The role of diet supplementation (Dynamisan) on the gene expression and diversity of superoxide dismutase and vitamins C, E and vanillylmandelic acid and lactate threshold among swimmers

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Abstract: Introduction: This research aim to determine the role of diet supplementation (Dynamisan) on the gene expression and diversity of superoxide dismutase and vitamins C, E and vanillylmandelic acid and lactate threshold among swimmers. Where, High intensity exercise induces oxidative stress, treatment with antioxidants such as vitamin C, E, and minerals in part against free radical-mediated damage in exercise. Where, its exceed and damage the muscle cells. Specially, Desoxyribonucleic acid (DNA), that causes a defect in cell ability to carry out its functions and destruction. **Methods:** 8 swimmers chosen from Ismailiy sports club. Laboratory blood tests were carried out to determine genotype, gene expression, vitamin C, E, and vanillylmandelic acid (VMA). Also the swimmers exposed to swimming lactate threshold (SLT) test to determine their performance. **Results:** there were statistical significant between pre and after training program. Where, the RNA improved 22.25 ± 6.02 vs. 36.13 ± 4.39 ng/µl, SOD3 0.33 ± 0.08 vs. 0.72 ± 0.07 mg/µl, SOD2 2.40 ± 0.16 vs. 3.97 ± 0.38 mg/µl, vitamin E 758.4 ± 25.08 vs. 824.3 ± 20.16 mg/dl, and vitamin C 2.24 ± 0.35 vs. 3.96 ± 0.57 mg/dl. also there were decreased in VMA 4.66 ± 0.61 vs. 3.06 ± 0.29 mg/l, 200m freestyle record 2.91 ± 0.23 vs. 2.52 ± 0.03, 400m freestyle 6.11 ± 0.05 vs. 5.67 ± 0.23 sec., and SLT1.35 ± 0.01 vs. 1.33 ± 0.02 min/100m. **Conclusion:** The diet supplementations include vitamin C, and E and other antioxidants enhance gene expression RNA, and superoxide dismutase SOD2, 3 that led to decrease VMA level, also improve swimming lactate threshold record.

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1- Introduction

High intensity exercise induces oxidative stress, treatment with antioxidants such as vitamin C, E, and minerals in part against free radical-mediated damage in exercise.

Where, its exceed and damage the muscle cells. Specially, Desoxyribonucleic acid (DNA), that causes a defect in cell ability to carry out its functions and destruction.[2, 19]

Then decrease the athlete ability to speed and anaerobic endurance, which demand from swimmer to use natural alternative (diet supplements) that contains an antioxidants from vitamins, minerals, and trace elements with essentials diet. that aids to increase energy, active chemical reaction, and modify biochemical variables for energy producer.[11]Swimmer needs all diet essentials to get the energy to expose training stress.[1]According to Dieter Reference Intake (DRI) tables, children are listed in two categories (because their nutrient needs differ): 1 to 3 years and 4 to 8 years of age. any person age 9 or older is listed as a male or female, with specific subcategories within each grouping: 9 to 13 years of age and 14 to 18 years of age for both males and females.7 Like adults, young athletes require sufficient nutrition to perform at their best. on average, females experience their most rapid linear growth spurt between 10 and 13 years of age, whereas males experience their rapid growth spurt between 12 and 15 years old.[31]Physical activity is an important component of development, energy recommendation, and intakes must be adjusted to meet the needs of particular athletic endeavors. The age period between (12 – 15 years) are the growth spurts and sexual maturation changes that occur during these developmental years, most noticeable are increase in height, body weight, and secondary sexual characteristics.[34]So, the young swimmers need to more and different energy than adults, and it increase with high intensity for health and performance as well as for proper growth and maturation.[24]Vitamins and minerals are also important to growth, development, and athletic performance, which consumption during physical exercise specially with increase in dehydration, that occurs in our area. That needs to focus on replacement the missing. because evaporation rate is inversely related to humidity, the ability of the body to dissipate heat through sweat decreases in hot and humid condition. overheating can result, which in turn can lead to heat illness. such as muscle (heat) cramps, exercise heat exhaustion, heat syncope, exertional hyponatremia, or heat stroke.[5]Ahmed (2002) illustrated a decrease in zinc concentration level, and increase in sodium, potassium, copper, and iron in urine, sweat, and blood after exercise on rowing ergometer for 2km.[3]

So, when you put the diet program it might include vitamins, minerals with protein, carbohydrate, and fat.[13]Where, the vitamins are

organic substances which athletes take as supplements because they believe that a supplement will compensate for poor eating.15fortunately, studies have suggested that young athletes are less likely to underconsume their daily requirements for vitamins than nonathletic.[27]The minerals are inorganic compounds and, like vitamins, are not a source of energy for body.[15] But it has important role in metabolism and this increase with athlete. where, young female athletes, in particular adolescents, may require higher calcium and iron intakes because of the greater risk of osteoporosis later in life and to menstruation.[16]

the researcher noticed that swimmers have weakness in their diet specially in the summer, due to high efforts and exercise intensity with young swimmers, they lost trace elements and minerals sequence to increase in temperature and humidity which effect on increase free radicals and decrease antioxidants that leads to reduce performance.

The research aim

The research aim to know the role of diet supplement (Dynamisan) onthe gene expression and diversity of Superoxide dismutase and vitamins C, E and Vanillylmandelic acid and lactate threshold among swimmers.

The research hypothesis

- There are statistical significant changes between pre and post measurement in the gene expression and diversity ofSuperoxide dismutase, vitamin C, and E for the sake of post measurement.
- There are statistical significant changes between pre and post measurement in vanillylmandelic acid level in urine for the sake of post measurement.
- There are statistical significant changes between pre and post measurement in 200m, 400m record, andlactate threshold level for the sake of post measurement.

2- Materials and methods

The researcher used experimental methodology by using one group, and geno type, VMD, and vitamin C, E analyses in Clinilab laboratory in Cairo by using SOD2,SOD3, VMA primers, and kits.

Where, any gene contains essential parts and nonessential parts. The nonessential parts may contain some changes between different individuals. There changes are conserved and called genotype, to detect genotype, PCR primers specific or a certain change are designed and used to detect the change. The primers specific for a particular change (or genotype) do not work with other changes (or genotype) so to detect it the following steps are performed:

1. Design primer or probes specific for each genotype.
2. DNA isolated from target cases of PCR amplification.
3. Analysis of PCR results on agarose gel electrophoresis of poly acrylamide gel.
4. Determination of each genotype and allele frequency by statistical analysis.

Gene are translated in the form of mRNA which is translated into proteins. These proteins could be hormones or enzymes or structural protein. So to measure gene expression the following steps are tracked:

1. Isolation of total RNA from the target tissue.
2. Selection of mRNA from total RNA.
3. Conversion of mRNA into cDNA by reverse transcription using oligo dt primers.
4. Enzymatic amplification of the level of cDNA which reflect the level of mRNA which in its turn represent the gene expression of the target protein.

Vitamin C was done using the method of Harris and Ray (1988).[9]

Vitamin E was estimated using the method of Baker and Frank (1968).[4]

The research sample

The sample was chosen from Ismailiy swimming team, and they consisted of 8 swimmers age (13.625±0.518 y), height (164.5±1.6 cm), and weight (55.5±2.2 kg).

Exposed the training program for 12 weeks by using diet supplement (Dynamisan) 1tablet daily.

3- The results

Table 1 illustrates there are statistical significant difference in 0.05 value between pre and post-test after training program by using diet supplement (Dynamisan) for the sake of post-test in genotype and gene expression.

Table 2 illustrates there are statistical significant difference in 0.05 value between pre and post-test after training program by using diet supplement (Dynamisan) for the sake of post-test in vitamin E, C, and VMA.

Table 3 illustrates there are statistical significant difference in 0.05 value between pre and post-test after training program by using diet supplement (Dynamisan) for the sake of post-test in time record 200,400m freestyle, andswimming lactate threshold.

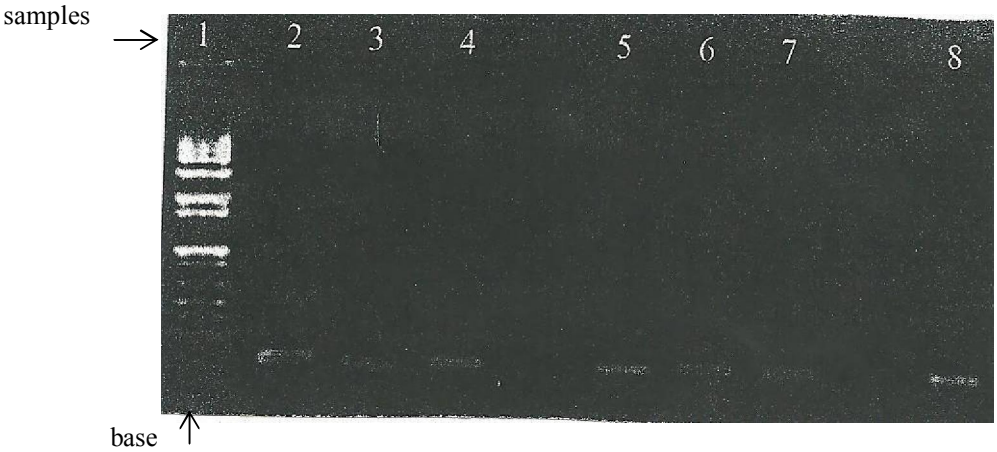

Fig. 1: The changes in RNA bands before using Dynamisan

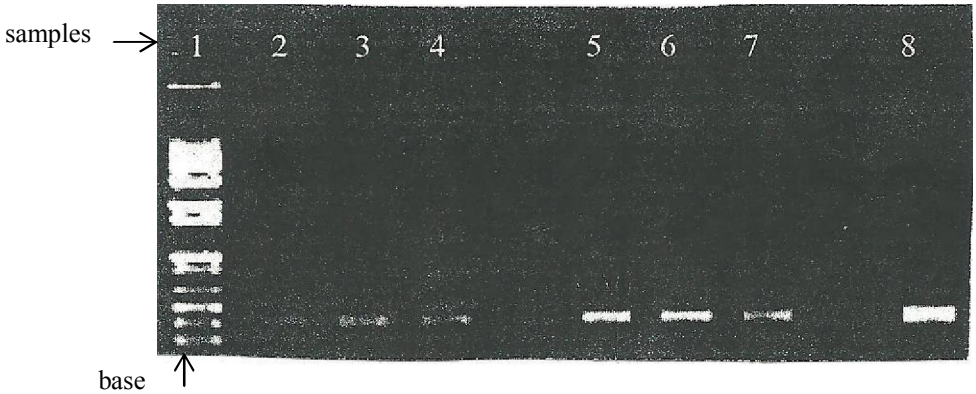

Fig. 2: The changes in RNA bands after using Dynamisan

Table (1): The statistical significant for genotype and gene expression before and after training program by using diet supplement (Dynamisan) N=8

statistical sign. / Variables	Measure. unit	Pre-test		Post-test		The difference between means	T test value	Improvement %
		mean	Std.	mean	Std.			
RNA	ng/µl	**22.25**	**6.02**	**36.13**	**4.39**	**-13.88**	**5.27***	20.63
SOD3	mg/µl	**0.33**	**0.08**	**0.72**	**0.07**	**-0.39**	**10.57***	1.83
SOD2	mg/µl	**2.40**	**0.16**	**3.97**	**0.38**	**-1.57**	**10.85***	0.75

Table T test value 0.05= 2.37

Table (2): The statistical significant for vitamins E, C, and VMA before and after training program by using diet supplement (Dynamisan), N=8

statistical sign. / Variables	Measure. unit	Pre-test		Post-test		The difference between means	T test value	Improvement %
		mean	Std.	mean	Std.			
Vitamin E	mg/dl	**758.4**	**25.08**	**824.3**	**20.16**	**-65.9**	**5.79***	757.3
Vitamin C	mg/dl	**2.24**	**0.35**	**3.96**	**0.57**	**-1.72**	**7.31***	0.47
VMA	mg/l	**4.66**	**0.61**	**3.06**	**0.29**	**1.6**	**6.72***	4.01

Table T test value 0.05= 2.37

Table (3): The statistical significant for time record for 200, 400m freestyle, and swimming lactate threshold before and after training program by using diet supplement (Dynamisan) N=8

statistical sign. Variables	Measure. unit	Pre-test		Post-test		The difference between means	T test value	Improvement %
		mean	Std.	mean	Std.			
200m freestyle	Sec.	**2.91**	**0.23**	**2.52**	**0.03**	**0.39**	**4.80***	2.04
400m freestyle	Sec.	**6.11**	**0.05**	**5.67**	**0.23**	**0.44**	**5.31***	5.18
Swimming lactate threshold	Min/100m	**1.35**	**0.01**	**1.33**	**0.02**	**0.02**	**3.49***	0.37

Table T test value 0.05= 2.37

4- The discussion

First: discus the results of 1st. hypothesis

Table 1 illustrated that there are a statistical significant differences in the gene expression and diversity of superoxide dismutase, vitamin C, and E for the sake of post measurement.

Henriette et al (2000) indicated that increase in gene expression may result to reproductive activation to group of protein as respond to exercise training which may lead to an increase in gene expression mRNA.[10]Heshmat (1999) definite that the high intensity training may leads to form free radicals that the human body try to defend through antioxidants, with increase the intensity training the free radicals reach to high increase to damage the muscle cells and red blood cells.[11]Ron Maughan & Glesson (2004) illustrated that one factor is common to all intensive training programs which is the generation of high reactive molecules called free radicals that can result in damage to cells and tissues. Hard exercise is associated with an increased level of free radicals because of the high rate of oxygen consumption.[28] William (2000) verified that exercise increases the generation of oxygen free radicals and lipid peroxidation. Strenuous exercise in a person who is unconditioned or unaccustomed to exercise will induce oxidative damage and result in muscle injury.[35]Also, Powers et al (1999) stated that cellular oxidants include a variety of reactive oxygen, nitrogen, and chlorinating species. It is well established that the increase in metabolic rate in skeletal muscle during contractile activity results in an increased production of oxidants. Failure to remove these oxidants during exercise can result in significant oxidative damage of cellular biomolecules.[25]Packer (1997) reported that if the ROS reach to more than antioxidants capacity to prevent its action, lipids, protein, and other DNA cell materials may exposed to oxidative damage.[22]It may be suggested that oral diet supplementation (Dynamisan) which include on vitamins and trace elements that made like antioxidant may due to increase in vitamin C, E level which effects on mRNA as a result to enhance SOD2,SOD3.

Thereby achieving the first hypothesis.

Secondly: discus the results of 2nd. Hypothesis

Table 2 illustrated that there are a statistical significant difference in VMA in swimmers urine after training program by using diet supplementation (Dynamisan) where it decreased than pre-test.

William (2000) indicated that aerobic exercise training strengthens the antioxidant defense system by increasing Superoxide dismutase. Vitamin C and, especially, vitamin E are shown to decrease the exercise-induced increase in the rate of lipid peroxidation. Also, vitamin E effects in augmenting the neutrophil response to eccentric exercise, and causes a greater increase in circularity creatine kinase activity, perhaps indicating increased skeletal muscle repair. Increased vitamin E intake has been associated with enhanced glucose tolerance and insulin action as well as improved lipoprotein status.[35]

Mari-Carmen et al (2008) reported that men taking 1.0g/d ascorbic acid (vitamin C) or the combination of ascorbic acid and α-tocopherol (vitamin E) had a markedly reduced increase in maximal oxygen uptake (Vo_2max) in response to 8wk of endurance training. They also reported that given rats ascorbic acid prevented adaptive increases in enzyme levels in skeletal muscle.[17]Several previous studies have shown that antioxidant vitamin supplementation decreases oxidative damage in human subjects.[8, 23, 30]

McBride & Kraemer (1999) stated that the high intensity training leads to increase free radicals production in skeletal muscles, which evaluated through increase malondialdehyde (MDA) levels in urine.[19]Where, free radicals are scavenged by antioxidants, however, when free radicals are produced in abundance and exceed the antioxidant capacity, these free radicals disrupt the cytoarchitectonic structure of cells such as proteins, lipids, enzymes, carbohydrates, nucleic acids and their compounds, and cause various metabolic and functional injuries. Oxygen radicals created in biological systems by various mechanisms and malondialdehyde (MDA). Superoxide dismutase (SOD) is an important enzyme that protects the cell from the harmful effects of O_2^{-}.[8]Heshmat & Nader (2003) indicated that exercise training leads to

increase metabolic process inside muscle cells through activate anabolic hormones such as growth hormone, also leads to enhance immune system through immune enzymes such as Superoxide dismutase 2, 3.[13]Shimaa (2012) suggested that increase in gene expression may due to anaerobic training that leads to fast deplete anaerobic energy. So, it leads to high produce of free radicals which play a role in stimulate antioxidants as enzymatic or as organic like vitamins.[32]Rousch (2013) indicated that endurance exercise can increase oxygen utilization from 10 to 20 times over the resting state. This greatly increases the generation of free radicals, prompting concern muscle damage and other tissues.[29]This study agree with Powers et al (2011) which indicated that both reactive oxygen species (ROS) and reactive nitrogen species (RNS) play important role in cell signaling pathways involved in muscle adaptation to exercise and the remodeling that occurs in skeletal muscle during periods of prolonged inactivity.[26]Neva et al (2003) contrasted that vitamin E supplementation (1200IU/day) after resistance exercise for 3 weeks, where they measured plasma MDA as a putative markers of free radical interaction with cell membranes, which has previously been shown to increase 24 hours after resistance exercise. There has been little evidence to date that indicates an improvement in exercise performance following vitamin E supplementation. But there are decreased in plasma MDA level.[21]Also, this study acceptable with study's Sikiru Lamina et al (2013) which illustrated that antioxidant supplementation such as vitamin C&E can reduce free radicals production and oxidation related to exercise.[33]It may be suggested that high intensity exercise, especially swimming lactate threshold test led to form free radicals in muscle cells pre-training program by using diet supplementation (Dynamisan) which has been used for 12 weeks. This supplementation led to enhance vitamins level for swimmers, where they could not replace it through nature sources in this age. wherefore, this diet supplement led to increase in increase in vitamin C, E, gene expression mRNA, and SOD2,3 which produced to decrease VMA level in urine.

Thereby achieving the second hypothesis.

Thirdly: discus the results of 3rd. Hypothesis

Table 3 illustrated that there are a statistical significant difference in 200m, 400m record, and swimming lactate threshold after training program by using diet supplementation (Dynamisan).

Claudi Zoppi et al (2006) indicated that exercise training is known to induce an increase in free radical production potentially leading to enhance muscle injury. Vitamin C and E are well known antioxidants that may prevent muscle cell damage. Where, the data demonstrated that vitamin C, and E supplementation in 10 soccer players may reduce lipid peroxidation and muscle damage during high intensity efforts, but did not enhance performance.[6]Mishima et al (2005) reported that exercise training is a basic element in healthy life, but nutrition that includes on antioxidants is play a mean role in this healthy life which leads to improve exercise performance and rapid recovery.[20]This study indicated that there are changes in swimming performance 200m, 400m record, and lactate threshold level perhaps as a result to improve aerobic capacity and depends on aerobic energy.

Thereby achieving the third hypothesis.

Conclusion

High intensity exercise like swimming lactate threshold test leads to increase in free radicals which measured through vanillylmandelic acid in urine. The diet supplementations include vitamin C, and E enhanced gene expression RNA, and superoxide dismutase SOD2, 3 that led to decrease VMA level.Also, improved 200m, 400m record, and swimming lactate threshold record.

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