MORPHOMETRY OF HISTOENZYMOLOGICAL TYPES OF MUSCULAR FIBERS OF RECTUS ABDOMINALIS MUSCLE OF ALCOHOLIZED RATS (RATTUS NORVEGICUS)

Nícolas Bertolaccini dos Santos[1]

Jesus Carlos Andreo[4]

Luis Henrique Rapucci Moraes[1]

[1]*Course on Biological Sciences, University of the Sacred Heart - USC.*

[2]*Course on Physical Therapy, University of the Sacred Heart - USC.*

[3]*Course on Veterinary Medicine – University of Marília - UNIMAR.*

[4]*Professor, – Graduated Course on Oral Biology – USC.*

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ABSTRACT

Taking into account that alcohol users represent a good portion of the human population, that the prolonged use of alcohol in excess causes multiplicity of both biochemical and morphological abnormalities, a study has been carried out on the effect of this drug in a mixture where alcohol represents more than 50% of the daily calories used by the rat, which presents a selective atrophy of Type II-fibers, similar to that in humans. Thus, a sample was collected from the rectus abdominalis muscle in five animals of each group: Control (C), Alcoholized (A) and Isocaloric (I). The samples were submitted to m-ATPase reactions (with acid and alkaline pre-incubations) and NADH-TR to classify the fibers according to the results obtained in these reactions. The results showed a significant difference in the frequency of Type-FG fibers, as well as in that of the type FOG, among the groups studied. As for the area, the results pointed to a significant difference just for the FG-type fibers, being smaller in the animals of group A and larger in those of group C. Based on this data, one may conclude that the alcoholization altered the size of Type-FG fibers and the frequency of fibers FG and FOG.

KEY WORDS: alcoholism; types of muscular fibers; rats – Rattus norvegicus; rectus abdominalis

INTRODUCTION

Alcoholism is considered a pharmacodependent disease by the World Health Organization (WHO) (FORTES; CARDO, 1991). In the literature there is no available data on the prevalence of alcoholics in Brazil but in some countries such as the USA the number is high – 7% of the total population, which means that 9 million Americans drink alcohol. It is important to note that most users deny this fact and thus the real number of those that use alcohol is usually greater than the reported figures.

The prolonged and abusive use of alcohol leads to several biochemical and electrophysiological abnormalities that are associated to liver diseases, neuromuscular system, heart and brain (SPENCER, 1986). According to Preedy et al. (1990) many metabolic pathways are modified and all organs are affected after acute or chronic exposition to alcohol.

The muscle system arises many interesting questions in research since it is the greatest organic system in the human body and responds quickly to alteration such as functional demand, nutritional status, innervation and irrigation (PREEDY et al, 1990; SPENCER, 1986).

Skeletal muscles of ethanol consumers can show acute and chronic modifications. In this sense, the physiopathological mechanism is probably related to alteration in the membrane flow, in the ionic pumps, depression in contractility, protein synthesis and genetic problems (FERNANDEZ-SOLA et al., 1996).

Effects of alcohol on muscles has been studied in many animals and mainly in man (FERRAZ et al., 1989; SHARMA et al., 1990; CHEN et al., 1991; PREEDY et al., 1994; SESTOFT et al.,1994; FERNANDEZ-SOLA et al., 1995) and rats (PREEDY et al., 1990; TROUNCE et al., 1990; SALYSBURY et al., 1992).

Alcoholic myopathy occurs in 1/3 to 1/2 of persons with abusive use of alcohol and it is characterized by selective atrophy of type II fibers whereas those of the type I are not affected (REILLY et al., 1998).

Such atrophy has been described in the *tibialis anterior* (SHARMA et al., 1990), soleus (PREDY et al., 1990), plantar (PREEDY et al., 1990), *gastrocnemius* (PREEDY et al., 1990), which has predominantly one type of fiber.

Rectus abdominalis has been considered a good control muscle since it contains the three types of myosin (I, IIA and IIB) (SCIOTE et al., 1994).

Histoenzimology and morphometry have been considered as two

excellent methods to evaluate alteration in the striated muscle system.

Data in the literature show that there is not enough information on the histoenzimologic characteristics of *rectus abdominalis*, mainly in alcoholic animals, although it is important to the posture of some animals, a good standard to some researches, and easily accessible for biopsies.

Taking the points mentioned above into account the objective of the present study is to observe, on the histoenzimologic point of view, the effect of alcohol in the area and frequency of different types of muscle fibers in this muscle.

MATERIAL AND METHODS

Samples of the *rectus abdominalis* were removed from 15 male Wistar rats (*Rattus novergicus*), approximately 3 months old and weighting about 200 g, provided by the biotery of the University of São Paulo – Campus Bauru.

90 days old animals were maintained in individual cages of stainless steel with individual eating and drinking devices to better control the solid and liquid intake. The room light was artificial and controlled by a timer in bright/dark cycle of 12 hours.

Animals were separated in three groups with five animals each as follows:

- Normal control group (N) – animals received tap water as liquid diet.
- Alcoholic group (A) – (animals submitted to chronic alcoholism) animals received ethylic alcohol 25% diluted in water.
- Isocaloric nutritional paired control group (I) – animals received water with sacarose in the liquid diet.

Animals of the three groups always received the same solid diet (ration Nuvilab CR 1, from NUVITAL) during the study.

The alcohol used was absolute ethylic alcohol from MERCK (CH_3CH_2O).

After the period of treatment the animals were sacrificed for removal of samples of the *rectus abdominalis* muscle.

The samples were kept at room temperature for 15 minutes and then immersed in frozen n-hexane at -70°C in liquid nitrogen for two minutes. The muscle samples were stored in liquid nitrogen until the processing.

From each sample several 10 mm slides transversal to the longitudinal axe were prepared. These slides were used in m-ATPase reactions (with acid and alkalin pre-incubation) following the methodology of Werneck

(1981) and NADH-Diaphorase (PEARSE, 1968) modified by Dubowitz and Brooke (1973) and stained by H&E (BEHMER et al., 1976).

Based on the result of the m-ATPase and NADH-TR reactions the fibers were classified as FG, FOG and SO according to Peter et al. (1972).

Many microscopic fields were randomly selected and 300 fibers were identified and classified. The area was calculated in 40 fibers of each type, in each slide for each animal.

Fiber counting and area calculation were done with a image analysis system model Image-Pro Plus version 4.1 connected to a Pentium III microcomputer.

Data on frequency and area from the different types of fibers were submitted to variance analysis to observe the presence or not of significant difference among them in the various studied groups. If any significant difference among them was detected by variance analysis, the Turkey test was used to identify the difference.

RESULT

Weight of animals (in grams) at the beginning of the study and in the moment of sacrifice, as well weight gain, can be seen in TABLE 1. The daily caloric intake is presented in TABLE 2.

	Normal		Alcoholic		Isocaloric	
Weight	mean	sd	mean	sd	mean	sd
Initial	271.2	28	258.3	18.6	276.68	27.2
Final	413.9	39	282.5	48.4	345.88	28.3
Weight gain in	**53.3**	14.8	**9.36**	18	25.4	**9.8**

TABLE 1 – Average weight in grams in the beginning of the study and in the moment of sacrifice. Gain of weight (%) in groups N, A and I.

	Normal		Alcoholic		Isocaloric	
	mean	sd	mean	sd	Mean	sd
Ration intake in g	23.26	1.43	10.49	1.53	9.34	1.57
Caloric intake	**63.97**		**28.80**		**25.69**	
Ration intake in g	49.65	4.82	22.84	3.70	21.41	4.03
Caloric intake	**0.0**		**32.44**		**30.71**	
Total caloric intake	**63.97**		**61.24**		**56.4**	

TABLE 2 – Average daily caloric intake by animals of groups N, A and I.

The nitrogen balance made in urine and stools revealed the following figures: Group N = 1.24 Group A= - 3 e group I = -3.5.

Blood samples revealed the presence of alcohol only in Group A with average 0.22g/l of blood (index for humans).

TABLE 3 – Results for m-ATPase and NADH-Tr reactions in the samples of *rectus abdominalis* from animals of groups N, A and I.

Fibers	m-ATPase pH 10.6 Fig. 1	m-ATPase pH 4.5 Fig. 2	m-ATPase pH 4.4 Fig. 3	NADH-Tr Fig. 4
1 Type FG	+++	++	+	+
2 Type FOG	+++	+	+	++
3 Type SO	+	+++	+++	+++

+ low reactivity ++ mild reactivity +++ intense reactivity

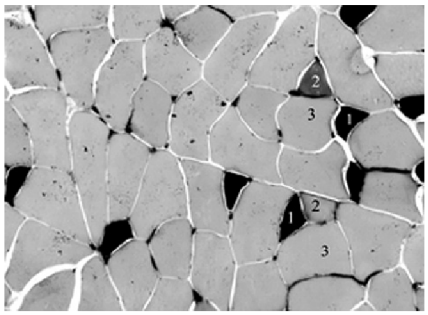

FIGURE 1 – *rectus abdominalis* muscle submitted to acid m-ATPase reaction (ph 4.4). 1=FG fiber; 2=FOG fiber and 3=SO fiber.

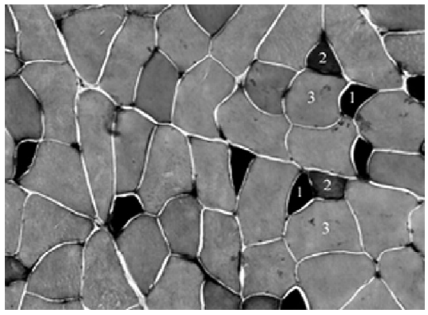

FIGURE 2 – *rectus abdominalis* muscle submitted to acid m-ATPase reaction (ph 4.5). 1=FG fiber; 2=FOG fiber and 3=SO fiber.

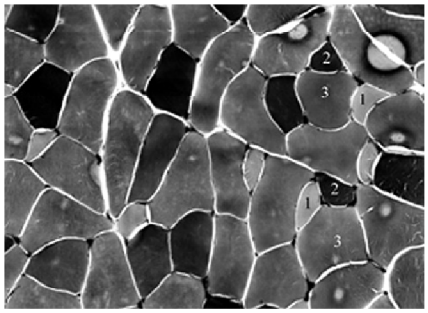

FIGURE 3 – *rectus abdominalis* muscle submitted to alkaline m-ATPase reaction (ph 10.4). 1 = FG fiber; 2 = FOG fiber and 3 = SO fiber.

FIGURE 4 – *rectus abdominalis* muscle submitted to NADH reaction. 1 = FG fiber; 2 = FOG fiber and 3 = SO fiber.

TABLE 4 – Area in μm² of the various fibers types found in the *rectus*

Fibers	Normal		Alcoholic		Isocaloric			
	mean	sd	mean	sd	mean	sd	F	P
FG	6.43 a	0.91	3.31 b	0.91	4.77 c	0.38	20.40	0.000*
FOG	2.72	0.82	1.87	0.46	1.90	0.19	3.78	0.053
SO	0.93	0.14	0.69	0.15	0.83	0.32	1.60	0.241

abdominalis muscle of animals from Groups N, A and I.

Values for fiber area are greater in Group N and smaller in Group A for all types of fibers. ANOVA test revealed significant difference among groups only for type FG fibers. However, values for type FOG fiber were close of a significant difference. The test of Turkey showed the difference found in the area of type FG fiber were among the groups N and A, N and I and A and I.

TABLE 5 – Frequency (in %) of the various fibers types found in the recto

Fibers	Normal		Alcoholic		Isocaloric			
	average	sd	average	sd	average	sd	F	P
FG	63.6^{a}	1.84	60.9^{ab}	4.09	57.7^{b}	5.59	4.04	0.045*
FOG	22.3^{a}	3.84	23.7^{ab}	2.18	28.3^{b}	5.16	4.08	0.044^{*}
SO	14.1	2.98	15.4	3.80	14.1	5.93	0.13	0.873

Groups with same letters do not show statistical significant difference among them.

abdominalis muscle of animals of groups N, A and I.

Concerning data on the frequency of the various types of fibers ANOVA revealed statistical significant difference among groups both for fibers FG and FOG. The test of Turkey showed that this difference was in groups N and I, both for FG and FOG fibers.

By means of Pearson correlation a correlation between the area of type FG fibers and the weight of animals was demonstrated.

DISCUSSION

There are few articles in the literature on the histoenzimology of *rectus abdominalis* muscle.

Martin (1985) conducted histoenzimologic studies in the muscle and has observed that the most frequent fibers were FG followed by FOG and then by SO. These data are in accordance with the findings of our study.

Haggmark and Thorstensson (1979) studied the muscles of the abdominal wall of humans (*rectus abdominalis*, *obliquus lateralis and medialis* and the *transversus abdominalis*), classifying fibers as I, IIA, IIB and IIC. According with these authors the frequency of fibers did not vary among muscles of the abdominal wall. The most frequent were those of type I followed by IIA, IIB and IIC. Regarding size, the small diameter was similar to all fibers, a finding that diverges from the present study.

The same muscle was studied by Sciote et al. (1994) that identified fiber I, IIA and IIB. Fibers of the IIB type were greater that IIA and I, in this sequence. These findings are similar to ours.

Ito (1998) studied the muscle *rectus abdominalis* of the Japanese monkey (*Macaca fuscata*). The most frequent fiber was type IIB followed by types I and IIA. These findings are not in accordance to ours.

In the literature there are several studies on the effects of alcohol in the striated muscle. Some of them where done in humans and other in animals. Among the latter, the rat was the most indicated as an ideal experimental model because it shows a selective atrophy for type II fibers similar to what happens to humans (TROUNCE et al., 1990; PREEDY et al., 1990; SALISBURY et al., 1992; PREEDY et al., 1994).

The results on the present study in what regards the atrophy, or the disturb in growth of type II fibers, which are of rapid contraction, this fact was also observed since the growth of fiber area in types FG and FOG was smaller in groups A and I than those of the group N. In the case of fibers FG the difference was significant among the studied groups.

In what regards the effect of alcohol on the slow contraction fibers, i.e. type I fibers, the literature shows conflicting data. Some authors consider these fibers as not being affected by use of alcohol (MARTIN et al., 1985; FERNÁDEZ-SOLÁ et al., 1995; REILLY et al., 1998), whereas others consider that fibers I can suffer a slight hipertrophia (PREEDY et al., 1989; SALISBURY et al., 1992; PREEDY & PETER, 1994). According to Preedy et al. (1994) in special circumstances a chronic alcoholic may show atrophy of type I fibers but always less than for type II fibers. In this study it was observed that the growth of slow contraction fibers (SO) was smaller in group A that in the other two groups, but the difference was not significant.

The preference for atrophy on slow contraction fibers due to alcoholism is considered by some authors as similar to what occurs in other metabolic miopathy such as denutrition. Therefore the mechanism that affects this type of fiber may be similar among themselves (PREEDY

et al., 1994).

This selective effect of atrophy of the rapid contraction fibers in alcoholic patients has been considered as independent from the nutritional status by some authors (MARTIN et al., 1985; SALISBURY et al., 1992). However, Carpenter and Karpati (1984) consider that in chronic alcoholics the development of atrophy of type II fiber may be due to other causes such as the superimposition of malnutrition. These authors refer in addition that in previous studies the atrophy of type II fibers was present in circa 30% of chronic alcoholics and that it was more frequent in patients showing superimposed malnutrition, peripheral neuropathy and other ethanol-related diseases.

In the present study a note should be made regarding the rats of the isocaloric group, which is considered as a control group in some other studies. Animals from this group had a small development when compared to rats with free food of the group N similar to those of Preedy et al. (1988) to whom this aspect can alter skeletic muscle fibers.

One should remember also, according to Bunout et al. (1987), that the alcoholic myopathy can be attributed, in part, to a negative nitrogen balance in alcoholic patients and that in this study the nitrogen balance was negative, both in the alcoholic and isocaloric groups.

In humans the chronic abuse of alcohol cause a reduction in the extraction of urine creatinine suggesting that the muscle mass is reduced as a whole ((DUANE; PETER, 1988). In the rat there is also a reduction of weight as a result of the long feeding with ethanol (six weeks) (PREEEDY; PETER, 1988 a and b; PREEDY et al., 1989; TROUNCE et al., 1990). In the present study animals of alcoholic group had an average body weight gain of 9.36% but two of these animals showed weight loss, as in the previously mentioned studies.

Another point that should be stressed is that the fibers of rapid contraction of the type FG, which showed a greater rate of atrophy, presented a positive correlation to the animals' weight of the studied muscle.

It was also observed in the present study that the isocaloric group, even having a smaller daily caloric intake than the alcoholic group (56.4 and 61.24 respectively) had a greater weight gain, although the difference was not significant from the statistical point of view.

Authors consider that fibers of rapid contraction that are mostly affected by alcohol, and thus showing a greater loss in size, are those of the IIB type (glicolytic anaerobic rapid contraction fibers) (TROUNCE et al., 1990; CHEN et al., 1991; PREEDY et al., 1994; SESTOFT et al., 1994; REILLY et al., 1998) what is similar to the finding of the present

study, in which the FG fiber showed a greater reduction in size among all studied muscles.

However, a question remains to be ansswered: to what extend does malnutrition affect the growth of muscle fibers? On should remember that animals of group I received a smaller amount of daily calories than animals of group A (56.4 and 61.2, respectively), the negative nitrogen balance was greater also in group I than in group A (-3.5 and -3.0, respectively) and even so the atrophy of FG fibers was smaller in group I animals that those in group A, showing a significant statistical difference.

CONCLUSION

From the results it is possible to conclude that rats submitted to alcoholization, in which alcohol represent more than 50% of the daily caloric intake, alter the size of FG fibers leading to a selective atrophy of these fibers in animals of the group A and alterations in the frequency of fibers of the types FG and FOG.

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