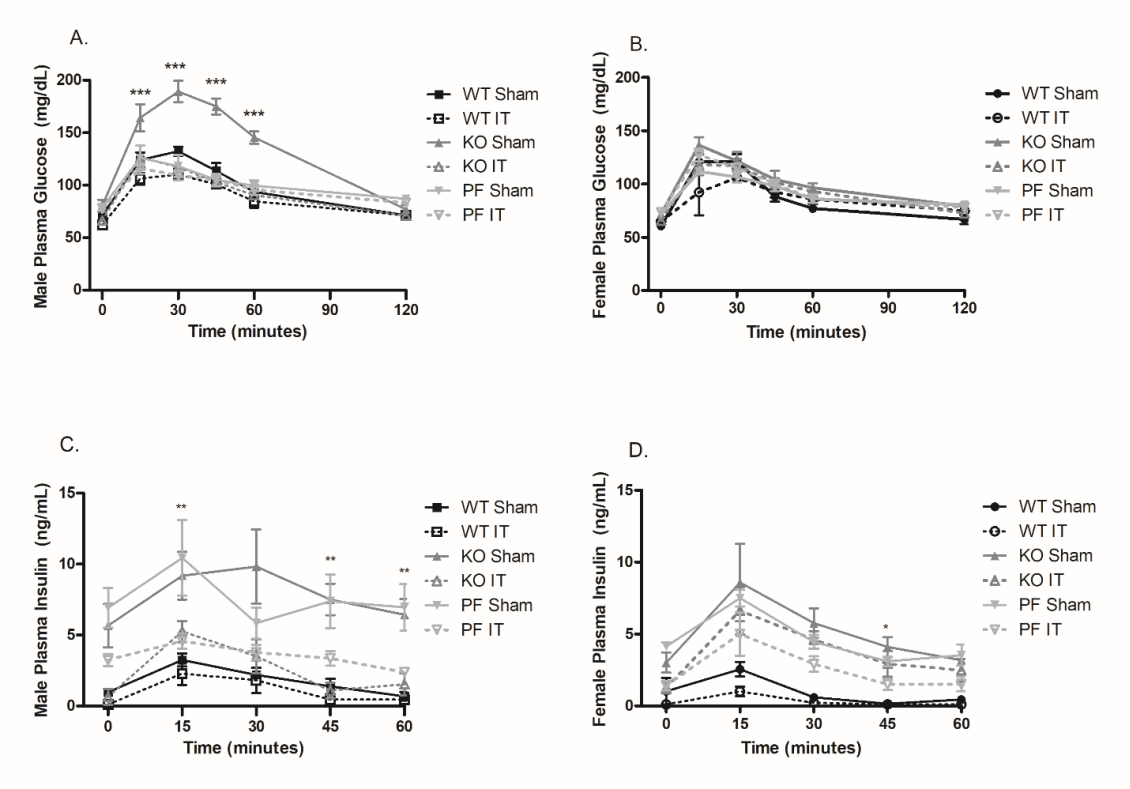
ANOVA with repeated measures.

Figure 2. Loss of *Mc4r* function in the rat induced obesity.

Male KO Sham, not female KO Sham, showed impaired glucose tolerance.

Glucose excursions in response to oral glucose administration were significantly higher in male KO Sham rats at 15, 30, 45, and 60 minutes, as compared with the excursions in the other five male groups (***p<0.001; Figure 3A), while glucose levels in all six female groups were not significantly different during the OGTT (Figure 3B). The secretion of insulin in the KO Sham and PF Sham groups was significantly higher than that in the WT Sham, WT IT, KO IT

and PF IT groups at 0, 45 and 60 minutes in males (**p<0.01, Figure 3C); the secretion of insulin in the KO Sham, KO IT, PF Sham and PF IT groups was significantly higher than that in the WT Sham and WT IT groups at 45 minutes in females (*p<0.05; Figure 3D). Glucose and



insulin were analyzed by two-way ANOVA with repeated measures.

Figure 3. Male KO Sham, not female KO Sham, showed impaired glucose tolerance.

IT surgery increased insulin sensitivity in both male and female rats independent of genotype

For both male and female rats, the insulin resistance in the KO Sham group was significantly higher than that in the KO IT group; the insulin resistance in the PF Sham group was significantly higher than that in the PF IT group (**p<0.01, *p<0.05; Figure 4A and 4B) on POD 28. The insulin resistance of male KO Sham and PF Sham is higher than that of female KO Sham and PF Sham. HOMA insulin resistance was analyzed by one-way ANOVA.

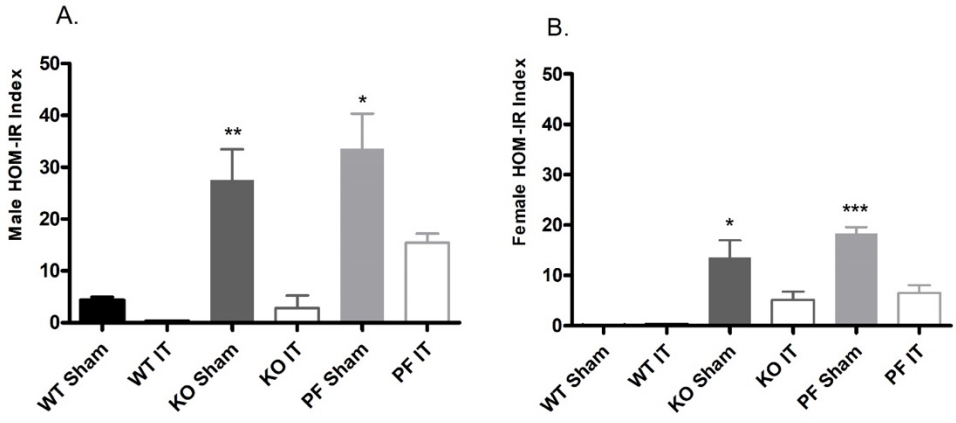


Figure 4. The insulin resistance.

IT surgery decreased both fat percentage and fat mass in female rats but not in male rats on POD 161

For male rats, both fat percentage (Figure 5A) and fat mass (Figure 5B) of KO Sham were significantly higher than those of the other five groups on POD 30 (***p<0.001); on POD 161, both fat percentage (Figure 5A) and fat mass (Figure 5B) of KO Sham, KO IT, PF Sham and PF IT groups were significantly higher than those of WT Sham and WT IT (***p<0.001). No significant difference in either fat percentage or fat mass was found between the WT Sham and WT IT, KO Sham and KO IT, and PF Sham and PF IT groups on POD 161.

For female rats, both fat percentage (Figure 5C) and fat mass (Figure 5D) of KO Sham group were significantly higher than those of the other five groups on POD 30 (*p<0. 05); both fat percentage (Figure 5C) and fat mass (Figure 5D) of KO IT and PF Sham were significantly higher than those of WT Sham, WT IT and PF IT on POD 30 (@p<0.01; Figure 5C). On POD 161, both fat percentage (Figure 5C) and fat mass (Figure 5D) of WT Sham and WT IT groups were significantly lower than those in the KO Sham, KO IT, PF Sham and PF IT groups (***p<0.001); both fat percentage (Figure 5C) and fat mass (Figure 5D) of WT IT, KO IT and PF IT groups were significantly lower than those of WT Sham, KO Sham and PF Sham groups, respectively ($p<0.05). Fat percentage and fat mass were analyzed by two-way ANOVA with repeated measures.

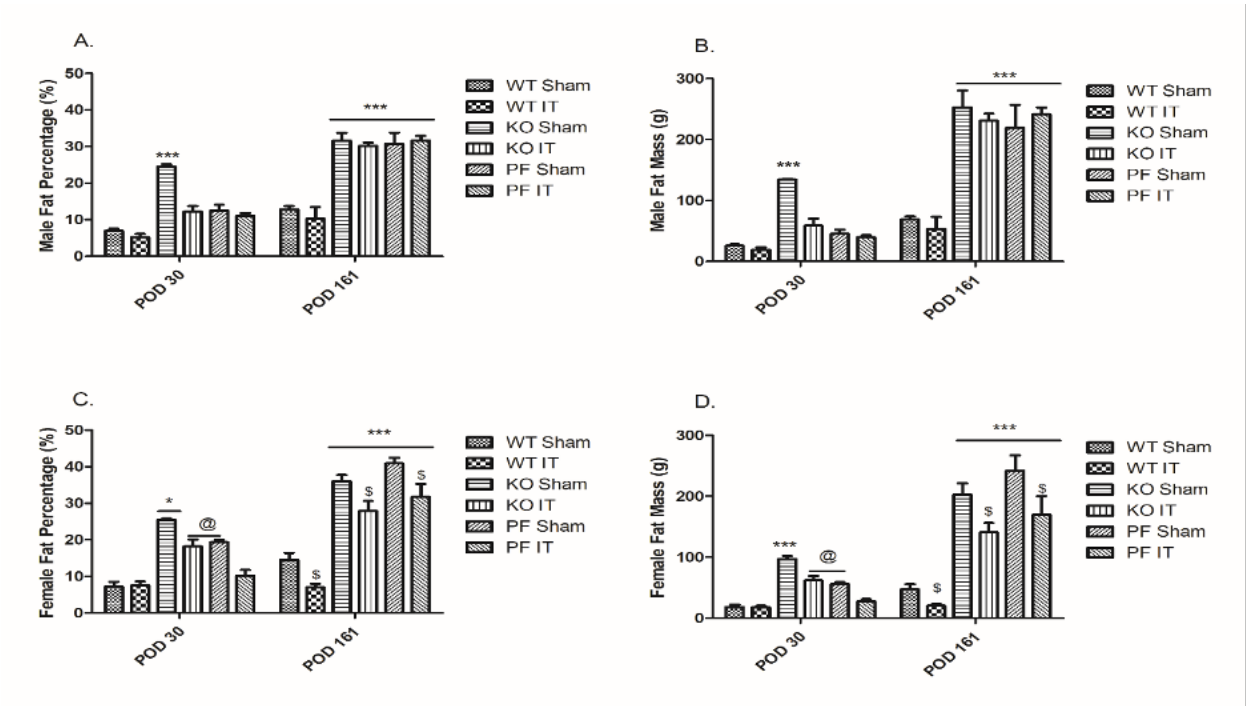


Figure 5. IT surgery decreased both fat percentage and fat mass in female rats, but not in male rats.

IT surgery did not affect lean percentage or lean mass

For male rats on POD 30, the lean percentage (Figure 6A) of WT Sham and WT IT was significantly higher than that in the KO Sham group (***p<0.001); on POD 161, the lean

percentage (Figure 6A) in WT Sham and WT IT was significantly higher than that of KO Sham, KO IT, PF Sham and PF IT groups (*p<0.05). The lean mass (Figure 6B) of KO Sham and KO IT was significantly higher than that of WT Sham, WT IT, PF Sham and PF IT on both POD 30 and POD 161 (***p<0.001, *p<0. 05).

For female rats on POD 30, the lean percentage (Figure 6C) of WT Sham and WT IT was significantly higher than that of KO Sham, KO IT and PF Sham (*p<0. 05); on POD 161, the lean percentage (Figure 6C) of WT Sham and WT IT was significantly higher than that of KO Sham, KO IT, PF Sham and PF IT (***p<0.001, *p<0.05). No significant differences were found in lean mass (Figure 6D) in all six groups on both POD 30 and POD 161. Lean percentage and lean mass were analyzed by two-way ANOVA with repeated measures.

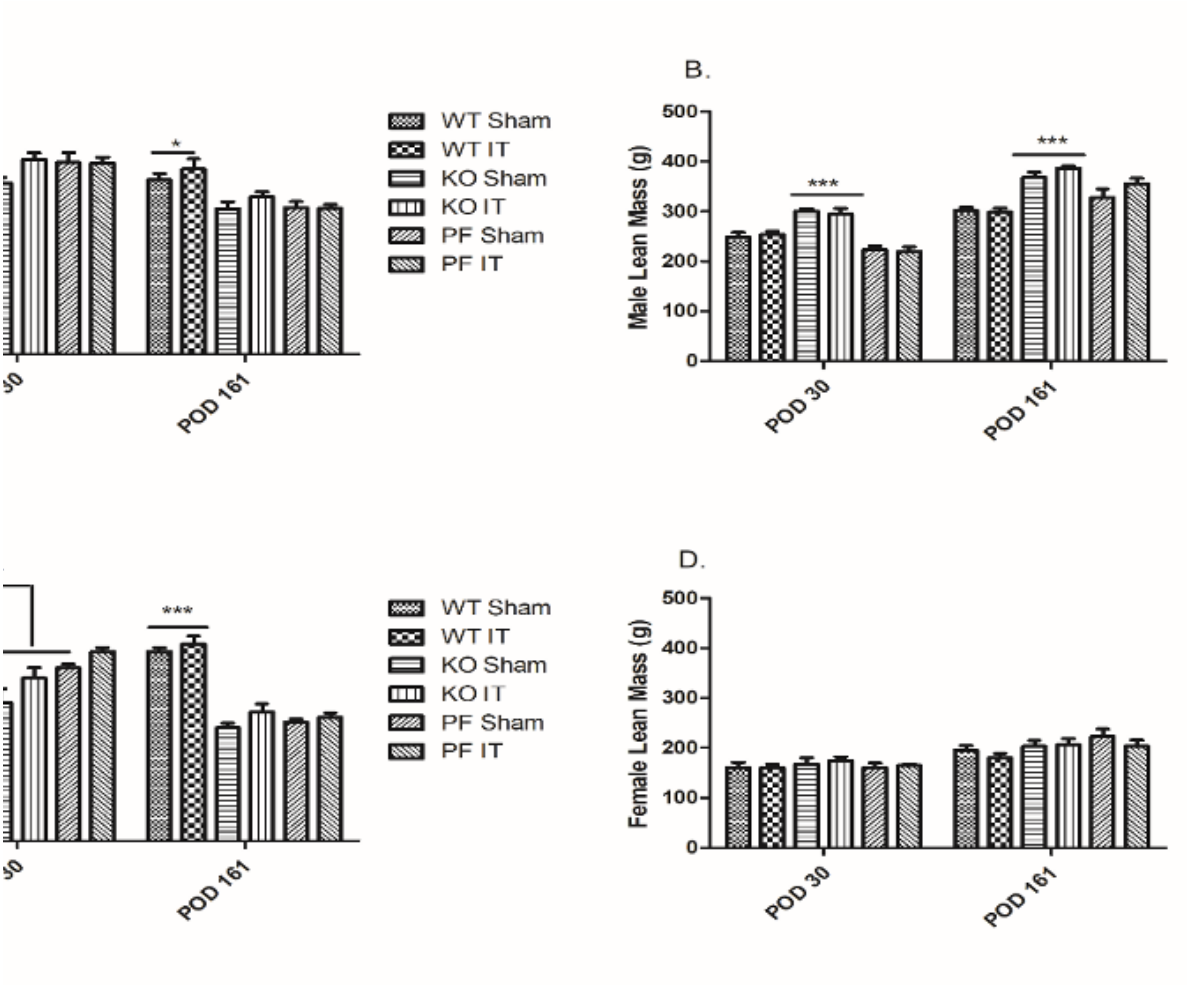


Figure 6. IT surgery did not affect lean percentage or lean mass.

DISCUSSION

Patients with pathogenic MC4R mutation show early-onset obesity and hyperphagia21, 22; We used rats, instead of mice, because the phenotype of Mc4r deficiency in mice, unlike MC4R deficiency in human and Mc4r deficiency in rats, is associated with hyperglycemia and hyperinsulinemia that precede the onset of obesity 4, 23.

Our data indicated that glucose tolerance of male KO rats was improved after IT surgery and IT surgery also increased the insulin sensitivity in both the male and female rats on about 30 days after the surgery, which suggests that Mc4r signaling might not be required for improved glucose tolerance or insulin sensitivity after IT surgery in both male and female KO rats. As we expected, the beneficial effect of glucose metabolism after IT surgery was independent of body weight loss15. Unlike the male KO Sham rats, female KO Sham rats did not show impaired glucose tolerance, which is consistent with the conclusion that males are more insulin resistant than females in both humans 24, 25 and different rodent models 26-29. In both women and female rats, the hormone estradiol (E2) has an anorexigenic function in the central nervous system through direct action in the hypothalamus and prevents the development of obesity and the onset of impaired glucose tolerance 30, 31. We also found that loss of Mc4r signaling led to insulin resistance in both male and female KO rats, which is also consistent with findings from previous studies 32, 33. Similar outcomes between PF and ad lib groups (Fig 4) suggested that IT surgery, but not the intermittent fasting (or meal feeding), may correct MC4R deficiency-induced insulin resistance.

We further expanded our study by adding both male and female PF groups to examine whether body weight is a factor affecting insulin resistance and whether IT surgery might increase insulin sensitivity in PF groups. We found that Mc4r mutation caused increased insulin resistance in both male and female PF rats on postoperative day 28 when the body weight and glucose of PF groups were almost the same as those of WT rats, which indicates that Mc4r might control the insulin resistance independent of body weight and glucose level. Additionally, IT surgery decreased insulin resistance significantly in both male and female PF rats. It might be due to the increased level of glucagon-like peptide 1 (GLP-1), fibroblast growth factor (FGF) 15/19 and FGF 21 after the IT surgery34, 35, 15, 36, 37.

Our results are consistent with previous studies that Mc4r mutation increased fat mass and/or percentage in male rats 13, 38; the mutation caused the same effect in female rats. IT surgery maintained the decreased fat mass and percentage in female rats, not male rats, on postoperative day (POD) 161; POD 161 in rats is equivalent to 18 years post surgery in humans 39. Therefore, the surgery has a lasting effect in female rats, not in male rats. This effect might be due to sexual dimorphism in fat accumulation; both human and rat females have a higher percentage of fat than age-matched males, but females accumulate more subcutaneous fat, whereas males accumulate more visceral fat 29. The mechanism by which IT surgery decreases the fat mass and percentage in only female rats needs further study.

In conclusion, our data suggest that Mc4r signaling is not necessary for the effect of ileal interposition on glucose homeostasis and insulin resistance; female rats, not male rats, maintained decreased fat mass and percentage for the duration of the study after IT surgery.

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REFERENCES

1. Cone RD. The central melanocortin system and energy homeostasis. Trends in Endocrinology & Metabolism. 1999;10(6):211-16.
2. Tao Y-X. The melanocortin-4 receptor: physiology, pharmacology, and pathophysiology. Endocrine reviews. 2010;31(4):506-43.
3. Berglund ED, Liu T, Kong X, Sohn J-W, Vong L, Deng Z, et al. Melanocortin 4 receptors in autonomic neurons regulate thermogenesis and glycemia. Nature Neuroscience. 2014 2014/07/01;17(7):911-13.
4. Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, et al. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell. 1997;88(1):131-41.
5. Farooqi IS, O'Rahilly S. Genetics of obesity in humans. Endocrine reviews. 2006;27(7):710-18.
6. Loos RJ. The genetic epidemiology of< i> melanocortin 4 receptor</i> variants. European journal of pharmacology. 2011;660(1):156-64.
7. Collet T-H, Dubern B, Mokrosinski J, Connors H, Keogh JM, de Oliveira EM, et al. Evaluation of a melanocortin-4 receptor (MC4R) agonist (Setmelanotide) in MC4R deficiency. Molecular metabolism. 2017;6(10):1321-29.
8. Buchwald H, Avidor Y, Braunwald E, Jensen MD, Pories W, Fahrbach K, et al. Bariatric surgery: a systematic review and meta-analysis. Jama. 2004;292(14):1724-37.
9. Pories WJ, Swanson MS, MacDonald KG, Long SB, Morris PG, Brown BM, et al. Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus. Ann Surg. 1995 Sep;222(3):339-50; discussion 50-2.
10. Aslan I, Ranadive SA, Ersoy BA, Rogers SJ, Lustig RH, Vaisse C. Bariatric surgery outcome in a patient with complete MC4R deficiency. International journal of obesity (2005). 2011;35(3):457.
11. Censani M, Conroy R, Deng L, Oberfield SE, McMahon DJ, Zitsman JL, et al. Weight loss after bariatric surgery in morbidly obese adolescents with MC4R mutations. Obesity. 2014;22(1):225-31.
12. Zechner JF, Mirshahi UL, Satapati S, Berglund ED, Rossi J, Scott MM, et al. Weight-independent effects of roux-en-Y gastric bypass on glucose homeostasis via melanocortin-4 receptors in mice and humans. Gastroenterology. 2013;144(3):580-90. e7.
13. Mul JD, Begg DP, Alsters SI, van Haaften G, Duran KJ, D'Alessio DA, et al. Effect of vertical sleeve gastrectomy in melanocortin receptor 4-deficient rats. American Journal of Physiology-Endocrinology And Metabolism. 2012;303(1):E103-E10.

14. Zhao P, Wendt D, Goodin SZ, Ravichandran S, Chouinard TE, Strader AD. Adaptation of Intestinal and Bile Acid Physiology Accompany the Metabolic Benefits Following Ileal Interposition in the Rat. Obesity Surgery.1-10.
15. Strader AD, Clausen TR, Goodin SZ, Wendt D. Ileal interposition improves glucose tolerance in low dose streptozotocin-treated diabetic and euglycemic rats. Obesity surgery. 2009;19(1):96-104.
16. Strader AD. Ileal transposition provides insight into the effectiveness of gastric bypass surgery. Physiology & behavior. 2006;88(3):277-82.
17. Buchwald H, Estok R, Fahrbach K, Banel D, Sledge I. Trends in mortality in bariatric surgery: a systematic review and meta-analysis. Surgery. 2007;142(4):621-35.
18. van Boxtel R, Vroling B, Toonen P, Nijman IJ, van Roekel H, Verheul M, et al. Systematic generation of in vivo G protein-coupled receptor mutants in the rat. The pharmacogenomics journal. 2011;11(5):326-36.
19. Obici S, Magrisso IJ, Ghazarian AS, Shirazian A, Miller JR, Loyd CM, et al. Moderate voluntary exercise attenuates the metabolic syndrome in melanocortin-4 receptor-deficient rats showing central dopaminergic dysregulation. Molecular metabolism. 2015;4(10):692-705.
20. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-19.
21. Lubrano-Berthelier C, Dubern B, Lacorte J-M, Picard F, Shapiro A, Zhang S, et al. Melanocortin 4 receptor mutations in a large cohort of severely obese adults: prevalence, functional classification, genotype-phenotype relationship, and lack of association with binge eating. The Journal of Clinical Endocrinology & Metabolism. 2006;91(5):1811-18.
22. Calton MA, Ersoy BA, Zhang S, Kane JP, Malloy MJ, Pullinger CR, et al. Association of functionally significant Melanocortin-4 but not Melanocortin-3 receptor mutations with severe adult obesity in a large North American case–control study. Human molecular genetics. 2009;18(6):1140-47.
23. Marie LS, Miura GI, Marsh DJ, Yagaloff K, Palmiter RD. A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors. Proceedings of the National Academy of Sciences. 2000;97(22):12339-44.
24. Nuutila P, Knuuti MJ, Mäki M, Laine H, Ruotsalainen U, Teräs M, et al. Gender and insulin sensitivity in the heart and in skeletal muscles: studies using positron emission tomography. Diabetes. 1995;44(1):31-36.
25. Mittendorfer B. Insulin resistance: sex matters. Current Opinion in Clinical Nutrition & Metabolic Care. 2005;8(4):367-72.
26. Clark JB, Palmer CJ, Shaw WN. The diabetic Zucker fatty rat. Proceedings of the Society for Experimental Biology and Medicine. 1983;173(1):68-75.
27. Zierath JR, Houseknecht KL, Gnudi L, Kahn BB. High-fat feeding impairs insulin-stimulated GLUT4 recruitment via an early insulin-signaling defect. Diabetes. 1997;46(2):215-23.
28. Li AC, Brown KK, Silvestre MJ, Willson TM, Palinski W, Glass CK. Peroxisome proliferator–activated receptor γ ligands inhibit development of atherosclerosis in LDL receptor–deficient mice. Journal of Clinical Investigation. 2000;106(4):523.
29. Macotela Y, Boucher J, Tran TT, Kahn CR. Sex and depot differences in adipocyte insulin sensitivity and glucose metabolism. Diabetes. 2009;58(4):803-12.

30. Clegg DJ, Brown LM, Woods SC, Benoit SC. Gonadal hormones determine sensitivity to central leptin and insulin. Diabetes. 2006;55(4):978-87.
31. Roepke TA. Oestrogen modulates hypothalamic control of energy homeostasis through multiple mechanisms. Journal of neuroendocrinology. 2009;21(2):141-50.
32. Fan W, Dinulescu DM, Butler AA, Zhou J, Marks DL, Cone RD. The central melanocortin system can directly regulate serum insulin levels. Endocrinology. 2000;141(9):3072-79.
33. Obici S, Feng Z, Tan J, Liu L, Karkanias G, Rossetti L. Central melanocortin receptors regulate insulin action. Journal of Clinical Investigation. 2001;108(7):1079-85.
34. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet. 2006 Nov 11;368(9548):1696-705.
35. Patti ME, Houten SM, Bianco AC, Bernier R, Larsen PR, Holst JJ, et al. Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism. Obesity. 2009;17(9):1671-77.
36. Mencarelli A, Renga B, D'Amore C, Santorelli C, Graziosi L, Bruno A, et al. Dissociation of intestinal and hepatic activities of FXR and LXRα supports metabolic effects of terminal ileum interposition in rodents. Diabetes. 2013;62(10):3384-93.
37. Yan K, Chen W, Zhu H, Lin G, Pan H, Li N, et al. Ileal transposition surgery decreases fat mass and improves glucose metabolism in diabetic GK rats: possible involvement of FGF21. Frontiers in physiology. 2018;9:191.
38. Mul JD, Boxtel R, Bergen DJ, Brans MA, Brakkee JH, Toonen PW, et al. Melanocortin receptor 4 deficiency affects body weight regulation, grooming behavior, and substrate preference in the rat. Obesity. 2012;20(3):612-21.
39. Andreollo NA, Santos EFd, Araújo MR, Lopes LR. Rat's age versus human's age: what is the relationship? ABCD Arquivos Brasileiros de Cirurgia Digestiva (São Paulo). 2012;25(1):49-51.