A Scanning Electron Microscopic Study on the Difference between White and Brown Adipose Tissues in the Rat
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ABSTRACT. Lipid globules in the white and brown adipose tissues in the rat were well preserved with prolonged postfixation in OsO₄. The difference between the surface of white adipose tissue and that of brown adipose tissue was observed in such a way that the surface of the brown adipocyte was lumpy owing to the presence of intracellular lipid globules, and that the surface of the white adipocyte was considerably smooth. After the matrix of lipid globules was dissolved during specimen preparation, the subgranal embedded in the matrix were disclosed on the inner surface of the lipid globules. The cut surface of the white adipocyte contained a large lipid droplet and the amount of cytoplasm was poor, whereas the brown adipocyte had several lipid droplets and many small granules in the cytoplasm.—KEY WORDS: adipose tissue, lipid globule, scanning electron microscopy.

The possibility of transformation of brown adipocytes into white ones is one of the key subjects in morphogenesis of intracellular lipid globules. At the transmission electron microscopic (TEM) level, the difference in structure of mitochondria and that in density of cytoplasm are indices of the difference between white and brown adipose tissues [4, 5]. In scanning electron microscopic (SEM) studies [2, 3, 6], however, the difference between white and brown adipocytes has not been well documented. The present study was attempted to compare white adipocytes with brown adipocytes in the rat at the SEM level, and to find a clue to distinction of these two types of adipocytes by their surface appearance.

A white adipose tissue from the outer femoral region and a brown adipose tissue from the interscapular region of each of 4 male Wistar rats (4 weeks old, 150–180 g in body weight) were fixed with perfusion of 2.5% glutaraldehyde in cacodylate buffer, followed by immersion in the same fixative for 3 hrs. Small pieces (about 1 mm³ in size) of each adipose tissue were postfixed in 1% OsO₄ buffered with 0.1 M sodium cacodylate at pH 7.4, and this postfixation was intentionally prolonged to 8–12 hrs for better preservation of lipid substances.

For freeze-fracture, the fixed specimens were immersed first in 25% and then in 50% dimethyl sulfoxide (DMSO) prior to fracture on the metallic stage cooled with liquid nitrogen.

The specimens were dehydrated through a graded series of ethanol and were briefly immersed in acetic isomyl to be exchanged with liquid carbon dioxide in a critical point dryer (HCP-2, HITACHI). The specimens were coated with evaporated gold and then examined with a scanning electron microscope (ALPHA-10, AKASHI) at 20 KV.

The size of adipocytes in the white adipose tissue (approximately 50 μm in diameter, Fig. 1) was much larger than that in the brown adipose tissue (approximately 15 μm in diameter, Fig. 2). The surface of the white adipocytes was comparatively smooth (Fig. 1), but that of the brown adipocytes was lumpy with intracellular lipid globules (Fig. 2). The network of collagen and reticular fibers covered the massive group of adipocytes.

Despite a considerably long fixation of the small white adipose tissues, lipid droplets were still dissolved in the central part of the tissues, leaving honeycomb structures (Fig. 3). At a higher magnification, small subglobules were recognized on the smooth surface of fractured lipid droplets (Fig. 4). Intercellular connective tissue fibers, which contained collagen and reticular fibers, covered the surface of adipocytes (Fig. 5).

In the brown adipose tissue, the relationship between cells was tight, and each cell had several lipid droplets and many small granules (Fig. 6). Lipid droplets in the brown adipocytes were uniformly preserved, without honeycomb structures as found in white adipocytes. The intercellular space of the brown adipocytes was narrower than that of the white adipocytes. After the removal of the matrix substance of lipid globules, small granules (0.2–0.8 μm in diameter) were revealed on the surfaces of the globules (Fig. 7).

A previous report from this laboratory suggested that the avian adipocytes could retain lipid droplets after prolonged fixation in OsO₄ [1]. In the present study, the size of specimens was further reduced to 1 mm³ and the period of fixation was increased. This modification was effective in preserving the lipid droplets except for those in the center of the specimens.

After fixation with OsO₄, the period of dehydration with ethanol was shortened. Smith and Jaret [6] employed a nonpolar solvent, ethylene glycol and ethylene glycol monomethyl ether, for dehydration. Propylene oxide was not required for the preparation of SEM samples, as it was for the preparation of TEM samples. Owing to its ability to dissolve lipid, propylene oxide would have a disadvantage to the preparation of adipose tissues. However, critical point drying (CPD) for TEM samples needs to use acetic isomyl which also dissolves lipid. Therefore, in the present study, either the period of immersion in acetic isomyl was minimized, or CPD was not entirely employed. During various stages of dissolution of the matrix of lipid droplets, small subgranules were recognized.

The size of lipid droplets in brown adipocytes was smaller than that of white adipocytes. The surface of brown adipocytes was lumpy owing to the presence of such small, round lipid droplets under the surface [3]. Thus, the difference between white and brown adipocytes
Fig. 1. The surface of the white adipocytes in the rat. Compared with that of brown adipocytes, the surface of each white adipocyte is smoother. Connective fibers cover the whole tissue. Bar=10 μm, × 760.
Fig. 2. The surface of the brown adipocytes in the rat. Intracellular lipid globules can be recognized from the cell surface. Bar=10 μm, × 2,600.
Fig. 3. The fractured surface of the white adipose tissue. The honeycomb structure in the central part of the tissue is due to dissolution of lipid substance during specimen preparation. Bar=10 μm, × 360.
Fig. 4. The surface of fractured lipid droplets in white adipose tissue. A small subglobule (arrow) is recognized in a lipid droplet. Bar=10 μm, × 1,300.
Fig. 5. The network of connective fibers between white adipocytes. Many pores exist in the fractured surface of lipid droplets. Bar=10 \mu m, \times 2,200.

Fig. 6. The fractured surface of the brown adipose tissue. The relationship between cells is tight. Many lipid droplets and small granules (probably mitochondria) are observed in the cytoplasm. Bar=10 \mu m, \times 2,700.

Fig. 7. Small subgranules on the inner surface of a lipid globule after dissolution of matrix substance. Bar=10 \mu m, \times 4,000.
was remarkable in view of the freeze-fractured surface. The white adipocyte included a large lipid droplet within its poor cytoplasm, whereas the brown adipocyte had several lipid droplets and small granules (probably mitochondria). An intercellular connective tissue, which contained collagen and reticular fibers together with nerve fibers and blood vessels, commonly covered the adipocytes of the white adipose tissue [2, 4].

REFERENCES