*J. Vet. Sci.* (2004), **5**(4), 295–302

JOURNAL OF
Veterinary
Science

# Changes in orexin-A and neuropeptide Y expression in the hypothalamus of the fasted and high-fat diet fed rats

**Eun Sung Park[1], Seong Joon Yi[2], Jin Sang Kim[3], Heungshik S. Lee[1], In Se Lee[1], Je Kyung Seong[1], Hee Kyung Jin[2], Yeo Sung Yoon[1,*]**

[1]*Department of Veterinary Anatomy and Cell Biology, College of Veterinary Medicine and Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea*
[2]*College of Veterinary Medicine, Kyungpook National University, Daegu 702-701, Korea*
[3]*Department of Physical Therapy, College of Rehabilitation, Daegu University, Daegu 705-714, Korea*

**This study was aimed to investigate the changes of orexin-A (OXA) and neuropeptide Y (NPY) expression in the hypothalamus of the fasted and high-fat diet fed rats. For the experiments, the male Sprague-Dawley (SD) rats were used as the model of high-fat diet-induced obesity. The mean loss of body weight (MLBW) did not show the linear pattern during the fasting; from 24 h to 84 h of fastings, the MLBW was not significantly changed. The numbers of OXA-immunoreactive (IR) neurons were decreased at 84 h of fasting compared with those in other five fasting subgroups. The NPY immunoreactivities in the arcuate nucleus (ARC) and the suprachiasmatic nucleus (SCN) observed at 84 h of fasting were higher than that observed at 24 h of fasting. The number of OXA-IR neurons of the LHA (lateral hypothalamic area) in the high-fat (HF) diet fed group was more increased than that of the same area in the normal-fat (NF) diet fed group. The NPY immunoreactivities of the ARC and the SCN were higher in HF group than those observed in the same areas of NF group. Based on these results, it is noteworthy that the decrease of the body weight during the fast was not proportionate to the time-course, implicating a possible adaptation of the body for survival against starvation. The HF diet might activate the OXA and the NPY in the LHA to enhance food intake.**

**Key words:** Arcuate nucleus, fasting, immunohistochemistry, lateral hypothalamus, neuropeptide Y, obesity, orexin-A, suprachiasmatic nucleus

*Corresponding author
Tel: +82-2-880-1264; Fax: +82-2-871-1752
E-mail: ysyoon@snu.ac.kr

## Introduction

Rising rate of obesity may be caused by the result of behavioral consequence of modern life; people have easy access to large amounts of palatable and high calorie food but they lack physical activity. However, such environment may affect the people in different ways. Some people are able to maintain a reasonable balance between energy input and energy expenditure, while others have a chronic imbalance that favors energy input, leading to overweight and obesity. It raises a question; what accounts for these differences between individuals?

The hypothalamus plays a major part in the regulation of the food intake. For instance, destruction of distinct hypothalamic regions, particularly the ventromedial nucleus (VMH) as well as the paraventricular and dorsomedial nucleus, induced hyperphagia [3,4,8,10,34,45,48]. In contrast, discrete lesions placed in the lateral hypothalamus reduced food intake [33,47]. The peptides-related actions on the feeding behavior of the hypothalamus could be divided into two classes: Corticotropin-releasing factor (CRF), cholecystokinin (CCK), neurotensin, cocaine- and amphetamine-regulated transcript, α-melanocyte-stimulating hormone (α-MSH), and vasopressin are anorexigenic [7,24,27,30], whereas NPY, galanin, agouti-related protein (AgRP), melanin-concentrating hormone (MCH), and the orexins are orexigenic, which stimulate food intake [16,36,38,46].

OXA (also known as hypocretin 1) is a novel neuropeptide that is known to be involved in the regulation of food intake and energy metabolism [18,19,25,36,42]. OXA is a 33-amino-acid peptide with two intramolecular disulfide bonds in the N-terminal region and orexin-B is a linear 28-amino-acid peptide [18,36]. Prepro-orexin, OXA peptide and the orexin 2 (OX2) receptor are predominant in the LHA [18,32,36], a center with a prominent role in feeding behavior [9]. OXA injected into the LHA stimulates feeding dose-dependently [19,42] and activates neurons in several

other areas involved of the hypothalamus in the regulation of feeding [28,29]. On the other hand, several studies reported other regulatory effects of OXA on the feeding conditions. For example, Mondal *et al*. reported that the OXA contents in the LHA increased after 48 h of fasting, but significantly decreased in other brain areas [26]. They suggested that OXA serve as neuromodulators and/or neurotansmitters that regulate feeding behavior through the interaction with diverse neural networks [26]. On the contrary, Taheri *et al*. reported that the OXA content in hypothalamic regions was not changed by fasting, suggesting that appetite regulation of the OXA may not be its main function [43].

NPY is a 36-amino-acid peptide discovered in the hypothalamus by Tatemoto in 1982 [44]. When NPY was administered into the paraventricular nucleus of the hypothalamus, NPY induced obesity with hyperphagia [39, 40]. Many studies suggest that NPY of hypothalamic origin, primarily produced in the ARC may be involved in the control of ingestive behavior [5,20,31,35]. Meanwhile, Kowalski *et al*. reported that 24 hours of maternal depriviation of food and water significantly increased the expression of preproNPY mRNA in pups on postnatal day (P) 2, P9, P12, and P15 by 14~31% [23].

The present study is to investigate the effect of the high-fat diet on the expression of OXA and NPY in the hypothalamus of the induced SD obese rats as well as the effect of the fasting on normal SD rats.

## Materials and Methods

### Animals and diets

Male Sprague-Dawley rats (260-280 g B.W., Samtako, Korea) were individually housed and maintained on a 12-h light-dark cycle (lights on at 06 : 00) at 22 ± 2°C with 40~50% relative humidity. Feed and tap water were provided *ad libitum*. The rats were divided into three groups with containing five rats, respectively; fasting (24, 36, 48, 60, 72 and 84 hs), HF, and NF diet fed groups. The compositions of the high-fat (30% fat) and normal diets are shown in Table 1 [13]. The high-fat and normal-fat diets were given to the rats for 14 days for each group.

### Tissue preparations

The rats were anesthetized with a mixture of xylazine hydrochloride (1 ml/kg, Rompun®, Bayer, Korea) and ketamin hydrochloride (1 ml/kg, Ketamin®, Yuhan, Korea), and then perfused intracardially with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). After perfusion, the brains were removed and post-fixed overnight in the same fixative solution at 4°C, and then cryoprotected by transferring to 30% sucrose in 0.1 M PB. All tissues were frozen in OCT embedding medium (Tissue-Tek, Sakura Finetek, USA) and stored at −70°C until cryostat sectioning.

**Table 1.** Composition of the experimental diets (g/kg)

| Constituents | Normal-fat diet | High-fat diet |
|---|---|---|
| Casein | 200 | 200 |
| Corn starch | 521 | 321 |
| Sucrose | 100 | 100 |
| Corn oil | 100 | 100 |
| Lard | - | 200 |
| Cellulose | 30 | 30 |
| DL-methionine | 2 | 2 |
| Mineral mix[a)] | 35 | 35 |
| Vitamin mix[b)] | 10 | 10 |
| Choline bitartrate | 2 | 2 |
| Gross energy content (kcal/g) | 4.25 | 5.20 |

[a]American institute of nutrition (AIN) mineral mix containing (g/kg): calcium phosphate diabasic 500, sodium chloride 74, potassium citrate 220, potassium sulfate 52, magnesium oxide 24, mangnous carbonate 3.5, ferric citrate 6, zinc carbonate 1.6, cupric carbonate 0.3, potassium iodate 0.01, sodium selenite 0.01, chrominium potassium sulfate 0.55.
[b]AIN vitamin mix containing (g/kg): thiamin HCl 0.6, riboflavin 0.6, pyridoxine HCl 0.7, niacin 3, calcium pantothenate 1.6, folic acid 0.2, biotin 0.02, vitamin B12 (0.1% trituration in mannitol) 1, dry vitamin A palmitate (500,000 U/g) 0.8, dry vitamin E acetate (500 U/g) 10, vitamin D3 trituration (4,000,000 U/g), 0.25, manadione sodium bisulfite complex 0.15.

### Immunohistochemistry

Hypothalamic nuclei were identified by using brain maps [41]. The brains were cut at 30 μm with the cryostat (Leica CM1850). The sections were rinsed in free floating with 0.01 M phosphate-buffered saline (PBS, pH 7.4), and then treated with 0.5% hydrogen peroxide in 0.01 M PBS for 15 min. The sections were washed with 0.01 M PBS five times for 7 min each, and nonspecific binding sites were blocked by incubation in 10% normal goat serum in 0.01 M PBS for 20 min at room temperature. The sections were incubated with primary antisera, rabbit polyclonal orexin-A antiserum (1 : 1000, Oncogene, USA) or rabbit anti-neuropeptide tyrosine polyclonal antibody (1 : 3000, Chemicon International, USA) overnight at 4°C. After incubation with the primary antibodies, the sections were rinsed in 0.01 M PBS five times for 7 min each and incubated for 2 h at room temperature with a secondary antibody (1 : 200, biotinylated goat anti-rabbit Ig G, DAKO, Denmark) for 2 h at room temperature, followed by a streptavidin-HRP (1 : 200, DAKO, Denmark) for 1 h at room temperature. The color reaction was developed by incubating sections with 0.05% 3' 3-diaminobenzidine tetrachloride (DAB, Sigma, USA) and 0.3% hydrogen peroxide in 0.01 M Tris buffer. The reaction was stopped by transferring the sections to 0.01 M PBS. The sections were washed with 0.01 M PBS for 35 min with five changes. Finally, the sections were mounted on gelatin-coated glass slides and examined with a Olympus U-SPT light microscope (Olympus, Japan).

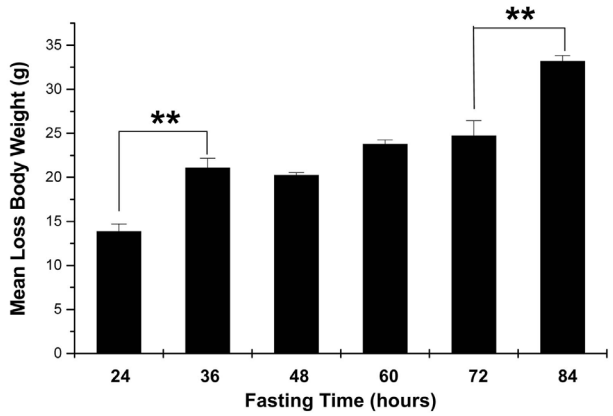


**Fig. 1.** Changes of the mean loss body weights in each fasting subgroup. Data were represented as means ± S.E.M. Five rats were used in each fasting subgroup. **; $p<0.01$.

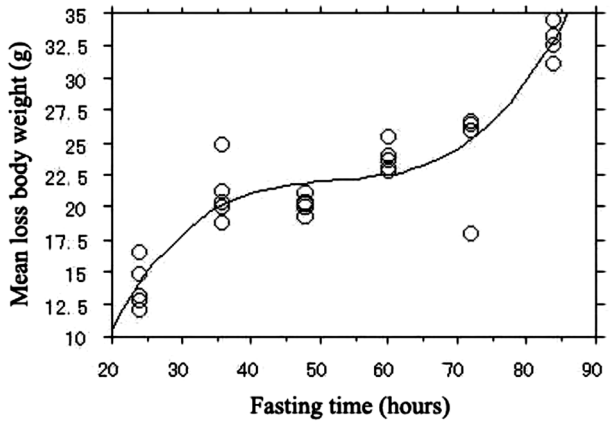


**Fig. 2.** Regression model of the mean loss body weights of each fasting subgroup.

### Statistical analysis

Statistical analyses of the data were performed using the StatView 4.5 (Abacus Concepts, USA) program. Student's *t* test was used for comparison of the two groups. In case of more than three groups, the statistical significance of differences was assessed by one-way ANOVA followed by Bonferroni-Dunnett's test. Results were represented as mean S.E.M. Differences were considered significant for $p < 0.05$.

## Results

### Changes of mean loss body weight in the fasting group

In the fasting group, the mean loss body weight (MLBW) of each subgroup (24, 36, 48, 60, 72, and 84 hs) were 13.9 ± 0.8 g, 21.1 ± 1.1 g, 20.3 ± 0.3 g, 23.8 ± 0.5 g, 24.7 ± 1.7 g, and 33.2 ± 0.6 g, respectively (Fig. 1). There was a significant difference in MLBW between 24 h and 36 h of fastings, and between 72 h and 84 h of fastings ($p < 0.01$, Fig. 1). The regression model of the MLBW showed a sigmoidal shape instead of a linear one for the fasting (Fig. 2).

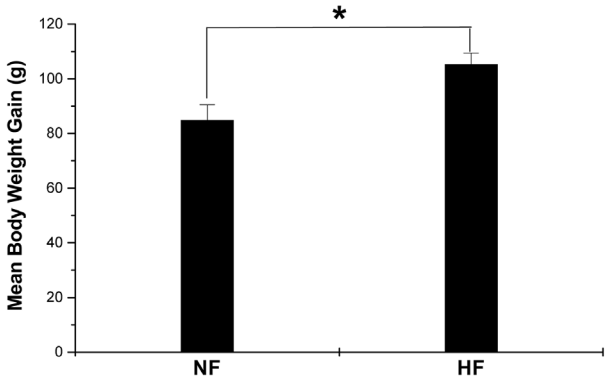


**Fig. 3.** Comparison of the mean body weight gain of the high-fat and normal-fat diet fed groups. Data were represented as means ± S.E.M. Five rats were used in each group. *; $p<0.05$.

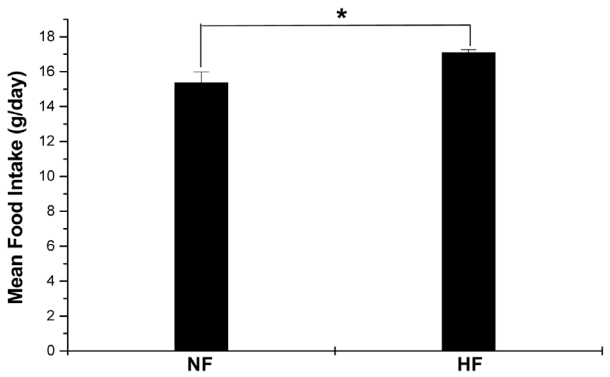


**Fig. 4.** Comparison of the mean food intake of the high-fat and normal-fat diet fed groups. Data represent means ± S.E.M. Five rats were used in each group. *; $p<0.05$.

### Changes of mean body weight gain and mean food intake in the high-fat and normal-fat diet fed groups

In the high-fat diet fed group, the mean body weight (MBW) increased from 229.9 ± 1.5 g to 335.3 ± 4.9 g, and the MBW gain was 105.4 ± 4.2 g. In the normal diet fed group, the MBW increased from 226.7 ± 1.6 g to 311.6 ± 7.2 g, and the MBW gain was 84.9 ± 5.6 g (Fig. 3). There was a significant difference in the mean food intake between the high-fat and normal-fat diet fed groups ($p < 0.05$, Fig. 4).

### Expression of OXA- and NPY- immunoreactivities in the fasting group

In the fasting group, OXA-IR neurons were confined in the LHA (bregma –2.45 ~ –2.85). The OXA-IR neurons were 13 to 30 μm in size, and multipolar and fusiform in shape. The neurons typically gave rise to 2~3 primary dendrites (Fig. 6). The NPY-IR neurons were observed in the ARC and the NPY-IR fibers in the SCN (Fig. 8). The NPY-IR neurons were 5 to 10 μm in size and mainly oval in shape (Fig. 8).

The mean number of OXA-IR neurons in the LHA of the fasting subgroups was 97.9 ± 5.2, 94.7 ± 9.9, 96.0 ± 5.3,

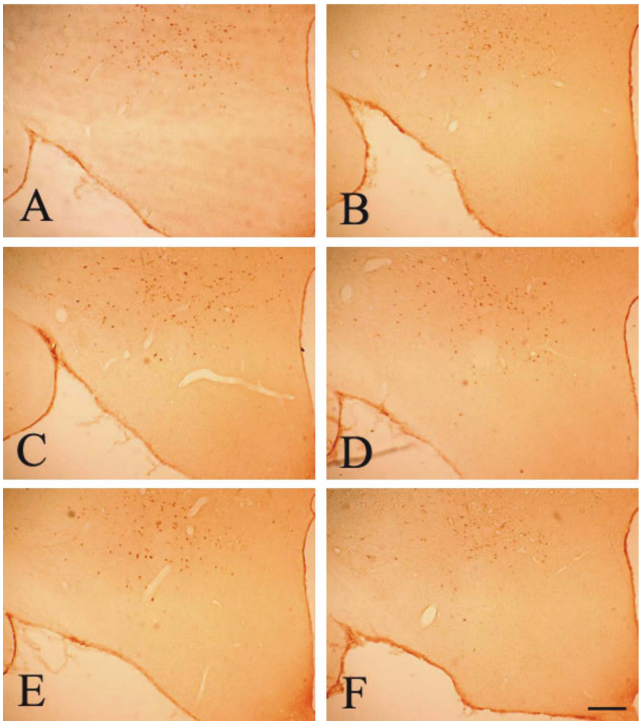


**Fig. 5.** Photomicrographs of the OXA-IR neurons in the LHA in each fasting subgroup. A; 24 h, B; 36 h, C; 48 h, D; 60 h, E; 72 h, F; 84 h, Bar = 300 µm.

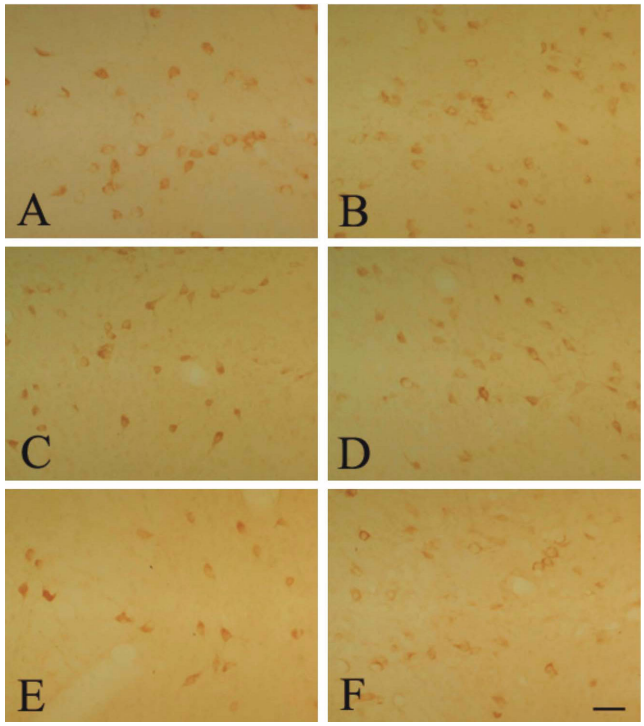


**Fig. 6.** Higher magnifications of Fig. 5; the OXA-IR neurons in the LHA in each fasting subgroup. A; 24 h, B; 36 h, C; 48 h, D; 60 h, E; 72 h, F; 84 h, Bar = 50 µm.

94.4 ± 2.8, 90.2 ± 3.2, and 51.0 ± 4.6. in 24, 36, 48, 60, 72, and 84 hs of fasting, respectively (Figs. 5 and 6). The mean numbers of OXA-IR cells of the LHA showed a significant decrease in 84 h fasting group compared with the other fasting groups ($p < 0.01$, Fig. 7). Using densitometry, NPY immunoreactivity per unit area in the ARC (0.01 $mm^2$) was 67.9 ± 0.9 and 88.9 ± 0.6 in 24 h and 84 h of fastings, respectively (Figs. 8A, B and 9). In the SCN, NPY immunoreactivity per unit area (0.01 $mm^2$) was 77.8 ± 3.8 and 88.9 ± 2.6 in 24 h and 84 h of fastings, respectively (Figs. 8C, D and 10).

### Expression of OXA- and NPY- immunoreactivities in the high-fat and normal diet fed groups

In the HF and NF diet fed groups, the OXA-IR neurons were observed in the LHA, and they were 13 to 30 µm in size and multipolar to fusiform in shape (Fig. 11). On the other hand, the NPY-IR cells were 5 to 10 µm in size and mainly oval in shape in the ARC (Fig. 13). The mean numbers of OXA-IR neurons in the LHA was 104.3 ± 6.2 and 68.4 ± 5.3, respectively, representing a significant difference between the mean numbers of OXA-IR neurons in the lateral hypothalami of the HF and the NF diet fed groups ($p < 0.01$, Figs. 11 and 12). NPY immunoreactivity of the ARC and the SCN was denser in the HF than in the same areas of the NF diet fed groups (Fig. 13). In the ARC, the mean NPY immunoreactivities of the HF and NF diet

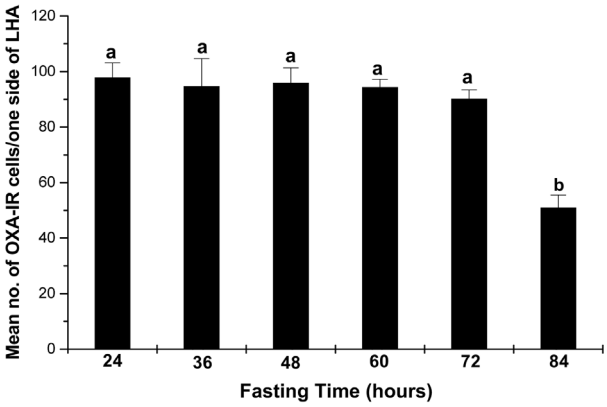


**Fig. 7.** The mean numbers of OXA-IR neurons in the LHA of each fasting subgroup. Bar not sharing a common letter was significantly different. $p<0.01$.

fed groups were 83.2 ± 1.6 and 70.2 ± 2.8, respectively, and 82.3 ± 2.3 and 51.1 ± 1.0 in the SCN, respectively. These results indicate that there was a significant difference in the mean NPY immunoreactivity of the ARC and the SCN between the HF and NF diet fed groups ($p < 0.01$, Figs. 14 and 15).

## Discussion

The present study was aimed to understand the changes of

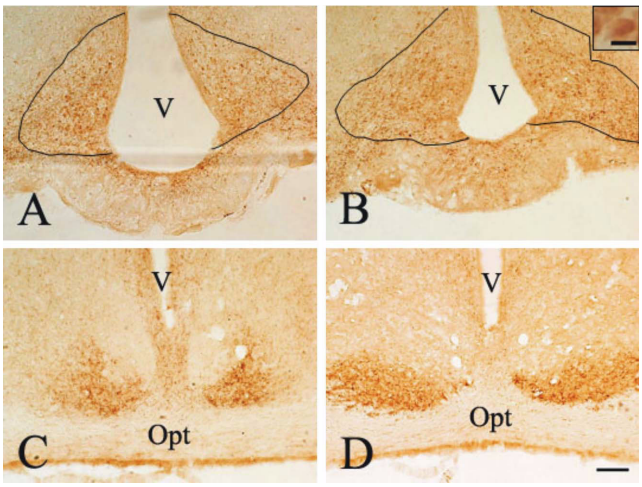


**Fig. 8.** Photomicrographs of the NPY immunoreactivity in the ARC and SCN in each fasting subgroup. The rectangle of B is a higher magnification of the NPY-IR neuron in the ARC (Bar = 10 μm). A and C; 24 h fasting, B and D; 84 h fasting. V; 3rd ventricle, Opt ; optic chiasm. Bar = 100 μm.

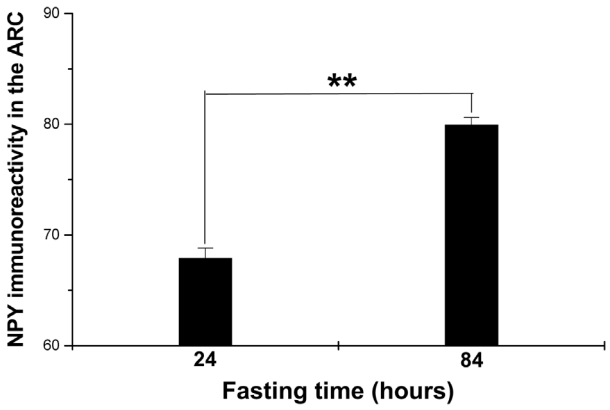


**Fig. 9.** The mean NPY immunoreactivity in the ARC of each fasting subgroup. **; $p<0.01$.

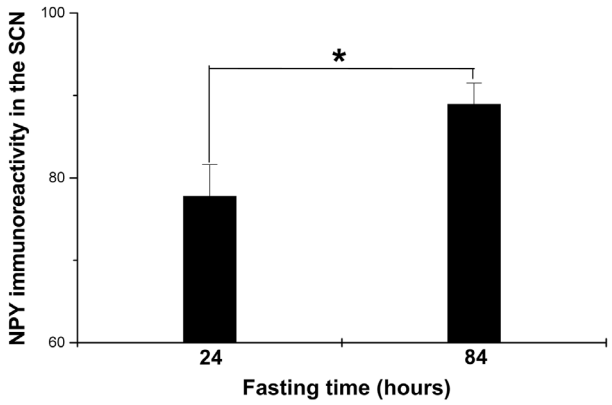


**Fig. 10.** The mean NPY immunoreactivity in the SCN of each fasting subgroup. *; $p<0.05$.

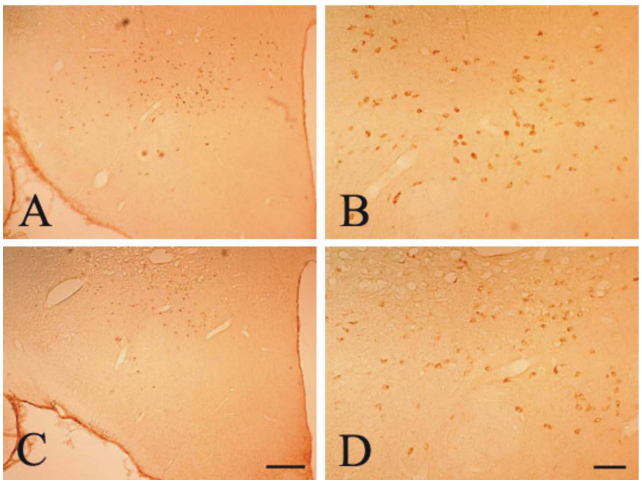


**Fig. 11.** Photomicrographs of the OXA-IR cells in the LHA (bregma −2.45~−2.85) of the HF (A and B) and NF (C and D) diet fed groups. B and D; higher magnifications of A and C. Bar in C = 300 μm, bar in D = 100 μm.

the OXA and NPY expressions in the hypothalamus of the fasted and high-fat diet induced obese rats. It was proposed that, among the variety of orexigenic peptides in the hypothalamus, OXA and NPY might play a pivotal role in the weight-gain or obesity.

Starvation is a threat to homeostasis that triggers adaptive responses [11,12,15,17,37]. Food deprivation for 2, 3, and 4 days decreased body weight by 15, 20, and 26% of the initial body weight in the male rats, respectively [36]. Ahima *et al*., also, reported that depriving male mice of food for 48 h caused a 16% fall of body weight [1]. In this study, the body weights of the male rats in 24, 36, 48, 60, 72, and 84 hs of fastings decreased by 5.9, 8.3, 8.4, 9.3, 10.2, and 13.2% of the initial body weight, respectively. In particular, although the result of Sahu *et al*.'s [35] was similar to that of Ahima *et al*.'s [1] in the food deprivation for 48 h, the result of the present study showed that the fasting for 48 hs decreased body weight by 8.4% of the initial body weight. The reason of the lower decrease rate of the body weight for the similar fasting preriod reported by Sahu *et al*.'s [35] may be the difference of the initial body weights.

It is noteworthy that the decrease of the body weight from fasting was not proportionate to the time-course, that is, the tendency of the decrease of the body weight during fasting was not linear but sigmoid in shape. This means that the fasting rats may adapt themselves to the starvation for survival.

Mondal *et al*. [26] reported that, after 48 h of fasting, the OXA and OXB contents of the LHA tended to increase as compared with the fed control rats. Also, rat hypothalamic prepro-orexin mRNA was up-regulated by 2.4-fold after 48 h fasting [36]. However, Taheri *et al*. reported that no significant difference in the content of the OXA was observed in any hypothalamic region of 48 h-fasted male rats compared with the fed control [43]. In the present study,

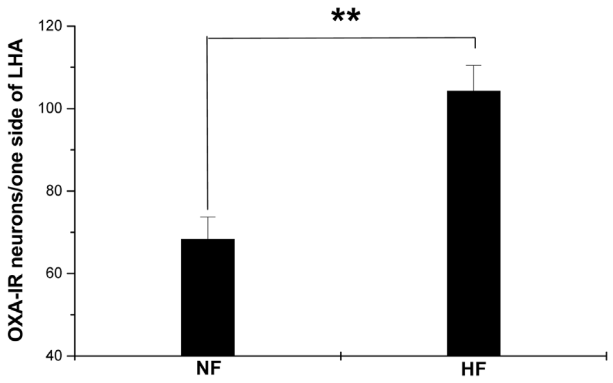


**Fig. 12.** The mean numbers of OXA-IR neurons in the LHA of the HF and NF diet fed groups. *; $p<0.01$.

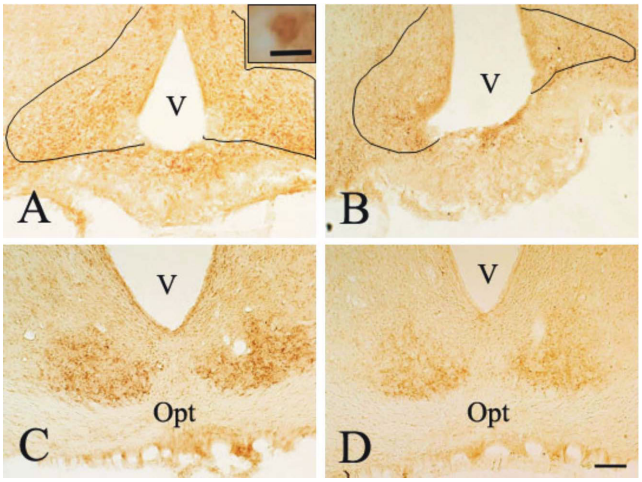


**Fig. 13.** Photomicrographs of the NPY immunoreactivity in the ARC and SCN in the HF (A and C) and NF (B and D) diet fed groups. The rectangle of A shows a higher magnification of NPY-IR neuron in the ARC (Bar=10 μm). V; 3rd ventricle, Opt; optic chiasm. Bar=100 μm.

almost all OXA-IR neurons were distributed bilaterally in the LHA at the level of median eminence (bregma –2.45 ~ –2.85); a few positive neurons were also noted in the dorsomedial hypothalamus adjacent to the 3rd ventricle. The number of the OXA-IR neurons of the LHA increased at the 24, 36, 48, 60, and 72 hs fastings compared with the fed control. On the other hand, at 84 h of fasting, the number of the OXA-IR neurons of the LHA decreased when compared with the fed control rats. Although there is a difference between the present results and those of Mondal *et al.* [26] in terms of the number and the contents of the OXA-IR neurons, the increase-tendency in the number of the OXA-IR neurons in the LHA of the fasting rats was consistent with the result of Mondal *et al.*'s [26]. In this study, from 24 h to 72 h of fastings, the number of OXA-IR neurons in the LHA was not significantly different, while the number of OXA-IR neurons was significantly decreased in 84 h of fasting rats.

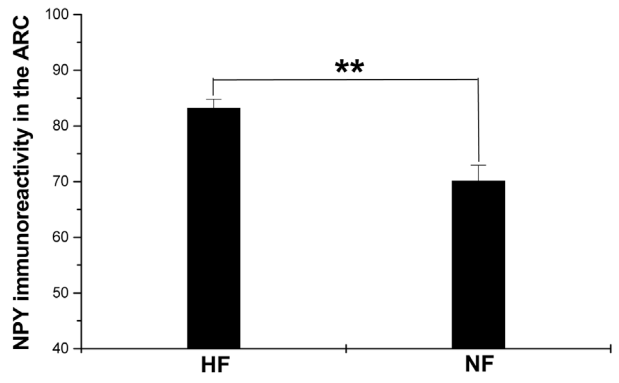


**Fig. 14.** The mean NPY immunoreactivity in the ARC of the HF and NF diet fed groups. **; $p<0.01$.

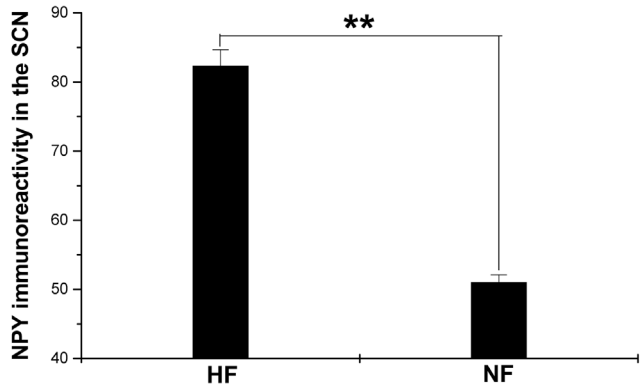


**Fig. 15.** The mean NPY immunoreactivity in the SCN of the HF and NF diet fed groups. **; $p<0.01$.

In the ARC, a site rich in NPY-producing perikarya, no change in NPY levels has been reported at day 2, but its levels rose significantly at day 3 and 4 after food deprivation [2,5,14,21,22,35]. In the present study, the NPY immunoreactivity of the ARC and SCN at 84 h of fasting increased compared with that of 24 h of fasting. It is consistent with the fact that a reduction in blood levels of leptin resulting from the fasting is detected by NPY neurons in the ARC and then these NPY neurons actively expresses NPY [6]. At present, it is difficult to interpret the facts that the NPY immunoreactivity of the SCN at 84 h of fasting was denser than that of 24 h of fasting, although the SCN has been already known as a site related to the circardian rhythm.

Taheri *et al.* [43] reported that no significant difference in the hypothalamic content of the OXA between the high-fat (45% fat) fed and low-fat fed control male Wistar rats (25.0 ± 2.0 versus 21.3 ± 2.0), despite a significantly greater average of body weight gain in the high-fat fed group (104 g versus 84.9 g, $p < 0.001$). Also, hypothalamic orexin mRNA expression was similar in the high (44.9% fat) and low (10% fat)-fat fed male *C57BL/6J* mice at all time points (1 day, 2, 7, 14 days) [49]. However, in the present study, the numbers

of the OXA-IR neurons in the LHA of the high-fat (30% fat) diet fed rats increased when compared with that of the normal-fat diet fed rats. On the other hand, Ziotopoulou *et al*. reported that after 2 days of high-fat feeding, NPYmRNA levels were significantly decreased both high-fat groups when compared with the low-fat fed group [49]. However, after 7 days, the expression of NPYmRNA returned to baseline and remained similar in the high-fat and low-fat groups at 14 days. However, in this study, the NPY immunoreactivity in the ARC and SCN of the HF diet fed rats was denser than that in the same sites of the NF fed rats.

These results suggest that the decrease of the body weight during the fasting was not proportionate to the time-course, implicating a possible adaptation of the body to starvation for survival. The increase of NPY expression in the ARC may be stimulated by the decrease of leptin in blood at 84 h of fasting, but not on the OXA. The expression of OXA and NPY may rise with obesity on a fat-rich diet. Thus high-fat appears to be a necessary component in the increased expression of OXA and NPY of the hypothalamus.

## Acknowledgments

This work was supported by grant No. R01-2000-000-00159-0 from Basic Research Program of the Korea Science and Engineering Foundation and partially supported by the Research Institute for Veterinary Science (RIVS), Seoul National University. Also, the authors would like to thank Helena Noh, a student from Philips Exeter Academy (Exeter, NH, USA) for reading our manuscript.

## References

1. **Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS.** Role of leptin in the neuroendocrine response to fasting. Nature 1996, **382**, 250-252.
2. **Allen YS, Adrian TE, Allen JM, Tatemoto K, Crow TJ, Bloom SR, Polak JM.** Neuropeptide Y distribution in the rat brain. Science 1983, **221**, 877-879.
3. **Anand BK, Brobeck JR.** Hypothalamic control of food intake in rats and cats. Yale J Biol Med 1951, **24**, 123-146.
4. **Aravich PF, Sclafani A.** Paraventricular hypothalamic lesions and medial hypothalamic knife cuts produce similar hyperphagia syndromes. Behav Neurosci 1983, **97**, 970-983.
5. **Bai FL, Yamano M, Shiotani Y, Emson PC, Smith AD, Powell JF, Tohyama, M.** An arcuato-paraventricular and -dorsomedial hypothalamic neuropeptide Y-containing system which lacks noradrenaline in the rat. Brain Res 1985, **331**, 172-175.
6. **Bear MF, Conners BW, Paradiso MA.** Neuroscience: exploring the brain, 2nd ed. pp. 528-533. Lippincott Williams and Wilkins, Baltimore, 2001.
7. **Beck B.** Cholecystokinin, neurotensin and corticotropin-releasing factor-3 important anorexic peptides. Ann Endocrinol 1992, **53**, 44.
8. **Bernardis LL, Berlinger LL.** The dorsomedial hypothalamic nucleus revised. Brain Res 1987, **434**, 321-381.
9. **Bernardis LL, Berlinger LL.** The lateral hypothalamic area revisited: ingestive behavior. Neurosci Biobehav Rev 1996, **20**, 189-287.
10. **Brobeck JR.** Mechanism of the development of obesity in animals with hypothalamic lesions. Physiol Rev 1946, **26**, 541-559.
11. **Bronson FH, Marsteller FA.** Effect of short-term food deprivation on reproduction in female mice. Biol Reprod 1985, **33**, 660-667.
12. **Cahill GFJr, Herrera MG, Morgan AP, Soeldner JS, Steinke J, Levy PL, Reichard GAJr, Kipnis DM.** Hormone-fuel interrelationships during fasting. J Clin Invest 1966, **45**, 1751-1769.
13. **Choo JJ, Shin HJ.** Body-fat suppressive effects of capsaicin through ß-adrenergic stimulation in rats fed a high-fat diet. Kor J Nutrition 1999, **32**, 533-539.
14. **Chronwall BM, DiMaggio DA, Massari VJ, Pickel VM, Ruggiero DA, O'Donohue TL.** The anatomy of neuropeptide-Y-containing neurons in rat brain. Neuroscience 1985, **15**, 1159-1181.
15. **Connors JM, DeVito WJ, Hedge GA.** Effects of food deprivation on the feedback regulation of the hypothalamic-pituitary-thyroid axis of the rat. Endocrinology 1985, **117**, 900-906.
16. **Crawley JN.** The role of galanin in feeding behavior. Neuropeptides 1999, **33**, 369.
17. **Dallman MF, Strack AM, Akana SF, Bradbury MJ, Hanson ES, Scribner KA, Smith M.** Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. Front Neuroendocrinol 1993, **14**, 303-347.
18. **de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS 2nd, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG.** The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci U S A 1998, **95**, 322-327.
19. **Dube MG, Kalra SP, Kalra PS.** Food intake elicited by central administration of orexins/hypocretins: identification of hypothalamic sites of action. Brain Res 1999, **842**, 473-477.
20. **Dube MG, Sahu A, Kalra PS, Kalra SP.** Neuropeptide Y release is elevated from the microdissected paraventricular nucleus of food-deprived rats: an in vitro study. Endocrinology 1992, **131**, 684-688.
21. **Everitt BJ, Hokfelt T, Terenius L, Tatemoto K, Mutt V, Goldstein M.** Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. Neuroscience 1984, **11**, 443-462.
22. **Hökfelt T, Johansson O, Goldstein M.** Chemical anatomy of the brain. Science 1984, **225**, 1326-1334.
23. **Kowalski TJ, Houpt TA, Jahng J, Okada N, Chua SCJr, Smith GP.** Ontogeny of neuropeptide Y expression in response to deprivation in lean Zucker rat pups. Am J Physiol 1998, **275**, R466-470.
24. **Kristensen P, Judge ME, Thim L, Ribel U, Christjansen**

**KN, Wulff BS, Clausen JT, Jensen PB, Madsen OD, Vrang N, Larsen PJ, Hastrup S.** Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature 1998, **393**, 72-76.
25. **Lubkin M, Stricker-Krongrad A.** Independent feeding and metabolic actions of orexins in mice. Biochem Biophys Res Commun 1998, **253,** 241-245.
26. **Mondal MS, Nakazato M, Date Y, Murakami N, Yanagisawa M, Matsukura S.** Widespread distribution of orexin in rat brain and its regulation upon fasting. Biochem Biophys Res Commun 1999, **256**, 495-499.
27. **Morley JE.** Neuropeptide regulation of appetite and weight. Endocr Rev 1987, **8**, 256.
28. **Mullett MA, Billington CJ, Levine AS, Kotz CM.** Hypocretin I in the lateral hypothalamus activates key feeding-regulatory brain sites. Neuroreport 2000, **11**, 103-108.
29. **Nambu T, Sakurai T, Mizukami K, Hosoya Y, Yanagisawa M, Goto K.** Distribution of orexin neurons in the adult rat brain. Brain Res 1999, **827**, 243-260.
30. **Owens MJ, Nemeroff CB.** Physiology and pharmacology of corticotropin-releasing factor. Pharmacol Rev 1991, **43**, 425.
31. **Park ES, Jo S, Yi SJ, Kim JS, Lee HS, Lee IS, Seo KM, Sung JK, Lee I, Yoon YS.** Effect of capsaicin on cholecystokimin and neuropeptide Y expressions in the brain of high-fat diet fed rats. J Vet Med Sci 2004, **66**, 107-114.
32. **Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS.** Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 1998, **18**, 9996-10015.
33. **Powley TL, Keesey RE.** Relationship of body weight to the lateral hypothalamic feeding syndrome. J Comp Physiol Psychol 1970, **70**, 25-36.
34. **Powley TL, Opsahl CH, Cox JE, Weingarten HP.** The role of the hypothalamus in energy homeostasis. In: Morgane PJ, Panskepp J. (eds.), Handbook of the hypothalamus. Part A: behavioral studies of the hypothalamus. pp. 211-298. Dekker, New York, 1980.
35. **Sahu A, Kalra SP, Crowley WR, Kalra PS.** Evidence that NPY-containing neurons in the brainstem project into selected hypothalamic nuclei: implication in feeding behavior. Brain Res 1988, **457**, 376-378.
36. **Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M.** Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998, **92**, 573-585.
37. **Schwartz MW, Dallman MF, Woods SC.** Hypothalamic response to starvation: implications for the study of wasting disorders. Am J Physiol 1995, **269**, R949-957.
38. **Stanley BG.** Neuropeptide Y in multiple hypothalamic sites controls eating behavior, endocrine, and autonomic systems for body energy balance. In: Colmers WF, Wahlestedt C. (eds.), Biology of Neuropeptide Y and related peptides. p. 457. Human Press, Totowa, 1993.
39. **Stanley BG, Kyrkouli SE, Lampert S, Leibowitz SF.** Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. Peptides 1986, 7, 1189-1192.
40. **Stanley BG, Leibowitz SF.** Neuropeptide Y: stimulation of feeding and drinking by injection into the paraventricular nucleus. Life Sci 1984, **35**, 2635-2642.
41. **Swanson LW.** Brain maps: structure of the rat brain, pp. 45-123. Elsevier Science, Netherlands, 1992.
42. **Sweet DC, Levine AS, Billington CJ, Kotz CM.** Feeding response to central orexins. Brain Res 1999, **821,** 535-538.
43. **Taheri S, Mahmoodi M, Opacka-Juffry J, Ghatei MA, Bloom SR.** Distribution and quantification of immunoreactive orexin A in rat tissues. FEBS Lett 1999, **457**, 157-161.
44. **Tatemoto K, Carlquist M, Mutt V.** Neuropeptide Y-a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature 1982, **296**, 659-660.
45. **Tokunaga K, Fukushima M, Kemnitz JW, Bray GA.** Comparison of ventromedial and paraventricular lesions in rats that become obese. Am J Physiol 1986, **251**, R1221-1227.
46. **Tritos NA, Maratos-Flier E.** Two important systems in energy homeostasis: melanocortins and melanin-concentrating hormone. Neuropeptides 1999, **33**, 339.
47. **van den Pol AN.** Lateral hypothalamic damage and body weight regulation: role of gender, diet, and lesion placement. Am J Physiol 1982, **242**, R265-274.
48. **Weingarten HP, Chang PK, McDonald TJ.** Comparison of the metabolic and behavioral disturbances following paraventricular- and ventromedial-hypothalamic lesions. Brain Res Bull 1985, **14**, 551-559.
49. **Ziotopoulou M, Mantzoros CS, Hileman SM, Flier JS.** Differential expression of hypothalamic neuropeptides in the early phase of diet-induced obesity in mice. Am J Physiol Endocrinol Metab 2000, **279**, E838-845.