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Chronic Alcohol Intake Induces the Oxidative Capacity of Brown Adipose Tissue in the Rat

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HUTTUNEN, P. AND M-L. KORTELAINEN. *Chronic alcohol intake induces the oxidative capacity of brown adipose tissue in the rat.* PHARMACOL BIOCHEM BEHAV **29**(1) 53–57, 1988.—The present study was carried out to elucidate the effect of long-term alcohol intake on the oxidative capacity of brown adipose tissue in the rat. Rats housed at room temperature were given water containing 10% ethanol for six months, while controls received water alone. Fully cold-acclimated rats (exposed to +4°C for 6 weeks) served as the second control group. Alcohol did not alter the food intake of the rats compared with the controls kept at room temperature, but it did cause a mean decrease of 8 ml in fluid consumption. There was no difference in the increase in body weight between the groups housed at room temperature. Body weight of the rats exposed to cold did not change during cold acclimation. No morphological liver changes were observed in alcohol-fed rats, but some changes related to long-term alcohol consumption were found in the myocardium. Chronic alcohol intake increased the quantity of brown adipose tissue and its protein content but changes were not as great as in the cold-acclimated rats nor did alcohol increase protein content per unit of the adipose tissue as did cold. On the other hand, the specific activity of mitochondrial cytochrome oxidase increased by 90% and that of succinate dehydrogenase by 130% in alcohol-fed rats, whereas specific activities of these enzymes displayed little or no change in the cold-acclimated rats. Results suggest that chronic alcohol ingestion induces the oxidative capacity of the interscapular brown adipose tissue in the rat, increasing the mass of BAT and specific activities of mitochondrial enzymes.

Ethanol Cold acclimation Interscapular brown adipose tissue Mitochondria Oxidative enzymes Succinate dehydrogenase Cytochrome oxidase

A previous study of ours showed that long-term ingestion of ethanol improves cold tolerance in the guinea-pig in the same way as does cold acclimation [12], the latter effect being due primarily to the change from a unilocular to a multilocular interscapular adipose tissue, which resembles typical brown adipose tissue [10]. Brown adipose tissue (BAT) is known to be the most important organ generating heat in non-shivering thermogenesis in mammals [6]. The principal mechanism for the generation of heat in BAT is through the proton conductance pathway which is located in the inner mitochondrial membrane and is associated to a specific 32000 protein. During cold adaptation an increase in the formation of 32000 polypeptide occurs and by the end of 3–4 weeks growth of BAT, mitochondrial proliferation, and the alteration in mitochondrial composition of BAT have reached completion [9].

The mechanism by which chronic ethanol intake improves cold tolerance is unclear. Being a stress factor and high-energy fuel, ethanol may stimulate the sympathetic activity of brown adipose tissue, as a high-fat and carbohydrate diet does [1, 2, 20, 21]. Chronic treatment of adult guinea-pigs, which do not possess typical brown adipose tissue, with catecholamines has been found to increase the oxidative capacity of the interscapular adipose tissue as does prolonged cold exposure [11].

The present study was carried out to elucidate a possible cross-adaptation between long-term alcohol intake and cold and to evaluate the role of the brown adipose tissue in such cross-adaptation. The guinea-pig, which we have used in our previous study, cannot be forced to drink alcohol and therefore the present study was carried out with the rat.

METHOD

Adult male rats (N=28) of the Sprague-Dawley strain ranging from 305 to 420 g were used in the present study. The rats were housed in individual cages and kept on a 12 hour light cycle. The animals were divided into three groups. Groups 1 and 2 were housed at an ambient temperature of 21–23°C for six months and group 3 was exposed to cold (+4°C) for six weeks. The cold exposed rats (N=8) were given pelleted food (Hankkija, Finland) and water ad lib and the second group of rats kept at room temperature (N=10) was fed similarly for six months. The first group (N=10) was given food, water and a solution of ethanol at a concentration of 10% (v/v) ad lib for two weeks, after which they received only water containing 10% ethanol and food ad lib for six months.

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TABLE 1

BODY WEIGHTS, RELATIVE WEIGHT OF THE INTERSCAPULAR BROWN ADIPOSE TISSUE AND TOTAL IBAT PROTEIN CONTENT

Groups	Body Weight (g) Initial	Final	BAT/Body Weight (mg/g)	Total Protein Content (mg)
Alcohol-fed rats	389 ± 35	538 ± 37	1.11 ± 0.28 (n=10) *	25.2 ± 6.9 (n=10) *
Controls at room temperature	368 ± 32	515 ± 97	0.51 ± 0.15 (n=10) *	11.6 ± 2.3 (n=8) *
Cold-acclimated	319 ± 16	327 ± 18	*1.97 ± 0.11 (n=8)	*63.3 ± 8.9 (n=8)

*$p<0.01$, Bonferroni test.
Values are means ± SD.

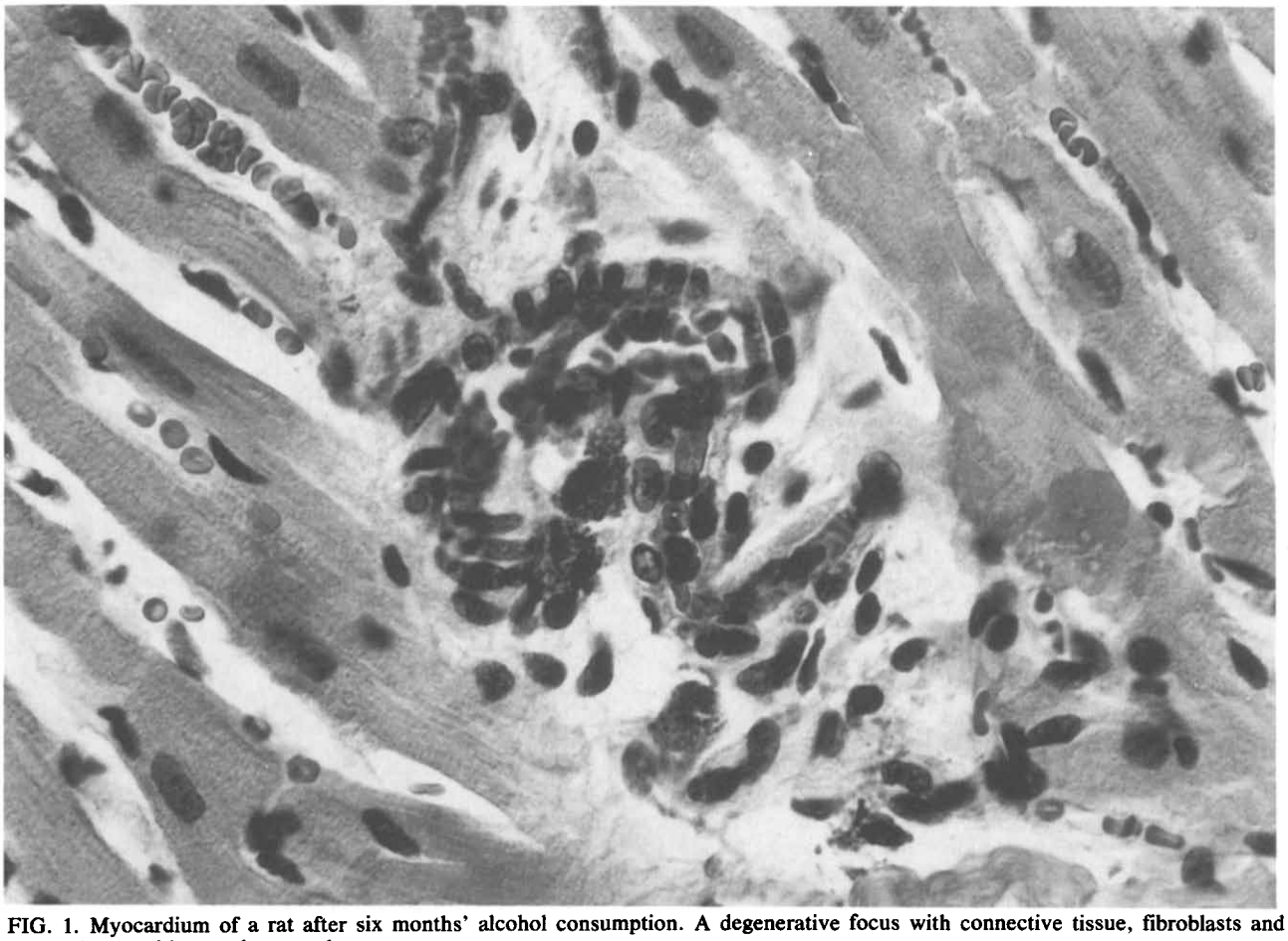

FIG. 1. Myocardium of a rat after six months' alcohol consumption. A degenerative focus with connective tissue, fibroblasts and macrophages with granular cytoplasm.

Samples

The rats were killed with CO_2 and the interscapular brown adipose tissue removed and dissected free from all recognizable white adipose tissue and other connective tissue under stereo-microscopy. The BAT was weighed and frozen in liquid nitrogen to be kept at −70°C until analyzed.

The heart and liver were removed and weighed and samples were taken for histological staining (haematoxylin-eosin). The heart sample was dissected transversely from the middle of both chambers and the brain tissue from the middle coronal line for haematoxylin-eosin staining. The stomach was emptied and verified and samples were taken for haematoxylin-eosin staining.

Isolation of Mitochondria of Brown Adipose Tissue

BAT mitochondrial were isolated according to Cannon

TABLE 2

THE SPECIFIC ACTIVITY OF CYTOCHROME OXIDASE (CYO) AND SUCCINATE DEHYDROGENASE (SDH) IN ISOLATED MITOCHONDRIA

Groups	CYO	SDH Actual	SDH Maximal
Alcohol-fed rats	2.66 ± 0.82 (n=10)	1.42 ± 0.37 (n=10)	1.52 ± 0.34 (n=10)
	*	*	*
Controls at room temperature	1.38 ± 0.50 (n=10)	0.60 ± 0.24 (n=8)	0.67 ± 0.22 (n=8)
Cold-acclimated	2.16 ± 0.83 (n=8)	*0.77 ± 0.21 (n=8)	*0.96 ± 0.28 (n=8)

CYO is expressed in μmol cytochrome c oxidized/min/mg protein and SDH in μmol succinate oxidized/min/mg protein.

*$p<0.01$, Bonferroni test.

Values are means ± SD.

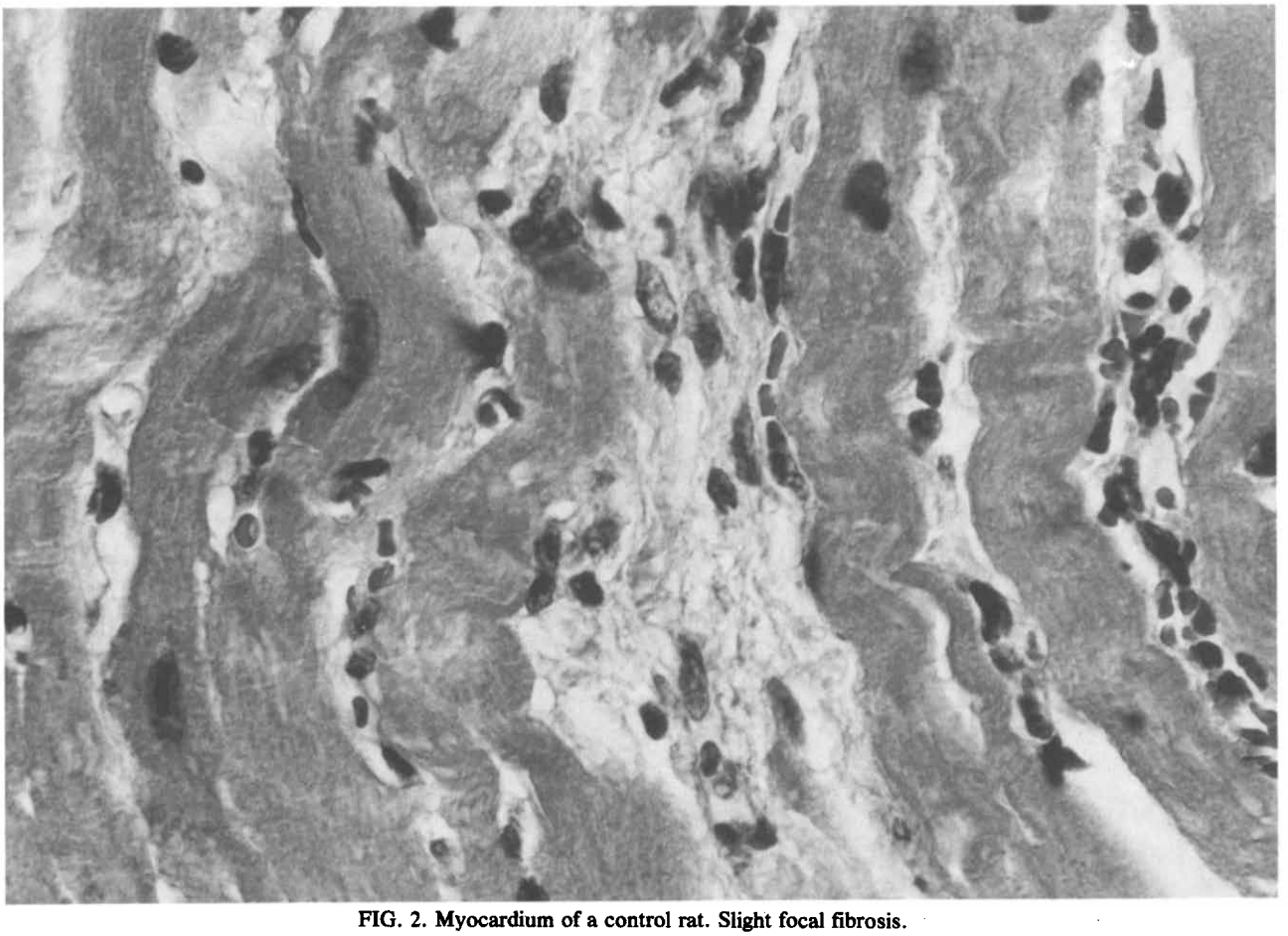

FIG. 2. Myocardium of a control rat. Slight focal fibrosis.

and Lindberg [4]. The BAT was homogenized in ice-cold 0.25 M sucrose containing 5 mM TES, pH 7.2. The homogenate was filtered through two layers of gauze and centrifuged at 8500×g for 10 min. The hard-packed fat layer and supernatant were discarded. The pellet, consisting of cell debris, nuclei and mitochondria, was resuspended in sucrose and the suspension centrifuged at 700×g for 10 min. The nuclear pellet was discarded and the supernatant centrifuged at 8500 for 10 min. The mitochondrial pellet was washed with sucrose.

Determination of Succinate Dehydrogenase and Cytochrome Oxidase Activity

Succinate dehydrogenase activity (SDH) in the isolated mitochondria was determined using phenazinemethosulphate and 2,6-dichloroindophenol as the electron acceptor

system, according to King [14]. In order to obtain the substrate-activated (maximal) enzyme activity, the suspension was pre-incubated at 37°C for 7 min in a mixture containing 50 μmol of potassium succinate in a total volume of 0.3 ml, after Kimura *et al.* [13]. The maximal enzyme activity was then determined from the incubated sample as described above.

Cytochrome oxidase activity (CYO) was determined spectrophotometrically by measuring the rate of aerobic oxidation of ferrocytochrome catalyzed by cytochrome oxidase at 21°C, according to Yonetani and Ray [25]. Cytochrome c was reduced using dithionite, and absorbing its excess with Sephadex. Protein was assayed by the method of Lowry *et al.* [18].

Histological Stainings

Routine haematoxylin-eosin staining methods were used on the brain, heart, liver and stomach samples [19].

Statistical Analysis

Analysis was performed using analysis of variance and the Bonferroni method.

RESULTS

Food intake did not differ significantly in rats receiving alcohol compared with the controls at room temperature, being in the alcohol-fed rats approximately 24±3 g a day and in the controls 26±3 g, respectively. The consumption of fluid in alcohol-fed rats was an average of 40±4 ml and in the controls 48±7 ml.

The average daily consumption of alcohol was 5 g/kg per rat, varying from 2 to 4 g/kg per day during the first month and from 5 to 7 g/kg in the last month.

There was no significant difference in body weight gained between the groups kept at room temperature. Body weight of rats exposed to cold did not change during cold-adaptation (Table 1).

Changes in Organs

The absolute and relative liver weights of the two groups of rats did not differ, nor did their absolute and relative heart weights. The HE stainings did not reveal any morphological liver changes in the rats which had consumed alcohol. They did have some changes in the myocardium, however, which may have been related to long-term alcohol consumption. These included focal disarrangement of myocardial fibres and slight fibrosis with the appearance of fibroblasts and macrophages (Fig. 1). The brain tissue samples did not reveal any morphological changes in the alcohol-treated rats, but the gastric mucosa was seen to be macroscopically irritated.

Brown Adipose Tissue

Chronic alcohol intake resulted in a doubling of the interscapular brown adipose tissue and of its total protein content whereas an increase in the relative weight of the IBAT in the cold-acclimated rats was four-fold compared with the controls at room temperature and protein content increased also per unit of tissue (Table 1). The specific activities of cytochrome oxidase and succinate dehydrogenase in isolated mitochondria increased in the alcohol-fed rats compared with the controls kept at room temperature, the latter, about 130% more markedly than the former, about 90%. In fully cold-acclimated rats the specific activities of these enzymes displayed little or no change (Table 2).

DISCUSSION

The present study shows that chronic alcohol intake increases the oxidative capacity of the interscapular brown adipose tissue in the rat as does prolonged cold exposure. The changes are characterized by growth in the adipose tissue and an increase in its protein content, which represent the physiological basis of cold adaptation. Changes induced by alcohol were not, however, as great as in fully cold-acclimated rats. Although the quantity of the BAT increased in both groups, the weight of the tissue per unit of body weight was significantly higher in cold-acclimated rats than in alcohol-fed ones, which might be due to the differences in the body weights. The failure of cold-acclimated rats to gain weight most probably resulted from an increased energy expenditure for thermogenic purposes. On the other hand, it has been shown that the weight of IBAT per unit of body weight in cold-acclimated rats remains constant after the first week of cold exposure [5]. The major difference between the groups was that alcohol did not lead to an increase in protein content per unit of tissue as did cold. On the other hand, specific activities of mitochondrial electron transport enzymes CYO and SDH were increased in alcohol-fed rats, whereas specific activities of these enzymes displayed little or no change in cold-acclimated animals. Although total activity of respiratory enzymes in BAT is known to be enhanced during cold-acclimation, there are controversial results on changes in specific activities of these enzymes. An increase seen in one study [23], was not seen in another ones [3,24].

The enhanced oxidative capacity of the IBAT induced by alcohol may, at least partly, explain the improved cold tolerance in our previous study [11] and the development of tolerance to ethanol-induced hypothermia during chronic ethanol treatment in the rat [17]. Although tolerance to ethanol-induced hypothermia has been found to develop even though the rats did not experience hypothermia during ethanol treatment, the possibility cannot be ruled out that the initial ethanol-induced hypothermia might cause long-lasting changes in BAT in the present study. On the other hand, ethanol intake for 14 days has been found to enhance only the activity of the proton conductance pathway, as does the initial phase of cold acclimation [9], but not the mass of BAT nor its protein content [22], as did alcohol intake for six months in our study.

In addition to cold adaptation, BAT is known to be a major effector of diet-induced non-shivering thermogenesis [1, 2, 20, 21]. The mass and protein content of BAT increase when rats are overfed with high-energy food [20,21] as also does the mitochondrial conductance pathway, which allows uncoupled respiration.

Repetitive chronic stress by means of immobilization has also been found to lead to improved tolerance to cold and an increase in the size and protein content in BAT in rats never previously exposed to cold [16] and alcoholization is reported to have increased the weight of BAT in emotionally stressed rats [7]. Hypertrophy of BAT has been observed in cold-adapted and overfed rats and in vivo measurements of tissue blood flow and oxygen extraction have revealed that both forms of thermogenesis result from sympathetic activation of the brown adipose tissue metabolism [26]. It is also

suggested that various stressful stimuli could activate the same neuroendocrine systems which are involved in the metabolic adaptation to cold [16]. It is possible that alcohol stimulates sympathetic activation in BAT and the hypertrophy and the increased oxidative capacity noted in the alcohol-fed rats may be due to just such an effect. Other endocrine systems connected with brown fat thermogenesis may also be affected by chronic alcohol consumption.

Although the present results show that long-term alcohol ingestion improves the oxidative capacity of the brown adipose tissue in the rat, it is possible that alcohol may involve thermogenic pathways in other tissues too. The microsomal system (MEOS) in the liver, the thermic effect of which is known to be greater than that of alcohol dehydrogenase [8], has been found to be enhanced in alcohol-fed guinea-pigs, for instance [15].

In conclusion, the major finding here is that long-term alcohol consumption increases the quantity of the interscapular brown adipose tissue in the rat, allowing an enhanced capacity for non-shivering thermogenesis as does cold-adaptation. These data suggest that prolonged consumption of alcohol will improve cold tolerance by increasing the oxidative capacity of BAT.

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