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Effect of Exercise Training and Vitamins E and C on Antioxidant Enzyme Activities and Lipid Peroxidation in Aging Rats

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Abstract

Background: There are many conditions that may affect the antioxidant enzyme activities, as aging and exercise. Moderate regular exercise up-regulate activities of antioxidant enzymes with depressant action on the lipid peroxidation processes, while strenuous exercise have a depressant effect on the activities of antioxidant enzymes with stimulatory action on the lipid peroxidation processes. Glutathione-S transferase (GST) and catalase are the major part of the specific antioxidant enzyme defence system response; malondialdehyde is the major indicator to lipid peroxidation processes which are affected by the exercise**.**
Aim of the work: To study the effect of moderate regular exercise and strenuous exercise with and without vitamin E or C supplementation on antioxidant defence system.
Material and Methods: Fifty six aging male albino rats were used in the present experiment. They were equally divided into seven groups. Group I: control group, Group II: moderate regular exercised group. Group III: strenuous exercised group. Group IV: moderate regular exercised group with vitamin E supplementation. Group V: strenuous exercised group with vitamin E supplementation, Group VI: moderate regular exercised group with vitamin C supplementation. Group VII: strenuous exercised group with vitamin C supplementation. The exercise was performed on a modified manual treadmill and the experiment continued for two months. Peripheral blood samples and muscular tissue samples were taken from all animal groups for estimation of glutathione-S transferase, catalase and malondialdehyde.
Results: The moderate regular exercise with or without vitamin E or C supplementation increased the antioxidant enzymes Glutathione-S transferase, catalase and decreased the malondialdehyde in serum and muscular tissue. The strenuous exercise decrease the antioxidant enzymes and increase the malondialdehyde production, while with vitamin E or C supplementation strenuous exercise enhanced the antioxidant enzymes and decreased the malondialdehyde production.
Conclusion: The moderate regular exercise enhance the antioxidant defence system and so inhibit the lipid peroxidation production, while the strenuous exercise inhibit the antioxidant defence system activity and increase the lipid peroxidation production. Supplementation with vitamin E or C is essential to enhance the antioxidant defence system activities that decrease lipid peroxidation.
Key words: Moderate regular exercise, strenuous exercise, antioxidant defence system, GST, catalase, and malondialdehyde**.**

Introduction

Oxidative stress is a physiological condition of elevated concentrations of reactive oxygen species (ROS) that cause molecular damage to vital structures and functions. Several factors influence the susceptibility to oxidative stress by affecting the antioxidant status or free oxygen radical generation .Oxidative damage to lipids, protein and DNA consequently decrease athletic performance ***(Deaton and Marlin, 2003).***

Free radicals are naturally produced in the body, both by normal cellular metabolism and as a result of disease process or through xenobiotic activities. They have the potential to elicit many of the tissue changes associated with toxicities and disease processes, but are also a consequence of such damage
(Kehrer et al., 2010).

Antioxidants enzymes act directly and indirectly to remove reactive organ species. One of the most important physiological antioxidants systems is the glutathione system in which glutathione peroxidise utilizes reduced glutathione as a hydrogen donor for the removal of peroxides ***(Hanachi et al.,2003)***.

Skeletal muscle is a highly malleable tissue that responds to changes in its pattern of activity or the mechanical and environmental stresses placed upon it. Reactive oxygen species are produced at various sites in skeletal muscle and there is increasing evidence that species play targeted roles in modulating redox-sensitive signalling pathways that are important to the muscle for making adaptation ***(Malcsolm,2009)***. Mitochondria, xanthine oxidize and phagocytes such as neutrophils may all contribute to free radical production ***(Sachdev and Davies, 2008).***

Regular physical exercise appears to retard or reverse age related functional decline and to delay the onset of age related diseases by attenuating potentially harmful oxidative damage and suppressing inflammatory processes even when performed at older ages ***(Goto and Radak, 2007).***

Ascorbic acid is involved in a number of biochemical pathways that are important to exercise metabolism and the health of exercising individuals. Exercise generally causes a transient increase in circulating ascorbic acid in the hours following exercise, but a decline below pre-exercise levels occurs in the days after prolonged exercise. These changes could be associated with increased exercise-induced oxidative stress ***(Peake, 2003).*** Vitamin E deficiency, which will increase oxidative stress, would augment the training-induced adaptation of antioxidant enzymes ***(Chang et al.,2007).***The daily moderate exercise with vitamin E and C supplementation may be beneficial for oxidative stress due to their role in reducing free radical production ***(Naziroglu et al.,2004).***

Material and Methods

The present study was carried out on 56 aging male albino rats, weighing 160-200 gm. The animals were divided equally into seven groups:

Group I: control group. Animals were kept in the used treadmill but did not exercise for a period of experiment of exercised performed according to ***(Somani et al., 1995)***.

Group II: moderate regular exercised group. Animals were kept in the a modified manual treadmill using special cage containing a septum and having a mobile smooth base made of plastic which allow manual rolling over a roller. After an acclimatisation period about one week, exercise was then performed at a rate of 10meters/minute for 30 min/day /5 days/ week for 8 weeks).

Group III: strenuous exercised group. Animals were exercised 5 days/ week for 8 weeks. The distance of exercise increased 10meters weekly till it reach 60 meters / minute till the end of the experiment.

Group IV: moderate regular exercised group with vitamin E supplementation. Animals received oral dose of vitamin E (4.28mg/kg/day), and exercised as in the previously mentioned regimen of moderate regular exercise.

GroupV: strenuous exercised group with vitamin E supplementation. Animals received oral dose of vitamin E, and exercised as in the previously mentioned regimen of strenuous exercise.

GroupVI: moderate regular exercised group with vitamin C supplementation. Animals received oral dose of vitamin C (21.4mg/kg/day), and exercised as in the previously mentioned regimen of moderate regular exercise.

GroupVII: strenuous exercised group with vitamin C supplementation. Animals received oral dose of vitamin C, and exercised as in the previously mentioned regimen of strenuous exercise.

Both vitamins were given through inter-oesephageal tube, the doses were determined according to the interspecies conversion table constructed by ***Paget and Barnes (1964).***

At the end of the experiment all animals were fasted for 12 hours, anesthetised by ether and, blood samples were collected from retro – orbital sinus. Muscular tissue samples were also taken. The collected samples were examined for:

- Quantitative estimation of glutathione-S-transferase (GST) by the UV method using kit from Bio-Diagnostic ***(Habig and Pabst, 1974)***.
- Estimation of catalase activity by calorimetric method using kits from Bio-Diagnostic ***(Aebi, 1984)***.
- Quantitative estimation of malondialdehyde by ELISA according to ***Yagi, (1998).***

Statistical analysis: All statistical analysis were computed using descriptive statistics including mean and SE for the outcome variables of interest. One-way analysis of variance with repeated measures was used for comparison of dependent variables. Differences were considered significant if $P < 0.05$.

Results

The antioxidant enzymes GST and catalase were significantly increased ($P<0.05$) in tissue and plasma of the moderate regular exercised groups without and with vitamin supplementation. On the other hand, GST was insignificantly changed in muscular tissue and plasma of the strenuous exercised group without vitamin supplementation. Both GST and catalse were significantly increased ($P<0.05$) in muscular tissue and plasma of strenuous exercised groups with vitamin E and vitamin C supplementation in comparison to control group. (Table 1, Fig 1, 2, 3, 4)

The malondialdehyde was insignificantly changed in the plasma and tissue of moderate regular exercised groups with and without vitamin supplementation compared to control group. Regarding the strenuous exercised groups, the malondialdehyde level showed a significant increase ($P<0.05$) in the plasma and tissue of the strenuous exercised group without vitamin supplementation as well as in tissue of both strenuous exercised groups with vitamin supplementation, but it was insignificantly changed in the plasma of the strenuous exercised groups with vitamin supplementation in comparison to control group.(Table 2, Fig 5,6)

Table (1): The changes of antioxidant enzymes GST in plasma (U/L), in tissue (U/g) and catalase in plasma (U/L), in tissue (U/g) in all studied groups.

	control		Exercised group		Exercised with vit E		Exercised with vit C	
	plasma	tissue	plasma	tissue	plasma	tissue	plasma	tissue
GST Mean±S.E	195.37± 47.24	0.38± 0.09						
GST moderate regular exerc Mean±S.E			260.7± 1 64.32	1.25± 0.33	384.4± 95.78	1.96± 0.42	303.1± 75.81	1.34±0.32
P value			P< 0.05*	P< 0.05*	P< 0.05*	P< 0.05*	P< 0.05*	P< 0.05*
GST strenuous exer. Mean±S.E			178.6± 47.5	0.21± 0.05	517.9± 128.6	2.10± 0.51	371.9± 83.25	1.10± 0.26
P value			P> 0.05†	P> 0.05†	P< 0.05*	P< 0.05*	P< 0.05*	P< 0.05*
catalase Mean±S.E	445.03± 93.77	0.48± 0.10						
catalase moder-ate regular exer Mean±S.E			485.3± 109.8	2.60± 0.70	597.3± 135.50	2.80 ± 0.77	617.4± 141.4	2.85±0.77
P value			P< 0.05*	P< 0.05*	P< 0.05*	P< 0.05*	P< 0.05*	P< 0.05*
catalase in strenuous exer. Mean±S.E P value			281.2± 71.28 P< 0.05*	0.23± 0.05 P< 0.05*	507.9± 108.31 P< 0.05*	2.20± 0.56 P< 0.05*	496.8± 96.61 P< 0.05*	2.17± 0.51 P< 0.05*

* significant †insignificant

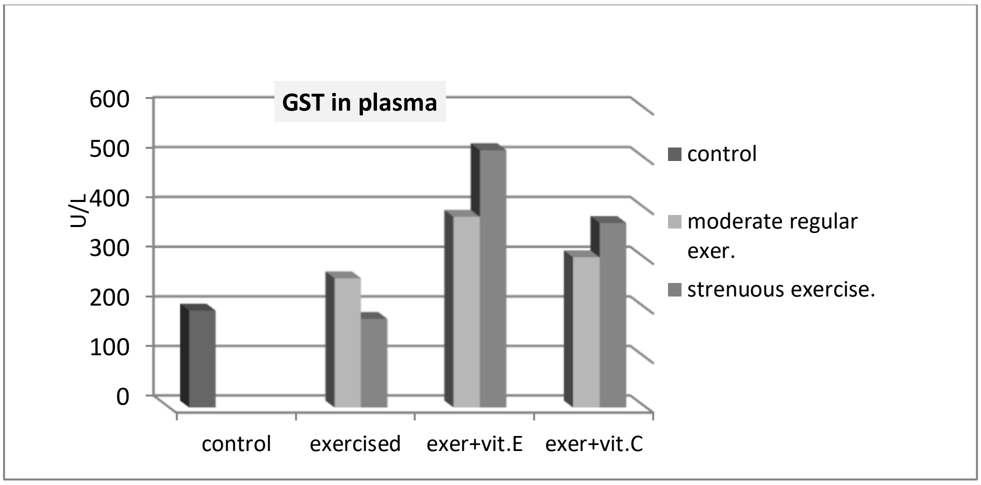

Fig (1): The GST (U /L) in plasma of all studied groups compared to control.

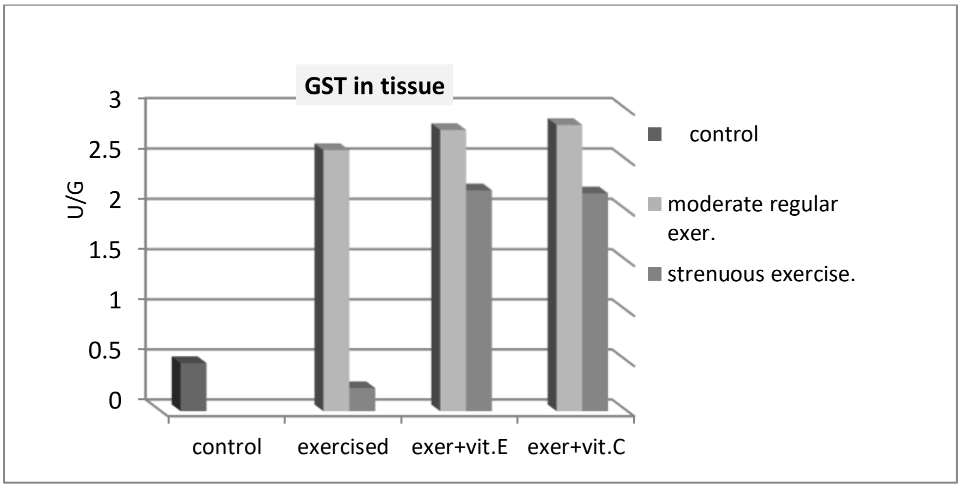

Fig (2): The GST (U /g) in tissue of all studied groups compared to control.

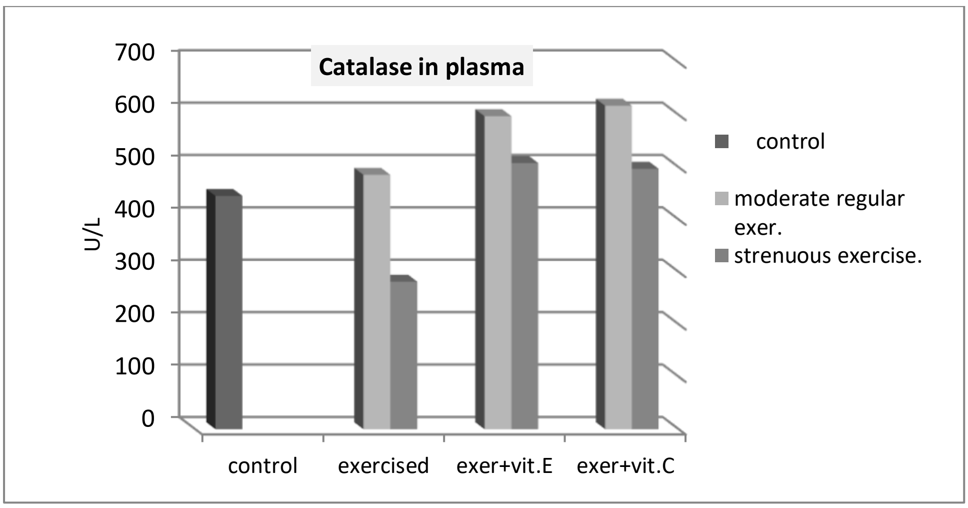

Fig (3): The catalase (U /L) in plasma of all studied groups compared to control.

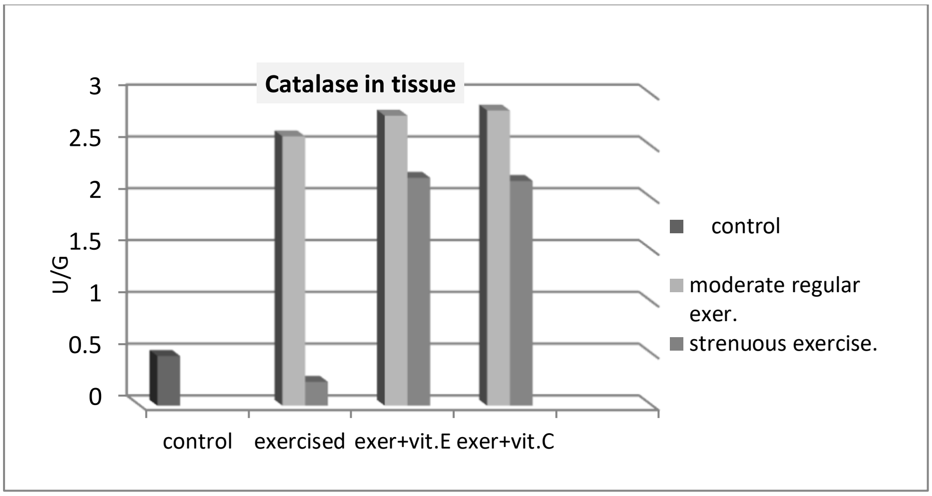

Fig (4): The catalase (U/g) in tissue of all studied groups compared to control.

Table (2): The changes of malondialdehyde in plasma (nmol/ml) and muscle (nmol/g) in all studied groups.

malondialdehyde / Group	Control		Exercised group.		Exercised group with vit E.		Exercised group with vit C.	
	In plasma	In muscle	In plasma	In muscle	In plasma	In muscle	In plasma	In muscle
.	3.06±0.78	3.2±0.85						
Moderat regular excersized Mean±S.E P value			4.4±0.85 P> 0.05†	5.01±1.12 P> 0.05†	2.05±0.49 P> 0.05†	2.6±0.49 P> 0.05†	2.09±0.46 P> 0.05†	2.9±0.52 P> 0.05†
Strenuous exercised Mean±S.E. P value			5.89±1.34 P< 0.05*	10. 5±2.3 P< 0.05*	3.12±0.70 P> 0.05†	4.7±0.9 P< 0.05*	4.16±0.94 P> 0.05†	5.9±1.08 P< 0.05*

† insignificant * significant.

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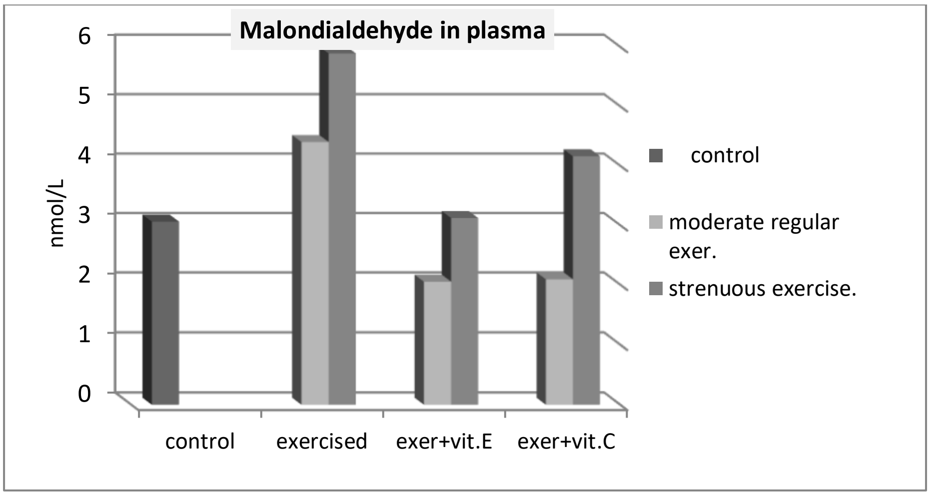

Fig (5): The malondialdehyde (nmol /L) in plasma of all studied groups compared to control.

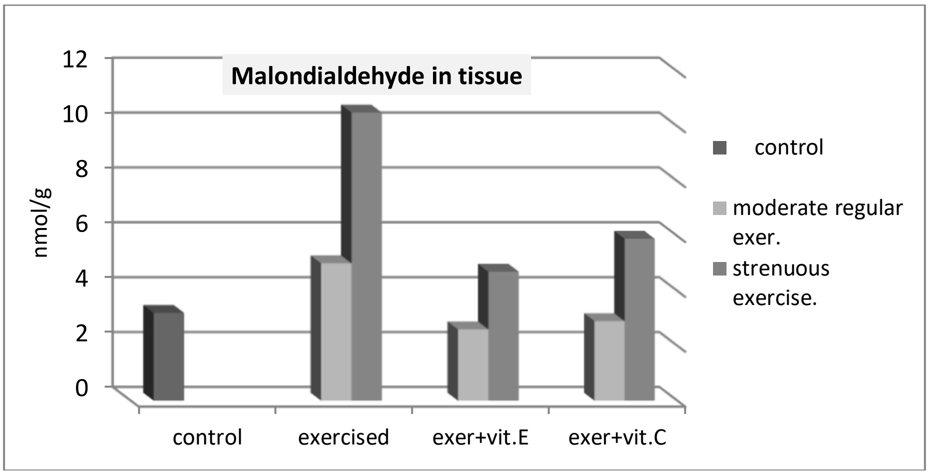

Fig (6): The malondialdehyde (nmol /g) in tissue of all studied groups compared to control.

Discussion

Regular exercise training is associated with numerous health benefits. It improves muscle strength and cardiorespiratory endurance. In addition, exercise reduces symptoms of depression and anxiety, attenuates cardiovascular and neurohumoral stress responses, and may also improve neurocognition ***(Blumenthal et al., 2007).***

Regular training is known to increase the accumulation of oxidative protein and DNA damage. The mild oxidative stress possibly elicited by regular exercise appears to manifest a hormesis-like effect in tissues, constituting beneficial mechanisms of exercise by adaptively upregulating various antioxidant mechanisms, including antioxidative and repair–degradation enzymes for damaged molecules. Importantly, the adaptation induced by regular exercise was effective even if initiated late in life ***(Goto et al., 2007)***.

Immune function in several body compartments exhibits signs of stress or suppression for a short period following prolonged endurance exercise. Performing routine moderate exercise stimulates the immune system, while strenuous exercise has been shown to cause immunosupression during recovery ***(Pedersen and Hoffman-Goetz, 2000).***

Dietary antioxidants are able to detoxify the peroxides produced during exercise, which could result in lipid peroxidation and they are capable of scavenging peroxyl radicals and therefore prevent muscle damage. Dietary antioxidant supplements are marketed to and used by athletes as a means to counteract the oxidative stress of exercise ***(Chong et al., 2007).***

Radak et al. (2005) hypothesized that decreased functional efficiency of antioxidants and antioxidant enzymes with advancement of age leads to increase free radical generations.

In the current experiment, the antioxidant enzymes GST and catalase were significantly increased ($P<0.05$) in tissue and plasma of the moderate regular exercised groups without and with vitamin supplementation being higher with vitamin supplementation. This coincide with the results of ***Husain(2003)***, who said that exercise conditioning resulted in up-regulation of catalase , glutathione peroxidase ,glutathione reductase and glutathione S transferase and depression of malonaldiahyde and lactate .

Ji et al. (2009) explained that useful signalling molecules are generated to regulate gene expression of proteins and enzymes that play a vital role in the normal muscle function and defence against dangerous effect of ROS. However ***Goto et al. (2007)*** suggested that regular exercise increased glutathione level which showed more than a two fold increase of the reduced form. ***Hanachi and Shemshaki (2010)*** in their study detected that the eccentric and concentric training show an improvement in blood antioxidant enzymes activities. It thus appears that cellular milieu is shifted to a less oxidative state suggesting a preventive effect by the exercise regimen even at old age.

Strenuous exercise may manifest an imbalance between ROS and antioxidant defence resulting in an oxidative stressful environment in the body. The extent of oxidative damage during physical exercise is determined not only by the level of free radical generation but also by the defence capacity of antioxidants**.** Prolonged or strenuous exercise of a concentric nature, then, can be associated with a metabolic stress rather than the mechanical stress linked with eccentric contraction **(*Hanachi and Shemshaki, 2010*).**

In this study, the strenuous exercised groups without vitamin supplementation GST was insignificantly changed in plasma and muscular tissue, while it was significantly increased ($p<0.05$) in plasma and muscular tissue in both strenuous exercised groups with vitamin E and vitamin C supplementation.

Catalase enzyme was significantly decreased ($p<0.05$) in plasma and muscular tissue of the strenuous exercised group without vitamin supplementation. While it was significantly increase in plasma and muscular tissue in both strenuous exercised groups with vitamin E and vitamin C supplementation.

This is in agreement with ***Taysi et al. (2008)*** who found that acute exhaustive exercise increase malondialdehyde level in untrained rats, decrease the activity of glutathione peroxidase, catalase and enzymatic plus non-enzymatic superoxide scavenger activity in untrained rats, however it did not affect glutathione S transferase, glutathione reductase , superoxidase dismutase. While ***Fisher and Gordon (2009)***, said that following a high intensity interval training there is an increase in the activities of superoxide dismutase, catalase, and glutathione peroxidase.

It was hypothesized by ***Brancaccio et al. (2010)*** that muscle tissue may be damaged following intense prolonged training as a consequence of both metabolic and mechanical factors. Functional disruption of sarcoplasmic reticulum and mitochondria has been reported after acute exercise (***Droge, 2002)***. Furthermore, changes in the biochemical structure and function of membrane lipids nucleic acids proteins have also been reported in working skeletal muscle immediately after strenuous exercise **(*Bloomer et al., 2005)*.**

In this study, the malondialdehyde level was insignificantly changed in the plasma and tissue of moderate regular exercised groups with and without vitamin supplementation compared to control. Regarding the strenuous exercised groups, the malondialdehyde level showed a significant increase ($P<0.05$) in the plasma and tissue of the strenuous exercised group without vitamin supplementation as well as in tissue of both strenuous exercised groups with vitamin supplementation. On the other hand, it was insignificantly changed in the plasma of the strenuous exercised groups with vitamin supplementation in comparison to control group. These results are in agreement with ***Sacheck and Blumberg (2001)***, who detected that bouts of intense exercise are associated with increase in lipid peroxidation, generation of malondialdehyde and the release of muscle enzymes**. *Muller et al. (2007)*** found that treadmill exercise increased level of lipid peroxidation in tissue of rats and they said that the increased level of malondialdehyde is a marker of oxidative stress in both mid-intensity and high intensity exercise.

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تأثير ممارسة الرياضة وفيتامينات (هـ) و (ج) على نشاط الإنزيمات مضادات الأكسدة وعلى أكسدة الدهون فى الجرذان المسنه

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يوجد العديد من العوامل التى تؤثر على نشاط الأنزيمات مضادة الأكسده مثل تقدم السن و الرياضه. وتعتبر الرياضه المنتظمه متوسطه الشده من إحدى العوامل المنشطة للإنزيمات مضادات الأكسده و هى أيضا تقلل إنتاج المواد الضارة الناتجه من أكسده الدهون. أما الرياضة العنيفة فلها تأثير عكسى حيث وجد انها مثبطه لأنتاج الأنزيمات مضادات الأكسده وتسبب زيادة أنتاج المواد الضارة الناتجه من أكسدة الدهون. وقد وجد ان أعطاء الڤيتامينات مضادات الأكسده أثناء ممارسه الرياضه ومع تقدم السن يساعد على تنبه جهاز الانزيمات مضادات الأكسده ويقلل من إنتاج المواد الضارة الناتجه من أكسده الدهون. ويعتبركل من الجلوتاثيون-أس-ترانسفيريز والكاتاليزمن الإنزيمات المضادة للأكسدة,كما أن المالونالدهايد من المواد التى تتكون نتيجة أكسدة الدهون .

ولقد صمم هذا البحث لدراسة تأثير ممارسه الرياضة المنتظمة متوسطة الشدة و العنيفة على نشاط جهاز الأنزيمات مضادات الأكسدة مع أوبدون التزود بفيتامين **(هـ)** و (ج) وتم إجراء البحث على ستة و خمسون من الجرذان البيضاء الذكور قسمت الى سبعة مجموعات

المجموعة الاولى : المجموعة الضابطة

المجموعةالثانية : التى مارست الرياضة المنتظمة متوسطة الشدة .

المجموعةالثالثه: التى مارست الرياضه العنيفة .

المجموعةالرابعه: التى مارست الرياضه المنتظمة متوسطة الشدة مع التزود بڤيتامين**(هـ)** يوميا

المجموعة الخامسه: التى مارست الرياضه العنيفة مع التزود بڤيتامين **(هـ)** يوميا

المجموعةالسادسه: التى مارست الرياضه المنتظمة متوسطة الشدة مع التزود بڤيتامين (ج) يوميا

المجموعةالسابعه: التى مارست الرياضه العنيفة مع التزود بڤيتامين (ج) يوميا

وقد تم إجراء البحث على مدار ثمانيه أسابيع و بعدها تم أخذ عينات من دم و عضلات جميع الجرذان بعد تخديرها ثم تم قياس مستوى كل من الجلوتاثيون-أس-ترانسفيريز والكاتاليزو المالونالدهايد فى هذه العينات. وقد أظهرت النتائج زيادة إنتاج الأنزيمات مضادات الأكسده ونقص إنتاج المالونالدهايد الناتج من أكسدة الدهون فى مجموعه الجرذان التى قامت بمارسة الرياضه المنتظمة المتوسطة الشدة. أما مجموعه الجرذان التى قامت بمارسة الرياضه العنيفة فقد نقص فيها إنتاج الأنزيمات مضادات الأكسدة و زاد إنتاج المالونالدهايد , كما وجد أن إعطاء الجرذان الڤيتامينات مضادات الأكسدة ساعد على تنبيه جهاز الأنزيمات مضادات الأكسدة وقلل من إنتاج المالونالدهايد الناتج من أكسدة الدهون .