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The effect of intermittent fasting and water restriction on myocardial ischemia/reperfusion-induced arrhythmia in rats

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Aim: To investigate the effect of intermittent fasting and water restriction on ischemia/reperfusion-induced arrhythmias.

Materials and methods: Six minutes of ischemia followed by 6 min of reperfusion was produced by the ligation and then releasing of the left coronary artery. Intermittent fasting and water restriction were applied during 1 month for 12 h/day. The duration, type, and incidence of arrhythmias during reperfusion and the survival rate at the end of reperfusion were determined and compared.

Results: The score of arrhythmia that was determined using the type and duration of arrhythmia did not show significant differences among groups. The arrhythmic period was significantly longer in animals subjected to 1 month of intermittent water and food restriction. The risk of ischemic zone was found to be significantly larger in the animals subjected to 1 month of normal feeding after 1 month of food and water restriction.

Conclusion: Intermittent fasting and water restriction was not found to be effective in decreasing the arrhythmia that occurred during 6 min of myocardial reperfusion in rats. Since there is no other study compatible with the present study, further research is required on the effect of intermittent fasting and water restriction on ischemia/reperfusion-induced arrhythmia and on the risk of an ischemic zone.

Key words: Myocardial ischemia, reperfusion, fasting, water restriction, arrhythmia

Introduction

Myocardial ischemia is a widespread cause of morbidity and a main reason for 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 each year (1). Many possible causes of sudden unexpected death are cardiogenic and occur by the lethal arrhythmias during either myocardial ischemia or reperfusion (2–5). That is why it is important to find factors increasing resistance to myocardial ischemia and reperfusion-induced arrhythmia. The changing of lifestyle or feeding habits may be more effective in the reduction or prevention of death from lethal arrhythmias. The physiological effects of various feeding activities such as short-term fasting, intermittent fasting, and caloric restriction have been investigated in different experimental models in animals (6–8). Dietary restriction has been shown to have several health benefits including reduced risk factors for cardiovascular disease, increased lifespan, reduced morbidity, decreased infarct size, and increased stress resistance (9–13). Intermittent fasting and water restriction have been shown to decrease cardiovascular risk factors, increasing insulin sensitivity, high density lipoprotein, and the cholesterol-to-high density

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lipoprotein ratio in humans (14,15). Although there are several clinical observations in healthy individuals, there has been no experimental study to evaluate the effect of intermittent fasting and water restriction on arrhythmias. This study has therefore provided experimental data related to the effects of intermittent fasting and water restriction on arrhythmias and the rate of sudden death following myocardial ischemia and reperfusion.

Materials and methods

Animals

Thirty male Sprague–Dawley rats, 8–9 months old, were used in this study. The rats were kept in 12 h of light and 12 h of dark (lights on at 0800 hours and off at 2000 hours), maintained under standard laboratory conditions, and individually caged. After 1 week of acclimatization, the animals were randomly separated into 3 groups, each containing 10 rats. Group 1 was the control, having no restrictions; group 2 had 1 month of food and water restriction between 2000 hours and 0800 hours for 12 h; and group 3 had 1 month of restriction followed by 1 month of normal diet. The third group was produced to investigate the possible effects of intermittent fasting and water restriction on ischemia/reperfusion-induced arrhythmias after the animals returned to a normal diet. All study protocol was approved by the ethical committee of Abant İzzet Baysal University, Bolu, Turkey.

Ischemia and reperfusion protocol

Experimental animals were anesthetized with sodium thiopental (60 mg/kg). The trachea was cannulated for artificial respiration. The left carotid artery was cannulated with a catheter to measure the blood pressure with a blood pressure transducer (SS 13 L, BIOPAC Systems, Santa Barbara, CA, USA). The catheter was filled with isotonic saline including heparin (500 IU/mL), but the animals were not heparinized. The chest was opened in the fourth intercostal space and the heart was exposed. A loose loop of 5-0 atraumatic silk was quickly placed around the left descending coronary artery (LAD). The heart was then carefully repositioned in the chest and artificial respiration was immediately restarted with an animal respirator (Ugo Basile Rodent Ventilator, Italy) at 60 strokes/min. Subcutaneous needle electrodes were placed under the skin to record the electrocardiogram (ECG; lead II). The animals were allowed to stabilize for 10 min, and then ischemia was produced for 6 min by tightening the silk with a bowknot around the coronary artery and reperfusion was produced for 6 min by loosening the bowknot.

At the end of the experiment, in surviving animals, heparin (500 IU/kg) was given intravenously and the heart was excised. After the retightening of the LAD, the heart was perfused through the aorta with solutions: first with 10 mL of NaCl and then with 2 mL of 96% ethanol for demarcation of the occluded and nonoccluded myocardial regions. Following the perfusion of the heart, the nonperfused area (red color, ischemic risk zone) was separated from the well-perfused area (white color, nonischemic myocardium mass) through a border line. The wet weights of the ischemic risk zone and the nonischemic myocardial mass were measured. The percentage of the nonperfused area in respect to the total weight of the ventricle, indicating the ischemic risk zone, was calculated. Determination of risk of ischemic area was done according to the methods suggested by Lepran et al (16).

The evaluation of the arrhythmias

The heart rate and blood pressure were determined from recorded ECGs at minutes 1, 3, and 5 during the ischemia and reperfusion. The incidence and total length of arrhythmias were calculated during 6 min of ischemia and 6 min of reperfusion. The arrhythmias were identified in accordance with the Lambeth Conventions (17) as ventricular tachycardia (VT), ventricular fibrillation (VF), and other types of arrhythmia including single ventricular extra beat, bigeminy, and salvos (Figure 1). The arrhythmic period that includes the time interval between the onset and offset of arrhythmias was measured.

An arrhythmia score was used to indicate the severity of arrhythmia based on the incidence and duration of arrhythmia by giving a grade to each animal as follows: 0 = no arrhythmia; 1 = ≤10 s of VT and/or other types of arrhythmias, no VF; 2 = 11 to 30 s of VT and/or other types of arrhythmias, no VF; 3 = 31 to 90 s of VT and/or other types of arrhythmias, no VF; 4 = 91 to 180 s of VT and/or other types of arrhythmias, and/or <10 s of reversible VF; 5 = ≥180

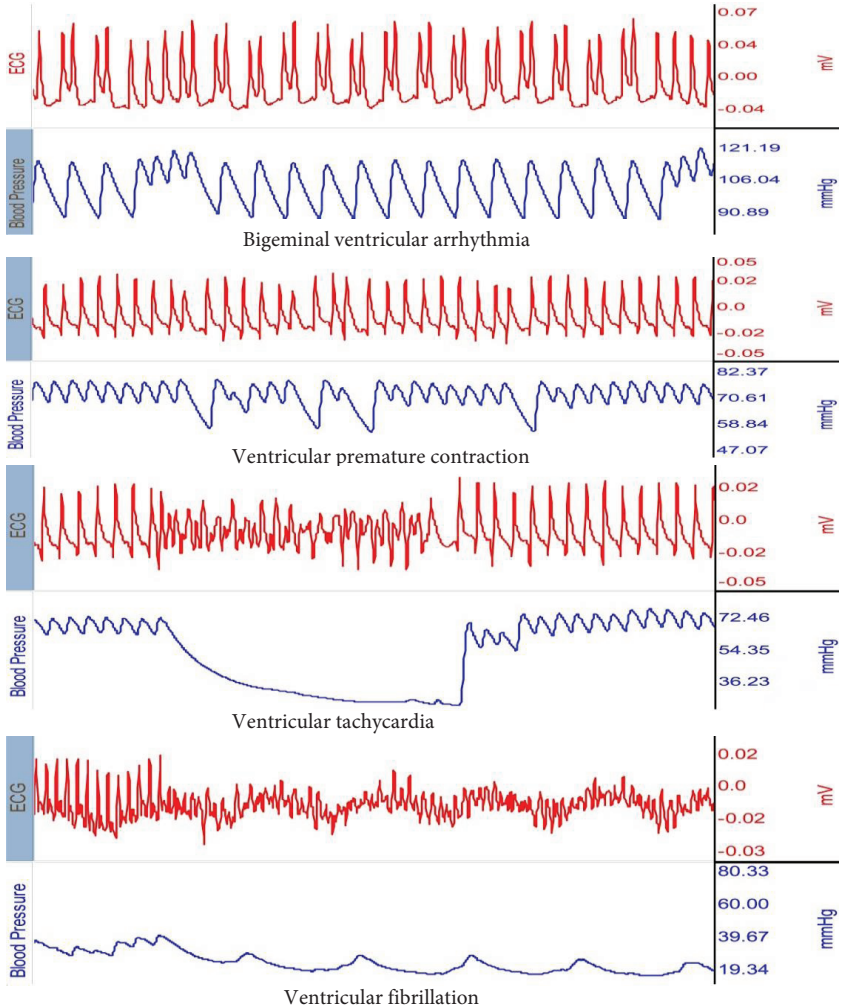

Figure 1. The types of arrhythmias and blood pressure recorded during reperfusion.

s of VT and/or other types of arrhythmias, and/or >10 s of reversible VF; 6 = irreversible VF.

Statistical analyses

Data were analyzed using SPSS 17. The mean and standard errors were determined for all parameters including heart rate, blood pressure, body weight, and arrhythmia score. Values were expressed as means ± standard errors. Data were analyzed by one-way analysis of variance combined with the least significant difference post hoc test. The incidences of arrhythmia were assessed by chi-square test (Fisher's exact test). Differences between means yielding a probability of $P < 0.05$ were considered statistically significant.

Results

Hemodynamic parameters

ST segment elevation and increased QRS complex amplitude and change in width were observed in all experimental animals after coronary artery ligation. ST segment elevation was accepted as a sign of ischemia in ECGs. The animals having no ST segment elevation and low blood pressure below 70 mmHg after the coronary artery ligation (5 animals) were excluded from evaluation. There was no significant change in mean blood pressure and heart rate among groups during reperfusion and coronary ligation (Table 1).

Table 1. Hemodynamic changes in anesthetized rats. Results are mean ± standard error of the surviving animals (N: number of animals). Heart rate (HR) in beats/min and mean arterial blood pressure (MBP) in mmHg were measured before 6 min of ischemia (basal), 5 min after coronary artery ligation (occlusion), and 5 min after release of occluded artery (reperfusion).

Group	N	Basal		Occlusion		Reperfusion	
		HR	MBP	HR	MBP	HR	MBP
Group 1 (control)	10	385 ± 5	104 ± 3	384 ± 5	91 ± 4	380 ± 6	96 ± 6
Group 2	10	396 ± 2	100 ± 5	383 ± 6	83 ± 7	383 ± 6	97 ± 6
Group 3	10	384 ± 6	104 ± 4	382 ± 6	91 ± 6	364 ± 10	92 ± 11

Arrhythmias during coronary artery ligation

Arrhythmias induced by ischemia generally started within 2–3 min following coronary artery ligation. There were no significant differences in the incidence of arrhythmias, arrhythmia score, and the survival rate among groups during coronary ligation. However, the arrhythmic period in group 2 was significantly longer compared to the control ($P < 0.05$) (Figure 2).

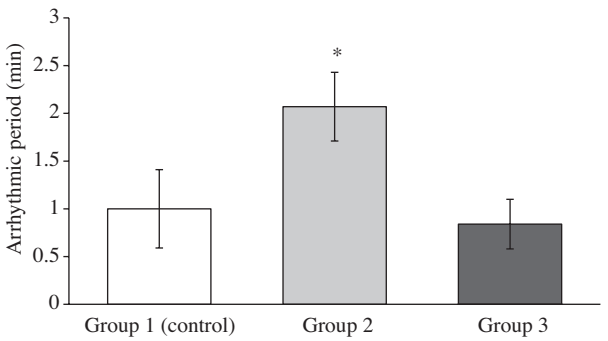

Figure 2. Arrhythmic period during 6 min of ligation. *$P < 0.05$, different from group 1 and group 3.

Arrhythmias during reperfusion

Reperfusion-induced arrhythmias appeared within 10–40 s after 6 min of myocardial ischemia. Total arrhythmia and length of arrhythmic attacks were not significantly different among groups (Figure 3). There were also no significant differences in the incidence of arrhythmias and arrhythmia scores (Table 2). However, ventricular fibrillation was only observed in group 3. Ischemic risk zones showed statistically significant differences in group 3 in respect to the control and group 2 ($P < 0.05$) (Figure 4).

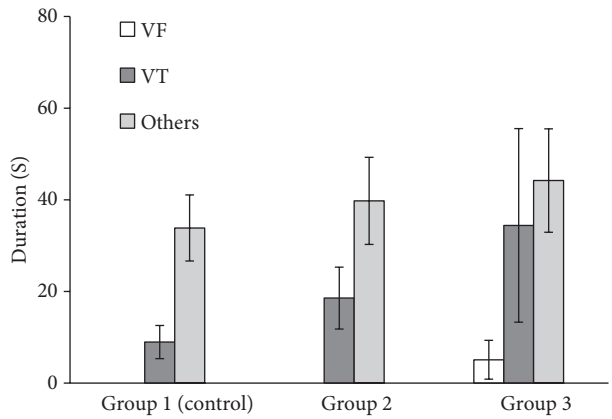

Figure 3. The types and length of arrhythmic attacks during reperfusion.

Body weight

Total body weight did not show any significant differences among groups before and after the restriction (data not shown).

Discussion

The results of the present study indicate that intermittent fasting and water restriction in rats decreases neither the incidence of arrhythmia nor the survival during reperfusion following myocardial ischemia. The length of dietary restriction is critical to get beneficial or adverse effects of the restriction in animals. It is not known how much time is required for beneficial effects (18).

Table 2. The incidence of arrhythmias during reperfusion after 6 min of coronary ligation in anesthetized rats. VF: ventricular fibrillation; VT: ventricular tachycardia; Others: arrhythmias including ventricular premature contraction, ventricular bigeminy, AV nodal arrhythmia, and salvo; N: number of animals at the beginning of reperfusion.

Group	N	Incidence of arrhythmia (n / %)					Arrhythmia score
		Survival (n / %)	VF	VT	Others	Bradycardia	
Group 1 (control)	10	10 / 100	0 / 0	7 / 70	10/100	0 / 0	2.70 ± 0.21
Group 2	10	10 / 100	0 / 0	8 / 80	10/100	0 / 0	2.90 ± 0.28
Group 3	10	10 / 100	3 / 30	8 / 80	10/100	0 / 0	3.30 ± 0.42

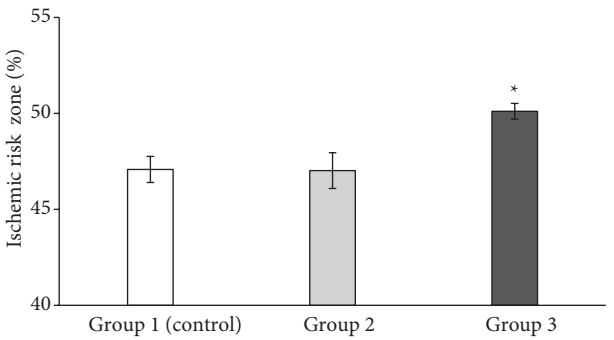

Figure 4. Ischemic risk zone. *P < 0.05, different from group 1 and group 2.

Dietary restriction with fasting every other day without water restrictions for 3 months reduced the ischemic damage in rats (11). We expected that this type of decreased ischemic damage could lead to the decreasing of reperfusion-induced arrhythmia after myocardial ischemia in our models, but it was not observed. The reperfusion-induced arrhythmia was not significantly different in the restricted groups in our study. This may be caused by different durations and lengths of the restriction, which may not be enough to show any beneficial effect on ischemia/reperfusion-induced arrhythmias.

In this study, the significant increase in arrhythmic period may result from alteration of the metabolism or circadian rhythm of animals in group 2. In other words, changes in eating habits and wake/sleep cycles may generate stress for animals. Nocturnal animals have been characterized by increased activity during the night and sleep during daytime. In our experimental model, animals in group 2 and group 3 accessed the food and water during daytime, which caused changes in biological rhythms. However, animals in group 3 may have had more stress than those in the other groups due to the 2 rounds of changes in feeding habits. The alternating period of anabolism and catabolism that occurs during intermittent fasting without water restriction may play an important role in triggering cellular stress resistance and the repair of damaged cells (19). However, we could not observe this kind of stress-induced protection on the ischemia and reperfusion arrhythmia in our fasting model.

There are some clinical investigations showing a reduction in body mass, body fat, and energy intake during intermittent fasting for 1 month (20–23). There are also similar studies showing either no change or slight increases in body mass (24,25). We could not observe any significant changes in total body weight between control animals and animals maintained for 1 month with water and food restrictions. The reason for no change in body weight among groups may be due to the adaptation of the animals to the new feeding habits.

In conclusion, no changes were observed in the incidence of arrhythmia, survival rates, and arrhythmia scores in animals during and after the restriction. Further studies should be performed to clarify the effect of food and water restriction on ischemia/reperfusion-induced arrhythmia.

Limitations

In the current study, the results have to be interpreted within the scope of some limitations. There was no significant effect of this feeding regime on the ischemia/reperfusion-induced arrhythmia. Only the

risk of ischemic zone was found to be significantly larger in this group. Since there are no identical studies in the literature, this result could not be exactly compared.

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