# Arcuate nucleus neurons are not essential for the preprandial peak in plasma ghrelin after neonatal monosodium glutamate treatment

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**Abstract.** The aim of the present study was to investigate whether the arcuate nucleus (ARC) was destroyed in mice treated neonatally with monosodium glutamate (MSG), and whether the ARC lesions affect the ghrelin level in the plasma and lipid mobilization in MSG-treated mice. The results revealed that MSG led to a marked reduction in ARC cresyl violet staining, tyrosine hydroxylase-immunoreactive (IR) neurons and neuropeptide Y-IR fibers, compared with saline controls. MSG-treated mice exhibited significantly increased body mass compared with saline controls, and MSG treatment did not prevent food deprivation-induced decrease in white adipose tissue mass compared with controls. Plasma ghrelin levels were significantly increased in MSG-treated mice that were fasted for 48 h, compared with the levels prior to fasting and re-feeding, and the preprandial peak of plasma ghrelin persisted in MSG-treated mice. In summary, the ARC was not found to be essential for food deprivation-induced lipid mobilization and preprandial peak in MSG-treated mice. However, this finding does not mean that ARC neurons do not contribute to food sensing and lipid mobilization under normal conditions, as compensatory mechanisms may have emerged after the ablation of ARC neurons.

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*Key words:* monosodium glutamate, ghrelin, arcuate nucleus, preprandial peak, lipid mobilization

## Introduction

Ghrelin was first identified in 1999 by Kojima *et al* as the endogenous ligand of the long-known growth hormone secretagogue receptor 1a (GHS-R1a) isoform (1). Ghrelin-positive X/A-like cells distributed throughout the gastric oxyntic mucosa (2,3) are the main source of circulating ghrelin (4), as demonstrated by the sharp decline in ghrelin levels following gastrectomy (5). Ghrelin has been also been detected in the central nervous system in the arcuate nucleus (ARC) of the hypothalamus (6), as well as in neurons adjacent to the third ventricle (7). The ARC is strongly implicated in the regulation of food intake. Ghrelin-containing neurons in the ARC send projections to neuropeptide Y (NPY) and agouti-related peptide (AgRP)-positive neurons (8). NPY and AgRP are orexigenic neuropeptides and are regulated by ghrelin (9). The peripheral injection of ghrelin was found to selectively activate NPY-containing neurons in the ARC in mice (10). Similarly, the intracerebroventricular (ICV) administration of ghrelin activates NPY/AgRP-expressing neurons and stimulates the expression of NPY and AgRP mRNA in the ARC (11). Total ghrelin levels are inversely correlated with the body mass index, as they increase in anorexic and cachectic patients and decrease under conditions of obesity (12). In humans and other mammals, ghrelin levels increase before meals, and decline rapidly postprandially (13,14). The postprandial suppression of plasma ghrelin has been considerably more extensively investigated compared with the preprandial peak. Although the physiological importance of this event remains unclear, the suppression of this orexigenic hormone may play a role in the satiating effect of ingested nutrients (15). Furthermore, the brain mechanism underlying the preprandial peak of plasma ghrelin remains unknown. ARC neurons are destroyed by neonatal administration of monosodium glutamate (MSG). The destruction of the ARC by neonatal administration of MSG leads to a significant decrease in the number of ARC neurons (16). This is an effect attributed to the underdeveloped blood-brain barrier (BBB) in this area, allowing MSG to penetrate the brain (16,17). Other areas with a weak BBB were destroyed by neonatal MSG administration, including the area postrema (AP) (16,18). One of the most notable effects of neonatal MSG treatment is obesity in

adult mice (16) and rats (19). The aim of the present study was to test whether ARC lesions affect the ghrelin level in the plasma and stomach in MSG-treated mice.

## Materials and methods

*Animals*. All animal experiments (total number of animals, 58) were conducted in accordance with the guidelines for animal care of Qingdao University. A total of 33 neonatal Kunming mice (obtained from the Laboratory Animal Center of Shandong University of Traditional Chinese Medicine; license: SCXK Lu 20050015) were subcutaneously injected into the dorsal dermis area, just below the interscapular region on days 1, 3, 5, 7 and 9 after birth with 10 $\mu$l MSG (Sigma-Aldrich; Merck KGaA, St. Louis, MO, USA) to deliver 4 mg/g (body mass), or with equivalent volumes of 0.9% saline. A 24.2% death rate occurred in MSG-treated pups, while there were no deaths among the 25 pups injected with saline. However, 6 animals (3 treated with MSG and 3 with saline) were excluded from the analysis, as their body mass index was low and >2 standard deviations from the mean. Finally, 22 animals were treated with MSG (10 male and 12 female) and 22 with saline (10 male and 12 female). At 4 weeks of age, the pups were weaned, bred and housed in groups according to treatment. The mice were housed in air-conditioned animal quarters, with lights on from 8:00 a.m. to 9:00 p.m., and were provided with food and water *ad libitum*.

*Food deprivation, serum and tissue harvesting*. Food intake was measured weekly after weaning. At 12 weeks of age, food was discontinued; in addition, the beddings were removed and replaced with new beddings. In order to measure the response to fasting, mice (n=8 per group) were provided with water (but no food) for 48 h. After fasting, food was provided. The body mass index was measured before and after food deprivation. Before fasting, at 48 h of fasting and after re-feeding, the animals were lightly anesthetized with diethyl ether and an orbital blood sample was collected for the measurement of serum ghrelin. Blood was collected into EDTA tubes containing 500 KIU of aprotinin, centrifuged at 4˚C for 15 min at 1,500 x g, and the separated serum was stored at -80˚C until use. All samples obtained from each subject were run in duplicate in the same assay. A commercially available mouse ghrelin EIA kit (Phoenix Pharmaceuticals, Belmont, CA, USA) was used. The sensitivity of the assay was 0.07 ng/ml. The intra- and inter-assay error was 5-10% and <15%, respectively. The remaining animals were divided into the before fasting (MSG, n=7; saline, n=7) and fasting for 48 h (MSG, n=7; saline, n=7) groups. The mice were deeply anesthetized with a lethal dose of pentobarbital (30 mg/kg, i.p.) before fasting or after fasting for 48 h. Then, the stomachs and brains were quickly removed and rinsed with double-distilled water. The stomach was deep-frozen in liquid nitrogen for reverse transcription (RT)-polymerase chain reaction or western blot analysis. The bilateral inguinal white adipose tissue (IWAT), bilateral retroperitoneal WAT (RWAT), bilateral gonadal WAT (GWAT) and interscapular brown adipose tissue depots were quickly removed and weighed.

*Perfusions and immunohistochemistry*. The animals were perfused with 25 ml isotonic saline, followed by 25 ml 4% paraformaldehyde in 0.01 M phosphate-buffered saline (PBS) solution (pH 7.2). The brains were quickly dissected out and stored in 4% paraformaldehyde, then transferred to 20% sucrose (0.1% sodium azide) for 24 h and 30% sucrose (0.1% sodium azide) for 48 h. The brains were embedded in optimal cutting temperature compound (Sakura Finetek USA, Inc., Torrance, CA, USA) and sectioned in the coronal plane on a freezing microtome (Kryostat 1720; Leica, Mannheim, Germany) at a thickness of 20 $\mu$m. Brain sections were stored in an ultra-cold freezer at -40˚C for immunohistochemistry staining.

For immunohistochemistry staining, the sections were first rinsed with distilled water and immersed in PBS for 5 min. Next, the sections were treated with a solution containing 3% $H_2O_2$ (V/V) and 0.5% Triton X-100 in 0.01 M PBS for 30 min to block endogenous peroxidase activity. Subsequently, the sections were treated with PBS containing 10% normal goat serum for 30 min to prevent non-specific binding of secondary antibodies, followed by incubation overnight at 4˚C with primary antibodies: Rabbit anti-NPY (dilution, 1:6,000; N9528), and mouse anti-tyrosine hydroxylase (TH; dilution, 1:4,000; T1299) (both from Sigma-Aldrich; Merck KGaA). The sections were next rinsed with PBS three times and incubated for 30 min at room temperature with biotin-conjugated secondary antibodies: Goat anti-mouse (TH; dilution, 1:500; sc-2039) and goat anti-rabbit (NPY; dilution, 1:500; sc-2040) (both from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Subsequently, the sections were rinsed with PBS three times and immersed in a horseradish peroxidase-conjugated streptavidin complex for 30 min at room temperature, then rinsed again with PBS three times. The immunoreaction was visualized by DAB staining (DAB substrate kit; Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) for 5 min and observed under an Olympus BX50 microscope (Olympus Corporation, Tokyo, Japan). The reaction was terminated by rinsing the sections with distilled water. The sections were covered by neutral balata after counterstaining and dehydration. Negative controls were determined by omission of the primary antibody. Three representative sections of ARC or AP were selected from each mouse. In each section, an area within the ARC or AP was selected for counting TH- or NPY-positive neurons and optical density analysis. The amount and mean optical density of TH- or NPY-IR fiber staining, as well as Nissl staining, were obtained using an Olympus BX50 microscope (Olympus Corporation); these were analyzed using image analysis software (Compix, Inc., Arizona, USA).

*RT of extracted tissue RNA*. Total RNA was extracted from the stomach tissues (~200 mg) of each mouse using TRIzol reagent, according to the manufacturer's instructions. RT was performed using the AMV Reverse Transcriptase system (Promega Corp., Madison, WI, USA). The ghrelin cDNA fragment (108 bp) was amplified with the following primers: Forward, 5'-TCAGGA GCTCAGTATCAGCAGCA-3' and reverse, 5'-GCCTGTCCG TGGTTACTTGTCA-3'; β-actin (171 bp) was amplified with the following primers: Forward, 5'-CATCCGTAAAGACCT CTATGCCAAC-3' and reverse, 5'-ATGGAGCCACCGATC CACA-3'. The DNA was immediately amplified with a single cycle at 94˚C for 5 min, followed by 30 cycles at 94˚C for 30 sec, 58˚C for 30 sec and 72˚C for 30 sec; and a final extension

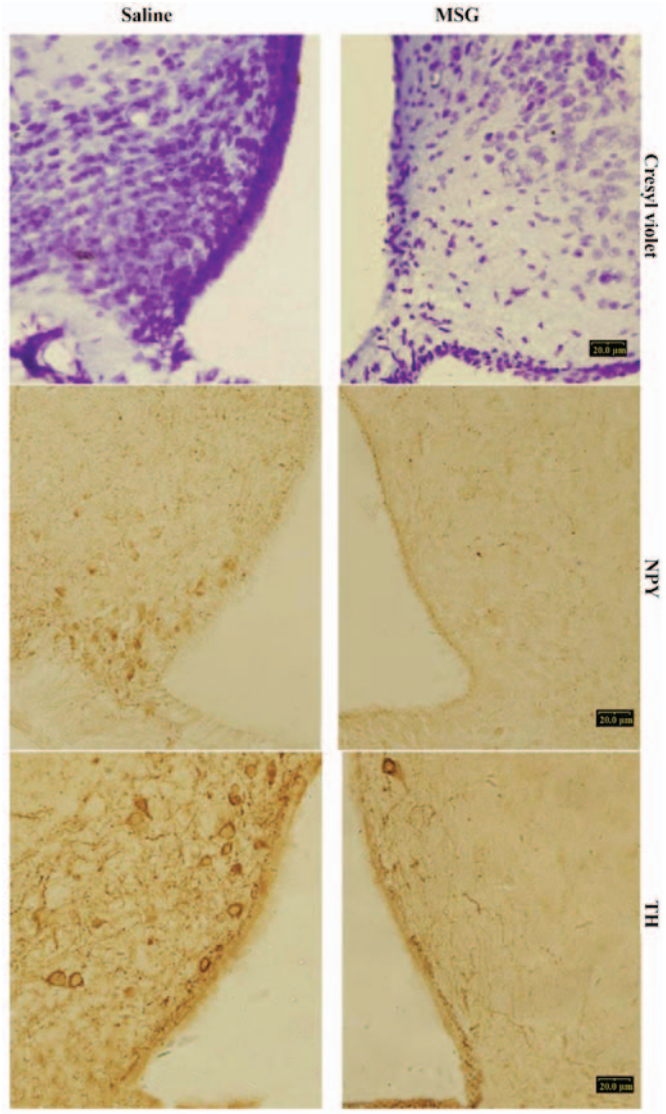


Figure 1. Photomicrographs of representative immunohistochemistry staining of the ARC in MSG (right)- and saline (left)-treated mice. There was a significant reduction in all histological measures, namely cresyl echt violet, NPY and TH (P<0.01). Bar, 20 $\mu$m. MSG, monosodium glutamate; ARC, arcuate nucleus; TH, tyrosine hydroxylase; NPY, neuropeptide Y.

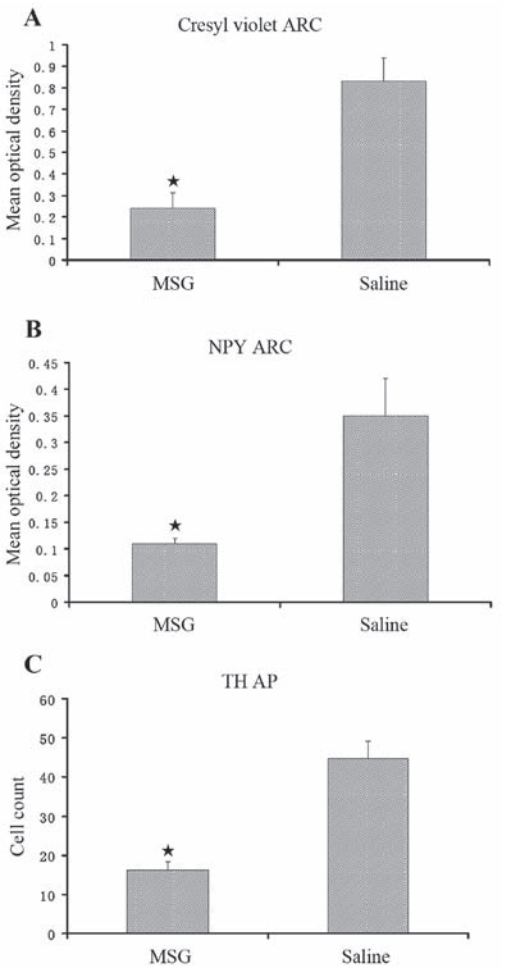


Figure 2. Relative optical density and the cell counts in ARC of MSG- and saline-treated mice. (A) Relative optical density of Nissl staining (cresyl violet) of the ARC in neonatally MSG- vs. saline-treated mice in adult life. Values are presented as means ± SEM. (B) Relative optical density of NPY-immunoreactive (IR) fibers of the ARC in neonatally MSG- vs. saline-treated mice in adult life. Values are presented as means ± SEM. (C) Counts of TH-IR cells of the ARC in neonatally MSG- vs. saline-treated mice in adult life. Values are presented as means ± SEM. *P<0.01, MSG vs. saline. NPY, neuropeptide Y; TH, tyrosine hydroxylase; ARC, arcuate nucleus; AP, area postrema; MSG, monosodium glutamate; SEM, standard error of mean.

step was performed at 72˚C for 10 min. Ethidium bromide stained gels were scanned and qualified using Tanon Image Software (Tanon 1600R; Tanon, Shanghai, China). Ghrelin mRNA levels were expressed as ratios to β-actin mRNA.

*Western blot analysis*. Western blot analysis was performed to detect the expression of proghrelin peptide in mouse gastric tissues. Tissue protein was extracted in lysis buffer (50 mmol/l Tris-HCl, 150 mmol/l NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 1 mmol/l EDTA and 1 mmol/l PMSF) with protease inhibitors (1 mg/ml pepstatin, 1 mg/ml aprotinin and 1 mg/ml leupeptin). Protein concentration was determined using the Bradford assay kit (Bio-Rad Laboratories, Hercules, CA, USA). Protein (20 $\mu$g) was boiled for 10 min in 4X loading buffer (250 mM Tris-Cl [pH 6.8], 2% sodium dodecyl sulfate, 10% glycerol, 20 mM dithiothreitol and 0.01% bromophenol blue), electrophoresed on 10% SDS-PAGE gels, and transferred by electroblotting onto nitrocellulose membranes. Following overnight blocking with 4% non-fat milk at 4˚C, the membranes were incubated with rabbit anti-ghrelin antibody (1:5,000; Phoenix Pharmaceuticals) and rabbit anti-β-actin (1:1,000; bs-0061R; BIOSS, Beijing, China) for 2 h at room temperature. After washing in Tris-buffered saline/Tween-20, the membranes were incubated with an anti-rabbit secondary antibody conjugated to horseradish peroxidase (1:10,000; sc-2040; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. Cross-reactivity was visualized using ECL western blot analysis detection reagents, and analyzed through scanning densitometry by the Tanon Image system. Proghrelin levels were expressed as ratios to β-actin.

*Statistical analysis*. Values are expressed as mean ± standard error of mean. Data were analyzed using two-way analysis of variance (ANOVA) [2x2: treatment (MSG/saline) x food deprivation/non-food deprivation group] with Bonferroni post hoc tests, when appropriate. Food intake was analyzed by using repeated measures ANOVA (MSG/saline) with

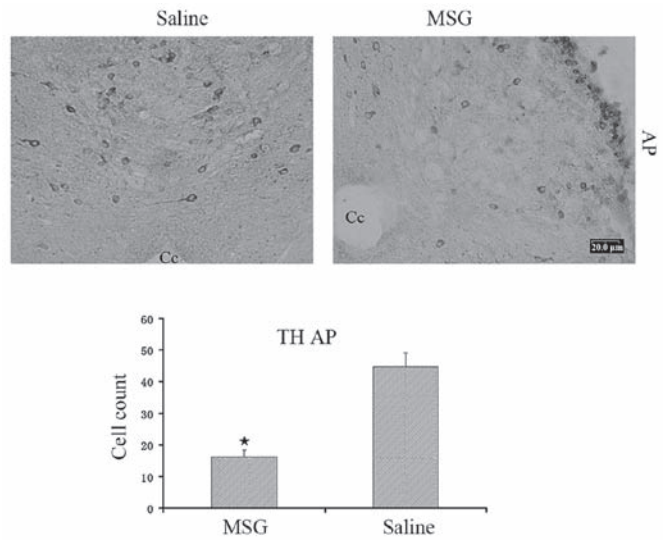


Figure 3. Photomicrographs and cell counts of representative IHC of TH cells in the AP of MSG- and saline-treated mice. TH-positive cells were significantly decreased in MSG- vs. saline-treated mice (*P<0.01). Bar, 20 $\mu$m. AP, area postrema; TH, tyrosine hydroxylase; IHC, immunohistochemistry; MSG, monosodium glutamate. Cc, central canal.

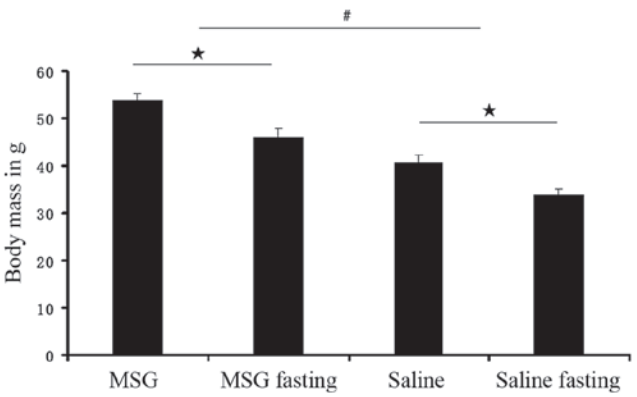


Figure 4. Body mass of MSG- and saline-treated mice. Values represent means ± standard error of the mean. #P<0.01, MSG vs. saline. *P<0.05 food deprivation effect within each group. MSG, monosodium glutamate.

Bonferroni post hoc tests. The remaining data were all analyzed by one-way ANOVA. P<0.05 was considered to indicate statistically significant differences. Statistical analyses were performed using SPSS for Windows, version 16.0 (SPSS, Inc., Chicago, IL, USA).

## Results

*Effects of MSG on ARC and AP neuroanatomy*. Compared with saline controls, MSG-treated mice exhibited significantly decreased cresyl violet (Nissl) staining (Fig. 1), NPY-IR cells and fibers (Figs. 1, 2A and B), and TH-positive cells (Figs. 1 and 2C) in the ARC (P<0.01). Furthermore, TH-positive cells were significantly reduced in the AP of MSG-treated mice (P<0.01, Fig. 3).

*Body and WAT pad masses, and food intake*. Body mass index was significantly increased in MSG-treated 3-month-old mice (P<0.01) compared with saline controls (Fig. 4), but the difference in food intake from 1- to 3-month-old mice between the two groups was not statistically significant (Fig. 5).

Compared with saline controls, the WAT pad mass was significantly increased in MSG-treated mice for all depots assayed (IWAT, RWAT, and GWAT; P<0.01; Fig. 7), as well as the total

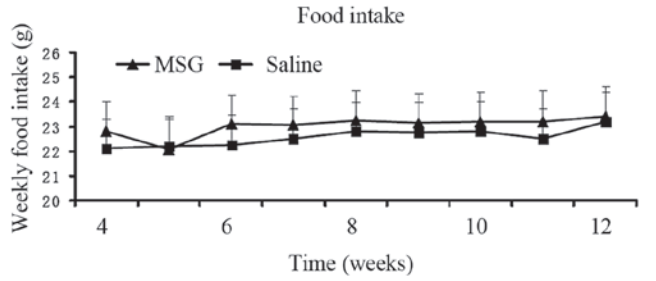


Figure 5. Weekly food intake of MSG- and saline-treated mice after weaning prior to food deprivation. Values represent means ± standard error of the mean. There was no significant difference in food intake from 1 to 3 months of age between the two groups. MSG, monosodium glutamate.

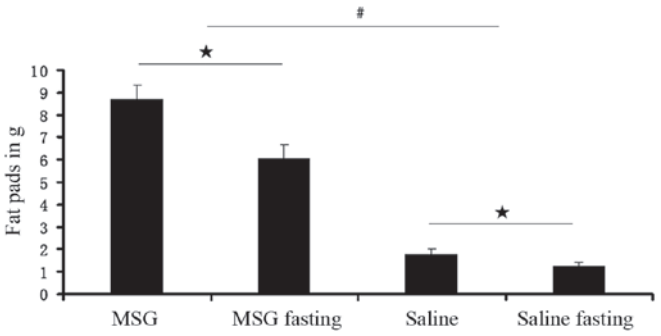


Figure 6. Total dissectible WAT mass of MSG- and saline-treated mice. Values represent means ± standard error of the mean. Fat mobilization occurred in both MSG- and saline-treated mice during fasting. #P<0.01, MSG vs. saline. *P<0.05, food deprivation effect within groups. WAT, white adipose tissue; MSG, monosodium glutamate.

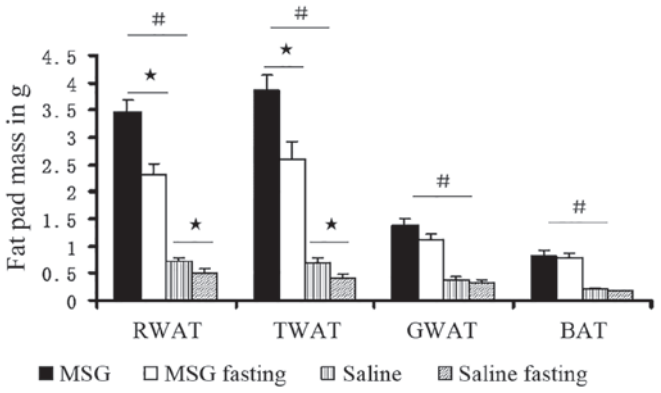


Figure 7. Fat pad mass of individual WAT in MSG- and saline-treated mice. Values represent means ± standard error of the mean. All WAT masses were significantly increased in MSG- vs. saline-treated mice. Only the bilateral inguinal WAT (IWAT) and bilateral retroperitoneal WAT (RWAT) masses were decreased by fasting, and this was observed in both MSG- and saline-treated mice. #P<0.01, MSG vs. saline; treatment effect. *P<0.05, food deprivation effect within groups. WAT, white adipose tissue; GWAT, gonadal WAT; BAT, interscapular brown adipose tissue; MSG, monosodium glutamate.

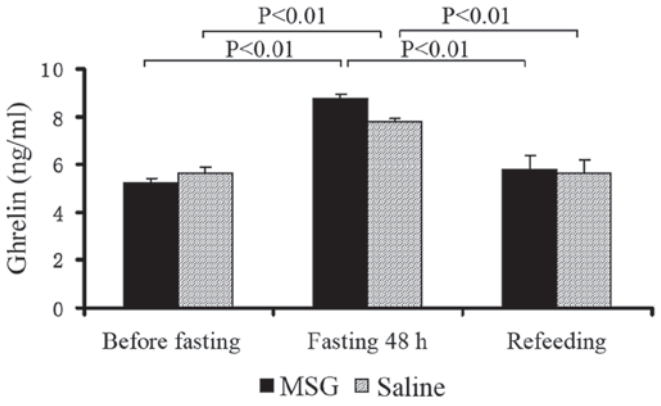


Figure 8. Plasma ghrelin concentrations of MSG- and saline-treated mice. Values represent means ± standard error of the mean. Plasma ghrelin levels were significantly increased in MSG- and saline-treated mice fasted for 48 h compared with the levels before fasting and refeeding (P<0.01), but there was no statistically significant difference between MSG-treated and saline-treated mice before fasting, after fasting for 48 h and refeeding. MSG, monosodium glutamate.

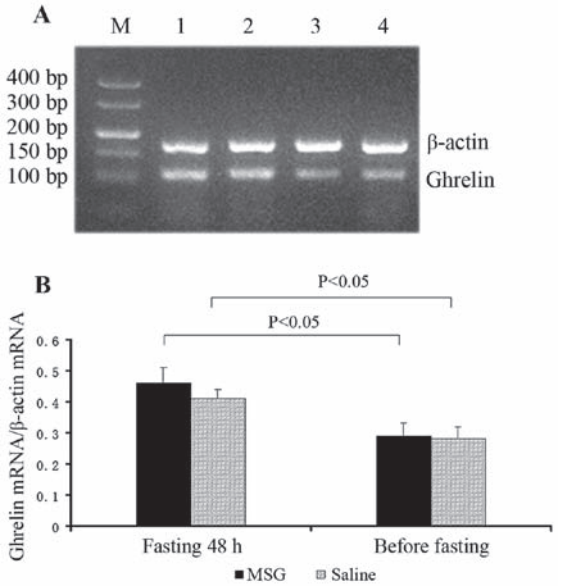


Figure 9. (A and B) Ghrelin mRNA expression in the stomach of MSG- and saline-treated mice. Values represent means ± standard error of the mean. The ghrelin mRNA expression in the stomach was significantly increased in MSG- and saline-treated mice fasted for 48 h compared with their respective counterparts before fasting (P<0.05), but there were no significant difference of ghrelin mRNA expression in the stomach between MSG-and saline-treated mice before and after fasting 48 h. M: maker; 1 and 2: fasting 48 h; 3 and 4: before fasting. 1 and 3-MSG, 2 and 4-saline. MSG, monosodium glutamate.

dissected WAT (P<0.01, Fig. 6). Both MSG- and saline-treated animals exhibited significant food deprivation-induced decreases in the three depots (IWAT, RWAT and GWAT; P<0.05), compared with their respective *ad libitum* fed counterparts (Fig. 7). As all depot assayed masses (IWAT, RWAT and GWAT) decreased (Figs. 6 and 7), neonatal MSG administration did not prevent food deprivation-induced lipid mobilization.

*Plasma ghrelin concentrations*. Plasma ghrelin levels were significantly increased in MSG- and saline-treated mice that were fasted for 48 h, compared with the levels before fasting

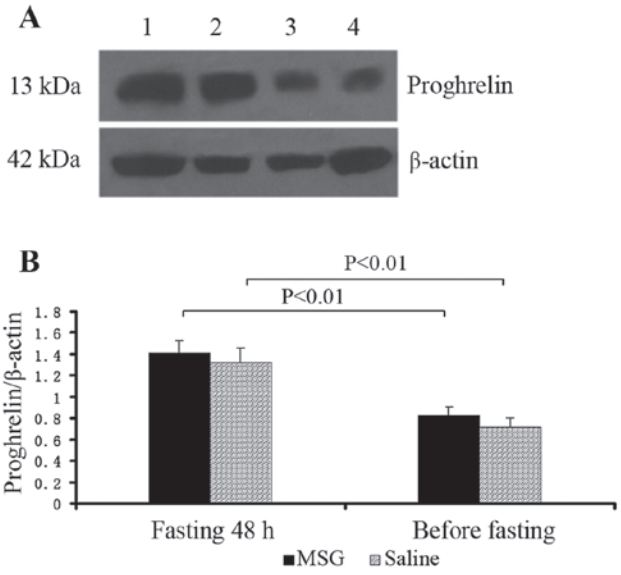


Figure 10. (A and B) Proghrelin expression in the stomach of MSG- and saline-treated mice. Values represent means ± standard error of the mean. The proghrelin protein expression in the stomach was significantly increased in MSG- and saline-treated mice fasted for 48 h compared with their respective counterparts before fasting (P<0.01), but there were no significant differences in proghrelin protein expression in the stomach between MSG- and saline-treated mice before and after fasting for 48 h. 1 and 2: fasting 48 h; 3 and 4: before fasting. 1 and 3-MSG, 2 and 4-saline. MSG, monosodium glutamate.

and after re-feeding (P<0.01); however, there was no significant difference between MSG- and saline-treated mice before and after fasting for 48 h, and during re-feeding (Fig. 8).

*Ghrelin mRNA expression*. Ghrelin mRNA expression in the stomach significantly increased in MSG- and saline-treated mice that were fasted for 48 h, compared with their respective counterparts before fasting (P<0.05). However, there was no significant difference in ghrelin mRNA expression in the stomach between MSG- and saline-treated mice before and after fasting for 48 h (Fig. 9).

*Proghrelin protein expression*. Proghrelin protein expression in the stomach significantly increased in MSG- and saline-treated mice that were fasted for 48 h, compared with their respective counterparts before fasting (P<0.01); however, there was no significant difference in proghrelin protein expression in the stomach between MSG-and saline-treated mice before and after fasting for 48 h (Fig. 10).

## Discussion

The role of ARC cells in altering energy intake is indisputable, with increasing emphasis placed on their involvement in energy expenditure (20,21). The activation of ARC neurons by energy-related stimuli is clear: ARC NPY (22) and AgRP gene expression (22,23) increases with food deprivation, while ARC pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript gene expression decreases (22,24). In the present study, ARC crystal violet staining was significantly decreased in MSG-treated mice, indicating overall cell loss, as well as significantly decreased ARC, NPY-IR and

TH-IR neurons. TH-IR cells were significantly decreased in the AP (Fig. 3). Therefore, there were distinct MSG-induced ARC and AP lesions. Our results revealed that the body mass index significantly increased in 3-month-old MSG-treated mice, compared with saline controls; however, there were no significant differences in food intake in 1- to 3-month-old mice between the two groups. The previously reported MSG-induced obesity without overeating (25) was similar to our results. The precise reason for the MSG-induced obesity in the present study is unknown, although one possible reason may be the decrease in energy expenditure. The ARC was revealed by transneuronal viral tract tracing using the pseudorabies virus (26-29) as a component of the sympathetic nervous system outflow circuit to WAT. Thus, ARC peptide systems appear to be likely candidates not only for the modulation of energy intake and expenditure, but may also participate in other energy-related responses, such as lipid mobilization. However, that MSG was found to induce the destruction of ARC, and food deprivation-induced lipid mobilization did not differ from that in saline controls. These data suggest that the ARC is not essential for food deprivation-induced lipid mobilization, but that the central nervous system contains sufficient neurocircuitry for such responses.

Ghrelin, a recently discovered peptide hormone, has been described as a 'hunger signal'. Ghrelin increases food intake when injected into either the forebrain or hindbrain ventricles and has been well-established to stimulate food consumption in both lean and obese humans (30), as well as food intake upon peripheral and brain injection in various naive animal species (31). In addition to regulating food consumption, ghrelin is also involved in body weight modulation. Chronic administration of this peptide leads to body weight gain in rodents, not only through increasing appetite, but also more prominently by promoting fat storage in WAT (32,33). Furthermore, total ghrelin levels are inversely correlated with body mass index, as these levels increase in anorexic and cachectic patients, and decrease under conditions of obesity (33,34). In the present study, it was observed that the body mass index significantly increased in 3-month-old MSG-treated mice compared with saline-treated controls; however, the plasma ghrelin levels were not significantly different between MSG-treated and saline-treated mice. Furthermore, there were no significant differences in food intake in 1- to 3-month-old mice between the two groups. Since there were no changes in plasma ghrelin levels, there were no changes in appetite in the two groups. Chronic alterations of ghrelin signaling pathways more prominently affect energy expenditure rather than food intake, although adaptive and compensatory regulatory mechanisms may also take place under conditions of chronically altered ghrelin signaling by genetic modifications (35). These data strongly support the hypothesis that the cause of obesity in MSG-treated mice is the decrease in energy expenditure. Hence, larger studies are required to confirm this hypothesis.

The ARC is strongly implicated in the regulation of food intake (8); ghrelin is also produced centrally in the ARC of the hypothalamus (6) and in neurons adjacent to the third ventricle (7). NPY and AgRP are orexigenic neuropeptides (9) regulated by ghrelin. Ghrelin, which is detected in neurons of the ARC, sends projections to NPY/AgRP neurons (7,36). Circulating ghrelin levels increase prior to a meal and decline postprandially in experimental animals and humans (37). The postprandial suppression of plasma ghrelin has been considerably more extensively investigated compared with the preprandial peak. The brain mechanism of the preprandial peak in plasma ghrelin remains unknown. If ARC does perform such a function, it would affect the preprandial peak of plasma ghrelin when destroyed. However, contrary to our hypothesis in the present study, plasma ghrelin levels significantly increased in MSG-treated mice that were fasted for 48 h, compared with levels prior to fasting and after re-feeding; however, the preprandial peak of plasma ghrelin continued to exist in MSG-treated mice. A recent study by Luquet *et al* (38) demonstrated that neonatal ablation of NPY/AgRP neurons had minimal effects on feeding, while their ablation in adults caused rapid starvation. Their results suggest that network-based compensatory mechanisms may develop following ablation of NPY/AgRP neurons in neonates, but these do not readily occur when these neurons become essential in adults. Luquet *et al* (39) also reported that the ablation of NPY/AgRP neurons in neonatal mice did not affect feeding in response to glucoprivation, while the feeding response to the ghrelin receptor agonist was completely abrogated. Their findings demonstrate that NPY/AgRP neurons are not necessary for generating or mediating the orexigenic response to glucose deficiency, but these neurons are essential for the feeding response to ghrelin and re-feeding on standard chow after fasting. Tamura *et al* (40) reported that the ICV administration of 1 $\mu$g ghrelin significantly increased 4 h food intake in normal controls, while this peptide did not increase food intake in MSG-treated rats. This indicates that feeding response to ghrelin requires an intact ARC. The primary action of ghrelin on appetite control is via the ARC, although it may act on another type of GHS-R, besides GHS-R1a. Faulconbridge *et al* (41) demonstrated that fourth ventricle ghrelin (150 pmol) injections increased Fos expression only in the nucleus of the solitary tract, but not in the ARC or PVN. This indicates that the ingestive response to caudal brainstem ghrelin administration does not depend on the activation of neurons in the PVN or ARC. Tamura *et al* (40) revealed that the ablation of ARC neurons by neonatal MSG treatment resulted in the loss of the appetite-stimulating effects of ghrelin, as well as the double knockout of the potent orexigenic neurotransmitters NPY and AgRP (42). Therefore, although ARC neurons are not essential for rodents to respond to food deprivation, this does not mean that they do not contribute to food sensing under normal conditions, since compensatory mechanisms may have emerged after the ablation of ARC neurons. The neonatal ablation of ARC neurons allows alternative mechanisms to develop, in order for rodents not to depend on these neurons for survival.

Ghrelin-positive X/A-like cells distributed throughout the gastric oxyntic mucosa (2,3) are the main source of circulating ghrelin (43), as demonstrated by the sharp decline in ghrelin levels following gastrectomy (5). We also investigated changes in ghrelin mRNA and preprotein levels in the stomach in the two groups of mice. In our results, ghrelin mRNA and preprotein (proghrelin) expression in the stomach significantly increased in MSG- and saline-treated mice that were fasted for 48 h, compared with their respective counterparts before fasting; however, there were no significant differences in ghrelin mRNA and preprotein expression

in the stomach between MSG-treated and saline-treated mice before and after fasting for 48 h. Ghrelin mRNA and proghrelin expression in the stomach significantly increased in the two groups of mice that were fasted for 48 h. Hence, the plasma ghrelin level was increased, and the preprandial peak of plasma ghrelin persisted in MSG-treated mice. After re-feeding, plasma ghrelin levels decreased in these two groups of mice. These data suggest that ARC is not essential for the preprandial ghrelin peak and postprandial suppression in MSG-treated mice.

In conclusion, our results demonstrated that MSG induced the destruction of the ARC, but food deprivation-induced lipid mobilization did not different from that in saline controls, and the preprandial peak of plasma ghrelin persisted in MSG-treated mice. Hence, ARC is not essential for normal food deprivation-induced preprandial ghrelin peak and lipid mobilization in MSG-treated mice. However, these findings do not mean that ARC neurons do not contribute to food sensing and lipid mobilization under normal conditions, as compensatory mechanisms may emerge following ablation of ARC neurons.

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