Lactation protects against myocardial ischemia-reperfusion injury in rats

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Some researchers have reported that lactation is effective in reducing cardiovascular disease risk factors. The purpose of this study was to investigate whether lactation may improve intrinsic tolerance against ischemia reperfusion (IR) injury. The rats were randomly divided into two groups ($n = 8$ in each group). In the lactation (Lact) group, the surgery was performed on postpartum day 21 (at the end of lactation period) and the results were compared with those of virgin female rats (control group). Cardiac IR injury was induced by means of left anterior descending coronary artery occlusion for 30 min followed by reperfusion for 120 min. Infarct size was measured using the staining agent 2,3,5-triphenyltetrazolium chloride. At the end of the experiment, Mean arterial pressure in the control group was significantly lower than that in the Lact group. Myocardial infarct size was significantly reduced in the Lact group ($23 \pm 3\%$ vs. $45 \pm 8\%$, $p < 0.05$ in the control group). Lactation reduced the extent of myocardial injury induced by ischemia and reperfusion. So, lactation may increase cardiac tolerance to ischemic injury.

Keywords: lactation, myocardial ischemia, reperfusion, preconditioning, cardioprotection

Recent researches have indicated that lactation has impacts on maternal health through effects on hypothalamic, autonomic, and cardiovascular functioning both during the breastfeeding period and after weaning. Lactation is associated with increased parasympathetic and decreased sympathetic activity (30), less atherogenic lipid profile and improved fasting glucose (18), decreased risk of developing hypertension (39), the metabolic syndrome (19), and cardiovascular disease. One may speculate that a complex hormonal cascade triggered by lactation can influence a number of different factors like blood pressure regulation, glucose homeostasis and insulin secretion, lipid homeostasis, and inflammatory processes (12).

The tissue damage caused by restoration of blood flow after a period of ischemia is known as ischemia reperfusion (IR) injury. The results from therapeutic targeting of the individual components of lethal myocardial IR injury, including oxidative stress, calcium overload, pH correction, and inflammation have been disappointing (21). So for several decades, there has been a concerted effort in cardiology to identify interventions to increase the heart resistance to infarction. In 1986, Murry et al. first introduced the concept of "conditioning" the myocardium to protect it against IR injury (32). Exposure to brief periods of ischemia (called ischemic preconditioning [IPC]), prior to prolonged ischemia, has been recognized as a powerful endogenous form of cardioprotection (21). However, this intervention was both invasive and impractical to apply, and non-invasive approaches for protecting the heart were sought such as exposure to a brief limb ischemia (remote IPC) (25),

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pharmacological agents, hyperoxic environment, exercise and so on. In experimental IR injury models, the protective effect of a wide range of hormones, including oxytocin (34), prolactin (26), estrogen (4), thyroid hormone (33), and endogenous substances, including NO (23), and heat shock proteins (14) including heme oxygenase-1 (24) has been shown. Due to substantial changes in the factors involved in the regulation of cardiovascular function during lactation, the purpose of the present study was to explore whether lactation may protect the heart against ischemia reperfusion (IR) injury in an *in vivo* rat model.

Materials and Methods

Animals
Female Wistar rats (weighing 200–250 g) were housed under standard conditions with free access to food and water. The investigation was approved by the animal and human ethics committee of Islamic Azad University (51601901119002).

Experimental protocols
The rats were randomly divided into two groups ($n = 8$ in each group). In the Lact group, each female was paired with a male until they looked visibly pregnant. On postpartum day 21 (at the end of lactation period), the rats underwent surgery. In the control group, each female was paired with another female. During this time (almost 42–45 days), virgin control rats were maintained in separate cages and then they underwent surgery. The rat estrous cycle is typically 4–5 days in length. Pregnancy lasts about 21–22 days. The pups are typically weaned by 21 days of age. The serum estradiol level fluctuations during various estrous cycle stages of rats do not influence cardiac IR injury and are not correlated with infarct size or arrhythmia. Because endogenous changes in reproductive hormones do not appear to be a confounding variable in IR studies (15), the serum estradiol level and estrous cycle stages of rats was not assessed.

Surgical procedure
The procedure used in the present study was described in our previous experiments (37). In brief, the animals were anesthetized with sodium pentobarbital (30 mg.kg^{-1}) and ketamin (50 mg.kg^{-1}) i.p. Rectal temperature was continuously monitored and maintained at 37 ± 0.5 °C. Following tracheotomy, the rats were ventilated with room air enriched with oxygen at a rate of 70 to 80 per min and tidal volume of 1 ml·kg^{-1} to maintain blood PO_2, PCO_2, and pH in the normal physiological range. A standard limb lead II electrocardiogram (ECG) was monitored and recorded throughout the experiment. Catheters were inserted into the left carotid artery and tail vein (Angiocat 23; Suppa, Tehran, Iran) for monitoring blood pressure and infusion of Evans blue solution, respectively. A left thoracotomy was performed and the pericardium was opened to expose the heart. A 6-0 silk suture was passed around the left anterior descending coronary artery (LAD) and its ends were threaded through a small plastic tube to form a snare. Following a stabilization period of 20 min, the artery was occluded for 30 min by clamping the snare against the surface of the heart. Ischemia was confirmed by regional cyanosis and ST elevation in the ECG. Reperfusion was established by releasing the snare for 120 min.

Determination of infarct size and area at risk

At the end of reperfusion, the LAD was re-occluded and 1 ml Evans Blue dye %1 (Sigma, St. Louis, MO) was injected into the tail vein to identify the area at risk (AAR). The heart was excised, rinsed of excess blue dye, trimmed of the atria and right ventricle, put in a matrix, wrapped in plastic foil and frozen at –20 °C (2 h). The left ventricle (LV) was cut into transverse slices of 2 mm thickness from the apex to the base. Tissue samples were then incubated in triphenyltetrazolium chloride 1% [TTC (Sigma)] solution in isotonic phosphated buffer, pH = 7.4, for 20 min at 37 °C, and subsequently fixed in 10% formalin for 1 h. Viable tissue was stained red by TTC, whereas infarcted tissue remained unstained (white). An image was obtained (canoscan LID 25) from both sides of each slice and all calculations from one heart (using Image Tool Software) were averaged into one value for statistical analysis. The total area at risk was expressed as a percentage of the left ventricles (AAR/LV). Infarct size (IS) was expressed as a percentage of the AAR (IS/AAR).

Assessment of ventricular arrhythmias

Ischemia-induced ventricular arrhythmias were determined in accordance with the Lambeth conventions (41): premature ventricular complexes (PVCs as a discrete and identifiable premature QRS complex (premature with respect to the P wave), ventricular tachycardia (VT) as a run of four or more PVC at a rate faster than the resting sinus rate and ventricular fibrillation (VF) as a signal for which individual QRS deflection can no longer be distinguished from one another. Complex forms (bigeminy and salvos) were added to PVCs count and not analyzed separately.

Statistical analysis

Statistical analysis was carried out using SPSS software (version 16). Data are expressed as mean ± SEM or as percent. Student's *t*-test or Fisher exact test was used to analyze the data. Values of $P < 0.05$ were considered as significant.

Results

Hemodynamic parameters

Table I summarizes the hemodynamic data. There were no significant differences at baseline values for heart rate (HR) and mean arterial blood pressure (MAP) between the groups. Ischemia caused a marked decrease in blood pressure. At the end of the experiment, MAP in the control group was significantly lower than that in the Lact group.

Table I. Hemodynamics parameters in experimental groups

Groups	Baseline		Ischemia		Reperfusion	
	HR	MBP	HR	MBP	HR	MBP
Control	331 ± 23	123 ± 13	341 ± 23	101 ± 22**	326 ± 16	91 ± 14** #
Lact	321 ± 16	124 ± 13	341 ± 43	112 ± 15*	335 ± 40	111 ± 14*

Control = Virgin female rats, Lact = Female rats that completed lactation period after parturition.
* $P < 0.05$, ** $P < 0.01$ compared with the baseline values within the same group
\# $P < 0.05$ compared with other group

Myocardial infarct size

The effect of lactation on myocardial infarct size is shown in Fig 1. The size of AAR did not differ between the two groups, indicating equal position of LAD occlusion. Myocardial infarct size was significantly reduced in the Lact group ($23 \pm 3\%$ vs. $45 \pm 8\%$, $p < 0.05$).
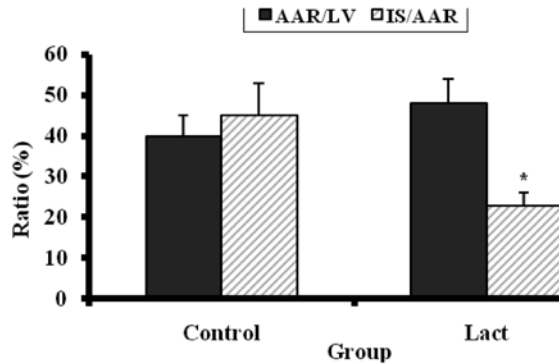

Fig. 1. Infarct size (IS) and area at risk (AAR) following 30-min ischemia and 120-min reperfusion in rats.
LV = left ventricle; * $P < 0.05$ compared with the other group.
Control = Virgin female rats, Lact = Female rats that completed lactation period after parturition

Ischemia-induced arrhythmias

The results of lactation on ischemia-induced arrhythmia are shown in Table II. During 30-min ischemia, the numbers of PVC, episodes of VT and duration of VT in the control group were 258 ± 35, 31 ± 6 and 31 ± 6 sec, respectively. The rate of these arrhythmias in the Lact group was reduced, but it was not significant. VF occurred nearly in 75% of rats in both groups. A rat (in the control group) died due to irreversible VF during ischemia. Mortality rate did not differ in this relatively small sample. There were no considerable reperfusion induced arrhythmias in the present work.

Table II. Incidence of ventricular arrhythmias during 30 min of ischemia in the Control and Lact groups

	PVC (n)	VT (n)	VT duration (sec)	VF (%)
Control group	258 ± 35	31 ± 6	31 ± 6	77.8%
Lact group	236 ± 30	28 ± 5	23 ± 6	75%

The numbers of premature ventricular complexes (PVC), the episodes of ventricular tachycardia (VT),
and VT duration (as means ± SEM) and the incidence of ventricular fibrillation (VF) (as percent) are shown.
Control = Virgin female rats, Lact = Female rats that completed lactation period after parturition

Discussion

The main findings of this study were: 1) A significant decrease in infarct size (IS) following 30-min of ischemia and 120 min of reperfusion was observed in the Lact group, 2) At the end of the lactation period, no significant changes were observed in hemodynamic parameters compared to the control group, 3) Ischemia induced arrhythmias were reduced in the Lact group, but no statistical significance was reached.

The incidence of ventricular arrhythmia, infarct size, and mortality positively correlated with the size of the ischemic area in animal models (6, 10). In this study, as in previous studies (8), VF occurred as a self-limited arrhythmia in 75% of rats in both groups. However, a rat (in the control group) died due to irreversible VF during ischemia.

The concept of therapeutic infarct size limitation was first promoted by Maroka et al. (29). Myocardial salvage is a potential strong endpoint for success in acute reperfused myocardial infarction (20). Our study indicated that the lactation can provide cardiac protection against IR injury by decreasing IS. Consistent with our findings, Faltová et al. showed that in lactating mothers the extent of isoprenaline-induced heart lesions and mortality were significantly decreased; moreover, the resistance of the right ventricle to anoxia was higher than that of virgin females (13).

The effect of lactation in IR injury model has not been studied so far. To our knowledge, this is the first animal experimental demonstration showing that lactation can mimic preconditioning. There were no significant changes in basal hemodynamic values on postpartum day 21. So, the protective effect of lactation on the myocardium is not related to alterations in hemodynamic parameters. It is a new benefit of breastfeeding in encouraging mothers to choose nursing.

Some clinical studies have established that there is a dose-dependent relationship between lactation and reduced risk of coronary heart disease (12, 36). Although the mechanisms are still debated, it has been hypothesized that lactation may influence several pituitary hormones and induce even long-term changes in the hypothalamic-pituitary axis (12). After parturition, large quantities of prolactin and oxytocin (OT) are released from the pituitary gland into the circulation in response to a suckling stimulus. Principal site of prolactin is the mammary gland. Few studies have investigated the potential role of prolactin in the increased risk of cardiovascular events, e.g. essential hypertension, the heart failure that accompanies Peripartum cardiomyopathy, and arrhythmias (7, 22). On the other hand, the positive influence of prolactin on the cardiovascular system was shown. Results of a study showed that administration of supraphysiological doses of prolactin isoforms could protect against sudden cardiac death as well as severe arrhythmias episodes during reperfusion (26). In general, experimental data and clinical observations remain contradictory.

OT synthesis and receptors are found in cardiac and vascular tissue (17). It has been reported that lactation alters maternal cardiovascular function, possibly through the actions of OT (31). Favourable effects of lactation on blood pressure have been attributed to OT (28). In animal models, the administration of OT leads to decreased blood pressure (35) and a reduction of myocardial infarct size in the rat (34). In addition, blockade of OT receptors by atosiban abrogates the cardioprotective effects of IPC (1).

The cardioprotective effects of glucocorticoids and adrenocorticotropin in IR injury have been experimentally demonstrated in animals (5, 40) and humans (16). Although a decline in the serum cortisol and adrenocorticotropin concentrations in the breast feeding women was found (2), there is a positive association between duration of lactation and cortisol levels in postmenopausal women (27), that means that both increased cortisol levels and increased duration of breast-feeding have protective roles in the cardiovascular system.

The release of OT and prolactin during suckling is associated with the vagus nerve activity (11). The results of previous studies have shown that vagus nerve stimulation (VNS) offers protection against ischemic brain (3) and myocardial (38) injury. The researchers found that cardioprotective effects of VNS including reduced infarct size and decreased

ventricular fibrillation episodes were abolished by muscarinic blockade (38). On the other hand, the protective signal of remote IPC reaches the heart through the vagus nerve and activation of muscarinic receptors (9).

Together these data suggest that lactation by a wide range of hormones and different pathways creates protective alterations in the cardiovascular function that have not yet been revealed. So, the mechanisms causing this protection remain to be explored. Also, it is necessary to reproduce this experiment to identify whether lactation also creates protection for other tissues against IR injury. The duration of the protective effect of lactation after weaning should also be determined. Future research is needed for the comparison of maternal females with and without breastfeeding.

In summary, lactation reduced the extent of myocardial injury induced by ischemia and reperfusion. So, there is evidence that lactation may increase cardiac tolerance to ischemic injury and is considered to be as a preconditioning stimulus in the lactating mothers.

Acknowledgement

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