

## Fatty Acid Levels in Striated Muscles of Chronic Alcohol-Fed Rats

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**Abstract**—Changes in the fatty acid levels in the cardiac and gastrocnemius muscles of rats that were chronically alcoholized for 3 and 6 months were studied using two methods of alcoholization: 30% ethanol-containing agar (method I) and a 5% ethanol-containing liquid diet with a balanced nutritional status (method II). In the control group, the fatty acid level in the cardiac muscle was considerably higher than that in the gastrocnemius muscle. In the animals that were alcoholized over a 3-month period using method I, a considerable increase in the levels of myristic, pentadecanoic, palmitic, stearic, and dihomo- $\gamma$ -linolenic acids and the total amount of fatty acids and a decrease in  $\omega$ -3 docosapentaenoic acid level were found in the cardiac muscle. After a 6-month period, during which rats were alcoholized using method I, an increase in the levels of palmitoleic and  $\omega$ -6 docosapentaenoic acids and a decrease in the levels of stearic, eicosadienoic, and arachidonic acids were found. The amount of  $\omega$ -3 docosapentaenoic acid in the myocardium, compared to that observed after a 3-month period during which rats were alcoholized, remained reduced compared to the control. In the gastrocnemius muscle of rats alcoholized for 3 months using method I, the amounts of myristic, vaccenic, dihomo- $\gamma$ -linolenic, and  $\omega$ -6 docosapentaenoic acid increased. Simultaneously, there was a tendency for the total amount of saturated, monounsaturated, and  $\omega$ -6 polyunsaturated fatty acids and the total amount of fatty acids to rise. After a 6-month alcoholization, a decrease in the levels of myristic, oleic, linoleic,  $\alpha$ - and  $\gamma$ -linolenic, and eicosadienoic acids, as well as the total amount of saturated,  $\omega$ -6 polyunsaturated fatty acids and the total amount of all fatty acids was found. When animals were alcoholized over a 3-month period using method II, a significant increase in the amount of dihomo- $\gamma$ -linolenic acid was detected in the cardiac and gastrocnemius muscles. The role of these changes in the muscle pathologies is discussed.

**Keywords:** chronic alcohol-fed rats, fatty acids, striated muscles

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### INTRODUCTION

Long-term chronic alcohol consumption leads to the development of the symptomatic complex of alcohol-induced destruction of both skeletal (alcoholic myopathy) and cardiac (alcoholic cardiomyopathy) muscles [1–3]. The development of alcoholic myopathy is accompanied by atrophy and weakness of the skeletal muscles [2, 3], whereas the development of alcoholic cardiomyopathy manifests itself as the development of myocardial hypertrophy. The main characteristics of alcohol-induced disorders in muscles are successfully simulated in animals, particularly in rats [4]. Recent studies have shown that an

increased activity of the calcium-activated protease calpain-1 contributes to the development of atrophy by reducing the contents of the giant sarcomeric and cytoskeletal proteins titin and nebulin in the skeletal muscles of rats that were chronically alcoholized for 6 months [5]. In the cardiac muscle of these animals, a tendency to the development of atrophic rather than hypertrophic changes was observed [6]. The development of alcohol-induced disorders in muscles is caused by an imbalance between the synthesis and degradation of proteins [3, 7]. Fatty acids (FAs) and their metabolites are also involved in the regulation of the weight of striated muscles [8–11].

Consumed alcohol is metabolized in two pathways, that is, oxidative and nonoxidative. Over 90% of the ethanol is oxidized with the involvement of alcohol

*Abbreviations:* FAs, fatty acids; FAEEs, fatty acid ethyl esters; DGLA, dihomo- $\gamma$ -linolenic acid.

dehydrogenase, cytochrome P450, and catalase to form acetaldehyde, a metabolite that is toxic to organs and tissues [12–15]. Acetaldehyde is oxidized by acetaldehyde dehydrogenase to acetate, which is metabolized in extrahepatic tissues such as muscles, heart, and brain [16] to acetyl-CoA, which can be used for the synthesis of fatty acids.

By the first of the two main nonoxidative mechanisms, ethanol is converted into ethanolamine, which is then used for the synthesis of phosphatidylethanolamine. By the second nonoxidative mechanism, which involves fatty acid ethyl ester synthase and acyl-CoA-ethanol-O-acyltransferase, ethanol is esterified with endogenous fatty acids to form fatty acid ethyl esters (FAEEs). Fatty acid ethyl esters and triacylglycerols are found in large amounts in cardiac and skeletal muscles [17–19].

Enzymes that synthesize FAEEs are found in the soluble and microsomal fractions of many tissues. The amount of FAEEs depends on the activity of hydrolases, which catalyze the cleavage of the ester bond in the FAEE molecule to form ethanol and FAs. FAEE hydrolases and FAEE themselves are present in lipid droplets, which are formed in the endoplasmic reticulum. Lipid droplets are intended for temporary or long-term storage of lipids primarily in the form of triacylglycerols and cholesterol esters [20, 21]. Elevated levels of triacylglycerols were detected in samples of the skeletal muscle vastus lateralis in patients with myopathy who consumed ethanol for a long period of time. In this case, the dominant FAs in triacylglycerols were palmitic and oleic acids. Histological analysis of m. vastus lateralis in patients with myopathy revealed an elevated content of lipid drops both between myofibrils and on the sarcolemma inside fibrils [22].

Accumulation of lipid droplets was detected in cardiomyocytes of rats that received alcohol with drinking water [17]. It was shown that lipid droplets were in direct contact with the mitochondria. Lipid analysis of the heart tissue showed a moderate accumulation of triacylglycerols and a significant (12-fold) increase in the FAEE content [17]. The toxic effects of FAEEs manifest themselves as inhibition of cell proliferation, destabilization of lysosomes, depolarization of mitochondria, and induction of apoptosis. It is not known definitely whether toxicity is exhibited by FAEEs themselves or by the FAs that are released as a result of their hydrolysis. It is believed that the FAs released as a result of FAEE hydrolysis lead to cellular dysfunction [20, 21].

Our previous studies showed the development of atrophic changes accompanied by an increased proteolysis of titin and nebulin in striated muscles of chronically alcoholized rats [4, 5]. It cannot be ruled out that the development of atrophy may be preceded by a change in the fatty acid composition of the muscle tissue.

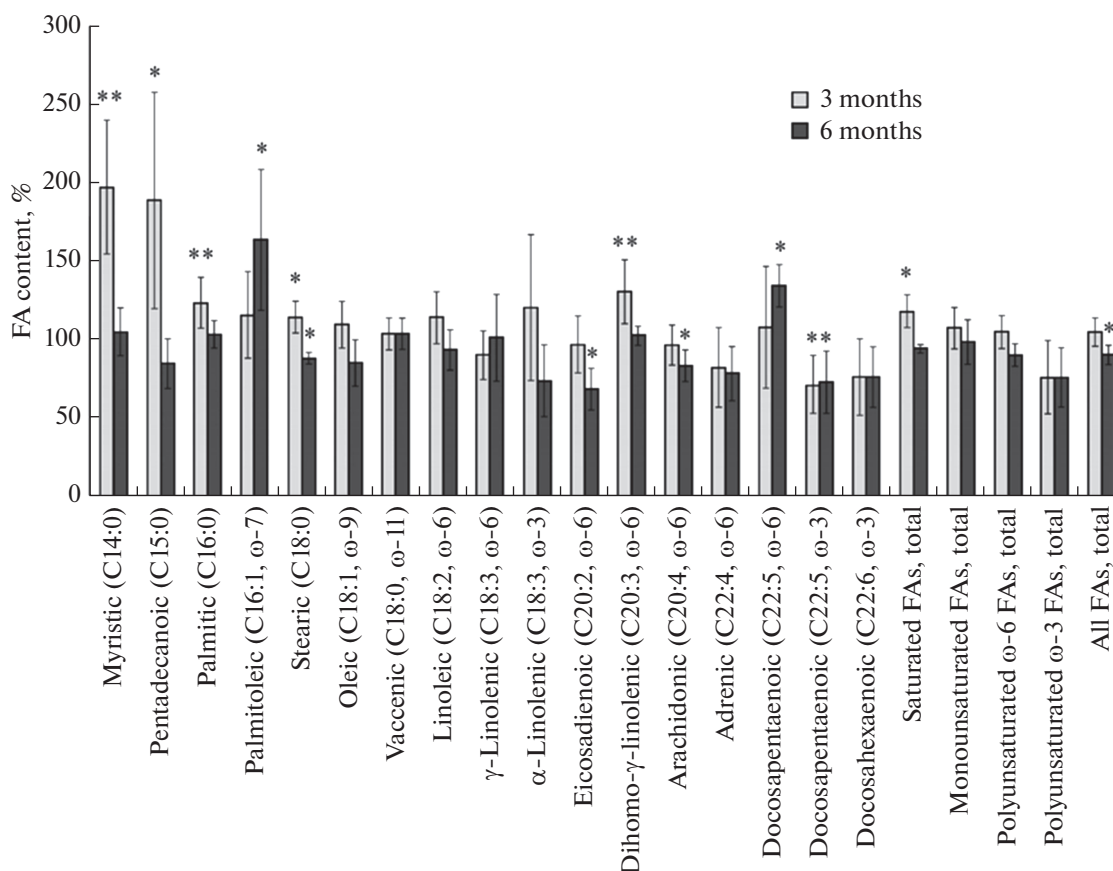
Thus, the aim of this study was to determine the fatty acid content in the cardiac and skeletal (m. gastrocnemius) muscles of rats that were alcoholized for 3 and 6 months.

## MATERIALS AND METHODS

**Chronic alcoholization of rats.** In this study, we used male Wistar rats. The weight of each animal at the beginning of the experiment was  $150 \pm 5$  g. The animals were kept in individual cages in the vivarium of the Institute of Theoretical and Experimental Biophysics and the Institute of Cell Biophysics, Russian Academy of Sciences (Pushchino, Moscow oblast). The experiments on animals were approved by the Biomedical Ethics Commission of the Institute of Theoretical and Experimental Biophysics and the Institute of Cell Biophysics of the Russian Academy of Sciences. The animals were chronically alcoholized using two methods. In method I [23], rats received alcohol in the form of a 10% aqueous ethanol solution and a 30% ethanol solution in agar blocks for 3 and 6 months (five animals in the control and alcohol-fed groups; the average daily ethanol consumption was  $26.6 \pm 3.0$  and  $23.4 \pm 2.0$  g kg<sup>-1</sup> day<sup>-1</sup>, respectively). In method II [24], rats received alcohol with a nutritionally balanced liquid feed containing 5% ethanol for 3 months (four animals in the control rats and alcohol-fed groups, the average daily ethanol consumption was  $19.1 \pm 2.0$  g kg<sup>-1</sup> day<sup>-1</sup>). The content of ethanol in the blood of the rat alcoholized by method II, which was measured using the Ethanol FS kit (DiaSys), was  $25.9 \pm 4.8$  mmol/L. The content of ethanol in the blood of the rats alcoholized by method I [23] was not measured.

In the simulation of alcoholic myopathy by method I using agar blocks, the addiction of animals to alcohol was developed gradually by increasing the ethanol content in drinking water and agar. The content of ethanol in water in the first 3 days and after 3 days until the end of the experiment was 5 and 10%, respectively. The ethanol content in agar was increased by 5% every 3 days (to a final alcohol concentration of 30%). After completion of the chronic alcoholization period, the animals were weighed and withdrawn from the experiment by euthanasia with Zoletil (Virbas Sante Animale, France) at a dose of 25 mg per 1 kg animal weight, which is 3 times greater than the therapeutic dose (7–8 mg/kg). The left ventricular myocardium and m. gastrocnemius were extracted and used in experiments. The muscles were frozen in liquid nitrogen and stored at  $-75^{\circ}\text{C}$ .

**Determination of the composition and contents of fatty acids in the studied muscles.** To determine the fatty acid composition we used striated muscle samples that were previously used to study the alcohol-induced changes in muscle proteins [5, 6]. Muscle tissue samples were homogenized in liquid nitrogen. An



**Fig. 1.** The fatty acid composition of the myocardium of rats that were alcoholized for 3 and 6 months by method I [23]. The control values of the FA content were taken as 100%. \*\*,  $p \leq 0.01$ , \*,  $p \leq 0.05$ .

aliquot of the homogenate (30–40 mg) in 0.9% NaCl solution containing 0.5% ionol (2,6-di-*tert*-butyl-4-methylphenol) was dried in a SpeedVac rotary vacuum concentrator (Savant Instruments, United States). The methyl esters of higher FAs were prepared as described previously [25]. Fatty acids were determined using a GC 3900 analytical gas chromatograph (Varian, United States) equipped with a flame ionization detector (detector temperature, 260°C). Fatty acids were separated using a silica capillary column (15 m × 0.25 mm × 0.3 μm) with a grafted stationary phase (Supelco, United States). The thermal analysis program was as follows: 90°C (0.5 min)–240°C (5 min) at a rate of 6°C per minute. Data were analyzed using Multichrom-1.5x software (ZAO Ampersed, Russia). The FA concentration was determined using an internal standard with a preliminary calculation of the respective calibration coefficients from the chromatograms of the mixture of the detected FAs with margaric acid (C<sub>17:0</sub>). For each sample, the absolute and relative contents of individual FAs were determined.

**Statistical analysis of data.** The significance of the differences between groups was evaluated using the nonparametric Mann–Whitney *U*-test. Differences between groups were considered significant at  $p \leq$

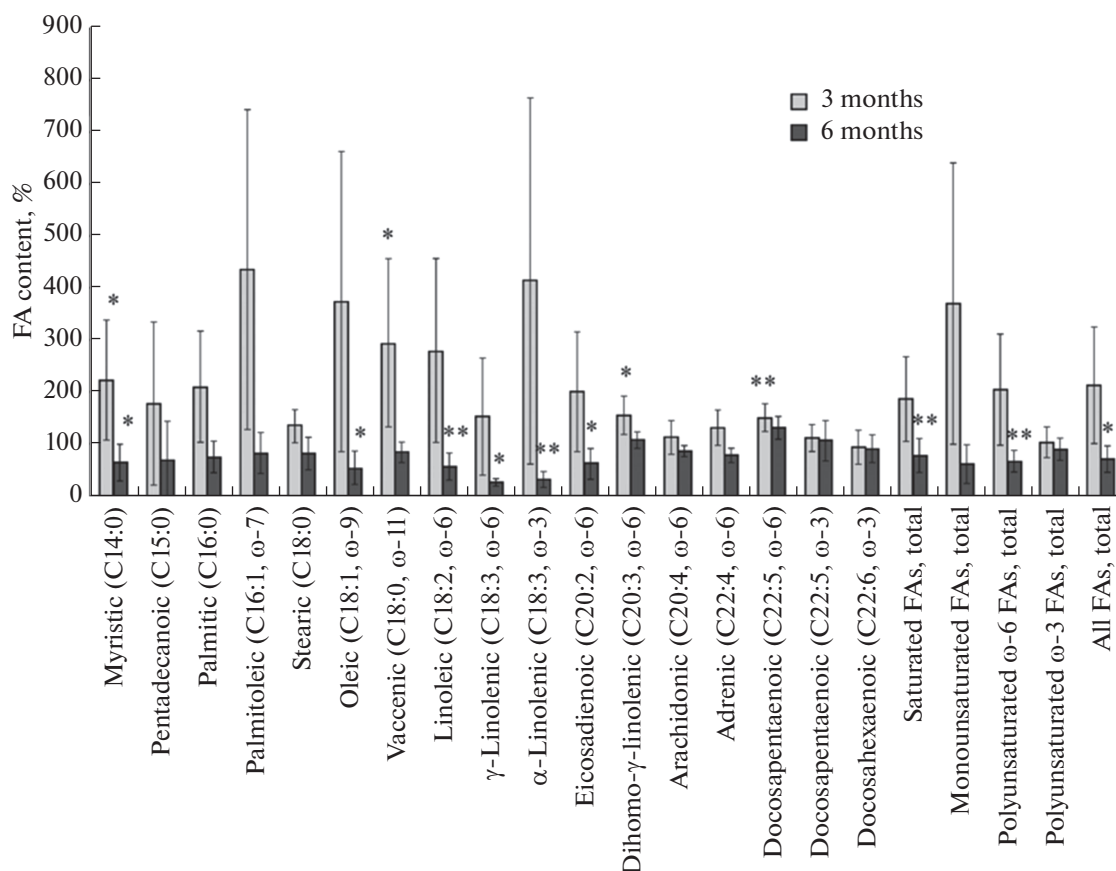
0.05. All data are represented as the mean value and the standard error of the mean.

## RESULTS

The composition and contents of FAs in the cardiac and gastrocnemius muscles of the control animals and rats alcoholized for 3 and 6 months according to method I are shown in Figs. 1 and 2 and in Tables 1 and 2. It was found that the total FA content in the myocardium of the control animals was higher (approximately twice) than that in the gastrocnemius muscle (Tables 1 and 2).

In the cardiac muscle of the rats that were alcoholized for 3 months by method I, we detected a significant increase in the contents of saturated FAs (myristic, pentadecanoic, palmitic, and stearic) and polyunsaturated dihomogamma-linolenic (DGLA) acid and, respectively, an increase in the total content of the saturated FAs (Fig. 1). The content of ω-3 docosapentaenoic acid decreased.

Alcoholization of animals for 6 months was accompanied by an increase in the contents of palmitoleic and ω-6 docosapentaenoic acids (Fig. 1) and a decrease in the contents of stearic, eicosadienoic, ara-



**Fig. 2.** The fatty acid composition in the gastrocnemius muscle of rats that were alcoholized for 3 and 6 months for I method [23]. Control value FA content taken as 100%. \*\*,  $p \leq 0.01$ , \*,  $p \leq 0.05$ .

chidonic, and  $\omega$ -3 docosapentaenoic acids in the myocardium of alcoholized rats.

In the gastrocnemius muscle of the rats that were alcoholized for 3 months by method I we detected an increase in the contents of myristic, vaccenic, DGLA, and  $\omega$ -6 docosapentaenoic acids (Fig. 2). The contents of palmitic, palmitoleic, oleic, and linoleic acids also tended to increase. This tendency was also observed for the total contents of saturated, monounsaturated, and  $\omega$ -6 polyunsaturated FAs and the total content of FAs (Fig. 2).

Alcoholization of animals for 6 months led to a decrease in the total FA content (Fig. 2) and a reduction in the contents of myristic, oleic, linoleic,  $\alpha$ - and  $\gamma$ -linolenic, and eicosadienoic acids, as well as in the total content of saturated and  $\omega$ -6 polyunsaturated FAs.

The compositions and contents of FAs in the myocardium and gastrocnemius muscle of the control animals and rats alcoholized for 3 months by method II are shown in Table 3. In the striated muscles of the control rats that consumed liquid feed containing no alcohol, the contents of monounsaturated oleic and vaccenic acids were higher than that in the muscles of the control animals that were kept on the agar diet

(Tables 1–3). This may be due to the high content of monounsaturated fats in the liquid feed.

An increase in the DGLA content was detected in the muscles of the rats that were alcoholized for 3 months by method II (Table 3).

## DISCUSSION

There is evidence that the accumulation of ethyl esters of saturated palmitic and monounsaturated oleic acids in tissues in the case of chronic alcohol consumption had less-pronounced toxic effects than the formation of ethyl esters of polyunsaturated FAs, which led to severe lesions of the liver due to peroxidation of unsaturated fatty acids [26, 27]. In particular, it was shown that an increase in the saturated FA content in the diet of alcohol-fed rats increased the resistance of liver cell membranes to oxidative stress, thereby reducing the alcohol-induced damage of the liver [28]. However, it was found that the saturated FAs that accumulate in tissues exhibit proinflammatory properties, because the incubation of cardiomyocytes (H9C2) with palmitic acid resulted in a gradual accumulation of intracellular lipids and, eventually, in cell death [29]. Using a  $C_2C_{12}$  muscle cell culture, it was

**Table 1.** The fatty acid composition ( $\mu\text{g}/\text{mg}$  tissue) in the myocardium of control rats

Fatty acid	Content	
	control (3 months)	control (6 months)
Myristic ( $C_{14:0}$ )	$0.035 \pm 0.007$	$0.072 \pm 0.026$
Pentadecanoic ( $C_{15:0}$ )	$0.013 \pm 0.003$	$0.025 \pm 0.007$
Palmitic ( $C_{16:0}$ )	$1.943 \pm 0.124$	$2.436 \pm 0.283$
Palmitoleic ( $C_{16:1, \omega-7}$ )	$0.119 \pm 0.014$	$0.157 \pm 0.042$
Stearic ( $C_{18:0}$ )	$3.806 \pm 0.266$	$3.915 \pm 0.343$
Oleic ( $C_{18:1, \omega-9}$ )	$0.794 \pm 0.103$	$1.097 \pm 0.169$
Vaccenic ( $C_{18:1, \omega-11}$ )	$0.893 \pm 0.043$	$0.878 \pm 0.104$
Linoleic ( $C_{18:2, \omega-6}$ )	$4.553 \pm 0.487$	$4.928 \pm 0.267$
$\gamma$ -Linolenic ( $C_{18:3, \omega-6}$ )	$0.039 \pm 0.009$	$0.0358 \pm 0.00914$
$\alpha$ -Linolenic ( $C_{18:3, \omega-3}$ )	$0.015 \pm 0.0029$	$0.0302 \pm 0.009$
Eicosadienoic ( $C_{20:2, \omega-6}$ )	$0.078 \pm 0.010$	$0.068 \pm 0.018$
Dihomo- $\gamma$ -linolenic ( $C_{20:3, \omega-6}$ )	$0.069 \pm 0.005$	$0.064 \pm 0.012$
Arachidonic ( $C_{20:4, \omega-6}$ )	$4.407 \pm 0.356$	$4.045 \pm 0.558$
Adrenic ( $C_{22:4, \omega-6}$ ) (docosatetraenoic)	$0.385 \pm 0.028$	$0.263 \pm 0.075$
Docosapentaenoic ( $C_{22:5, \omega-6}$ )	$0.639 \pm 0.033$	$0.287 \pm 0.082$
Docosapentaenoic ( $C_{22:5, \omega-3}$ )	$0.345 \pm 0.065$	$0.285 \pm 0.065$
Docosahexaenoic ( $C_{22:6, \omega-3}$ )	$2.342 \pm 0.195$	$2.328 \pm 0.634$
Saturated FAs, total	$5.797 \pm 0.379$	$6.448 \pm 0.527$
Monounsaturated FAs, total	$1.806 \pm 0.134$	$2.131 \pm 0.288$
Polyunsaturated $\omega-6$ FAs, total	$10.169 \pm 0.793$	$9.689 \pm 0.975$
Polyunsaturated $\omega-3$ FAs, total	$2.702 \pm 0.221$	$2.643 \pm 0.703$
All FAs, total	$20.4758 \pm 1.499$	$20.911 \pm 2.216$

Respective experimental animals were alcoholized during 3 and 6 months according to method I [23];  $p \leq 0.05$ .

shown that an enhanced level of palmitic acid induced the secretion of proinflammatory interleukin-6 and tumor necrosis factor  $\alpha$  [30, 31]. It was also shown that the treatment of cells with saturated palmitic and stearic acids induced the expression of cyclooxygenase-2 (COX-2) and subsequent formation of the proinflammatory prostaglandin  $E_2$ , whereas the unsaturated FAs inhibited the COX-2 expression induced by palmitic acid [32]. Endoplasmic reticulum stress induced by palmitic acid, which resulted in the activation of caspases, proteolysis, and autophagy, was also prevented by the unsaturated oleic and docosahexaenoic acids [33, 34]. Oleic acid also prevented an increased formation of reactive oxygen species in mitochondria and inhibited the expression of tumor necrosis factor  $\alpha$  and interleukin-6 mRNA in myocytes [35].

It is believed that the opposite effects of oleic and palmitic acids are associated with the accumulation of palmitate as a component of diacylglycerols and oleate as a component of triacylglycerols. Histological studies showed that the treatment of cardiomyoblasts

(H9C2) with palmitic and oleic acids leads to different results. In the cells incubated with oleic acid, lipid droplets had more distinct contours. Conversely, the incubation of cells with palmitic acid disrupted the lipid droplets, which had a diffuse color. Their number was significantly lower, despite the increased accumulation of intracellular lipids. The palmitic acid-induced lipid accumulation on the endoplasmic reticulum caused a severe stress of the latter, eventually leading to cell death [36].

The increased content of saturated FAs in the myocardium after 3-month alcoholization of rats observed in our experiments was possibly a defensive response of cells against lipid peroxidation, to which the saturated FAs are resistant. However, the myocardium and the gastrocnemius muscle contain significant amounts of palmitic acid, which exhibits proinflammatory properties. It can be assumed that the increased content of this acid in the myocardium and the trend towards its increase in the gastrocnemius muscle of the rats that were alcoholized for 3 months

**Table 2.** The fatty acid composition ( $\mu\text{g}/\text{mg}$  tissue) in the gastrocnemius muscle of the control rats

Fatty acid	Content	
	control (3 months)	control (6 months)
Myristic ( $C_{14:0}$ )	$0.133 \pm 0.0467$	$0.159 \pm 0.049$
Pentadecanoic ( $C_{15:0}$ )	$0.061 \pm 0.042$	$0.079 \pm 0.042$
Palmitic ( $C_{16:0}$ )	$2.358 \pm 0.383$	$2.819 \pm 0.604$
Palmitoleic ( $C_{16:1, \omega-7}$ )	$0.212 \pm 0.085$	$0.277 \pm 0.179$
Stearic ( $C_{18:0}$ )	$1.192 \pm 0.057$	$1.121 \pm 0.123$
Oleic ( $C_{18:1, \omega-9}$ )	$1.104 \pm 0.399$	$1.778 \pm 0.948$
Vaccenic ( $C_{18:1, \omega-11}$ )	$0.243 \pm 0.039$	$0.337 \pm 0.115$
Linoleic ( $C_{18:2, \omega-6}$ )	$2.028 \pm 0.419$	$3.335 \pm 1.448$
$\gamma$ -Linolenic ( $C_{18:3, \omega-6}$ )	$0.015 \pm 0.006$	$0.046 \pm 0.075$
$\alpha$ -Linolenic ( $C_{18:3, \omega-3}$ )	$0.023 \pm 0.010$	$0.057 \pm 0.039$
Eicosadienoic ( $C_{20:2, \omega-6}$ )	$0.039 \pm 0.007$	$0.055 \pm 0.028$
Dihomo- $\gamma$ -linolenic ( $C_{20:3, \omega-6}$ )	$0.046 \pm 0.011$	$0.056 \pm 0.027$
Arachidonic ( $C_{20:4, \omega-6}$ )	$1.451 \pm 0.286$	$1.304 \pm 0.262$
Adrenic ( $C_{22:4, \omega-6}$ ) (docosatetraenoic)	$0.102 \pm 0.015$	$0.106 \pm 0.033$
Docosapentaenoic ( $C_{22:5, \omega-6}$ )	$0.133 \pm 0.018$	$0.068 \pm 0.019$
Docosapentaenoic ( $C_{22:5, \omega-3}$ )	$0.134 \pm 0.013$	$0.114 \pm 0.007$
Docosahexaenoic ( $C_{22:6, \omega-3}$ )	$0.852 \pm 0.053$	$0.910 \pm 0.177$
Saturated FAs, total	$3.743 \pm 0.455$	$4.179 \pm 0.679$
Monounsaturated FAs, total	$1.559 \pm 0.464$	$2.392 \pm 1.226$
Polyunsaturated $\omega-6$ FAs, total	$3.814 \pm 0.569$	$4.970 \pm 1.695$
Polyunsaturated $\omega-3$ FAs, total	$1.016 \pm 0.065$	$1.082 \pm 0.173$
All FAs, total	$10.133 \pm 1.447$	$12.623 \pm 3.486$

Respective experimental animals were alcoholized during 3 and 6 months according to method I [23];  $p \leq 0.05$ .

by method I contributed to the development of atrophy of the gastrocnemius muscle [5] and to the revealed trend to the development of atrophy of the myocardium [6] in the rats that were alcoholized for 6 months. The observed decrease in the content of  $\omega-3$  docosapentaenoic acid in the myocardium of rats that were alcoholized by method I for 3 and 6 months (Fig. 1) was apparently due to the necessity of maintaining a certain level of docosahexaenoic acid, whose precursor it is. The metabolites of docosahexaenoic acid exhibit antiinflammatory properties [37, 38], which is important to combat the inflammation caused by palmitic acid. This may explain the absence of adverse changes and development of atrophy in the cardiac muscle samples of the rats that were alcoholized for 3 months [6].

The decrease in the content of stearic acid after a 6 month alcoholization of rats was apparently due to the increase in the content of palmitoleic acid mediated by  $\Delta 9$ -desaturase. The elevated level of palmitoleic acid in the myocardium detected in our experiments may be a harbinger of its pathological changes. It is believed that an elevated level of palmitoleic acid in lymphocytes is a marker of polymyositis, which is characterized by progressive muscle weakness and muscle inflammation [39].

The decrease in the contents of eicosadienoic and arachidonic acids in the cardiac muscle of rats that were alcoholized for 6 months was possibly due to the increased content of  $\omega-6$  docosapentaenoic acid. These acids are precursors in the chain of synthesis of  $\omega-6$  docosapentaenoic acid. It was shown that the  $\omega-6$

**Table 3.** The fatty acid composition ( $\mu\text{g}/\text{mg}$  tissue) in the myocardium and gastrocnemius muscle of the control and experimental rats that were alcoholized for 3 months according to method II [24]

Fatty acid	Content in the myocardium		Content in the gastrocnemius muscle	
	control	experiment	control	experiment
Myristic ( $\text{C}_{14:0}$ )	0.129 $\pm$ 0.012	0.162 $\pm$ 0.013	0.107 $\pm$ 0.015	0.099 $\pm$ 0.010
Pentadecanoic ( $\text{C}_{15:0}$ )	—	—	—	—
Palmitic ( $\text{C}_{16:0}$ )	2.286 $\pm$ 0.084	2.569 $\pm$ 0.222	2.338 $\pm$ 0.323	2.405 $\pm$ 0.245
Palmitoleic ( $\text{C}_{16:1, \omega-7}$ )	0.058 $\pm$ 0.009	0.077 $\pm$ 0.016	0.095 $\pm$ 0.037	0.086 $\pm$ 0.019
Stearic ( $\text{C}_{18:0}$ )	3.815 $\pm$ 0.235	3.931 $\pm$ 0.305	1.107 $\pm$ 0.132	1.225 $\pm$ 0.188
Oleic ( $\text{C}_{18:1, \omega-9}$ )	2.738 $\pm$ 0.431 <sup>#</sup>	2.635 $\pm$ 0.167	3.459 $\pm$ 0.712 <sup>#</sup>	3.025 $\pm$ 0.394
Vaccenic ( $\text{C}_{18:1, \omega-11}$ )	2.652 $\pm$ 0.199 <sup>#</sup>	2.556 $\pm$ 0.366	1.612 $\pm$ 0.234 <sup>#</sup>	1.865 $\pm$ 0.233
Linoleic ( $\text{C}_{18:2, \omega-6}$ )	0.009 $\pm$ 0.003	0.008 $\pm$ 0.004	0.005 $\pm$ 0.003	0.009 $\pm$ 0.004
$\gamma$ -Linolenic ( $\text{C}_{18:3, \omega-6}$ )	0.012 $\pm$ 0.003	0.010 $\pm$ 0.001	0.013 $\pm$ 0.004	0.012 $\pm$ 0.002
$\alpha$ -Linolenic ( $\text{C}_{18:3, \omega-3}$ )	0.019 $\pm$ 0.013	0.024 $\pm$ 0.006	0.023 $\pm$ 0.005	0.024 $\pm$ 0.006
Eicosadienoic ( $\text{C}_{20:2, \omega-6}$ )	0.042 $\pm$ 0.006	0.042 $\pm$ 0.007	0.026 $\pm$ 0.003	0.032 $\pm$ 0.012
Dihomo- $\gamma$ -linolenic ( $\text{C}_{20:3, \omega-6}$ )	0.053 $\pm$ 0.007	0.080 $\pm$ 0.016*	0.032 $\pm$ 0.007	0.051 $\pm$ 0.016*
Arachidonic ( $\text{C}_{20:4, \omega-6}$ )	3.993 $\pm$ 0.155	3.888 $\pm$ 0.356	1.163 $\pm$ 0.165	1.261 $\pm$ 0.162
Eicosadienoic ( $\text{C}_{20:5, \omega-3}$ )	0.004 $\pm$ 0.002	0.004 $\pm$ 0.0004	0.004 $\pm$ 0.001	0.007 $\pm$ 0.003
Adrenic ( $\text{C}_{22:4, \omega-6}$ ) (docosatetraenoic)	0.251 $\pm$ 0.027	0.231 $\pm$ 0.039	0.095 $\pm$ 0.019	0.111 $\pm$ 0.036
Docosapentaenoic ( $\text{C}_{22:5, \omega-6}$ )	0.892 $\pm$ 0.245	1.147 $\pm$ 0.089	0.249 $\pm$ 0.076	0.389 $\pm$ 0.135
Docosapentaenoic ( $\text{C}_{22:5, \omega-3}$ )	0.118 $\pm$ 0.012	0.130 $\pm$ 0.027	0.076 $\pm$ 0.013	0.084 $\pm$ 0.023
Docosahexaenoic ( $\text{C}_{22:6, \omega-3}$ )	1.035 $\pm$ 0.075	1.074 $\pm$ 0.176	0.484 $\pm$ 0.093	0.507 $\pm$ 0.073
Saturated FAs, total	6.229 $\pm$ 0.291	6.66 $\pm$ 0.474	3.551 $\pm$ 0.451	3.729 $\pm$ 0.407
Monounsaturated FAs, total	5.449 $\pm$ 0.439	5.269 $\pm$ 0.541	5.167 $\pm$ 0.959	4.000 $\pm$ 0.519
Polyunsaturated $\omega$ -6 FAs, total	5.251 $\pm$ 0.410	5.403 $\pm$ 0.418	1.582 $\pm$ 0.2332	1.865 $\pm$ 0.356
Polyunsaturated $\omega$ -3 FAs, total	1.177 $\pm$ 0.093	1.231 $\pm$ 0.205	0.585 $\pm$ 0.107	0.619 $\pm$ 0.103
All FAs, total	18.105 $\pm$ 0.846	18.563 $\pm$ 1.524	8.782 $\pm$ 5.637	11.188 $\pm$ 1.234

<sup>#</sup>, Differences were significant with respect to the contents of oleic and vaccenic acids in the myocardium and gastrocnemius muscles of the rats that served as a control for the animal that were alcoholized for 3 months according to method I (see Tables 1 and 2). \*  $p \leq 0.05$  vs. control..

and  $\omega$ -3 forms of docosapentaenoic acids are strong inhibitors of sphingosylphosphorylcholine-induced spasm of vascular smooth muscles (in particular, the smooth muscles of human coronary artery) [40]. The increase in the content of  $\omega$ -6 docosapentaenoic acid in the myocardium after alcoholization for 6 months is probably a defensive response of the myocardium against the detrimental effects of alcohol.

The increase in the content of myristic acid in the gastrocnemius muscle after alcoholization of rats for 3 months may be due to increased incorporation of glucose into muscle cells. It was demonstrated in  $\text{C}_2\text{C}_{12}$  cells that myristic acid enhances the insulin-independent glucose incorporation into myocytes by increasing the expression of diacylglycerol kinase  $\delta$ . Palmitic and stearic acids, for which a significant increase in content was detected in our experiments, did not have such an effect [41]. In addition, it was

shown that myristoylation of the pentapeptide Gblin, an inhibitor of ubiquitin ligase Cbl-b, prevented the glucocorticosteroid-stimulated atrophy of skeletal muscles [42]. The increase in the content of myristic acid in this period of alcoholization of animals is probably a defensive response against the atrophic changes in the gastrocnemius muscle [43].

The cause of the increased content of vaccenic acid in the gastrocnemius muscle of rats that were alcoholized for 3 months by method I is unclear. The metabolic effects of vaccenic acid are poorly understood. It is known that *cis*-vaccenic acid is synthesized from palmitoleic acid by the enzyme elongase-5. Using HepG2 cells, it was shown that this acid is involved in the regulation of the mTORC2-Akt-FoxO1 signaling pathway [44].

The decrease in the contents of oleic and linoleic acids in the gastrocnemius muscle after alcoholization

of rats for 6 months might be associated with the atrophic changes in the gastrocnemius muscle. It was shown that oleic and linoleic acids function as endogenous ligands of receptor 1 of free fatty acids and induce the proliferation of human trachea smooth muscle cells [45]. A decrease in the contents of these acids indicates a decline in proliferation and is consistent with the previous data on the atrophic changes in this muscle [5]. The reduced content of  $\alpha$ -linolenic acid, which is a precursor for the synthesis of docosapentaenoic and docosahexaenoic acids, was apparently due to the necessity of maintaining the contents of these acids at a certain level. The decrease in the content of eicosadienoic acid after alcoholization of animals for 6 months may be associated with a decrease in the content of linoleic acid, which is a precursor of its synthesis.

A distinctive feature of the studied muscles of rats that received alcohol for 3 months by both method I and II is the significant increase in the DGLA content. Dihomo- $\gamma$ -linolenic acid performs various functions in cellular metabolism. The metabolites of this acid exhibit primarily antiinflammatory properties. An increase in the content of DGLA in relation to the content of arachidonic acid indicates a decrease in the endogenous production of arachidonic acid by  $\Delta 5$ -desaturase and a reduction in the synthesis of its proinflammatory metabolites such as series 2 prostaglandins and series 4 leukotrienes. Dihomo- $\gamma$ -linolenic acid is the substrate for the COX-2-catalyzed synthesis of series 2 prostaglandins, which exhibit antiinflammatory properties. Introduction of this acid to the diet of humans and animals selectively increased the synthesis of prostaglandins (in particular, the antiinflammatory prostaglandin E1). By binding to corresponding receptors through a G protein, prostaglandin E1 triggers the signaling mechanisms that stimulate the expression of a number of genes through the activation of transcription factors, thereby causing positive effects during a number of diseases [46, 47]. Experiments on mice showed that DGLA had a cardioprotective effect as a result of its oxidation by 12-lipoxygenase to form 12-(S)hydroxy-8Z,10E,14Z-eicosatrienoic acid (12(S)-HETrE), which inhibits activation of platelets and thrombosis [48]. In addition to the series 1 prostaglandins, DGLA is converted into series 3 leukotrienes, which also exhibit antiinflammatory properties. These leukotrienes also inhibit the action of arachidonic acid metabolites [46]. The increase in the content of DGLA in all muscle tissue samples after alcoholization of animals for 3 months (Figs. 1, 2, Table 3) is apparently indicative of the activation of a defensive mechanism that is common to all muscle groups. This assumption is consistent with the absence of atrophic changes and increased proteolysis of titin and nebulin in the myocardium and gastrocnemius muscle of rats that were alcoholized for 3 months [6, 43].

It should be noted that the contents of monounsaturated oleic and vaccenic acids in the myocardium of both the control rats and the rats that were alcoholized for 3 months by method II (Table 3) increased compared to the contents of these acids in the myocardium and gastrocnemius muscle of the rats that were alcoholized for 3 months by method I (Figs. 1, 2; Tables 1, 2). It was shown that oleic acid can affect gene expression [49]. In our experiments, an increased content of this acid was observed against the background of an increased expression of titin gene in the myocardium and gastrocnemius muscle of the rats that were chronically alcoholized by method II [6, 43].

The results of this study suggest that the changes in the contents of FAs in chronic alcohol intoxication precede the development of atrophic and other adverse changes in striated muscles.

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