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Leptin signaling in vagal afferent neurons supports the absorption and storage of nutrients from high-fat diet

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Abstract

Objective: Activation of vagal afferent neurons (VAN) by postprandial gastrointestinal signals terminates feeding and facilitates nutrient digestion and absorption. Leptin modulates responsiveness of VAN to meal-related gastrointestinal signals. Rodents with high-fat diet (HF) feeding develop leptin resistance that impairs responsiveness of VAN. We hypothesized that lack of leptin signaling in VAN reduces responses to meal-related signals, which in turn decreases absorption of nutrients and energy storage from high-fat, calorically dense food.

Methods: Mice with conditional deletion of the leptin receptor from VAN (Nav1.8-Cre/LepR$^{fl/fl}$; KO) were used in this study. Six-week-old male mice were fed a 45% HF for 4 weeks; metabolic phenotype, food intake, and energy expenditure were measured. Absorption and storage of nutrients were investigated in the refed state.

Results: After 4 weeks of HF feeding, KO mice gained less body weight and fat mass that WT controls, but this was not due to differences in food intake or energy expenditure. KO mice had reduced expression of carbohydrate transporters and absorption of carbohydrate in the jejunum.

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We do not have no conflict of interest.

KO mice had fewer hepatic lipid droplets and decreased expression of *de novo* lipogenesis-associated enzymes and lipoproteins for endogenous lipoprotein pathway in liver, suggesting decreased long-term storage of carbohydrate in KO mice.

Conclusions: Impairment of leptin signaling in VAN reduces responsiveness to gastrointestinal signals, which reduces intestinal absorption of carbohydrates and *de novo* lipogenesis resulting in reduced long-term energy storage. This study reveals a novel role of vagal afferents to support digestion and energy storage that may contribute to the effectiveness of vagal blockade to induce weight loss.

Keywords

Gut brain axis; Leptin resistance; High fat diet; Obesity; Lipogenesis; Glucose absorption

Introduction

Activation of the parasympathetic nervous system promotes digestion and integrates the postprandial response to a meal. The vagus nerve is the major parasympathetic neural circuit connecting visceral organs and the central nervous system. The vagal pathway is bidirectional, comprising afferent neurons that sense peripheral signals and conduct this information to the nucleus of the solitary tract (NTS) and efferent neurons that send commands from the dorsal nucleus of vagus nerve (DMV) to peripheral organs [1]. To coordinate digestion and absorption, vagal afferent and efferent neurons comprise neural reflexes that are involved in the regulation of gastrointestinal motility and secretion. Gastric distension stimulates muscular vagal afferents to induce a vago-vagal reflex relaxation of gastric smooth muscle to accommodate the meal [2]. Food particles in the stomach also stimulate mucosal vagal afferent neurons, and induce a vago-vagal reflex to delay gastric emptying, to ensure efficient digestion and absorption in the small intestine [3]. During the intestinal phase, nutrients induce secretion of gut hormones, such as cholecystokinin (CCK), from enteroendocrine cells. CCK acts primarily on vagal afferent neurons to initiate a vago-vagal reflex slowing of gastric emptying [4] and also stimulates secretion of pancreatic enzymes and bile acids that facilitates digestion and absorption of proteins and fats [5].

Leptin secreted by adipocytes and gastric endocrine cells is involved in long-term regulation of energy homeostasis via actions on the hypothalamus and other neural components, including vagal afferent neurons [6,7]. The leptin receptor (LepR) is expressed by vagal afferent neurons (VAN) that innervate the stomach and duodenum [8–11]. Studies of cultured VAN from rats show that leptin evokes Ca2+ influx and induces depolarization [12,13]. In the stomach, leptin potentiates the mechanosensitivity of mucosal VAN after feeding but down-regulates the activity of muscular vagal afferent during fasting [14]. In addition, *in vitro* studies show that leptin synergizes CCK-evoked Ca^{2+} influx in VAN [11–13] and *in vivo* enhances the effectiveness of CCK in suppressing food intake [15]. In rodents with high-fat diet (HF) feeding, VAN develop leptin resistance that leads to reduced gastric vagal mechanosensitivity [14], CCK-induced satiety [16] and hyperphagia. We have previously shown that mice with conditional deletion of the LepR from VAN (Nav1.8-Cre/$LepR^{fl/fl}$; KO) are hyperphagic, have an increase in body weight and adiposity, and do not respond to CCK [17].

Taken together, these findings suggest that leptin acts at the level of the vagal afferent pathway to support the physiological response to meal ingestion.

In the present study, we used KO mice to test the hypothesis that lack of leptin signaling in VAN reduces responses to meal-related signals, which in turn decreases the absorption of nutrients and storage of energy from a HF. Firstly, we provide further evidence for impaired integrity of VAN response to CCK and gastric mechanostimulation in chow-fed KO mice. Secondly, we fed wild-type control (WT) and KO mice HF for 4 weeks and examined the HF-induced obese phenotype, including weight gain, fat accumulation, and glucose tolerance. To determine the net energy balance, we measured 24-hr food intake and energy expenditure by indirect calorimetry. Lastly, we determined the intestinal absorption of carbohydrates and fats, and evaluated the energy deposition of excess carbohydrates in liver and adipose tissue.

Materials and Methods

Animals

All studies were approved by UC Davis Institutional Animal Care and Use Committee or the South Australian Health and Medical Research Institute Animal Ethics Committee. KO mice were generated by crossing of $LepR^{fl/fl}$ mice and Nav1.8-Cre mice; $LepR^{fl/fl}$ mice were used for WT [17,18]. Mice were housed in a facility maintained at 22°C under 12–12 hr light-dark schedule. Six-week old male mice were fed either low-fat chow diet (PicoLab Mouse Diet 20 #5053, LabDiet, St. Louis, MO), or HF containing 45% kcal fat and 17% kcal sucrose (D14110103, Research Diets Inc, New Brunswick, NJ) for 4 weeks before the studies unless specifically stated otherwise. Mice were allowed ad libitum access to food and water unless specifically stated otherwise. Body composition was measured using X-ray absorptiometry (DEXA). In terminal experiments, tissues were collected in different conditions as specified below.

Histological Staining

Tissues were processed and stained with hematoxylin and eosin (H&E), Oil Red O, and Periodic acid-Schiff (PAC). Details of staining, imaging, and quantification are described in Supplementary Materials and Methods.

Real-Time PCR

Fresh frozen tissues were homogenized, and total RNA was extracted with TRIzol (Invitrogen, Waltham, MA) following the manufacturer's instructions. One μg of total RNA was used for synthesizing cDNA with the iScript cDNA synthesis kit (BIO-RAD, Hercules, CA). Real-time PCR was performed with the QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, Waltham, MA) with PowerUp SYBR Green Master Mix (Applied Biosystems, Waltham, MA). The sequence of primers used in real-time PCR is listed in Table S1. Gene expression was analyzed by $2^{-\Delta\Delta CT}$ and normalized by β-actin and Rplp0 mRNA expression.

Measurement of Food Intake and Energy Expenditure

HF-fed mice were individually housed and acclimated to monitoring chambers for 2 days prior to the data collection. Food intake, physical activity, and O_2/CO_2 exchange were monitored by Comprehensive Laboratory Animal Monitoring system (CLAMS, Columbus Instruments, Columbus, OH) for 3 days. The data from the second day was used for analysis, and the parameters for CLAMS and analysis were described previously [19].

Ussing Chamber

HF-fed mice were overnight fasted and refed for 1 hr. Mid-jejunum was dissected and mounted in an Ussing chamber. The tissues were allowed to reach equilibrium for 30 min, and baseline short-circuit current was recorded. Glucose and mannitol (10mM) were added into the mucosal and serosal side, respectively, and the short-circuit current at equilibrium was recorded after 5 min. The glucose-evoked current was calculated from the difference between baseline and final equilibrium. Details are described in Supplementary Materials and Methods.

Statistics

Randomization and blinding were used in experimental design for animal studies, histology process, images analysis, and analytical measurements. All experiments were replicated at least two times in the laboratory. Comparisons between WT mice and KO mice were calculated using two-tailed unpaired t-tests, unless specified otherwise. Two-way ANOVA with Bonferroni's multiple comparisons test was performed to analyze the data of weekly body weight, cumulative food intake, glucose tolerance test, and insulin tolerance test. Statistical analyses were performed with GraphPad Prism 8.0 (GraphPad Software, La Jolla, CA). All results are presented as mean ± SEM, with the following significance levels: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Results

Conditional deletion of LepR in VAN reduces responsiveness to peripheral meal-related signals

CCK-induced c-fos expression in second-order neurons located in the hindbrain via activation of unmyelinated vagal C-fibers from the gastrointestinal tract is synergized by leptin [20,21]. Exogenous CCK was administered to low-fat chow diet fed mice and the hindbrain was immunostained for c-fos and isolectin-IB4 to label C-fibers (Fig. S1A-C). There was a significant reduction of CCK-induced c-fos in NTS and DMV in KO (Fig. S1D). This suggests deletion of LepR in VAN decreased CCK-induced activation of VAN and potentially impaired the vago-vagal reflex circuit. The density of unmyelinated vagal C-fibers in the area postrema (AP) and NTS was reduced in KO mice, suggesting that impaired gut-brain axis might be due to reduced projections of VAN (Fig. S1E). Activation of the vago-vagal reflex circuit by CCK inhibits gastric emptying. To evaluate the integrity of this circuit, low-fat chow-fed mice were orally gavaged with a liquid diet containing acetaminophen (1 % w/v); the rate of gastric emptying was measured by appearance of acetaminophen in the plasma. Fifteen min after oral gavage, plasma levels of acetaminophen

were higher in KO mice, suggesting that gastric emptying was faster in KO mice (Fig. S1F). Moreover, administration of the CCK A receptor antagonist, devazepide (100 μg/Kg, ip) increased the rate of gastric emptying in WT but not KO mice. This suggests that CCK-induced inhibition of gastric emptying was impaired in KO mice. The mechanosensitivity of gastric mucosal VAN is potentiated by leptin in *ab libitum* feeding [14]. To examine the mechanosensitivity of individual gastric vagal afferents in KO mice, the effect of leptin on gastric afferent activity was measured *in vitro*. In low-fat chow-fed WT mice, leptin significantly increased mechanosensitivity to mucosal stroking, but this leptin-induced potentiation was not observed in chow-fed KO mice (Fig. S2). Overall, these data suggest that KO mice have a reduced response to peripheral meal-related signals, which impairs vago-vagal reflex regulation of gastric emptying.

KO mice are resistant to HF-induced obese phenotype

To evaluate the effect of impairment of vagal sensitivity to gastrointestinal signals on HF feeding, 6-week-old male mice were fed HF for 4 weeks. WT mice had a significant weight gain after 2 weeks of HF; in comparison there was no significant increase in body weight in KO mice (Fig. 1A). After 4 weeks of HF feeding, body composition was measured by DEXA and tissues were collected for histology. Compared to KO mice, WT mice had a higher total fat mass, with no difference in lean mass between groups (Fig. 1B). Both mass and adipocyte size of epididymal and inguinal fat pad were higher in WT mice (Fig. S3). In addition, WT mice had a higher number of lipid droplets in the liver (Fig. 1C), but no significant immune cell infiltration was observed (Fig. S4A). WT mice had higher expression of Ccl2 in liver, but there was no difference in proinflammatory markers, including Il1b, Il6 and Tnf (Fig S4B). These data suggest that the accumulation of lipid droplets in the liver led to mild hepatocellular injury in WT mice. Furthermore, WT mice developed glucose intolerance and insulin resistance, possibly due to greater fat accumulation in adipose tissues and liver (Fig. S5). These results suggest that impairment of vagal sensitivity metabolically protects KO mice from HF-induced weight gain, fat accumulation, and glucose intolerance.

No difference in overall food intake and energy expenditure

To study the mechanism of this metabolic protection in KO mice, food intake and energy expenditure were measured using CLAMS from male mice fed HF diet for 4 weeks. There was no difference in 24 hr-averaged food intake (Fig. 2A-B). However, KO had increased food intake at the first 3 hr of dark phase and decreased food intake at the last 3 hr of dark phase, suggesting that KO mice tend to consume most food at the beginning of the dark phase and eat less towards the end (Fig. 2C). These data showed that although impairment of vagal sensitivity in the gastrointestinal tract does not affect total consumption of HF, it does impact on the pattern and timing of food intake. In addition, there was no difference in 24 hr-averaged energy expenditure (Fig. 2D-E). ANCOVA analysis for averaged energy expenditure in either light phase or dark phase showed no difference (Fig. S6A-B). However, KO had higher energy expenditure for the first 3 hr of the dark phase and lower energy expenditure at the last 3 hr of dark phase (Figure 2F and Fig. S6C-D). This difference in energy expenditure might be primarily due to the concomitant change in physical activity (Fig. S7).

Reduced the jejunal glucose absorption in HF-fed KO mice

Rodents with ablation of VAN by capsaicin do not upregulate jejunal glucose transport in response to dietary carbohydrate, and this is due to lack of upregulation of sodium-glucose linked transporter (SGLT1) in the jejunum [22,23]. To determine whether the impairment of vagal sensitivity in KO mice leads to malabsorption of dietary carbohydrate, we performed an *ex vivo* study to examine glucose transport through SGLT1 in Ussing chambers. Mice fed HF for 4 weeks were fasted overnight followed by *ad libitum* feeding for 1 hr and the mid-jejunum was mounted in Ussing chambers. Addition of glucose to the mucosal chamber increased the short circuit current in jejunal tissue from both WT and KO ; however, the increase in short circuit current was 2 times higher in WT than KO mice, suggesting jejunal SGLT1-mediated glucose transport is reduced in KO mice (Fig. 3A). The upregulation of SGLT1 in jejunum is mediated by sweet taste receptors, T1R2/T1R3, and this effect is blunted by capsaicin vagal deafferentation [24]. To determine whether reduced responsiveness to gastrointestinal signals impairs glucose-evoked upregulation of carbohydrate transporters, HF-fed mice fasted for 6 hr followed by oral gavage of glucose (3 mg/Kg, after 15 min). KO mice had significantly less expression of Sglt1, Glut2 and Glut5 in jejunum (Fig. 3B). These results suggest that impaired vagal sensitivity to gastrointestinal signals in KO reduces meal-evoked upregulation of carbohydrate transporters in the jejunum, which in turn leads to malabsorption of dietary carbohydrate.

Mice with a global deletion of CCK malabsorb fat and are also resistant to HF-induced obesity [25]. To determine whether the decreased response to CCK in KO mice leads to defects in fat absorption, mice were fed with HF containing 5% fat as sucrose polybehenate and the amount of fecal fatty acid was measured by mass spectrometry [26]. The intestinal fat absorption was slightly higher in KO mice (94% vs 91%) compared with WT mice, but there was no difference in the amount or composition of unabsorbed fatty acid in the feces (Fig. S8A-B). KO mice that were fasted overnight followed by a refeed for 3 hr had higher expression of apolipoproteins, Apob and Apoa4, in jejunum, but there was no difference in other genes related to intestinal fat absorption compared to WT mice (Fig. S8C). Taken together, it suggests that less weight gain in HF-fed KO mice is likely due to reduced absorption of carbohydrate but not fat.

Reduced hepatic glycogenesis and systemic lipid deposition in HF-fed KO mice

After meal consumption, carbohydrate is stored as glycogen in the liver and muscle and excess carbohydrate is converted into fatty acids by *de novo* lipogenesis in liver and adipose tissue [27]. To determine whether reduced carbohydrate absorption in KO compared to WT mice alters metabolism of carbohydrate in liver and adipose tissue, mice fed HF for 4 weeks were fasted overnight followed by refeeding for 3 hr. KO mice had a lower expression of Pklr and G6pc, but higher expression of Gys2 (Fig. 4A). This suggests that hepatic gluconeogenesis and glycolysis is reduced in KO mice on a HF, but hepatic glycogenesis is higher (Fig. S9). However, the amount of glycogen storage in liver was lower in KO mice (Fig. 4B). These data suggest that decreased carbohydrate absorption in KO mice leads to a delay in replenishing hepatic glycogen after overnight fasting. In addition to delayed hepatic glycogenesis, KO mice had lower expression of critical enzymes in lipogenesis, Scd1 and Acaca, in liver (Fig. 4C). However, there was no difference in the expression of Srebp1,

suggesting the downregulation of hepatic lipogenesis in KO mice is not due to lack of regulation sterol-sensing mechanism. Accompanied with reduced lipogenesis, KO mice had lower expression of proteins associated with endogenous lipoprotein pathway, including Apob, Apoe and ldlr, in liver (Fig. 4D). The expression of Acaca and Lpl, an enzyme mediated uptake of triglycerides from lipoprotein, in epididymal white adipose tissue (WAT) was lower in KO mice; the expression of Acaca was lower in inguinal WAT (Fig. S10). Taken together, the data suggest that reduced carbohydrate absorption in KO mice results in the majority of the substrate going into the glycogenesis pathway and limiting the conversion of carbohydrate to lipid in both the liver and adipose tissue.

Discussion

Leptin signaling promotes energy expenditure and facilitates the suppression of food intake, which under normal conditions leads to negative energy balance, in response to an energy surplus. However, the development of leptin resistance in peripheral neurons, such as VAN, and in neurons in the CNS during ingestion of HF leads to a failure of this response to positive energy balance [28,29]. Reduced leptin signaling has been considered as a key factor causing energy imbalance leading to obesity. However, the present study reveals an unexpected effect of leptin signaling in VAN on energy homeostasis (Fig. 5). The data show that defective vagal transmission of gastrointestinal signals leads to glucose malabsorption and lower systemic energy storage from a HF, which ameliorates the diet-induced obese phenotype, including fat accumulation, hepatic steatosis and glucose intolerance, during the early stage of HF feeding.

As discussed in the introduction, leptin modulates sensitivity of vagal afferent to gastrointestinal signals. However, it should be noted that a recent study using chromogenic *in situ* hybridization suggests that only 3% of VAN in the mouse express the LepR and could not demonstrate leptin-induced JAK-STAT signaling in the nodose ganglion [30]. Whilst it is hard to reconcile these findings with other data showing a role for the leptin receptor in VAN, it is clear in the present study, that conditional deletion of the LepR in VAN provides strong evidence to support the concept that leptin signaling is essential for the peripheral endings of VAN to respond to the gastrointestinal signals, CCK and gastric mucosal mechanostimulation, and transmit this information from the gut to the brain. This defect in vagal afferent transmission leads to, decreased CCK-induced activation of brainstem neurons, an absence of leptin-induced potentiation of gastric mucosal receptor to mucosal stroking, and increased gastric emptying of a liquid nutrient meal. Here we also demonstrate that lack of the LepR in VAN leads to a significant reduction in the projection of vagal C-fibers to sites of termination in the brainstem. This finding is similar to observations in previous studies showing that leptin signaling promotes neuronal projection to the arcuate nucleus of the hypothalamus during development [31–33]. The neuronal projection of agouti-related peptide neurons and VAN is decreased in HF-fed adult mice, suggesting the plasticity of neuronal projections can be modulated by diets in adulthood; however, whether this is solely mediated by leptin is not known [34,35]. It is possible that the alteration in the function of the vagal afferent pathway in the KO mice is due to this decreased vagal projection on gastrointestinal tract and even loss of VAN. There was no discernible difference in preparation of measuring mechanosensitivity of gastric vagal afferents between WT and KO

mice, and no clear evidence for differences in neuronal cell number in the nodose ganglion in our previous publication [17]. However, neuronal counts in the nodose ganglion and staining of gastrointestinal projection of vagal afferent are desirable to fully understand the role of leptin in VAN.

Preclinical and clinical studies show that ablation of the vagus nerve leads to significant weight loss in obese animals or human subjects [36]. Rats with either chemical vagal deafferentation of the upper gastrointestinal tract or subdiaphragmatic vagotomy are resistant to diet-induced weight gain [37]. In addition, ablation of the vagus nerve in obese rodents improves glucose intolerance [38,39], hepatic steatosis [40,41], and adipose tissue expansion [42,43]. Morbidly obese patients with ablation of the vagus nerve have significant weight loss one year after the operation [44]. Recently, electrical blockade of the vagus nerve has been used for obesity treatment. More than half of obese patients had significant weight loss and improvement of metabolic risk factors after vagal nerve blockade [45,46]. However, the mechanism by which vagal nerve blockade facilitates weight loss in obese patients is not fully understood. In this study, we show reduced responsiveness of VAN to meal-related gastrointestinal signals efficiently impairs intestinal carbohydrate absorption and long-term energy storage during HF feeding. This provides a potential mechanism for how manipulation of the vagus nerve, by electrical blockade, may contribute to weight loss in obese patients.

Duodenal infusion of glucose, fructose, or saccharin leads to a significant increase in the amount of SGLT1 protein; this upregulation is mediated by the 5-HT_3 receptor and vagal afferents [24]. The activation of T1R2/T1R3 on L cells induces the release of GLP-1 that further triggers the release of serotonin from enterochromaffin cells [47]. Serotonin via 5-HT_3 receptor has been shown to activated vagal afferent neurons and increases glutamatergic neurotransmission from VAN to secondary neurons located in hindbrain [48]. Thus, it appears that L cells and enterochromaffin cells sense dietary carbohydrate, and the signal is mediated by vagal afferents resulting in upregulation of SGLT1 in the small intestine to facilitate carbohydrate absorption. Here we show that ablation of leptin signaling in VAN reduces postprandial SGLT1-mediated glucose transport in jejunum. We also showed that expression of SGLT1, GLUT2 and GLUT5 after oral gavage of glucose was lower in KO than wild-type mice. These results suggest that leptin signaling is necessary to support the vagal function in responding to gut signals that acutely induce expression of carbohydrate transporters and facilitate absorption. The reduced intestinal absorption of glucose limits the amount of substrate that can replenish hepatic storage of glycogen after the meal. Furthermore, the reduction in substrate decreases the *de novo* lipogenesis in both liver and adipose tissues in KO mice that impairs long-term energy storage. It is interesting to note that when mice are ingesting a chow diet, there is an increase in body weight due to an increase in food intake. If there is also a decrease in carbohydrate absorption in chow-fed KO mice, this is clearly not limiting weight gain.

Subdiaphragmatic vagotomy reduces *de novo* lipogenesis and production of lipoprotein in the liver which ameliorates hepatic steatosis and hyperlipidemia in mice fed a HF for 12 weeks [41]. In addition, mice with subdiaphragmatic vagotomy have increased postprandial release of GLP-1, and authors speculate increased direct action of GLP-1 on hepatocytes

reduces hepatic lipogenesis. Another study demonstrates that subdiaphragmatic vagotomy reduced hepatic triglyceride, adipocytic lipogenesis, and adipocyte proliferation in mice with disruption of melanocortin 4 receptor signaling in the CNS, suggesting that vagal nerves mediate the signals upregulating lipogenesis in liver and adipose tissue [43]. In contrast, the vagal efferent pathway mediated hypothalamic signals, including GLP-1 and fatty acid, that downregulate hepatic lipogenesis and secretion of lipoprotein [49,50]. Therefore, to date, the role of the vagal pathway in influencing hepatic lipogenesis remains unclear. In the present study, we demonstrate that defective vagal transmission of gastrointestinal signals in KO mice could reduce hepatic lipogenesis and lipoproteins during HF feeding, that is similar to previous study with subdiaphragmatic vagotomy [41]. However, we did not observe an increase in glucose-induced or diet-induced GLP-1 in KO mice fed a HF for 4 weeks (data not shown), suggesting the indirect GLP-1 pathway is unlikely to be the mechanism that downregulates hepatic lipogenesis in KO mice. Whether peripheral signals acting on vagal afferent are involved in the direct regulation of hepatic lipid metabolism needs to be determined.

In conclusion, we demonstrate that lack of leptin signaling in VAN leads to reduced sensitivity of vagal afferent pathway to gastrointestinal signals. This results in a dysregulation of expression of intestinal carbohydrate transporters and malabsorption of glucose from a HF, which has an impact on global *de novo* lipogenesis and energy deposition. These findings, while still correlative, emphasize the importance of leptin in the vagal pathway, that supports digestion, nutrient absorption, and energy storage during ingestion of HF. This pathway may provide a potential mechanism for how manipulation of the vagus nerve, for example electrical blockade, may contribute to weight loss in people with obesity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations:

AP	area postrema
AUC	area under the curve
CCK	cholecystokinin

CLAMS	comprehensive laboratory animal monitoring system
CNS	central nervous system
DEXA	X-ray absorptiometry
DMV	dorsal nucleus of vagus nerve
GLP-1	glucagon-like peptide 1
HF	high-fat diet
LepR	leptin receptor
NTS	nucleus of the solitary tract
PNS	peripheral nervous system
SGLT1	sodium-glucose linked transporter
VAN	vagal afferent neurons

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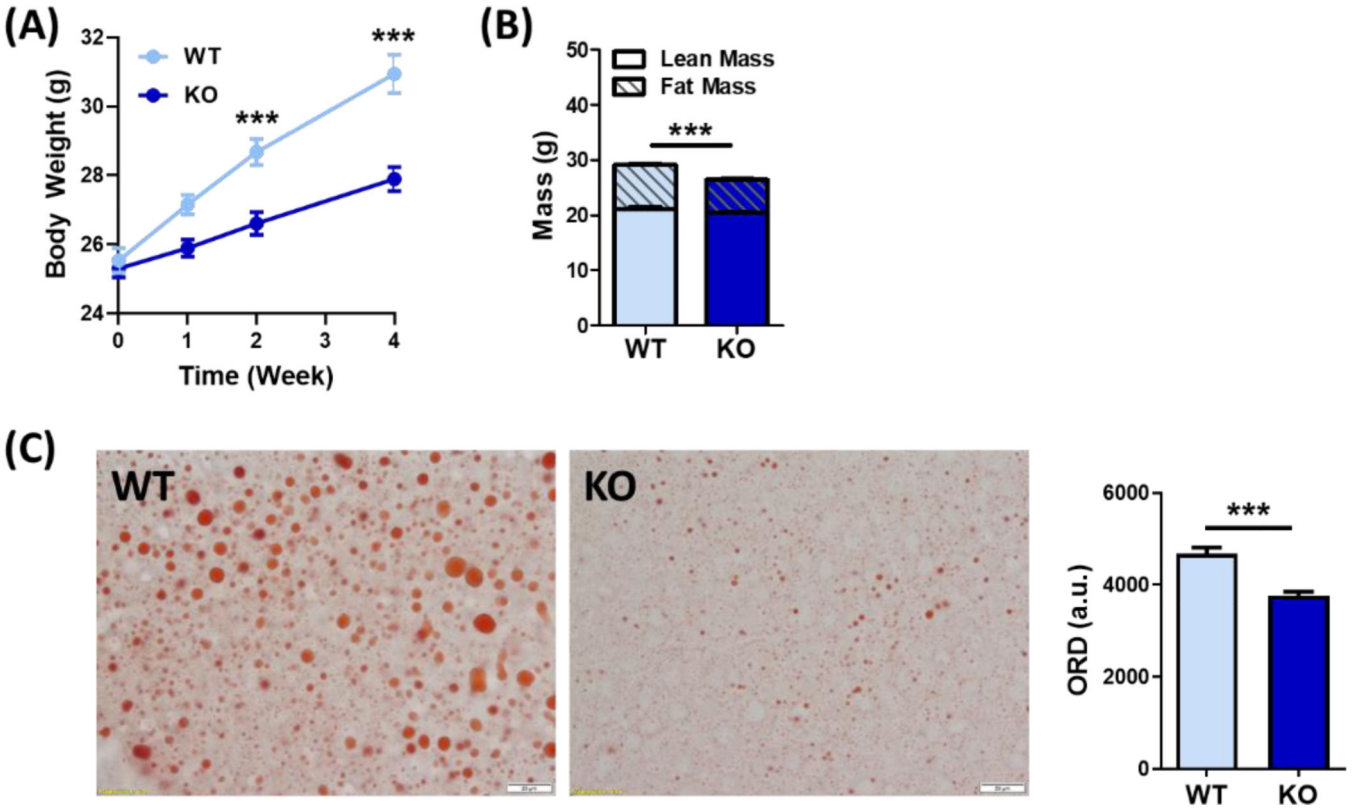

Figure 1. KO mice were resistant to high-fat diet-induced obese phenotype.

Mice were fed HF for 4 weeks; body weight was measured weekly and analyzed by two-way ANOVA with Bonferroni's multiple comparisons test (A). Body composition was measured by DEXA after 4 weeks of HF (B). Liver were collected at necropsy following *ab libitum* feeding. Lipid droplets in liver was measured using oil red O staining; 6 replicates per mouse (C). N = 9 per group, data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$.

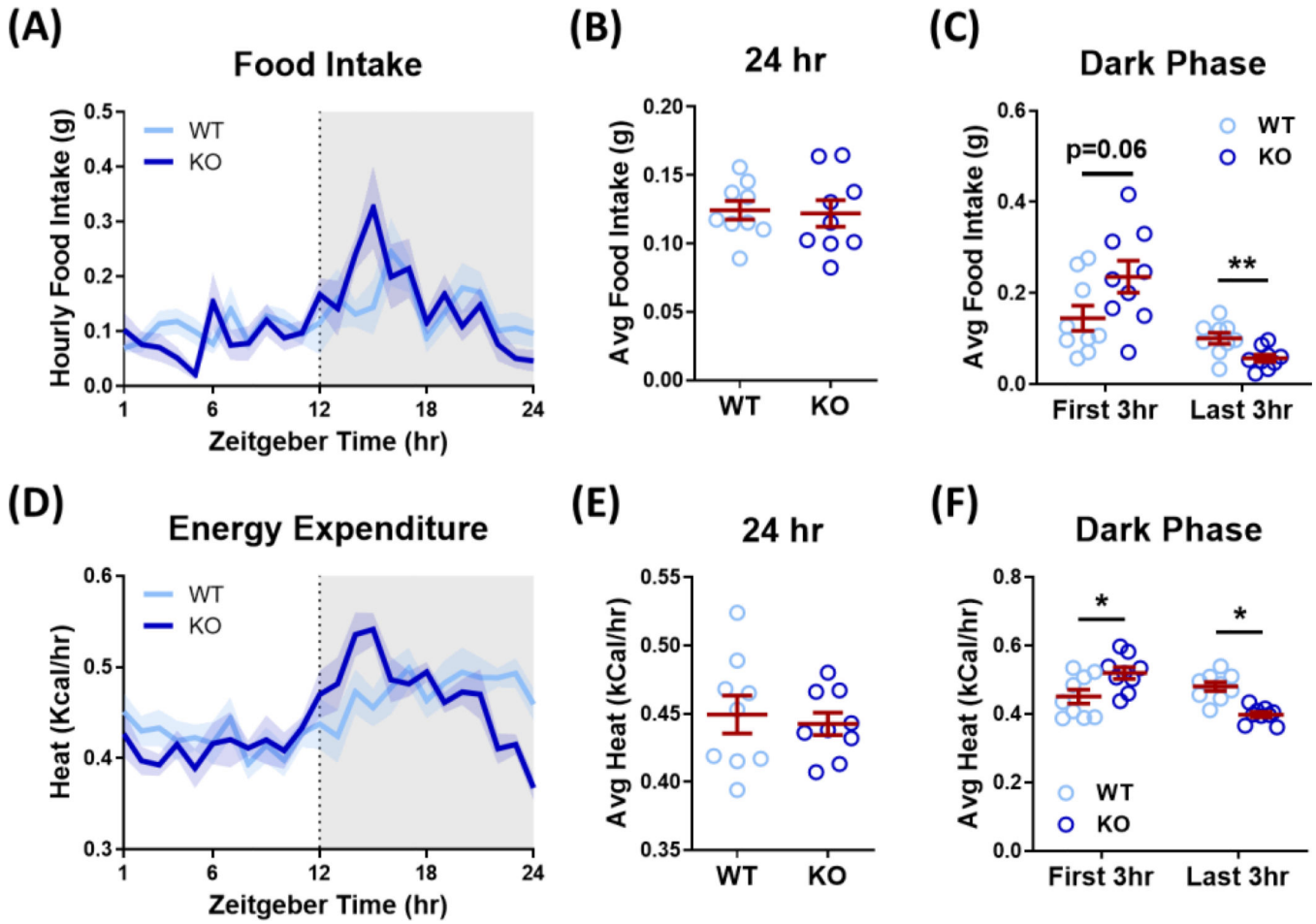

Figure 2. No difference in overall food intake and energy expenditure.

Food intake and energy expenditure (EE) was monitored using CLAMS system after 4 weeks of HF. Hourly food intake (A), 24 hr-averaged food intake (B) and dark-phase food intake (C) were analyzed. Hourly EE (D), 24 hr-averaged EE (E) and dark-phase EE (F) were analyzed. N = 9 per group, data are presented as mean ± SEM. *p < 0.05, **p < 0.01.

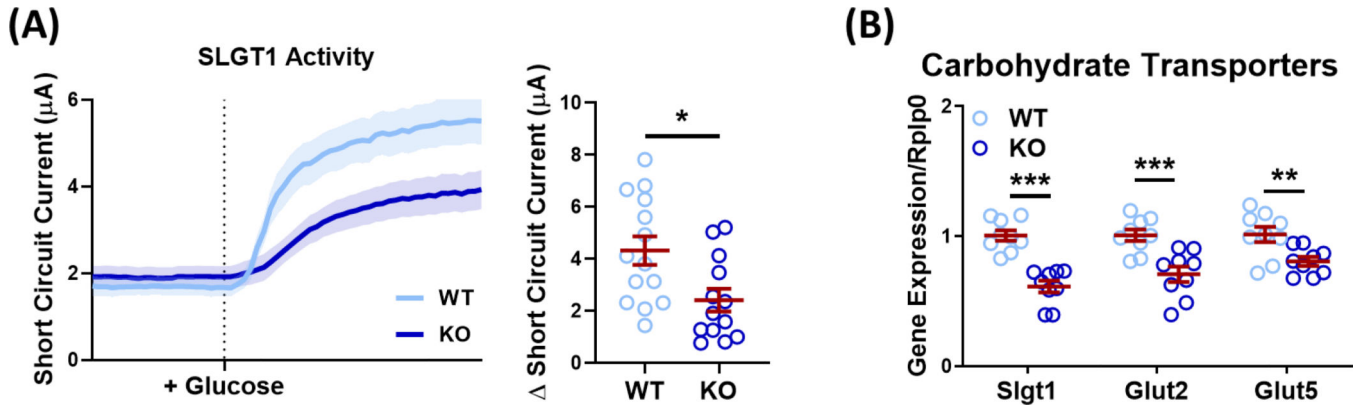

Figure 3. Decreased absorption of monosaccharide in the jejunum in KO mice.

Mice were fasted overnight and refed for 1 hr after 4 weeks of HF. Short circuit current conducted by sodium-glucose transport 1 (SGLT1) and glucose-evoked current was measured in mid-jejunum by Ussing chambers (2–4 replicates per mouse, N = 13–14 per group) (A). Expression of carbohydrate transporters was measured in jejunal tissue, N = 9 per group (B). Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

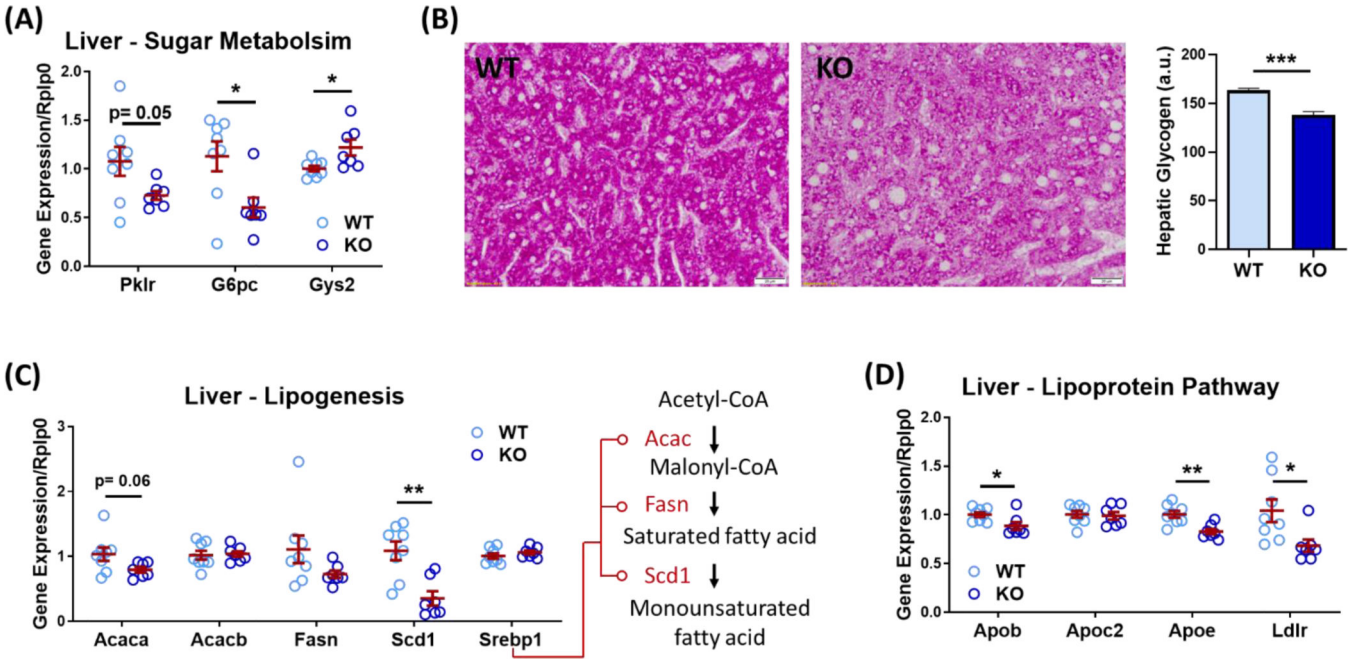

Figure 4. Delayed storage of hepatic glycogen and decreased lipogenesis in liver KO mice.

Mice were fasted overnight and refed for 3 hr after 4 weeks of HF. Expression of sugar metabolism associated genes was measured in liver (A). Periodic acid-Schiff stain for hepatic glycogen, 6 replicates per mouse (B). Expression of *de novo* lipogenesis associated genes was measured in liver (C). Expression of endogenous lipoprotein pathway associated genes was measured in liver (D). N = 7–8 per group, data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

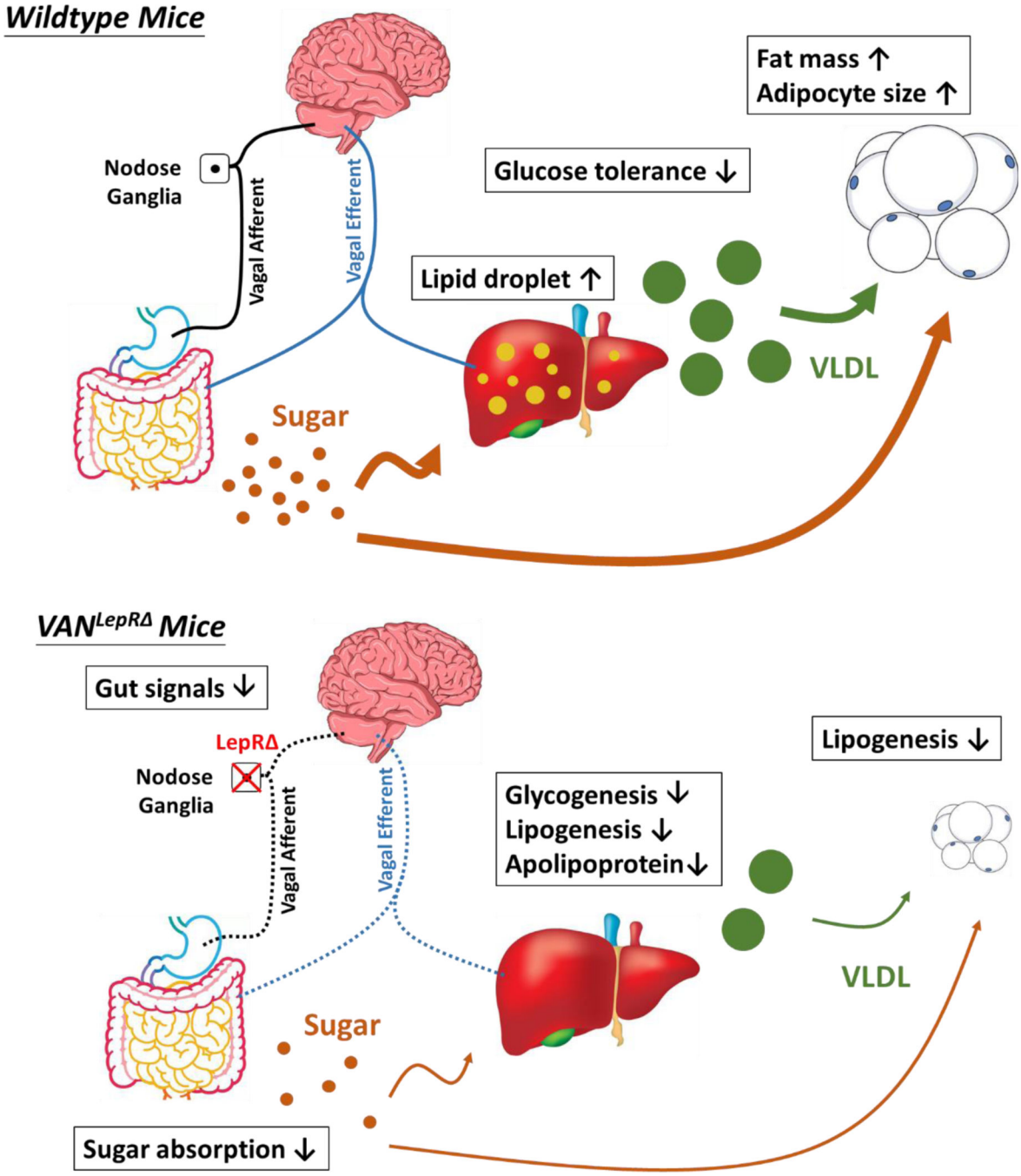

Figure 5. Illustration of the effect of leptin signaling in VAN on supporting nutrients absorption and long-term energy storage during HF feeding.

Deletion of leptin receptor in VAN reduces responsiveness to gastrointestinal signals, which leads to a decrease in intestinal absorption of carbohydrate from HF. The storage of carbohydrate in hepatocytes and adipocytes is decreased in KO mice, with a reduction in glycogenesis, *de novo* lipogenesis, and production of apolipoproteins.