ORIGINAL ARTICLE

EFFECT OF ASCORBIC ACID ON FATIGUE OF SKELETAL MUSCLE FIBRES IN LONG TERM COLD EXPOSED SPRAGUE DAWLEY RATS

Aneeqa Rashid, Umar Ali Khan*, Muhammad Ayub**

Department of Physiology, Wah Medical College, Wah Cantt, *Al-Nafees Medical College & Hospital, Islamabad, **Ayub Medical College, Abbottabad, Pakistan

Background: On exposure to prolonged cold temperature, the body responds for effective heat production both by shivering and non-shivering thermogenesis. Cold exposure increases the production of reactive oxygen species which influence the sarcoplasmic reticulum Ca^{++} release from the skeletal muscles and affect their contractile properties. The role of ascorbic acid supplementation on force of contraction during fatigue of cold exposed skeletal muscles was evaluated in this study. **Method:** Ninety healthy, male Sprague Dawley rats were randomly divided into three groups of control (I), cold exposed (II), and cold exposed with ascorbic acid 500 mg/L supplementation mixed in drinking water (III). Group II and III were given cold exposure by keeping their cages in ice-filled tubs for 1 hr/day for one month. After one month, the extensor digitorum longus muscle was dissected out and force of contraction during fatigue in the skeletal muscle fibres was analysed on a computerised data acquisition system. **Results:** The cold exposed group showed a significant delay in the force of contraction during fatigue of skeletal muscle fibres compared to control group. Group III showed easy fatigability and a better force of contraction than the cold exposed group. **Conclusions:** Ascorbic acid increases the force of contraction and decreases resistance to fatigue in the muscles exposed to chronic cold.

Keywords: Ascorbic acid, cold stress, fatigue, skeletal muscles

INTRODUCTION

All living organisms are facing the challenges of vast diversity in environmental temperatures for their survival.[1] Prolonged exposure to cold induces increase in heat production and enhances tolerance to cold. Studies have proved an enhanced thermogenic capacity under hypoxia and stress. This is achieved through both shivering and non-shivering thermogenesis (NST).[2]

Skeletal muscle, which represents a large percentage of body mass, significantly contributes to thermogenesis. Shivering is the earliest and most primitive response towards increase heat generation.[3] It is the increased skeletal muscle activity that generates heat.[4] However, if the exposure is prolonged, more effective and long lasting heat generating system is activated.[5] There is increased metabolism in skeletal muscles generating heat without contraction or non-shivering thermogenesis (NST).[6] It is linked with the energy turnover associated with the release and reuptake of Ca^{++} by intracellular membrane system of muscle, the sarcoplasmic reticulum (SR). The reuptake of released Ca^{++} back into the SR involves sarcoplasmic reticulum calcium dependant ATPase (SERCA) and hydrolysis of ATP which releases energy.[7]

Stress induced by cold exposure increases the activity of sympathetic nervous system.[3] Epinephrine has been found to activate thermogenesis, showing that skeletal muscle is involved in cold-induced thermogenesis.[7] Sympathetic stimulation causes ß-oxidation in skeletal muscles and enhances energy production by mitochondria.[2] In short term durations, this is helpful but prolonged stimulation has deteriorating effects.[8] Increased ß-oxidation of fatty acetyl-CoA molecules causes formation of reactive oxygen species (ROS) which lead to physical damage to mitochondria, cytoskeleton and other cell membrane organelles.[9] Prolonged stimulation of skeletal muscle also causes inhibition of SERCA and reduction in Ca^{++} accumulation in SR.[10] These pro-oxidants increase the sensitivity of certain muscle proteins, e.g., dihydropyridine receptors and ryanodine calcium channels. Furthermore, they hinder nitric oxide over-activity, which may inhibit excitation contraction coupling through direct inhibition of myosin ATPase.[11]

Intense or prolonged contractile activity can reduce muscle force output that occurs after cessation of activity. This pattern of events is referred to as muscle fatigue.[12]

Normally, oxidants are produced in the cells during metabolic activity and are combated by intracellular antioxidants.[9] Biological antioxidants react with free radicals or precursor metabolites converting them into less reactive molecules and preventing or delaying oxidation of biological molecules.[13]

In conditions of increased stress like in smokers, older patients or persons experiencing environmental stress from heat, cold or radiation, the production of free radical species is dramatically increased.[14] Therefore, the requirement for antioxidants is amplified and without exogenous intake, it becomes difficult to prevent oxidative damage to the cells.[15]

Ascorbic acid is regarded as the most important water-soluble antioxidant in plants, animals and humans. It can donate a hydrogen atom and form a relatively stable ascorbyl free radical.[16] Ascorbate is a critical component of the oxidant shield in skeletal muscle, being actively accumulated by muscle endothelium.[17] As an antioxidant, it can rejuvenate the antioxidant, alpha tocopherol and both reduce the destructive process of lipid peroxidation of cytomembranes.[18]

Different studies have suggested cold exposure induced derangements in skeletal muscle fibres, but their relationship along with ascorbic acid is not emphasised in literature. The present study was designed to investigate the effect of ascorbic acid supplementation on fatigue of skeletal muscle fibres in cold exposed rats.

MATERIAL AND METHODS

It was a randomised control trial. Nine weeks old healthy, male Sprague-Dawley rats, weighing 200±25 grams were included in this study. Female rats were not selected as their monthly cyclical changes affect the stress induced. Diseased rats or those which developed any disease during the study period were excluded from the study. Ninety rats were randomly divided into 3 groups with 30 rats in each group.

These rats Group-I were fed on standard diet and tap water. They were kept at room temperature at 22±3 °C.[19] Rats of Group-II were fed on standard diet and tap water. They were exposed to cold environment between 8–14 °C for 1 h/day for one month by keeping their cages in ice-filled tubs and recording the temperature by thermometer.[19] Rats of Group-III were fed on standard diet. They were given ascorbic acid (Vitamin C Ascorbic acid MERCK, Research grade Cat No. 500074) supplement in a dose of 500 mg/L mixed in drinking water.[20] They were exposed to cold environment between 8–14 °C for 1 h/day for one month by keeping the cages in ice-filled tubs.[19]

After four weeks of study, the rats were ether anesthetised in glass jars, then placed dorsally on wooden board and extensor digitorum longus muscle (EDL) was dissected out by opening the femoral and anterior tibial compartments (Figure-1). Muscles were fixed on the muscle holder of the PowerLab® (Figure-2). Muscle fatigue was determined by stimulating the muscle for 30 seconds with supramaximal velocity having 5 seconds of rest for 5 minutes. Graphical events as well as calculations were obtained with the help of LabTutor®.

Data was analysed using SPSS-16.0. Mean and standard deviation was calculated for the decline of force during muscle fatigue in all the three groups. The statistical significance of differences across the groups was determined by applying one way student *t*-test and $p \leq 0.05$ was considered significant.

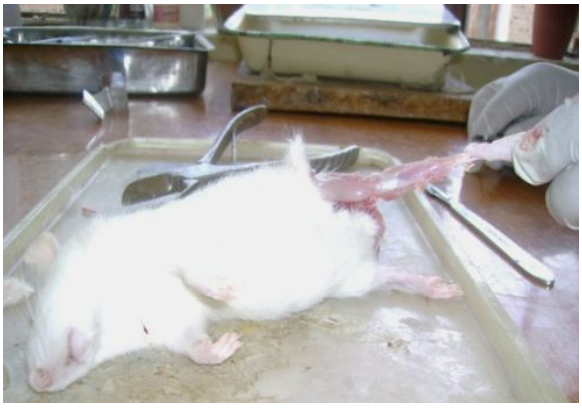

Figure-1: Dissection of extensor digitorum longus

Figure-2: Muscle setup on PowerLab®

RESULTS

The study comprised of 90 rats divided into 3 groups. Comparison of fatigue (decline in force) among control group, cold exposed group and cold exposed with ascorbic acid treated group is shown in Tables-1, 2 and 3. The cold exposed group had deranged force of contraction and less fatigability than the control group. The third group which was cold exposed supplemented with ascorbic acid showed better force of contraction and less resistance to fatigue. The results were close to the control group.

Figure-3 shows the trend of decline in force among all the three groups together at 0, 1, 2, 3, 4 and 5 minutes interval. The cold exposed group showed the minimum decline in force, i.e., an increased resistance to fatigue, followed by the cold exposed with ascorbic acid treated group, while the control group had the maximum decline in force except for at 0 and 1 minutes.

Table-1: Comparison of force fatigue (decline in force) between control and 4 weeks cold exposed group

Fatigue Time	Force of fatigue (Mean±SD)		*P*
	Control group	Cold exposed group	
At 0 min	0.253±0.001	0.246±0.002	0.000**
At 1 min	0.159±0.001	0.133±0.002	0.000**
At 2 min	0.129±0.001	0.161±0.003	0.000**
At 3 min	0.106±0.001	0.118±0.002	0.000**
At 4 min	0.083±0.002	0.094±0.002	0.000**
At 5 min	0.048±0.001	0.075±0.002	0.000**

** $p<0.01$ is taken as highly significant

Table-2: Comparison of force fatigue (decline in force) between cold exposed group and cold exposed with ascorbic acid supplement group after four weeks

Fatigue Time (min)	Force of fatigue Mean±SD)		*P*
	Cold exposed group	Cold exposed with ascorbic acid supplement group	
At 0 min	0.246±0.002	0.255±0.005	0.000**
At 1 min	0.133±0.002	0.155±0.002	0.000**
At 2 min	0.161±0.003	0.132±0.003	0.000**
At 3 min	0.118±0.002	0.110±0.006	0.000**
At 4 min	0.094±0.002	0.089±0.003	0.000**
At 5 min	0.075±0.002	0.065±0.002	0.000**

**$p<0.01$ is taken as highly significant

Table-3: Comparison of force of fatigue (decline in force) between control group and cold exposed with ascorbic acid supplement group after four weeks

Fatigue Time	Force of fatigue (Mean±SD)		*p*
	Control group	Cold exposed with ascorbic acid supplement group	
At 0 min	0.253±0.001	0.255±0.005	0.043*
At 1 min	0.159±0.001	0.155±0.002	0.000**
At 2 min	0.129±0.001	0.132±0.003	0.000**
At 3 min	0.106±0.001	0.110±0.006	0.002**
At 4 min	0.083±0.002	0.089±0.003	0.000**
At 5 min	0.048±0.001	0.065±0.002	0.000**

*$p<0.05$ is taken as significant, **$p<0.01$ is taken as highly significant

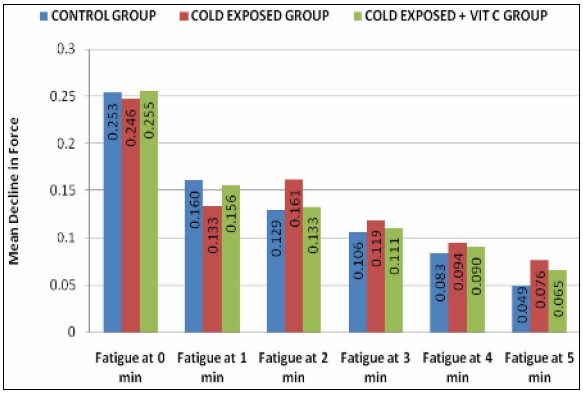

Figure-3: Distribution of decline in force during fatigue in three groups showing resistance to fatigue in the cold exposed group at 2, 3, 4 and 5 minutes

DISCUSSION

We assessed the force of contraction of skeletal muscles during the development of fatigue among the control, cold exposed and cold exposed along with ascorbic acid supplement group. Our findings showed a deranged force of contraction in the cold exposed group compared to the control group. The ascorbic acid supplemented group showed better force of contraction and less resistance to fatigue. These findings are comparable with other studies.

Comparable results were obtained by Nomura *et al.* They noted a fall in the tension development and an improved fatigue resistance in the cold exposed group.[19] The results are consistent with the fact that cold stress causes accumulation of oxidants and derangements in the Ca^{++} release by the sarcoplasmic reticulum of muscles. Thus, the contractility of the muscles is delayed.

Study conducted by Supinski *et al* supports our findings. They demonstrated that infusion of the antioxidant, N-acetylcysteine, in rabbits resulted in a remarkable preservation of force production during fatigue at low frequency stimulations of diaphragm.[21] The underlying cause of this response is that antioxidants change the redox tone of certain metabolic enzymes responsible for preservation of energy status within the cell. This occurs particularly during conditions of imbalance in energy supply and utilisation as occurs during fatigue.

Clanton and his fellows from Ohio also observed that oxidative stress, with increased lipid and protein oxidation products, produced a net reduction in tetanic force development in response to low frequencies of stimulation which was improved by the use of antioxidants like N-acetylcysteine, superoxide dismutase (SOD) and catalase.[22] Their findings support the results of our study.

Kroese and his co-workers showed results similar to our observations. They found that long-term cold preservation of isolated skeletal muscles resulted in increased intracellular calcium levels, following increased contraction, and an overproduction of oxygen free radicals.[23] They added calcium antagonists and antioxidants to the bathing solution and observed an improvement in muscle function and a preservation of the muscle cell membrane integrity.

Experiments conducted by Brutton *et al* on isolated muscles of rodents have determined that the primary mechanism causing fatigue in fast twitch fibres are decreased SR Ca^{++} and decreased Ca^{++} sensitivity.[24]

McKenna *et al* found out that antioxidant N-acetylcysteine (NAC) supplementation increased the time to fatigue in humans during submaximal cycling exercise.[25] These findings also support our results. Their analysis of muscle biopsies suggested that the improved performance could be due to preserved function of Na^{+}-K^{+} ATPase. Thus, indicating that antioxidants improve muscle performance by stabilising cell membranes.

CONCLUSION

Prolonged cold exposure to skeletal muscle cells leads to derangement in their contractile properties while adequate supplementation with ascorbic acid increases the force of contraction and reduces the resistance during fatigue.

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Address for Correspondence:

Dr. Aneeqa Rashid, House No. 20, St 45, F-7/1 Islamabad, Pakistan. **Tel:** +92-51-52613215, **Cell:** +92-300-5103813
Email: dr.aneeqa@hotmail.com