Effect of Calorie Restricted Diet on Brown Adipose Tissue in Mice

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Calorie restricted diet (50% food intake of control animals) for 3 weeks decelerated weight gain in laboratory mice, reduced the weight of abdominal fat, and decreased the rate of oxygen consumption by brown adipose tissue. The relative weight of interscapular brown fat and protein content in it did not differ from the control. DNA content in brown fat in mice kept on calorie restricted diet increased by 93% compared with the control.

Key Words: *brown and white adipose tissue; calorie restricted diet*

Periods of undernutrition caused by seasonal conditions or climatic disasters are usual phenomena in wildlife. At the population level, the animals successfully adapt to such environmental conditions by reducing energy expenditure in the non-vital tissues and organs [14]. In small animals, reduction of core temperature and increase in the duration and number of torpid episodes help to overcome energy deficit [4,8]. In light of this, the impact of nutritional energy deficiency on brown adipose tissue (BAT), a specialized organ of thermogenesis in mammals [2], is of particular interest.

In rodents subjected to the short-term complete food deprivation (24-72 h), virtually all parameters reflecting current intensity of heat generation and its potential capacities are reduced: basal and norepinephrine-stimulated respiration, lipolysis, activity of cytochrome oxidase, expression of the gene encoding uncoupling protein 1, the key element of thermogesis mechanism, and binding of purine nucleotides to this protein [2,3,6,9,14]. However, the data on the content and functional activity of BAT during prolonged limited food deprivation are scanty and ambiguous. The diet restricted to 50-70% *ad libitum* food intake, but with the same proportion of the major nutrients, is called calorie restricted diet. In male Wistar rats kept on calorie restricted diet, the level of thermogenesis-active uncoupling protein decreased to the middle of the 1st month [9], and the absolute and relative weight of BAT decreased by the end of the 3rd month [12]. On the contrary, in Fisher 344 rats the same diet for 2 and 6 months led to a 2-fold increase in absolute and relative weight of BAT in comparison with that in age-matched controls [10], while in BN/CrlBR rats it increased uncouples respiration of BAT mitochondria in state 4 [7]. The cause of these interstrain differences is still unclear. Since rats of the latter two strains have substantially lower body weight than Wistar rats, we can hypothesize that the differences are related to initially different levels of heat loss.

The data on the effect of calorie restricted diet on thermogenic properties of BAT in smaller rodents could clarify the situation. It was therefore necessary to explain the influence long-term calorie restricted nutrition on the energy and plastic metabolism in BAT of laboratory mice.

MATERIALS AND METHODS

The study was performed on 1.5-month-old outbred male ICR mice (nursery of Vector Research Center) kept under vivarium conditions at 23±1°C (experiments were performed with strict adherence to principles of the Helsinki Declaration). The animals received bal-

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anced pelleted food for laboratory rodents (BioPro). The amount of food per mouse in the control group was determined every day and experimental animals received 50% food (by weight) consumed by controls; access to water was not restricted. At the end of the 3-week experiment, the animals were decapitated. Brown fat was isolated from the interscapular depot and white adipose tissue (WAT) from perigonadal fat pads.

The intensity of energy exchange was evaluated by the rate of oxygen consumption at 30.0±0.5°C in a flow metabolimeter [1]. Oxygen consumption by BAT was recorded in a standard suspension of tissue fragments in 1.2-ml measuring cell using an N5972 oxygen transducer.

The content of DNA and protein was evaluated in tissue homogenates in 0.01 M Tris-HCl buffer with 0.001M EDTA, pH 7.4, at a tissue–buffer ratio of 30-40 mg per 0.5 ml. Protein content was determined by the method of Lowry after a preliminary solubilization of the homogenate with a solution containing sodium dodecyl sulfate and NaOH. DNA content was calculated from the results of spectrophotometry of acid hydrolysates prepared from deproteinized homogenate at 270 and 290 nm [1].

Statistical significance of differences between mean values was assessed by Student's *t* test.

RESULTS

Body weight of animals kept on calorie restricted diet did not change by the end of the experiment, whereas in the control group it increased by 30%. The differences in body weight between the control and experimental groups after 3 weeks attained 8.5 g ($p<0.001$; Table 1).

Integral indicator of energy exchange, rate of O_2 consumption, did not differ in animals receiving food *ad libitum* and calorie restricted mice. Bearing in mind that O_2 consumption by skeletal muscles and internal organs is reduced in starving animals [5], this fact can be explained by reduced weight of metabolically inert white fat [10]. In our experiment, the amount of abdominal fat was significantly reduced in experimental mice in comparison with the control. For the quantitative characteristics of white fat depots, easily isolated perigonadal fat pads were used; its total weight in experimental mice decreased by 2.25 times compared with that in controls (Table 1).

Visual assessment of the main BAT depots also showed reduced volume of this tissue in fasting mice compared with controls, but to a much lesser extent. Quantitative assessment was performed for interscapular BAT depot, its total weight decreased by only 18% (Table 1). The relative weight and protein content remained practically unchanged, but the rate of oxygen consumption by interscapular BAT samples from experimental mice was reduced 2-fold in comparison with that in controls (Table 2).

Despite reduction of BAT stores, the amount of DNA in tissue samples of experimental mice increased by 2.5 times, and total DNA content in the interscapular depot increased by 93% (Table 2). This clearly indicates acceleration of cell proliferation induced by calorie restricted diet and BAT enrichment with small young adipocytes.

TABLE 1. Effect of Calorie Restricted Diet on Body Weight, Energy Balance, and Fat Weight in Mice (*M*±*m*)

Parameter	Group	
	control	experiment
Body weight, g	30.25±0.58 (*n*=31)	21.75±0.74** (*n*=31)
Rate of oxygen consumption by mouse, liter/h×kg$^{0.75}$	0.86±0.03 (*n*=14)	0.88±0.04 (*n*=13)
Total weight of perigonadal WAT, mg	307.73±26.08 (*n*=15)	136.60±10.43** (*n*=15)
Relative weight of perigonadal WAT, %	1 .01±0.07 (*n*=15)	0.61±0.04** (*n*=15)
Total weight of interscapular BAT, mg	92.73±4.07 (*n*=31)	76.39±5.01* (*n*=31)
Relative weight of interscapular BAT,%	0.305±0.010 (*n*=31)	0.335±0.018 (*n*=31)

Note. Here and in Table 2: *$p<0.05$, **$p<0.001$ in comparison with the control group.

TABLE 2. Effect of Calorie Restricted Diet on Energy Exchange and Plastic Metabolism in Interscapular BAT

Parameter	Group	
	control	experiment
Rate of oxygen consumption, μmol/min×g	368±82 (*n*=7)	164±38* (*n*=7)
Protein, g/mg	50.24±2.81 (*n*=16)	53.81±3.70 (*n*=15)
DNA, μg/mg	0.63±0.07 (*n*=16)	1.58±0.20** (*n*=15)
Total DNA, μg	57.47±5.71 (*n*=16)	110.77±13.93** (*n*=15)

Decreased size of adipocytes in white fat depots under the influence of calorie restricted diet was previously reported [15], and the possibility to enhance adipogenesis and increase the content of young cells in the fat depots was discussed. Intensification of cell proliferation in brown fat of mice feeding calorie restricted diet was first observed by us.

Thus, newly formed adipocytes were smaller compared to adipocytes of mice fed *ad libitum*. This is crucial for understanding of the biological role of accelerated proliferation. First, additional plastic material and increased energy expenditure are not required for new formation of adipocytes. Hence, the formation of cell reserve is not burdensome for starving animals. Second, increased cellularity leads to increased total membrane area per unit of tissue weight. Increased membrane area provides a potential springboard for a sharp increase in metabolite influx and functional activity in specific situations. For example, in case of possible violations of temperature homeostasis, BAT cell reserve can accommodate the rapid tissue growth and increase in heat production. The role of expanded "cellular springboard of thermogenesis" appears to be particularly significant for animals in natural environment, where periods of undernutrition often coincide with the cold season [14].

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