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Effect of Training on High Sensitive C-Reactive Protein and Blood Lipids Responses in Rats

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Abstract: This study was designed to investigate the effect of continuous and intermittent trainings on high sensitive C-reactive protein (hs-CRP) and blood lipids(LDL-C and HDL-C). The menopause, obese, wistar$_{14848}$ rats, (325± 4.93 g, 21 months old, at least three months after menopause), were randomly divided into the control, continuous or intermittent training groups. Training program was carried out for five days a week for 6 and/or 12 weeks. Blood samples were drawn at three phases of baseline, 6 wk and 12 wk for evaluate (hs-CRP) and LDL-C and HDL-C. Results showed that hs-CRP was insignificantly reduced in both training groups after 6 weeks (P= 0.08 and 0.351), but its reduction was significant after 12 weeks in both groups (P= 0.003 and P< 0.001). However, there were no significant different between continuous and intermittent training groups after 6 and 12 weeks. Decrease in LDL-C and increase in HDL-C following continuous and intermittent training represents an important intervention to reduce hs-CRP and therefore, can be result in the anti-inflammatory and cardio-protective effects. Also, both types of training can be useful in lowering high sensitive C-reactive protein and blood lipids.

Key words: Training · HS-CRP · Blood lipids · Rats

INTRODUCTION

Senility is a great problem in industrial countries. One problem due to senility is atherosclerosis and it has been predicted to be the major disease by 2020 [1], The past decade has been characterized by growing interest in the idea that atherosclerosis is an inflammatory disease and systemic inflammation has a main role in developing atherosclerosis [2-7]. There is growing evidence that the development of the atherosclerotic plaque is associated with inflammation [5,8]. Thus, researchers have paid considerable attention to the inflammatory markers to prediction of the cardiovascular diseases. There are many inflammatory markers but high sensitive C-reactive protein (hs-CRP) is the most sensitive inflammatory marker for prediction of cardiovascular risk [6,9-11]. Therefore, according to strong relationship between this inflammatory marker and incidence of cardiovascular diseases, any treatment reducing this marker can decrease the risk of cardiovascular diseases. Numerous factors can affect this marker. Many researchers have shown that amount of hs-CRP in elderly [5,8,12-14], females [1,12,15] and obesity [2,4,5,7,16,17], is higher than youth, male and active persons. Growing evidence suggests that over 80% of cardiovascular diseases have non congenital etiology and are due to life style specially lack of activity [3]. Researchers reported amount of regular physical activity is inversely related to C-reactive protein (CRP) in a healthy elderly population [2,9,10], Stewart *et al.* [14], examined the influence of a 12-wk exercise training program on CRP concentrations in the healthy young and old humans. Results showed serum CRP can decrease with training in both old and young subjects. Similarly, Kuo *et al*. [9], reported higher circulating levels of CRP are independently associated with lower Vo_2max in men without coronary heart disease (CHD). However, Davis *et al.* [18] and Rawson *et al*. [13] could not report any relationship between exercise training and hs-CRP values. Moreover, results of some researches indicate that the amount of hs-CRP can be increased after a single long-term activity, such as marathon [15], or high-intensity anaerobic training [11]. Due to hormonal changes female

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are at the risk of cardiovascular diseases after menopause [12,18,19], Investigations have shown that hs-CRP can be changed significantly after menopause [12]. Although studies provide evidence that regular exercise may reduce CRP levels [9,14], in our knowledge, there is not any research on the effect of both continuous and intermittent trainings in a controlled condition in animal subjects. Therefore, the purpose of this study was to investigate the comparison of the effects of 6 and 12 weeks continuous and intermittent trainings on the hs-CRP and blood lipid include; low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C). Thus an essential question is: can the continuous training get useful effects of intermittent training (exercises periods with rest intervals)? On the other word, intermittent training can be a replacement for continuous training in community? given the positive relationship observed between accumulation of lipids within the artery wall and atherogenesis process [4,8] and on the other hand, since C-reactive protein (CRP) has been proposed as a marker of inflammatory and an independent risk factor for cardiovascular disease that has been positively associated with blood lipid [17], we examined the hypothesis that substantial loss in blood lipids would reduce CRP levels in postmenopausal female rats. Understanding the effect of training on blood lipid and finally, inflammatory markers may provide insight into the potential of exercise as a therapeutic option to reduce CRP.

MATERIALS AND METHODS

Animals and Experimental Design: This experimental protocol was approved by Department of physiology, University of Tehran and followed the guidelines established by the American Physiological Society for the use of animals in research (20). Fifty six female $wistar_{14848}$ rats aged 21 months with at least three months after menopause were obtained from the laboratory of animal bearing and multiplying at the Pasture institute of Iran. The animals were familiarized with laboratory environment and running on treadmill and then were randomly assigned to one of three experimental groups including control, training continuous and intermittent group. The experimental animals were subdivided into 7 subgroups (every group consist of 8 rats) (Table 1).

Each rat was housed in single standard cages of polycarbonate (20×15×15), made in Pasture institute of Iran, in a large air-conditioned room with controlled temperature of 22±2 °C with a light- dark cycles 12:12 hours and humidity of % 50±5. According to information from the pollution determination station of Iranian meteorological organization, air pollutants with consideration of pollutant standard index (PSI) were in normal range. The animals were obtained and cared for in guiding procedures in the Care and Use of Animals, prepared by the Council of the American physiological Society.

Rats were fed with a standard rat chow provided by Pars Institute for animal and poultry factory with a daily regimen of 10 g /100 body weight for every rat, Also, water was available *ad libitum*.

Training Methodology: After rats were housing for adaptation, in second week all rats had familiarization training on a motor-driven treadmill. Familiarization training included five times walking and running at speed 5-8 m/min for 5-10 minute. It has been shown that this program does not induce to noticeable changes in aerobic capacity [21]. Most animals ran voluntarily, but for those that did not, mild electrical stimulation (10 volts at a constant 0.055 amperes) was used to encourage the animals to run. We are replicating the research protocol in the present study with a previously-reported training regimen [12].

Training Protocol consisted of a 12 weeks training, five times/week that on basis of overload principle were progressed to end of training program. In general, in both training groups, training program intensity started at 12 m/min in the first and second week and increased 1 m/min from 3^{rd} to 12^{th} weeks. Duration of endurance training was increased to 10 min at the first day to 80 min in early 11^{th} week and then was maintained. Duration of intermittent training program was the same except that, it was done in the first four weeks in two sets and in 5^{th} to 8^{th} weeks in three sets and in 9^{th} to 12^{th} in four sets. Resting interval ratio of training to training was 1:1/4. Two training groups initially were running at 7 m/min for 3 min and then running speed was increased 2 m/min until target speed. At the end of training speed was decreased inversely to initial speed to cool down. All training programs have been done on a treadmill with 0% grade at an estimated work rate of approximately equals to 50-75 % VO_2max [21,22]. Total training distance plus warm up and cool down in every group was approximately 74010 meters.

Blood Sampling and Laboratory Analysis: For the

determination of circulating levels of hs-CRP, HDL-C and LDL-C, one groups of rats at baseline, three groups at 6 wk (following of 6 weeks) and other three groups at 12 wk (after 12 weeks) in same conditions were sacrificed. All groups were anesthetized with ether and sacrificed after 12-14 hours overnight fasting and 24 hours after the last session of training. Blood samples were taken from heart. Then coagulated samples were centrifuged for biochemical analysis. Serums were separated and thereafter values of hs-CRP and other variables (LDL-C and HDL-C) were measured. The serum high-sensitive C-reactive protein (hs-CRP) concentrations were determined by Latex particle-enhanced Immunoturbidimetric assay on a Hitachi 912 automated analyzer using reagents from Diasorin (Stillwater, MN). The latex particles coated with anti-human CRP antibody aggregates with serum or plasma CRP, forming immune complexes. The formed immune complexes caused increased turbidity measured at 572 nm, which is proportional to the concentration of CRP in the serum. The serum high-sensitive C-reactive protein concentration was determined from CRP standards of known concentration [1]. Furthermore, fasting serum high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations were measured by an enzymatic colorimetric method, as previously described by Dabidi Roshan and *et al*. [23]. The assay sensitivity for the 2 tests was 1 mg/dL.

Statistical Analysis: Results are expressed as means ± SE. significance differences of biochemical measurements were analyzed by using repeated measure and ANOVA tests to identify differences in phases and among groups, respectively. When appropriate, a Tukey post hoc test was applied, also. Statistical significance was accepted at $P<0.05$.

RESULTS

Table 1, shows means and standard deviations of hs-CRP, LDL-C and HDL-C in the Control, continuous and intermittent aerobic training groups in the various stages of research. Six weeks of the continuous and intermittent aerobic training had no significant effect on hs-CRP ($P=0.08$ and 0.351, respectively). In addition, after administration of the 6 weeks training, there were no significantly difference in hs-CRP level between the continuous and intermittent groups($P=0.936$). Furthermore, in the continuous and intermittent groups, although, amount of hs-CRP had significantly decrease after 12 weeks of training, as compared to the baseline ($P=0.000$ and 0.001, respectively), no significantly difference was detected between the trained groups ($P=0.427$). In contrast, hs-CRP level in control group gradually increased significantly after the 6 and 12 weeks, as compared to the training groups ($P< 0.001$)(Fig. 1).

Amount of LDL-C decreased significantly in both the training groups after 6 wk and 12 wk and in control group increased significantly. In addition, amount of LDL-C in control group gradually increased significantly during the 6 and 12 weeks, as compared to that observed in the training groups (Fig. 2). Furthermore, after 6 and 12-wk of training, no significant differences were detected in the resting LDL-C levels between the trained animals ($P=0.912$ and 0.810, respectively). In the other words, after 12-wks of adaptive training the resting LDL-C level decreased, as compared to the sedentary, control group ($P< 0.001$).

The 6 and 12 weeks exercise resulted in a significant increase in HDL-C levels, as compared to the control, sedentary group, while, in control group was detected decreased significantly. In addition, there were no differences in HDL-C both the continuous and intermittent aerobic groups after the 6 and 12 weeks

Table 1: hs-CRP, LDL-C and HDL-C in the control, continuous and intermittent groups

		Training methods					
groups		Control		Continuous		Intermittent	
Variables	baseline	6 wk	12 wk	6 wk	12 wk	6 wk	12 wk
Weight(g)	326±5	323±4	319±6	324±4	325±5	325±4	324±5
hs-CRP (mg/dl)	0.37±0.08	0.38±0.02	0.42±0.02 *†	0.36±0.01 ¥	0.32±0.01 *†¥	0.36±0.01 *¥	0.33±0.01 *†¥
LDL-C(mg/dl)	17.6±2	18.7±2 *	20.7±2.1 *†	15.6±1.7 *¥	11.8±1.8 *†¥	16±1.3 *¥	12.4±1.7 *†¥
HDL-C(mg/dl)	55.3±2.4	50.6±2.5 *†	45.1±2.3 *†	60.2±1.5 *¥	64.4±1.3 *†¥	59.4±2 *¥	62.2±2.1 *†¥

Values are means ± SD. hs-CRP, high sensitive C-reactive protein; LDL-C, low- density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

… Significant difference from baseline($P<0.05$). ? significant difference from before phase ($P<0.05$). ¥ significant difference, as compared to control($P< 0.001$).

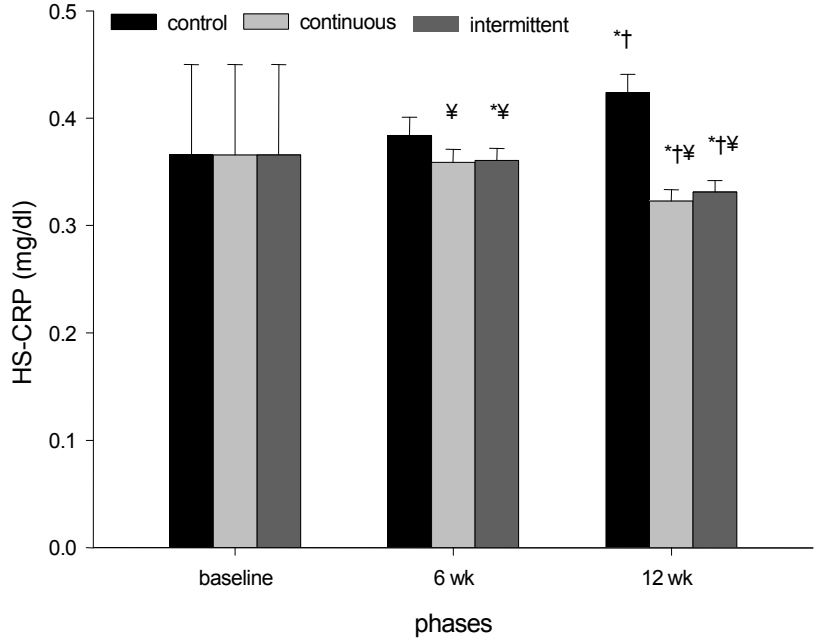

Fig. 1: Serum hs- CRP concentrations in the control and experimental groups at baseline, after 6 weeks (6 wk) and after 12 weeks (12 wk), Values are means ± SD. † significant difference from baseline (P<0.05). * significant difference from before phase (P<0.05). ¥ significant difference, as compared to control(P< 0.001).

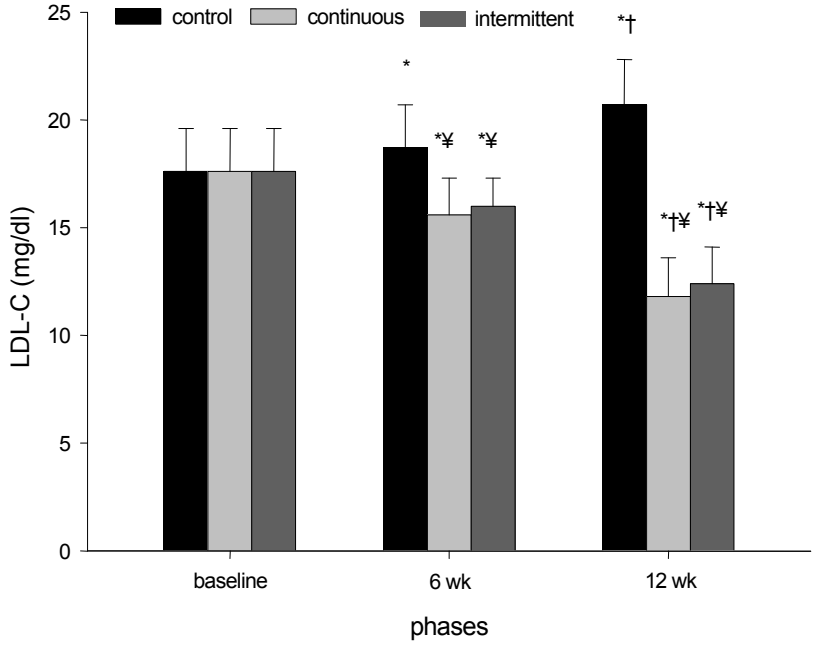

Fig. 2: Serum LDL-C concentrations in the control and experimental groups at base line, after 6 weeks (6 wk) and after 12 weeks (12 wk). Values are means ± SD. † Significant difference from baseline(P<0.05). * significant difference from before phase (P<0.05). ¥ significant difference, as compared to control(P< 0.001).

exercise (P=0.769 and 0.093, respectively). However, in both the continuous and intermittent groups, amount of HDL-C had significant increase after the 12 weeks of training (P< 0.000 and 0.001, respectively). In contrast, amount of HDL-C in control group gradually decreased significantly from the baseline level to 12 weeks, as compared to that observed in the training groups (P< 0.001)(Fig. 3).

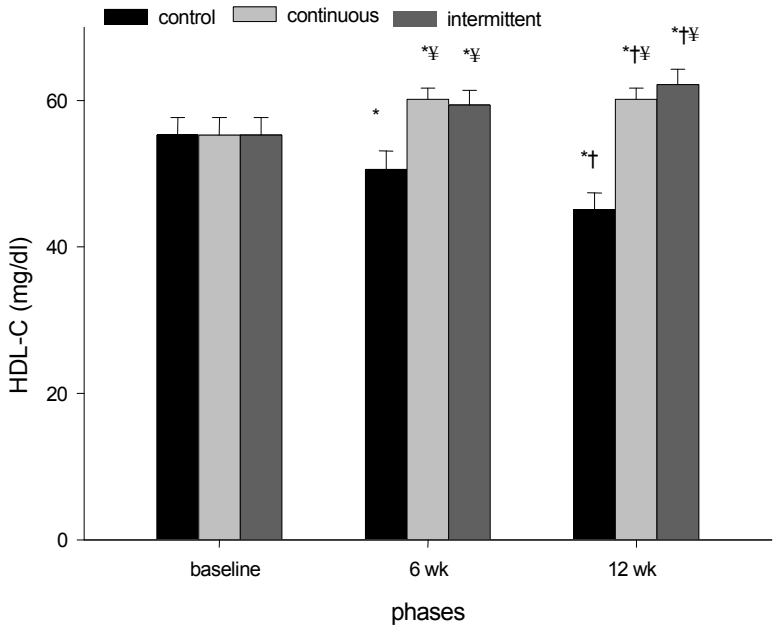

Fig. 3: Serum HDL-C concentrations in the control and experimental groups at baseline, after 6 weeks (6 wk) and after 12 weeks (12 wk).Values are means ± SD. † Significant difference from baseline(P<0.05). * significant difference from before phase (P<0.05). ¥ significant difference, as compared to control(P< 0.001).

DISCUSSION

We demonstrated that hs-CRP level in the control group gradually significantly increased after 6 wk and 12 wk. In contrast, hs-CRP levels in the continuous and intermittent aerobic training groups decreased, but were not significant in the first six weeks of training. However, the 12 weeks of the continuous and intermittent aerobic training resulted in significantly decrease in hs-CRP levels, as compared to the baseline and/ or control group.

These finding confirm the results reported by others that regular physical activities and cardiovascular fitness were associated with a lower level of inflammatory markers [2,5,9,10,16,24,25-,8]. Within the first six weeks of training, the decrease in CRP was not considerable. This may indicate the effectiveness of the length, intensity and the duration of exercise on CRP. In this study, the duration of exercise in the seventh week was 45 min at a speed of $m.m^{-1}$ that has gradually increased to 80 min at a speed of 23 $m.m^{-1}$ by the last session of training. Previous studies indicated that intensive activity particularly eccentric actions and prolonged sessions resulted in an increase in CRP. Thus, adaptation to training probably can be related to significant changes in the hs-CRP in the last six periods of training. Davis *et al.* [18] and King and Carek [5] have confirmed the effect of intensity and duration of training on inflammatory markers.

Elevated CRP has been associated with infectious viral pathogens [10,29,30]. however, enhanced natural killer (NK) cell activity may confer a resistance to acute infections in fit individuals [12], High levels of physical activity and fitness are associated with improved insulin sensitivity and lower levels of body fat and oxidized LDL-C [5,18,19], These factors may be noninfectious triggers for elevated CRP [10], Higher fitness levels appear to have an anti-inflammatory effect that may be a mechanism for lowering CHD and type 2 diabetes risks [5,9,14,21,25]. In contrast, it was demonstrated resistance training can not affect blood lipids profile, so it has no substantial effect on hs-CRP [31]. Also, research findings have shown that high-intensity anaerobic training, especially, eccentric exercise can stimulate acute phase response (APS) and hs-CRP [11].

Many researchers have reported that there is a high association between the cardiovascular fitness and the level of CRP [2,5,9,10,16,25 -28]. Continuous exercise has beneficial cardiovascular effect on the prevention and treatment of cardiovascular system and causes an increase in protective capacity of cardiovascular system in humans [10,15,27] and animals [19,32] and consequently life quality. The protective role of physical activity on cardiovascular morbidity has, mainly, attributed to its favorable effects on “traditional”

coronary risk factors, like body mass composition and arterial blood pressures in people with hypertension as well as to inflammatory process [5,8]. Furthermore, exercise affects cardiovascular system indirectly by increasing blood volume and stroke volume and decrease in viscosity [5], increase in protective antioxidant [16,23], increase Vo_2max [2,4,5,9,10,14,21,25,26,28] and changes in blood lipids [2,3,4,9,17,21,28,32]. Low levels of high density lipoprotein (HDL) cholesterol and high levels of low density lipoprotein (LDL) cholesterol have been identified as independent coronary risk factors [8,29]. Atherosclerosis is an inflammatory disease. Since, high LDL-C concentration is the risk factor for atherosclerosis, atherogenesis has been considered by increase in the accumulation of lipids within the artery wall [4,8]. On the contrary, given the anti-inflammatory effect of aerobic training and relationship between cardiovascular fitness with lower rate of hs-CRP and role of obesity and blood lipids in induce atherosclerosis and inflammation, it can be concluded that continuous exercise and decrease in LDL-C and increase in HDL-C led to decrease in hs-CRP.

HDL-C is negatively correlated with all of the makers except albumin. For example, information from cross-sectional research shows that for every 1 mg/dl increase in HDL-C, cardiovascular risk decreases about 2% and 3% in males and females, respectively [5]. Many researches also have shown that continuous aerobic training decreases the harmful lipids in humans [2,13,17] and also in animals [5,19], Furthermore, pro-inflammatory cytokines such as IL-6 and TNF-α are released in significant amounts from adipose tissue, particularly visceral adipose tissue [2,5,20,29,30]. These release from adipose tissue is augmented by increased sympathetic stimulation, which is down-regulated by regular physical activity [5,30]. TNF-α is a potent stimulator of IL-6 production and IL-6 is a potent stimulator of CRP production [2,21]. Although this issue was not measured in current study, the effect of regular exercise on TNF-α and IL-6 levels may be responsible for reduced lower CRP in rats with higher levels of fitness. Kuo *et al.* (9) have suggested higher circulating levels of IL-6, CRP and fibrinogen are independently associated with lower VO_2max in asymptomatic men. Also, Physical activity is associated with lesser degrees of central obesity [15]. With sympathetic stimulation, cytokines released from lipid tissues are increased and it has been shown that training causes sympathetic stimulation and the adaptation to endurance training leads to parasympathetic stimulation [2].

CONCLUSION

In summary, aerobic exercise training appears to positively influence the concentrations of hs-CRP and blood lipids. However, the available evidence indicates that low cardiovascular fitness are associated with a pro-inflammatory profile and the presence of increased risk factors such as; hs-CRP and LDL-C, while aerobic exercise training elicit a protective role relative to this profile. One of the limitations of the present study was the inaccessibility to the technique for measuring dual-energy X-ray absorption for analyzing the body composition in animals. Therefore, more controlled research for different age groups using appropriate techniques is needed.

REFERENCES

1. Jayachandran, M., H. Okano, R. Chatrath, W.G. Owen, J.P. Mcconnell and V.M. Miller, 2004. Sex-Specific changes in platelet aggregation and secretion with sexual maturity in pigs. Journal Applied Physiology, 97: 1445-1452.
2. Church, T.S., C.E. Barlow, C.P. Earnest, J.B. Kampert, E.L. Priest and S.N. Blair, 2002. Associations between cardiorespiratory fitness and C-reactive protein in men. Arteriosclerosis, Thrombosis and vascular Biology, 22: 1869-1876.
3. Davis, E., D.G. Edwards Brubaker, P.H. Philips, T.C. Leeuwenburgh and R.W. Braith, 2002. Lipid profiles and plasma C-reactive protein levels in patients entering cardia rehabilitation. Medicine Science Sport Exercise, 34(5): 180.
4. Donovan, G.O. and A. Owen, 2005. Changes in cardiorespiratory fitness and coronary heart disease risk factors following 24 weeks of moderate-or high-intensity exercise of equal energy cost. Journal Applied Physiology, 98(5): 1619-1625.
5. Geffken, D.F., M. Cushman, G.L. Burke, J.F. Polak, P.A. Sakkinen and R.P. Tracy, 2001. Association between physical activity and markers of inflammation in a healthy elderly population. American journal of epidemiology, 153: 242-250.
6. Ridker, P.M., N. Rifai, L. Rose, J.E. Buring and N.R. Cook, 2002. Camparison of C-reactive protein and LDL cholesterol levels in the prediction of first cardiovascular events. New England Journal Medicine, 347: 1557-1565.

7. Stauffer, B.L., G.L. Hoetzer, D.T. Smith and C.A Desouza, 2004. Plasma C-reactive protein is not elevated in physically active postmenopausal women taking hormone replacement therapy. Journal Applied Physiology, 96: 143-148.
8. Corrado, E., M. Rizzo, I. Muratori, G. Coppola and S. Novo, 2008. Older age and markers of inflammation are strong predictors of clinical events in women with asymptomatic carotid lesions. Menopause, 15(2): 240-247.
9. Kuo, H.K., C.J. Yen, J.H. Chen, Y.H. Yu and J.F. Bean, 2007. Association of cardiorespiratory fitness and levels of C-reactive protein: data from the National Health and Nutrition Examination Survey 1999-2002. International Journal Cardiology, 114: 28-33.
10. Lamonte, M.J., L. Durstine, F.G. Yanowitz, T. Lim, K.D. DuBose, P. Davis and B.E. Ainsworth, 2002. Cardiorespiratory fitness and C-reactive protein among a Tri-ethnic sample of women. *Circulation,* 106: 403-406.
11. Meyer, T., H.H. Gabriel, M. Mratz, H.J. Muller and W. Kindermann, 2001. Anaerobic exercise induces moderate acute phase response. Medicine Science Sport Exercise, 33(4): 549-55.
12. Gaeini, A.A., N. Rahnama and V. Dabidi Roshan, 2008. The effect of continuous training on C-reactive protein of wistar14848 rats. Gazz Medicine Italia Archive Science Medicine, 167: 221-229.
13. Rawson, E.S., P.S. Freedson, S.K. Osganian, C.E. Matthws, G. Reed and I.S. Okene, 2003. Body mass index, but not physical activity, is associated with C-reactive protein. Medicine Science Sport Exercise, 35(7): 1160-1166.
14. Stewart, L.K., M.G. Flynn, W.W. Campbell, B.A. Craig, J.P. Robinson, K.L. Timmerman, *et al.,* 2007. The Influence of Exercise Training on Inflammatory Cytokines and C-Reactive Protein. Medicine Science Sport Exercise, 39(10): 1714-1719.
15. Hiller, W.D.B., L.M. Dierenfield, P.S. Douglas, M.L. Otool, E.E. Fortess, D.S. Yamada, *et al.,* 2003. C-reactive protein levels before and after endurance exercise. Medicine Science Sport Exercise, 35(5): 121.
16. Abramson, J.L. and V. Vaccarino, 2002. Relationship between physical activity and inflammation among apparently healthy middle-aged older us adults. Archive International Medicine, 162(11): 1286-1292.
17. Tchernof, A., A. Nolan, C.K. Sites, P.A. Ades and E.T. Poehlman, 2002. Weight loss reduces C-reactive protein levels in obese postmenopausal women. Circulation, 105(5): 564.
18. Davis, J., M. Murphy, T. Trinick, E. Duly, A. Nevill and G. Davison, 2007. Acute effects of walking on inflammatory and cardiovascular risk in sedentary post-menopausal women. Journal Sports Science, 17: 1-7.
19. Durstine, J.L., K.A. Kenno and R.E. Shepherd, 1985. Serum lipoproteins of the Zuckers rat in response to an endurance running program. Medicine Science Sport Exercise, 17(4): 567-573.
20. Whihe, W.H., 1987. The laboratory rat. In T. Pool (Ed.). UFAW Handbook on the care and management of laboratory animals. 6th Ed. Longman scientific and technical, Harlow, UK.
21. Lawler, J.M., S.K. Powers, J. Hammeren and A.D. Martin, 1993. Oxygen cost of treadmill running in 24-month-old Fischer-344 rats. Medicine Science Sport Exercise, 25(11): 1259-1264.
22. Naito, H.S.K., H.A.D. Powers and J. Aoki, 2001. Exercise training increases heat shock protein in skeletal muscles of old rats. Medicine Science Sport Exercise, 33(5): 729-734.
23. Dabidi Roshan, V., M. Assali, A. Hajizadeh Moghaddam, M. Hosseinzadeh and J. Myers, 2011. Exercise Training and Antioxidants: Effects on Rat Heart Tissue Exposed to Lead Acetate. International Journal of Toxicology, 30(2): 190-196.
24. Lavie, C.J., T.S. Church, R.V. Milani and C.P. Earnest, 2011. Impact of physical activity, cardiorespiratory fitness and exercise training on markers of inflammation. Journal Cardiopulmonary Rehabilitation Prevention, 31(3): 137-145.
25. King, C. and F. Carek, 2003. Inflammatory markers and exercise: Differences related to exercise type. Medicine Science Sport Exercise, 35(4): 575-581.
26. Mattusch, F., B. Dufaux, O. Heine, I. Mertens and R. Rost, 2000. Reduction of the plasma concentration of C-reactive protein following nine months of endurance training. International Journal Sport Medicine, 21: 21-24.
27. Muylaert, S.J., T.S. Church, S.N. Blair and S.N. Facsm, 2003. Cardiorespiratory fitness (CRF) and C-reactive protein in premenopausal women, Medicine Science Sport Exercise, 35(5): 69.
28. Reis, J.P., M.J. Lamonte, B.E. Ainsworth and J.L. Durstine, 2003. C-reactive protein and cardiorespiratory fitness in an adult population. Medicine Science Sport Exercise, 35(5): 68.

29. Albert, C.M., N. Rifai and P.M. Ridker, 2002. Prospective study of C-reactive protein, Homocysteine and plasma lipid levels as predictors of sudden cardiac death. Circulation, 105(22): 2595.
30. Il'yasova, D., A. Ivanova, J.D. Morrow, M. Cesari and M. Pahor, 2008. Correlation between two markers of inflammation, serum C-reactive protein and interleukin 6 and indices of oxidative stress in patients with high risk of cardiovascular disease. Biomarkers, 11: 1-11.
31. Nicklas, B.J., W. Ambrosius, S.P. Messier, G.D. Miller, B.W. Peninx, R.F. Loeser, *et al.,* 2004. Pahor Diet-induced weight loss, exercise and chronic inflammation in older, obese adults: a randomized controlled clinical trial. American Journal Clinical Nutrition, 79(4): 544-551.
32. Fiebig, R.G., J.M. Hollander, D. Ney, R. Boileau, E. Jeffery and L.L. JI, 2002. Training down-regulates fatty acid synthase and body fat in obese zucker rats. Medicine Science Sport Exercise, 34(7): 1106-1114.