Original Paper

Interaction of Exercise and Alcohol on Energy Metabolic Profiles in the Skeletal Muscle Fibres of Male Albino Rat with Reference to Aging

Chennaiah K, Mallikarjuna K, Sudarsanam P, Sathyavelu Reddy K*

ABSTRACT

Exercise together with chronic ingestion of ethanol produces physiological and morphological alterations in skeletal muscle. The present study has been carried out to investigate the combined effect of exercise and ethanol ingestion on selected energy metabolic profiles of skeletal muscle fibres with reference to age induced changes. Wistar strain albino rats of two age groups (3 months & 18 months) were divided into four groups - Group I, sedentary control (SC); Group II, exercise (ExT) (30 min, speed of 23 m/min/day/5 days/week for a period of 8 weeks); Group III, ethanol treated (Et) (20% ethanol, 2 gm/kg body weight); Group IV, exercise trained + ethanol treated (ExT + Et) as mentioned in Groups II and III. The animals were sacrificed after 24 hours of the last treatment by cervical dislocation, and the skeletal muscle fibres of gastrocnemius (GN) and soleus (SOL) were isolated from the hind limbs, and selected energy metabolic profiles such as carbohydrates, glycogen, and free amino acids were estimated. The total carbohydrate content, glycogen and FAA are significantly elevated with ExT and also with combination treatment. However, the same parameters were decreased with ethanol intoxication in both skeletal muscle fibres when compared with sedentary control rats. The results suggest a beneficial role of exercise in preventing ethanol-induced toxicity.

Key Words: Exercise, Alcohol, Ethanol, Skeletal Muscle, Carbohydrates, Glycogen, Free Amino Acids, Rat

Introduction

Exercise influences oxidative metabolism, and produces reactive oxygen species (ROS) which appear to play a key role in changing the membrane fatty acid composition, permeability, and leakage of enzymes and chemotactive factory that lead to the repair process.[1] **Meites** reported that regular exercise in elderly human decreases incidence of heart diseases, improves lung function and reduces bone loss.[2] During exercise, oxygen consumption by the body can increase by as much as 20 fold.

Regularly performed exercise induces a number of physiological adaptations in skeletal muscle. One of the most important adaptations to occur is the increase in the capacity of the oxidative pathways, reflected by an increase in mitochondrial density, and increase in the maximal activities of a number of mitochondrial enzymes of the TCA cycles and oxidative pathways. Exercise at a given oxygen uptake after training, results in less of a decrease in the high-energy phosphates, a smaller increase in Pi creatine, and ADP[3], and this is believed to provide a reduced stimulus to glycogenolysis and glycolysis, and increase the reliance on fat catabolism during exercise.

The intake of alcohol influences the energy metabolism of skeletal muscle by a mechanism in which a disturbed metabolism of lactate occurs in skeletal muscle.[4] Both ethanol and its metabolic by-product, aldehyde have been shown to reduce protein synthesis in skeletal muscle.[5,6] A characteristic feature of chronic alcohol consumption

*(*Author for correspondence*) Division of Exercise Physiology, Department of Zoology, Sri Venkateswara University, Tirupati - 517 502, Andhra Pradesh, India

is a wasting of skeletal muscle.[7] Even acute alcohol intoxication can cause reversible skeletal muscle dysfunction.[8] Alcohol induced oxidative stress is linked to the metabolism of ethanol. Three metabolic pathways of ethanol have been described in the human body: alcohol dehydrogenase (ALD), microsomal ethanol oxidation system (MEOS), and catalase systems. Each of these pathways could cause degradation of essential complete molecules in the cells, including fat molecules, proteins, and DNA. Excessive ROS have been implicated in many other major diseases, including atherosclerosis, Parkinsons's disease, Alzheimer's disease, destruction of joints, and cataracts.[9,10] This study was designed to quantify changes in the skeletal muscle of rat attributed to alcohol and exercise.

Materials and Methods

Male, pathogen-free, Wistar strain albino rats aged 3 months, and 18 months were housed in clean polypropylene cages, six in each, in a temperature controlled room (27 ± 2^0C), with photoperiod of 12hrs light and 12hrs dark cycle. The rats were fed with standard laboratory chow, and water was provided *ad libitum*. The age-matched rats were divided into four groups of six in each group - Group I: sedentary control (SC), Group II: exercise trained (ExT), Group III: ethanol treated (Et), and Group IV: ExT+Et. The second group of rats was subjected to treadmill exercise 30 min/day, maintaining running speed of 23m/min for 5 days/week for a period of 8 weeks. The third group of rats received 20% of ethanol at the dose of 2g/kg body weight, and the fourth group received both exercise training and ethanol for a period of 8 weeks, as described for groups II and III. The animals were sacrificed after 48 hours of last training session, and skeletal muscle fibres of gastrocnemius (GN) and soleus (SOL) were isolated from the hind limbs. The tissues were washed with cold saline, immediately immersed in liquid nitrogen, and stored at -80^0C for biochemical assays. The selected energy metabolic profiles such as carbohydrates, glycogen, and free amino acids were estimated by using the methods of **Carrol et** al,[11] **Kemp & Van Hejnigen**,[12] and **Moore & Stein**[13] respectively. Total carbohydrates, and glycogen content were expressed as mg of glucose/gm wet weight of the tissue, and free amino acid content was expressed in mg of free amino acid per gram wet weight of tissue.

Statistical analysis:

Statistical analysis was carried out using INSTAT software. The data were analyzed for the significance, and the results were presented as P value.

Results

In this study it was noticed that the total carbohydrate levels increased marginally in both skeletal muscles with aging, exercise training, and also with combination treatments, but the same content decreased with ethanol intoxication. In GN muscle, by exercise training it was increased 1% in the younger rats (Y), 4% in older (O); and with combination treatment, 2% in Y, and 3% in O was observed. In SOL muscle, by exercise training it was increased 8% in Y, and 14% in O; whereas in combination treatment, it was 7% in Y, and 8% in O. We observed a significant and non-significant depletion in the total carbohydrate levels, in both (GN, SOL) skeletal muscle fibres with ethanol treatment (Table 1).

Due to exercise training, and also with combination treatment, elevated levels of glycogen were observed in both the muscles (GN, SOL) of young (Y) and old (O) male albino rats. The elevation of glycogen with exercise training in GN muscle was 16% in Y, 14% in O; and with combination treatment (ExT+Et) by 123% in Y, 59% in O was observed. In SOL muscle, exercise induced elevation was 25% in Y, 35% in O, and in combination treated group 32% in Y, 20% in O. However, a significant decrease was observed in ethanol treated (Et) rats by 19% in Y, and 29% in O (Table 2).

The total free amino acid content was significantly increased in both gastrocnemius and soleus muscle fibres due to exercise training in both young and old rats, except in the case of GN (by -41%) of young rats. It was noticed that a significant depletion in free amino acid content occurred in the gastrocenemius fibres due to ethanol treatment. However, in old rats the depleted FAA content due to ethanol treatment was elevated more in Gastrocnemius than the Soleus muscle due to interaction of exercise and ethanol (Table 3).

Discussion

Skeletal muscle (SM) accounts for up to 40% of body weight,[14] and is a major determinant of whole body energy expenditure, even at rest, because of its larger mass. A contracting muscle exerts demand for extra energy, and the ability to maintain energy and redox status varies considerably among different muscle types.[15] In the present study, it was observed that aging results in a slight

Table 1: Albino Rats - Changes in Total Carbohydrate due to Exercise Training (ExT), Ethanol Treatment (Et) and their Interaction (2 months) over the Sedentary Control (SC) in Gastrocnemius (GN) and Soleus (SOL) Muscles

S.No.	Type of the skeletal muscle	Age	Treatment			
			SC	ExT	Et	ExT+Et
1.	GN	3 months	126.01 ±2.79	127.59@ ±2.32 (+1.25)	125.86@ ±2.53 (- 0.11)	129.50** ±1.07 (+2.76)
		18 months	128.11 ±0.69	134.13* ±2.83 (+4.69)	125.23* ±1.338 (- 2.24)	131.96* ±2.20 (+3.00)
2.	SOL	3 months	116.88 ±2.55	126.77* ±1.25 (+8.46)	116.24@ ±1.76 (- 0.54)	125.33* ±1.89 (+7.22)
		18 months	119.15 ±0.54	136.78* ±1.19 (+14.79)	115.88** ±2.08 (-2.74)	129.05* ±2.38 (+8.30)

Values are expressed in mg/gm wet weight of the tissue
All values are ± SD of six individual observations
Values in parentheses denote per cent change over respective sedentary control
** Values are significant at P < 0.001*
***Values are significant at P < 0.05*
@Values are non significant

Table 2: Albino Rats - Changes in Glycogen Content due to Exercise Training (ExT), Ethanol Treatment (Et) and their Interaction (2 months) over the Sedentary Control (SC) in Gastrocnemius (GN) and Soleus (SOL) Muscles

S.No.	Type of the Skeletal muscle	Age	Treatment			
			SC	ExT	Et	ExT+Et
1.	GN	3 months	1.26 ±0.11	1.47@ ±0.28 (+16.66)	1.01* ±0.29 (-19.84)	2.82* ±0.21 (+123.81)
		18 months	1.16 ±0.04	1.33@ ±0.15 (+14.65)	0.81* ±0.31 (-29.31)	1.85* ±0.24 (+59.48)
2.	SOL	3 months	1.56 ±0.14	1.95@ ±0.36 (+25.00)	1.21* ±0.14 (-22.43)	2.06@ ±0.41 (+32.05)
		18 months	1.45 ±0.24	1.97* ±0.30 (+35.86)	0.97** ±0.27 (-33.10)	1.75@ ±0.55 (+20.68)

Values are expressed in mg/gm wet weight of the tissue
All values are ± SD of six individual observations
Values in parentheses denote per cent change over respective sedentary control
** Values are significant at P < 0.001*
***Values are significant at P < 0.05*
@Values are non significant

Table 3: Albino Rats - Changes in Total Free Amino Acid Content due to Exercise Training (ExT), Ethanol Treatment (Et) and their Interaction (2 months) over the Sedentary Control (SC) in Gastrocnemius (GN) and Soleus (SOL) Muscles

S.No.	Type of the Skeletal muscle	Age	Treatment			
			SC	ExT	Et	ExT+Et
1.	GN	Young	10.21 ±0.88	5.94* ±2.16 (- 41.82)	5.62* ±1.56 (-44.95)	8.22* ±1.35 (-19.49)
		Old	5.05 ±0.79	7.13* ±0.69 (+41.18)	4.48** ±0.53 (-11.28)	7.32* ±0.95 (+44.95)
2.	SOL	Young	4.32 ±0.61	8.22* ±0.48 (+90.27)	4.18@ ±0.73 (-3.24)	5.46** ±1.59 (+26.38)
		Old	4.31 ±1.23	4.53@ ±1.20 (+5.10)	3.96@ ±0.52 (- 8.12)	4.71@ ±1.24 (+9.28)

Values are expressed in mg/gm wet weight of the tissue
All values are ± SD of six individual observations
Values in parentheses denote per cent change over respective sedentary control
** Values are significant at P < 0.001*
***Values are significant at P < 0.05*
@Values are non significant

elevation in total carbohydrates in both gastrocnemius and soleus muscles which may be due to decreased metabolic utilization in the older animals. Age-related slowing down and impairment in carbohydrate metabolism appear to play a role in the expression of cellular senescence.[16] The significant decrease in total carbohydrate levels in the selected skeletal muscles (GN and SOL) of older rats after ethanol treatment suggests possible utilization of carbohydrates to meet the energy demand during ethanol toxicity. A similar pattern of changes in carbohydrate levels has been reported in brain and other tissues of rats during ethanol intoxication. This is possible due to the products of alcohol metabolism that inhibit the formation of glucose from other compounds such as amino acids, etc. These findings support the present results in that the utilization of carbohydrates is more in older rats than the younger ones (**Table 1**). Younger rats can more readily maintain high levels of oxygen consumption accompanied by a more efficient use of fats as an energy source as compared to older ones.[17]

Glycogen levels would be expected to increase in GN and SOL of rats after two months exercise training due to increased glycogenesis or gluconeogenesis,[18,19] to meet the energy demands of the tissue. In the present investigation, it was observed that the glycogen content was more in SOL than the GN of exercised rats, which may be attributed to functional adaptations of the fibres. The increase in the glycogen content may be due to decreased utilization of glycogen for energy release, since fats are preferred over carbohydrates, or it may be due to increased synthesis of the same from non-carbohydrate sources, or both. The elevation in the skeletal muscle glycogen concentration also accounts for the decrease in blood glucose during endurance exercise.[20]

In the present investigation, it was observed that the muscle glycogen levels significantly decreased (**Table 2**) due to aging. The decrease in the glycogen content with advancement of age may be due to augmented glycogen degradation, through glycolysis or due to decreased synthesis of glycogen during aging. Glycogen levels generally decrease with advancement of age.[21] **Peters et al** have reported that ethanol and its byproducts are associated with reduced glycogen re-synthesis in skeletal muscle.[22] Reduced levels of glycogen also could lead to reduced glucose availability and glycolysis in cells. Studies of the intact liver, whole liver hepatocytes,[23] and periportal and perivenous hepatocytes[24] have demonstrated that glycogen levels are greatly reduced in livers of chronic alcohol consumers. Similarly, in skeletal muscle fibres

the reduced glycogen levels may be attributed to reduced glucose availability and glycolysis. Both the muscles (GN and SOL) showed similar trend of changes in glycogen content, which reveals no specific fibre type alterations in the glycogen content in the muscle fibres.

The total FAA content increased significantly in both skeletal muscles due to exercise training. Similar increase in the amino acid levels during muscular work has been reported in the muscle of frog,[25,26] and rat,[27,28] and the plasma of human subjects.[29] The total FAAs in skeletal muscles arise from a balance between a number of factors. Muscle provides much larger portions of the total FAA pool of the body than does liver.[30] The total amino acid turnover per hour is also much greater for muscle than for liver.

Besides these, the enhanced level of FAA may be due to ammonia intoxication.[31] In the present study, total free amino acid content was decreased in the rats of both age groups subjected to ethanol treatment. This decrease may be due to the effect of ethanol products on the FAA content in the plasma. Similar reports are available under acute ethanol administration conditions.[33] However, contradictory reports are also available regarding the influence of ethanol on total free amino acid pool. It was observed that the concentration of ethanol load may influence the amino acid pool. The low levels of FAA in the muscle fibres due to ethanol treatment may also be due to high utilization of these to carbohydrate sources via gluconeogenesis pathway to meet the energy demand under the influence of alcohol intoxication. In the present study, we observed an elevation of FAA pool in the muscle fibres due to combination of exercise and ethanol, suggesting that exercise training enhances the supply of FAA content to counter the ethanol toxicity. This is more pronounced in older age groups than in the young.

To sum up, the present findings suggest that exercise training augments the energy supply in rats. It was also observed that there is decreased activity of energy metabolism enzymes due to ethanol treatment. In the rats subjected to combination treatment, the enzyme activity rose similar to the control or exercised rats. Thus, exercise training for 2 months seems to be highly beneficial in upregulation of the decreased energy metabolism resulting from ethanol intoxication.

REFERENCES

1. Yu BP. Cellular defences against damage from reactive oxygen species. Physiol Rev. 1994; 74: 139-162.
2. Meites J. Anti aging interventures and their neuroendocrinal aspects in mammals. J Reprod Fertil. 1993; 46: 1-9.
3. Green HJ, Ball-Burnett M, Chin ER, Dux L, Pette D. Time dependent increases in Na^+-K^+ ATPase content of low frequency-stimulated rabbit muscle. Febs Lett. 1992; 310: 129-131.
4. Shiraishi K, Watanabe M, Motegi S, Nagaoka R, Matsuzaki S, Ikemoto H. Influence of acute alcohol load on metabolism of skeletal muscles - Expired gas analysis during exercise. Alcoholism Clin Exper Res. 2003; 27: 76S-78S.
5. Preedy VR, Keating JW, Peters TJ. The acute effects of ethanol and acetaldehyde on rates of protein synthesis in type I and type II fibre-rich skeletal muscles of the rat. Alcohol. 1992; 27: 241-51.
6. Reilly ME, Mc Koy G, Mantle D, Peters TJ, Goldspink G, Preedy VR. Protein and mRNA levels of the myosin heavy chain isoforms Ibeta, IIa, IIx and IIb in type I and type II fibre-predominant rat skeletal muscles in response to chronic alcohol feeding. J Muscle Res Cell Motil; 2000; 21: 763-773.
7. Preedy VR, Salisbury JR, Peters TJ. Alcoholic muscle disease: features and mechanisms. J Pathol. 1994; 173: 309-315.
8. Eichner ER. Ergolytic drugs in medicine and sports. Am J Med. 1993; 94: 205-211.
9. Knight J. Free radicals: Their history and current status in aging and disease. Ann Clinical Lab Sci. 1998; 28: 331-346.
10. Kehrer JP. Free radicals as mediators of tissue injury and disease. Crit Rev Toxicol. 1993; 23: 21-28.
11. Carrol NV, Longley RW, Roe JH. Glycogen determination in liver and muscle by use of anthrone reagent. J Biol Chem. 1956; 220: 583-593.
12. Kemp A, Van Hejnigen MK. A colorimetric micromethod for the determination of glycogen in tissues. Biochem J. 1954; 56: 646-652.
13. Moore S, Stein WH. A modified ninhydrin reagent for the photometric determination of amino acids and released compounds. J Biochem. 1954; 211: 907-913.
14. Snyder WS, Coole MJ, Karhausen LR, Nasset ES, Howells GP, Tipton IH. Report of the Task Group on Reference Man. 1984. Oxford, UK. 146-147.

15. Venkataiah A. Effect of Exercise Training on Age-related Antioxidant Defense Mechanisms in Rat Skeletal Muscles. 1995. PhD thesis, SV University, Tirupati, Andhra Pradesh, India.
16. Tollefsbol TO. Gene expression of carbohydrate metabolism in cellular senescence and aging. Mol Biol Med. 1987; 4: 251-263.
17. Somani SM, Buckenmeyer P, Dube SN, Mandalayawala RH, Verhulst SJ, Knowlton RG. Influence of age and caloric expenditure during exercise. Ind J Clin Pharmacol Ther Toxicol. 1992; 30: 1-6.
18. Hughes VA, Fiatarone MA, Fielding RA, Ferrara CM, Elahi D, Evans WJ. Long term effects of a high carbohydrate diet and exercise on insulin action in older subjects with impaired glucose tolerance. Amer J Clin Nutr. 1995; 62: 426-433.
19. Dhahbi JM, Mote PL, Wingo J, Tillman JB, Walford RL, Spindler SR. Calories and aging alter gene expression for gluconeogenic, glycolytic and nitrogen metabolizing enzymes. Amer J Physiol. 1999; 277: E352-E360.
20. Bilwanath M. Biochemical Changes in Selected Parameters of Young and Old Rats to Endurance Exercise Training. 1996. MPhil dissertation, SV University, Tirupati (AP), India.
21. Takahashi A, Philpot DE, Miguel J. Electronic microscopic studies on aging. J. Geront. 1970; 25: 228-228.
22. Peters T, Nicolovski S, Raja G, Palmer TN, Fournier P. Ethanol acutely impairs glycogen repletion in skeletal muscle following high intensity short duration exercise in the rat. Addiction Biol. 1996; 7: 289-295.
23. Van Horn CG, Cunningham CC. Contributions of dietary carbohydrate and ethanol to alterations in liver glycogen levels and glycolytic activity. Alcohol. 1999; 19: 139-144.
24. Baio DL, Czyz CN, Van Hon CG. Effect of chronic ethanol consumption on respiratory and glycolytic activities of rat periportal and perivenous hepatocytes. Archives of Biochemistry and Biophysics. 1998; 350: 193-200.
25. Rajendra W, Indira K, Swami KS. Effect of electrical stimulation on ammonia detoxification in amphibian skeletal muscle. Curr Sci. 1980; 49: 681-683.
26. Bhargava D. Fatigue induced changes in carbohydrate and protein metabolism and their modulation by pyridoxal-5-phosphate in the gastrocnemius muscle of *Bufo melanostictus*. 1982. PhD thesis, SV University, Tirupati (AP), India.
27. Dohm GL, Beecher GR, Warren RQ, Williams RT. Influence of exercise on free amino acid concentrations in rat tissue. J Appl Physiol. 1981; 50: 41-44.
28. Krishna Mohan P, Indira K, Rajendra W. Protein degradation in functionally different muscles of rat during exhaustive exercise. Indian J Exp Biol. 1985; 23: 655-657.
29. Pivarnik JM, Leeds EM, Wilkerson JE. Effects of endurance exercise on metabolic water production and plasma volume. J Appl Physiol 1984; 56: 613-618.
30. Nelson DL, Cox MM. In: Lehninger's Principles of Biochemistry, 3rd edn, 2000. Macmillan Press Ltd., Hampshire, UK. 873.
31. Krishan Mohan Reddy P. Metabolic Modulations of Fatigue with Special Reference to Lactate and Ammonia Metabolism in Different Skeletal Muscle Fiber Types of Albino Rat. 1986. PhD thesis, SV University, Tirupati, AP, India.
32. Milakofsky L, Miller JM, Vogel WH. Effects of acute ethanol administration on rat plasma amino acids and related compounds. Biochem Pharmacol. 1986; 35: 3885-3888.
33. Green RS, MacDermid RG, Scheig Rl, Hajjar JJ. Effect of ethanol on amino acid absorption across in-vivo rat intestine. Amer J Physiol 1981; 241: G176-181