Exercise Effects on Menopause-Induced Changes in Heart of Ovariectomized Rats

Necip Fazıl Kishali
Department of Physical Education and Sport,
School of Physical Education and Sport Sciences, Ataturk University, 25240 Erzurum, Turkey

Abstract: The menopause is characterized by the progressive decrease in estrogenic secretion and associated with bio-psycho and social changes which in turn impairs quality of life. This experiment was conducted to determine if exercise training has a protective role against the deleterious effects of aging in ovariectomized rats. Control rats and ovariectomized rats 12 weeks after surgery were subjected to a 4 weeks treadmill-running program. Subgroups established were young animals, old animals, ovariectomized animals, young exercise-trained animals, old exercise-trained animals and ovariectomized exercise-trained animals. In exercise groups, rats were subjected to treadmill exercise during which time each rat walked on a motor-driven treadmill at 15 m min⁻¹ speed and 15° incline once every 2 days during 10 days for three courses as 5, 10 and 15 min day⁻¹ totally for 30 days. In ovariectomized rat, heart tissue was remarkably dens fibrosis and an increase in necrotic cell density, possibly resulting from myocyte defect. Findings of old exercise-trained animal group more resembled young animal group rather than ovariectomized animal group and ovariectomized exercise-trained animal group.

Key words: Exercise, heart, menopause, myocyte, ovariectomy, rats, Turkey

INTRODUCTION

The menopause is defined by the World Health Organization (WHO) as the point in time of permanent cessation of menstruation due to loss of ovarian function (Burger et al., 2002). A progressive decrease in estrogenic secretion during this period is associated with bio-psycho and social changes which in turn impair quality of life (Chedraui et al., 2009). Estrogen is a very important regulatory factor in all target structures of body which have two Estrogen Receptor (ER) subtypes denoted ER-α and ER-β. They are intracellular proteins that are members of a large superfamily of proteins and act as ligand-activated transcription factors (Katzenellenbogen et al., 2000). In estrogen deficiency as in menopause, execution of estrogen-mediated a lot of cellular or subcellular functions will not be possible in normal conditions to impair the regulation of ER-α and ER-β expressions.

To be related to any kind of activity in the body with others directly or indirectly in this case, itself of many physiological processes regulated via estrogens are only be affected but also cause other negatives depending on the former. For example, menopausal period may be accompanied by many undesirable situations such as memory impairment (Dumas et al., 2010), depression (Dormonen et al., 2011), vasomotor and cardiovascular symptoms (Szmulowicz and Manson, 2011) and increasing oxidative stress and metabolic disorders (Behr et al., 2011). Many questions remain to be answered are the hypothalamus and other brain centers also involved? How can we predict it. Why do some women suffer from these health problems more than others. Exactly how is the process of menopause involved in the increased risk for these health problems and conditions. Knowing these answers is likely to lead to better interventions and treatments that will delay, attenuate or even prevent altogether the decline in quality of life that accompanies menopause (Bellino and Wise, 2003).

When looking from another aspect how may be it affected other structure in the body such as heart, liver, kidney (etc. in terms of histopathology) are studies performed about menopause up to now enough for understanding it. Although, Cardiovascular Disease (CVD) constitution at any age, emerging evidence suggests that age and time since menopause deteriorate on cardiovascular outcomes. However, Manson et al. (2003) have reported that in clinical study coronary heart disease results were presented by time since menopause, the hazard ratios increased with greater distance from menopause (Manson et al., 2003). Also clinical studies showed that women are defended from CVD before menopause, suggesting an advantageous action of endogenous estrogens (Dubey et al., 2005). Addition of all these data on the vasculoprotective action of estrogens was also clearly demonstrated in all animal models of early atheroma (Clarkson and Appt, 2005; Holm et al., 1999). However, some beneficial effect of exogenous estrogens was inquired after the study of the heart and estrogen/progestin replacement study (Hulley et al., 1998) and the women’s health initiative.
(Rossouw et al., 2002). These studies performed that
declining estrogen levels and removing the protective effects of
menopause which is impossible to be stop without
hormonal therapy. Is one of solution ways physical
exercise in addition to the other treatment protocols
proven benefits. We know that there are close
relationship between physical exercise and disease rate.

If menopause is accepted as a complex of abnormal
conditions or a kind of disease and also estrogen
deficiency is considered as an oxidative stress producing
source, it is likely to try to connect a positive correlation
between menopause and physical exercise. Also physical
inactivity and ageing are widely showed appreciation of
as risk factors for the result of developing of coronary
artery disease. A female rat underwent ovariectomy
immitating a woman in the postmenopausal period. Therefore in
this study, we tried to answer three questions:

- Does exercise training affect the in ovariectomized
  hearts?
- Can exercise training prevent changes in cardiac
  histological findings in ovariectomized rat hearts?
- Can exercise training have a protective role against
  the deleterious effects of aging?

**MATERIALS AND METHODS**

**Animals and experimental groups:** Forty-eight adult
female Wistar albino rats, weighing 200-220 g were
obtained from Atatürk 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 Atatürk University local animal care
committee. Rats were housed in standard plastic cages on
sawdust bedding in an air-conditioned room at 22±1°C
under controlled lighting (14 h light 10 h dark cycle).
Standard rat chow and tap water were given ad libitum.
The rats were randomly divided into 6 groups with each
group containing eight rats:

- Young animal group (4-6 months)
- Old animal group (24-26 months)
- Ovariectomized animal group (9 months)
- Young exercise-trained animal group (4-6 months)
- Old exercise-trained animal group (24-26 months)
- Ovariectomized exercise-trained animal group
  (9 months) (Table 1)

**Ovariectomy surgery:** Bilateral ovariectomy was
performed as follows:

- Anesthetize rats with an intraperitoneal injection of
  20 mg kg⁻¹ thiopental sodium
- Shave the fur over the dorsal lumbar area
- Disinfect the skin with Betadine followed by an
  alcohol rinse
- A longitudinal incision (0.5-1 cm) was made in the
  midline area of the lower abdomen and the ovaries
  were removed
- Use cautery to control any bleeding
- Stitch the muscle with 3-0 absorbable sutures and
  use stainless steel wound clips (2-3 each side) to
  close the skin incision (Kharode et al., 2008;
  Albayrak et al., 2009)

After ovariectomy, rats were given 25 mg kg⁻¹
metamizol sodium as analgesic for 2 days and
1.75 mg kg⁻¹ amoxicillin once daily (i.m.) for 1 week to
protect the host against infection.

**Treadmill exercise:** All exercise-trained rat groups were
habituated to treadmill exercise during which time each rat
walked on a motor-driven treadmill at 15 m min⁻¹ speed
and 15° incline once every two days during 10 days for
three courses as 5, 10 and 15 min day⁻¹ totally for 30 days
(Delp et al., 1993; Kwak et al., 2010; Choi et al., 2010).

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<tr>
<th>Groups</th>
<th>1-10 days (once every other day)</th>
<th>11-20 days (once every other day)</th>
<th>21-30 days (once every other day)</th>
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<tr>
<td>Young (4-6 months old)</td>
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<td>Intaperitoneal thiopental Sodium 50 mg kg⁻¹</td>
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<td>Old (24-26 months old)</td>
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<td>Ovariectomized (9 months old)</td>
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<td>Young exercise-trained (4-6 months old)</td>
<td>15 min⁻¹ speed and 15° incline</td>
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<td>Old exercise-trained (24-26 months old)</td>
<td>5 min day⁻¹</td>
<td>10 min day⁻¹</td>
<td>15 min day⁻¹</td>
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<td>Ovariectomized exercise-trained (9 months old)</td>
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*No exercise*
**Tissue preparation processes:** At the end of the experiment, the rats were killed by an overdose of a general anesthetic (thiopental sodium, 50 mg kg⁻¹) and the heart samples were dissected out immediately and transferred into a 10% formaldehyde solution for light microscopy. On the following day, the samples were waited into same fixative (buffered formalin) for 24 h in room temperature.

After that routine preparation of samples according to conventional light microscopy they were dehydrated in graded alcohol series, embedded in paraffin wax and serially sectioned using a Leica RM2125RT microtome (Leica Microsystems, Wetzlar, Germany) and stained with Hematoxylin-Eosin (H and E) and Periodic Acid-Schiff (PAS). All sections were studied and photographed by a light photomicroscope (Olympus BH 40) (Altinkaynak et al., 2008; Halici et al., 2008).

**RESULTS**

**Conventional light microscopy by H and E and PAS**

**Young animal group (4-6 months):** The three layers that constitute the heart wall, the endocardium, myocardium and epicardium were detected very well. The endocardium that constitute three distinct components called as a single layer of squamous endothelial cells, subendothelial layer, containing elastic and collagen fibers and smooth muscle cells and subendocardial layer including small blood vessels, nerves and Purkinje fibers were in normal appearances. The myocardium which is the muscular wall of the heart, attached the myocardium to the fibrous cardiac skeleton had no abnormalities.

Cardiac muscle cells, main component of the myocardium shown in normal features in myocytes such as a cross-striated banding pattern, centrally located pale-staining nuclei, endomysial connective tissue containing a rich capillary network and intercalated disks found at the interface between adjacent cardiac muscle cells. Epicardium, the outermost layer of heart wall is also called visceral layer of the pericardium that is composed of a simple squamous epithelium known as a mesothelium were in normal appearances (Fig. 1a-f (group 1) and 2a-d (group 2)).

**Old animal group (24-26 months):** In old animal group, some of findings including such as protection structural integrity (Fig. 1a-f (group 1) and 2a-d (group 2)) were

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**Fig. 1:** Light microscopic photomicrograph of all groups (H and E)
similar to those seen in young animal group (Fig. 1a-f (group 1) and 2a-d (group 1)) and other findings such as an increase in fibrotic area and necrotic cell density and remarkable prominence in basement membrane thickness and amount of connective tissue elements were similar to those seen in ovariectomized animal group (Fig. 1a-f (group 3) and 2a-d (group 3)).

Ovariectomized animal group (9 months): In ovariectomy groups as in old animal group there were similar findings, except for presence of very dense fibrosis and an increase in necrotic cell density as well as distribution of normal appearance of heart tissue (Fig. 1a-f (group 3) and 2a-d (group 3)).

Young exercise-trained animal group (4-6 months): It was not observed significant change, all finding were similar to young animal group (Fig. 1a-f and 2a-d (group 4)).

Old exercise-trained animal group (24-26 months): It was determined a series of changes in the direction of improvement such as a decrease tendency in fibrotic area and necrotic cell density. It was also shown normalization in basement membrane thickness and amount of connective tissue elements (Fig. 1a-f and 2a-d (group 5)). Findings of old exercise-trained animal group (Fig. 1a-f and 2a-d (group 5)) more resembled young animal group (Fig. 1a-f and 2a-d (group 1)) rather than
ovariectomized animal group (Fig. 1a-f and 2a-d (group 3)) and ovariectomized exercise-trained animal group (Fig. 1a-f and 2a-d (group 6)).

**Ovariecotomized exercise-trained animal group (9 months):** Findings of this group were similar to old exercise-trained animal group (Fig. 1a-f and 2a-d (group 6)).

**DISCUSSION**

In this study, we evaluated some histopathological findings of myocardial structure in an *in vivo* menopausal rat model upon exercise training. Results from the present study in ovariecotomized rats provided important and novel evidence for exercise training as a cardioprotective alternative in ovarian sex hormone-deficient condition. The study points to a possibility that regular running with moderate intensity could normalize the alteration in type of cardiomyocyte with ovariectomy. The initial results suggested overall harm from hormone replacement therapy, leading to a dramatic worldwide decrease in its use and concerns from clinicians and regulatory authorities. The study indicates the future important of exercise training as an alternative strategy. The American heart association scientific statement, recently has published the evidence for the efficacies of physical activity in the prevention and treatment of cardiovascular disease (Thompson *et al.*, 2003). Now-a-days, there is evidence for confidence of increased exercise for the patients; additional physiological and basic study is needed to improve the scientific meaning to support the importance of such a recommendation. In the present study ovariectomy groups, other findings were almost similar to old animal group except two observations. One of them was very dens fibrosis and an increase in necrotic cell density.

Other was distribution of normal appearance of heart tissue. Finding of old exercise-trained animal group more resembled young animal group rather than ovariectomized animal group and ovariectomized exercise-trained animal group.

Scheuer (1982) discussed the hypothesis that physical exercises are beneficial to the heart also chronic repeated exercise increases myocardial vascularity and protects the myocardium against ischemic insult (Scheuer, 1982).

White *et al.* (1998) have reported that exercise induced cardiac capillary growth but this event rapidly followed by vascular remodeling which leads to an increase in the number and size of arterioles (White *et al.*, 1998). Thus, reports on exercise-induced angiogenesis which focus on capillary density fail to convey the global picture of exercise-induced increase in cardiac blood supply.

Ho *et al.* (1983) presented that coronary vascular cast weights increased in relation to heart weight only after endurance rats were trained for 16 weeks by exercises and training (Ho *et al.*, 1983). Treadmill training for long period increased the maximum coronary blood flow in dogs (Laughlin, 1985) and increased the number of coronary resistance arterioles in pigs (Bresch *et al.*, 1986). Bronikowski *et al.* (2003) showed that exercise prevented from progress the effects of ageing on heart muscle by limiting changes in gene expression (Bronikowski *et al.*, 2003).

There is now a critical mass of data to support that age or time since menopause importantly influences the benefit-risk ratio associated with heart histopathological changes, especially with respect to very dens fibrosis and an increase in necrotic cell density causes myocyte defect outcomes.

Clinically, the use of treadmill exercises would be too early to suggest for effective treatment of cardiovascular disease but at least exercise training as an additional treatment which is more common in late phase menopause. Additional research is needed to help postmenopausal women in decision making.

**CONCLUSION**

In this study, the use of treadmill exercises would be too early to suggest for effective treatment of cardiovascular disease but at least exercise training as an additional treatment which is already more common in late phase menopause

**REFERENCES**


