

Brown Adipose Tissue in Amyotrophic Lateral Sclerosis

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Abstract: The prevalence of brown adipose tissue (BAT) in the periadrenal adipose tissue was studied in 20 autopsy cases of amyotrophic lateral sclerosis (ALS). In 19 of 20 cases, the periadrenal BAT in focal or diffuse distribution was observed. Histometry of BAT revealed average occupancy rates of 21.6% and 4.2% in ALS and age-matched control cases, respectively ($p < 0.01$). Since emaciation and chronic hypoxia in those ALS patients were most likely related to severe muscle wasting and respiratory involvement, it is speculated that an increase in BAT occupancy rates may be secondary to altered thermogenesis which was augmented by chronic hypoxia.

Key words: brown adipose tissue, amyotrophic lateral sclerosis

INTRODUCTION

The major sites of thermogenesis in humans are believed to be the skeletal muscle, liver, and brown adipose tissue (BAT). BAT develops in neonate and the early stages of life as an important site of non-shivering thermogenesis (NST) and diet-induced thermogenesis (Lean and James, 1986). The brown adipocyte is a characteristic small multilocular cell with an abundance of ovoid or pleomorphic mitochondria with complete transverse cristae. However, characteristic multiloculated BAT recedes in adulthood and occasional microfoci are then observed in the perirenal and retroperitoneal regions (Heaton 1972). It is a well known fact that the skeletal muscle plays an important role in shivering thermogenesis, but the involvement of skeletal muscle in human adult NST is still controversial (Dubosi-Ferriere and Chinet, 1981; Astrup *et al.*, 1985). Several reports have suggested that skeletal muscle contributes largely to NST in large mammals including adult humans (Astrup *et al.*, 1985; Schaefer *et al.*, 1982). The nature of the cellular mechanism that might be responsible for muscle-mediated NST is unclear. In a previous report, we described well developed BAT in Duchenne's progressive muscular dystrophy, a myogenic muscular disease (Ito *et al.*, 1988). We speculated that the BAT was activated by chronic hypoxia secondary to respiratory muscle involvement and to compensate for the poor thermogenesis of severely damaged striated muscle including skeletal and cardiac muscle. This observation raised the question of the occurrence of BAT in neurogenic muscle involvement diseases. Amyotrophic lateral sclerosis

(ALS) is a well known motor neuron disease resulting in severe skeletal muscle atrophy.

The purpose of this study is to evaluate the occurrence of BAT in patients with ALS with severe neurogenic atrophy. Necropsy sections of fat were chosen for retrospective review from the periadrenal area because it has been shown that BAT persists in this anatomic site until the latest decades of life.

MATERIALS AND METHODS

Twenty cases of ALS were selected from consecutive autopsy cases in the National Kawatana Hospital. The diagnosis of ALS was confirmed on the basis of clinical symptoms and autopsy findings. All cases showed skeletal muscle atrophy featuring neurogenic group of fascicular atrophy. The atrophic spinal cords exhibited massive neuronal cell loss in the anterior horn and demyelination and gliosis of the lateral and anterior columns. The patients' ages ranged from 12 to 76 years (mean 54.4 ± 14.8). Fifteen cases were male and five were female. For the age-matched controls, 16 cases with a mean age of 55.8 ± 8.2 years were selected. Thirteen cases were male and three were female. Subjects with pheochromocytoma (Warren and Chute, 1972), severe congestive heart failure (Shellock *et al.*, 1985), cor pulmonale (Teplitz and Lim, 1974), and previous history of prolonged cold-exposure (Huttunen *et al.*, 1981) were excluded from the control group because BAT is typically associated with these conditions. In order to evaluate the severity of skeletal muscle atrophy, the emaciation index (EI) was calculated in each case on the basis of the ideal body weight, that is, $[\text{body weight} - (\text{height} - 100) \times 0.9] / (\text{height} - 100) \times 0.9$. This formula indicated deviation percentages from the ideal body weight. The EI was -27.8 ± 14.1 and $2.4 \pm 10.6\%$ in ALS and age-matched controls respectively. The age-matched controls were selected cases which satisfied the EI within $\pm 20\%$. For retrospective evaluation, necropsy sections of fat were selected from the periadrenal area because it is part of the normal brown fat pad distribution in mammals. The histologic sections were prepared by fixation of tissues in 4% formaldehyde, processing in graded alcohols, embedding in paraffin, sectioning at $3\mu\text{m}$, and staining with hematoxylin-eosin. The periadrenal adipose tissue was photographed on no less than six fields at total magnification of 25 in each case. Quantitatively, the occupancy rate of BAT in the periadrenal adipose region was calculated by the Leitz's image analyzer. The formula was as follows; characteristic multiloculated BAT area/characteristic BAT and unilocular (white adipose tissue like) area $\times 100\%$. Subsequently, the correlation between the EI and occupancy rate was examined in the ALS and control groups together. Electron microscopic observation was applied in two cases of ALS. Cause of death and blood gas level were investigated on the clinical charts. The values of arterial oxygen tension and arterial carbon dioxide tension were measured with or without a respirator.

Student's *t* test or χ^2 test were used for statistical analysis in this study. The data were expressed as mean \pm SD.

RESULTS

Table 1 shows the results of the present study. Multiloculated brown adipose tissue was consistently observed in the periadrenal adipose tissue in 95% of cases of ALS in comparison with 63% of age-matched controls. However, the interindividual variation was pronounced, the number of multiloculated brown adipocytes varied from single cells scattered among unilocular adipocytes to large cell clusters of confluent multiloculated brown adipocytes. In ALS cases BAT distribution was diffuse or focal, but in age-matched control cases it was very scanty. Two kinds of BAT were present in the periadrenal region as reported previously in Duchenne's muscular dystrophy (Ito *et al.*, 1988). One was oxyphilic granular brown adipocytes with complete or almost complete fat depletion, and the other was multilocular adipocytes characteristic of stimulated BAT with numerous small or large fat vacuoles. The majority of brown adipocytes observed in this study were cells with multivacuolated cytoplasm (Fig. 1). Granular brown adipocytes with eosinophilic cytoplasm and centrally located nuclei were encountered in five cases of ALS (Fig. 2). Electron microscopy revealed an abundant number of characteristic BAT mitochondria with well developed complete transverse cristae in the brown adipocytes of ALS cases (Fig. 3). On the contrary, nonstimulated unilocular adipose tissue was indistinguishable from white adipose tissue. The average stimulated BAT occupancy rate value was 21.6 ± 22.2 and $4.2 \pm 6.2\%$ in ALS and age-matched controls respectively ($P < 0.01$). A correlation existed between stimulated BAT occupancy rate and EI in ALS ($r = -0.466$, $p < 0.05$, $n = 20$) (Fig. 4).

In ALS, arterial oxygen tension ranged from 30.9 to 81.2 mmHg, with a mean of 67.2 ± 16.7 mmHg, and arterial carbon dioxide tension ranged from 36.8 to 84.0 mmHg, with a mean of 55.1 ± 14.3 mmHg. Clinical histories and blood gas findings indicated that many cases of ALS fell in under the category of carbon dioxide narcosis. Commonly, ALS patients died of respiratory failure due to respiratory muscle atrophy.

Table 1. Summary of EI, BAT incidence and BAT occupancy rate in ALS

| Group | No. | Sex | Age | EI | BAT | BAT Occupancy |
|---------|-----|------|------|---------|--------------|---------------|
| | | M/F | | | Incidence % | Rate % |
| ALS | 20 | 15/5 | 54.4 | -27.8** | 95.0 (19/20) | 21.6* |
| Control | 16 | 13/3 | 55.8 | 2.4 | 63.0 (10/16) | 4.2 |

EI, emaciation index; Data are expressed as mean value. *, ** indicates significant differences at $p < 0.01$ and $p < 0.001$ respectively. Mean BAT occupancy rate was calculated from all individuals in a group.

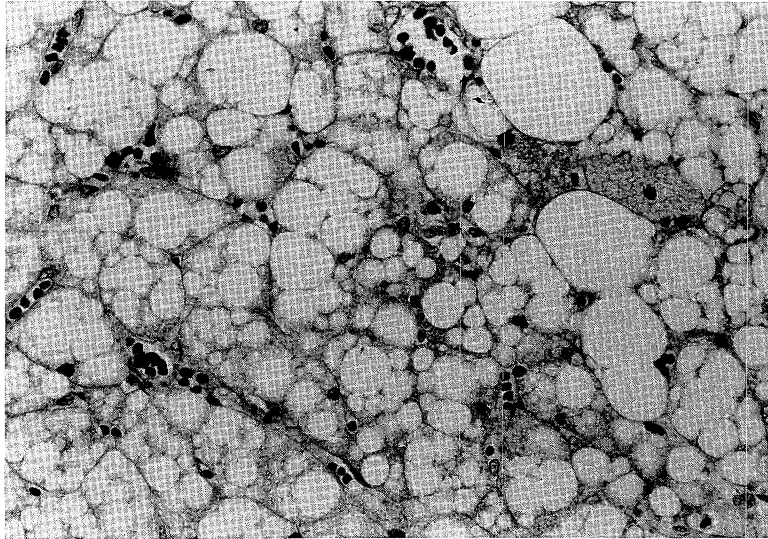


Fig. 1. Clusters of multilocular brown adipocytes were present in the periadrenal region in patients with ALS. (hematoxylin-eosin, $\times 200$)

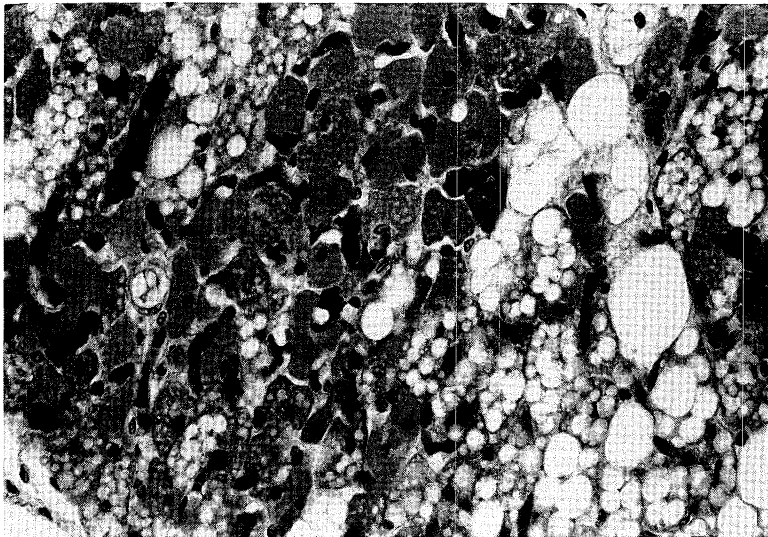


Fig. 2. Typical brown adipocytes with eosinophilic granular cytoplasm and centrally placed nuclei were present in a few cases of ALS. (hematoxylin-eosin, $\times 200$)

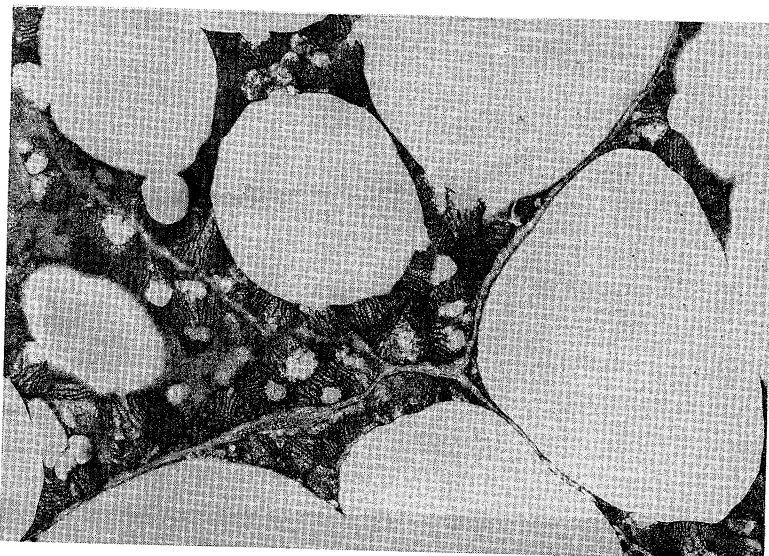


Fig. 3. Abundant mitochondria and well developed cristae were observed in the cytoplasm of brown adipocytes (uranyl-lead double staining, $\times 5,000$)

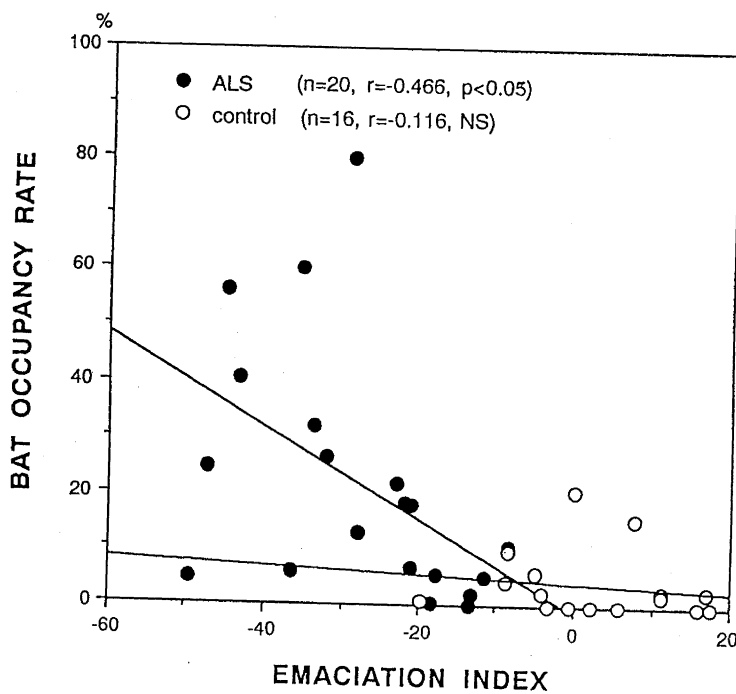


Fig. 4. A correlation existed between the emaciation index and the BAT occupancy rate in ALS was existed ($r = -0.446$, $p < 0.05$). The closed circle indicates ALS cases and the open circle represents age-matched controls.

DISCUSSION

BAT is highly innervated with noradrenergic fibers and activated by noradrenaline released from regional nerve terminals, especially via beta receptors (Lever *et al.*, 1986). Multiloculated BAT is normally well developed in small animals and mammalian neonates. Using the unique uncoupling metabolic processes (Rothwell and Stock, 1984), neonatal humans and other small mammals utilize BAT to generate body heat during the vulnerable period after birth. The total BAT mass has been estimated to comprise 2 to 5% of body weight in the human neonate (Lean and James, 1986). A number of studies on human neonate have been carried out, but the investigation of the presence and significance of BAT in the adult is a relatively recent subject (Shellock *et al.*, 1986; Seydoux, 1983). The distribution of BAT in humans was described in detail by Heaton (1972). Tanuma *et al.* (1976) also reported the prevalence of BAT in Japanese patients with various conditions.

Teplitz and Lim (1974) have described the transformation of perirenal white adipose tissue into BAT in cases of severe chronic hypoxemia, a condition which is also accompanied by an increased level of circulating catecholamines. Patients with ALS have increased levels of the neurotransmitter norepinephrine in blood and cerebrospinal fluid (Zeigler *et al.*, 1980a; Zeigler *et al.*, 1980b), and this increment is particularly marked in patients bed-ridden with advanced disease. This may be related to chronic hypoxemia induced by respiratory muscle involvement in the advanced stage of ALS. It is suggested that BAT transformation in ALS results from chronic hypoxemia. But the authors raise the questions as to whether BAT transformation could also be a compensation for thermogenesis that is lost consequent to severe muscular involvement, because skeletal muscle plays a major role in the thermogenic action of beta-agonist. It is generally accepted that skeletal muscle participates in the thermogenic response to cold and catecholamine in rodents and humans. Astrup *et al.* (1985) reported that skeletal muscle can account for about 50% of the ephedrine-induced thermogenesis but no important thermogenic function of human BAT in healthy volunteer has been detected in the adult. In muscle from adults with ALS, the basal activity of adenylyl cyclase was lower than normal (Mawatari *et al.*, 1974). It is natural that degenerated skeletal muscle could not produce enough heat by shivering and NST. Previously, we reported a high prevalence of BAT in patients with Duchenne's progressive muscular dystrophy (Ito *et al.*, 1988). We considered that severe skeletal muscle atrophy results in poor thermogenesis and induces BAT transformation for compensation of myogenic heat production.

It is concluded that an increase in activated BAT transformation may be secondary to altered thermogenesis which was augmented by chronic hypoxia since emaciation and chronic hypoxia in ALS patients were most likely related to severe muscle wasting and respiratory involvement.

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