ISSN 0362-1197, Human Physiology, 2015, Vol. 41, No. 6, pp. 625–628. © Pleiades Publishing, Inc., 2015.
Original Russian Text © T.L. Nemirovskaya, B.S. Shenkman, O.E. Zinovyeva, Yu.N. Kazantseva, N.D. Samkhaeva, 2015, published in Fiziologiya Cheloveka, 2015, Vol. 41, No. 6, pp. 65–69.

Development of Clinical and Morphological Signs of Chronic Alcoholic Myopathy in Men with Prolonged Alcohol Intoxication

T. L. Nemirovskaya[a, b], B. S. Shenkman[b], O. E. Zinovyeva[c], Yu. N. Kazantseva[c], and N. D. Samkhaeva[c]

[a] *Moscow State University, Moscow, Russia*
e-mail: nemirovskaya@bk.ru
[b] *Institute of Medico-Biological Problems, Russian Academy of Sciences, Moscow, Russia*
[c] *Sechenov First Moscow State Medical University, Moscow, Russia*

Received May 14, 2015

Abstract—Chronic alcoholic myopathy (ChAM) develops in 40–60% of alcohol-abusing patients and is accompanied by a lower performance, proximal paresis, and skeletal muscle atrophy. It is still unclear whether the duration or amount of alcohol consumption is important for ChAM development. The time course of the pathological process in skeletal muscle is also unknown. Male patients with and without alcoholic myopathy were evaluated for the duration of alcohol abuse, ethanol intake, morphological characteristics of *m. quadriceps vastus lateralis*, and the plasma content of insulin-like growth factor 1 (IGF-1). ChAM was found to develop after 10 years of alcohol abuse. Proximal paresis was observed only in patients with muscle fiber atrophy and was associated with a slow to fast transformation of the myosin phenotype. A decrease in plasma IGF-1 was detected in early ChAM, including stages without clinical signs of proximal paresis or morphological signs of muscle fiber atrophy.

Keywords: chronic alcoholic myopathy, muscle fiber atrophy, insulin-like growth factor 1
DOI: 10.1134/S0362119715060043

INTRODUCTION

Chronic alcoholic myopathy (ChAM) is an alcoholic disease form that is characterized by a progressive weakening and hypotrophy of proximal muscle groups of the limbs, the legs being affected more often and the arms involved upon disease progression; difficulty waking; and painful cramps of leg muscles [1]. Atrophy usually affects locomotor muscles and involves mostly the fibers that express fast myosin II isoforms [2, 3]. The changes are found in both humans [2–4] and animals [5, 6]. Experiments with animals have focused mostly on fast muscles (*m. gastrocnemius* and *m. plantaris*), which almost totally lack slow muscle fibers. Atrophy of the postural *m. soleus* has not been observed in alcoholic rats [7]. It has been assumed that type I fibers are protected from the ethanol effect and, consequently, are not affected by atrophy [8]. Yet only few studies have been performed in human subjects with chronic alcoholic intoxication, and atrophy of type I muscle fibers is still an open question. Studies focusing on changes in myosin phenotype are also few in the available literature, and their results are discrepant. For instance, a decrease in myosin heavy chain mRNA has been observed in both fast *m. plantaris* and slow *m. soleus* in alcoholic rats [7], while the fast myosin isoform protein contents have not changed. No difference in the proportion of fast and slow muscle fibers in *m. deltoideus* has been observed between patients with a 25-year history of alcohol abuse and control subjects [9]. However, a predominance of type I muscle fibers has been assumed as a histological criterion of ChAM in [9], the assumption contradicting the experimental results obtained in animals [7]. We studied the changes in myosin phenotype in patients with chronic alcoholic intoxication and collated the time course of morphological characteristics of *m. vastus lateralis* with the development of clinical signs of ChAM. An attempt was made to establish whether the duration of alcohol abuse or the amount of ethanol consumption is responsible for ChAM development. A better understanding of these problems will help to develop a strategy for preventing alcoholic myopathy.

METHODS

We examined patients with chronic alcoholic intoxication who were hospitalized to the Kozhevnikov Clinic for Nervous System Diseases. The study was approved by an ethics committee (extract from Minutes no. 0908 dated November 13, 2008 of the Interacademic Ethics Committee). The study involved 18 male patients aged 29 to 59 years (mean age 47.7 ± 2.0 years) and 12 age-matched healthy male volunteers (a control group). All of the subjects signed an informed consent to participation in the study.

Table 1. Muscle strength evaluation

Grade	Characteristics
0	Active movements and visible contractions of the muscle are not observed
1	Contractions of the muscle are just detectable (stirring is preserved)
2	Movements of the muscle are possible only when the resistance of gravity is eliminated
3	The strength of the muscle is moderately decreased. The patient is capable of holding the limb against gravity and resisting the force applied by the investigator, but the resistance is easy to overcome
4	Muscle strength is slightly lower. The patients resists well the force applied by the investigator, but the resistance can still be overcome (the muscle yields upon testing)
5	Muscle strength is preserved in full and corresponds to the gender-, age-, and constitution-related norm. The investigator cannot overcome the muscle resistance

The inclusion criteria for the study were as follows: (1) at least a 3-year history of regular alcohol drinking and (2) consumption of no less than 4 ethanol units per day and no less than 14 ethanol units per week. The exclusion criteria were: (1) a refusal to participate in the study; (2) a newly diagnosed infection (including virus hepatitis forms, syphilis, and HIV infection); (3) a newly diagnosed disorder that may lead to polyneuropathy (diabetes mellitus, thyroid disorders, oncology diseases, etc.); (5) a newly diagnosed hereditary myopathy; (6) a newly diagnosed disorder that may lead to myopathy (diabetes mellitus, thyroid disorders, oncology diseases, renal failure, systemic connective tissue disorders, etc.); and (7) uncontrolled concomitant somatic conditions.

The daily alcohol consumption of the patients was estimated in alcohol units, one alcohol unit corresponding to 10 mL of pure (96%) ethanol. The mean daily consumption was 17.5 ± 1.0 alcohol units in the test group, ranging from 10.6 to 31.6 alcohol units per day. All subjects of the control group denied alcohol abuse and lacked acute or chronic diseases and signs of damage to peripheral nerves and skeletal muscles at the time of testing. A clinical neurological examination of the subjects included motor, sensory, and coordination testing. In motor testing, we evaluated the muscle tone and muscle strength in the proximal and distal regions of the limbs upon isometric exercise, using a six-grade scale (Table 1).

Functional tests with walking on toes, walking on heels, and squatting were performed to detect a subclinical decrease in muscle strength of the limbs.

Laboratory testing was aimed at detecting concomitant conditions and included a total blood count and blood chemistry, in particular, liver aminotransferases (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)), γ-glutamyltransferase (GGT), and creatine phosphokinase (CPK). In addition, insulin-like growth factor 1 (IGF-1) was assayed in the blood plasma by ELISA.

Muscle tissue samples of the alcoholic subjects were taken from the lateral head of *m. quadriceps femoris* (*vastus lateralis*) by open biopsy under local anesthesia with 1% novocaine. Needle biopsy was performed in the control group. The *vastus lateralis* biopsy samples were examined pathomorphologically and immunohistochemically. Signs of muscle fiber destruction (necrosis), excess formation of connective (fibrosis) and adipose tissues, and leukocyte infiltration (inflammation) were checked after staining with hematoxylin–eosin. Primary monoclonal antibodies to type I and type II myosin heavy chains (MHCs) were from Novocastra Laboratories (dilution 1 : 40) and Sigma (United States, dilution 1 : 100 with PBS). Tissue sections were incubated in a moisture chamber at 37°C for 60 min, washed thrice with PBS for 5 min. Secondary FITC-conjugated polyclonal anti-mouse immunoglobulin goat antibodies (Sigma, United States) were applied at a 1 : 100 dilution with PBS. The sections were incubated for 60 min, washed thrice with PBS for 5 min, and mounted in the Fluoromount-G medium (Southern Biotech). The preparations were photographed at magnification ×400, using a Leica Q500MC microscope (Germany) and a Leica DC300F camera. Fibers expressing different MHC isoforms were counted and the mean cross-sectional area (CSA) of muscle fibers was measured using LeicaQwin software. To estimate the relative portions of the fast and slow MHC isoforms, at least 200 fibers of each type were scored. At least 100 muscle fibers of each type were examined to obtain the mean CSA.

RESULTS

The alcoholic patients were divided into two subgroups depending on the history of alcohol abuse. In subgroup 1 ($n = 6$), the mean duration of excessive drinking was 9.6 ± 1.6 years. The proportion of different fibers in this group did not significantly differ from that in the control group (Table 2). A clinical neurological examination did not detect any cases of weakness in proximal limb regions or hypotrophy of upper or lower limb girdle muscles; i.e., clinical signs of myopathy were absent.

Table 2. Cross-sectional area (μm^2) and muscle fiber proportion in patients with chronic alcoholic intoxication and control subjects

Parameter	Control group ($n = 12$)	Subgroup 1 (without MF atrophy)	Subgroup 2 (with atrophy of type II MFs)
Type I MF CSA	4882 ± 152	4319 ± 359	4871 ± 540
Type II MF CSA	4878 ± 161	4661 ± 246	3351 ± 213*
Type I MF, %	42.1 ± 3.9%	36.7 ± 4.4%	31.5 ± 2.0%*
Type II MF, %	51.0 ± 3.4%	57.5 ± 4.8%	56.6 ± 2.4%
Hybrid MFs, %	6.9 ± 1.7%	5.7 ± 1.1%	11.8 ± 2.6%

CSA, cross-sectional area; MF, muscle fiber.

* The difference from the control is significant at $p < 0.05$.

In subgroup 2 ($n = 12$), the mean duration of alcohol intoxication was 14.6 ± 2.0 years. Atrophy of type II fibers and fast to slow muscle fiber transformation were observed in these patients (Table 2). Neurologically, lower proximal paraparesis was observed in ten patients, including one patient with a score below 3–3.5, six patients with a score below 4, and three patients with muscle weakness by functional tests. Muscle weakness in the shoulder girdle with a score below 3.5–4 was additionally observed in three patients having lower proximal paraparesis with a score below 3, suggesting a generalized skeletal muscle involvement and proximal tetraparesis.

Blood chemistry. The IGF-1 concentration in the blood plasma significantly differed between the chronic alcoholics (subgroups 1 and 2) and the control subjects (143.3 ± 15.7 and 94.8 ± 7 ng/mL vs. 216 ± 63.6 ng/mL in the control group, $p < 0.05$). It should be noted that both of the alcoholic subgroups displayed significantly lower IGF-1 concentrations. Yet a greater decrease in IGF-1 was observed in the subgroup of patients with morphologically verified muscle fiber atrophy. Plasma GGT was substantially elevated in all of the patients. The mean GGT was 184.2 ± 40.6 units/L in the patient group and 19.0 ± 3.2 units/L in the control group. A moderate increase in liver aminotransferases (AST and ALT) of the blood plasma was additionally found in the two patient subgroups. The mean levels were 47.9 ± 5.3 units/L (vs. 19.8 ± 5.9 units/L in the control group) for ALT and 43.5 ± 6.4 units/L (vs. 19.0 ± 2.3 units/L in the control group) for AST. The higher plasma levels observed not only for GGT, but also for AST and ALT point to a long-term toxic effect of ethanol and its metabolites on the lifer function. The CPK level was generally within the normal range in the patients. The mean CPK was 81.0 ± 9.7 units/L (64.0 ± 7.6 units/L in the control group). The result suggests no destructive change (necrosis) in muscle fibers. Histochemistry confirmed the absence of necrosis and inflammatory infiltration for all *m. quadriceps vastus lateralis* biopsy samples.

DISCUSSION

Type II muscle fibers are predominantly affected by atrophy, as has been observe in experimental animals and patients with chronic alcoholic intoxication [2–4]. However, it is unclear whether the amount of alcohol consumption or the duration of alcoholic intoxication contributes to the changes. We have previously examined both male and female patients, but the sample size was insufficient for the total sample to be stratified by gender. In this study, we examined a random sample that included only male alcoholic patients. A comparison showed that subgroups 1 and 2 were similar in the age and daily alcohol intake of the patients. It should be noted that the daily alcohol consumption of our patients was substantially higher than considered allowable by the WHO [10]. Thus, the patients were at high risk of various forms of alcoholic disease, including ChAM, according to WHO recommendations. The duration of alcohol abuse substantially differed between the subgroups, being greater in subgroup 2. Type II muscle fiber atrophy was observed only in the subgroup 2 patients, whose daily alcohol consumption was much the same as in subgroup 1. Therefore, ChAM develops gradually and the extent of atrophy depends on the duration of alcohol abuse. An earlier study has demonstrated atrophy of type II muscle fibers, but the duration of drinking history was not compared, and the alcohol dosage was reported for the total patient sample [11]. Another study has focused on a correlation analysis within an alcoholic sample, and a decrease in size of type II muscle fibers was correlated with the amount of alcohol consumption and the duration of alcohol intoxication [12]. Although the daily consumption and the duration of alcohol abuse did not differ in patients with different myopathy severity, a dose-dependent effect was assumed for ethanol action on skeletal muscles. We did not observe such an effect in our sample because the daily alcohol consumption was similarly high in both of the subgroups. Muscle fiber atrophy was not detected in subgroup 1, but the IGF-1 concentration in the blood plasma was already decreased in these patients. IGF-1 is thought to be a marker of protein synthesis [13, 14].

A decrease in IGF-1 in our patients agrees with the results obtained in experimental animals [15]. Yet we were the first to observe a decrease in IGF-1 prior to atrophy development in muscles. A decrease in IGF-1 was accompanied by laboratory signs of damage to the liver in our patients. It is known that hepatocytes synthesize more than 95% of the total IGF-1. Chronic alcohol intoxication decreases the circulating IGF-1 level because the rates of IGF-1 synthesis and secretion in the liver grow lower. A significant decrease in IGF-1 was observed in the subgroup of patients without myopathy (i.e., several years before the onset of atrophy), indicating that IGF-1 is not the only factor responsible for the pathology.

A decrease in the portion of type I muscle fibers (Table 2) with a slightly increased portion of hybrid muscle fibers was observed in the subgroup 2 patients. We were the first to observe this phenomenon. The phenomenon has not been detected so far in patients with chronic alcoholic intoxication [9, 11, 12]. A predominance of type I muscle fibers has been described for several samples, but significant results have not been reported. Discrepant data on changes in myosin phenotype have been obtained in experiments with animals [7]. It is thought that rat *m. soleus* is protected from the toxic effects of ethanol and its metabolites, so that fast muscles are usually examined in rats. A clinico-morphological analysis showed that patients with morphologically verified atrophy of muscle fibers had weakness and hypotrophy of lower girdle and femoral muscles as clinical signs of ChAM. Proximal muscle group weakness varying in severity was observed only in subgroup 2 patients with atrophy of type II muscle fibers. Motor defects were absent in patients without muscle fiber atrophy.

CONCLUSIONS

(1) Chronic alcoholic myopathy develops gradually, and the severity of atrophy depends on the duration of alcohol abuse.

(2) Males with a lower duration of alcoholic intoxication (9.6 ± 1.6 years) lack the clinical signs of myopathy and signs of muscle fiber atrophy and transformation, but display a decrease in IGF-1 in the blood plasma.

(3) As alcoholic intoxication increases in duration (14.6 ± 2.0 years), clinical signs of myopathy develop and grow in severity, type II muscle fibers are affected by atrophy, and a slow to fast transformation of muscle fibers occurs in males.

ACKNOWLEDGMENTS

This work was supported by the Russian Science Foundation (project no. 14-15-00392).

REFERENCES

1. Zinov'eva, O.E. and Shenkman, B.S., Alcoholic myopathy, *Nevrol. Zh.*, 2007, no. 5, p. 4.
2. Shenkman, B.S., Zinov'eva, O.E., Maslova, G.A., et al., Atrophy of slow and fast fibers of locomotor muscles in patients with chronic alcoholism: A clinical physiological analysis, *Tekhnol. Zhiv. Sist.*, 2009, no. 8, p. 3.
3. Freilich, R., Kirsner, R., Whelan, G., et al., Quantitative measure of muscle strength and size in chronic alcoholism: An early indication of tissue damage, *Drug Alcohol Rev.*, 1996, vol. 15, p. 277.
4. Hanid, A., Slavin, G., Mair, W., et al., Fiber type changes in striated muscle of alcoholics, *J. Clin. Pathol.*, 1981, vol. 34, p. 991.
5. Preedy, V.R. and Peters, T.J., The effect of chronic ethanol ingestion on protein metabolism in type-I- and type-II-fire-rich skeletal muscles of the rat, *Biochem. J.*, 1988, vol. 254, p. 631.
6. Steiner, J.L. and Lang, C.H., Dysregulation of skeletal muscle protein metabolism by alcohol, *Am. J. Physiol. Endocrinol. Metab.*, 2015, Mar. DOI: 10.1152/ajpendo.00006.2015.
7. Reilly, M.E., McKoy, G., Mantle, D., et al., Protein and mRNA levels of the myosin heavy chain isoforms (β, IIa, IIx and IIb in type I and type II fiber-predominant rat skeletal muscles in response to chronic alcohol feeding, *J. Muscle Res. Cell Motil.*, 2000, vol. 21, no. 8, p. 763.
8. Adachi, J., Asano, M., Ueno, Y., et al., Alcoholic muscle disease and biomembrane perturbations (review), *J. Nutrit. Biochem.*, 2003, vol. 14, no. 11, p. 616.
9. Fernández-Solà, J., García, G., Elena, M., et al., Muscle antioxidant status in chronic alcoholism, *Alcohol. Clin. Exp. Res.*, 2002, vol. 26, no. 12, p. 1858.
10. *WHO International guide for monitoring of alcohol consumption and related harm*, http:/who int/publication/en.International Center for Alcohol Policies (ICAP). The ICAP Blue Book, Module 20: Standard Drinks.
11. Estruch, R., Sacanella, E., Fernandez-Sola, J., et al., Natural history of alcoholic myopathy: A 5-year study, *Alcohol. Clin. Exp. Res.*, 1998, vol. 22, no. 9, p. 2023.
12. Nicolás, J.M., García, G., Fatjó, F., et al., Influence of nutritional status on alcoholic myopathy, *Am. J. Clin. Nutr.,* 2003, vol. 78, p. 326.
13. Lang, C.H., Frost, R.A., Svanberg, E., et al., IGF-1/IGFBP-3 ameliorates alterations in protein synthesis, eIF4E availability, and myostatin in alcohol-fed rats, *Am. J. Physiol. Endocrinol. Metab.*, 2004, vol. 286, p. 916.
14. Yang, S., Alnaqeeb, M., Simpson, H., et al., Changes in muscle fiber type, muscle mass and IGF-I gene expression in rabbit skeletal muscle subjected to stretch, *J. Anat.*, 1997, vol. 190, p. 613.
15. Fernandez-Solà, J., Preedy, V.R., Lang, C.H., et al., Molecular and cellular events in alcohol-induced muscle disease, *Alcohol. Clin. Exp. Res.,* 2007, vol. 31, no. 12, p. 1953.

Translated by T. Tkacheva