Morphometry of Fine Structural Alterations of Hepatocytes of Japanese Monkeys under Fasting Stress

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ABSTRACT. The fine structural alterations of hepatocytes of Japanese monkeys under 4 days of fasting stress were analyzed morphometrically. One of the conspicuous alterations was the enlargement of mitochondria. The average diameter of mitochondria in fasting group increased to approximately 1.89-fold of that in control group, though their number did not change. The number of peroxisomes was 1.36-fold of that in control, though their area did not change. In addition, many of r-ER were swollen and were vesiculated. The appearance of bundle of actin-like stress fiber also increased in the fasting animals. The glycogen area as well as liver weight decreased in fasting group. — KEY WORDS: fasting stress, hepatocyte, monkey.

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Although there are some publications on the morphological alteration of liver under various stressors [4, 5, 20–25], no study on monkey liver has been reported. This experiment was to evaluate size (area) and number of mitochondria and peroxisomes by morphometry as a series of experiments on peroxisome induction by stress [24].

Of 20 Japanese monkeys (average body weight was 4.8 kg and average liver weight was 120 g), 12 of them were allowed to drink only water for 4 days, while 8 control animals were fed primate diet (PS, Oriental Yeast Co., Ltd.) *ad libitum*.

Under the code of animal experiment of Yamaguchi University, animals were anesthetized with overdose of ketalar (Sankyo Co.). The weight of liver was measured and the pieces of liver samples were fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer at pH7.4 followed by post-fixation with 1% OsO_4 for electron microscopy. Then, specimens were dehydrated in ethanol, infiltrated by propyleneoxide, and embedded in the mixture of epon 812 resin. Ultrathin sections were stained with ulanyl acetate and lead citrate. At least 3 blocks of resin were randomly selected from each animal and more than 5 electron micrographs for 1 block were taken at the same magnification (× 9,000). Both the number and the area of mitochondrion and peroxisome were measured by a computer-aided pattern analyzer (Olympus-PIAS PC-9801).

Compared with the fine structure of normal hepatocytes of control Japanese monkeys (Fig. 1), the most conspicuous ultrastructural alteration in hepatocytes of fasting monkeys was the enlargement of mitochondria (Fig. 2). The matrix of giant mitochondria was denser than that of normal ones and the cristae were irregular and occasionally displaced to the margin of mitochondrion (Fig. 2).

The dilatation or vesiculation of r-ER was also conspicuous in the fasting animal. The dilated rER was aggregated in the hepatocyte cytoplasm (Fig. 3). The stress fibers were often identified (Fig. 4). The number of glycogen granules decreased remarkably in the fasting hepatocytes.

The differences in size (area) and number of mitochondria (Table 1) and those of peroxisomes (Table 2) between fasting and control groups indicated that the area of mitochondria and number of peroxisomes significantly increased, while the number of mitochondria and area of peroxisomes were unchanged.

The percentage of liver weight to body weight decreased significantly from 2.54% (control) to 2.06% (fasting) (Fig. 5). The short term (24 hr) starvation induced a significant decrease in the weight and volume of hepatocytes of rats [5]. About 95% of hepatocyte atrophy was due to the decrease in glycogen and endoplasmic reticulum membranes, and about 5% was the depletion of lipid droplets [5]. The decrease in liver weight may be interpreted as the fasting-induced enhancement of protein degradation in rat liver[5]. Glycogen area also decreased in fasting hepatocytes in this experiment. In rats, the precipitous fall of glycogenic activity during the transition from the fed to fasting states was associated with a transient increase in plasma glucagon [23]. The fall of portal venous concentration of glucose and secretion of glucagon act as signals to initiate liver glycogen metabolism characteristic of the fasting state [23]. In the fasting conditions, epinephrine plays an essential role in stress-induced hyperglycemia [25].

The average diameter of mitochondria in unit area was approximately 1.89-fold in fasting animals, though the number of mitochondria did not change. The deformation of cristae, increase of matrix granules and the fusion of outer membranes of adjacent mitochondria [16] were related to the pathology of mitochondria [14, 16]. A series of biochemical changes caused by fasting stress were associated with the giant mitochondria. Fasting shifted the subcellular distribution of the enzyme toward mitochondria and refeeding in previously fasted rats shifted the distribution of the enzyme toward cytoplasm [1, 19]. Protein, vitamin and related nutritional deficiencies were also related to giant mitochondria. The effects of feeding a protein-free diet were reported on the mitochondrial respiration and on phospholipid fatty acids of mitochondria in the rat liver. The decrease in P/O (adenosine diphosphate (ADP) molecules consumed/oxygen atoms consumed during ADP-stimulated respiration) of mitochondria might be

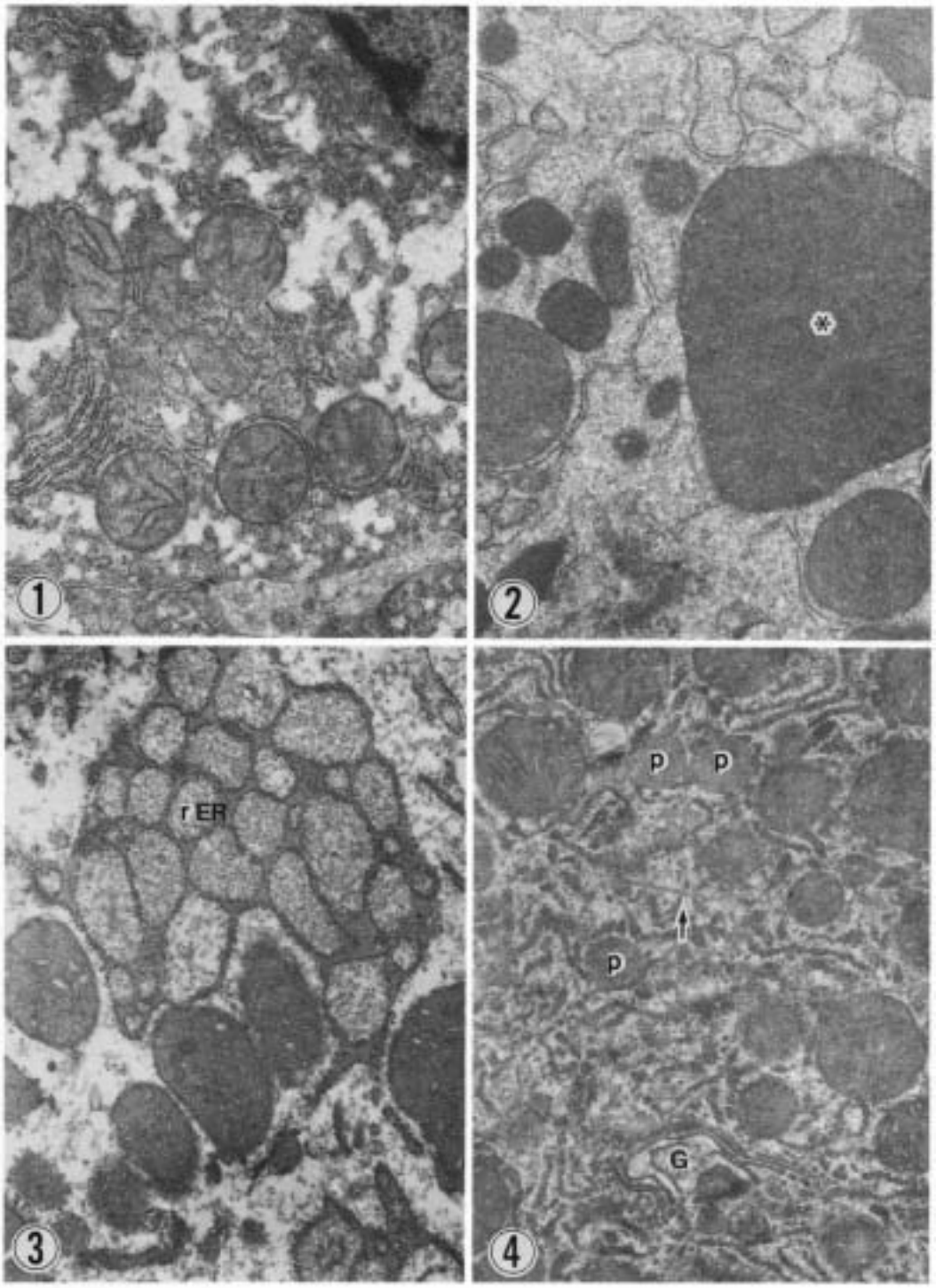

Fig. 1. Fine structure of a normal hepatocyte of control Japanese monkey. × 18,000.
Fig. 2. A megamitochondrion (*) in a fasting monkey hepatocyte. × 17,500.
Fig. 3. An aggregated r-ER (rER) in a hepatocyte of a fasting monkey. × 24,000.
Fig. 4. Stress fibers (arrow) in the cytoplasm of a hepatocyte of a fasting monkey. P: peroxisome, G: Golgi complex. × 17,500.

closely related to their enlargement [18]. The concentration of potassium ions in the mitochondria under fasting stress lowers by 20–30% at an average as compared to the control [6]. An essential fatty acid deficiency, enforced by the

Table 1. Number and area of mitochondria in μm^2 cytoplasm of hepatocytes in fasting and control groups (mean ± SE)

Group	Number of mitochondria	Area of mitochondria (μm^2)
stress	14.738 ± 0.789[a)]	3.665 ± 0.451[c)]
control	13.448 ± 1.016[b)]	1.944 ± 0.141[d)]

The *t*-tests were conducted between stress and control groups, respectively. a,b) P>0.05 and c,d P<0.05; There was a significant difference in mitochondrial size (area), though not in number of mitochondria.

Table 2. Number and area of peroxisome in μm^2 cytoplasm of hepatocytes in fasting and control groups (mean ± SE)

Group	Peroxisomal number	Peroxisomal areas (μm^2)
stress	0.319 ± 0.019[a)]	0.023 ± 0.0013[c)]
control	0.235 ± 0.0083[b)]	0.022 ± 0.0018[d)]

The *t*-tests were conducted between stress and control groups respectively. a,b P<0.05 and c,d P>0.05; There was a significant difference in number of peroxisome, though not in area of peroxisome.

presence of transfatty acid in the diet, was most likely the determining factor for the development of megamitochondria. In the fasting condition, the long-chain fatty acids can be transported through the inner mitochondrial membrane to the enzymes of fatty acid oxidation and ketogenesis [7].

Various conditions including starvation, cause the dilation of r-ER in hepatocytes. The ER hypertrophy (Fig. 3) was an earliest response of cells to stress. Endoplasmin in the endoplasmic reticulum is one of stress proteins [10, 11]. The starvation stress can induce the synthesis of the glucose-regulated proteins, which is involved in the stabilization of membrane-bound and secreted proteins by forming soluble complexes utilizing ATP [13].

In this experiment, the number of peroxisomes was significantly increased in fasting group, though the area of peroxisome did not change. However, both the number and the area of peroxisome in the renal tubule cells increased significantly in fasting monkeys [24]. The increase of peroxisomal number was associated with the increase of enzymes involved in the β-oxidation of fatty acid [15, 17].

When cells are exposed to heat or chemical stress, the expression of genes for heat-shock proteins (HSPS) is enhanced and stress proteins are accumulated in cells to tolerate against the additional stress and adapt to the new circumstances [3, 9]. Caloric restriction elicited stress proteins 27, 34, 70, and 90 in the hypothalamus [2]. In cultured hepatocytes, stress fibers, which are related to the stress proteins [8, 12], are often recognized. Although the relations between stress fiber, stress proteins, and target proteins, are beyond the scope of this report, the bundles of actin-like filaments (Fig. 4) are conspicuous in hepatocytes of animals under fasting stress.

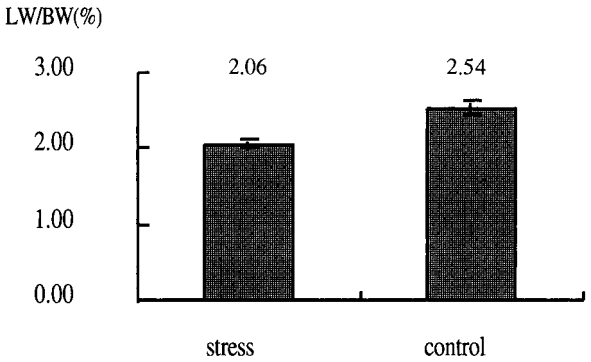

Fig. 5. Percentage of liver weight (LW) to body weight (BW). The mean values of percentage of LW/BW in the fasting groups and the control groups were 2.06% and 2.54%, respectively. With the *t*-test examination, there was a significant difference between fasting and control groups (P<0.05).

REFERENCES

1. Allred, J.B., Roman-Lopez, C.R., Pope, T.S. and Goodson, J. 1985. *Biochem. Biophys. Res. Commun.* 129: 453–460.
2. Aly, K.B., Pipkin, J.L., Hinson, W.G., Feuers, R.J., Duffy, P.H., Lyn-Cook, L. and Hart, R.W. 1994. *Mech. Ageing Dev.* 76: 11–23.
3. Andres, J. 1994. *Przegl. Lek.* 51: 246–248 (in Polish).
4. Babcock, M.B. and Cardell, R.R. 1974. *Am. J. Anat.* 143: 399–438.
5. Belloni, A.S., Rebuffat, P., Gottardo, G., Meneghelli, V., Coi, A., Mazzocchi, G. and Nussdorfer G.G. 1988. *J. Submicroscop. Cytol. Pathol.* 20: 751–757.
6. Chykhlova, E.A., Kudzina, L. Iu. and Evtodienko, Iu.V. 1982. *Ukrain. Biokhim. Zhurnal* 54: 190–193 (in Russian).
7. Foster, D.W. and McGarry, J.D. 1982. *Ciba F. Symp.* 87: 120–131.
8. Huot, J., Houle, F., Spitz, D.R. and Landry, J. 1996. *Cancer Res.* 56: 273–279.
9. Kato, K., Ito, H. and Inaguma, Y. 1996. *Nihon Shinkei Seishin Yakurigaku Zasshi* 16: 1–9 (in Japanese).
10. Koch, G.L.E., Macer, D.R.J. and Wooding, F.B.P. 1988. *J. Cell Sci.* 90: 485–491.
11. Koch, G., Smith, M., Macer, D. and Webster, P. 1986. *J. Cell Sci.* 86: 217–232.
12. Landry, J. and Hout, J. 1995. *Biochem. Cell Biol.* 73: 703–707.
13. Lee, A.S. 1987. *Curr.Opin. Cell Biol.* 4: 267–273, 1992.
14. Makita, T. 1993. pp. 505–517. *In*: Electron Microscopic Cytochemistry and Immunocytochemistry in Biomedicine (Ogawa, K. and Barka, T. eds.), CRC Press, Ann Arbor.
15. Makita, T. 1995. *Int. Rev. Cytol.* 160: 303–352.
16. Makita, T. and Sasaki, K. 1979. *Cytobios* 25: 183–192.
17. Masterd, C. and Crane, D. 1992. pp. 363–398. *In*: Membranology and Subcellular Organelles (Bitter, E.E. ed.), JAI Press, Greenwich.
18. Miyazawa, E. and Kametaka, M. 1990. *J. Nutr. Sci. Vitaminol.* 36: 251–263.
19. Ozawa, T. and Hanaki, A. 1985. *Biochem. Biophys. Res. Commun.* 129: 461–466.
20. Pokrovskii, A.A., Tashev, T.A., Krystev, L.P., Tutelian, V.A. and Krovskii, L.V. 1976. *Vopro. Pitan.* 2: 26–31 (in Rus-

sian).
21. Salas, M. and Tuchweber, B. 1978. *Exp. Geront.* 13: 213–225.
22. Salas, M., Tuchweber, B. and Kourounakis, P. 1980. *Pathol. Res. Pract.* 167: 217–233.
23. Shikama, H., Yajima, M. and Ui, M. 1980. *Biochem. Biophys. Acta* 631: 278–288.
24. Wresdiyati, T. and Makita, T. 1995. *Pathophysiology* 2: 177–182.
25. Yamada, F., Inoue, S., Saotoh, T., Tanaka, K., Satoh, S. and Takamura Y. 1993. *Endocrinology* 132: 2199–2205.