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Increasing hypothalamic nucleobindin 2 levels and decreasing hypothalamic inflammation in obese male mice via diet and exercise alleviate obesity-associated hypogonadism

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ABSTRACT

To explore the role of nesfatin-1 in regulating male reproductive function during energy balance variation, we employed an obese mouse model which was first induced by a high-fat diet (HFD) and followed by interventions of a normal diet (ND) and/or moderate exercise, and then serum reproductive hormones of male mice, hypothalamic nucleobindin 2 (NUCB2)/nesfatin-1, inflammatory factors, and gonadotropin-releasing hormone (GnRH) levels were tested. Our findings showed that both serum nesfatin-1, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T) levels and hypothalamic *NUCB2*/nesfatin-1 and *Gnrh* mRNA levels were reduced, whereas, the mRNA and protein levels of hypothalamic tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, inhibitor kappa B kinase β (IKKβ), and nuclear factor (NF)-κB were increased in obese male mice. Diet, exercise, and diet combined with exercise interventions reversed the decreases in serum nesfatin-1, FSH, LH, and T levels; increased hypothalamic *NUCB2*/nesfatin-1 and *Gnrh* mRNA levels; and reduced hypothalamic TNF-α, IL-1β, IKKβ, and NF-κB levels. These changes were accompanied by reduced adiposity, and these effects were more obvious in the diet combined with exercise group. Overall, our findings suggested that the hypogonadotropic hypogonadism associated with obesity may be induced by reduced hypothalamic NUCB2/nesfatin-1 levels, which attenuated the stimulatory effect on GnRH directly or indirectly by suppressing its anti-inflammatory effect in the brain. Diet and/or exercise interventions were able to alleviate the hypogonadotropic hypogonadism associated with obesity, potentially by increasing hypothalamic NUCB2/nesfatin-1 levels.

1. Introduction

Within the past four decades, the prevalence of obesity has nearly tripled worldwide (Collaboration, 2016), and the growth rate of global mean body mass index in men is higher than that in women (Collaboration, 2016). Moreover, associated with the increased prevalence of obesity in men, the prevalence rates of oligospermia, azoospermatism, and induced infertility in men have also increased (Bieniek et al., 2016; Ramaraju et al., 2018). Although the underlying mechanisms are unclear, some studies have suggested associations with late-onset male hypogonadism, which is characterized by low levels of serum testosterone and related symptoms, such as poor libido, erectile dysfunction, depression, lack of motivation, lethargy, and muscle weakness, which seriously affects quality of life (Corona et al., 2015). Further studies have also shown that obesity not only reduces serum testosterone levels, but also induces lower levels of serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and higher levels of serum estradiol, which characterize hypogonadotropic hypogonadism (Corona et al., 2013; Dandona and Dhindsa, 2011; Rabijewski et al., 2013). Thus, hypogonadotropic hypogonadism associated with obesity is considered a major cause of subfertility in obese men.

The mechanisms underlying hypogonadotropic hypogonadism associated with obesity are complex and have not been fully elucidated. Recent studies have shown showed that some satiety regulators, such as leptin and ghrelin, also act as important players in the regulation of reproductive function (Boggio et al., 2013; Muccioli et al., 2011). Nesfatin-1, a recently identified satiety molecule, was also found to play important roles in the maintenance of energy balance (Chen et al.,

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2015; Oh et al., 2006) and regulation of reproductive function (Catak et al., 2014). In vitro and in vivo studies have confirmed that nesfatin-1 administration not only increases mRNA and protein levels of gonadotropin-releasing hormone (GnRH), Lhβ, and Fshβ (Hatef and Unniappan, 2017) but also promotes LH and FSH secretion in adult male rats (Garcia-Galiano et al., 2010). These studies suggest that nesfatin-1 could regulate male reproductive function by directly acting on GnRH neurons in the hypothalamus and gonadotropes in the pituitary, thereby stimulating the secretion of GnRH, LH, and FSH. Notably, serum nesfatin-1 levels in obese young and adult men were found to be significantly decreased compared with those in normal weight counterparts (Abaci et al., 2013; Guo et al., 2014), and the protein level of nesfatin-1 was also found reduced in the hypothalamus of Tsumura Suzuki obese diabetic mice (Miyata et al., 2012). Thus, the reduced serum nesfatin-1 level associated with obesity may induce decreases in hypothalamic nesfatin-1 levels, which further reduce GnRH levels and eventually impair reproductive function in obese men.

Nesfatin-1 has recently been shown to exert anti-inflammatory effects both in the brain and in the digestive system of rats or mice (Ozturk et al., 2015; Ravussin et al., 2018; Tang et al., 2012). In contrast, chronic low-grade inflammation associated with obesity, particularly inflammation in the hypothalamus (Kalin et al., 2015; Nakata et al., 2017; Tran et al., 2016), has been confirmed as a critical mechanism in the development of male hypogonadism (Gautier et al., 2013). Previous studies have shown that a high-fat diet (HFD) not only increases levels of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, in the hypothalamus of mice (Thaler et al., 2012) but also induces the mRNA expression and phosphorylation of inhibitor kappa B kinase (IKKβ), and promotes the degradation of inhibitor kappa B alpha (IκBα) in the hypothalamus (Ropelle et al., 2010; Thaler et al., 2012), which results in activation of the nuclear factor (NF)-κB pathway in the hypothalamus. Unfortunately, activation of the IKKβ/NF-κB pathway not only inhibits *Gnrh* mRNA expression in the mediobasal hypothalamus of mice (Zhang et al., 2013) but also reduces hypothalamic GnRH immunopositivity and decreases FSH, LH, and testosterone (T) levels in the plasma of male rabbits (Filippi et al., 2009; Morelli et al., 2014). However, it is unclear whether nesfatin-1 can promote GnRH expression and increase reproductive hormones by inhibiting inflammation in the hypothalamus of men.

Diet and/or exercise interventions are effective for reducing adiposity and reversing the decreases in LH, FSH, and T levels in obese male rodents (Palmer et al., 2012; You et al., 2013); however, the mechanisms mediating these effects are not fully understood. Recently, findings from studies in obese men or male mice have shown that, serum nesfatin-1 levels can be increased by diet intervention (Guo et al., 2014) or exercise (Ahmadizad et al., 2015; Chaolu et al., 2011). In addition, diet and/or exercise can reduce hypothalamic levels of pro-inflammatory cytokines (Diane et al., 2015; Masson et al., 2015; Ropelle et al., 2010; Yi et al., 2012), increase the expression of IκBα, and suppress the activation of IKKβ/NF-κB in the hypothalamus of obese rats (Diane et al., 2015; Ropelle et al., 2010).

Accordingly, in this study, we aimed to test the hypothesis that nucleobindin 2 (NUCB2)/nesfatin-1 may promote GnRH expression directly and/or indirectly in the hypothalamus of males during diet and/or exercise interventions to improve the reproductive functions of males.

2. Materials and methods

2.1. Laboratory animals and breeding environment

In this study, 55 male C57BL/6J mice (4–5 weeks old) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) under permit number SCXK (Beijing) 2016-0011. Mice were maintained in a breeding room at a temperature of 22 ± 3 °C and a humidity of 50% ± 10% under a 12:12-h light-dark cycle, and food and water were fed ad libitum. In each cage, there were no more than five mice. All animal experiments in this study were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Ethics Committee of Shenyang Sport University.

2.2. Diet and animal grouping

After 1 week of acclimation, 10 mice were placed on a normal diet (ND) and 45 mice were placed on an HFD. The HFD had been shown to induce obesity in male mice in previous studies (Palmer et al., 2012; Yi et al., 2017). The components of the two diets used in this study were described previously (Palmer et al., 2012). Briefly, the fat and carbohydrate contents were 6.0% and 30.5%, respectively, in the ND and 21.0% and 15.5%, respectively, in the HFD by weight. The digestible energy from lipids was 13.9% for the ND and 40.0% for the HFD. The fat content was derived from canola oil in the ND and clarified butter in the HFD. The other components of the two diets were identical. The total digestible energy was 3.89 kcal/g for the ND and 4.72 kcal/g for the HFD. Diets were purchased from Jianmin Company Ltd. (Shenyang, China). After 10 weeks, the body weights of all mice in the HFD group were > 120% of the mean body weight in the ND group, with the exception of three mice that did not develop obesity; thus, the animal obesity model was effectively established (Chandler et al., 2005). The three mice that did not develop obesity were excluded from the HFD group.

After the obesity model was successfully established, mice in the HFD group were randomly allocated into one of four groups: the obesity control (OC) group (n = 10), obesity with ND (ON) group (n = 10), obesity and moderate exercise (OE) group (n = 11), and obesity with ND and moderate exercise (ONE) group (n = 11). The intervention period lasted for 8 weeks, during which time the mice in the OC and OE groups were fed the HFD, and mice in the ON and ONE groups were fed the ND. The body weights of mice in the four groups did not differ significantly at the beginning of the three interventions (p > .05). Mice

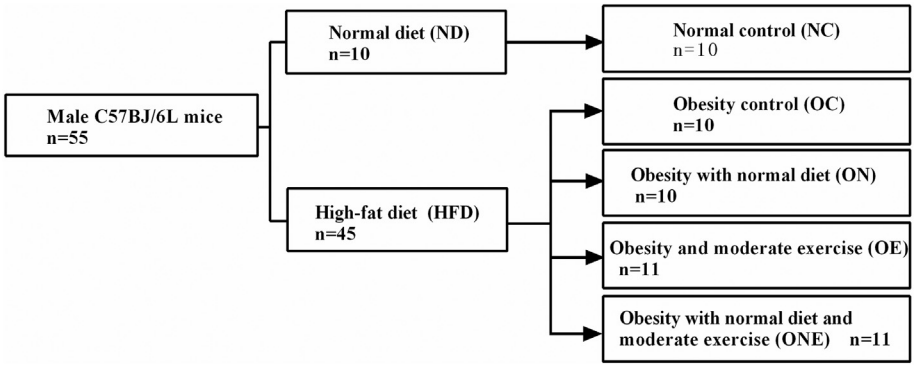

Fig. 1. Animal grouping. Mice were allocated to the normal diet (ND; n = 10) and high-fat diet (HFD; n = 45) groups at random. After 10 weeks of diet intervention, three mice that did not develop obesity were excluded from the HFD group, and the remaining 42 mice were further assigned to the obesity control (OC; n = 10), obesity with ND (ON; n = 10), obesity and exercise (OE; n = 11), and obesity with ND and exercise (ONE; n = 11) groups.

that were fed the ND during the initial feeding period were designated as the normal control (NC) group and continued to receive the ND during the interventional period (Fig. 1). Individual body weight measurements were performed weekly during both the initial and interventional periods.

2.3. Exercise protocol

A moderate-load exercise was used in the OE and ONE groups (Lee et al., 2015). Briefly, mice in the OE and ONE groups were acclimated to treadmill running during the first 2 weeks, and the treadmill running speed and duration were gradually increased from 20 min/day at a speed of 10 m/min to 60 min/day at a speed of 20 m/min at the end of week 2. The final exercise protocol was 60 min/day at a speed of 20 m/min, 6 days/week, with 0% incline, for 6 weeks (trained at the beginning of the dark cycle, from 18:00 to 19:00).

2.4. Sample collection

At 36–48 h after the last session of exercise (for washing out the acute effects of the last session of exercise on the measurements) (Lee et al., 2011) in the OE and ONE groups (with fasting for 12 h), mice were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight; Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). Mice were sedated, and blood was immediately collected from the tail vein. Serum was separated for measurement of hormones. All groups of mice were decapitated, and hypothalamus tissues were harvested, immediately frozen in liquid nitrogen, and then stored in a −80 °C freezer for subsequent mRNA analysis. The adipose tissues that surrounded the testis, kidneys, and mesentery were also collected and weighed to determine the abdominal fat content. Six mice in each of the NC, OC, and ON groups and seven mice in each of the OE and ONE groups were used for harvesting of serum, hypothalamus tissues, and adipose tissues. The remaining four mice in each group were used for immunohistochemical analysis.

2.5. Hormone measurement

Serum levels of FSH, LH, total T, and nesfatin-1 were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocols on a Thermo Fisher Multiskan GO 1510 (Thermo Fisher Scientific, Finland). All mouse ELISA kits were obtained from Shanghai Enzyme-linked Biotechnology Co., Ltd. The detection limits for the three kits were as follows: FSH, 2.5–80 mIU/mL; LH, 0.25–8.0 ng/mL; T, 0.75–24 ng/mL; nesfatin-1, 12–400 pg/mL. The intra-assay coefficient of variation (CV, %) was < 10%, and the inter-assay CV (%) was < 15% for all four ELISA kits.

2.6. Real-time polymerase chain reaction (PCR)

Total RNA was isolated from the mouse hypothalamus using RNA Isolater Total RNA Extraction Reagent (R401-01; Vazyme Biotech Co., Ltd., Nanjing, Jiangsu, China). For each sample, 1.0 μg total RNA was reverse transcribed into cDNA using a GoScript Reverse Transcription System kit (A5001; Promega, Madison, WI, USA) according to the manufacturer's instructions on an Applied Biosystems Veriti 96-Well Thermal Cycler (Applied Biosystems, Singapore). Amplification was conducted using a GoTaq qPCR Master Mix kit (A6001; Promega) on an Applied Biosystems StepOne Real-Time PCR System (Applied Biosystems) according to the manufacturer's instructions. The primers used in this study were as follows: *Nucb2*, forward, 5′-GTC ACA AAG TGA GGA CGA GAC TG-3′ and reverse, 5-TGG TTC AGG TGT TCA AAC TGC TTC-3′; *Gnrh 1*, forward, 5′-AGG AGC TCT GGA AAG TCT GAT-3′ and reverse, 5′-AAT GTT ATA CTC GGG TGT TGT GGA T-3′; *Tnf-α*, forward, 5′-ACG GCA TGG ATC TCA AAG AC-3′ and reverse, 5′-GTG GGT GAG GAG CAC GTA GT-3′; *Il-1β*, forward, 5′-GCT GCT TCC AAA CCT TTG AC-3′ and reverse, 5′-AGC TTC TCC ACA GCC ACA AT-3′; *Nf-kb p65*, forward, 5′-GAC CTG GAG CAA GCC ATT AG-3′ and reverse, 5′-CAC TGT CAC CTG GAA GCA GA-3′; *Ikkβ*, forward, 5′-GCC TTA TGA ACG AGG ACG AG-3′ and reverse, 5′-CTG TCT GGG CTT CCA CTC A-3′; and 18s ribosomal RNA (*18s rRNA*), forward, 5′-GGC GGC TTG GTG ACT CTA GAT AAC-3′ and reverse, 5′-CCT GCT GCC TTC CTT GGA TGT G-3′. All primers were designed and synthesized by Sangon Biotech (Shanghai) Co., Ltd. All real-time PCR assays were conducted in triplicate, and mRNA levels were normalized to the level of the internal control *18s rRNA* in the same sample. Data were analyzed based on the $2^{-\triangle\triangle Ct}$ method.

2.7. Immunohistochemistry (IHC)

In this experiment, four mice in each group were used. After the mice were anesthetized, fixation-perfusion was performed with ice-cold 4% paraformaldehyde/phosphate-buffered saline (PBS). Brains were harvested and immersed in 4% paraformaldehyde/PBS for 24 h at 4 °C. The brains were then dehydrated in increasing concentrations of ethanol and embedded in paraffin blocks. Brain tissue blocks were cut into 7-μm-thick sections at the coronal plane using a microtome (Leica Biosystems Ltd., Germany). Sections were mounted onto slides, deparaffinized with xylene, rehydrated with decreasing concentrations of ethanol, and then rinsed with PBS. Slides were immersed in citrate antigen retrieval buffer for antigen hot retrieval in a microwave oven. For immunostaining, an Ultra Sensitive SP (Mouse/Rabbit) IHC Kit (Maxim, Fuzhou, Fujian, China) was used. Briefly, sections were first incubated with 3% H_2O_2 for 30 min at room temperature to quench endogenous peroxidase and then blocked with goat serum working solution for 30 min at room temperature. Next, tissues were incubated overnight at 4 °C with the following primary antibodies: rabbit anti-NUCB2 polyclonal antibodies (diluted 1:500; Bioss, Beijing, China), rabbit anti-GnRH1 polyclonal antibodies (diluted 1:1000; ABclonal, Wuhan, Hubei, China), rabbit anti-TNF-α polyclonal antibodies (diluted 1:1000; ABclonal), rabbit anti-IL-1β polyclonal antibodies (diluted 1:1000; ABclonal), rabbit anti-phospho-IKKβ (Tyr188) polyclonal antibodies (diluted 1:500; Bioss), and rabbit anti-NF-κB p65 polyclonal antibodies (diluted 1:500; Bioss). Antigen specificity of primary antibodies was confirmed with negative controls in which primary antibodies were replaced with PBS. After washing with PBS for five times, sections were incubated with the secondary antibody (biotinylated goat anti-rabbit immunoglobulin G) for 10 min at room temperature, and a streptavidin-peroxidase conjugate was then applied according to the manufacturer's instructions. To visualize staining, sections were stained with diaminobenzidine solution for 30s. Sections were then counterstained with hematoxylin, dehydrated with increasing concentrations of ethanol, cleared in xylene, and mounted with neutral resins. Images were captured by a camera connected with a light/fluorescence microscope (Leica Microsystem Ltd.) after the same exposure time. Quantitative histological analysis of NUCB2/nesfatin-1, TNF-α, IL-1β, phospho-IKKβ, and NF-κB p65 pixel intensities was automatically performed on four successive sections from each animal using Image Pro Plus software 6.0 (Media Cybernetics Inc., USA). For quantitative histological analysis, only images captured at the mediobasal hypothalamus were included, with a focus on the arcuate nucleus (Arc). The results are shown as the average integrated optical density of images, and the NC group was set at 1. The results in the other groups were then determined as the fold change relative to the NC group.

2.8. Statistical analysis

All data were expressed as means ± standard errors of the means (SEMs), and all data analyses were performed in SPSS 18.0 (SPSS, Chicago, IL, USA). Student's *t*-tests were used to compare the data between the NC and OC groups, and one-way analysis of variance with least significant difference post-hoc tests were performed for statistical

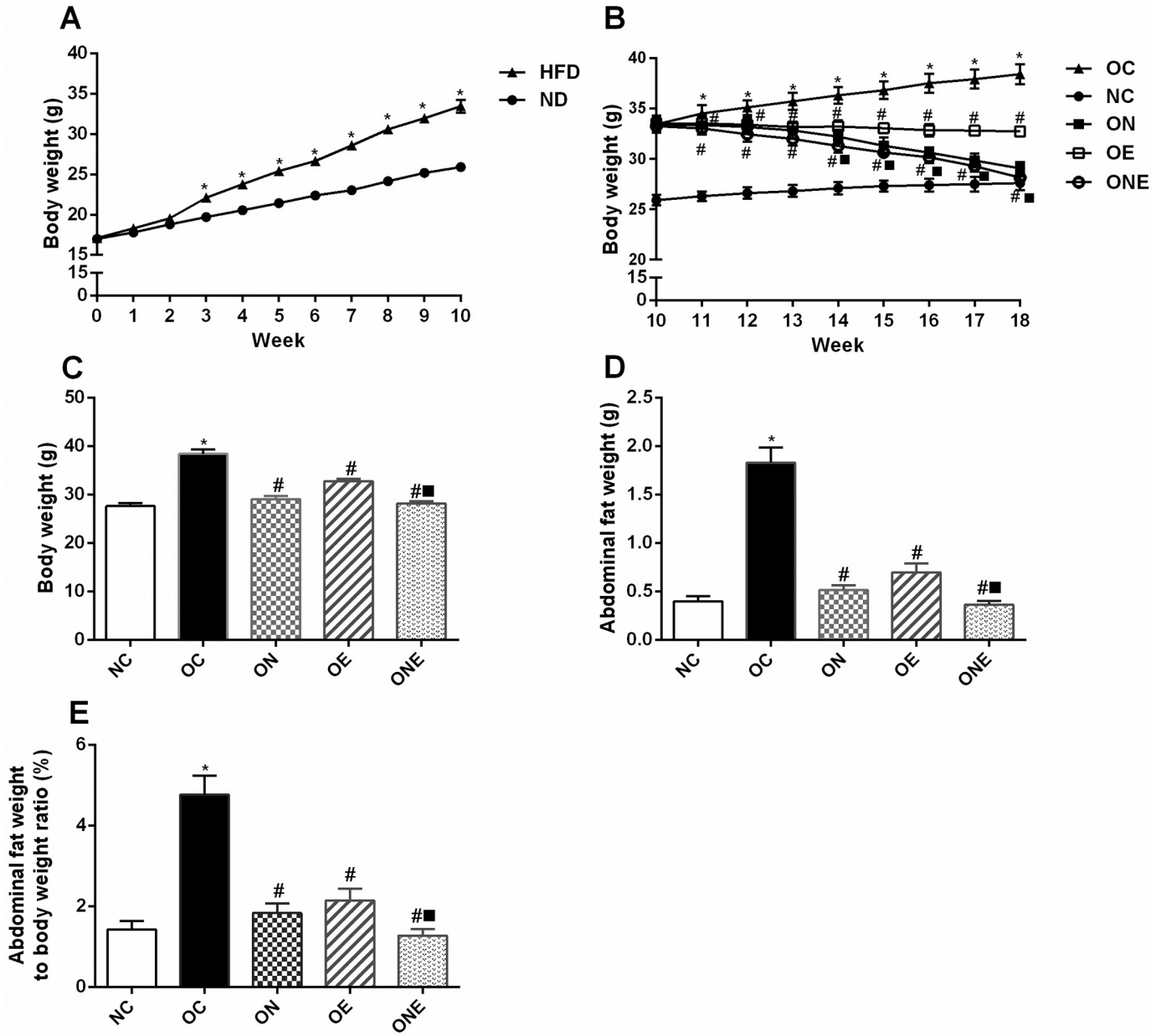

Fig. 2. Effects of diet and exercise on body weights, abdominal fat masses, and abdominal fat mass to body weight ratios. All data are expressed as means ± SEMs. A. Time-dependent increases in body weight in the pre-intervention period, $n = 55$. B. Time-dependent changes in body weight in the post-intervention period, $n = 52$. C. Effects of diet and exercise on body weights, $n = 52$. D. Effects of diet and exercise on abdominal fat masses, $n = 32$. E. Effects of diet and exercise on abdominal fat mass to body weight ratios, $n = 32$. $^{*}P < .05$ compared with the NC group. $^{\#}P < .05$ compared with the OC group. $^{\blacksquare}P < .05$ compared with the OE group.

analysis of data between the OC, ON, OE, and ONE groups. If the variance was heterogeneous, Mann-Whitney *U* tests were used. Correlation analysis was performed using the Pearson correlation. Results with *P* values of < 0.05 were considered significant.

3. Results

3.1. Effects of diet and exercise on body weight and abdominal fat mass

At the end of week 10 on the HFD, the average body weight of the mice on the HFD was greater than that of mice on the ND by 29.15% (33.45 g versus 25.90 g; Fig. 2A), up to the standard of obesity model level in mice (Chandler et al., 2005). After continuing with the HFD for 8 weeks, body weights, abdominal fat masses, and abdominal fat mass to body weight ratios increased significantly in the OC group compared with that in the NC group ($P < .05$, Fig. 2B–E). All interventions were able to reduce body weights, abdominal fat masses, and abdominal fat mass to body weight ratios significantly compared with those in the OC group ($P < .05$, Fig. 3C–E). In addition, when compared with the OE group, mice in the ONE group showed significantly reduced body weights, abdominal fat masses, and abdominal fat mass to body weight ratios ($P < .05$, Fig. 2B–E). In terms of weight loss effect, diet combined with exercise was the most effective, and exercise alone was not as effective as diet alone.

3.2. Effects of diet and exercise on the serum hormonal milieu

Male mice in the OC group had significantly lower levels of serum LH, FSH, T, and nesfatin-1 than those in the NC group ($P < .05$, Fig. 3A–D). Serum levels of LH, FSH, T, and nesfatin-1 were significantly increased in all intervention groups compared with those in the OC group ($P < .05$, Fig. 3A–D), except for nesfatin-1 levels in the

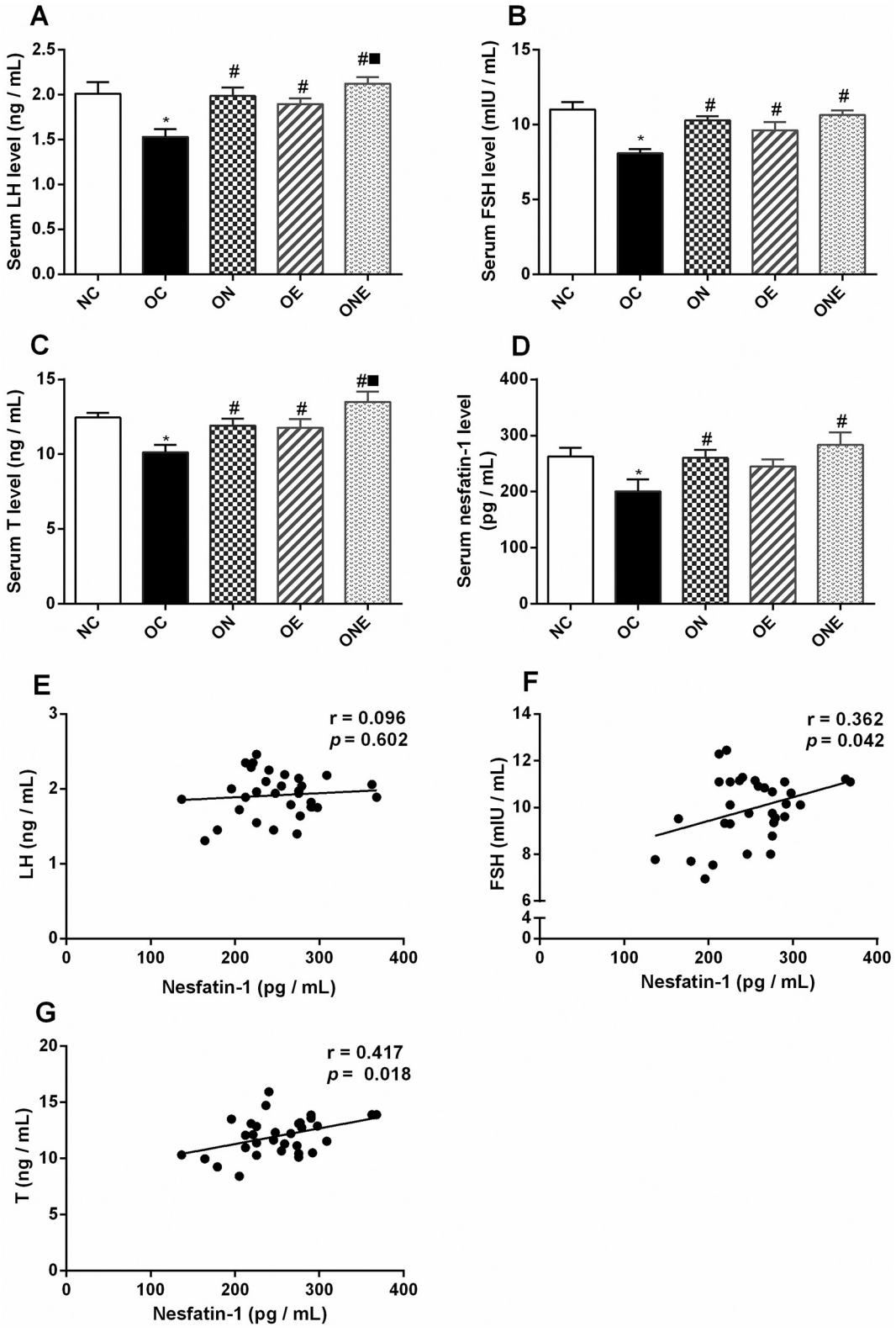

Fig. 3. Effects of diet and exercise on the serum hormonal milieu. A–D. Effects of diet and/or exercise on the levels of LH, FSH, T, and nesfatin-1 in the serum. Values are means ± SEMs; $n = 32$. $^{*}P < .05$ compared with the NC group. $^{\#}P < .05$ compared with the OC group. $^{\blacksquare}P < .05$ compared with the OE group. E–G. Correlation of serum nesfatin-1 levels with LH, FSH, and T levels.

OE group, which tended to increase ($P > .05$, Fig. 3D). In addition, mice in the ONE group showed significantly increased LH and T levels compared with those in the OE group ($P < .05$, Fig. 3A and C). Among the three types of interventions, increases in serum reproductive hormones and nesfatin-1 levels were the highest in the ONE group, followed by the ON group. In all mice, serum nesfatin-1 levels were positively correlated with FSH and T levels ($P < .05$, Fig. 3F and G).

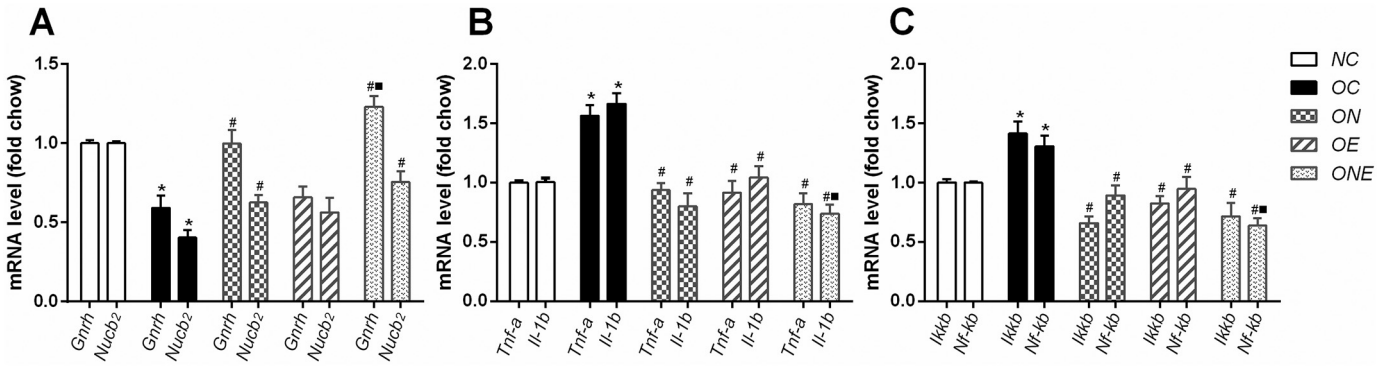

Fig. 4. Effects of diet and exercise on mRNA levels of *Gnrh*, *Nucb2,* and inflammatory factors in the hypothalamus. mRNA levels are presented as fold changes relative to the NC group. Values are means ± SEMs. n = 32. $^{*}P < .05$ compared with the NC group. $^{\#}P < .05$ compared with the OC group. $^{\bullet}P < .05$ compared with the ON group. $^{\blacksquare}P < .05$ compared with the OE group.

3.3. Effects of diet and exercise on mRNA levels of Nucb2, Gnrh, and inflammatory factors in the hypothalamus

Compared with the NC group, mRNA levels of *Gnrh* and *Nucb2* were significantly decreased ($P < .05$, Fig. 4A), whereas mRNA levels of pro-inflammatory cytokines (*Tnf-α* and *Il-1β*) and inflammatory signaling pathway components (*Ikkβ* and *Nf-κb*) were significantly increased in the hypothalamus of male mice in the OC group ($P < .05$, Fig. 4B and C). After diet and/or exercise interventions, the mRNA levels of *Gnrh* and *Nucb2* were significantly increased in the ON and ONE groups when compared with those in the OC group ($P < .05$, Fig. 4A). In contrast, mRNA levels of *Tnf-a*, *Il-1β*, *Nf-κb,* and *Ikkβ* were significantly reduced in all intervention groups compared with those in the OC group ($P < .05$, Fig. 4B and C). In addition, when compared with mice in the OE group, mice in the ONE group showed significantly increased *Gnrh* mRNA levels ($P < .05$, Fig. 4A), but significantly reduced *Il-1β* and *Nf-κb* mRNA levels ($P < .05$, Fig. 4B and C). In the three intervention groups, increases in *Gnrh* and *Nucb2* mRNA levels were the highest in the ONE group, in which the mRNA levels of the inflammatory factors were the lowest.

3.4. Effects of diet and exercise on protein levels of nefatin-1, GnRH and inflammatory factors in the hypothalamus

For immunohistochemical analysis, neurons showing positive staining for nesfatin-1, GnRH, and inflammatory factors were found to be localized in the mediobasal hypothalamus, particularly in the Arc (Fig. 5A–F). Moreover, levels of nesfatin-1, GnRH, inflammatory factors (TNF-α and IL-1β), and NF-κB pathway components (phospho-IKKβ and NF-κB p65), expressed as the fold change in average integrated optical density compared with the NC group, were evaluated (Fig. 5G–L). The results showed that mice in the OC group exhibited decreased levels of nesfatin-1 and GnRH ($P < .05$, Fig. 5G–H), but increased levels of TNF-α, IL-1β, phospho-IKKβ, and NF-κB p65 compared with those in the NC group ($P < .05$, Fig. 5H–L). After diet and/or exercise interventions, nesfatin-1 levels were significantly increased in the ON and ONE groups, and GnRH levels were significantly increased in the ONE group compared with those in the OC group ($P < .05$, Fig. 5G–H). Moreover, mice in the ONE group showed significantly increased GnRH levels compared with the ON and OE groups ($P < .05$, Fig. 5H). All interventions significantly reduced TNF-α, IL-1β, phospho-IKKβ, and NF-κB p65 levels compared with those in the OC group ($P < .05$, Fig. 5G–J), with the exception of TNF-α in the OE group and IL-1β in the ON and OE groups ($P > .05$, Fig. 5G and H). Finally, mice in the ONE group showed significantly decreased TNF-α and phospho-IKKβ levels compared with mice in the OE group ($P < .05$, Fig. 5G and I) and significantly decreased phospho-IKKβ levels compared with mice in the ON group ($P < .05$, Fig. 5I).

4. Discussion

The findings in this study showed that exposure to an HFD for 18 weeks led to obesity, which was associated the hypogonadotropic hypogonadism, reduced serum and hypothalamic NUCB2/nesfatin-1 levels, increased hypothalamic pro-inflammatory factors and NF-κB pathway components levels, and reduced hypothalamic GnRH levels. Diet and/or exercise interventions were effective in reducing adiposity and improving hypogonadotropic hypogonadism-associated obesity; moreover, decreased serum and hypothalamic NUCB2/nesfatin-1 levels, increased inflammatory factors and NF-κB pathway components levels, and reduced hypothalamic GnRH levels associated with obesity were also reversed by diet and/or exercise interventions.

GnRH, which is mainly secreted by the neurons in the preoptic area of the hypothalamus and in the mediobasal hypothalamus, is essential for the normal reproductive function of mammals (Stamatiades and Kaiser, 2018). GnRH acts on gonadotrope cells in the anterior pituitary to increase the production and secretion of the gonadotropin hormones LH and FSH (Stamatiades and Kaiser, 2018). In obesity, both mRNA levels and immunopositivity of GnRH were decreased in the hypothalamus of obese male rabbits (Morelli et al., 2014). Consistent with these previous findings, we showed that GnRH positive neurons mainly located in the mediobasal hypothalamus, particularly in the Arc. In addition, the mRNA and protein levels of GnRH in the hypothalamus of obese male mice were significantly decreased compared with those in normal weight male mice, accompanied by decreased serum levels of LH, FSH, and T in obese mice. These findings indicated that the reduced mRNA and protein levels of GnRH in the hypothalamus of obese mice directly induced decreases in serum reproductive hormones levels, although the mechanisms through which GnRH levels were reduced in obesity are still unknown.

As was observed in previous studies, nesfatin-1 positive neurons were distributed in many hypothalamic nuclei, particularly in the Arc, which is located in the mediobasal hypothalamus and is implicated in the control of energy balance and reproductive function (Chen et al., 2015; Goebel-Stengel and Wang, 2013; Oh et al., 2006). Thus, nesfatin-1 was first identified as a satiety peptide in many hypothalamic nuclei and was found to play important roles in controlling appetite and energy balance (Oh et al., 2006). Later, NUCB2/nesfatin-1-positive neurons were found to be co-localized with GnRH in murine hypothalamic (GT1–7) cells and in the hypothalamic perikarya of mice, and nesfatin-1 intervention was found to increase the mRNA and protein levels of GnRH in murine hypothalamic (GT1–7) cells and Lhβ in murine pituitary (LβT2) cells (Hatef and Unniappan, 2017). In addition, nesfatin-1 administration was also found to promote the secretion of LH and FSH in the serum of adult male rats (Garcia-Galiano et al., 2010). Moreover, treatment of cultured testis tissues and Leydig cells with nesfatin-1 increased T levels (Gao et al., 2016; Garcia-Galiano et al., 2012). These

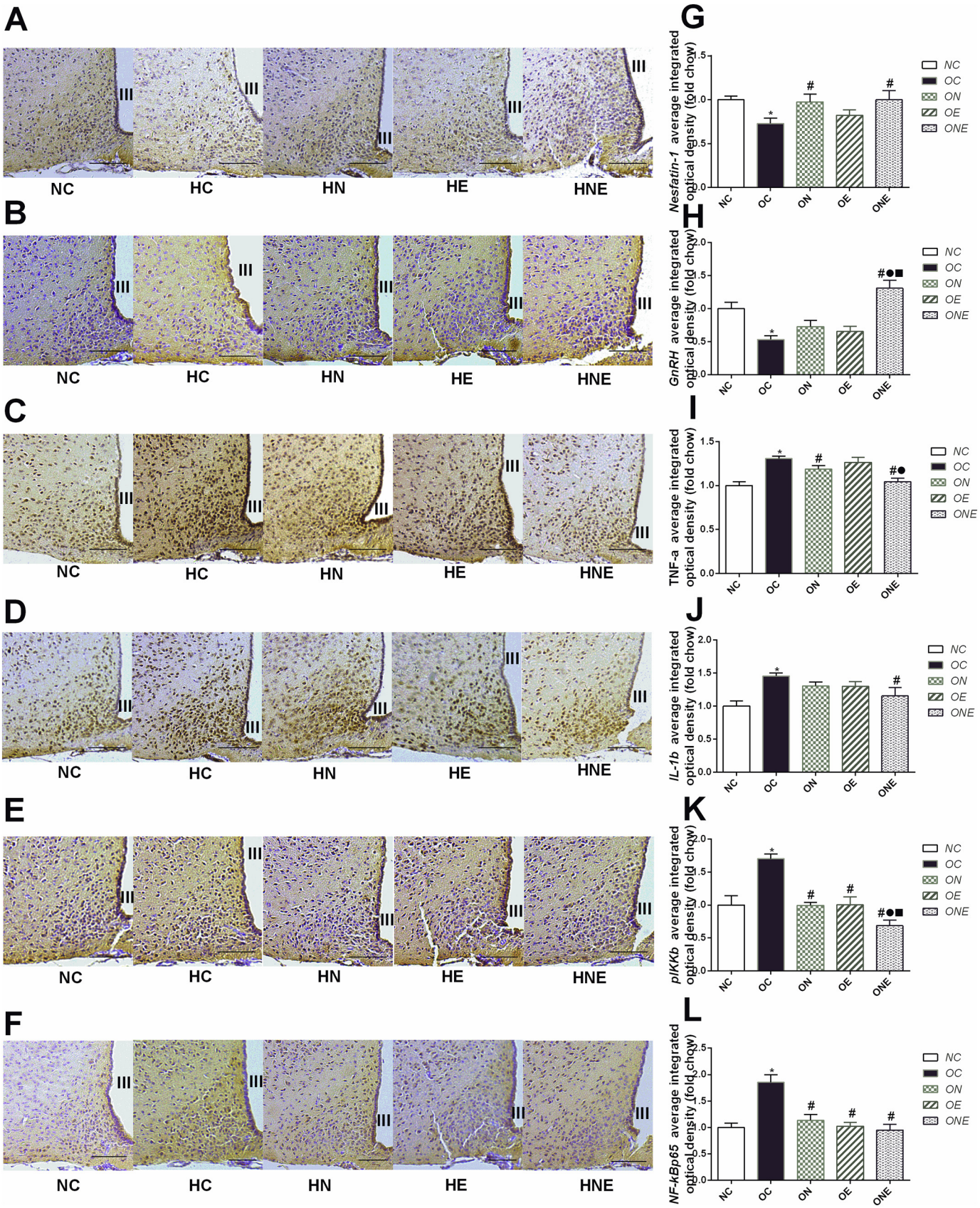

Fig. 5. Effects of diet and exercise on the average integrated optical densities of nesfatin-1, GnRH, and inflammatory factors in the hypothalamus. A–F show representative images of nesfatin-1, GnRH, and inflammatory factors. G–L show the average integrated optical density levels of nesfatin-1, GnRH, and inflammatory factors and are presented as fold changes relative to those in the NC group. Values are means ± SEMs. $n = 20$. $^{*}P < .05$ compared with the NC group. $^{\#}P < .05$ compared with the OC group. $^{\bullet}P < .05$ compared with the ON group. $^{\blacksquare}P < .05$ compared with the OE group. Scale bars, 100 μm.

findings indicated that, nesfatin-1 could stimulate LH, FSH, and T secretion in males by directly acting on all levels of the hypothalamic-pituitary-testicular axis. In our study, serum nesfatin-1 levels in obese mice were significantly reduced compared with that in NC group, similar to previous findings in obese men (Abaci et al., 2013; Guo et al., 2014). Notably, nesfatin-1 can cross the blood-brain barrier without saturation (Pan et al., 2007), and cerebrospinal fluid NUCB2/nesfatin-1 is significantly positively associated with plasma NUCB2/nesfatin-1 (Tan et al., 2011). Thus, in this study, the decreases in serum nesfatin-1 levels in obesity were likely to induce changes in NUCB2/nesfatin-1 expression in the hypothalamus of obese male mice. Indeed, we found that NUCB2/nesfatin-1-immunopositive neurons were mainly distributed in the mediobasal hypothalamus, particularly in the Arc, similar to the previous description of NUCB2/nesfatin-1 distribution in the hypothalamus of mice (Oh et al., 2006). Moreover, the mRNA and protein levels of NUCB2/nesfatin-1 in the hypothalamus of obese mice were decreased compared with those in NC mice. As described above, compared with NC mice, the mRNA and protein levels of GnRH were decreased in the hypothalamus of obese mice, and it is likely that decreases in serum and hypothalamic NUCB2/nesfatin-1 levels may directly attenuate stimulatory effects on GnRH expression, which could further reduce serum LH, FSH, and T levels in obese male mice.

Nesfatin-1 exerts anti-inflammatory effects in the rat brain (Ozsavci et al., 2011; Tang et al., 2012). Moreover, activation of the IKKβ/NF-κB pathway could reduce mRNA levels of *Gnrh* in the hypothalamus of mice (Zhang et al., 2013). Thus, levels of pro-inflammatory factors (TNF-α and IL-1β) and IKKβ/NF-κB pathway components (IKKβ and NF-κB) were also evaluated to determine whether the regulatory effects of NUCB2/nesfatin-1 on GnRH were related to its anti-inflammatory effects in the hypothalamus. The results showed that positive neurons for TNF-α, IL-1β, phospho-IKKβ, and NF-κB were mainly distributed in the Arc, similar to the distribution of NUCB2/nesfatin-1-positive neurons in this study. After semi-quantitative analysis, we found that the mRNA and protein levels of TNF-α, IL-1β, NF-κB, and the phosphorylation of IKKβ were increased in obese mice compared with that in NC mice. Thus, it is likely that diet-induced obesity may attenuate the anti-inflammatory effects in the brain by decreasing hypothalamic NUCB2/nesfatin-1 level; the IKKβ/NF-κB pathway was then activated and GnRH mRNA and protein levels were reduced.

To further elucidate the potential mechanism of nesfatin-1 in hypothalamic regulation of reproductive function, diet and/or exercise interventions were employed in obese mice, and we examined whether hypogonadotropic hypogonadism could be alleviated by diet and/or exercise interventions in obese mice. As mentioned above, diet and exercise interventions are effective in reducing adiposity, but it is still unclear whether hypogonadotropic hypogonadism associated with obesity could be affected by diet and/or exercise interventions. In previous studies, the effects of dietary intervention on adiposity reduction and alleviation of hypogonadotropic hypogonadism associated with obesity have been consistent; that is, a moderate reduction in calorie intake can reverse low T levels in obese men and male mice (Palmer et al., 2012; Schulte et al., 2013) and can increase serum sex hormone-binding globulin levels (Khoo et al., 2013; Schulte et al., 2013) and the T to estradiol ratio in obese men (Schulte et al., 2013). In this study, the energy content in the diet of mice decreased from 4.72 to 3.89 kcal/g when the dietary intervention was applied, and both the body weights and abdominal fat masses were significantly decreased, accompanied by increases in serum productive hormone (LH, FSH, and T) levels in obese mice after dietary intervention. These findings indicated that dietary intervention alleviated hypogonadotropic hypogonadism in male mice caused by obesity and effectively reduced adiposity. However, previous studies of the effects of exercise on serum reproductive hormones in obese men or male animals have not been consistent; that is, although most studies have reported that exercise can increase serum T levels in obese men or rodents (Rosety et al., 2017; Yi et al., 2017; You et al., 2013), some studies have shown that exercise decreases serum LH, FSH, and T levels in obese men (Safarinejad et al., 2009), or has no effect on decreased T and increased estradiol levels in obese men (Yi et al., 2017). These inconsistent results may be related to variations in exercise load or volume, as the findings in our previous study showed that moderate-volume exercise could effectively reverse the decreased T and increased estradiol levels in obese mice, but this effect was not found after high-volume exercise (Yi et al., 2017). Thus, moderate load exercise was adopted in this study. In addition, to further determine whether diet combined with exercise intervention was more effective for alleviating hypogonadotropic hypogonadism, 8 weeks of diet combined with exercise was also intervened in obese male mice. After intervention, 8 weeks of exercise and diet combined with exercise not only effectively reduced abdominal fat masses and body weights in obese mice, but also reversed decreases in serum LH, FSH, and T levels induced by obesity, similar to findings in previous studies (Armamento-Villareal et al., 2016; Palmer et al., 2012; You et al., 2013). In studies of the effects of the three interventions on hypothalamic GnRH levels, we found that diet alone significantly increased *Gnrh* mRNA levels, diet combined with exercise significantly increased GnRH mRNA and protein levels, and the effects were more obvious in the diet combined with exercise intervention group. However, increases in the mRNA and protein levels of GnRH in the exercise alone group were not significant, and these results may be related to the minor effects of this intervention on weight loss in obese mice (weight loss induced in the exercise alone, diet alone, and diet combined with exercise groups were 61.96%,71.84%, and 80.13%, respectively). Interestingly, serum productive hormones and hypothalamic GnRH levels affected by the three interventions were consistent with the trends in weight loss. These findings suggested that diet alone and diet combined with exercise could alleviate hypogonadotropic hypogonadism associated with obesity by reversing the decreased GnRH levels in the hypothalamus of obese mice and that diet combined with exercise was more effective. Nevertheless, it was unclear whether the changes in hypothalamic GnRH levels were related to changes in serum, particularly hypothalamic NUCB2/nesfatin-1 levels. Therefore, serum and hypothalamic NUCB2/nesfatin-1 levels in the mice of the three intervention groups were also evaluated; the results showed that exercise alone did not alter serum and hypothalamic NUCB2/nesfatin-1 levels, whereas diet alone and diet combined with exercise significantly increased serum and hypothalamic NUCB2/nesfatin-1 levels, with the most obvious effects in the diet combined with exercise group. These findings for NUCB2/nesfatin-1 in the diet and/or exercise mice groups were opposite those in OC group, although trends for hypothalamic NUCB2/nesfatin-1 levels were similar to hypothalamic GnRH and serum productive hormones levels in mice in the three intervention groups. Based on these findings, NUCB2/nesfatin-1 may act directly on GnRH neurons in the hypothalamus to regulate male reproductive function.

In this study, we showed that all three interventions reduced the mRNA and protein levels of TNF-α, IL-1β, IKKβ, and NF-κB in the hypothalamus, particularly in the Arc, of obese male mice. Moreover, mRNA and protein levels of NUCB2/nesfatin-1 and GnRH were reversed by diet and/or exercise with the most significant effects observed in the diet combined with exercise intervention. The trends in hypothalamic levels of NUCB2/nesfatin-1 and GnRH were consistent with those in serum reproductive hormones after the three treatments in mice. In addition, long-term diet intervention has been shown to be successful for reversing the increases in astrocyte and microglial activation in the Arc of obese mice (Berkseth et al., 2014) and to significantly reduce the levels of TNF-α and NF-κB in the Arc of juvenile obese rats. Moreover, long-term exercise can markedly decrease microglial activation specifically in hypothalamic Arc regions (Yi et al., 2012) and inhibit activation of the IKKβ/NF-κB pathway in the hypothalamus of mice (Ropelle et al., 2010). Based on these previous findings, the findings in our study indicated that nesfatin-1 may indirectly regulate male reproductive function through its anti-inflammatory effects.

However, because the antibody used in this study for NUCB2/nesfatin-1 cannot discriminate unprocessed/processed forms of the peptide, nesfatin-1 protein levels in the hypothalamus of obese mice and after diet and/or exercise interventions were not able be determined. In addition, because nesfatin-1 is cleaved from NUCB2 by prohormone convertase (PC) 1/3 and PC 2, and mutation of the *PCSK1* gene (which encodes PC 1/3) may also exhibit hypogonadotropic hypogonadism (Stijnen et al., 2016), it is possible that the hypogonadotropic hypogonadism associated with obesity may induced by impairment of hypothalamic PC1/3 and PC 2 expression. Moreover, in this study, we were not able to further verify the potential mechanisms underlying the role of nesfatin-1 in regulating reproductive function by exogenous administration of nesfatin-1 in vivo or in vitro. Thus, further studies are needed to elucidate these mechanisms.

5. Conclusions

Taken together, the findings of this study showed that exposure to an HFD for 18 weeks enhanced obesity, impaired hypothalamic/pituitary/testicular axis activity and induced hypogonadotropic hypogonadism in male mice. The underlying mechanisms may be related to decreases in hypothalamic NUCB2/nesfatin-1 levels caused by obesity, which attenuated the stimulatory effect on hypothalamic GnRH expression directly or indirectly by suppressing its anti-inflammatory effects in the brain. Diet and exercise interventions were able to alleviate the hypogonadotropic hypogonadism associated with obesity, potentially by increasing hypothalamic NUCB2/nesfatin-1 levels. However, further studies are needed.

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Conflicts of interest

All of the authors declare no conflicts of interest in this study.

Declarations of interest

None.

References

Abaci, A., Catli, G., Anik, A., Kume, T., Bober, E., 2013. The relation of serum nesfatin-1 level with metabolic and clinical parameters in obese and healthy children. Pediatr. Diabetes 14, 189–195. https://doi.org/10.1111/pedi.12009.

Ahmadizad, S., Avansar, A.S., Ebrahim, K., Avandi, M., Ghasemikaram, M., 2015. The effects of short-term high-intensity interval training vs. moderate-intensity continuous training on plasma levels of nesfatin-1 and inflammatory markers. Horm. Mol. Biol. Clin. Invest. 21, 165–173. https://doi.org/10.1515/hmbci-2014-0038.

Armamento-Villareal, R., Aguirre, L.E., Qualls, C., Villareal, D.T., 2016. Effect of lifestyle intervention on the hormonal profile of frail, obese older men. J. Nutr. Health Aging 20, 334–340. https://doi.org/10.1007/s12603-016-0698-x.

Berkseth, K.E., Guyenet, S.J., Melhorn, S.J., Lee, D., Thaler, J.P., Schur, E.A., Schwartz, M.W., 2014. Hypothalamic gliosis associated with high-fat diet feeding is reversible in mice: a combined immunohistochemical and magnetic resonance imaging study. Endocrinology 155, 2858–2867. https://doi.org/10.1210/en.2014-1121.

Bieniek, J.M., Kashanian, J.A., Deibert, C.M., Grober, E.D., Lo, K.C., Brannigan, R.E., Sandlow, J.I., Jarvi, K.A., 2016. Influence of increasing body mass index on semen and reproductive hormonal parameters in a multi-institutional cohort of subfertile men. Fertil. Steril. 106, 1070–1075. https://doi.org/10.1016/j.fertnstert.2016.06.041.

Boggio, V., Cutrera, R., Carbone, S., Scacchi, P., Ponzo, O.J., 2013. Leptin inhibits the reproductive axis in adult male Syrian hamsters exposed to long and short photoperiod. Reprod. Biol. 13, 203–208. https://doi.org/10.1016/j.repbio.2013.07.002.

Catak, Z., Aydin, S., Sahin, I., Kuloglu, T., Aksoy, A., Dagli, A.F., 2014. Regulatory neuropeptides (ghrelin, obestatin and nesfatin-1) levels in serum and reproductive tissues of female and male rats with fructose-induced metabolic syndrome. Neuropeptides 48, 167–177. https://doi.org/10.1016/j.npep.2014.04.002.

Chandler, P.C., Viana, J.B., Oswald, K.D., Wauford, P.K., Boggiano, M.M., 2005. Feeding response to melanocortin agonist predicts preference for and obesity from a high-fat diet. Physiol. Behav. 85, 221–230. https://doi.org/10.1016/j.physbeh.2005.04.011.

Chaolu, H., Asakawa, A., Ushikai, M., Li, Y.X., Cheng, K.C., Li, J.B., Zoshiki, T., Terashi, M., Tanaka, C., Atsuchi, K., Sakoguchi, T., Tsai, M., Amitani, H., Horiuchi, M., Takeuchi, T., Inui, A., 2011. Effect of exercise and high-fat diet on plasma adiponectin and nesfatin levels in mice. Exp. Ther. Med. 2, 369–373. https://doi.org/10.3892/etm.2011.199.

Chen, X., Shu, X., Cong, Z.K., Jiang, Z.Y., Jiang, H., 2015. Nesfatin-1 acts on the dopaminergic reward pathway to inhibit food intake. Neuropeptides 53, 45–50. https://doi.org/10.1016/j.npep.2015.07.004.

Collaboration, N.R.F, 2016. Trends in adult body-mass index in 200 countries from 1975 to 2014 a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. Lancet 387, 1377–1396. https://doi.org/10.1016/S0140-6736(16)30054-X.

Corona, G., Rastrelli, G., Monami, M., Saad, F., Luconi, M., Lucchese, M., Facchiano, E., Sforza, A., Forti, G., Mannucci, E., Maggi, M., 2013. Body weight loss reverts obesity-associated hypogonadotropic hypogonadism: a systematic review and meta-analysis. Eur. J. Endocrinol. 168, 829–843. https://doi.org/10.1530/EJE-12-0955.

Corona, G., Vignozzi, L., Sforza, A., Mannucci, E., Maggi, M., 2015. Obesity and late-onset hypogonadism. Mol. Cell Endocrinol. 418 Pt 2, 120–133. https://doi.org/10.1016/j.mce.2015.06.031.

Dandona, P., Dhindsa, S., 2011. Update: Hypogonadotropic hypogonadism in type 2 diabetes and obesity. J. Clin. Endocrinol. Metab. 96, 2643–2651. https://doi.org/10.1210/jc.2010-2724.

Diane, A., Pierce, W.D., Mangat, R., Borthwick, F., Nelson, R., Russell, J.C., Heth, C.D., Jacobs, R.L., Vine, D.F., Proctor, S.D., 2015. Differential expression of hypothalamic, metabolic and inflammatory genes in response to short-term calorie restriction in juvenile obese- and lean-prone JCR rats. Nutr. Diabetes 5, e178. https://doi.org/10.1038/nutd.2015.28.

Filippi, S., Vignozzi, L., Morelli, A., Chavalmane, A.K., Sarchielli, E., Fibbi, B., Saad, F., Sandner, P., Ruggiano, P., Vannelli, G.B., Mannucci, E., Maggi, M., 2009. Testosterone partially pmeliorates metabolic profile and erectile responsiveness to PDE5 inhibitors in an animal model of male metabolic syndrome. J. Sex. Med. 6, 3274–3288. https://doi.org/10.1111/j.1743-6109.2009.01467.x.

Gao, X., Zhang, K., Song, M., Li, X., Luo, L., Tian, Y., Zhang, Y., Li, Y., Zhang, X., Ling, Y., Fang, F., Liu, Y., 2016. Role of Nesfatin-1 in the reproductive axis of male rat. Sci. Rep. 6, 32877. https://doi.org/10.1038/srep32877.

Garcia-Galiano, D., Navarro, V.M., Gaytan, F., Tena-Sempere, M., 2010. Expanding roles of NUCB2/nesfatin-1 in neuroendocrine regulation. J. Mol. Endocrinol. 45, 281–290. https://doi.org/10.1677/JME-10-0059.

Garcia-Galiano, D., Pineda, R., Ilhan, T., Castellano, J.M., Ruiz-Pino, F., Sanchez-Garrido, M.A., Vazquez, M.J., Sangiao-Alvarellos, S., Romero-Ruiz, A., Pinilla, L., Dieguez, C., Gaytan, F., Tena-Sempere, M., 2012. Cellular distribution, regulated expression, and functional role of the anorexigenic peptide, NUCB2/nesfatin-1, in the testis. Endocrinology 153, 1959–1971. https://doi.org/10.1210/en.2011-2032.

Gautier, A., Bonnet, F., Dubois, S., Massart, C., Grosheny, C., Bachelot, A., Aube, C., Balkau, B., Ducluzeau, P.H., 2013. Associations between visceral adipose tissue, inflammation and sex steroid concentrations in men. Clin. Endocrinol. 78, 373–378. https://doi.org/10.1111/j.1365-2265.2012.04401.x.

Goebel-Stengel, M., Wang, L., 2013. Central and peripheral expression and distribution of NUCB2/nesfatin-1. Curr. Pharm. Des. 19, 6935–6940.

Guo, Y., Xing, M., Sun, W., Yuan, X., Dai, H., Ding, H., 2014. Plasma nesfatin-1 level in obese patients after acupuncture: a randomised controlled trial. Acupunct. Med. 32, 313–317. https://doi.org/10.1136/acupmed-2014-010530.

Hatef, A., Unniappan, S., 2017. Gonadotropin-releasing hormone, kisspeptin, and gonadal steroids directly modulate nucleobindin-2/nesfatin-1 in murine hypothalamic gonadotropin-releasing hormone neurons and gonadotropes. Biol. Reprod. 96, 635–651. https://doi.org/10.1095/biolreprod.116.146621.

Kalin, S., Heppner, F.L., Bechmann, I., Prinz, M., Tschop, M.H., Yi, C.X., 2015. Hypothalamic innate immune reaction in obesity. Nat. Rev. Endocrinol. 11, 339–351. https://doi.org/10.1038/nrendo.2015.48.

Khoo, J., Ling, P.S., Tan, J., Teo, A., Ng, H.L., Chen, R.Y.T., Tay, T.L., Tan, E., Cheong, M., 2013. Comparing the effects of meal replacements with reduced-fat diet on weight, sexual and endothelial function, testosterone and quality of life in obese Asian men. Int. J. Impot. Res. 26, 61–66. https://doi.org/10.1038/ijir.2013.36.

Lee, H., Chang, H., Park, J.Y., Kim, S.Y., Choi, K.M., Song, W., 2011. Exercise training improves basal blood glucose metabolism with no changes of cytosolic inhibitor B kinase or c-Jun N-terminal kinase activation in skeletal muscle of Otsuka Long-Evans Tokushima fatty rats. Exp. Physiol. 96, 689–698. https://doi.org/10.1113/expphysiol.2011.057737.

Lee, S., Kim, M., Lim, W., Kim, T., Kang, C., 2015. Strenuous exercise induces mitochondrial damage in skeletal muscle of old mice. Biochem. Biophys. Res. Commun. 461, 354–360. https://doi.org/10.1016/j.bbrc.2015.04.038.

Masson, G.S., Nair, A.R., Silva Soares, P.P., Michelini, L.C., Francis, J., 2015. Aerobic training normalizes autonomic dysfunction, HMGB1 content, microglia activation

and inflammation in hypothalamic paraventricular nucleus of SHR. Am. J. Physiol. Heart Circ. Physiol. 309, H1115–H1122. https://doi.org/10.1152/ajpheart.00349.2015.

Miyata, S., Yamada, N., Kawada, T., 2012. Possible involvement of hypothalamic nucleobindin-2 in hyperphagic feeding in Tsumura Suzuki obese diabetes mice. Biol. Pharm. Bull. 35, 1784–1793.

Morelli, A., Sarchielli, E., Comeglio, P., Filippi, S., Vignozzi, L., Marini, M., Rastrelli, G., Maneschi, E., Cellai, I., Persani, L., Adorini, L., Vannelli, G.B., Maggi, M., 2014. Metabolic syndrome induces inflammation and impairs gonadotropin-releasing hormone neurons in the preoptic area of the hypothalamus in rabbits. Mol. Cell. Endocrinol. 382, 107–119. https://doi.org/10.1016/j.mce.2013.09.017.

Muccioli, G., Lorenzi, T., Lorenzi, M., Ghe, C., Arnoletti, E., Raso, G.M., Castellucci, M., Gualillo, O., Meli, R., 2011. Beyond the metabolic role of ghrelin: a new player in the regulation of reproductive function. Peptides 32, 2514–2521. https://doi.org/10.1016/j.peptides.2011.10.020.

Nakata, M., Yamamoto, S., Okada, T., Yada, T., 2017. AAV-mediated IL-10 gene transfer counteracts inflammation in the hypothalamic arcuate nucleus and obesity induced by high-fat diet. Neuropeptides 62, 87–92. https://doi.org/10.1016/j.npep.2016.11.009.

Oh, I.S., Shimizu, H., Satoh, T., Okada, S., Adachi, S., Inoue, K., Eguchi, H., Yamamoto, M., Imaki, T., Hashimoto, K., Tsuchiya, T., Monden, T., Horiguchi, K., Yamada, M., Mori, M., 2006. Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature 443, 709–712. https://doi.org/10.1038/nature05162.

Ozsavci, D., Ersahin, M., Sener, A., Ozakpinar, O.B., Toklu, H.Z., Akakin, D., Sener, G., Yegen, B.C., 2011. The novel function of nesfatin-1 as an anti-inflammatory and antiapoptotic peptide in subarachnoid hemorrhage-induced oxidative brain damage in rats. Neurosurgery 68, 1699–1708. https://doi.org/10.1227/NEU.0b013e318210f258.

Ozturk, C.C., Oktay, S., Yuksel, M., Akakin, D., Yarat, A., Kasimay Cakir, O., 2015. Anti-inflammatory effects of nesfatin-1 in rats with acetic acid - induced colitis and underlying mechanisms. J. Physiol. Pharmacol. 66, 741–750.

Palmer, N.O., Bakos, H.W., Owens, J.A., Setchell, B.P., Lane, M., 2012. Diet and exercise in an obese mouse fed a high-fat diet improve metabolic health and reverse perturbed sperm function. Am. J. Physiol. Endocrinol. Metab. 302, E768–E780. https://doi.org/10.1152/ajpendo.00401.2011.-Male.

Pan, W., Hsuchou, H., Kastin, A.J., 2007. Nesfatin-1 crosses the blood-brain barrier without saturation. Peptides 28, 2223–2228. https://doi.org/10.1016/j.peptides.2007.09.005.

Rabijewski, M., Papierska, L., Zgliczynski, W., Piatkiewicz, P., 2013. The incidence of hypogonadotropic hypogonadism in type 2 diabetic men in Polish population. Biomed. Res. Int. 2013, 767496. https://doi.org/10.1155/2013/767496.

Ramaraju, G.A., Teppala, S., Prathigudupu, K., Kalagara, M., Thota, S., Kota, M., Cheemakurthi, R., 2018. Association between obesity and sperm quality. Andrologia 50. https://doi.org/10.1111/and.12888.

Ravussin, A., Youm, Y.H., Sander, J., Ryu, S., Nguyen, K., Varela, L., Shulman, G.I., Sidorov, S., Horvath, T.L., Schultze, J.L., Dixit, V.D., 2018. Loss of nucleobindin-2 causes insulin resistance in obesity without impacting satiety or adiposity. Cell Rep. 24 (1085–1092), e6. https://doi.org/10.1016/j.celrep.2018.06.112.

Ropelle, E.R., Flores, M.B., Cintra, D.E., Rocha, G.Z., Pauli, J.R., Morari, J., de Souza, C.T., Moraes, J.C., Prada, P.O., Guadagnini, D., Marin, R.M., Oliveira, A.G., Augusto, T.M., Carvalho, H.F., Velloso, L.A., Saad, M.J., Carvalheira, J.B., 2010. IL-6 and IL-10 anti-inflammatory activity links exercise to hypothalamic insulin and leptin sensitivity through IKKbeta and ER stress inhibition. PLoS Biol. 8, e1000465. https://doi.org/10.1371/journal.pbio.1000465.

Rosety, M.Á., Díaz, A.J., Rosety, J.M., Pery, M.T., Brenes-Martín, F., Bernardi, M., García, N., Rosety-Rodríguez, M., Ordoñez, F.J., Rosety, I., 2017. Exercise improved semen quality and reproductive hormone levels in sedentary obese adults. Nutr. Hosp. 34, 603. https://doi.org/10.20960/nh.549.

Safarinejad, M.R., Azma, K., Kolahi, A.A., 2009. The effects of intensive, long-term treadmill running on reproductive hormones, hypothalamus-pituitary-testis axis, and semen quality: a randomized controlled study. J. Endocrinol. 200, 259–271. https://doi.org/10.1677/JOE-08-0477.

Schulte, D., Hahn, M., Oberhäuser, F., Malchau, G., Schubert, M., Heppner, C., Müller, N., Güdelhöfer, H., Faust, M., Krone, W., Laudes, M., 2013. Caloric restriction increases serum testosterone concentrations in obese male subjects by two distinct mechanisms. Horm. Metab. Res. 46, 283–286. https://doi.org/10.1055/s-0033-1358678.

Stamatiades, G.A., Kaiser, U.B., 2018. Gonadotropin regulation by pulsatile GnRH: signaling and gene expression. Mol. Cell. Endocrinol. 463, 131–141. https://doi.org/10.1016/j.mce.2017.10.015.

Stijnen, P., Ramos-Molina, B., O'Rahilly, S., Creemers, J.W., 2016. PCSK1 mutations and human endocrinopathies: from obesity to gastrointestinal disorders. Endocr. Rev. 37, 347–371. https://doi.org/10.1210/er.2015-1117.

Tan, B.K., Hallschmid, M., Kern, W., Lehnert, H., Randeva, H.S., 2011. Decreased cerebrospinal fluid/plasma ratio of the novel satiety molecule, nesfatin-1/NUCB-2, in obese humans: evidence of nesfatin-1/NUCB-2 resistance and implications for obesity treatment. J. Clin. Endocrinol. Metab. 96, E669–E673. https://doi.org/10.1210/jc.2010-1782.

Tang, C.H., Fu, X.J., Xu, X.L., Wei, X.J., Pan, H.S., 2012. The anti-inflammatory and anti-apoptotic effects of nesfatin-1 in the traumatic rat brain. Peptides 36, 39–45. https://doi.org/10.1016/j.peptides.2012.04.014.

Thaler, J.P., Yi, C.X., Schur, E.A., Guyenet, S.J., Hwang, B.H., Dietrich, M.O., Zhao, X., Sarruf, D.A., Izgur, V., Maravilla, K.R., Nguyen, H.T., Fischer, J.D., Matsen, M.E., Wisse, B.E., Morton, G.J., Horvath, T.L., Baskin, D.G., Tschop, M.H., Schwartz, M.W., 2012. Obesity is associated with hypothalamic injury in rodents and humans. J. Clin. Invest. 122, 153–162. https://doi.org/10.1172/jci59660.

Tran, D.Q., Tse, E.K., Kim, M.H., Belsham, D.D., 2016. Diet-induced cellular neuroinflammation in the hypothalamus mechanistic insights from investigation of neurons and microglia. Mol. Cell. Endocrinol. 438, 18–26. https://doi.org/10.1016/j.mce.2016.05.015.

Yi, C.X., Al-Massadi, O., Donelan, E., Lehti, M., Weber, J., Ress, C., Trivedi, C., Muller, T.D., Woods, S.C., Hofmann, S.M., 2012. Exercise protects against high-fat diet-induced hypothalamic inflammation. Physiol. Behav. 106, 485–490. https://doi.org/10.1016/j.physbeh.2012.03.021.

Yi, X., Gao, H., Chen, D., Tang, D., Huang, W., Li, T., Ma, T., Chang, B., 2017. Effects of obesity and exercise on testicular leptin signal transduction and testosterone biosynthesis in male mice. Am. J. Phys. Regul. Integr. Comp. Phys. 312, R501–R510. https://doi.org/10.1152/ajpregu.00405.2016.

You, T., Disanzo, B.L., Arsenis, N.C., 2013. Aerobic exercise training attenuates obesity-related hypogonadism in male rats. Med. Sci. Sports Exerc. 45, 1244–1251. https://doi.org/10.1249/MSS.0b013e318285816c.

Zhang, G., Li, J., Purkayastha, S., Tang, Y., Zhang, H., Yin, Y., Li, B., Liu, G., Cai, D., 2013. Hypothalamic programming of systemic ageing involving IKK-beta, NF-kappaB and GnRH. Nature 497, 211–235. https://doi.org/10.1038/nature12143.