THE EFFECT OF INTERMITTENT FASTING AND WATER RESTRICTION ON ARRHYTHMIAS INDUCED BY ISCHEMIA-REPERFUSION IN RATS

SALİH TUNÇ KAYA

JUNE 2011

THE EFFECT OF INTERMITTENT FASTING AND WATER RESTRICTION ON ARRHYTHMIAS INDUCED BY ISCHEMIA-REPERFUSION IN RATS

by
SALİH TUNÇ KAYA

THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
THE ABANT IZZET BAYSAL UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE
IN
THE DEPARTMENT OF BIOLOGY

June 2011

Approval of the Graduate School of Natural and Applied Science

Prof. Dr. Nihat ÇELEBİ
Director

I certify that this thesis satisfies all the requirements as a thesis for degree of Master of Science.

Prof. Dr. Mehmet Tekin BABAÇ
Head of Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for degree of Master of Science in Biology.

Prof. Dr. Ömer BOZDOĞAN
Supervisor

Examining Committee Members

1. Prof. Dr. Ömer BOZDOĞAN .....

2. Prof. Dr. Cihan DEMİRCİ TANSEL .....

3. Doç. Dr. Fatma TÖRE .....

ABSTRACT

THE EFFECT OF INTERMITTENT FASTING AND WATER RESTRICTION ON ARRHYTHMIAS INDUCED BY ISCHEMIA-REPERFUSION IN RATS

KAYA, Salih Tunç
Master of Science, Department of Biology
Supervisor: Prof. Dr. Ömer BOZDOĞAN

JUNE 2011, 74 pages

Ischemia and reperfusion induced arrhythmias such as ventricular tachycardia or ventricular fibrillation are one of the most important causes of death in myocardial ischemia. That is why, it is important to find factors increasing resistance against myocardial ischemia induced by sudden occlusion of coronaries in human being. The changing of life style or feeding habit may be more effective in the reduction or prevention of death from cardiovascular disease instead of using drug. Dietary restriction, including caloric restriction, intermittent fasting or alternative day fasting may be protective in the decreasing the incidence of death due to the coronary artery disease in human being. Recently, researchers have focused on the various effects of intermittent fasting. It has been shown that intermittent fasting can reduce cardiovascular disease. In literature, there could not found any research related with the effect of both intermittent fasting and water restriction on the ischemia and reperfusion induced arrhythmias in rats.

Thirty male rats in 8-9 months old were used in this study. Six minutes ischemia was produced by ligation of left coronary artery which was followed by six minute reperfusion by releasing of this artery. Control group was fed ad libitum. IFW1 group was subjected to intermittent fasting and water restriction for one month. IFW2 group was maintained one month intermittent fasting and water restriction followed by one month normal feeding.ECG and arterial blood pressure were recorded during ischemia and reperfusion. Blood glucose level was also measured before ligation. The blood pressure and heart rate during ischemia and reperfusion did not show a significant difference among groups. Similarly, there was no significant difference in arrhythmia score calculated from duration and type of arrhythmia. But arrhythmic period during ischemia in IFW1 was longer in respect to other groups. Blood glucose level significantly decreased in animals maintained on food and water intermitted restriction. In conclusion, it was found that the intermittent fasting and water restriction that is experimental model of Ramadan fasting did not have any effect on the severity of arrhythmia induced by ischemia and reperfusion. Further studies should be made to research the effect of other restriction models on the ischemia or reperfusion induced arrhythmia and the survival rate of animals after acute coronary artery occlusion.

Key Words: Myocardial Ischemia, Reperfusion, Fasting, Water Restriction, Arrhythmia

ÖZET

SIÇANLARDA ARALIKLI AÇLIĞIN VE SUSUZLUĞUN İSKEMİ-REPERFÜZYON İLE UYARILAN ARİTMİLER ÜZERİNE ETKİSİ

KAYA, Salih Tunç
Yüksek Lisans, Biyoloji Bölüm
Tez Danışmanı: Prof. Dr. Ömer BOZDOĞAN

Haziran 2011, 74 sayfa

İskemi ve reperfüzyon ile uyarılan ventriküler taşikardi veya ventriküler fibrilasyon gibi aritmiler miyokardiyal iskemiden kaynaklı ölümün en önemli sebeplerinden birisidir. Bu nedenle insanlarda koronerlerin ani tıkanmasıyla oluşan miyokardial iskemiye karşı direnci artıracak faktörlerin bulunması önemlidir. Yaşam stilini veya beslenme alışkanlıklarını değiştirmek kardiyovasküler hastalıklar sonucu oluşan ölümleri azaltmada veya önlemede kullanılan ilaçlardan daha etkili olabilir. Kalori kısıtlaması, aralıklı açlık veya alternatif gün açlığını kapsayan diyet kısıtlaması insanlarda koroner arter hastalıkları yüzünden oluşan ölümlerin azaltılmasında koruyucu olabilir. Son zamanlarda, araştırmacılar aralıklı açlığın çeşitli etkileri üzerine odaklanmaktadır. Aralıklı açlığın kardiyovasküler hastalıkları azaltabildiği gösterilmiştir. Literatürde aralıklı açlığın ve su kısıtlamasının iskemi-reperfüzyon ile uyarılan aritmiler üzerine etkisini gösteren çalışmaya rastlanmamıştır.

Bu çalışmada 8-9 aylık otuz sıçan kullanıldı. Sol koroner arterin ligasyonu ile 6 dakikalık iskemi, bunu takiben arterin açılmasıyla 6 dakikalık reperfüzyon oluşturulmuştur. Kontrol grubuna istediği kadar su ve besin sağlandı. IFW1 grubu bir ay süreyle aralıklı açlık ve su kısıtlamasına maruz bırakıldı. IFW2 grubu bir ay aralıklı açlık ve su kısıtlamasına maruz bırakılmasını takiben, bir ay normal beslendi. İskemi ve reperfüzyon sırasında EKG ve arteriyal kan basıncı kaydedildi. Ligasyondan önce kan glikoz seviyesi'de ölçüldü. İskemi ve reperfüsyon sırasındaki kan basıncı ve kalp atım oranı gruplar arasında belirgin bir farklılık göstermedi. Benzer olarak, aritmilerin süresi ve çeşidi kullanılarak hesaplanan aritmi skorunda belirgin bir fark gözlenmedi. Ancak, IFW1'de iskemi sırasındaki aritmik periyot diğer gruplara göre daha uzundu. Aralıklı açlığa ve su kısıtlamasına maruz bırakılan hayvanlardaki kan glikoz seviyesi belirgin bir şekilde düştü. Sonuç olarak, Ramazan açlığının deneysel modeli olan aralıklı açlık ve su kısıtlamasının iskemi ve reperfüzyonla uyarılan aritmilerin şiddeti üzerine etkisinin olmadığı bulundu. İlerideki çalışmalarda, iskemi reperfüzyon aritmilerinin ve akut koroner arter tıkanmasını takiben oluşan ölüm oranının azaltılmasında diğer kısıtlama modellerinin etkisinin araştırılması gerekmektedir.

Anahtar Kelimeler: Miyokardiyal İskemi, Reperfüzyon, Açlık, Su Kısıtlaması, Aritmi

TO MY PARENTS

İBRAHİM KAYA and MAKBÜLE KAYA

ACKNOWLEDGMENT

I express sincere appreciation to my supervisor, Prof. Dr. Ömer BOZDOĞAN for his guidance, encouragement, great help and unceasing interest throughout the research. His guidance and experience has influenced not only the document at hand but, also my way of thinking as a biologist and a person.

To my family, I would like to thanks for their support and love. And also I would like to express my special thanks to Özlem SAĞIR for her endless support, patience and for motivating me towards my goal.

I wish to thank Assist. Prof. Dr. Barış GÜLCÜ for various tactics and great support. Also, I want to convey my deepest thanks to my friends, Barış YILDIZ for his help in feeding of rats.

Last but not least I would like to thank all of those who have given me new ideas, support and those who have showed interest in this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Myocardial ischemia is a widespread cause of morbidity and main reason of sudden death in the developed countries of the world. It is estimated that 3.8 million men and 3.4 million women die due to coronary heart disease every year (Yellon and Hausenloy, 2007). Most of the possible causes of sudden unexpected death are cardiogenic origin which led to the lethal arrhythmias occurring during either myocardial ischemia or reperfusion (Reimer and Ideker, 1987). It has been suggested that this type of arrhythmias and mortality rate is higher in obese individuals (Fisler, 1992). Recently, the physiological effects of various feeding activities such as short term fasting, intermittent fasting, caloric restriction has been investigated in different experimental models in animals. Nowadays the importance of dietary factors on health has been increasingly appreciated. Dietary restriction has been shown to have several health benefits including reduced risk factors for cardiovascular disease, increased stress resistance, reduced morbidity, and increased life span (Carlson and Hoelzel, 1945; Aksungor-Benli et al., 2005; Mattson and Wan, 2005; Wan et al., 2010). The decreased cardiovascular risk factor is seem to be most important, since more than 50 % of sudden death in industrial nations result from acute myocardial infarction (Akao et al., 2007). It has been shown in a study that the maintaining intermittent fasting with one day interval but not water restrictions during 3 months decrease infarct size in rats (Ahmet et al., 2005).

Intermittent fasting and water restriction have shown to decrease cardiovascular risk factors including increasing insulin sensitivity, increasing high density lipoprotein and improving the cholesterol-high density lipoprotein ratio in human being (Pedersen et al., 1999; Aksungar-Benli et al., 2005). Intermittent fasting have also beneficial effect that lowers left ventricular end diastolic volume, left ventricle systolic volume and heart rate in rats (Wan et al, 2003; Ahmet et al., 2005). Although cardioprotective effect of intermittent fasting has been studied, the underlying mechanisms of this protection have not been clarified. Some researchers suggest that the oxidative stress play a considerable role in the aging and cardiovascular diseases associated with mortality. Modification of oxidative stress during dietary restriction can affect disease process (Mattson, 2008). Another assumption is that nutritional status of organism before a possible ischemia is an important issue in determining of postishemic conditions of myocardium. For example, increasing glycogen content after fasting is possible causes of cardiac protection against global ischemia (Schneider and Taegtmeyer, 1991).

In the world, some population voluntarily restrains from eating and drinking. For example, during the month of Ramadan Muslims do not eat and drink between sunrise and sunset. This fasting regime is intermittent and do not include caloric restriction. There is no experimental study to evaluate the effect of such intermittent fasting on animal models. Although there are several clinical studies performed in healthy individuals during Ramadan in Muslim countries, the results do not always seem to be comparable.

The reason of incompatible results arise from differences in nutritional habits, the changes in length and the temperature of fasting day due to seasonal occurrence of the Ramadan month (Roky et al., 2004).

The aim of present study is to clarify the role of intermittent fasting and water restriction on ischemia-reperfusion induced arrhythmias. The effects of intermittent fasting and water restriction on arrhythmias caused by ischemia and reperfusion are going to be investigated for the first time in this study. Our hypothesis in this study is to determine the effects of the intermittent fasting and water restriction simulated as Ramadan fasting on ischemia and reperfusion induced arrhythmias.

1.1. ANATOMY AND HISTOLOGY OF HEART

The heart, a muscular pump, serves two functions. First one is to collect blood from the tissues of the body and pump it to the lungs. Second one is to collect blood from the lungs and pump it to all tissues of the body. The heart is located in the thoracic cavity between right and left lung and rest on the superior surface of the diaphragm. The apex of heart is its anterior surface, which is formed by the tip of left ventricle and rest on the diaphragm. Posterior surface of heart is the base of heart formed by atria. The structures surrounding and protecting heart is named as pericardium. Pericardium has important functions in term of proper function of heart. It confines the heart to its location in the mediastium, an anatomical region extending from the sternum to vertebral column, and it facilities the movement of heart by the help of pericardial fluid which prevent friction as heart beat. Pericardium has double layers; parietal layer (outer) and visceral layer (inner).

The internal anatomy of the heart shows four chambers. Two chambers are atria functioning as collecting chambers. The other two chambers called as ventricles serves as pumping chambers that have stronger contraction than atria. The right atrium receives blood from three veins: superior vena cava, inferior vena cava, and coronary sinus. The right atrium empties blood to right ventricle by passing through the opening of antrioventricular valve named as tricuspid valve. The function of valve generally is to prevent blood backflow. Then right ventricle pumps the deoxygenated blood to lung through pulmonary semilunar valve. Left atrium receive oxygenated blood from lungs. Blood passes from left atrium into the left ventricle through the bicuspid valve or mitral valve. Contraction of left ventricle pumps the blood to the aorta by opening of aortic valve named as aortic semilunar valve **(Figure 1. 1).**

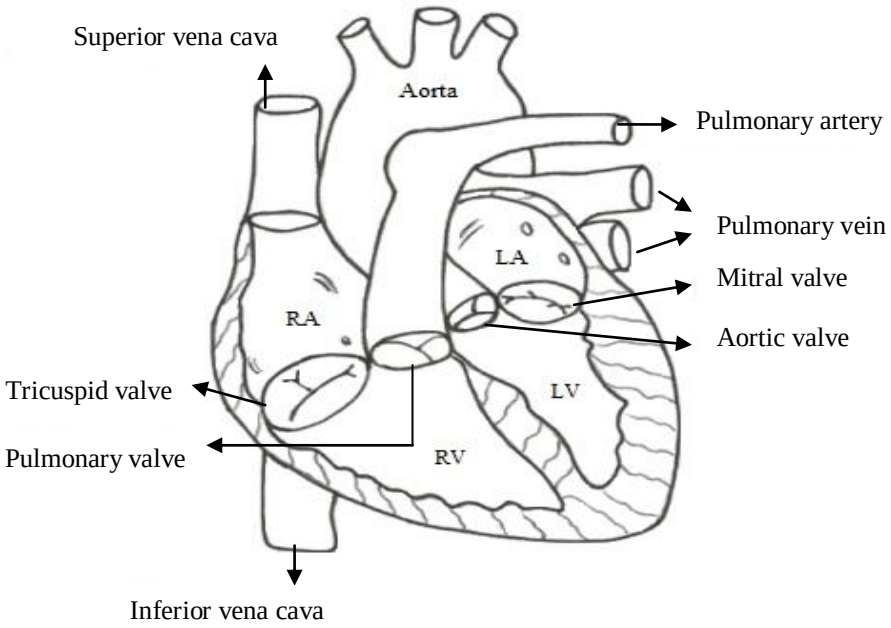

Figure 1. 1. Anatomy of the heart

Myocardium receives its important nutrients from a network of coronary blood vessels. Two coronary arteries are originated from ascending aorta; left and right coronary artery **(Figure 1.2 and Figure 1.3).** Left coronary artery is divided into two branches as left anterior descending coronary (LAD) and circumflex artery. The left anterior descending coronary artery supply a small part of anterior region of interventricular septum by branches which anastomose with those of septal artery. The circumflex artery, another branch of the left coronary artery, runs on the anterior surface of left ventricle. The circumflex artery nourishes mainly the left margin and posterior surface of left ventricle. The right coronary artery lies on the surface of the right ventricle and is directed first to the right side then downwards to end on the posterior surface of that ventricle near apex.

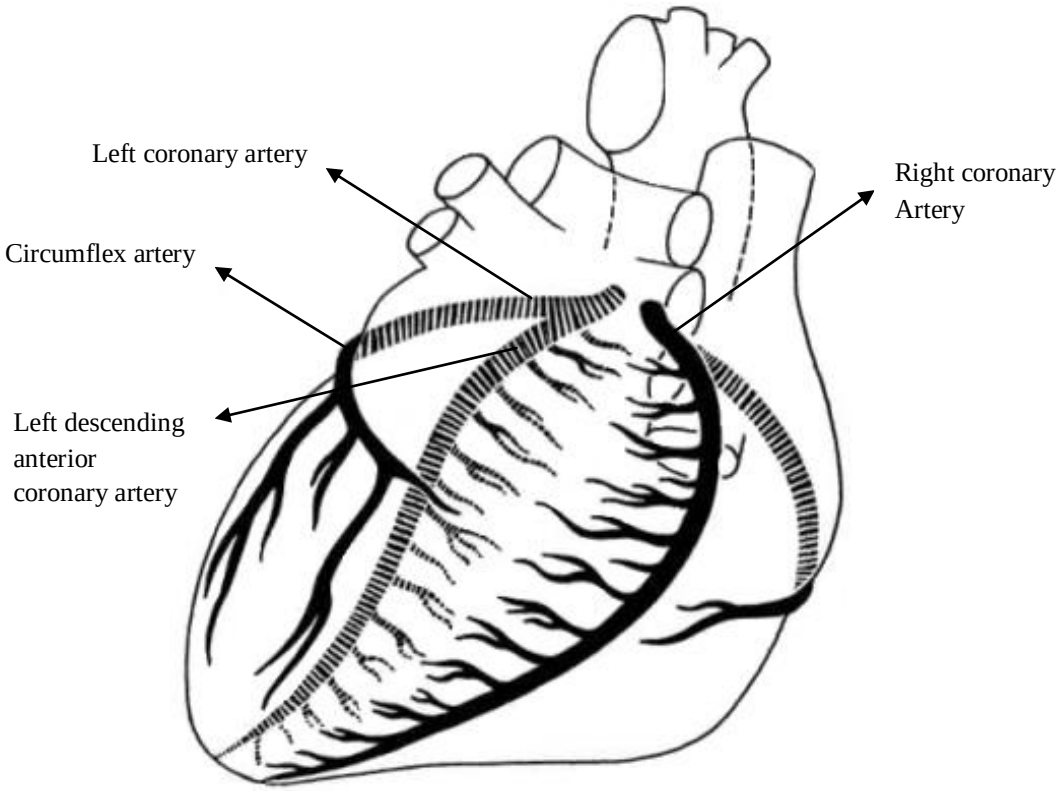

Figure 1. 2. Schematic representation of posterior view of the rat heart showing coronary arteries (modified from Ahmed et. al., 1978)

Therefore, posterior surface of heart of rat receive blood from circumflex branch of left coronary artery and also from right coronary arteries. In contrast to left coronary artery, the right coronary artery is smaller and supplies smaller area of myocardium in right side (Ahmed et al., 1978).

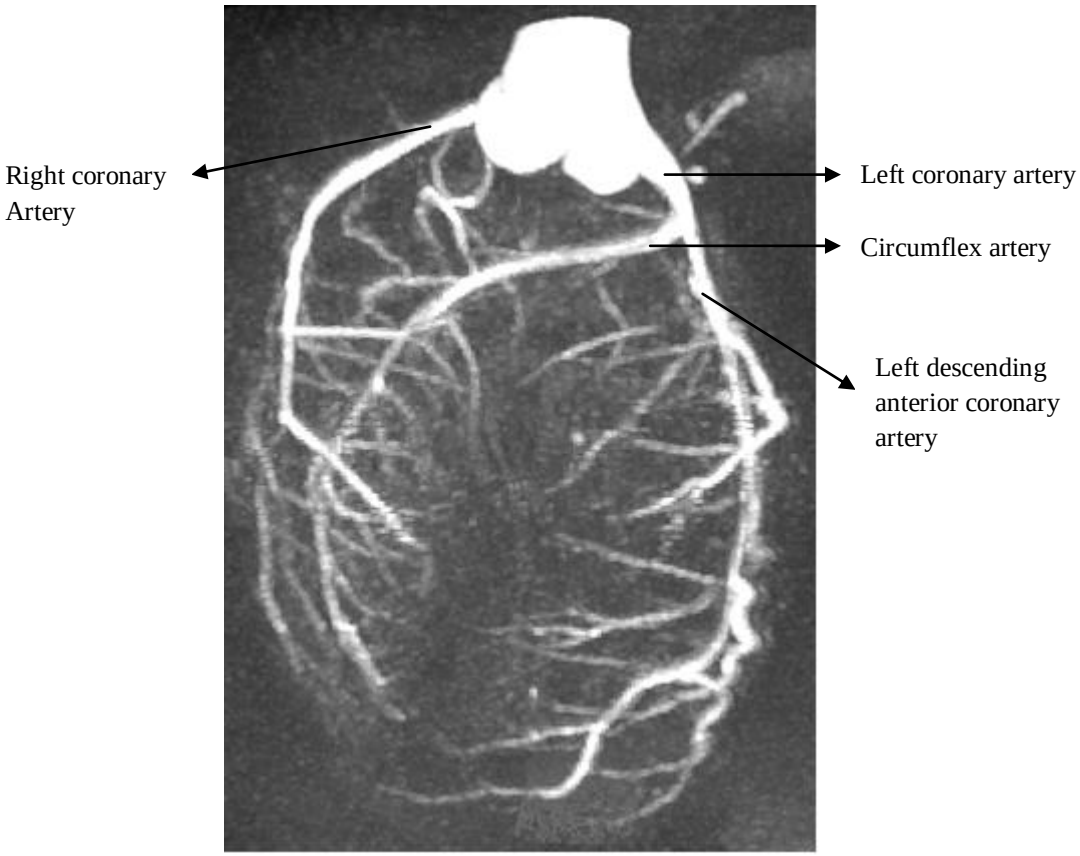

Figure 1. 3. Angiographic illustration of the coronary artery tree in the rat heart (From Waller et al., 2003)

The heart's wall is composed of three layers: epicardium, myocardium and endocardium. Endocardium covers the inner surfaces of the heart, including valves. Cells in this layer are tightly joint each other by tight junction. Myocardium, middle muscular layer, is consisted of cardiac muscle cells or myocytes, and it is the thickest of three layers. Myocardium is also responsible for contractile activity of cardiac muscle.

Cardiac muscle cells contain more mitochondria, and they are joined by complex intercellular junctions called as intercalated disks. There are specialized junctions along the intercalated disk such as gap junctions, which improve conduction. Epicardium is the outermost layer of the heart wall, which is also known as visceral layer of the serous pericardium. When body gets overweight, excess fat accumulates in this layer, which usually occurs in obese individuals (Tortora and Derrickson, 2009).

1.2. AUTOMATIC EXCITATION OF HEART AND CARDIAC CONDUCTION SYSTEM

There is autorhythmic activity in heart, which is provided by autorhythmic cells. Autorhythmic cells are the source of electrical activity of heart. They continuously generate action potential which induces heart contractions. Autorhythmic cells continue to generate action potential even if all nerves innervating the heart have been cut because they have ability of self-excitation Primary automatic cells of the heart are mainly found in specialized regions known as sinoatrial node, atrioventricular node. Automatic cells in sinoatrial node control automatic excitation of heart. Sinoatrial node is located in the right atrial wall just inferior and lateral to opening of superior vena cava. This node is called as pacemaker of heart (Bozdoğan, 2004). Sinoatrial node cells do not have a stable resting potential. In other words, they repeatedly depolarize to threshold level. Electrical activity or action potential is propagated to the right atria by interatrial pathway and to the left atria by Bachman's band that results in atrial contraction. After atrial contraction, action potential is travelled from sinoatrial node to atrioventricular node via a specialized pathway-internodal tract. Atrioventricular node is located in inferior posterior region of interatrial septum,

which impulse conduction is slowed. Thus, conduction delay occurs in the atrioventricular node. This delay has important physiologic consequences. It allows sufficient time for completion of atrial depolarization, contraction and emptying atrial blood into the ventricules before ventricular contraction (Guyton and Hall, 2006). From atrioventricular node, the action potential enter atrioventricular bundle which is located in the interatrial septum. Atrioventricular bundle is also known as bundle of His. This bundle is only site where action potential is travelled from the atria to ventricles. Then, action potential enters both right and left bundle braches, which extend through interventricular septum toward apex of heart. The bundle branches divides into extensive branches called Purkinje fibers. They conduct the action potential toward the ventricular myocardium, resulting in ventricular contraction **(Figure 1. 4)**

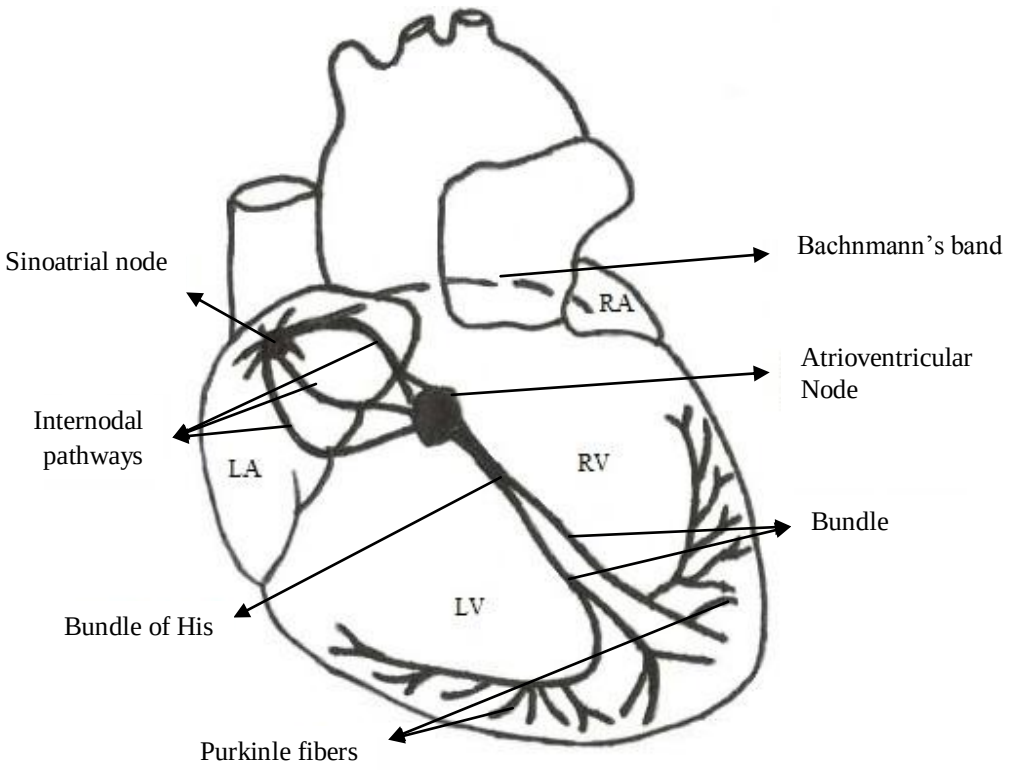

Figure 1. 4. Cardiac Conduction System

1. 3. ELECTROPHYSIOLOGICAL PROPERTIES OF CARDIAC CELL

Cardiomyocytes are excitable cells having the capacity to contract after excitation. The ion channels, ions pumps, concentration of ions (Na^+, K^+, CI^-, Ca^{2+}) in and outside of cell determine the membrane potential or membrane excitability across cell membrane. Depolarizations and action potential in myocardial cells occur by the Na^+ influx and repolarization by K^+ efflux from the cells. Two types of action potential are defined in myocardial cells **(Figure 1. 5).**

First one is nonpacemaker action potential that occurs in contractile cells. Second one is pacemaker action potential that occurs in nodal cells having automatic depolarizations. Contractile cells and nodal cells show differences in duration of resting potential and action potential. Nonpacemaker cells, such as atrial and ventricular cells have very rapid depolarizing phase, short plateau region and a true resting membrane potential whereas automatic cells have no plateau phase. The duration of action potential in sinoatrial and atrial muscle are about 150 ms whereas it is about 250 ms in ventricular muscle, and is about 300 ms in Purkinje fibers. Action potential in nonpacemaker cells is divided into five phases; Phase 0, phase 1, phase 2, phase 3, and phase 4 (Jaye et al., 2010). Phase 0 refers to rapid phase of depolarization due to the rapid increase in Na^+ and Ca^{2+} influx. Phase 1 is initiation repolarization caused by a small transient chloride flow into the cells and outward K^+ current via fast-activating and transient potassium channels and inactivation of Na^+ channels. Plateau or refractory period is called as phase 2 that mainly Ca^{2+} current occur and depolarized potentials is maintained. Increase in outward K^+ current and Ca^{2+} conductance through L-type calcium channels is responsible in the formation of this period.

After plateau phase, rapid repolarization occurs by the closing of slow Ca^{2+} channels, which is the phase 3. Phase 4 is the end of repolarization that the cells reach the resting state. At the end of phase 4, the membrane potential is near to K^{+} equilibrium potential.

Automatic cells have spontaneous action potentials. Unlike other cells, action potential in automatic cells is mainly carried out by slow, inward Ca^{2+} currents. Pacemaker action potentials are categorized into three phases: 0, 3 and 4. Phase 0, upstroke of action potential; result from an increase in Ca^{2+} conductance which drives the membrane potential toward the Ca^{2+} equilibrium potentials. Phase 3 in nodal cells is the repolarization phase caused by a rise in K^{+} conductance resulting in an outward K^{+} current. Following phase 3, phase 4 appear, which is responsible for automaticity. It is caused by increased Na^{+} conductance (Costanzo, 2007).

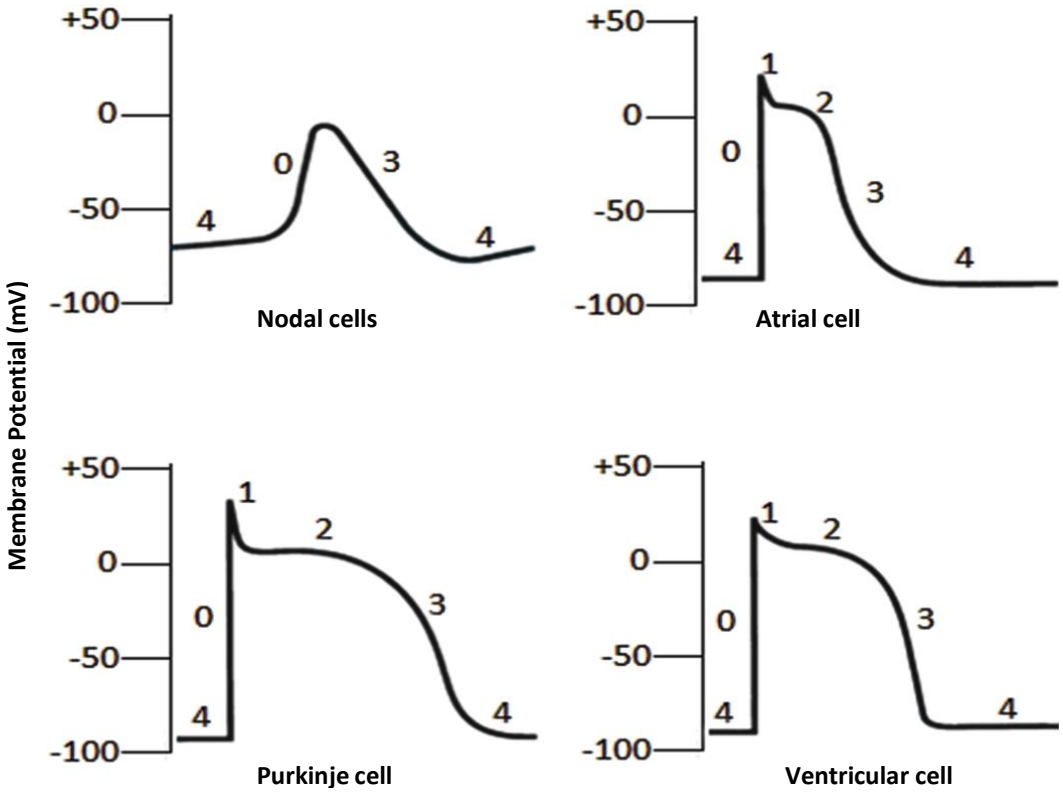

Figure 1. 5. Action potential in pacemaker and nonpacemaker cells

1. 4. CARDIAC ARRHYTHMIAS

Heart is a muscular pump of cardiovascular system that regulates its rhythmic automatic activity. This rhythmic activity is achieved by well coordinated impulse generation and conduction through heart. Cardiac arrhythmias occur due to any abnormality in conduction system or autonomic activity of heart or both. This type of abnormality occurs following ischemia or reperfusion of myocardial cells. In rats various arrhythmias can be observed in response to ischemia and reperfusion.

1. 4. 1. Sinus Rhythm

Normal sinus rhythm indicates that the rhythm is generated at a constant rate by the sinus node. There is a normal P wave followed by ORS-T waves on ECG. Heart rates in rats are about 300 to 450 beat/minutes, and mean arterial blood pressure normally ranges from 80 to 120 mmHg.

1. 4. 2. Sinus Arrhythmia

Impulse is originated from sinoatrial node but the interval between QRS wave are different. Underlying mechanism of this arrhythmia is irregular generation of automatic stimuli from SA node. In human being, it is usually associated with respiration. Heart rate increases during inspiration whereas heart rate decreases during expiration. In dogs, sinus arrhythmias can be normal and commonly seen rhythms (Martin, 2007). However, it is rare in rats, which can be seen depending on degree of anesthesia.

1. 4. 3. Sinus Bradycardia

Bradycardia is defined as slow heart rate. In human being, sinoatrial node discharges less than 60/min. In rats, heart rate below 250 beats/minutes is accepted as bradycardia. Increased vagal tone, hypothyroidism, hypothermia, ischemia are the possible causes of sinusal bradycardia. During sinus bradycardia, P waves, QRS and T wave are normal in appearance. This arrhythmia can normally be observed in sleep, in athletes and healthy young adult human being (Bender et al., 2011)

1. 4. 4. Sinus Tachycardia

Tachycardia refers to a heart rate that exceeds the normal resting heart rate. Sinus tachycardia is usually the body's normal reaction to stress, including fever, dehydration, or blood loss (shock). High sympathetic drive may also lead to sinus tachycardia. Normal P waves and shortened PR interval are electrocardiographic signs of sinus tachycardia. In rats, heart rate is higher than 500 beat/minutes in sinusal tachycardia.

1. 4. 5. Atrial Fibrillation

Atrial fibrillation is a supraventricular arrhythmia. In clinical practice, atrial fibrillation is the most common arrhythmia; however, atrial fibrillation is not usually observed in rat in response to myocardial infarction (Nishida et al., 2010). Atrial fibrillation is associated with old age, hypertension and ischemic heart in patient (Heeringa et al., 2006). No P wave and normal ORS complex but at irregular interval are electrocardiographic features of this abnormality. In contrast to ventricular fibrillation, atrial fibrillation is not a serious problem. However, formation of blood clotting in atrium is the risk associated with atrial fibrillation because blood can stagnate in nonbeating atrium (Downey, 2003).

1. 4. 6. Ventricular Tachycardia

Ventricular tachycardia is a serious rhythm disturbance that originates below the atrioventricular node. In this rhythm, QRS wave is widen and the rhythm is irregular. Ventricular tachycardia can be observed electrocardiographically in two forms; monomorphic and polymorphic ventricular tachycardia (Hudson et al., 2003).

If ventricular tachycardia originates from a single ectopic focus, it is termed as monomorphic ventricular tachycardia. In this type, the shapes of QRS are identical with each other. However, if ventricular tachycardia originates from multiple ectopic focuses, it is called as polymorphic ventricular tachycardia that is characterized with different QRS shapes **(Figure 1. 6).** In rats, ventricular tachycardia usually may be seen both during ischemia and reperfusion and as a result of sustained tachycardia it turns to ventricular fibrillation.

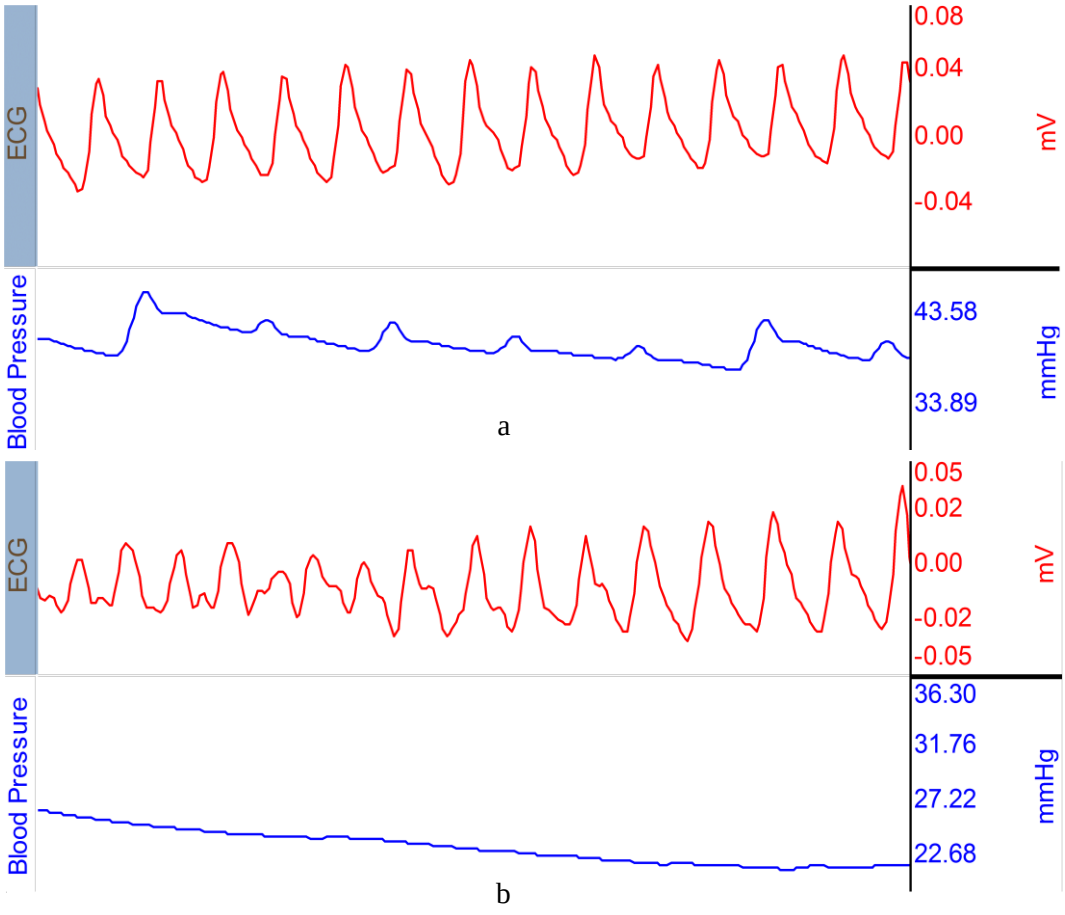

Figure 1. 6. Forms of Ventricular Tachycardia; a: monomorphic ventricular tachycardia, b: polymorphic ventricular tachycardia.

1. 4. 7. Ventricular Fibrillation

Ventricular fibrillation is the most dangerous type of arrhythmias, which is a major cause of sudden cardiac death. Ventricular fibrillation usually follows ventricular tachycardia. There is no significant coordinated contraction to produce cardiac output in the ventricular fibrillation and heart sounds cannot be heard. The blood pressure gradually falls toward zero, and the ECG shows no normal waves, there are irregular larger or smaller rapid waves (Cheung et al., 1993). Although sustained ventricular fibrillation result in death in human beings, spontaneous defibrillation occur after the long lasting fibrillation in rats.

1. 4. 8. Premature Contractions

Premature contraction occurs due to additional extra stimuli in or following regular beats. Premature contraction is named according to the origin of extra stimuli driving the heart. Contractile activity of the heart driven by extra stimuli is also known as extra systole, premature contraction or ectopic beat. Ectopic focus can be seen in the atria, ventricles, or the AV node (Guyton and Hall, 2006).

1. 4. 8. 1. Atrial Premature Contraction

Contraction originated from an ectopic focus in atria rather than SA node is referred to atrial premature contraction (APC). Early P wave, with an unusual shape, and normal QRS complex can be observed during atrial premature contraction in ECG (Bender et al., 2011). However, it is difficult to recognize this type of arrhythmia in ECG recorded from rats.

1. 4. 8. 2. Ventricular Premature Contraction

Ectopic beat which occurs anywhere in ventricle lead to ventricular premature contraction. Ventricular premature contraction is the most common type of arrhythmias seen in rats during ischemia-reperfusion injury. In rat, there is no P wave, and QRS complex is unusual shaped in ECG. Premature ectopic beat can occur single or three or more in pair. If it does comprise at least five consecutive beats, it is referred to as tachycardia (Martin, 2007).

1. 5. MECHANISM OF ARRHYTHMIAS

Cardiac arrhythmias are defined as irregular rhythmic activity of heart. Underlying mechanisms of cardiac arrhythmias is so complex that exact mechanism of some type of arrhythmias is still unclear. However, the mechanisms responsible for cardiac arrhythmias can generally divided into two categories as like abnormal impulse formation and reentry. Abnormal impulse generation is derived from abnormal automaticity or triggered activity.

1. 5. 1. Abnormal Automaticity

Spontaneous action potential is defined as automaticity that is a normal feature of sino-atrial node, atrio-ventricular node and His- Purkinje fibers. However, only cells found in sino-atrial node can control the heart rate and initiate heart beat under normal physiological conditions because they have a higher automaticity or a faster pacemaker activity. All other pacemaker is referred to as subsidiary or latent pacemaker. In normal condition, subsidiary pacemaker is suppressed. The 4^{th} phase of slow depolarization in automatic cells play important role in pacing the heart (de Bakker Jacques and van Rijen Harold, 2008).

Any changes in automatic depolarization alter the firing rate of pacemaker cell, thus causing rhythm disturbance. Autonomic nervous system have influential role in modulating phase 4. Enhanced sympathetic stimulation increases the rate of phase 4 depolarization, thereby enhancing subsidiary pacemaker activity. On the other hand, parasympathetic stimulation slows down the rate of phase 4 depolarization. Also, acetylcholine released from parasympathetic nerve hyperpolarizes the cell. Another factor leading to latent pacemaker or abnormal automaticity may be due to the increased interstitial collagen deposition or reduced connexin expression that causes the diminishing cell-to cell coupling. Hypokalemia and ischemia which reduce the activity of Na-K ATPase and increasing phase 4 diastolic depolarization, are also crucial factor affecting normal automaticity. Sinus tachycardia, atrial tachycardia, sinus bradycardia and accelerated idioventricular rhythm all may result from abnormal impulse formation (Tomaselli and Roden, 2005; de Bakker Jacques and van Rijen Harold, 2008).

1. 5. 2. Triggered Activity

Triggered activity is an abnormal impulse generation taking place during or following action potential that is also called as afterdepolarization. Two types of afterdepolarization are present: early afterdepolarization and delayed afterdepolarization **(Figure 1. 7).** If afterdepolarization occurs during repolarization of a next action potential or phase 3, it is known as early afterdepolarization. Early afterdepolarization appear when heart rate is slow and action potential duration is long (January et al., 1991). In contrast, delayed afterdepolarization is observed after repolarization or after phase 4 of action potential.

Main reason of delayed afterdepolarization is calcium overload such as during digitalis intoxication, increased beta adrenergic stimulation and reperfusion after a period of ischemia. Triggered activity causes atrial tachycardia, atrial fibrillation and ventricular tachycardia (Fozzard, 1992; Janse and Rosen, 2006).

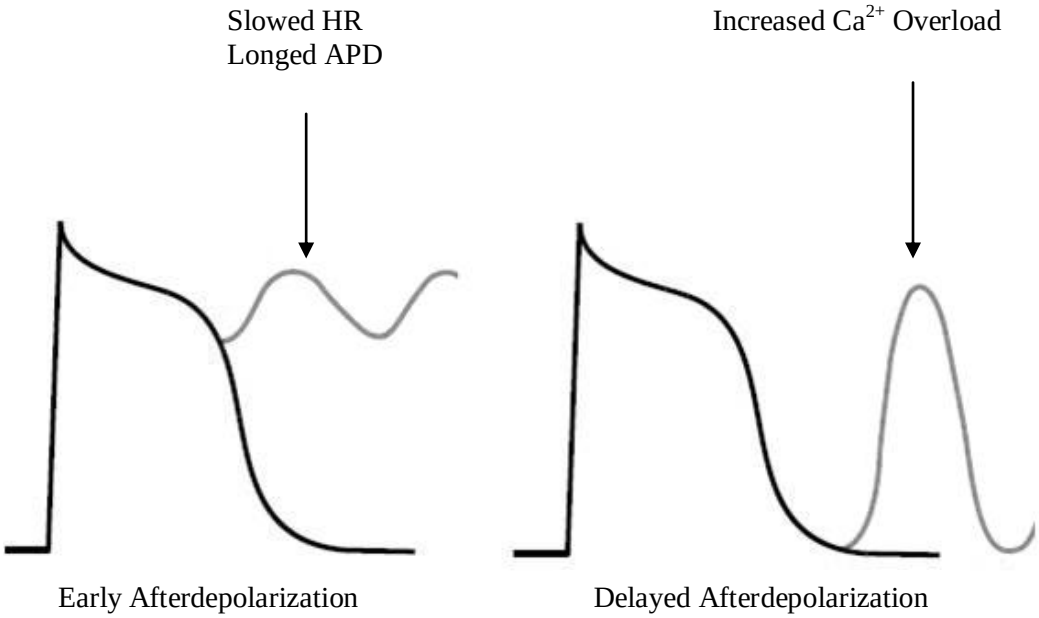

Figure 1. 7. Early and Delayed afterdepolarization (HR: Heart Rate, APD: Action Potential Duration)

1. 5. 3. Re-entrant Circuit

Reentry that is common causes of arrhythmias seen following ischemia and reperfusion occurs by reentrant impulse originating from ischemic area. Slow conduction of impulses through ischemic region excites the ventricles before sinusal impulses and reentrant impulses predominate. Re-entrant circuit continues to excite the myocardium that has just recovered from refractoriness until sinusal rhythm change.

Formation of reentry depends on the combination of conduction velocity and refractory period. It means that reduced refractory time and a low conduction velocity can make myocardium more susceptible to reentrant circuit. Most arrhythmias; atrial fibrillation, polymorphic VT, and ventricular fibrillation are caused by reentrant mechanism (Jafile et al., 2009).

1. 4. CELLULAR CHANGES FOLLOWING ISCHEMIA AND REPERFUSION

Myocardial ischemia occurs as a result of decreased blood flow to myocardium by a partial or complete blockage of coronary artery. The decrease in blood flow reduces oxygen supply and other factors essential for metabolic activity of cardiac cells. As a result of myocardial ischemia and reperfusion, important biochemical and electrophysiological changes occur. It is important to know these changes to cope with possible abnormalities.

Mitochondrial oxidative phoshorylation supply most of ATP needed for excitation-contraction coupling and other energy dependent reactions in cardiac muscle. With the loss of oxygen to heart, this activity ceases, and major source of ATP production is lost. Following, myocardial cell turns to perform anaerobic glycolysis in order to produce ATP. This results in accumulation of H^+ and lactate, which leads to acidosis. In addition, Na^+/K^+ ATPase is inhibited due to decline in ATP production. Consequently, Na^+ increases in myocardial cells whereas K^+ decline. Under normal conditions, Ca^{2+} ion is extruded for three Na^+ ions entering the myocytes by Na^+/Ca^{2+} exchanger. Then, excess Na^+ is actively washout by Na^+/K^+ ATPase. However, under conditions of ischemic or elevated intracellular Na^+, Na^+/Ca^{2+} exchanger does work in reverse direction. As a result, intracellular Ca^{+2} ions rise up (Cascio, 2001; Murphy and Steenbergen, 2007).

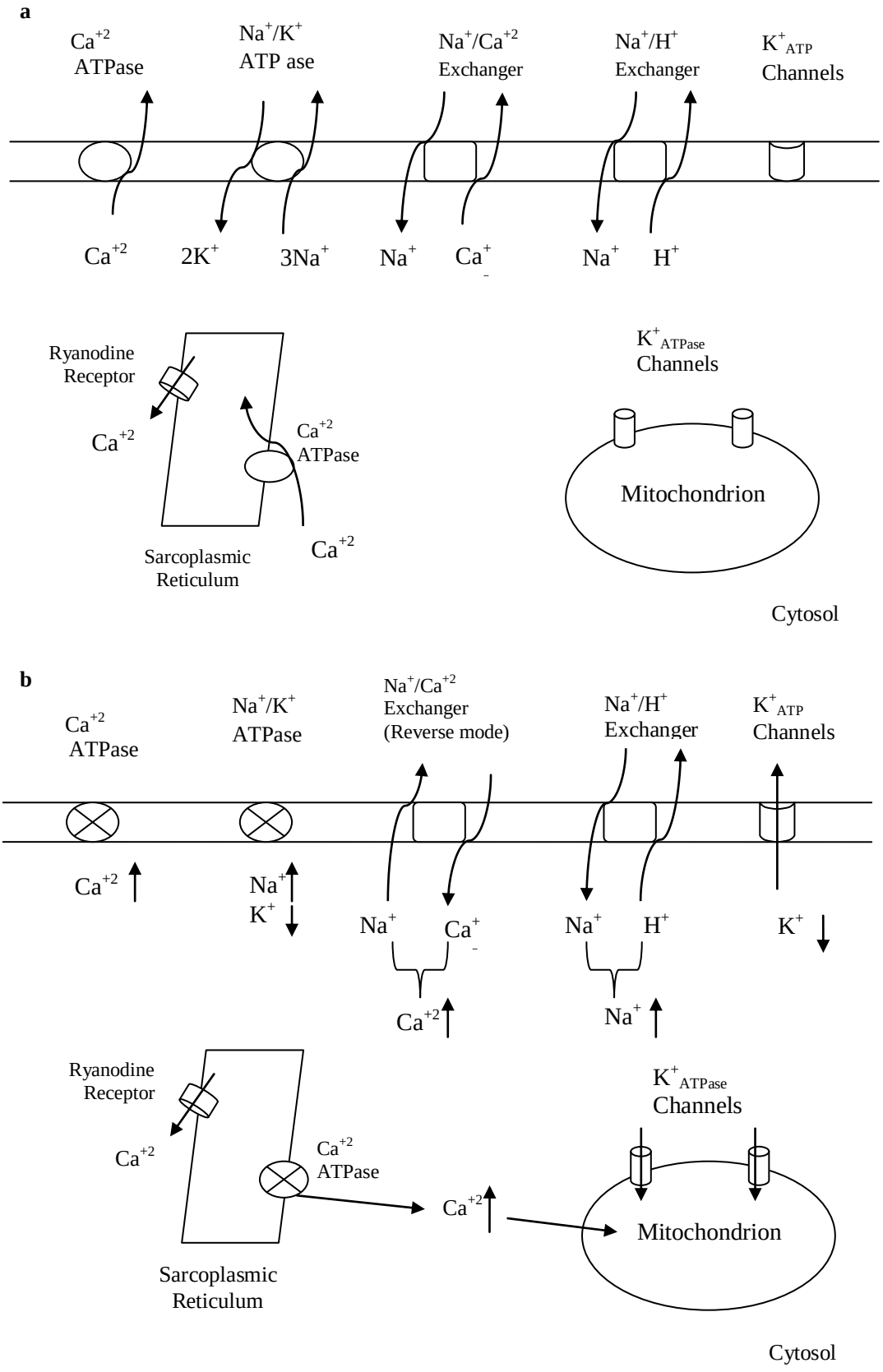

Figure 1. 8. Ionic changes under (a) normal and (b) ischemic condition

Increased Ca^{+2} ions in internal environment induce activation of proteases which causes alternation in contractile protein, decreases sensitivity to Ca^{+2}, lead to phospholipids degradation with release of lysophospholipid and free fatty acid. Otherwise, the rise in Ca^{+2} concentration may lead to activation of enzymes such as calpains involved in initiating apoptosis and necrosis (Murphy and Steenbergen, 2007). In the ischemic conditions, toxic oxygen species and free radicals are also generated from ischemic endothelial cells, and activated leukocytes that induce peroxidative damage to fatty acids of membrane phospholipids (Buja, 2005). The ionic changes during normal and ischemic conditions of myocardial cells are summarized in **Figure 1.8.**

Reperfusion injury refers to tissue damage resulting from reflow of blood to ischemic cell. Reperfusion is necessary to recovery of myocardial cells and maintaining cardiac functions; however, reperfusion itself leads to dysfunctions such as myocardial stunning-temporary loss of myocardial contractility, arrhythmias, irreversible injury (Goldhaber and Weiss, 1992; Piper et al., 1998; Moukarbel et al., 2004). There are several causes for the formation of reperfusion injury. Upon reperfusion, reactive oxygen species including superoxide anion, hydroxyl radical, hydrogen peroxide increase that is thought to be main factor causing reperfusion injury. Close interaction between reactive oxygen species and cell membrane lipids results in abnormalities in contraction and irreversible injury (Park and Lucchesi, 1999). After ischemic period, reoxygenation of cell leads to rapid energy production. Resynthesis of ATP from aerobic respiration enable cell to balance disturbed ionic concentration by reactivating Ca^{2+}-ATPase and Na^{+}/K^{+} ATPase pump.

Consequently, excess Na^+ is removed from cytosol of myocardial cell and cytosolic Ca^{2+} concentration decrease due to its entering into sarcoplasmic reticulum (SR) in the early phase of reperfusion. However, in later period of reperfusion when capacity of SR to Ca^{2+} level in cytosol is full, a cycle in which Ca^{2+} release from and uptake into SR is initiated. This cycle leads to Ca^{2+} overload induced hypercontraction.

Another important issue is that cell tries to renormalize H^+ gradient by action of Na^+/H^+ exchanger and Na^+/HCO_3^- symporter. As a result, Na^+ enters into cytosol and H^+ is excluded from cell. Entrance of Na^+ into cell activate the Na^+/Ca^{2+} exchange mechanism, which result in Ca^{2+} overload causing contraction abnormality (Piper et al., 1998; Piper et al., 2003).

1. 7. REPERFUSION ARRHYTHMIAS

Reperfusion induced arrhythmias is more severe and lethal in respect to ischemia induced arrhythmias. In human being, thrombolytic therapy following coronary occlusion provides reperfusion to ischemic myocardium, but lead to more myocardial damage and arrhythmias. In this case, these arrhythmias appear to occur as a result of increased heterogeneity in reperfused myocardial cells. Most of the death in the acute myocardial infarction occurs due to arrhythmia following reperfusion (Grech and Ramsdale, 1994).

1. 7. 1. Mechanism of Reperfusion Arrhythmias

Underlying causes of reperfusion arrhythmias have not been exactly understood. Lethal arrhythmias such as ventricular tachycardia and ventricular fibrillation occur intensively during reperfusion (Guo et al., 2005).

Several mechanisms have been suggested to be involved in generation of reperfusion induced arrhythmias. Both the heterogeneity and rapidity of the recovery from ischemia is believed to be two major factors in the occurrence of arrhythmias during reperfusion. Heterogeneity of electrical recovery results in reentry or enhanced ventricular automaticity (Corr and Witkowski, 1983). During rapid reperfusion after ischemic period, action potential duration and refractory period decline. Increased heterogeneity in duration of action potential around the ischemic zone is thought to facilitate ventricular fibrillation by enhancing reentry (Wit and Janse, 2001). It has been demonstrated that initiation of nonsustained ventricular tachycardia related with reperfusion of ischemic area results from non-reentrant mechanisms. Triggered activity and abnormal automaticity have been thought to be possible non-reentrant mechanism contributing to nonsustained ventricular tachycardia. But the sustained ventricular tachycardia during reperfusion occurs due to both reentrant and non reentrant mechanisms (Pogwizd and Corr, 1987).

Reactive oxygen species have important role in genesis of reperfusion-induced arrhythmia. It was reported that the pretreatment with allopurinol, inhibiting the xanthine oxidase which is an enzyme responsible for production of free radicals decreases incidence of reperfusion arrhythmias (Manning et al., 1984). It was also suggested that in isolated langendorff perfused heart, the occurrence of severe arrhythmia results from enhanced production of H_2O_2 during first minutes of reperfusion. Excess free oxygen radicals may exert their arrhythymogenic effect by inhibiting Na^+/K^+ ATPase activity and subsequently Ca^{2+}overload (Ravingerova et al., 1999).

1. 7. 2. Factors effecting reperfusion arrhythmias

Duration of occlusion is one of the most important factors determining severity of reperfusion arrhythmias. It has been suggested that in vivo ischemia-reperfusion model, the 5-6 minutes ischemia followed by 5-6 min reperfusion result in more severe arrhythmias than 15-16 minutes ischemia and reperfusion period. It has been shown that the incidence of ventricular tachycardia and ventricular fibrillation is significantly more in respect to longer ischemia and reperfusion (Kane et al., 1984).

Heart rate is thought to have an effect on intensity of reperfusion arrhythmias. It has been shown that rapid heart rate trigger reperfusion induced arrhythmias, especially ventricular fibrillation while slow heart rate is protective against arrhythmia induced by reperfusion (Murdock et al., 1980).

Ion concentrations inside and outside of the myocardium is important in development of arrhythmias during reperfusion. Depending on extracellular sodium concentration, duration of reperfusion arrhythmias shows variability. Low sodium concentration can decrease the intensity of reperfusion induced arrhythmias by preventing sodium and calcium entrance into myocardium and by leading myocardial potassium loss (Tosaki et al., 1989).

Gender differences are also important factor effecting reperfusion induced arrhythmias. Most recently, it has been showed that arrhythmic activity during 6 minutes reperfusion is significantly lower in female than that in male (Gonca and Bozdoğan, 2010).

1. 8. GENERAL EFFECTS OF INTERMITTENT FASTING AND WATER RESTRICTION

Various dietary regimens such as caloric restriction, alternate day fasting, intermittent fasting or Ramadan fasting have been extensively studied. Caloric restriction is a form of diet that restricts the caloric intake. Alternate day fasting is an approach in which overall caloric intake may not be limited, but the frequency of food consumption increases on alternate days (Varady and Hellerstein 2007). Intermittent fasting is also another regimen that feeding frequency is reduced (Ahmet et al., 2005; Mattson and Wan, 2005). In different experimental models, various forms of intermittent fasting have been studied. For examples, the effect of 24 h intermittent fasting has been performed in one or twice a week (Thomas et al., 2010; Buschemeyer et al., 2010). Ramadan fasting is voluntarily refraining of eating, drinking during daytime, which is thought to be unique model of intermittent fasting in human being (Aksungar-Benli et al., 2005). Ramadan fasting regimen during religious day Ramadan in Muslim people have been applied in 18 % of the world's population (Reilly and Waterhouse, 2007). The period and duration in which intermittent fasting can be different according to research models. The adverse or beneficial effect of intermittent fasting may depend on the duration and period of restriction, sex and age.

The general effects of intermittent fasting on organisms have been studied in many researches. It was shown in an early study that intermittent fasting prolonged the life span of rats (Carlson and Hoelzel, 1945). In the protocols of this study, intermittent fasting was applied as 1 day fasting in 4 day, 1 day fasting in 3 day and 1 day fasting in 2 day.

In this study, intermitted fasting was started at the age of 42 days rats and was continued until rats die. At the end of this study, it was shown that the longevity of animals increased in all fasting group in respect to control animals. It is interesting that longest life span was observed in rat fasted 1 day fasting in 2. The increased life span due to intermittent fasting was also observed in several other studies (Goodrick et al., 1983; Anson et al. 2003). There are two mechanisms which are thought to be responsible for increased life span by intermittent fasting are a decreased oxyradical production that reduces the amount of oxidative damage to cells and increases cellular stress resistance (Mattson and Wan, 2005). A significant decrease in generation of reactive oxygen species associated with a rise in spleen mitochondrial superoxide dismutase activity in mice maintained on alternative day fasting was shown in another study (Anson et al., 2003; Descamp et al., 2005).

Intermittent fasting can change circadian rhythms controlled by suprachiasmatic nuclei (SCN), the site of body clock depending on food availability time. Depending on circadian food intake analysis, intermittent fasting in which food is given to mice every other day for 24 h beginning at time of lights on during 3 week have been shown to decrease nocturnal peak of food consumption and also declined expression of some genes responsible for circadian activity (Froy et al. 2009). Intermittent diet restriction also play important role on the circadian variation of biological variables. For example, the restriction resulted in changing of circadian profile of gastric pH, plasma gastrin, insulin, glucose and calcium in blood plasma in human being in Ramadan (Iraki et al., 1997).

Various psychological changes occur in human being during intermittent fasting and dehydration. Irritability increases during daytime fasting, especially in smoker during intermittent fasting in Ramadan (Kadri et al., 2000). Besides, sleep habit was altered during Ramadan intermittent fasting. It was reported that intermittent fasting lead to delay in sleep onset associated with an increase in nocturnal body temperature and decreased sleep duration (Roky et al., 2001). The authors in this study suggested that the observed effect can be result from late meal consumption. Most recently, it has been suggested that in addition to shift in meal times, there are other factors can affect sleep pattern and circadian rhythms during Ramadan fasting (BaHamman et al., 2010). The authors have documented that the peak of body temperature and maximum energy expenditure are delayed in young healthy men. The most commonly observed problems in healthy person during Ramadan intermittent fasting is headaches. Headache frequency increase in proportional to duration of fasting (Leiper et al., 2003).

1. 8. 1. The Effects of Intermittent Fasting and Water Restriction on Heart and Circulation

The effect of intermittent fasting on cardiovascular system has been studied in vitro and in vivo studies. It was investigated the relationship between every other day fasting or intermittent fasting and cardiovascular functions by using telemetry system. Dietary restriction during one month resulted in decreased heart rate and systolic blood pressure compared to control rats fed ad libitum, especially in dark period; however, these parameters returned to control values when animals return to normal diets (Mager et al., 2006).

In a myocardial infarction model, it was reported that rats maintained on intermittent fasting regime had less infarct size at 24 hours after coronary artery ligation. Apoptotic activity and neutrophil infiltration in the area at risk was found to decrease. Depending on this finding, it was suggested that antiapoptotic and anti-inflammatory effect of restriction seemed to be responsible for reduction in infarct size. Also, many factors related to cardiac functions during acute disorder were documented. Left ventricular end diastolic volumes, left ventricle systolic volume was decreased in intermittent fasting groups (Ahmet et al., 2005). In this study, animals subjected to dietary restriction was found interestingly to have the decreased heart size. Most recently, different from the above results, the end systolic volume has been found to increase during the 3 month intermittent fasting diet in rats (Wan et al., 2010). In this study, circulating adiponectin, a hormone secreted from adipose tissue, has been elevated in diet restricted rats. In addition to antiapoptotic and anti-inflamatory effects, increased adiponectin level have been thought to be responsible for cardioprotective effect of intermittent fasting regime but it requires further experiments**.** Intermittent fasting was also reported to exhibit an important improvement in cardiovascular stress adaption in response to cold-water swim stress or immobilization stress (Wan et al, 2003). Fasting of animals for 16 h was reported to preserve adenine nucleotide content of myocardial cells during ischemia, normalize post ischemic glucose utilization, shorten recovery period, and decrease ischemic membrane damage after 15 minutes of total global ischemia (Schneider and Taegtmeyer, 1991). The cardioprotective effect of fasting is most likely due to the increased glycogen content of fasting heart**.**

There are limited studies to show the exact effects of fasting and hypodehydration on cardiovascular event in Ramadan intermittent fasting model. The studies that try to clarify the relationship between Ramadan fasting and cardiac events have been studied in various researches in different countries. That is why; it is difficult to compare the results obtaining from these studies, because the duration of Ramadan fasting change depending on the geographical site and season. A clinical study by Temizhan et al. (1999) suggested that the person having acute coronary heart disease hospitalized at emergency center of Ankara Numune Hospital between 1991 and 1997 was significantly lower during Ramadan. Another clinical study on 162 patients fasting during one month was revealed that the beginning of acute myocardial infarction symptoms such as unstable angina show significant differences between nonfasting and fasting individuals (Al Suwaidi et al., 2006). The occurrence of acute myocardial infarction was higher in human being having intermittent fasting during Ramadan that was also observed in above study. The investigators of this study suggested that the changes in food intake and/or sleep time during Ramadan intermittent fasting may explain the circadian rhythm of acute cardiac events. Besides, it was reported that the majority of patients with stable cardiac disease may have fasting during Ramadan without detrimental effect to the patients, because it was observed no significant changes in any of the hematological or biochemical parameters in patients with coronary artery disease, valvular heart disease and congestive heart failure (Chamsi-Pasha and Ahmed, 2004).

1. 8. 2. Biochemical Changes Occurring During Intermittent Fasting and Water Restriction

Several studies have been performed to show biochemical and cellular changes induced by fasting and refeeding cycle. In human being, the effect of Ramadan fasting which includes intermittent fasting and water restriction on oxidative stress parameters has been studied. Oxidative stress is defined as chemical or metabolic generation of reactive oxygen species such as superoxide radicals, hydrogen peroxide, singlet oxygen, lipid peroxidation (Jones, 2008). Lipid peroxidation was found to be decreased during Ramadan intermittent fasting (Ibrahim et al., 2008). The occurrence of lipid peroxidation has been determined by the increased level of malondialdehyde (MDA) in serum.

The intermittent fasting regime has beneficial effects on health. High density lipoprotein is known to play important role in uptake and transport of cholesterol to liver which is thought to be cardioprotective (Imaizumi et al., 2008). Changes in serum low-density and high density lipoprotein in response to Ramadan fasting stress has been shown in many studies. In a clinical study performed in healthy individuals, it was indicated that LDL reduced in the 15^{th} day and 30^{th} day of restriction but HDL increased (Qujeq et al., 2002). In another clinical study, it was demonstrated that high density lipoprotein level increased during one month intermittent fasting (Aksungar-Benli et al., 2005). In the same study, it was also observed that homocysteine level, a risk factor for cardiovascular diseases (Bonaa et al., 2006), decreased in healthy fasting volunteers. Interleukin-6 was found to be decreased during Ramadan fasting and remained depleted 20 days after restriction.

This indicates some positive effects of Ramadan fasting on inflammatory status of the body (Aksungar-Benli et al., 2007). Recently, it has observed that serum white blood cells count, interleukin-2, interleukin-8, and tumor necrosis factor-α decrease in healthy and obese individuals after Ramadan fasting (Unalacak et al., 2011). Concentrated urine formation is a sign of Ramadan fasting. Born et al. (1979) suggested that Ramadan intermittent fasting did result in increased haematocrit, serum albumin, and serum creatine. All of which indicate hypohydration due to water deprivation (Leiper et al., 2003). Fasting with fluid restriction was observed to change the lipid profile; total serum cholesterol level, apolipoprotein A-1 and apolipoprotein B increased (Campbell et al., 1994).

During dietary restriction, many significant differences in biochemical parameters and anabolic and catabolic reactions occur due to dehydration that has been observed experimentally (Kale et al., 2009). In the above study, it was observed that neutrophils, hemoglobin, platelets and basophiles concentration gradually increased in male rats in increasing fasting time.

1. 8. 3. Hormonal Changes Occurring During Intermittent Fasting and Water Restriction

Intermittent fasting with water restriction can cause rhythmic changes in secretion of hormones. In young male individual, one month intermittent food deprivation resulted in decreased testosterone and follicle stimulating hormone level (Mesbahzadeh et al., 2005). Contrary to this report, Bogdan et al. (2001) found that 24 h mean concentration of testosterone did not change but the time of hormone peak delayed during Ramadan.

Fasting was shown to modify the activity of hypothalamic neuropeptides through the signal molecules such as mitogen-activated protein kinases (MAPKs) and cAMP/ calcium-responsive element binding protein (CREB). MAPKs are serine/threonine protein kinases including extracellular signal-regulated kinases 1/2 (ERK) and p38, which is activated by various stimuli such as growth factors, cytokines, ischemia, heat shock. It has been demonstrated that fasting induces neuropeptide Y, increase phoshorylation of ERK, CREB and p38 in brain. Fasting induced phosphorylation of these protein kinases are associated with a decrease in the blood glucose concentration. By refeeding ad libitum for 1 h and 2 h after 48 hours fasting lead to attenuation of fasting induced phoshorlylation of ERK and CREB (Ueyama et al., 2004). Induction of diabetes in rats resulted in increase in expression of p38 level. Eight week intermittent food deprivation was reported to prevent the increase of p38 level (Tikoo et al., 2007).

Basal levels of ACTH and corticosterone have been shown to increase in rats maintained on the every other day fasting (Wan et al., 2003). Similarly, it was documented that serum corticosterone concentration in mice was elevated by fasting and returned to normal level by refeeding (Ueyama et al., 2004). It is known that cortisol is released when body is under stress for reasons such as trauma, infection, exposure to extreme temperature. The effects of cortisol is to help body to cope with short-term stressor by increasing blood glucose levels and promoting the use of fatty instead of glucose for energy production. It was shown that cortisol level and ACTH level in people increased in response to stress from fasting and water restriction (El-Migdadi et al., 2002).

Also, maintaining on one month intermittent fasting and water restriction was reported to cause changes in circadian pattern of cortisol. It was found that serum cortisol level increased in the afternoon and rise in the morning in fasting human (Bogdan et al., 2001).

Leptin is one of the most important adipose derived hormones that plays crucial role in regulation of energy metabolism, reduction of body fat mass. Repeated fasting and refeeding are known to increase capacity of fat storage in adipose tissue as an adaptive response to fasting (Kim et al., 2003). In this study, the adaptive response of leptin in repeated cycles of 24 h fasting and 24 h refeeding for 42 days was investigated in rats. The results indicated that repeated feeding followed by fasting lead to a chronic increase in plasma leptin level. It is interesting that significant rise in leptin secretion was observed after 26 days**.** In a clinical study in obese and lean healthy female, it was observed that serum leptin level increased by 39% in lean and 37% in obese individuals in one month Ramadan intermittent fasting (Kassab et al., 2003). Although leptin is associated with caloric intake and fat deposition, it was demonstrated that leptin was not significantly changed in trained young men during intermittent fasting (Bouhlel et al., 2008). Another study related with circadian patterns of leptin revealed that mean leptin concentration did not change during prolonged Ramadan fasting but a significant shift with 5 h delay in the leptin peak was observed (Bogdan et al, 2005). 48 hours fasting reduced leptin level in both young and old-age male Wistar rats (Kmiec et al., 2005). But, 24 hours refeeding period following 48 hours fasting resulted in reversion of leptin level to control value in young animals but not in old rats.

Adiponectin is another hormone secreted from adipose tissue, which increases the insulin sensitivity by stimulating fatty acid oxidation and decreases plasma triglycerides**.** Adiponectin levels in both young and old male rats did not change in response to 48 hours fasting followed by 24 hours refeeding period (Nedvidkova et al., 2005). However, it was demonstrated that adiponectin concentration was twofold greater in the plasma of rats maintained on every other day fasting for 3 months (Wan et al., 2010).

Thyroid hormones are also affected by fasting and refeeding. It was showed that there were no significant differences in thyroid- stimulating hormone (TSH), thyroxin (T4) and triiodothyronine (T3) level in women in pre and post fasting period, although THS level significantly increased within normal range in man (Demirbas et al., 2001). It was reported that Ramadan intermittent fasting did not change the pattern of free T3 and T4 while significant changes in time-related variations occurred in TSH; decreasing midnight and increasing afternoon (Bogdan et al., 2001).In contrast to above studies, it was demonstrated that T4 level decreased significantly in both genders during Ramadan fasting (Ahmedinejad et al., 2006). However, TSH level was found to be increased significantly only in male subjects.

Melatonin plays important role in various physiological processes such as circadian entrainment, blood pressure regulation, oncogenesis, seasonal reproduction and immune function (Altun and Ugur-Altun, 2007). In an extensive study, it is found that one month diurnal intermittent fasting resulted in a decrease in the 24 h mean serum level of melatonin, and lead to delay in time of the night peak (Bogdan et al., 2001).

2. MATERIALS AND METHOD

2. 1. Animals

In this study, 30 Male Sprague-Dawley, 8-9 months old rats were used. The rats were maintained under standard laboratory conditions. The animals were kept in 12-h light/12-h dark (lights on at 8 a.m. and off at 8 p.m.). Animals were individually caged. After one week of acclimination, the animals were randomly separated into three groups, each containing 10 rats. The intermittent fasting and water restriction were applied in 7 months old rats during one month. No food and water restriction were applied in control group which were fed ad libitium. Group 1 animals (IFW1) were maintained on intermittent fasting and water restriction for a month between 8 pm and 8 am for 12 hours (12 h of fasting and 12 h of refeeding). Group 2 animals (IFW2) were exposed to intermittent fasting and water restriction similar to group 1 and then it is followed by normal diet for one month. The reason for the formation of second group was to investigate the further effects of intermittent fasting and water restriction on the ischemia reperfusion induced arrhythmias after the animals returned to normal diet. All study protocol was approved by ethical committee of Abant Izzet Baysal University, Bolu, Turkey and all animals were treated in adherence to all of the guiding principles in the care and use of animals together with the recommendation from the Declaration of Helsinki.

2. 2. Ischemia-Reperfusion Protocol

Experimental animal was anaesthetized with sodium thiopental (60 mg/kg) administrated intraperitoneally and taken to operation table that heated automatically at 37 ° C. Blood glucose levels from tail vein was determined by blood testing equipment (OK meter Blood Glucose Meter, Taiwan) in all groups of animals before operation. The blood glucose concentration was expressed as milligrams of glucose for deciliter of blood. Left carotid artery was cannulated by catheter filled with heparin (25000UI/100ml) diluted 1/10 with saline for measuring systemic blood pressure (Harvard Model 50-8952 transducer). Tracheotomy that is the opening a cannular airway through an incision from trachea was performed using a polythene cannula for artificial respiration. The Chest was opened through a left thoracotomy by cutting the fourth and fifth ribs about 2 mm away from the sternum. After the incisition of pericardium, heart was exposed by a gentle pressure on the right side of the rib cage. A loose loop of 5-0 autramatic silk was quickly placed around the left anterior descending coronary artery (LAD) about 2mm from its origin. Then the heart was carefully repositioned in the chest and artificial respiration was immediately restarted with an animal respirator (Ugo Basile Rodent Ventilator, Italy) in 60–strokes/minute. Subcutaneous needle electrodes were placed under the skin to record standard electrocardiogram. Afterwards, animals were remained for 10 minute to stabilize blood pressure and heart rate. Six minutes ischemia was made by tightening the silk thread around the coronary by a bowknot, and six minutes reperfusion was achieved by loosening the bowknot.

In this operation any animals having arrhythmias or a sustained decrease in blood pressure to less than 70 mm Hg before ligation was discarded. The arterial blood pressure, bipolar ECG were recorded during 6 minutes ischemia and 6 minutes reperfusion and the recordings was stored in computer for later analysis.

2. 3. Determination of Risk of Infarct Zone

At the end of experiment, the survived animals was heparinized with 1/10 heparin (25000 IU/100 ml) via tail vein for 1 ml/100 mg body weight. Respirator was closed and the heart of the animal was get out by cutting the aorta. The heart was then perfused first with 10 ml serum physiologic, then with 2 ml ethyl alcohol to determine risk of infarct zone. Following the perfusion of heart, the non-perfused area (red color) was separated from the well perfused area (white color) through border line **(Figure 2. 1).** The non-perfused myocardium was cut and weighed. The percentage of non-perfused area in respect to the total weight of ventricle was calculated that is called as the risk of infarct area.

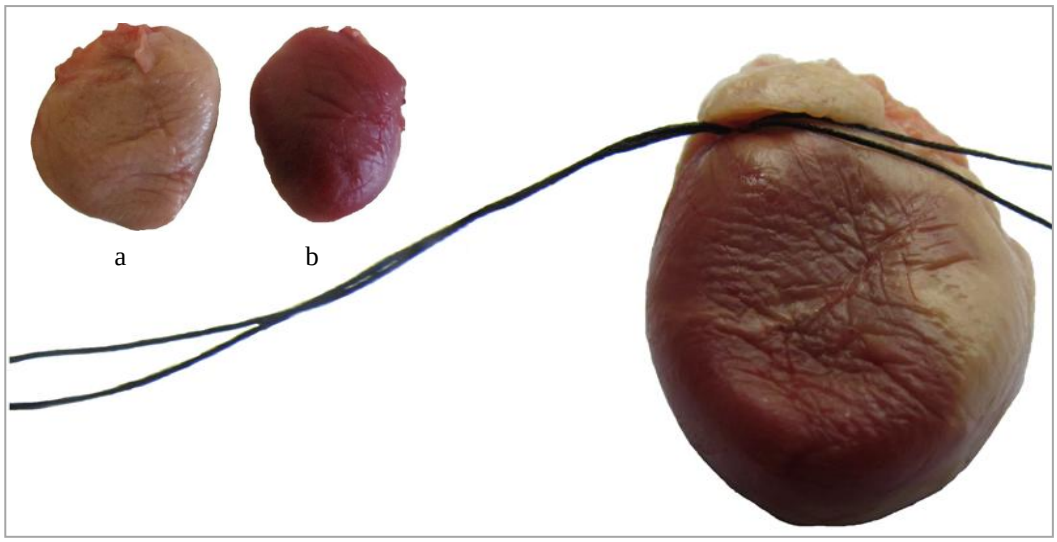

Figure 2. 1. Appearance of heart after perfusion with ethanol; a: perfused area, b: non-perfused area

2. 4. The Evaluation of the Arrhythmias

The heart rate and blood pressure were determined from recorded ECG at 1, 3, 5, minute during the ischemia and reperfusion. The incidence and total length of arrhythmias was calculated from recorded ECG during 6 minute of ischemia and 6 minutes of reperfusion. The arrhythmias were identified as ventricular tachycardia (VT), ventricular fibrillation (VF), and other type of arrhythmia including single ventricular extra beat, bigemini and salvos **(Figure 2. 2).**

The onset and offset of VT, VF and other types of arrhythmias were also measured. The sinusal tachycardia is differentiated from ventricular tachycardia according to heart rate and blood pressure. The arrhythmias are designated as sinusal tachycardia when the heart rate was 500/min to 600/min and the blood pressure was higher than 70 mmHg. However, ventricular tachycardia is described when the heart rate was above 600/min and blood pressure was lower than 50 mmHg **(Figure 2. 3).**

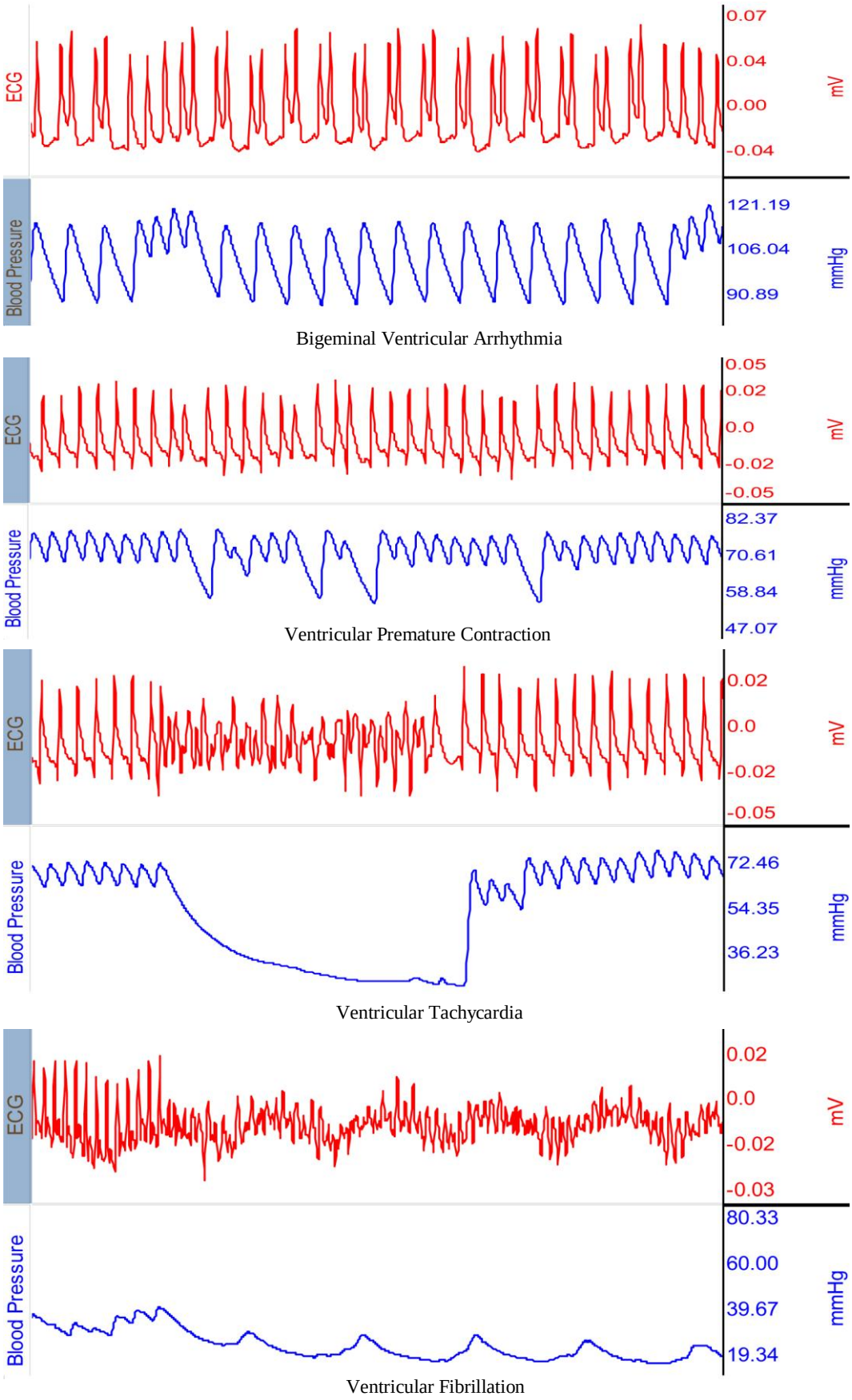

Figure 2. 2. Types of arrhythmias and blood pressure recorded during reperfusion

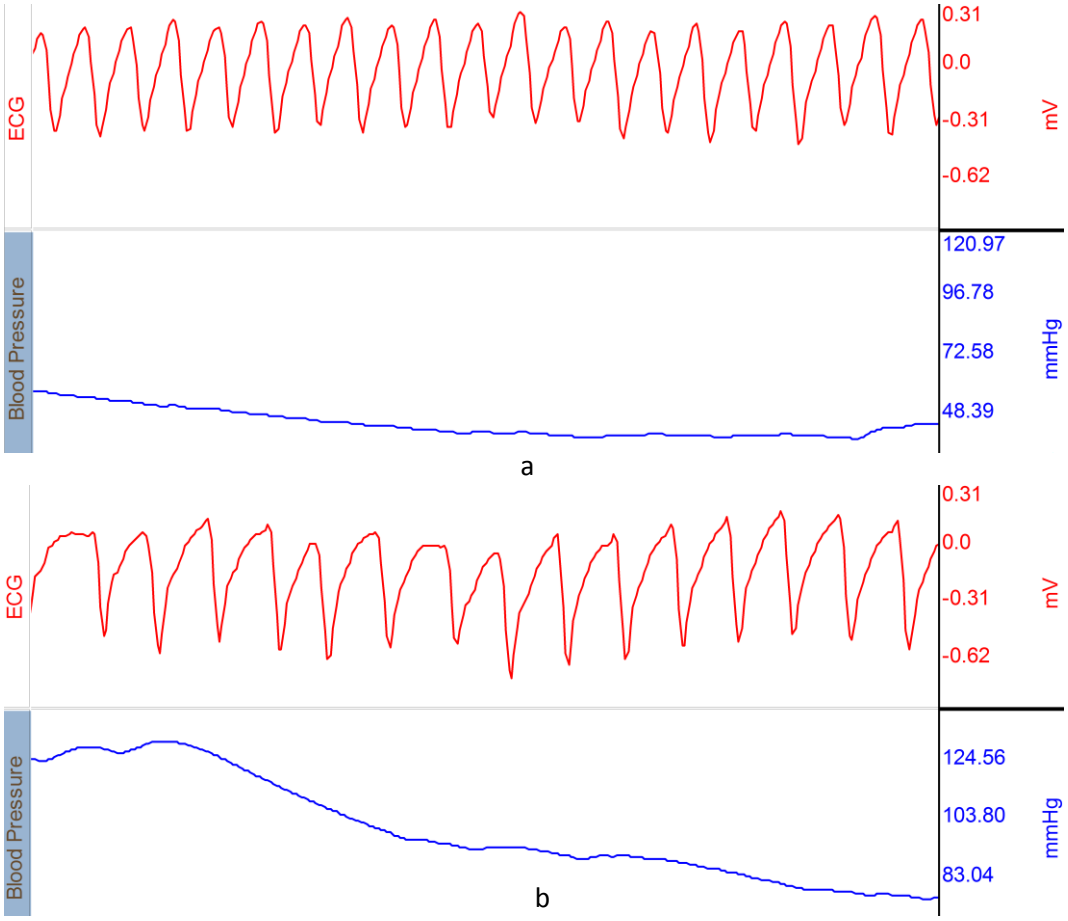

Figure 2. 3. The ECG pattern and blood pressure during (a) ventricular and (b) sinusal tachycardia.

An arrhythmia score was determined using the incidence and duration of arrhythmias for each animal in accordance with the Lambeth conventions (Walker et al., 1988) as follows; 0 = No arrhythmias; 1 = < 10 sec VT or other arrhythmias; 2 = 11–30 sec VT or other arrhythmias; 3 = 31–90 sec VT or other arrhythmias; 4 = 91–180 sec VT or other arrhythmias and / or < 10 sec reversible VF; 5 = > 180 sec VT or other arrhythmias and / or > 10 sec reversible VF; 6 = irreversible VF.

2. 5. Statistical Analyses

Data were analyzed using SPSS (SPSS Statistical Software, SPSS Inc., USA, and Ver. 17.0). The mean and standard errors were determined for all parameters including heart rate, blood pressure and body weight and blood glucose level. Data were analyzed by the one-way analysis of variance (ANOVA) combined with the LSD post hoc. test. The incidences of arrhythmia were assessed by Fisher exact test. Values were expressed as mean ± standard errors (S. E.). Differences between means yielding a probability $P< 0.05$ were considered as statistically significant.

3. RESULTS

ST segment elevation and alternation of QRS complex were observed in all experimental animals after coronary artery ligation. ST segment elevation was accepted as a sign of ischemia in electrocardiogram **(Figure 3. 1).** In unsuccessfully ligated animals, ST segment elevation was not present and these animals were excluded from evaluation.

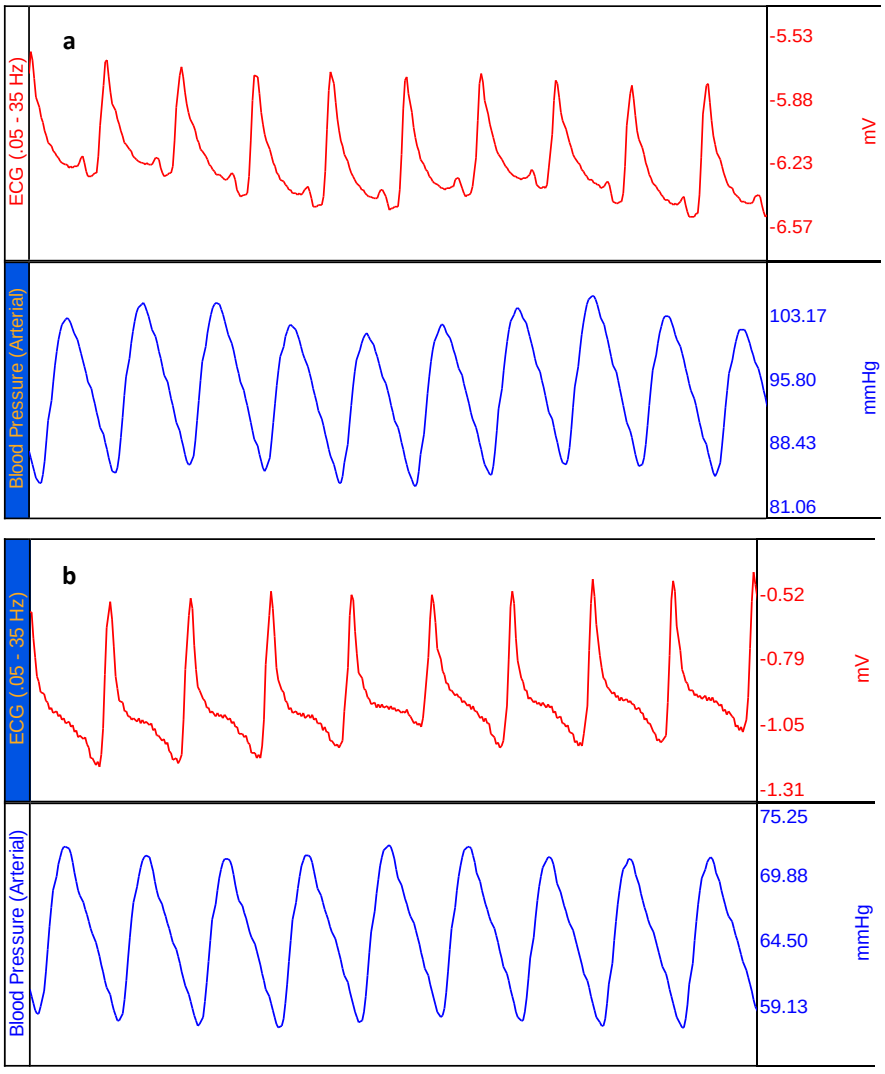

Figure 3. 1. ST segment elevation; a: before and b: after ligation

3. 1. Blood Pressure and Heart Rate

Blood pressure decreased rapidly following coronary artery ligation in all experimental groups **(Figure 3. 2, Table 1).** The mean blood pressure in anaesthetized rats was not significantly different among all three groups during ligation. Mean arterial blood pressure varied from 70 to 105 mmHg during ligation.

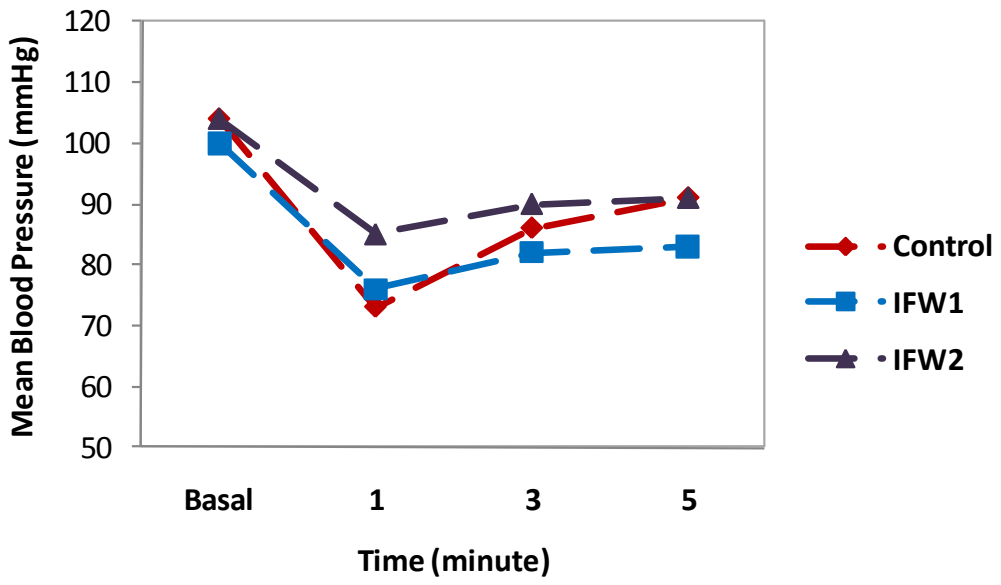

Figure 3. 2. Mean blood pressure during coronary artery ligation

Mean value of heart rate in IFW1 groups before ligation was higher than the control and IFW2 groups **(Figure 3. 3, Table 1).** However, this difference was not statistically significant. Heart rate was ranged from 380 to 396 beats/min.

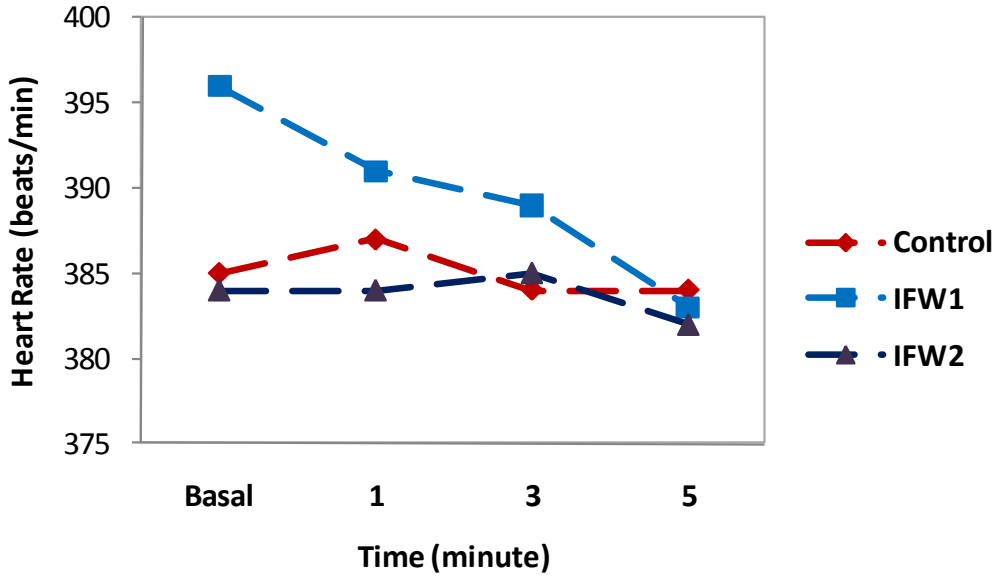

Figure 3. 3. Heart rate during coronary artery ligation

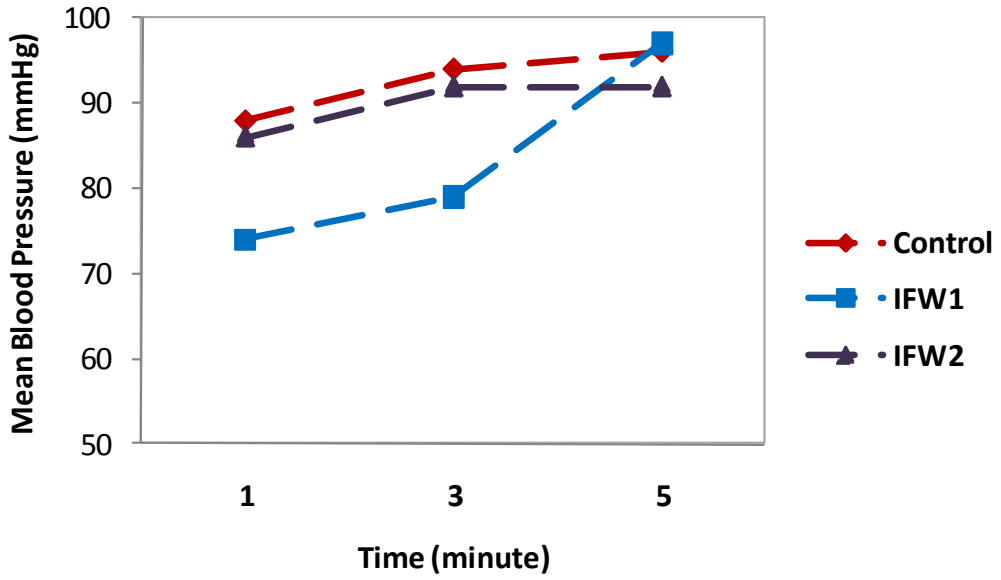

Figure 3. 4. Mean blood pressure during reperfusion

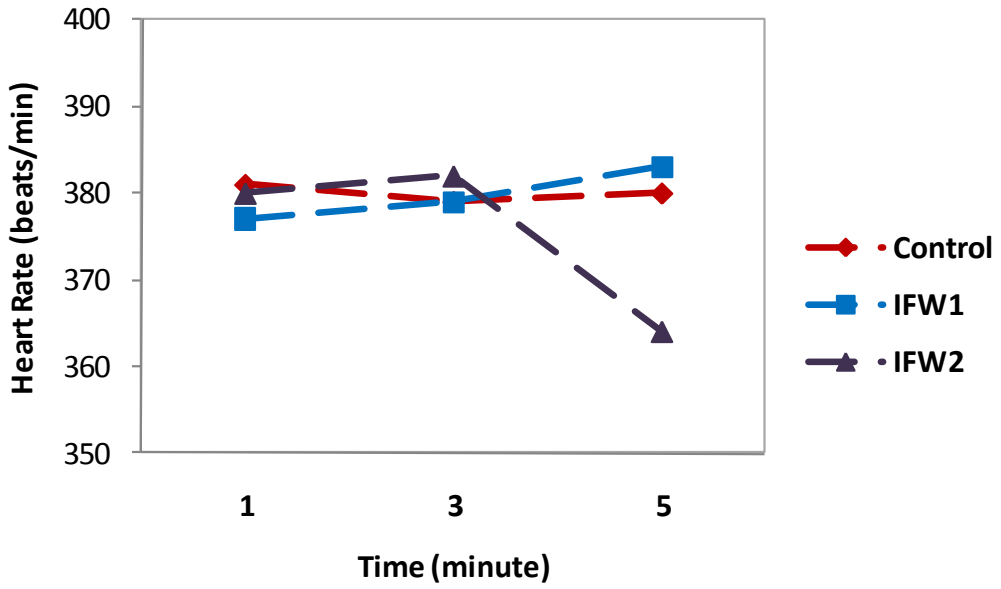

Figure 3. 5. Heart rate during reperfusion

Following reperfusion, mean blood pressure gradually increased in all experimental groups**.** In the initial phase of reperfusion (1st min), the mean blood pressure of IFW2 group was lower in contrast to other groups, and the mean blood pressures reached almost to basal value at the end of reperfusion **(Figure 3. 4)**. However, the difference in blood pressure among groups during reperfusion was not significant **(Table 2).** Besides, mean baseline value of heart rate in all groups was approximately in same range **(see Table 2).** But, heart rate of IFW2 group decreased gradually after 3rd minute of reperfusion but again this was not significant from other groups **(Figure 3. 5).**

3. 2. Arrhythmias and Risk of Infarct Zone

Arrhythmias generally started at about 2-3 min following coronary artery ligation **(Figure 3. 6.).** Arrhythmic period that is the difference between the start and the end of arrhythmia during 6 min. ligation in IFW1 group, was significantly longer compared to control and IFW2 ($P< 0.05$) (**Figure 3. 7, Table 3).** However, arrhythmic period during reperfusion was not found significantly different among experimental groups **(Figure 3. 8, Table 4).**

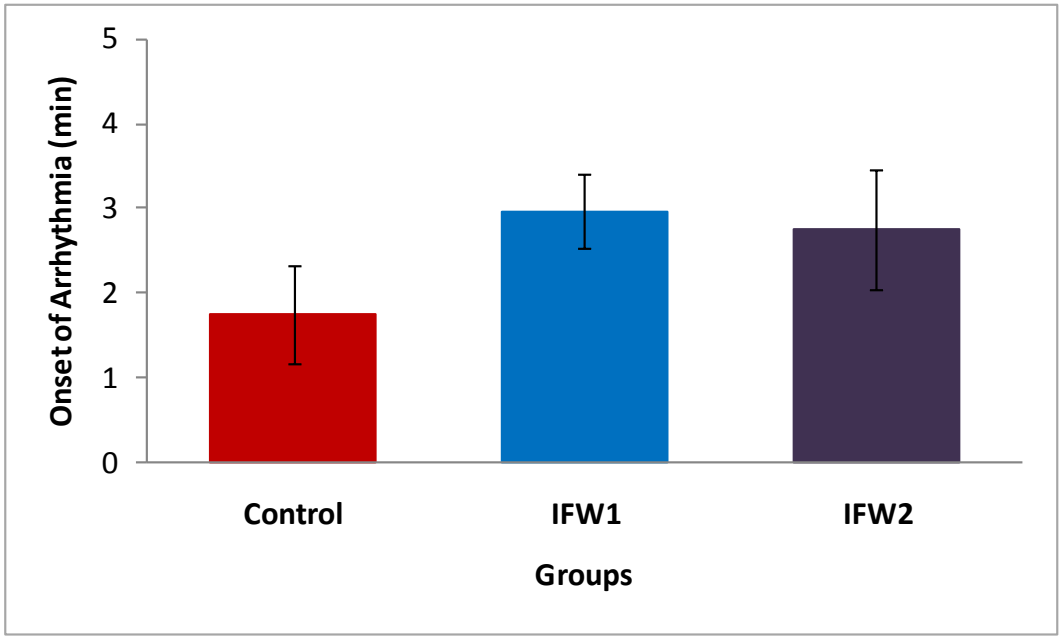

Figure 3. 6. Appearance of arrhythmias after 6 minute of ligation.

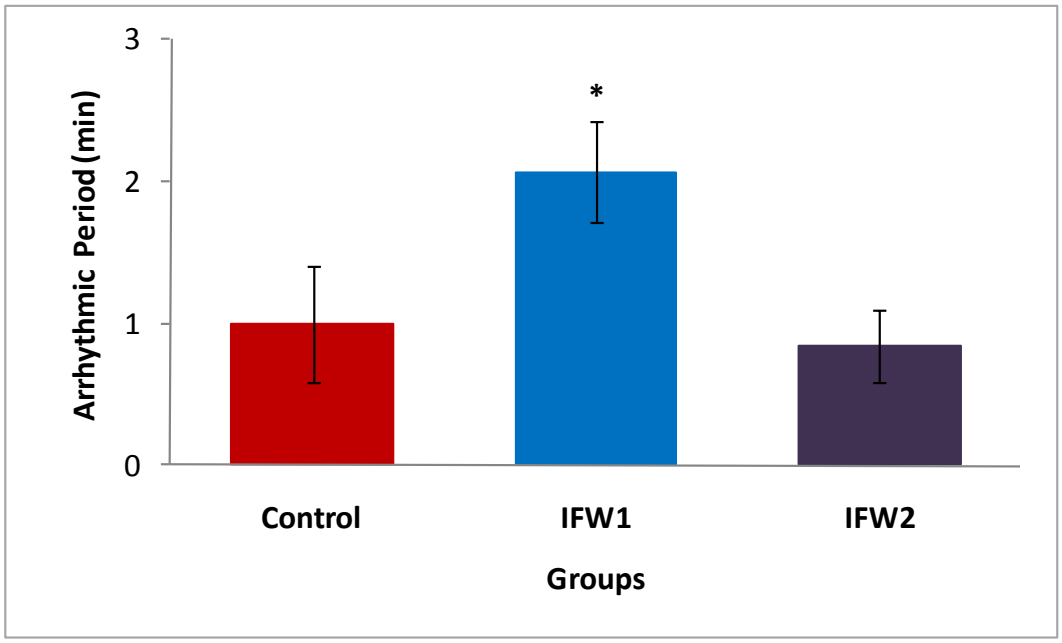

Figure 3. 7. Arrhythmic period during 6 minute of ligation
(*$P<0.05$; different from control and IFW2)

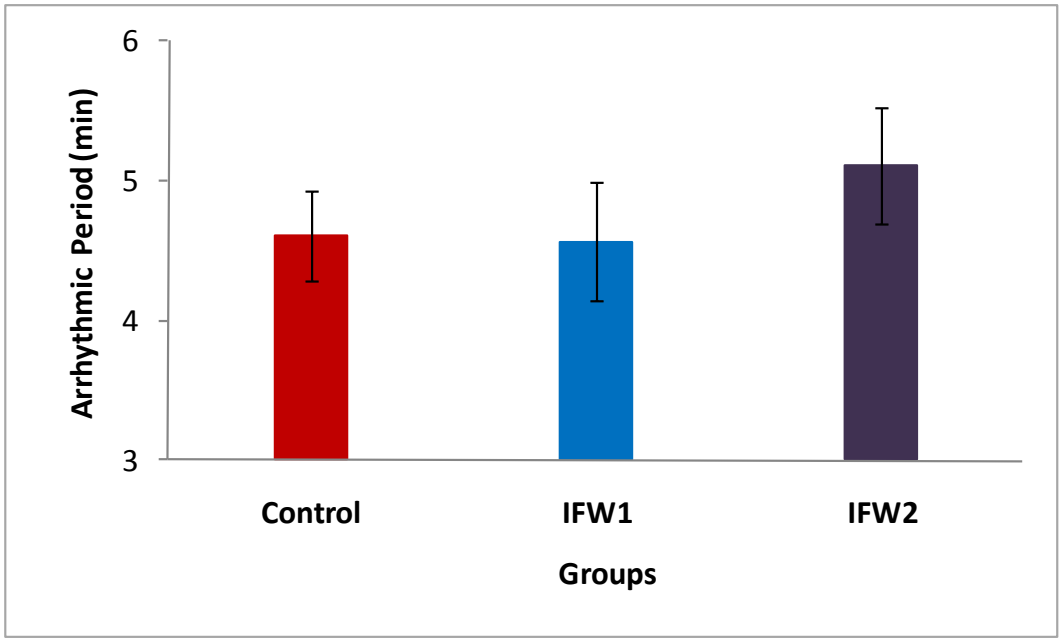

Figure 3. 8. Arrhythmic period during reperfusion

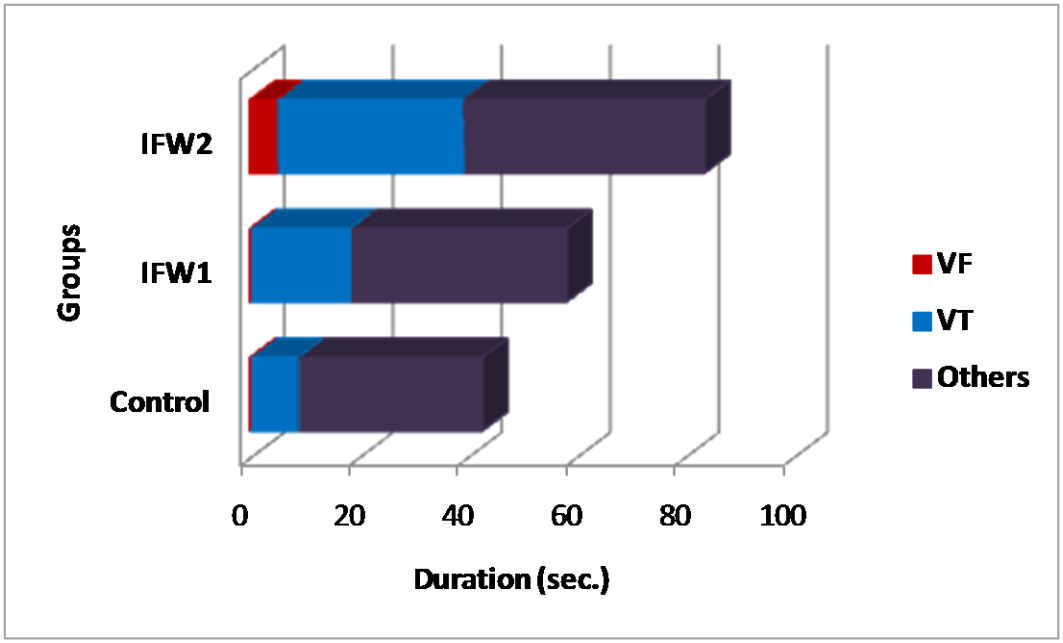

Figure 3. 9. The types and length of arrhythmic attacks during reperfusion

All types of arrhythmias including ventricular fibrillation, ventricular tachycardia and ventricular premature contractions were observed during 6 min. of reperfusion following 6 min. of ischemia. The total arrhythmia among groups was not significantly different **(Figure 3. 9).** Also, there were no significant differences in the incidence of arrhythmias among groups. Ventricular fibrillation was observed only in IFW2 groups **(Table 5).** Risk of infarct zone that indicates area at risk, showed statistically significant differences in IFW2 in respect to control and IFW1 groups ($P< 0.05$) **(Figure 3. 10, Table 6).**

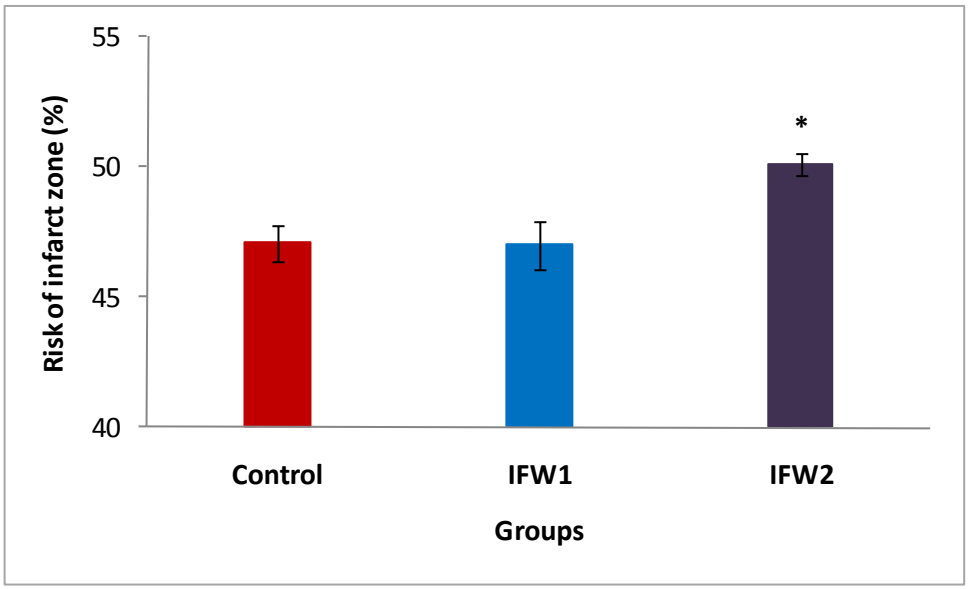

Figure 3. 10. Risk of Infarct Zone (*$P< 0.05$; different from control and IFW1)

3. 3. Biochemical Finding

After dietary restriction, blood glucose concentration was significantly lower in IFW1 compared to other two groups ($P<0.05$) **(Figure 3.11)**. Blood glucose closed to control value in animals of IFW2 group following normal feeding for one months after one month intermittent fasting **(Table 6)**.

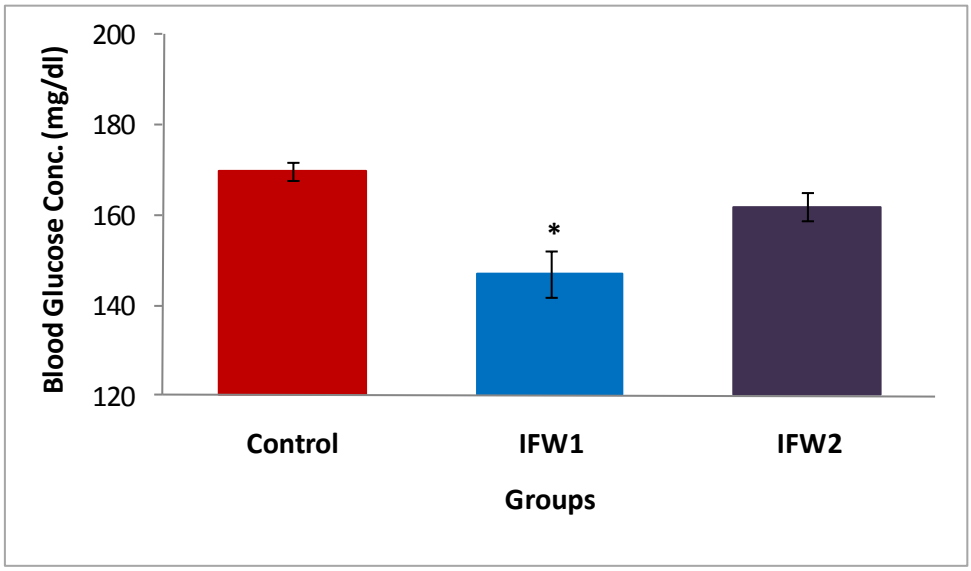

Figure 3. 11. Blood glucose level before coronary artery ligation
(*$P<0.05$; different from control and IFW2)

3. 4. Body Weight

Changes in body weight before and after dietary restriction were not significantly different between control and IFW1 group. However, body weight significantly increased in IFW2 groups after returning to the normal feeding habits at the end of one month (*P<0.05*) **(Figure 3. 12, Table 7)**.

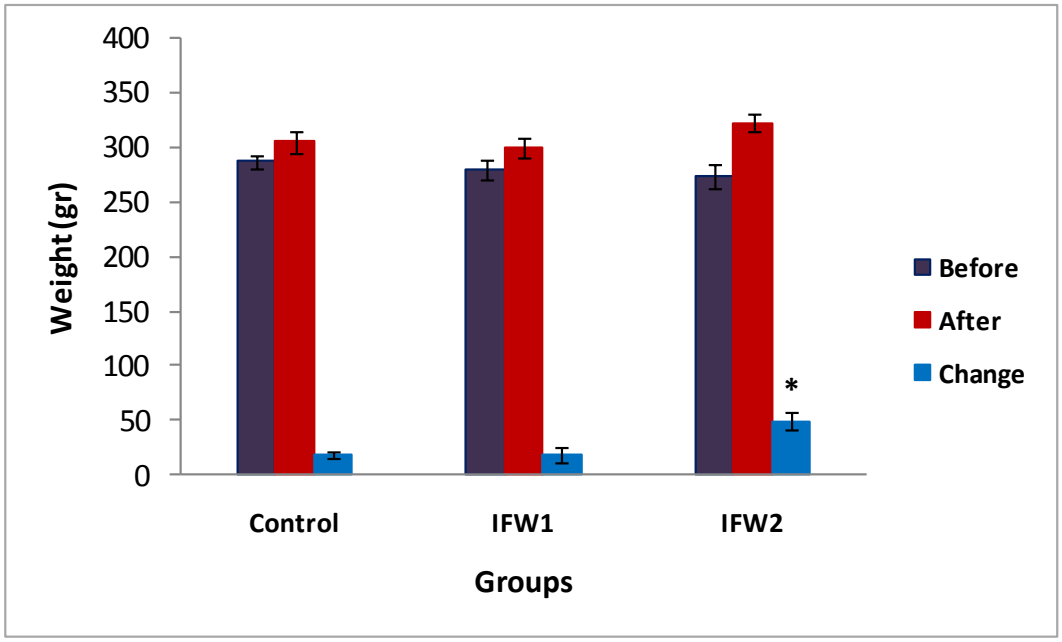

Figure 3. 12. Body weight change before and after dietary restriction
(**P<0.05*; different from control and IFW1)

4. DISCUSSION

The possible cardio protective effect of dietary restrictions including caloric restriction, every other day fasting without water restriction is not entirely clear. The effect of Ramadan intermittent fasting including water restriction on cardiovascular disorders has also not been exactly clarified. In clinical studies it has been observed that the intermittent fasting with water restriction results in increased HDL concentration decreased LDL level, increased insulin sensitivity. All of which has been thought to be protective against cardiovascular disease. However, there is controversy about the effect of intermittent fasting on the occurrence of cardiovascular disease in human being. In one of the clinical studies, it was demonstrated that acute myocardial infarction increased in fasting individuals during Ramadan. But, in another study, it was shown that Ramadan intermittent fasting had not any beneficial effect or detrimental effect on cardiac disease (Chamsi-Pasha and Ahmed, 2004). Unfortunately, there were no experimental studies researching the effect of intermittent fasting with water restriction. Although our study is not exactly comparable with the above study, our findings support the intermittent fasting and water restriction have no detrimental or protective effect against arrhythmias and survival rate of animals.

Our results demonstrated that the restriction did not affect the basal blood pressure and heart rate in anesthetized rats. Basal value of blood pressure and heart rate was not significantly different among groups. However, in previous studies, heart rate and blood pressure measured by telemetry was found to be decreased in rats subjected on every other day fasting without water restriction (Mager et al., 2006). The difference of our results may be due to the different experimental protocol and the type of restriction.

The length of dietary restriction is critical to show beneficial or adverse effect of the restriction on animals. It is exactly not known how much time is required for beneficial effects (Mitchell et. al., 2010). For examples, in Ramadan intermittent fasting, Muslims are approximately subjected to 12 hours fasting and 12 hours refeeding period. As mentioned before, duration of restriction can show differences depending on seasons, geographical locations. It is important to eliminate such differences for obtaining comparable results. There are no identical experimental restriction model with Ramadan fasting. However, different model of dietary restriction such as alternate day fasting, caloric restriction, intermittent day fasting and different length of the restriction such as 24 h fasting/24 hours refeeding, 48 h fasting/24 hour refeeding have been investigated in vivo and in vitro animal studies. For example, dietary restriction with every other day fasting without water restriction for 3 months reduced the ischemic damage to heart in rats (Ahmet et al., 2005). According to the findings of previous studies, we expected less arrhythmia following ischemia and reperfusion. But it did not happen in the present study.

This may be caused by different duration and length of the food and water restriction, which may not be enough to show any beneficial effect on ischemia-reperfusion induced arrhythmias. In our experiment, animals were maintained on 12 hours fasting and 12 hours refeeding period. Because it could not be found any significant differences in incidence of arrhythmia or arrhythmia score between nonfasting and fasting animal.

In this study it was found that the severity of arrhythmia increased non significantly in IFW2 group which was fed ad libitum for one month after one month restriction. The arrhythmic period during ischemia in IFW1 increased in respect to other groups. Increased arrhythmia in IFW2 group and increased arrhythmic period in IFW1 group may result from changes of metabolism or circadian rhythm of animals. In other words, animals can change their eating habit and awake/sleep cycle, resulting in a stress factor during intermittent fasting. It is known that rats are nocturnal animals characterized by activity during the night and sleeping during daytime. In our experimental design, animals in IFW1 and IFW2 groups could access to their nutrition during daytime, indicating great changes in biological rhythms. But animals in IFW2 groups had more stress than the other groups depending on the changes of feeding habit in two times. It has suggested that alternating period of anabolism and catabolism which occurs during intermittent fasting without water restriction may play an important role in triggering in cellular stress resistance and the repair of damaged cells (Martin et al., 2006). However, we could not observed this kind of stress induced protection on the ischemia and reperfusion induced arrhythmia in the animals having one month intermitted fasting followed by one month normal diet.

Recent studies have shown that animals maintained on dietary restriction without water restriction have less infarct size induced by myocardial ischemia (Ahmet et al., 2005; Katare et al., 2009). It has been revealed that asHIF-1, BDNF, and VEGF which are angiogenic factors are up-regulated in fasted heart and responsible for decreased infarct size. It was showed that the arterioles and capillary density increased in fasted myocardium. In response to ischemia, whether true angiogenesis or the recruitment of coronary collateral or both are responsible for the enhancement of blood flow to the ischemic area is not clear (Simons and Ware, 1996; Tabibiazar and Rockson, 2001). In the present study, area at risk was not found to be different between control and IFW1 groups; however area at risk was significantly higher in IFW2 group than the control and IFW1 groups. One explanation for high risk of infarct zone in IFW2 group may be related to the development of new collateral. Returning to normal feeding activity after one month dietary restriction may lead to changes in either process of angiogenesis or recruitment of coronary collateral in IFW2 groups. Stress induced by fasting and turning of the animals to the normal feeding may result in increased or decreased in biochemical parameter and rhythmic activity of hormones responsible for angiogenesis or collateral development.

The changes in energy balance are not consistent between studies. There are many investigations that have shown a reduction in body mass, body fat and a decrease in energy intake during the intermittent fasting for one month (Yucel et al., 2004; Ziaee et al., 2006; Bouhlel et al., 2008; Mansi, 2007). There are also many studies showing either no change or slight increase in body mass in human being (Finch et al., 1998; Beltaifa et al., 2002; Ibrahim et al., 2008).

In animal model of intermittent fasting with water ad libitum, it has been noted that body weight did not change in C57BL/6 mice and OF1 mice subjected to every other day fasting (Anson et al., 2003; Descamp et al., 2005). It was shown that body weight decreased during first 2-3 weeks and gradually increased over 8 week of restriction with water and the final body weight in control and fasting group did not change (Mager et al., 2006). Similarly, we could not observe any significant changes in body weight between control animals and animals maintained on one month water and food restriction (IFW1 groups). Otherwise the body weight of both groups increased approximately 18 g at the end of one month. The reason of no change in body weight in IFW1 group even after fasting period may be due to adaptation of animals to the new feeding habit. However, body weight in IFW2 group was increased as 49 g that was significantly different from control and IFW1 groups. Body weight gain in this group was expected results because animals in IFW2 group were aged one month than the others. The other factor may be stress induced over feeding in following months after food and water restriction.

The fasting and water restriction resulted in lowering blood glucose level from 170 mg/dl to 147 mg/dl in our study. The blood glucose level returned to baseline value that was approximately 162 mg/dl in IFW2 group. It is known that the fasting and refeeding cycle plays an important role in myocardial metabolism. When animals are unsuccessful in their forage during their active period, glucose uptake and utilization decrease in peripheral tissues such as heart, instead they use circulating fatty acids as substrates for ATP generation (Bray and Young, 2008).

It has been demonstrated that fasting increase myocardial glycogen content, which reduce injury and improve recovery of myocardium during and after ischemia (Schneider and Taegtmeyer, 1991). The authors suggest the higher ADP content in fasted heart may be important contributor factor for the recovery of myocardium after ischemia, since increased ADP provide a readily available substrate for rephosphorylation during reperfusion. In another study in isolated rat heart, the glycogen utilization increased, lactate level and creatine kinase releases decreased in animals having 24 hours. fasting (Schaefer and Ramasamy, 1997). In this study, it was suggested these changes may include in the protective effect of fasting against ischemic injury. Although we did not measure the above mentioned parameters, no beneficial or harmful effect of intermittent fasting and water restriction on ischemia-reperfusion induced arrhythmia was observed.

5. CONCLUSION

The dietary restriction similar to Ramadan fasting applied experimentally to the rats in this study did not alter blood pressure and heart rate. Besides, it was not observed any changes in the incidence of arrhythmia, survival rates, and arrhythmia scores in animals maintained on one month dietary restriction. Our hypothesis that the intermittent fasting and water restriction through one month will decrease ischemia and reperfusion induced arrhythmia in rats is not proved by the present data. This results support the clinical observations that the acute myocardial disorders did not change in hospitalized person having Ramadan fasting. There are no studies found related with the intermittent fasting and water restriction simulated as Ramadan intermittent fasting in animals. Therefore, the result of present study was not directly compared with the previous studies. Since there are various studies suggesting the myocardial damage decreased in the model of every other day fasting or the intermittent fasting without water restriction, further studies should be performed to clarify the effect of such restriction models on the ischemia or reperfusion induced arrhythmia.

Table 1. The heart rate and mean blood pressure during 6 minute of coronary artery ligation in anesthetized rats

Groups	N	Heart Rate (BPM)				Mean Blood Pressure (mmHg)			
		Basal	1 min	3 min	5 min	Basal	1 min	3 min	5 min
Control	10	385±5	387±5	384±5	384±5	104±3	73±4	86±5	91±4
IFW1	10	396±2	391±7	389±6	383±6	100±5	76±5	82±6	83±7
IFW2	10	384±6	384±6	385±7	382±6	104±4	85±6	90±8	91±6

N: The number of animals. Basal: Before coronary artery ligation and 1, 3, 5 min after coronary artery ligation.

Data are mean ± SE.

Table 2. The heart rate and mean blood pressure during 6 minute reperfusion in anesthetized rats

Groups	N	Heart Rate (BPM)			Mean Blood Pressure (mmHg)		
		1 min	3 min	5 min	1 min	3 min	5 min
Control	10	381±7	379±6	380±6	88±5	94±5	96±6
IFW1	10	377±7	379±9	383±6	74±6	79±10	97±6
IFW2	10	380±5	382±7	364±10	86±8	92±9	92±11

N: The number of animals. 1, 3, 5 min during reperfusion.

Data are mean ± SE.

Table 3. The duration of the arrhythmias during 6 minute of coronary artery ligation in anesthetized rats

Groups	N	Appearance of arrhythmia (min)	Arrhythmic period (min)	Length of arrhythmic attacks (s)				
				VF	VT	Other	Total	Bradycardia
Control	10	1,75±0,58	1,00±0,41	0	0	7,11±1,88	7,11±1,88	0
IFW1	10	2,97±0,44	2,07±0,36*	0	0	9,18±2,52	9,18±2,52	0
IFW2	10	2,75±0,70	0,84±0,26	0	0	8,62±1,85	8,62±1,85	0

N: The number of animals at the beginning of ligation; VF: Ventricular fibrillation; VT: Ventricular Tachycardia; Other: Ventricular premature contraction, bigemini, AV nodal arrhythmia and salvos.

Data are mean ± SE

*$P<0.05$; different from other groups

Table 4. The duration of the arrhythmias during 6 minute reperfusion in anesthetized rats

Groups	n	Appearance of arrhythmia (min)	Arrhythmic periods (min)	Length of arrhythmic attacks (seconds)				
				VF	VT	Other	Total	Bradycardia
Control	10	0,08±0,35	4,61±0,32	0	8,96±3,61	33,86±7,21	42,78±9,74	0
IFW1	10	0,04±0,01	4,57±0,43	0	18,56±6,75	39,77±9,50	58,30±10,99	0
IFW2	10	0,37±0,19	5,11±0,42	6,28±4,24	34,42±21,13	44,21±11,29	84,36±27,35	0

N: The number of animals at the beginning of the reperfusion; VF: Ventricular fibrillation; VT: Ventricular Tachycardia; Other: Ventricular premature contraction, bigemini, AV nodal arrhythmia and salvos.

Data are mean ± SE

Table 5. The incidence of the arrhythmias during 6 minute reperfusion in anesthetized rats

Groups	N	Survival (n/%)	Incidence of arrhythmia (n/%)				Arrhythmia score
			VF	VT	Other	Bradycardia	
Control	10	10/100	0/0	7/70	10/100	0/0	2,70±0,21
IFW1	10	10/100	0/0	8/80	10/100	0/0	2,90±0,28
IFW2	10	10/100	3/30	8/80	10/100	0/0	3,30±0,42

N: The number of animals at the beginning of the reperfusion; VF: Ventricular fibrillation; VT: Ventricular Tachycardia; Other: Ventricular extra beat, bigemini and salvos.

Data are mean ± SE

Table 6. The risk of infarct zone (%) occurring during 6 minute of coronary artery ligation in anaesthetized rats

Groups	N	Risk of Infarct Area (%)
Control	10	47,08±0,68
IFW1	10	47,02±0,93
IFW2	10	50,11±0,41*

N: The number of animals.

Risk of Infarct Area (%): Risk of infarct area / all of the heart

Data are mean ± SE

*$P<0.05$; different from other groups

Table 7. Blood glucose concentration before coronary artery ligation anesthetized rats

Groups	N	Glucose (mg/dl)
Control	10	170,0±2,02
IFW1	10	147,5±5,32*
IFW2	10	162,6±3,22

N: The number of animals survived

Data are mean ± SE

* $P<0.05$; different from other groups

REFERENCES

Ahmed S. H., Rakhawy M. T., Abdalla A. and Assaad E. I. 1978**.** The comparative anatomy of the blood supply of cardiac ventricles in the albino rat and guinea-pig. Journal of Anatomy 126: 51-57

Ahmedinejad Z., Ziace V., Rezae M., Yarmohammadi L.,Shaikh H., Bozongi F., Yousefi R. and Behjatiardekani J. 2006. The effect of Ramadan fasting on Thyroid Hormone Profile: A Cohort Study. Pakistan Journal of Biological Sciences 9: 1999-2002.

Ahmet I., Wan R., Mattson M. P., Lakatta E. G. and Talan M 2005. Cardioprotection by Intermittent Fasting in Rats. Circulation 112: 3115-3121.

Akao M., Takeda T., Kita T., Kume T. and Akaike A. 2007. Serofendic Acid, a Substance Extracted from Fetal Calf Serum, as a Novel Drug for Cardioprotection. Cardiovascular Drug Reviews 25: 333–341.

Aksungar-Benli F., Eren A., Ure S., Teskin O. and Ates G. 2005. Effects of Intermittent fasting on Serum Lipid Levels, Coagulation Status. Annals of Nutrition and Metabolism 49: 77-82.

Aksungar-Benli F., Topkaya A. E. and Akyildiz M. 2007. Interleukin-6-Reactive Protein and Biochemical Parameters during Prolonged Intermittent Fasting. Annuals of Nutrition and Metabolism 51: 88-95.

Al Suwaidi J., Bener A., Gehani A. A., Behair S., Mohanadi D. AL., Salam A. and Binali HA Al. 2006. Does circadian pattern for acute cardiac events presentation vary with fasting? Journal of Postgraduate Medicine 52: 30-33.

Altun A. and Ugur-Altun B. 2007. Melatonin: therapeutic and clinical utilization. International Journal of Clinical Practice 61 (5): 835-845.

Anson R. M., Guo Z., Cabo R., Lyun T., Rios M., Hagepanos A., Ingram D. K., Lane M. A. and Mattson M. P. 2003. Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from caloric intake. Proceedings of the National Academy of Sciences 100: 6216-6220.

BaHammam A., Alrajeh M., Albabtain M. and Baham S. 2010. Circadian pattern of sleep, energy expenditure, and body temperature healthy men during the intermittent fasting of Ramadan. Appetite 54:426-429.

Beltaifa L. , Bouguerra R. , Ben Slama C. , Jabrane H. , El-Khadhi A. , Ben Rayana M. C. and Doghri T. 2002. Food intake, and anthropometrical and biological parameters in adult Tunisians during fasting at Ramadan. Eastern Mediterranean Health Journal 8: 603-611.

Bender R. J., Russell S. K., Rosenfeld E. L. and Chaundry S. 2011. Oxford American Handbook of Cardiology. Oxford University Press, New York. Pp: 358-360.

Bogdan A., Bouchareb B. and Touitou Y. 2001. Ramadan fasting alters endocrine and neuroendocrine circadian patterns. Meal-time as synchronizer in humans? Life Sciences 68: 1607-1615.

Bogdan A., Bouchareb B. and Touitou Y. 2005. Response of circulating leptin to Ramadan daytime fasting: a circadian study. British Journal of Nutrition 93:515-518.

Bonaa K. H., Njolstad I., Ueland P. M., Schirmer H., Tverdal A., Steigen T., Wang H., Nordrehaug J. E., Arnesen E. and Rasmussen K. 2006. Homocysteine Lowering and Cardiovascular Events after Acute Myocardial Infarction. The New England Journal of Medicine 354:1578-1588.

Bouhlel E., Denguezli M., Zauoli M., Tabka Z. and Shephard R. 2008. Ramadan Fasting's Effect on Plasma Leptin, Adiponectin concentration, and Body Composition in Trained Young Men. International Journal of Sport Nutrition and Exercise Metabolism 18: 617-627.

Bozdoğan Ö. 2004. Fizyoloji. Palme Company, 2nd edition. Pp: 16-19, 191-197.

Bray M. S. and Young M.E. 2008. Diurnal variations in myocardial metabolism. Cardiovascular Research 79: 228-237.

Buja L. M. 2005. Myocardial ischemia and reperfusion injury. Cardiovascular Pathology 14:170– 175

Buschemeyer W. C., Klink J. C., Mavropoulos J. C., Poulton S.H., Demark-Wahnefried W., Hursting S.D., Cohen P., Hwang D., Johnson T. L. and Freedland S. J. 2010. Effect of intermittent fasting with or without caloric restriction on prostate cancer growth and survival in SCID mice. The Prostate 70: 1037-1043

Campbell N. R., Wickert W., Magner P. and Shumak S. L. 1994. Dehydration during fasting increases serum lipids and lipoproteins. Clinical and Investigative Medicine 17:570-576.

Carlson A. D. and Hoelzel F. 1945. Apparent prolongation of the life span of rats by intermittent fasting. Journal of Nutrition 363-375.

Cascio W. E. 2001. Myocardial Ischemia: What Factors Determine Arrhythmogenesis. Journal of Cardiovascular Electrophysiology 12: 726-729

Chamsi-Pasha H. and Ahmed W. H. 2004. The effect of fasting in Ramadan on patients with heart disease. Saudi Medical Journal 25:47-51.

Cheung P. H., Pugsley M. K. and Walker M. J. A. 1993. Arrhythmia Models in the Rat. Journal of Pharmacological and Toxicological Methods 29,:179-184

Corr P. B. and Witkowski F. X. 1983. Potential electrophysiologic mechanisms responsible for dysrhythmias associated with reperfusion of ischemic myocardium.Circulation 68:16-24

Costanzo L. S. 2007. Physiology (Board Review Series). Wolters Kluwer Company, 4. Edition, Pennsylvania, USA. Pp.:73-77

de Bakker Jacques M. T. and van Rijen Harold V. M. 2008. Mechanisms of ventricular tachycardia: underlying pathological and physiological abnormalities. In: Wang J. P., Al-Ahmad A., Hsia H. H. and Zei P. (Editors). Ventricular Arrhythmias and Sudden Cardiac Death, Blackwell Publishing, Massachusetts, USA. Pp.: 18-21

Demirbas B., Temizhan A. C, Sahin C., Karakurt I., Tandogan F., Gürsoy I., Serter G. and Aral R. 2001. Ramadan Fasting and Thyroid Hormon Profile. Medical Network Klinik Bilimler ve Doktor 7: 730-732.

Descamp O., Riondel J., Ducros V. and Roussel A-M. 2005. Mitochondrial production of reactive oxygen species and incidence of age-associated lymphoma in OF1 mice: Effect of alternate-day fasting. Mechanism of Ageing and Development 126: 1185-1191.

Downey M. J. 2003. The Electrocardiogram. In: Johnson L. R. (Editor) Essential Medical Physiology, Elsevier Inc., Third Edition, USA. Pp:197-200

El-Migdadi F., El-Akawi Z., Abudheese R. and Bashir N. 2002. Plasma levels of adrenocorticotropic hormone and cortisol in people living in an environment below sea level (Jordan valley) during fasting in the month of Ramadan. Hormonal Research 58: 279–282.

Finch G. M., Day J. E., Razak A., Welch D. A. and Rogers P. J. 1998. Appetite changes under free-living conditions during Ramadan fasting. Appetite 31: 159-170.

Fisler J. S. 1992. Cardiac effects of starvation and semi starvation diets: safety and mechanisms of action. The American Journal of Clinical Nutrition 56: 230-234.

Fozzard H. A. 1992. Afterdepolarization and triggered activity. Basic Research in Cardiology 87 (2):105-113

Froy O., Chapnik N., and Miskin R. 2009. Effect of intermittent fasting on circadian rhythms in mice depends on feeding time. Mechanism of Ageing and Development 130: 154-160

Goldhaber J. I. and Weiss J. N. 1992. Oxygen Free Radicals and Cardiac Reperfusion Abnormalities. Hypertension 20:118-127

Gonca E. and Bozdoğan O. 2010. Both Mitochondrial KATP Channel opening and Sarcolemmal KATP Channel Blockage Confer Protection Against Ischemia/Reperfusion-Induced Arrhythmia in Anesthetized Male Rats. Journal of Pharmacological Therapeutics 15: 403-411

Goodrick C. L., Ingram D. K., Reynolds M. A., Freeman J. R. and Cider N. L. 1983. Effects of intermittent feeding upon growth, activity, and lifespan in rats allowed voluntary exercise. Experimental Aging Research 9: 203-209

Grech E. D. and Ramsdale D. R. 1994. Termination of reperfusion arrhythmia by coronary artery occlusion. British Heart Journal 72: 94-95

Guo R., Gao X., Wang W., Wang H., Zhang F., Zhang Y. and Zhu G. 2005. Tempol reduces reperfusion-induced arrhythmias in anesthesized rats. Pharmacological Research 52: 192-198.

Guyton A. C. and Hall J. C. 2006. Textbook of Medical Physiology. 11th Edition. W. B. Saunders Company. Philadelphia/New York. Pp: 147-157.

Heeringa J., Van der Kuip D. A., Hofman A., Kors J. A., Van Herpen G., Stricker B. H., Stijnen T., Lip G. Y. H. and Witteman J. C. M. 2006. Prevalence, Incidence and Lifetime Risk of Atrial Fibrillation: the Rotterdam Study. European Heart Journal 27: 949−953.

Hudson K. B., Brady W. J., Chan T. C., Pollack M. and Harrigan R. A. 2003. Electrocardiographic Manifestations: Ventricular Tachycardia. The Journal of Emergency Medicine 25:303-314.

Ibrahim H. W., Habib M. H., Jarar H. A. and Al Baz A. S. 2008. Effect of Ramadan Fasting on Markers of Oxidative Stress and Serum Biochemical Markers of Cellular Damage in Healthy Subjects. Annuals of Nutrition and Metabolism 53: 175-181.

Imaizumi S., Miura S., Nakamura K., Kiya Y., Uehara Y., Zhang B., Matsuo Y., Urata H., Ideishi M., Rye K., Sata M. and Saku K. 2008. Antiarrhythmogenic Effect of Reconstituted High-Density Lipoprotein Against Ischemia/Reperfusion in Rats. Journal of the American College of Cardiology 51:1604-1612.

Iraki L., Bogdan A., Hakkou F., Amrani N., Abkari A. and Touitou Y. 1997 Ramadan Diet Restriction Modify the Circadian Time Structure in Humans. A Study on plasma Gastrin, Insulin, Glucose and Calcium and on Gastric pH. The Journal of Clinical Endocrinology and Metabolism 82: 1261-1273.

Jafile J., Delmar M., Anumonwo J., Berenfeld O. and Kalifa J. 2009. Basic Cardiac Electrophysiology for the Clinician. Blackwell Publishing, 2. Edition, England. Pp.: 152-160

Janse M. J. and Rosen M. R. 2006. History of Arrhythmias. In: Kass R. K. and Clancy C.E. (Editors). Basis and Treatment of Cardiac Arrhythmias, Springer, Berlin, Germany. Pp.: 15-18

January C. T., Chau V. and Makielski J. C. 1991. Triggered activity in the heart: Cellular Mechanism of early after depolarization. European Heart Journal 12; 4-9

Jaye D. A., Xiao Y. and Sigg D. C. 2010. Basic Cardiac Electrophysiology: Excitable Membranes. In: Sigg D. C., Laizzo P. A., Xiao Y. and He B. (Editors). Cardiac Electrophysiology Methods and Models. Springer, New York, USA. Pp.: 41-45.

Jones P. Dean. 2008. Radical-free biology of oxidative stress**.** American Journal of Physiology - Cell Physiology 295: 849-868.

Kadri N., Tilane A., El Batal M., Taltit Y., Tahiri S. M. and Moussauoi D. 2000. Irritability during the Month of Ramadan. Psychosomatic Medicine 62:280-285

Kale V. P., Joshi G. S., Gohil P. B. and Jain M. R. 2009. Effect of fasting duration on clinical pathology results in Wistar rats. Veterinary Clinical Pathology 38:361-366

Kane K. A., Parratt J. R. and Williams F. M. 1984. An investigation into the characteristics of reperfusion induced arrhythmias in the anaesthetized rat and their susceptibility to antiarrhythmic agents. British Journal of Pharmacology 82: 349-357

Kassab S., Abdul-Ghaffar T.,Nagalla D. S., Sachdeva U. and Nayar U. 2003. Serum leptin and insulin levels during chronic diurnal fasting. Asia Pacific Journal of Clinical Nutrition 12: 483-487.

Katare R. G., Kakinuma Y., Arikawa M., Yamasaki F. and Sato T. 2009. Chronic intermittent fasting improves the survival following large myocardial ischemia by activation of BDNF/VEGF/PI3K signaling pathway. Journal of Molecular and Cellular Cardiology 46: 405–412

Kim Y. W. and Scarpace P. J. 2003. Repeated fasting/refeeding elevates plasma leptin without increasing fat mass in rats. Physiology and Behavior 78: 459-464.

Kmiec Z., Pokrywka L., Kotlarz G., Kubasi J., Szutowicz A. and Mysliwski A. 2005. Effects of Fasting and Refeeding on Serum Leptin, Adiponectin and Free Fatty Acid Concentrations in Young and Old Male. Gerontology 51: 357-362.

Leiper J. B, Molla A. M. and Molla A. M. 2003. Effects on health of fluid restriction during fasting in Ramadan. European Journal of Clinical Nutrition 57:30-38

Mager D. E., Wan R., Brown M., Cheng A., Wareski P., Abernethy D. R. and Mattson M. P. 2006. Caloric restriction and intermittent fasting alter spectral measures of heart rate and blood pressure variability in rats. The Journal of the Federation of American Societies for Experimental Biology 20: 631-637.

Manning A. S., Coltart D. J. and Hearse D. J. 1984. Ischemia and Reperfusion-Induced Arrhythmias in the Rat: Effects of Xanthine Oxidase Inhibition with Allopurinol. Circulation Research 55: 545-548

Mansi K. M. S. 2007. Study the Effects of Ramadan Fasting on the Serum Glucose and Lipid Profile among Healthy Jordanian Students. American Journal of Applied Sciences 4 (8): 565-569.

Martin B., Mattson M. P. and Maudsley S. 2006. Caloric restriction and intermittent fasting: Two potential diets for successful brain aging. Ageing Research Reviews 5: 332-353.

Martin M. 2007. Small Animal ECGs: An introductory guide. Blackwell publishing, 2nd edition, Oxford, UK. Pp: 9-10

Mattson M. P. 2008. Dietary factors, hormesis and health. Ageing Research Reviews 7: 43-48.

Mattson M. P. and Wan R. 2005. Beneficial effects of intermittent fasting and caloric restriction on cardiovascular and cerebrovascular systems. Journal of Nutritional Biochemistry 16: 129-137.

Mesbahzadehn B., Ghiravani Z. and Mehrjoofard H. 2005. Effect of Ramadan Fasting on Secretion of Sex Hormones in healthy single males. La Revue de Sante de la Mediterranee orientale 11: 1120-1223.

Mitchell J. R., Verweij M., Brand K., van de Ven M. Goemaere N., van den Enge S., Chu T., Forrer F., Müller C., de Jong M., van Ucken W., Uzermans J. N. M., Hoeijmakers J. H. J. and de Bruin R. W. F. 2010. Short-term dietary restriction and fasting precondition against ischemia reperfusion injury in mice. Aging Cell 9: 40-53

Moukarbel G. V., Ayoub C. M. and Abchee A. B. 2004. Pharmacological therapy for myocardial reperfusion injury. Current Opinion in Pharmacology 4: 147–153

Murdock D. K., Loeb J. M., Euler D. E., and Randall W.C. 1980. Electrophysiology of Coronary Reperfusion: A Mechanism for Reperfusion Arrhythmias. Circulation 61; 175-182

Murphy E. and Steenbergen C. 2007. Ion Transport and Energetics During Cell Death and Protection. Physiology 23: 115-123.

Nedvídková J., Smıtka K., Kopský V. and Hainer V. 2005. Adiponectin, an Adipocyte-Derived Protein. Physiological Research 54: 133-140.

Nishida K., Michael G., Dobrev D. and Nattel S. 2010. Animal models for atrial fibrillation: clinical insights and scientific opportunities. Europace 12:160–172

Park J. L. and Lucchesi B. R. 1999. Mechanisms of Myocardial Reperfusion Injury. Annals Thoracic Surgery 68: 1905–1912

Pedersen C. R., Hageman I., Bock T. and Buschard K. 1999. Intermittent feeding and fasting reduces diabetes incidence in BB rats. Autoimmunity 30: 243-250.

Piper H. M., Gardia-Dorada D. and Ovize M. 1998. A fresh look at reperfusion injury. Cardiovascular Research 38: 291-200.

Piper H. M., Meuter K. and Schafer C. 2003. Cellular Mechanisms of Ischemia-Reperfusion Injury. Annals Thoracic Surgery 75**:** 644–648

Pogwizd S. M. and Corr P. B. 1987. Electrophysiologic mechanism underlying arrhythmia due to reperfusion of ischemic myocardium. Circulation 76: 404-426

Qujeq D., Bijani K., Kalavi K., Mohiti J. and Aliakbarpour H. 2002. Effect of Ramadan fasting on serum low-density and high-density lipoprotein-cholesterol concentrations. Annals of Saudi Medicine 22: 297-299.

Ravingerova T., Slezak J., Tribulova N., Dzurba A., Uhrik B. and Ziegelhöffer A. 1999. Free oxygen radicals contribute to high incidence of reperfusion-induced arrhythmias in isolated heart. Life Sciences 65: 1927-1930

Reilly T. and Waterhouse J. 2007. Altered sleep – wake cycles and food intake: The Ramadan model. Physiology and Behavior 90: 219 – 228

Reimer K. A. and Ideker R. E. 1987. Myocardial Ischemia and Infarction: Anatomic and Biochemical Substrates for Ischemic Cell Death and Ventricular Arrhythmias. Human Pathology 18: 462-475

Roky R., Chapotot F., Hakkou F., Benchekroun M. T. and Buguet A. 2001. Sleep during Ramadan intermittent fasting. European Sleep Research Society 10: 319-327.

Roky R., Houti I., Moussamih S., Qotbi S. and Aadil N. 2004. Physiological and Chronobiological changes during Ramadan Intermittent Fasting. Annals of Nutrition and Metabolism 48: 296-303.

Schaefer S. and Ramasamy R. 1997. Glycogen utilization and ischemic injury in the isolated rat heart. Cardiovascular Research 35: 90-98.

Schneider C. A. and Taegtmeyer H. 1991. Fasting in vivo delays myocardial cell damage after brief period of ischemia in the isolated working rat heart. Circulation research 68: 1045-1050

Simons M. and Ware J. A. 1996. Food for starving hearts. Nature Medicine 2: 534-539.

Tabibiazar R. and Rockson S. G. 2001. Angiogenesis and the ischemic heart. European Heart Journal 22:903-918.

Temizhan A., Dönderici O., Ouz D. and Demirbas B. 2003. Is there any effect of Ramadan fasting on acute coronary heart disease? International Journal of Cardiology 70: 149-153.

Thomas J. A., Antonelli J. A, Lloyd J. C., Masko E. M., Poulton S. H., Phillips T. E., Pollak M. and Freedland S. J. 2010. Effect of Intermittent Fasting on Prostate cancer tumor growth in a mouse model. Prostate Cancer and Prostatic Diseases 13: 350-355.

Tikoo K., Tripathi N. D., Kabra G. D., Sharma V. and Gaikwad B. A. 2007. Intermittent fasting prevents the progression of type I diabetic nephropathy in rats and changes the expression of Sir2 and p53. FEBS LETTERS 581:1071-1078.

Tomaselli G. and Roden D. M. 2005. Molecular and Cellular Basis of Cardiac Electrophysiology. In: Lenehan A. and Shreiner J. (Editors). Electrophysiological Disorders of the Heart, Elsevier Inc., Philadelphia, United States of America. Pp.: 21-25

Tortora G. and Derrickson B. H. 2009. Principles of Anatomy and Physiology. JohnWiley and Sons, 12 Editions, Asia. Pp.: 717-732.

Tosaki A., Koltai M. and Braquet P. 1989. Effects of low extracellular sodium concentration on reperfusion induced arrhythmias: changes in the myocardial sodium, potassium and calcium contents in isolated guinea pig hearts. Cardiovascular Research 23: 993-1000.

Ueyama E., MorikawaY., YasudaT. and Senba E. 2004. Attenuation of fasting-induced phosphorylation of mitogen-activated protein kinases (ERK/p38) in the mouse hypothalamus in response to refeeding. Neuroscience Letters 371: 40–44

Unalacak M., Kara I. H., Baltaci D., Erdem O. and Bucaktepe P. G. 2011. Effects of Ramadan fasting on biochemical and hematological parameters and cytokines in healthy and obese individuals Metabolic Syndrome and Related Disorders 9:157-161

Varady K. A. and Hellerstein M. K. 2007. Alternate-day fasting and chronic disease prevention: a review of human and animal trials. American Journal of Clinical Nutrition 86: 7-13.

Walker M. J. A., Curtis M. J., Hearse D. J., Campbell R. W. F., Janse M. J., Yellon D. M., Cobbe S. M., Coker S. J., Harness J. B., Harron D. W. G., Higgins A. J., Julian D. G., Lab M. J., Manning A. S., Northover B. J., Parratt J. R., Riemersma R. A., Riva E., Russell D. C., Sheridan D. J., Winslow E. and Woodward B. 1988. The Lambeth Conventions: guidelines for the study of arrhythmias in ischemia, infarction, and reperfusion. Cardiovascular Research 22: 441-455.

Waller C., Hiller K. H., Albrecht M., Hu K., Nahrendorf M., Gattenlöhner S., Haase A., Ertl G. and Bauer W. R. 2003. Microvascular adaptation to coronary stenosis in the rat heart in vivo: a serial magnetic resonance imaging study. Microvascular Research 66: 173-182

Wan R., Ahmet I., Brown M., Cheng A., KamimuraN., Talan M. And Mattson M. P. 2010. Cardioprotective effect of intermittent fasting is associated with an elevation of adiponectin levels in rats. Journal of Nutritional Biochemistry 21: 413–417.

Wan R., Camandola S. and Mattson M. P. 2003. Intermittent Food Deprivation Improves Cardiovascular and Neuroendocrine Responses to Stress in Rats. Nutritional Neurosciences 133: 1921-1929.

Wit A. L. and Janse M. J. 2001. Reperfusion arrhythmias and sudden cardiac death: A century of progress toward an understanding of mechanisms. Circulation Research 89: 741-743.

Yellon D. M. and Hausenloy D. J. 2007. Myocardial Reperfusion Injury. The New England Journal of Medicine 357 (11): 1121-1235

Yucel A., Degirmenci B., Acar M., Albayrak R. and Haktanır A. 2004. The effect of Fasting Month of Ramadan on the Abdominal Fat Distribution: Assessment by Computed Tomography. Tohoku University Medical Press 204: 179-187.

Ziaee V., Razaei M., Ahmadinejad Z., Shaikh H., Yousefi R., Yarmohammadi L., Bozorgi F. and Behjati M. J. 2006. The changes of metabolic profile and weight during Ramadan fasting. Singapore Medical Journal 47: 409-414