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# The Occurrence of Brown Adipose Tissue in Outdoor Workers

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**Summary.** Histochemical reactions and activities of mitochondrial enzymes in adipose tissue around the neck arteries and in pericardium were studied in men who had been outdoor workers in northern Finland. The purpose was to study the occurrence of brown fat in workers having been exposed to cool or cold ambient temperature. Indoor workers of the same age were used as controls.

Histochemically, no mitochondrial enzyme reactions were seen in the adipose tissues taken from the indoor workers, whereas some outdoor workers had some multilocular adipose tissue, mostly around the neck arteries. Biochemical parameters also showed increased enzyme activities of aerobic energy metabolism in the adipose tissue of these people.

The present results suggest that working in the cold can retain brown adipose tissue in "strategic" places in human adults.

**Key words:** Cold-exposure – Mitochondrial enzymes of adipose tissue – Human adults

## Introduction

In most small animals shivering is replaced by the more economical non-shivering thermogenesis (NST) during long-term cold exposure, and this type of cold acclimation is also seen in the small neonates of larger species, including man (Brück 1961). NST involves calorigenesis via increases in the metabolic activity of brown adipose tissue and the mechanical activity of the heart and respiratory muscles (Foster and Frydman 1978).

It has been claimed that human adults have no typical brown adipose tissue and that shivering is their most important mechanism for generating heat when exposed to the cold. Studies in primitive human beings living naked or poorly clothed in cold environments nevertheless show that man behaves like other species and adapts to the cold through certain metabolic changes (LeBlanc

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1975). Prolonged exposure to cold also reduces shivering in white men, but it is suggested that this adaptation is acquired through habituation (Brück 1976).

Brown adipose tissue has been observed in some human adults (Hassi 1977), and it seems that these individuals have spent more time outdoors than is usual in modern technological society. This observation has led us to study the possible occurrence of brown adipose tissue in human adults who have been working outdoors in northern Finland where the ambient temperature is cool or cold for most of the year. One part of their acclimation to cold could consist of heat production in the brown adipose tissue, which has been observed around the neck arteries of such people at necropsy. Histochemical and biochemical parameters in necropsy samples of adipose tissue support such a hypothesis, i.e., increased activities of the enzymes of aerobic energy metabolism are found in these people.

## Material and Methods

The material, collected at necropsies carried out at the Department of Forensic Medicine, University of Oulu, concerns human adults over 25 years of age (25–63 years) who had been outdoor workers or otherwise were known to spend a considerable time outdoors: lumberjacks, painters, timbermen, farmers and skid row alcoholics, and a further 10 indoor workers of the same age as controls. The adipose tissue around the neck arteries and in the pericardium was excised. The histochemical reactions of the enzymes studied do not change significantly during the first seven days postmortem (Hassi 1977), and the interval for the present samples varied from 1–5 days.

For the histochemical enzyme reactions a portion of each sample was frozen in liquid nitrogen and cut into 10 μ sections at −25° C. Modifications of the methods of Nachlas et al. and Hess et al. were used for the determination of succinate dehydrogenase (SD) and β-hydroxybutyrate dehydrogenase (HBD) respectively (see Barka and Anderson 1965). Cytochrome oxidase (CYO) was demonstrated by the method of Burstone and monoamine oxidase (MAO) following the tetrazolium method of Glenner et al. (see Barka and Anderson 1965). The percentage of multilocular enzyme positive fat cells was estimated microscopically without knowledge of the origin of the sample.

The intensity of the oxidative enzyme reactions as described above forms a reliable way of estimating differences in metabolic activities of white and brown fat tissue (Fawcett 1952; Menschik 1953; Hassi 1977).

In order to obtain more accurate knowledge of the enzyme activities the following biochemical methods were also used: The mitochondria from the adipose tissue samples were immediately isolated according to Bukowiecki and Himms-Hagen (1976), and a sample was homogenized in 0.25 M sucrose-0.001 M EDTA-0.01 M Tris-buffer, pH 7.4, and centrifuged twice for 10 min at 900 *g*. The supernatant was then centrifuged for 10 min at 5,600 *g* and the mitochondria were washed twice with the isolation medium. They were then suspended in the isolation medium for determination of the activity of SD and CYO and the protein content. SD activity was determined using phenazinemethosulphate and 2,6-dichloroindophenol as the electron acceptor system, according to King (1967). Succinate stimulated maximal activity was determined by the method of Kimura et al. 1967. CYO activity was determined spectrophotometrically by measuring the rate of aerobic oxidation of ferrocytochrome catalyzed by cytochrome oxidase at 21° C (Yonetani and Ray 1965). The protein concentration was determined by the method of Lowry et al. (1951).

## Results

No positive histochemical enzyme reactions for SD, HBD, CYO, and MAO were observed in the samples of adipose tissue in the controls, whereas these

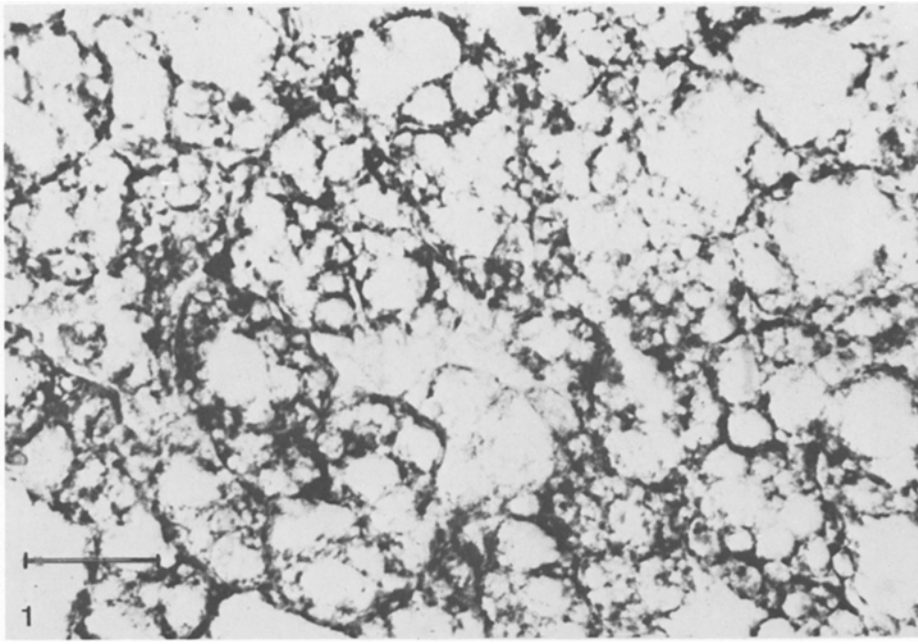


**Fig. 1.** Brown adipose tissue from the neck of a 48 years old timberman. Plenty of multilocular fat cells with strong reaction of $\beta$-hydroxybutyrate dehydrogenase. 250×. Same tissue had a positive cytochrome oxidase reaction, too

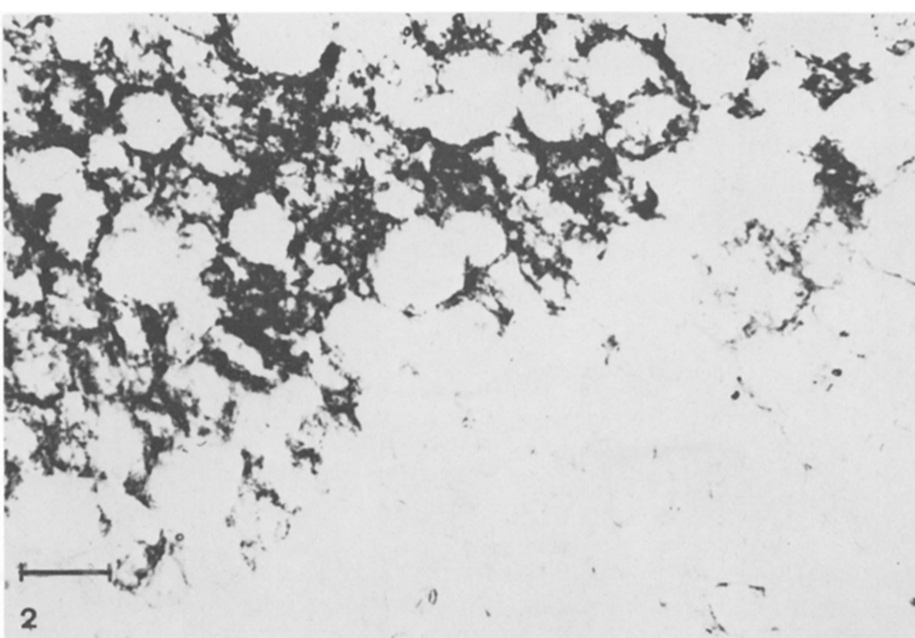


**Fig. 2.** Adipose tissue from the neck of a 25 years old lumberjack. Upper left there is brown fat with multilocular cells having strong reaction of succinate dehydrogenase. Lower right there is white fat with unilocular cells devoid of enzyme reaction. 160×

**Table 1.** Histochemical reactions of mitochondrial oxidative enzymes (β-hydroxybutyrate and succinate dehydrogenases, cytochrome and monoamine oxidases) and activities of mitochondrial succinate dehydrogenase and cytochrome oxidase in the adipose tissues of human adults

| Occupation | Age (years) | Postmortem time (days) | Percentage of enzyme-positive fat cells in the specimens | | | | | | | | Enzyme activities μmol/min/100 g of wet weight | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pericardial | | | | Cervical paravascular | | | | Pericardial | | Cervical paravascular | |
| | | | HBD | SD | CYO | MAO | HBD | SD | CYO | MAO | SD | CYO | SD | CYO |
| Lumberjack | 25 | 2 (December) | – | – | – | – | ++ | + | ++ | ++ | 8.32 | 2.42 | 13.47 | 4.59 |
| Lumberjack | 27 | 3 (August) | – | – | – | – | + | + | – | – | No sample | | No sample | |
| Lumberjack | 30 | 1 (February) | No sample | | | | ++++ | ++++ | ++++ | ++ | No sample | | 3.23 | No sample |
| Lumberjack | 34 | 5 (March) | – | – | – | – | ++ | ++ | – | + | N | N | N | 0.02 |
| Timberman | 48 | 4 (May) | No sample | | | | +++ | +++ | +++ | +++ | No sample | | No sample | |
| Farmer | 58 | 2 (February) | No sample | | | | + | + | – | + | No sample | | No sample | |
| Farmer | 63 | 3 (January) | No sample | | | | – | – | – | – | No sample | | N | N |
| Farmer (retired) | 47 | 4 (April) | No sample | | | | – | – | – | – | No sample | | N | N |
| Farmer (retired) | 60 | 5 (January) | – | – | – | – | – | – | – | – | N | N | N | N |
| Painter | 49 | 2 (December) | – | – | – | – | – | – | – | – | N | N | N | N |
| Skid row alcoholic | 56 | 1 (January) | + | – | – | ++ | +++ | +++ | ++ | +++ | 2.77 | 4.74 | 7.15 | 2.53 |

Enzyme positive fat cells – = 0%
+ = 5–15%
++ = 20–40%
+++ = 50–70%
++++ = 80–100%
N = no measurable activity

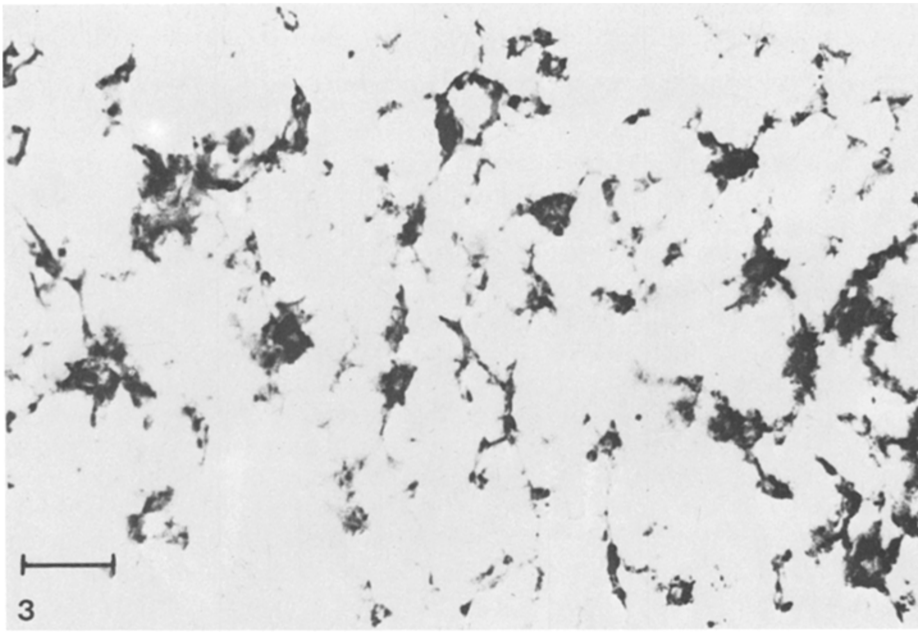


**Fig. 3.** Brown adipose tissue having both multilocular and unilocular fat cells with positive reaction of monoamine oxidase. (White fat was always negative.) The sample is from a 60 years old skid row alcoholic. 160×. The bar in the pictures is 100 $\mu$

reactions were observed in seven out of eleven cases among the outdoor workers (Table 1 and Figs. 1–3). Positive fat cells were mostly seen in the samples taken from adipose tissue around the carotic arteries. 20–90% of the cervical paravascular adipose tissue cells were multilocular in lumberjacks who had been actively working and had died in the middle of the winter, but only about 10% of the adipose cells were multilocular in a lumberjack who had died in August. About 60% of the cervical paravascular cells were multilocular in a timberman and a skid row alcoholic. Neither the painter nor the lumberjacks or the farmers who had retired had any multilocular adipose cells. Mitochondrial protein and SD and CYO activity were found in the same cases as the positive histochemical enzyme reactions (Table 1).

## Discussion

Under cold conditions, newborn infants generate heat by non-shivering thermogenesis, a mechanism similar to that observed in animals (Brück 1961). The human neonate has no compact pad of interscapular adipose tissue, but brown fat is distributed close to the vital organs (Aherne and Hull 1964). The number of fat cells in the brown adipose tissue begins to fall after the first perinatal year, and the adult human being has little or no such tissue left. The prevalence of brown adipose tissue in human adults aged 25 years or more in northern Finland has been found to be about 10% (Hassi 1977). The present human adults who had been indoor workers had not retained any multilocular fat, whereas some outdoor workers such as lumberjacks and a timberman, as well as a skid row alcoholic, had some multilocular adipose tissue, mostly around the carotic arteries. The presence of histochemical reactions and activities of mitochondrial enzymes suggests that working in the cold can cause man to reactivate or retain the oxidative metabolism of the adipose tissue around the neck, at least. The amount of the multilocular adipose tissue in the lumberjacks was the greater, the longer they had been exposed to cold recently. Thus the lumberjack who had died in August did not have so much multilocular adipose tissue as the one who had died in February. This points partly to a disappearance of the mitochondrial enzyme reactions with a return to warm conditions. The same phenomenon was also observed with retired farmers.

Adaptation to cold in human is thought to involve central nervous habituation (Brück 1976), although the enhanced response to noradrenaline in Japanese women (Itoh 1974) provides some evidence of NST in man, first suggested by Davis (1961) and Joy (1963). Non-shivering thermogenesis, achieved by the enhanced utilization of lipids has been found to develop in men who are adapted to the winter cold in Hokkaido (Doi et al. 1979) and the present results point to the possibility that one aspect of NST in man could consist of heat production in brown adipose tissue, which is observed in "strategic" places in persons who need cold acclimation. Similar phenomena occur during cold-acclimation in adult guinea-pigs, which have no multilocular brown adipose tissue when reared under warm conditions (Hirvonen et al. 1973, Huttunen and Kinnula 1979).

The metabolic activity in brown adipose tissue is much higher than in white adipose tissue, and the vascular connection between the brown adipose tissue and other organs is important to its thermogenetic function. A vascular connection between cervical brown adipose tissue and the vertebral canal has been found in the human neonate (Aherne and Hull 1964). With external cooling, the temperature of the cervical brown adipose tissue and vertebral canal increases, and heat flows to the spinal cord, suppressing shivering. Although a marked elevation in heat production occurs, decreased shivering, an essential feature of cold acclimation, is reported in men adapted to the winter cold in Hokkaido (Doi et al. 1979), which means a saving of energy stores for physical work.

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