

# The effect of fasting on the ultrastructure of the hypothalamic arcuate nucleus in young rats

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*In the present study, we described ultrastructural changes occurring in the neurons of the hypothalamic arcuate nucleus after food deprivation. Young male Wistar rats (5 months old, n = 12) were divided into three groups. The animals in Group I were used as control (normally fed), and the rats in Groups II and III were fasted for 48 hours and 96 hours, respectively. In both treated groups, fasting caused rearrangement of the rough endoplasmic reticulum forming lamellar bodies and membranous whorls. The lamellar bodies were rather short in the controls, whereas in the fasting animals they became longer and were sometimes participating in the formation of membranous whorls composed of the concentric layers of the smooth endoplasmic reticulum. The whorls were often placed in the vicinity of a very well developed Golgi complex. Some Golgi complexes displayed an early stage of whorl formation. Moreover, an increased serum level of 8-isoprostanes, being a reliable marker of total oxidative stress in the body, was observed in both fasting groups of rats as compared to the control. (Folia Morphol 2009; 68, 3: 113–118)*

**Key words:** arcuate nucleus, fasting, membranous whorls, isoprostanes

## INTRODUCTION

The arcuate nucleus of the hypothalamus (ARH) is located in the lateral wall of the third ventricle above the median eminence. ARH takes part in the regulation of food intake, energy expenditure, and body weight regulation. There has been no report describing the effect of fasting on the formation of membranous whorls composed of concentric layers of the rough endoplasmic reticulum (RER) and Golgi complexes. However, they can be observed in the arcuate neurons after treatment with morphine, colchicine, or mercury salts [3, 5, 10]. The function of whorls is still unclear. Membranous whorls associated with RER and Golgi complexes were found in 5–16% of the neurons in mercury-treated animals [10]. Brawer [1] and Price et al. [13] also observed the appearance of

membranous whorls in the ARH neurons of a male rat on the fourth day after castration. Ford et al. [4] reported whorl proliferation in morphine-treated rats as early as at 24 hours. The aim of the present study was to determine whether short-term fasting induces ultrastructural changes in the neurons of the ARH.

## MATERIAL AND METHODS

### Animals

Inbred male Wistar rats, aged 5 months (body weight  $338 \pm 22$  g, mean  $\pm$  SD; n = 12) were used. The animals were housed 2 per cage, and they were maintained according to a controlled light schedule (with the light on during 7:00–19:00) at  $20 \pm 1^\circ\text{C}$ . They were fed with standard chow containing (w/w)

13% protein, 55.5% carbohydrate, 2.5% lipid, 1% calcium, 0.75% phosphates, and 27% indigestible compounds (Labofeed B, Kcynia, Poland). The animals were cared for and treated according to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. The study was approved by the Local Ethical Committee for Animal Experiments in Gdansk, Poland.

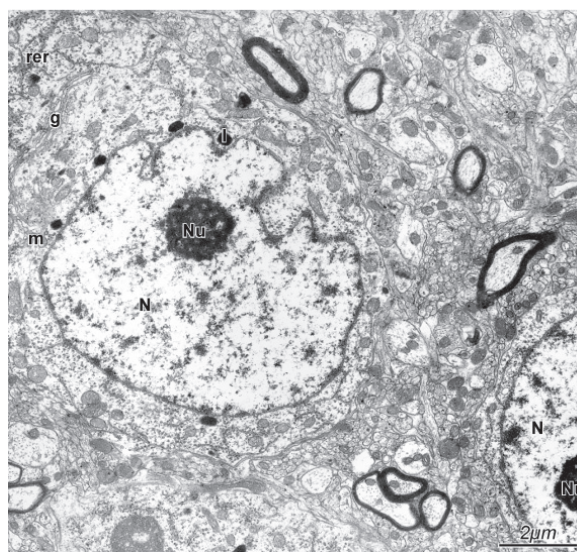
#### Dietary manipulation and sampling

The control rats (Group I) were fed *ad libitum* and sampled at the same time as the fasting animals. The fasting rats were sacrificed at 8:00 after having been starved for 48 hours (Group II) or 96 hours (Group III), respectively. Food consumption was calculated per 2 rats being housed together, and a body weight change was determined for each rat individually. The average body weight of the rats in groups I, II, and III was equal to  $342 \pm 27$  g,  $302 \pm 21$  g, and  $268 \pm 17$  g, respectively (mean  $\pm$  SD;  $n = 4$  in each group). The rats in Group I consumed  $10.8 \pm 1.1$  g chow per 100 g body weight over 24 hours. The rats were provided with water *ad libitum*.

#### Ultrastructural study

The animals were anaesthetized with 10% ketamine and were perfused transcardially with a mixture of 4% paraformaldehyde and 1% glutaraldehyde dissolved in 0.05 M phosphate buffer (pH 7.4) supplemented with 0.3%  $\text{CaCl}_2$ . After perfusion, the brains were removed from the skulls and stored in the same fixative at 4°C overnight. Next, hypothalamic tissue blocks were cut down on a vibratome 1000S (Leica, Germany) into serial coronal slices (500  $\mu\text{m}$  thick). Small tissue samples comprising arcuate nucleus were taken bilaterally from the rat brain under control of a binocular stereomicroscope. The samples were left within the fixative for 3 hours and then post-fixed in 1% osmium tetroxide for 2 hours. After dehydration with alcohols and propylene oxide, the specimens were embedded in Epon 812.

Semithin Epon sections were cut on a Reichert Om U3 ultramicrotome and then stained with 1% toluidine blue for light microscope examination in order to identify the arcuate nucleus. After the required area had been confirmed, ultrathin sections were then cut. The sections were stained with uranyl acetate and lead citrate and examined under a JEM 1200 EX II electron microscope (JEOL, Japan).



**Figure 1.** Control (normally fed) young rat. Neurons in the arcuate nucleus are separated by neuropile. The cytoplasm contains Golgi complex (g), randomly arranged strands of the rough endoplasmic reticulum (rer), mitochondria (m), and lysosomes (l). The large nucleus (N) exhibits a prominent nucleolus (Nu) lying near the slightly invaginated nuclear envelope. Bar: 2  $\mu\text{m}$ .

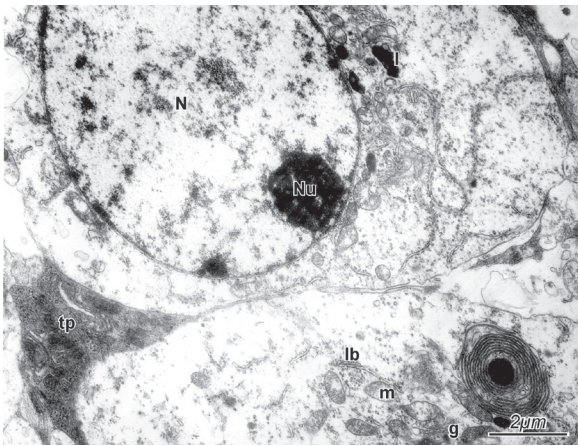
#### Immunochemistry

Serum 8-isoprostane was measured using commercially available ELISA kits (Cayman, Ann Arbor, MI, USA).

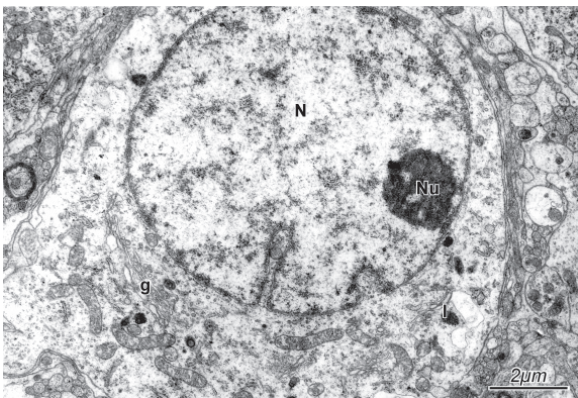
## RESULTS

#### Group I (fed control)

Neurons of the arcuate nucleus were round in shape and contained a large centrally placed nucleus (Fig. 1). The nuclear envelope was often invaginated. The invaginations usually contained ribosomes, sometimes mitochondria or lysosomes. The prominent nucleolus was frequently localized eccentrically. A thin band of cytoplasm contained several patches of Golgi complexes in the perinuclear region while the RER was sparse. Other organelles including ribosomes and mitochondria were distributed quite evenly in the cytoplasm. There were also few lysosome-like bodies present in the examined neurons. Short lamellar bodies, composed of two parallel cisternae of the RER, were occasionally observed in the cytoplasm. Membranous whorls, consisting of closely apposed concentric cisternae of the smooth endoplasmic reticulum (RE), were rarely found in the control animals.



**Figure 2.** Young rat fasted for 48 hours. The large nucleus (N) with prominent nucleolus (Nu) lying close to the nuclear envelope. At the bottom, the neuronal cytoplasm contains the membranous whorl near the Golgi complex (g). Mitochondria (m), lamellar body (lb), lysosomes (l), tanocyte process (tp). Bar: 2  $\mu$ m.



**Figure 3.** Young rat fasted for 96 hours. The arcuate neuron contains the large and round invaginated nucleus (N) with the prominent nucleolus (Nu) placed near the nuclear envelope. Extensive Golgi complexes (g) are arranged into incomplete perinuclear ring. Lysosomes (l). Bar: 2  $\mu$ m.

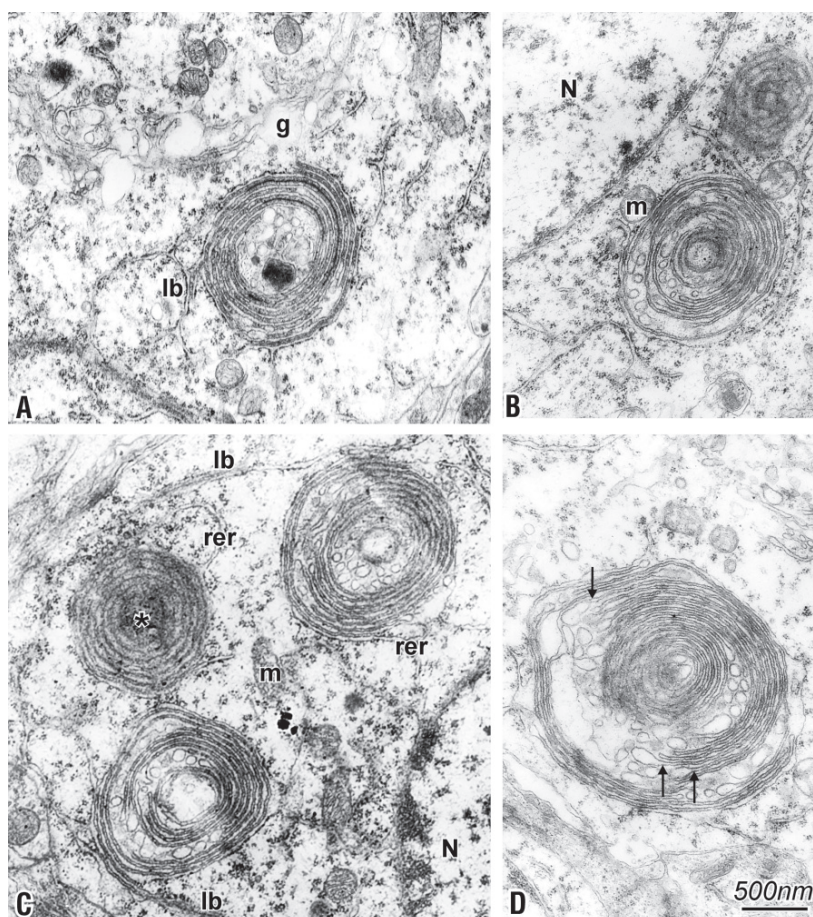
### Groups II and III (having fasted for 48 h and 96 h, respectively)

In both groups of fasted animals, the neurons of the arcuate nucleus were characterized by large, invaginated nuclei. The prominent nucleoli were often situated close to the nuclear envelope (Figs. 2, 3) or its invaginations as compared to the control rats. Furthermore, food deprivation, both for 48 hours and 96 hours, resulted in various rearrangements of the RER in the arcuate neurons. Lamellar bodies were observed (Figs. 2,

4A, C, 5A, 6A) among other ultrastructural changes. They were formed by two cisternae of the RER attached closely to each other. Their outer membranes were irregularly studded with ribosomes. The narrow cleft between two adjacent membranes was often filled with moderately dense material. In the control they were rather short, whereas in the fasting animals they became longer. Some of them curved to participate in the formation of membranous whorls (Figs. 4A, C, 6A) composed of concentric layers of the smooth endoplasmic reticulum. The outermost layers of the membranous whorls were covered with ribosomes and often appeared to be continuous with the RER cisternae. Different forms of the membranous whorls were detected in arcuate neurons. Most of them of regular structure (Figs. 4A–C, 6A) consisted of several layers arranged concentrically, usually around a cytoplasmic core. Sometimes small vesicles were observed between the layers. One to three membranous whorls were observed in a single cross section. Moreover, some of the membranous whorls present in the cytoplasm of arcuate neurons exhibited more vesiculated membranes. They were larger, multilayered, and often composed of semicircular incomplete rings with vacuoles budding off the ends of the smooth ER cisternae (Figs. 4D, 6B). One whorl was usually observed within a single cell. Occasionally, interconnected whorls were also stated (Fig. 5A). The outer membranes of two adjacent whorls were directly connected and were found to continue with the cisternae of RER. Membranous whorls were often seen in close association with the well-developed Golgi region. Some Golgi complexes were arranged into more or less complete circular structures (Figs. 5B, 6C). Their cisternae were extremely dilated with numerous clear vesicles in the vicinity. Such unique neuronal morphology was rarely found in the control animals. In addition, grouped lysosomes containing dark granules or lucent vacuoles were often noticed in close relationship with Golgi complexes.

Serum 8-isoprostane levels in the rats fasting for 96 hours (Group III) were equal to 13 ng/mL, whereas in the animals fasting for 48 hours (Group II) they averaged 15 ng/mL, in both cases being significantly higher in comparison to the fed control (Group I) level of 3 ng/mL ( $p < 0.01$ ; Fig. 7). There was no statistically significant difference between the animals fasting for 48 hours and 96 hours (Groups II and III).





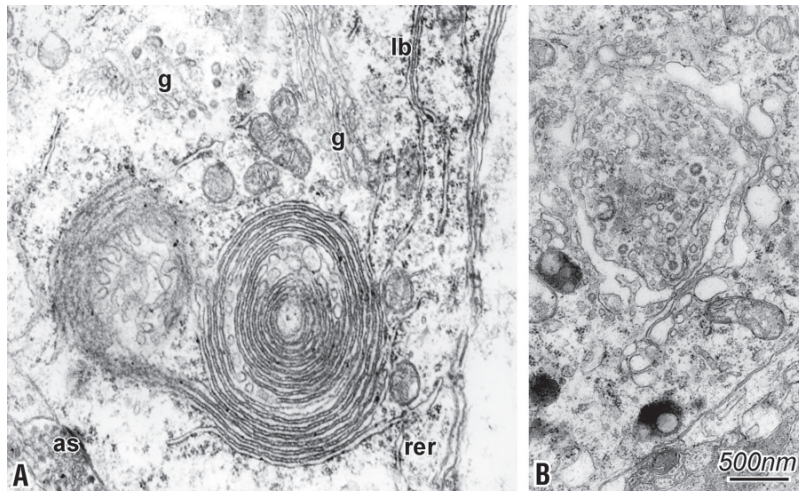
**Figure 4.** Young rat fasted for 48 hours. **A.** Lamellar body (lb) taking part in the formation of the membranous whorl localized near the extensive Golgi complex (g) in an arcuate neuron. Note the electron dense material between cisternae. Part of the cytoplasm is trapped in the centre of the whorl; **B.** Two membranous whorls resulting from the involution of the smooth endoplasmic reticulum in an arcuate neuron. Note the parts of cytoplasm trapped between layers and in the centre of the whorl. Nucleus (N), mitochondrion (m); **C.** A cluster of small whorled bodies in the arcuate neuron. One of them is blurred because of an oblique sectioning technique (asterisk). There is a continuation of the outermost layers which usually contain ribosomes with the rough endoplasmic reticulum cisternae (rer). In two of these structures the cisternae are partly fragmented into vesicles. The upper lamellar body (lb) is continuous with cisternae of the rough endoplasmic reticulum, whereas the lower one (lb), with the whorled body. Nucleus (N), mitochondrion (m); **D.** Large whorled body consisting of about 18 membranes. Vacuoles can be observed extending from the ends of the smooth endoplasmic reticulum cisternae (arrowheads). Bar: 500 nm.

## DISCUSSION

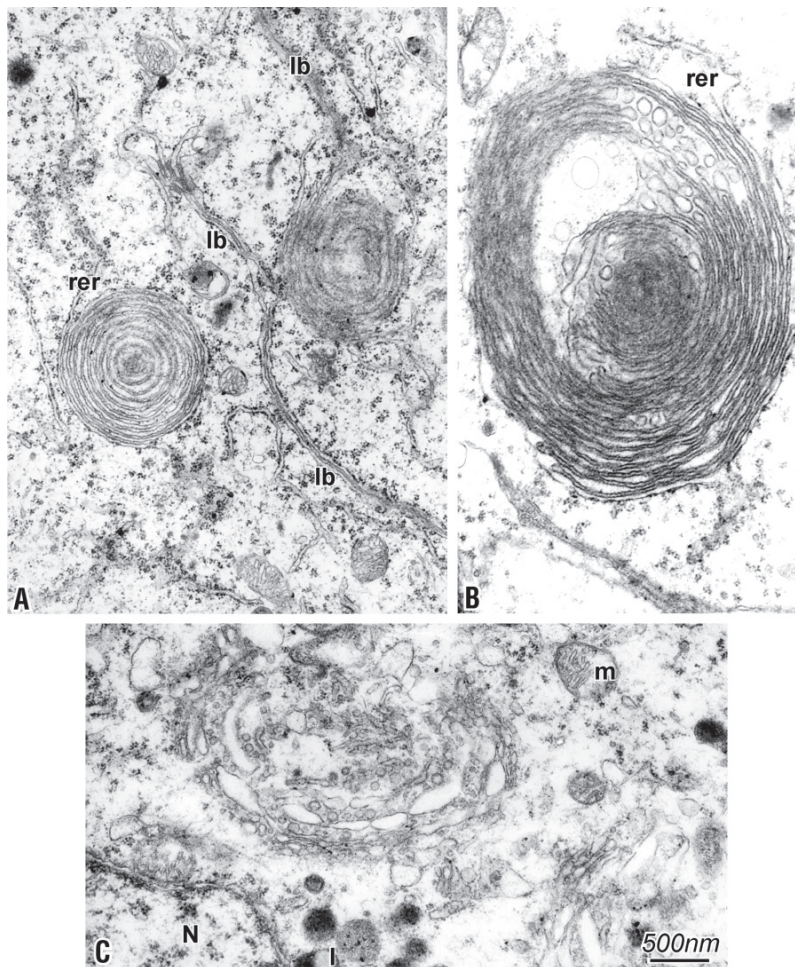
Our data constitute the first report on morphological changes in arcuate neurons during short-term fasting. In a previous electron microscopic study of the arcuate nucleus performed on rats 1 month after ovariectomy, Zambrano and de Robertis [15] noticed hyperactivity of the RER characterized only by dilated cisternae. We have already described sole dilatation of the RER cisternae and expansion of Golgi complexes in the hypothalamic paraventricular nucleus of male rats after fasting [9]. The present study was focused upon neurons of the arcuate nucleus. Under normal conditions, we noted only occasional membranous whorls there, whereas they were more often seen in ependymal tanyocyte pro-

cesses [2]. Naik [12] rarely observed similar structures in normal male rats. King et al. [6] noted that the formation of membranous whorls was dependent on the oestrous cycle being most intense during the diestrus. According to the authors, high plasma levels of oestrogen may activate the arcuate neurons, and thus the formation of whorls. These structures were also observed in the arcuate neurons of male rats treated with morphine [4], and they could be reversed with testosterone injection. Some investigators [1, 4, 6, 13] suggested that the whorls could be the sites of luteinizing hormone-releasing hormone synthesis. This hypothesis was later confirmed by immuno-electron microscopy [11]. Van Houten and Brawer [14] reported membranous whorls to

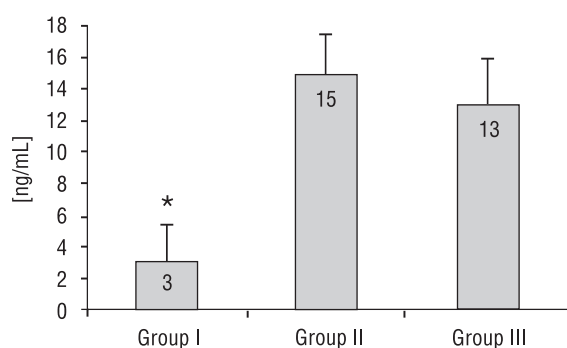




**Figure 5.** Young rat fasted for 48 hours; **A.** The two whorled bodies are continuous with one another and they are localized near the vesicular Golgi complexes (g). Some of the membranes are blurred because of the oblique cutting angle. Axosomatic synapse (as) identify cell as a neuron, lamellar body (lb), rough endoplasmic reticulum (rer); **B.** The extensive Golgi complex is formed into closed circular profile. Within this structure there are numerous small vesicles. Bar: 500 nm.



**Figure 6.** Young rat fasted for 96 hours; **A.** The cytoplasm of the arcuate neuron contains two whorled bodies typical of those commonly present in the arcuate neurons of rats deprived of food for 48 hours. The body on the left is continuous with the cisternae of the rough endoplasmic reticulum (rer), whereas the right one, with the lamellar bodies (lb); **B.** The large membranous whorl with incomplete outer cisternal rings. The outermost rings are continuous with the cisternae of rough endoplasmic reticulum (rer). Vesicles can be seen extending from the ends of the inner rings; **C.** Large Golgi complex rather vesicular in structure with some tendency to circle. Nucleus (N), mitochondrion (m), lysosome (l). Bar: 500 nm.



**Figure 7.** Serum 8-isoprostane levels in the young fasting rats as compared to the fed control (\* $p < 0.01$ ). Control rats (Group I); rats fasting for 48 hours (Group II); and fasting for 96 hours (Group III).

occur commonly in the ventrolateral part of the ventromedial nucleus (VMN) in normal female rats, presuming that they might be involved in the synthesis of luteinizing hormone-releasing hormone. As reported in our previous paper [8], the ultrastructural changes in the VMN after fasting did not include whorls, most probably because the samples were taken from the central part of the VMN. Kiss [7] observed the endoplasmic reticulum forming lamellar and concentric bodies in the hypothalamic VMN of male rats after repeated immobilization stress. In addition to that, we observed an early stage of membranous whorl formation from Golgi complexes in the initial phase of fasting. It was probably too short for expression of typical morphology of the whorls. In our material, we often observed whorls being continuous with the RER and localized near Golgi regions. Similarly, in the arcuate nucleus. Lamperti et al. [10] noticed whorls associated with the RER and Golgi network in mercury-treated female hamsters. Direct comparison of our study with the results of the cited authors does not seem possible due to major differences in experimental conditions.

We observed a significant increase of the serum level of 8-isoprostanes (over 4-fold), being a marker of *in vivo* lipid peroxidation, in the two groups of fasting rats as compared to the fed controls. Moreover, the formation of membranous whorls observed in ARH of both fasting rat groups correlated with a marked increase in 8-isoprostane serum levels, being a widely accepted parameter of the total bodily oxidative stress.

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