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CLINICAL INVESTIGATION

Lipid Profile and Lipid Peroxidation Pattern Pre and Post Exercise in Coronary Artery Disease

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Abstract: Increased aerobic metabolism during exercise is a potential source of oxidative stress, which plays an important role in the development of atherosclerotic disease, while antioxidants delay or prevent various steps in atherosclerosis. The aim of this study was to identify the effect of oxidative stress induced by the TMT (Treadmill exercise Test) and the lipid peroxidation level as and lipid profile in blood, which may help in confirming the diagnosis of CAD (Coronary Artery Disease) in borderline cases of TMT results. This may help in future for early detection and possible prevention of atherosclerosis especially in people with a family history of CAD.

Fifty-two male and female cases were selected with ages ranging from 35-65years. The control group consisted of 30 subjects. Measuring the maximal exercise capacity was done by using a motor driven treadmill or bicycle ergometer in the upright position. 5ml of venous blood was withdrawn from each subject just before the exercise test and another 5ml. sample half an hour after finishing the test. The biochemical tests included: Total Cholesterol, Triglycerides, HDL (High Density Lipoproteins), VLDL (Very Low Density Lipoproteins), LDL-(Low Density Lipoproteins), cholesterols, and MDA (Malondialdehyde).

There was a drop in post-exercise LDL level as well as a decrease in the levels of TG (triglycerides), total-cholesterol, and VLDL. HDL was only slightly increased in the post-exercise state. MDA had higher levels in female than in male patients. All results were discussed thoroughly.

Restoring the antioxidant status of the body may have a preventive role in the formation of atherosclerosis and in the management of myocardial complications of ischemic heart disease.

Key Words: Lipid Profile, Lipid Peroxidation, Coronary Artery Disease, TMT.

Introduction

Oxidative stress plays an important role in the development of atherosclerotic disease, while antioxidants may delay or prevent various steps in atherosclerosis (1).

Oxidized low-density lipoproteins (LDL) particles are strongly linked to the pathophysiology of atherogenesis, and descriptive prospective cohort. Case control studies suggest that high dietary intake of antioxidant vitamins (A & C) and polyphenolic antioxidants (flavonoids) which are naturally present in vegetable, fruits, tea and wine are associated with a reduction in the incidence of coronary heart disease events (2,3).

Heavy physical exercise increases oxygen consumption and potentially initiates enhanced formation of reactive oxygen species (ROS) (4).This in turn leads to oxidative stress and cellular damage if not properly counteracted. The increase in malondialdehyde (an oxidative stress marker), released after intracoronary platelet aggregation might be a biochemical marker of coronary artery disease (5). Oxygen derived free radicals after temporary coronary occlusion causes myocardial stunning. Hypothetically, free radicals may either directly depress contraction or do so by increasing cytosolic calcium, for example by stimulating sodium hydrogen transport with subsequent sodium calcium transport

inhibition. Thus free radicals interact with calcium ions (6).

Exercise is a common physiological stress used to elicit cardiovascular abnormalities not present at rest and to determine the adequacy of cardiac function (7).

As exercise progresses, skeletal muscle blood flow is increased, oxygen extraction increases by as much as threefold, total calculated peripheral resistance decreases, but systolic and mean arterial and pulse pressure usually increase (8).

In the post-exercise phase, haemodynamic parameters return to baseline within minutes of termination. Vagal reactivation is an important mechanism after exercise and is accelerated in well-trained athletes but blunted in patients with chronic heart failure (9).

The aim of this study is to identify the effect of oxidative stress induced by the TMT (Treadmill exercise Test) on the lipid peroxidation level as well as lipid profile in blood, which may help in confirming the diagnosis of CAD (Coronary Artery Disease) in borderline cases of TMT results. People should be considered at high risk especially when they have high lipid peroxidation after an exercise test. This may help in future for early detection and possible prevention of atherosclerosis especially in people with a family history of CAD.

Materials and Methods

Selection of subjects

Fifty-two cases were selected (28 males and 24 females) with ages ranging from 35-65 years. All patients had coronary artery disease confirmed by coronary angiography. The control group consisted of 30 subjects (15 males and 15 females) who were selected from those attending cardiology consultation clinics and from volunteers. Their ages ranged between 20-50 years. All subjects were instructed not to take any cholesterol lowering drugs and stop taking β-blockers or calcium channel blocker 3 days before the exercise test. Blood sample were taken immediately before and half an hour after finishing the exercise test. The serum levels of triglycerides, cholesterol, HDL (high density lipoproteins, LDL (low-density lipoproteins), VLDL (very low- density lipoproteins), and MDA (malondialdehyde) were measured before and after exercise.

Exercise testing

Measuring the maximal exercise capacity was done using a motor driven treadmill device (Exercise testing system, Marquette 2000, Marquett Electronics, USA). Bruce (modified) Protocol was applied (8). The length of time a subject is able to continue exercise by any of the progressive continuous protocols correlates with the subjects' maximal oxygen consumption. Exercise endurance in males was about 11.5 ± 0.5 min and in females 7.6 ± 0.3 min.

Blood Samples

Five ml of venous blood were withdrawn from each subject just before the exercise test and another 5ml. sample half an hour after finishing the test. The samples were centrifuged for 15min at 3000 rpm in order to provide an appropriate amount of serum to use in measuring the biochemical tests which included: Total Cholesterol, Triglycerides, HDL, VLDL, LDL, and MDA.

Lipid profile

Serum total cholesterol (TC) and triglyceride (TG) were measured by enzymatic methods using kits from bioMérieux (France).

Serum high-density lipoprotein (HDL) was determined by precipitation with phosphotungstate-$MgCl_2$ solution followed by an enzymatic method, for determination of cholesterol in the supernatant using kits supplied by bioMérieux Company – France.

Serum Very Low Density Lipoproteins (VLDL) and Low Density Lipoproteins (LDL) were calculated by the Friedwald formula (10).

$$LDL - C = TC - [HDL - C + VLDL]$$

$$LDL - C = TC - [HDL - C + TG/5]$$

The formula is only valid at serum triglyceride concentration of less than 400mg/100ml.

Malondialdehyde (MDA) measurement

Lipid peroxidation was estimated according to the method described by Rehncrona et al.. Measurement of MDA (Malondialdehyde), a secondary product of lipid peroxidation, was based on the colorimetric reaction with thiobarbituric acid.

Results

The results shown in Table 1 will be discussed in regards to the changes between the pre- and post-exercise data to show whether they are significant or not.

Table 1 shows the following:

- The mean malondialdehyde level value showed a significant rise in the post-exercise samples of both groups ($P < 0.05$).
- A significant drop in the level of LDL in the post-exercise levels of the control group and in the post-exercise levels of the patient group was found.
- A significant rise in the high density lipoprotein level of post exercise was found in the patient group.
- On the other hand a significant drop in the level of very low density lipoprotein was found to exist in post-exercise control and patient groups both males and females.
- A significant decrease in the level of triglycerides in the post-exercise samples of the control group and in the post-exercise level of the patient group was noticed.
- In the post-exercise control group and in the post-exercise patient group a significant decrease in the level of total cholesterol can be seen.

There was a significant correlation between low density lipoproteins and malondialdehyde only in the post-exercise male control group Figure 1 and only in the pre-exercise female patients' Figure 2.

Significant correlation was found to exist between high density lipoprotein and malondialdehyde only in the pre-exercise male control group as seen in Figure 3.

Significant correlation between very low density lipoprotein and malondialdehyde exist in the post-exercise male control group, seen in Figure 4.

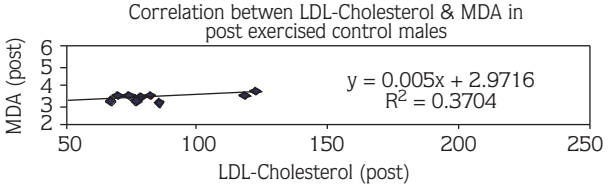

Figure 1. Correlation between LDL and MDA in post-exercised males in control group.

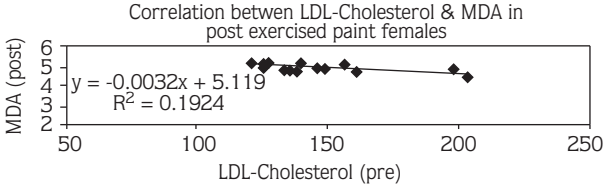

Figure 2. Correlation between LDL and MDA in pre-exercised females in patient group.

Discussion

Myocardial ischemia is usually considered to result mainly from atherosclerosis and thromboembolism or surgical interventions (coronary bypass surgery or from cardiac transplantation) and PTCA (Percutaneous transluminal coronary angioplasty) causing a reduction in regional blood flow and myocardial oxygen deprivation

Table 1. Mean Values of parameters studied for males and females in control and patient groups pre- and post exercise

Parameter	Patients mean ± SD				Controls mean ± SD			
	Males n = 28		Females n = 24		Males n = 15		Females n = 15	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
MDA mmol/L	3.968 ± 0.2607	4.16 ± 0.3407	4.661 ± 0.1635	5.663 ± 0.2254	3.102 ± 0.2708	3.382 ± 0.197	2.870 ± 0.195	3.170 ± 0.254
Triglycerides mg/dl	163.95 ± 5.915	139.95 ± 9.358	138.5 ± 8.12	118.1 ± 10.315	104.7 ± 24.935	85.3 ± 12.454	80.4 ± 18.845	66 ± 18.36
Cholesterol mg/dl	207.05 ± 13.646	184.41 ± 16.746	209.65 ± 19.104	186.65 ± 22.169	197.5 ± 17.614	156.9 ± 18.615	179.9 ± 15.559	156.8 ± 16.396
HDL mg/dl	40.818 ± 5.687	43.591 ± 5.7126	39.5 ± 5.472	42.35 ± 5.3927	52.8 ± 8.134	58.1 ± 8.45	44.5 ± 7.306	49 ± 6.912
LDL mg/dl	199 ± 13.49	112.9 ± 18.543	142.45 ± 22.262	120.681 ± 25.036	105.76 ± 19.907	81.74 ± 23.871	119.32 ± 15.863	94.6 ± 16.503
VLDL mg/dl	32.8 ± 1.18	28 ± 1.87	27.7 ± 1.62	23.6 ± 2.06	21 ± 4.98	17 ± 2.49	16.1 ± 3.76	13.2 ± 3.67

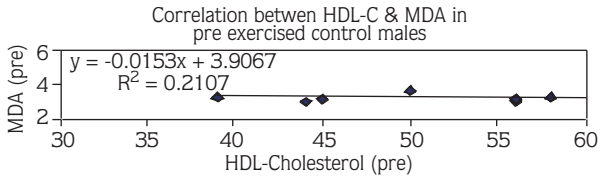

Figure 3. Correlation between HDL and MDA in pre-exercised male in control group.

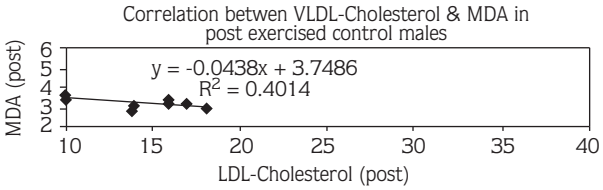

Figure 4. Correlation between VLDL and MDA in post-exercised males in control group.

(11). If the occlusion is total or multivessels are involved, ischemia may then occur at rest (12). If the narrowing inside the coronary artery lumen becomes critical (> 60% of the lumen), as assessed by coronary angiography, then symptoms of oxygen deprivation will occur at times of stress or exercise (increased oxidative stress) (13,14).

It has been shown that 2%-5% of the oxygen used for aerobic metabolism in the mitochondrial respiratory chain is converted to oxygen free radicals under physiological conditions (15). Exercise elevates oxygen consumption above resting levels, so it is likely that the generation of oxygen free radicals is elevated also leading to increased lipid peroxidation. Oxygen free radicals generated in vivo react with polyunsaturated fatty acids and form new radicals (peroxyradicals) which initiates a chain reaction of lipid peroxidation in the presence of oxygen, so malondialdehyde level is an indicator of free radical generation in the human body. Plasma contains sufficient polyunsaturated fatty acids and is thought to reflect the systemic status of lipid peroxidation in humans.

Oxygen free radicals are highly reactive species that can cause a wide spectrum of cell damage including lipid peroxidation, enzyme inactivation, and DNA damage. Oxidative stress is a major factor in the production of reactive oxygen species and lipid peroxidation, which influences the pathogenesis and prognosis of atherosclerosis.

The mean malondialdehyde level value showed a significant rise in the post-exercise samples of both groups ($P < 0.05$), Table 1.

This rise after exercise in the female patients (19%) is more than the rise in the male patients (16%), which could be due to a wider deprivation of antioxidants in the female group of patients. This elevation of malondialdehyde was consistent with that reported by McMurray et al. (1992) (16). It probably reflects an increased lipid peroxidation initiated by free radicals reaction with biological membranes. A large body of evidence suggests that hyperlipidemia is a known risk factor in the development of premature atherosclerosis and ischemic heart disease (1). The rapid production of oxygen free radicals by ischemic myocardium triggers a later secondary effect such as depletion of protective antioxidants and enzymes, with higher lipid hyperoxide level, tends which to inactivate prostacycline synthase, enhancing the formation of arachidonic acid by prostaglandins and thromboxane A2-synthase which is associated with enhanced platelet aggregation and migration of peroxide forming leukocytes to the region causing more injury to the vessel and myocytes. Post exercise and ECG resolution concluded that the prolonged post ischemia dysfunction was subsequent to the increased free radicals activity during ischemia and oxidative stress. Mangaro in 1993 (17) stressed the role of inducibility of antioxidant defense in the ischemic preconditioning process. From the previous speculation and the present observation it might be postulated that the high malondialdehyde level in ischemic heart disease cases is attributed to circulating fractions of membrane lipids peroxidation products in addition to serum lipids. Decreased serum total antioxidant status is associated with increased malondialdehyde levels in coronary atherosclerosis (18).

A significant drop in the level of LDL in the post-exercise levels of the control group and in the post-exercise levels of the patient group can be seen in the Table (Table 1).

As seen in the Table, the percentage decrease is greater in male patient group than females (-76%). Low density lipoproteins are formed from very low density lipoproteins in the liver and distributed to the blood stream. By liposis, triglycerides are removed and low density lipoproteins are formed which contain high amounts of cholesterol. These molecules are small

enough to pass through the vascular endothelium, bind to specific low density lipoprotein receptors on cell membranes, and enter the cells by active intake (19). The cholesterol within the cell is needed for membrane growth and repair; in the liver they form bile acids. So low density lipoproteins are a major vehicle in distributing cholesterol from the liver to other tissues.

A significant rise in the high density lipoprotein level in the post exercise period was found in the patient group (Table 1)

High density lipoprotein particles are large and rich in cholesterol, and has the reverse action of low density lipoprotein by transporting cholesterol from the periphery to the liver. High density lipoproteins are formed in the liver by an enzyme system in the Golgi apparatus and are rich in unesterified cholesterol but in the plasma they become more enriched with cholesterol (19,20).

On the other hand a significant drop in the level of very low density lipoprotein was found to exist in post-exercise control and both male and female, patient groups Table 1.

A significant decrease in the level of triglycerides in the post-exercise samples of the control group and in the post-exercise level of the patient group can be seen in Table 1.

Resistance training and moderate aerobic exercise increase serum testosterone levels of untrained subjects after 15-20 min. Testosterone increases the formation of high density lipoprotein, in males and in females, which increases the metabolism of triglycerides (20). The utilization of triglycerides by myocardial cells for energy production accounts for the decrease in the level of triglycerides in the post-exercise blood samples.

In the post-exercise control group and in the post-exercise patient group a significant decrease in the level of total cholesterol can be seen in Table 1.

All circulating cholesterol in the blood is contained in lipoproteins. Control of body level of cholesterol depends on the rate of excretion in the bile as cholesterol or bile salts in relation to the rate of synthesis in the liver from Acetyl-CoA, which is regulated by feed back inhibition on HMGCoA reductase (hydroxyl-methyl-glutary-CoA reductase) by excess cholesterol. High diet cholesterol causes decreased synthesis and the excess is excreted in bile. The excreted bile alts are more efficiently reabsorbed than dietary cholesterol.

Conclusion

Oxidative stress generates free radicals and oxidants that play a role in increasing lipid peroxidation, as confirmed by the high levels of MDA, of serum lipids and fractions of membrane lipids. The higher level of MDA in female patients than males may indicate a state of wider deprivation of antioxidants in females developing coronary artery disease and may be related to the hormonal imbalance in females at the age of developing ischemia.

The drop in LDL level post exercise, due to high lipid peroxidation during stress, is very much pronounced in the male patient group, which may be an effect of sex hormones and higher levels of hemoglobin in blood. The decrease in levels of triglycerides, total cholesterol and VLDL indicates a state of high lipid utilization for energy production during exercise by myocardial cells and skeletal muscles. HDL is only slightly increased at post-exercise, which may result from increasing testosterone level during exercise.

The combination of treadmill exercise testing and estimation of lipid peroxidation can assist in conformation of borderline treadmill result cases and indicate the prognostic risk for such cases and may stress the need for antioxidants supplementation in healthy human beings having a low antioxidant status.

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References

1. Liebson PR. and Amsterdam EA. Coronary Heart Disease, part 1 Primary prevention. Dis Mon 45: 497-571, 1999.

2. Kushi LH., Folsom AR., Prineas RJ., *et al.* Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. *N Engl J Med* 334:1156, 1996.

3. Yusuf S, Dagenais G, Pogue J *et al.* Vitamin E supplementation and cardiovascular events in high risk patients. *N Engl J Med* 342:154-160, 2000.

4. Leeuwenburgh C., and Heinecke J. W. Oxidative stress and antioxidants in exercise. *Cur Medc Chem* 8: 829-838, 2001.

5. Rabago-Velasco M, Cortez-Valero H, Anguilar-Parado E *et al.* Plasma MDA in patients with Type 2 diabetes and in patients with CAD. *Gac Med Mex* 136(1): 23-30, 2000.

6. Bolli R. and Marban E. Molecular and Cellular Mechanisms of Myocardial Stunning. *J Physiol Rev* 79: 609-634, 1999.

7. Frolicher VF. and Myers J. Exercise and the Heart, 3rd edition, Philadelphia, WBSaunders, 1999.

8. McArdle WD, Kath FI, Katch VL. Essentials of exercise physiology, 2nd edition, Lippincott Williams & Williams, 2000.

9. Imai K, Sato H, Hori M *et al.* Recovery after exercise is accelerated in athletes but blunted in patients with chronic heart failure. *J Am Coll Cardiol* 24: 1529, 1994.

10. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of LDL-Chol. In plasma without use of the preparative ultra centrifugation. *Clin Chem* 18: 499-509, 1972.

11. Rymik TM *et al.* Independent Prognostic significance of ischemic ST segment response limited to recovery from treadmill exercise in asymptomatic subjects. *Circulation* 97: 2117, 1998

12. Pederson BK *et al.* The cytokines response to strenuous exercise. *Can J Physiol Pharm* 76: 505-511, 1998.

13. Granot E, Kohen R. Oxidative stress in childhood in health and disease states. *Clin Nutr* 23: 3-11, 2004.

14. Singh BN. Detection, quantification and clinical significance of silent myocardial ischemia in coronary artery disease. *Am J Cardiol* 58, 1B, 1986.

15. Yasuhiro N *et al.* Oxidative stress is related to exercise in patients with heart failure. *Am Heart J* 135: 115-120, 1998.

16. McMurray *et al.* Evidence for oxidative stress in unstable angina. *Br Heart J* 68: 154-157, 1992.

17. Mangaro DT. Myocardial stunning. *J Card Sur* 8: 204-213, 1993.

18. Arlt S, Kontush A, Muller-Thomsen T, Beisiegel U. Lipid peroxidation as a common pathomechanism in coronary heart disease and Alzheimer disease. *Z Gerontol Geriatr*. 34: 461-5, 2001.

19. Campbell PN. and Smith AD. Biochemistry Illustrated. 4th edition, Churchill Livingstone, 2000.

20. Guyton AC. Textbook of Medical Physiology. 9th edition, Philadelphia, W.B.Saunders, 1995.