

Association between Testicular Atrophy and Muscular Atrophy after Ethanol Administration

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ABSTRACT

Ethanol produces multiple organ injuries, including liver damage, muscle atrophy, and testicular atrophy. Because androgenic steroid hormones are produced by testicular Leydig cells, ethanol-induced testicular atrophy may affect androgenic steroid hormones and produce an anabolic effect. The aim of the present study was to investigate whether ethanol-induced testicular atrophy affects the increase in protein catabolism and muscular atrophy. Male Sprague-Dawley rats were pair-fed a high-fat, low-protein diet containing ethanol or isocaloric dextrose for 6 weeks. Testicular weight and levels of plasma and testicular testosterone were measured. Urinary 3-methylhistidine was measured as a marker of protein catabolism. The sizes of type I (soleus muscle) and type II (anterior tibial muscle) muscle fibers were analyzed morphometrically. The gastrocnemius was examined with electron microscopy. Testicular atrophy and decreased plasma levels of testosterone were observed in ethanol-fed rats. The urinary 3-methylhistidine/creatinine ratio was higher in ethanol-fed rats than in control rats. The mean diameters of type I and II muscle fibers were less in ethanol-fed rats than in control rats. Electron microscopy showed injury to structures of the gastrocnemius in ethanol-fed rats. These results suggest that ethanol-induced testicular atrophy is associated with an increase in protein catabolism, resulting in muscular atrophy.

(Jikeikai Med J 2003 ; 50 : 37-43)

Key words : testosterone, protein catabolism, testicular atrophy, muscular atrophy, ethanol administration

INTRODUCTION

Ethanol produces multiple organ injuries, including liver damage, muscle atrophy¹, and testicular atrophy². Testicular atrophy is found in chronic alcoholics, in whom skeletal muscle atrophy is also prevalent³. In addition, in most patients with alcoholic liver cirrhosis a gonadal disorder develops which may be involved in the hypogonadism and feminiza-

tion phenotype, which includes gynecomastia, palmar erythema, and testicular atrophy, observed in these patients⁴. However, these symptoms appear in alcoholics without cirrhosis or without apparent liver disease⁵. Therefore, hypogonadism might be induced by ethanol and its metabolites rather than by liver disease alone. We have reported testicular atrophy and reduced serum testosterone levels in chronic alcoholics². An experimental animal model has also

Received for publication, December 9, 2002

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shown that ethanol reduces plasma testosterone concentrations⁶. Inhibition of testosterone biosynthesis during ethanol intoxication is mediated directly or indirectly by ethanol and its metabolites both inside and outside the testes. However, the mechanisms of testicular atrophy and testosterone reduction in alcoholics remain poorly understood.

Ethanol overconsumption is a common cause of skeletal myopathy, which is present in one third to two thirds of alcoholics^{7,8}. Several mechanisms of alcoholic myopathy can be considered. In addition to the effects of ethanol, multiple additional factors, including electrolyte disturbances, malnutrition, peripheral neuropathy, and chronic heart failure, may contribute to muscle weakness in alcoholics¹. Although malnutrition might also contribute to skeletal muscle atrophy and decreased muscle strength, alcoholics can have myopathy without malnutrition⁹. A recent study has also found that skeletal muscle function in alcoholic cirrhosis is independent of polyneuropathy but is related to the severity of muscle wasting as reflected by lean body mass¹⁰.

Androgens are produced by testicular Leydig cells. Ethanol and its metabolite acetaldehyde reduce binding of luteinizing hormone to Leydig cells, thereby inhibiting the enzymes responsible for the production of sex hormones¹¹. Alcoholics also exhibit a significant decrease in the free-androgen index⁴, which is the ratio of total testosterone to sex hormone-binding globulin, and is a sensitive indicator of abnormal androgen status¹². Ethanol-induced testicular atrophy might reduce the plasma and testicular levels of androgenic steroid hormones, thereby leading to catabolic effects.

However, the mechanisms responsible for muscle atrophy and testicular atrophy are still unknown. The relation between muscle injury and testicular atrophy also remains unclear. The aim of present study was to investigate whether ethanol-induced testicular atrophy is associated with increased protein catabolism and muscular atrophy after chronic ethanol administration.

METHODS

Animals: Male Sprague-Dawley rats weighting 150 to 180 g were fed a liquid diet containing 20% of calories from fat, 8% from protein, and 42% from ethanol (ethanol-fed rats, $n=6$) for 6 weeks. Control rats ($n=6$) were pair-fed isocaloric amounts of the same diet but with ethanol replaced by isocaloric dextrose. The ethanol concentration was increased gradually from 20% to 42% of calories during the first 2 weeks and maintained at 42% until the end of the experiments. Because body weights increased, total caloric intake was also believed to have increased. The animal protocol was approved by our institutional criteria for the care and use of laboratory animals, which are in accordance with the National Institutes of Health Guidelines.

Plasma analysis: Rats were killed at 6 weeks, and blood was collected into ice-cold, heparinized tubes. Plasma was separated with centrifugation. Plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), mitochondrial-AST (m-AST), and glutamate dehydrogenase (GLDH) were measured. Plasma levels of testosterone were also measured with radioimmunoassay¹³.

Measurement of the urinary excretion of 3-methyl histidine: Rats were fed in metabolic cages, and daily urine was collected. Urinary 3-methyl histidine (3-MH) was measured as a marker of protein catabolism with high-performance liquid chromatography¹⁴. The ratio of urinary 3-MH to urinary creatinine (3-MH/Cr) was measured.

Analysis of the size of muscle fibers and weight of muscle and testis: The gastrocnemius and testes were rapidly dissected out and weighed. Muscles were fixed in formalin. The sizes of the soleus (type I fibers) and anterior tibial (type II fibers) muscles were analyzed morphometrically with hematoxylin-eosin staining and histochemical staining of NADH tetrazolin reductase and adenosine triphosphatase¹⁵.

Electron-microscopic analysis of muscle: The gastrocnemius was fixed in 2% glutaraldehyde, post-fixed in 1% buffered osmium tetroxide, dehydrated, and embedded in epoxy resin (Araldite, Vantico AG, Basel, Switzerland). Ultrathin sections were cut,

stained with uranyl acetate and lead citrate, and examined with a electron microscope. Micrographs of selected fields were obtained at a range of magnifications.

Statistical analysis: All data are expressed as means \pm SEM. Student's *t* test was used to assess the significance of differences. A *p* value less than 0.05 was considered to indicate statistical significance.

RESULTS

Body weights did not differ significantly between the control rats and the ethanol-fed rats.

Plasma levels of AST, ALT, m-AST, and GLDH were significantly higher in ethanol-fed rats than in control rats (Table 1).

The 3-MH/Cr ratio was significantly higher in ethanol-fed rats (0.108 ± 0.005) than in control rats (0.069 ± 0.009 , $p < 0.005$).

Both testicular weight and plasma levels of testosterone were significantly lower in ethanol-fed rats as than in control rats (Table 1).

The mean diameters of both type I (soleus) and type II (anterior tibial) muscle fibers were lower in ethanol-fed rats than in control rats (Fig. 1 and 2). The weight (g) of the gastrocnemius was significantly lower in ethanol-fed rats than in control rats (1.12 ± 0.10 vs. 1.46 ± 0.03 , $p < 0.005$).

Testicular weight and gastrocnemius weight were positively correlated ($p < 0.025$, Fig. 3).

Electron microscopy showed that in ethanol-fed rats muscle fiber architecture was rough and disturbed, with enlarged and irregular giant mitochondria.

Table 1. Plasma liver enzymes, testicular weight and plasma testosterone level in ethanol-fed rats and control rats

| | Control | Ethanol-fed | <i>p</i> value |
|-----------------------|------------------|-----------------|----------------|
| AST | 61 \pm 7 | 95 \pm 10 | $p < 0.001$ |
| ALT | 27 \pm 4 | 46 \pm 7 | $p < 0.001$ |
| m-AST | 12.1 \pm 1.4 | 16.7 \pm 3.9 | $p < 0.05$ |
| GLDH | 3.1 \pm 0.1 | 7.3 \pm 1.3 | $p < 0.001$ |
| Testicular weight (g) | 2.72 \pm 0.05 | 2.42 \pm 0.08 | $p < 0.05$ |
| Testosterone (ng/ml) | 21.08 \pm 4.12 | 9.52 \pm 1.63 | $p < 0.025$ |

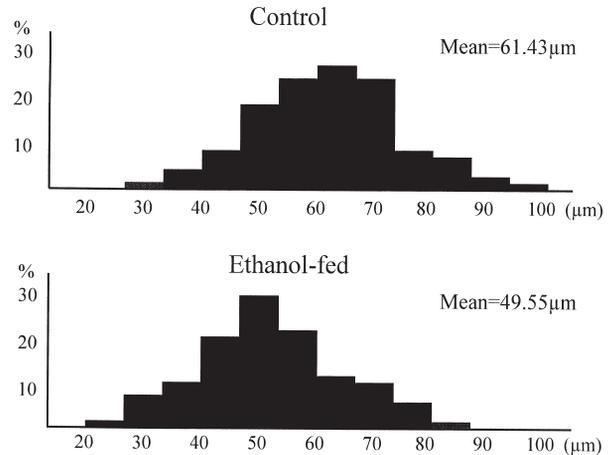


Fig. 1. Diameters of type I (soleus muscle) muscle fibers in ethanol-fed rats and control rats.

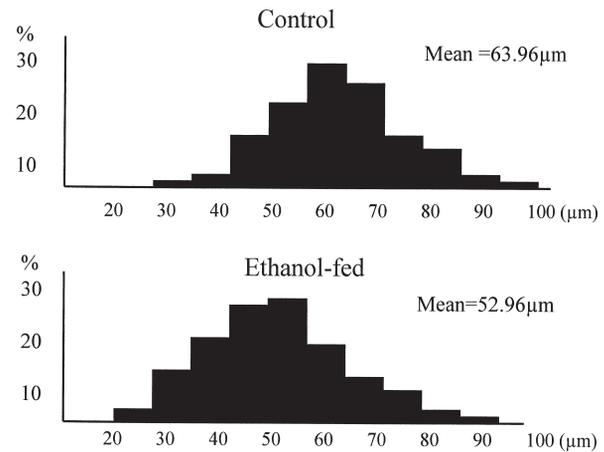


Fig. 2. Diameters of type II (anterior tibial muscle) muscle fibers in ethanol-fed rats and control rats.

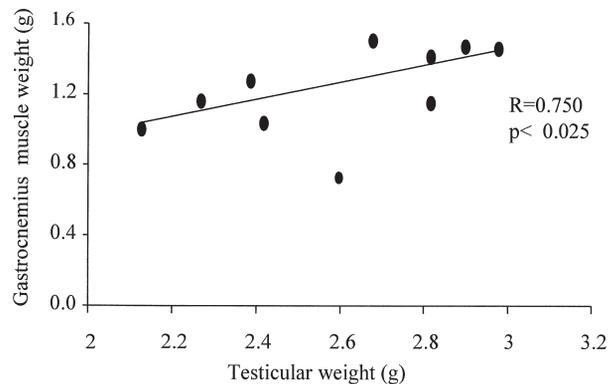


Fig. 3. Correlation between testicular weight and gastrocnemius weight in rats fed liquid diets containing ethanol or dextrose for 6 weeks.

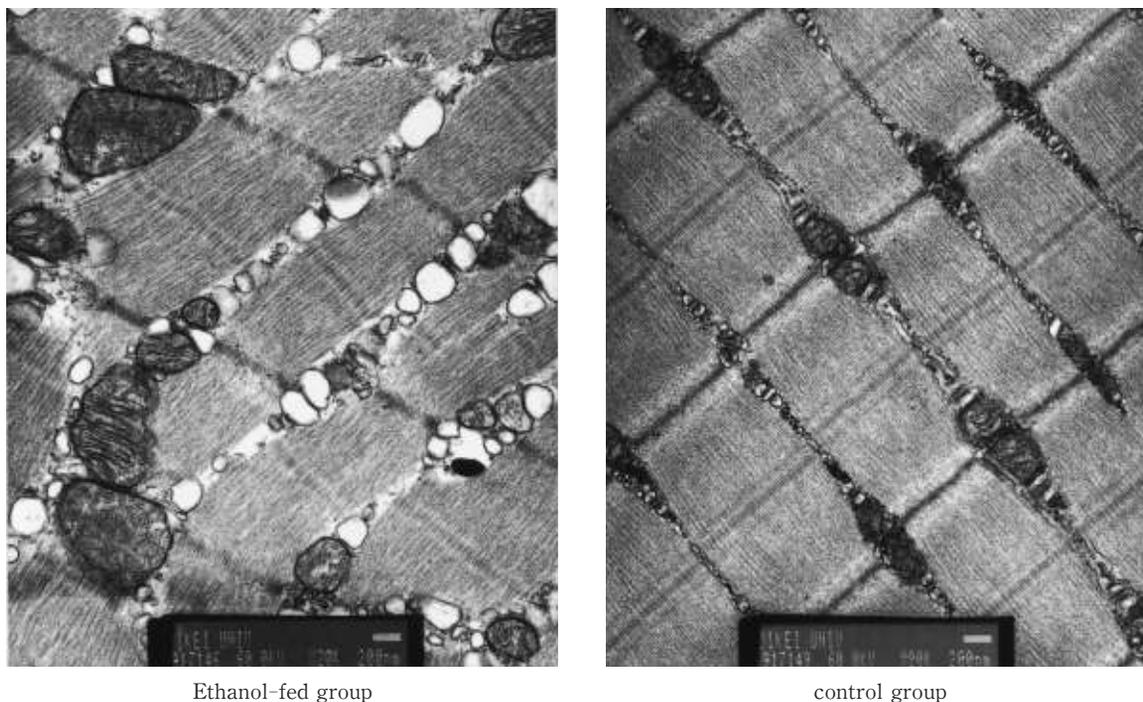


Fig. 4. Electron microscopic analysis of muscle.

In addition, T-tubules were swollen and the margin between the A and I bands was unclear, but the Z band units were normal (Fig. 4).

DISCUSSION

We found that testicular weight, muscular weight, and plasma levels of testosterone were significantly lower in ethanol-fed rats than in control rats. Ethanol-induced testicular atrophy may be associated with the increase in protein catabolism, resulting in muscular atrophy. We have previously reported that testicular atrophy is manifested as decreased testicular size and reduced serum testosterone levels in chronic alcoholics². Similar changes were produced in the present study by feeding rats ethanol for 6 weeks. Alcohol induces a profound hypoandrogenization, with reduction in serum testosterone levels and androgen receptor activity⁶, together with a substantial decrease in androgen-dependent hepatic estrogen-metabolizing enzyme activity which leads to increased serum estrogen levels⁶. Increased estrogen levels may be involved in feminization and clinical features, such as gynecomastia, palmar erythema, and

testicular atrophy, in alcoholics.

The mechanisms of the reduced testosterone level and testicular weight in alcoholics remain unclear but may be related to the alcohol dehydrogenase (ADH) activity of the testis. ADH is found in testicular interstitial tissue and the liver¹⁶ and is increased in the testes of rats chronically fed ethanol¹⁷. Nicotinamide adenine dinucleotide (NAD⁺) is a coenzyme for ADH and is also related to the synthesis of testosterone. In fact, the metabolism of ethanol, or, more specifically, ethanol-derived acetaldehyde and ethanol-induced NAD⁺ deficiency, has been suggested as the primary cause of reduced testosterone synthesis^{18,19}. Decreases in the testicular NAD⁺/NADH ratio induced by ethanol metabolism in testicular ADH may be involved in the reduction of testosterone synthesis and testicular weight. The results of another study, in which acetaldehyde inhibited testosterone biosynthesis, suggest that acetaldehyde rather than ethanol may be the primary mediator of inhibited steroidogenesis²⁰. These suggestions are based on the observation that acetaldehyde directly inhibits testosterone biosynthesis and that the addition of NAD⁺ reverses ethanol-induced inhibition. Increased ADH

levels in the testis may also affect the conversion of vitamin A to retinol, the active form of vitamin A, because ADH mediates this conversion. Retinol is necessary for spermatogenesis and normal testicular function^{21,22}. Induction of ethanol metabolism in testicular ADH may inhibit the conversion of vitamin A, leading to reduced testosterone levels and testicular weight. A recent study found that alcoholics with the ADH²¹/2¹ genotype are more susceptible to alcohol-induced testicular atrophy than are those with the ADH²²/2² genotype²³.

Chronic myopathy is a common complication of alcoholism, and continued alcohol abuse is generally reflected in deterioration of muscle strength and the appearance of histologic injury to muscle¹. However, because muscle strength does not return to normal levels in almost half of sober patients, alcohol myopathy is only partially reversible²⁴. Therefore alcoholic myopathy is the critical complication that should be resolved early in alcoholic-associated organ injury. In the present study, the diameter of skeletal muscle fibers decreased and muscle injury was demonstrated with electron microscopy in ethanol-fed rats. Several previous studies have reported that the type II fiber-predominant plantaris muscle is more vulnerable to the detrimental effects of ethanol than is the type I fiber-predominant soleus^{25,26}. A factor underlying the differences between type I and II muscle fibers may be the sensitivity to various hormones that affect each type of muscle fiber differently²⁶. However, in our study the diameters of both type I and II muscle fibers were significantly reduced in ethanol-fed rats. Why both types of muscle fiber atrophied in the ethanol-fed rats is unclear. Type I muscle fibers may atrophy under some conditions of alcohol misuse. The maturity of the rats at the start of the study may have had some bearing on the result. The initial body weight of rats (approximately 150 g) in our study was greater than that in one previous study (approximately 99 g)²⁷. The maturity of muscles may differ significantly between the experiments. This difference in muscle maturity may explain why both types of muscle fiber were atrophic in our ethanol-fed rats. However, another study has shown that similar differential effects between the two fiber types can be

obtained in both immature and mature rats²⁶. More likely, the presence of marked atrophy of type I fibers in some studies but not others may be due to differences in the nutritional status of the rats or the composition of the liquid^{6,27}. We found atrophy of both type I and II fibers and elevations of liver enzymes in serum after feeding rats a high-fat low-protein diet with 42% of calories from ethanol. Low-protein ethanol diets exacerbate muscle abnormalities²⁸.

3-MH is a component of the muscle-fiber proteins actin and myosin. 3-MH, which is liberated by resolution of muscular fiber protein, is not used for protein synthesis and is eliminated in the urine²⁹. Therefore, the urinary excretion of 3-MH indicates the rate of skeletal muscle catabolism. In the present study, the weight of the gastrocnemius was significantly lower and urinary 3-MH/creatinine ratio was significantly higher in ethanol-fed rats than in control rats; these findings suggest increased protein catabolism. Moreover, testicular weight and gastrocnemius weight were positively correlated.

However, the relation between the reduced levels of testosterone and muscle atrophy in ethanol-fed rats remains unclear. Older men show decreased serum testosterone levels, proximally predominant muscle wasting, and decreased muscle power on formal myometry³⁰. Testosterone replacement therapy decreases muscle atrophy and improves muscle power³¹. Our data suggest that under some conditions, such as consumption of a high-fat, low-protein diet, long-term ethanol administration causes testicular atrophy, resulting in a decrease in the plasma and testicular levels of testosterone. These changes may be associated with the increase in protein catabolism, resulting in muscular atrophy.

In conclusion, although the pathogenesis of alcohol-induced testicular atrophy and muscle atrophy has been considered to be multifactorial and attributable to both nutritional factors and the toxic effects of ethanol and its metabolites, the results of our study suggest that testicular atrophy and testosterone degradation induced by ethanol metabolism and its metabolite cause muscle atrophy mediated by protein catabolism.

Acknowledgements: The authors wish to express their profound appreciation of the kind advice provided by Professor Gotaro Toda. An abstract of this study was published in the proceeding of a symposium at the 2000 ISBRA Meeting (Alcohol Clin Exp Res 2001; 25: 54S-59S).

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