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Response of Ischemia- Modified Albumin (IMA) Plasma to the Time of Intensive Exercise (Morning and Evening) in Male Patients with Cardiovascular Disease

Poorya Pasavand [1], Mohsen Salesi [2], Amin Mehrabi [3], Maryam Amirazodi [4], Maryam Koushki [2]

1. Department of Sport Physiology, Larestan Branch, Islamic Azad University,Larestan, Iran
2. Department of Exercise Physiology, Shiraz University, Shiraz, Iran
3. Department of Exercise Biochemistry and Exercise Metabolism, Kish International Campus, University of Tehran, Kish, Iran
4. Department of Exercise Physiology, Shiraz University International Division, Shiraz University, Shiraz, Iran

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***Corresponding author:**
Amin Mehrabi. Department of Exercise Biochemistry and Exercise Metabolism, Kish International Campus, University of Tehran, Kish, Iran

Phone: +989128899502

Fax: +982188964130

Email: mehrabi64@ut.ac.ir

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Abstract

Introduction: Heart markers such as albumin are used for the fast and assured diagnosis of heart problems. However, the amount of this factor in response to exercise and in different times of the day is not determined. Therefore, the present study aimed to investigate the effect of training time (morning and evening) on ischemia-modified albumin (IMA) plasma levels in male patients with cardiovascular disease.

Methods: 15 male patients with cardiovascular disease were chosen to participate in this quasi-experimental study. Before the outset of exercise protocol under specialist supervision, pre-test blood samples were taken from subjects at 7:00 in the morning. After taking blood samples, all participants took part in an exercise test until exhaustion. This test was designed based on Bruce protocol including 7 stages and each of these 3 minute stages followed predetermined speed and grade. One week later, subjects went through the same process at 7:00 in the evening. Paired sample t- test was used to analyze and compare pre-test and post-test means of each training period (morning and evening) and compare the mean of both times in the morning and in the evening. SPSS.20 was used to analyze study data.

Results: there is no significant difference between pre-test means during morning and evening exercises (P=0.292). In addition to this, no significant relation was observed between post-test mean and three-hour-after test in the morning and evening, (P=0.185) and (P=0.250), respectively.

Conclusion: Based on this study it could be said that circadian rhythm and circadian cycle have no severe effect on chemical responds and cardiac risk factors. Thus, cardiovascular patients can engage in their desired exercise during morning and evening.

Keywords: Training Time, Ischemia- Modified Albumin, Exercise, Cardiovascular Disease

Introduction

Cardiovascular diseases (CVD) are major causes of fatality in developed countries. According to the World Health Organization, the responsibility of 17.1 million deaths around the world falls on CVD (1). The most suitable way to oppose cardiovascular issues is to identify and modify all the major risk factors of the disease. In recent decades, research has specifically put more emphasis on lipids and their related compounds in healthy and sick plasma and, thus, more risk factors were highlighted (2-4). So far, many have tried to identify the best predictive markers for cardiovascular diseases. However, in some cases, patients with normal traditional risk factors (especially blood lipoproteins) were seen to suffer from cardiovascular issues (5).

Nowadays, new markers such as cardiac troponin I (cTnI), cardiac troponin T (cTnT) and creatine kinase MB isoenzyme (CK-MB) are used for the evaluation of myocardial cell damage (6). This said, researchers seek more accurate and sensitive markers to predict the risk of cardiovascular diseases. Among newly presented markers related to the fast identification of cardiovascular issues we could point to pregnancy- associated plasma protein *A* (*PAPP-A*), myeloperoxidase, glycogen phosphorylaze-BB and ischemia modified albumin (7-9). IMA is an acute ischemia marker validated by the U.S. Food and Drug Administration (FDA). Terminal amines (N-terminal) of human serum Albumin (aa585, 5kDa/66, HAS) includes four binding sites for connecting with metal ions such as cobalt, copper and nickel which is in form of strong binding with three amino acid residues in the N-terminal region (Asp-Ala-His) as well as binding to one lysine residue (Lys4). Reduction in oxygen supply results in local acidosis and production of free radicals. In this case, copper and zinc ions, which are normally bound to plasma proteins, disconnect from their binding site to protein and enter blood circulation as free ions and, in turn, N-terminal a albumin forms new bindings with these metals. This modified form of albumin is known as IMA. Research shows that 30 minutes after ischemia, copper and iron concentrations are, respectively, 50 and 15 times higher than their pre-ischemic concentration and, thus, can be considered as criteria for a quick diagnosis of medical issues and application of necessary therapeutic interventions (7). Many recent studies have presented evidence on the predictive effects of IMA and show that an increase in IMA is not specific to cardiovascular patients; compared to healthy individuals, the level of this important factor after brain trauma and pulmonary embolism can also increase significantly in patients (10- 16). Results show that exercise can also be an effective factor on reaching different levels of this myocardial damage marker. A significant of related studies have investigated the changes in Albumin levels caused by ischemia which show an increase or no change in levels of this marker after exercise and also its return to normal levels in different individuals (17- 19). On the other hand, another factor which has attracted much less attention is the time of exercise. Various reports show that most cardiovascular attacks happen during the day and at specific times, especially early in the morning (20- 22). Therefore, it is possible to say that the performance of body during or after exercise in different times of the day is not similar. Researchers believe human body performance is highest during evening and close to night hours since the core body temperature and metabolism rate are close to their highest levels (23). Thus, it gives rise to the question that ِduring what hours of the day after exercise, levels of risk factors related to cardiovascular risk factors are higher?. During the process of assessing relevant literature, it was observed that many studies have focused on the effects of the time of exercise on different physiological and cardiovascular risk factors such as platelets (20), lipoproteins, immunoglobulins, cortisol (24, 25), heart rate (26), myoglobin and creatine kinase (27) and troponin T (28); however, among the present studies, there is a lack of evidence on the effects of the time of exercise on Ischemia Modified Albumin (IMA). Based on the results of the existing literature it is obvious that the relationship between exercise and myocardial damage markers such as ischemia modified ischemia has significant importance; and considering the lack of any report on effects of exercise in different hours of the day on ischemia modified albumin in human body, the importance of conducting this study is strictly evident. Therefore, on the one hand, to find the answer for ِwhether concentration levels of cardiovascular risk factors are affected by morning and evening exercise coursed?" and, on the other hand, by considering the importance of preventing cardiovascular diseases and effect of exercise on public health, we sought to investigate the

effects of intensive exhausting physical practice on ischemia modified albumin (IMA), one of the cardiovascular risk factors, in male patients suffering from cardiovascular diseases during morning and evening. For this purpose, we aimed to clarify the role of day/night cycle on one of the predictive risk factors of cardiovascular diseases in order to identify the most optimal time for training.

Methods

This research is a semi-experimental one. Study sample included cardiovascular patients of Emam Reza hospital in Shiraz. Among this sample, 15 male patients suffering from cardiovascular disease and visiting a cardiologist for their normal checkup were chosen for the purpose of this study. All participants were aged from 36 to 57. Also, they were non-athletic, non-smoker and interested to take part in the study. Therefore, since all participants were willing and had the specified criteria to be considered in the study, the sampling method was voluntary. At first, during an explanatory session, the participants were clearly informed about the purpose and objective of the study, our exercise protocol and how the tests were designed and performed. Then, their anthropometric measurements were taken. Personal information and consent forms were also signed. The participants were informed that in case of disinterest to continue their participation in the study, they can be excluded whenever they desired. Demographic characteristics of the participants are shown in Table 1. On the day of the experiment, blood samples were taken from participants at 7:00 in the morning and, then, the exercise protocol was applied with the physician present. After taking blood samples, all participants took part in an exercise test until exhaustion. This test was designed based on Bruce protocol including 7 stages and each of these 3 minute stages followed predetermined speed and grade. Blood samples were taken immediately after exercise and three hours after exercise. Plasma was immediately separated from blood samples in the laboratory using a Centrifuge machine with 2000 to 3000 rpm and samples were kept in 30 degrees celsius until further testing. After one week, participants were asked to repeat the same protocol at 7:00 in the evening. Levels of ischemia modified albumin were measured using its specific ELISA kit and, also, albumin cobalt binding test provided by Eastbioharm corporation. Descriptive and inferential statistics were used for the statistical analysis of the data. Descriptive statistics were used to calculate the mean, standard deviation and, also graphs. In addition, dependent sample t-test was used for inferential analysis of the data and means of the pre- and post-tests for each time (morning and evening), also the pre- and post-tests in morning and evening were compared.

Results

Results of the present study in Table 2 show that there is no relation between IMA levels in the pre-test and immediately post-test in the morning (P=0.228) and, also, there is no relation between IMA levels in the pre-test and three hours after the test in the morning (P=0.391) . Moreover, there is no significant relation between IMA levels in the pre-test and post-test (immediately) in the evening (P=0.190) and, also, there is no relation between IMA levels in the pre-test and three hours post-test in the evening (P=389). In addition to this, results point to lack of any significant relation between the obtained means for pre-test (P=0.292), post-test (P=0.185) and 3 hours after (P=0.250) in both morning and evening (Fig.1). IMA mean values in different times are included in Table 2. The results further show that there is only a significant difference between participantsب VO_{2max} levels and maximum heart rate in the morning and evening (P<0.001) which points to an increase in VO_{2max} levels and maximum heart rate during evening (Table 3).

Table 1. Demographic characteristics of the participants

Parameters	Number	Mean	Standard deviation	Min.	Max.
Age	15	52.2	6.22	36	57
Height (cm)	15	175.6	8.11	170	183
Weight (kg)	15	76.8	12.48	60	95
Body Mass Index (Kg/m^2)	15	26.3	4.36	19.8	29.3
Waist circumference (cm)	15	97	5.41	103.6	117
Hip circumference (cm)	15	100	3.83	104.1	115

Table 2. Descriptive findings concerning ischemia modified Albumin (IMA) in the morning and evening

Variable	Testing time	Number	Min	Max	Mean	Standard deviation
IMA (Morning)	Pre-test	15	27.80	96.60	54.12	23.99
	Post-test (immediately)		31.80	99.10	57.50	25.52
	Post-test (3 hours later)		32.10	95.10	55.35	23.62
IMA (Evening)	Pre-test	15	28.60	96.80	45.70	21.18
	Post-test (immediately)		30.20	96.60	47.25	19.73
	Post-test (3 hours later)		30.50	96.00	46.62	19.58

Table 3. Descriptive findings concerning the practice test in the morning and evening

	Variable	Number	Minimum	Maximum	Mean	Standard deviation
Morning	VO_{2max} ($_{ml/kg/min}$)	15	22.30	39.50	31.6	6.36
	MAXMETS($_{ml/kg/min}$)	15	6.70	32.10	11.7	6.04
	$MAXDBT_{(Mm\ Hg)}$	15	60	90	78.6	8.33
	$MAXSBT_{(Mm\ Hg)}$	15	120	159	143.6	10.20
	MAXHR	15	120	170	151.2	18.34
Evening	VO_{2max} ($_{ml/kg/min}$)	15	34.20	74.30	39.6	5.20
	MAXMETS($_{ml/kg/min}$)	15	10.30	13.90	12.8	1.13
	$MAXDBT_{(Mm\ Hg)}$	15	60	90	79.3	8.83
	$MAXSBT_{(Mm\ Hg)}$	15	130	158	143.67	9.82
	MAXHR	15	120	190	167.8	21.06

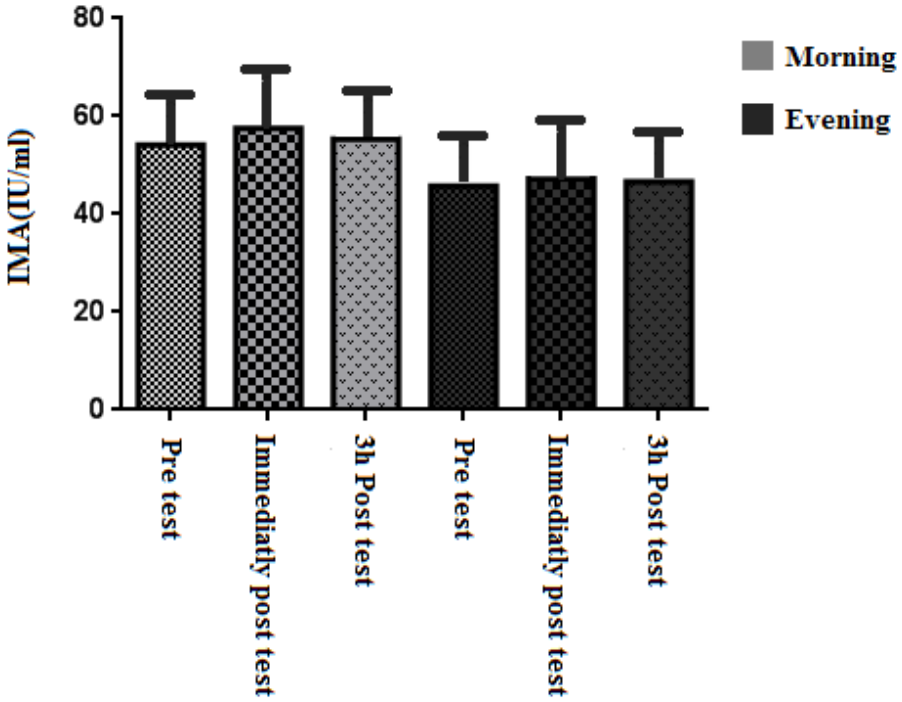

Fig 1. IMA mean in the morning and evening

Discussion

Based on the results of this study, there is no relation between IMA levels in the pre-test and immediately after the test in the morning. In addition, no significant difference was observed between IMA levels in the pre-test and three-hour post-test. Moreover, IMA levels in evening pre-test and immediately post-test at the same time showed no significant change. Also, comparison of evening pretest IMA levels with the results of 3-hour post-test in the evening pointed to the same results. IMA plasma levels in the morning pre-test were higher than the evening; however, these changes had no significant statistical importance. Based on the gathered data, there is no significant difference between the pretest and three-hour post-test means in both morning and evening. IMA is a biochemical marker for the quick diagnosis of acute coronary syndrome (ACS). Combined with cardiac marker panel, IMA can provide the opportunity to quickly identify ACS. Note that an increase in the blood circulation IMA concentration is not specific to myocardial ischemia, but it can be produced through all ischemic processes in the body (29). Considering the kinetics of IMA, studies on patients undergoing angioplasty, in whom ischemia is under control, show the process of IMA production. These studies show that there is a sudden increase in IMA levels after ischemia while after 6 hours the levels decline and reach normal levels within 24 hours. IMA elevation occurs earlier than an increase in levels of cardiac troponin and Natriuretic Peptide, and even earlier at just the onset of plaque rupture (30, 31). Since blood IMA level increases just minutes after the onset of ischemia and returns to normal within 6-12 hours, it has significant importance in the diagnosis of acute ischemia before necrosis. Sports related studies on the effect of exercise on IMA levels show that exercise can affect this factor. Prior research on this topic present contradictory results which were influenced by many factors. Clark *et al.* (2010) study on 20 male wrestlers with an average age of 28 showed that one wrestling training session results in increased levels of IMA in

professional wrestlers compared to their IMA levels before exercise (32). In another similar study on 12 healthy male patients, IMA changes after an ischemia test were evaluated and results pointed to a significant increase in IMA after ischemia; however, after 30 minutes, the IMA levels returned to their normal value. The researchers concluded that IMA has a meaningful relation with albumin, while there is no relation between IMA and lactic acid (19). Despite the fact that participants of these studies were both healthy and athletic, the changes in IMA showed significant increase in its levels. In other words, considering the intense activity and training of professional wrestlers, levels of this myocardial damage marker increased significantly. Also, ischemia in leg muscles of healthy individuals caused an increase in IMA. It is possible to say that the sensitive nature of this marker results in increased levels of IMA after intense activity (professional training of wrestlers) or mild activity (leg muscles ischemia) since even in our study we observed the insignificant increase of this important and sensitive marker after intense training (sports test) in cardiovascular patients. Therefore, we conclude that exercise intensity and in general, mild or intense exercise with long or short periods cannot influence this marker in blood since, as reported in prior studies, healthy individuals (in Falkensammer *et al.* study, 2007) and professional athletes (in Clarck *et al.* study, 2010) and cardiovascular patients (the present study), levels of this marker have increased in blood. However, this increase in level was insignificant in our study. On the other hand, Apple *et al.* (2002) investigated the effects of a marathon race on ischemia modified albumin in 19 marathon runners. They took blood samples before, immediately after and 30 minutes, 24 hours and 48 hours after the activity. Results showed that there was a decrease in IMA levels immediately after race, but 24-48 hours later the levels increased. They concluded that abnormal amount of IMA is directly related to skeletal muscle ischemia (17). Moreover, the study by Sbarouni *et al.* (2006) on 40 patients with coronary artery disease showed that exercise on a treadmill results in decreased levels of IMA at the peak of exercise and, after one hour, IMA levels return to normal in both positive and negative tests. They concluded that IMA changes do not reflect myocardial ischemia (18). On the same topic, we could also mention another study by Roy *et al.* (2004). The study was conducted on 23 PVD patients. IMA levels were evaluated in three time periods, before, immediately after and one hour after exercise test. Results pointed to the decreased levels of this factor immediately after exercise while IMA levels increased to their normal value one hour after the test (34). Since participants in both Sbarouni *et al.* (2006) and Roy *et al.* (2004) are similar to our participants (cardiovascular patients) and all three studies used the same exercise protocol, the dissimilarity in results can be due to the number of participants, the average time of the test and the level of the induced ischemia. Time is a factor in determining practice size; therefore, it seems that the average time of practice on treadmill had significant effects on inducing ischemia in both myocardial tissue and other actively involved tissues. Considering this possibility, it could be said that time of exercise has caused the distinction in results. Another factor is the similar sampling time in these studies (immediately after and one hour after in Sbarouni et.al. study; before, immediately after and one hour after in Roy et.al study; and before, immediately after and three hours after in the present study). In addition to this, Van der Zee *et al.* (2005) divided 38 patients experiencing chest pains and susceptible to coronary artery disease into two groups with ischemia and without myocardial ischemia. Participants of this study underwent training on cycle ergometer with 30w intensity and, after every 2 minutes, 25w was added to the intensity. Training would stop the moment participants experienced chest pain during exercise or when their heart rate reached 85% of their maximum heart rate based on their age. The

researchers did not find any significant difference between the IMA levels of two groups; however, at the peak of their exercise, IMA levels of both groups were lower than their base values. Moreover, at different times, IMA levels of both groups after training were almost similar (34). Results of this study showed that there is no difference between IMA levels of healthy individuals and patients suffering from ischemia caused by cardiovascular diseases which points to the possibility that IMA does not necessarily secrete because of myocardial disorders. On the other hand, although exercise may result in mild ischemia, the levels of IMA were expected to rise significantly, however, as these studies indicate, sports induced ischemia (although mild) does not result in an increase in this factor. Nevertheless, our study shows that exercise to the point of exhaustion (sports test) caused an insignificant increase in this factor. Despite this, the results of these two studies (the present study and Van der Zee *et al.)* show that even after all the decreasing and increasing changes, IMA levels return to their normal rate. Bakula *et al.* (2016) also divided their participants into two groups, the ischemia group with 43 cardiovascular patients (experiment group) and control group with 22 healthy individuals. During a 6 hour practice test, IMA levels were measured after 5, 30 and 60 minutes and through the next 5 hours, these levels were measured every 60 minutes. IMA level in ischemia group up to 3 and 4 hours after practice test showed significant increase; however, in the control group, IMA level 5 minutes after the test had a significant decrease. This value was stable until 30 minutes after test, but after one hour IMA level returned to its base value (35). Similar to our study, the results of this study showed that IMA levels in cardiovascular patients increase after a practice test; however, this increase in IMA level was not significant in the present study. Note that, in healthy individuals, IMA levels returned to normal after 5 minutes. It could be said that IMA response in healthy individuals is much faster. Since IMA is a biochemical factor, the biochemical response of healthy individuals may be quicker and better than patients. Considering the time of practice, the results of our study show that whether cardiovascular patients exercise in the morning or in the evening, it makes no difference on their biochemical cardiac markers, such as IMA and they can freely choose any time of the day for exercise. It should be noted that the level of participantsب stress before and during practice, their nutritional conditions and their daily activities were among the limitations of our study. In addition, the half-life of this protein in the presence of ischemia should also be taken into account. As was mentioned before, IMA is not specific to cardiovascular patients and cardiac ischemia and, thus, it may be observed in different tissues as a results of ischemia (10-16). Therefore, more studies should focus on the response of this protein to exercise in patients suffering from underlying diseases. Finally, considering the results of this study, it could be said that biochemical responses to exercise depend on intensity, duration, type of participants and their conditions. However, there is a possibility that the day-night cycles do not have a significant effect on biochemical responses of myocardial tissues to intense training and cardiovascular patients can exercise in the morning or the evening as desired.

Conclusion

It seems that Based on this study it could be said that circadian rhythm and circadian cycle have no severe effect on chemical responds and cardiac risk factors. Thus, cardiovascular patients can engage in their desired exercise during morning and evening.

Ethical issues

Not applicable.

Authors' contribution

All authors equally contributed to the writing and revision of this paper.

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