Metabolic Brain Disease (2019) 34:747–761
https://doi.org/10.1007/s11011-019-0392-x

ORIGINAL ARTICLE

Selective inhibition of intestinal 5-HT improves neurobehavioral abnormalities caused by high-fat diet mice

Qi Pan[1,2,3] · Qiongzhen Liu[1,2] · Renling Wan[1,2,3] · Praveen Kumar Kalavagunta[1,2,3] · Li Liu[1,2,3] · Wenting Lv[1,2,3] · Tong Qiao[4] · Jing Shang[1,2,3,5] · Huali Wu[1,6]

Received: 22 February 2018 / Accepted: 28 January 2019 / Published online: 1 April 2019

Abstract

Recent literature reported the adverse effects of high-fat diet (HFD) on animal's emotional and cognitive function. An HFD-induced obesity/hyperlipidemia is accompanied by hormonal and neurochemical changes that can lead to depression. The important roles of gut-derived serotonin (5-Hydroxytryptamine, 5-HT) during this processing have been increasingly focused. Hence, to determine the potential role of gut-derived serotonin, HFD model was established in C57BL/6 mice. At the 4th week of feeding, a pharmacologic inhibitor of gut-derived 5-HT synthesis LP533401 (12.5 mg/kg/day), simvastatin (SIM) (5 mg/kg/day) and benzafibrate (BZ) (75 mg/kg/day) were administered for two weeks by oral gavage. Then, intraperitoneal glucose tolerance test (IPGTT), open field test (OFT), tail suspension test (TST), forced swim test (FST), sucrose preference test (SPT) were used to evaluate metabolic and neurobehavioral performances. Immunohistochemical staining, real-time quantitative PCR and other methods were to explore possible mechanisms. It was found that HFD feeding and drug treatments had some significant effects on neurobehaviors and brain: (1) All administrations reduced the total cholesterol (TC) and triglyceride (TG) parametric abnormality caused by HFD. LP533401 and SIM could significantly improve the impaired glucose tolerance, while BZ had no significant effect. (2) LP533401, SIM and BZ alleviated depression-like behavior of HFD mice in OFT, TST, FST and SPT. (3) LP533401 and SIM reversed the inhibition of Tryptophan Hydroxylase 2, Tph2 gene expression and the activation of Indoleamine 2,3-dioxy-Genase, IDO expression in HFD-treated brain, whereas BZ did not. (4) LP533401, SIM and BZ restored the inhibitory expression of 5-HT_{1A} receptor in HFD hippocampus. Conclusions: Selective inhibition of intestinal 5-HT can attenuate depressive-like behavior, reduce 5-HT_{1A}R impairment in hippocampus and correct abnormal 5-HT pathway in brain while ameliorating HFD-induced glucose intolerance. Further experiments are warranted to define the adequate strategy of targeting peripheral 5-HT for the treatment of such co-morbidity.

Keywords High-fat diet (HFD) · Peripheral 5-HT · Obesity · Behavioral changes · Glucose intolerance · Hippocampal 5-HT_{1A} receptors

Abbreviations

5-HT	5-Hydroxytryptamine
AUC	Area under the curve
BBB	Blood-brain barrier
BDNF	Brain derived neurotrophic factor
BZ	Benzafibrate
CREB	cAMP-response element binding
DAB	Diaminobenzidine
EC	Enterochromaffin
FST	Forced swim test

✉ Jing Shang
shangjing21cn@163.com

✉ Huali Wu
wu5254307@163.com

[1] State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, China

[2] Jiangsu Key Laboratory of TCM Evaluation and Translational Research, China Pharmaceutical University, Nanjing 211198, China

[3] School of Traditional Chinese Pharmacy, China Pharmaceutical University, Nanjing 211198, China

[4] Vascular Surgery Department, Nanjing Drum Tower Hospital, Nanjing 210008, China

[5] Qinghai Key Laboratory of Tibetan Medicine Pharmacology and Safety Evaluation, Northwest, Xining, Qinghai, China

[6] Department of Pharmacology, China Pharmaceutical University, Nanjing 210009, China

GI	Gastrointestinal
HFD	High-fat diet
HMG-COA	Hydroxymethylglutaryl coenzyme A
IDO	Indoleamine 2,3-dioxy-Genase
IPGTT	Intraperitoneal glucose tolerance test
IRS-1	Insulin receptor substrate-1
LPS	Lipopolysaccharide
LRP5	LDL-receptor related protein 5
NPY	Neuropeptide Y
OFT	Open Field Test
PET	Positron Emission Computed Tomography
PPARα	Peroxisome proliferator activated receptor α
SIM	Simvastatin
SPT	Sucrose preference test
TC	total cholesterol
TG	triglyceride
Tph	Tryptophan Hydroxylase
TST	Tail Suspension Test

Introduction

Metabolic diseases such obesity, type-2 diabetes, metabolic syndrome, atherosclerosis, cerebrovascular diseases etc., with high morbidity and mortality are becoming worldwide health problems. Epidemiological studies revealed that among metabolic diseases the prevalence of depression is 6.6% with a lifetime prevalence of 16.2% (Kessler et al. 2003). Clinical studies revealed that these two diseases are interrelated. For example, compared to the general population, the incidence of depression among patients with type-2 diabetes is twice, and this depression may in turn lead to poor blood glucose control (Lustman and Clouse 2005), foot ulcers (Ismail et al. 2007), poor compliance of drug and dietary therapy (Knol et al. 2006), and so on in diabetic patients. A meta-analysis showed that depression is closely related to insulin resistance (Kan et al. 2013). Other metabolic diseases, such as obesity (Luppino et al. 2010), metabolic syndrome (Skilton et al. 2007; Dunbar et al. 2008; Kawada 2013), atherosclerosis (Tiemeier et al. 2004; Manev and Manev 2007) etc., were to have two-way concurrent phenomenon with depression. However, the underlying mechanisms are yet to be resolved.

In recent years, a large amount of literature revealed the adverse effects of high-fat diet (HFD) on the animal's emotional and cognitive function, and confirmed that metabolic diseases could induce cognitive deficits such as depression and Alzheimer's disease in animals (Schachter et al. 2017). Sharma et al, revealed that elevated levels of BDNF in nucleus accumbens, increased CREB phosphorylation and depression-like behavior can be observed in HFD fed mice (Sharma and Fulton 2013). Andre et al, reported that HFD could induce anxiety and spatial cognitive dysfunction without depression-like behavior in mice, but significantly aggravate hippocampal and hypothalamic inflammation induced by LPS (André et al. 2014). Similarly, a study by Boitard et al. revealed that HFD amplified hippocampal inflammation stimulated by LPS and induced spatial cognitive impairment (Boitard et al. 2014), though it did not influence the expression of peripheral and central pro-inflammatory cytokines under basal conditions. Interestingly, the study found that these changes were significant only in adolescent mice but not adult mice under basal conditions or LPS stimulation. In addition, insulin resistance (Arnold et al. 2014) and leptin resistance (Valladolidacebes et al. 2013) in brain are also associated with HFD-induced cognitive dysfunction, which is accompanied with plasticity impairment and morphological abnormalities in hippocampal synapses.

Intestinal tract is the main organ to absorb exogenous glucose and the first barrier to maintain glucose homeostasis. Animal studies have shown that HFD could alter intestinal flora balance, such as decreasing bifidobacterium and increasing intestinal endotoxin concentration (Cani et al. 2007b). Simultaneously, HFD increases intestinal mucosal permeability and in turn elevates circulating LPS levels (Cani et al. 2008), contributing into chronic and low-grade systemic inflammation which eventually leads to metabolic disorders and insulin resistance (Cani et al. 2007a; Ding et al. 2010) along with neurobehavioral deficiencies (Hao et al. 2012). Based upon these factors, we hypothesized that intestine is an important mediator of neuro-behavioral dysfunction induced by metabolic disorders. 5-hydroxytryptamine (5-HT) or serotonin which is a monoamine neurotransmitter is predominantly concentrated in the gastrointestinal tract, platelets and central nervous system. Almost 90% 5-HT in our body is synthesized in specialized enteroendocrine cells within the gastrointestinal (GI) mucosa called enterochromaffin (EC) cells and regulates gut motility (Kim and Camilleri 2000). 5-HT in brain is closely related with emotion, diet and sleep. The synthesis of 5-HT requires the rate-limiting enzyme tryptophan hydroxylase (Tph) which exists as two isoforms (Tph1, Tph2). Tph1 is predominantly localized in gut EC cells as well as other non-neuronal sources, while Tph2 is largely expressed in CNS neurons. 5-HT, being an important neurotransmitter in the brain-gut axis regulates brain-intestine interaction (Vanner et al. 2006). Gut 5-HT will be released by EC cells in response to mechanical stimulation, parasympathetic activation, chemical substances (acids or bases), nutrients such as fatty acids, peptides, glucose and so on (Bertrand and Bertrand 2010). Further, extra intestinal microenvironment changes such as inflammation (Wang et al. 2007; Manocha et al. 2013), acidification (Kellum et al. 1984) increase the number of EC cells leading to an increase in 5-HT synthesis and release.

For a long time, we have focused on the important effects of gut 5-HT on the modulation of peripheral energy metabolism, bone formation (Yadav et al. 2008; Karsenty and Gershon 2011), hepatic regeneration (Gershon 2013), and

adaptation to food deprivation (Sumara et al. 2012). Because adipocytes express Tph1, 5-HTT, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, it can regulate their own proliferation and differentiation as well as leptin secretion in circulation (Kinoshita et al. 2010; Stunes et al. 2011). Studies proved that 5-HT could promote lipolysis and hepatic gluconeogenesis via 5-HT_{2B} receptors and inhibit glucose uptake. During periods of fasting, intestinal 5-HT synthesis increases which subsequently elevates the circulating 5-HT levels, and enhances fat mobilization and hepatic gluconeogenesis to maintain the energy supply (Sumara et al. 2012). Therefore, EC-derived 5-HT on glycolipid metabolism is of great significance. Our previous studies showed that Tph1 was up-regulated and LDL-receptor related protein 5 (LRP5), which is the key upstream negative regulation gene of Tph1 expression, was down-regulated in HFD mice. The 5-HT immunohistochemical results of duodenum also suggested that HFD can increase intestinal 5-HT content (Fig. 2). In addition, peripheral 5-HT acts as a fundamental mediator of depression-like behaviors induced by stress (Gul et al. 2017; Wang et al. 2017a). So, the main purpose of this study is to investigate whether peripheral intestinal 5-HT is involved in the development of HFD induced depression-like behavior. If yes, what are the underlying molecular mechanisms?

n order to deal with the above problems, we detected the corresponding changes of mice's glycolipid metabolism and neurobehavior after HFD and LP533401 administration which selectively inhibited intestinal 5-HT, so as to clarify whether the intestinal EC-derived 5-HT could participate in HFD-induced neurobehavioral abnormalities. We chose simvastatin (SIM) and benzafibrate (BZ) as positive controls because they are common clinically hypolipidemic agents. SIM is a hydroxymethylglutaryl coenzyme A (HMG-COA) reductase inhibitor and inhibits cholesterol synthesis while BZ is a non-selective peroxisome proliferator activated receptor α (PPARα) agonist (Blednov et al. 2015; Ferguson et al. 2014) with a strong anti-inflammatory effect and promotes the degradation of triglycerides (Magliano et al. 2013; Terasawa et al. 2015). These two drugs were reported to significantly reverse the neurobehavioral disorders in HFD animals (Wang et al. 2017b).

Materials and methods

Animals

Male C57BL/6 mice (5–6 weeks old, 15-17 g), were purchased from the Changzhou Cavens Experimental Animal Co., Ltd. All animals were acclimated for one week under the following conditions: the room temperature was 23 ± 1 °C; humidity was 50 ± 5% with a 12-h light/dark cycle (lights on at 7: 00 a.m. and off at 7: 00 p.m.). During this period, food and water were provided ad libitum.

Experimental design

After acclimated for one week, all animals were screened by open field test. 36 mice with similar weight and spontaneous activity were randomly divided into 6 groups: (1) Control group (normal diet, ND + vehicle), $n = 6$; (2) ND + LP533401 group, n = 6; (3) model group (HFD + vehicle), n = 6; (4) HFD + LP533401 group, n = 6; (5) HFD + BZ group (HFD + BZ), n = 6; (6) HFD + SIM group (HFD + SIM), $n = 6$. ND and HFD were provided by the Jiangsu Xietong Medical and Biological Corporation (Nanjing, China). The HFD contained 506.8 kcal/100 g (23.7% fat, 23.0% protein and 53.3% carbohydrate). The normal diet contained 360 kcal/100 g (13.3 g/100 g from main saturated fat, 26.2 g/100 g from protein, and 60.5 g/100 g from carbohydrate). LP533401 is a specific Tph1 inhibitor that inhibits intestinal 5-HT synthesis without affecting brain 5-HT levels, the dose being 12.5 mg/kg/day. BZ and SIM are common clinical hypolipidemic drugs. The dose of BZ was 75 mg/kg/day, the dose of SIM was 5 mg/kg/day, and the medium was 0.5% CMC-Na. The drugs were administrated by oral gavage.

Body weight and food intake were recorded throughout the experiment. Animals' metabolic states were assessed by intraperitoneal glucose tolerance test (IPGTT), serum triglyceride (TG) and total cholesterol (TC). Behavioral states were evaluated by open field test (OFT), forced swim test (FST), tail suspension test (TST) and sucrose preference test (SPT). OFT reflects the animals' spontaneous activity and emotional responsiveness. FST and TST reflect the presence or absence of desperate behavior on animals. SPT reflects whether animals lack pleasure. The process design was as shown in Fig. 1. All behavioral tests were performed on the same animals. All experimental protocols were carried out in accordance with the standards of the Guide for the Care and Use of Laboratory Animals and approved according to the Animal Experimentation Ethics Committee of the China Pharmaceutical University.

Intraperitoneal glucose tolerance test (IPGTT)

At the end of the experiment, all animals were fasted overnight. D-glucose (supplier Sigma-Aldrich, St. Louis, MO) was administered (2 g/kg BW) intraperitoneally as an aqueous solution (2% *w*/*v*) and blood glucose levels were determined with a glucometer (TRUEresult®, Nipro Diagnostics, Fort Lauderdale, FL) by serial tail bleeds at 0, 15, 30, 60 and 120 min after administration. The area under the blood glucose concentration-time curve for 2 h after D-glucose loading (AUC, 0–2 h) was used to measure the glucose tolerance.

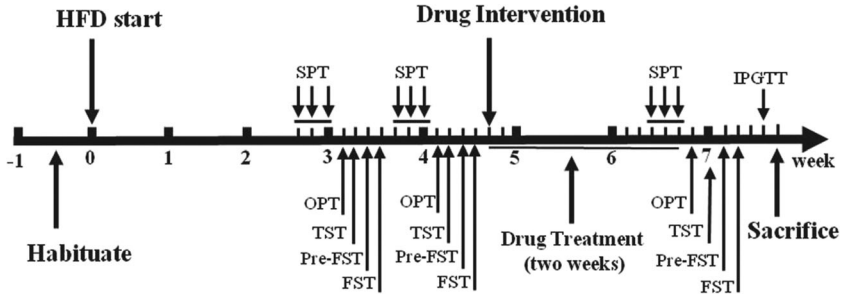


Fig. 1 An overview of the design for the experiment. Animals were acclimated for 1 week before the experiments and randomly distributed into the corresponding experimental groups. After four weeks of high-fat feeding, the animals' metabolic state was indicated by intraperitoneal glucose tolerance test (IPGTT), while behavioral state was assessed using an open field test (OFT), forced swimming test (FST), sucrose preference test (SPT) and tail suspension test (TST). These tests were repeated at the third, fourth and seventh week of HFD feeding

Behavioral tests

Open field test (OFT)

According to Budni et al. (Budni et al. 2007) the OFT was usually performed to measure spontaneous activity in mice. The apparatus was a square, walled arena (100 cm × 100 cm × 40 cm) with black walls and floor. The floor was marked into 25 equal squares, and the central nine squares were defined as the center area. The test was conducted from 9:00 am to 11:00 am. Mice were placed in the center area of the open-field and allowed to explore freely for 6 min. The number of crossing (with the four paws), the number of rearing (posture sustained with hind-paws on the floor) and the frequencies of grooming (including washing or mouthing of forelimbs, hind-paws, face, body and genitals) were manually counted by observers blind to the test conditions. The number of crossings represents the locomotion activity, rearings represents explorative activity and grooming represents emotional status. After each test the arena was cleaned with a 10% ethanol solution.

Tail suspension test (TST)

TST was performed as per previous report (Cunha et al. 2013). In brief, an adhesive tape was fixed to the mouse tail (distance from the tip of the tail 1-2 cm) and hooked to a horizontal ring stand bar placed 50 cm above the floor. All animals were suspended for 5 min in a visually isolated area without hearing and visual "communication". The test sessions were video-taped for scoring. Mice with several escape attempts interspersed with immobility periods during which they hung passively and completely motionless. The immobility time was recorded by observers blind to the treatment conditions. The scores were expressed as percentages of time.

Forced-swim test (FST)

FST was carried out as per reports with slight modifications (Porsolt et al. 1977). Mice were separately placed in an open cylindrical container (diameter = 20 cm, height = 50 cm) consisted of approximately 30 cm water at 24 ± 2 °C. The test contained two phases: a 15-min pre-swimming experiment and an official 5-min swimming test after 24 h. The test procedure was recorded with a camera. The total immobility time (passive, floating-like behavior) without active behavior (swimming, diving and climbing) was measured during the 5 min period by independent observers blinded to the experiment.

Sucrose preference test (SPT)

Test uses the mouse's preference to the taste of sugar to simulate the human sense of interest and the decline in sugar consumption can simulate the decline in interest as the core of depression symptoms. Animals were first trained to drink 1% sucrose solution from two bottles (48 h before the formal experiment). Twenty-four hours later, the animals were allowed free access to 1% sucrose (*w*/*v*) and water solution in two bottles. To avoid bottle side preference, the two bottles were switched. The amounts in the two bottles were measured after 24 h and the sucrose preference was calculated according to the following formula:

$$\text{Sucrose preference}\,(\%) = \text{sucrose intake}\,(\text{g})/$$

$$(\text{sucrose intake}\,(\text{g}) + \text{water intake}\,(\text{g})) \times 100$$

Serum and tissue collection

All animals were fasted overnight, and blood was collected from the eyeball. Then the blood was centrifuged (4000 rpm, 5 min) and the serum was separated and stored at −20 °C. After the blood was collected, the mice were sacrificed by direct cervical dislocation and their brains and duodenums were removed on ice and stored at −70 °C. Some of brains and duodenums were removed after mice heart perfusion and placed in 4% paraformaldehyde for further fixation for immunohistochemistry.

Serum triglyceride(TG)⊠total cholesterol (TC) and 5-HT test

The serum levels of TG and TC were measured according to the manufacturer's protocols. (Nanjing Jiancheng Corp., Nanjing, China). The serum 5-HT was measured by LC-MS according to previous studies (Wu et al. 2014).

Immunohistochemistry

Subset of the mice ($n = 3$ per treatment group) were perfused with saline and fixed with a freshly prepared solution consisting of 4% paraformaldehyde in phosphate buffer (pH 7.4). Their brains and duodenums were dissected and stored overnight in the same fixative solution. The tissues were then transferred to a 30% sucrose solution for cryoprotection. The fixed brain tissues were embedded in paraffin and were sectioned coronally into 4-μm slices for immunohistochemical staining. Immunohistochemical staining of the 5-HT_{1A} receptor (1:200, ab85615, Abcam, Cambridge, UK) and 5-HT (1:200, ab16007, Abcam, Cambridge, UK) was performed as per previous report (Wu et al. 2016, 2018). Diaminobenzidine (DAB) was used as the chromogen and the stained sections were analyzed using bright field microscopy under an Olympus-BX53 biological microscope.

Q-PCR

RNA was extracted using Trizol (Invitrogen) and first-strand cDNA synthesis was performed using Advantage RT-for-PCR (TaKaRa, China). Expression of 5-HT_{1A} receptor, Tph2, SERT (Slc6a4), IDO was assessed by using quantitative PCR. Primers for these genes were as follows: 5-HT_{1A} receptor (forward, 5′- ACT CCC TGC TCA ACC CAG TTA TTT A -3′, and reverse, 5'-CAC TCT TCC TCC ACT TCT TCC TTC T -3′),TpH2 (forward, 5′- TCT ACA CCC CGG AAC CAG ATA CA -3′, and reverse, 5′- CTC CCA GAG ACG CTA AGC CTA TC -3′), SERT (forward, 5′- GCT CAT CTT CAC CAT TAT CTA CTT C -3′, and reverse, 5′- AGT TTC TGC CAG TTG GGT TTC -3′), IDO (forward, 5′- CCT GGT TTT GAG GTT TTC GTG TA -3′, and reverse, 5′- AAG GTT TCA GCA TTA AGA AGG TTG -3′), GAPDH (forward,5′- ACT CCC TGC TCA ACC CAG TTA TTT A -3′, and reverse, 5′- ACT CCC TGC TCA ACC CAG TTA TTT A -3′,). Transcripts were all amplified by 40 cycles of the following: 95 °C for 30s (denaturation), 60 °C for 30s (annealing) and 72 °C for 30s (extension). For quantitative PCR, cDNA was amplified using iQ SYBR Green Supermix (TaKaRa, China) in an MJ Research Chromo4 System (Bio-Rad Laboratories). All reactions were performed in triplicate.

Statistical analysis

All data were expressed as means ± SD. Statistical analysis was performed with One-Way Analysis of Variance followed by Tukey's post hoc test for multiple comparisons tests. Graphs were completed with GraphPad Prism 5 software. Significant differences were accepted when $P < 0.05$.

Results

Effect of LP533401 on intestinal and serum5-HT synthesis in mice

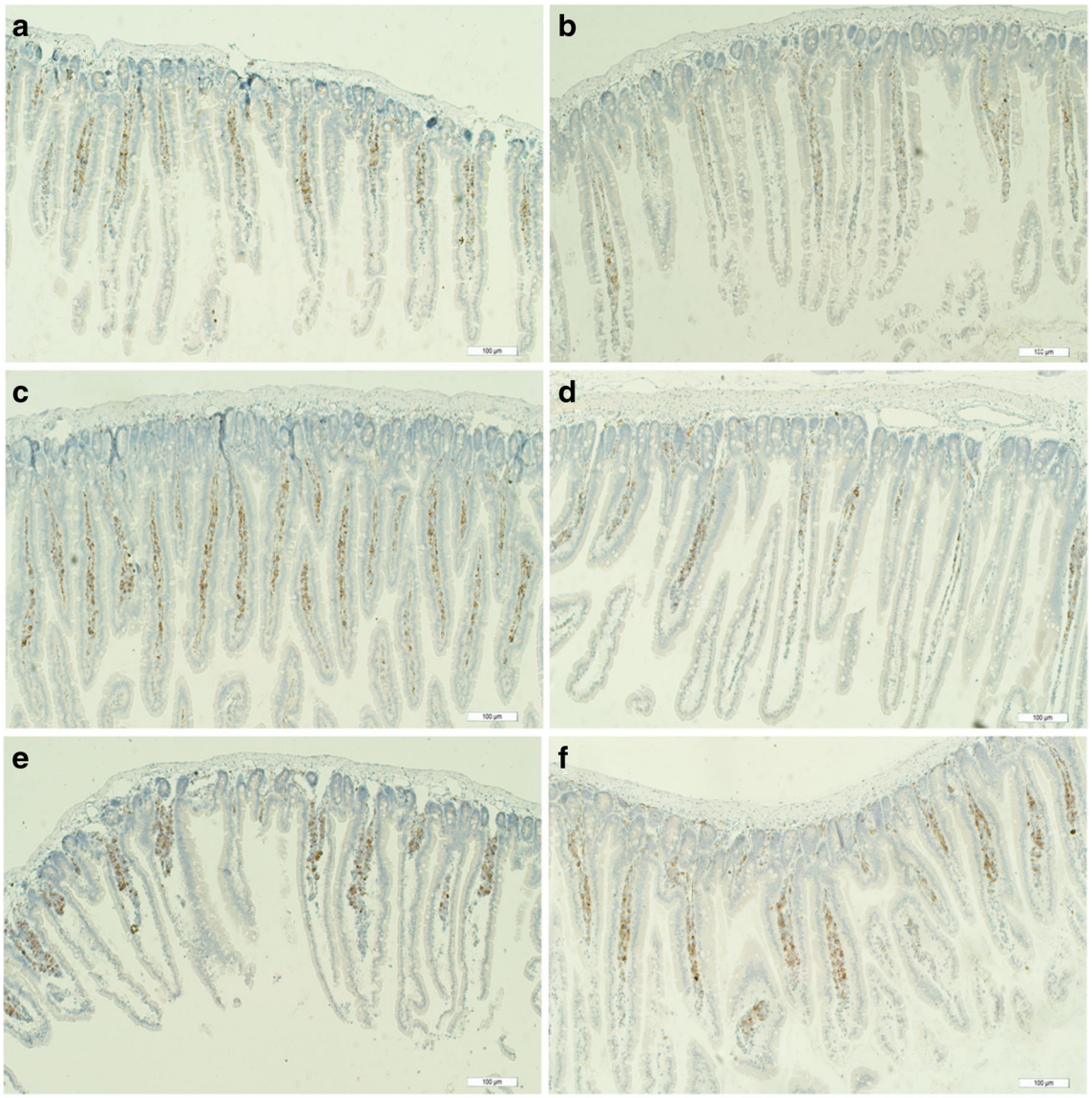
As shown in Fig. 2, compared to ND + vehicle group, the number of 5-HT immunoreactive cells in the duodenum were increased by 9.84% in HFD + vehicle group. ND mice treated with LP533401 had around 20% decrease in duodenum 5-HT levels (Fig. 2b g). After LP533401 treatment, 5-HT immunoreactive cells in the duodenum were decreased by 9.22% than their HFD counter parts (Fig. 2d and g) suggesting that LP533401 could reduce duodenum 5-HT immunoreactive cells. SIM and BZ interventions both reduced the number of 5-HT immunoreactive cells by 25.46% and 15.23% respectively (Fig. 2e, f and g).

In HFD + vehicle group, serum 5-HT levels were increased by 37% compared with the ND + vehicle group, indicating that the HFD feeding could significantly increase circulating 5-HT levels. After LP533401, BZ and SIM treatments, these circulating 5-HT levels were decreased by 32.40%, 24.33% and 22.99%, respectively (Fig. 2h).

Effect of selective inhibition of intestinal 5-HT synthesis on food intake, body weight, acute blood glucose control and lipid metabolism in HFD mice

There was no significant difference in food intake between groups, so daily calorie intake of HFD mice was about 40% higher than ND mice (Fig. 3a). HFD mice weighed heavier from week 1 to week 7 (Fig. 3c and d). After LP533401 intervention, the increase body weight continued to be observed but with a slower rate (Fig. 3c and d), suggesting that inhibiting peripheral 5-HT could not down-regulate the weight gain. However, SIM intervention displayed the decreased body weight on HFD animals and BZ did not (Fig. 3c and d).

Hyperglycemia and insulin resistance are the manifestations of endocrine abnormalities in all obese animals (Kan et al. 2013). After short-term HFD, even though the mice showed normal fasting blood glucose and insulin, they still had acute blood glucose clearance impairment in the glucose tolerance test (Luppino et al. 2010), indicating that glucose tolerance could sensitively reflect the metabolism and endocrine disorders. In HFD + vehicle



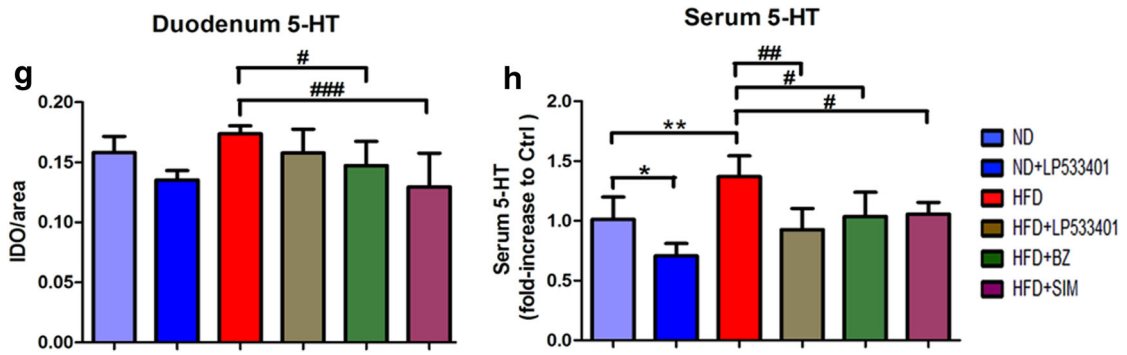


Fig. 2 Duodenal 5-HT expression of mice in each group. Shown are representative photomicrographs of 5-HT staining of duodenum. **a** ND + vehicle group (control), **b** ND + LP533401 group, **c** HFD + vehicle group (model), **d** HFD + LP533401 group, **e** HFD + BZ group, **f** HFD + SIM group. Magnification, 100×. Scale bar, 100 μm. **g** The content of serotonin in duodenum. (H) The content of serotonin in serum. Data are presented as the mean ± SD($n = 6$). **P<0.01,compared with the control group; #$P < 0.05$, ##P<0.01, ###P<0.001 compared with the model group

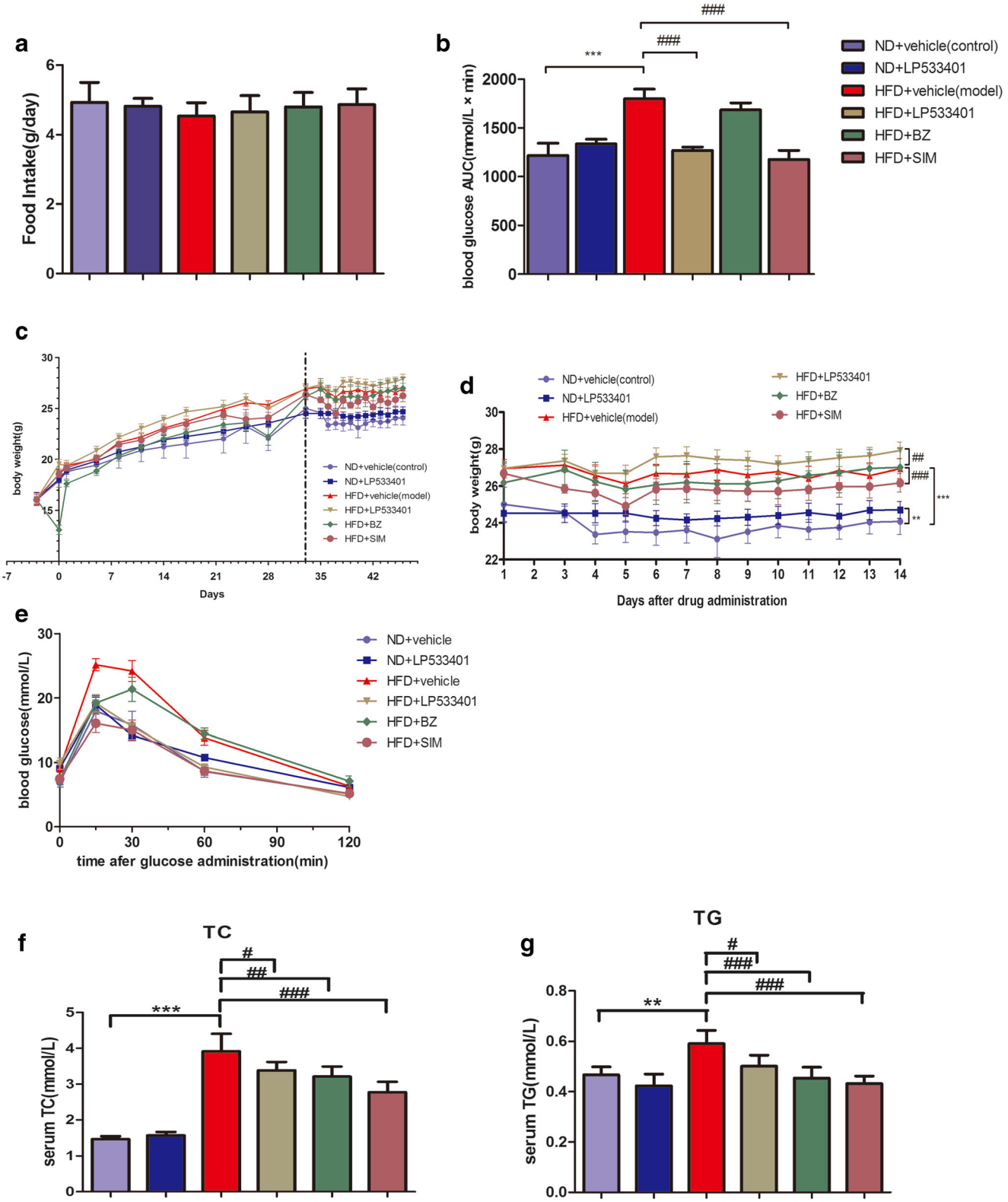


Fig. 3 Effects of selective inhibition of gut-derived serotonin synthesis on the animals' body weight and acute blood glucose control. **a** Animals' mean daily food intake. **b** Animals' body weight throughout the study. **c** Animals' body weight during drug treatment. **d** Blood glucose levels during an IPGTT. **e** The area under the blood glucose concentration-time curve for 2 h (AUC 0–2 h) in an IPGTT. **f** Total triglyceride (TG). **g** Total cholesterol (TC). Data are presented as the mean ± SD (n = 6). **P<0.01, ***P<0.001 compared with the control group; #$P < 0.05$, ##P<0.01, ###P<0.001 compared with the model group

group, the integrated area under the curve (AUC) for GTT showed a 47.90% increase (1800.5 mmol/L × min) (Fig. 3b and e) but was stood at a standard level (1217.47 mmol/L × min) in ND fed mice, suggesting that HFD fed mice developed impaired glucose tolerance. After LP533401 treatment for 2 weeks, these impaired mice revealed a normal level (1270.5 mmol/L × min) of the blood glucose AUC. For positive control, SIM intervention completely restored the impaired glucose tolerance to the normal level (1178.3 mmol/L × min) which BZ could not (Fig. 3b and e).

The levels of blood lipids (TG; TC) in the HFD + vehicle group were significantly increased: TG and TC were increased by approximately 27% and 160% respectively. Compared to HFD mice, drug-treated mice showed lower levels of blood lipids: LP533401, BZ and SIM decreased TG levels by 15.37%, 23.16% and 26.95%, and TC levels by 13.52%, 17.99% and 29.21% respectively (Fig. 3f and g).

Effect of selective inhibition of intestinal 5-HT synthesis on behavioral performance of HFD mice

In 6th week, HFD treatment significantly decreased sucrose intake (Table 1) by 44.1%. In addition, the number of crossings in OFT were decreased by 43.59% (Fig. 4a) and the immobility time in both TST (Fig. 4b) and FST (Fig. 4c) were markedly elevated by 106.31% and 176.04% respectively, indicating that "depression-like" behaviour was fully developed in HFD fed mice. Treating these 6 week HFD fed mice with LP533401 significantly decreased the increase in immobility time elicited by HFD in both TST (31.75%) and FST (28.80%) (Fig. 4b and c) while the number of crossings and sucrose intake weren't found to be affected as observed in OFT and SPT (Fig. 4a and Table 1). Similarly, the positive drugs SIM and BZ were found to ameliorate these depression-like behaviors as shown in TST and FST whereas they weren't found to have any effect on their number of crossings and sucrose intake as shown from the OFT and SPT experiment results (Fig. 4 and Table 1).

Effect of selective inhibition of intestinal 5-HT synthesis on brain 5-HT system of HFD mice

Brain 5-HT system has attracted considerable attention in the research of depression and antidepressant therapy (Stockmeier 2003). In depression patients, brain 5-HT content was found to be decreased. Antidepressant drugs such as monoamine oxidase inhibitors and 5-HT reuptake inhibitors can decrease brain 5-HT metabolism or transport and increase its level, thus contributing to an antidepressant effect. In addition, a variety of 5-HT receptors, especially 5-HT_{1A} receptor, are involved in processing the antidepressant effect (Carr and Lucki 2011), and these receptors exist simultaneously in the brain and the gastrointestinal tract.

Tph2 is the rate limiting enzyme for 5-HT synthesis in neuron and IDO is the limiting enzyme of the tryptophan-canine urea pathway. Literature reveals that IDO activation could produce neurotoxic canine urea which is closely related to the development of HFD-induced depressive-like behavior (André et al. 2014). Our study revealed that a simple HFD feeding had no obvious effects on brain 5-HT receptor system (date not shown), but it could inhibit the expression of Tph2 by 11.5% (Fig. 5b) and significantly activate the expression of IDO by 52.85% (Fig. 5c). Under HFD condition, LP533401 could drastically block the abnormalities of Tph2 and IDO expression, which might be an important mechanism of LP533401-induced anti-depressant effect (Fig. 5b and C). SIM therapy in HFD fed mice largely elevated the mRNA expression of 5-HT_{1A} (Fig. 5a), Tph2 and SERT (Fig. 5b and d), and decreased IDO expression by 43.13% (Fig. 5c) but weren't observed in BZ therapy. It is presumed that LP533401 increased the synthesis and transport rates of brain 5-HT and remained steady of 5-HT microenvironment.

Effect of selective inhibition of intestinal 5-HT synthesis on 5-HT_{1A} receptor expression in hippocampus of HFD mice

Our data showed that not only HFD but also drug intervention had no effect on the expression of 5-HTR (1~7) system in the whole brain (data not shown). Our recent research revealed the critical role of hippocampal 5-HT_{1A} receptor in developing

Table 1 Effects of selective inhibition of gut-derived serotonin synthesis on the sucrose preference of mice

Time	Sucrose Preference(%)					
	ND + vehicle	ND + LP533401	HFD + vehicle	HFD + LP533401	HFD + BZ	HFD + SIM
3 weeks	75.96	69.50	66.22	64.25	62.12	60.39
4 weeks	61.64	65.88	49.42	43.45	44.44	41.09
6 weeks	64.37	56.56	35.96	40.40	47.95	63.61

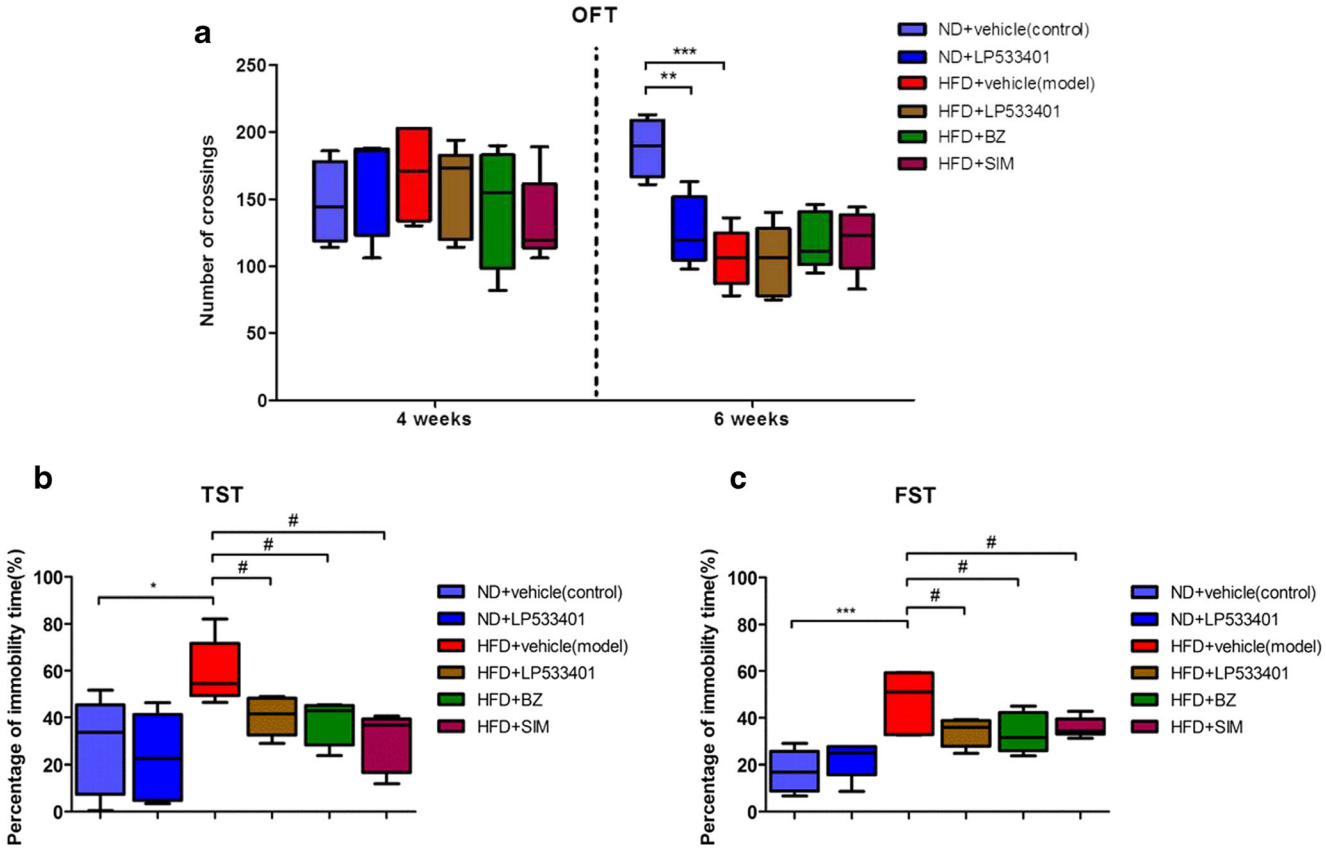


Fig. 4 Effects of selective inhibition of gut-derived serotonin synthesis on the behavioral performance of mice. **a** Number of crossings in the open field test. **b** Immobility time in the tail suspension test. **c** Immobility time in the forced swim test. Each column represents the median with ranges. Data are presented as the mean ± SD ($n = 6$). *$P<0.05$; **$P<0.01$; ***$P<0.001$ compared with the control group; #$P<0.05$ compared with the model group

HFD-induced depression-like behaviors (Wu et al. 2018). Therefore, we were intended to evaluate 5-HT_{1A} receptor expression in the hippocampal region of ND fed, HFD fed and drug treated HFD fed mice. As shown in Fig. 6a and b, immunohistochemical staining of hippocampus revealed that compared to ND fed mice, expression of 5-HT_{1A} receptor in CA1, CA3 and dentate gyrus (DG) regions of HFD fed mice were reduced by 15.51%, 15.30% and 8.08% respectively. Further, after LP533401 administration, hippocampal 5-HT_{1A} receptor inhibition was partially/completely reversed and it was increased by 15.18% (CA1), 10.06% (CA3), and 19.13% (DG) (Fig. 6a and b). Positive drugs (SIM, BZ) both enhanced the protein expression of 5-HT_{1A} receptor. These above data suggested that the inhibition of peripheral 5-HT synthesis minimized the HFD induced neurobehavioral disorders possibly through the up-regulation of hippocampal 5-HT_{1A} receptor expression.

Discussion

5-HT can promote lipolysis and hepatic gluconeogenesis and inhibit glucose uptake; Peripheral 5-HT attributed to metabolic dysfunction not only by increasing gluconeogenesis and fasting blood glucose levels, but also by acting on fat cells to mobilize free fatty acids and glycerol (Martin et al. 2017). In vitro studies have showed that 5-HT could induce the degradation of insulin receptor substrate-1 (IRS-1), leading to insulin resistance (Sharma and Fulton 2013). Our recent study found that HFD increased duodenal and serum 5-HT. LP533401 is a peripherally restricted Tph1 inhibitor and only inhibits intestinal and circulating 5-HT synthesis without affecting the brain 5-HT level (Liu et al. 2008; Krevvata et al. 2014) and intestinal 5-HT plays a key role in functional bowel disease (represented by irritable bowel syndrome) (Shi et al. 2008) and osteoporosis. LP533401 treatment in mice (12.5 mg/kg/day) reduced the serum 5-HT by 35% -40% (Fig. 2h) which is consistent with previous reports a phenomenon which could be used for the prevention and treatment of osteoporosis (Yadav et al. 2010). Further, LP533401 treatment slightly decreased the increased 5-HT in the duodenum, but its effect on serum 5-HT was considerable (Fig. 2g). The relatively low dose of LP533401 may be the reason for the least inhibition of duodenum 5-HT, while high dosage of 30 mg/kg/day (Oh et al. 2015) and 100 mg/kg/day (Sumara et al. 2012) were known to inhibit it efficiently. In addition, we quantified 5-HT levels by semi-quantitative

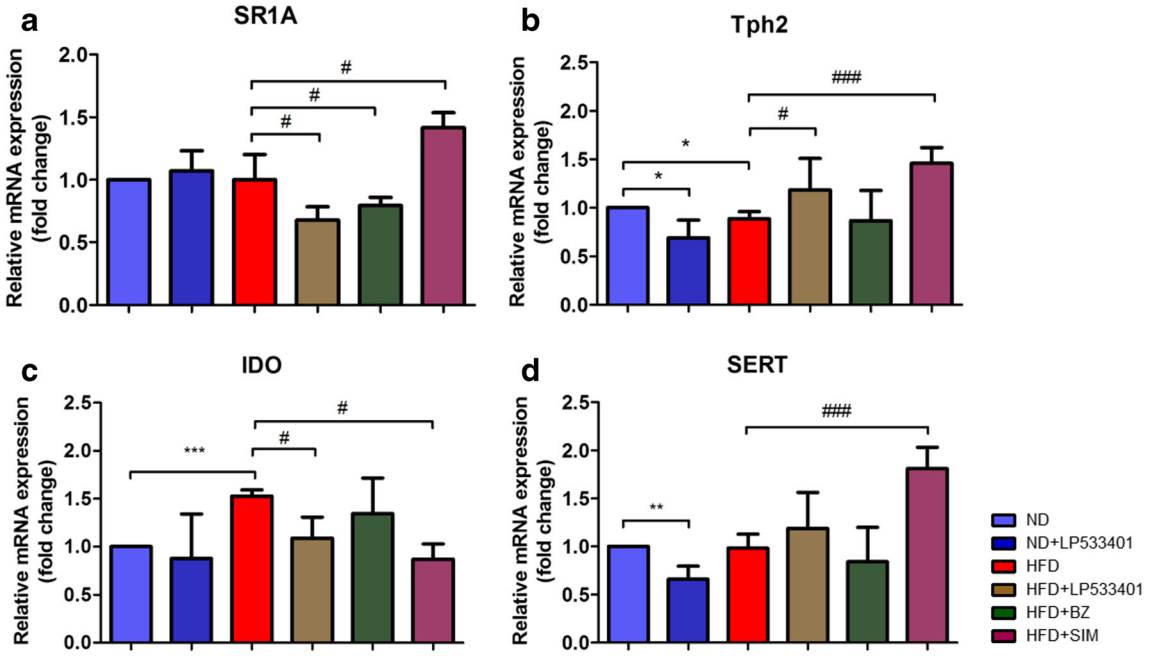


Fig. 5 Effect of selective inhibition of intestinal 5-HT synthesis on brain 5-HT system of HFD mice. **a** Effects of selective inhibition of gut-derived serotonin synthesis on the expression of 5-HT_{1A} receptors in the brain. **b** Effects of selective inhibition of gut-derived serotonin synthesis on Tph2 in the brain. **c** Effects of selective inhibition of gut-derived serotonin synthesis on IDO in the brain. **d** Effects of selective inhibition of gut-derived serotonin synthesis on SERT in the brain. Each colum represents the mean ± SD ($n = 6$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the control group; #$P < 0.05$, ###$P < 0.001$ compared with the model group

scanning of duodenal immunohistochemistry images, the accuracy of which was limited (Fig. 2g and h). BZ and SIM reversed the HFD induced 5-HT increase in duodenum and serum, but not as effectively as LP533401 (Fig. 2g and h). Meanwhile, when reducing intestinal 5-HT levels, effects on metabolism and neurobehavior in HFD mice would be assessed by detecting body weight, blood glucose, TC, TG and depression-like behaviors.

Selective inhibition of intestinal 5-HT affects glucose tolerance through a variety of peripheral mediators and the most likely way is to improve insulin resistance. Recent animal studies have shown that improving insulin resistance and glycemic control are implicated with a relief of mood-cognitive deficits induced by HFD. Though peripheral 5-HT is not able to cross the blood-brain barrier, it can regulate leptin and ghrelin, both of which cross the blood-brain barrier and mediate satiety and hunger signals through neuropeptide Y (NPY) and melanocytes Cortical (POMC) neurons in the hypothalamus (Valassi et al. 2008; Haleem 2014). Therefore, in this study, selective inhibition of intestinal 5-HT could not down-regulate weight gain in mice (Fig. 3c and d), but it apparently attenuated glucose tolerance impairment in HFD fed mice (Fig. 3b and e), which may regulate to leptin and ghrelin.

HFD is known to induce anxiety- and depression-like behaviors in mice (Krishna et al. 2016). Zemdegs et al. integrated the metabolic and emotional parameters using Z-score, and found that there was a significant positive correlation between metabolic and emotional changes after both 12 and 16 weeks of HFD mice (Zemdegs et al. 2016). In this experiment, we carried out behavioral experiments (OFT, TST, FST and SPT) in normal, HFD fed and drug treated mice models. FST and TST are the most commonly used methods to predict for behavioral despair with high predictive validity (Porsolt et al. 1977; Cunha et al. 2013). Similarly, OFT is effective to examine locomotor and explorative activity in rodents (Budni et al. 2007). SPT reflects the pleasure states of animals. They are all the most commonly used parameters to measure the development of depression like behaviors and to examine the antidepressant effects of drug and drug like molecules, so we choose the same behavioral tests to illustrate the degree of depression in HFD fed mice and effect of our experimental compouds.

Our study demonstrated that HFD induced depression-like behavior evidenced by the decreased locomotor activity, loss of explorative interests in OFT, increased immobility time in TST and FST, and decreased sucrose preference in SPT (Fig. 4 & Table 1). Our results showed that there was no significant difference in the number of crossings between groups in OFT at the 4th week but was significantly decreased in the HFD group in the 6th week. This may be due to the fact that mice were still fresh to the palatable HFD at the 4th week and additionally, body's self-repairing effects may be compensating for neurobehavioral damage to some extent. When being fed with HFD upto the 6th week, mice lost their freshness and neurobehavioral damage may have exceeded the self-repair ability and so serious neurological disorder was observed in the OFT at the 6th week. Our previous study (Wu et al. 2018)

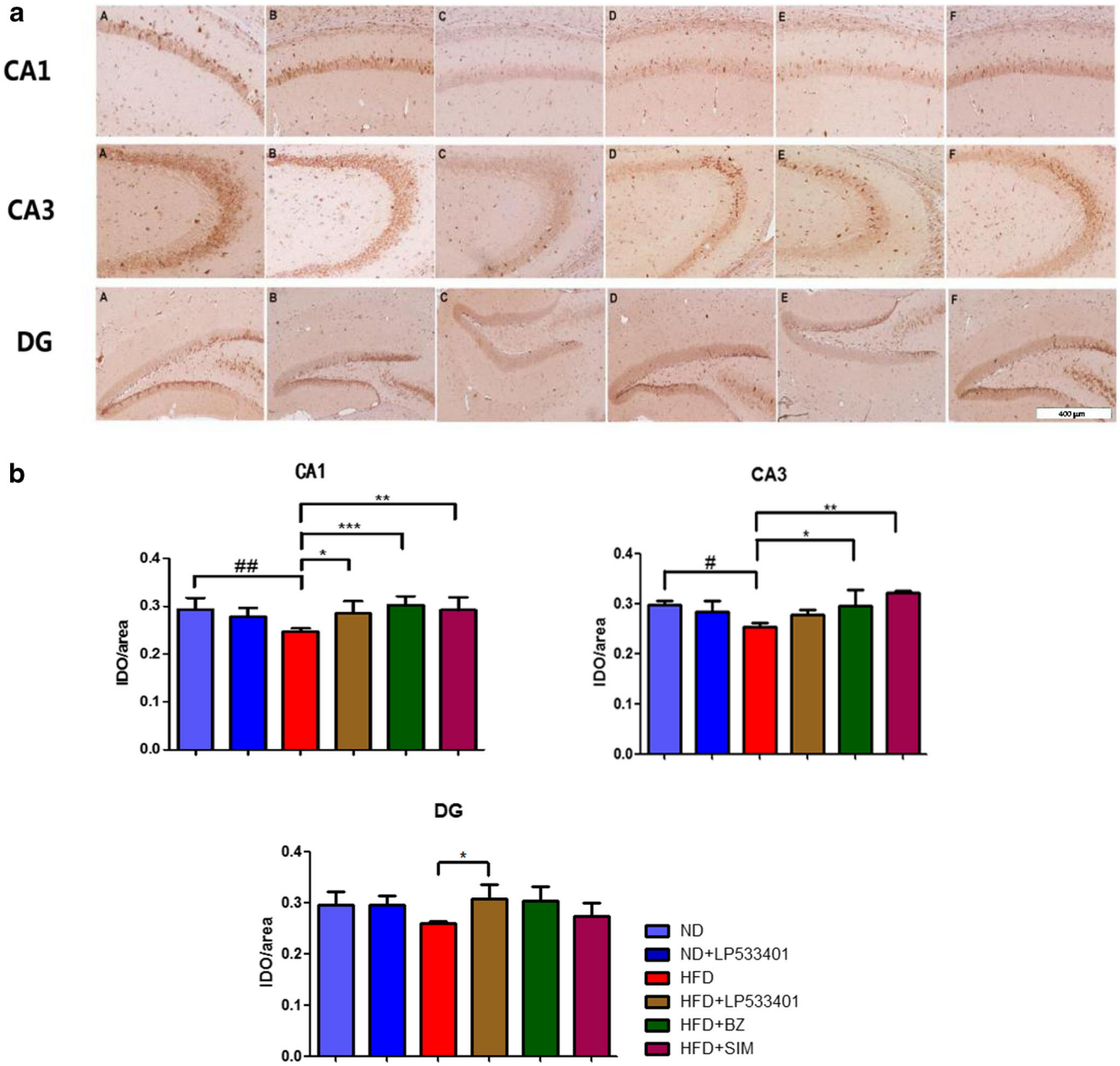


Fig. 6 Effects of selective inhibition of gut-derived serotonin synthesis on the 5-HT$_{1A}$receptor expression of the hippocampal CA1, CA3, DG subregion. **a** Representative photomicrographs of 5-HT$_{1A}$ receptor immunohistochemistry of brain sections. **a**. ND + vehicle group (control), **b**. ND + LP533401 group, **c**. HFD + vehicle group (model), **d**. HFD + LP533401 group, **e**. HFD + BZ group, **f**. HFD + SIM group. Magnification, 100×. Scale bar, 400 μm. **b** 5-HT$_{1A}$ expression of the hippocampal CA1, CA3, DG subregion. Each colum represents the mean ± SD ($n = 6$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the control group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ compared with the model group

also showed that 6 weeks of HFD feeding could develop moderate cytoarchitectural abnormalities and reduced neuron density in the hippocampus, which are closely related to cognitive functions and regulation of emotions (Arcego et al. 2017). Therefore the OFT results at the 4th and the 6th week were rather different.

Accumulating studies indicated that HFD caused depression-like behavior because of a desensitization of 5-HT-activated Akt / GSK3β signal pathway and the impairment of cell proliferation in DG (Papazoglou et al. 2015). Moreover, this depressive phenotype was associated with decreased extracellular 5-HT levels in the hippocampus and increased sensitivity of 5-HT$_{1A}$ autoreceptor in the dorsal raphe (Zemdegs et al. 2016), which was consistent with our recent finding (Wu et al. 2018). From peripheral aspects, it was reported that LPS was produced by gut microflora and inflammatory cytokines were likely to respond and mediate the effects of HFD on brain function and behavior (Schachter et al. 2017). These inflammatory cytokines could

directly cross blood-brain barrier (BBB) into the brain, affect brain 5-HT function and develop some behavioral anomalies. However, effect of changes in peripheral 5-HT in developing such co-morbidities wasn't studied earlier. 5-HT was the key mediator of the brain-gut connection (Kim and Camilleri 2000). Central nervous system regulates peripheral intestinal motor, sensory activity or metabolism (and vice versa) through neurotransmitters. GI symptoms might result from dysregulation of this brain-gut mediation (Kim and Camilleri 2000). The substance P, NO, 5-HT, and other substances influenced pain sensation, GI motility, emotional behavior, immunity and metabolism (Kaneko and Goto 2013). To test the hypothesis whether peripheral 5-HT was associated with the etiology of HFD-induced depression, we chose a pharmacologic inhibitor of peripheral serotonin synthesis, LP533401, with which HFD mice was treated. Interestingly, we found that selective inhibition of intestinal 5-HT counteracted depressive-like behaviors (Table 1 & Fig. 4), reversed impaired glucose tolerance, serum TC and TG levels which were increased in response to HFD (Fig. 3b, f and g), suggesting that changes in peripheral 5-HT concentrations could mediate either development and/or curative effects in metabolic and psychiatric disorders.

Both SIM and BZ are clinical hypolipidemic drugs and SIM is more prominent than BZ in improving behavioral impairments and anhedonia, probably due to difference in their neurobiological mechanisms (Wang et al. 2017b). SIM is an HMG-COA reductase inhibitor and inhibits cholesterol synthesis. Studies have suggested that SIM could restore serum triglycerides to normal levels and effectively relieve anxiety/depression-like behaviors and cognitive dysfunction in HFD animals. SIM also had anxiolytic, antidepressant and "puzzle" effects on normal animals (Can et al. 2012) and antidepressant effects on animals with depression like bahaviors (Lin et al. 2014), suggesting that its anti-depressant effects were independent of its lipid-lowering effect. BZ is a non-selective PPAR agonist with a potent anti-inflammatory effect, accelerating the degradation of triglycerides (Magliano et al. 2013; Terasawa et al. 2015) through which it might be reducing inflammation to have anti-depressant effect.

qPCR results showed that LP533401 treatment had no effect on brain 5-HTR system functions, but dramatically inhibited HFD induced activation of IDO in brain (Fig. 5 & Fig. 6). Brain IDO could be activated by the pro-inflammatory cytokines LPS, TNF-α and IFN-γ resulting in the development of sickness behavior and depression-like behavior (Dantzer et al. 2008). Therefore, it is reasonable to speculate that the antidepressant effect of LP544401 by selectively inhibiting intestinal 5-HT might be due to inhibition of these pro-inflammatory cytokines and subsequent inhibition of IDO activation.

The SIM's antidepressant mechanism may be similar to the selective serotonin reuptake inhibitor fluoxetine's (Santos et al. 2012). Our study showed that SIM had a very broad and obvious effect on the brain 5-HT system (data not shown), which was consistent with the speculation above. In addition, SIM inhibited HFD-induced brain IDO activation, indicating that its anti-depressant effect was also related to its anti-inflammatory effect. Statins have been reported to degrade the secretion of IL-1 and TNF-α in spinal cord injury and ischemic stroke (Balduini et al. 2003). SIM attenuated cerebral vascular endothelial inflammatory response and decreased hippocampal neuroinflammation in rats following Traumatic Brain Injury (Wang et al. 2014; Lim et al. 2017). SIM could readily cross the blood-brain barrier (Sierra et al. 2010) with well-defined pharmacokinetic activity (Schachter 2005). Along with antidepressant-like effects, SIM was found to have potential neuroprotective effect in both short and chronic HFD (Can et al. 2012; Elbatsh 2015) with which our present results are in consistent with.

Serum LC-MS analysis to detect changes in the metabolites in the upstream and downstream of 5-HT and TRP-KYN pathways revealed that there was no significant difference in TRP, KYN, 5-HTP and 5-HIAA between HFD group and ND group. We also examined brain tissue to detect 5-HT metabolites but none of the above were detected including 5-HT. Differential disorders of lipid metabolites (including palmitic amide, oleamide, 8,9-dimethyl-5,11,14- eicosatrienoic acid, etc.) were detected and all administration groups have produced callback effect. Numerous documents have indicated that patients with major depressive disorder (MDD) exhibit significant deficits in long-chain omega-3 (LCn-3) fatty acids, including eicosapentaenoic acid and docosahexaenoic acid (DHA) (Assies et al. 2010; Lin et al. 2010). Preclinical evidence suggested that dietary-induced elevations in LCn-3 fatty acid status increased 5-HT concentrations in rat frontal cortex (Chalon et al. 1998), attenuated the decrease of 5-HT content in stressed frontal cortex (Vancassel et al. 2008), and decreased depression-like behavior in FST (Jr et al. 2005). Cholesterol is an essential constituent of the human brain, and the brain is the cholesterol-richest organ containing about 20% of the body's total cholesterol (Orth and Bellosta 2012). The close association of Low cholesterol levels with impulsivity and depression (Vevera et al. 2003) suggested a basis for the potential role of cholesterol within the CNS as a factor in the underlying neuropsychiatric pathology (Freemantle et al. 2013). A study also confirmed the changes in serotonin transmission caused by simvastatin in its clinical brain cholesterol-lowering therapy (Vevera et al. 2016). Low cholesterol and altered 5-HT activity may be causal (Buydensbranchey et al. 2000). Therefore, altered lipid delivery in the brain is closely related to the anabolism of central 5-HT. We speculated that, in our study, HFD and the administration groups might alter brain lipid substrate to affect central 5-HT.

5-HT_{1A} receptors are widely distributed as postsynaptic receptors in the hippocampus, the cortex, the amygdala and the hypothalamus, and are also the most pivotal cytosolic autoreceptors of the nucleus pulposus. Current researches

have demonstrated that depression worsened 5-HT_{1A} receptor activity. Some studies suggested that depression might be elicited by 5-HT_{1A} autoreceptors hypersensitivity. The detection of brain 5-HT_{1A} receptors through Positron Emission Computed Tomography (PET) found that hippocampal 5-HT_{1A} receptor binding broadly decreased and presynaptic membrane 5-HT_{1A} receptor expression in the syncytial nucleus of brain stem abnormally increased among patients with severe depression. A large number of researches proposed that 5-HT dysfunction was commonly recognized as one of the pathogenesis of depression and 5-HT_{1A} receptor was critical in its pathophysiology (Kaufman et al. 2016). Our previous studies also demonstrated the increased apoptosis in hippocampal neurons, plasticity, and decreased expression of 5-HT_{1A} receptors in HFD mice (Wu et al. 2018), confirming our results that HFD induced depression-like symptoms. The selective inhibition of gut-derived 5-HT as well as lipid-lowering drug can significantly reverse the decrease of 5-HT_{1A} expression in hippocampus (Fig. 6), proving that selectively inhibiting intestinal 5-HT could ameliorate neurological behavioral disorders elicited by the metabolic dysfunction.

In conclusion, our data indicated that selective inhibition of peripheral 5-HT hinder HFD-induced depressive progression, and meanwhile can facilitate some metabolic disorders. This effect was likely to propose a combined therapeutical strategy of targeting peripheral 5-HT which not only treats metabolic diseases but also mediates anti-depressant effect.

Acknowledgements This study was supported by One Hundred Person Project of The Chinese Academy of Sciences, Applied Basic Research Programs of Qinghai Province (Y229461211); Science and Technology Plan Projects in Xinjiang (2014AB043); 2017 CMA-L'OREAL China Skin/Hair Grant (No.S2017140917); Prospective Joint Research Project of Jiangsu Province (BY2016078-02); The Open Project of State Key Laboratory of Natural Medicines (No. 3144060130); The National Natural Science Foundation of China (No. 81874331) and Science and Technology Plan Projects in Qinghai Province (2015-ZJ-733).

Compliance with ethical standards

Conflict of interest The authors have declared that there are no conflicts of interest.

References

André C, Dinel AL et al (2014) Diet-induced obesity progressively alters cognition, anxiety-like behavior and lipopolysaccharide-induced depressive-like behavior: focus on brain indoleamine 2,3-dioxygenase activation. Brain Behav Immun 41(4):10–21

Arcego DM, Toniazzo AP et al (2017) Impact of high-fat diet and early stress on depressive-like behavior and hippocampal plasticity in adult male rats. Mol Neurobiol (6):1–14

Arnold SE, Lucki I, Brookshire BR, Carlson GC, Browne CA, Kazi H, Bang S, Choi BR, Chen Y, McMullen MF, Kim SF (2014) High fat diet produces brain insulin resistance, synaptodendritic abnormalities and altered behavior in mice. Neurobiol Dis 67(7):79–87

Assies J, Pouwer F, Lok A, Mocking RJT, Bockting CLH, Visser I, Abeling NGGM, Duran M, Schene AH (2010) Plasma and erythrocyte fatty acid patterns in patients with recurrent depression: a matched case-control study. PLoS One 5:e10635

Balduini W, Mazzoni E et al (2003) Prophylactic but not delayed administration of simvastatin protects against long-lasting cognitive and morphological consequences of neonatal hypoxic-ischemic brain injury, reduces interleukin-1beta and tumor necrosis factor-alpha mRNA induction, and does not affect endothelial nitric oxide synthase expression. Stroke 34(8):2007–2012

Bertrand PP, Bertrand RL (2010) Serotonin release and uptake in the gastrointestinal tract. Auton Neurosci 153(1–2):47–57

Blednov YA, Benavidez JM, Black M, Ferguson LB, Schoenhard GL, Goate AM, Edenberg HJ, Wetherill L, Hesselbrock V, Foroud T, Adron Harris R (2015) Peroxisome proliferator-activated receptors α and γ are linked with alcohol consumption in mice and withdrawal and dependence in humans. Alcohol Clin Exp Res 39(1):136–145

Boitard C, Cavaroc A, Sauvant J, Aubert A, Castanon N, Layé S, Ferreira G (2014) Impairment of hippocampal-dependent memory induced by juvenile high-fat diet intake is associated with enhanced hippocampal inflammation in rats. Brain Behav Immun 40:9–17

Budni J, Gadotti VM, Kaster MP, Santos ARS, Rodrigues ALS (2007) Role of different types of potassium channels in the antidepressant-like effect of agmatine in the mouse forced swimming test. Eur J Pharmacol 575(1–3):87–93

Buydensbranchey L, Branchey M, Hudson J, Fergeson P (2000) Low HDL cholesterol, aggression and altered central serotonergic activity. Psychiatry Res 93:93–102

Can ÖD, Ulupınar E et al (2012) The effect of simvastatin treatment on behavioral parameters, cognitive performance, and hippocampal morphology in rats fed a standard or a high-fat diet. Behav Pharmacol 23(5):582–592

Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmee E, Cousin B, Sulpice T, Chamontin B, Ferrieres J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R (2007a) Metabolic Endotoxemia initiates obesity and insulin resistance. Diabetes 56(7):1761–1772

Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, Gibson GR, Delzenne NM (2007b) Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 50(11):2374–2383

Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R (2008) Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 57(6):1470–1481

Carr GV, Lucki I (2011) The role of serotonin receptor subtypes in treating depression: a review of animal studies. Psychopharmacology 213(2–3):265–287

Chalon S, Delion-Vancassel S, Belzung C, Guilloteau D, Leguisquet AM, Besnard JC, Durand G (1998) Dietary fish oil affects monoaminergic neurotransmission and behavior in rats. J Nutr 128:2512–2519

Cunha MP, Pazini FL, Oliveira Á, Machado DG, Rodrigues ALS (2013) Evidence for the involvement of 5-HT1A receptor in the acute antidepressant-like effect of creatine in mice. Brain Res Bull 95:61–69

Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW (2008) From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 9(1):46–56

Ding S, Chi MM, Scull BP, Rigby R, Schwerbrock NMJ, Magness S, Jobin C, Lund PK (2010) High-fat diet: Bacteria interactions promote intestinal inflammation which precedes and correlates with obesity and insulin resistance in mouse. PLoS One 5(8):e12191

Dunbar JA, Reddy P, Davis-Lameloise N, Philpot B, Laatikainen T, Kilkkinen A, Bunker SJ, Best JD, Vartiainen E, Kai Lo S, Janus

ED (2008) Depression: an important comorbidity with metabolic syndrome in a general population. Diabetes Care 31(12):2368–2373
Elbatsh MM (2015) Antidepressant-like effect of simvastatin in diabetic rats. Can J Physiol Pharmacol 93(8):649–656
Ferguson LB, Most D, Blednov YA, Harris RA (2014) PPAR agonists regulate brain gene expression: relationship to their effects on ethanol consumption. Neuropharmacology 86(1):397–407
Freemantle E, Chen GG, Cruceanu C, Mechawar N, Turecki G (2013) Analysis of oxysterols and cholesterol in prefrontal cortex of suicides. Int J Neuropsychopharmacol 16:1241–1249
Gershon MD (2013) 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract. Curr Opin Endocrinol Diabetes Obes 20(1):14–21
Gul S, Saleem D, Haleem MA, Haleem DJ (2017) Inhibition of hormonal and behavioral effects of stress by tryptophan in rats. Nutr Neurosci:1–9
Haleem DJ (2014) Investigations into the involvement of leptin in responses to stress. Behav Pharmacol 25(5–6):384
Hao JX, Han M et al (2012) Relationship between intestinal mucosal inflammation and mental disorders in patients with irritable bowel syndrome. Zhonghua Yi Xue Za Zhi 92(32):2247–2251
Ismail K, Winkley K, Stahl D, Chalder T, Edmonds M (2007) A cohort study of people with diabetes and their first foot ulcer: the role of depression on mortality. Diabetes Care 30(6):1473–1479
Jr CW, Mague SD, Parow AM, Stoll AL, Cohen BM, Renshaw PF (2005) Antidepressant-like effects of uridine and omega-3 fatty acids are potentiated by combined treatment in rats. Biol Psychiatry 57:343–350
Kan C, Silva N, Golden SH, Rajala U, Timonen M, Stahl D, Ismail K (2013) A systematic review and meta-analysis of the association between depression and insulin resistance. Diabetes Care 36(2):480–489
Kaneko H, Goto H (2013) Functional gastrointestinal disorders (FGID): progress in diagnosis and treatments. Topic II. Current status and future prospective of medical care of the representative disorders; 4. Pathophysiology, diagnosis and treatment of irritable bowel syndrome. Nihon Naika Gakkai Zasshi 102(1):70–76
Karsenty G, Gershon MD (2011) The importance of the gastrointestinal tract in the control of bone mass accrual. Gastroenterology 141(2):439–442
Kaufman J, Delorenzo C et al (2016) The 5-HT1A receptor in major depressive disorder. Eur Neuropsychopharmacol 26(3):397–410
Kawada T (2013) Comment on: Pan et al. bidirectional association between depression and metabolic syndrome: a systematic review and meta-analysis of epidemiological studies. Diabetes care 2012;35: 1171–1180. Diabetes Care 36(2):E27–E27
Kellum JM, Donowitz M, Cerel A, Wu J (1984) Acid and isoproterenol cause serotonin release by acting on opposite surfaces of duodenal mucosa. J Surg Res 36(2):172–176
Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, Rush AJ, Walters EE, Wang PS (2003) The epidemiology of major depressive disorder. JAMA 289(23):3095–3105
Kim DY, Camilleri M (2000) Serotonin: a mediator of the brain-gut connection. Am J Gastroenterol 95(10):2698–2709
Kinoshita M, Ono K, Horie T, Nagao K, Nishi H, Kuwabara Y, Takanabe-Mori R, Hasegawa K, Kita T, Kimura T (2010) Regulation of adipocyte differentiation by activation of serotonin (5-HT) receptors 5-HT2AR and 5-HT2CR and involvement of MicroRNA-448-mediated repression of KLF5. Mol Endocrinol 24(10):1978–1987
Knol MJ, Twisk JW et al (2006) Depression as a risk factor for the onset of type 2 diabetes mellitus. A meta-analysis. Diabetologia 49(5): 837–845
Krevvata M, Silva BC, Manavalan JS, Galan-Diez M, Kode A, Matthews BG, Park D, Zhang CA, Galili N, Nickolas TL, Dempster DW, Dougall W, Teruya-Feldstein J, Economides AN, Kalajzic I, Raza A, Berman E, Mukherjee S, Bhagat G, Kousteni S (2014) Inhibition of leukemia cell engraftment and disease progression in mice by osteoblasts. Blood 124(18):2834–2846
Krishna S, Lin Z, de la Serre CB, Wagner JJ, Harn DH, Pepples LM, Djani DM, Weber MT, Srivastava L, Filipov NM (2016) Time-dependent behavioral, neurochemical, and metabolic dysregulation in female C57BL/6 mice caused by chronic high-fat diet intake. Physiol Behav 157:196–208
Lim SW, Shiue YL, Liao JC, Wee HY, Wang CC, Chio CC, Chang CH, Hu CY, Kuo JR (2017) Simvastatin therapy in the acute stage of traumatic brain injury attenuates brain trauma-induced depression-like behavior in rats by reducing Neuroinflammation in the Hippocampus. Neurocrit Care 26(1):122–132
Lin PY, Huang SY, Su KP (2010) A meta-analytic review of polyunsaturated fatty acid compositions in patients with depression. Biol Psychiatry 68:140–147
Lin PY, Chang AY et al (2014) Simvastatin treatment exerts antidepressant-like effect in rats exposed to chronic mild stress. Pharmacol Biochem Behav 124(124):174–179
Liu Q, Yang Q, Sun W, Vogel P, Heydorn W, Yu XQ, Hu Z, Yu W, Jonas B, Pineda R, Calderon-Gay V, Germann M, O'Neill E, Brommage R, Cullinan E, Platt K, Wilson A, Powell D, Sands A, Zambrowicz B, Shi ZC (2008) Discovery and characterization of novel tryptophan hydroxylase inhibitors that selectively inhibit serotonin synthesis in the gastrointestinal tract. J Pharmacol Exp Ther 325(1):47–55
Luppino FS, de Wit LM, Bouvy PF, Stijnen T, Cuijpers P, Penninx BWJH, Zitman FG (2010) Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. Arch Gen Psychiatry 67(3):220
Lustman PJ, Clouse RE (2005) Depression in diabetic patients: the relationship between mood and glycemic control. J Diabetes Complicat 19(2):113–122
Magliano DC, Bargut TC et al (2013) Peroxisome proliferator-activated receptors-alpha and gamma are targets to treat offspring from maternal diet-induced obesity in mice. PLoS One 8(5):e64258
Manev H, Manev R (2007) 5-lipoxygenase as a possible biological link between depressive symptoms and atherosclerosis. Arch Gen Psychiatry 64(11):1333
Manocha M, Shajib MS, Rahman MM, Wang H, Rengasamy P, Bogunovic M, Jordana M, Mayer L, Khan WI (2013) IL-13-mediated immunological control of enterochromaffin cell hyperplasia and serotonin production in the gut. Mucosal Immunol 6(1):146–155
Martin AM, Young RL, Leong L, Rogers GB, Spencer NJ, Jessup CF, et al (2017) The diverse metabolic roles of peripheral serotonin. Endocrinology 158(5):1049–1063
Oh C-M, Namkung J, Go Y, Shong KE, Kim K, Kim H, Park B-Y, Lee HW, Jeon YH, Song J, Shong M, Yadav VK, Karsenty G, Kajimura S, Lee I-K, Park S, Kim H (2015) Regulation of systemic energy homeostasis by serotonin in adipose tissues. Nat Commun 6 (1). https://doi.org/10.1038/ncomms7794
Orth M, Bellosta S (2012) Cholesterol: its regulation and role in central nervous system disorders. Cholesterol 2012:292598
Papazoglou IK, Jean A, Gertler A, Taouis M, Vacher CM (2015) Hippocampal GSK3β as a molecular link between obesity and depression. Mol Neurobiol 52(1):363–374
Porsolt RD, Le Pichon M et al (1977) Depression: a new animal model sensitive to antidepressant treatments. Nature 266(5604):730–732
Santos T, Baungratz MM et al (2012) Behavioral interactions of simvastatin and fluoxetine in tests of anxiety and depression. Neuropsychiatr Dis Treat 8(8):413–422
Schachter M (2005) Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. Fundam Clin Pharmacol 19(1):117–125
Schachter J, Martel J et al (2017) Effects of obesity on depression: a role for inflammation and the gut microbiota. Brain Behav Immun
Sharma S, Fulton S (2013) Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry. Int J Obes 37(3):382–389
Shi ZC, Devasagayaraj A, Gu K, Jin H, Marinelli B, Samala L, Scott S, Stouch T, Tunoori A, Wang Y, Zang Y, Zhang C, Kimball SD, Main AJ, Sun W, Yang Q, Nouraldeen A, Yu XQ, Buxton E, Patel S, Nguyen N, Swaffield J, Powell DR, Wilson A, Liu Q (2008) Modulation of peripheral serotonin levels by novel tryptophan

hydroxylase inhibitors for the potential treatment of functional gastrointestinal disorders. J Med Chem 51(13):3684–3687

Sierra S, Ramos MC et al (2010) Statins as neuroprotectants: a comparative in vitro study of lipophilicity, blood-brain-barrier penetration, lowering of brain cholesterol, and decrease of neuron cell death. J Alzheimers Dis 23(2):307–318

Skilton MR, Moulin P, Terra JL, Bonnet F (2007) Associations between anxiety, depression, and the metabolic syndrome. Biol Psychiatry 62(11):1251–1257

Stockmeier CA (2003) Involvement of serotonin in depression: evidence from postmortem and imaging studies of serotonin receptors and the serotonin transporter. J Psychiatr Res 37(5):357–373

Stunes AK, Reseland JE, Hauso Ø, Kidd M, Tømmerås K, Waldum HL, Syversen U, Gustafsson BI (2011) Adipocytes express a functional system for serotonin synthesis, reuptake and receptor activation. Diabetes Obes Metab 13(6):551–558

Sumara G, Sumara O, Kim JK, Karsenty G (2012) Gut-derived serotonin is a multifunctional determinant to fasting adaptation. Cell Metab 16(5):588–600

Terasawa T, Aso Y, Omori K, Fukushima M, Momobayashi A, Inukai T (2015) Bezafibrate, a peroxisome proliferator-activated receptor α agonist, decreases circulating CD14(+)CD16(+) monocytes in patients with type 2 diabetes. Transl Res 165(2):336–345

Tiemeier H, Van DW et al (2004) Relationship between atherosclerosis and late-life depression: the Rotterdam study. Arch Gen Psychiatry 61(4):369

Valassi E, Scacchi M, Cavagnini F (2008) Neuroendocrine control of food intake. Nutr Metab Cardiovasc Dis 18(2):158–168

Valladolidacebes I, Fole A et al (2013) Spatial memory impairment and changes in hippocampal morphology are triggered by high-fat diets in adolescent mice. Is there a role of leptin? Neurobiol Learn Mem 106(6):18–25

Vancassel S, Leman S, Hanonick L, Denis S, Roger J, Nollet M, Bodard S, Kousignian I, Belzung C, Chalon S (2008) N-3 polyunsaturated fatty acid supplementation reverses stress-induced modifications on brain monoamine levels in mice. J Lipid Res 49:340–348

Vanner SJ, Meerveld GV et al (2006) Fundamentals of Neurogastroenterology: basic science. Gastroenterology 130(5): 1391–1411

Vevera J, Žukov I, Morcinek T, Papežová H (2003) Cholesterol concentrations in violent and non-violent women suicide attempters. Eur Psychiatry 18:23–27

Vevera J, Valeš K, Fišar Z, Hroudová J, Singh N, Stuchlík A, Kačer P, Nekovářová T (2016) The effect of prolonged simvastatin application on serotonin uptake, membrane microviscosity and behavioral changes in the animal model. Physiol Behav 158:112–120

Wang H, Steeds J, Motomura Y, Deng Y, Verma-Gandhu M, el-Sharkawy RT, McLaughlin JT, Grencis RK, Khan WI (2007) CD4+ T cell-mediated immunological control of enterochromaffin cell hyperplasia and 5-hydroxytryptamine production in enteric infection. Gut 56(7):949–957

Wang KW, Chen HJ et al (2014) Simvastatin attenuates the cerebral vascular endothelial inflammatory response in a rat traumatic brain injury. Ann Clin Lab Sci 44(2):145–150

Wang J, Liu Y, Li L, Qi Y, Zhang Y, Li L, Teng L, Wang D (2017a) Dopamine and serotonin contribute to Paecilomyces hepiali against chronic unpredictable mild stress induced depressive behavior in Sprague Dawley rats. Mol Med Rep 16(4):5675–5682

Wang H, Zhou J et al (2017b) Simvastatin and Bezafibrate ameliorate emotional disorder induced by high fat diet in C57BL/6 mice. Sci Rep 7(1)

Wu HL, Pang SL, Liu QZ, Wang Q, Cai MX, Shang J (2014) 5-HT1A/1B receptors as targets for optimizing pigmentary responses in C57BL/6 mouse skin to stress. PLoS One 9:e89663

Wu H, Feng J, Lv W, Huang Q, Fu M, Cai M, He Q, Shang J (2016) Developmental neurotoxic effects of percutaneous drug delivery: behavior and neurochemical studies in C57BL/6 mice. PLoS One 11(9):e0162570

Wu H, Liu Q, Kalavagunta PK, Huang Q, Lv W, An X, Chen H, Wang T, Heriniaina RM, Qiao T, Shang J (2018) Normal diet vs high fat diet - a comparative study: behavioral and neuroimmunological changes in adolescent male mice. Metab Brain Dis 33:177–190

Yadav VK, Ryu JH, Suda N, Tanaka KF, Gingrich JA, Schütz G, Glorieux FH, Chiang CY, Zajac JD, Insogna KL, Mann JJ, Hen R, Ducy P, Karsenty G (2008) Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. Cell 135(5):825–837

Yadav VK, Balaji S, Suresh PS, Liu XS, Lu X, Li Z, Guo XE, Mann JJ, Balapure AK, Gershon MD, Medhamurthy R, Vidal M, Karsenty G, Ducy P (2010) Pharmacological inhibition of gut-derived serotonin synthesis is a potential bone anabolic treatment for osteoporosis. Nat Med 16(3):308–312

Zemdegs J, Quesseveur G, Jarriault D, Pénicaud L, Fioramonti X, Guiard BP (2016) High-fat diet-induced metabolic disorders impairs 5-HT function and anxiety-like behavior in mice. Br J Pharmacol 173(13): 2095–2110