The Effect of Astaxanthin and Regular Training on Dynamic Pattern of Oxidative Stress on Male under Strenuous Exercise

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Abstract. Strenuous physical activity induced Reactive Oxygen Species (ROS) level in human body that can be measured by serum Malondialdehyde (MDA) level. Malondialdehyde is product of lipid peroxidation process that describe as oxidative damage of lipid biomolecule by reactivity of reactive oxygen species. Still the dynamic pattern of Malondialdehyde (MDA) level under strenuous exercise not fully understood. Potent antioxidant such as Astaxanthin and training may be altered the level of MDA. The purpose of this study is to understand effect of astaxanthin to MDA dynamic pattern during male after strenuous physical activity. It was a double blind experimental study, conducted on thirty young male age, divided into untrained and trained groups. Supplement Astaxanthin was given to 15 subject as well as placebo for one week after supplementation, subjects were tested with anaerobic strenuous physical activity. The values were analyzed with ANOVA test followed by Duncan test showed that in every groups, mean MDA before test was similar, start increase significantly after tested, begin decrease at 6 hour post test and back to baseline at 24th hour post-test ($p<0.05$), except for group of untrained male with placebo still increase twice from baseline. The lowest mean of MDA was found on group trained male with Astaxanthin supplementation and the highest was found on group of untrained male with placebo ($p<0.05$). These findings support that Astaxanthin and training might have positive effect to oxidative stress condition without altered its dynamic pattern in male after strenuous physical activity

1. Introduction

An increase in macromolecule oxidation has been demonstrated following both aerobic and anaerobic exercise of sufficient intensity. The generation of reactive oxygen and nitrogen species (RONS), such as singlet oxygen (.O), superoxide radical (O_2-), hydroxyl radical (OH), and peroxynitrite (ONO_2-) occur as a consequence of normal cellular metabolism and seem to be increased under psychological and physical stress conditions. In anaerobic exercise (e.g., resistance, isometric, eccentric, sprint training or just acute muscle contraction with heavy load (90% 1 RM)) however, other pathways of ROS generation exist including ischemia-reperfusion, xanthine and NADPH oxidase production, prostanoid metabolism, phagocytic respiratory burst activity, disruption of iron containing proteins, and altered calcium homeostasis.

The production of ROS via these pathways may result partly from eccentric muscle actions, which cause muscle injury.[4] Resistance training is reported to have many benefits, such as weight control, prevention of osteoporosis, improvement of cardiovascular risk factors, and prevention of injury. However excessive resistance training program may increase oxidative stress and cellula[r] damage. There are two theories supporting the concept that resistance exercise could lead to an increas[e] the production of oxygen free radicals in active muscle sites. A widely supported hypothesis involv[es] the ischemia reperfusion injury. Intense muscle contractions can result in a temporary decrease[d] blood flow and oxygen availability and subsequent ischemia. Following contraction (muscle [re]laxation), reperfusion produces an abundant reintroduction of O2 and results in the formation of [O2]- radical. Mechanical stress is another hypothesis used to explain an increase in ROS. In particular, eccen[tr]ic acute exercise, which includes high levels of force, was shown to result in muscle tissue d[amage]. This [ini]tiates the inflammation process that eventually produces oxygen free radicals and li[pid] perox[ida]tion, o[r] its product is malondialdehyde (MDA).

These free radicals are neutralized by an elaborate antioxidant [de]fense syst[em] con[sis]ting o[f] [en]zymes such as catalase, superoxide dismutase, glutathione peroxida[se] and nu[mer]ous non-[en]zymatic antioxidants, including vitamins A, E and C, glutathione, ubiquinone [and] flavonoi[ds]. Exerci[se] can cause an imbalance between ROS and antioxidants, which is referred to as [ox]idative stress which lead to produce amount of malondialdehyde. Exercise training seems [to] reduce the [ox]idative stress of exercise, such that trained athletes show less evidence of lipid perox[id]ation for a given [bout] of exercise and an enhanced defense system in relation to untrained subjects. Whether the body's natural antioxidant defense system is sufficient to counteract the increase in reactive oxygen species with exercise or whether additional exogenous supplements are needed is not known.

Astaxanthin as an exogenous antioxidant, [is] a powerful antioxidant supplement. Astaxanthin exhibits strong ROS scavenging activity, protects against lipid peroxidation and oxidative damage of cell membranes, cells, and tissues. [15-18] these supplement may suppress the increasing of lipid peroxidation product such as malondialdehyde (MDA) during acute muscle contraction with heavy load in trained and untrained male. The comparison of Astaxanthin effect on MDA in trained and untrained male have not been fully described in the literature.

2. Purpose of Study

Therefore, the aim of this study is to compare the Astaxanthin supplementation effect on MDA changing in 24 hours between trained and untrained male after acute muscle contraction with heavy load.

3. Method

3.1. Exercise Protocol

On the 8th day, the subjects did 7 different physical test such as leg Press, leg curls, leg extension, rowing, bench press, military press, and two arm curl with load 90% from 1 RM. 4 repetition, 3 sets.

3.2. Collection of Blood Samples

Blood sampling were collected at pretest, immediate posttest, 6th hour's posttest and 24th hour's posttest.

3.3. Statistic

Plasma samples were used for MDA (mmol/ml). The MDA's values were expressed as mean ± SE, they were assessed using homogeneity test (levene test) and normality test (Kolmogorov-Smirnov). Independent samples t-test was used for comparison of corresponding values between the groups. The values were analyzed with ANOVA test and followed by Duncan test to determine whether there is a specific different among groups.

4. Result

4.1. Subject Physical Characteristics

Mean of physical characteristics subjects such as age (years), height (cm), weight (kg), body mass index (BMI), and fitness level subjects based on the strength of the muscles when lifting loads up to 90% of 1 RM listed in the table 1.

Table 1. Subject Physical Characteristic

Groups	Groups							
	A		B		C		D	
	χ	± s	χ	± s	χ	± s	χ	± s
Age(Year)*	21,14	± 1,35	20,88	± 1,64	18,38	± 0,92	1[illegible]86	± 2,48
Height(cm)*	174,4	± 8,85	173,1	± 3,31	165,0	± [illegible]	1[illegible],5	± 3,78
Weight(Kg)#	70,57	± 17,48	68,75	± 5,85	[illegible]75	± [illegible]	[illegible],7	± 7,43
IMT(Kg/m^2)#	23,11	± 4,75	22,64	± 1,21	[illegible]	± 2,[illegible]	21,46	± 1,92
Leg Press(Kg)*	171,43	± 41,58	132,25	± 17,75	107,[illegible]	± 17,33	98,14	± 9,87
Leg Curls(Kg)*	74,14	± 13,90	68,38	± 9,[illegible]	63,25	± 3,[illegible]	58,71	± 10,89
Leg Extension(kg)*	137,57	± 17,78	114,88	± [illegible]05	82,38	± [illegible]45	74,14	± 12,48
Rowing(Kg)*	65,29	± 10,06	54,50	± [illegible]80	39,[illegible]	± 8,44	41,29	± 6,80
Bench Press(Kg)*	65,30	± 17,27	54,60	± [illegible]9	39,7[illegible]	± 8,44	41,30	± 10,86
Military Press(Kg)*	74,86	± 13,90	69,[illegible]	± 9,[illegible]	49,[illegible]	± 12,27	40,00	± 7,66
Two Arm Curls(Kg)*	49,00	± 6,61	[illegible],00	7,46	[illegible]8	± 8,96	33,71	± 7,52

Note : * Significantly difference (p<0.05)
Not Significant
Group A: Trained +Astaxanthin Su[illegible]men C : U[illegible]ained +Astaxanthin Suplemen
B : Trained +plac[illegible]o D : Un[illegible] + Placebo

4.2. Comparative Betwee[illegible]ined and [illegible]rained Male

On trained male, the result sho[illegible]significant [illegible]ference between Astaxanthin & placebo effects on MDA mean level at immedi[illegible]ly posttes[illegible]11,55±2,34 vs. 16,33±7,09mmol/ml)(p= 0,01),6th hours posttest (5,58±3,32vs7,73[illegible],06mm[illegible]l) (p=0[illegible]24thhours posttest (4,25±1,86 vs. 5,14±1,07mmol/ml(p= 0,027). In untra[illegible]d male[illegible]e result is also significantly different between astaxanthin & placebo effects on MDA level [illegible]imm[illegible]ately [illegible]sttest (18, 98 vs. 20,09mmol/ml (p= 0,047), 6th hours posttest (9, 31 [illegible]mmol/[illegible]0,048), [illegible]h hours posttest (5, 02 vs. 8,14mmol/ml (p= 0,048).

Table 2. MDA Measurement Results (mean)

Groups	[illegible]re test (mmol/ml)		Post test (mmol/ml)		6th h Post test (mmol/ml)		24th h Post test (mmol/ml)	
	χ	± s	χ	± s	χ	± s	χ	± s
[illegible]A	5.96	± 1,82	11,55	± 2.34	5,58	± 3,32	4,25	± 1,86
	4,39	± 1,77	16,33	± 7.09	7,73	± 3,06	5,14	± 1,07
C	4,69	± 1,79	18,98	± 6,04	9,31	± 2,80	5,02	± 1,04
D	4,66	± 2,92	20,09	± 4,68	12,74	± 3,45	8,14	± 2,03

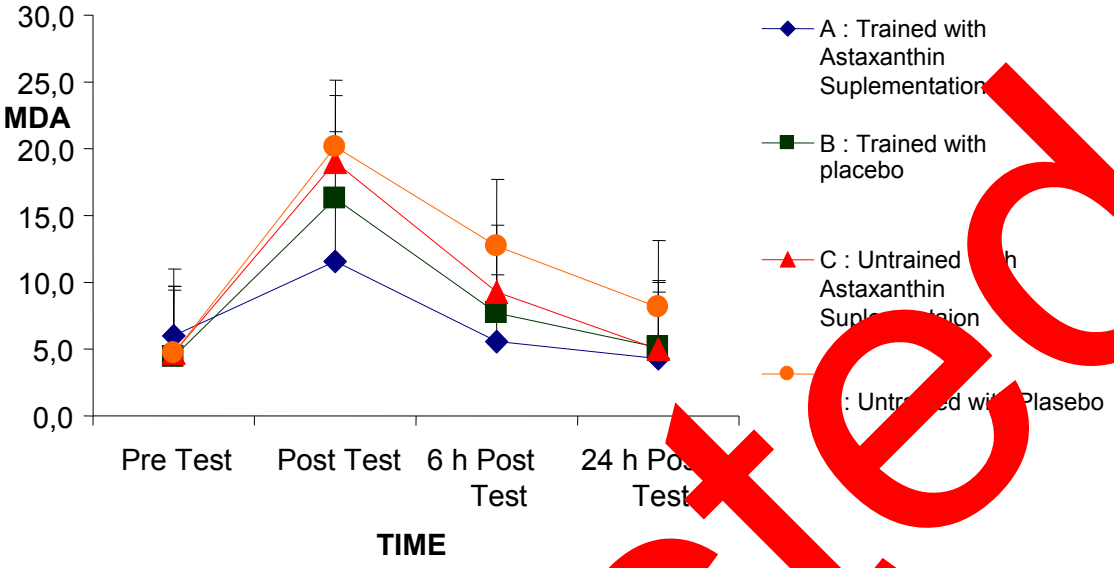

Figure 1. Dynamic Pattern of Oxidative Stress

The ANOVA test and Duncan test shows that MDA level is significantly different group A at immediate posttest (11,55±2,34 vs. 16,33±7,09 vs. 18,98±6,04 vs. 20,09±6,03)(p< 0,03), 6th hours posttest (5,58±3,32 vs. 7,73±3,06 vs. 9,31 ± 2,80 vs. 12,74 ±3,45)(p<0,002), 24th hours posttest (4,25±1,86 vs. 5,14±1,07vs 5,02± 1,04vs 8,14±2,03)(p<0,000). The findings show that Astaxanthin supplementation and training will protects body from ROS better than untrained person.

Table 3. ANOVA Test Result on MDA Measurement

Time Measurement	F test	p value
Pretest	0, 779	0.506
Post test	3,474	0.030*
6th hour post test	6,485	0,002**
24th hour post test	8,854	0,000**

Note : * significantly different (p< 0,05)
** significantly different (p< 0,01)

Table 4. Duncan Test Result on MDA Measurement

Groups	Duncan Test			
	x Pre test	*x* Post test	*x* 6th h post test	*x* 24th h post test
A	5.96 A	11,55 a	5,58 a	4,25 a
B	4.39 A	16,33 ab	7,73 ab	5,14 a
C	4,69 A	18,98 b	9,31 b	5,02 a
D	4,66 a	20,09 b	12,74 c	8,14 b

5. Discussion

From the physical characteristic of the subject (Table 1) illustrated that the heaviest load can be lifted by a trained group. This is possible because the group exercises often used to lift weights and body functions and muscle contractions have been accustomed to the heavy load.

From the above study showed that physical exercise may increase the levels of reactive ygen species in accordance with previous research by Guzel et al. Astaxanthin and exercise has a po e effect on the increase in MDA levels but did not affect dynamic pattern after strenuous physica After a strenuous physical activity, mean MDA in each group experienced a significant incr se, began to decline at 6 hours after the activity and return to the initial value approaching 24 hours a physical tests except in groups of untrained without Astaxanthin supplementation where the mean leve f MDA was increased by 2 times the initial value. After the tests of strenuous physical acti the m value of the lowest MDA is the group of trained with Astaxanthin supplementatio nd the hest group of untrained men without Astaxanthin supplementation. This is in releva with p ous esearch which states that the maximum levels of Astaxanthin are 8 hours a er supple ntati

The previous study was conducted on 32 healthy subjects we n a s dose of mg of Astaxanthin, Astaxanthin is known to reach maximum levels at 8 h after ad istrati and has a half-life of 15.9 hours, but in this study this pattern also occurred in the u ined group whom receiving placebo although the mean value of MDA remain higher 2 ti e nor value. At 24-hours after physical activity the mean MDA value return to initial MD mean value, it m because the body was adapt to ROS formation. Adaptation mechanism is portant in th overall antioxidant defense system, body will produces antioxidant enzyme to preven ody from R exogenous antioxidant or supplementation will enhance the enzyme function therefo e increas of ROS in the body could be suppressed.

In this study, subjects who received sup mentat with Astaxanthin has minimal increased of MDA level compared to subject without sup ement on. axanthin is one component of fat-soluble carotenoids and work on cell membran by r uci he act of singlet oxygen radicals and breaking the chain initiation, propagation and mination the fo ation of ROS and can boost the immune system and antioxidant enzyme is result por previous research that supplementation Astaxanthin is able to in ase he ac y of glutathione peroxidase, catalase, and the MnSOD in mice balb / c. In this study the de se of plas MDA levels in trained and untrained groups are significantly different ($p<0.05$). It is s pe the Papa statement (1999), factors that affect oxidative stress are diet, environment and training. R lar traini led to an increase in antioxidant endogen exercise. Other study in mice that high in sity print 30 second 6 repetition led to an increase MDA level compare to cont , howev after 12 w k sprint training shows decreasing the level MDA without any antioxidant sup ement n.

6. Conc sion

These f ings show that Astaxanthin supplementation and regular training may protects body from free radic untrained males. Astaxanthin improves oxidant status of trained and untrained males wi ut altering t ynamic pattern of oxidative stress under strenuous exercise.

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