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Immunohistochemical detection of leptin in interscapular brown adipose tissue of hypothyroid rats

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Leptin is protein hormone involved in regulation of many physiological processes including energy expenditure [1]. In mammals, adipose organ is the most important source of leptin. White adipose tissue (WAT) synthesizes and secretes leptin in proportion to body fat, while contribution of brown adipose tissue (BAT) is somewhat controversial and generally considered as less important. In rodents, acting via hypothalamic receptors, leptin enhances sympathetic input in BAT, stimulates uncoupling protein-1 synthesis and energy dissipation [2,3].

Thyroid hormones (THs) regulate numerous cellular processes in all tissue types. BAT thermogenic function is closely related to optimal THs supply. In systemic hypothyroidism, in the presence of even minimal circulating THs, BAT-deiodinase 2 provides sufficient triiodothyronine locally and protects BAT from hormone deficiency [4]. Given that leptin and THs are engaged in regulation of BAT thermogenesis and bearing in mind that role of brown adipocytes in leptin production is not clearly defined, the aim of this study was to investigate the pattern of leptin immunoexpression in BAT of systemic hypothyroid rats.

Male Wistar rats (130-150 g) maintained under standard laboratory conditions were used. The animals from the experimental group (n=8) were made hypothyroid by drinking methimazole solution (Sigma, St. Louis, MO, USA) (0.02% in tap water), for three weeks. The other animals (n=6) were untreated controls. At the end of the experiment interscapular BAT was isolated and routinely prepared for light microscopy. Leptin-immunoreactivity was assessed on 5 μm thick paraffin sections by avidin-biotin peroxidase method, using rabbit primary polyclonal anti-leptin and secondary goat anti-rabbit antibodies (both at 1:200) (SantaCruz Biotechnology, Santa Cruz, CA, USA).

Leptin-positive cells were observed in BAT of both control and hypothyroid rats. They could be classified into three morphological categories: (a) multilocular adipocytes containing few large lipid droplets, (b) unilocular adipocytes commonly present in BAT and (c) classic multilocular brown adipocytes (Figure 1A, B and C). Immunopositive adipocytes (a) and (b) were seen in both control and experimental group, while positive adipocytes (c) were noticed almost exclusively in hypothyroid rats. Leptin-positive cells were noticeably more abundant in BAT of hypothyroid rats.

It has been reported previously that in rodents serum levels of THs and leptin are inversely regulated [5]. Increase in circulating leptin in hypothyroid rats was explained by increased leptin synthesis in WAT depots [6]. Relatively frequent occurrence of leptin-positive brown adipocytes in interscapular BAT of hypothyroid rats observed in this study suggests the possibility that aforementioned leptin increment may at least in part be the result of enhanced synthesis in BAT. Modulation of leptin synthesis in brown adipocytes is probably caused by intensified lipogenesis characteristic for BAT in hypothyroidism [7]. Considering that, leptin is also able to exert direct effects on brown adipocytes [8] and that such effects are in general sense positively correlated with their function, observed local increase in leptin expression may support thermogenic activity of BAT in systemic hypothyroidism.

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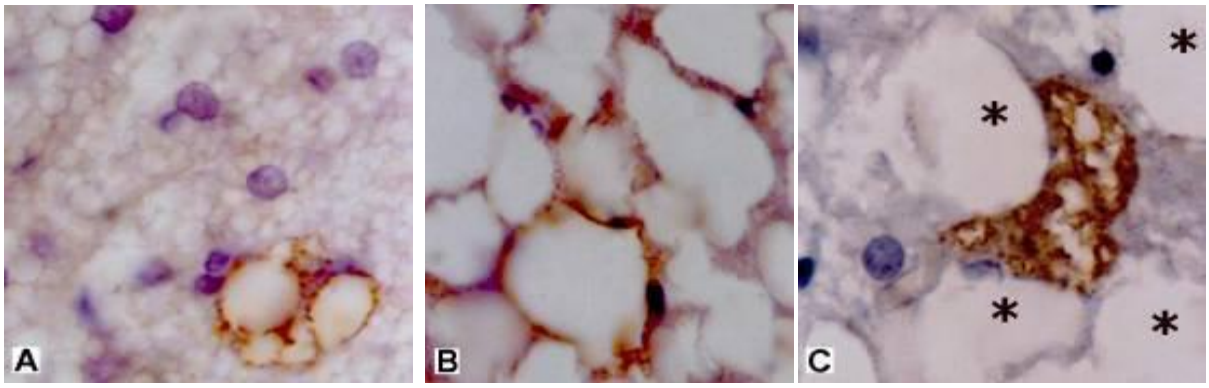

Figure 1. Types of adipocytes showing leptin-immunopositivity: multilocular adipocytes containing few large lipid droplets, observed in BAT of both control and hypothyroid rats (A), unilocular adipocytes observed in BAT of both control and hypothyroid rats (B) and classic brown adipocyte observed almost exclusively in hypothyroid rats. Note leptin-negative unilocular adipocytes closely apposed to leptin-positive brown adipocyte (✱). Magnification 100 x orig.