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RESEARCH REPORT

Expression of gastrointestinal nesfatin-1 and gastric emptying in ventromedial hypothalamic nucleus- and ventrolateral hypothalamic nucleus-lesioned rats

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Abstract

AIM: To determine the expression levels of gastrointestinal nesfatin-1 in ventromedial hypothalamic nucleus (VMH)-lesioned (obese) and ventrolateral hypothalamic nucleus (VLH)-lesioned (lean) rats that exhibit an imbalance in their energy metabolism and gastric mobility.

METHODS: Male Wistar rats were randomly divided into a VMH-lesioned group, a VLH-lesioned group, and their respective sham-operated groups. The animals had free access to food and water, and their diets and weights were monitored after surgery. Reverse transcription-polymerase chain reaction and immunostaining were used to analyse the levels of NUCB2 mRNA and nesfatin-1 immunoreactive (IR) cells in the stomach, duodenum, small intestine, and colon, respectively. Gastric emptying was also assessed using a modified phenol red-methylcellulose recovery method.

RESULTS: The VMH-lesioned rats fed normal chow exhibited markedly greater food intake and body weight gain, whereas the VLH-lesioned rats exhibited markedly lower food intake and body weight gain. NUCB2/nesfatin-1 IR cells were localised in the lower third and middle portion of the gastric mucosal gland and in the submucous layer of the enteric tract. Compared with their respective controls, gastric emptying was enhanced in the VMH-lesioned rats (85.94% ± 2.27%), whereas the VLH lesions exhibited inhibitory effects on gastric emptying (29.12% ± 1.62%). In the VMH-lesioned rats, the levels of NUCB2 mRNA and nesfatin-1 protein were significantly increased in the stomach and duodenum and reduced in the small intestine. In addition, the levels of NUCB2 mRNA and nesfatin-1 protein in the VLH-lesioned rats were decreased in the stomach, duodenum, and small intestine.

CONCLUSION: Our study demonstrated that nesfatin-1 level in the stomach and duodenum is positively correlated with body mass. Additionally, there is a positive relationship between gastric emptying and body mass. The results of this study indicate that gastrointestinal nesfatin-1 may play a significant role in gastric mobility and energy homeostasis.

Key words: Nucleobindin; Nesfatin-1; Gastrointestinal tract; Gastric emptying; Ventromedial hypothalamic nucleus; Hyperphagia; Obesity; Anorexia

Core tip: We report that nesfatin-1 level in the stomach and duodenum is positively correlated with body mass. To our knowledge, this is the first time to detect the nesfatin-1 level in the duodenum, small intestine and colon with traditional obese and lean subjects induced by nuclei lesions. Additionally, the data of gastric nesfatin-1 expression are an objective supplement to the previous report. Notably, we also show a positive relationship between gastric emptying and body mass. Further, it is possible that the expression level of gastrointestinal nesfatin-1 may be used to diagnose the gastric mobility disorder and metabolism disturbance.

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INTRODUCTION

The energy balance and homeostasis of the body are maintained when the energy intake equals the energy expenditure over an extended period of time with a steady-state plateau of body weight, including the adaptive adjustment of appetitive factors and gastrointestinal mobility. Anabolic and catabolic neuropeptides acting at either cerebral or peripheral sites contribute to the regulation of the energy balance[1-3].

Nesfatin-1, which is an 82-amino-acid peptide derived from nucleobindin-2 (NUCB2), was initially identified as an anorectic hypothalamic neuropeptide by Oh-I and coworkers[4]. As a brain-gut peptide, nesfatin/NUCB2 is expressed in appetite-control hypothalamic nuclei, such as the supraoptic nucleus (SON), the paraventricular nucleus, the arcuate nucleus, the ventrolateral nucleus (VLH) of the hypothalamus, and the nuclei of the solitary tract and zona incerta of rats[5,6]. Additionally, peripheral tissues, such as the oesophagus, gastrointestinal tract, liver, endocrine pancreas, adipose, serum, and testis, exhibit multiple peptidergic correlations[7-10]. The intracerebroventricular administration of nesfatin-1 reduces dark-phase feeding in a dose-dependent manner, whereas the injection of an antibody that neutralises nesfatin-1 stimulates appetite in mice[4]. The chronic administration of nesfatin-1 into the periphery significantly decreases body weight gain in rodents. Thus, it was proposed that the systemic or local administration of nesfatin-1 and its analogues may treat obesity in the clinic[11].

In previous years, many efforts have been made to document the expression of NUCB2/nesfatin-1 in hypothalamic areas with essential roles in food intake control[5,6,12,13]. Notably, central nesfatin-1 can influence gastric mobility and feeding behaviour, and this hypothesis is supported by the inhibition of gastric emptying observed after the central administration of the peptide[7,14]. It is possible that gastric mobility participates in the process underlying imbalances in the energy metabolism correlated with nesfatin-1. The electrolytic lesions of the bilateral ventromedial nuclei and ventrolateral nuclei in the hypothalamus are known to cause obese and lean rats, respectively[15]. Unlike congenitally obese and lean animals, the lesioned rats present an increased and decreased body weight gain, respectively[16-18]. In this study, to assess the mechanisms regulating body weight homeostasis, we hypothesise that nesfatin-1 in the gastrointestinal tract exhibits functional relevance with ventromedial hypothalamic nucleus (VMH)-lesioned polyphagia or obesity and VLH-lesioned anorexia or frailty.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (240-250 g) were purchased from the Qingdao Institute for Drug Control and housed in a temperature-controlled environment with a 12-h light/12-h dark cycle with access to laboratory chow and water *ad libitum*. All of the rats were allowed to adapt to the laboratory for 7-10 d before being used in the experiments. Our study protocol was approved by the Qingdao University Animal Care Committee.

VMH and VLH lesions

For the VMH and VLH surgical procedures, the rats were anaesthetised with 10% chloral hydrate (0.3 mL/100 g *i.p.*) The coordinate was selected from the atlas of Paxinos and Watson. The flat-skull position of the rats was achieved when the incisor bar was lowered 3.3 ± 0.4 mm below the horizontal zero. The stereotaxic coordinates of the VMH were 2.8 mm posterior to the bregma, 0.6 mm lateral to the midline, and 9.6 mm ventral to the top of the skull. For the VLH, the electrode was placed 1.3 mm posterior to the bregma, 0.18 mm lateral to the midline, and 8.9 mm ventral to the skull. Bilateral VMH and VLH were produced by passing an anodal direct current (2 mA for 10 s) through the exposed tips of insulated nickel-cadmium electrodes (0.3 mm at the tip). The control animals received sham VMH lesions (no current was passed through the electrode). After surgery, the animals had free access to food and water, and their diet and weight were monitored. On day 21 post lesion, the brain of each rat was removed, fixed in paraformaldehyde, sectioned, pathologically stained, and examined microscopically to confirm the sites of VMH and VLH. Those animals in which the nuclei were not destroyed on both sides were not analysed.

Measurement of gastric emptying

Gastric emptying was assessed using a modified phenol red-methylcellulose recovery method. The rats were deprived of food but had free access to tap water for 16 h before the experiments. The non-absorbable dilution,

which consisted of continuously stirred 1.5% methylcellulose (wt/vol) with phenol red (50 mg/100 mL), was given intragastrically through a stainless steel feeding tube equipped with a 2-mL syringe to conscious rats. The rats were sacrificed 15 min later through CO_2 inhalation. The abdominal cavity was opened, the gastric cardia and pylorus were clamped, and the stomach was removed, rinsed, and placed into 20 mL of distilled water. The gastric contents were homogenised with 20 mL of 0.5 mol/L NaOH solution through electric agitation for 1 min and allowed to settle for 60 min at room temperature. Then, 5 mL of the supernatant was added to 0.5 mL of 20% trichloroacetic acid (wt/vol), and the mixture was centrifuged for 10 min (3500 rpm). The standard 0% emptying was defined by homogenisation through the above-mentioned extracorporeal operation. All of the samples were analysed by spectrophotometry at 560 nm to determine the absorbance values. Gastric emptying was calculated as percent emptying = (1 - absorbance of test sample/absorbance of standard).

Immunohistochemical and immunofluorescence staining

After the animals were sacrificed, the stomach, duodenum, small intestine, and colon were quickly resected, transferred to 4% paraformaldehyde, and fixed at 4 ℃ for 24 h. After dehydration through an ethanol-xylene series, the specimens were embedded in paraffin. The paraffin sections were then cut, dewaxed, rehydrated through a graded xylene-alcohol series (once in each of the following: 95% ethanol, 70% ethanol, and 50% ethanol; 5 min in each solution; 25 ℃), and subjected to antigen retrieval in citrate buffer (pH 6.0). The endogenous peroxidase was inactivated with 3% hydrogen peroxide in PBS for 10 min. The sections were subsequently incubated with the primary rabbit anti-mouse antibody (1:1000 dilution; H-003-22, Phoenix Pharmaceuticals) for 1 h at room temperature. After washing, the tissue was incubated with a goat anti-rabbit antibody (30 min; PV-6001, Beijing, Zhongshan Jinqiao). The sections were finally coverslipped with neutral gum mounting and observed and photomicrographed under an optical microscope (CX31, Olympus Corp, Tokyo, Japan). In addition, the number of nesfatin-1-positive cells was counted and compared between groups. The percentage of nesfatin-1-positive cells was determined using Image Pro Plus software (Media Cybernetics, United States)

For immunofluorescent double-staining, the sections were prepared as described above, incubated with rabbit polyclonal anti-nucleobindin antibody (ab125260, Abcam Corp.) and goat anti-rabbit antibody (ZF0311, Beijing, Zhongshan Jinqiao), and analysed by fluorescence microscopy.

Real-time RT-PCR analysis of NUCB2 mRNA expression

The gastric, duodenal, small intestinal, and colonic tissues were removed quickly after the animals were sacrificed, immediately immersed into RNA preserving solution, and conserved at -80 ℃. The total RNA was extracted from the samples using the TRIzol® RNA isolation reagent according to the manufacturer's instructions, and the concentration and purity of the mRNA were verified by spectrophotometry and agarose gel electrophoresis, respectively. Only those samples with an absorption ratio (OD 260 nm/OD 280 nm) greater than 1.7 were used for cDNA synthesis. The cDNA was obtained using a reverse transcription kit (Takara Bio Inc.) according to the supplier's manual and directly used as templates for PCR amplification. For the analysis of normalised mRNA expression, β-actin was used as a housekeeping gene. The primer sequences were as follows: β-actin forward, 5'-GGAGAT-TACTGCCCTGGCTCCTA-3' and reverse, 5'-GACT-CATCGTACTCCTGCTTGCTG-3'; NUCB2 forward, 5'-CAGTTTGAA-CACCTGAAC-CACCA-3' and reverse, 5'-TCATGCC-GAGTCCGGT-CATA-3'. The amplification of the genes consisted of 40 cycles of predenaturation at 95 ℃ for 30 s, denaturation at 95 ℃ for 3 s, primer annealing at 60 ℃ for 30 s, and elongation at 72 ℃ for 30 s and was performed on a real-time PCR instrument (RotorGene 3000, Australia).

Statistical analysis

The statistical significance for all of the analyses was determined by one-way ANOVA followed by Scheffe's *F*-test, and data were analysed using an independent *t* test. All of the data in the figures are expressed as mean ± SE of the mean. Differences with a P value less than 0.05 were considered significant.

RESULTS

Effects of VMH and VLH on food intake and body weight

The daily food intake and body weight gain were monitored and calculated on days 3, 7, 14, and 21 after the operation, and the lesions were confirmed by pathological staining (Table 1, Figure 1). The data from those rats with misplaced lesions were discarded. As shown in Table 1, the rats with bilateral VMH lesions consumed more food and gained greater weight than the sham-operated rats, whereas the VLH-lesioned group exhibited the opposite trend. The diversities in the weight tended to be further enlarged with continued growth (Figure 1), and the differences between the matched groups reached statistical significance ($P < 0.01$), which is consistent with previous observations in rodents[16,17]. The total body weight and ingestion observed in the VLH-sham rats (sham-VLH-lesioned rats) were somewhat higher than those observed in the VMH-shams, but this difference was not significant ($P > 0.05$).

Gastric emptying rate of phenol red solution

The amount of phenol red recovered from the stomach was negatively correlated with the gastric empting rate. Compared with the calculated output of gastric emptying for each control animal, the VMH-lesioned rats exhibited increased values (85.94% ± 2.27% *vs* 61.66% ± 2.28%, P

Table 1 Daily food intake and cumulative body weight gain on days 3, 7, 14, and 21 post ventromedial hypothalamic nucleus and ventrolateral hypothalamic nucleus lesioning in rats

	Daily food intake (g)				Body weight gain (g)			
	Day 3	Day 7	Day 14	Day 21	Day 3	Day 7	Day 14	Day 21
VMH	33.46 ± 1.96	35.85 ± 1.88	33.42 ± 1.25	36.21 ± 1.51	35.02 ± 3.46	70.07 ± 3.51	118.72 ± 3.23	184.36 ± 4.28
Sham 1	24.71 ± 1.89[b]	26.39 ± 0.82[b]	27.73 ± 0.95[b]	25.26 ± 1.76[b]	8.14 ± 3.47[b]	31.40 ± 2.48[b]	68.57 ± 2.31[b]	111.02 ± 3.34[b]
VLH	8.50 ± 1.20	16.16 ± 0.83	17.44 ± 1.54	17.25 ± 1.20	0.58 ± 1.08	18.35 ± 1.15	45.05 ± 2.58	66.43 ± 2.34
Sham 2	26.31 ± 0.86[b]	29.06 ± 1.69[b]	29.30 ± 0.51[b]	26.89 ± 1.01[b]	18.57 ± 2.44[d]	37.54 ± 2.48[d]	81.80 ± 2.29[d]	116.72 ± 3.25[d]

The values are mean ± SE (n = 6 rats per group). [b]P < 0.01 *vs* sham-operated rats; [d]P < 0.01 *vs* sham-operated rats. VLH: Ventrolateral nucleus; VMH: Ventromedial hypothalamic nucleus.

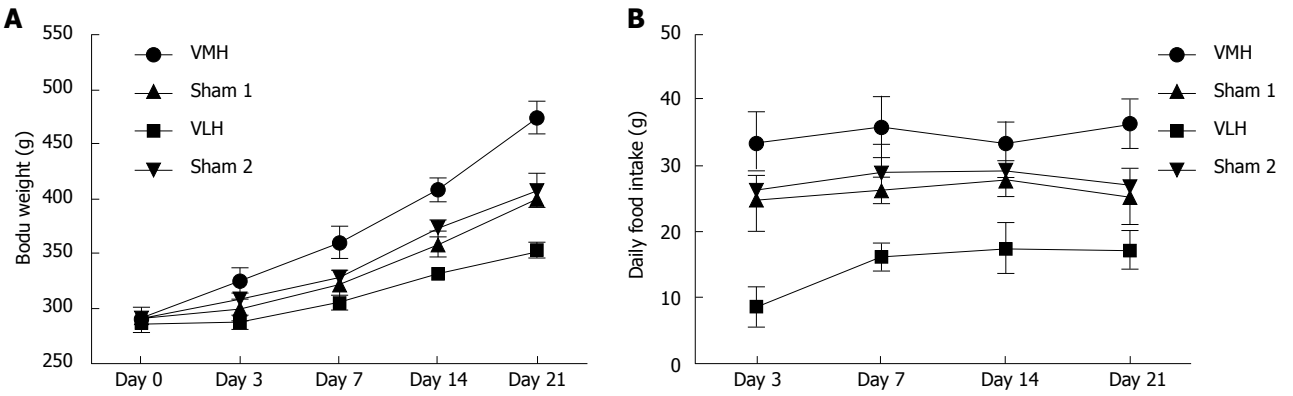

Figure 1 Changes in the body weight and daily food intake between hypothalamic nucleus-lesioned and sham-operated rats. A: Body weight on days 3, 7, 14, and 21 post the operation; B: Daily food intake on days 3, 7, 14, and 21 post the operation. VMH: Ventromedial hypothalamic nucleus; VLH: Ventrolateral hypothalamic nucleus.

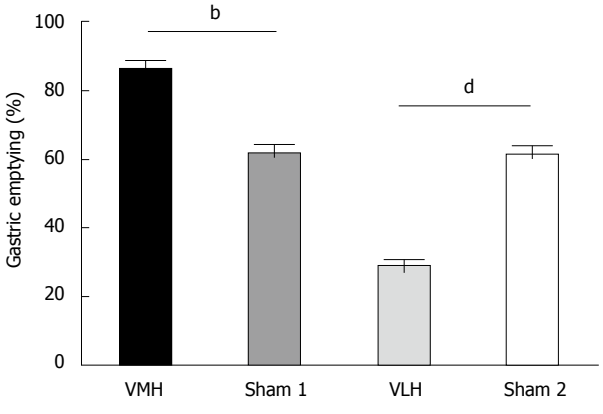

Figure 2 Effects of ventromedial hypothalamic nucleus and ventrolateral hypothalamic nucleus lesions on gastric emptying rate in rats. The values represent mean ± SE (n = 6); [b]P < 0.01 and [d]P < 0.01 *vs* sham-operated rats. VMH: Ventromedial hypothalamic nucleus; VLH: Ventrolateral hypothalamic nucleus.

< 0.01), whereas the VLH-lesioned rats exhibited lower gastric emptying values (29.12% ± 1.62% *vs* 61.08% ± 2.46%, P < 0.01; Figure 2).

Immunohistochemistry and immunofluorescence of NUCB2/Nesfatin-1 in gastrointestinal tissues

The immunolocalisation of the nesfatin-1 peptide in the stomach, duodenum, and small intestine of rats is shown in Figure 3. To reduce the feasibility of any unspecific reaction induced by the goat antibody and to confirm the location of the protein, immunofluorescence (Figure 4) was also examined. Figure 3 shows that NUCB2/nesfatin-1 IR cells were mainly localised in the lower two-thirds of gastric mucosal glands (A-D), Paneth cells of the duodenum (E-H), and the small intestine (I-L) (brown stating). The same localisation was also obtained by immunofluorescence (green fluorescence; Figure 4). In our experiment, the immunostaining of NUCB2/nesfatin-1 immunoreactive cells was undetectable in the colon tissue. In VMH-lesioned rats, the proportion of nesfatin-1-positive cells amounted to 24.74% ± 3.54% per observation field in the stomach (Figure 3A), 17.40% ± 1.52 % in the duodenum (Figure 3E), and 11.01% ± 1.54% in the small intestine (Figure 3I) compared with the stomach (16.57% ± 1.77%; Figure 4B), duodenum (12.24% ± 1.34%; Figure 4F) and small intestine (16.16% ± 1.41%; Figure 4J; P < 0.01) of the control rats. The VLH-lesioned rats showed a decreased percentage of nesfatin-1-positive cells in the stomach (Figure 3C), duodenum (Figure 3G), and small intestine (Figure 3K) than the control group (Figure 3D, H, and L; 8.53% ± 1.59% *vs* 15.79% ± 1.83%, 8.03% ± 0.98% *vs* 12.06% ± 1.74%, and 11.18% ± 1.36% *vs* 15.37% ± 14.17%, respectively; P < 0.01). The immunofluorescence results tend to correspond with the diversities in the nesfatin-1 peptide observed between different groups by immunohistochemistry. To further investigate the differences in NUCB2/nesfatin-1 protein in the nuclei-lesioned rats and the control groups, real-time PCR techniques were used to quantify the NUCB2

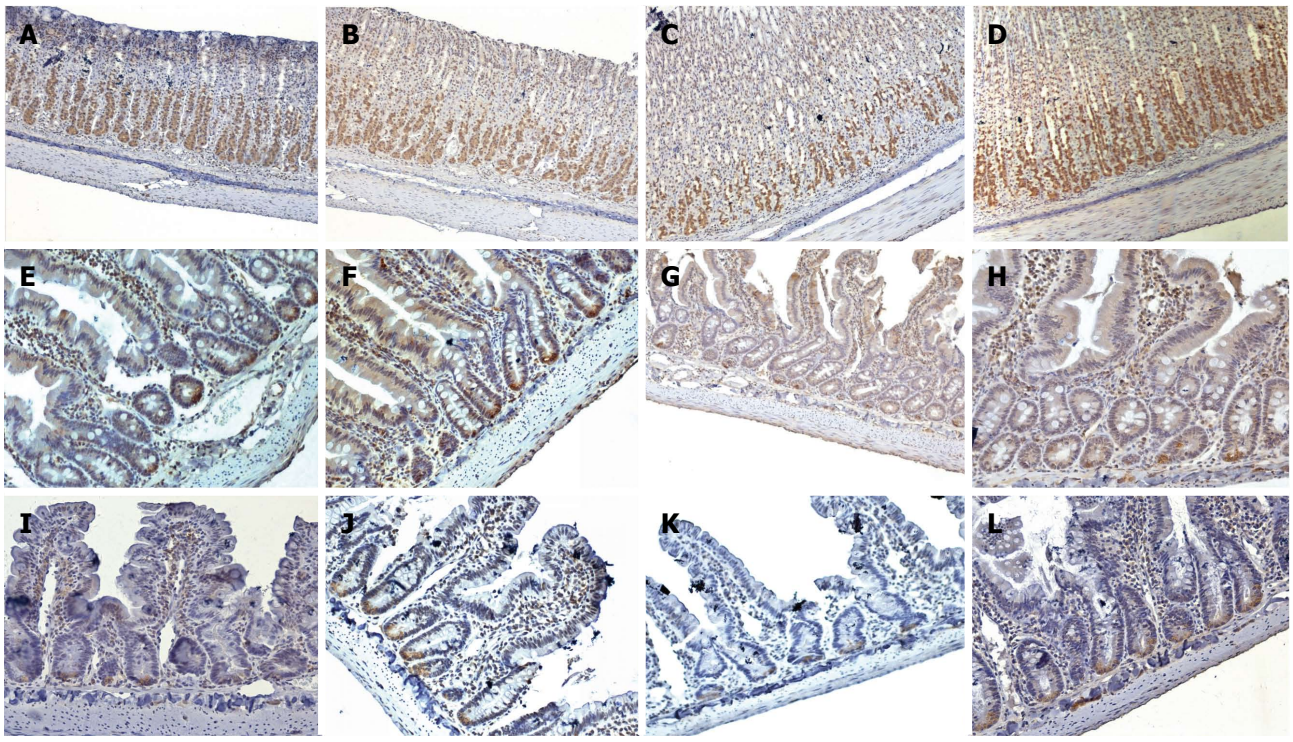

Figure 3 Immunohistochemical localisation of nesfatin-1 in the gastrointestinal tissues. A-D: Nesfatin-1 IR cells (brown) in the stomach of VMH-lesioned, VMH-sham, VLH-lesioned, and VLH-sham rats, respectively; E-H: Nesfatin-1 IR cells (brown) in the duodenum of VMH-lesioned, VMH-sham, VLH-lesioned, and VLH-sham rats, respectively; I-L: Nesfatin-1 IR cells (brown) in the small intestine of VMH-lesioned, VMH-sham, VLH-lesioned, and VLH-sham rats, respectively. All of the magnifications are × 200 with the exception of the stomach, which is × 100. VMH: Ventromedial hypothalamic nucleus; VLH: Ventrolateral hypothalamic nucleus; IR: Immunoreactive.

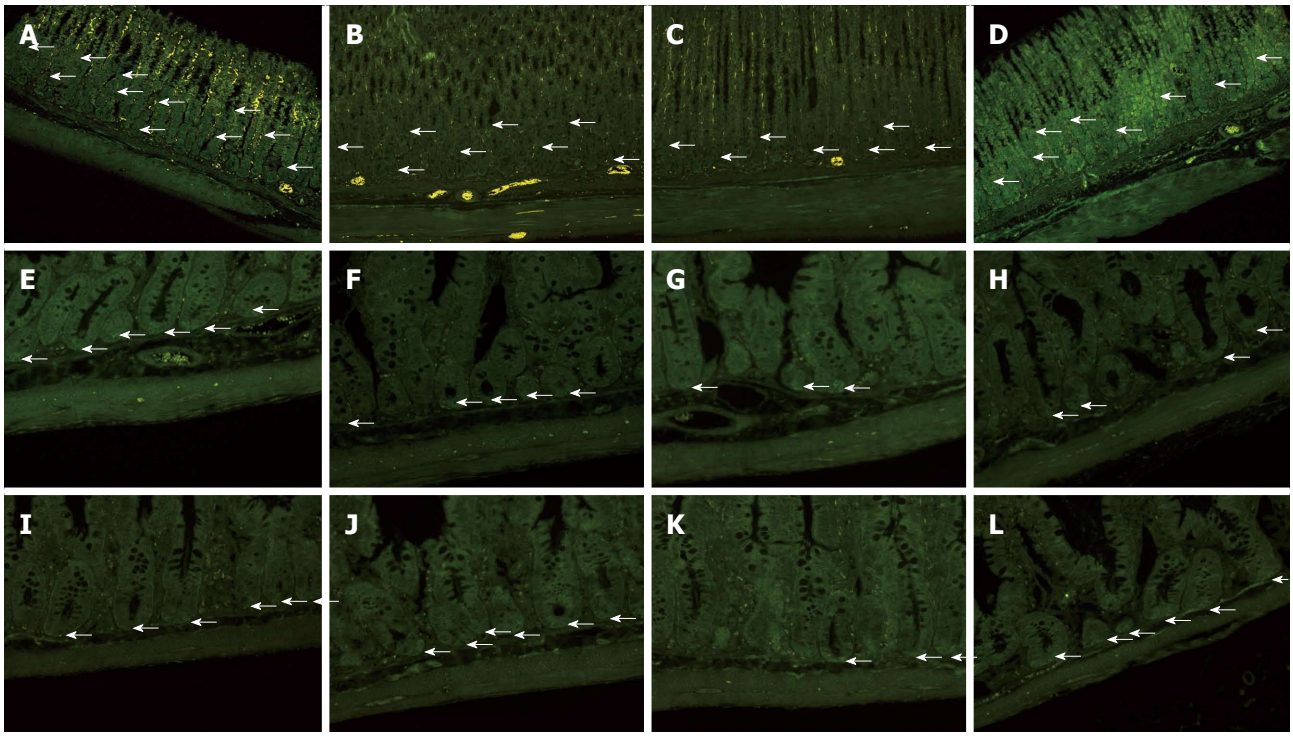

Figure 4 Immunofluorescence localisation of nesfatin-1 in the gastrointestinal tissue. A-D: Nesfatin-1 IR cells (arrow; enhanced green) in the stomach of VMH-lesioned, VMH-sham, VLH-lesioned, and VLH-sham rats, respectively; E-H: Nesfatin-1 IR cells (arrow; enhanced green) in the duodenum of VMH-lesioned, VMH-sham, VLH-lesioned, and VLH-sham rats, respectively; I-L: Nesfatin-1 IR cells (arrow; enhanced green) in the small intestine of VMH-lesioned, VMH-sham, VLH-lesioned, and VLH-sham rats, respectively. All of the magnifications are × 200 with the exception of the stomach, which is × 100. VMH: Ventromedial hypothalamic nucleus; VLH: Ventrolateral hypothalamic nucleus; IR: Immunoreactive.

mRNA levels.

Expression level of NUCB2 mRNA in gastrointestinal tissues

To investigate the physical changes in NUCB2 mRNA expression, the relative expression ratio was determined by real-time PCR analyses[19]. As shown in Figure 5, the expression levels of the target gene in the stomach and duodenum of VMH-lesioned rats were 2.022 and 2.960 higher than those observed in the control animals ($P < 0.05$; Figure 5A and B). In contrast, the levels in the small intestine and colon were 0.476 and 0.268 compared with the controls ($P < 0.05$; Figure 5C and D). In the VLH-lesioned group, the NUCB2 mRNA levels were significantly decreased in the stomach, duodenum, and small intestine (0.271-fold, 0.278-fold, and 0.534-fold, respectively; $P < 0.05$). The NUCB2 concentration in the colon tended to be slightly lower in the operated animals, but these differences did not reach statistical significance ($P > 0.05$).

DISCUSSION

To the best of our knowledge, nesfatin-1 is a potent anorexigen with a reproducible food intake-reducing effect. It has been reported that the number of nesfatin-1 IR cells is positively correlated with BMI in the gastric oxyntic mucosa of obese patients, as shown by immunofluorescence[20]. In addition, Ogiso *et al*[21] reported that plasma nesfatin-1 levels are significantly lower in restricting-type anorexia nervosa patients than in control patients. Therefore, we hypothesised that there is difference in the expression of nesfatin-1 in gastrointestinal tissues between polyphagous/obese and anorectic/lean rats. To confirm this hypothesis, rats pretreated with VMH and VLH lesions, which were induced through a direct current, were compared to the controls. In the present study, we identified the presence of NUCB2 mRNA and nesfatin-1 IR cells in rat gastrointestinal tissues by RT-PCR and immunostaining. It was shown that the NUCB2/nesfatin-1 IR cells detected in the stomach, duodenum, and small intestine are in agreement with previous reports that showed that nesfatin-1/NUCB2 IR cells are localised in the lower third and middle portion of the gastric mucosal gland and in submucous layer of the duodenum in SD rats and ICR mice[7,8,22]. The present study provides the first demonstration that the levels of NUCB2 mRNA and nesfatin-1 protein are increased in the stomach and duodenum of VMH-lesioned rats compared with their sham-operated controls. However, in the VLH-lesioned rats with a lower body weight, the levels of NUCB2 mRNA and nesfatin-1 protein were significantly reduced compared with their controls. Interestingly, our observations showed that the expression of nesfatin-1 in the small intestine is significantly decreased in both VMH- and VLH-lesioned rats. Taken together, these observations indicate that peripheral nesfatin-1 from gastrointestinal tissues is participated to regulate the energy expenditure under obese and lean conditions.

The hypothalamus is the main centre for energy balance, where numerous neuropeptides and transmitters are released to participate in the control of essential body functions. We have confirmed the previous findings that VMH and VLH can be considered "feeding" and "hunger centres"[15-18]. The VMH-lesioned rats exhibited significant increases in daily food intake and body weight compared with the sham-operated rats, whereas these measurements were evidently decreased in the VLH-lesioned group compared with the controls. Therefore, it is likely that the variation in body weight is due to altered ingestion. The data show that the gastric emptying rate is distinctly elevated by 85.94% in the VMH-lesioned group compared with the sham-operated rats (61.66%) and markedly reduced by 29.12% in the VLH-lesioned group compared with the controls (61.08%), and these results are similar to those found in a previous study[23]. Cummings *et al*[24] reported that an increased gastric volume induced by a lower gastric emptying rate can result in suppression of food intake, and this finding is similar to the observations found in our study. It has also been reported that the gastroduodenal motility of mice is inhibited after the intracerebroventricular administration of nesfatin-1[14,25] and that the suppressing effect on gastric emptying is dose-dependent[26]. The underlying mechanisms may involve alterations in the expression of nesfatin-1. Sakaguchi *et al*[27] found reduced sympathetic activity and enhanced vagal activity in VMH-lesioned rats. Activated cholinergic neurons in the gastric submucosal, myenteric plexuses, and subsequent vagal gastric nerve may stimulate the gastric secretory and motor functions[28]. Therefore, it is possible that hypothalamic nucleus lesions and nesfatin-1 exert an influence on gastric mobility by adjusting vagal activity. To confirm the regulatory mechanism of nesfatin-1 in gastric mobility, further neuroendocrine characterisation of the brain-gut axis is essential.

As a "satiety molecule", NUCB2/nesfatin-1 was recently studied in the peripheral organs and not just in the brain. Ramanjaneya *et al*[29] found increased NUCB2/nesfatin-1 expression in adipose tissue in obesity. The analysis of obese patients detected a positive correlation between gastric nesfatin-1 IR cells and BMI[20]. Accordingly, it was recently reported that the plasma nesfatin-1 levels are significantly lower in AN-R (restricting-type anorexia nervosa) patients[21]. A similar tendency of increased expression of NUCB2/nesfatin-1 in both subcutaneous and visceral fat tissues in VMH-lesioned rats was also observed previously[30]. Our study describes a novel relationship between the expression of nesfatin-1 in gastrointestinal tissue and body mass. The analysis of our data from the stomach and duodenum samples led us to speculate that the elevation and decrease of nesfatin-1 levels in obese and lean rats may be a compensatory upregulation and downregulation to compensate for the abnormal metabolism. It is possible that the nesfatin-1-driven positive feedback may play a physiological role in the regulation

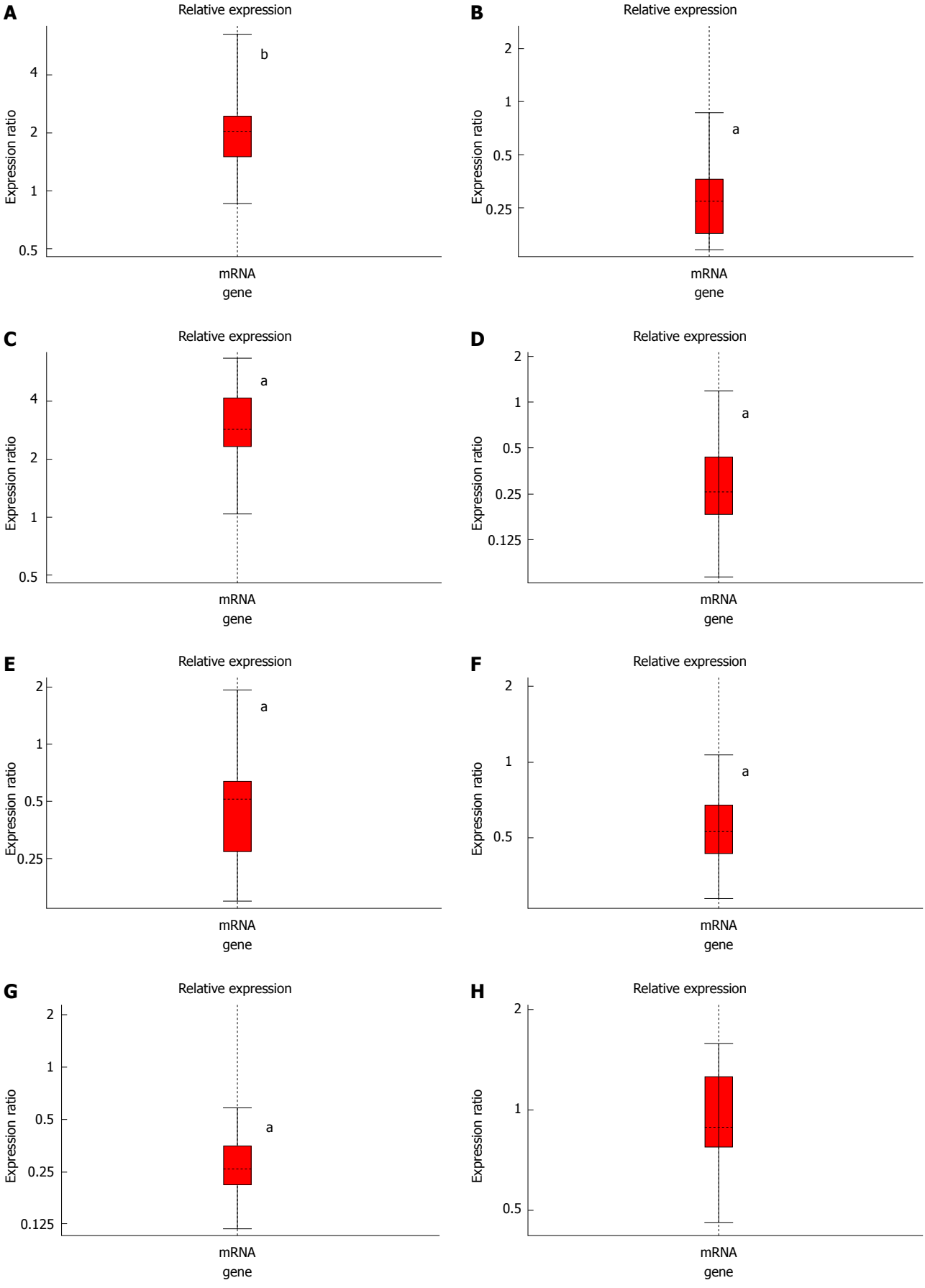

Figure 5 Relative expression levels of nucleobindin-2 mRNA in the stomach (A, B), duodenum (C, D), small intestine (E, F), and colon (G, H) between ventromedial hypothalamic nucleus- or ventrolateral hypothalamic nucleus-lesioned rats and the respective sham-operated rats. Each tissue represents mean ± SE in the model rats and control groups ($n = 6$); $^{b}P < 0.01$, $^{a}P < 0.05$ *vs* sham-operated rats. VMH: Ventromedial hypothalamic nucleus; VLH: Ventrolateral hypothalamic nucleus.

of the metabolic disturbance imposed by obese and lean conditions and the maintenance of homeostasis. However, our study reveals no clear significance of nesfatin-1 in the small intestine and colon. Future studies are needed to determine whether the variations in the nesfatin-1 levels are the cause or consequence of metabolic disorder.

In addition, it should be mentioned that conflicting results have been reported regarding the association between BMI and nesfatin-1 levels. Abaci *et al*[31] found that the serum nesfatin-1 level in obese children is significantly lower compared to that in healthy subjects. Tsuchiya *et al*[32] presented data that are in agreement with a negative correlation between nesfatin-1 and body mass index in non-obese males. It was also reported that the cord blood nesfatin-1 levels are decreased in LGA (large for gestational age) compared to AGA (appropriate for gestational age) foetuses[33]. Based on the study conducted by Stengel *et al*[7], we hypothesised that the nesfatin-1 in the circulation is secreted from gastric and pancreatic tissues. Studies in mice have provided strong evidence that nesfatin-1 traverses the blood-brain barrier bidirectionally in a non-saturable manner[34,35]. In addition, there is a significant linear relationship between the cerebrospinal fluid and plasma nesfatin-1/NUCB-2 levels in both obese and lean subjects[36]. Thus, it can be hypothesised that the nesfatin-1 levels in the cerebrospinal fluid and plasma are negatively correlated with the levels of this protein in the stomach and duodenum. However, further studies on the functional relevance of central and peripheral nesfatin-1 are needed to address this question.

In conclusion, this study provides the first demonstration of the expression of peripheral nesfatin-1 in gastrointestinal tissues in VMH-lesioned/obese rats and VLH-lesioned/lean rats. Notably, the present study demonstrates that the nesfatine-1 levels in the stomach and duodenum are upregulated under conditions of a positive energy balance (obesity) and downregulated under conditions of a negative energy balance (lean). Additionally, there is a positive correlation between gastric emptying and body mass. Therefore, we hypothesised that nesfatin-1 may participate in the regulation of gastric mobility by adjusting the vagal activity. These data are supported by numerous previous observations and provide new insight regarding the physiological relevance of nesfatin-1, gastric mobility, and metabolic disturbances that result in obese and lean conditions. This study also gives rise to numerous unanswered questions on the physiological regulation of nesfatin-1. Further investigation of the biochemical and physiological functions of nesfatin-1 will help improve our understanding of the mechanisms of energy homeostasis.

COMMENTS

Background

Nesfatin-1 was originally identified as a hypothalamic neuropeptide derived from nucleobindin-2 (NUCB2) that exhibits the ability to suppress food intake. The intracerebroventricular administration of nesfatin-1 reduces dark-phase feeding in a dose-dependent manner, whereas the injection of an antibody that neutralises nesfatin-1 stimulates appetite in mice

Research frontiers

In addition to its central anorexigenic activity, the expression patterns of NUCB2/nesfatin-1 in peripheral tissues, such as adipose and serum, were recently reported. However, it is not known whether gastrointestinal nesfatin-1 is correlated with energy balance and gastric mobility.

Innovations and breakthroughs

This study showed that the nesfatine-1 levels in the stomach and duodenum are upregulated under conditions of a positive energy balance (obesity) and downregulated under conditions of a negative energy balance (lean). Notably, there is also a positive correlation between gastric emptying and body mass.

Applications

The identification here about the relevance of gastrointestinal nesfatin-1 and gastric motility in obese or lean subject will help to improve our understanding of the mechanisms of gastric dysfunction and metabolism disturbance. Knowledge of nesfatin-1 may facilitate clinical diagnosis of the gastric mobility disorder and metabolic disturbances.

Peer review

The authors did a complicated experiment to investigate changes of nesfatin-1 in gastrointestinal tissues of ventromedial hypothalamic nucleus (VMH)-lesioned rats and ventrolateral hypothalamic nucleus (VLH) -lesioned rats. A phenomenon that peripheral nefastin-1 may participate in regulation of energy balance in VMH-lesioned and VLH-lesioned rats was observed, but the authors did not express their ideas using simple and legible writing.

REFERENCES

1 **Szekely M**, Petervari E, Balasko M. Thermoregulation, energy balance, regulatory peptides: recent developments. *Front Biosci* (Schol Ed) 2010; **2**: 1009-1046 [PMID: 20515839 DOI: 10.2741/S116]

2 **Woods SC**, Seeley RJ. Adiposity signals and the control of energy homeostasis. *Nutrition* 2000; **16**: 894-902 [PMID: 11054594 DOI: 10.1016/S0899-9007(00)00454-8]

3 **Székely M**, Szelényi Z. Regulation of energy balance by peptides: a review. *Curr Protein Pept Sci* 2005; **6**: 327-353 [PMID: 16101437 DOI: 10.2174/1389203054546343]

4 **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. Identification of nesfatin-1 as a satiety molecule in the hypothalamus. *Nature* 2006; **443**: 709-712 [PMID: 17036007 DOI: 10.1038/nature05162]

5 **Kohno D**, Nakata M, Maejima Y, Shimizu H, Sedbazar U, Yoshida N, Dezaki K, Onaka T, Mori M, Yada T. Nesfatin-1 neurons in paraventricular and supraoptic nuclei of the rat hypothalamus coexpress oxytocin and vasopressin and are activated by refeeding. *Endocrinology* 2008; **149**: 1295-1301 [PMID: 18048495 DOI: 10.1210/en.2007-1276]

6 **Maejima Y**, Sedbazar U, Suyama S, Kohno D, Onaka T, Takano E, Yoshida N, Koike M, Uchiyama Y, Fujiwara K, Yashiro T, Horvath TL, Dietrich MO, Tanaka S, Dezaki K, Oh-I S, Hashimoto K, Shimizu H, Nakata M, Mori M, Yada T. Nesfatin-1-regulated oxytocinergic signaling in the paraventricular nucleus causes anorexia through a leptin-independent melanocortin pathway. *Cell Metab* 2009; **10**: 355-365 [PMID: 19883614 DOI: 10.1016/j.cmet.2009.09.002]

7 **Stengel A**, Goebel M, Yakubov I, Wang L, Witcher D, Coskun T, Taché Y, Sachs G, Lambrecht NW. Identification and characterization of nesfatin-1 immunoreactivity in endocrine cell types of the rat gastric oxyntic mucosa. *Endocrinology* 2009; **150**: 232-238 [PMID: 18818289 DOI: 10.1210/en.2008-0747]

8 **Zhang AQ**, Li XL, Jiang CY, Lin L, Shi RH, Chen JD, Oomura Y. Expression of nesfatin-1/NUCB2 in rodent digestive system. *World J Gastroenterol* 2010; **16**: 1735-1741 [PMID: 20380005 DOI: 10.3748/wjg.v16.i14.1735]

9 **Foo KS**, Brauner H, Ostenson CG, Broberger C. Nucleobindin-2/nesfatin in the endocrine pancreas: distribution

and relationship to glycaemic state. *J Endocrinol* 2010; **204**: 255-263 [PMID: 20032201 DOI: 10.1677/JOE-09-0254]
10 **García-Galiano D**, Pineda R, Ilhan T, Castellano JM, Ruiz-Pino F, Sánchez-Garrido MA, Vazquez MJ, Sangiao-Alvarellos S, Romero-Ruiz A, Pinilla L, Diéguez C, Gaytán F, Tena-Sempere M. Cellular distribution, regulated expression, and functional role of the anorexigenic peptide, NUCB2/nesfatin-1, in the testis. *Endocrinology* 2012; **153**: 1959-1971 [PMID: 22334726 DOI: 10.1210/en.2011-2032]
11 **Shimizu H**, Oh-I S, Okada S, Mori M. Nesfatin-1: an overview and future clinical application. *Endocr J* 2009; **56**: 537-543 [PMID: 19461159 DOI: 10.1507/endocrj.K09E-117]
12 **Brailoiu GC**, Dun SL, Brailoiu E, Inan S, Yang J, Chang JK, Dun NJ. Nesfatin-1: distribution and interaction with a G protein-coupled receptor in the rat brain. *Endocrinology* 2007; **148**: 5088-5094 [PMID: 17627999 DOI: 10.1210/en.2007-0701]
13 **Foo KS**, Brismar H, Broberger C. Distribution and neuropeptide coexistence of nucleobindin-2 mRNA/nesfatin-like immunoreactivity in the rat CNS. *Neuroscience* 2008; **156**: 563-579 [PMID: 18761059 DOI: 10.1016/j.neuroscience.2008.07.054]
14 **Atsuchi K**, Asakawa A, Ushikai M, Ataka K, Tsai M, Koyama K, Sato Y, Kato I, Fujimiya M, Inui A. Centrally administered nesfatin-1 inhibits feeding behaviour and gastroduodenal motility in mice. *Neuroreport* 2010; **21**: 1008-1011 [PMID: 20827224 DOI: 10.1097/WNR.0b013e32833f7b96]
15 **Anand BK**, Brobeck JR. Hypothalamic control of food intake in rats and cats. *Yale J Biol Med* 1951; **24**: 123-140 [PMID: 14901884]
16 **Bray GA**. Hypothalamic and genetic obesity: an appraisal of the autonomic hypothesis and the endocrine hypothesis. *Int J Obes* 1984; **8** Suppl 1: 119-137 [PMID: 6398803]
17 **Penicaud L**, Larue-Achagiotis C, Le Magnen J. Endocrine basis for weight gain after fasting or VMH lesion in rats. *Am J Physiol* 1983; **245**: E246-E252 [PMID: 6351633]
18 **Zenina OY**, Kromin AA. Effects of electrical stimulation of the hunger center in the lateral hypothalamus and food reinforcement on impulse activity of the stomach in rabbits under conditions of hunger and satiation. *Bull Exp Biol Med* 2012; **153**: 808-815 [PMID: 23113291 DOI: 10.1007/s10517-012-1832-3]
19 **Pfaffl MW**. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001; **29**: e45 [PMID: 11328886 DOI: 10.1093/nar/29.9.e45]
20 **Stengel A**, Hofmann T, Goebel-Stengel M, Lembke V, Ahnis A, Elbelt U, Lambrecht NW, Ordemann J, Klapp BF, Kobelt P. Ghrelin and NUCB2/nesfatin-1 are expressed in the same gastric cell and differentially correlated with body mass index in obese subjects. *Histochem Cell Biol* 2013; **139**: 909-918 [PMID: 23515787 DOI: 10.1007/s00418-013-1087-8]
21 **Ogiso K**, Asakawa A, Amitani H, Nakahara T, Ushikai M, Haruta I, Koyama K, Amitani M, Harada T, Yasuhara D, Inui A. Plasma nesfatin-1 concentrations in restricting-type anorexia nervosa. *Peptides* 2011; **32**: 150-153 [PMID: 20937336 DOI: 10.1016/j.peptides.2010.10.004]
22 **Mohan H**, Unniappan S. Ontogenic pattern of nucleobindin-2/nesfatin-1 expression in the gastroenteropancreatic tissues and serum of Sprague Dawley rats. *Regul Pept* 2012; **175**: 61-69 [PMID: 22387700 DOI: 10.1016/j.regpep.2012.02.006]
23 **Duggan JP**, Booth DA. Obesity, overeating, and rapid gastric emptying in rats with ventromedial hypothalamic lesions. *Science* 1986; **231**: 609-611 [PMID: 3511527 DOI: 10.1126/science.3511527]
24 **Cummings DE**, Overduin J. Gastrointestinal regulation of food intake. *J Clin Invest* 2007; **117**: 13-23 [PMID: 17200702 DOI: 10.1172/JCI30227]
25 **Goebel-Stengel M**, Wang L, Stengel A, Taché Y. Localization of nesfatin-1 neurons in the mouse brain and functional implication. *Brain Res* 2011; **1396**: 20-34 [PMID: 21555116 DOI: 10.1016/j.brainres.2011.04.031]
26 **Stengel A**, Goebel M, Wang L, Rivier J, Kobelt P, Mönnikes H, Lambrecht NW, Taché Y. Central nesfatin-1 reduces dark-phase food intake and gastric emptying in rats: differential role of corticotropin-releasing factor2 receptor. *Endocrinology* 2009; **150**: 4911-4919 [PMID: 19797401 DOI: 10.1210/en.2009-0578]
27 **Sakaguchi T**, Bray GA, Eddlestone G. Sympathetic activity following paraventricular or ventromedial hypothalamic lesions in rats. *Brain Res Bull* 1988; **20**: 461-465 [PMID: 3293706 DOI: 10.1016/0361-9230(88)90135-9]
28 **Taché Y**, Yang H, Miampamba M, Martinez V, Yuan PQ. Role of brainstem TRH/TRH-R1 receptors in the vagal gastric cholinergic response to various stimuli including sham-feeding. *Auton Neurosci* 2006; **125**: 42-52 [PMID: 16520096 DOI: 10.1016/j.autneu.2006.01.014]
29 **Ramanjaneya M**, Chen J, Brown JE, Tripathi G, Hallschmid M, Patel S, Kern W, Hillhouse EW, Lehnert H, Tan BK, Randeva HS. Identification of nesfatin-1 in human and murine adipose tissue: a novel depot-specific adipokine with increased levels in obesity. *Endocrinology* 2010; **151**: 3169-3180 [PMID: 20427481 DOI: 10.1210/en.2009-1358]
30 **Osaki A**, Shimizu H, Ishizuka N, Suzuki Y, Mori M, Inoue S. Enhanced expression of nesfatin/nucleobindin-2 in white adipose tissue of ventromedial hypothalamus-lesioned rats. *Neurosci Lett* 2012; **521**: 46-51 [PMID: 22641054 DOI: 10.1016/j.neulet.2012.05.056]
31 **Abaci A**, Catli G, Anik A, Kume T, Bober E. The relation of serum nesfatin-1 level with metabolic and clinical parameters in obese and healthy children. *Pediatr Diabetes* 2013; **14**: 189-195 [PMID: 23346951 DOI: 10.1111/pedi.12009]
32 **Tsuchiya T**, Shimizu H, Yamada M, Osaki A, Oh-I S, Ariyama Y, Takahashi H, Okada S, Hashimoto K, Satoh T, Kojima M, Mori M. Fasting concentrations of nesfatin-1 are negatively correlated with body mass index in non-obese males. *Clin Endocrinol* (Oxf) 2010; **73**: 484-490 [PMID: 20550530 DOI: 10.1111/j.1365-2265.2010.03835.x]
33 **Boutsikou T**, Briana DD, Boutsikou M, Kafalidis G, Piatopoulou D, Baka S, Hassiakos D, Gourgiotis D, Malamitsi-Puchner A. Cord blood nesfatin-1 in large for gestational age pregnancies. *Cytokine* 2013; **61**: 591-594 [PMID: 23178145 DOI: 10.1016/j.cyto.2012.10.029]
34 **Pan W**, Hsuchou H, Kastin AJ. Nesfatin-1 crosses the blood-brain barrier without saturation. *Peptides* 2007; **28**: 2223-2228 [PMID: 17950952 DOI: 10.1016/j.peptides.2007.09.005]
35 **Price TO**, Samson WK, Niehoff ML, Banks WA. Permeability of the blood-brain barrier to a novel satiety molecule nesfatin-1. *Peptides* 2007; **28**: 2372-2381 [PMID: 18006117 DOI: 10.1016/j.peptides.2007.10.008]
36 **Tan BK**, Hallschmid M, Kern W, Lehnert H, Randeva HS. 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* 2011; **96**: E669-E673 [PMID: 21252251 DOI: 10.1210/jc.2010-1782]

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