# Energy Metabolism and Biochemical Features of Adipose Tissues in ICR Mice after Long-Term Calorie-Restricted Diet

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Long-term calorie-restricted diet (8 weeks, 60% of control food intake) was followed by an increase in thermogenic activity of interscapular brown fat. The relative amount of DNA and protein and the rate of oxygen consumption increased and tissue-specific marker of brown fat (uncoupling protein UCP1) appeared in significantly reduced deep-pink abdominal adipose tissue.

**Key Words**: *brown and white adipose tissue; uncoupling protein 1; nutrition*

Food deprivation for 1-2 days is followed by a significant suppression of the specific thermogenic mechanism in mitochondria of the brown adipose tissue (BAT) of laboratory animals and inhibition of the main metabolic pathways contributing to its functional responses [5,6,8,10]. Energy consumption in BAT decreases, which contributes to economy of internal resources in the body [5]. However, the data on energetic and thermogenic activity of BAT during partial food restriction for a long time are ambiguous [9,11-13]. Our previous experiments on ICR mice have shown that calorie-restricted diet for 3 weeks was followed by reduction of thermogenesis in BAT and increase in cell proliferation and tissue cellularity [1]. Apparently, BAT response is as adaptive metabolic response associated with resource saving and optimization of organism thermogenic activity. Thus, this response should partly save thermogenic activity of BAT.

The changes in thermogenic parameters of BAT during longer experimental exposures are interesting for better understanding of adaptive rearrangements induced by food deprivation. High plasticity of adipose tissues, the presence of brown adipocyte precursors, and appearance of mature brown adipocytes in the white fat depot are widely discussed [4,7,14], therefore the data on thermogenic activity of white adipose tissue are necessary for full analysis.

Here we studied the effects of 8-week calorie-restricted diet on plastic and energy metabolism in adipose tissues of ICR mice.

## MATERIALS AND METHODS

Experiments were performed on 6-week-old male ICR mice (Vektor breeding center). The experiments were conducted in accordance with the Declaration of Helsinki on animal welfare. The mice were housed at 23±2°C and feed balanced granulated food for laboratory rodents (BioPro). Food consumption per mouse in the control group was determined daily and experimental group animals received 60% of this amount. The mice were housed individually during the experiment. Vitamin and trace element deficiency caused by food restriction was compensated by adding vitamin-mineral solution to drinking water. Experiment lasted for 8 weeks; no deaths of experimental animals were observed.

After the end of the experiment, the animals were decapitated; BAT was isolated from the interscapular depot and white adipose tissue from perigonadal pads of abdominal fat (AF). The intensity of energy metabolism was evaluated by the rate of $O_2$ consumption by standard tissue fragment suspension at 37°C [2]. The

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content of DNA and protein was evaluated in tissue homogenates in 0.01 M Tris-HCl buffer with 1 mM EDTA (pH 7.2) [2]. Uncoupling protein UCP1 was identified by immunoblotting. Tissue homogenates were analyzed by SDS-PAAG (12.5%) electrophoresis in a Laemmly buffer [13]; 100 μg protein was applied to each row. BSA (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), and lactoalbumin (14.4 kDa) served as molecular weight markers. The proteins were transferred from the gel to nitrocellulose membrane (pore size 0.2 μ) by a semidry method in 25 mM Tris-glycine buffer (pH 8.5) with 20% ethanol and 0.1% sodium dodecyl sulfate at 3 W for 30 min. Rabbit antibodies to synthetic N-terminal UCP1 peptide served as the primary antibodies; goat anti-rabbit IgG antibodies conjugated with alkaline phosphatase served as the secondary antibodies. Incubation with antibodies and band development were conducted in accordance to Sigma Aldrich instructions.

The significance of differences between the control and experimental groups was evaluated by nonparametric Student's *t* test for independent samples.

## RESULTS

The initial body weight of animals in the control and experimental groups was similar (24.86±0.80 and 25.99±0.61 g, respectively). At the end of the experiment, body weight of control and experimental mice was 43.56±0.92 and 31.61±0.72 g respectively, so mouse body weight in the experimental group was lower by 38% ($p<0.001$).

The total weight of the interscapular BAT depot in the experimental group was higher by 33%, but its relative weight was similar to the control weight (Table 1). The relative DNA content was higher by 52% ($p<0.05$), total absolute DNA content increased insignificantly. Hence, the mean size of adipocytes decreased, while the total number of cells in the interscapular BAT in experimental mice was not less than in the control group. Protein concentration was similar in these groups. The rate of $O_2$ consumption by BAT (per tissue weight unit) increased in experimental animals (Table 1). These data suggest that thermogenic activity of BAT increased in calorie-restricted mice (in comparison with animals receiving food *ad libitum*), despite the fact that total BAT store in these animals was lower.

Analysis of AF response to long-term calorie restriction revealed equal (~3-fold) increase in DNA and protein content and $O_2$ consumption rate in AF of experimental animals (Table 1). By these parameters, AF of calorie-restricted mice was similar to interscapular BAT, whereas in control animals, DNA and protein content and the rate of $O_2$ consumption in AF were lower than in BAT by 2, 5, and ~5 times, respectively. On the one hand, these results are consistent with the data on a decrease in adipocyte size and a decrease in the number of cells in AF (per weight unit) in calorie-restricted animals [3]. On the other hand, metabolic activity of AF obviously increased. AF and subcutaneous fat in experimental mice turned dark-pink, which is typical of BAT. Uncoupling protein UCP1, a typical marker of brown adipocytes, was detected in the larg-

**TABLE 1.** Amount and Biochemical Parameters of Adipose Tissues of ICR Mice

| Parameter | BAT | | AF | |
|---|---|---|---|---|
| | control group | experimental group | control group | experimental group |
| Absolute weight, mg | 108.53±7.31 (*n*=15) | 72.73±5.92** (*n*=15) | 510.20±47.32 (*n*=15) | 88.15±17.52** (*n*=13) |
| Relative weight, % | 0.24±0.01 (*n*=15) | 0.22±0.02 (*n*=15) | 1.15±0.09 (*n*=15) | 0.40±0.09** (*n*=13) |
| DNA, μg/mg | 0.48±0.07 (*n*=11) | 0.74±0.10* (*n*=14) | 0.21±0.04 (*n*=13) | 0.62±0.10** (*n*=9) |
| DNA, μg/depot | 48.22±8.22 (*n*=11) | 61.01±12.11 (*n*=15) | 107.02±17.33 (*n*=13) | 85.70±24.61 (*n*=10) |
| Protein, μg/mg | 58.70±6.11 (*n*=14) | 60.62±8.51 (*n*=13) | 9.94±1.41 (*n*=9) | 33.90±8.53* (*n*=7) |
| Rate of $O_2$ consumption, nmol/min×mg | 1.16±0.08 (*n*=15) | 1.63±0.20* (*n*=15) | 0.24±0.03 (*n*=15) | 0.88±0.18** (*n*=14) |

**Note**. *$0.05 \le p < 0.01$, **$p<0.001$ in comparison with the control.

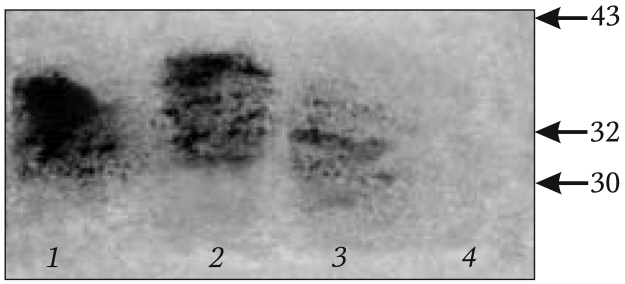


**Fig 1**. Uncoupling protein UCP1 antigen in the adipose tissues of animals receiving food *ad libitum* (control group) and calorie-restricted mice (8 weeks; experimental group; representative blot). Interscapular BAT: *1*) control; *2*) experimental; perigonadal white adipose tissue: *3*) experimental group; *4*) control. Arrows: molecular weights markers and UCP1 band (32 kDa).

est perigonadal accumulation of AF (Fig. 1). In control mice, no UCP1 was found in the perigonadal fat (at least at the level of method sensitivity). It is known that UCP1 mRNA and immunoreactive UCP1 can be detected in the abdominal white fat depot soon after birth and UCP1-positive multiocular adipocytes can be found by immunohistochemistry [14]. The expression of UCP1 gene ceased at the age of 20-60 days depending on mouse strain, but low temperatures and injections of $\beta_3$-adrenomimetics reverse this process and induce the appearance of UCP1-positive cells in FT and subcutaneous fat of mature mice [4,6,14]. The appearance of UCP1 in AF of calorie-restricted mice suggests that long-term calorie restriction similarly to low temperatures stimulates the processes of cell differentiation in fat depot towards BAT adipocytes. Further immunohistochemical and molecular studies are required for verification of this assumption.

Therefore, our findings suggest that long-term calorie restriction activates the total metabolic activity of the adipose tissues and their specific thermogenic activity. What is the physiological significance of these changes? Taking into account that the main function of BAT is thermal generation, these changes are undoubtedly related to the maintenance of the body heat balance. In light of this, the fact that the temperature in our experiment was 23°C has great significance, as it was below the thermoneutral range 28-30°C for small homoiothermal animals. Thus, heat balance in these animals can be maintained only during active function of thermogenesis mechanisms. Probably, long-term chronic calorie restriction limits the possibility of contractile thermogenesis, or this process become very consumptive due to total loss of subcutaneous fat. Therefore, BAT activation is a compensatory adaptive response.

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