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HACETTEPE UNIVERSITY

GRADUATE SCHOOL OF HEALTH SCIENCES

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DOCTORAL DISSERTATION

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APPROVAL

This dissertation has been approved by the committee above in conformity to the regulations and bylaws of Hacettepe University Graduate Program and has been accepted by the Board of Directory of the Institute of Health Sciences.

Prof. Ersin FADILLIOĞLU, MD, PhD
Institute Director

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ABSTRACT

AZADPOUR, N. The effects of 10 weeks of aerobic exercise training on angiotensin (ACE) and adrenergic receptor beta 2 (ADRB2) gene expression, circulating level of angiotensin II and flow mediated dilation in obese postmenopausal women with prehypertension. Graduate School of Health Sciences Sport Sciences and Technology Doctoral Dissertation, Ankara, 2015. Menopause, obesity, physical inactivity and genetic predisposition are the major risk factors for high blood pressure (BP) in women. In postmenopausal women (PMW), reduction in estradiol and estrogen/testosterone ratio as well as obesity leads to activation of sympathetic nervous system, renin-angiotensin system, and endothelial dysfunction that are associated with hypertension. Moderate intensity aerobic exercise training (AET) is known as important modality in the prevention and treatment of hypertension. The purpose of this study was to determine the effect of 10 weeks AET on BP, ACE and ADRB2 gene expression, Ang II plasma level, and flow-mediated dilation (FMD) in obese PMW with prehypertension. Twenty four obese PMW (aged 50-70 years; BMI $\geq$ 30 kg/m^2 and BP less than 139/89 mmHg) randomly assigned into two groups: Control (n=12) and Exercise (n=12). Exercise group performed AET (25-40 min/day, 3 days/week at 50-70% of heart rate reserve) for 10 weeks. Body composition, $VO_{2\ max}$, BP, ACE and ADRB2 gene expression, Ang II plasma level and FMD were measured before and after training program. General linear model for repeated measures (2×2) (time × group) ANOVA was used to analyze treatment effects and group differences. Systolic and diastolic BP were significantly reduced after AET. There was a marked increase in ADRB2 and a significant decrease in ACE mRNA levels. Also, Ang II plasma level was reduced and FMD was improved after AET. Results showed a significant inverse correlation between ADRB2 with systolic and diastolic BP, ACE and Ang II, and positive correlation with FMD. Also, there was a significant positive correlation between ACE and both systolic and diastolic BP, and an inverse correlation with FMD. Significant positive correlations of Ang II with systolic and diastolic BP and an inverse correlation with ADRB2 were observed. Our results showed that 10 weeks moderate intensity AET modulates ACE and ADRB2, decrease Ang II plasma levels and improves endothelial function in obese PMW and these alterations are associated with reduction in BP (4.6% and 2.4%, respectively) and improvement in body composition.

Key words: Prehypertension, ACE, ADRB2 gene expression, Angiotensin II, Flow- mediated dilation, Obese postmenopausal women, Exercise training

ÖZET

AZADPOUR, N. 10 Haftalık aerobik egzersiz programının postmenapozal, obez, prehipertansif kadınlarda ACE ve ADRB2 gen ekspresyonu, plazma anjiyotensin II ve akıma bağlı dilatasyon üzerine etkisi. Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü Spor Bilimleri ve Teknolojisi Programı Doktora Tezi, Ankara, 2015. Menapoz, obezite, fiziksel inaktivite ve genetik yatkınlık kadınlarda hipertansiyon için temel risk faktörlerindendir. Postmenapozal kadınlarda, hipertansiyonla ilişkili olarak estradiol ve östrojen/testosteron oranındaki azalmanın sempatik sinir sistemi ve renin-anjiyotensin sistemi aktivasyonuna ve endotelyal fonksiyon yetersizliğine yol açtığı ileri sürülmüştür. Diğer taraftan orta şiddetli aerobik egzersize katılımın hipertansiyonu önlenmesi ve control edilmesinde önemli bir yöntem olduğu bilinmektedir. Bu nedenle, bu çalışmanın amacı 10 haftalık orta şiddetli egzersiz programının postmenapozal obez, prehipertansif kadınlarda anjiyotensin dönüştürücü enzim (ACE) ve beta 2 adrenerjik reseptör (ADRB2) mRNA gen ekspresyonu, plazma anjiyotensin II (Ang II) düzeyi ve akıma bağlı dilatasyon (FMD) üzerine etkisini incelemektir. Bu çalışmaya 24 obez postmenopozal kadın (50-70 yaş arası; BKİ≥ 30 kg/m^2 ve kan basıncı <139/89) katılmıştır. Katılımcılar rastgele yöntemle kontrol grubu (n=12) ve egzersiz grubu (n=12) olmak üzere iki gruba ayrılmıştır. Egzersiz grubu 10 haftalık orta şiddetli egzersiz programına (25-40 dk/gün, 3 gün/hafta, %50-70 rezerv kalp atım hızı aralığında) katılmıştır. Egzersiz programından önce ve sonra vücut kompozisyonu, $VO_{2\ max}$, kan basıncı, ACE and ADRB2 gen ekspresyonu, Ang II plazma düzeyi and FMD ölçümleri tekrarlanmıştır. Egzersiz etkisi ve gruplar arasındaki farklılığı analiz etmek amacıyla 2x2 (Grup x Zaman) tekrarlı ölçümlerde ANOVA yöntemi kullanılmıştır. Egzersiz grubundaki postmenopozal obez kadınlarda sistolik ve diastolik kan basıncı anlamlı olarak azalmıştır. ADRB2 anlamlı olarak artarken, ACE mRNA düzeylerinde anlamlı bir azalma görülmüştür. Egzersiz grubunda Ang II plazma seviyeleri azalmıştır. Ayrıca FMD anlamlı olarak artış göstermiştir. Sonuçlar, ADRB2 ve sistolik kan basıncı, diastolik kan basıncı, ACE ve Ang II arasında negatif korelasyon olduğunu; ADRB2 ile FMD arasında ise pozitif korelasyon olduğunu göstermektedir. Ayrıca ACE ile hem sistolik hem de diastolik kan basıncı arasında anlamlı pozitif korelasyon, ACE ile FMD arasında ise negatif korelasyon olduğu belirlenmiştir. Ang II ile sistolik ve diastolik kan basıncı arasında anlamlı pozitif korelasyon görülürken, Ang II ile ADRB2 arasında negatif korelasyon görülmüştür. Bu çalışmanın bulguları, postmenapozal prehipertansif obez kadınlarda 10 haftalık orta şiddetli aerobik egzersizin ACE ve ADRB2'yi etkilediğini, Ang II plazma seviyelerini azalttığını ve endotelyal fonksiyonu iyileştirdiğini göstermiştir. Bu değişiklikler kan basıncının azalması (%4.6 ve %2.4) ve vücut kompozisyonunun iyileşmesi ile birlikte görülmüştür.

Anahtar kelimeler: Prehipertansiyon, ACE, ADRB2 gen ekspresyonu, anjiyotensin II, akıma bağlı dilatasyon, obez postmenapozal kadınlar, egzersiz

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ABBREVIATIONS

ACE	: Angiotensin Converting Enzyme
ADRB2	: Beta 2 Adrenergic Receptor
αARs	: Alpha Adrenergic Receptor
Adrenoceptors	: Adrenergic Receptors
Ang II	: Angiotensin II
Ang I	: Angiotensin I
ARs	: Adrenergic Receptors
AT1	: Angiotensin II Receptor Type 1
βARs	: Beta-adrenergic Receptors
βARK1	: Beta-adrenergic Receptor Kinase 1
BF%	: Body Fat Percentage
BMI	: Body Mass Index
BP	: Blood Pressure
cAMP	: Cyclic Adenosine Monophosphate
cDNA	: Complementary DNA
cGMP	: Cyclic Guanosine Monophosphate
CV	: Coefficient of Variation
CVD	: Cardiovascular Disease
DBP	: Diastolic Blood Pressure
eNOS	: Endothelial Nitric Oxide Synthase
FMD	: Flow-Mediated Dilation
GPCR	: G Protein-Coupled Receptor
GRK	: G Protein-Coupled Receptor Kinases
GRK2	: G Protein-Coupled Receptor Kinase 2
HTN	: Hypertension
mRNA	: Messenger Ribonucleic Acid
NADH	: Nicotinamide Adenine Dinucleotide

NAPDH	: Nicotinamide Adenine Dinucleotide Phosphate
NO	: Nitric Oxide
NOS	: Nitric Oxide Synthase
IPAQ-S	: International Physical Activity Questionnaire-Short Form
PAR-Q	: Physical Activity Readiness Questionnaire
PKA	: Protein Kinase A
Qpcr	: Quantitative Real-Time Polymerase Chain Reaction
SBP	: Systolic Blood Pressure
SNS	: Sympathetic Nervous System
TMB	: Tetramethylbenzidine
RAS	: Renin-Angiotensin System
ROS	: Reactive Oxygen Species
VO_2 max	: Maximal Oxygen Uptake
VSMCs	: Vascular Smooth Muscle Cells

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CHAPTER 1
INTRODUCTION

Cardiovascular disease (CVD) is the major cause of death in the world (1). Hypertension (HTN) is the most important risk factor for CVDs (2). In addition, prevalence of HTN in postmenopausal women is higher than compared with men (3). Meanwhile, current evidence have shown that the risk of cardiovascular death, myocardial infarction and stroke in women with pre-hypertension is higher compared to normotensives. Diseases associated with HTN have chronic disabling nature and mostly lead to repeated hospitalization. Moreover, expensive drugs are required for the treatment of these diseases. Although the prevalence of HTN in postmenopausal women is higher than in age-matched men (4), HTN in postmenopausal women has received little attention and less well controlled than men.

The major risk factors for HTN include pre-hypertension, aging, obesity and physical inactivity (5). In addition to the environmental factors, emerging evidence have revealed genetic basis of HTN (6). However, the genetic factors involved in HTN, particularly in postmenopausal women are not fully investigated.

HTN results from a disturbance in blood pressure (BP) regulation systems such as sympathetic nervous system (SNS) (7), renin-angiotensin system (RAS) (8) and endothelial function. In postmenopausal women, it seems that a reduction in estradiol and in the estrogen to testosterone ratio after menopause lead to activation of SNS, RAS and endothelial dysfunction (9). On the other hand, weight gain or obesity a common condition in postmenopausal women is also associated with an increased activation of the SNS and RAS, and endothelial dysfunction (10,11).

Recent studies have revealed that alterations in the genetic components of BP regulation systems may influence HTN. In this regard, angiotensin converting enzyme (ACE) (RAS component) and β2 adrenergic receptor (ADRB2) (SNS component) have been implicated as candidate genes involved in the induction of HTN (12,13). ACE converts angiotensin I to angiotensin II (Ang II) that is a potent vasoconstrictor, and

ADRB2 is involved in vasodilation. Therefore, both of these genes have critical role in BP regulation.

Additionally, activation of the RAS and SNS have been implicated in obesity-related HTN (14) and studies have also revealed an important role for circulating level of Ang II (an important component of RAS) in obesity (15) and obesity-induced HTN (16). Meanwhile, synergistic role of Ang II and sympathetic activity in obesity associated HTN has been demonstrated (17). Furthermore, due to the lack of estrogen, menopause in women has been associated with endothelial dysfunction which is a well-demonstrated independent predictor of cardiovascular events in hypertensive postmenopausal women (18). Endothelial function is most widely assessed by flow-mediated dilation (FMD) in the brachial artery which is accepted as an index of nitric oxide (NO)-mediated endothelium-dependent vasodilator function in humans (19).

On the other hand, increased level of BP acts as a biomechanical stress that influences on blood cells. Since, blood cells include many genes involved in SNS, RAS, inflammation and oxidative stress; they can response to changes in their environment with alterations in the expression of these genes. In this way, it has been suggested that blood cells can provide disease-specific data (20).

On the other side, aerobic exercise training, particularly moderate intensity exercise is known as one of the most important factors in reducing risk of mortality and in the management of HTN (21). Furthermore, studies have suggested that individuals with pre-hypertension without clinical evidence of CVD are generally treated with non-pharmacologic therapies such as increased physical activity. Possible mechanisms involved in mediating BP-lowering effect of regular exercise include reduction in activity of SNS and RAS, attenuated endothelial dysfunction, and decreased abdominal visceral fat (22).

Although the BP lowering effect of exercise training through its effects on SNS, RAS and endothelial function has been well documented, research on the effect of exercise on BP regulating genes is limited. It has been reported that reduction in plasma norepinephrine (as an index of systemic SNS activity) following exercise training is associated with decrease in SNS activity in hypertensive individuals (23). Decreased

norepinephrine at the synapse results in decreased vascular resistance and lowers BP. Also, insulin resistance has close association with SNS activity and exercise training can reduce SNS activity due to the improvement in insulin sensitivity. Furthermore, because renin and Ang II play important role in vasoconstriction and BP regulation, exercise training can decrease BP by modulating these components. However, the effects of exercise training on modulation of these components are not consistent. In addition, exercise training improves endothelial function due to increased bioavailability of NO and vasodilation. Swift et al (24) have reported improvement in FMD following 6 months physical activity in postmenopausal women with pre-hypertension and HTN. Moreover, exercise training reduces BP due to reduction in visceral fat (25).

Nevertheless, there are few studies on the effects of exercise on genes involved in BP control. For example, a recent study by Tartibian et al (26) reported increased ADRB2 gene expression and decreased ACE gene expression following 8 weeks exercise training in leukocytes of untrained middle-aged men. Litwin et al (27) indicated inhibition of ACE expression in leukocytes of hypertensive children after 6 months non-pharmacological intervention (physical activity and dietary guideline). Mousa et al (28) shown that exercise training prevents the increase in Ang II. Yet, there are inconsistent results about the role of ADRB2 in HTN (29) and effect of exercise on expression level of this gene (30,31).

Given the positive effects of physical activity on the treatment of pre-hypertension and HTN in postmenopausal women and considering that to date there is no study investigating the role of exercise training on expression level of genes involved in the induction of HTN in postmenopausal women, it is logical to hypothesize that exercise training program may induce alterations in these genes and improves endothelial dysfunction and Ang II levels in this group. These alterations may be associated with reduction in BP in obese postmenopausal women with pre-hypertension.

1.1. Research Problem

Whether 10 weeks moderate intensity exercise training affects ACE and ADRB2 gene expression levels in obese postmenopausal women? Whether 10 weeks exercise training affects FMD and Ang II circulating level and decrease BP in this group?

1.2. Aim of the Study

The first aim of the study was to investigate the effects of a 10 weeks of moderate intensity exercise training on gene expression of ACE and ADRB2, and circulating level of Ang II in obese postmenopausal women with pre-hypertension. The second aim of the study was to determine the effect of 10 weeks of exercise training on FMD and BP in the same group.

1.3. Hypothesis

10 weeks of moderate intensity exercise training will;

1. Increase ADRB2 gene expression in leukocytes of obese postmenopausal women with pre-hypertension.
2. Decrease ACE gene expression in leukocytes of obese postmenopausal women with pre-hypertension.
3. Decrease circulating levels of Ang II in obese postmenopausal women with pre-hypertension.
4. Improve FMD of the brachial artery in obese postmenopausal women with pre-hypertension.

1.4. Significance of the Study

Obesity and menopause are associated with HTN. But, HTN has not been thoroughly investigated in obese postmenopausal women. Recently, the role of genetic components of SNS and RAS (such as ACE and ADRB2) in high BP have been received much attention. Regular physical activity has been effective in the prevention and treatment of HTN. However, some studies have reported that exercise training has no effect on BP in obese postmenopausal women (32). On the other hand, in hypertensive

patients, blood peripheral leukocytes represent differential expression of genes involved in BP control (33). Interestingly, treatment of hypertensive patients with pharmacological interventions such as ACE-inhibitor has corrected these perturbations in leukocytes gene expression. Accordingly, we can hypothesize that the effect of physical activity in lowering BP, at least in part, mediated through alteration in genes involved in BP control system. To our knowledge, this is the first study investigating expression of genes involved in BP regulation in leukocytes of obese postmenopausal women. Indeed, this study indicates effects of moderate intensity exercise training on gene markers involved in BP regulation. It is possible that this approach can give information about hypertensive patients, which not effectively respond to non-pharmacological treatment (such as physical activity and dietary intervention).

CHAPTER 2
REVIEW OF LITERATURE

CVDs are the number one cause of death worldwide (1). Studies have shown that more people die annually from CVDs than from any other cause (1). The number of people, who die from CVDs, mainly from heart disease and stroke, will increase to reach 23.3 million by 2030 (1).

Studies have identified modifiable and non-modifiable risk factors for CVDs. Non-modifiable risk factors include gender, aging, family history of heart disease, menopause and race. Modifiable risk factors include high BP, physical inactivity (3), obesity, smoking, excessive alcohol consumption , hyperlipidemia and diabetes mellitus.

Among the modifiable cardiovascular risk factors, high BP or hypertension has been recognized as a major risk factor for CVDs such as stroke, coronary heart disease, heart failure, peripheral arterial disease, and kidney failure. According to the World Health Organization (34) HTN is the number one risk factor for mortality Hence, the purpose of this study was to determine the effects of aerobic exercise training on BP and several factors involved in BP regulation in postmenopausal obese women with pre-hypertension. This chapter will present a review of the literature regarding all relevant topics of the study, including: HTN in postmenopausal women; SNS and RAS as major mechanisms involved in BP control (with attention to systemic and genetic factors involved in the systems), and endothelial function; importance of exercise in reducing BP with special attention to the effect of exercise training on genes involved in BP control systems and endothelial dysfunction in obese postmenopausal women.

2.1. Hypertension

HTN is defined as systolic blood pressure (SBP) greater or equal to 140 mmHg or diastolic blood pressure (DBP) greater or equal to 90 mmHg. However, the Joint National Committee on High Blood Pressure (35) identified a new category of BP in adults termed pre-hypertension. Pre-hypertension indicates SBP 120-139 mmHg or DBP 80-89 mmHg. Therefore, it is estimated that high percent of adults falls into this category.

In this regard, a community-based prospective cohort study (Framingham Study) (36) has indicated that the risk of CVDs was greater among men and women with “high-normal” BP (130 to 139 mmHg systolic or 85 to 89 mmHg diastolic) compared with individuals with BP < 120/80 mmHg.

Additionally, study by Qureshi et al (37) has shown that pre-hypertension has been related to an increased risk of myocardial infarction and coronary artery disease but not stroke. Therefore, pre-hypertension should not be ignored as it can lead to high BP.

2.2. Types of Hypertension

HTN is classified as primary or secondary. Primary or "essential" HTN accounts for 95% of persons diagnosed with HTN. Although, it has no known cause a variety of environmental factors (such as obesity, sedentary lifestyle, lack of physical activity, smoking, diabetes, high levels of salt intake), physiological factors such as aging, as well as genetic factors are involved (38). Secondary HTN has multiple etiologies, including renal, vascular, and endocrine causes and accounts for 2-10% of the cases.

2.3. Prevalence and Risk Factors for Hypertension

Aging is the major risk factor of pre-hypertension and HTN, and studies (4) have shown that BP increases with age in both men and women. In this regard, Framingham Heart Study (39) has demonstrated that more than 90% of normotensive adults in midlife develop HTN in their lifetime.

BP is typically lower in premenopausal women than in men. However, after menopause, the prevalence of HTN in women is higher than in aged-match men (3), with 41% of postmenopausal women becoming hypertensive (National Center for Health Statistics) (3). In fact, aging remains one of the most important determinants for postmenopausal HTN (40). Worldwide, 25% of adult women are hypertensive. Furthermore, Women's Health Initiative study (41), involving over 60.785 postmenopausal women followed for 7.7 years, has reported an increased risk of myocardial infarction, stroke and cardiovascular death in women with pre-hypertension compared to normotensive counterparts.

Growing evidence have shown that aging-induced decrease in NO bioavailability and increase in oxidative stress result in endothelial dysfunction and subsequent HTN (42). However, in postmenopausal women, hormonal changes (estrogen deficiency) are associated with endothelial dysfunction and high BP (43).

Obesity is also one of the most prevalent risk factors for development of HTN, and studies (44) have indicated that at least 75% of the incidence of HTN is related directly to obesity. Moreover, several studies (44) have found close association between abdominal visceral fat with BP.

Indeed, obesity through abdominal visceral fat leads to SNS overactivity that is associated with increase in renin and Ang II generation. Elevated levels of these components increase adrenal aldosterone formation and sodium retention. Also, increased visceral fat is associated with elevation in inflammatory markers and oxidative stress, which result in decreased endothelial vasodilation (45).

Along with aging and obesity, physical inactivity has been identified as a major risk factor for CVDs including HTN. Strong evidence indicates that low levels of physical activity are associated with an increase in the incidence of HTN. A detailed review of literature on the relation between exercise and BP has been presented in the following sections under the heading of "*2.6. Effect of Exercise Training on Arterial Blood Pressure*".

2.4. Pathogenesis of Hypertension

The pathogenesis of HTN is multifactorial. It is a complex interaction between genetic factors, lifestyle and environmental influences, and disturbances in vascular structure and neurohumoral control mechanisms. However, an increase in peripheral vascular resistance is a hallmark of established HTN. Although, several mechanisms have been suggested for increased peripheral resistance in HTN, however, abnormalities in BP regulation systems such as the SNS (7) and/or in the RAS (8) and endothelial dysfunction (46) are of great importance. On the other hand, recent studies have shown that genetic variations involved in these regulatory systems could play a role in the induction of HTN. In this regard, emerging evidence has identified a variety of candidate genes in HTN as

well as their interaction with one another. Among these hypertension-predisposition genes investigated, genes involved in the RAS and SNS have attracted much attention (13). A detailed literature review of the factors associated with HTN follows.

2.4.1. The Role of Sympathetic Nervous System on Blood Pressure

The sympathetic arm of autonomic nervous system plays an important role in the regulation of BP through alterations in cardiac output, vascular tone, and renal sodium reabsorption. The role of SNS in short-term regulation of BP through baroreflex mechanisms is well established. However, recent studies (47) have documented the important role of SNS in long term regulation of BP.

Indeed, SNS regulates BP through different neural systems, known as the adrenergic receptors (adrenoceptors or ARs). These receptors affect blood vessels and heart by changing vascular tone. Furthermore, several studies (48) have suggested increased SNS activity as the primary precursor of HTN in both humans and animal models of the disease.

On the other hand, aging is associated with overactivity of the SNS (49) and elevated plasma catecholamine levels. Several mechanisms have been suggested for aging-induced increase in SNS includes a loss of central inhibitory pathways in the brainstem, aging-induced decrease in vessels distensibility and aging-associated increase in fatness (due to inflammatory mediators).

As the SNS acts through the adrenergic receptors and the effect of exercise training on ADRB2 expression has been investigated in the present study, a detailed discussion of these receptors is essential.

Sympathetic Adrenergic Receptors

Sympathetic adrenergic receptors are the components of SNS that play a significant role in the regulation of cardiovascular system. They are a class of G protein-coupled receptors (GPCRs) that are targets of the catecholamines, especially norepinephrine and epinephrine. Many cells possess these receptors, and the binding of a catecholamine to the receptor will generally stimulate the SNS. There are two main groups

of adrenergic receptors, alpha (α) and beta (β), with several subtypes (50). α adrenergic receptors (αARs) have the subtypes α1 and α2; and β adrenergic receptors (βARs) have the subtypes β1, β2, β3 and β4. Activation of α-adrenergic receptors with agonists causes vasoconstriction and a rise in BP, whereas activation of β-adrenergic receptors by specific agonists leads to vasodilatation and a consecutive fall in BP (50). Indeed, epinephrine stimulates both α- and β-adrenoreceptors, causing vasoconstriction and vasodilation, respectively. β adrenergic receptors are more sensitive to epinephrine than α-receptors. Therefore, low levels of circulating epinephrine result in vasodilation through the stimulation of β adrenergic receptors which leads to decreased peripheral vascular resistance and BP. On the other hand, high levels of circulating epinephrine result in vasoconstriction due to the activation of α-adrenergic receptors. This can be explained with presence of more α1 receptors than β receptors in peripheral vessels (Figure 2.1).

β1ARs are the dominant subtypes in the heart that are equally sensitive to epinephrine and norepinephrine (51). Generally, β1ARs promote cardiac chronotropic and inotropic responses (increasing heart rate and contractile force, respectively). While, β2ARs are responsible for vasodilation and have less sensitivity to norepinephrine than to epinephrine. Accordingly, studies have documented that the β2AR (ADRB2) is the primary adrenergic receptor that induces vasodilation in humans and impairment of ADRB2-mediated vasorelaxation has been reported both in human hypertensive patients (52) and in animal models of HTN (53). Since the activation of β2 adrenergic receptor leads to decreased BP, effect of exercise on ADRB2 gene expression has been investigated in this study. Hence, a detailed literature on β2 adrenergic receptors was presented below.

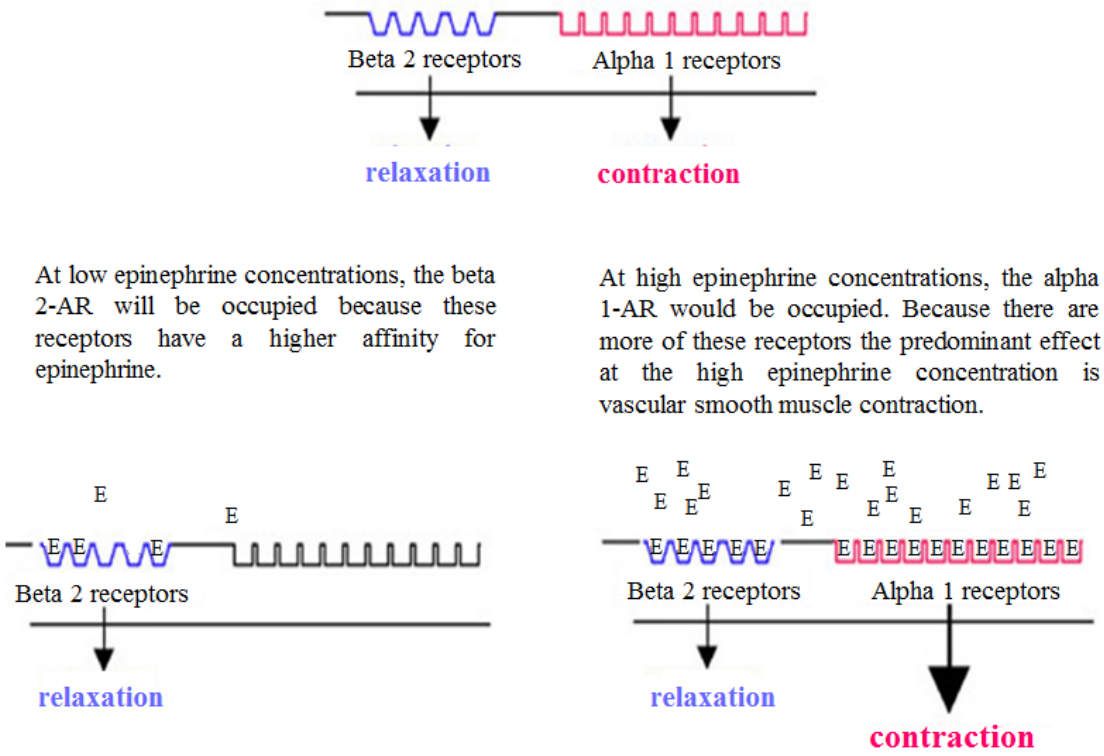

Figure 2.1. Effect of catecholamines on vascular smooth muscle. Adapted from (54).

β2 Adrenergic Receptors and Gene Expression

β2 adrenergic receptors are present in different cell types such as lymphocytes, vascular smooth muscle cells (VSMCs) and skeletal muscle cell and have various functions including modulation of hormone release and metabolic control. Moreover, they are essential regulators of cardiovascular homeostasis through activation of VSMCs and induction of vasorelaxation.

ADRB2 is the most abundant vascular ARs subtype (55) that has been identified as the predominant subtype mediating vasodilator response (56). Hence, ADRB2 play an important role in the regulation of the cardiovascular system (57).

Since ADRB2 mediates vasodilation in vascular smooth muscle, impairment of ADRB2 protein function may result to increased peripheral vascular resistance and HTN (58). In this regard, several studies (59,60) have shown that this receptor has been implicated in the pathogenesis of HTN and can be considered as a potential candidate gene

for the development of essential HTN. However, little is known about the genetic expression of ADRB2 in different human tissues in the context of clinical HTN.

For instance, recently Dungan et al (13) reported that patients with HTN had significantly less expression of ADRB2 gene (2.76-fold) in arterial tissue samples compared to normotensive subjects. They concluded that impairment of vasorelaxation in HTN can be due to down regulation of ADRB2 gene expression. Others (61) have shown that overexpression of ADRB2 in endothelium of spontaneously hypertensive rat carotid improved impaired adrenergic vasorelaxation. It has been also reported that ADRB2 density in cultured fibroblasts was correlated with human salt sensitivity, which is an important factor involved in the pathogenesis of essential HTN (62).

Another recent study (63) has shown a weak but statistically insignificant decrease in the mRNA levels of ADRB2 in lymphocytes of hypertensive compared with normotensive individuals. Besides, expression levels of ADRB2 in these patients significantly and inversely correlated with 24 h diastolic BP values that reflect direct participation of this subtype in the regulation of diastolic BP. However, several previous studies (29,64) have reported an increased density of ARRB2 in lymphocytes from hypertensive patients.

In addition, one study (65) on pregnant women has indicated a reduced number of functional ADRB2 in mononuclear leukocytes of pregnant women with pre-eclampsia compared with an increased density of functional ADRB2 in normal pregnant women that can be one of the several factors involved in pathogenesis of pre-eclampsia (increased peripheral vascular resistance in pre-eclampsia), while the increased number of functional ADRB2 may relate to the vasodilatation seen in normal pregnancy. On the other hand, aging is associated with alteration in β adrenergic receptors in human and animals.

Aging-induced Alteration in β Adrenergic Receptors

Experimental findings have revealed an age-associated decrease in βAR-mediated responsiveness in both human heart (66) and dorsal hand vein (67,68). In addition, studies in animals have reported aging-induced decrease in βAR mediated vasodilatation in rat skeletal muscles arterioles (69), rat aorta (70) and rat cardiac muscle (71). Also, Xiao et

al (72) reported aging-associated reduction in β1 adrenergic receptor (ADRB1) and ADRB2 subtype densities in rat ventricular myocytes. Aging-associated reduction in β adrenergic vasorelaxation in these studies is associtaed with reduction in the number or sensitivity of smooth muscle β receptors.

Vascular tone is regulated by both VSMCs and endothelial cells (by releasing different vasodilators and vasoconstrictors from endothelial cells) (73). As ADRB2 is expressed in both VSMCs and endothelial cells, it plays a critical role in age-mediated decline in vasoreactivity. In fact, aging-induced reduction in adrenergic responsiveness impairs vasodilatation and increases total peripheral resistance which has been suggested as the underlying mechanism for HTN in elderly (74).

Meanwhile, there is also evidence that prolonged exposure of ADRB2 to high levels of catecholamines (as observed in aging) leads to down-regulation and desensitization of these receptors.

Adrenergic Receptor Desensitization

Receptor desensitization refers to the process by which a receptor decreases its response to a signaling molecule when that agonist is in high concentration. Desensitization acts as an important protective mechanism in preventing overstimulation of the β2AR and possible organ damage. Desensitization occurs due to receptor phosphorylation, receptor internalization and receptor uncoupling. Importantly, receptor uncoupling refers to the uncoupling of the β2-AR and its second messenger system.

Several mechanisms involved in desensitization process. G protein-coupled receptor kinases (GRK) family play major role in this process. The β-adrenergic receptor kinase 1 (βARK1 or GRK2) member is a GRK that controls βARs signaling via receptor phosphorylation and results in desensitization. Increased expression of βARK1 has resulted in reduction in β adrenergic receptor function in the failing human heart (75). Also, lymphocyte levels of βARK1 mirror alterations in failing human heart and lower level of βARK1 is related with improved βAR signaling after treatment in these patients (76). Moreover, studies (77) have reported β-AR signaling dysfunction (desensitization) and decreased vasorelaxation in aged rat aorta due to up-regulation of βARK-1. Studies

(78) in spontaneously hypertensive rats suggest that up-regulation of βARK-1 in vasculature and lymphocytes might account for the impairment of βAR-mediated vasodilation observed in HTN.

There is evidence that βARK1 is expressed in endothelium. On the other hand, βARs vasorelaxation in the most of vasculatore involved in the determination of total peripheral resistance is largely endothelium dependent. Therefore, βARK1 plays important role in the control of the vascular tone.

Responsible Mechanisms for ADRB2-stimulated Vasodilation

The multiple intracellular signaling pathways involved in vasodilatory response to ADRB2 stimulation (Figure 2.2). β2ARs in vascular smooth muscle have a high binding affinity for circulating epinephrine. Stimulation of ADRB2 due to binding with epinephrine leads to coupling this receptor with G protein (guanine nucleotide–binding protein), which activates adenylyl cyclase and leads to formation of cyclic adenosine monophosphate (cAMP). cAMP activates protein kinase A (PKA) which reduces intracellular Ca^{2+} concentration in VSMC, thus promotes vasodilation (79).

In addition, studies (80) have shown the presence of cAMP-independent pathway that induces β2ARs-mediated vasorelaxation through activation of K^+ channels. Opening of these channels causes cell hyperpolarization and promotes the closure of voltage-dependent Ca^{2+} channels and subsequent relaxation of smooth muscle.

Moreover, it has been reported that the endothelium-derived NO increases smooth muscle cyclic guanosine monophosphate (cGMP) level and potentiates βARs mediated vasorelaxation through an increase in cAMP levels (81).

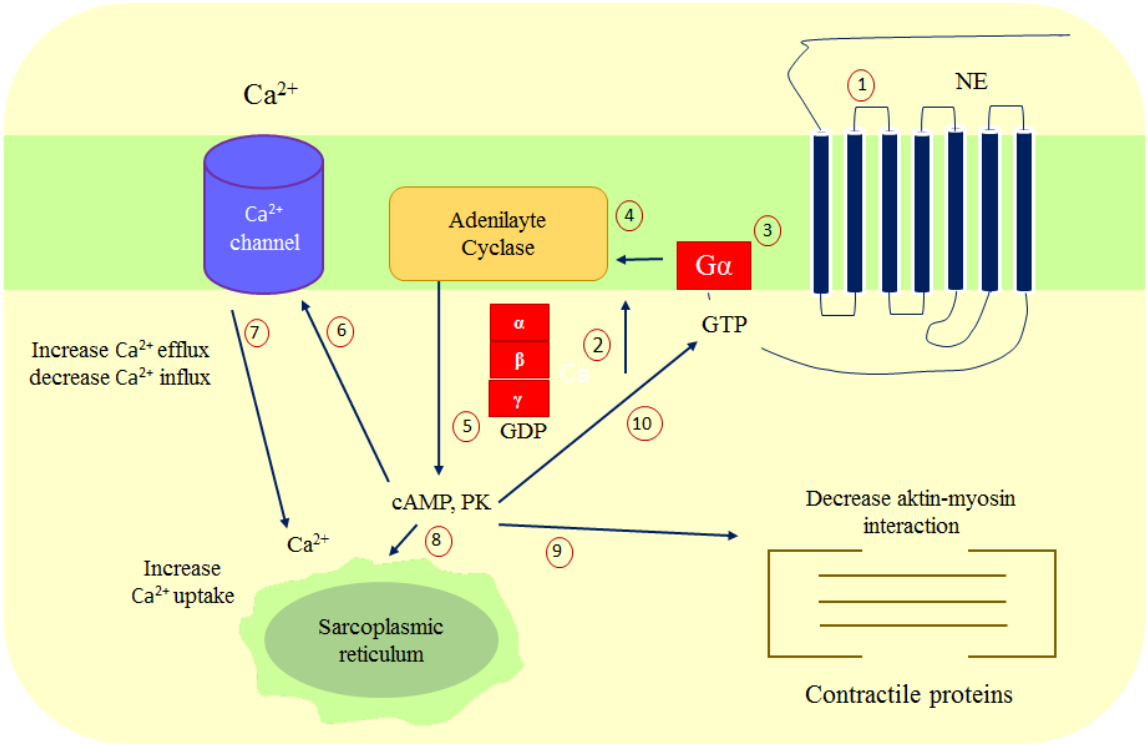

Figure 2.2. Effect of β2 receptor activation on smooth muscle. Adapted from (54).

As mentioned previously, there is an age-associated decrease in the ability of βAR to respond to agonists at the cellular level. However, studies have documented a modulatory effect of exercise training in these modifications. Since the ADRB2 has been expressed in human VSMCs, it is able to increase vascular relaxation at rest and during exercise. Impact of exercise on sympathoadrenergic system is well known and the regulation of this system plays critical role in physiological adaptation to exercise. In this regard, the effect of physical activity on gene expression of ADRB2 will be discussed in more detail later on under the subheading of "*2.6.3. Effect of Exercise Training on Beta 2 Adrenergic Receptor and Gene Expression*".

On the other hand, previous studies have revealed an interaction between SNS and RAS and sympatho-modulatory role of RAS in BP regulation (82). It has been reported that Ang II stimulates sympathetic nerve outflow in human and animals (due to modulation of norepinephrine release and α adrenergic receptor) (83).

2.4.2. The Role of Renin-Angiotensin System in the Regulation of Blood Pressure

In addition to the SNS, RAS plays a critical role in the regulation of BP and fluid balance in the body. When blood volume or sodium is low, or blood potassium is high, kidney releases renin hormone. Renin converts angiotensinogen synthesized by the liver to the hormone Ang I. The ACE converts Ang I into Ang II. Indeed, the RAS is the body's most important long-term BP regulation system, and ACE and Ang II are the important members of this system (Figure 2.3). Below a detailed review of literature was presented on ACE and Ang II.

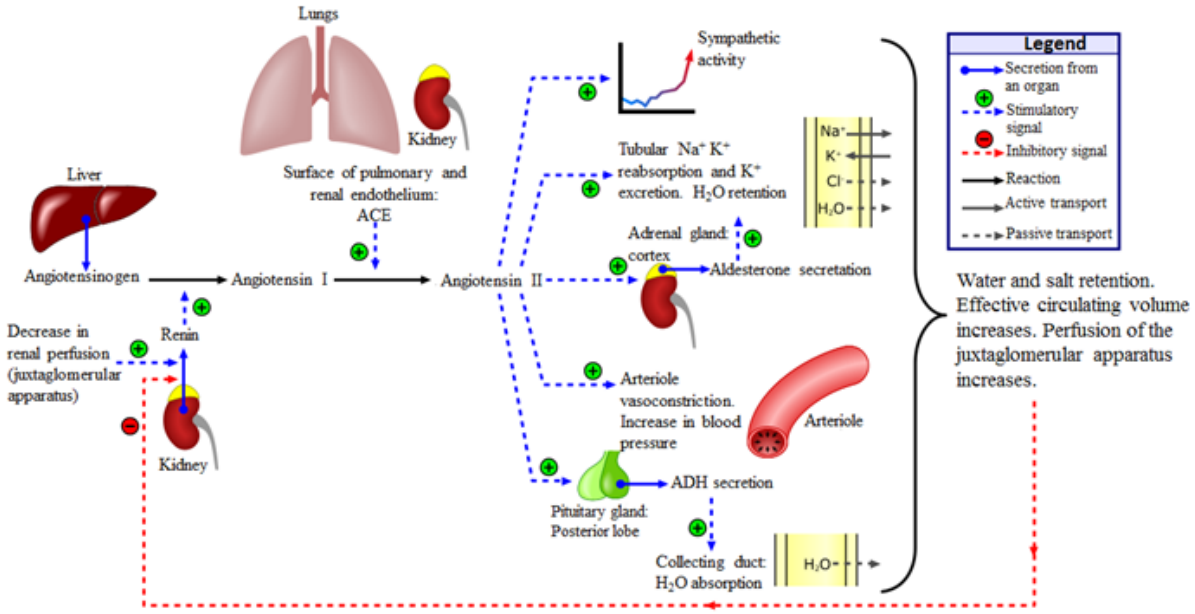

Figure 2.3. Renin angiotensin system. Adapted from (84).

Angiotensin-Converting Enzyme

ACE is secreted in the lungs and kidneys by endothelial cells of the blood vessels. It plays a central role in cardiovascular homeostasis. This enzyme indirectly increases BP by converting Ang I to Ang II (an extremely powerful vasoconstrictor) and by degrading bradykinin (a potent vasodilator). This critical role of ACE in the induction of HTN has been demonstrated in human and animal models of HTN.

Chandra et al (85) reported increased levels of ACE gene expression and protein in whole blood cells of patients with essential HTN compared to the normotensive control group. Also, Shiota et al (86) indicated an increased ACE gene expression in the aorta of hypertensive rats. Furthermore, in recent study by Litween et al (27), ACE mRNA expression in leukocytes of hypertensive children was higher than normotensive children. In human subjects, higher levels of plasma ACE have been associated with increased carotid wall thickness (87). In addition, one study (88) have demonstrated positive correlation between serum ACE with plasma Ang II in men. Similarly, increased level of vascular ACE activity was found to be associated with the increase in BP in hypertensive rats (86). Moreover, recent studies (89) using transgenic mice have indicated that the level of ACE gene expression plays an important role in arterial pressure regulation. On the other hand, genetic variations of components of the RAS such as ACE have been shown to be associated with susceptibility to essential HTN (90). In this regard, studies (91) suggest that in hypertensive but not normotensive subjects, ACE genotypes are involved in the regulation of aortic rigidity.

For this reason, ACE is considered as the first-line treatment for HTN. Indeed, inhibition of ACE by ACE inhibitors results in decreased formation of Ang II and decreased degradation of bradykinin, leading to arterial and venous dilation, and subsequent decrease in BP. In experimental studies (92,93), ACE inhibition have been associated with reduced Ang II levels in blood and tissues of rats and humans.

Thus, the ACE gene is also a candidate gene for essential HTN in humans. The effect of exercise training on ACE gene expression will be detailed under the heading of "*2.6.4. Effect of Exercise Training on Angiotensin Converting Enzyme and Gene Expression*".

Angiotensin II

In addition to ACE, Ang II has been known to play important role in the pathophysiology of CVD including HTN, heart failure, cardiac remodeling, myocardial infarction and left ventricular hypertrophy. Ang II is a potent constrictor of all blood vessels that acts on the musculature of arteries, increases the peripheral resistance and so

elevates BP. It should also be noted that vasoconstriction results from Ang II action on the angiotensin receptors. Indeed, Ang II increases BP by stimulating angiotensin II receptor type 1 (AT1 receptor) located on vascular smooth muscle and causes vasoconstriction. Of course, AT1 receptors are ubiquitously distributed throughout the body, including blood vessels, heart, kidney, adrenal gland, liver, brain and lung. One study (94) has reported that Ang II causes HTN and cardiac hypertrophy through its receptors (AT1) in the kidney. Therefore, down regulation of these receptors can modulate Ang II function. Furthermore, aging is associated with increase in circulating level of Ang II as wells as AT1 receptor density (95).

As mentioned previously, there is an interaction between RAS and SNS. In this regard, studies have shown a relationship between resting sympathetic nerve activity and plasma Ang II in animals (96). Also, a cross-talk between AT1 receptors and α1 adrenergic receptor subtype of SNS has been documented in elderly rat, which is pronounced in aging (97). Others (98) have revealed that the Ang II potentiates α-adrenergic vasoconstriction in the elderly human. Indeed, aging-associated increase in Ang II-mediated vasoconstriction, at least in part mediated through α adrenergic receptor.

On the other hand, increased circulating level of Ang II has been observed in obese hypertensive adults (99) and children (100) that is associated with high BP. Because, subcutaneous adipose tissue is known as the main source of Ang II and drains Ang II directly into circulation and can influence vasculature (101).

Other studies (102) have reported that Ang II activates blood monocytes in a dose dependent manner and results in subendothelial infiltration of these cells, which is possible mechanism in initiation of endothelial injury.

In addition, Ang II is involved in signaling pathways that lead to production of reactive oxygen species (ROS) and inflammation, which both of them play important role in HTN (103). Ang II increases ROS production in endothelial and vascular cells. There is the relation between Ang II-induced oxidative stress and CVDs. Ang II induces oxidative stress that plays an important role in the pathogenesis of HTN, especially in obesity-related HTN. Ang II activates vascular NADH/NADPH oxidase and promotes ROS, which can increase nitric oxide synthase (NOS) uncoupling in endothelial cells and

VSMCs (104). Uncoupling of NOS results in endothelial dysfunction and further increase in ROS production, and subsequently leads to increase in vasoconstriction. In addition, Ang II stimulates xanthine oxidase activation in endothelial cells, which indicates a novel mechanism of endothelial oxidative stress in response to Ang II (105).

More previous studies on the effect of exercise on circulating level of Ang II has been conducted in acute exercise protocols. These effects will be presented later under the heading of "*2.6.5. Effect of Exercise Training on Angiotensin II*".

2.4.3. The Role of Endothelial Dysfunction in Blood Pressure

Endothelium is known to play a critical role in vascular function. Endothelial dysfunction is defined as an imbalance between vasodilating and vasoconstricting factors produced by the endothelium. It is primary risk factor in the development of HTN and increases the risk of CVDs (46).

Endothelial function is most widely assessed by FMD in the brachial artery which is accepted as an index of NO-mediated endothelium-dependent vasodilator function in humans (19). FMD represents the endothelium-dependent relaxation of a conduit artery due to an increased blood flow. Growing evidence shows that FMD predicts cardiovascular events.

Several mechanisms involved in endothelial dysfunction in HTN and obesity-induced HTN. Decreased NO bioavailability due to impairment of endothelium-dependent vasodilation has been reported to play major role (106), but increased sympathetic activity and overactivation of RAS is of importance. NO acts as a vasodilator that increases blood flow and lowers BP, hence play critical role in endothelium. Furtheremore, NO is involved in the central regulation of sympathetic activity in humans, meaning that both neuronal and eNOS may contribute to the regulation of vasomotor vascular tone (107).

On the other hand, growing evidence indicates that endothelial dysfunction is associated with an increase in ROS and oxidative stress that results in NO uncoupling (inactivation). Likewise endothelial dysfunction is associated with decrease in antioxidant defense in hypertensive individuals. The overactivity of SNS is a common feature of

obesity that links to increase in BP. In this regard, one study (108) has reported that sympathetic stimulation reduces endothelium-dependent flow-mediated vasodilation. Additionally, another study (109) has shown that activation of vascular ADRB2 causes vasodilatation due to endothelium-dependent and -independent mechanisms, and that vasodilatation induced by ADRB2 activation is impaired in obese hypertensive individuals.

In addition to the aforementioned mechanisms, overactivity of RAS has been associated with endothelial dysfunction. In this regard, Ang II is contributed to endothelial dysfunction due to the induction of intracellular ROS in VSMCs and enhancement of the tyrosine phosphorylation of endothelial nitric oxide synthase (eNOS) through AT1 receptor and oxidative stress. This leads to uncoupling (eNOS generates O_2^- instead of NO) and attenuation of NO production and endothelium-dependent vasodilatation. AT1 receptors have major role in ROS generation and are critically involved in vascular remodeling processes leading to CVD. Indeed, these receptors induce NADH/NADPH oxidase that is the most important source of ROS (such as superoxide) in the vasculature. Recent studies have shown that endothelial dysfunction in aorta of spontaneously hypertensive rats is associated with increased activity of NADH/NADPH oxidase (110). On the other hand, aging is associated with endothelial dysfunction and decline in FMD in healthy individuals and in patients with essential HTN.

In addition to aging, menopause is associated with endothelial dysfunction due to a lack of estrogen in this phase of life (111). In this regard, studies (111) reported that in both normotensive and essential hypertensive women, menopause is associated with the onset or worsening of aging-associated endothelial dysfunction, respectively. Also, recent findings show that hypertensive postmenopausal women have abnormal endothelium dependent vascular function (112). On the other hand, prospective data (113) indicate that endothelial dysfunction acts as a promoter of HTN in apparently healthy postmenopausal women.

The effects of exercise on endothelial function will be presented later under the heading of *"2.6.6. Effect of Exercise Training on Flow-Mediated Dilation".*

2.5. Hypertension in Postmenopausal Women

HTN is probably the most important cardiovascular risk factor in postmenopausal women. Although responsible mechanisms for the postmenopausal HTN have not been elucidated, it is likely that underlying mechanisms may be similar in men and women. The difference is that, the lack of estrogen can exaggerate the underlying defects in postmenopausal women. The mechanisms responsible for postmenopausal HTN include changes in estrogen/androgen ratios, activation of the SNS (114) and RAS (115), oxidative stress (116), endothelial dysfunction and presence of obesity (117) (Figure 2.4).

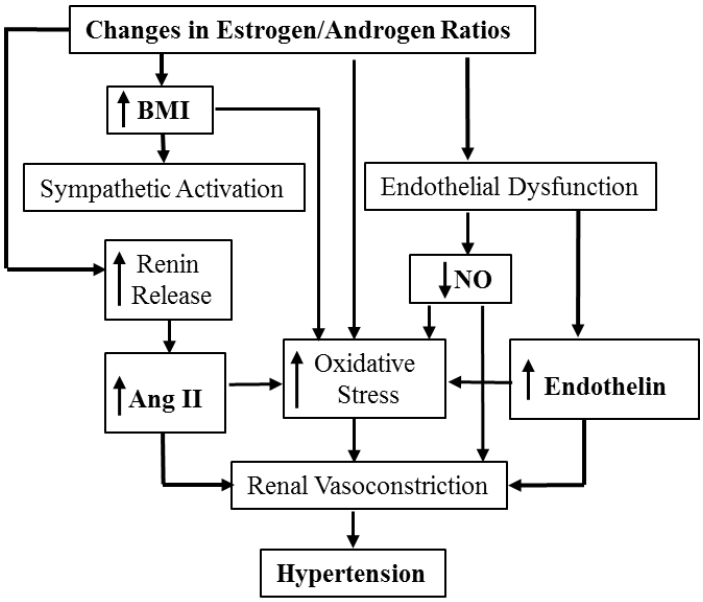

Figure 2.4. Menopause and hypertension. Adapted from Coylewright et al (45).

2. 5. 1. Menopause, Sympathetic Nervous System and Hypertension

Estrogen regulates BP through decrease in SNS activity (118). In animal models of HTN, it has been demonstrated that in old female animal, HTN is in part due to the activation of the SNS (114). In postmenopausal women short-term transdermal estrogen replacement therapy has reduced sympathetic nerve activity.

2.5.2. Menopause, Renin Angiotensin System and Hypertension

Estradiol has protective role in cardiovascular system by modulating components of RAS. So it can decrease expression of AT1 receptors in vessels and kidney (119), as well as reduces ACE activity (120). In this regard, Licy et al (121) have reported that RAS through AT1 receptors is an important mechanism involved in the pathophysiology of HTN in postmenopausal rat.

2.5.3. Menopause, Oxidative Stress and Hypertension

Estrogen decreases ROS formation through modulation of enzymes involved in oxidative stress (such as superoxide dismutase and NADH/NADPH oxidase). Therefore, estrogen deficiency in menopause can be associated with increased oxidative stress, vasoconstriction and HTN (122).

2.5.4. Menopause, Endothelial Dysfunction and Hypertension

Several studies have shown abnormal endothelial function in hypertensive postmenopausal women. Indeed, aging is associated with reduction in endothelium-dependent vasodilation (assessed by FMD) in hypertensive individuals. But, postmenopausal women have shown the highest rate of decrease in FMD (111). Also, it should be noted that in normotensive women, aging-induced endothelial dysfunction is only observed after menopause. Decreased endothelial-dependent vasodilation in postmenopausal women is related to decrease in NO production that improves with estrogen therapy (123).

2.5.5. Menopause and Obesity-induced Hypertension

Generally, menopause is related with a tendency to gain weight and weight gain is associated with increase in BP. The prevalence of obesity has been reported as high as 40% in postmenopausal women. Moreover, at least 75% of the incidence of HTN is related directly to obesity.

Relationship between body weight and BP has been demonstrated by Framingham Heart Study in the 1960s (124). Also, other population-based studies have shown close

relationship between excess adipose mass and HTN in men and women (125). Others have reported that weight gain lead to increased arterial pressure (126). Thus, excess weight gain is a good predictor for future development of HTN. Although the mechanisms of obesity-induced HTN are not fully understood, increased activation of SNS and RAS play key roles (Figure 2.5).

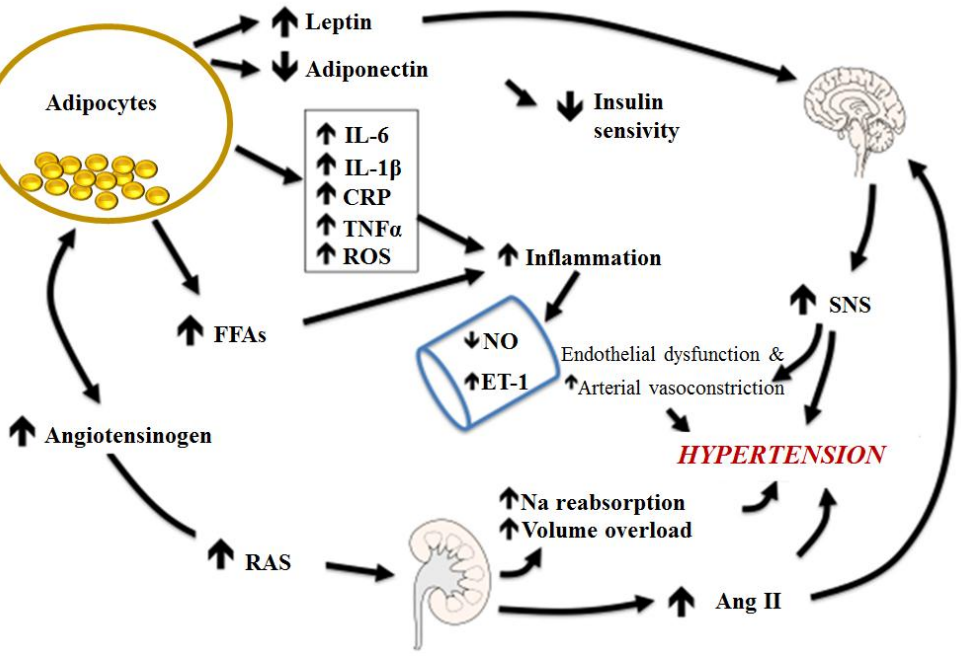

Figure 2.5. Mechanisms of obesity-induced hypertension. Adapted from Kotsis (15).

In this regard, the most recent evidence suggests that increased SNS activity contributes to obesity-induced HTN (127,128). Indeed, even modest weight gain in non-obese subjects is associated with increased SNS activity. Also, over activity in SNS is related to abdominal visceral fat (129).

In addition, human obesity is associated with increased leptin and insulin levels and several studies have reported that insulin and leptin stimulates the SNS. Furthermore, Hogarth et al (130) have reported that sympathetic activity in postmenopausal women is higher compared with premenopausal women. Considering that aging and obesity

increases SNS activity independently, it is reasonable to assume that the combination of these two factors in postmenopausal women might result in a greater activation of the SNS. Moreover, a large number of studies have shown that RAS is activated in obesity.

Possible underlying mechanisms include increased level of renin (due to stimulation of SNS) and production of Ang II; and production of angiotensinogen and Ang II in adipose tissue (131). These alterations result in increased adrenal aldosterone production and sodium retention. Several studies (132) have reported that Ang II increases sympathetic nervous activity through actions on both central and peripheral sites in human and animal with chronic heart failure.

On the other hand, synergistic role of Ang II and sympathetic activity in obesity-induced HTN has been demonstrated in several studies (11,17). Indeed, in obesity-related HTN, elevated level of Ang II increases sodium reabsorption (by direct effects on the kidney) as well as activates SNS, which both lead to increased BP. Accordingly, in an effort to find strategies for the prevention or treatment of HTN, studies have indicated that physical activity plays an important role in the etiology, prevention, and treatment of HTN and pre-hypertension.

However, the role of physical activity in all of the components of systems involved in BP control such as SNS, RAS and endothelial function is not completely clear. In the following section, the effect of exercise training on BP in pre-hypertensive and hypertensive postmenopausal women will be detailed under the heading of "*2.6.1. Effect of Moderate Intensity Exercise Training on Pre-Hypertension and Hypertension in Obese Post-Menopausal Women"*. Also, mechanisms involved in BP control have been reviewed under the heading of "*2.6.2. Exercise-Induced Mechanisms Involved in Reducing Blood Pressure".*

2.6. Effect of Exercise Training on Arterial Blood Pressure

Several investigations have demonstrated that regular exercise training not only reduces the incidence of HTN, but also lowers BP in hypertensive subjects. Randomized controlled trials have indicated that exercise training reduces BP up to 11 and 8 mmHg, for systolic and diastolic pressures, respectively, in 75% of hypertensive individuals of

both genders (21). On the other hand, moderate intensity exercise seems to be the most effective modality for reducing BP in hypertensive patients (21).

2.6.1. Effect of Moderate Intensity Exercise Training on Pre-Hypertension and Hypertension in Obese Post-Menopausal Women

Numerous studies have revealed that exercise training decreases cardiac risk factors such as HTN in postmenopausal women. In this regard, Wong et al (133) reported that 8 weeks stretch training reduces peripheral and central blood pressure and vascular sympathetic activity in obese postmenopausal women with pre-hypertension and hypertension. Others (134) indicated the effect of whole-body vibration exercise training (6 weeks) in reducing aortic blood pressure in postmenopausal women with pre-hypertension or HTN. Also, Jessup et al (135) reported that 16-weeks of endurance exercise training inhibits aging-associated increase in BP in older men and women.

In addition, significant reduction in systolic and diastolic BP was observed in postmenopausal women following 6 months of moderate intensity exercise training at 50% heart rate reserve (136). Also, Khalid et al (137) reported reduction in systolic and diastolic BP after 8 weeks moderate intensity exercise training in obese postmenopausal women. Furthermore, Goldie et al (138) reported reduction in systolic and diastolic BP in pre- and postmenopausal women with HTN following 12-week low-intensity exercise training (walking). Others (139) have shown improvement in BP following 8 weeks moderate intensity exercise training in postmenopausal hypertensive women.

2.6.2. Exercise-Induced Mechanisms Involved in Reducing Blood Pressure

Several mechanisms involved in exercise-induced reduction in BP. Mean arterial pressure is determined by cardiac output and total peripheral resistance, thus, reduction in arterial pressure after chronic exercise training is mediated by a decrease in these variables. In fact, studies have documented that decrease in total peripheral resistance is the primary mechanism by which resting BP is decreased after exercise training. It should be noted that training-induced reductions in vascular resistance are mediated by neurohumoral and structural adaptations and alteration in vascular responsiveness (22).

With regard to neurohumoral adaptations, exercise-induced alterations in SNS and RAS activity play a major role in reducing BP following training. It has been shown that exercise decreases SNS activity and increases baroreflex sensitivity. In addition, studies show that exercise training may reduce sympathetic nerve activity at rest in normotensive subjects. Furthermore, data from well-controlled animal studies suggests that exercise training reduces resting and reflex control of sympathetic outflow in normal animals (140).

On the other hand, there is evidence that exercise training may reduce activation of the RAS (141). Ang II is a powerful vasoconstrictor and regulator of blood volume. Therefore, exercise training-induced reduction in Ang II would likely be a contributor to reduced BP. In addition, animal studies have shown that chronic exercise lowered plasma Ang II concentration in chronic heart failure (96), which may contribute to the decrease in sympathetic nerve activity after exercise in patients with chronic heart failure.

Furthermore, considerable evidence suggests that exercise training induces structural adaptations include vascular remodeling (such as increased length, cross-sectional area, and diameter of already existing arteries and veins) and angiogenesis (new vessel growth). Therefore, increased total cross-sectional area of resistance vessels due to these alterations might be a possible mechanism to lower peripheral resistance and reduced resting BP.

Concerning the vascular adaptations, improvement in endothelial function due to the alteration in vascular responsiveness to the adrenergic receptors (such as α and β adrenoceptors) and also endothelium-dependent vasodilator and vasoconstrictor substances are of importance.

Moreover, numerous studies (142) have documented that endothelium-derived NO in peripheral vessels modulates vascular resistance and BP. In hypertensive subjects endothelium-dependent dilation is impaired and exercise training improves endothelium-dependent vasorelaxation due to an increase in NO bioavailability (143). These findings have been supported with experimental studies using normotensive and spontaneous hypertensive rats.

In addition to the described mechanisms for training-induced reduction in BP, genetic factors may be involved in the antihypertensive effects of exercise training that is

related to polymorphism of genes. The effects of genetic factors on BP have been viewed at rest and during exercise and the results obtained from the Heritage family study confirmed this view (144). For example, one study has reported that polymorphism of apolipoprotein E and ACE gene in hypertensive individual is associated with different training-induced alterations in resting BP (145).

Although BP lowering effect of exercise is well-documented, the effects of regular exercise training on different components of these systems in pre-hypertensive or hypertensive subjects have not been completely clarified. In this regard, studies examining the effects of exercise training on B2ADR, ACE, Ang II, and FMD have been presented in the following paragraphs.

2.6.3. Effect of Exercise Training on Beta 2 Adrenergic Receptor and Gene Expression

There are few studies investigating the effect of exercise on ADRB2 gene expression or density following different protocols of acute or chronic exercise training (26). Also, no consensus exists in the literature regarding changes in sensitivity or density of ADRB2 on different tissues following exercise training.

Physical activity was shown to be inversely (146,147), positively (31,148), or not related to (149,150) the density of β-ARs on immune cells in both human and animal studies.

Others have shown that 10 weeks treadmill running (151,152) and 6 weeks swimming exercise (153) resulted in decreased number of myocardial ADRB2 and reduced sympathomimetic effects in rats. Frey et al (150) investigated the effect of progressive exhaustive exercise on lymphocyte βARs in normal individuals and reported increased receptors density. Another study (154) found that 6 weeks swimming exercise (5days/wk) restored aging-associated reduction in βAR density of carotid arteris to the levels observed in young rat carotids. Moreover, one study (71) has shown that 12 weeks treadmill exercise training restored cardiac β-AR density in old rats.

A new study by Tartibian et al (26) reported an increase in ADRB2 mRNA expression following 8 weeks moderate intensity exercise training (intensity of 50–65%

maximal HR, 40–50 min/day, 4 days/week) in untrained normotensive and pre-hypertensive (SBP < 130 mmHg and DBP < 85 mmHg) middle-aged men. In addition, they have shown that increase in ADRB2 gene expression is associated with decrease in BP.

Also, Ogasawara et al (155) have indicated acute exercise-induced increase in the density of cell surface β-ARs in rat adipocytes.

2.6.4. Effect of Exercise on Angiotensin Converting Enzyme and Gene Expression

The effects of exercise training on RAS components are not completely understood. In this regard, there is little information on the effects of exercise on ACE gene expression in hypertensive human. In a recent study, Litwin et al (27) assessed the effect of 6 months non-pharmacological treatment (dietary advice and physical activities) on ACE mRNA expression in leukocytes of hypertensive children and reported inhibition of ACE gene expression and decrease in AT1 receptor mRNA. Also, decrease in ACE expression level is associated with reduction in BP and decrease in metabolic abnormalities. More recently, Tartibian et al (26) reported decrease in ACE mRNA expression in leukocytes of normotensive and pre-hypertensive untrained middle-aged men following 4 and 8 weeks of exercise training, which associated with increase in ADRB2 gene expression.

Animal studies (156) have shown that 8 weeks exercise training following post-myocardial infarction in rat mitigates the expressions of ACE in cardiac muscle. In addition, 3 weeks treadmill exercise (30 min/day, 6 days/week) has normalized brain ACE expression in rabbit with chronic heart failure (157). Fernandes et al (158) reported a decrease in ACE mRNA in rat cardiac muscle following moderate/high volume swimming exercise training.

2.6.5. Effect of exercise on Angiotensin II

Limited studies have investigated the effects of exercise training on Ang II levels and most of the studies have focused on Ang II response to acute bout of exercise. Also,

more studies have conducted in animals. For instance, Wei et al (100) reported decreased circulating levels of Ang II following 6 weeks aerobic exercise training include basketball, swimming and jogging at 60% -70% of maximum HR (40 min/days, 5 days/week) in obese children, which is associated with reduction in BP. Another study (159) found reduction in resting levels of Ang II following 16 weeks endurance exercise training (40% to 70% of peak oxygen uptake) in chronic heart failure patients.

However, Hespel et al (160) reported non-significant decrease in Ang II plasma levels after 4 months training (2.5 h/week) in sedentary men. Furthermore, one study (161) conducted in obese postmenopausal women, did not show any change in Ang II circulating levels following a 16-week weight-loss program.

But, acute bouts of exercise are associated with transient elevation in plasma levels of Ang II. For example, exhaustive graded exercise test on the bicycle in endurance trained runners and non-athletes is associated with increased plasma levels of Ang II, which exercise-induced enhancement in Ang II was less steep in the athletes (162). Similar results have also been found in animal models.

For instance, Liu et al (96) have reported that chronic exercise training lowered the elevated Ang II plasma levels in the rabbit with chronic heart failure. Others (163) have shown a reduction in plasma and skeletal muscles levels of Ang II following 8 weeks moderate intensity aerobic exercise training (5 days/week) in rats with chronic heart failure. Brothers et al (164) found exercise-induced inhibition of Ang II vasoconstriction in human thigh muscle following one-legged knee-extensor exercise with low and moderate workload. In addition, Pereira et al (165) have reported that 8 weeks running exercise training (60 min, 5 days/week) reduced cardiac Ang II levels and prevented cardiac dysfunction in a genetic model of sympathetic hyperactivity-induced heart failure in mice. Another study by Mousa et al (28) showed that 4 weeks treadmill running prevented the increase in plasma Ang II levels in rabbits with chronic heart failure. Also, one study investigated the effect of 10 weeks of swimming exercise (60 min, 5 days/week) on obese Zucker rats and observed a reduction in plasma level of Ang II (166). In addition, moderate/high volume swimming exercise training has been resulted in a decrease in Ang II levels in rat cardiac muscle (158).

2.6.6. Effect of Exercise Training on Flow-Mediated Dilation

Another mechanism explaining exercise-induced improvement in cardiovascular system is through the role of exercise on the preservation of endothelial function and improvement of FMD. Regular exercise training has been shown to improve endothelial function in normotensive and hypertensive individuals (143). Moreover, the improvement in FMD with exercise training has been reported in patients with coronary artery disease, coronary heart disease, adolescents and spontaneously hypertensive rats (167).

Several mechanisms involved in improvement of endothelial function following regular aerobic exercise training. One possible mechanism that exercise improves endothelial-dependent vasorelaxation in HTN is due to increase in blood flow and shear stress. Exercise-induced shear stress improves endothelial dependent vasodilation by increase in NO bioavailability or decrease in NO inactivation. It has well known that NO plays critical role in regulation of vascular tone.

Also, exercise training promotes angiogenesis that is related to reduction in peripheral resistance and decrease in BP (168). In addition, exercise training improves endothelium-dependent vasodilators such as prostaglandins and endothelium-derived hyperpolarizing factor (EDHF), and this way affects on endothelial function and HTN. On the other hand, exercise training increases blood flow and shear stress; results in NO and prostacyclin production, which improves endothelium-dependent vasodilation.

2.7. Leukocytes as Useful Tool for Gene Expression Studying

Studies have documented that peripheral blood cells can display disease-specific gene expression signatures that are accurate enough to identify relevant patient subgroups (169). In this regard, studies suggest that circulating blood cells of CVDs human may mirror the same abnormalities occurring in the heart (170). In support of this view, Chon et al (33) studied changes in gene expression of leukocytes from untreated and treated hypertensive patients. Their findings indicated the differential expression of several genes involved in regulation of BP in untreated hypertensive group; but, treatment of BP to normal values corrected these disturbances in treated group. Other studies have reported

up-regulation of ACE in leukocytes of adults (85) and children (27) with primary HTN compared with normotensives. Meanwhile, up-regulation of many of genes involved in oxidative stress and inflammation in peripheral blood leukocytes of patients with arterial HTN have been demonstrated (20). Therefore, the results of these studies demonstrate that alterations in expression of genes involved in the pathogenesis of CVD such as HTN could be observed in peripheral blood leukocytes.

In addition, peripheral blood leukocytes contain all elements of the RAS (171) and adrenergic receptors (172), and thus can be used as useful model for monitoring changes in RAS and SNS components especially in less accessible target tissues such as heart and vascular smooth muscles. Also, one study (79) has reported that the number of βARs on lymphocytes is related to the number of βARs on the myocardial tissue.

This can be considered as the activation of peripheral blood leucocyte in the early stage of HTN. Additionally, studies have documented the presence of preactivated peripheral blood monocytes in hypertensive patients and suggested that Ang II may be directly involved in the process of monocyte activation (173).

The reason for that is unknown but some mechanisms can be considered. HTN is associated with increased vascular oxidative stress that leads to increased shear-stress (174) and oxidative stress on leukocytes. On the other hand, leukocytes are sensitive both to oxidative stress and to Ang II (175). It is possible that leukocytes respond to these disturbances (increased mechanical force and oxidative stress) with alteration in genes involved in HTN.

Given the positive effects of exercise on treatment of pre-hypertension and HTN in menopause and considering that to date there is no study investigating the role of exercise on the expression of candidate genes involved in the induction of HTN in obese postmenopausal women, it is logical to hypothesize that exercise training program may induce alterations in these genes and improves endothelial dysfunction and HTN in this group.

CHAPTER 3
METHODS

3.1. Subjects

Twenty-four healthy, sedentary post-menopausal obese women (age 50-70 years and BMI $\geq$ 30 kg/m^2), diagnosed with pre-hypertension by a physician (SBP 120-139 or DBP 80-89 mmHg) (Canadian Hypertension Education Program) (176), were recruited to participate in this study. Natural menopause was defined as at least 12 consecutive months of amenorrhea (177).

Eligibility of the subjects was determined according to the medical history (Appendix 1) and to the results of medical screening. Inclusion and exclusion criteria were as follows:

The inclusion criteria: 1) 50 to 70 years of age; 2) menopause for at least 1 year; 3) obese, defined as having a BMI between 30 and 40 kg/m^2; 4) maintenance of stable body weight (less than 10 percent body weight change) for at least 12 months prior to enrollment; 5) sedentary, consistent with the American College of Sports Medicine guidelines (178), was defined as performance of fewer than two sessions per week of regular aerobic exercise; and 6) having systolic and diastolic BP between 120-139/80-89 mmHg.

The exclusion criteria: 1) presence of any significant chronic disease as well as acute disease, including infections in 6 weeks preceding the enrollment; 2) use of medications that could alter study results, including antihypertensive, lipid-lowering or anticoagulant medications; 3) hormone replacement therapy; 4) any history of CVD; 5) diabetes mellitus; 6) tobacco use (past 6 months); 7) current abuse of alcohol; and 8) current participation in a formal weight loss program during the study period.

In initial screening visit, all participants received a detailed explanation of the potential benefits and possible risks associated with participation in the study. After obtaining informed consent (Appendix 2) and subjects' personal and familial medical history (Appendix 1), all subjects underwent an initial medical screening and completed a Physical Activity Readiness Questionnaire (PAR-Q) (Appendix 3) (179).

The PAR-Q consists of seven “yes” or “no” questions which are designed to identify the subjects for whom physical activity might be inappropriate (178). Written approval from family physician was obtained for participants who answered “yes” to one or more questions on the PAR-Q. This study was approved by ethics committee of Hacettepe University (No. GO 14/608 - 32) (Appendix 4).

3.2. Experimental Design

Subjects reported to the laboratory twice for both pre- and post-training measurements. Sample size of thirty participants was estimated from previous studies in the literature. Because there was a dropout probability, a total of 36 obese postmenopausal women were initially recruited to the study and randomly divided into two groups: Control group (n=18) and exercise group (n=18). However, 24 subjects completed 10-week training program (Control: 12, Exercise: 12). Exercise group participated in a 10-week, supervised moderate intensity exercise training program (MIET), 25-40 min/day, 3 days/week at 50-70% of each subject’s heart rate reserve. Subjects in control group were instructed to maintain their current physical activity level and dietary habits during the study. All subjects underwent identical tests before and after the 10 week training program. Pre- and post-training evaluations including anthropometric measurements, heart rate and BP measurements, cardiorespiratory fitness test, laboratory tests (blood sampling) and FMD were performed 4 days before and 48 hours after the training program.

All tests carried out in the morning between 7:00 and 10:00 am to avoid circadian rhythm variances. All subjects were asked to abstain from any physical activity for 72 hours. Laboratory tests (blood sampling for evaluating of Ang II plasma level and gene expression of ACE and ADRB2) and FMD test were performed after 12 hour overnight fasting conditions. VO_{2max} was determined following consumption a light breakfast at least 2 hour before testing. Blood samples were analyzed at Farabi laboratory in Tehran and the flow-mediated dilation were measured at Fatemi radiology center in Ardebil.

3.3. Data Collection

3.3.1. Anthropometrics and Body Composition

Body compositions of the subjects were measured to determine the eligible subjects as well as body composition changes throughout the study. In this respect, body weight, height, BMI, body fat percentage, fat mass, fat free mass, waist and hip circumferences and waist to hip ratio were measured before and after the exercise training program.

Body weight was measured to the nearest 0.01 kg using a digital scale (GS 420 tara, Beurer, Germany) with subjects only lightly dressed. Height was determined to the nearest 0.1 cm with a stadiometer (SECA 222; SECA, Germany) while the participants were standing barefoot. Body mass index (BMI) was calculated as the ratio of body weight divided by the square of the height in meters (kg/m^2).

The waist circumference was measured with a Gulick tape measure at the midpoint between the last rib and iliac crest with participants in a standing position (180). Hip circumference was measured at the maximal protrusion of the greater trochanter (181). Waist to hip ratio (WHR) was calculated by dividing the waist circumference to hip circumference.

Body fat percentage (BF%) was measured by bioelectrical impedance analysis (Omron HBF-306C) (182) in the morning following a 12 hour overnight fasting. This device was chosen because it is portable, safe, and is both a valid and reliable measure of body composition (183). Fat mass was calculated by multiplying total body weight (kg) by percent body fat divided by 100. Fat free mass was calculated as total weight (kg) minus fat mass (kg).

3.3.2. Determination of Physical Activity

The International Physical Activity Questionnaire-Short Form (IPAQ-S) (self-administered) was used to determine the current physical activity level of the subjects (184) (Appendix 5). We used Persian version of IPAQ that studies have demonstrated its' reliability and validity for assessing total physical activity in Iranian individuals (185).

It is a reliable questionnaire developed in the young and middle-aged adults (15-69 years) and is used to assess habitual physical activity during the past 7 days. This questioner consists of 7 items which record the frequency and duration of walking, moderate and vigorous physical activities as well as time spent on sitting, in the last week. Data from these items were converted to metabolic equivalent scores (MET-minutes/week) for each type of activity, by multiplying the number of minutes spent on each activity by the specific MET score for that activity. According to the IPAQ scoring system, score ≤ 600 MET-min/weeks are considered inactive.

3.3.3. Resting Heart Rate and Blood Pressure

Resting heart rate was measured using a heart rate monitor (Polar T31, Finland) after 20 min of seated position.

Brachial systolic and diastolic BPs were measured on the right arm, after 20 min of sitting position, using an automatic BP monitor (HBP-1300, Omron Healthcare, Inc.). Three consecutive BP were measured (one minute between measurements), and the resting BP was determined as the average of the three measurements (186).

3.3.4. Cardiorespiratory Fitness Test

To determine subjects' baseline cardiorespiratory fitness levels as well as the effect of 10-week training program on cardiorespiratory fitness subjects were underwent a Bruce treadmill test (187) four days before and 48 hours after the training program.

Bruce protocol is one of the most common methods for estimating VO_{2max}. The standard Bruce test consists of 7 stages each lasting 3 minutes. It starts at 1.7 mph with a 10% grade. The grade and speed of treadmill are increased at 3 minutes intervals (see Bruce protocol chart in table 3.1).

Table 3.1. Bruce protocol chart.

Stage	Duration (min)	Speed (mph)	Grade (%)
1	3	1.7	10
2	3	2.5	12
3	3	3.4	14
4	3	4.2	16
5	3	5.0	18
6	3	5.5	20
7	3	6.0	22

The test was performed on a treadmill (NordicTrack, USA) under the direct supervision of an attending cardiologist for each participant. During the test, heart rate, BP, 12 leads ECG and ratings of perceived exertion were monitored and recorded (Appendix 6).

The whole test consisted of warm-up, main test and cool down periods. First, participants performed a 3 minute warm up period on the treadmill at approximately 40% of age-predicted maximal heart rate. During the warm-up period, the test procedures were again explained.

Participants were asked to identify their perceived level of exertion every minute and at the end of the test by indicating a number between 6 and 20 from a visual chart (188) (Appendix 7). The test was terminated when the following criteria were present:

1) Possible effort of the subjects as judged by the attending cardiologist (189).
2) Rating of perceived exertion ≥ 18 on the Borg scale of 6–20.
3) Maximal HR > 90% of age-predicted maximal HR (206 - 0.88 × age) (190).

An active cool-down period for 3–5 minutes was done following the completion of the test. Time to peak exhaustion was also recorded. Duration of the test was the test score which was used to estimate the VO_{2max} value. VO_{2max} of the participants were calculated by using the following equation:

VO_{2max}= (4.38 x T) - 3.9 (3. 1)

where, T= time (maximum time of effort up to exhaustion) (191).

3.3.5. Exercise Training Program

The participants underwent a 10-week training protocol with three weekly sessions under the direct supervision of an exercise physiologist and a medical doctor. The training program was progressive in nature, with increases in training intensity and duration occurring throughout the 10-week period. All exercise sessions began with a 5-minute warm-up at approximately 40% of age-predicted maximal heart rate and ended with a 5-minute cool-down at a continually declining intensity.

Exercise intensity was based on each subject's target heart rate calculated from the Karvonen equation (192):

[(heart rate reserve × intensity) + resting heart rate], (3. 2)

Where heart rate reserve ($HR_{reserve}$) is the maximal heart rate (HR_{max}), obtained from each subject's VO_{2max} test, minus resting heart rate (HR_{rest}).

Exercise target heart rate= (% exercise intensity) × (HR_{max}–HR_{rest}) +HR_{rest} (3. 3)

In the first two weeks of the study, subjects in exercise group walked or jogged on a treadmill (25 min/day, three times/week) at 50% of each subject's $HR_{reserve}$. During the third and fourth week, as their exercise tolerance improved, the training volume was increased to 30 minutes at 55% of $HR_{reserve}$ and then was progressed to 35 minutes at 60% $HR_{reserve}$ in the fifth and sixth weeks. In the last four weeks, subjects will train 40 minutes at 70% $HR_{reserve}$.

Adherence to the exercise prescription was documented through the use of polar heart rate monitors. Subjects received feedback if training intensities were either too high or low in comparison with desirable intensities. All exercise sessions began between 0400 and 0600 PM.

3.3.6. Determination of Dietary Intake

To determine the possible impact of dietary intake on the results of the study as well as any change in the diet throughout the study, nutrient intake of each subject was assessed via 3-day food records in the first and last weeks of exercise program (over 2 weekdays and 1 weekend day) (Appendix 8). All subjects were instructed by a trained registered dietitian to keep a record of food intake and to maintain their usual diet during the study. Dietary intake data were analyzed by a dietary analysis software (Nutritionist Pro V. 4.1.0, Axxya Systems, Stafford, TX) to determine total energy intake, protein, CHO and lipid intake of the subjects.

3.3.7. Flow-Mediated Dilation (FMD)

Flow-mediated dilation (FMD) was performed to determine the effect of 10 weeks exercise training on endothelial function in subjects by a trained sonographer. FMD test was performed in the morning following 12 h overnight fasting and after the subjects had rested in supine position for 20 min in a quiet temperature controlled room (22–25°C).

For FMD measurement, the brachial artery was imaged longitudinally by a linear array vascular ultrasound transducer (Seimens Sequoia Model #256, frequency 8 MHz, range 5–8 MHz) above the antecubital fossa of the dominant arm and brachial artery diameter were recorded for 1 minute. After the baseline resting scan, a blood pressure cuff was placed at the level of the mid-forearm, and inflated to 50 mmHg above resting blood pressure (> 200 mmHg) until no blood flow was detected through the brachial artery with the Doppler probe, and this pressure was continued for 5 minutes. Then, cuff was released and diameter recording was performed 30 second before cuff deflation and continued for 3 minutes post-deflation (193). Flow-mediated dilation was calculated as the percentage change in brachial diameter from baseline to the maximal post-deflation diameters according to the following equation:

$$\text{FMD} = (\text{post deflation diameter} - \text{resting diameter})/\text{resting diameter} \times 100 \quad (3.4)$$

According to Vogel (194), normal values for FMD is vasodilation > 10% (response to release of a 5 minutes arterial occlusion relative to baseline).

3.3.8. Blood Sampling and Assays

Blood samples were collected to determine plasma Ang II levels, and ACE and ADRB2 gene expression in leukocytes. Five milliliters of peripheral venous blood were taken from the antecubital vein using standard venipuncture methods after 12 h of overnight fast (between 7:00 and 8:00 am) by a registered nurse. Samples were collected into the Ethylenediaminetetraacetate (EDTA) vial. About 1 ml of whole blood were used for RNA isolation. Plasma were separated by centrifugation of the remaining volume of blood at 3000g for 10 min. Remaining plasma were stored in a refrigerator (-80°) until further analysis.

Angiotensin II Analysis

The 96-well plates precoated with angiotensin II specific antibody were used to measure angiotensin II in plasma (RayBio® Angiotensin II Enzyme Immunoassay Kit; RayBiotech, Inc., Norcross, GA, USA) (195). 100 µl anti-Angiotensin II antibody were added to each well and incubated for 1.5 hours at room temperature and then followed by 4 times washing with 200 µL of 1x Wash Buffer. Subsequently 100 µl standard or sample were added to each well and incubated for 2.5 hours at room temperature with gentle shaking and followed by washing 4 times. 100 µl prepared streptavidin solution was added to each well and incubated for 45 minutes, and then washed 4 times. Subsequent, 100 µl TMB One-Step Substrate Reagent was added per well and incubated for 30 minutes at room temperature. Finally, 50 µl Stop Solution was added to each well and absorbance was immediately read at 450 nm. The assay minimum detectable concentration of angiotensin II is 2.62 pg/ml and detection range is 0.1-1,000 pg/ml. Intra- and Inter-assay CV (%) were <10% and <15%, respectively.

Leukocyte RNA Isolation

RNA was extracted from whole blood using the QIAamp RNA Blood Mini Kit (50) (QIAGEN, Maryland, US, *Catalog Number 52304*) according to the manufacturer's instructions (196). At first, 1.5 ml of whole blood was mixed with 5 ml of erythrocyte lysis buffer and the remaining cells were pelleted by centrifugation. The leukocytes were then lysed with RLT buffer and homogenized with the QIAshredder. The homogenized lysate was mixed with one volume (600 µl) of 70% ethanol and applied to the QIAamp RNA mini spin column. The column was washed once with RW1 buffer and twice with RPE buffer. Finally, the RNA was eluted with 2 × 60 µL of RNase-free water. The quantity of total RNA (µg/ml) was measured by spectrophotometry at 260 nm, and purity of total RNA was evaluated by measuring the A260/A280 ratio. A ratio greater than 1.8 indicates high purity of the corresponding RNA sample. The RNA samples were treated with DNase I (Ambion) to remove any genomic DNA. The resulting RNA was used as template for cDNA synthesis.

cDNA Synthesis

First-strand cDNA was synthesized from isolated total RNA using a cDNA synthesis kit (RevertAid™ First Strand cDNA Synthesis Kit, Fermentas, Germany) according to the standard protocol for first strand cDNA synthesis (197).

Briefly, first strand cDNA synthesis reaction was performed in a 20 µl reaction mixture containing 100 ng of total RNA, 4 µl 5X reaction buffer, 2 µl 10 mMdNTP Mix, 12 µl nuclease-free water, 1 µl RiboLock™ RNase Inhibitor (20 u/µl), 1 µl RevertAid™ M-MuLV Reverse Transcriptase (200 u/µl) and 15 pmol of each gene specific primers. Reaction mixtures were incubated for 5 minutes at 25°C followed by 60 minutes at 42°C and the reactions were terminated by heating at 70°C for 5 minutes. Then samples were stored in -70°C for further analysis. First strand cDNA products obtained from this kit were used directly as templates for RT-PCR reactions.

Real-Time PCR (qPCR)

The gene (mRNA) expression levels of ACE and ADRB2 were analyzed by quantitative real-time polymerase chain reaction (PCR) assay using SYBR Green master mix kit (Takara) and with the use of a real-time PCR system (Rotor-Gene 6000; Corbett, Sydney, Australia) according to manufacturer's recommendation.

The SYBR green dye binds to double stranded DNA during the PCR amplification. As the cDNA amplification occurs during the PCR reaction the fluorescence increases secondary to increase amount of SYBR green bound to DNA.

Primer pairs for PCR were designed with Primer 3 software and were synthesized by Bioneer (Germany). Housekeeping gene β-actin was used as an internal control. The primer sequences used for ACE and B2ADR gene amplification were:

ACE Forward 5'-CGCAGAGCTACAACTCCAGCGCC-3'
ACE Reverse 5'-CCCCAGGCCTCCGCAAACTC-3'
ADRB2 Forward 5'-GGAACTGGCAGGCACCGCGA-3'
ADRB2 Reverse 5'-GAGCGGACGCCTGGAAGCC-3'
β actin Forward 5'-TCCCTGGAGAAGAGCTACG-3'
β actin Reverse 5'-GTAGTTTCGTGGATGCCACA-3'

The reaction mixture (PCR mixture) consisted of 2µl of cDNA template, 10 µl of SYBR Green master mix, 7.4µl DEPC treated water, 0.3µl of forward primer (10 pmol/ µl) and 0.3µl of reverse primer (10 pmol/ µl) in total volume of 20 µl (198). Negative controls included samples without cDNA. All steps were performed on ice. The PCR mixture was then transferred into a plastic 96-well plate with optical adhesive covers, and placed onto a real-time PCR machine.

The PCR profile (thermal cycling condition) used was as follows: 95 °C for 10 min as an initial denaturation step, followed by 40 cycles of denaturation at 95 ˚C for 15 sec, annealing at 57 ˚C for 30 sec, extension at 60 ˚C for 60 sec. A final elongation for 15 min at 37 °C ended the profile.

After the thermo-cycling (following amplification), samples were subjected to melting curve analysis to insure that only a single product was formed. The mRNA level of interested genes was measured by the Delta-Delta-Threshold Cycle (ΔΔCT) method and was normalized to β-actin as an internal control. The Delta-Delta-Threshold cycle (DDCT) equation was used to compare the expression of genes of interest in terms of fold difference according to the formula (199):

$$\text{Fold Change} = 2^{-\Delta(\Delta CT)} \quad (3.5)$$

$$\text{Where } \Delta CT = CT_{target} - CT_{\beta\text{-actin}}, \text{ and } \Delta(\Delta CT) = \Delta CT_{treatment} - \Delta CT_{control} \quad (3.6)$$

3.4. Data Analysis

All data were analyzed using SPSS statistical software (version 22.0). Data were tested for normal distribution by the Kolmogorov-Smirnov test. Descriptive statistics were calculated to demonstrate the groups' baseline demographic characteristics. Baseline differences between groups were determined using unpaired t test.

A general linear model of 2×2 (Time × Group) repeated-measures analysis of variance (RM-ANOVA) was used to determine differences between groups. The effect over time (pre- and post-test intervention) was treated as the within-subjects factor, and the differences between the exercise and control groups were treated as the between-subjects factor.

Partial Eta squared (η^2) was used to calculate effect sizes for significant main effects and interactions according to Hopkins (2002) guidelines (200). For this mean, the following standards used to determine the magnitude of mean effect size: 0.2–0.6 represented a small effect size; 0.6–1.2, a medium effect size; and >1.2, a large effect size.

Pearson's correlation coefficients were calculated for the gained scores (difference between post-test and pre-test) to determine the association between the changes in main variables of FMD, Ang II, ACE, and ADRB2 with systolic and diastolic BP. The significance level was set as $p<0.05$.

CHAPTER 4

RESULTS

The purpose of this study was to examine the effects of 10 weeks moderate intensity aerobic exercise training on BP and some of factors involved in BP regulation in obese PMW with prehypertension. In this chapter findings of the study on subjects' demographic variables, variables regarding the experimental design (effects of training on cardiorespiratory fitness and body composition, and changes in food intake throughout the study), systolic and diastolic BP, circulating Ang II, mRNA levels of ACE and ADRB2 in leukocytes, and FMD were presented.

4.1. Baseline Comparison of Experimental Groups with Regard to Demographic, Body composition and Cardirespiratory Fitness Variables

Comparisons of baseline group differences in demographic, body composition and physiological variables were presented in Table 4.1. According to the Student t test analysis no significant differences were noted between the groups at baseline ($p>0.05$), except fat free mass and waist to hip ratio ($p<0.05$) (Table 4.1). These finding indicates that experimental groups were similar with respect to most of the variables measured before the training. While fat free mass was higher and waist to hip ratio was lower in exercise group compared to control group.

Table 4.1. Baseline demographic variables and physiological measures of participants in control and exercise groups.

	Control (n=12)	Exercise (n=12)		
	Mean ± SD	**Mean ± SD**	**t**	**p**
Demographic variables				
Age (years)	56.58 ± 4.17	57.58 ± 4.29	-0.579	0.569
Menopause time (years)	9.33 ± 4.29	9.92 ± 3.99	-0.345	0.733
Anthropometric variables				
Height (cm)	162.25 ± 4.99	160.71 ± 4.47	0.797	0.434
Weight (kg)	82.39 ± 5.35	83.03 ± 5.51	-0.290	0.774
BMI (kg/m^2)	31.29 ± 1.40	32.15 ± 1.78	-1.313	0.203
Body fat (%)	40.75 ± 2.86	39.25 ± 2.73	1.312	0.172
Fat mass (kg)	33.71 ± 4.48	32.72 ± 4.40	0.545	0.591
Fat free mass (kg)	48.97 ± 1.14	50.23 ± 1.36	-3.268	0.004
Waist circumference (cm)	108.58 ± 7.57	107.96 ± 8.90	0.185	0.855
Hip circumference (cm)	119.08 ± 8.35	120.04 ± 8.95	-0.271	0.789
Waist to hip ratio	0.91 ± 0.01	0.90 ± 0.02	2.142	0.044
Physiological measures				
Resting heart rate (bpm)	85.00 ± 2.13	83.00 ± 2.73	2.000	0.058
Maximum heart rate (bpm)	153.92 ± 4.66	153.08 ± 3.78	0.481	0.635
Time to exhaustion (min)	6.05 ± 1.22	6.06 ± 1.30	0.190	0.851
$VO_{2\ max}$ (ml/kg/min)	23.11 ± 6.23	22.64 ± 5.71	0.190	0.851

BMI, body mass index; BP, blood pressure; $VO_{2\ max}$, maximal oxygen uptake

To determine the effects of exercise training on body composition, cardiorespiratory fitness, BP and factors involved in the regulation of BP (FMD, Ang II, ACE, ADRB2) a general linear model of 2x2 repeated measures of ANOVA was used. The findings were presented below under subheadings.

4.1.1. Effect of Exercise Training on Body Composition

With regard to body composition, body weight, height, BMI, body fat percentage, fat mass, fat free mass, waist and hip circumferences and waist to hip ratio were evaluated before and after the training program. Table 4.2 presents comparison of the differences in body composition variables from pre- to 10 weeks post-exercise between control and exercise groups. Only statistics for *Time* × *Group* interaction were illustrated in the table, however, statistics for *Time* and *Group* effects were reported in the text.

RM-ANOVA conducted on body weight revealed a significant main effect of *Time* [$F_{(1, 22)}$ =106.31, p=0.000, Partial Eta η^2 =0.829] and *Time* × *Group* interaction [$F_{(1, 22)}$ =295.82, p=0.000, Partial Eta η^2 =0.931] indicating that body weight decreased significantly in exercise group (Figure 4.1A). However, no main effect for *Group* was noted [$F_{(1, 22)}$ =0.17, p=0.684, Partial Eta η^2 =0.008] indicating no significant difference between the two groups.

Significant main effects of *Time* [$F_{(1, 22)}$ =116.12, p=0.000, Partial Eta η^2 =0.841] and *Time x Group interaction* [$F_{(1, 22)}$ =308.61, p=0.000, Partial Eta η^2 =0.933] were found. But, there was no significant main effect for *Group* [$F_{(1, 22)}$ = 0.16, p=0.690, Partial Eta η^2 =0.007]. These results revealed that BMI decreased in exercise group (Figure 4.1B) while there was no significant difference between the two groups.

RM-ANOVA conducted on fat percent revealed significant main effects of *Time* [$F_{(1, 22)}$ =54.71, p=0.000, Partial Eta η^2 =0.713], *Group* [$F_{(1, 22)}$ =13.78; p=0.001, Partial Eta η^2 =0.385], and *Time x Group interaction* [$F_{(1, 22)}$ =73.21; p=0.000, Partial Eta η^2 =0.769] indicating that fat percent decreased significantly in exercise group compared to control group after exercise training (Figure 4.1C).

Table 4.2. Comparison of the differences in body composition variables from pre- to 10 weeks post-exercise training between control and exercise groups.

Variables	Pre training	Post training	Time × Group interaction		
			F	P	η^2
Weight (kg)					
Control	82.39 ± 5.35	83.01 ± 5.38	295.82	0.000	0.931
Exercise	83.03 ± 5.51	80.54 ± 5.47			
BMI (kg/m^2)					
Control	31.29 ± 1.40	31.52 ± 1.33	308.61	0.000	0.933
Exercise	32.15 ± 1.78	31.19 ± 1.77			
Body fat (%)					
Control	40.75 ± 2.86	41.08 ± 2.81	73.21	0.000	0.769
Exercise	39.25 ± 2.73	34.67 ± 2.39			
Fat mass (kg)					
Control	33.71 ± 4.48	33.98 ± 4.45	96.09	0.000	0.814
Exercise	32.72 ± 4.40	28.01 ± 3.59			
Fat free mass (kg)					
Control	48.97 ± 1.14	48.43 ± 1.21	28.10	0.000	0.561
Exercise	50.23 ± 1.36	52.54 ± 2.51			
Waist circumference (cm)					
Control	108.58 ± 7.57	109.08 ± 7.22	65.71	0.000	0.749
Exercise	107.96 ± 8.90	106.58 ± 8.98			
Hip circumference (cm)					
Control	119.08 ± 8.35	119.24 ± 8.20	4.73	0.041	0.177
Exercise	120.04 ± 8.95	119.04 ± 9.05			
Waist-hip ratio					
Control	0.91 ± 0.01	0.92 ± 0.01	3.19	0.088	0.127
Exercise	0.90 ± 0.02	0.89 ± 0.02			

BMI: body mass index; η^2: partial eta squared

Significant main effects of *Time* [$F_{(1, 22)}$ =76.40, p=0.000, Partial Eta η^2 =0.776] and *Time* × *Group interaction* [$F_{(1, 22)}$ =96.09; p=0.000, Partial Eta η^2 =0.814] were found

for fat mass while no main effect was observed for *Group* [$F_{(1, 22)}$ =4.12; p=0.055, Partial Eta η^2 =0.158]. These results revealed that fat mass decreased in exercise group (Figure 4.1D), however, there was no significant difference between the two groups.

Significant main effects of *Time* [$F_{(1, 22)}$ =17.25, p=0.000, Partial Eta η^2 =0.439], *Group* [$F_{(1, 22)}$ =21.29; p=0.000, Partial Eta η^2 =0.492], and *Time* × *Group interaction* [$F_{(1, 22)}$ =28.10; p=0.000, Partial Eta η^2 =0.561] was observed for fat free mass suggesting that exercise training significantly increased fat free mass in exercise group compared to control group (Figure 4.1E).

RM-ANOVA performed on waist circumference showed significant main effects of *Time* [$F_{(1, 22)}$ =14.31, p=0.001, Partial Eta η^2 =0.394] and *Time* × *Group interaction* [$F_{(1, 22)}$ =65.71; p=0.000, Partial Eta η^2 =0.749], while no main effect for *Group* [$F_{(1, 22)}$ =0.22; p=0.645, Partial Eta η^2 =0.10]. These results suggest that waist circumference decreased in exercise group (Figure 4.1F) while there was no significant difference between the two groups.

A significant *Time* × *Group* interaction [$F_{(1, 22)}$ =4.73, p=0.041, Partial Eta η^2 =0.177] was found for hip circumference revealing that change in hip circumference was significantly different in exercise and control groups (Figure 4.1G). Figure 4.1G illustrating the *Time* × *Group* interaction for hip circumference clearly indicates that it increased in control group while decreased in exercise group. However, the magnitude of this difference was very small (Eta η^2 =0.177). In addition, there was no significant main effect of *Time* [$F_{(1, 22)}$ =2.57, p=0.123, Partial Eta η^2 =0.104] or *Group* [$F_{(1, 22)}$ = 0.012, p=0.914, Partial Eta η^2 =0.001] (Figure 4.1G) indicating that hip circumference changes over time in control and exercise groups was not significant.

A significant main effect of *Group* [F (1, 22) = 6.46, p=0.019, Partial Eta η^2 =0. 0.227)] for WHR indicated that WHR in exercise group was lower significantly compared to control group (Table 4.2, Figure 4.1H). Although figure 4.1H illustrates a trend of WHR increase in control and decrease in exercise groups, no significant main effect for *Time* [F (1, 22) = 0.36, p=0.557, Partial Eta η^2 =0.016)] or *Time* × *Group* interaction [F (1, 22) =

3.19, p=0.088, Partial Eta η^2 =0.127)] was noted which indicates that the magnitude of these changes was not significant.

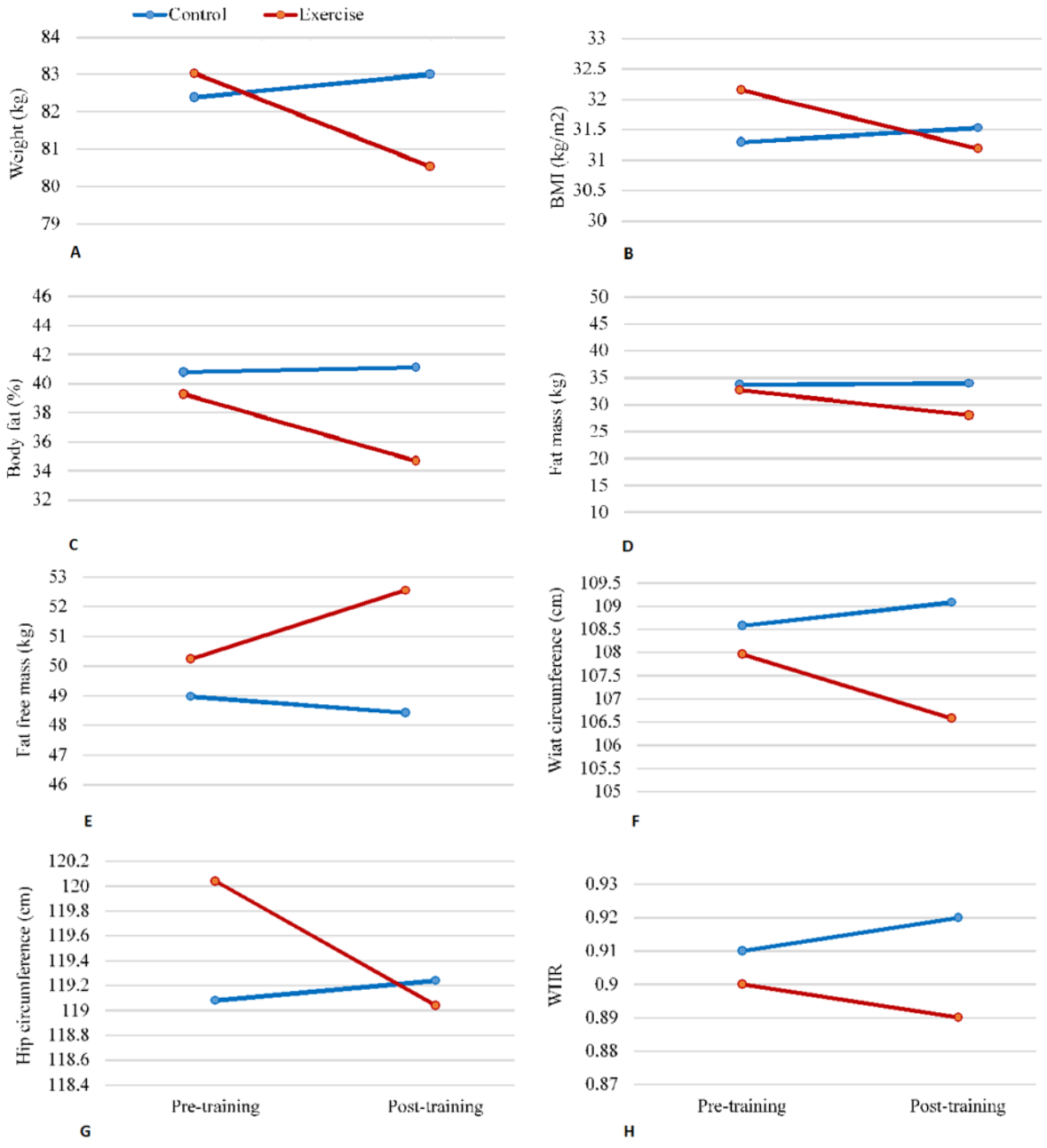

Figure 4.1. Changes in body composition variables in time with respect to experimental groups.

4.1.2. Effect of Exercise Training on Cardiorespiratory Fitness

To determine baseline cardiorespiratory fitness level of the subjects and the effectiveness of exercise training program, subjects underwent a Bruce protocol test during which maximal heart rate and time to exhaustion was determined and maximal oxygen uptake was calculated by a prediction equation. In table 4.3 descriptive statistics and RM-ANOVA results were summarized on these parameters as well as resting heart rate.

Table 4.3. Comparison of the differences in cardiorespiratory fitness variables from pre- to 10 weeks post-exercise training between control and exercise groups.

Variables	Pre training	Post training	Time × Group interaction		
			F	P	η^2
Resting HR (bpm)					
Control	85.00 ± 2.13	86.00 ± 1.35	248.77	0.000	0.919
Exercise	83.00 ± 2.73	77.00 ± 2.13			
Maximal HR (bpm)					
Control	153.92 ± 4.66	154.00 ± 5.05	28.40	0.000	0.563
Exercise	153.08 ± 3.78	150.17 ± 3.86			
Time to exhaustion (min)					
Control	6.17 ± 1.42	6.05 ± 1.22	45.12	0.000	0.672
Exercise	6.06 ± 1.30	7.23 ± 1.17			
$VO_{2\ max}$ (ml/kg/min)					
Control	23.11 ± 6.23	22.60 ± 5.36	45.11	0.000	0.672
Exercise	22.64 ± 5.71	27.78 ± 5.14			

$VO_{2\ max}$, maximal oxygen uptake; HR, Heart rate; η^2, partial eta squared

RM-ANOVA conducted on resting HR revealed a main effect of *Time* [$F_{(1, 22)}$ = 126.92, p=0.000, Partial Eta η^2 =0.852], and a significant main effect of *Group* [$F_{(1, 22)}$ = 42.25, p=0.000, Partial Eta η^2 =0.658], suggesting that resting HR decreased after exercise training. In addition, there was a significant *Time* × *Group* interaction [$F_{(1, 22)}$ = 248.77, p=0.000, Partial Eta η^2 =0.919], indicating that exercise training significantly decreased resting HR in exercise group compared to control group (Figure 4.2A).

RM-ANOVA conducted on maximal HR revealed a main effect of *Time* [$F_{(1, 22)}$ = 25.33, p=0.000, Partial Eta η^2 =0.535) and a significant *Time* × *Group* interaction [$F_{(1, 22)}$ = 28.40, p=0.000, Partial Eta η^2 =0.563], (Figure 4.2B). But, there was no significant main effect for *Group* [$F_{(1, 22)}$ = 1.76, p=0.199, Partial Eta η^2 =0.074]. These findings suggest that maximal HR decreased in exercise group (Figure 4.2B) while there was no significant difference between the two groups.

Similarly significant main effects of *Time* [$F_{(1, 22)}$ = 30.34, p=0.000, Partial Eta η^2 =0.580] and *Time* × *Group* interaction [$F_{(1, 22)}$ = 45.11, p=0.000, Partial Eta η^2 =0.672], (Figure 4.2C) were found for $VO_{2\ max}$. However, no significant main effect was observed for *Group* [$F_{(1, 22)}$ = 1.09, p=0.308, Partial Eta η^2 =0.047]. These findings indicate that exercise training resulted in significant increase in $VO_{2\ max}$ while there was no significant difference between the two groups.

Similar to VO_2 max, significant main effects of *Time* [$F_{(1, 22)}$ = 30.35, p=0.000, Partial Eta η^2 =0.580] and *Time* × *Group* interaction [$F_{(1, 22)}$ = 45.12, p=0.000, Partial Eta η^2 =0.672], were observed for time to exhaustion revealing a significant increase in time to exhaustion in exercise group (Figure 4.2D). However, no significant main effect was observed for *Group* [$F_{(1, 22)}$ = 1.09, p=0.308, Partial Eta η^2 =0.047] showing that magnitude of the changes in experimental groups was not different.

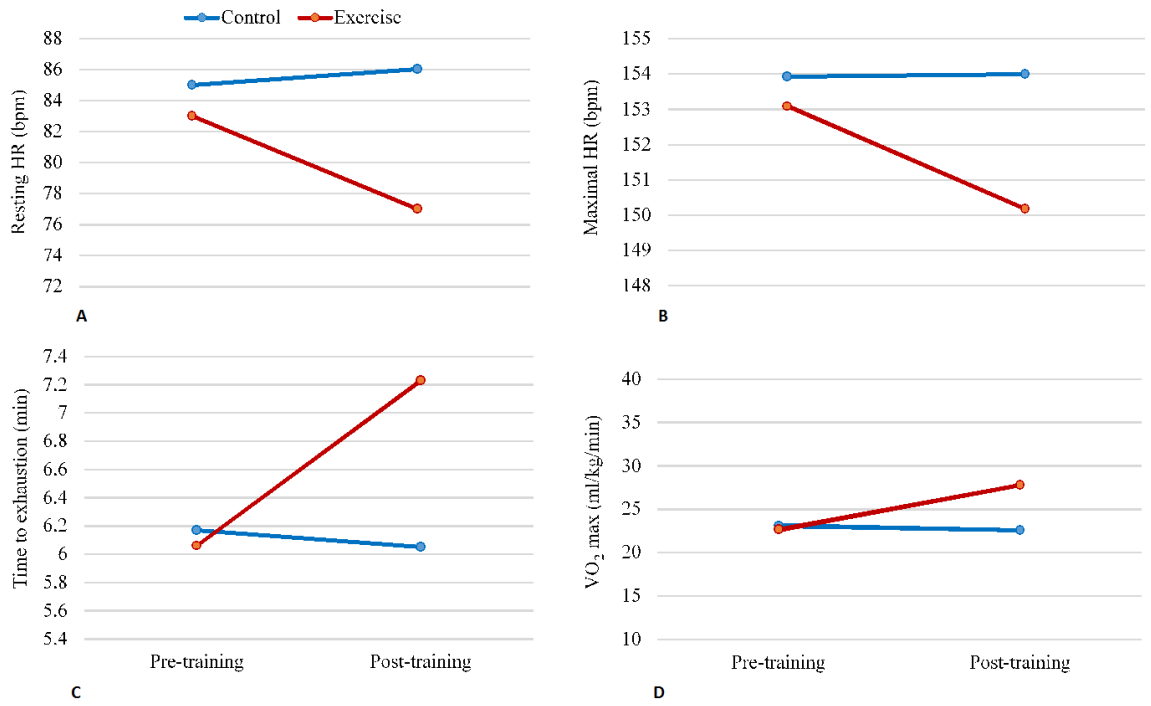

Figure 4.2. Changes in resting BP, maximal HR, time to exhaustion and VO_2 max in time with respect to experimental groups.

4.2. Food Intake

There was no significant difference in food intake variables between groups at baseline or within groups after 10 weeks of exercise training (table 4.4). These results indicate that two groups were similar in food intake at the beginning and at the end of the study, and no change has occurred in food intake over time. In table 4.4. descriptive data and *Time × Group interaction* statistics of RM-ANOVA results for food intake variables were presented. Since this data was collected just to check food intake no graph or explanations for each variable was presented.

Table 4.4. Comparison of the differences in total energy intake, protein, CHO and lipid intake from pre- to 10 weeks post-exercise between control and exercise groups.

Variables	Pre training	Post training	Time × Group interaction		
			F	P	η^2
TEI (kcal/day)					
Control	2134.08 ± 272.49	2136.83 ± 228.64	0.01	0.912	0.001
Exercise	2135.67 ± 86.95	2134.75 ± 284.11			
Protein (g)					
Control	100.92 ± 26.23	97.92 ± 29.67	0.000	0.958	0.000
Exercise	101.67 ± 35.58	99.17 ± 41.18			
CHO (g)					
Control	282.42 ± 70.20	284.92 ± 72.79	0.52	0.478	0.023
Exercise	283.67 ± 66.35	281.92 ± 65.75			
Lipid (g)					
Control	79.67 ± 25.46	82.33 ± 25.44	1.6	0.219	0.068
Exercise	81.42 ± 26.41	79.25 ± 27.83			

TEI, total energy intake; CHO, carbohydrate

4.3. Blood Pressure and Factors Involved in Blood Pressure Regulation

Baseline values for BP, plasma Ang II, FMD, ACE and ADRB2 mRNA levels in leukocytes have been presented in table 4.5. Independent t-test revealed no significant differences between the groups before 10 weeks of aerobic exercise training ($p>0.05$).

Table 4.5. Comparison of baseline values of systolic and diastolic BP, FMD, Ang II, ACE and ADRB2 between control and exercise group.

Variables	Control (n=12)	Exercise (n=12)		
	Mean ± SD	Mean ± SD	t	p
Systolic BP (mmHg)	129.58 ± 3.85	127.92 ± 4.74	0.946	0.354
Diastolic BP (mmHg)	82.33 ± 1.30	82.00 ± 1.54	0.573	0.572
FMD (%)	5.78 ± 0.52	6.02 ± 0.71	-0.938	0.358
Ang II (pg/ml)	14.08 ± 1.36	14.03 ± 1.48	0.099	0.922
ACE (fold change)	1.19 ± 0.22	1.23 ± 0.21	-0.500	0.657
ADRB2 (fold change)	0.95 ± 0.17	0.89 ± 0.43	0.467	0.645

BP, blood pressure; FMD, flow mediated dilation; Ang II, angiotensin II; ACE, angiotensin converting enzyme; ADRB2, β2 adrenergic receptor

4.3.1. Effect of Exercise Training on BP, FMD, Ang II, ACE and ADRB2

Table 4.5 illustrates comparison of the differences in SBP, DBP, FMD, Ang II, ACE and ADRB2 from pre- to 10 weeks post-exercise between control and exercise groups.

Table 4.6. Comparison of the differences in SBP, DBP, FMD, Ang II, ACE and ADRB2 from pre- to 10 weeks post-exercise training between control and exercise groups.

Variables	Pre training	Post training	Time × Group interaction		
			F	P	η^2
SBP (mmHg)					
Control	129.58 ± 3.85	129.67 ± 3.77	52.17	0.000	0.703
Exercise	127.92 ± 4.74	122.08 ± 3.58			
DBP (mmHg)					
Control	82.33 ± 1.30	82.83 ± 0.72	23.57	0.000	0.517
Exercise	82.00 ± 1.54	80.00 ± 0.43			
FMD (%)					
Control	5.78 ± 0.52	5.31 ± 0.49	249.67	0.000	0.919
Exercise	6.02 ± 0.71	11.20 ± 1.12			
Ang II (pg/ml)					
Control	14.08 ± 1.36	14.15 ± 1.15	215.08	0.000	0.907
Exercise	14.03 ± 1.48	7.62 ± 0.62			
ACE (fold change)					
Control	1.19 ± 0.22	1.66 ± 0.43	400.32	0.000	0.948
Exercise	1.23 ± 0.21	-4.87 ± 0.90			
ADRB2 (fold change)					
Control	0.95 ± 0.17	0.99 ± 0.38	104.49	0.000	0.826
Exercise	0.89 ± 0.43	2.76 ± 0.55			

SBP, systolic blood pressure; DBP, diastolic blood pressure; FMD, flow mediated dilation; Ang II, angiotensin II; ACE, angiotensin converting enzyme; ADRB2, β2 adrenergic receptor; η^2, partial eta squared;

RM-ANOVA conducted on mean SBP revealed significant main effects of *Time* [$F_{(1, 22)}$ =49.27, p=0.000, Partial Eta η^2 =0.69], *Group* [$F_{(1, 22)}$ =8.52, p=0.008, Partial Eta η^2 =0.28] and interaction of *Time* × *Group* [$F_{(1, 22)}$ =52.17, p=0.000, Partial Eta η^2 =0.70] (Figure 4.3A) for SBP. These findings suggested that exercise training significantly decreased SBP compared to control group.

Similarly, RM-ANOVA conducted on mean DBP revealed significant main effects of *Time* [$F_{(1, 22)}$ =8.49, p=0.008, Partial Eta η^2 =0.28], *Group* [$F_{(1, 22)}$ =19, p=0.000, Partial Eta η^2 =0.46] and interaction of *Time x Group* [$F_{(1, 22)}$ =23.57, p=0.000, Partial Eta η^2 =0.52], (Figure 4.3B) indicating that exercise training significantly decreased DBP in exercise group compared to control group. Figure 4.3C and 4.3D are the bar-graphs presenting SBP and DBP of the experimental groups, respectively, before and after exercise training.

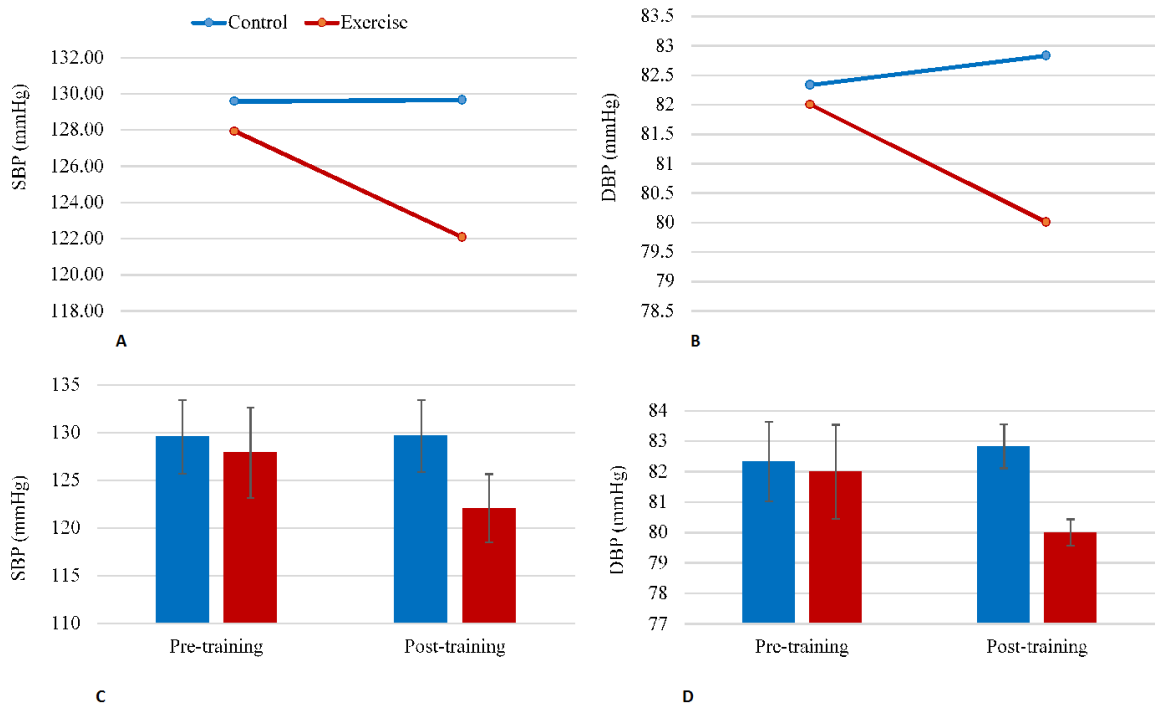

Figure 4.3. Comparison of the differences in systolic and diastolic blood pressures from pre- to 10 weeks post-exercise between control and exercise groups.

RM-ANOVA conducted on FMD revealed significant main effects of *Time* [$F_{(1, 22)} = 173.14$, p=0.000, Partial Eta $\eta^2 = 0.887$], *Group* [$F_{(1, 22)} = 148.70.67$, p=0.000, Partial Eta $\eta^2 = 0.871$] and interaction of *Time* × *Group* [$F_{(1, 22)} = 249.67$, p=0.000, Partial Eta $\eta^2 = 0.919$], (Figure 4.4A) suggesting that exercise training significantly increased FMD in exercise group compared to control group.

Similarly significant main effects of *Time* [$F_{(1, 22)} = 205.98$, p=0.000, Partial Eta $\eta^2 = 0.904$), *Group* [$F_{(1, 22)} = 56.84$, p=0.000, Partial Eta $\eta^2 = 0.721$), and *Time x Group* interaction [$F_{(1, 22)} = 215.08$, p=0.000, Partial Eta $\eta^2 = 0.907$], (Figure 4.4B) were found for Ang II revealing that exercise training significantly decreased Ang II plasma level in exercise group compared to control group. Figure 4.4C and 4.4D are the bar-graphs presenting FMD and Ang II of the experimental groups, respectively, before and after exercise training.

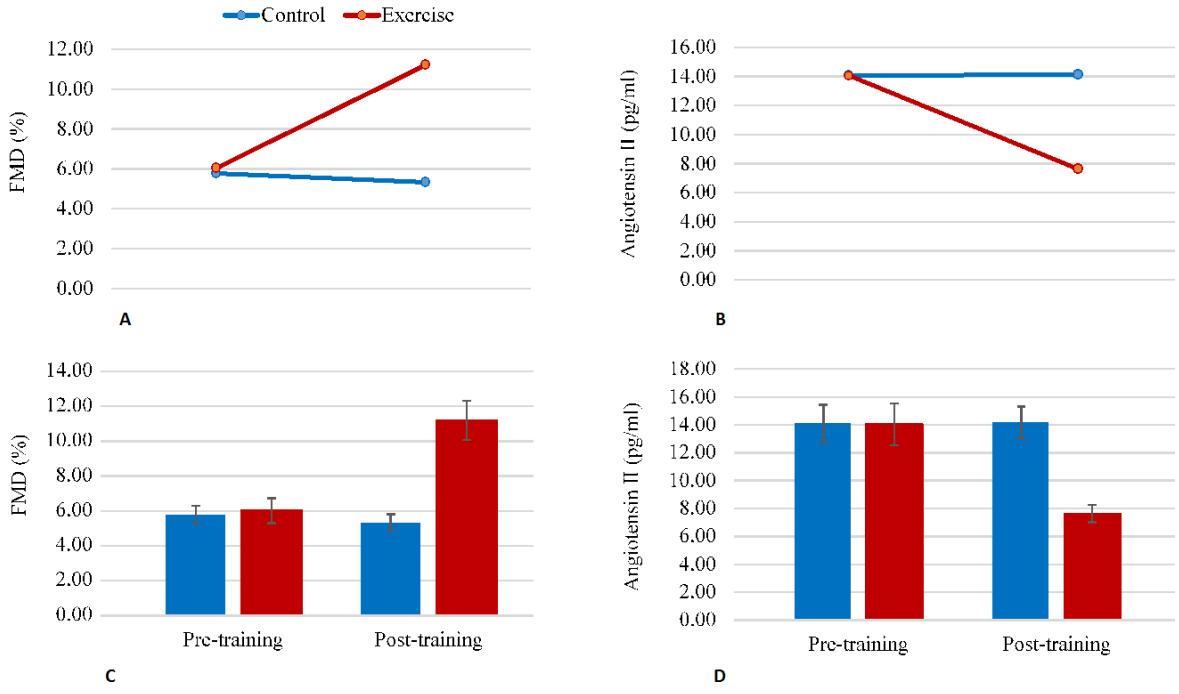

Figure 4.4. Comparison of the differences in Ang II from pre- to 10 weeks post-exercise between control and exercise groups.

RM–ANOVA performed on the ACE highlighted significant main effects of *Time* [$F_{(1, 22)}$ = 292.20, p=0.000, Partial Eta η^2 =0.930), *Group* [$F_{(1, 22)}$ = 580.97, p=0.000, Partial Eta η^2 = =0.964), and *Time* × *Group* interaction [$F_{(1, 22)}$ =400.32, p=0.000, Partial Eta η^2 =0.948], (Figure 4.5A) suggesting that exercise training significantly decreased ACE in exercise group compared to control group.

RM-ANOVA conducted on ADRB2 revealed significant main effects for *Time* [$F_{(1, 22)}$ =110.83, p=0.000, Partial Eta η^2 =0.834] and *Group* [$F_{(1, 22)}$ = 36.12, p=0.000, Partial Eta η^2 =0.621], and interaction of *Time x Group* interaction [$F_{(1, 22)}$ =104.49, p=0.000, Partial Eta η^2 =0.826], (Figure 4.5B). These findings showed that exercise training significantly increased ADRB2 in exercise group compared to control group. Figure 4.5C and 4.5D are the bar-graphs presenting ACE and ADRB2 of the experimental groups, respectively, before and after exercise training.

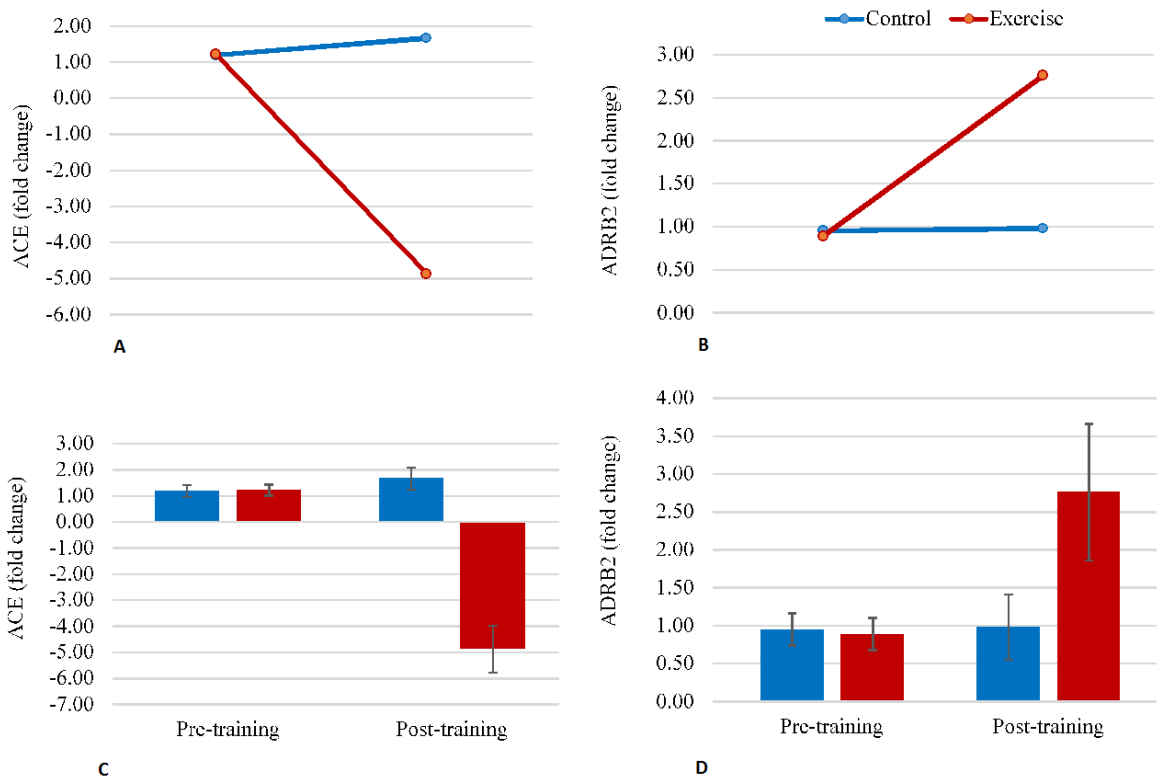

Figure 4.5. Comparison of the differences in ACE and ADRB2 mRNA from pre- to 10 weeks post-exercise between control and exercise groups.

4.4. Associations among Blood Pressure and Factors Involved in Blood Pressure Regulation

To determine the associations among blood pressure and the factors involved in BP regulation Pearson product-moment correlation analysis was used. The results were presented in Table 4.7. Significant inverse associations were found between ADRB2 mRNA level with both SBP ($r=-0.778$, $p<0.05$) and DBP ($r=-0.553$, $p<0.05$) after 10 weeks of exercise training. In addition, ADRB2 mRNA level was associated with both FMD ($r=0.857$, $p<0.05$) and ACE ($r=-0.863$, $p<0.05$). Also, the correlation between ADRB and Ang II ($r=-0.856$, $p>0.05$) was statistically significant.

ACE mRNA level was positively correlated to both SBP ($r=0.861$, $p<0.05$) and DBP ($r=0.764$, $p<0.05$) and inversely associated to FMD ($r=-0.967$, $p<0.05$). In addition, significant positive relationship was found between ACE and Ang II ($r=0.941$, $p > 0.05$).

A strong negative relationship was found between FMD and both SBP ($r=-0.746$, $p<0.05$) and DBP ($r=-0.656$, $p<0.05$). Additionally, FMD inversely correlated with Ang II ($r=-0.915$, $p<0.05$). Furthermore, a significant positive correlation was found between Ang II and both SBP ($r=0.849$, $p<0.05$) and DBP ($r=0.675$, $p<0.05$).

Table 4.7. Correlations (r) between Change in FMD, Ang II, ACE mRNA, B2ADR mRNA and change in systolic and diastolic blood pressure after 10 weeks of intervention ($^{*}p< 0.05$).

	SBP	**DBP**	**FMD**	**Ang II**	**ACE**	**ADRB2**
SBP	-					
DBP	0.776*	-				
FMD	-0.746*	-0.656*	-			
Ang II	0.849*	0.675*	-0.915*	-		
ACE	0.861*	0.764*	-0.967*	0.941*	-	
ADRB2	-0.778*	-0.553*	0.857*	-0.856*	-0.863*	-

CHAPTER 5
DISCUSSION

The main findings of the present study are that the 10 weeks of aerobic exercise training in obese pre-hypertensive postmenopausal women decreased systolic and diastolic BP, which was accompanied by significantly reduced Ang II plasma levels, decreased ACE and increased ADRB2 mRNA expression in leukocytes, and enhanced FMD. These findings indicate the beneficial effects of moderate intensity exercise training in the modulation of risk factors of CVD in obese postmenopausal women with pre-hypertension that is associated with alteration in genes involved in regulation of BP and improvement in endothelial function. The following section will discuss the details of the effect of 10 weeks of aerobic exercise training on these indices.

5.1. Effect of Exercise Training on Systolic and Diastolic Blood Pressure

Our results revealed that 10 weeks of moderate intensity exercise training decreased systolic (4.6%) and diastolic (2.4%) BP in obese postmenopausal women with pre-hypertension. There are growing evidence showing that aerobic exercise training promotes beneficial effects on the prevention and treatment of HTN. These findings are consistent with the results of Zaros et al (136) reporting a decline in systolic and diastolic BP following 6 month moderate intensity exercise training (50% of HRR, 60 min, 3 day/week) in obese postmenopausal women with stage 1 HTN (SBP, 140-159 and DBP, 90-99 mmHg). This result also agrees with the results of Khalid et al (137), revealing that 8 weeks moderate intensity exercise (walking on treadmill with 60-70% of MHR, 20 min, 3 day/week) decreased systolic and diastolic BP by 16.2% and 9.5%, respectively in obese postmenopausal women with HTN. Others (139) have shown that 8 weeks aerobic exercise training (treadmill exercise at intensity of 100% maximal lactate steady state, 30 to 40 min, 3 day/week) in overweight postmenopausal women with HTN (using antihypertensive medications) resulted in approximately 3.8% and 5.9% decrease in systolic and diastolic BP, respectively. Indeed, reduction in BP following moderate intensity exercise training has been reported in numerous well-controlled studies (201).

According to these studies, magnitude of decline is dependent to the initial level of BP. Individuals with high BP have shown a higher reduction compared with normotensives both in systolic and diastolic BP. On the contrary, there are studies (202) reporting no decrease in BP following aerobic exercise training (despite significant improvement in cardiorespiratory fitness) in healthy but overweight postmenopausal women with HTN and pre-hypertension. However, it is possible that the variability in the results of studies is related to design of training program, duration of program, inclusion criteria and initial condition of subjects.

Reduction in total peripheral resistance is the primary mechanism involved in decreasing of BP following exercise training. Training-induced reductions in vascular resistance are mediated by neurohumoral and structural adaptations and alteration in vascular responsiveness. Our results indicated that 10 weeks of exercise training resulted to alteration in RAS components (ACE gene expression and Ang II plasma level), ADRB2 expression and FMD. Therefore, it is possible that these alterations have modulated BP in our subjects.

On the other hand, potential effect of exercise training on NO formation has been well established. In this regard, recently published papers (136,137) have shown significant reduction in BP of hypertensive postmenopausal women following aerobic exercise training with different durations (8 weeks, 3 month, 6 month), which is associated with increase in NO levels. Therefore, exercise-induced enhancement in NO production can be one of the possible mechanisms that reduced BP in our subjects.

In addition to the described mechanisms for reduction in BP, we must consider the role of genetic variation in modification of BP response to exercise. For example, in response to exercise training, hypertensive individuals with different alleles for apolipoprotein E gene have indicated different changes in resting BP (145).

However, we should also note the fact that regulation of BP as well as exercise-induced response to BP, essentially are mediated through an interaction between these mechanisms. Therefore, the aim of this study was to investigate the effect of 10 weeks of aerobic exercise training on some of the potential markers involved in BP reduction in obese postmenopausal women with pre-hypertension. In this regard, we evaluated the

effect of exercise training on ADRB2 as a component of SNS, ACE and Ang II as the components of RAS as well as FMD as an index of endothelial function in this group.

5.2. Effect of Exercise Training on ADRB2 Gene Expression in Leukocytes

The results of our study indicated that 10 weeks of aerobic exercise training significantly increased mRNA level of ADRB2 in leukocytes of obese postmenopausal women, which was accompanied by reduced systolic and diastolic BP compared with sedentary control. This finding supports the results of the study by Tartibian et al (26) indicating an increased level of ADRB2 in leukocytes of healthy sedentary middle-age men following 8 weeks of moderate intensity exercise training, which was accompanied by decrease in BP.

In addition, there are studies reporting increased density of βARs following acute prolonged aerobic exercise in both healthy subjects (203) and heart failure patients (204). Furthermore, Maisel et al (205) determined increased number of lymphocyte βARs after treadmill exercise to exhaustion in healthy individuals and patients with congestive heart failure which was mediated by epinephrine. Also, findings of increased βARs density following various types of exercise training have been reported in animal models. For example, results of this study is in accordance with findings of Leosco et al (154) that reported increased βARs density and sensitivity following 6-week swimming exercise training (5 days/week) in aged-rat carotid arteries. Same group in another study (71) indicated that 12 weeks treadmill running exercise resulted in improved βAR responsiveness in the myocardium of aged rat.

Impact of exercise training on sympathoadrenergic system is well known and the regulation of this system plays critical role in physiological adaptation to exercise. Some of these adaptatory responses are mediated by decreased catecholamines levels (206) and reduction in sympathetic activity through alteration in adrenergic receptor density and responsiveness (207). Also, exercise-induced improvements in cardiovascular function in elderly are related to increased cardiovascular βAR responsiveness (208,209), which probably resulting from circulating epinephrine-mediated stimulation of the ADRB2. For instance, studies (210) have reported decrease in resting levels of epinephrine following

12 weeks exercise training in postmenopausal women. Therefore, decrease in catecholamine levels can be one possible mechanism involved in enhancement of ADRB2. It is likely that reduction in catecholamine level resulted in up-regulation of ADRB2 in vessels and has increased vasodilation and decreased peripheral resistance, which led to a reduced BP in our subjects. In this regard, exercise-induced up-regulation of βARs has previously been demonstrated, and expression of these genes may be an early adaptive response of SNS to exercise (211).

On the other hand, Leosco et al (71,154) reported aging-induced impairment in βAR vasorelaxation in cardiac and carotid arteries of rat, which restored with exercise training. Also, they reported an exercise-induced reduction in βARK-1 protein levels in trained old rats to the levels similar to those observed in young animals. Therefore, at least, a part of βAR-induced vasorelaxation following exercise training can be mediated by decreased expression level of βARK1. We did not measure expression of βARK1 in leukocytes. Nevertheless, increase in ADRB2 due to reduction in expression of βARK1 may be one possible mechanism for exercise-induced vasodilation and reduction in BP observed in our subjects.

In addition, considering that aerobic exercise training activates cAMP that mediates vasodilation, and that cAMP also stimulates the expression of ADRB2 mRNA (212), which also affects vasodilation responses, this may be another possible mechanism involved in the reduction of BP.

Furthermore, it has been reported that moderate intensity exercise training increases plasma concentration of cyclic guanosine monophosphate (cGMP) (a second messenger of NO) in elderly women (213). cGMP induces vascular smooth muscle relaxation that leads to vasodilation. Therefore, this mechanism may also contribute to the reduction of BP.

Furthermore, relation between lipolysis and ADRB2 expression has been documented. A moderate weight loss results in a higher adipose cell lipolytic efficiency which is associated with increased ADRB2 in adipose tissue (214). We have shown a significant decrease in body fat percentage (11.7%) in exercise group. Therefore, it is

possible that moderate intensity exercise activated the β2-adrenergic receptor in the lipolysis pathway.

Moreover, there is a relation between ADRB2 and NO generation. In support of this, studies (215) have shown that stimulation of ADRB2 and elevation of cyclic AMP resulted in an increase in NO production through the L-arginine/NO system and promotion of vasorelaxation in human umbilical vein. Therefore, ADRB2 induced-increase in NO level can be another possible mechanism that resulted in improvement in vasodilation and reduction in BP in our subjects.

In addition, in our study there was a significant negative (inverse) correlation between ADRB2 with systolic ($r=-0.778$, $p<0.05$) and diastolic ($r=-0.553$, $p<0.05$) BP after 10 weeks training. Similarly, Oliver et al (63) reported a significant inverse correlation between lymphocyte ADRB2 and 24 h DBP in hypertensive patients. This suggests a direct participation of ADRB2 in the regulation of BP. Considering that ADRB2 vasodilatation is a critical component in the modulation of vascular resistance, a possible explanation for this correlation is that a higher expression of ADRB2 in vessels (mirrored in leukocytes) can decrease vascular resistance and reduce BP.

Moreover, pharmacological inhibition of ACE has increased β adrenergic receptor density and decreased βARK1 protein expression and plasma levels of norepinephrine (216). Our results indicated decline in ACE gene expression as well as significant negative correlation between ACE and ADRB2 ($r=-0.863$, $p<0.05$). Therefore, ACE decrease-induced enhancement in ADRB2 can be possible mechanism in vasodilation and resultant reduction in BP.

Altogether, the observed reduction in systolic and diastolic BP in our study could be resulted from alteration in ADRB2 gene expression that induced functional and physiological changes in the vasculature. On the other hand, there is an interaction between components of SNS and RAS in the regulation of BP. Among these, ACE as an important component of RAS has attracted considerable attention.

5.3. Effect of Exercise Training on ACE Gene Expression in Leukocytes

ACE is one of the important components of the RAS that increases BP due to conversion of Ang I to Ang II and degradation of bradykinin.

On one hand, studies have demonstrated aging-induced overactivity of RAS as one of the underlying mechanisms for HTN in postmenopausal women. On the other hand, RAS is activated in obesity. Therefore, it is logical that decrease in ACE level can modulate HTN in obese postmenopausal women. Nevertheless, up to now, pharmacologic interventions (ACE inhibitors) have been used extensively for ACE inhibition and treatment of HTN, but there is little information on the impact of various types of exercise (as a non-pharmacological intervention) on ACE gene expression in humans, and most of the studies have been conducted on animals.

Our results showed a significant decrease in mRNA levels of ACE in leukocytes of obese postmenopausal women with pre-hypertension, which is accompanied by significantly reduced systolic and diastolic BP. This result is consistent with findings of Tartibian et al (26) that reported decrease in ACE mRNA level in leukocytes of middle-aged sedentary men after 4 weeks of moderate intensity exercise training, which is accompanied by BP reduction. In addition, this result is in agreement with findings of Litwin et al (217) that revealed inhibition of ACE expression in the leukocytes of hypertensive children and a decrease in BP following 6 months non-pharmacological treatment (dietary advice and physical activities).

Animal studies have reported exercise training-induced normalization of ACE expression in cardiac muscle (8 weeks of treadmill running) (156) and brain (3 week of treadmill running) (157) of animals following post-myocardial infarction and chronic heart failure. Also, Fernandes et al (158) have shown a decrease in ACE mRNA in rat cardiac muscle following 10 weeks of high volume swimming exercise training.

There are several possible mechanisms that could explain the effect of exercise training on ACE mRNA expression and its association with BP. First, changes in body composition including decrease in body weight, fat mass and fat percent may be related to the alterations in ACE expression with subsequent decrease in BP (218). It is also important to note that adipose tissue promotes an inflammatory response in obesity, and

obesity-induced inflammation plays a role in the development of HTN. It is likely that activation of peripheral blood leukocytes in hypertensive individuals might be due to nonspecific inflammatory reaction in addition to increased shear stress, which induced alteration in leukocytes gene expression profile. Exercise can modulate shear stress. In our study 10 weeks exercise training resulted in significant decrease in body weight (3%), fat percent (11.7%) and fat mass (14.4%) in obese postmenopausal women. Therefore, we can consider the possibility that the exercise-induced decline in fat mass resulted in the modulation of inflammatory responses in peripheral blood cells and decrease in ACE mRNA expression. Because ACE increases BP due to the conversion of Ang I to Ang II (potent vasoconstrictor), decrease in ACE level results in decrease in vascular resistance and thus reduces BP.

Other possible mechanism is related to interaction between SNS and RAS (219,220). Exercise training decreases elevated SNS activity. Thus there is possibility that exercise-induced improvement in SNS resulted in decreased overactivity of RAS and decrease in ACE mRNA levels.

In addition, different mechanisms could also be involved in decreased ACE mRNA expression. Evidence (221) shows that macrophages (major leukocyte types) secrete Ang II via an ACE-dependent mechanism, and Ang II is involved in vasoconstriction. In the present study, 10 weeks exercise training decreased both ACE mRNA expression and circulating levels of Ang II, which both of them may be associated with reduction in BP in our subjects.

Furthermore, it has been demonstrated that exercise activates endothelial NO productions due to increased shear stress (222). High levels of shear stress alter endothelial cell function by suppressing ACE gene and protein expression in vitro and in vivo (223). Therefore, exercise-induced increase in shear stress could have modulated NO production and ACE activity, and subsequently reduced BP. Also, one study has reported that inhibition of ACE increases cardiac NO levels via the accumulation of bradykinin in canine ischemic myocardium (224). It is likely that exercise has increased NO levels due to decrease in ACE levels, and NO has essential role in lowering of BP.

Taken together, our results confirm the data obtained from previous studies (26,27) by indicating that exercise training alters gene expression levels of some of genes involved in BP regulation in peripheral blood cell in hypertensive subjects that is accompanied by reduction in BP.

5.4. Effect of Exercise Training on Circulating Level of Ang II

Aging-induced and obesity-induced HTN are associated with overactivity of RAS and alterations in its components. Ang II is one of the most important components of RAS which increases total peripheral resistance and leads to high BP. Several studies have reported elevated plasma Ang II levels in obese hypertensive adults (99) and children (100) compared with normotensives. However, limited studies have investigated the effects of exercise training on Ang II levels in humans especially in obese postmenopausal women.

Our results showed that 10 weeks of aerobic exercise training resulted in a significant reduction in circulating levels of Ang II in obese postmenopausal women, which was associated with decreased BP. Considering important role of Ang II in induction of HTN, this finding suggests that moderate intensity exercise training may be an alternative and effective non-pharmacological treatment of HTN in the early stages. Our findings are consistent with previous studies that have reported exercise training-induced reduction in plasma Ang II levels. However, the most of these studies conducted in humans with cardiac heart failure and animal models. For example, it has been reported (159) that 16 weeks of endurance exercise training (walking at 40% to 70% of peak VO_2, 3 times/week) resulted in significant decrease in resting levels of Ang II in patients with chronic heart failure. Also, one recently published study (100) reported decrease in Ang II plasma levels after 6 weeks aerobic exercise training (60% to 70% of MHR, 40min, 5 days/week) in obese children.

Several studies have reported reduction in plasma Ang II levels following moderate intensity exercise training (treadmill running for 4 and 8 weeks) in rabbit and rat with chronic heart failure (28,96,163). Barretti et al (166) indicated reduction in Ang II level after 10 weeks of swimming exercise (60 min, 5 days/week) on obese Zucker rats.

Others (225) have shown that 16 weeks moderate-intensity exercise training attenuated Ang II plasma concentrations, systolic and diastolic BP in spontaneously hypertensive rats compared with sedentary counterparts. Fillho et al (226) reported that 8 weeks of swimming training decreased Ang II plasma levels in normotensive Wistar rats and spontaneously hypertensive rats. Also, 8 weeks swimming exercise decreased Ang II plasma levels in both young and old rats, which was associated with reduction in BP (227).

In addition to the effect on circulating level, 8 weeks running exercise has also decreased cardiac Ang II levels and prevented cardiac dysfunction in a genetic model of sympathetic hyperactivity-induced heart failure in mice (165). Also, both moderate and high volumes of swimming exercise have been associated with a decrease cardiac Ang II levels in rats (158). Moreover, 8 weeks swimming training has decreased Ang II-induced coronary vasoconstriction in ovariectomized rat (with increased Ang II-induced vasoconstriction in the coronary vascular bed) (228).

One possible mechanism mediating exercise-induced decrease in circulating levels of Ang II may be due to decrease in ACE activity in response to exercise training. Our study revealed that 10 weeks exercise training decreased ACE mRNA levels in leukocytes of obese postmenopausal women with pre-hypertension. Since, ACE is the main enzyme responsible for conversion of the Ang I to Ang II, it is likely that exercise-induced reduction in ACE level may decrease BP through modulation of Ang II circulating levels. In this regard, our results showed a significant positive correlation between ACE and Ang II ($r=0.941$, $p < 0.05$).

This fact has been observed in study by Agarwal et al (225) that reported 16 weeks exercise training in spontaneously hypertensive rats decreased systolic and diastolic BP, which is associated with reduction in plasma Ang II levels, and brain ACE and Ang II level. Furthermore, Pereira et al (165) reported that a decreased cardiac Ang II level in exercise-trained mice was paralleled by reduced cardiac ACE activity.

On the other hand, considering the role of Ang II in regulation of renal excretion of water and electrolyte, exercise-induced increase in urinary sodium excretion could also attributed to pressure-lowering effects of exercise. We cannot rule out the possibility, because we did not measured urinary sodium excretion in our subjects. But, other studies

(229) have confirmed these findings. It has been reported that long term exercise training decreases BP and increases fractional urinary sodium excretion in trained spontaneously hypertensive rats, compared with sedentary counterparts (229).

In addition, the function of Ang II in regulation of BP has been mediated due to AT1 receptors and down regulation of these receptors can modulate Ang II function. It seems that exercise exerts a modulatory effect on Ang II receptor expression. Several studies have reported exercise-induced decrease in expression of AT1 receptors in the entire kidney (229) and brain (central nervous system) (225) of trained spontaneously hypertensive rats. Others have shown a significant up regulation AT1 receptor in the brain of chronic heart failure rats that was normalized by exercise training (230). Therefore, 10 weeks exercise training could be effective in modulating of these receptors that resulted in decrease in total peripheral resistance and reduction in BP.

On the other hand, studies have shown a relationship between renal sympathetic nerve activity and plasma Ang II in animals (96). Ang II increases sympathetic nerve activity due to actions on both central and peripheral sites. Increased renal sympathetic nerve activity (due to increase vasoconstriction and renin release) contributes to maintenance of HTN. Therefore, it is rational to assume that exercise training-induced decrease in Ang II level results in decline in sympathetic nerve activity and modulation of BP. For example, Mousa et al (28) reported decrease in Ang II plasma levels and AT1 receptor in brain that resulted in decreased renal sympathetic nerve activity in rabbit with chronic heart failure. In study by Roveda et al (231) 4 month exercise training program reduced sympathetic nerve activity in patients with chronic heart failure. Also, one longitudinal study (232) on never-treated hypertensive patients has reported that 4 month aerobic exercise training elicited a significant reduction of both BP and resting muscle sympathetic nerve activity, and also improved sympathetic baroreflex. But, Ang II level has not been measured in that study. Therefore, exercise-induced Ang II-dependent decrease in sympathetic nerve activity could be related to reduce BP in the present study. Taken together, these findings suggest that aerobic exercise training modulates the RAS and SNS, which results in decrease in circulating levels of Ang II. This mechanism, at least in part, may contribute to pressure-lowering effect of exercise training.

As mentioned earlier, Ang II causes oxidative stress that increases vasoconstriction and leads to high BP. Exercise training decreases vascular oxidative stress through inactivation of NADPH and increased production of NO. Therefore, it is likely that one of the potential mechanisms explaining BP lowering effect of 10 weeks exercise training is related to reduction in oxidative stress.

Considering the overactivity of RAS in obesity-related HTN, it is possible that exercise training could have modified RAS components due to its effect on body composition. In this regard, one study conducted in obese postmenopausal women demonstrated that 16 weeks diet and exercise intervention reduced body weight and fat percentage (5% and 10%, respectively) and resulted in meaningful decline in RAS components in plasma and adipose tissue, which may contribute to reduction in BP (161). Interestingly, they did not find any difference in the systemic Ang II levels at the baseline and after training program between obese and lean women, while the renin, ACE and angiotensinogen plasma levels were higher in obese postmenopausal women. However, their study showed direct relation between body weight and fat mass with circulating levels of RAS.

In addition, study by Al-Daghri et al (99) revealed positive association between systolic and diastolic BP with BMI and Ang II levels. 10 weeks of exercise training program in our study resulted in reduction in Ang II plasma level and is accompanied by 3% weight loss; 11.7% reduction in fat percent and 14.4% reduction in fat mass. Also, we found a positive correlation between systolic and diastolic BP with Ang II at the baseline and after 10 weeks training program. Therefore, decrease in BP in our subjects can be attributed to decrease in Ang II plasma levels (decreased activity level of RAS) and also decrease in body weight and fat mass. It is likely that reduction in fat % and fat mass has modulated Ang II levels due to effect on adipose tissues.

In the present study, we demonstrated for the first time to our knowledge that in obese postmenopausal women with pre-hypertension, 10 weeks of aerobic exercise training decreases Ang II plasma levels. Also, this decrease is positively correlated with systolic and diastolic BP.

5.5. Effect of Exercise Training on Flow Mediated Dilation

Obesity, menopause and HTN are associated with endothelium dysfunction that plays an important role in the pathogenesis of vascular disease (46).

At the same time, role of exercise training on the preservation of endothelial function as well as improvement of FMD (as an index of endothelium function) is well established (233). However, there are few studies investigating the impact of exercise training on endothelial function and FMD in obese postmenopausal women with pre-hypertension.

Our study showed significant improvement in FMD (6.02 ± 0.71 before vs. 11.20 ± 1.12 after exercise, p=0.001) following 10 weeks of aerobic exercise training in obese postmenopausal women with pre-hypertension compared with sedentary controls. This result is consistent with findings of Swift et al (24) that reported increase in FMD following 6 month exercise (treadmill running and ergometer at intensity of 50% VO_2 peak with different energy expenditure per week) in hypertensive overweight/obese postmenopausal women. It is important to note that participants in this study taking different types of antihypertensive medications include ACE inhibitors, diuretics, hormone replacement therapy and β-blockers.

Our result is also in line with previous studies (234) which reported increase in FMD following 8 weeks moderated intensity exercise training in postmenopausal women (normal weight and normal BP). In addition, there are several studies (235,236) reporting exercise-induced improvement in FMD in animal models of HTN and in patients with essential HTN as well as in healthy individuals.

One possible mechanism for exercise-induced improvement in endothelial function is through an increase in NO bioavailability or decrease in NO inactivation. Exercise training increases vascular shear stress due to enhanced blood flow, which in turn up-regulates eNOS, and thus increases NO production. Although we did not assess the production of NO, it is plausible that the improvement in endothelial function in our subjects could be mediated by an increase in exercise-induced NO bioavailability.

In addition, it has been documented that moderate intensity exercise training decreases oxidative stress, inhibits oxidative stress-induced degradation of NO and

improves antioxidant defense system (such as superoxide dismutase) through inactivation of NADH/NADPH in vasculature, and in this way improves endothelial function. Therefore, exercise-induced reduction in oxidative stress is another possible mechanism that may be contributes to improvement of FMD in our subjects.

Considering that the degree of endothelial dysfunction is related to the severity of HTN (Panza, Casino et al. 1993), it would be reasonable to assume that reduction in blood pressure will improve endothelial function. In our study there was a strong negative correlation between systolic ($r=-0.746$, $p<0.05$) and diastolic ($r=-0.656$, $p<0.05$) blood pressure with FMD after cessation of training program. Therefore, we cannot exclude the possibility that a reduction in BP may be involved in improvement of endothelial function.

Additionally, ADRB2 causes vasodilation due to endothelium-dependent and -independent mechanisms. Therefore, increased ADRB2 in our study could be possible mechanism involved in the improvement of endothelial function and FMD. We demonstrated that 10 weeks of exercise training increased ADRB2 and FMD in obese postmenopausal women. In addition, we found positive correlation between ADRB2 and FMD ($r=0.857$, $p<0.05$). Therefore, we can propose that increased level of ADRB2 in our subjects is associated with reduction in BP, which is resulted in improvement in endothelial function and FMD.

On the other hand, we shouldn't ignore the effect of weight loss in FMD improvement. In this regard, studies (167) have demonstrated a relation between FMD and weight loss. They reported that 4-month exercise and weight-loss program (dietary intervention) resulted in significant improvement in FMD in overweight and obese patients with chronic heart disease. But, greater weight reduction was associated with a greater improvement in FMD. In our study subjects in exercise group had 3% weight loss. Therefore, we can purpose that weight loss may be one of the factors that affected on FMD improvement.

Furthermore, the role of Ang II in decreasing FMD in skeletal muscle arterioles of mice has already been reported (237). This mechanism may contribute to the endothelial dysfunction observed in CVD that is associated with increased activity of the RAS and elevated redox stress.

Taken together, it seems that prevention of overactivity of the SNS and RAS is likely to decline total peripheral resistance and to increase BP and subsequent improve FMD.

5.6. Effect of Exercise Training-induced Body Composition Changes on Blood Pressure

Several clinical interventions in humans have demonstrated significant impact of weight loss on BP. In support of this, recently published meta-analysis reported that 1 kg reduction in body weight was associated with an approximate 1mmHg drop in blood pressure (238). Our study showed a significant decrease of body weight (3 %), fat mass (14.4%) and fat percent (11.7%) in the exercise group compared with pre-training values. Previous studies have shown that aerobic exercise is the optimal mode of exercise for reducing body mass and fat mass (239). The results concerning body weight are consistent with the results of a randomized controlled trial which reported that regular exercise (such as brisk walking) results in reduced body weight and body fat in overweight and obese postmenopausal women (240). Different mechanisms have been suggested to explain the blood pressure-lowering effect of weight loss include decrease in SNS activity, an improvement in insulin resistance, and a normalization of the aldosterone-renin interrelationship. Therefore, we can conclude that 10 weeks aerobic exercise training has reduced BP due to alteration in SNA and RAS. Also, it has been effective in reducing of body weight and fat percent.

5.7. Effect of 10 Weeks Aerobic Exercise Training on Cardiovascular Fitness

The results of the present study indicated significant improvement in VO_2 max (22.7 %) obtained during a graded exercise test (27.78 ± 5.14 ml/kg/min in post-training vs. 22.64 ± 5.71 ml/kg/min in pre-training). Also, time to exhaustion has been increased in exercise group (19.3 %) (7.23 ± 1.17 min post exercise vs. 6.06 ± 1.30 min before exercise). This finding is consistent with results of previous studies (202,241) indicating that regular moderate intensity exercise improves VO2 max in sedentary, overweight/obese postmenopausal women with elevated BP. Furthermore, a number of previous studies (242) have suggested that high cardiorespiratory fitness is associated with

lower levels of total and abdominal obesity independent of BMI. We conclude that in addition to exercise-induced cardiovascular adaptation that leads to improvement in cardiorespiratory fitness, significant decrease in body fat mass and fat percent as well as increase in lean body mass can be related to increased VO_2 max observed in our study.

CHAPTER 6
CONCLUSION AND SUGGESTIONS

6.1. Conclusion

The results of this study showed that 10 weeks of moderate intensity exercise training decreased systolic and diastolic BP and improved cardiovascular fitness and body composition in obese postmenopausal women with pre-hypertension. Also, these results clearly revealed that reduction in systolic and diastolic BP are associated with reduction in ACE mRNA and Ang II plasma levels as well as increase in ADRB2 mRNA expression. Improvement in FMD was another important finding of this study. In the present study, for the first time, we demonstrated that 10 weeks aerobic exercise training in obese postmenopausal women with pre-hypertension is associated with alteration in gene expression markers of BP. Our findings confirm the previous studies reporting that gene profile in leukocytes of hypertensive patients can be altered with treatment. To our knowledge, so far only one study investigated ACE and ADRB2 mRNA expression and BP changes at the same time, which was conducted on middle-aged men. However, no study has been conducted in obese postmenopausal women with pre-hypertension.

6.2. Suggestions

The present study had some limitations. First, we only studied mRNA expression of ADRB2 and ACE. Thus, someone could argue that there is no guarantee that the changes in gene expression have been translated into protein expression. Second, due to difficulty in participant recruitment, we were unable to have a lean control and lean exercise training group. Furthermore, this study focused only on obese postmenopausal women with pre-hypertension. Thus, the findings of this study cannot be generalized to other populations, such as young men and women with high BP. In addition, as the type and intensity of exercise may influence the activation of these genes, it is recommended that different type and intensity of exercise be investigated. In conclusion, further studies are warranted to deal with the genes involved in BP regulation in different age and sex

groups, different stages of HTN, as well as the effect of various intensities and duration of exercise programs on BP in both men and women.

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Appendix 1

Medical History and Screening Form

Participant Code:		Date:
Name:	Age:	Contact phone numbers:
Sex:	Height (cm):	Family physician name:
SBP:	Weight (kg):	Family physician telephone:
DBP:	Menopause time:	Occupation:

Present Medical History

Check those questions to which you answer yes (leave the others blank).

- ☐ Has a doctor ever said your blood pressure was too high?
- ☐ Do you ever have pain in your chest or heart?
- ☐ Are you often bothered by a thumping of the heart?
- ☐ Do you ever notice extra heartbeats or skipped beats?
- ☐ Are your ankles often badly swollen?
- ☐ Do cold hands or feet trouble you even in hot weather?
- ☐ Has a doctor ever said that you have or have had heart trouble, an abnormal electrocardiogram, heart attack or coronary?
- ☐ Do you suffer from frequent cramps in your legs?
- ☐ Do you often have difficulty breathing?
- ☐ Do you sometimes get out of breath when sitting still or sleeping?
- ☐ Has a doctor ever told you your cholesterol level was high?
- ☐ Has a doctor ever told you that you have an abdominal aortic aneurysm?
- ☐ Has a doctor ever told you that you have critical aortic stenosis?

Do you now have or have you recently experienced:

- ☐ Chronic, recurrent or morning coughs?
- ☐ Episode of coughing up blood?
- ☐ Increased anxiety or depression?
- ☐ Problems with recurrent fatigue, trouble sleeping or increased irritability?
- ☐ Migraine or recurrent headaches?
- ☐ Swollen or painful knees or ankles?
- ☐ Swollen, stiff or painful joints?
- ☐ Pain in your legs after walking short distances?
- ☐ Musculoskeletal problems?

☐ Stomach or intestinal problems, such as recurrent heartburn, ulcers, constipation or diarrhea?
☐ Glaucoma or increased pressure in the eyes?
☐ An infection such as pneumonia accompanied by a fever?
☐ Significant unexplained weight loss?
☐ A fever, which can cause dehydration and rapid heartbeat?
☐ A deep vein thrombosis (blood clot)?
☐ A hernia that is causing symptoms?
☐ Foot or ankle sores that won't heal?
☐ Persistent pain or problems walking after you have fallen?

Are you on any type of hormone replacement therapy?
.....
List any prescription medications you are now taking:
…….
List any other medical or diagnostic test you have had in the past two years:
……..
Did you any change more than 10% of your normal body weight in the last year?
……..
Do you engage in regular physical activity more than 2 times during a week?
……

Past Medical History

Check those questions to which your answer is yes (leave others blank).

☐ Heart attack if so, how many years ago?
☐ Rheumatic Fever
☐ Diseases of the arteries
☐ Heart murmur
☐ Varicose veins
☐ Stroke
☐ Diphtheria
☐ Scarlet Fever
☐ Thyroid problems
☐ Pneumonia
☐ Bronchitis
☐ Injuries to back, arms, legs or joint
☐ Arthritis of legs or arms
☐ Diabetes or abnormal blood-sugar tests
☐ Phlebitis (inflammation of a vein)
☐ Dizziness or fainting spells
☐ Epilepsy or seizures
☐ Infectious mononucleosis
☐ Nervous or emotional problems
☐ Anemia
☐ Asthma
☐ Abnormal chest X-ray
☐ Other lung disease
☐ Bone fractures

Familial Diseases

Have you or your blood relatives had any of the following (include grandparents, aunts and uncles, but exclude cousins, relatives by marriage and half-relatives)?

Check those to which the answer is yes (leave other blank).

- ☐ Heart attacks under age 50
- ☐ Strokes under age 50
- ☐ High blood pressure
- ☐ Elevated cholesterol
- ☐ Glaucoma
- ☐ Diabetes
- ☐ Asthma or hay fever
- ☐ Heart operations
- ☐ Obesity
- ☐ Leukemia or cancer under age 60

Other Heart Disease Risk Factors

Have you ever smoked cigarettes or a pipe?	□ Yes	□ No
Do you ever drink alcoholic beverages?	□ Yes	□ No
Do you usually add salt at the table?	□ Yes	□ No

Appendix 2

Informed Consent Form for Exercise Group

Dear participant,

You are being invited to participate in a research study directed by Assistant Professor Dr. Şükran Nazan Koşar in Hacettepe University Faculty of Sport Sciences, Associate Professor Dr. Bakhtyar Tartibian in Urmia University and physiotherapist Noushin Azadpour in Ardabil University of Medical Sciences.

Menopausal women have decreased vasodilator capacity of vessels due to hormonal changes and obesity that increases the risk of hypertension in this group of women. However, regular moderate intensity exercise training reduces the risk of hypertension and related risk factors. Blood pressure lowering effect of exercise training is associated with positive effects on nervous system, improvement of vascular function and decrease in intra-abdominal fat. In addition, recent studeis have demonstrated the impact of exercise training on genes involved in blood pressure regulation.

Therefore, the aim of this study is to investigate the effect of 10 weeks of moderate intensity aerobic exercise training in some possible genetic, hormonal and structural factors involved in blood pressure regulation in obese postmenopausal women with pre-hypertension. The results of this study will be published in an academic journal. We hope that the findings of this study to be useful to clarify the effect of exercise training on the regulation of blood pressure in obese postmenopausal women with pre-hypertension.

If you agree to participate in the study, medical history will be taken and you will undergo medical examination. Then, you will be participated in 10 weeks of moderate intensity aerobic exercise training under direct supervision of exercise physiologist.

Three days before and 48 hours after training program, you will undergo tests for the measuement of body fat percentage, waist and hip circumference, and also test for the determination of maximal oxygen uptake. Also, your vascular function will be measured and 5 mililiter blood samples will be taken from your antecubital vein following 12 hours overnight fasting. Furthermore, you will complete physical activity questionnaire and 3 day food record before and after 10 weeks of training program.

The exercise training program will be performed 3 days per week for 10 weeks. In the first weeks, you will exercise for 25 minutes. Then, time will be increased gradually in the later weeks and will be maintained at 40 minutes during the last four weeks. The intensity of the

exercise will be determined specifically for each subject and will be controlled by a heart rate monitor.

At least one week prior to these tests, you will be asked to avoid participation in exercise and consumption of alcohol and caffeine. During the 10 weeks of exercise training, you will be asked to refrain from making changes in your diet and in physical activity level.

All information obtained during the course of this study is strictly confidential and your anonymity will be protected at all times. All data will be available only to the researchers and you. This information will be used only for educational and research purposes. During this study, the privacy of your information will be approached with great care and respect.

The results will not be reported immediately since knowing the results does not provide any benefit to you. If you wish, you will be told the results of your tests at the end of study. There will be no costs for participating in the research. Also, you will not be paid for your participation.

Potential risks and discomfort

The methods used for the determination of your waist and hip circumference as well as body fat percentage have not any risk for you. Measurement of maximal oxygen uptake tests will be performed on a treadmill. The degree of difficulty on the treadmill test will be increased every 3 minutes interval and test will be continued until exhaustion. Your maximal effort during determination of maximal oxygen uptake is extremely important for the reliability of the findings obtained from the study.

During the test, you may feel fatigue, increase in your heart rate, breathless and sweating. But, these are normal body responses to exercise. In addition, during the test, heart rate, blood pressure, 12 leads ECG and ratings of perceived exertion will be monitored and recorded. Test will be performed under direct supervision of a cardiologist. You may feel tired or even exhausted after the test.

During blood sampling, you may feel temporary pain due to needling. Blood sampling will be performed in a hygienic manner. The material used for others will not be used in any way for you.

Doppler sonography will be used to measure changes in brachial artery diameter (by a specialist sonographer). Ultrasound studies will be performed in the morning, after that you had rested in supine position for 30 min in a quiet temperature controlled room. During this test a blood pressure cuff will be placed on the forearm and inflated to 50 mmHg above SBP for 5

minutes. This can cause discomfort, due to the pressure applied to your arm, but there is no health risk for you.

During the exercise training, exercise intensity will be monitored through heart rate monitor placed around your chest. During each exercise session, your heart rate and breathing frequency will increase and you will sweat. You may feel tired at the end of the training sessions. However, these are normal physiological responses to exercise.

With regard to the above-mentioned notification, these are potential risks that may be experienced during participation in this study. However, every precaution will be taken to minimize possible problems by the preliminary examination and constant surveillance during tests and training sessions. Participation in this study is completely voluntary. You can refuse to participate in this study. If you decide to participate, you are free to withdraw at any time.

If you want to get more information about the study or if you encounter any questions, you can contact with the researchers conducting the study, Associate Professor Dr. Bakhtyar Tartibian (Tel: +98 9126090551) and physiotherapist Noushin Azadpour (Tel: +989144521533).

Participant / Patient's Declaration

I have been given sufficient information about the study conducted by principal researcher Assistant Professor Dr. Ş. Nazan Koşar and Associate Professor Dr. Bakhtyar Tartibian and physiotherapist Noushin Azadpour. After obtaining this information, I have been invited to participate in this study.

If I participate in this study, I believe that all my information obtained during the course of this study will be kept confidential. I have got enough confidence that my personal information will be protected during the use of research results for educational and scientific purposes.

I can withdraw from this study at any time without giving a reason. However, in order to avoid of any problem in research process, it is appropriate that I already inform to researchers that I withdraw from study. Morover, the researchers drop out me from the study provided that this doesn’t affect my medical condition negatively.

In case of injury arising from participation in the study, first aid will be provided. If additional medical care is necessary, payment will be provided by researchers.

In case of any health problem related to participation in study, I should contact with researchers telephone number or addresses.

Principal Researcher	**Assistant Researcher**	**Assistant researcher**
Assist. Prof. Dr.	Physical therapist	Assoc. Prof.
Ş. Nazan Koşar	Noushin Azadpour	Dr. Bakhtyar Tartibian
Work phone: 2976890/117	Work phone: +984512251401	Work phone: +984432753174
Mobile: 05387612924	Mobile: +989144521533	Mobile: +98 9126090551

By signing this form, I hereby agree to the following:

1. The aim of the study was explained to me.
2. My participation in this study is completely voluntary.
3. My questions were answered adequately.
4. My participation in this study is completely voluntary. I have free choice to participate or not participate in this study. I have not encountered a coercive behavior to participate in this research.

I have understood the purpose of the research and all the explanations made to me in detail. By signing this consent form, I am indicating that I agree to participate in this study. I accept the invitation for participation in this study in great satisfaction and volunteering. A signed copy of this form will be given to me.

Participant	**Interview witnesses**
Name & Surname:	Name & Surname:
Adress:	Adress:
Tel:	Tel:
Sign:	Sign:

Informed Consent Form for Control Group

Dear participant,

You are being invited to participate in a research study directed by Assistant Professor Dr. Şükran Nazan Koşar in Hacettepe University Faculty of Sport Sciences, Associate Professor Dr. Bakhtyar Tartibian in Urmia University and physiotherapist Noushin Azadpour in Ardabil University of Medical Sciences.

Menopausal women have decreased vasodilator capacity of vessels due to hormonal changes and obesity that increases the risk of hypertension in this group of women. However, regular moderate intensity exercise training reduces the risk of hypertension and related risk factors. Blood pressure lowering effect of exercise training is associated with positive effects on nervous system, improvement of vascular function and decrease in intra-abdominal fat. In addition, recent studeis have demonstrated the impact of exercise training on genes involved in blood pressure regulation.

Therefore, the aim of this study is to investigate the effect of 10 weeks of moderate intensity aerobic exercise training in some possible genetic, hormonal and structural factors involved in blood pressure regulation in obese postmenopausal women with pre-hypertension. The results of this study will be published in an academic journal. We hope that the findings of this study to be useful to clarify the effect of exercise training on the regulation of blood pressure in obese postmenopausal women with pre-hypertension.

If you agree to participate in the study, medical history will be taken and you will undergo medical examination.

Within the next 10 weeks, you will be asked not to change your eating habits and physical activity levels. Three days before and 48 hours after 10 weeks, you will undergo tests for the measuement of body fat percentage, waist and hip circumference, and also test for the determination of maximal oxygen uptake. Also, your vascular function will be measured and 5 mililiter blood samples will be taken from your antecubital vein following 12 hours overnight fasting. Furthermore, you will complete physical activity questionnaire and 3 day food record before and after 10 weeks. At least one week prior to these tests, you will be asked to avoid participation in exercise and consumption of alcohol and caffeine.

All information obtained during the course of this study is strictly confidential and your anonymity will be protected at all times. All data will be available only to the researchers and you.

This information will be used only for educational and research purposes. During this study, the privacy of your information will be approached with great care and respect.

The results will not be reported immediately since knowing the results does not provide any benefit to you. If you wish, you will be told the results of your tests at the end of study. There will be no costs for participating in the research. Also, you will not be paid for your participation.

Potential risks and discomfort

The methods used for the determination of your waist and hip circumference as well as body fat percentage have not any risk for you. Measurement of maximal oxygen uptake tests will be performed on a treadmill. The degree of difficulty on the treadmill test will be increased every 3 minutes interval and test will be continued until exhaustion. Your maximal effort during determination of maximal oxygen uptake is extremely important for the reliability of the findings obtained from the study.

During the test, you may feel fatigue, increase in your heart rate, breathless and sweating. But, these are normal body responses to exercise. In addition, during the test, heart rate, blood pressure, 12 leads ECG and ratings of perceived exertion will be monitored and recorded. Test will be performed under direct supervision of a cardiologist. You may feel tired or even exhausted after the test.

During blood sampling, you may feel temporary pain due to needling. Blood sampling will be performed in a hygienic manner. The material used for others will not be used in any way for you.

Doppler sonography will be used to measure changes in brachial artery diameter (by a specialist sonographer). Ultrasound studies will be performed in the morning, after that you had rested in supine position for 30 min in a quiet temperature controlled room. During this test a blood pressure cuff will be placed on the forearm and inflated to 50 mmHg above SBP for 5 minutes. This can cause discomfort, due to the pressure applied to your arm, but there is no health risk for you.

With regard to the above-mentioned notification, these are potential risks that may be experienced during participation in this study. However, every precaution will be taken to minimize possible problems by the preliminary examination and constant surveillance during tests and training sessions. Participation in this study is completely voluntary. You can refuse to participate in this study. If you decide to participate, you are free to withdraw at any time.

If you want to get more information about the study or if you encounter any questions, you can contact with the researchers conducting the study, Associate Professor Dr. Bakhtyar Tartibian (Tel: +98 9126090551) and physiotherapist Noushin Azadpour (Tel: +989144521533).

Participant / Patient's Declaration

I have been given sufficient information about the study conducted by principal researcher Assistant Professor Dr. Ş. Nazan Koşar and Associate Professor Dr. Bakhtyar Tartibian and physiotherapist Noushin Azadpour. After obtaining this information, I have been invited to participate in this study.

If I participate in this study, I believe that all my information obtained during the course of this study will be kept confidential. I have got enough confidence that my personal information will be protected during the use of research results for educational and scientific purposes.

I can withdraw from this study at any time without giving a reason. However, in order to avoid of any problem in research process, it is appropriate that I already inform to researchers that I withdraw from study. Morover, the researchers drop out me from the study provided that this doesn't affect my medical condition negatively.

In case of injury arising from participation in the study, first aid will be provided. If additional medical care is necessary, payment will be provided by researchers.

In case of any health problem related to participation in study, I should contact with researchers telephone number or addresses.

Principal Researcher	**Assistant Researcher**	**Assistant researcher**
Assist. Prof. Dr.	Physical therapist	Assoc. Prof.
Ş. Nazan Koşar	Noushin Azadpour	Dr. Bakhtyar Tartibian
Work phone: 2976890/117	Work phone: +984512251401	Work phone: +984432753174
Mobile: 05387612924	Mobile: +989144521533	Mobile: +98 9126090551

By signing this form, I hereby agree to the following:

1. The aim of the study was explained to me.
2. My participation in this study is completely voluntary.
3. My questions were answered adequately.

4. My participation in this study is completely voluntary. I have free choice to participate or not participate in this study. I have not encountered a coercive behavior to participate in this research.

I have understood the purpose of the research and all the explanations made to me in detail. By signing this consent form, I am indicating that I agree to participate in this study. I accept the invitation for participation in this study in great satisfaction and volunteering. A signed copy of this form will be given to me.

Participant	**Interview witnesses**
Name & Surname:	Name & Surname:
Adress:	Adress:
Tel:	Tel:
Sign:	Sign:

Appendix 3
Physical Activity Readiness Questionnaire (PAR-Q)

Name: …….. Date:……....

Wight (kg): …….. Height (cm):……. Age: ……

Physicians name: ……. Phone: …….

	Questions	Yes	No
1	Has your doctor ever said that you have a heart condition and that you should only perform physical activity recommended by a doctor?		
2	Do you feel pain in your chest when you perform physical activity?		
3	In the past month, have you had chest pain when you were not performing any physical activity?		
4	Do you lose your balance because of dizziness or do you ever lose consciousness?		
5	Do you have a bone or joint problem that could be made worse by a change in your physical activity?		
6	Is your doctor currently prescribing any medication for your blood pressure or for a heart condition?		
7	Do you know of any other reason why you should not engage in physical activity?		

If you have answered "Yes" to one or more of the above questions, consult your physician before engaging in physical activity. Tell your physician which questions you answered "Yes" to. After a medical evaluation, seek advice from your physician on what type of activity is suitable for your current condition.

Appendix 4

T.C.
HACETTEPE ÜNİVERSİTESİ
Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu

Sayı : 16969557-12.01

01 Aralık 2014

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi : 26.11.2014 ÇARŞAMBA
Toplantı No : 2014/17
Proje No : GO 14/608 (Değerlendirme Tarihi: 26.11.2014)
Karar No : GO 14/608 – 32

Üniversitemiz Spor Bilimleri Fakültesi öğretim üyelerinden Yrd.Doç.Dr.Şükran Nazan KOŞAR'ın sorumlu araştırmacısı olduğu, Nooshin AZADPOUR'un tezi olan Yrd.Doç.Dr.Bakhtyar TARTİBİAN ile birlikte çalışacakları GO 14/608 kayıt numaralı ve ***"10 Haftalık Aerobik Egzersiz Programının Postmenopozal, Obez, Prehipertansif Kadınlarda ACE ve ADRB2 Gen Ekspresyonu, Plazma Anjiyotensin II ve Akıma Bağlı Dilatasyon Üzerine Etkisi"*** başlıklı proje önerisi araştırmanın gerekçe, amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş olup, tıbbi etik açıdan uygun bulunmuştur.

1.Prof. Dr. Nurten Akarsu	(Başkan)	8.Prof. Dr. Rahime Nohutçu	(Üye)
İZİNLİ 2. Prof. Dr. Nüket Örnek Buken	(Üye)	9. Prof. Dr. R. Keksal Özgül	(Üye)
3. Prof. Dr. M. [illegible]n Sara	(Üye)	10. Prof. Dr. Ayşe Lale Doğan	(Üye)
4. Prof. Dr. Sevda F. Müftüoğlu	(Üye)	11. Doç. Dr. S. Kutay Demirkan	(Üye)
5. Prof. Dr. Cenk Sökmensüer	(Üye)	12 Prof. Dr Leyla Dinç	(Üye)
6. Prof. Dr. Volga Bayrakçı Tunay	(Üye)	13. Yrd. Doç. Dr. H. Hüsrev Turnagöl	(Üye)
İZİNLİ 7. Prof. Dr. Ali Düzova	(Üye)	14. Av. Meltem Onurlu	(Üye)

Hacettepe Üniversitesi Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu
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Ayrıntılı Bilgi için:

Appendix 5

International Physical Activity Questionnaire

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ days per week

☐ No vigorous physical activities ⟶ Skip to question 3

2. How much time did you usually spend doing vigorous physical activities on one of those days?

_____ hours per day

_____ minutes per day

☐ Don't know/Not sure

Think about all the moderate activities that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and make you breathe harder than normal. Think only about those physical activities that you somewhat did for at least 10 minutes at a time.

_____ days per week

☐ No moderate physical activities ⟶ Skip to question 5

4. How much time did you usually spend doing moderate physical activities on one of those days?

_____ hours per day

_____ minutes per day

☐ Don't know/Not sure

Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

_____ days per week

☐ No walking ⟶ Skip to question 7

6. How much time did you usually spend walking on one of those days?

_____ hours per day

_____ minutes per day

☐ Don't know/Not sure

The last question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the last 7 days, how much time did you spend sitting on a week day?

_____ hours per day

_____ minutes per day

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

Appendix 6

Bruce Protocol Data Collection Form

Subjects name:……. ……. Date: ……

Age (years): …… Height (cm):…… Weight (kg):……

Age-predicted HR_{max}: ……. beat/min; Equation: (206 – (0.88 × age))

90% of age-predicted HR_{max}: …….. beat/min

Resting HR: …….. beat/min Maximal HR: ……..beat/min

Resting BP: …… mmHg

Time to exhaustion (min): …….. $VO_{2\ max}$ (ml/mg/min): ……

Time (min)	**Speed** (mph)	**Grade** (%)	**HR** (bpm)	**BP** (mmHg)	**RPE**
1	1.7	10			
2					
3				*	*
4	2.5	12			
5					
6				*	*
7	3.4	14			
8					
9				*	*
10	4.2	16			
11					
12				*	*
13	5.0	18			
14					
15				*	*

*Where BP and RPE were recorded.

Appendix 7

Borg's Rate of Perceived Exertion Scale

Borg's Scale	Perceived Exertion
6	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (heavy)
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

Appendix 8

Three-Day Food Record Form

Name:…….. Date: …… Weight (kg): ……

Instructions:

Do not change your eating habits while keeping your 3-day food record.

Tell the truth. Record what you really eat so that an accurate analysis can be made.

Fill out the following food record form for 3 days, including 2 weekdays and one weekend day.

Time	Food/Beverage Ingested	Amount (cups, tablespoons, slices, pieces, etc.)

Basic rules to remember:

- Write down everything! Keep your food record form with you all days long and write down everything you eat and drink. A piece of candy, a handful of potato ships, a can of soda, or a small bagel may not seem like much, but calories in these foods can add up.
- Record what you eat right away! Don't depend on your memory at the end of the day. Record what you eat and drink as you go, meaning you should carry a pen and paper or use a list on your phone, etc.
- Be specific. Make sure you include "extras," such as cheese on your sandwich or vegetables, butter, and salad dressings.
- Estimate amounts. If you have a bowl of cereal or ice cream, measure out or estimate the actual amount (rather than writing "bowl" of cereal or "bowl" of ice cream).