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Exercise Prevents Cardiovascular Dysfunction by Suppression of some Pro-Inflammatory Cytokines

[1]Mariam A. Mahmoud, [1]Asmaa F. Galal, [23,] Asser I. Ghoneim, [4]Amani E. Khalifa, [5]Osama A. Elshabrawy

[1]Toxicology and Narcotics Department, National Research Center, Egypt
2Department of Pharmacology, Toxicology & Clinical Pharmacy, Faculty of Pharmacy, Damanhour University, Damanhour, Egypt.
[3]Department of Pharmacology and Therapeutics, Faculty of Pharmacy, Beirut Arab University, Beirut, Lebanon
[4]Professor of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, seconded as The Strategic Planning Consultant for the Board of 57357 Children Cancer Hospital in Egypt
[5]Pharmacology Department, National Research Center, Egypt

ABSTRACT

Physical exercise is well recognized as important strategy for reducing the risk of chronic heart disease, and recent research has focused on its role in the inflammatory profile beside the effect on the lipid profile. However, the mechanisms by which exercise training (swimming) improve cardiovascular function have not been fully clarified. In the present study we aimed to evaluate the effect of 10 weeks exercise on the proinfammatory cytokine TNF-α and lipid profile and the link between inflammation and lipid metabolism. Male rats were assigned to a sedentary control group and swimming group (three times per week for 10 weeks). Swimming exercise induced a decrease in plasma LDL. In addition, there was a reduction in cardiac TNF- α levels.

***Key words**:* Swimming - exercise - lipid profile - TNF-α.

Introduction

Today, physical exercise is considered as a life style practice that generally has beneficial effects on many of the physiological processes involved in the primary prevention of cardiovascular diseases. Recently, the use of physical exercise as a non-pharmacological therapy has emerged for treatment of hypertension (Sosner *et al.* 2014), and coronary heart disease (Tang *et al.* 2014). Moreover, regular aerobic exercise protects endothelial function and reduces cardiovascular risk associated with aging (Walker *et al.* 2014).

Current public health recommendations state that all people over 2 years of age should accumulate 30 min of moderate – intensity endurance – type physical activity on most (preferably all) days of the week. The physiological response to exercise varies depending on either a single or acute bout of exercise, as occurs during exercise test, or chronic adaptations that occur in response to repetitive bouts of exercise training, as occurs with cardiac rehabilitation (Myers *et al.* 2009).

Both clinical observations and basic research have indicated a potential link between inflammation and lipid metabolism. Tumor necrosis factor-α (TNF-α) act as a key cytokine that affects and mediates intermediary metabolism, and a close relationship between TNF-α and lipid metabolism is supported by several studies (Hanrui *et al.* 2009).

TNF-α is a strong biological driver of the metabolic syndrome, which is characterized by abdominal obesity, hypertension, a reduced level of HDL, elevated triacylglycerols and high-fasting glucose, and constitutes an important risk factor in atherosclerosis and Type 2 diabetes (Bruunsgaard *et al.* 2005).Keller *et al.*(2004) have reported that TNF-α over expression returned to normal levels after 1 h of acute swimming exercise in TNFR (TNF receptor)-knockout mice. In addition, chronic exercise appears to suppress pro-inflammatory factors, such as TNF-α, C-reactive protein (CRP) and interleukin-6 (IL-6), and augment anti-inflammatory factors, including IL-4, IL-10, TGF-β (transforming growth factor-β) and adiponectin, even though these results showed discrepancies according to the modes, intensity and time duration of exercise (Bruunsgaard H *et al.* 2005, Flynn *et al.* 2007, de Lemos *et al.* 2007).

In the present study we aimed to evaluate the effect of 10 weeks exercise on the proinfammatory cytokine TNF-α and lipid profile and the link between inflammation and lipid metabolism.

Corresponding Author: Mariam A. Mahmoud, Toxicology and Narcotics Department, National Research Center, Egypt.
E-mail: mariam_pharm@hotmail.com

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Material and methods

Animals:

The experiments were carried out using adult male albino rats, weighing 120-140 g. Animals were obtained from the animal house (National Research Centre, Cairo, Egypt). All animals were housed under conventional laboratory conditions throughout the period of experimentation and fed standard laboratory pellets (20 % proteins, 5 % fats, 1 % multivitamins) and allowed free access to tap water. Animals were allowed at least one week of acclimatization before using them. Experimental protocols were approved by the Research Ethical Committee of the National Research Centre (Cairo, Egypt).

Study design:

Rats were randomly divided into two groups; 10 rats each, sedentary control group and swimming exercise group. Rats in the exercise group were gradually trained to swim for 2 weeks in a cylindrical swimming pool with a diameter and height of 50 and 70 cm, respectively, in water depth of 30-45 cm. Within 2 weeks, the daily exercise time was gradually increased from 10 to 30 min. After this period, air was supplied from the bottom of the pool to make a current to force the rat to swim continuously. The water temperature was maintained near 28°C. Exercise time was increased gradually from 30-60 min, 5 days/ week for 8 weeks (Kanda and Hashizume, 1998).

After 24 hours from the last day of the experiment, blood samples were withdrawn from retro-orbital venous plexus under ether anesthesia for the biochemical parameters to be done (lipid profile),and then sacrificed by decapitation, and hearts were collected, weighed and prepared for TNFα mesurments.

Cardiac tissue TNFα determination:

Hearts were isolated, washed by saline, dried on filter paper then were immediately weighed to avoid dryness then heart tissues were homogenized in phosphate buffer solution (20% w/v).Quantitative determination of TNFα was detected by a sandwich ELISA method with rat TNFα kit (Bio Vendor Laboratorní medicína), and absorbance was read at 450nm.

Serum analyses:

Serum was obtained by centrifuging blood at 3000 rpm for 10 min, aliquoted and stored at -20ºC for enzyme assays. Serum levels of total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were determined according to manufacturer's instructions on Shimadzu UVPC 240 v3.9 spectrophotometer (Shimadzu, Koyoto, Japan).

Statistical Analysis:

Values were expressed as means ± S.E. Comparisons between means were carried out using t- test. A probability level of less than 0.05 was accepted as being significant in all types of statistical tests. Graph pad INSTAT software (version 3) was used to carry out all statistical tests.

Results:

Cardiac TNFα content:

Rats subjected to swimming exercise for 8 weeks showed decrease in TNFα levels by about 30% as compared to corresponding sedentary control group.

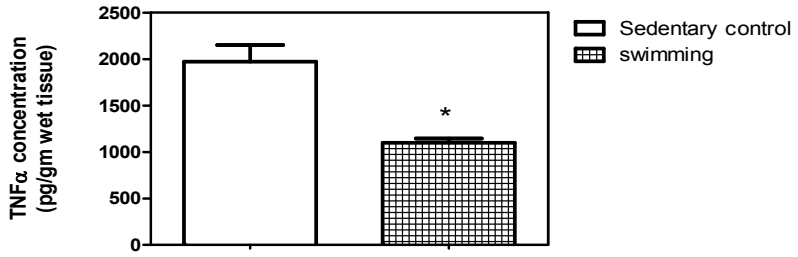

Fig. 1: Effect of nandrolone decanoate on heart TNFα in sedentary and exercised rats.
* Significantly different from sedentary control group.

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Lipid profile:

Rats subjected to swimming exercise for 8 weeks showed decrease in LDL levels by about 40 % as compared to corresponding sedentary group rats.

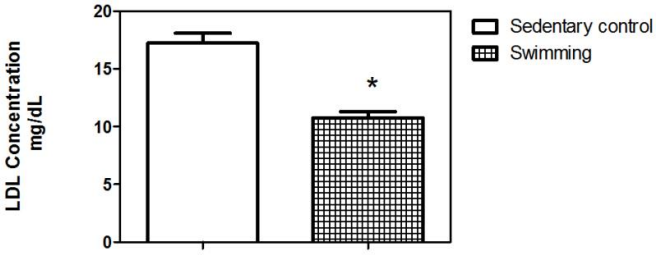

Fig. 2: Effect of nandrolone decanoate on plasmaLDL in sedentary and exercised rats.
* Significantly different from sedentary control group.

Table 1: Effect of swimming exercise on plasma TC, HDL, TG and LDL.

Group	TC(mg/dl)	HDL(mg/dl)	TG(mg/dl)	LDL(mg/dl)
Sedentary control	64.96 ± 2.07	34.5 ± 2.35	73.43 ± 2.01	17.23 ± 0.086
Swimming control	64.98 ± 3.11	40.21 ± 1.73	65.28 ±1.4	10.55* ± 0.77

* Significantly different from sedentary control group.

Discussion:

The main finding of the present study is that moderate physical exercise protocol adopted in our study produces anti-inflammatory response accompanied by decrease in LDL levels. These events further support the protective effect of exercise on cardiovascular system.

TNFα release is one of the earliest deleterious events in response to a variety of forms of cardiac injury; the released TNFα can both directly damage the myocardium and trigger the inflammatory cascade involved in subacute ischemia-reperfusion injury (Meldrum, 1998; Frangoginmis *et al.* 2002).

In patients with hyperlipidaemia, TNF-α levels correlated significantly with the concentrations of VLDL (very-low-density lipoprotein) -triacylglycerol (triglyceride) and -cholesterol, and negatively with HDL (high-density lipoprotein)-cholesterol (Jovinge *et al.* 1998). Simvastatin and atorvastatin decrease TNF-α level in subjects with hyperlipidaemia and hypercholesterolaemia (Ascer *et al.* 2004, Zubelewicz-Szkodzinska *et al.* 2004, Marketou *et al.* 2006). Furthermore, patients with type IIa and IIb dyslipidaemia have an abnormal pattern of TNF-α. TNF-α blockade could significantly affect lipid metabolism. Short-term administration of adaluminab, a fully human anti-TNF-α monoclonal antibody, to patients with active RA (rheumatoid arthritis), significantly increased HDL-cholesterol concentrations; in addition, the atherogenic index decreased (Popa *et al.* 2005).

Administration of TNF-α has been demonstrated to directly interfere with the plasma lipid level and metabolic pathways. In mice, administration of TNF-α result in an acute increase in plasma triacylgycerol concentrations of 85%, and inhibition of TNF-α activity blocked the increase in serum triacylglycerols that is characteristically observed after LPS (lipopolysaccharide) treatment (Memon *et al.* 1993, Feingold *et al* 1994).

Observational data from large population cohort studies consistently show an association between physical activity and inflammation. Specifically, lower inflammatory biomarker concentrations are observed in individuals who report performing more frequent and more intense physical activity, including leisure and non-leisure time physical activity (Hsu *et al.*2009).

The exact mechanisms by which physical activity may reduce inflammation are not entirely understood, there are some data pointing to factors that may contribute to an effect of repeated bouts of muscle contraction leading to improvements in inflammatory status over time. These factors include: 1) shifts in monocyte phenotype, specifically reductions in immune cell production of inflammatory mediators, with exercise training, 2) immune function adaptations that occur locally in exercised skeletal muscle, and 3) exercise-induced adaptations in intracellular generation of reactive oxygen species (ROS)(Beavers *et al.* 2010).

Physical exercise induces expression of growth factors and pro- and anti-inflammatory cytokines within circulating leukocytes, perhaps preparing the organism to respond to a variety of stressor imposed by exercise (Gokhale *et al.* 2007).Exercise training-induced improvements in inflammatory status may also result from the modulation of intracellular signaling pathways and cellular function that are mediated by nitric oxide (NO) and ROS(Zanesca *et al.* 2007).

In one study, aerobic exercise training in adults at high risk for ischemic heart disease resulted in a 58% decrease in mononuclear cell production of atherogenic cytokines (INFγ, TNFα and IL-1α), while the production of atheroprotective cytokines (IL-10, IL-4, and transforming growth factor beta-1 (TGFβ1)) increased by 36%. Exercise training also reduces TNFα production by monocytes, in healthy older men and women. Similarly, in healthy young adults, higher-intensity aerobic exercise training reduces stimulated production of TNFα by monocytes (Beavers *et al.* 2010). Thus, these data point to an adaptive down-regulation of cytokine release from innate immune cells in response to regularly performed muscular contraction.

However, it is now evident that an acute inflammatory response plays a major role in the training adaptations observed in exercised muscles. IL-6 release from muscle increases up to 100-fold during contractile exercise and its production results in increased systemic anti-inflammatory cytokines (IL-1 receptor antagonist and IL-10), but decreased TNFα and IL-1β production (Pederson *et al.* 2003). There are also data to suggest that the exercise-induced increase in IL-6 inhibits TNFα production in the presence of low-grade inflammation (Starkie 2003). Thus, acute exercise activates an immune response, but the effects are primarily anti-inflammatory and serve to enhance lipid and glucose metabolism. In turn, regular/chronic exercise can lead to lower basal levels of circulating inflammatory markers, as well as reduce the inflammatory response to acute exercise.

Regular and moderate exercise training have been associated with a reduced risk of CVD partially because of an improvement in the lipoprotein profile(La Rosa, 1992). However, the amount of exercise training required to obtain benefits is debatable (Blair *et al.* 2004). In general, onlyhigh-intensity exercise training or long-term endurance exercise programme are able to improve overall lipoprotein profile, including elevation of HDL-c and reduction in TG levels in humans (Seals *et al.* 1984; Kraus *et al.* 2002; Halverstadt *et al.* 2007).

The effect of exercise on lipid levels is an area of active research. A meta-analysis of 95 studies, most of which were not randomized controlled trails, concluded that exercise leads to a reduction of 6.3% in total cholesterol, 10.1% in LDL cholesterol, and 5% increase in HDL cholesterol. These improvements in lipid profile have a favorable effect on cardiovascular risk (Shephard *et al.* 1999).

Current study showed that rats subjected to swimming exercise for 8 weeks showed decrease in LDL-c levels by 38.8% as compared to corresponding sedentary group rats.

In another study by Aparicio (2013), resistant training groups presented lower, but not significantly, plasma TC and TG and significantly higher HDL-C concentrations, affect that confirms the highly contrasted effects of resistance training on lipid profile (Williams *et al,* 2007). This better plasma lipid profile in general, could have a protective effect on cardiovascular diseases (Houston *et al* 2009).

In conclusion, regular exercise contributes to the prevention of cardiovascular dysfunction by controlling traditional cardiovascular risk factors, including suppression of LDL -cholesterol levels, and suppressing TNF-α, which is the main pro-inflammatory cytokine.

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