Development of the Epididymal Adipose Tissue in Monosodium Glutamate-Induced Obese Mice

Masaharu OCHI,1 Kyoko FUKUHARA,1 Tadashi SAWADA,1 Takanori HATTORI,2 and Tomoichi KUSUNOKI1

1 Department of Pediatrics, Kyoto Prefectural University of Medicine, Kamikyo-ku, Kyoto 602, Japan
2 Department of Pathology, Shiga Medical School, Otsu, Shiga 520–21, Japan

(Received March 2, 1988)

Summary Obesity was induced in neonatal mice by subcutaneous injections of monosodium glutamate (MSG) at an early neonatal stage. The process of adipocyte formation was studied comparatively in the developing epididymal adipose tissue of the MSG-treated mice and in normal mice during the period from the 6th to the 100th postnatal day. Tritiated thymidine autoradiographic studies showed that cell proliferation activity was the highest on the 6th postnatal day both in the MSG-treated and the control mice. In normal mice, however, cell proliferation took place less frequently after 6 days and had almost ceased after 49 days. In the obese mice, as evidenced by relatively high labeling indices, cell proliferation continued to occur even after 49 days. Ultimately there was no difference in the number of adipocytes counted by Hirsch’s method in the MSG-treated and the control mice at the 100th postnatal day. The storage of fat droplets became more noticeable in obese mice than in normal mice after 35 days. The mean size of fat droplets of the obese mice was twice as large as that in normal mice on the 49th postnatal day. These results indicate that the MSG-induced obesity is of the hypertrophic type.

Key Words monosodium glutamate (MSG), hypothalamic lesion, obesity, epididymal adipose tissue, [3H]thymidine, autoradiography, fat cell enlargement, fat cell proliferation

Obesity can be induced in experimental animals by injuring the hypothalamus (1). The ventromedial nucleus of hypothalamus can be damaged by administration of goldthioglucose (2–4) or by an electric coagulation method (5). These methods have been used on adult animals, and it has been shown that the animals become obese by overproduction of fat in individual fat cells. The number

1 越智雅晴，福原京子，沢田 淳，2 服部隆則，1 楠 智一
of fat cells is not increased, and the obesity is therefore referred to as a hypertrophic type (6, 7).

Recently, another model of the hypothalamic obesity has been introduced by injection of monosodium glutamate (MSG) (8). MSG injection causes degeneration and necrosis of neurons in several nuclei of the hypothalamus. Although the animals receive a hypothalamic injury during the neonatal period, they become obese after puberty (9). There is a time lag after the hypothalamic injury before obesity is manifested in these animals. During this period, the fatty tissue develops. But, how the fatty tissue develops, or whether there is any difference in development of the tissue between MSG-treated and normal animals, is open to question.

In the present study, we investigated cell proliferation of the developing adipose tissue of the MSG-treated mice and compared it with that of normal mice. The epididymal adipose tissue, commonly used for the study of fat metabolism, was studied. Epididymal adipose tissue is formed after birth, and it develops to a measurable size in a relatively short period, so that changes in size and weight can be followed easily. Two methods were used to study cell proliferation: \[^{3}H\]thymidine autoradiography (\[^{3}H\]TdR ARG) and cell counting of the tissue by Hirsch's method (10).

MATERIALS AND METHODS

In this study, 180 male mice (Jcl-ICR) ranging in age from 6 to 100 days old were used. Ninety mice were rendered obese by subcutaneous injection of 10% monosodium glutamate (MSG), 2 mg/g of body weight every 24 h for the first five postnatal days. The other 90 mice were injected with saline solution and served as the control. After weaning at 18 days old, they were allowed access to food and water ad libitum.

1) Experiment 1. In the first experiment, we examined cell proliferation of the epididymal adipose tissue at different ages by \[^{3}H\]thymidine autoradiography \((^{3}H)TdR\) ARG) using a flash-labeling technique. Six mice from each age group from both MSG-treated and control groups of 6, 10, 16, 21, 28, 35, 42, 49, 56, 70, and 100 day olds were given a single injection of \[^{3}H\]TdR containing 5 µCi per gram of body weight. They were weighed and killed 30 min after injection (Fig. 1, a and b, Fig. 2). The unilateral (left side) epididymal adipose tissue of each mouse was weighed (Fig. 3) and, thereafter, cut into regional segments (head, body and tail). The body was cut further into smaller pieces, which were fixed in 4% glutaraldehyde in cacodylate buffer adjusted to pH 7.4 for 1 h. Specimens were then rinsed in several changes of cacodylate buffer over a period of 1 h and postfixed in 2% osmium tetroxide for 2 h. The tissue was dehydrated in a graded ethanol series and embedded in epoxy resin. Semithin sections of 1 micron in thickness were mounted on glass slides, dipped in KODAK NTB2 nuclear emulsion, and developed in FD-111 after 4 weeks exposure. Sections were stained by toluidine blue.

In our flash-labeling autoradiographs (30 min after a single injection of \[^{3}H\]-
Tdr), the mean grain count of the labeled cells was 43 (33–35). In the flash-labeling experiment, the total labeling index of the epididymal adipose tissue was scored except in 70- and 100-day-old mice. The labeling index was shown by the percentage of the labeled cells in a total count of the observed cells (about 1,000 cells for each mouse). Simultaneously labeled cells were classified into 4 groups: adipocytes (f), endothelial cells (e), poorly differentiated mesenchymal cells with no specific features (m), and other cells (o), which contained fibroblasts and blood cells. The percentage of each cell group in a total count of the labeled cells was calculated (Fig. 4). For the same specimens that were scored by the labeling index, we measured the fat cell dimension by the Manual Optical Picture Analyzing System MOP/AM03 (Fig. 5).

2) Experiment 2. In the second experiment, normal and MSG-treated mice of 28, 40, 70, and 100 days old were used. Each mouse was weighed and killed (Fig. 1, a and b, Fig. 2). Unilateral epididymal adipose tissue was weighed (Fig. 3). The number of adipocytes per mg wet weight of epididymal fat pad was estimated by the method of Hirsch and Gallian (10) (Fig. 6). The sizing of isolated fat cells was done under the microscopic observation. The diameter of 1,000–2,000 cells were estimated in each mouse and the dimensions of fat cells were calculated from the mean diameter (Fig. 7).

RESULTS

1) Growth curve and development of the epididymal fat pad

The MSG-treated mice were smaller than the control mice in nasoanal length at all ages examined (Fig. 1a). They were lighter than normal mice from 6 to 49 days old, but afterwards their weight increased rapidly and they became obese (Fig. 1b). The ratio of weight to nasoanal length of the MSG-treated ones, which showed the degree of obesity, was larger than that of the control mice after 56 days of age (Fig. 2).

The wet weight of the epididymal adipose tissue in the MSG-treated mice was less than that of the control mice until 21 days, but it increased progressively afterwards and became about three times that of the control mice at 70 days old (Fig. 3).

2) Cell proliferation in the epididymal adipose tissue

The first experiment revealed kinetics of cell proliferation in the adipose tissue. Around the epididymis, only loose connective tissue was seen before the 6th postnatal day. The tissue consisted of several types of cells. Poorly differentiated mesenchymal cells and endothelial cells were commonly seen, and occasionally adipocytes with small fat droplets were found. From 6 days after, immature adipocytes with multilocular fat droplets increased and fat cells with unilocular fat droplets appeared.

The flash-labeling experiment has shown that cell proliferation occurred most
Fig. 1. Growth curves in normal and MSG-treated mice: (a) nasoanal length, cm; and (b) body weight, g.

J. Nutr. Sci. Vitaminol.
actively on the 6th postnatal day both in the MSG-treated and the control mice. The labeling index of the epididymal adipose tissue as a whole was an average 12.0% (±4.7%) in the MSG-treated mice and 16.2% (±4.9%) in the control mice. Most of the labeled cells were endothelial cells and poorly differentiated mesenchymal cells. The adipocytes with large fat droplets were not labeled, but immature cells with small fat droplets were occasionally labeled.

The labeling indices of the mesenchymal cells in the flash-labeling experiment decreased after 6 days and cell proliferation almost ceased after 49 days in the control mice. On the other hand, in the MSG-treated mice, cell proliferation was less active than that of the control mice from 6 to 21 days, and after this period, cell proliferation in the MSG-treated mice was more active and continued to occur even after 49 days, as evidenced by relatively high labeling indices (Fig. 4). In 70 and 100 day olds, we could find no labeled cells in the control mice, but we could find a few labeled cells in MSG-treated mice. Most of the labeled cells were poorly differentiated mesenchymal cells, but they were too few to be included in the labeling indices.

3) Cellularity in the developing epididymal adipose tissue

The cell number of total epididymal fat tissue was counted by the method of Hirsch and Gallian (10) in the second experiment. The number of cells in the MSG-
Fig. 3. Wet weights of epididymal adipose tissues in normal and MSG-treated mice.

Fig. 4. The frequency and classification of labeled cells in the epididymal adipose tissue of normal and MSG-treated mice at different ages by flash labeling. Asterisk indicates a significantly different value ($p<0.005$) from the corresponding control value by Student's $t$-test. Mean±SEM.

*J. Nutr. Sci. Vitaminol.*
Fig. 5. The dimensions of fat cells measured with Manual Optical Picture Analyzing System MOP/AM03 from enlarged photograph of epididymal adipose tissue in normal and MSG-treated mice.

Fig. 6. Number of total epididymal fat cells in normal and MSG-treated mice. Asterisk indicates a significantly different value from the corresponding control value by Student's t-test. Mean±SEM.

treated mice was fewer than that of the control mice at 28 days ($6.8 \times 10^5 \pm 1.8 \times 10^5$ vs. $9.7 \times 10^5 \pm 1.8 \times 10^5$, $0.01 < p < 0.02$) and at 40 days ($10.9 \times 10^5 \pm 1.8 \times 10^5$ vs. $15.0 \times 10^5 \pm 2.1 \times 10^5$, $p < 0.01$), but there was no statistical significance by Student's t-test at 70 days and 100 days (Fig. 6).

4) The process of cell enlargement in the epididymal adipose tissue

In the first experiment we measured the dimensions of adipocytes with Manual Optical Picture Analyzing System MOP/AM03 from enlarged photographs of epididymal adipose tissue. There were no remarkable differences in the mean dimensions between the MSG-treated and the control mice until the mice were 28 days old; however, after 35 days the size of cells became larger in MSG-treated mice.
than in normal mice, reaching twice their size at the 49th day (Fig. 5). These results correspond to the data in the 2nd experiment (Fig. 7, a and b). As for the size of the isolated adipocytes, there was no difference between the MSG-treated and the control mice of 28 days old. The mean diameters and dimensions of the adipocytes became larger in the MSG-treated mice than in the controls. These results showed that the increase of the wet weight of epididymal adipose mass in MSG-treated mice was due to enlargement of each fat cell and not by an increase in cell number.

DISCUSSION

Neonatal mice were rendered obese by subcutaneous injections of MSG for the first five postnatal days in this study. Tanaka et al. reported that neurons with pyknotic nuclei were found in the nucleus preopticus, n.suprachiasmaticus, n.arcuatus, n.ventromedialis, n.premamillaris dorsalis and ventralis, n.prelateralis mamillaris, and n.mamillaris medialis and lateralis in mice treated with MSG every 24 h for the first five postnatal days (9). Accordingly, the MSG-treated mice are hypothalamic obese mice. The MSG-treated mice showed a short stature, indicated by a shorter nasoanal length, but they gained weight excessively compared to controls, becoming obese after 56 days, although it must be emphasized that they remained lighter in weight than the control mice until the 56th day (Fig. 1, a and b, Fig. 2). The rapid increase of their weight after 56 days appeared to be mainly due to excess development of the adipose tissue (Fig. 3). The mechanism of this excess fatty tissue formation has been discussed by many investigators. However, there were no.
reports which studied adipocyte formation from the cell kinetics point of view. In the present study, the development of fatty tissue was examined through cell proliferation in epididymal adipose tissue.

Around the epididymis, only loose connective tissue was seen in 6-day-old mice both in the control and MSG-treated mice. The tissue consisted of several cell types; poorly differentiated mesenchymal cells and endothelial cells were a major component, and a few fat cells with small fat droplets could already be seen. In the flash-labeling experiment in both the MSG-treated and the control mice, most of the labeled cells were endothelial cells and poorly differentiated mesenchymal cells. Proliferation of the former cell type contributes to the development of vascular system of the tissue; proliferation of the latter cell type is suspected to result in the production of fat cells (partially of fibroblasts and immature endothelial cells) (11).

In the control mice, cell proliferation in the epididymal fatty tissue was most active on the 6th postnatal day. The labeling indices of the tissue gradually decreased thereafter, and they became less than 1% after 49-56 days (Fig. 4). These findings indicate that cell proliferation in the epididymal fatty tissue of normal mice almost stops after 56 days; in other words, the number of fat cells in the tissue seems to be then fixed (12). On the other hand, cell proliferation occurred less actively in the MSG-treated mice than in the control mice during postnatal days 6 to 28. However, after 35 days, the cell proliferation rate became higher in the MSG-treated mice, and it was shown that cell proliferation continued to occur even after 56 days, as evidenced by relatively high labeling indices (2-3%). In these MSG-treated mice, the ratio of the labeled poorly differentiated mesenchymal to other labeled cell types was higher than that of the control mice. These findings suggest that the number of fat cells became fixed later in the MSG-treated mice than in the normal mice. However, it was shown by Hirsch’s method that the number of fat cells in adult age was ultimately about the same for both the MSG-treated and the control mice.

Both in the MSG-treated and the control mice, lipid accumulation in individual fat cells appeared to occur rapidly after 6 postnatal days, and unilocular fat cells increased in number during 6-28 days. After 28 days, the size of the lipid droplet increased in obese mice more than in normal mice (Figs. 5 and 7). Since it has been shown that the number of fat cells is not different between the MSG-treated and the control mice, the obesity seen in the MSG-treated mice must be caused by hypertrophy of individual fat cells. Therefore, the MSG-induced hypothalamic obesity is a hypertrophic type.

Factors influencing the development of obesity in MSG-treated mice are scarcely known. It seems less probable that the hypothalamic injury may directly influence cell proliferation activity of the tissue. Although the cell proliferation rate was higher after 28 days in the MSG-treated mice, this may not have been caused directly by hypothalamic injury, when we consider that the prolonged cell proliferation seen in the MSG-treated mice may contribute to cell replacement of the tissue. The fatty tissue in the MSG-treated mice consists of extremely larger fat cells,
and it is possible that the larger fat cells may have a shorter survival than the normal cells. This view is partially supported by the existence of fat necrosis, followed by infiltration of inflammatory cells in the fatty tissue of old obese mice (13, 14). Therefore, the obesity induced by MSG hypothalamic injury can be considered primarily to be a metabolic disorder (15–17).

REFERENCES

1) Bray, G. A., and York, D. A. (1979): Hypothalamic and genetic obesity in experimental animals; an autonomic and endocrine hypothesis. Physiol. Rev., 59, 717–809.
2) Marshall, N. B., Barrnett, R. J., and Mayer, J. (1955): Hypothalamic lesions in goldthioglucose injected mice. Proc. Soc. Exp. Biol. Med., 90, 240–244.
3) Brecher, G., Laqueur, G. L., Cronkite, E. P., Edelman, P. M., and Schwartz, I. L. (1965): The brain lesion of goldthioglucose obesity. J. Exp. Med., 121, 395–401.
4) Debons, A. F., Krimsky, I., From, A., and Cloutie, R. J. (1940): Goldthioglucose induction of obesity: significance of focal gold deposits in hypothalamus. Am. J. Physiol., 219, 1403–1408.
5) Hetherington, A. W., and Ranson, S. W. (1940): Hypothalamic lesions and adiposity in the rat. Anat. Rec., 78, 149–172.
6) Hirsch, J., and Han, P. W. (1969): Cellularity of rat adipose tissue: effects of growth, starvation and obesity. J. Lipid Res., 10, 77–82.
7) Johnson, P. R., Zucker, L. M., Cruce, J. A. F., and Hirsch, J. (1971): Cellularity of adipose depots in the genetically obese Zucker rat. J. Lipid Res., 12, 706–714.
8) Olney, J. W. (1969): Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. Science, 164, 719–721.
9) Tanaka, K., Shimada, M., Nakao, K., and Kusunoki, T. (1978): Hypothalamic lesion induced by injection of monosodium glutamate in suckling period and subsequent development of obesity. Exp. Neurol., 62, 191–199.
10) Hirsch, J., and Gallian, E. (1968): Methods for the determination of adipose cell size in man and animals. J. Lipid Res., 9, 110–119.
11) Ochi, M., Sawada, T., and Hattori, T. (1987): Tritiated thymidine autoradiographic study on postnatal development of epididymal adipose tissue in the normal mouse. Anat. Embryol., 177, 139–145.
12) Johnson, P. R., and Hirsch, J. (1972): Cellularity of adipose depots in six strains of genetically obese mice. J. Lipid Res., 13, 2–11.
13) Soret, M. G., Kupiecki, F. P., and Wyse, B. M. (1974): Epididymal fat pad alterations in mice with spontaneous obesity and with chemically induced obesity. Diabetologica, 10, 639–648.
14) Hausberger, F. X. (1966): Pathological changes in adipose tissue of obese mice. Anat. Rec., 154, 651–660.
15) Furukawa, N., Kusunoki, T., and Iwashima, A. (1981): Active PDH rises before the onset of hyperinsulinemia in MSG-treated obese mice. J. Chron. Dis. Therap. Res., 21, 270–274.
16) Cameron, D. P., Cutbush, L., and Opat, F. (1973): Effects of monosodium glutamate induced obesity in mice on carbohydrate metabolism in insulin secretion. Clin. Exp. Pharmacol. Physiol., 5, 41–51.
17) Redding, T. W., Schally, A. V., Arimura, A., and Wakabayashi, I. (1971): Effect of monosodium glutamate on some endocrine functions. Neuroendocrinology, 8, 245–255.