Effect of Semi-Compulsory Alcoholization on Brown Fat in Mice

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 132, No. 12, pp. 621-623, December, 2001
Original article submitted June 28, 2001

We studied brown adipose tissue isolated from mice receiving 20% ethanol as a single source of fluid. *In vitro* O_2 utilization by brown adipose tissue decreased after one month of forced alcohol intake, but surpassed the control after 3 months of ethanol drinking. The absolute and relative weight of brown adipose tissue also increased at this term. The rate of *in vivo* O_2 utilization also decreased during the first experimental month and returned to normal after 3 months.

Key Words: *brown adipose tissue; thermogenesis; ethanol; alcoholization*

Brown adipose tissue (BAT) is a specialized organ of noncontractile thermogenesis, which is activated by high-caloric diet thereby decelerating fat deposition [7,8]. Chronic intake of ethanol, a highly caloric product, can produce energy surplus and up-regulate thermogenesis in BAT. However, the data on thermogenesis in BAT during alcoholization are scarce and ambiguous. On the one hand, some data indicate that synthesis of uncoupling protein and genesis of mitochondria are down-regulated in mouse BAT adipocytes after 1-month alcohol drinking [5]. On the other hand, BAT of alcoholized animals demonstrates signs of activation [4,6]. We hypothesized that this difference can be explained by different duration of alcohol intake in the cited works. Therefore, our aim was to study thermogenesis in BAT during various periods of alcohol intake.

MATERIALS AND METHODS

Experiments were carried out on albino male mice aged 1.0-1.5 month at the start of the experiments. The mice were housed under natural day-night cycle at 24°C and maintained on a standard ration including 20-25% protein, 6.5% fat, and 60% carbohydrates. During 3-week experiment the only source of fluid was ethanol in increasing concentrations (from 5 to 20%). The control mice received water. The intensity of energy metabolism was assessed by the rate of oxygen consumption (Vo_2) at 30-32°C using a flow metabolimeter [2].

Brown fat was isolated from the interscapular depot. Thermogenesis in BAT and liver was evaluated by Vo_2 in tissue fragments [1]. The measurements were performed at 37°C in a 1.2-ml measuring cell under constant stirring. Oxygen was determined with an N5972 oxygen transducer (Poland) working as a galvanic element. Isolation and incubation media were aerated with 95% O_2 and contained (in mM: 10.0 phosphate buffer; 138.0 NaCl; 4.0 KCl; 1.4 $MgSO_4$; 10.0 glucose; 40 g/l bovine serum albumin; pH 7.4). The total weight of tissue in the cell was 15-30 mg. Oxygen absorption was recorded for 5 min, which corresponded to linear fragment of oxygen absorption curve. Water content in tissue was calculated as the difference of wet and dry sample, which was dried for 24 h at 105°C. The data were processed statistically using Student's *t* test.

RESULTS

Chronic ethanol intake was accompanied by pronounced changes in heat production by mouse brown fat, but direction of these changes depended on the duration of alcohol drinking. After the first month, oxygen consumption by BAT was below the control by 31%, but after 3 months it surpassed the control by 38% (Table 1). Other parameters characterizing

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TABLE 1. Effect of Forced Alcohol Intake on Interscapular BAT in Mice (*M*±*m*)

Parameter	Alcohol intake, months					
	1			3		
	control	experiment	Δ, %	control	experiment	Δ, %
BAT weight, g	0.156±0.012 (9)	0.151±0.010 (10)	-3.2	0.112±0.005 (19)	0.129±0.008 (15)	15**
%	0.417±0.040 (9)	0.377±0.025 (10)	-9.4	0.294±0.010 (19)	0.336±0.020 (15)	15**
Water content in BAT, %	28.36±1.50 (11)	24.50±3.01 (10)	-13.6	29.96±1.29 (17)	33.80±1.69 (13)	11
Vo_2, μM/min per 1 g BAT	0.83±0.10 (12)	0.57±0.10 (10)	-31**	0.98±0.10 (19)	1.35±0.10 (13)	38*

Note. The number of mice is given in parentheses. *p<0.05, **0.05<p<0.01.

thermogenic in BAT remained unchanged after the first month, but after 3 months of alcohol drinking the absolute and relative weight of interscapular BAT increased. Energy metabolism in animals drinking ethanol for one month decreased by 18% (Fig. 1), but then it gradually increased and returned to the control after the third month of alcohol intake.

It is well known that liver also contributes to the non-contractile thermogenesis. Our experiments showed that oxygen consumption by liver fragment from control and experimental mice did not differ during 3 months (0.71 μM/min/g). This suggests that liver does not play a role in modification of energy metabolism in alcoholized animals.

Taking this inference into consideration, we hypothesized that decceleration of energy metabolism is related to down-regulation of BAT thermogenesis, which, in turn, results from disturbances in protein synthesis and expression of RB1 gene in mouse adipocytes during 3-4-week ethanol drinking [5]. Inhibition of BAT thermogenesis is not the only cause of down-regulation of energy metabolism in animals. Decreased motor activity of experimental mice during the first 3-5 weeks of alcoholization can also contribute to these changes. Low motor activity and down-regulation of BAT thermogenesis during this period resulted in obesity (after 1 month of ethanol intake body weight in experimental mice increased by 19% compared to the control, p<0.01). However, no differences in the body weights were found between the control and experimental groups during experimental months 2 and 3.

To observed up-regulation of BAT thermogenesis during long-term alcohol intake is of particular importance. First, it coincides with adaptation to alcohol [3] manifested in deceleration of body weight gain and recovery of energy metabolism in alcohol-treated mice. Second, similar rates of energy metabolism and body weight in experimental and control mice observed after 3 months of the experiment unambiguously attest to restoration of the balance between energy production and dissipation in alcoholized animals. Therefore, pronounced up-regulation of BAT thermogenesis is counterbalanced by its down-regulation in some other organ or tissue. It can be hypothesized that up-regulation of BAT thermogenesis is a compensation of diminished energy metabolism and heat production caused by ethanol in some other structures.

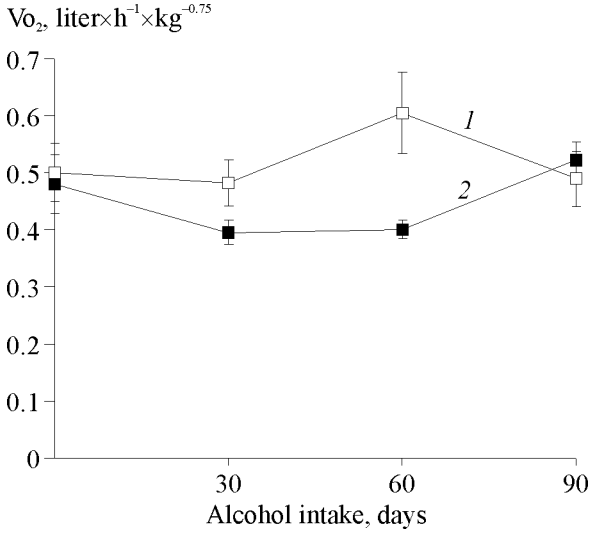

Fig. 1. Oxygen consumption (Vo_2) in control mice (*1*) and under conditions of forced alcohol intake (*2*).

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