

ABANT İZZET BAYSAL UNIVERSITY
THE GRADUATE SCHOOL OF NATURAL AND APPLIED
SCIENCES
DEPARTMENT OF BIOLOGY



THE EFFECTS OF EXERCISE AND NUTRITION ON
ISCHEMIA AND REPERFUSION ARRHYTHMIAS IN RATS

DOCTOR OF PHILOSOPHY

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BOLU, FEBRUARY 2016

APPROVAL OF THE THESIS

The effects of exercise and nutrition on ischemia and reperfusion arrhythmias in rats submitted by **Selçuk YAŞAR** in partial fulfillment of the requirements for the degree of Doctor of Philosophy in **Department of Biology, Abant İzzet Baysal University** by,

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ABSTRACT

THE EFFECTS OF EXERCISE AND NUTRITION ON ISCHEMIA AND REPERFUSION ARRHYTHMIAS IN RATS

PHD THESIS

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**ABANT İZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF
NATURAL AND APPLIED SCIENCES**

DEPARTMENT OF BIOLOGY

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The risk of coronary artery disease (CAD) increases with aging and death occurs due to the arrhythmias following coronary artery occlusion. In recent years, researchers have focused on the effects of physical exercise and diet for decreasing of these deaths. However, the effect of nutrition together with physical exercise and food restriction (FR) on the arrhythmias induced by ischemia and reperfusion was not studied more in experimentally produced myocardial infarction. That is why in this research the effect of exercise, different nutrition and food restriction alone and together on ischemia reperfusion (I/R) induced arrhythmia in male and female was aimed to be studied.

148 Male and 142 female Sprague-Dawley rats were used in this study. Exercise was made by swimming in 45min / day, food restriction by decreasing food in %50 during six weeks. Animals were fed by two different food; standard food and protein rich food. Ischemia was produced by the ligation of left coronary artery in 6 minutes and reperfusion by releasing of this artery in 15 minutes.

The score of arrhythmia, ventricular premature contraction and the duration of total arrhythmia decreased during ischemia in male having exercise and also in exercised combined with food restriction in rats fed with standard diet ($P < 0.05$). However in females, this effect was only seen in combination group ($P < 0.05$). The amount of superoxide dismutase (SOD) increased in male groups that applied exercise and combination ($P < 0.05$). Otherwise, there was no difference in ATP dependent potassium (K_{ATP}) channel protein in exercise and exercise with food restriction male group in respect to control male, but in females it was increased in both condition. No changes in arrhythmia during reperfusion

period were observed in male and female in exercise or exercise and food restriction groups in respect to their control.

Different effect of protein rich diet on the ischemia-induced arrhythmia was observed. Protein rich diet was not effective on the ischemia-induced arrhythmia in male but it increased the arrhythmia in female following exercise combined with food restriction group. Otherwise, in reperfusion period, protein rich diet increased the arrhythmia in male having exercise, but no change in arrhythmia was observed in female in this period. The amount of SOD was not significantly increased either in male and female having exercise alone or together with the food restriction in protein rich diet. But K_{ATP} channel protein increased in exercised alone and with food restriction applied groups only in male.

Increasing in the amount of SOD was effective alone in reducing the arrhythmias during ischemia without increasing K_{ATP} channels in males. This result supports the previous studies. In female, the increase of SOD was not effective on arrhythmias alone but this time the increasing number of K_{ATP} channel was effective in reducing the arrhythmias occurred during ischemia. The protein-rich feeding eliminates the protective effect against arrhythmia in male and female having long-term exercise firstly, was found in this study.

KEYWORDS: Ischemia, Reperfusion, Arrhythmia, Diet, Food restriction, Exercise.

ÖZET

**SIÇANLARDA EGZERSİZ VE BESLENMENİN İSKEMİ VE
REPERFÜZYON ARİTMİLERİ ÜZERİNE ETKİSİ
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Koroner arter hastalığı riski ve koroner arter tıkanması sonucu oluşan aritmilere bağlı ölümler yaşlanmayla birlikte artmaktadır. Son yıllarda araştırmacılar fiziksel egzersiz ve diyetin bu ölümlerin azaltılmasındaki etkileri üzerinde yoğunlaşmışlardır. Ancak fiziksel egzersizle birlikte diyetin ve besin kısıtlamasının deneysel miyokart enfarktüsüyle iskemi ve reperfüzyonu takiben oluşan aritmiler üzerine etkisini araştıran çok az çalışma bulunmaktadır. Bu çalışmada, fiziksel egzersiz ve beslenmenin erkek ve dişide iskemi ve reperfüzyonu takiben oluşan aritmiler üzerine etkisinin araştırılması amaçlanmıştır.

Bu çalışmada 148 erkek ve 142 dişi Sprague-Dawley sıçan kullanıldı. Günde 40 dakikalık yüzme egzersizi ve 6 haftalık %50 besin kısıtlaması uygulandı. Hayvanlar iki farklı; standart besin ve yüksek proteinli besin, ile beslendi. Sol koroner arter bağlanılarak 6 dakika iskemi ve bağ çözülerek 15 dakika reperfüzyon yapıldı.

Standart yemle beslenen erkek grupta iskemi sırasında saptanan aritmi skoru, ventriküler prematüre atımlar ve toplam aritmi süreleri sadece egzersiz grubunda ve egzersizle besin kısıtlamasının birlikte yapıldığı kombinasyon grubunda azaldı ($P < 0.05$). Fakat bu etki dişilerde sadece kombinasyon grubunda görüldü ($P < 0.05$). Erkeklerde SOD miktarı egzersiz ve kombinasyon uygulanan gruplarda arttı ($P < 0.05$). Buna karşın erkeklerde K_{ATP} kanal sayıları kontrol, egzersiz ve kombinasyon grupları arasında farklı değildi, fakat dişilerde K_{ATP} kanal sayıları her iki durumda da arttı. Reperfüzyon periyodu süresince hem erkekte hem de dişide egzersiz ve besin kısıtlamasının aritmiler üzerine belirgin bir etkisi olmadı.

Proteinden zengin beslenmenin iskemi reperfüzyon aritmileri üzerine erkek ve diřide farklı etkileri görüldü. Proteinden zengin beslenme erkeklerde iskemi ile uyarılan aritmiler üzerine etkili deęilken, diřilerde bu aritmiler kombinasyon grubunda artmıřtır. Buna karřın proteinden zengin diyet egzersiz uygulanan erkeklerde reperfüzyon ile uyarılan aritmeleri artırmıřtır, fakat diřilerde bu periyotta aritmiler kontrol grubundan farklı olmamıřtır. Proteinden zengin beslenen erkek ve diřilerde egzersiz grubunda ve kombinasyon grubunda SOD miktarındaki artış anlamlı bulunmamıřtır. Fakat K_{ATP} kanal proteinleri sadece egzersizin tek bařına ve besin kısıtlamasıyla birlikte uygulandıęı erkek gruplarda artmıřtır.

SOD miktarının artışı erkeklerde iskemi sırasında oluřan aritmileri azaltmada, K_{ATP} kanallarının artışına baęlı olmaksızın tek bařına etkili bulunmuřtur. Bu sonuç önceki alıřmaları desteklemektedir. Diřilerde ise SOD artışı aritmiler üzerine tek bařına etkili bulunmamıřtır. Diřilerde iskemi sırasında oluřan aritmileri azaltmada K_{ATP} kanal sayısındaki artışın daha etkili olduęu görülmüřtür. Proteinden zengin beslenme erkeklerde egzersizle saęlanan koruyucu etkiyi ortadan kaldırırken diřilerde ise olumsuzla evirdięi ilk kez bu alıřmada ortaya konmuřtur.

ANAHTAR KELİMELEER: Miyokardiyal iskemi, Reperfüzyon, Aritmi, Beslenme, Besin kısıtlaması, Egzersiz.

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LIST OF ABBREVIATIONS AND SYMBOLS

AA	: After application
ALT	: Alanine transaminase
ANOVA	: Analysis of Variance
AST	: Aspartate transaminase
ATP	: Adenosine Triphosphate
BA	: Before application
BP	: Blood Pressure
BUN	: Blood Urea Nitrogen
Brdc	: Bradycardia
°C	: Celsius degree
Ca²⁺	: Calcium ion
CAD	: Coronary Artery Disease
CK-MB	: Creatine Kinase-MB
CV	: Cardiovascular
CVD	: Cardiovascular Disease
DNA	: Deoxyribonucleic acid
ECG	: Electrocardiogram
EDTA	: Ethylenediaminetetraacetic acid
ELISA	: Enzyme-linked immunosorbent assay
eNOS	: Endothelial Nitric Oxide Synthase
EX	: Exercise
EX+FR	: Combination of exercise and 50% food restriction
FR	: 50% food restriction
g	: Gram
Grp	: Glucose-regulated protein
H⁺	: Hydrogen ion
HDL	: High Density Lipoprotein Cholesterol
HR	: Heart rate
HSP	: Heat Shock Protein
I/R	: Ischemia / Reperfusion
IPC	: Ischemic preconditioning
IU	: International unit

K⁺	: Potassium ion
K_{ATP}	: ATP dependent Potassium channel
kg	: Kilogram
L	: Liter
LAD	: Left Anterior Descending
LDH	: Lactate Dehydrogenase
Lig	: Ligation
LSD post hoc	: Least Significant Difference post hoc test
M	: Mean
mg	: Milligram
min	: Minute
Mito K_{ATP}	: Mitochondrial ATP Dependent Potassium Channel
ml	: Milliliter
mmHg	: Millimeter Mercury
MnSOD	: Manganese Superoxide Dismutase
mtDNA	: Mitochondrial Deoxyribonucleic acid
NO	: Nitric Oxide
NOS	: Nitric oxide synthase
PBS	: Phosphate buffer solution
pH	: Power of Hydrogen
PRF	: Protein rich food
Rep	: Reperfusion
ROS	: Reactive oxygen species
ROZ	: Risk of Infarct Zone
Sarc K_{ATP}	: Sarcolemmal K _{ATP}
SE	: Standard Error
SOD	: Superoxide Dismutase
SPSS	: Statistical Package for the Social Sciences
SRF	: Standard rat food
SUR 2A	: Sulphonylurea receptor 2A
U	: Enzyme unit
VF	: Ventricular fibrillation
VPC	: Ventricular premature complexes
VT	: Ventricular Tachycardia

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1. INTRODUCTION

In developed countries, %50 of sudden death occurred due to coronary artery disease (Goldstein, 1974). The main cause of these sudden deaths are fatal arrhythmias occurred following ischemia and reperfusion. The risk of death due to the cardiovascular disease (CVD) increases with aging (Wei, 1992). The cellular and metabolic changes occurred in myocardial cells with aging (Pollack et al., 2002). Researchers have focused on especially the effect of physical exercise and different diet to reduce the risk of coronary artery disease that increasing with aging (Boengler, 2009). In research, short-term or long-term exercise and different diet have been applied to the animals and looked for arrhythmia and death after coronary ligation in experimental animals (Aubin et al., 2010; Billman et al., 1984; Charnock et al., 1985; Frasier et al., 2011; Kaya and Bozdoğan, 2012; Posel et al., 1989; Šnorek et al., 2012; Végh and Parratt, 2005). Cardiovascular (CV) risk factors are; aging, obesity, stress, type 2 diabetes, hypertension, smoking, sedentary lifestyle and genetic factors (Jugdutt, 2010). It was demonstrated in clinical studies that exercise increases protective effect against the cardiovascular diseases (Berlardinelli et al., 1999, Hertzeanu et al., 1993; Kilavuori et al., 1995) and arrhythmias occurred following ischemia reperfusion produced in various experimental studies (Frasier et al., 2011; Nagy et al., 2004; Noakes et al., 1983; Powers et al., 1998). There are a few studies that indicates the number of myocardial K_{ATP} channels as like antioxidant enzyme activity increases following exercise (Brown et al., 2005a, 2005b). However, there was no study on comparing the effect of exercise on K_{ATP} channel, antioxidant defense together with ischemia or reperfusion induced arrhythmia in both male and female.

It is known that food restriction is effective in reducing the cardiovascular risk factors. Also, it was reported that the antioxidant activity increased with food restriction (Pamplona and Barja, 2006; Rippe et al., 2010; Sreekumar et al., 2002). But, there was no research investigating the effect of food restriction on the number of K_{ATP} channels and there are only a few studies investigating the effect of food or caloric restriction on ischemia reperfusion induced arrhythmias (Kaya and

Bozdoğan, 2012). Abete et al. (2002) reported that food restriction restored the protective effect of the ischemic preconditioning (IPC) against arrhythmias that decreases with aging observed in invitro studies. In another study performed in vivo, it was found that three-day food restriction was not effective on the duration of ventricular premature contraction (VPC) and ventricular tachycardia (VT) during ischemia, but decreased the duration of VPC and VT during reperfusion (Šnorek et al., 2012). In another previous study, not unsustainable but intermittent fasting was found not to be effective on ischemia reperfusion arrhythmias, Kaya and Bozdoğan Ö, (2012).

There are several studies investigating the effect of different type of diet on the I/R arrhythmias in experimental animals (Aubin et al., 2010; Charnock et al., 1985; McLennan et al., 1988; Murnaghan, 1981). However, the effects of soy protein-rich diet on I/R arrhythmias is not known and was not studied before. And also the effect of food restriction alone and with the combination of exercise on I/R induced arrhythmia in both normal and more soy protein including food were not investigated.

1.1 Aging and Heart

Post mitotic cells decrease with aging (Adams et al., 2001; Anversa et al., 1986). This decreasing does occur by the necrosis and apoptosis (Kajstura et al., 1996). Various metabolic and physiological changes in tissues occur with aging. These changes are; the increase in fibrosis, oxidative stress and damage, free oxygen radicals, metabolic end products and advanced glycosylation, angiotension and endothelin, telomere shortening, mitochondrial dysfunctions and damaging repair mechanisms (Jugdutt, 2010).

1.1.1 Metabolic and Functional Changes in Aging Heart

Cellular deteriorations occur with aging. It is not quite understood yet but mitochondrial damage may cause this deteriorations (Hagen et al., 2002). Aging occurs as a result of accumulation of the errors by the free radicals on mitochondrial DNA (mtDNA). Defected mtDNA causes some errors on the encoded polypeptides

and produces dysfunctional proteins causes deficiency on the mitochondrial respiratory chain complex. If complex are defective, more radicals produced and errors on mtDNA increases (Barja and Herrero, 2000; Beckman and Ames, 1999). As a result of these defects, apoptosis is triggered and improves aging process, where it could serve to eliminate nonfunctional, harmful, abnormal, or misplaced cells, especially in advanced age (Barja and Herrero, 2000; Sastre et al, 2000; Warner, 1999).

Animal studies report a significant reduction in contraction and relaxation occurs in aged animals compared to younger animals (Schwartz et al., 1991). The heart is dependent on high-energy phosphates for contraction and relaxation (Hagen et al., 2002; Katz, 1988). Some researchers reported that slow cardiac relaxation occurs by the mitochondrial decay due to the decreasing rate of Ca^{2+} removal from the cytosol (Frolkis et al., 1988; Negretti et al., 1993). There are many reason of deterioration of the diastolic functions. These are decreasing number of myocyte, increasing stiffness in myocardial wall, accumulation of Ca^{2+} in the extracellular matrix and prolonged relaxation (Fares and Howlett, 2010; Sanders et al., 2009).

Some researchers suggest that cardiac output and cardiac index decreases with age, but some researchers suggest no differences (Abete et al., 1999; Bujak et al., 2008; Onose et al., 1999; Shannon et al., 1991; Slotwiner et al., 1998). Another study implied that some deteriorations occur in systolic functions with aging (Slotwiner et al., 1998). Many studies report that heart rate (HR) decreases with exercise in aging (Carter et al., 2003b; De Meersman, 1993). However, some researchers reported that sympathetic activity increased with aging (Ng et al., 1993; Ziegler et al., 1976).

1.1.2 Ischemia and Reperfusion Induced Arrhythmia in Aged Heart

Cardiac damage increases during ischemia and reperfusion in aging (Lesnefsky and Hoppel, 2003). Aging is a main risk factor on the ischemic heart disease. Apoptosis and infarct size are increased more in senescent mice due to the ischemia and reperfusion (Azhar et al., 1999).

In our previous study, arrhythmias increased during 30 minutes of ischemia in female rats due to the aging, but it was not seen in male rats (Bozdoğan et al., 2013). The effect of the number of ATP dependent potassium channels is very important on the arrhythmias induced by myocardial ischemia and reperfusion. The number of K_{ATP} channels decreases in females, but not in males with aging (Jovanovic and Jovanovich, 2004). Protective effect of ischemic preconditioning against the ischemia reperfusion induced arrhythmia decreases with aging (Fenton et al., 2000).

1.2 The Effect of Exercise on Heart

In many studies, it was reported that exercise is a cardioprotective in human (Hull et al., 1994; Morris et al., 1980; Siscovick et al., 1984) and animals (Demirel et al., 2001; Powers et al., 1998; Powers et al., 2008). Morris et al. (1980) reported that physical exercise reduces the incidence of myocardial infarction. In other studies, some researchers were reported that the exercise provide more survival following heart attack in respect to sedentary person (Hull et al., 1994; Siscovick et al., 1984). In studies, it was reported that regular exercise improves the myocardial tolerance against the I/R injury (Demirel et al., 2001; Powers et al., 1998; Powers et al., 2008) although there are some studies indicating opposite (Pina et al., 2003; Siscovick et al., 1984; Tanasescu et al., 2002).

1.2.1 The Effect of Exercise to Metabolic and Functional Changes in Heart

Regular application of exercise improves the myocardial tolerance for both male and female old and young animals against I/R (Brown et al., 2005b; Bowles and Starnes, 1994; Demirel et al., 1998; Demirel et al., 2001; Hamilton et al., 2004; Hoshida et al., 2002; Lennon et al., 2004; Libonati et al., 1997; Powers et al., 2001; Powers et al., 2004; Quindry et al., 2005; Starnes et al., 2003; Taylor et al., 1994; Yamashita et al., 1999). Exercise protects the mitochondria and cardiac myocytes against I/R induced oxidative stress (Hamilton et al., 2001; Powers et al., 1998). This cardioprotection is observed at short and long term (Demirel et al., 2001; Powers et al., 1998; Quindry et al., 2005; Yamashita et al., 1999). Previous studies show that exercise protects the heart against myocardial stunning, myocardial cell death and

I/R arrhythmias. Some other studies showed that the length of exercise was not important in its protective effects (Demirel et al., 2001; Hamilton et al., 2001; Lennon et al., 2004; Powers et al., 1998). Therefore, that short term (weeks) or long term (months) exercise produced similar protection in heart. However, exercise-induced cardioprotection disappear after about 18 days (Lennon et al., 2004).

1.2.1.1 Cardioprotective Effect of Exercise

1.2.1.1.1 Angiogenesis

Long-term regular exercise induces angiogenesis that was shown in some experimental animals (Brown, 2003; Laughlin et al., 1998). However, in some other studies, it was observed that exercise did not induce collateral formation in animals having 1-5 days exercise (Yamashita et al., 1999). Therefore, it can be said that exercise is not only factor to induce angiogenesis.

1.2.1.1.2 Cardiac Heat Shock Proteins

Heat shock proteins (HSPs) are produced in stress conditions and they protect the cell. Application of exercise increases the expression of HSP72 about in 3-5 times than normal (Demirel et al., 2003). Experimental results indicate that the over expression of HSP72 creates the myocardial protection against I/R injury (Hutter et al., 1996; Jayakumar et al., 2001). HSP72 protect the myocardium against I/R injury also protect the cell against the apoptosis (Steel et al., 2004). However, in some studies it has been shown that exercise creates the cardioprotective effect without increment of HSPs (Hamilton et al., 2001; Taylor et al., 1999). Therefore, HSP alone is not enough to provide complete myocardial protection in stress condition.

1.2.1.1.3 Sarc K_{ATP} Channels

Sarcolemmal ATP dependent potassium (sarcK_{ATP}) channels are located in sarcolemma of cardiac myocytes (Noma, 1983). Many researchers said that opening of these channels during ischemia was cardioprotective (Gross and Peart, 2003). If these channels open, potassium exits out of the cell. Cardiac action potential duration

is shortening with the acceleration of phase 3 repolarization when the sarcolemmal K_{ATP} channels open. In this way, protection occurs against the I/R damage. Action potential duration shortening inhibit Ca^{2+} entry into the cell through the L-Type Ca^{2+} channels and prevent the Ca^{2+} overload (Gross and Peart, 2003). Brown et al. (2005a) reported that exercise increased the sarcolemmal K_{ATP} channel protein expression in myocytes. In the above study, sarcolemmal K_{ATP} channels were blocked during I/R in exercise and it was observed that the protective effect of exercise was eliminated by blockage of K_{ATP} channel (Brown et al., 2005a). These results suggest that increased sarcolemmal K_{ATP} channels expression by exercise has an important role in cardioprotection (Powers et al., 2008).

1.2.1.1.4 Mito K_{ATP} Channels

Mitochondrial ATP dependent potassium (mito K_{ATP}) channels are located in the inner membrane of the mitochondria (Inoue et al., 1991). These channels also make cardioprotective effects like sarcolemmal channels. Opening of mitochondrial K_{ATP} channel increases the pH of the mitochondrial matrix, decreases reactive oxygen species (ROS) production which occurs during reperfusion and the accumulation of Ca^{2+} decreases in ischemia and the mitochondrial energy production increases following reperfusion (Costa et al., 2006; O'Rourke, 2004). There is also another result that was shown by research that the mitochondrial K_{ATP} channel blockers do not have cardioprotection against I/R (Brown et al., 2005a).

1.2.1.1.5 Myocardial Antioxidants

Increasing of ROS production is an important factor for the formation of cardiac damage during myocardial I/R. Overexpression of myocardial antioxidant is associated with the increasing of ROS production. Elevation of antioxidant protects the heart against the I/R damage (Adlam et al., 2005; Chen et al., 1998). It was reported in many studies that both long-term and short-term exercise increased the myocardial antioxidant capacity (Demirel et al., 2001; French et al., 2006; Hamilton et al., 2001; Hamilton et al., 2003; Hoshida et al., 2002; Powers et al., 1993; Powers et al., 1998; Yamashita et al., 1999). It was shown in these studies that exercise increased the myocardial MnSOD, glutathione peroxidase, and catalase activity. In

contrast, some researchers said that it did not increase the myocardial levels of antioxidant enzymes (Dickson et al., 2008). It was shown in various studies that the MnSOD activity increased in animals having different exercise training protocol (Brown et al., 2003). Additionally, it was reported that increasing of MnSOD activity in exercise reduces the I/R arrhythmias and infraction (Brown et al., 2003; Hamilton et al., 2004; Powers et al., 1998).

1.2.2 The Effect of Exercise to the Arrhythmia Induced by Ischemia and Reperfusion

In the first phase of Ischemia, impulse transmission slows down at the ischemic region and ectopia is developed from ischemic region and generates ectopic impulse. During the reperfusion, more severe arrhythmia occurs. Because the electrical inhomogeneities increased in reperfusion that it causes the formation of many irregular ectopic focus and increasing more severe arrhythmia. Reperfusion causes the intracellular Ca^{2+} overload and the ROS generation in myocardial cells (Kramer et al., 1994). ROS production is very effective for the formation of reperfusion damage and arrhythmias, and in the final stage leads to the cell death (Bernauer, 1991). Releasing of large amounts of adenosine and its derivatives causes the formation of ventricular fibrillation (VF) during reperfusion (Bernauer, 1991). ROS production causes the ionic disorders by the damaging ion channel protein.

Studies reported that exercise is protective against the I/R injury (Powers et al., 1998). This protection was reported to be mediated by reducing myocyte death, myocardial oxidative injury, and cardiac contractile dysfunction (Bowles and Starnes, 1994; Demirel et al., 1998; Demirel et al., 2001; Hamilton et al., 2001; Hamilton et al., 2003; Powers et al., 1998; Yamashita et al., 2001). Some researchers revealed that exercise increased the myocardial tolerance in I/R (Demirel et al., 2001; Powers et al., 1998; Powers et al., 2008). Some others reported that exercise was not effective (Fuller and Nutter, 1981) and few of them said that exercise reduced the myocardial tolerance in I/R (Korge and Mannik, 1990). Mechanism of I/R arrhythmia formation is complex and depends on the duration of ischemia. The main causes of I/R arrhythmias is unstable ionic distribution in myocytes. This situation depends on the decreasing in cellular energy level and increasing ROS. Reduction of

I/R arrhythmias is associated with the increasing amount of SOD or SOD activity (French et al., 2006; Hamilton et al., 2004; Powers et al., 1998). In previous studies, it was reported that the exercise increases the amount of the antioxidant enzyme and its activity (Demirel et al., 2001; French et al., 2006; Hamilton et al., 2001; Hamilton et al., 2003; Hoshida et al., 2002; Powers et al., 1993; Powers et al., 1998; Yamashita et al., 1999). The increasing MnSOD activity reduces score of arrhythmia observed following ischemia and reperfusion (Hamilton et al., 2004). Some studies reported that exercise increases the number of cardiac K_{ATP} channel (Brown et al., 2005a, 2005b in press.). Normally these channels are closed so that it opens during ischemia and provide K^+ out flux. In this way, electrical inhomogeneity and formation of arrhythmia reduces. Additionally, exercise increases the parasympathetic activity or decreases the sympathetic activity (Billman, 2002; Blomquist and Saltin, 1983). Researchers reported that the heart rate is lower in exercised group than the sedentary (Billman et al., 1984; Hull Jr et al., 1994; Kokkinos et al., 1995). That means less arrhythmia observed in exercise group might be depend on decreasing heart rate.

1.3 The Effect of Dietary Changes in Myocardial Functions

Cardiovascular deterioration occurs due to the physical inactivity, cellular degradation caused by smoking, drinking alcohol and eating habits in advanced ages (Mozaffarian et al., 2012). These deteriorations are oxidative damage, vascular disease, ischemic injury, left ventricular hypertrophy and heart failure (Grieve et al., 2004; Ide et al., 1999; Judge et al., 2005; Rodriguez-Manas et al., 2009; Seddon et al., 2007). The main CV risk factors are type 2 diabetes, hypertension, hypercholesterolemia and obesity (Clarke et al., 2009). The most important way of the prevention of coronary artery disease is diet regime. In recent years, researchers studied the effect of the life style and the diet on the CAD (Corella and Ordovás, 2014). There is a significant correlation between plasma proteins, dietary stored fats and CAD (Payne, 2012). Recently researchers has focused on food restriction (Abete et al. 2002; Dolinsky and Dyck, 2010; Rippe et al., 2010), calorie restriction (low carbohydrate rich fiber diet) (van der Meer et al., 2007), high protein (Blouet et al., 2006) and vegetable protein (Soenen et al., 2012) diets in the prevention of the cardiovascular diseases.

1.3.1 Food Restriction

The effect of FR on CAD is commonly studied. Three common types of food restriction; long-term, short-term and intermittent fasting is used in animal models. FR decreases infarct size, increases lifespan, increases resistance against stress, and reduces morbidity and inflammation (Carlson and Hoelzel, 1946; Cui et al., 2013; Csiszar et al., 2009; Han et al., 2012; Takatsu et al., 2013; Wang et al., 2014).

1.3.1.1 The Effect of Food Restriction to Metabolic and Functional Changes in Heart

Food restriction prolonged the healthy lifespan and this effect is shown in both FR and intermittent fasting (Anson et al., 2003; Bodkin et al., 2003; Goodrick et al., 1990). The FR studies was performed in rodents, monkeys and humans (Bodkin et al., 2003; Shinmura et al., 2015; Van der Meer et al., 2007; Wan et al., 2003). FR not only prolonged lifespan, it also shows cardioprotective effect against CAD by activating the molecular signals increasing resistance in the cellular stress (Dolinsky et al., 2010; Giani et al., 2008; McCurdy and Cartee, 2005). This cardioprotective effect may be provided by the increasing resistance against oxidative stress, reducing mitochondrial ROS production, reducing inflammation and fibrosis, increasing insulin sensitivity, improvement of glucose tolerance and decreasing of insulin and glucose concentration in blood plasma (Corella and Ordovás, 2014; Dolinsky and Dyck, 2011; Mattson and Wan, 2005).

It has also been reported that FR reduce the oxidative damage and levels of oxidative stress (Pamplona and Barja, 2006; Shinmura, 2011; Sung and Dyck, 2012). The elevated levels of low-density lipoprotein (LDL) and low levels of high density lipoprotein (HDL) are risk factors for CVD (Pan and Chiang, 1995). Studies suggest that FR decrease LDL cholesterol and increase HDL cholesterol levels (Raeini-Sarjas et al., 2001). It has been reported that FR reduces triglyceride levels (Meckling et al., 2002). FR reduces atherosclerosis and inflammation by reducing leukocytes and tumor necrosis factor (Spaulding et al., 1997).

The cardioprotective effect of IPC decreases with aging but, this cardioprotective effect was maintained by food restriction in senescent rats that was

shown in a previous study (Abete et al. 2002). Ahmet et al. (2005) reported that ischemic damage decreased in food restricted in rats.

Food restriction decreases the blood pressure (BP) by the way of reducing sympathetic activity (Mattson and Wan, 2005). In a study performed in rats was reported that intermittent fasting decreased BP and heart rate via the increased parasympathetic tone (Wan et al., 2003). FR regulates hypertension, another important factor on CAD, decreasing systolic and diastolic blood pressure. Food restriction shows vasodilatory effect against hypertension by increasing the nitric oxide (NO) production and the activity of endothelial nitric oxide synthesis (eNOS) (Mattagajasingh et al., 2007; Zanetti et al., 2004). Thus, food restriction has protective effect on the left ventricular hypertrophy by decreasing hypertension.

1.3.1.2 The Effect of Food Restriction to the Arrhythmia Induced by Ischemia and Reperfusion

It is known that food restriction is cardioprotective and increase lifespan. In addition, food restriction and intermittent fasting decrease the infarct size and protect the heart against I/R injury (Edwards et al., 2010; Šnorek et al., 2012). It was reported that food restriction decreased the mitochondrial induced ROS production and by the way it decreases reperfusion injury (Pamplona and Barja, 2006; Shinmura, 2011; Sung and Dyck, 2012). There are very few studies found in literature about the effect of food restriction on the arrhythmias induced by I/R. For this reason, the effect of food restriction on the ischemia reperfusion induced arrhythmias is not clearly known. In a previous study performed on rats, intermittent fasting was not effective on the I/R induced arrhythmias at the end of four weeks (Kaya and Bozdoğan, 2012). Even, it was suggested that the arrhythmic period increased by intermittent mentioned in above study. Snorek et al. (2012) was reported that three day food restriction decreased the VPC and VT induced by reperfusion, but it did not effective on VPC and VT induced by ligation. Three-day food restriction also reduced the infarct size. In another in vitro study performed in rats, nutrient restriction in perfused fluid showed cardioprotective effect on the arrhythmias occurred following I/R and FR had been effective on the stabilization of Ca^{2+} cycling (Wang et al., 2012).

1.3.2 The Effect of High Soy Protein Diet to Metabolic and Functional Changes in Heart

There are few studies found in literature that research the effect of protein rich feeding on healthy subjects. The researchers especially focused on different dietary patterns against obesity and diabetes mellitus (Hill et al., 1991; Jeor et al., 2001). Long-term protein rich feeding increases satiety and causes weight loss (Boden et al., 2005). Additionally, thermic effect of protein rich feeding is more than the carbohydrate feeding which reduces energy demand (Westerterp et al., 1999). Therefore high protein diet causes weight loss.

Researchers reported that increasing protein ratio in diet reduces body fat and improves glucose homeostasis (Gannon et al., 2003; Gannon and Nuttall, 2004; Goodpaster et al., 1999; Lacroix et al., 2004).

Proteins are the main components required for the structure and functioning of the body. Proteins may function as enzymes, hormones, antibodies, structural components in the body (Jeor et al., 2001). Proteins are also associated with the regulation of insulin and glucose level and muscle building. Daily protein demand increases with physical exercise, pregnancy and old age (Campbell et al., 1994).

Two type of protein rich feeding have been used commonly in research. These are vegetable (Mikkelsen et al., 2000) and animal based protein feeding (Hegsted, 1986). Animal based protein rich feeding has some negative consequences, such as elevated total cholesterol and LDL cholesterol (Hegsted, 1986; Jeor et al., 2001; Larosa et al., 1980).

Most of previous studies demonstrate the relation between protein rich feeding and weight loss (de Castro et al., 2009; St-Onge et al., 2007). High protein diet more effective than low fat diet in the weight loss in short term feeding (Brehm et al., 2003; Yancy et al., 2004). The weight loss occurred in the first few days following protein rich feeding by its anorectic effect and fluid loss (Jeor et al., 2001; St-Onge et al., 2007).

Soy protein rich feeding regulate the lipid profile in human having risk for coronary heart disease (Girgih et al., 2013). Soy based diet decreased the common

cancer and CAD in Asian populations (Barnes, 1998). Soy is a source of plant derived isoflavones. Major soy based isoflavones are genistein, daidzein and glycitein. Their structures are similar to the structure of estrogen and it may bind to the estrogen receptors (Zhai et al., 2001). It has been reported that estrogen was cardioprotective against I/R injury in both male (Zhai et al., 2000a) and female (Zhai et al., 2000b). Cardioprotective effect of isoflavones occurs due to its antioxidant activity (Barbosa et al., 2011; Ferramola et al., 2012; Morel et al., 1993). In a study performed in rats, soy protein rich feeding increased catalase activity in liver and SOD activity in erythrocytes (Barbosa et al., 2011). However, in another study performed in rats, SOD activity increased with soy based diet (Hagen et al., 2009). On the contrary, some studies suggest that SOD activity did not change in soy protein rich diet (Ferramola et al., 2012).

In previous studies, both the total and LDL cholesterol was decreased and HDL cholesterol was increased in young women that fed with containing soy protein food (Wang et al., 1995). In some other studies, protein rich feeding decreases the total cholesterol and LDL cholesterol (Westman et al., 2002). However, some other researchers reported that protein rich feeding was not effective on total cholesterol and LDL cholesterol (Kappagoda et al., 2004; St-Onge et al., 2007).

1.3.3 The Effect of High Soy Protein Diet on the Arrhythmia Induced by Ischemia and Reperfusion

There was no study found that studying the effect of protein rich feeding on the arrhythmias following ischemia and reperfusion. However, the injection of up to 1 mg/kg genistein that is soy isoflavones decreased the infarct size and the incidence of VF and VPC following reperfusion (Deodato et al., 1999). Furthermore, some researchers have reported that soy isoflavones increased the antioxidant capacity (Barbosa et al., 2011, Morel et al., 1993). It was known that increasing antioxidant capacity reduces the arrhythmias.

Zhai et al. (2001) suggest that the Ca^{2+} accumulation was higher in ovariectomized and in female rats having low phytoestrogen diet in rats produced global ischemia produced rats. This result shows that soy phytoestrogens prevent the Ca^{2+} accumulation. The prevention of Ca^{2+} accumulation reduces the infarct size. It

is also known that arrhythmias following ischemia depend on the size of ischemia (Yaşar et al., 2015).

1.4 The Effect of Combination of Exercise and Food Restriction on Heart

It is known that, regular physical exercise and food restriction alone are effective against coronary heart diseases and arrhythmias (Abete et al. 2002; Berlardinelli et al., 1999; Nagy et al., 2004; Powers et al., 1998). However, researchers thought that the combination of physical exercise and food restriction would be more effective in the prevention of the risk of coronary artery diseases. The combination of food restriction and exercise training was studied commonly in overweight man or in subjects having risk of CAD (Baba et al., 1999; Brehm et al., 2003; Foster et al., 2003; Hu, 2005; Leidy et al., 2007; Soenen et al., 2012). However, the combination of food restriction and intensive exercise may cause negative consequences in the heart was also reported (Sugizaki et al., 2006). There is no any study on the effect of combination of exercise and food restriction related to the I/R arrhythmias. Therefore, no information is available on the effect of this application on the arrhythmias yet.

1.4.1 The Effect of Combination of Exercise and Food Restriction on the Metabolic and Functional Changes in Heart

The cardioprotective effects of both food restriction and physical exercise individually are known but, the effects of the combination of both exercise and FR are not clarified yet. Previous study reported that the combination of food restriction and exercise improved the diastolic BP, LDL cholesterol and insulin sensitivity but this improvement was not observed in FR alone (Larson-Meyer et al., 2010).

Researchers reported that the combination of exercise and food restriction improved insulin sensitivity (Roberts et al., 2006), reduced serum lipids and markers of oxidative stress and inflammation (Mokdad et al., 2003) and also reduced ROS production while increasing NO production (Mokdad et al., 2001).

The combination of exercise and food restriction decreased the total cholesterol, HDL, LDL cholesterol, triglyceride and blood glucose in man with diabetes mellitus (Roberts et al., 2002; Roberts et al., 2006). In addition, it increased the antioxidant/oxidant ratio in a previous study (Roberts et al., 2006). Atherosclerosis formation may be decreased with combination of exercise and food restriction (Roberts et al., 2006).

The combination of regular exercise and low calorie diet is reported to be effective in reducing coronary heart disease in both sexes (Wood et al., 1991). The combination of food restriction and exercise increases resistance to cardiac ischemia (Broderick et al., 2001).

1.5 The Effect of ATP Dependent Potassium Channels in Heart

ATP dependent potassium channels were discovered in cardiac myocytes (Noma, 1983) and then it was identified in pancreatic β cells, skeletal and smooth muscle, neuronal cells and kidney. In normal conditions, K_{ATP} channels are in closed state. However, these channels open when ATP level decreases in cells under pathological conditions such as ischemia. There are two subtypes of K_{ATP} channels in cardiac cells. These are sarcolemmal K_{ATP} channels that located on sarcolemmal membrane (Noma, 1983) and mitochondrial K_{ATP} channels that located on mitochondrial inner membrane (Inoue et al., 1991).

K_{ATP} channels are effective on the preventing arrhythmias and decreasing infarct size. Physicians are reported that K_{ATP} channels are more in female than male (Ranki et al., 2001). High amount of K_{ATP} channels are more cardioprotective.

K_{ATP} channels cause K^+ loss from the cells by opening of these channels under ischemic conditions. This situation cause rapid repolarization on the cells (Giudicelli et al., 1991). Because of the amount of entering Ca^{2+} to cell decrease, the contraction of myocytes is prevented (Tosaki and Hellegouarch, 1994). The inhibition of the cell contraction demonstrates the use of less ATP. Thus, intracellular ATP is protected. In previous studies, it was reported that opening of K_{ATP} channels decreased infarct size (Brown et al., 2005a; Quindry et al., 2010). Physicians suggest that sarc K_{ATP} channels are effective on the size of infarct area

(Brown et al., 2005a). Furthermore, some researchers report that the opening of sarc K_{ATP} channels during ischemia decreases the VF and VT due to the increase in the amount of extracellular K^+ (D'Alonzo et al., 1994). The mito K_{ATP} channels are effective on decreasing arrhythmias following ischemia and reperfusion (Brown et al., 2005a; Brown et al., 2005b; Quindry et al., 2010). Opening of the mito K_{ATP} channels cause K^+ influx to the matrix region in the mitochondria and it activates K/H exchanger. K^+ ions excreted out and H^+ ions taken in by K/H exchanger. Increased acidity in mitochondria decreases production of ATP (Oldenburg et al., 2002). Accordingly, less ATP production, ROS production is also reduced from mitochondria. Previous studies have reported that ROS increased the cardiac arrhythmias. It was indicated that the reduction of ROS production is effective in preventing arrhythmias by mito K_{ATP} channels.

Researchers reported that K_{ATP} channels increased with exercise and it were found to be cardioprotective by decreased both arrhythmias and infarct size (Brown et al., 2005a; Brown et al., 2005b; Quindry et al., 2010). There are few studies that focus on the effect of food restriction on K_{ATP} channels. However, the effect of food restriction on K_{ATP} channels was not clarified yet.

2. AIM AND SCOPE OF THE STUDY

The purpose of this study was to investigate the effects of physical exercise, food restriction, protein-rich feeding separately and together on the arrhythmias occurred following I/R and death in male and female. The relation of the amount of SOD level, the myocardial K_{ATP} channel protein, biochemical and hematological value with the I/R induced arrhythmias were also investigated in this study. The comparable effect of food restriction and exercise on these arrhythmias in male and female was also studied.



3. MATERIALS AND METHODS

In this study, 12 months old, 142 female and 148 male Sprague- Dawley rats were used. The animals were kept in 12 hours light / 12 hours dark (lights on at 8 a.m. and off at 8 p.m.). Animals were fed with standard pellet rat food that contains 20% soy protein until 12 months old. Animals were individually caged after 12 months old and divided into two main groups. First group were fed with standard pellet rat food (SRF) which contain normal protein and second group also were fed with special pellet rat food containing 35% soy protein designated as high protein (Protein rich food (PRF)). The list of component of rat food was shown in Table 3.1 and both standard and protein rich pellet rat food contents were shown in Table 3.2 and Table 3.3. Both two main groups were separated into four subgroups. These subgroups were control, 50% food restriction (FR), exercise (EX) and the combination of exercise and 50% food restriction (EX+FR). Experiment was performed in both male and female rats and totally 16 groups were produced (Table 3.4).

All study protocol was approved by Animal Research Local Ethics Committee (ID number: 2011/44) of Abant Izzet Baysal University, Bolu, Turkey (Appendix A).

Table 3.1. The materials used in the preparation of rat food.

Component List		
Soybean meal	Wheat bran	Phosphate
Bonkalit	Animal and vegetable oil	Calcium Carbonate
Corn	Molasses	Salt
Rice bran	Dicalcium	Vitamin-mineral mix

Table 3.2. Standard rat food content.

% of Analytical compounds					
	%		%		%
Crude protein	20	Crude ash	7,93	Calcium	1,18
Crude fiber	3,57	Lysine	1,55	Phosphorus	0,82
The crude oil	5,79	Methionine	0,49	Sodium	0,24
Trace Elements mg/kg					
E2 Iodine	0,8	E4 Copper	10	E6 Zinc	50
E3 Cobalt	0,15	E5 Manganese	50	E8 Selenium	0,15
The additives IU/kg					
Vitamin A	22200	Vitamin D	4850		

Table 3.3. Protein rich rat food content.

% of Analytical compounds					
	%		%		%
Crude protein	35	Crude ash	7,93	Calcium	1,18
Crude fiber	3,57	Lysine	1,55	Phosphorus	0,82
The crude oil	5,79	Methionine	0,49	Sodium	0,24
Trace Elements mg/kg					
E2 Iodine	0,8	E4 Copper	10	E6 Zinc	50
E3 Cobalt	0,15	E5 Manganese	50	E8 Selenium	0,15
The additives IU/kg					
Vitamin A	22200	Vitamin D	4850		

Table 3.4. Experimental groups.

Experimental groups	Number of Animals
1. SRF Group (Standard Rat Food Which Contains 20% Soy protein)	
Control	19 Male + 17 Female
50% Food Restriction (FR)	15 Male + 18 Female
Exercise (EX)	18 Male + 18 Female
Exercise + 50% Food Restriction (EX+FR)	20 Male + 21 Female
2. PRF Group (Special Rat Food Which Contains 35% Soy protein)	
Control	18 Male + 16 Female
50% Food Restriction (FR)	17 Male + 16 Female
Exercise (EX)	18 Male + 18 Female
Exercise + 50% Food Restriction (EX+FR)	23 Male + 18 Female

3.1 Experimental Groups

3.1.1 Control Groups

In this group, 19 male and 17 female rats fed with standard rat food and 18 male and 16 female rats fed with protein rich special rat food were used. After the individually caged, animals are maintained during 7 days for nutrition and environmental adaptation. In this adaptation period, food and top water were given as ad libitum. At the end of the 7 days, the animals were weighed and the value saved

as basal weight. In these groups, food restriction and exercise were not applied to the animals and the animals were fed as ad libitum for 6 weeks.

3.1.2 Food Restriction Groups

In this group, 15 male and 18 female rats fed with standard rat food and 17 male and 16 female rats fed with protein rich special rat food were used. After individually caged, animals are maintained during 7 days for nutrition and environmental adaptation. In this adaptation period, food and top water were given ad libitum. Consumed food by the animals was calculated from daily weighed initial and final food given to the animals. At the end of 7 days, animals were weighed and the value saved as a basal weight. After this time, food was given to animals by decreasing 50% of daily eaten food / day for 6 weeks. Top water was given ad libitum.

3.1.3 Exercise Group

In this group, 18 male and 18 female rats fed with standard rat food and 18 male and 18 female rats fed with protein rich special rat food were used. After the individually caged, the animals were awaited 7 days for adaption of nutrition and swimming exercise. In this adaptation period, food and top water were given as ad libitum. Animals were swam by increasing 5 minutes until 40-minute in a day for adaptation to the swimming exercise. Following adaptation, 40-minute swimming exercise was made for 6 weeks (5 days/week). Food and water were given ad libitum. Swimming exercise was applied in a water tank 75 cm width, 80 cm height. The water in the tank was 50 cm height and 28-30 degree centigrade.

3.1.4 Exercise + Food Restriction Group

In this group, 20 male and 21 female rats fed with standard rat food and 23 male and 18 female rats fed with protein rich special rat food were used. After the individually caged, the animals were awaited 7 days for adaption to the nutrition and swimming exercise. In this adaptation period, food and top water were given as ad libitum. Animals were swam by increasing 5 minutes until 40-minute in a day for

adaptation to the swimming exercise. At the same time of swimming exercise, food given to the animals daily were weight and the consumed food during the exercise was calculated from initial and final food given to the animals. At the end of 7 days of adaptation period, animals were weighed and this weight was taken as basal weight. After this term, food given to animals during adaptation period decreased in %50 and this food regime was maintained for 6 weeks together with 40-minute swimming exercise (5 days/week). Top water was given ad libitum.

Surgical operation was applied in all groups of animals following days later from the end of the 6 weeks of food restriction and swimming exercise. Blood was taken for biochemical analysis and hearts were taken for immunohistochemical analysis in four animals from all groups which have no surgical operation.

3.2 Surgical Operation

Animals were anesthetized with urethane (1.7 g/kg). Trachea was cannulated for artificial respiration. The catheter for carotid cannulation was filled with heparin (25000UI/100ml) diluted with 1/10 saline and inserted into the right carotid artery for measuring the blood pressure (Harvard Model 50-8952 transducer). Artificial respiration was started using an animal ventilator (Ugo Basile Rodent Ventilator, Italy) that set in air 0.9 ml/100 grams body weight at a rate of 60 strokes/minute and subcutaneous needle electrodes were placed under the skin to record standard electrocardiogram (ECG) before the opening of chest. Then ECG recording was started. The chest was opened by cutting the fourth rib for thoracotomy and pericardiotomy were performed to expose the heart. Heart was exposed by applying gentle pressure on the rib cage. A 5/0 silk suture was quickly placed around the branch of left coronary artery, ramus interventricularis (LAD) and then the heart was replaced in the chest and artificial respiration was started using an animal ventilator.

The animal was allowed to stabilize blood pressure and heart rate for five minutes. The six minutes of ligation was made by tightening the silk by producing a bowknot and fifteen minutes of reperfusion was produced by releasing the silk through the bowknot. ECG and blood pressure were recorded during ischemia and reperfusion by computerized recording system (Biopac System Inc Goleta CA USA, Turkish representative Commat-Ankara / Turkey) using BSL Pro 3.7 software. In this

operation animals having arrhythmias or less blood pressure below 70mmHg before ligation was discarded. Recording and animal ventilator were stopped at the end of reperfusion. Animals having no respiration following the stop of ventilator were discarded and these animals were not evaluated.

3.3 Determination of Risk of Infarct Zone

At the end of experiment, the survived animals were heparinized with 1/10 heparin (25000 IU/100 ml) via carotid artery, ml/100 mg body weight. The heart was excised out via cutting the aorta and the silk around of the left coronary artery was retightened. The heart was fist perfused with 10 milliliters isotonic sodium chloride solution, then 2 milliliters ethanol to determine occluded myocardium. Non-perfused area that remained red was separated from the perfused area that seemed white in color (Figure 3.1). The perfused and non perfused myocardium were separated from each other by cutting razor blade and weighed. The weight of non-perfused area was measured alone and together with the perfused area. The percentage of non-perfused area were calculated in respect to whole weight of ventricles and designated as a risk of infarct zone ($\text{Weight of non-perfused area} \times 100 / \text{Whole weight of ventricle}$).

3.1 Recording

ECG and blood pressure were recorded during six minutes ischemia and fifteen minutes reperfusion by computerized recording system (Biopac System Inc Goleta CA USA, Turkish representative Commat-Ankara / Turkey). The types of arrhythmias were determined according to the Lambeth Convention (Walker et al., 1988) from recorded ECG. An arrhythmia score were defined in respect to the duration and the type of arrhythmia observed during ischemia and reperfusion in all animals (Lepran et al., 1983).

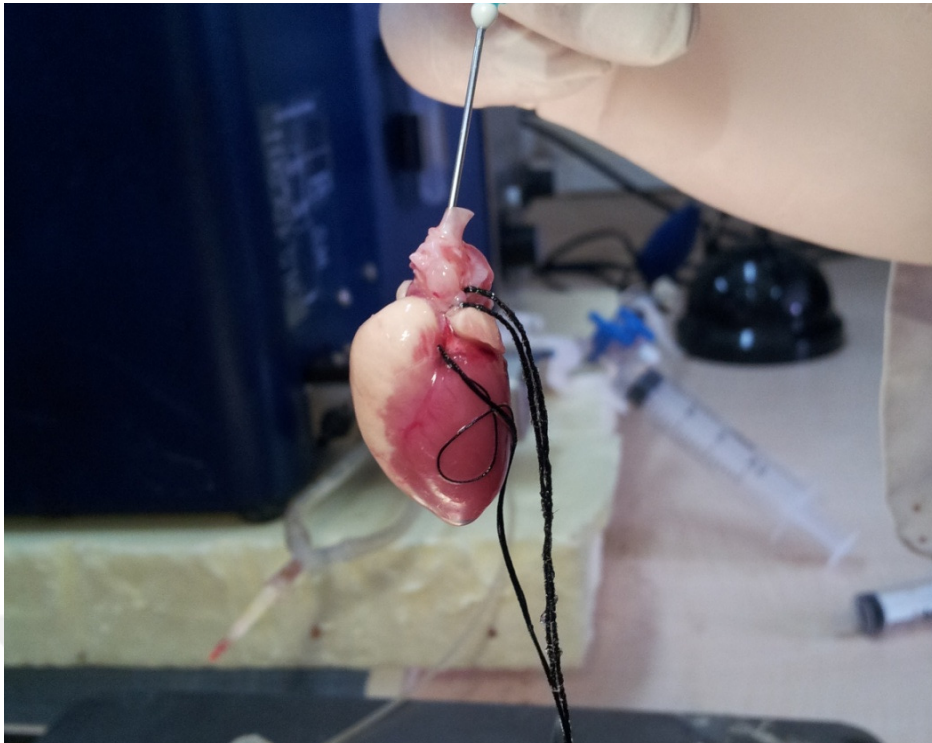


Figure 3.1 Determination of risk of infarct zone

3.2 The Evaluation of the Arrhythmias

Blood pressure, heart rate, the incidence of arrhythmias, the type and the lengths of arrhythmias, the total length of arrhythmias and the score of arrhythmias were determined from the stored ECG recordings. Heart rate and blood pressure were determined from recorded ECG at one minute before ligation (basal) and at 1st, 3th, 5th, minutes following ligation, beginning of reperfusion and 1st, 3th, 5th, 10th, 15th minutes of reperfusion. Arrhythmias were classified as ventricular tachycardia (VT), ventricular fibrillation (VF) and other types of arrhythmias (ventricular premature contraction (VPC)), ventricular bigeminy, ventricular salvo, etc) (Walker et al., 1988). The onset of arrhythmia and the end of arrhythmia during ischemia and reperfusion were determined. Arrhythmic period was determined as length (second) covering the differences between the onset and the end of arrhythmia. The arrhythmias was designated as sinus tachycardia when the heart rate was 500 to 600 and blood pressure was higher than 70 mmHg and as ventricular tachycardia when the heart rate was 500-600/min and blood pressure lower than 40 mmHg and as

ventricular fibrillation when the blood pressure abruptly decrease to the less than 20 mmHg.

An arrhythmia score was determined according to the incidence and duration of arrhythmias for each animal as follows; 0 = no arrhythmias; 1 = < 10 second VT or other arrhythmias, no VF; 2 = 11-30 second VT or other arrhythmias, no VF; 3 = 31-90 second VT or other arrhythmias, no VF; 4 = 91-180 second VT or other type of arrhythmias and / or < 10 second reversible VF; 5 = >180 second VT or other arrhythmias and/or >10 second reversible VF; 6 = irreversible VF.

3.3 Biochemical Analysis

In all experimental groups, 4-7 rats without surgical operation were used for biochemical analysis, (see Table 4.1, 4.2). These animals were anesthetized first with ketamine and xylazine (0.1ml/0.1ml) and then blood was taken by 10 ml syringe directly from heart on the chest. One milliliter of blood samples were collected into sterile vacuum tubes with EDTA for complete blood cell count. Complete blood count was studied by routine tests by cell counter (Cell-DYN 3700). Six milliliters of blood samples were taken in to the four eppendorf tubes having no EDTA (1.5 ml) and was waited for 20 minutes and then were centrifuged at 4 °C with 4000/min spin for 20 minutes on HETTICH EBA 12R Centrifuge for separation of serum. Blood serum samples were separated from the blood cells and stored in deep freeze (-80 °C) for biochemical analysis. Glucose, high density lipoprotein cholesterol (HDL), triglycerides, total cholesterol, creatinine, blood urea nitrogen (BUN), Aspartate transaminase (AST), Alanine transaminase (ALT), Lactate dehydrogenase (LDH), Creatine Kinase-MB (CK-MB), urea and albumin were measured by routine biochemical tests by (Architect c8000) in Abant İzzet Baysal University Faculty of Medicine, Biochemistry Department. Super Oxidase Dismutase (SOD) levels were measured in blood serum by using commercial ELISA kit (Rat Super Oxidase Dismutase ELISA Kit, Sunred Biological Technology Co.) according to the manufacturer's instructions on ELISA test (Stat Fax 2600 washer and Stat Fax 4700 micro strip reader).

3.4 Immunohistochemical Analysis

Hearts from 4-7 rats without surgical operation from all groups were taken and perfused with tyrode solution from aorta. Then ventricular tissues were fixed with 10% neutral buffered formalin. Fixed tissues were dehydrated in ethanol, cleared in xylene and embedded in paraffin blocks. The sections in 4 micrometer were cut from paraffin blocks. Ventricular myocardium sections were stored at 37 °C for one night. Tissue sections were deparaffinized and hydrated with xylene and ethanol (xylene (2x), 100%, 95%, 80%, 70%, 50%, 30% ETOH - 5 min each). Antigen retrieval procedure was applied in citrate buffer (Abcam, ab64236) by heating in microwave oven for 15 minutes. After the tissue sections were washed via Phosphate buffer solution (PBS), hydrogen peroxide block was applied (Abcam, ab80437) to tissue sections for 5 minutes. Tissue sections were rinsed two times via PBS. Then the sections were incubated with the primary antibody sulphonylurea receptor 2A (SUR 2A) (SAB2100013, Sigma Aldrich, USA) that was diluted 1/100 ratio by antibody dilution buffer during one hour. SUR 2A is a regulatory subunit of K_{ATP} channel. Secondary antibody (Abcam, ab80437) was applied during 30 minutes on the tissue sections after that was rinsed three times by PBS. Than tissue sections were incubated for five minutes by Diaminobenzidine. Tissue sections were rinsed by distilled water. Counterstain was applied on the tissue sections by Mayer hematoxylin (Abcam, ab128990) for 3 minutes. Tissue samples were washed two times by water. Finally, tissue samples were covered by coverslide after dehydration. Then tissue samples were observed by light microscopy and then they were photographed.

3.5 Statistical Analysis

Data were analyzed using SPSS statistical programme (IBM Statistics version 21). The mean and standard errors were determined by one way analyse of variance (ANOVA) combined with the LSD post hoc. test for all parameters including ventricular weights, heart rate, blood pressure, duration of arrhythmias, hematological and biochemical values. The survival rate and the incidence of arrhythmia were compared by the chi-square test (Fisher exact test, two tailed).

Values were considered statistically significant at $P < 0.05$. Data were presented as mean $\pm$ standard error ($M \pm SE$).



4. RESULTS AND DISCUSSIONS

4.1 RESULTS

In this study, 290 male and female rats were used. 52 of them were died due to natural causes during feeding or drowning during swimming exercise. 20 Rats were died due to respiratory arrest or blood loss during surgical operation. Successful results were taken from 106 male and 112 female rats (Table 4.1, Table 4.2). ST segment elevation and QRS changes were observed in all animals following the ligation. These changes were not seen in animals having unsuccessful ligation. Blood pressure decreased immediately after the ligation in all groups.

4.1.1 CONTROL GROUPS

In these groups of male and female rats, animals were fed with both standard and special rat food without exercise and food restriction. Comparison was made for all parameters with control male and female animals fed with Standard rat food (SRF) or Protein rich food (PRF). Control groups for standard rat food and protein rich food in both genders were different.

4.1.1.1 Heart Rate and Blood Pressure

Heart rate was found to be not different between control groups before ligation in male and female rats fed with normal or protein rich diet (Figure 4.1). In PRF group, heart rate was lower than SRF group in male during reperfusion. Heart rate in SRF female group was lower than SRF male group at 1st, 3rd, 5th and 10th minutes of reperfusion. Heart rate in PRF female was higher than PRF male group during reperfusion (Table 4.3). Protein rich diet decreased heart rate in male but not female during reperfusion.

Basal blood pressure in both normal and protein rich diet group was not different between male and female (Figure 4.2). In some time during the reperfusion period, blood pressure was lower in PRF male group than SRF.

Table 4.1. Number of male animals used in the experiment.

Gender	Protein ratio	Groups	Number of animals	Number of died animals during practice	Number of survived animals after practice	Operation	N	Number of died animals during operation	n	Total number of died animals	Number of survived animals after surgical operation
Male	20% (SRF)	Control	19	4	15	I/R	8	0	8	4	15
						Biochemistry	7	0	7		
		FR	15	0	15	I/R	9	1	8	1	14
						Biochemistry	6	0	6		
		EX	18	0	18	I/R	12	2	10	2	16
						Biochemistry	6	0	6		
		EX+FR	20	8	12	I/R	8	0	8	8	12
						Biochemistry	4	0	4		
	35% (PRF)	Control	18	3	15	I/R	11	2	9	5	13
						Biochemistry	4	0	4		
		FR	17	4	13	I/R	9	1	8	5	12
						Biochemistry	4	0	4		
		EX	18	3	15	I/R	11	3	8	6	12
						Biochemistry	4	0	4		
		EX+FR	23	10	13	I/R	9	1	8	11	12
						Biochemistry	4	0	4		
		Total	148	32	116	0	116	10	106	42	106

N: Number of animals before surgical operation, n: Number of animals after surgical operation, SRF: Standard rat food, PRF: Protein rich food, I/R: Ischemia reperfusion, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction

Table 4.2. Number of female animals used in the experiment.

Gender	Protein ratio	Groups	Number of animals	Number of died animals during practice	Number of survived animals after practice	Operation	N	Number of died animals during operation	n	Total number of died animals	Number of survived animals after surgical operation
Female	20% (SRF)	Control	17	1	16	I/R	10	0	10	1	16
						Biochemistry	6	0	6		
		FR	18	1	17	I/R	11	3	8	4	14
						Biochemistry	6	0	6		
		EX	18	1	17	I/R	11	0	11	1	17
						Biochemistry	6	0	6		
		EX+FR	21	8	13	I/R	9	0	9	8	13
						Biochemistry	4	0	4		
	35% (PRF)	Control	16	1	15	I/R	11	2	9	3	13
						Biochemistry	4	0	4		
		FR	16	2	14	I/R	10	2	8	4	12
						Biochemistry	4	0	4		
		EX	18	1	17	I/R	13	3	10	4	14
						Biochemistry	4	0	4		
		EX+FR	18	5	13	I/R	9	0	9	5	13
						Biochemistry	4	0	4		
		Total	142	20	122		122	10	112	30	112

N: Number of animals before surgical operation, n: Number of animals after surgical operation, SRF: Standard rat food, PRF: Protein rich food, I/R: Ischemia reperfusion, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction

Table 4.3. The heart rate (Beat / minute) in the control groups of rats fed with protein rich and standard food

Groups	N	Basal	Ligation	Lig 1 st	Lig 3 rd	Lig 5 th	Reperfusion	Rep 1 st	Rep 3 rd	Rep 5 th	Rep 10 th	Rep 15 th
SRF Male Control (a)	4-7	343±17	343±16	350±12	338±25	328±26	346±8	363±17	360±11	364±14	357±18	339±13
PRF Male Control (b)	5-9	338±17	334±19	335±20	319±18	317±22	274±20 ^a	268±27 ^a	281±27 ^a	277±25 ^a	272±25 ^a	266±24 ^a
SRF Female Control (c)	7-9	345±10	337±11	337±11	325±10	311±8	307±11	303±11 ^a	307±10 ^a	305±5 ^a	292±9 ^a	299±13
PRF Female Control (d)	6-9	343±18	345±20	357±19	355±17	346±20	329±23 ^b	338±20 ^b	338±18 ^b	344±14 ^b	330±17 ^b	336±16 ^b

N: Number of animals (HR was not determined following minutes of I/R because of ventricular arrhythmia in some rats), SRF: Standard rat food, PRF: Protein rich food, Basal: Before coronary ligation, Lig 1st, Lig 3rd, Lig 5th: 1st, 3rd, 5th minutes after the coronary ligation. Rep 1st, Rep 3rd, Rep 5th, Rep 10th, Rep 15th: 1st, 3rd, 5th, 10th, 15th minutes after the coronary reperfusion. ^aP < 0.05 : Compared to SRF male control, ^b P < 0.05 : Compared to PRF male control. Results: mean ± SE.

Table 4.4. The arterial blood pressure (mmHg) in the control groups of rats fed with protein rich and standard food

Groups	N	Basal	Ligation	Lig 1 st	Lig 3 rd	Lig 5 th	Reperfusion	Rep 1 st	Rep 3 rd	Rep 5 th	Rep 10 th	Rep 15 th
SRF Male Control (a)	4-7	113±12	82±8	77±8	71±11	79±11	94±13	81±6	92±11	89±10	78±9	82±8
PRF Male Control (b)	5-9	105±4	89±4	81±4	78±5	74±5	67±4 ^a	73±5	74±7	71±7	72±7	68±7
SRF Female Control (c)	7-9	117±5	98±8	88±8	86±7	83±7	80±4	82±8	79±8	80±7	83±6	90±7
PRF Female Control (d)	6-9	131±4 ^b	106±8	98±8	94±10	96±9	96±9 ^b	83±6	88±6	80±5	80±7	83±9

N: Number of animals (BP was not determined following minutes of I/R because of ventricular arrhythmia in some rats), SRF: Standard rat food, PRF: Protein rich food, Basal: Before coronary ligation, Lig 1st, Lig 3rd, Lig 5th: 1st, 3rd, 5th minutes after the coronary ligation. Rep 1st, Rep 3rd, Rep 5th, Rep 10th, Rep 15th: 1st, 3rd, 5th, 10th, 15th minutes after the coronary reperfusion. ^aP < 0.05: Compared to SRF male control, ^bP < 0.05: Compared to PRF male control. Results: mean ± SE.

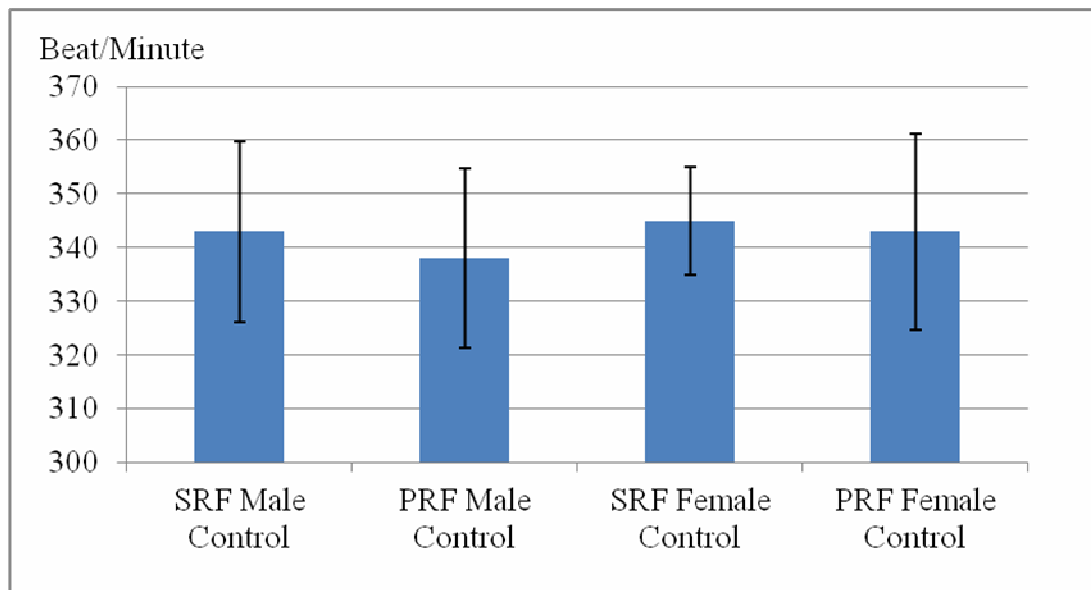


Figure 4.1. The heart rate before ligation in the control groups of rats fed with protein rich and standard food

SRF: Standard rat food, PRF: Protein rich food.

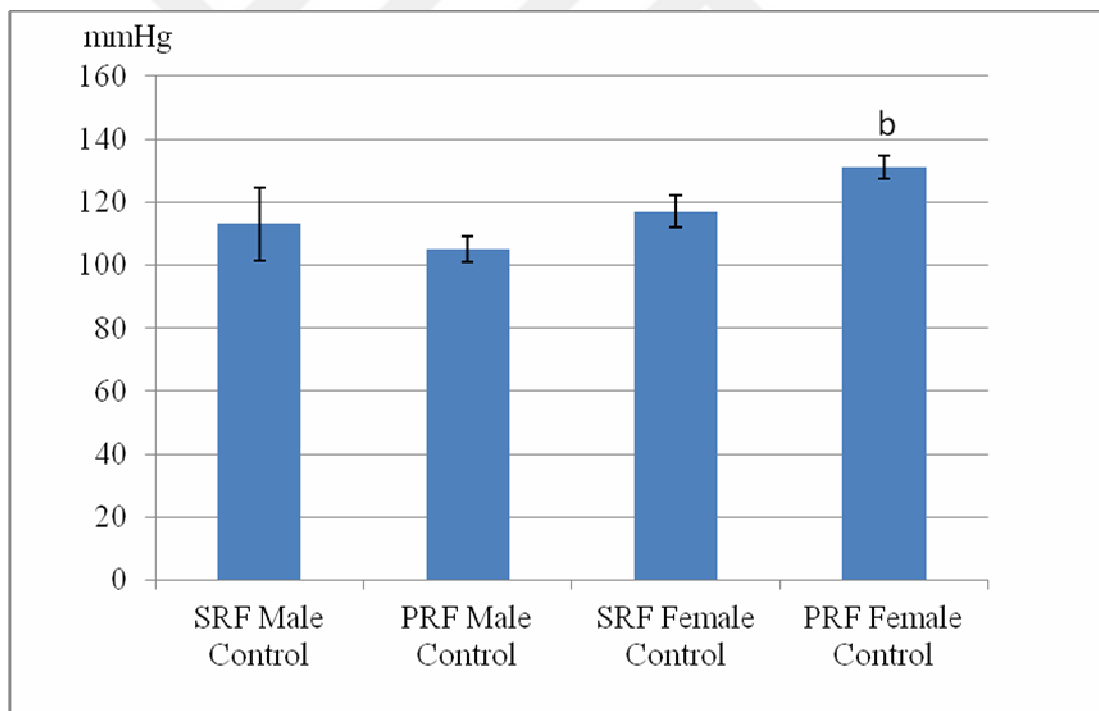


Figure 4.2. The blood pressure before ligation in the control groups of rats fed with protein rich and standard food

SRF: Standard rat food, PRF: Protein rich food, ^bP<0.05: Compared to PRF male control.

4.1.1.2 The duration of arrhythmias, the score of arrhythmia, the onset and the end of arrhythmias, the arrhythmic period, the incidence of arrhythmias and the survival rate of animals during six minutes of ischemia in rats fed with protein rich and standard rat food

Duration of VF and VT was not significantly different in the control groups during six minutes of ischemia. Other types of arrhythmias and duration of total arrhythmias was higher in SRF male control group in respect to other groups (Figure 4.3). The score of arrhythmia was not different in the control group during six minutes of ligation (Figure 4.4). The onset and offset of arrhythmias were not significantly different among control group during six minutes of ischemia (Figure 4.5).

The length of the arrhythmic period was significantly lower in PRF male and PRF female control groups in respect to SRF male and female groups during ischemia (Figure 4.6). The incidence of arrhythmias and survival rate of animals were not different in the control animals during ischemia (Table 4.5).

4.1.1.3 The duration of arrhythmias, the score of arrhythmia, the onset and the end of arrhythmias, the arrhythmic period, the incidence of arrhythmias and the survival rate of animals during fifteen minutes of reperfusion in rats fed with protein rich and standard rat food

The duration of arrhythmias and total arrhythmias were not different between control groups during fifteen minutes of reperfusion (Figure 4.7). The score of arrhythmia was not significantly different among control groups during reperfusion (Figure 4.8). The onset and the end of arrhythmia were not significantly different between control groups during fifteen minutes of reperfusion (Figure 4.9). The length of arrhythmic period was not different in the control animals during reperfusion (Figure 4.10).

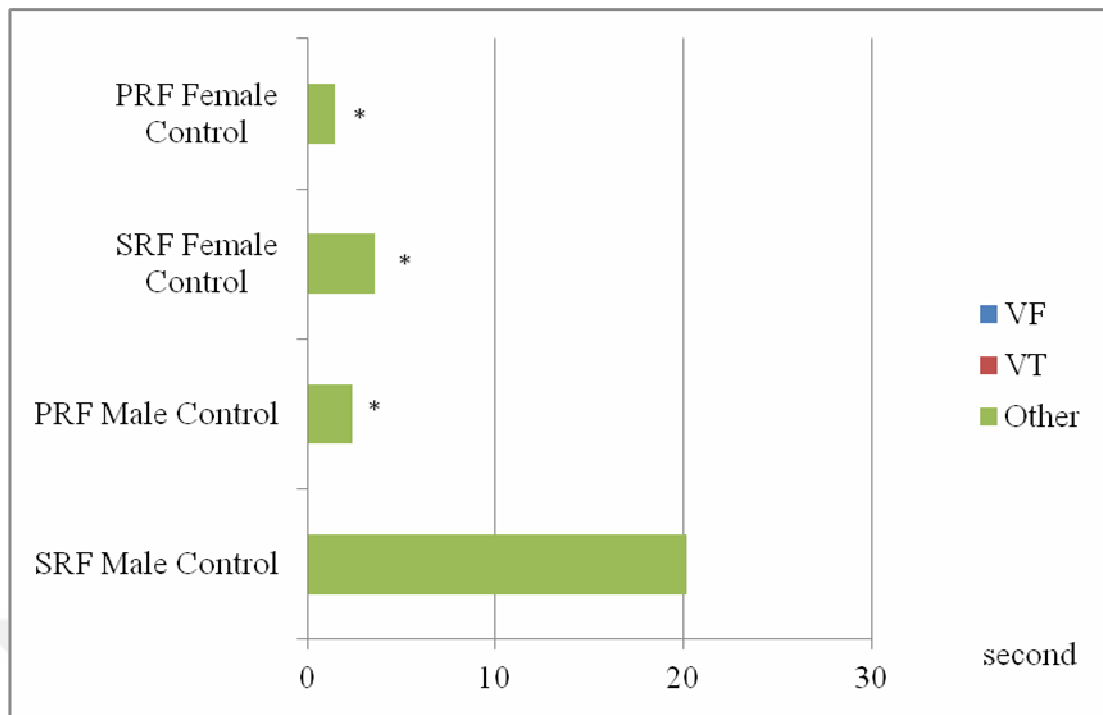


Figure 4.3. The duration of arrhythmias in the control groups of rats fed with protein rich and standard rat food during ligation.

SRF: Standard rat food, PRF: Protein rich food, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Ventricular premature contractions, *P<0.05: Compared to SRF male control.

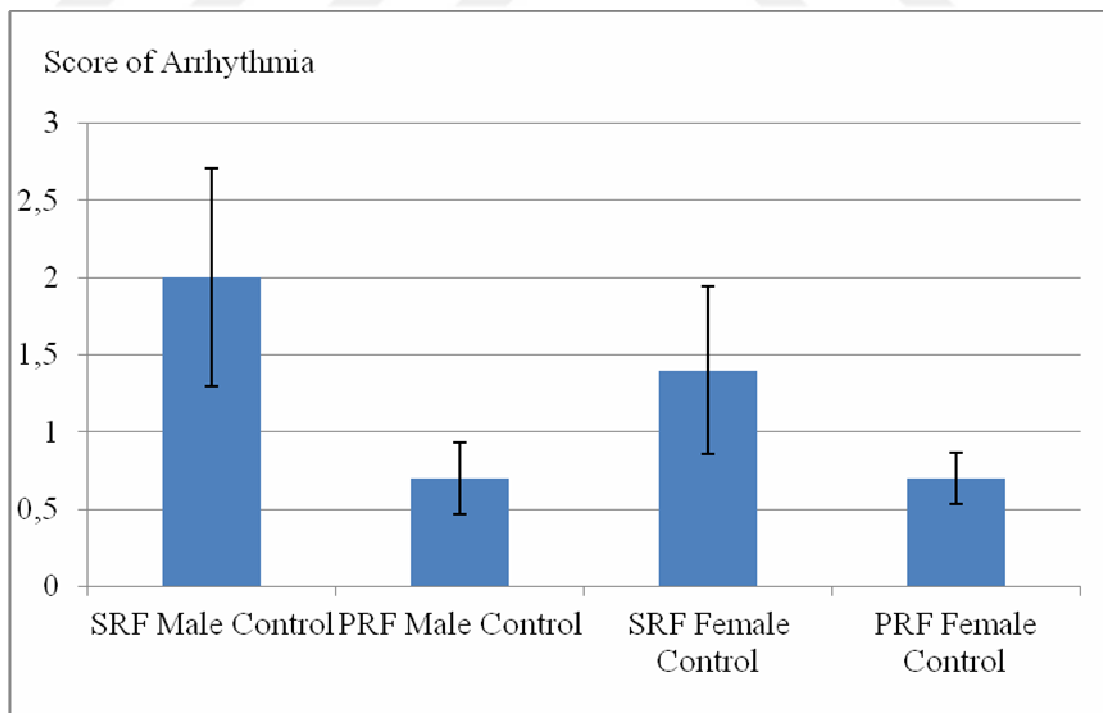


Figure 4.4. The score of arrhythmias in the control groups of rats fed with protein rich and standard rat food during ligation.

SRF: Standard rat food, PRF: Protein rich food.

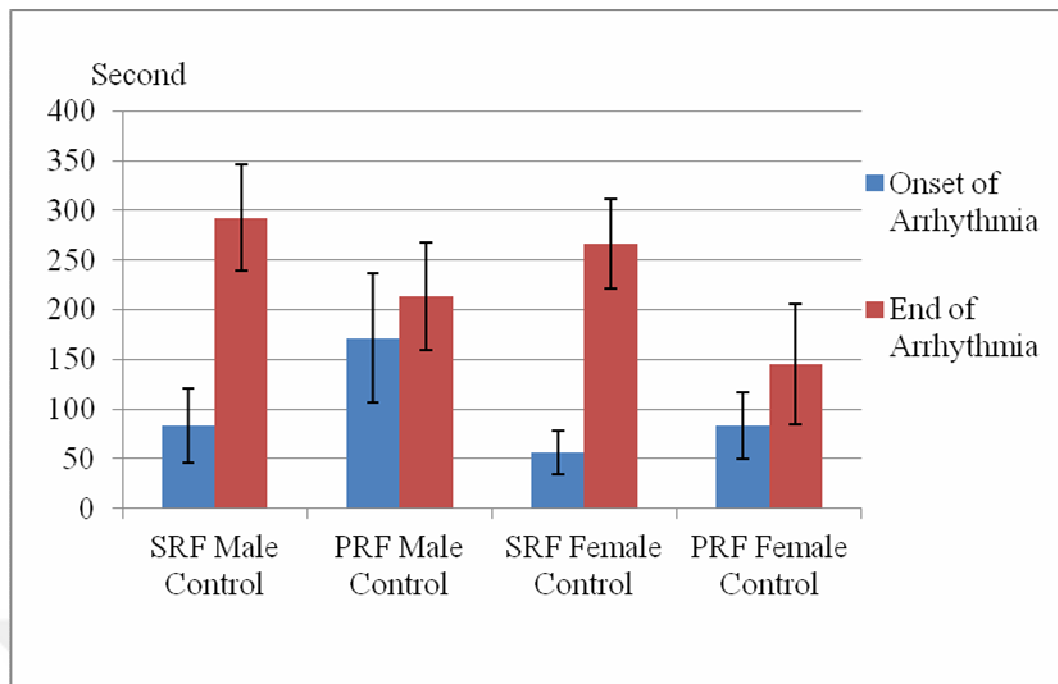


Figure 4.5. The onset and the end of arrhythmias in the control groups of rats fed with protein rich and standard rat food during ligation.

SRF: Standard rat food, PRF: Protein rich food.

Table 4.5. The survival rate of animals and the incidence of arrhythmias during six minutes of ischemia in the control groups of rats fed with protein rich and standard rat food.

Groups	N	Survival (n / %)	Incidence of arrhythmia (n / %)			
			VF	VT	Other	Bradycardia
SRF Male Control (a)	8	7/88	1/13	1/13	6/75	0/0
PRF Male Control (b)	9	9/100	0/0	0/0	5/56	0/0
SRF Female Control (c)	10	9/90	1/10	1/10	8/80	0/0
PRF Female Control (d)	9	9/100	0/0	0/10	6/67	0/0

N: The number of animals at the onset of ligation, n/%: The number of animals / The percentage of animals, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.), SRF: Standard rat food, PRF: Protein rich food.

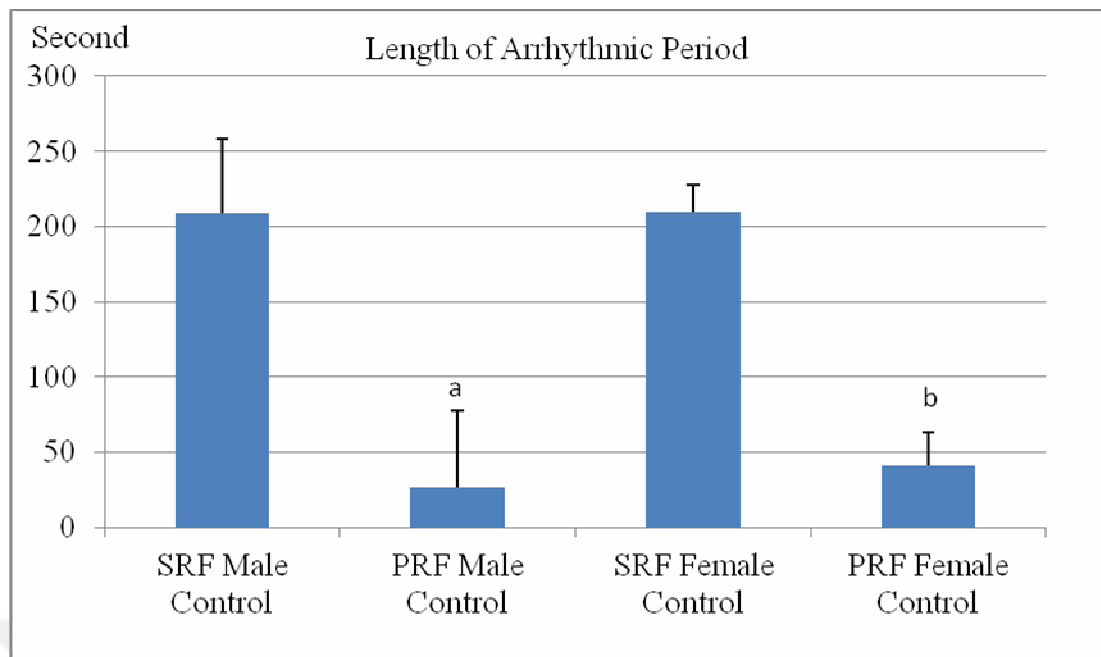


Figure 4.6. The length of arrhythmic period in the control groups of rats fed with protein rich and standard rat food during ligation.

SRF: Standard rat food, PRF: Protein rich food, ^aP < 0.05: Compared to SRF male control, ^bP < 0.05: Compared to SRF female control.

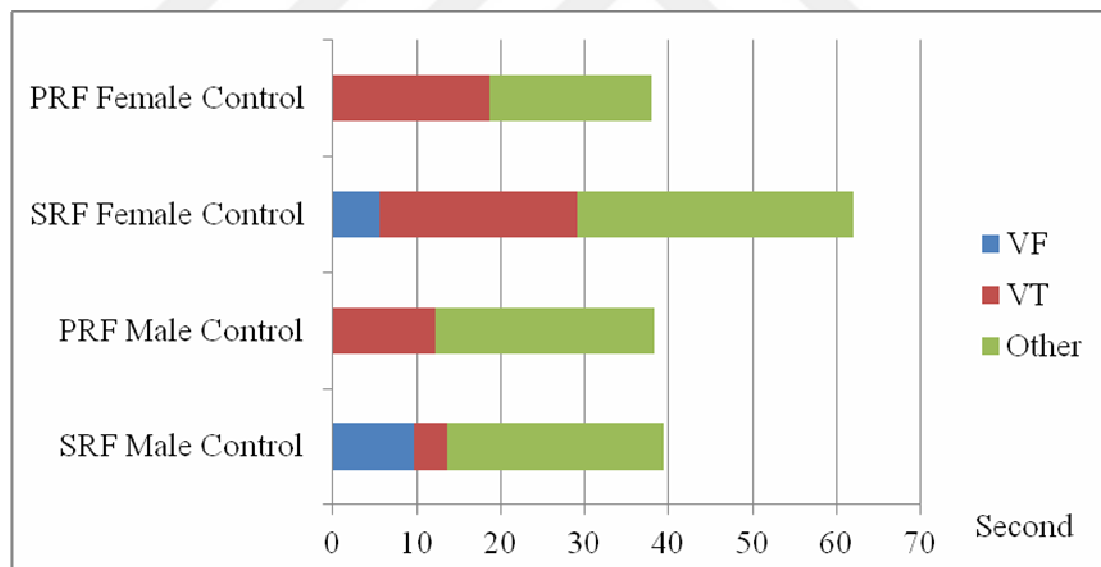


Figure 4.7. The duration of arrhythmias in the control groups of rats fed with protein rich and standard rat food during reperfusion.

SRF: Standard rat food, PRF: Protein rich food VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.).

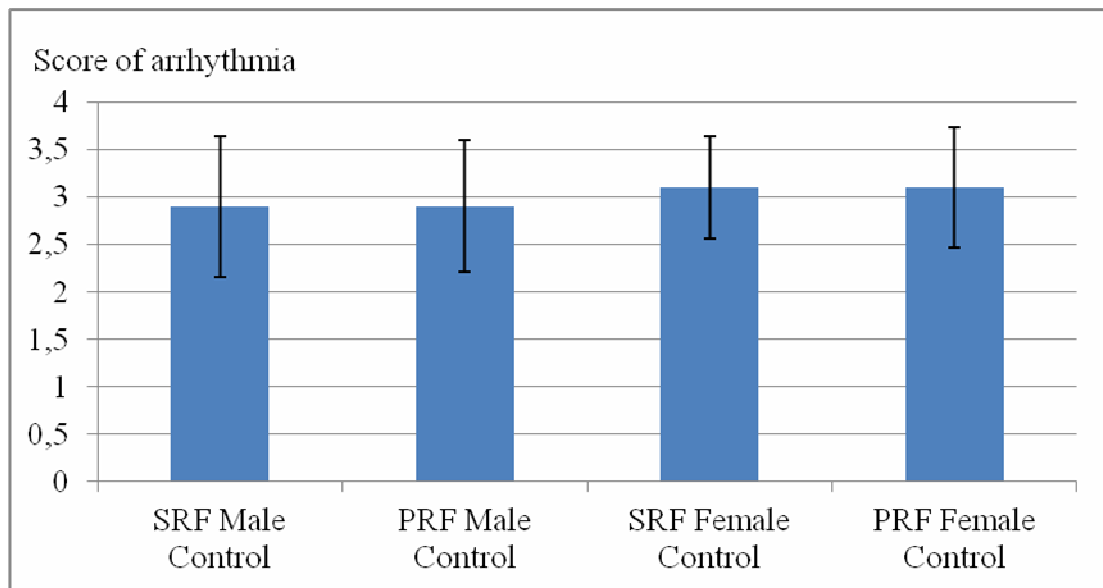


Figure 4.8. The score of arrhythmia in the control groups of rats fed with protein rich and standard rat food during reperfusion.

SRF: Standard rat food, PRF: Protein rich food.

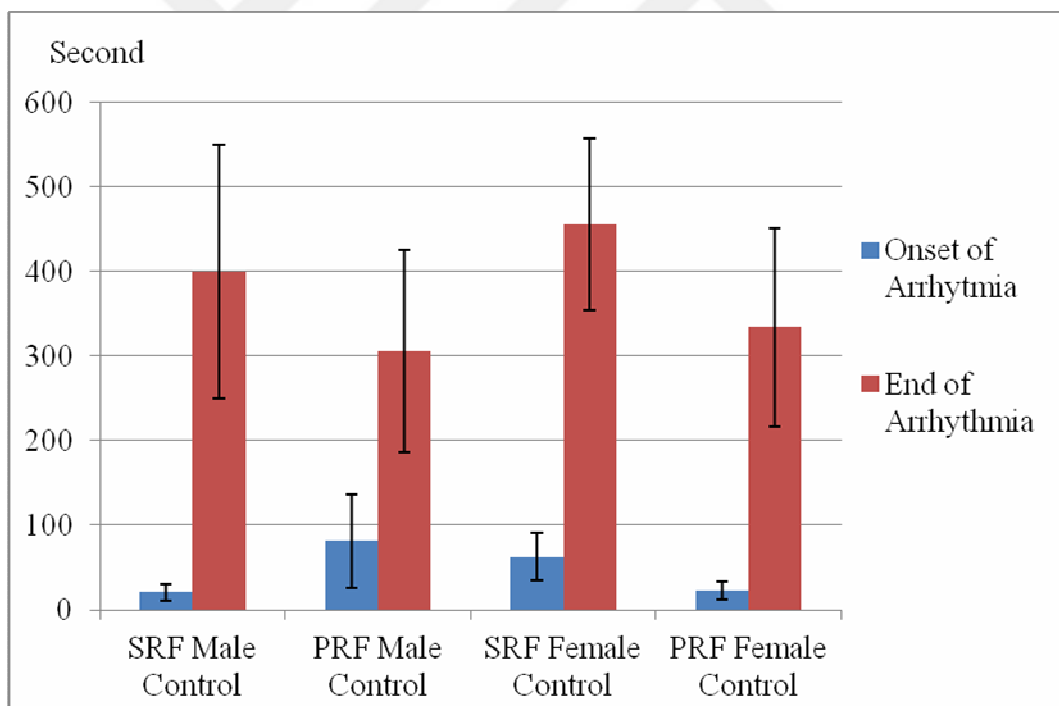


Figure 4.9. The onset and the end of arrhythmias in the control groups of rats fed with protein rich and standard rat food during reperfusion.

SRF: Standard rat food, PRF: Protein rich food.

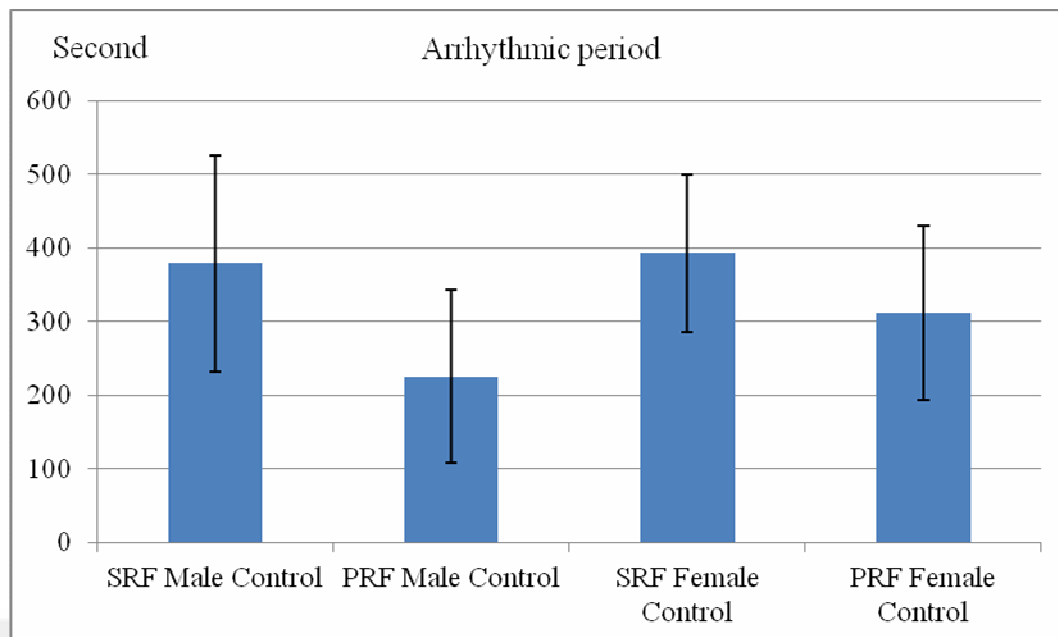


Figure 4.10. The length of arrhythmic period in the control groups of rats fed with protein rich and standard rat food during reperfusion.

SRF: Standard rat food, PRF: Protein rich food.

The incidence of VT in PRF female group was significantly higher than SRF female group ($P < 0.05$) and PRF male control group ($P < 0.01$) during reperfusion. Survival rate, incidence of VF, other types of arrhythmia and bradycardia were not different between control groups during reperfusion (Table 4.6).

4.1.1.4 Body weights, percentage of risk of infarct zone and ventricular weights

Body weights, percentage of risk of infarct zone and ventricular weights of animals were not significantly different in the control groups of animals (Table 4.7).

4.1.1.5 The hematological results: leukocytes, erythrocytes and hemoglobin

The number of leukocytes in PRF female control group were significantly lower than PRF male control group ($P < 0.05$). The amount of erythrocytes and hemoglobin was not different between control animals (Table 4.8).

Table 4.6. The survival rate of animals and the incidence of arrhythmias during fifteen minutes of reperfusion in the control groups of rats fed with protein rich and standard rat food.

Groups	N	Survival (n / %)	Incidence of arrhythmia (n / %)			
			VF	VT	Other	Bradycardia
SRF Male Control (a)	7	6/86	2/29	2/29	7/100	2/29
PRF Male Control (b)	9	7/78	2/22	2/22	9/100	0/0
SRF Female Control (c)	9	9/100	2/22	3/33	9/100	2/22
PRF Female Control (d)	9	7/78	2/22	7/78 ^{bb c}	9/100	1/11

N: The number of animals at the onset of ligation, n/%: The number of animals / The percentage of animals, SRF: Standard rat food, PRF: Protein rich food, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.), ^{bb}P < 0.01: Compared to PRF male control, ^cP < 0.05: Compared to SRF female control.

Table 4.7. Body weights, percentage of risk of infarct zone and ventricular weights in the control groups of rats fed with protein rich and standard rat food.

Groups	N	Body weight BA (g)	Body weight AA (g)	Risk of infarct zone %	N	Ventricular weight (g)
SRF Male Control (a)	8	397±16	396±17	51±1	8	1.1±0.05
PRF Male Control (b)	9	366±16	378±17	47±1	8	1.1±0.06
SRF Female Control (c)	10	268±11	284±11	49±2	10	0.9±0.05
PRF Female Control (d)	9	244±9	245±11	48±1	8	0.8±0.05

N: The number of animals, SRF: Standard rat food, PRF: Protein rich food, BA: Before application, AA: After application (Application: swimming exercise, food restriction and high protein diet).

Table 4.8. The hematological results in the control animals fed with high protein and standard rat food.

Groups	N	Leukocytes	Erythrocytes	Hemoglobin (g/dl)
SRF Male Control (a)	4	6.47±1	7.52±0.2	13.72±0.3
PRF Male Control (b)	4	10.97±2.4	7.21±0.5	13.43±0.6
SRF Female Control (c)	5	4.64±0.9	6.96±0.2	13.58±0.3
PRF Female Control (d)	4	5.36±1.2 ^b	7.29±0.2	13.5±0.2

N: The number of animals, SRF: Standard rat food, PRF: Protein rich food, ^bP < 0.05.

4.1.1.6 The biochemical results; Glucose; HDL, Triglyceride, Total Cholesterol; Creatinine, BUN, Urea, Albumin, AST, ALT, LDH, CK-MB; SOD in the control groups of rats fed with protein rich and standard rat food

The amount of glucose in PRF male control group was significantly lower than SRF male control group (P<0.05). But there was no significant difference in glucose level between SRF and PRF group of females. The amount of HDL

cholesterol in SRF female control group was significantly higher than SRF male control group ($P<0.05$). The amount of total cholesterol in PRF female control group was significantly lower than SRF female control group ($P<0.05$). The amount of BUN in PRF male control was significantly higher than SRF male control group ($P<0.05$). The amount of albumin in SRF female control group was significantly higher than PRF female control group ($P<0.05$). PRF diet increased the amount of urea in blood serum significantly in male ($P<0.05$) and nonsignificantly in female rats. The amount of triglyceride, creatinine, AST, ALT, LDH and CK-MB were not different between control animals (Table 4.9). There was no difference between control groups in the amount of SOD (Figure 4.11).

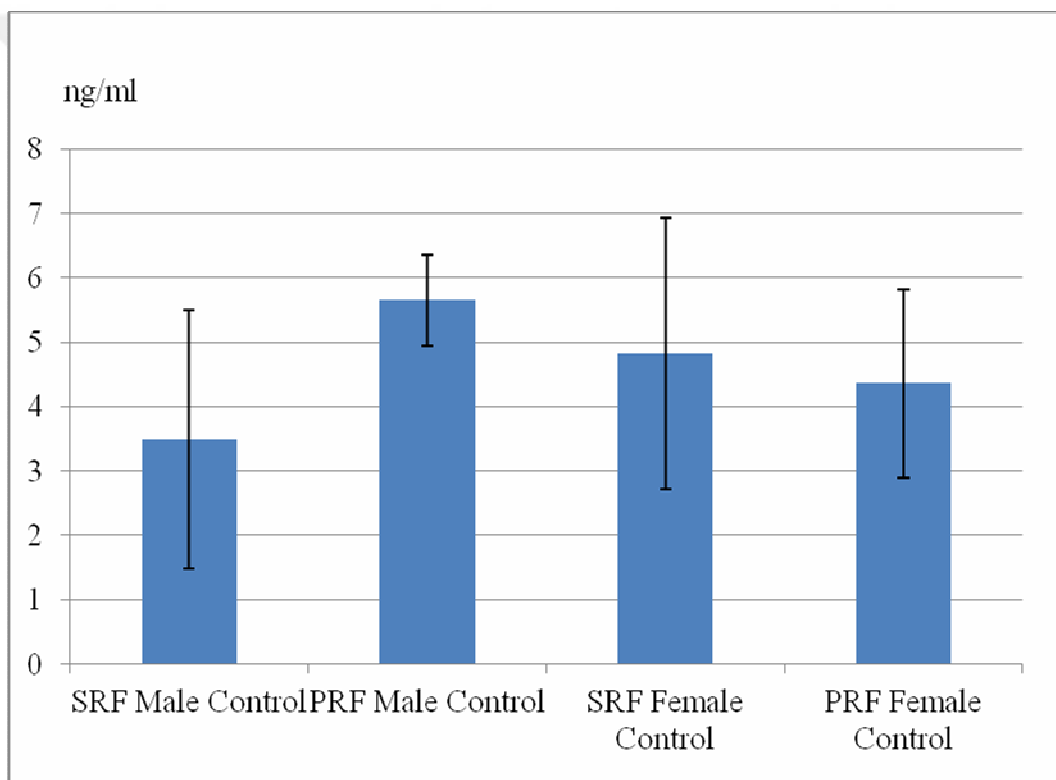


Figure 4.11. The amount of SOD in the control groups of rats fed with protein rich and standard rat food.

SRF: Standard rat food, PRF: Protein rich food.

4.1.1.7 The results of immunohistochemical analysis

The density of immunostaining of SUR2A in myocardial cell was not different between the control groups. (Figure 4.12)

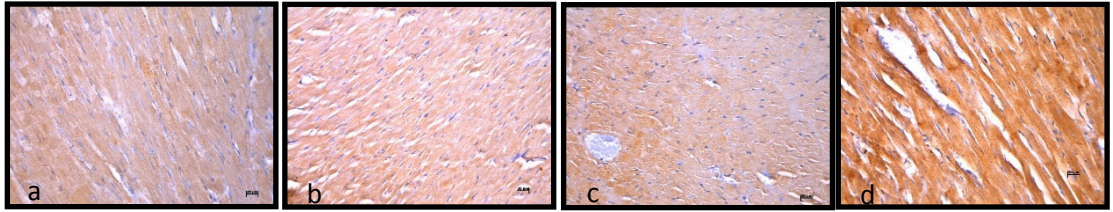


Figure 4.12. The results of immunohistochemical analysis in the control groups of rats fed with protein rich and standard rat food.

a: SRF male control, b: PRF male control, c: SRF female control, d: PRF female control.

Table 4.9. The biochemical results in the control rats fed with protein rich and standard rat food.

Groups	N	Glucose (mg/dl)	HDL (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Creatinine (mg/dl)	BUN (mg/dl)	AST (U/L)	ALT (U/L)	LDH (U/L)	CK_MB (U/L)	Albumin (g/dl)	Urea (mg/dl)
SRF Male Control (a)	6	300±20	19.8±1	56±9	57±5	0.55±0,04	18±1	214±48	83±7	821±124	435±58	2.7±0,2	39±1
PRF Male Control (b)	4	175±20 ^a	22.7±1	37±7	60±4	0.52±0,04	30±5 ^a	170±43	68±3	1390±365	704±217	2.1±0,1	63±12 ^a
SRF Female Control (c)	6	264±29	26.5±2 ^a	56±11	70±7	0.53±0,03	19±3	108±10	56±8	453±184	287±83	3.6±0,4 ^a	40±5
PRF Female Control (d)	4	213±26	20.6±1	79±25	51±6 ^c	0.52±0,01	23±3	194±118	63±20	1057±649	682±405	2.9±0,2 ^b	50±6

SRF: Standard rat food, PRF: Protein rich food, N: The number of animals. HDL: High density lipoprotein cholesterol, BUN: Blood urea nitrogen, AST: Aspartate transaminase, ALT: Alanine transaminase LDH: Lactate dehydrogenase, CK-MB: Creatine Kinase-MB, ^aP < 0.05: Compared to SRF male control, ^bP < 0.05: Compared to PRF male control, ^cP < 0.05: Compared to SRF female control. Results: mean ± SE.

4.1.2 Male Rats Fed With Standard Rat Food (SRF Male Groups)

In this section, the comparison of all parameters were made between control, exercise, food restriction and exercise + 50% food restriction groups in male rats fed with standard rat food.

4.1.2.1 The heart rate and the blood pressure in male rats fed with standard rat food

Food restriction decreased the heart rate at basal, 1st minute of ligation and during reperfusion as it compared to control rats ($P<0.05$). The heart rate in exercise group was lower at 5th, 10th, and 15th minutes of reperfusion when compared with control rats ($P<0.05$). The heart rate in EX+FR group was lower than the control and EX groups before and during ligation and reperfusion ($P<0.05$) (table 4.10).

The blood pressure in FR group was significantly lower than the control group during reperfusion ($P<0.05$). The blood pressure in EX group was higher than the control group at ligation ($P<0.05$) (Table 4.11).

4.1.2.2 The duration of arrhythmias, the score of arrhythmia, the onset and the end of arrhythmias, the arrhythmic period, the incidence of arrhythmias and the survival rate of animals during six minutes of ischemia in male rats fed with standard rat food

The duration of ventricular fibrillation and ventricular tachycardia were not different among groups during six minutes of ligation. The other types of arrhythmia and total arrhythmias in EX and EX+FR groups were lower than the control group during ischemia ($P<0.05$). In EX and EX+FR groups, the score of arrhythmia was significantly lower with respect to control group ($P<0.05$) (Table 4.12).

The arrhythmic period, the onset and offset of arrhythmia did not significantly change in control animals fed with normal diet in respect to the exercise and food restriction and from combination group (Figure 4.13). The incidence of arrhythmia and the survival rate of animals were not different among SRF male animals during ischemia (Table 4.13).

Table 4.10. The heart rate (Beat / minute) in male rats fed with standard rat food

Groups	N	Basal	Ligation	Lig 1 st	Lig 3 rd	Lig 5 th	Reperfusion	Rep 1 st	Rep 3 rd	Rep 5 th	Rep 10 th	Rep 15 th
SRF Male Control (a)	4-7	343±17	343±16	350±12	338±25	328±26	346±8	363±17	360±11	364±14	357±18	339±13
SRF Male FR (b)	6-8	267±19 ^a	288±16	279±26 ^a	283±28	250±28	250±24 ^a	241±34 ^a	244±33 ^a	259±18 ^a	230±17 ^a	204±17 ^a
SRF Male EX (c)	6-10	328±16	350±16	342±17	323±24	316±23	318±25	315±28	331±35	292±23 ^a	284±20 ^a	276±16 ^a
SRF Male EX+FR (d)	7	255±24 ^{ac}	270±44 ^c	228±25 ^{ac}	216±27 ^{ac}	192±19 ^{ac}	209±25 ^{ac}	198±22 ^{ac}	193±24 ^{ac}	198±23 ^{ac}	179±19 ^{ac}	181±22 ^{ac}

N: Number of animals (HR was not determined following minutes of I/R because of ventricular arrhythmia in some rats), SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, Basal: Before coronary ligation, Lig 1st, Lig 3rd, Lig 5th: 1st, 3rd, 5th minutes after the coronary ligation. Rep 1st, Rep 3rd, Rep 5th, Rep 10th, Rep 15th: 1st, 3rd, 5th, 10th, 15th minutes after the coronary reperfusion. ^aP < 0.05: Compared to SRF male control, ^cP < 0.05: Compared to SRF male EX. Results: mean ± SE.

Table 4.11. The arterial blood pressure (mmHg) in male rats fed with standard rat food

Groups	N	Basal	Ligation	Lig 1 st	Lig 3 rd	Lig 5 th	Reperfusion	Rep 1 st	Rep 3 rd	Rep 5 th	Rep 10 th	Rep 15 th
SRF Male Control (a)	4-7	113±12	82±8	77±8	71±11	79±11	94±13	81±6	92±11	89±10	78±9	82±8
SRF Male FR (b)	6-8	100±7	77±5	70±6	70±9	63±8	64±7 ^a	79±11	70±8	77±12	71±9	64±8
SRF Male EX (c)	6-10	124±5	103±4 ^a	93±7	90±8	88±9	77±6	87±9	94±6	81±7	86±7	83±4
SRF Male EX+FR (d)	7	105±6	89±4	71±4 ^c	67±6	65±5	70±6	73±8	70±7	74±7	65±7	64±7

N: Number of animals, SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, Basal: Before coronary ligation, Lig 1st, Lig 3rd, Lig 5th: 1st, 3rd, 5th minutes after the coronary ligation. Rep 1st, Rep 3rd, Rep 5th, Rep 10th, Rep 15th: 1st, 3rd, 5th, 10th, 15th minutes after the coronary reperfusion. ^aP < 0.05: Compared to SRF male control, Results: mean ± SE.

Table 4.12. The score and the duration of arrhythmias during six minutes of ischemia in male rats fed with standard rat food

Groups	N	The duration of arrhythmia (second)					n	Score of arrhythmia
		VF	VT	Other	Total	Bradycardia		
SRF Male Control (a)	7	0	0	20.20±11.6	20.20±11.6	0	8	2±0.7
SRF Male FR (b)	8	0	0.1±0.1	7.88±3.1	7.98±3.1	17.5±17.7	8	1.1±0.3
SRF Male EX (c)	10	0	0.09±0.09	3.80±2.3 ^a	3.89±2.4 ^a	0	10	0.7±0.2 ^a
SRF Male EX+FR (d)	7	0	0.79±0.79	1.92±0.6 ^a	2.71±1.1 ^a	0	7	0.7±0.2 ^a

N: The number of animals, SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.), ^aP < 0.05: Compared to SRF male control, Results: mean ± SE.

Table 4.13. The survival rate of animals and the incidence of arrhythmias during six minutes of coronary ligation in male rats fed with standard rat food

Groups	N	Survival (n / %)	Incidence of arrhythmia (n / %)			
			VF	VT	Other	Bradycardia
SRF Male Control (a)	8	7/88	0/0	0/0	5/63	0/0
SRF Male FR (b)	8	8/100	0/0	1/13	6/75	1/13
SRF Male EX (c)	10	10/100	0/0	1/10	6/60	0/0
SRF Male EX+FR (d)	7	7/100	0/0	1/13	5/71	0/0

N: The number of animals, SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.).

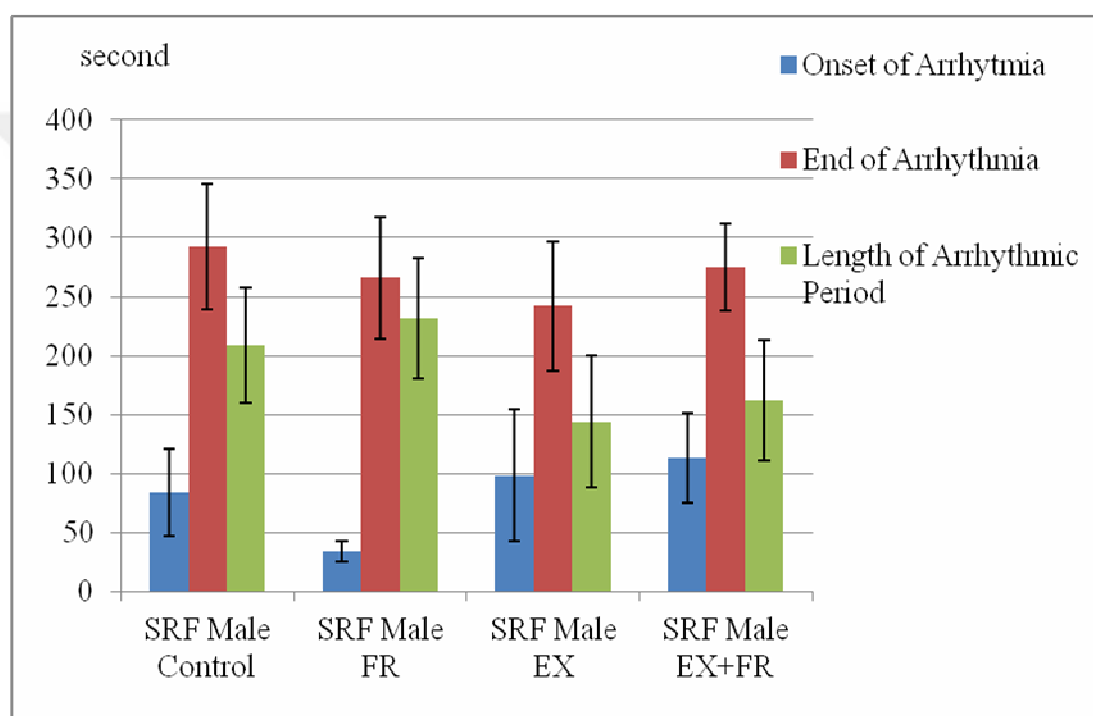


Figure 4.13. The onset and the end of arrhythmias and length of arrhythmic period during ligation in male rats fed with standard rat food

SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction.

4.1.2.3 The duration of arrhythmias, the score of arrhythmia, the onset and the end of arrhythmias, the arrhythmic period, the incidence of arrhythmias and the survival rate of animals during fifteen minutes of reperfusion in male rats fed with standard rat food

The duration and score of arrhythmias were not different among groups during reperfusion (Table 4.14). The onset of arrhythmia, the end of arrhythmia and

the length of arrhythmic period were not different among groups during reperfusion (Figure 4.14).

The survival rate of animals at the end of reperfusion was not different among groups. The incidence of VT in FR group was higher than the control and EX+FR groups ($P<0.05$). The incidence of VF, other types of arrhythmias and the bradycardia were not different among groups during fifteen minutes of reperfusion (Table 4.15).

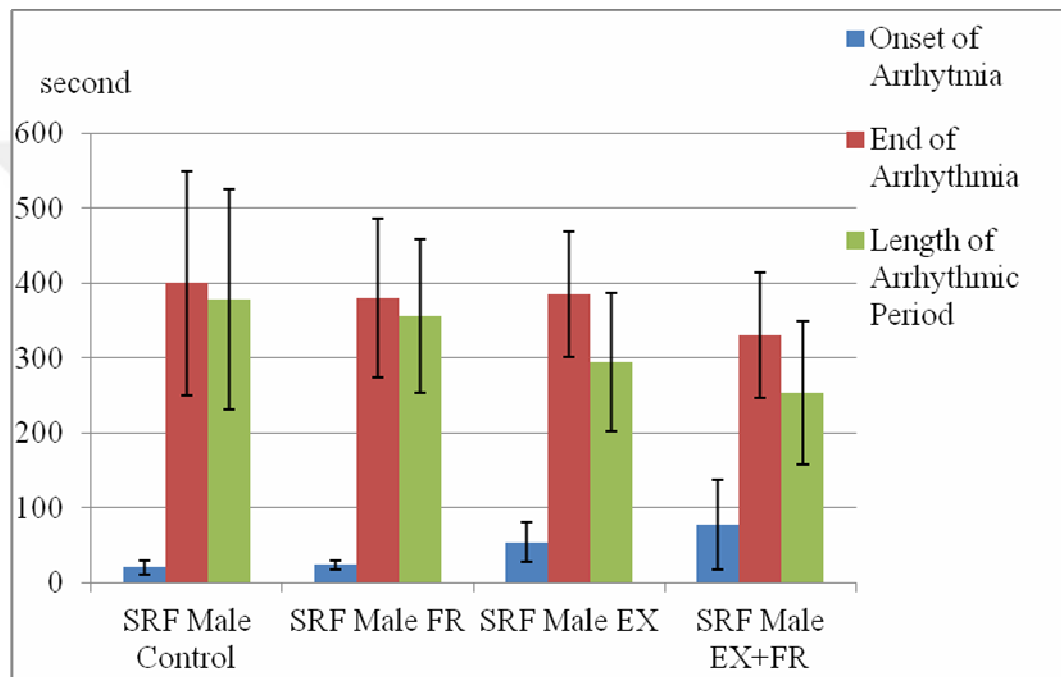


Figure 4.14. The onset and the end of arrhythmias and the duration of arrhythmic period during reperfusion in male rats fed with standard rat food

SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction.

Table 4.14. The score and the duration of arrhythmias during fifteen minutes of reperfusion in male rats fed with standard rat food

Groups	N	The duration of arrhythmia (second)					n	Score of arrhythmia
		VF	VT	Other	Total	Bradycardia		
SRF Male Control (a)	7	9.6±9.6	4±4	25.82±11.8	39.40±19.8	20.2±13.4	7	2.9±0.7
SRF Male FR (b)	8	4.1±4.1	16.2±7.1	32.60±12.8	52.9±16.2	24.4±24.4	8	2.8±0.4
SRF Male EX (c)	9	1.9±1.2	13.8±6.3	24.50±11.6	40.1±15.3	0±0	10	2.6±0.6
SRF Male EX+FR (d)	7	0±0	0.3±0.3	6.67±2.3	6.9±2.5 ^b	0±0	8	1.8±0.6

N: The number of animals, SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.), ^bP < 0.05: Compared to SRF male FR.

Table 4.15. The survival rate of animals and the incidence of arrhythmias during fifteen minutes of coronary reperfusion in male rats fed with standard rat food

Groups	N	Survival (n / %)	Incidence of arrhythmia (n / %)			
			VF	VT	Other	Bradycardia
SRF Male Control (a)	7	6/86	2/29	1/14	6/86	2/29
SRF Male FR (b)	8	8/100	1/13	6/75 ^a	8/100	1/13
SRF Male EX (c)	10	9/90	2/20	4/40	8/80	0/0
SRF Male EX+FR (d)	8	7/88	1/13	1/13 ^b	6/75	0/0

N: The number of animals, SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.), ^aP < 0.05: Compared to SRF male control, ^bP < 0.05: Compared to SRF male FR.

4.1.2.4 Body weights, the percentage of risk of infarct zone and the ventricular weights in male rats fed with standard rat food

The body weights of animals before six weeks exercise and food restriction were not different among male rats that fed with containing normal protein ratio. The body weights of animals after six weeks applications in food restricted animals were lower than the control group (P<0.05). The percentage of risk of infarct zone in exercise group was significantly lower than the control group (P<0.05). The total ventricular weight in exercise group was higher than the control and EX+FR group (P<0.05). The total weight of ventricle in FR group was lower than the control group (P<0.05) (Table 4.16).

Table 4.16. Body weights, percentage of risk of infarct zone and ventricular weights in male rats fed with standard rat food

Groups	N	Body weight BA (g)	Body weight AA (g)	Risk of infarct zone %	N	Ventricular weight (g)
SRF Male Control (a)	8	397±16	396±18	51±1	8	1.1±0.05
SRF Male FR (b)	8	433±24	312±16 ^a	54±3	8	0.9±0.07 ^a
SRF Male EX (c)	10	445±16	434±23	44±3 ^a	9	1.4±0.07 ^a
SRF Male EX+FR (d)	8	412±24	300±18 ^{ac}	46±1	8	1±0.06 ^c

N: The number of animals, SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, BA: Before application, AA: After application (Application: swimming exercise, food restriction and high protein diet). ^aP < 0.05: Compared to SRF male control, ^cP < 0.05: Compared to SRF male EX.

4.1.2.5 The hematological results: leukocytes, erythrocytes and hemoglobin in male rats fed with standard rat food

The number of leukocytes in EX+FR group was lower than the control group ($P<0.05$). The amount of hemoglobin and erythrocytes were not different among groups ($P<0.05$) (Table 4.17).

Table 4.17. The hematological results in male rats fed with standard rat food

Groups	N	Leukocytes	Erythrocytes	Hemoglobin
SRF Male Control (a)	4	6.47±1	7.52±0.2	13.72±0.3
SRF Male FR (b)	6	5.28±0.7	7.97±0.2	14.4±0.2
SRF Male EX (c)	5	4.74±0.7	7.59±0.4	14.28±0.4
SRF Male EX+FR (d)	4	3.03±0.4 ^a	7.94±1	13.9±1.3

N: The number of animals, SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, ^a $P < 0.05$: Compared to SRF male control.

4.1.2.6 The biochemical results; Glucose; HDL, Triglycerides, Total Cholesterol; Creatinine, BUN, Urea, Albumin, AST, ALT, LDH, CK-MB; SOD in male rats fed with standard rat food

The amount of glucose and triglycerides in FR group was lower and the amount of HDL cholesterol was higher than the control group ($P<0.05$). The amount of HDL cholesterol in EX+FR was higher than the control and EX groups ($P<0.05$). The amount of creatinine in EX+FR group was lower than the control group ($P<0.05$). The amount of albumin in EX+FR group was significantly higher than the other groups ($P<0.05$) (Table 4.18). The amount of SOD in EX and EX+FR group was significantly higher than the control group ($P<0.05$) (Figure 4.15).

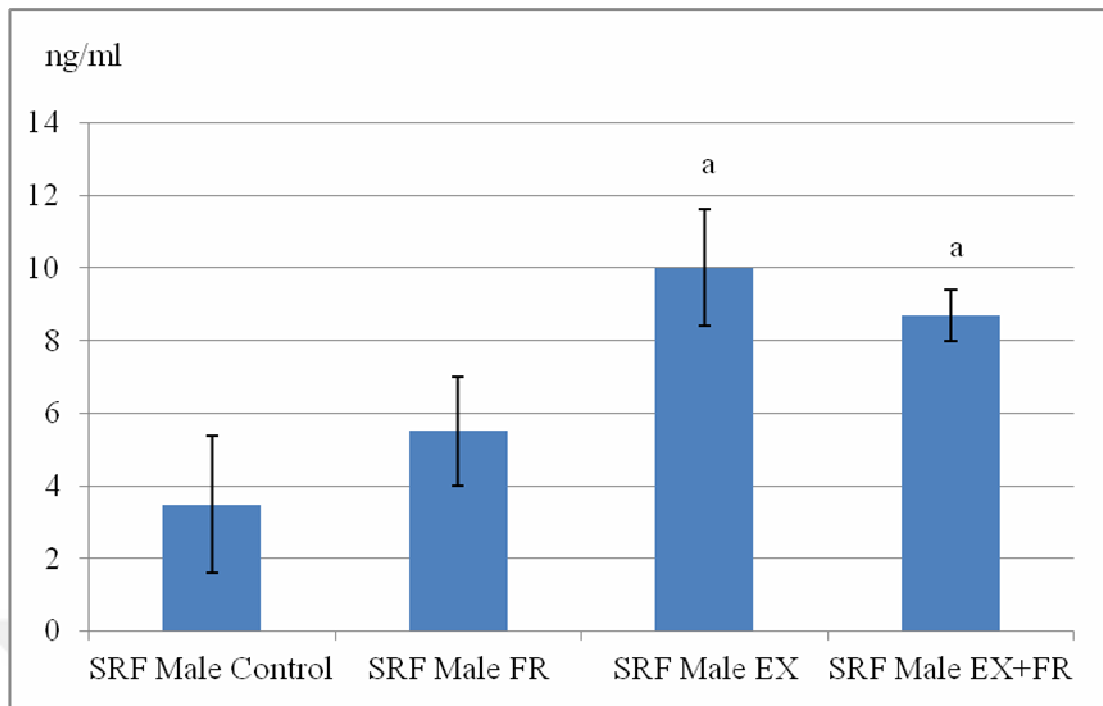


Figure 4.15. The amount of SOD in male rats fed with standard rat food

SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, ^aP<0.05: Compared to control.

4.1.2.7 The results of immunohistochemical analysis in male rats fed with standard rat food

The density of immunostaining of SUR2A in myocardial cell was not different among groups (Figure 4.16).

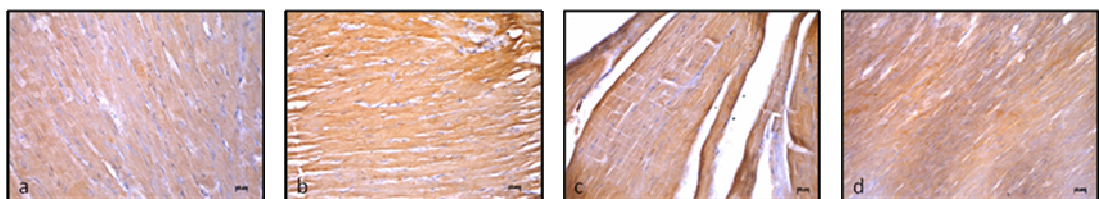


Figure 4.16. The results of immunohistochemical analysis in male rats fed with standard rat food

a: SRF male control, b: SRF male FR, c: SRF male EX, d: SRF male EX+FR.

Table 4.18. The biochemical results in male rats fed with standard rat food

Groups	N	Glucose (mg/dl)	HDL (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Creatinine (mg/dl)	BUN (mg/dl)	AST (U/L)	ALT (U/L)	LDH (U/L)	CK_MB (U/L)	Albumin (g/dl)	Urea (mg/dl)
SRF Male Control (a)	6	300±20	19.8±1	56±9	57±5	0.55±0.04	18±1	214±48	83±7	821±124	435±58	2.7±0.2	39±1
SRF Male FR (b)	5	174±33 ^a	26.2±3 ^a	30±4 ^a	69±10	0.48±0.01	22±2	132±18	70±3	492±91	1327±1069	2.7±0.2	47±4
SRF Male EX (c)	6	331±57	22.1±1	52±10	55±4	0.54±0.03	19±1	268±89	85±16	894±144	442±40	2.7±0.2	41±2
SRF Male EX+FR (d)	4	228±35	29.6±3 ^{ac}	40±7	73±8	0.45±0.02 ^a	23±4	181±84	85±25	811±255	577±182	3.3±0.1 ^{abc}	49±9

SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, N: The number of animals. HDL: High density lipoprotein cholesterol, BUN: Blood urea nitrogen, AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase, CK-MB: Creatine Kinase-MB, ^aP < 0.05: Compared to SRF male control, ^bP < 0.05: Compared to SRF male FR, ^cP < 0.05: Compared to SRF male EX. Results: mean ± SE.

4.1.3 Female Rats Fed With Standart Pellet Rat Food (SRF Female Groups)

In this section, the comparison of all parameters were made between control, exercise, food restriction and exercise + food restriction groups in female rats fed with standard rat food.

4.1.3.1 The heart rate and the blood pressure in female rats fed with standard rat food

Similarly to male, food restriction decreased the heart rate, before and during ligation and during reperfusion as it compared to control rats in female ($P<0.05$). The heart rate in EX+FR group was lower than the control group before and during ligation and reperfusion and it was also lower than the EX group during ligation and reperfusion ($P<0.05$) (Table 4.19).

The blood pressure in EX+FR group was lower than EX group at 1st, 3rd, 5th minutes of ligation and during reperfusion ($P<0.05$). The blood pressure in EX+FR group was lower than FR group at 3rd, 5th, 10th, 15th minutes of reperfusion ($P<0.05$). The blood pressure in EX+FR group was lower than control group at 5th, 10th, 15th minutes of reperfusion ($P<0.05$) (Table 4.20).

Table 4.19. The heart rate (Beat / minute) in female rats fed with standard rat food

Groups	N	Basal	Ligation	Lig 1 st	Lig 3 rd	Lig 5 th	Reperfusion	Rep 1 st	Rep 3 rd	Rep 5 th	Rep 10 th	Rep 15 th
SRF Female Control (a)	7-9	345±10	337±11	337±11	325±10	311±8	307±11	303±11	307±10	305±5	292±9	299±13
SRF Female FR (b)	5-8	271±18 ^a	257±18 ^a	252±20 ^a	237±28 ^a	216±30 ^a	220±31 ^a	236±31	256±28	262±25	254±29	248±29
SRF Female EX (c)	8-11	326±26	343±27	335±27	332±30	320±31	309±32	314±38	318±37	303±29	299±24	301±23
SRF Female EX+FR (d)	6-9	273±16 ^a	255±23 ^{ac}	245±26 ^{ac}	224±26 ^{ac}	203±27 ^{ac}	217±25 ^{ac}	189±28 ^{ac}	211±25 ^{ac}	212±24 ^{ac}	180±18 ^{abc}	185±20 ^{ac}

N: Number of animals, SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, Basal: Before coronary ligation, Lig 1st, Lig 3rd, Lig 5th: 1st, 3rd, 5th minutes after the coronary ligation. Rep 1st, Rep 3rd, Rep 5th, Rep 10th, Rep 15th: 1st, 3rd, 5th, 10th, 15th minutes after the coronary reperfusion. ^aP < 0.05: Compared to SRF female control, ^bP < 0.05: Compared to SRF female FR, ^cP < 0.05: Compared to SRF female EX. Results: mean ± SE.

Table 4.20. The arterial blood pressure (mmHg) in female rats fed with standard rat food

SRF Female Groups	N	Basal	Ligation	Lig 1 st	Lig 3 rd	Lig 5 th	Reperfusion	Rep 1 st	Rep 3 rd	Rep 5 th	Rep 10 th	Rep 15 th
SRF Female Control (a)	7-9	117±5	98±8	88±8	86±7	83±7	80±4	82±8	79±8	80±7	83±6	90±7
SRF Female FR (b)	5-8	126±13	85±5	80±6	79±7	77±9	75±8	78±9	94±6	90±7	88±6	92±7
SRF Female EX (c)	8-11	132±3	99±5	101±5	97±6	95±6	82±7	89±10	88±9	84±7	83±8	82±9
SRF Female EX+FR (d)	6-9	123±3	86±4	80±4 ^c	72±5 ^c	66±6 ^c	68±7	63±6 ^c	62±6 ^{bc}	59±5 ^{abc}	56±4 ^{abc}	57±3 ^{abc}

N: Number of animals, SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, Basal: Before coronary ligation, Lig 1st, Lig 3rd, Lig 5th: 1st, 3rd, 5th minutes after the coronary ligation. Rep 1st, Rep 3rd, Rep 5th, Rep 10th, Rep 15th: 1st, 3rd, 5th, 10th, 15th minutes after the coronary reperfusion. ^aP < 0.05: Compared to SRF female control, ^bP < 0.05: Compared to SRF female FR, ^cP < 0.05: Compared to SRF female EX, Results: mean ± SE.

4.1.3.2 The duration of arrhythmias, the score of arrhythmia, the onset and the end of arrhythmias, the arrhythmic period, the incidence of arrhythmias and the survival rate of animals during six minutes of ischemia in female rats fed with standard rat food

The duration of ventricular fibrillation and ventricular tachycardia were not different among groups during six minutes of ligation in female fed with standard rat pellet food. The other types of arrhythmia and total arrhythmias in EX+FR groups were lower than FR group during ischemia ($P < 0.05$). The score of arrhythmia was not different among groups (Table 4.21).

Table 4.21. The score and the duration of arrhythmias during six minutes of ischemia in female rats fed with standard rat food

Groups	N	The duration of arrhythmia (second)					n	Score of arrhythmia
		VF	VT	Other	Total	Brdc		
SRF Female Control (a)	9	0	0	3.58±1.4	3.58±1.4	0	10	1.4±0.5
SRF Female FR (b)	8	0	0	10.45±6.3	10.45±6.3	0	8	1±0.4
SRF Female EX (c)	11	0	0	1.75±0.6	1.75±0.6	0	11	0.6±0.1
SRF Female EX+FR (d)	9	0	0	1.62±0.7 ^b	1.62±0.7 ^b	0	9	0.6±0.2

N: The number of animals, SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.), Brdc: Bradycardia, ^b $P < 0.05$: Compared to SRF female FR, Results: mean ± SE.

The end of arrhythmia in EX+FR group was later than in control group ($P < 0.05$). The arrhythmic period and the onset of arrhythmias were not different among groups in six minutes of ligation (Figure 4.17). The incidence of arrhythmias and survival rate of animals were not different in animals during ischemia (Table 4.22).

Table 4.22. The survival rate of animals and the incidence of arrhythmias during six minutes of coronary ligation in female rats fed with standart rat food

Groups	N	Survival (n / %)	Incidence of arrhythmia (n / %)			
			VF	VT	Other	Bradycardia
SRF Female Control (a)	10	9/90	1/10	1/10	8/80	0/0
SRF Female FR (b)	8	8/100	0/0	0/0	4/50	0/0
SRF Female EX (c)	11	11/100	0/0	0/0	7/64	0/0
SRF Female EX+FR (d)	9	9/100	0/0	0/0	5/56	0/0

N: The number of animals, SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.).

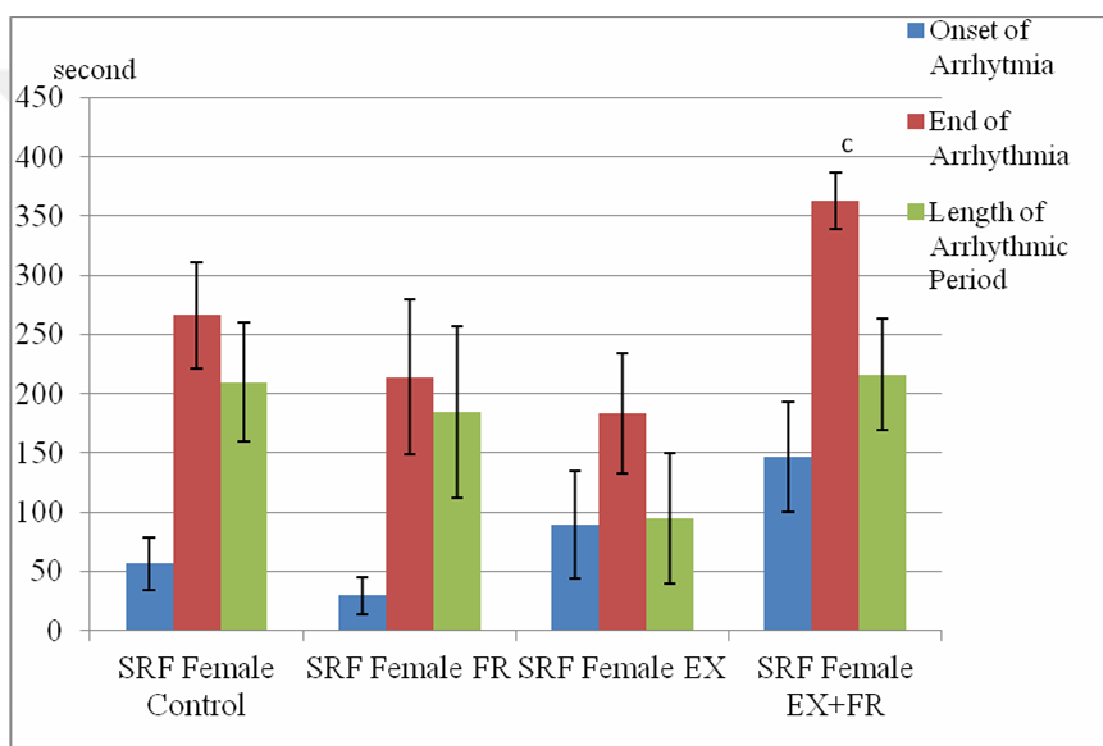


Figure 4.17. The onset and the end of arrhythmias and the length of arrhythmic period during ligation in female rats fed with standart rat food

SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction ^cP < 0.05: Compared to EX.

4.1.3.3 The duration of arrhythmias, the score of arrhythmia, the onset and the end of arrhythmias, the arrhythmic period, the incidence of arrhythmias and the survival rate of animals during fifteen minutes of reperfusion in female rats fed with standard rat food

The duration of VT, VF, bradycardia and total length of arrhythmias were not different among groups during reperfusion. The other types of arrhythmias during reperfusion was higher in EX+FR group according to EX group ($P < 0.05$). The score of arrhythmia was not different among female groups (Table 4.23). The onset and offset of arrhythmia and the length of arrhythmic period were not different among groups during reperfusion (Figure 4.18).

The survival rate of animals at the end of reperfusion was not different among groups. The incidence of VF, VT, Other types of arrhythmias and bradycardia were not different among groups during fifteen minutes of reperfusion (Table 4.24).

Table 4.23. The score and the duration of arrhythmias during fifteen minutes of reperfusion in female rats fed with standard rat food

Groups	N	The duration of arrhythmia (second)					n	Score of arrhythmia
		VF	VT	Other	Total	Brdc		
SRF Female Control	9	5.5±4.3	23.6±13.4	32.9±9.9	62±24.3	25±21.5	9	3.1±0.5
SRF Female FR	8	3.3±3.3	15.1±7.7	24.6±8.7	43±13.2	57.8±57.8	8	2.6±0.5
SRF Female EX (c)	10	3.1±2.9	15.1±7.7	18.7±5.8	37±7.4	0±0	11	2.9±0.4
SRF Female EX+FR	8	0±0	5.4±5	49.7±14.1 ^c	55.2±14.3	18.1±18.1	9	3±0.5

N: The number of animals, SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.), Brdc: Bradycardia, ^cP < 0.05: Compared to SRF female EX, Results: mean ± SE.

Table 4.24. The survival rate of animals and the incidence of arrhythmias during fifteen minutes of coronary reperfusion in female rats fed with standard rat food

Groups	N	Survival (n / %)	Incidence of arrhythmia (n / %)			
			VF	VT	Other	Bradycardia
SRF Female Control (a)	9	9/100	2/22	3/33	9/100	2/22
SRF Female FR (b)	8	8/100	1/13	3/38	8/100	1/13
SRF Female EX (c)	11	10/91	3/27	8/73	10/91	0/0
SRF Female EX+FR (d)	9	8/89	1/11	3/33	8/89	1/11

N: The number of animals, SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.).

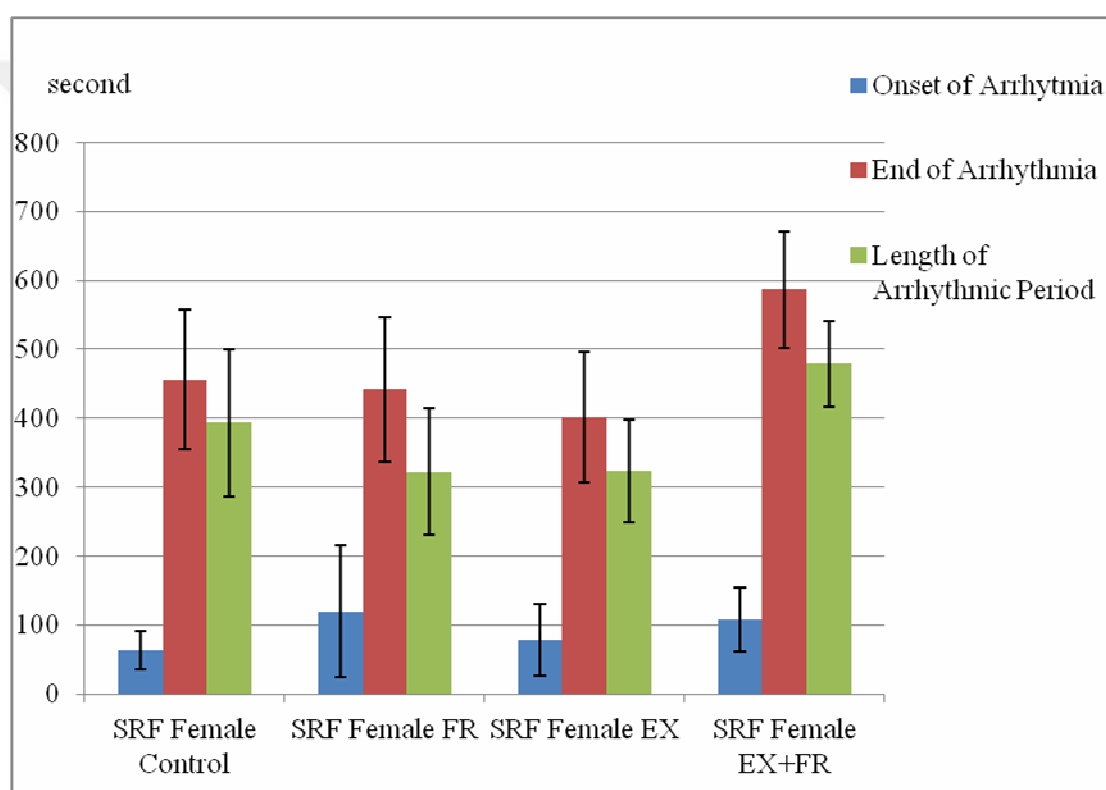


Figure 4.18. The onset and the end of arrhythmias and length of arrhythmic period during reperfusion in female rats fed with standard rat food

SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction.

4.1.3.4 Body weights, the percentage of risk of infarct zone and the ventricular weights in female rats fed with standard rat food

The body weights of animals before six weeks exercise and food restriction were not different among female rats that fed with containing standard rat food having normal protein. The body weights of animals after six weeks applications in food restricted animals were lower than control and exercise groups ($P < 0.05$). The percentage of risk of infarct zone was not different among groups. The total ventricular weight in FR group was lower than control group ($P < 0.05$) (Table 4.25).

Table 4.25. Body weights, percentage of risk of infarct zone and ventricular weights in female rats fed with standard rat food

Groups	N	Body weight BA (g)	Body weight AA (g)	Risk of infarct zone %	Ventricular weight (g)
SRF Female Control (a)	10	268±11	284±11	49±2	0,9±0,04
SRF Female FR (b)	9	277±18	219±12 ^a	53±2	0,7±0,07 ^a
SRF Female EX (c)	11	286±12	294±11	48±2	1±0,03
SRF Female EX+FR (d)	9	272±12	210±8 ^{ac}	49±1	0,8±0,05

N: The number of animals, SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, BA: Before application, AA: After application (Application: swimming exercise, food restriction and high protein diet). ^aP < 0.05: Compared to SRF female control, ^cP < 0.05: Compared to SRF female EX.

4.1.3.5 The hematological results: leukocytes, erythrocytes and hemoglobin in female rats fed with standard rat food

The numbers of leukocytes were not different among groups. The amount of hemoglobin and erythrocytes were lower in EX+FR group according to other groups ($P < 0.05$) (Table 4.26).

Table 4.26. The hematological results in female rats fed with standard rat food

Groups	N	Leukocytes	Erythrocytes	Hemoglobin
SRF Female Control (a)	5	4,64±0,9	6,96±0,2	13,58±0,3
SRF Female FR (b)	6	3,88±0,3	7,63±0,3	14,48±0,2
SRF Female EX (c)	5	4,08±0,9	7,43±0,5	13,64±0,5
SRF Female EX+FR (d)	3	3,01±0,1	3,98±1,7 ^{abc}	9,2±3,7 ^{abc}

N: The number of animals, SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, ^aP < 0.05: Compared to SRF female control, ^bP < 0.05: Compared to SRF female FR, ^cP < 0.05: Compared to SRF female EX.

4.1.3.6 The biochemical results; Glucose; HDL, Triglycerides, Total Cholesterol; Creatinine, BUN, Urea, Albumin, AST, ALT, LDH, CK-MB; SOD in female rats fed with standard rat food

The amount of glucose in FR, EX and EX+FR groups were lower than that in control group ($P<0.05$). The amount of total cholesterol and triglyceride was lower in FR and EX+FR groups when it was compared to control group ($P<0.05$). The amount of creatinine was lower in EX+FR group when compared to other groups ($P<0.05$). The amount of AST was higher in EX and EX+FR groups when it was compared to control group ($P<0.05$). The amount of HDL cholesterol, BUN, ALT, LDH, CK-MB, albumin and urea was not different among groups (Table 4.27). The amount of SOD was also not different among groups (Figure 4.19).

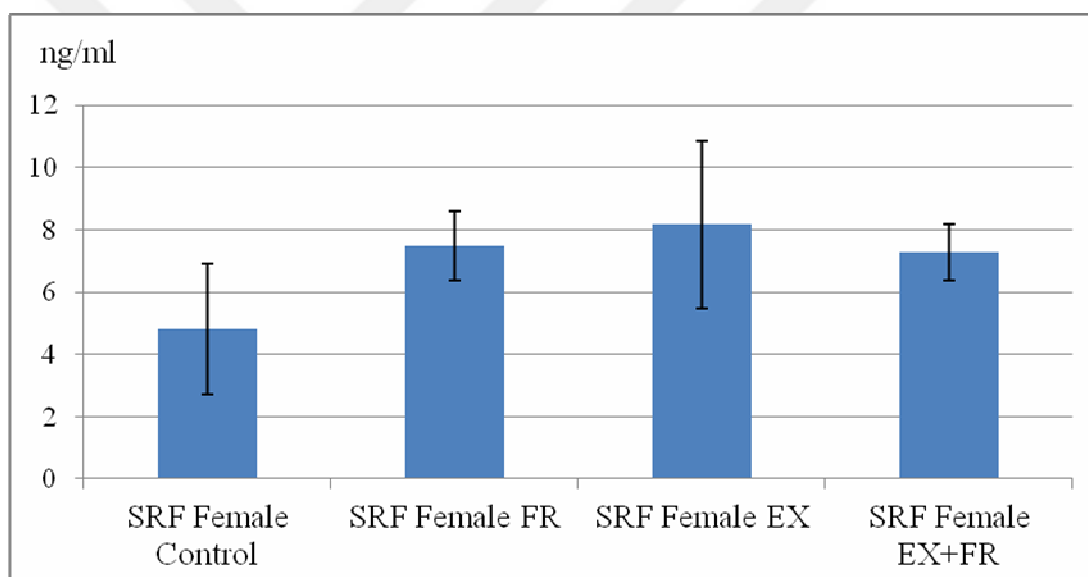


Figure 4.19. The amount of SOD in female rats fed with standard rat food

SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction.

4.1.3.7 The results of immunohistochemical analysis in female rats fed with standard rat food

The density of immunostaining in myocardial cell marked with SUR 2A in EX and EX+FR was higher than the control groups (Figure 4.20).

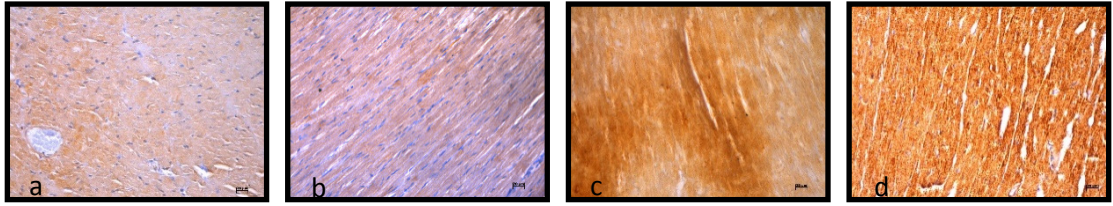


Figure 4.20. The results of immunohistochemical analysis in female rats fed with standart rat food

a: SRF female control, b: SRF female FR, c: SRF female EX, d: SRF female EX+FR.

Table 4.27. The biochemical results in female rats fed with standard rat food

Groups	N	Glucose (mg/dl)	HDL (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Creatinine (mg/dl)	BUN (mg/dl)	AST (U/L)	ALT (U/L)	LDH (U/L)	CK_MB (U/L)	Albumin (g/dl)	Urea (mg/dl)
SRF Female Control (a)	6	264±29	26.5±1.8	56±11	70±7	0.53±0.03	19±3	108±10	56±8	453±184	286.6±82	3.6±0.4	40±5
SRF Female FR (b)	6	137±10 ^a	22.4±1.8	31±6 ^a	49±3 ^a	0.48±0.02	24±3	119±16	54±7	424±55	244.7±42	3±0.2	51±6
SRF Female EX (c)	6	180±17 ^a	24.9±0.9	49±3	65±4	0.53±0.03	20±1	263±67 ^a	77±19	1178±356	761.7±411	3.5±0.1	42±2
SRF Female EX+FR (d)	4	127±34 ^a	22.4±4.7	32±6 ^a	50±6 ^a	0.41±0.02 ^{abc}	30±10	263±70 ^a	86±2	1156±355	706.8±185	3.2±0.3	65±20

SRF: Standard rat food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, N: The number of animals. HDL: High density lipoprotein cholesterol, BUN: Blood urea nitrogen, AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase, CK-MB: Creatine Kinase-MB, ^aP < 0.05: Compared to SRF female control, ^bP < 0.05: Compared to SRF female FR, ^cP < 0.05: Compared to SRF female EX. Results: mean ± SE.

4.1.4 Male Rats Fed With Containing High Protein Special Rat Food Diet (PRF Male Groups)

In this section, the comparison of all parameters were made between control, exercise, food restriction and exercise + food restriction groups in male rats fed with protein rich food.

4.1.4.1 The heart rate and the blood pressure in male rats fed with protein rich food

Food restriction decreased the heart rate at ligation and 1st minute of ligation as it compared to control rats ($P<0.05$). The heart rate in EX group was higher than the control group ($P<0.05$). The heart rate in EX+FR group was lower than the other groups at basal and 1st minutes of ligation and it was lower than the control and EX groups at ligation, 3rd and 5th minutes of ligation and it was lower than the EX group at, 1st, 10th and 15th minutes of reperfusion ($P<0.05$) (Table 4.28).

The blood pressure in FR group was significantly lower than the control group at 1st minutes of ligation ($P<0.05$). The blood pressure in EX group was significantly higher than the control group at basal, 5th minutes of ligation and during reperfusion except 1st minutes of reperfusion ($P<0.05$). The blood pressure in EX+FR group was significantly lower than the EX group during ligation and reperfusion period except 15th minutes of reperfusion ($P<0.05$) (Table 4.29).

Table 4.28. The heart rate (Beat / minute) in male rats fed with protein rich food

Groups	N	Basal	Ligation	Lig 1 st	Lig 3 rd	Lig 5 th	Reperfusion	Rep 1 st	Rep 3 rd	Rep 5 th	Rep 10 th	Rep 15 th
PRF Male Control (a)	5-9	338±17	334±19	335±20	319±18	317±22	274±20	268±27	281±27	277±25	272±25	266±24
PRF Male FR (b)	4-8	302±19	281±16 ^a	288±14 ^a	285±14	269±14	277±15	260±21	282±34	259±16	266±14	261±11
PRF Male EX (c)	3-8	303±16	316±17	319±14	312±15	305±15	304±13	373±42 ^a	323±26	319±17	321±15	305±17
PRF Male EX+FR (d)	3-7	236±17 ^{abc}	265±14 ^{ac}	234±17 ^{abc}	240±15 ^{ac}	233±18 ^{ac}	246±15 ^c	217±22 ^c	208±33	251±28	247±29 ^c	237±15 ^c

N: Number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, Basal: Before coronary ligation, Lig 1st, Lig 3rd, Lig 5th: 1st, 3rd, 5th minutes after the coronary ligation. Rep 1st, Rep 3rd, Rep 5th, Rep 10th, Rep 15th: 1st, 3rd, 5th, 10th, 15th minutes after the coronary reperfusion. ^aP < 0.05: Compared to PRF male control, ^bP < 0.05: Compared to PRF male FR, ^cP < 0.05: Compared to PRF male EX. Results: mean ± SE.

Table 4.29. The arterial blood pressure (mmHg) in male rats fed with protein rich food

Groups	N	Basal	Ligation	Lig 1 st	Lig 3 rd	Lig 5 th	Reperfusion	Rep 1 st	Rep 3 rd	Rep 5 th	Rep 10 th	Rep 15 th
PRF Male Control (a)	5-9	105±4	89±4	81±4	78±5	74±5	67±4	73±5	74±7	71±7	72±7	68±7
PRF Male FR (b)	4-8	105±6	80±3	66±4 ^a	63±5	61±4	61±5	69±12	70±4	72±6	78±7	74±5
PRF Male EX (c)	3-8	120±6 ^a	98±6	91±6	90±6	90±6 ^a	88±6 ^{abd}	94±2	110±15 ^{abd}	110±9 ^{abd}	101±7 ^{abd}	92±8 ^a
PRF Male EX+FR (d)	3-7	109±6	82±4 ^c	68±5 ^c	66±6 ^c	63±6 ^c	57±4	57±7 ^c	61±3	75±11	73±10	73±6

N: Number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, Basal: Before coronary ligation, Lig 1st, Lig 3rd, Lig 5th: 1st, 3rd, 5th minutes after the coronary ligation. Rep 1st, Rep 3rd, Rep 5th, Rep 10th, Rep 15th: 1st, 3rd, 5th, 10th, 15th minutes after the coronary reperfusion. ^aP < 0.05: Compared to PRF male control, ^bP < 0.05: Compared to PRF male FR, ^cP < 0.05: Compared to PRF male EX, ^dP < 0.05: Compared to PRF male EX+FR. Results: mean ± SE.

4.1.4.2 The duration of arrhythmias, the score of arrhythmia, the onset and the end of arrhythmias, the arrhythmic period, the incidence of arrhythmias and the survival rate of animals during six minutes of ischemia in male rats fed with protein rich food

The duration of arrhythmias and the score of arrhythmias were not different among groups during six minutes of ligation ($P<0.05$) (Table 4.30). The onset of arrhythmia was not different among groups ($P<0.05$). The end of arrhythmia in EX+FR group was lower than FR group ($P<0.05$). The length of arrhythmic period was higher in FR group as it compared to the control and the EX+FR group ($P<0.05$) (Figure 4.21). The incidence of arrhythmias and the survival rate of animals were not different among groups during ischemia (Table 4.31).

Table 4.30. The score and the duration of arrhythmias during six minutes of ischemia in male rats fed with protein rich food

Groups	N	The duration of arrhythmia (second)					n	Score of arrhythmia
		VF	VT	Other	Total	Bradycardia		
PRF Male Control (a)	9	0	0	2.38±1.5	2.38±1.5	0	9	0.7±0.2
PRF Male FR (b)	8	0	0	15.31±10.9	15.31±10.9	1.15±1.1	8	1.4±0.4
PRF Male EX (c)	8	0	0	6.08±3.1	6.08±3.1	0	8	1±0.3
PRF Male EX+FR (d)	7	0	0	3±1.3	3±1.3	0	8	1.8±0.6

N: The number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.), Results: mean ± SE.

Table 4.31. The survival rate of animals and the incidence of arrhythmias during six minutes of coronary ligation in male rats fed with protein rich food

Groups	N	Survival (n / %)	Incidence of arrhythmia (n / %)			
			VF	VT	Other	Bradycardia
PRF Male Control (a)	9	9/100	0/0	0/0	5/56	0/0
PRF Male FR (b)	8	8/100	0/0	0/0	7/88	1/13
PRF Male EX (c)	8	8/100	0/0	0/0	6/75	0/0
PRF Male EX+FR (d)	8	7/88	1/13	1/13	8/100	0/0

N: The number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.).

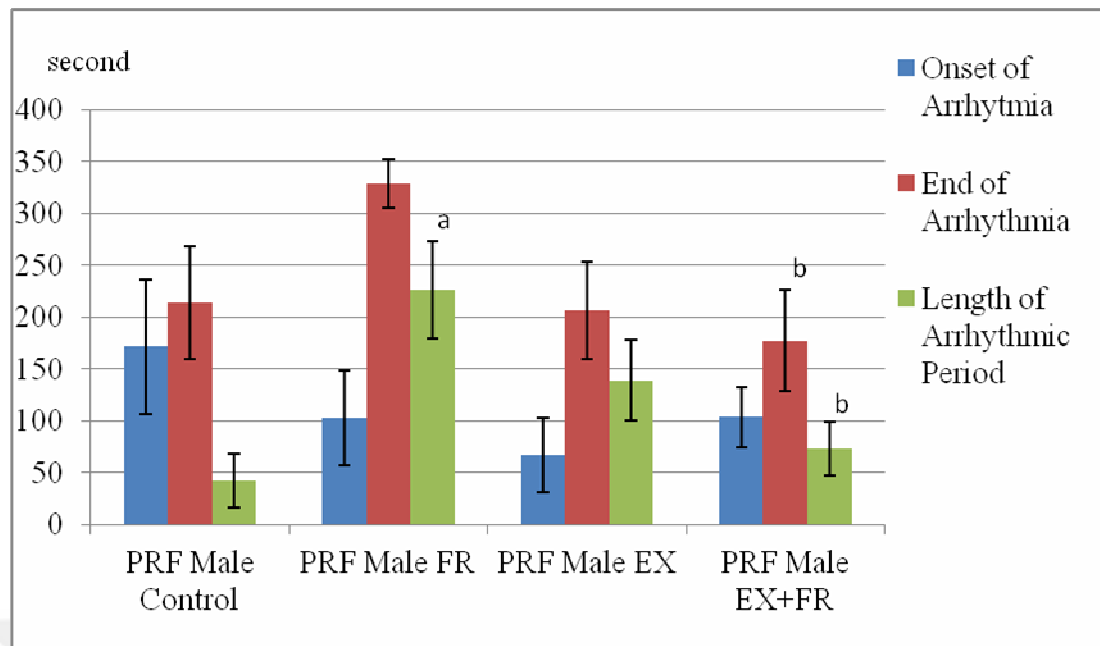


Figure 4.21. The onset and the end of arrhythmias and the length of arrhythmic period during ligation in male rats fed with protein rich food

PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, ^aP < 0.05: Compared to control, ^bP < 0.05: Compared to FR.

4.1.4.3 The duration of arrhythmias, the score of arrhythmia, the onset and the end of arrhythmias, the arrhythmic period, the incidence of arrhythmias and the survival rate of animals during fifteen minutes of reperfusion in male rats fed with protein rich food

The duration of VF in the EX group was significantly higher than the other groups ($P < 0.05$). The duration of VT and total arrhythmias and the score of arrhythmias were significantly higher than the control group ($P < 0.05$) (Table 4.32). The onset of arrhythmia and the length of arrhythmic period were not different among groups during reperfusion. The offset of arrhythmia in EX+FR group was longer than the control ($P < 0.05$) (Figure 4.22). The incidence of VF in EX group was significantly higher than the control group ($P < 0.05$). The incidence of VT, other types of arrhythmia and bradycardia and the survival rate of animals were not different among groups (Table 4.33).

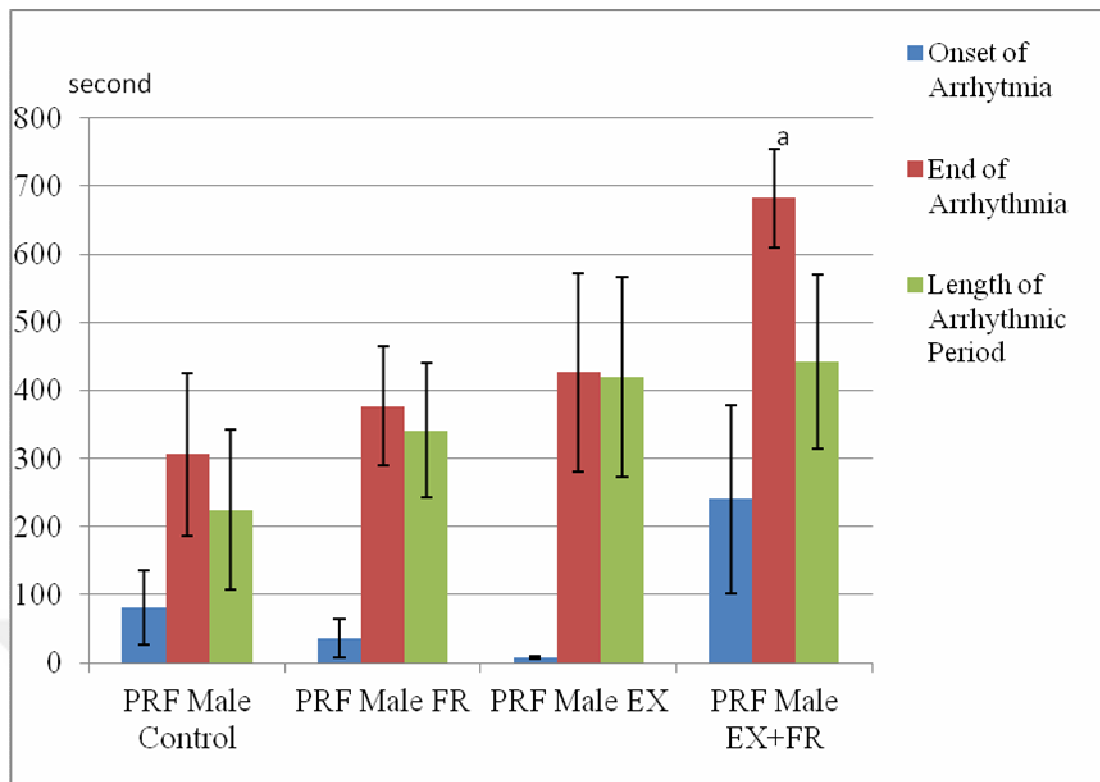


Figure 4.22. The onset and the end of arrhythmias and length of arrhythmic period during reperfusion in male rats fed with protein rich food

PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, ^aP < 0.05: Compared to control.

Table 4.32. The score and the duration of arrhythmias during fifteen minutes of reperfusion in male rats fed with protein rich food

Groups	N	The duration of arrhythmia (second)					n	Score of arrhythmia
		VF	VT	Other	Total	Bradycardia		
PRF Male Control (a)	7	0±0	12.2±7	26.1±16.8	38.3±21.4	0±0	9	2.9±0.7
PRF Male FR (b)	7	3.1±2.1	22.6±13.1	36.1±11.8	61.8±18.6	85.8±61	8	3.4±0.6
PRF Male EX (c)	6	50.8±14.4 ^{abd}	59±20.8 ^a	35±14.2	144.8±42.5 ^a	0±0	8	4.8±0.6 ^a
PRF Male EX+FR (d)	7	7.4±6.9	34.9±14.5	62.9±23.4	105.3±28.9	13.1±13.1	7	3.4±0.6

N: The number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.), ^aP < 0.05: Compared to PRF male control, ^bP < 0.05: Compared to PRF male FR, ^dP < 0.05: Compared to PRF male EX+FR.

Table 4.33. The survival rate of animals and the incidence of arrhythmias during fifteen minutes of coronary reperfusion in male rats fed with protein rich food

Groups	N	Survival (n / %)	Incidence of arrhythmia (n / %)			
			VF	VT	Other	Bradycardia
PRF Male Control (a)	9	7/78	2/22	5/56	9/100	0/0
PRF Male FR (b)	8	7/88	3/38	5/63	8/100	2/25
PRF Male EX (c)	8	6/75	7/88 ^a	7/88	8/100	0/0
PRF Male EX+FR (d)	7	7/100	2/29	5/71	7/100	1/14

N: The number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.), ^aP < 0.05: Compared to SRF male control.

4.1.4.4 Body weights, the percentage of risk of infarct zone and the ventricular weights in male rats fed with protein rich food

The body weights of animals before six weeks exercise and food restriction were not different among male rats that fed with containing high protein diet. The body weights of animals after six weeks applications in food restricted animals were lower than the control and exercise groups (P<0.05). The percentage of risk of infarct zone was not different among groups. The total weight of ventricle in FR and EX+FR group was lower than the other groups (P<0.05) (Table 4.34).

Table 4.34. Body weights, percentage of risk of infarct zone and ventricular weights in male rats fed with protein rich food

Groups	N	Body weight BA (g)	Body weight AA (g)	Risk of infarct zone %	N	Ventricular weight (g)
PRF Male Control (a)	9	366±16	378±17	47±1,4	8	1.1±0.06
PRF Male FR (b)	8	333±11	270±10 ^a	49±1,5	8	0.8±0.06 ^{acd}
PRF Male EX (c)	8	370±12	380±13	49±1,5	8	1.2±0.04
PRF Male EX+FR (d)	8	360±18	266±6 ^{ac}	50±2,2	8	0.9±0.04 ^{abc}

N: The number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, BA: Before application, AA: After application (Application: swimming exercise, food restriction and high protein diet). ^aP < 0.05: Compared to PRF male control, ^bP < 0.05: Compared to PRF male FR, ^cP < 0.05: Compared to PRF male EX, ^dP < 0.05: Compared to PRF male EX+FR.

4.1.4.5 The hematological results: leukocytes, erythrocytes and hemoglobin in male rats fed with protein rich food

The number of leukocytes and erythrocytes and the amount of hemoglobin were not different among male rats that fed with protein rich food (Table 4.35).

Table 4.35. The hematological results in male rats fed with protein rich food

Groups	N	Leukocytes	Erythrocytes	Hemoglobin
PRF Male Control (a)	4	10.97±2.5	7.21±0.5	13.43±0.6
PRF Male FR (b)	4	7.77±0.9	7.14±1.7	12.65±3
PRF Male EX (c)	3	5.89±1.7	7.8±0.2	14.83±0.2
PRF Male EX+FR (d)	4	7.05±1	8.18±0.1	15.17±0.3

N: The number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction.

4.1.4.6 The biochemical results; Glucose; HDL, Triglycerides, Total Cholesterol; Creatinine, BUN, Urea, Albumin, AST, ALT, LDH, CK-MB; SOD in male rats fed with protein rich food

The amount of glucose in EX group was higher than the other groups ($P<0.05$). The amount of HDL cholesterol in EX+FR group was higher than EX group ($P<0.05$). The amount of total cholesterol in EX group was lower than in the other groups ($P<0.05$). The amount of albumin in EX+FR group was significantly higher than the control and FR groups ($P<0.05$). The amount of albumin in EX group was significantly higher than in control group ($P<0.05$) (Table 4.36). The amount of SOD was not different among groups (Figure 4.23).

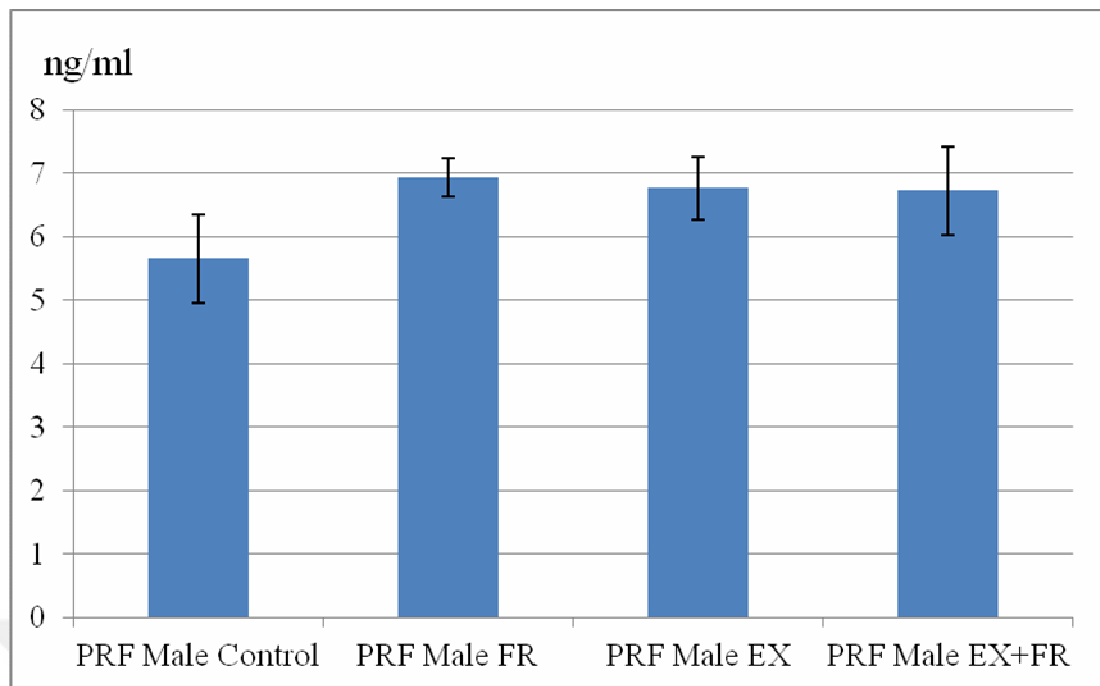


Figure 4.23. The amount of SOD in male rats fed with protein rich food

PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction.

4.1.4.7 The results of immunohistochemical analysis in male rats fed with protein rich food

The density of immunostaining of SUR2A in myocardial cell was significantly higher in EX and EX+FR groups than the control group (Figure 4.24).

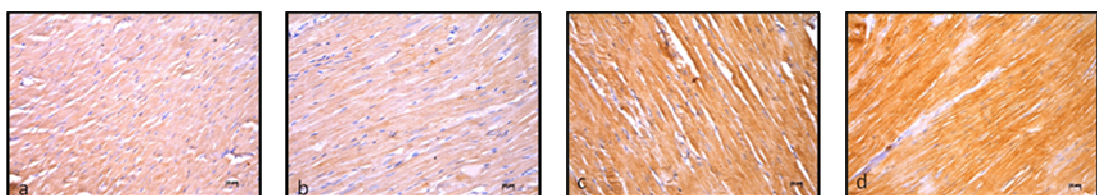


Figure 4.24. The results of immunohistochemical analysis in male rats fed with protein rich food

a: PRF male control, b: PRF male FR, c: PRF male EX, d: PRF male EX+FR.

Table 4.36. The biochemical results in male rats fed with protein rich food

Groups	N	Glucose (mg/dl)	HDL (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Creatinine (mg/dl)	BUN (mg/dl)	AST (U/L)	ALT (U/L)	LDH (U/L)	CK_MB (U/L)	Albumin (g/dl)	Urea (mg/dl)
PRF Male Control (a)	4	175±20	22.7±1.3	37±7	60±4	0.52±0.04	30±6	170±44	68±3	1390±365	704±217	2.1±0.1	63±12
PRF Male FR (b)	4	138±22	22.2±3.6	50±14	61±5	0.45±0.04	34±10	231±95	105±39	821±120	458±59	2.2±0.2	74±21
PRF Male EX (c)	4	273±17 ^{abd}	18.3±1.4	38±9	48±3 ^{abd}	0.52±0.02	22±1	286±79	82±16	1473±363	906±291	2.6±0.1 ^a	46±1
PRF Male EX+FR (d)	4	163±10	26.9±1.2 ^c	38±4	64±2	0.45±0.01	24±2	119±21	61±7	951±251	367±98	2.8±0.1 ^{ab}	52±5

PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, N: The number of animals. HDL: High density lipoprotein cholesterol, BUN: Blood urea nitrogen, AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase, CK-MB: Creatine Kinase-MB, ^aP < 0.05: Compared to PRF male control, ^bP < 0.05: Compared to PRF male FR, ^cP < 0.05: Compared to PRF male EX, ^dP < 0.05: Compared to PRF male EX+FR, Results: mean ± SE.

4.1.5 Female Rats Fed With Containing High Protein Diet (PRF Female Groups)

In this section, the comparison of all parameters were made between control, exercise, food restriction and exercise + 50% food restriction groups in female rats fed with protein rich food.

4.1.5.1 The heart rate and the blood pressure in female rats fed with protein rich food

The heart rate in FR and EX+FR groups was significantly lower than the control group before ligation, during ligation and reperfusion ($P<0.05$). The heart rate before ligation, at 1st, 3rd and 5th minutes of ligation and 5th, 10th, 15th minutes of reperfusion in EX group was lower than the control group ($P<0.05$) (Table 4.37).

The blood pressure in FR group was significantly lower than in the control group at basal to 3rd minutes of reperfusion ($P<0.05$). The blood pressure in EX+FR group was lower than the control group ($P<0.05$) and it was higher than the FR group at ligation, at 1st minute of ligation, and at 1st and 5th minutes of reperfusion ($P<0.05$) (Table 4.38).

4.1.5.2 The duration of arrhythmias, the score of arrhythmia, the onset and the end of arrhythmias, the arrhythmic period, the incidence of arrhythmias and the survival rate of animals during six minutes of ischemia in female rats fed with protein rich food

The duration of VF, VT and bradycardia were not different among groups. The duration of the other types of arrhythmia, total length of arrhythmia in the EX+FR group were significantly higher than the other groups ($P<0.05$). The score of arrhythmia in EX+FR group was significantly higher than the other groups ($P<0.05$) (Table 4.39).

Table 4.37. The heart rate (Beat / minute) in female rats fed with protein rich food

Groups	N	Basal	Ligation	Lig 1 st	Lig 3 rd	Lig 5 th	Reperfusion	Rep 1 st	Rep 3 rd	Rep 5 th	Rep 10 th	Rep 15 th
PRF Female Control (a)	6-9	343±18	345±20	357±19	355±17	346±20	329±23	338±20	338±18	344±14	330±17	336±16
PRF Female FR (b)	5-8	262±17 ^a	268±18 ^a	269±26 ^a	269±24 ^a	247±19 ^a	236±26 ^a	245±17 ^a	228±27 ^a	225±25 ^a	214±18 ^a	226±14 ^a
PRF Female EX (c)	6-10	287±18 ^a	304±14	300±16 ^a	301±15 ^a	297±15 ^a	291±18	299±25	287±23	280±18 ^a	266±20 ^a	274±20 ^a
PRF Female EX+FR (d)	5-9	260±16 ^a	272±14 ^a	277±13 ^a	278±11 ^a	271±12 ^a	241±18 ^a	240±19 ^a	252±18 ^a	253±16 ^a	244±16 ^a	236±16 ^a

N: Number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, Basal: Before coronary ligation, Lig 1st, Lig 3rd, Lig 5th: 1st, 3rd, 5th minutes after the coronary ligation. Rep 1st, Rep 3rd, Rep 5th, Rep 10th, Rep 15th: 1st, 3rd, 5th, 10th, 15th minutes after the coronary reperfusion. ^aP < 0.05: Compared to PRF female control. Results: mean ± SE.

Table 4.38. The arterial blood pressure (mmHg) in female rats fed with protein rich food

Groups	N	Basal	Ligation	Lig 1 st	Lig 3 rd	Lig 5 th	Reperfusion	Rep 1 st	Rep 3 rd	Rep 5 th	Rep 10 th	Rep 15 th
PRF Female Control (a)	6-9	131±4	106±8	98±8	94±10	96±9	96±9	83±6	88±6	80±5	80±7	83±9
PRF Female FR (b)	5-8	112±5 ^a	77±7 ^a	65±5 ^a	65±5 ^a	67±6 ^a	59±5 ^a	61±6 ^a	60±7 ^a	60±7	59±6	60±7
PRF Female EX (c)	6-10	121±5	95±5	90±6	88±6	87±6	81±6	101±8	93±9	90±9	88±9	91±7
PRF Female EX+FR (d)	5-9	117±5 ^a	97±7 ^b	87±7 ^b	85±8	84±9	78±11	88±9 ^b	81±7	91±10 ^b	83±10	82±10

N: Number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, Basal: Before coronary ligation, Lig 1st, Lig 3rd, Lig 5th: 1st, 3rd, 5th minutes after the coronary ligation. Rep 1st, Rep 3rd, Rep 5th, Rep 10th, Rep 15th: 1st, 3rd, 5th, 10th, 15th minutes after the coronary reperfusion. ^aP < 0.05: Compared to PRF female control, ^bP < 0.05: Compared to PRF female FR. Results: mean ± SE.

Table 4.39. The score and the duration of arrhythmias during six minutes of ischemia in female rats fed with protein rich food

Groups	N	The duration of arrhythmia (second)					n	Score of arrhythmia
		VF	VT	Other	Total	Bradycardia		
PRF Female Control (a)	9	0	0	1.48±0.8	1.48±0.8	0	9	0.7±0.2
PRF Female FR (b)	8	0	0	0.60±0.3	0.60±0.3	0	8	0.4±0.2
PRF Female EX (c)	10	0	0	1.11±0.7	1.11±0.7	0	10	0.6±0.2
PRF Female EX+FR (d)	9	0	0	7.66±2 ^{abc}	7.66±2 ^{abc}	0	9	1.2±0.2 ^{abc}

N: The number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.), ^aP < 0.05: Compared to PRF female control, ^bP < 0.05: Compared to PRF female FR, ^cP < 0.05: Compared to PRF female EX. Results: mean ± SE.

The onset and offset of arrhythmia were not different among groups (P<0.05). The length of arrhythmic period in EX+FR group was significantly higher than in the control group (P<0.05) (Figure 4.25).

The incidence of arrhythmia and the survival rate of animals were not different among groups during ischemia (Table 4.40).

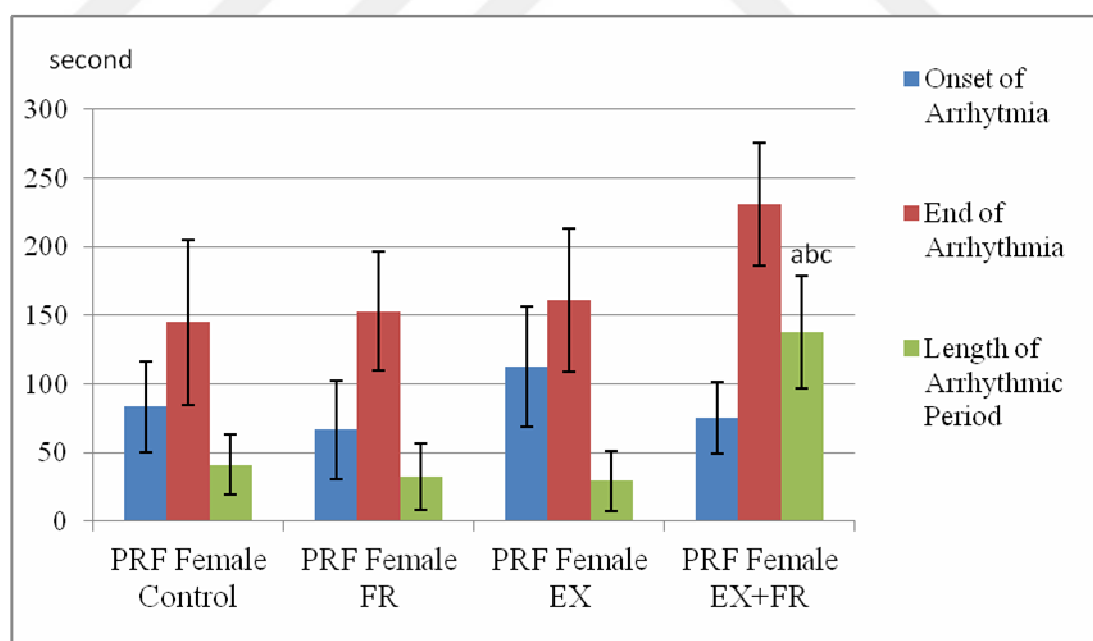


Figure 4.25. The onset and the end of arrhythmias and the length of arrhythmic period during ligation in female rats fed with protein rich food

PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, ^aP < 0.05: Compared to control, ^bP < 0.05: Compared to FR, ^cP < 0.05: Compared to EX.

Table 4.40. The survival rate of animals and the incidence of arrhythmias during six minutes of coronary ligation in female rats fed with protein rich food

Groups	N	Survival (n / %)	Incidence of arrhythmia (n / %)			
			VF	VT	Other	Bradycardia
PRF Female Control (a)	9	9/100	0/0	0/0	6/67	0/0
PRF Female FR (b)	8	8/100	0/0	0/0	3/38	0/0
PRF Female EX (c)	10	10/100	0/0	0/0	6/60	0/0
PRF Female EX+FR (d)	9	9/100	0/0	0/0	8/89	0/0

N: The number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.).

4.1.5.3 The duration of arrhythmias, the score of arrhythmia, the onset and the end of arrhythmias, the arrhythmic period, the incidence of arrhythmias and the survival rate of animals during fifteen minutes of reperfusion in female rats fed with protein rich food

The duration of arrhythmias and total arrhythmias were not different among groups during fifteen minutes of reperfusion (Table 4.41). The score of arrhythmia was not also different among groups ($P < 0.05$) (Table 4.41).

The onset and the end of arrhythmia were not different between groups of animals during fifteen minutes of reperfusion. The length of arrhythmic period observed during reperfusion was not different among groups (Figure 4.26). The incidence of arrhythmias and the survival rate of animals were not also different among groups during reperfusion ($P < 0.05$) (Table 4.42).

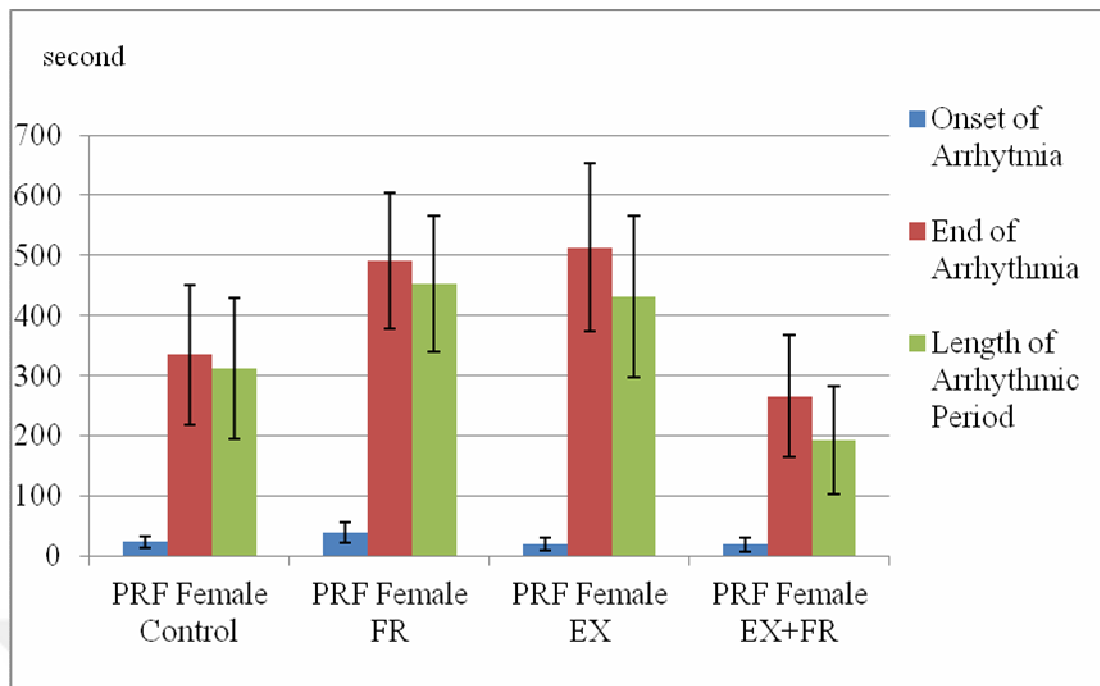


Figure 4.26. The onset and the end of arrhythmias and the length of arrhythmic period during reperfusion in female rats fed with protein rich food

PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction.

Table 4.41. The score and the duration of arrhythmias during fifteen minutes of reperfusion in female rats fed with protein rich food

Groups	N	The duration of arrhythmia (second)					n	Score of arrhythmia
		VF	VT	Other	Total	Bradycardia		
PRF Female Control (a)	7	0	18.7±7.8	19.26±10	38±15	2.9±2.9	9	3.1±0.6
PRF Female FR (b)	6	0	5.5±3.3	17.67±9	23.10±12	0±0	8	2.8±0.8
PRF Female EX (c)	8	5.5±4.4	27.9±13.4	38.8±22.5	72.3±32	0±0	10	3.1±0.7
PRF Female EX+FR (d)	9	1.6±1.6	29.5±17.4	25.74±12.2	56.9±26.6	44.4±44.4	9	2.2±0.7

N: The number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.).

Table 4.42. The survival rate of animals and the incidence of arrhythmias during fifteen minutes of coronary reperfusion in female rats fed with protein rich food

Groups	N	Survival (n / %)	Incidence of arrhythmia (n / %)			
			VF	VT	Other	Bradycardia
PRF Female Control (a)	9	7/78	2/22	7/78	9/100	1/11
PRF Female FR (b)	8	6/75	2/25	5/63	8/100	0/0
PRF Female EX (c)	10	8/80	4/40	8/80	9/90	0/0
PRF Female EX+FR (d)	9	9/100	1/11	4/44	7/78	1/11

N: The number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, VF: Ventricular fibrillation, VT: Ventricular tachycardia, Other: Other types of arrhythmias (ventricular premature contraction (VPC), ventricular bigeminy, ventricular salvo, etc.).

4.1.5.4 Body weights, the percentage of risk of infarct zone and the ventricular weights in female rats fed with protein rich food

The body weights of animals before six weeks exercise and food restriction applications were not different among female rats that fed with containing high protein diet. The body weights of animals after six weeks applications in food restricted animals were lower than the control and exercise groups ($P < 0.05$). The percentage of risk of infarct zone was not different among groups. The total weight of ventricle in FR group was lower than the control group ($P < 0.05$). The total weight of ventricle in EX+FR group was lower than the EX group ($P < 0.05$) (Table 4.43).

Table 4.43. Body weights, percentage of risk of infarct zone and ventricular weights in female rats fed with protein rich food

Groups	N	Body weight BA (g)	Body weight AA (g)	Risk of infarct zone %	N	Ventricular weight (g)
PRF Female Control (a)	9	244±9	245±11	48±1	8	0.8±0.05
PRF Female FR (b)	8	234±10	194±9 ^a	51±2	8	0.6±0.03 ^a
PRF Female EX (c)	10	259±6	261±6	52±1	9	0.9±0.05
PRF Female EX+FR (d)	9	254±10	204±8 ^{ac}	50±2	9	0.7±0.04 ^c

N: The number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, BA: Before application, AA: After application (Application: swimming exercise, food restriction and high protein diet). ^a $P < 0.05$: Compared to PRF female control, ^c $P < 0.05$: Compared to PRF female EX.

4.1.5.5 The hematological results: leukocytes, erythrocytes and hemoglobin in female rats fed with protein rich food

The amount of hemoglobin and the number of erythrocytes were not different among groups. The number of leukocytes were lower in EX+FR group according to control and EX groups ($P < 0.05$) (Table 4.44).

Table 4.44. The hematological results in female rats fed with protein rich food

Groups	N	Leukocytes	Erythrocytes	Hemoglobin
PRF Female Control (a)	3	5.36±1.2	7.29±0.2	13.5±0.2
PRF Female FR (b)	3	3.6±1	7.14±0.6	13.8±1
PRF Female EX (c)	4	4.49±0.5	6.57±0.2	13.5±0.4
PRF Female EX+FR (d)	3	1.72±0.7 ^{ac}	6.84±0.3	13.4±0.3

N: The number of animals, PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, ^a $P < 0.05$: Compared to PRF female control, ^c $P < 0.05$: Compared to PRF female EX.

4.1.5.6 The biochemical results; Glucose; HDL, Triglycerides, Total Cholesterol; Creatinine, BUN, Urea, Albumin, AST, ALT, LDH, CK-MB; SOD in female rats fed with protein rich food

The amount of glucose in FR group was lower than the female control group ($P < 0.05$). The amount of glucose in EX+FR group was lower than EX group ($P < 0.05$). The amounts of triglyceride and creatine in FR and EX+FR groups were significantly lower than the control group ($P < 0.05$). The amounts of BUN and urea in FR group were higher than the control group ($P < 0.05$). The amounts of HDL cholesterol, total cholesterol, AST, ALT, LDH, CK-MB and albumin were not different among groups (Table 4.45). The amount of SOD in FR group was higher than the control group ($P < 0.05$) (Figure 4.27).

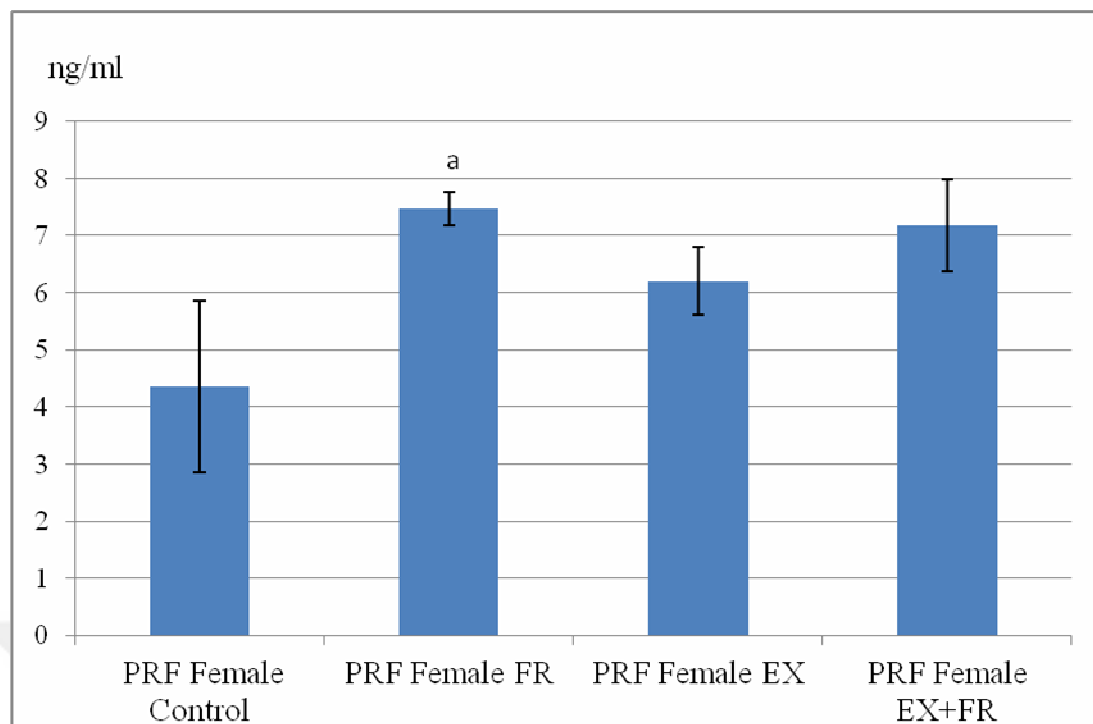


Figure 4.27. The amount of SOD in female rats fed with protein rich food

PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction. ^bP < 0.05: Compared to PRF female FR.

Table 4.45. The biochemical results in female rats fed with protein rich food

Groups	N	Glucose (mg/dl)	HDL (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Creatinine (mg/dl)	BUN (mg/dl)	AST (U/L)	ALT (U/L)	LDH (U/L)	CK_MB (U/L)	Albumin (g/dl)	Urea (mg/dl)
PRF Female Control (a)	4	213±26	20.6±3.8	79±25	51±6	0.52±0.01	23±3	194±118	63±20	1057±649	682±405	2.9±0.2	50±6
PRF Female FR (b)	4	93±35 ^a	27.5±4.8	20±3 ^a	62±9	0.43±0.05 ^a	44±9 ^a	173±40	88±16	1163±384	594±147	2.5±0.3	95±20 ^a
PRF Female EX (c)	4	260±28	18.1±1.2	48±8	43±4	0.51±0.01	21±1	109±29	45±4	580±295	367±173	2.9±0.04	46±3
PRF Female EX+FR (d)	4	144±18 ^c	22.9±0.8	22±6 ^a	52±4	0.43±0.02 ^a	32±6	210±54	81±5	992±397	426±187	3±0.1	69±14

PRF: Protein rich food, FR: 50% Food restriction, EX: Exercise, EX+FR: Exercise + 50% Food restriction, N: The number of animals. HDL: High density lipoprotein cholesterol, BUN: Blood urea nitrogen, AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase, CK-MB: Creatine Kinase-MB, ^aP < 0.05: Compared to PRF female control, ^cP < 0.05: Compared to PRF female EX, Results: mean ± SE.

4.1.5.7 The results of immunohistochemical analysis in female rats fed with protein rich food

The density of immunostaining of SUR2A was not different in myocardial cell among groups (Figure 4.28).

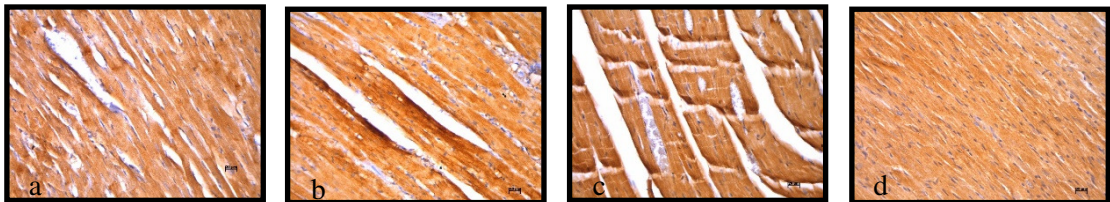


Figure 4.28. The results of immunohistochemical analysis in female rats fed with protein rich food

a: PRF female control, b: PRF female FR, c: PRF female EX, d: PRF female EX+FR.

4.2 DISCUSSIONS

4.2.1 The Heart Rate and The Blood Pressure

In this study, 50% food restriction decreased the heart rate at basal and during I/R period compared to control rats, only exception was protein rich fed male rats. This result was supported with the previous studies (Mager et al., 2006; Thomas et al., 1993; Wan et al., 2003). But there were found other studies indicating food restriction was not effective on heart rate in mice (Kobara et al., 2015; Shinmura et al., 2015) and rats (Makino et al., 2015; Kaya and Bozdoğan, 2012) and in clinical trials (Van der Meer et al., 2007). Decreased heart rate might be depending on the reduction of the autonomic activity in heart (Mattson and Wan, 2005). Food restriction reduced the sympathetic activity in rodent and human (Young and Landsberg, 1982). In addition, researchers suggest that food restriction decreased sympathetic activity or increased vagal tone (Mager et al., 2006; Mattson and Wan, 2005).

Food restriction decreased the blood pressure in protein rich fed female rats, but not in the group having food restriction with standard food. Although there are no study found studied with protein rich food, it was shown that food restriction with standart food decreased blood pressure in mice (Shinmura et al., 2015), rats (Mager et al., 2006; Thomas et al., 1993; Van Ness et al., 1997; Wan et al., 2003) and monkeys (Mattison et al., 2003) and in clinical trials (Van der Meer et al., 2007). Food restriction was also shown to decrease the blood pressure in spontaneously hypertensive rats (Dolinsky et al., 2010; Overton et al., 1997). According to a clinical study performed in healthy man, very low calorie diet decreased diastolic blood pressure but it was not effective on reducing systolic blood pressure (Van der Meer et al., 2007). In a previous review, it was reported that blood pressure decreased with caloric restriction because of increased vasodilatation induced by NO production from endothelial cells (Dolinsky and Dyck, 2011). In most of the studies mentioned above indicates the decreasing effect of blood pressure with restriction in male or female. But in our study and one of the previous study having intermittent fasting (Kaya and Bozdoğan, 2012), no decreasing effect of food restriction on BP was observed. Possible reason of this differences might be depend on different time length of food restriction. Why the blood pressure decreased in rats fed with protein rich diet but not with standart diet in female is open to further studies and could not be clarified with result of the present study.

In present study, there was no apparent effect of exercise training on HR and BP. There were different effect of long-term exercise on the heart rate and blood pressure in rats fed with protein rich and standard diet. First of all, exercise decreased the heart rate in rats fed with protein rich diet in female. But, exercise training increased BP at baseline and during reperfusion in protein rich fed male rats compared to sedentary control. There were no studies found comparing the effect of exercise on the heart rate and blood pressure in rats fed with protein rich and standard diet.

In our studies, similarly to result of some previous studies, exercise training was not effective on HR at baseline in both male and female rats fed with standard rat food (Brown et al., 2005a; French et al., 2008; Frasier et al., 2011; Quindry et al., 2005). However, some researchers report that long-term endurance exercise training

decreases the heart rate in man and experimental animal (Billman et al., 1984; Hull Jr et al., 1994; Kokkinos et al., 1995).

In our study similarly to heart rate, exercise training was not found to be effective on BP before (Brown et al., 2005a; Frasier et al., 2011; French et al., 2008; Quindry et al., 2005) and during I/R (Brown et al., 2005b; Chicco et al., 2007). But in other studies it was found that exercise was not found to be effective to change blood pressure, but during I/R period exercise was found to increase blood pressure (Brown et al., 2003; Demirel et al., 2001; Powers et al., 1998). In clinical studies, it was observed that the blood pressure decreases following exercise training in hypertensive people (Pattyn et al., 2013; Kokkinos et al., 1995), and exercise reduces the risk of hypertension in healthy people (Blair et al., 1984). Exercise training causes changes in ANS activity like as food restriction. These changes are reported as decreasing sympathetic activity and increasing parasympathetic activity (Billman, 2002; Blomqvist, 1983; Carter et al., 2003b). Exercise training has been shown to increase production of NO and NOS expression, antioxidant enzyme levels, and improve endothelial function (Hambrecht et al., 1998). However, it was also observed that the exercise increased the HR and BP following minutes of reperfusion compared to sedentary controls in some other studies (French et al., 2006). In some of the above studies, indicating the decreasing effect of blood pressure due to the exercise that was different from our study might be related with different type and the length of exercise applied.

In some studies performed in hypertensive and obese people, the combination of exercise training and food restriction decreased systolic and diastolic BP (Roberts et al., 2002; Wood et al., 1991). Similarly, both HR and BP were decreased in the combination of exercise and food restriction groups in both female and male rats fed with standard diet. Otherwise, the BP did not change in protein rich fed male rats, but it decreased in female.

4.2.2 The duration of arrhythmias, the score of arrhythmia, the onset and the end of arrhythmias, the arrhythmic period, the incidence of arrhythmias and the survival rate of animals during ischemia reperfusion period.

Exercise training improves myocardial performance during I/R. This amelioration is seen in both short and long-term physical exercise (Demirel et al., 2001; Powers et al., 1998; Powers et al., 2008). The effect of exercise training on the arrhythmias are being researched in both animal experiments and clinical trials (Blain et al., 2005; Nagy et al., 2004; Posel et al., 1989; van der Werf et al., 2011). Exercise training improves cardiac functions and reduces arrhythmias in heart failure patients (Berlardinelli et al., 1999; Kilavuori et al., 1995). Billman et al. (1984) first demonstrated that the daily exercise prevent ventricular fibrillation occurred following acute ischemia in dogs. And it was also reported that exercise training increases VF threshold in invitro rat heart after coronary occlusion (Noakes et al., 1983; Posel et al., 1989). In addition, repeated exercise training decreased the incidence of VF and increased the survival rate of dogs after I/R (Végh and Parratt, 2005). Despite of these positive results, there are other studies indicating that exercise did not affect on the incidence of VF (Hamilton et al., 2003) as like in our study.

In our study, exercise decrease the duration VPC, total arrhythmia and the score of arrhythmia in ischemic period in male rats fed with standard food. In the literature, most of the studies conducted in male fed with standard food similarly found less ventricular arrhythmia in ischemic period (Nagy et al., 2004; Noakes et al., 1983; Posel et al., 1989; Végh and Parratt, 2005). In the present study, no effect of exercise was shown on the ischemia-induced arrhythmia in female. Powers et al., (1998) was found similar result as like our's that ventricular arrhythmia in ischemic period was not different in respect to control. Similarly, but comprising both male and female in human it was found that six months exercise decreased frequency and severity of arrhythmia in clinical research (Hertzeanu et al., 1993).

In reperfusion period, the response of heart to exercise in both sexes changed. Exercise was not found to be effective on the reperfusion-induced arrhythmias in

female and male rats. This result was different and did not support the previous study (Noakes et al., 1983; Posel et al., 1989). Oppositely, in the above studies, exercise decreased the score of arrhythmia and the incidence of VF during reperfusion period. The different result of our study from previous ones might be depend on different exercise model and time length used. On the other hand, Powers et al., (1998), found that exercise did not change the incidence of VT but decreased the incidence of VPC in reperfusion period in female.

The findings of the present study that the survival rate of animals was not significantly increased with exercise training compared to sedentary control after the ischemia reperfusion were similar with the result of Frasier et al., (2011).

The underlying mechanism of why the ventricular arrhythmia decrease in ischemic period in male fed with standard diet could not be explained with the result of this study. But in the literature, it has been reported by researchers that physical exercise decreased the arrhythmias due to the regulatory effect of exercise training on cardiac autonomic nervous system (Billman, 2002). Researchers also suggest that, the protective effect of exercise against arrhythmias due to the increasing effect of exercise on the MnSOD activity (Hamilton et al., 2004). In our study similarly it was shown that decreased the arrhythmia during ischemic period in male was might be associated with the increased amount of SOD level.

Protein rich diet changed the effect of exercise and food restriction on the ischemia reperfusion induced arrhythmia. Exercise training was not effective on the arrhythmias during ischemia but, increased the duration of VT, VF, and total arrhythmias and the incidence of VF and score of arrhythmia during reperfusion in protein rich fed male rats. However, this increase was not observed in the combination of exercise and food restriction group in protein-rich fed male rats. Exercise training alone was not effective on the ischemia and reperfusion arrhythmias in protein-rich fed female rats. The combination of exercise and food restriction increased the duration of VPC and total arrhythmias, score of arrhythmia and arrhythmic period during ischemia but this increase was disappeared on the reperfusion period in protein rich fed female rats compared to control group. The cause of the increasing of arrhythmias with protein rich feeding in exercised rats are not known yet, because there was no study found investigating the effect of protein

rich feeding on I/R arrhythmias. However, in some studies conducted with standard food diet, long-term endurance exercise was mentioned to increase the risk of cardiac arrhythmias (Pina et al., 2003; Siscovick et al., 1984; Tanasescu et al., 2002). And also long term endurance exercise training increased atrial fibrillation (Aizer et al., 2009; Molina et al., 2008; Mont et al., 2008). In some studies, it has been shown that the exercise increases the ventricular arrhythmias (Biffi et al., 2002; Ector et al., 2007; Heidbuchel et al., 2003). Researchers have also reported that the source of exercise-induced atrial fibrillation and ventricular arrhythmias is right ventricle (Aizer et al., 2009; Biffi et al., 2002; Heidbuchel et al., 2003; Molina et al., 2008; Mont et al., 2002; Mont et al., 2008). The mechanism of increasing ventricular arrhythmias due to exercise that was shown in male during reperfusion period could not explained with the result of present data. It was mentioned that endurance exercise causes volume overload in the heart and regional over expression of growth factors and increases collagen deposition in the right ventricle but not in the left ventricle (Benito et al., 2011; Modesti et al., 2004). Cardiac fibrosis and myocardial deterioration induce electrical inhomogeneity and promote reentrant circuits and arrhythmogenesis (Burstein and Nattel, 2008).

In the present study, food restriction increased the incidence of VT in male rats that fed with standard rat food. However, food restriction was not found to be effective on the other parameters of cardiac arrhythmias in male rats (Kaya and Bozdoğan 2012; Šnorek et al., 2012). There was no identical study found in the literature indicating the effects of food restriction on the ischemia reperfusion arrhythmias. However, there are a few studies that research the effect of food or caloric restriction on the arrhythmias. In one of the study which used different method from the present study, it was indicated that Nutrient restriction decreases the reperfusion arrhythmias in rats (Wang et al., 2012). Again in the other studies, it was differently indicated that the food restriction was effective for restoring the effect of ischemic preconditioning which disappear in older age (Abete et al. 2002).

In female rats that fed with standard rat food, both food restriction and exercise training alone was not effective on the arrhythmias. However, the combination of food restriction and exercise training decreased the duration of VPC and total arrhythmias in this group. There was no study in the literature about the

effect of the combination of food restriction and exercise on ischemia reperfusion arrhythmias.

Food restriction also was not effective on the arrhythmias in protein rich fed female and male rats. But, protein rich feeding reversed the decreasing effect of the combination of food restriction and exercise training on the arrhythmias; protein rich feeding increased duration of VPC and the score of arrhythmia in female rats.

4.2.3 Body weights, percentage of risk of infarct zone and ventricular weights

Protein-rich feeding did not cause weight loss in our study and previous studies (Jenkins et al., 1999; Peiyong et al., 2001; St-Onge et al., 2007). Otherwise, there are some studies showing that protein-rich diet leads to weight loss. (Baba et al., 1999; Brehm et al., 2003; Farnsworth et al., 2003; Foster et al., 2003; Hu, 2005; Leidy et al., 2007; Samaha et al., 2003; Skov et al., 1999; Soenen et al., 2012; Yancy Jr et al., 2004). It was reported that soy protein-rich feeding increased the energy expenditure more than carbohydrate-rich feeding in over weight men (Mikkelsen et al., 2000). Some researchers were reported that protein-rich feeding caused to weight loss because of being greater thermic effects and leading to satiety (Hu, 2005). The different effect of protein rich feeding on weight loss might be depend on the type (vegetable or animal protein) and duration of protein rich feeding in this study.

Food restriction caused to weight loss that was found in the present study supports previous findings (Abete et al., 2002; Drew et al., 2003; Edwards et al., 2010; Heilbronn et al., 2006; Larson-Meyer et al., 2010; Mager et al., 2006). Likewise, some studies were reported the weight loss occur when combination of exercise and food restriction were applied (Larson-Meyer et al., 2006; Redman et al., 2007; Larson-Meyer et al., 2010). Food restriction decreased the ventricular weight shown previously was also seen in this present study (Abete et al., 2002; Edwards et al., 2010).

Body weight did not change due to the exercise in male and female in our study and previous studies (Brown et al., 2005b; Chicco et al., 2007; French et al., 2006; Oh et al., 1993; Quindry et al., 2005). But in some research it was found that

exercise increases body weight (Brown et al., 2003; Brown et al., 2005a), but some others informed that exercise decreased the body weight (Chicco et al., 2007; French et al., 2006). Researchers mentioned that different effect of exercise depends on gender. Chicco et al., (2007) and Brown et al., (2007) were found that exercise was not effective in female, but it reduced the body weight in male. In another study, exercise reduced the body weight in older while it was not effective in younger (Quindry et al., 2005).

In our study, exercise increased the ventricular weight in male rats that fed with standard rat food, but it was not effective on body weight in rats fed with protein rich diet (Benito et al., 2011; Brown et al., 2005a; Frasier et al., 2011; Powers et al., 1998). But in some other studies it was suggested that exercise was not effective for increasing heart weight (Brown et al., 2005b; Chicco et al., 2007; French et al., 2006; Oh et al., 1993; Quindry et al., 2005).

No changes were seen in the risk of infarct zone in animals applied food restriction and the infarct size was not determined because of short ischemia in this study. The infarct size decreased with food restriction was shown in previous studies (Edwards et al., 2010; Šnorek et al., 2012).

In our study, it was observed that exercise reduced the risk of infarct zone only in males fed with normal diet (Brown et al., 2005a; French et al., 2008) but not in female and protein rich fed male and female (Yamashita et al., 1999). In this group, high ventricular weight might be effective on decreasing risk of infarct zone. We did not measure the infarct size because of short ischemic period. But most of the researcher indicates that exercise decreases infarct size (Brown et al., 2003; Brown et al., 2005b; Chicco et al., 2007; Hamilton et al., 2003). Exercise is effective on reducing infarct size due to the increase of coronary angiogenesis (McElroy et al., 1978).

4.2.4 The biochemical analysis; Glucose; HDL, Triglycerides, Total Cholesterol; Creatinine, BUN, Urea, Albumin, AST, ALT, LDH, CK-MB; SOD

In our study, food restriction non significantly increased the amount of SOD level in blood serum in all rats, but significantly increased in protein rich fed females. Researchers reported that food restriction decreased the reactive oxygen species (Pamplona and Barja, 2006; Shinmura, 2011; Sung and Dyck, 2012). But some researchers report that food restriction was not effective on the ROS production in the heart (Drew et al., 2003). Decreasing antioxidant activity with aging is increased by food restriction (Pamplona and Barja, 2006). Rippe et al. (2010) suggest that MnSOD activity in old mice was significantly lower than young mice and food restriction increased MnSOD activity in old mice although no change was observed in young mice. This was also supported by other researchers, suggesting that food restriction increased the amount of MnSOD level (Dolinsky and Dyck, 2011; Sreekumar et al., 2002). Caloric restriction in 60% was not effective on MnSOD expression at baseline (before ischemia), but it increased the MnSOD expression at first and second hours of the reperfusion in rats (Chandrasekar et al., 2001).

In the present study, exercise training increased the amount of SOD level in blood serum, but significant increasing was shown in only male rats that fed with standard rat food. The score and the duration of arrhythmias were also lower in this group of animal. The same results were shown in the combination of exercise and food restriction group that fed with standard rat food. This reduction in arrhythmias may be associated with increasing the amount of SOD. Food restriction and physical exercise was effective in reducing ROS production in advanced age was reported in previous studies (Bo et al., 2013). Many studies reported that physical exercise increases the activity of MnSOD (Demirel et al., 2001; French et al., 2006; Hamilton et al., 2001; Hamilton et al., 2003; Hoshida et al., 2002; Powers et al., 1998; Powers et al., 1993; Yamashita et al., 1999). But in some other studies, it was also observed that exercise was not effective on MnSOD activity (Brown et al., 2005b; Dickson et al., 2008). The effect of exercise on MnSOD activity depends on the duration and type of exercise (Brown et al., 2003). Additionally the effect of exercise on SOD

activity depends on the age of experimental animals. Physical exercise was not effective on MnSOD activity in young animals, but it increased the MnSOD activity in old animals (Quindry et al., 2005). It was shown that MnSOD expression was higher in females than males (Brown et al., 2005b). Researchers reported that physical exercise decreases I/R arrhythmias (Hamilton et al., 2004; Powers et al., 1998) and infarct size (Brown et al., 2003) by increasing MnSOD expression and activity. But this effect of exercise might be related with the increasing of the K_{ATP} channel expression (Brown et al., 2005a).

In our study, there was no effect of exercise on the LDH level. Lactate dehydrogenase level in blood serum increases due to cell damage. LDH levels in the blood may be increased because of exercise that leads to the damage of the skeletal muscle and cardiac muscle cells. Previous studies have reported that the endurance exercise increased the amount of LDH and LDH activity (Oláh et al., 2015; Powers et al., 1998; Xiao, 2015). However, this elevation was reduced to normal levels again in the period following the exercise training (Armstrong et al., 1983). Some researchers also suggested that regular exercise reduces the increased LDH activity (Papadopoulos et al., 1968). This different observation might be related with the exercise program and the procedure for blood sampling that was performed after one day following exercise.

It was reported that food restriction reduces the LDH level (Shinmura et al., 2005; Shinmura, 2011). However, in our study there was no effect of food restriction on the serum LDH level. This different observation might be related with the food restriction program in this study.

In our study, exercise was not effective on HDL cholesterol levels. HDL cholesterol levels were increased with the combination of exercise and food restriction in male rats. Low HDL cholesterol level in blood is associated with increasing risk of coronary artery disease (Assmann and Schulte, 1992; Couillard et al., 2001; Pan and Chiang, 1995). HDL cholesterol levels are higher in females than in males (Kokkinos and Fernhall, 1999). Regular endurance exercise increases HDL cholesterol levels in blood plasma (Durstine and Haskell, 1994; Hardman, 1999; Hussien and Shoman, 2013; Kodama et al., 2007; Thompson and Rader, 2001;).

HDL cholesterol level increased with food restriction alone and combination of food restriction and exercise which has been reported previously (Fontana et al., 2004; Larson-Meyer et al., 2010). Our study shows that food restriction increased the level of HDL cholesterol in only males that fed with standard rat food but it was not effective in other groups.

In present study, triglyceride levels decreased with exercise alone in only male rats that fed with standard food. However, triglyceride levels decreased in females when food restriction and exercise applied together and only food restriction alone. High level of triglycerides is a risk factor for coronary artery disease. Regular physical exercise reduces triglyceride levels that have been reported in several studies (Hussien and Shoman, 2013; Taylor et al., 2004; Thompson and Rader, 2001; Thompson et al., 1980). In previous studies it has been reported that both food restriction alone (Fontana et al., 2004; Gresl et al., 2001; Rippe et al., 2010; Shinmura et al., 2005) and combination with exercise decreases triglyceride levels (Larson-Meyer et al., 2010). Our study shows that food restriction was more effective for decreasing triglyceride level in females than males.

In our study, exercise did not have any effect on the amount of total cholesterol between the groups of animals as in previous studies (Hurter et al., 1972; Lehtonen and Viikari, 1978) except in male rats that fed with high-protein diet. But the amount of total cholesterol is reduced by regular exercise in previous studies (Hussien and Shoman, 2013; Lennon et al., 1983; Taylor et al., 2004; Thompson et al., 1980).

In present study, decreasing effect of food restriction on the total cholesterol level was shown in female rats that fed with standard rat food, and also it was shown in combination of food restriction and exercise group in female rats that fed with standard rat food. Decreasing effect of food restriction on the total cholesterol level was reported in previous studies (Fontana et al., 2004), but in another previous studies, food restriction was not effective on total cholesterol level (Rippe et al., 2010). However, some researchers suggest that food restriction was not effective on total cholesterol level, but it decreased the total cholesterol level in senescent subjects (Shinmura et al., 2005).

In our study, exercise alone and combination with food restriction increased the amount of AST level only in female rats that fed with standard rat food, but exercise was not effective on the amount of ALT level in all groups of animals. In addition, food restriction was not effective on the amount of AST and ALT levels in all groups of animals. The amount of aspartate transaminase increases with injury on the neural cells in the brain, skeletal and heart muscle and liver cells, but the amount of alanine transaminase is more specific on the liver cell damage. The amount of AST and ALT levels was reduced with exercise in blood (Hussien and Shoman, 2013), but some researchers report that it was increased with physical exercise (Chen et al., 2014; Li et al., 2014). The results of some previous studies that performed in animals with food restriction support our results (Matsuzaki et al., 2001).

In our study, CK-MB levels in blood serum were unchanged. The elevation of the total creatine kinase shows the damage of the heart and skeletal muscles. The elevation of CK-MB indicates the myocyte damage due to the coronary artery occlusion in the heart. The increase of CK levels due to strenuous exercise was reported in previous studies (Chen et al., 2014).

In the present study, food restriction alone decreased the amount of serum creatinine in females that fed with high protein diet, but it did not change the amount of serum creatinine level in other groups. Previous studies reported that food restriction was not effective on the serum creatine level in rats (Pinto et al., 2012) and adult and old monkeys (Ingram et al., 1990). But long term food restriction in 30 weeks increased the serum creatinine levels in dogs (Lawler et al., 2007). Different effect of food restriction on serum creatine level might depend to the type and the duration of food restriction in this study.

In our study, exercise alone was not effective on the amount of creatine level in blood serum, but it decreased the amount of creatinine level in blood serum in combination of food restriction and exercise group of animals having standard rat food diet but not other protein rich diet. Exercise reduces the level of serum creatinine (Aparicio et al., 2014, Chen et al., 2014). In contrast, Li et al. (2014) found that exercise increased the level of serum creatinine. Different effect of exercise on serum creatinine level might depend to type and duration of exercise.

In the present study, exercise was not effective on the amount of BUN and urea in blood serum. Some researchers reported that physical exercise decreased the amount of BUN and urea (Chen et al., 2014), whereas some researchers reported that it increased the amount of BUN and urea (Aparicio et al., 2014; Li et al., 2014). Different effect of exercise on BUN and urea might depend to type and duration of exercise.

In the present study, the food restriction decreased the amount of blood glucose in all groups except in male rats that fed with protein rich food. Decreasing effect of food restriction on the amount of blood glucose level was also shown in the combination of exercise with food restriction groups in females. It was reported that physical exercise improves insulin sensitivity and also increases the Glucose transporter type 4 content in skeletal muscle and increases glucose uptake into the muscle (Borghouts and Keizer, 1999; Goodyear and Kahn, 1998). Blood glucose level was decreased with exercise training in previous studies (Andrew et al., 1990). In the present study, exercise training alone decreased the blood glucose in females that fed with standard rat food, but it increased the blood glucose in male rats fed with protein rich food. It is known that the amount of blood glucose level decreases with food restriction (Fontana et al., 2004; Gresl et al., 2001; Rippe et al., 2010; Shinmura et al., 2005). However, it was reported that the amount of blood glucose level did not change with 25% dietary restriction (Heilbronn et al., 2006). These results suggest that food restriction is more effective on decreasing blood glucose in females than in males.

4.2.5 The immunohistochemical analysis

The effect of protein rich diet on K_{ATP} channels was studied for the first time in this study. There was no effect of protein rich feeding on the density of SUR 2A protein of K_{ATP} .

In the present study, physical exercise increased the density of SUR 2A protein of K_{ATP} in both group fed with standard or protein rich diet in male rats. This might be related to the reduction of the risk of infarct zone suggested in the previous studies (Brown et al., 2005a, 2005b). The number of K_{ATP} channels is important for the size of infarction. Chicco et al. (2007) was reported that the infarct size was

reduced by short-term exercise, but it was increased significantly by using of the K_{ATP} channel blocker. The number of K_{ATP} channels was found higher in females than males (Ranki et al., 2001). Physical exercise performed quick protection in males compared to females (Brown et al., 2005a, Brown and Moore, 2007). $mitoK_{ATP}$ channels decreases the ischemia reperfusion induced arrhythmias and $sarcK_{ATP}$ channels reduces the ischemic cell death (Brown et al., 2005a, 2005b; Quindry et al., 2010). The reduction of arrhythmias during ischemia in exercised rats that was observed in the present study, may be related with the increase of the number of K_{ATP} channel due to exercise. The effective factor for the prevention of arrhythmias was $mitoK_{ATP}$ channels (Quindry et al., 2010; Quindry and Hamilton, 2013). But, the findings of the present study do not have any evidence about the type of K_{ATP} channel protein. Further research is required whether exercise increases the expression of sarcolemmal or mitochondrial K_{ATP} channel protein.

In the present study, food restriction alone is not effective on the K_{ATP} channels, but the increasing effect of exercise on these channels was increased more than with the food restriction in female rats. There was no study researched the effect of food restriction on the number of K_{ATP} channels yet. However, cardioprotective effect of food restriction was researched in a Langendorf study by using both non-selective and selective K_{ATP} channel blocker (Shinmura et al., 2005). Previous study reported that food restriction was cardioprotective in other ways without the influence of K_{ATP} channel (Shinmura, et al., 2005).

5. CONCLUSIONS AND RECOMMENDATIONS

Food restriction decreased the heart rate in both male and female at baseline and during ischemia reperfusion period. Food restriction decreased the arterial blood pressure only in female. There was no significant effect of exercise on the heart rate and the arterial blood pressure. The reason of less blood pressure in female could not be clarified with the result of this study. But food restriction was more effective to decrease blood pressure in female than male. In later studies, the role of eNOS activity induced by food restriction can be investigated in both sexes.

The body weight and also the ventricular weight decreased in all animals having food restriction. The risk of infarct zone was only decreased in exercised group of males having standard diet. But similar result was not seen in male and female rats having protein rich diet.

The amount of glucose decreased in food restricted males and females, but decreasing effect of food restriction on the amount of glucose disappeared with exercise and/or protein rich feeding in males. Likewise, the amount of triglyceride was reduced in both male and female animals following food restriction. This results shows that food restriction is more effective in maintaining blood glucose and plasma lipid profiles.

The amount of SOD increased with both food restriction and physical exercise, but this increase was not significant in most of the groups. Because the amount of SOD was studied in four animals in each group, this increase were not statistically different. The number of animals in groups can be increased in future studies.

The exercise increased the density of SUR2A protein of K_{ATP} channel in myocardial cells but, food restriction alone was not effective on the density of SUR 2A. In this study, the SUR2A channel proteins were analyzed by immunohistochemical staining so that quantitative result was not obtained. But the

amount of SUR2A should be also studied analytically by another techniques, western blott analysis.

This study clarified that protein rich diet might be harmful to ischemia reperfusion induced arrhythmias in animals having exercise. Exercise is generally protective against the ischemia-induced arrhythmia especially in male but not reperfusion-induced arrhythmia. Further research is required to answer why the exercise did not decrease the reperfusion-induced arrhythmia. On the other hand, opposite to the expectation that was made at the beginning of the research, no effect of food restriction was seen on the ischemia reperfusion induced arrhythmia in both male and female. In addition, the protection produced by exercise against ischemia-induced arrhythmia abolished with the food restriction. Why the exercise was more effective to decrease arrhythmia in male than female needs further research.

Protein rich diet was shown to decrease ischemia induced arrhythmia and arrhythmic period in male and nonsignificantly in female in respect to standard diet. The reason of this was not clarified with the result of this study. It is needed further research. In reperfusion why these protection disappear should be clarified with further research. Even the incidence of ventricular tachycardia increased in reperfusion in protein rich feeding especially in female. Why the protein rich diet induce more arrhythmia in reperfusion period in female. Can we say that the protein rich diet is helpful for ischemia reperfusion induced arrhythmias? For ischemia may be yes but for reperfusion is not. In later studies, the effect of protein rich diet only in long term ischemia can be investigated also on the infarct size. No relation was found between increased arrhythmia and blood cholesterol and triglyceride and other blood parameters in reperfusion in female but not male fed with protein rich diet. Cholesterol and triglyceride did not increase with the protein rich diet and also no change was observed in hemodynamics and liver enzymes; AST, ALT, LDH and CK-MB. Superoxide dismutase enzyme did not change with protein rich diet and no difference was found between male and female.

In standard diet, exercise decreased the ischemia reperfusion induced arrhythmia in male but not female. Furthermore, food restriction alone did not have any effect on the ischemia reperfusion arrhythmia in male and female. Food restriction did not change the effect of exercise on these arrhythmias in male but it

decreased the ischemia-induced arrhythmia in female. Exercise is not significantly effective to decrease arrhythmia in female. That means exercise, generally; was effective to decrease ischemia-induced arrhythmia in both sexes. But food restriction was not protective against ischemia induced arrhythmia was shown first in this study. Otherwise exercise and food restriction alone or in combination was not found to be effective to decrease reperfusion-induced arrhythmia in both male and female. Exercise alone and with food restriction increased serum SOD level in male but not in female. This might be indicator that stress induced by exercise and food restriction is more in male than female and this makes male rats be more protected against ischemia induced arrhythmia than female. Anyway, lesser arrhythmia seen during ischemia in male than female might be explained by the more SOD level in male. Protein rich diet has unfavorable effect on the arrhythmias. Because, exercise alone or together with food restriction did not changed ischemia induced arrhythmia in male and female in protein rich feeding. Even ischemia induced arrhythmia significantly increased in female during reperfusion. Similarly, arrhythmia increased significantly during reperfusion in exercised male in protein rich feeding. The protein rich diet induces more arrhythmia in reperfusion period in male and it did not change in female. SOD level was not different after exercise in male and female in protein rich feeding. Therefore, there is no relation of more arrhythmia in male than female with SOD level in reperfusion period. Other blood parameters were also not so different between male and female in response to exercise. Food restriction in protein rich diet group does not have any significant effect on the reperfusion-induced arrhythmia. Similarly, in male reperfusion induced arrhythmia was not different from control in combination group including exercise and food restriction. In this case it seems that exercise have decreasing effect on the reperfusion induced arrhythmia with food restriction. The underlying mechanism of this combined effect to decrease reperfusion induced arrhythmia in the combination of food restriction and exercise group in respect to food restriction should be researched further. In protein rich food group, food restriction significantly increased SOD level in female but not male. In combination of food restriction and exercise, SOD level was decreased also but nonsignificantly in respect to control. In protein rich diet group, food restriction might be helpful to decrease the deleterious effect of exercise on the reperfusion-induced arrhythmias.

As a result of this study, it could be said that exercise might be helpful to decrease ischemia induced arrhythmia, protein rich diet is not so good in this case. Food restriction alone could not be liked at least to decrease ischemia or reperfusion induced arrhythmia. Both food restriction and exercise again does not seen more favorable to decrease ischemia or reperfusion induced arrhythmia in protein rich diet but favorable in standard diet. There was no significant relation found between biochemical findings and hemodynamics value in respect to the changes of food regime and arrhythmias.



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APPENDICES

7. APPENDICES

Appendix A



T.C.
ABANT İZZET BAYSAL ÜNİVERSİTESİ
Hayvan Araştırmaları Yerel Etik Kurulu

Sayı :B.30.2.ABÜ.0.05.05-050.01.04-141

09/12/2011

Konu: Kararlar.

Sayın; Prof. Dr. Ömer BOZDOĞAN
A.İ.B.Ü Tıp Fakültesi
A.İ.B.Ü Fen Edebiyat Fakültesi Öğretim Üyesi

Hayvan Araştırmaları Yerel Etik Kurulu tarafından 2011/44 no.lu'' Sıçanlarda beslenme ve egzersizin iskemi reperfüzyon aritmileri üzerine etkileri '' isimli çalışmanız etik olarak uygun bulunmuştur.

Prof. Dr. Ayşel KÜRKNER
(Başkan)

Prof. Dr. Erol AYAZ
(Üye)

Prof. Dr. Ömer BOZDOĞAN
(Üye)

Prof. Dr. Mehmet YAZICI
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Doç. Dr. Azra BOZCAARMUTLU
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List of Publications

1. Bozdoğan Ö, Kaya ST, Yaşar S, Orallar H. Effect of ATP-dependent channel modulators on ischemia-induced arrhythmia change depending on age and gender. Exp Biol Med. 238, 1170-1179, 2013.
2. Yaşar S, Bozdoğan Ö, Kaya ST, Soytürk Orallar H. The effects of ATP-dependent potassium channel opener; pinacidil, and blocker; glibenclamide on the ischemia induced arrhythmia change in partial and complete ligation of coronary artery in rats. Iran J Basic Med Sci 2015; 18: 188-193.
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