Exercise prevents ethanol-induced oxidative stress: regulation of selected dehydrogenase activities in the skeletal muscle fibers of male albino rat with reference to aging

K. CHENNAIAH, K. KHALINDAR BASHA, R. SIVASANKAR, K. RAMAIAH AND K. SATHYAVELU REDDY

ABSTRACT
Alcohol consumption is associated with several injuries of organs, including liver, lung, heart as well as skeletal muscle; as a result of numerous physiological, morphological and functional changes occur in the tissues. Wistar strain male albino rats of two age groups 3 months (young) and 18 months (old) were divided into four groups, Group I sedentary control (SC); Group II, exercise trained (ExT) (30 min, at a 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 described in group II and group III. The animals were sacrificed after 24 hours of the last treatment by cervical dislocation and the skeletal muscle fibres such as gastrocnemius (GN) and soleus (SOL) were isolated from the hind limbs of rats and selected enzymes such as succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and isocitrate dehydrogenase activities were assayed. The activities of SDH, LDH and MDH were increased in both skeletal muscle fibers of ExT group. However, a significant decrease was observed in the same parameters with ethanol intoxication in both skeletal muscle fibers when compared to their sedentary control group. From the results obtained in this study, we conclude that 8 weeks exercise training is beneficial to the animal in preventing the ethanol induced toxicity.

Key words: Exercise, Alcohol, Skeletal muscle fibers, Dehydrogenases, Albino rat

Exercise physiology is one of the active fields of research in modern days, since it presents the material essential for understanding relevant changes in various mechanisms of the body that occur during the onset of exercise. As the body is subjected to repeated bouts of exercise, long term adaptation in the bodily function occur (Wilmore, 1982). Exercise can be accomplished only through a series of complex interactions within the body involving all body systems. Exercise increase the oxygen consumption rate by 10 to 15 folds and may result in oxidative stress due to increased production of reactive oxygen species. It also enhances the antioxidant enzyme activity (AOE). Exercise generates adenosine as a break down product of ATP and provides cytoprotection against oxidative stress. Arthur Simman (1988) reported increased blood cell composition in exercising animal. It is generally accepted that the increase in mitochondrial oxidative capacity that occurs with training leads to the altered metabolic response to exercise. Spina et al. (1996) have shown an increase in the activity of some mitochondrial enzymes during short-term training and have associated, with this smaller increase in blood lactate and lower respiratory exchange ratio values during sub maximal exercise after training. However, Phillips et al. (1996) have shown that the metabolic adaptations will occur before increase in enzyme activities during the same type of training.

Alcohol abuse is associated with severe damage of several biological functions and activities within the cell (Harper and Krill, 1990; Lieber, 1991), including electrolyte homeostasis (Gandhi and Ross, 1988). Chronic intake of ethanol produces alterations in several tissues, including skeletal muscle. In chronic alcohol patients, progressive wasting and weakness of proximal muscle groups seems to be associated with lesions of skeletal muscle (Urbano-Marquez et al., 1989). Ethanol has been described as producing atrophy of human type II fibres (Martin and Peter, 1985) and sometimes necrosis (Urbano-Marquez et al., 1985) in patients affected by alcohol. While some authors consider that the direct effect of ethanol on Chennaiah, K., Khalindar Basha, K., Sivasankar, R., Ramaiah, K. and Sathyavelu Reddy, K. (2011). Exercise prevents ethanol-induced oxidative stress: regulation of selected dehydrogenase activities in the skeletal muscle fibers of male albino rat with reference to aging. Asian J. Animal Sci., 6(1): 8-13.
skeletal muscle fibers is responsible, others believe that the alterations may be a secondary effect of ethanol consumption, whose primary effects, apart from liver dysfunction, are malnutrition and disturbances to the nervous system (Morrison et al., 1990).

Aging is associated with a progressive decline in muscle performance, characterized by decreased muscle strength and endurance capacity in both humans (Larsson et al., 1979) and animals (Carmeli and Reznick, 1994). Although the reduction in muscle size could account for much of the reduction in muscle strength (Rodgers and Evans, 1993), the mechanism(s) underlying the reduced aerobic capacity is less clear. When animal models of aging have been used, a decrease of mitochondrial oxidative enzyme activities in skeletal muscle homogenates (Stump et al., 1977; Hansford, 1983) as well as in isolated mitochondria (Sugiyama et al., 1993; Desai et al., 1996). Some evidences implicates oxidative damage of cellular constituents in aging, as well as in the pathogenesis of the degenerative diseases of later years (Ames et al., 1993; Viner et al., 1996). Reactive oxygen intermediates are potentially damaging to nucleic acids, lipids, and proteins (Esser and Martin, 1995). This study was undertaken to discovery the beneficial role of exercise on young age as well as old age, especially for alcoholic subjects.

MATERIALS AND METHODS

Male, pathogenic free Wistar strain albino rats aged 3 months (young) and 18 months (old) were housed in a clean poly propylene cages, six in each, in a temperature controlled room (27 ± 2°C) 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, they are; group I sedentary control (SC), group II exercise trained (ExT), group III ethanol treated (Et) and group IV (ExT+Et). The group II rats were subjected to treadmill exercise for 30 min/day maintaining running speed of 23m/min for 5 days/week for a period of 8 weeks. Group III rats received 20% of ethanol with a dose of 2gm/kg body weight and group IV rats received both exercise training and ethanol for a period of 8 weeks as described in group II and group III. The animals were sacrificed after 24 hrs of last training session and skeletal muscle fibers such as gastrocnemius (GN) and soleus (SOL) were isolated from the hind limbs and the tissues were, washed with cold saline, immediately immersed in liquid nitrogen, and stored at –80°C for biochemical assays. The selected enzymes such as SDH, LDH and MDH activities were assayed using the methods of Nachlas et al. (1960) with slight modifications as suggested by Pramelamma and Swami (1975). The activities was expressed in ì moles of formazan formed / mg protein / hour.

Statistical analysis:

Statistical analysis has been carried out using INSTAT software. The data was analyzed for the significance; the results were presented with the P-value.

RESULTS AND DISCUSSION

From the data it can be visualized that there was an increase in the activity of SDH in GN muscle fibres of young (32%) and old (8%) exercised rats. Whereas, in case of SOL we observed an increase of 16% in young and 6% in old aged rats (Table 1). The ethanol treatment diminishes the SDH activity in both the muscle fibres of young and old rats. SDH is known to be altered under several physiological and pathological conditions such as

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Type of skeletal muscle</th>
<th>Age</th>
<th>Treatments</th>
<th>SC</th>
<th>ExT</th>
<th>Et</th>
<th>ExT+Et</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>GN</td>
<td>Young</td>
<td></td>
<td>1.89±0.15</td>
<td>2.51**±0.24</td>
<td>1.62**±0.23</td>
<td>1.74±0.20</td>
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<tr>
<td></td>
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<td>1.68±0.27</td>
<td>1.82**±0.15</td>
<td>1.34*±0.17</td>
<td>1.77**±0.16</td>
</tr>
<tr>
<td>2.</td>
<td>SOL</td>
<td>Young</td>
<td></td>
<td>1.54±0.12</td>
<td>1.79*±0.12</td>
<td>1.14*±0.10</td>
<td>1.43*±0.23</td>
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<tr>
<td></td>
<td></td>
<td>Old</td>
<td></td>
<td>1.23±0.18</td>
<td>1.31*±0.21</td>
<td>0.73*±0.16</td>
<td>0.87*±0.06</td>
</tr>
</tbody>
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All the values are ± SD of six individual observations
Values in parentheses denote per cent change over respective sedentary control
* and ** indicate significance of values at P=0.05 and 0.001, respectively
@ Values are non significant.
homic, hyperthermic and exhaustive (Umadevi, 1992; Sairaja, 1997). The SDH activity was significantly higher in exercised rat tissues than those of no exercised in both the age groups. The increased SDH activity during exercise training indicates increased aerobic efficiency of tissues. In consonance with these results, Hammeren et al. (1992) and Sullivan et al. (1995) observed a significant increase in SDH activity of different tissues in both young and old female rats after exercise training. SDH activity was significantly higher in the muscle tissues of exercised animals than that of age matched sedentary controls. The increase in SDH activity during exercise training indicates increased efficiency of tissues (Hammeren et al., 1992; Jhansi, 1998).

Thus, the results of the present study confirm earlier observations. The decrease in SDH activity due to alcohol stress condition indicates reduction in the conversion of succinate to fumarate resulting in decreased oxidative metabolism. From the data it can be observed that a significant decrease in the activity of SDH in ethanol treated rats of both age groups. Waring et al. (1981, 1982) correlated the inhibition of SDH activity by alcohol to the changes in mitochondrial membrane. During stress conditions diversion of phosphoenolpyruvate leads to increased formation of fumarate resulting in the product inhibition of SDH (Moorthy, 1983). Butler et al. (1985) reported a decrease in SDH activity level in the heart of rat on chronic treatment with ethanol. Cederbaum et al. (1976) also reported a decrease in SDH activity following the administration of acetaldehyde to rats. The results of the present study further confirm these earlier reports.

Thus, the decreased SDH activity observed in the tissues of both age groups of rats treated with alcohol indicate depressed oxidative metabolism in mitochondria. The combination treatment with ethanol and exercise training could marginally up regulate the SDH activity in GN of old rats. However, a significant decrease in SDH activity was noticed in SOL muscle fibres of old rats. Thus, differential response of SDH activity was observed in GN and SOL muscle fibres in the present study.

In the present investigation a decrease in LDH activity was observed in skeletal muscles of old rats (Table 2) which confirms earlier findings. Lowered LDH enzyme activity during aging (Talesara and Mohini, 1978) suggest that the individual has to rely more on alternate pathway other than oxidative metabolism for production of metabolic energy presumably on anaerobic glycolytic pathway as evinced by lactic acid accumulation (Sairaja, 1997). The decrease in oxygen consumption during aging (Shock, 1962), might have been shift the metabolism towards anaerobic glycolysis resulting in the increased lactate/pyruvate ratio.

The considerable increase in the LDH activity after sub maximal exercise training indicates the possible shift in the metabolic emphasis from anaerobiosis to aerobic i.e., the NAD-LDH actively helps in the efficient conversion of lactate to pyruvate and its subsequent utilization in TCA cycle oxidative reactions. The changes in the activities of enzymes related to energy metabolism suggest that increased glycolysis to pyruvate formation and increased fatty acid oxidation can simultaneously provide energy for contractile muscle during exercise. In the present study, LDH activity was increased with acute exercise training in the young rats by 33% in GN and 79% in SOL. Exercise training increases the activity of the enzymes involved in glucose phosphorylation. According to Dubouchand et al. (2000) and Parra et al. (2000) the lactate dehydrogenase activity elevated significantly during sub maximal cycling and endurance exercise training. A decrease in LDH activity with age and increase during regular exercise (Jill and Thomas, 1991) indicates regular exercise can prevent the loss of

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<th>Et</th>
<th>ExT+Et</th>
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<tr>
<td>1.</td>
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<td>1.67±0.24</td>
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<td>2.41±1.20</td>
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<td>(+44.31)</td>
<td>(+7.78)</td>
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<td></td>
<td></td>
<td>Old</td>
<td></td>
<td>0.79±0.22</td>
<td>0.82±0.29</td>
<td>1.24±1.14</td>
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<td>(+56.96)</td>
<td>(+24.05)</td>
</tr>
<tr>
<td>2.</td>
<td>SOL</td>
<td>Young</td>
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<td>0.84±0.40</td>
<td>1.51±0.63</td>
<td>1.18±0.9</td>
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<td>(+40.47)</td>
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<tr>
<td></td>
<td></td>
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<td>0.53±0.17</td>
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<td>0.91±0.21</td>
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<td></td>
<td>(+35.84)</td>
<td>(+71.69)</td>
<td>(+43.39)</td>
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All the values are ± SD of six individual observations.

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Values are non significant.

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catalytic efficiency of the enzyme systems during old age. The present findings also attest the fact that exercise training can improve the reduced catalytic efficiency of old age rat muscle enzyme by 4% in GN and 35% in SOL. In the alcohol metabolism acetaldehyde is a metabolite of alcohol-oxidation is considered to be a highly toxic compound. In the present study in ethanol treated rats, the LDH activity was increased in GN of young age rats by 44% and 57% in old age rats. In SOL muscle of rats, the LDH activity was increased in GN of young age rats by 40% and 72% in old age rats.

The activity of LDH enzyme in tissues of rats treated with ethanol indicates increased conversion of lactate to pyruvate. Lieber et al. (1975) reported increased lactic acid content following the administration of alcohol to rats. Lactate the end product of glycolysis, is formed and further metabolized only by LDH. However, an increase in lactate levels and decrease in pyruvate content in rats treated with ethanol was reported by Kiessling (1962). The considerable increase in the LDH activity after ethanol-treatment in the rat tissues indicates active conversion of lactate to pyruvate. Kershenobich et al. (1981) attributed the increased formation of lactate levels from pyruvate to the increased NADH/NAD ratio during alcohol metabolism. It has been shown that the hypoxic condition would prevail in animals treated with several stress conditions (Eto, 1974). The present data also indicate increased sensitivity of rat tissues to acetaldehyde toxicity due to ethanol metabolism with advancement of age. In the present study, the combination treatment also witnessed an increase in LDH enzyme activity in both the muscle fibres which indicates the up regulation of energy metabolism towards the formation of pyruvate, thereby to increase in energy supply to meet the energy demand under stress conditions.

The activity levels of MDH indicate the status of prevailing oxidative metabolism. Hence, an attempt was made to examine the specific activity of MDH in the selected tissues of young and old age rats as a consequence of exercise training, ethanol treatment and exercise + ethanol treatment. The changes in the activity levels of MDH in the tissues of GN and SOL were studied in young and old age rats. From the data obtained, it can be inferred that there was a significant increase in MDH activity in the tissues of rat during aging and after endurance exercise, however this increase was more pronounced during exercise training. The per cent elevation in MDH activity in response to exercise training was more in GN of young rats than in SOL of the same. Due to ethanol treatment the activity was decreased, where as with the combination treatment the decreased activity due to ethanol treatment was shifted to increasing (Table 3).

The decrease in specific activity of MDH in tissues of all age groups of rats as a consequence of ethanol treatment suggests decreased utilization of malate. The reduced levels of TCA cycle intermediates may also be due to the decrease in MDH activity during ethanol-treatment. An increase in proteolytic activity during alcohol intoxication may also be responsible for the decreased MDH activity (Klatskin, 1961).

Mitochondrial swelling and alterations in the permeability properties reported in the tissues under alcohol treatment could also be considered as causative factors (Orrego et al., 1981; Miyakawa et al., 1985). Similar inhibition of MDH activity was reported in animals under toxic conditions (Tripathi and Shukla, 1990; Reddy and Yellamma, 1991). Inhibition of SDH and MDH activities observed in the present study suggests the prevalence of hypoxic condition in tissues and reduction

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<th>Ext</th>
<th>Et</th>
<th>Ext+Et</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>GN</td>
<td>Young</td>
<td>0.88±0.09</td>
<td>1.74±0.27</td>
<td>0.82±0.25</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Old</td>
<td>0.74±0.25</td>
<td>0.85±0.19</td>
<td>0.72±0.27</td>
<td>0.77±0.17</td>
</tr>
<tr>
<td>2.</td>
<td>SOL</td>
<td>Young</td>
<td>1.73±0.29</td>
<td>1.94±0.37</td>
<td>1.55±0.56</td>
<td>1.87±0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Old</td>
<td>0.92±0.22</td>
<td>1.29±0.18</td>
<td>0.86±0.29</td>
<td>1.15±0.18</td>
</tr>
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</table>

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Values in parentheses denote per cent change over respective sedentary control
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* Values are non significant.
in mitochondrial oxidative metabolism in tissues of rat administered with ethanol. Alterations in the activities of TCA cycle enzymes cause mitochondrial dysfunction and integrity ultimately leading to energy crisis during induced ethanol and exercise training. From the present study we report that the combination treatment with ethanol and exercise training exhibit a beneficial recovery of MDH activity in both the muscle fibres to an extent of 10% increase.

Conclusion:

The SDH, LDH, MDH, enzyme activities were up regulated due to combination treatment in both the skeletal muscle fibres of both age groups. The augmentation of these enzyme systems due to 2 months treadmill exercise training would provide a significant advantage to overcome various pathological and physiological processes that occur in old age. This investigation draws a conclusion stating that this much of intensity treadmill exercise to the old age as well as young age male subjects may be beneficial, especially for the alcoholic subjects to improve the metabolic efficiency and thereby to improve the health status and life span.

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LITERATURE CITED


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