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Effect of exercise training on livers of young, old, and ovariectomized rats

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ABSTRACT

The menopause is a mid life event, characterized by estrogenic deficiency and associated with bio-psycho and social changes which impair quality of life. In addition, exercise can be described as a body activity that enhances or maintains physical fitness and overall life quality. This experiment was conducted to determine if exercise training has a protective role against the deleterious effects of aging in ovariectomized rats. In this study, thirty-six *Wistar albino* rats were used. The rats were divided into six subgroups. Subgroups consisted of young rats, old rats, ovariectomized rats, young exercise-trained rats, old exercise-trained rats and ovariectomized exercise-trained rats. Control rats and ovariectomized rats 12 weeks after surgery were subjected to a 4 week treadmill-running program. In exercise groups, the rats were subjected to treadmill exercise during the time each rat walked on a motor-driven treadmill for 15 m min-1 speed and 15° incline once every 2 days for a period of 10 days over three courses for 5, 10 and 15 mins per day, totally for 30 days. In the stereological analysis of this study, the numerical density of the control groups were found significantly higher compared to ovariectomized and/or training groups ($p<0.05$). On the other hand, the liver cell numbers of ovariectomized exercise-trained group was found to be lesser than other trained and ovariectomized groups. In addition, the cell density of ovariectomized group estimated as a little higher than other groups except for control group ($p<0.05$).The stereological results revealed that ovariectomy and training can decrease the hepatocyte density in ovariectomized rats.

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1. Introduction

The increase in lifespan has increased the focus on menopause which leads physiological alterations in elderly women. Menopause is the permanent end of menstruation due to cessation of ovarian follicular activity (i.e. the absence of oocytes in the ovaries) (Burger et al., 2002). It begins around the age of 50 years and is characterized by at least 12 months of amenorrhea (Gracia et al., 2005). Menopause can be described as transition from a potentially reproductive phase of a woman to non-reproductive phase of life.

This period is an inevitable part of every woman's life, changes in hormone levels might lead to complaints and health consequences especially during peri- and post menopause (Appling et al., 2007; MacLennan, 2009).

The most common menopausal symptoms include hot flashes, night sweats, fatigue, body aches and pains and mood changes (Greene, 2008; Warren, 2007) and these symptoms often persist for several years in the postmenopausal period. Levels of hormones involved in ovarian function vary greatly during menopause. These modifications are associated with the elevated risk of several pathologies such as cardiovascular disease, diabetes, insulin sensitivity independent of obesity, metabolic syndrome, obesity, hypertension, dyslipidemia, non-alcoholic fat liver disease, and others. Estrogen deficiency also can lead increasing of plasma lipid levels by creating metabolic syndrome (Godsland, 2005). High cholesterol levels are important risk factors for liver injury and steatohepatitis (Kamada et al., 2011). The prevalence and severity of liver injury and non-alcoholic steatohepatitis show acceleration after menopause (Koruk et al., 2003; Ruhl and Everhart, 2004).

Postmenopausal women also show an increasing preva-

lence of liver disorders dependent on estrogen deficiency (Unal et al. 2011). So, the estrogen deficiency itself and estrogen deficiency-induced hypercholesterolemia should accelerate the severity of liver disorders in postmenopausal women (Hagymási et al., 2009; Suzuki and Abdelmalek, 2009).

Because of estrogen levels decline, many tissue and organs (muscular, bone, adipose tissue, brain and liver) are affected. In recent years many strategies were established to prevent and reverse the effects of menopause (Unal et al., 2010a). Hormone replacement therapy (HRT), diet and physical exercise have been recommended. (Dubnov et al., 2007; Hagey and Warren, 2008) One of the healthiest components of lifestyle is physical activity. It has been known as promotion of all aspects of human health including menopause (Pines and Berry, 2007; Zanesco and Zaros, 2009). The results of a study by Hagberg et al. indicated that high-intensity endurance training had a greater effect on increase in total complaints and regional body fat values than HRT in postmenopausal women (Hagberg et al., 2000). Restrictive diets often have a negative effects on muscle mass. Considering that, resistance training seems to be a logical choice with its beneficial effects in postmenopausal women (Bemben et al., 2000). In preventing menopause-related osteoporosis and sarcopenia resistance training exercise has been demonstrated as effective substitute for hormone replacement therapy (Maddalozzo et al., 2007). Also, it has been suggested that resistance training has the potential to prevent the development of insulin resistance and may reduce the risk of non-insulin dependent diabetes mellitus in obese sedentary postmenopausal women (Ryan et al., 1996).

Many studies have reported the association between inflammation and exercise in healthy subjects (Fried et al., 1998; Fallon et al., 2001; Geffken et al., 2001). Also, some studies suggested the anti-inflammatory effects of exercise by reduction of C-reactive protein (CRP) concentration (Mattusch et al., 2000; Fallon et al., 2001; Starkie et al., 2003) and cytokines (IL-1β, TNF and etc.) production (Keller et al., 2001; Ostrawski et al., 1999). The possible relation between regular exercise and inflammatory activity, which protect against chronic low-grade inflammation, has not yet been officially confirmed. In addition, the benefits of regular exercise on cardiovascular stress and systemic low-grade inflammation were reported in many studies (Pinto et al., 2012; Vieira et al., 2012). In light of these data, the present study was designed to investigate effects of resistance training on liver injury during the ovariectomy (OVX)-induced postmenopausal period in rats. We examined the effects of menopause on the livers of rats using two distinct methods: Histopathological detection with the help of a light microscope, quantitative analyses by means of stereological tools.

2. Material and methods

Animal model and exercise

Thirty six adult female *Wistar albino* rats, weighing 200-220 g were obtained from Ataturk University Experimental Animal Laboratory of Medicinal and Experimental Application and Research Center. Animal experiments and procedures were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by Ataturk University local animal care committee.

Rats were housed in standard plastic cages on sawdust bedding in an air-conditioned room at 22±3 °C under controlled lighting (14 h light 10 h dark cycle). Standard rat chew and tap water were given ad libitum. At the beginning of the study, thirty-six *Wistar albino* rats were divided into six subgroups and each group consisted of six animals. Subgroups consisted of young rats, old rats, ovariectomized rats, young exercise-trained rats, old exercise-trained rats and ovariectomized exercise-trained rats.

Ovariectomy procedure

For the ovariectomized and ovariectomized-exercise groups, under sterile conditions, the animals (n=6) were anesthetized with 25 mg/kg sodium thiopental injected intra-peritoneally. First, for each rat, the fur of the lower abdomen was shaved and then sterilized with anti-septic and a longitudinal incision (0.5-1 cm) was made in the midline area of the lower abdomen.

Next, a small peritoneal incision was made and the ovaries were removed. The incisions were sutured after the operation. The rats were then transferred to the care laboratory. Food and water were provided ad libitum throughout the experiments. Twelve weeks after surgically ovariectomized rats were subjected to exercise program as shown Table 1.

Light microscopic evaluation

At the end of the study, all animals were sacrificed under the ether anesthetize and liver tissues were removed and placed in 10% neutral buffered formaldehyde. After fixation period, the liver tissues were dehydrated in alcohol series, cleaned in xylene and embedded in paraffin blocks.

Histopathological evaluations

The paraffin blocks were cut 5 μm for histopathological analysis and then these sections were stained with haematoxylin-

Table 1. Exercise program and experimental program

Groups	1-10 days (once every other day)	11-20 days (once every other day)	21-30 days (once every other day)	32 days
Young (4-6 months old)	Resting period	Resting period	Resting period	Sacrificed
Old (24-26 months old)	Resting period	Resting period	Resting period	Sacrificed
Ovariectomized (9 months old)	Resting period	Resting period	Resting period	Sacrificed
Young exercise-trained (4-6 months old)	15 m speed and 15° incline 5 min a day	15 m speed and 15° incline 10 min a day	15 m speed and 15° incline 15 min a day	Sacrificed
Old exercise-trained (24-26 months old)	15 m speed and 15° incline 5 m a day	15 m speed and 15° incline 10 min a day	15 m speed and 15° incline 15 min a day	Sacrificed
Ovariectomized exercise-trained (9 months old)	15 m speed and 15° incline 5 min a day	15 m speed and 15° incline 10 min a day	15 m speed and 15° incline 15 min a day	Sacrificed

eosin staining.

All serial sections (approximately 10 section for each group) were analyzed for histopathological changes under the light microscopy. Evaluations and scoring of the liver changes in terms of histopathology were inflammatory cell infiltration, degenerative cells and sinusoidal dilatation level. The scores were derived semi-quantitatively using light microscopy on the preparations from each animal, and were reported as follows: none = –, mild = +, moderate = ++, and severe = +++.

Stereological analysis

The serially sectioned livers tissues for each animal was counted using optic dissector counting method at a stereology workstation for stereological analyses as described previously (Unal et al., 2010b). Each hepatocyte was counted by software of the image analysis system (Stereo Investigator 9.0, MicroBrightField; USA) according to the unbiased counting rules of the optical dissector (Howard and Reed, 1998). The number of hepatocyte estimation via optic dissector method was seen in Fig 1.

3. Results

In stereological estimations, there was increased hepatocyte number in the non-trained OVX group compared with remaining groups ($p<0.05$). Hepatocyte numbers of training groups (young and old) were significantly increased when compared with hepatocyte number of non-trained groups (old and young) ($p<0.05$). The number of hepatocytes in non-trained young group were lower than that found in non-trained old group ($p<0.05$). Thereafter, the lowest hepatocyte number was found in control and non-trained young groups. All results of hepatocyte densities for all groups were shown in Table 2.

In the histopathological evaluations, inflammatory cell densities of non-trained and trained OVX groups were more intensive than remaining groups, but the highest histopathology was observed in the non-trained OVX group. Degenerative cell densities also were found higher in the non-trained and trained OVX groups. Although, there was not found any

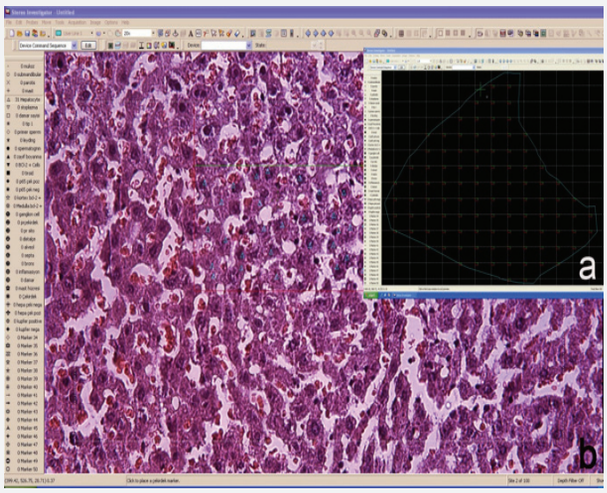

Fig. 1. Illustration of hepatocyte number estimation via stereologic optic dissector method, a: screen view of counting with higher magnification and b: drawing contour lines on the liver section.

Table 2. Numerical hepatocyte densities (cell/mm3) of all groups

Groups	Hepatocyte density
Group 1 (young rats)	207.200 ± 3730[a]
Group 2 (old rats)	313.000 ± 8150[b]
Group 3 (OVX)	298.200 ± 1521[c]
Group 4 (young-trained)	363.200 ± 9400[b]
Group 5 (old-trained)	384.200 ± 4120[b]
Group 6 (OVX-trained)	353.600 ± 5430[b]

All results expressed as mean ± standard deviation. The footnote letters in the same column indicate significant differences between groups; Mann Whitney-U Test was used ($p<0.05$). The results of numerical density expressed as mean ± standard deviation.

histopathology in the non-trained young and old groups, mild degenerative cells was observed in the trained old group. In addition, exercises decreased the inflammatory cell, degenerative cell densities and sinusoidal dilatation in the trained-OVX group compared to non-trained OVX group (Fig. 2). All scores of histopathological evaluations were presented in Table 3.

4. Discussion

The major finding of the present study is that increased number of hepatocyte and ameliorative histopathology in the training groups is determined by exercise training. Exercise training is emerging as an important method of therapy against inflammation mediated damages. From the data of the present study, a causal relationship cannot be clearly determined. However, several effects of exercise training are potentially inhibited the degenerative changes in the ovariectomized rat's livers.

The increasing knowledge about effects of exercise led to focus on beneficial effects on cardiovascular system in last decade (Yasuda et al., 2004; Duncker and Bache, 2008). However, some recent studies have indicated the effects of exercise on inflammatory mediators (Petersen and Pedersen, 2005). Thereafter, during inflammation, some mediators induced acute phase reactants such as C-reactive protein (CRP) concentration and cause an increase in some systemic cytokines (Ross, 1999). CRP plays an important role both in the production of anti-inflammatory cytokines and in the inhibiting the synthesis of pro-inflammatory cytokines (Pue et al., 1996). The effects of exercise training may consequently improve liver damages by inhibiting inflammation.

Previous studies reported that estrogen has some roles

Table 3. Scores of histopathological evaluations for all groups

Groups	Inflammatory Cell Infiltration	Degenerative Cell Density	Sinusoidal Dilatation
Group 1 (non-trained young rats)	-	-	–
Group 2 (non-trained old rats)	-	+	–
Group 3 (non-trained OVX)	+++	++	++
Group 4 (trained young)	-	-	-
Group 5 (trained old)	-	-	+
Group 6 (trained OVX)	+	++	+

The scores were reported as follows: none = –, mild = +, moderate = ++, and severe = +++.

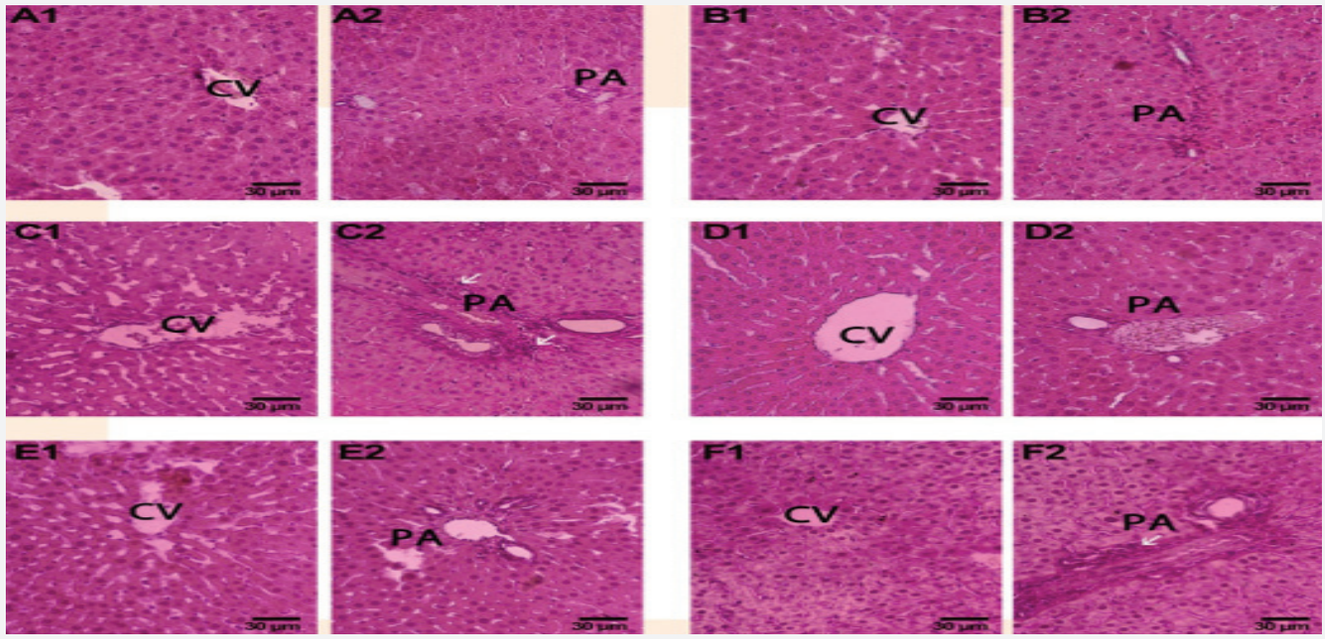

Fig. 2. The portal area and central vein around of liver sections for all groups, A1-2: group 1 (non-trained young rats), B1-2: group 2 (non-trained old rats), C1-2: group 3 (non-trained OVX rats), D1-2: group 4 (trained young rats), E1-2: group 5 (trained old rats), and F1-2: group 6 (trained OVX rats), CV: central vein, PA: portal area, and arrows: inflammatory cell infiltration, Haematoxylin-Eosin staining.

in the liver and kidney metabolisms, which indicated the changed estrogen concentration, lead to damages in the liver (Kim et al., 1997). Findings of OVX groups revealed the estrogen deficiency led to cause damages in the liver tissue, however exercise showed beneficial effects on liver tissues of ovariectomized rats. Here exercise can shows protective effects on inflammation mediated tissue damages.

In the stereological estimations, increased hepatocyte numbers were found in the trained young and old groups than their non-trained groups ($p<0.05$). Some studies reported the simulative effects of exercise on producing of hepatocyte growth factor (HGF) (Ono et al., 1997; Yasuda et al., 2004) as well as exercise training induce the angiogenesis (Richardson et al., 2000). HGF has ability to induce the cell mitosis, motility, and anti-apoptotic activities in a variety of cells during the tissue regeneration (Boros and Miller, 1995). Thus, interventions that enhance exercise training could be beneficial effects in the regeneration of liver via induction of HGF producing and angiogenesis. Exercise training is a potential approach in term of anti-inflammatory effects. However, further studies are required to clarify the possible benefits of exercise training in the body including liver metabolism.

Apart from other groups, exercise training decreased the hepatocyte numbers in the trained OVX group ($p<0.05$). Previous studies indicated the exercise induced HGF production by mesenchymal cells (Boros and Miller, 1995). Because this factor is an important role in the tissue regeneration, during exercise HGF secretion may lead to an increase in hepatocyte degeneration and induced the tissue regeneration. Therefore, OVX group hepatocyte number can be reduced by exercise training depending on tissue regeneration.

In conclusion, the present study demonstrates the effects of exercise on the liver histopathology and hepatocyte number in the young, adult, and ovariectomized rats. Although the beneficial effects of exercise training are still little known, our findings encourage further studies on this subjects.

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